Handbook of hydrocolloids

Second edition

Edited by
G. O. Phillips and P. A. Williams

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Preface

The first edition of the *Handbook of Hydrocolloids* was published in 2000 and was exceptionally well received. It has been used as the substantive reference book in the subject. It was not meant to be a textbook, but a convenient reference to provide the relevant information readily and at the same time authoritatively. The chapters were all written by top specialists in their fields. Since the year 2000, the subject has moved on with remarkable speed. The dominant industrial influence has been the succession of mergers and acquisitions. The names of very many of the companies which participated over the years have either disappeared or now form part of bigger amalgamations. The specialist single product producer has given way to the global multi-ingredient suppliers. Now it is the global giants who dominate the ever increasing technological industry. While such progress is, I suppose, inevitable, it has taken its toll on individuals who are finding it increasingly difficult to keep pace with changing materials, technologies, loyalties and names. Much corporate memory has been lost in these frantic changes. In this Second Edition we have kept this in mind and now provide a single and reliable reference volume where hydrocolloids structure, functionality, synergistic behaviour, applications and regulatory aspects are brought together.

These are the new improvements we have taken to ensure that we fully meet the core and developing hydrocolloid areas:

- First, all chapters in the first edition were re-visited and, where necessary, these chapters were up-dated.
- Now there is a much greater emphasis on the protein hydrocolloids. New chapters have therefore been included on egg proteins and vegetable proteins (soybean, pea, wheat, and other related protein isolates) and the more recent information about fish gelatin has been added.

- We have increased the coverage of microbial polysaccharides, with a new chapter on the newly emerging microbial polysaccharides such as pullulan, scleroglucan, elsinan, levan, alternan, etc., which are now moving out of the scientific novelty area into practical use.
- The developing role of the exudate gums has been recognized, with a new chapter on gum ghatti included. This old gum is rapidly making a resurgence, particularly as a clean label natural emulsifier and adhesive. New sections have been added also on mesquite gum and larchwood arabinogalactans.
- Protein-hydrocolloid complexes are now extensively studied in order to gain synergy from both components. A new chapter is, therefore, included to chart these new developments.
- There is a new chapter on the function of hydrocolloids as emulsifiers and the factors which determine the stabilization and long-term stability of the emulsions.
- The customer is now not only demanding convenience but also healthier foods. The functional foods, or nutraceuticals, have now come of age. Sales of such foods have risen exponentially. Obesity, calorific value, fat replacement, glycaemic index and dietary fibre are at the forefront of the medical and political platform. A new chapter has been added to acknowledge this vital new emphasis in using hydrocolloids in foods. Information has also been added about health aspects where relevant to other updated chapters.
- There is a complete new chapter on the extraction, structure, analysis, physical chemical properties, technology, applications and health benefits of arabinoxylans.

Without question it can prove rewarding to become well aquainted with the hydrocolloids described in this book. The tumultuous growth associated with the revolution already referred to is both confusing and perplexing. The choice of hydrocolloids is larger but fewer and fewer companies are in a position to provide them because, as they become larger, each company tries to provide the widest possible range of hydrocolloids. Due to the rash of recent mergers, a single company can now provide galactomannans, guar and locust bean gum, pectins, alginates, carrageenans, xanthan and gelatin. Added to this, the technological developments are leading to the crossing of traditional boundaries. Carrageenan is challenging the functionality of gelatin. Starch is trying to replicate the behaviour of gum arabic, and so on. Therefore, there is no substitute for studying the hydrocolloids individually and objectively, in order to avoid this over-concentration of expertise and supply. We hope that this handbook in its new and enlarged form will assist in this respect. We welcome any comments or suggestions. We certainly hope that it will assist all levels of reader, from the student to the experienced scientist, to understand this rapidly growing, enjoyable yet challenging subject.

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Contents

1

Introduction to food hydrocolloids

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Abstract: This introductory chapter provides an overview of the source, market, functional characteristics and regulatory aspects of hydrocolloids used in foods. Examples of a number of different food products are given and the role of the hydrocolloids in controlling their texture and properties is discussed. A brief introduction to the rheological properties of the various types of hydrocolloids is provided and comparisons of the viscosity and viscoelastic behaviour are given. Many hydrocolloids are able to form gels in response to changes in temperature and/or in the presence of ions. The various mechanisms by which gelation occurs are summarised. The interactions that occur in systems containing mixtures of hydrocolloids are considered and combinations demonstrating synergistic behaviour are highlighted. The role of hydrocolloids as dietary fibre is an area of increasing importance because of the associated benefits for health and this area is briefly reviewed.

Key words: source of hydrocolloids, hydrocolloids, market, regulatory aspects, rheological properties, viscosity, storage and loss moduli, food products, dietary fibre, health benefits.

1.1 Introduction

The term ‘hydrocolloids’ is commonly used to describe a range of polysaccharides and proteins that are nowadays widely used in a variety of industrial sectors to perform a number of functions including thickening and gelling aqueous solutions, stabilising foams, emulsions and dispersions, inhibiting ice and sugar crystal formation and the controlled release of flavours, etc. The commercially important hydrocolloids and their origins are given in Table 1.1.

Table 1.1 Source of commercially important hydrocolloids

Botanical	
trees	cellulose
tree gum exudates	gum arabic, gum karaya, gum ghatti, gum tragacanth
plants	starch, pectin, cellulose
seeds	guar gum, locust bean gum, tara gum, tamarind gum
tubers	konjac mannan
Algal	
red seaweeds	agar, carrageenan
brown seaweeds	alginate
Microbial	xanthan gum, curdlan, dextran, gellan gum, cellulose
Animal	Gelatin, caseinate, whey protein, soy protein, egg white protein, chitosan

The food industry, in particular, has seen a large increase in the use of these materials in recent years. Even though they are often present only at concentrations of less than 1% they can have a significant influence on the textural and organoleptic properties of food products. Some typical examples of foods containing hydrocolloids are shown in Fig. 1.1, clearly demonstrating the widespread application of these materials. The specific hydrocolloids used in the production of the individual products shown are:

- baked beans contain modified corn starch as a thickener
- hoi-sin sauce contains modified corn starch as a thickener
- sweet and sour sauce contains guar gum as a thickener
- Sunny Delight fruit drink contains modified starch as an emulsifier with carboxymethyl cellulose (CMC) and xanthan gum as thickeners
- the Italian dressing includes xanthan gum as a thickener
- 'light' mayonnaise contains guar gum and xanthan gum as fat replacers to enhance viscosity
- the yoghurt incorporates gelatin as a thickener rather than a gelling agent
- the mousse contains modified maize starch as a thickener with guar gum, carrageenan and pectin present as 'stabilisers'
- the Bramley apple pies contain modified maize starch with sodium alginate as gelling agent
- the fruit pie bars contain gellan gum and the blackcurrant preserve and redcurrant jelly contain pectin as gelling agents

Fig. 1.1 Examples of food products containing hydrocolloids.

- the trifle contains xanthan gum, sodium alginate and locust bean gum as 'stabilisers', modified maize starch as a thickener and pectin as a gelling agent.

The changes in modern lifestyle, the ever growing awareness of the link between diet and health and new processing technologies have led to a rapid rise in the consumption of ready-made meals, functional foods and the development of high fibre and low-fat food products. In particular, numerous hydrocolloid products have been developed specifically for use as fat replacers in food. This has consequently led to an increased demand for hydrocolloids. The world

hydrocolloids market is valued at around $4.4 billion p.a. with a total volume of about 260,000 tonnes. The market has been growing at the rate of 2–3% in recent years.

Hydrocolloid selection is dictated by the functional characteristics required but is inevitably influenced by price and security of supply. It is for these reasons that starches (costing typically < US$1/kg) are the most commonly used thickening agents. It is interesting to note here, however, that xanthan gum (~US$12/kg) is becoming the thickener of choice in many applications. This is because xanthan gum has unique rheological behaviour and its increased use has led to strong competition between supplier companies ensuring that the price has remained at reduced levels. Xanthan gum forms highly viscous, highly shear thinning solutions at very low concentrations and the viscosity is not influenced to any great extent by changes in pH, the presence of salts and temperature. The high viscosity at low shear enables the gum to prevent particle sedimentation and droplet creaming and the shear thinning characteristics ensure that the product readily flows from the bottle after shaking. This explains its widespread application in sauces and salad dressings.

Gelatin is by far the most widely used gelling agent, although with the increasing demand for non-animal products and in particular the Bovine Spongiform Encephalopathy (BSE) outbreak in the UK, prices have increased significantly over recent years. There is currently considerable interest in alternative sources of gelatine, notably fish skins, and in the development of gelatin replacements. The carrageenan market has also been very competitive over recent years due to the introduction of cheaper lower refined grades (Processed Euchema Seaweed, PES), which can be used effectively where gel clarity is not important. The price differential between carrageenan and PES has decreased and the use of carrageenans has increased markedly since its use in meat and poultry products was recently approved. A further development has been the introduction of kappa/iota carrageenan hybrids which have potential to provide novel functionality. The gum arabic market has traditionally been erratic due to price fluctuations and security of supply and much effort has been directed at finding alternatives. A number of starch-based substitutes (for example, succinylated starch) were introduced some years ago as alternatives in the emulsification of flavour oils and recent work has been concerned with using pectin (notably sugar beet pectin) as a gum arabic replacement. It has been shown that the emulsification properties of gum arabic and pectin are due to the small amount of protein (2–3%) which is present as in integral part of their structure. This has led to significant interest in the development of polysaccharide–protein complexes (gum arabic lookalikes) which can be formed by a mild heat treatment through the Maillard reaction and by electrostatic interaction. Gellan gum was approved for food use in Japan in 1988 but much later in the USA and Europe, and is beginning to establish its own niche markets. An overview of the hydrocolloids market is given in Figs 1.2(a) and 1.2(b).

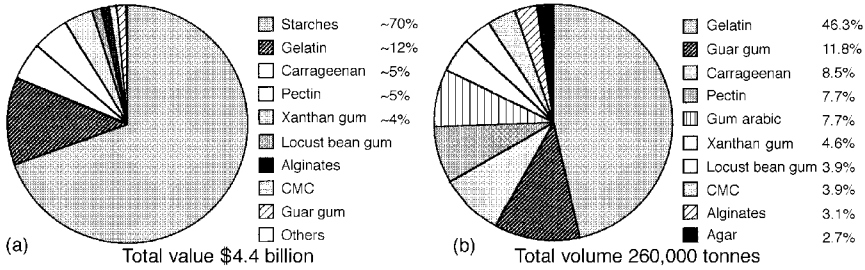

Fig. 1.2 (a) Value of world market for individual hydrocolloids; (b) Volume of world market for individual hydrocolloids.

1.2 Regulatory aspects

Food hydrocolloids do not exist as a regulatory category in their own right, rather they are regulated either as a food additive or as a food ingredient. With the exception of gelatin, however, the vast majority of food hydrocolloids are currently regulated as food additives.

1.2.1 International

The most widely accepted fully international system to regulate the safety of food additives is that set up by a Joint FAO/WHO Conference on Food Additives in September 1955, which recommended that the two organisations collect and disseminate information on food additives. Since that time more than 600 substances have been evaluated and provided with specifications for purity and identity by the Joint/WHO Expert Committee on Food Additives (JECFA).

JECFA was first established in the mid-1950s by the FAO and WHO to assess chemical additives in food on an international basis. In the early 1960s the Codex Alimentarius Commission (CAC), an international inter-governmental body, was set up with the primary aims of protecting the health of the consumer and facilitating international trade in food commodities. When CAC was formed, it was decided that JECFA would provide expert advice to Codex on matters relating to food additives. A system was established whereby the Codex Committee on Food Additives and Contaminants (CCFAC), a general sub-committee, identified food additives that should receive priority attention, which were then referred to JECFA for assessment before being considered for inclusion in Codex Food Standards. Specialists invited to serve as members of JECFA are independent scientists who serve in their individual capacities as experts and not as representatives of their governments or employers. The objective is to establish safe levels of intake and to develop specifications for identity and purity of food additives. The reports of the JECFA meetings are published in the WHO Technical Report Series. The toxicological evaluations, which summarise the data that serve as the basis for safety assignments, are

published in the WHO Food Additive Series. The specifications are published in the FAO Food and Nutrition Paper Series.

The procedure, therefore, is for JECFA to consider the specification of any given additive and to recommend to the Codex Committee for Food Additives and Contaminants (CCFAC) that this be adopted. If they agree after further consideration by all Member States at a Plenary Session, the specification can be confirmed by the full Codex Commission. The ultimate for a food additive, therefore, is to be included into the *Codex General Standard for Food Additives*. The procedure can prove lengthy, controversial and expensive, since all interested parties can input objections or amendments. However, once accepted, the food additive has world-wide currency.

The detailed procedures are described in *Codex Alimentarius* (General Requirements) Second Edition (Revised 1995). Thus *Codex Alimentarius* is a collection of internationally adopted food standards presented in a uniform manner. The food standards aim at protecting consumers' health and ensuring fair practice in the food trade. Once accepted, an international number is allocated to the additive which is an acknowledgement of its acceptability. It must be stressed that there are food additives which stay at the JECFA specification level and that this advisory specification is the authority for its use, at the conditions given, until the full acceptance by Codex is given. Gum arabic is one such example, a food hydrocolloid which has been used for more than 2000 years but only finally gained full Codex specification in June 1999.

1.2.2 The European system

Clearance of food hydrocolloids by the European Commission was first introduced in 1995 under Directive 95/2/EU for Food Additives other than colours and sweeteners. This is known as the Miscellaneous Additives Directive (MAD), which provides authorisation for a large number of additives from the hydrocolloid group. The majority of these are authorised for general use in foods to Quantum Satis (QS) levels given in Annex 1 of the Directive. Starches, the vast majority of gums, alginates and celluloses enjoy this wide authorisation.

Almost immediately following adoption of the original Directive, the Commission began working on proposed amendments, largely to take account of market developments that had not been taken into account in the last stages of the lengthy and complicated legislative process. The historical development of the process must be referred to in order to understand the almost unintelligible machinery adopted by the European Commission in its work. The ground rules for food additives harmonisation were set out in the form of a framework Directive, 89/107/EEC adopted in 1988 (and amended by the European Parliament and Council Directive 94/34/EC). It instructs the Council to adopt in subsequent follow-up Directives

- a list of additives to be authorised
- a list of foods to which the additives may be added and the levels of use,

which gives delegated powers to the Commission to adopt

- specifications for each additive
- where necessary, methods of analysis and procedures for sampling.

A number of general criteria for the use of additives in food are also set out. According to the criteria, food additives may be authorised only if

- a reasonable technological need can be demonstrated
- they present no hazard to health at the levels proposed
- they do not mislead the consumer.

Evidence of the need for an additive which, incidentally, plays no part in approvals in the USA, must be provided by the user of the additive, that is the food manufacturer, not the supplier or manufacturer of the additive. The criteria also stipulate that all food additives must be kept under 'continuous observation and re-evaluated whenever necessary in the light of changing conditions of use and new scientific observation'.

The EC process acknowledges the JECFA system and in the most unintelligible legal language adopted by the Commission adds the following:

- Whereas Directive 78/663/EEC should be repealed accordingly:
- Whereas it is necessary to take into account the specification and analytical techniques for food additives as set out in the Codex Alimentarius as drafted by JECFA:
- Whereas food additives, if prepared by production methods or starting materials significantly different from those included in the evaluation of the Scientific Committee for Food, or if different from those mentioned in this Directive, should be submitted for evaluation by the Scientific Committee for Food for the purposes of a full evaluation with emphasis on the purity criteria.

It is surprising that any progress was made at all with all the accompanying bureaucracy.

After a most highly political first amendment to the MAD intended to clear Processed Eucheuma Seaweed (E407a) (see Directive 96/85/EC of 19 December 1996), a second amendment was introduced which affected many hydrocolloids. Member States were required to implement the provisions of this Directive in the year 2000. Thus at this time the new authorisation introduced by the current Directive 98/72/EC came into force in all EU Member States. This Directive provides for E401 Sodium alginate, E402 Potassium alginate and E407 Carrageenan, E440 Pectin, E425 Konjac and E412 Guar. Each has controlling conditions associated with the approval.

1.2.3 Other trade blocks

While the Codex Alimentarius Commission is the ultimate specification and can provide for approval throughout the world, each country (outside the EU) is free to adopt its own standards. In the USA, for example, the United States Food

Chemicals Codex (FCC) also has currency. The FCC is an activity of the Food and Nutrition Board of the Institute of Medicine that is sponsored by the United States Food and Drug Administration (FDA). The current specification of hydrocolloids are to be found in the Fourth Edition (1996). Japan too has its own specifications which include many of the food additives particular to Japan.

1.2.4 The international numbering system for food additives (INS)

INS has been prepared by the Codex Committee on Food Additives in order to be able to identify food additives in ingredient lists as an alternative to the declaration of the specific name. The INS is intended as an identification system for food additives approved for use in one or more member countries. It does not imply toxicological approval by Codex.

There is an equivalence with the EU system of E numbers, albeit that the EU system is more restricted. Where both INS and E numbers are available they are interchangeable. The list of INS numbers is given in Tables 1.2 and 1.3.

1.3 Thickening characteristics

Hydrocolloids are widely used to thicken food systems and a much clearer understanding of their rheological behaviour has been gained over the last thirty years or so, particularly through the development of controlled stress and controlled strain rheometers capable of measuring to very low shear rates ($<10^{-3}\,s^{-1}$). The viscosity of polymer solutions shows a marked increase at a

Table 1.2 INS numbers for modified starches

Modified starch*	INS number
Dextrin (roasted starch)	1400
Acid treated starch	1401
Alkali treated starch	1402
Bleached starch	1403
Oxidised starch	1404
Monostarch phosphate	1410
Distarch phosphate	1412
Phosphated distarch	1413
Acetylated starch	1414
Starch acetate	1420
Acetylated distarch adipate	1422
Hydroxypropyl starch	1440
Hydroxypropyl distarch phosphate	1442
Starch sodium octenyl succinate	1450
Starch, enzyme treated	1405

*The Codex General Standard for labelling of pre-packaged foods specifies that modified starches may be declared as such in a list of ingredients. However, certain countries require specific identification and use these numbers.

Table 1.3 INS numbers for hydrocolloids

Polysaccharide	INS number	Function
Alginic acid	400	Thickening agent, stabiliser
Sodium alginate	401	Thickening agent, stabiliser, gelling agent
Potassium alginate	402	Thickening agent, stabiliser
Ammonium alginate	403	Thickening agent, stabiliser
Calcium alginate	404	Thickening agent, stabiliser, gelling agent, antifoaming agent
Propylene glycol alginate (propane-1,2-diol alginate)	405	Thickener, emulsifier, stabiliser
Agar	406	Thickener, stabiliser, gelling agent
Carrageenan (including furcelleran)	407	Thickener, gelling agent, stabiliser, emulsifier
Processed Euchema Seaweed	407a	Thickener, stabiliser
Bakers yeast glycan	408	Thickener, gelling agent, stabiliser
Arabinogalactan	409	Thickener, gelling agent, stabiliser
Locust bean gum	410	Thickener, gelling agent
Oat gum	411	Thickener, stabiliser
Guar gum	412	Thickener, stabiliser and emulsifier
Tragacanth gum	413	Emulsifier, stabiliser, thickening agent
Gum arabic (Acacia gum)	414	Emulsifier, stabiliser, thickener
Xanthan gum	415	Thickener, stabiliser, emulsifier, foaming agent
Karaya gum	416	Emulsifier, stabiliser and thickening agent
Tara gum	417	Thickener, stabiliser
Gellan gum	418	Thickener, gelling agent and stabiliser
Gum ghatti	419	Thickener, stabiliser, emulsifier
Curdlan gum	424	Thickener, stabiliser
Konjac flour	425	Thickener
Soybean hemicellulose	426	Emulsifier, thickener, stabiliser, anticaking agent
Pectin	440	Thickener, stabiliser, gelling agent, emulsifier
Cellulose	460	Emulsifier, anticaking agent, texturiser, dispersing agent
Microcrystalline cellulose	460 (i)	Emulsifier, anticaking agent, texturiser, dispersing agent
Powdered cellulose	460(ii)	Anticaking agent, emulsifier, stabiliser and dispersing agent
Methyl cellulose	461	Thickener, emulsifier, stabiliser
Ethyl cellulose	462	Binder, filler
Hydroxypropyl cellulose	463	Thickener, emulsifier stabiliser
Hydroxypropyl methyl cellulose	464	Thickener, emulsifier stabiliser
Methyl ethyl cellulose	465	Thickener, emulsifier, stabiliser, foaming agent
Sodium carboxymethyl cellulose	466	Thickener, stabiliser, emulsifier
Ethyl hydroxyethyl cellulose	467	Thickener, stabiliser, emulsifier
Cross-linked sodium carboxymethyl cellulose	468	Stabiliser, binder
Sodium carboxymethyl cellulose, enzymatically hydrolysed	469	Thickener, stabiliser

Certain hydrocolloids not included are either classed as food ingredients (e.g., gelatin) or have yet to complete the regulatory process.

critical polymer concentration, commonly referred to as C^*, corresponding to the transition from the so-called 'dilute region', where the polymer molecules are free to move independently in solution without interpenetration, to the 'semi-dilute region' where molecular crowding gives rise to the overlap of polymer coils and interpenetration occurs. For polymer molecules, C^* occurs at a volume fraction above 1.0, which is in contrast to the case, for example, of hard spheres which give rise to a dramatic increase in viscosity at a volume fraction of ~0.6. C^* is a function of the hydrodynamic volume of the polymer and is given by the following relationship:

$$C^* = a/[\eta] \qquad 1.1$$

where a is an integer which varies for different polysaccharides. For random coil polymers, a has a reported value of between 1 and 4.

Figure 1.3 shows the log of the zero shear viscosity (η_0) as a function of polymer concentration. The slopes of the lines for the dilute and semi-dilute regions are typically 1.4 and 3.3 respectively for random coil polymers. Polysaccharide solutions normally exhibit Newtonian behaviour at concentrations well below C^*, i.e. their viscosity is independent of the rate of shear; however, above C^* non-Newtonian behaviour is usually observed. A typical viscosity–shear rate profile for a polymer solution above C^* is given in Fig. 1.4 and shows three distinct regions: (a) a low-shear Newtonian plateau, (b) a shear-thinning region and (c) a high-shear Newtonian plateau. At low shear rates, the rate of disruption of entanglements is less than the rate of re-entanglement and hence viscosity is independent of shear. Above a critical shear rate, dis-

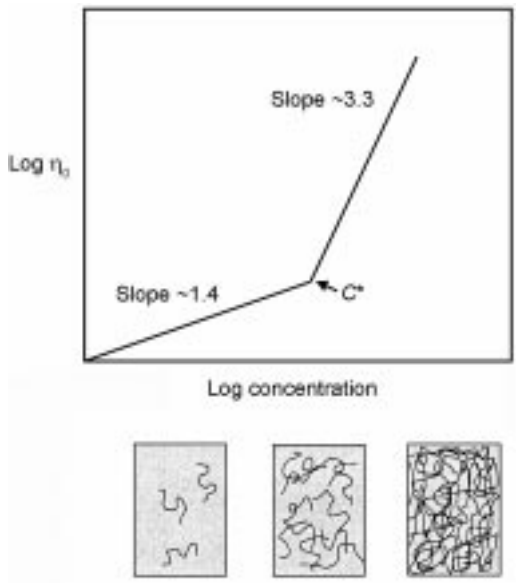

Fig. 1.3 Log of the zero shear viscosity as a function of polymer concentration.

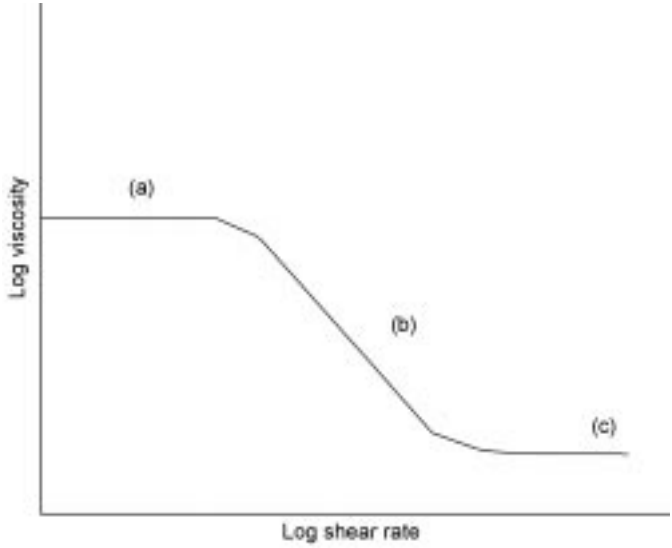

Fig. 1.4 Typical viscosity–shear rate profile for a polymer solution above C^*.

entanglement predominates and the viscosity drops to a minimum plateau value at infinite shear rate.

A number of empirical mathematical models have been developed to describe the flow characteristics. The most widely used model to describe the whole shear rate range is the Cross equation:

$$\eta = \eta_\infty + \frac{\eta_0 - \eta}{1 + (\tau\gamma)^m} \qquad 1.2$$

where η is the viscosity at a given shear rate, η_∞ is the infinite shear viscosity, η_0 is the zero shear viscosity, τ is a shear-dependent constant denoting the onset of shear thinning, γ is shear rate and m is an exponent quantifying the degree of shear thinning. m has a value of 0 for a Newtonian solution and increases to 1 with increased shear thinning.

The viscosity of polymer solutions is influenced significantly by the polymer molecular mass as illustrated in Fig. 1.5 which gives the shear viscosity of a series of guar gum samples as a function of shear rate. The viscosity shear rate dependency increases with increasing molecular mass and the shear rate at which shear thinning occurs shifts to lower values.

In addition to molecular mass effects, the hydrodynamic size of polymer molecules in solution is significantly influenced by molecular structure. Linear, stiff molecules have a larger hydrodynamic size than highly branched, highly flexible polymers of the same molecular mass and hence give rise to a much higher viscosity. This is illustrated in Fig. 1.6 which shows the viscosity–shear rate profiles for xanthan gum, carboxymethyl cellulose (CMC), dextran, guar gum and gum arabic. Xanthan consists of linear chains of β1,4-linked glucose residues

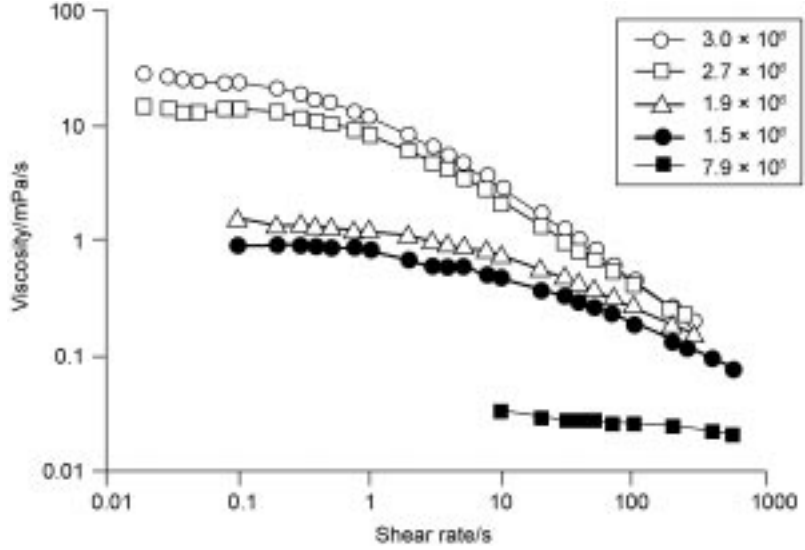

Fig. 1.5 Viscosity–shear rate profiles for 1% solutions of guar gum of varying molecular mass.

with a trisaccharide side chain attached on every other glucose unit. The molecules adopt an ordered double helical conformation at room temperature and hence are very stiff (persistence length, q, > 100 nm). Xanthan gum has a unique rheological profile with a very high viscosity at low shear rates (providing good suspending properties) but exhibiting extensive shear thinning characteristics

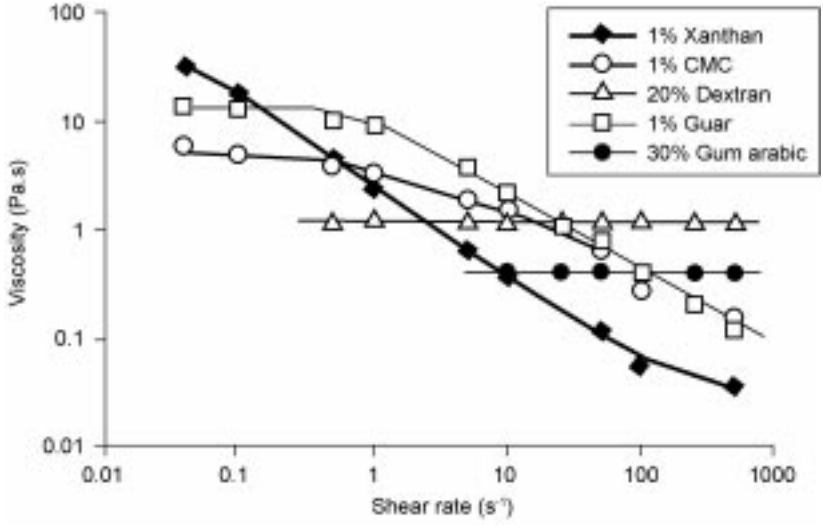

Fig. 1.6 Viscosity–shear rate profiles for 1% xanthan gum, 1% CMC, 1% guar gum, 20% dextran and 30% gum arabic.

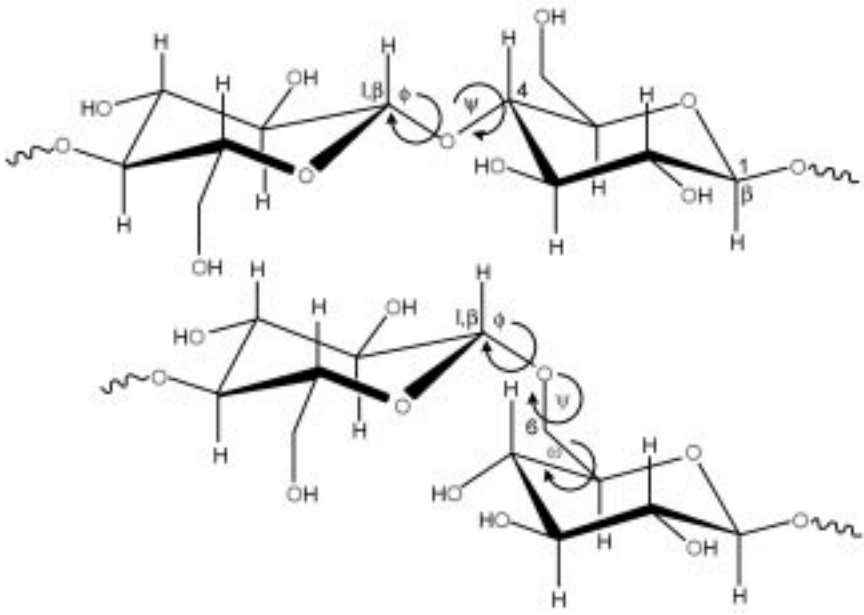

Fig. 1.7 Disaccharide repeat units showing 1,4 (top) and 1,6 (bottom) glycodisic linkages. ψ, φ and ω represent the bonds of rotation.

(readily flows on shearing). This behaviour is probably due to weak associations formed between the molecular chains. CMC consists of linear chains of β1,4-linked glucose residues while guar gum consists of linear chains of β1,4-linked mannose residues with approximately half having a galactose residue attached through α1,6-linkages. Both adopt relatively stiff ribbon-like conformations (q ~ 10–30 nm). They have typical viscosity–shear rate profiles exhibiting a high viscosity Newtonian plateau at low shear and shear thin above a critical shear rate. Dextran consists of linear chains of α1,6-linked glucose residues with some branching (linked 1,3 and 1,4). It has a very flexible compact structure since the (1,6) glycosidic linkage has three bonds of rotation between the glucose residues rather than two as is the case for other glycosidic bonds (Fig. 1.7). Significant interpenetration of molecular chains does not occur even at concentrations of 20% and hence the viscosity–shear rate profile exhibits Newtonian characteristics. Similar behaviour also occurs for gum arabic which has a very highly branched structure. It should be noted, however, that although the viscosity of dextran and gum arabic is much less than the viscosity of the 1% xanthan, CMC and guar solutions at low shear, they are greater at high shear rates.

Charged polymers have a higher viscosity than non-ionic polymers of similar molecular mass because their molecular coils are expanded as a consequence of intramolecular charge repulsions. Addition of electrolyte or adjustment of the pH to reduce the degree of dissociation of the charged groups normally leads to compaction of the coils and a significant drop in viscosity. The main hydrocolloid thickeners used in food products are listed in Table 1.4.

Table 1.4 Main hydrocolloid thickeners

Xanthan gum Very high low-shear viscosity (yield stress), highly shear thinning, maintains viscosity in the presence of electrolyte, over a broad pH range and at high temperatures.
Carboxymethyl cellulose High viscosity but reduced by the addition of electrolyte and at low pH.
Methyl cellulose and hydroxypropyl methyl cellulose Viscosity increases with temperature (gelation may occur) not influenced by the addition of electrolytes or pH.
Galactomannans (guar and locust bean gum) Very high low-shear viscosity and strongly shear thinning. Not influenced by the presence of electrolyte but can degrade and lose viscosity at high and low pH and when subjected to high temperatures.

1.4 Viscoelasticity and gelation

Hydrocolloid solutions are viscoelastic and can be characterised by the magnitude and frequency dependence of the storage and loss modulii, G' and G'' respectively. In dilute solutions below C^* where intermolecular entanglement does not occur, most polymers show G'' greater than G' over much of the frequency range. Both G' and G'' show significant frequency dependence, G' is proportional to the frequency, ω, while G' is proportional to ω^2, hence at higher frequencies $G' > G''$. At higher concentrations, in the entanglement region above C^*, G' and G'' are still frequency dependent but G' is greater than G'' over a broader range of frequencies.

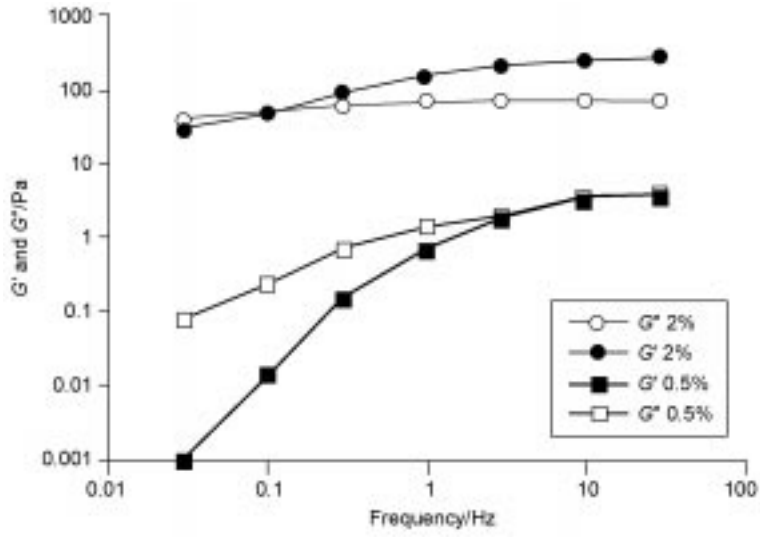

Fig. 1.8 G' and G'' of 0.5% and 2.0% guar gum solutions as a function of frequency.

This is illustrated in Fig. 1.8 which shows the frequency dependence for guar gum solutions at concentrations of 0.5% and 2.0%.

Some polysaccharides, notably xanthan gum, have a tendency to undergo weak intermolecular chain association in solution leading to the formation of a three-dimensional network structure. The junction zones formed can be readily disrupted even at very low shear rates and the network structure is destroyed. In these systems $G' > G''$ over a broad frequency range and both have a reduced frequency dependence. This is illustrated in Fig. 1.9(a) which shows the

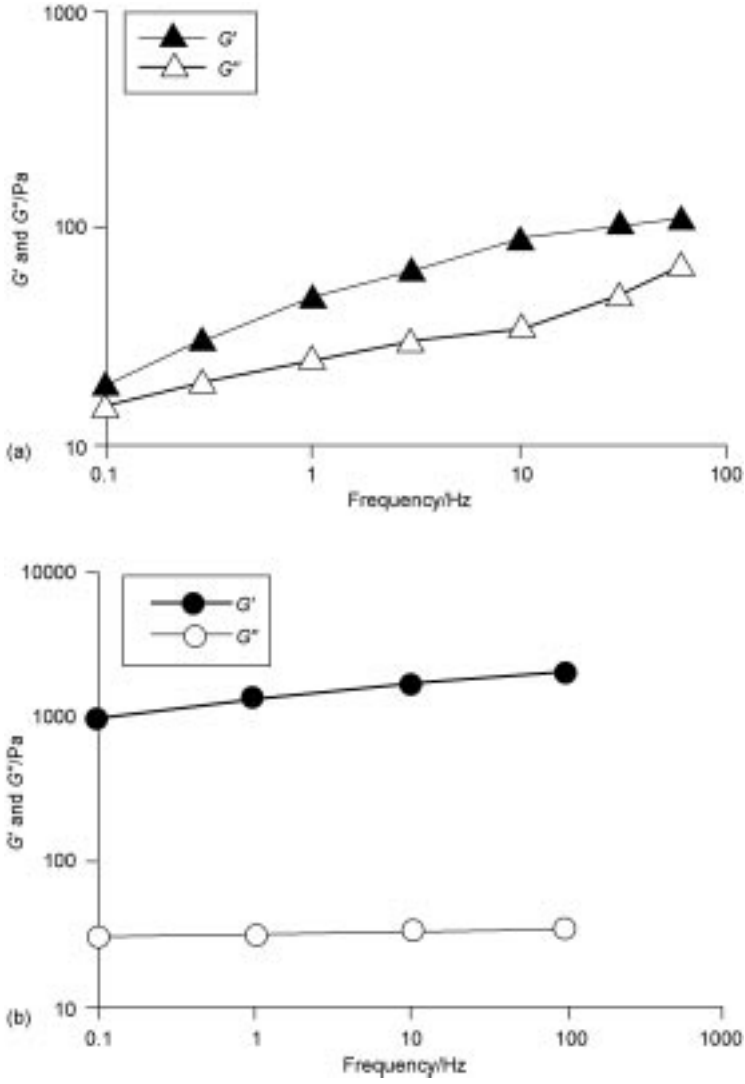

Fig. 1.9 (a) G' and G'' of 1% xanthan gum solution as a function of frequency; (b) G' and G'' of 1.5% amylose gels as a function of frequency.

mechanical spectrum for a 1% solution of xanthan gum and is typical for 'weak gels'. Other polysaccharides, for example amylose, agarose, carrageenan and gellan gum, can form stable intermolecular regions of association (referred to as junction zones) and as a consequence strong gel structures are produced. For these systems $G' \gg G''$ and both are virtually independent of frequency over this frequency range (Fig. 1.9(b)).

Hydrocolloid gels are referred to as 'physical gels' because the junction zones are formed through physical interaction, for example, by hydrogen bonding, hydrophobic association, cation-mediated crosslinking, etc., and differ from synthetic polymer gels which normally consist of covalently crosslinked polymer chains.

Some hydrocolloids form thermoreversible gels and examples exist where gelation occurs on cooling or heating. Some form non-thermoreversible gels. In such cases gelation may be induced by crosslinking polymer chains with divalent cations. Gels may be optically clear or turbid and a range of textures can be obtained. Gel formation occurs above a critical minimum concentration which is specific for each hydrocolloid. Agarose, for example, will form gels at concentrations as low as 0.2%, while for acid-thinned starch, a concentration of ~15% is required. Gel strength increases with increasing concentration. Molecular mass is also important. It has been shown that gel strength increases significantly as molecular mass increases up to ~100,000 but then becomes independent of molecular mass at higher values. The principal hydrocolloid gelling agents are listed in Table 1.5 and a comparison of their relative gel textures is illustrated in Fig. 1.10. An increase in brittleness is usually accom-

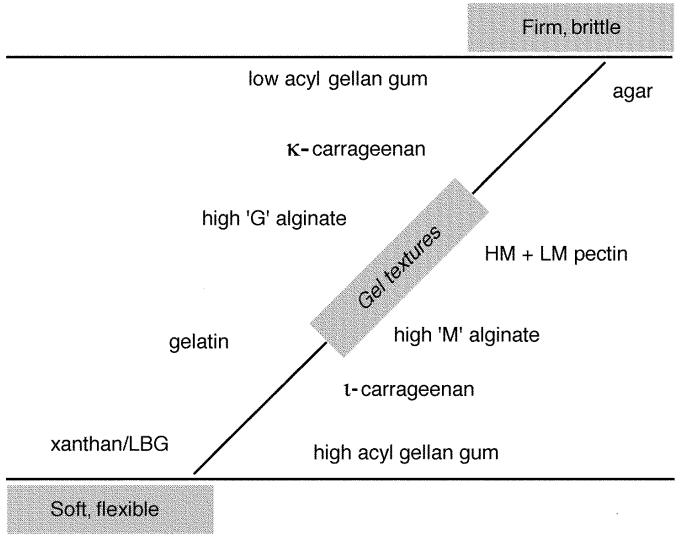

Fig. 1.10 Qualitative comparison of the textures of gels produced by different hydrocolloids.

Table 1.5 Main hydrocolloid gelling agents

1. Thermoreversible gelling agents

Gelatin
Gel formed on cooling. Molecules undergo a coil-helix transition followed by aggregation of helices.

Agar
Gel formed on cooling. Molecules undergo a coil-helix transition followed by aggregation of helices.

***Kappa* Carrageenan**
Gel formed on cooling in the presence of salts notably potassium salts. Molecules undergo a coil-helix transition followed by aggregation of helices. Potassium ions bind specifically to the helices. Salts present reduce electrostatic repulsion between chains promoting aggregation.

***Iota* Carrageenan**
Gel formed on cooling in the presence of salts. Molecules undergo a coil-helix transition followed by aggregation of helices. Salts present reduce electrostatic repulsion between chains promoting aggregation.

Low methoxyl (LM) pectin
Gels formed in the presence of divalent cations, notably calcium at low pH (3–4.5). Molecules crosslinked by the cations. The low pH reduces intermolecular electrostatic repulsions.

Gellan gum
Gels formed on cooling in the presence of salts. Molecules undergo a coil-helix transition followed by aggregation of helices. Salts reduce electrostatic repulsions between chains and promote aggregation. Multivalent ions can act by crosslinking chains. Low acyl gellan gels are thermoreversible at low salt concentrations but non-thermoreversible at higher salt contents (> 100mM) particularly in the presence of divalent cations.

Methyl cellulose and hydroxypropylmethyl cellulose
Gels formed on heating. Molecules associate on heating due to hydrophobic interaction of methyl groups.

Xanthan gum and locust bean gum or konjac mannan
Gels formed on cooling mixtures. Xanthan and polymannan chains associate following the xanthan coil-helix transition. For locust bean gum the galactose deficient regions are involved in the association.

2. Thermally irreversible gelling agents

Alginate
Gels formed on the addition of polyvalent cations notably calcium or at low pH (< 4). Molecules crosslinked by the polyvalent ions. Guluronic acid residues give a buckled conformation providing an effective binding site for the cations (egg box model).

High methoxyl (HM) pectin
Gels formed at high soluble solids (e.g. 50% sugar) content at low pH < 3.5. The high sugar content and low pH reduce electrostatic repulsions between chains. Chain association also encouraged by reduced water activity.

Konjac mannan
Gels formed on addition of alkali. Alkali removes acetyl groups along the polymer chain and chain association occurs.

Locust bean gum
Gels formed after freezing. Galactose deficient regions associate.

panied by an increase in the tendency to undergo syneresis and is attributed to an increase in the degree of aggregation of molecular chains.

1.5 Synergistic combinations

Mixtures of hydrocolloids are commonly used to impart novel and improved rheological characteristics to food products and an added incentive is a reduction in costs. Classic examples include the addition of locust bean gum to *kappa* carrageenan to yield softer more transparent gels and also the addition of locust bean gum to xanthan gum to induce gel formation. The nature of the synergy can be due to association of the different hydrocolloid molecules or to phase separation. The various effects that can occur are summarised schematically in Fig. 1.11.

If the two hydrocolloids associate then precipitation or gelation can occur. Oppositely charged hydrocolloids (e.g. a protein below its isoelectric point and an anionic polysaccharide) will associate and may form soluble or insoluble complexes depending on the pH, mixing ratio and ionic strength of the solution. There is evidence to show that some stiff polysaccharide molecules (xanthan gum, carrageenan, etc.) will associate with galactomannans and glucomannans leading to gel formation. If the two hydrocolloids do not associate, as is commonly the case, then at 'low' concentrations they will appear to exist as a single homogeneous phase while at higher concentrations they will separate in time into two liquid phases each enriched in one of the hydrocolloids. The phase separation process involves the formation of 'water-in-water' emulsions which consist of droplets enriched in one hydrocolloid dispersed in a continuous phase enriched in the other. Whether the hydrocolloid is present in the dispersed or continuous phase depends on the relative concentrations. If either or both of the hydrocolloids can form gels independently, then phase separation and gelation will occur simultaneously. The characteristics of the resultant gel will depend on the relative rates of these two processes. Careful selection of hydrocolloid type and concentration can, therefore, lead to the formation of a broad range of gel textures and this is currently an area receiving considerable attention.

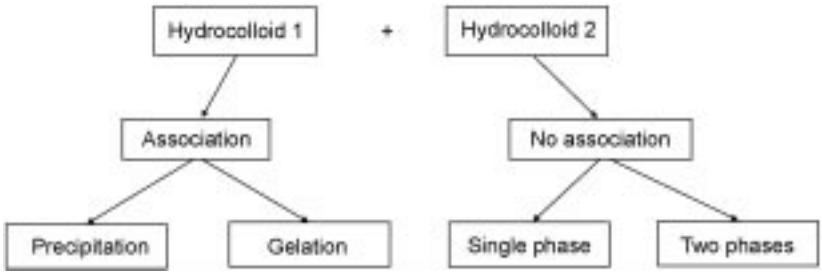

Fig. 1.11 Schematic representation of the interactions that occur in solutions containing mixtures of hydrocolloids.

1.6 Hydrocolloid fibres

Because there is a growing belief throughout the world that natural fibre foods are an integral part of a healthy lifestyle, food producers source an increasing proportion of their raw materials from nature itself. There is a growing demand from an increasingly health-conscious consumer for reduced fat and enhanced fibre foods of all types. If this can be achieved using materials which have low calorific value, further health benefits will result. Foods containing such ingredients will need to match the quality of the original product and without adverse dietary effects. This target cannot be achieved without the scientific use of thickeners, stabilisers and emulsifiers, particularly of the 'natural type'. This calls for fibres, which can interact with water to form new textures and perform specific functions, which itself requires the use of hydrocolloids. In 1998 the world market for such hydrocolloids of the fibre type was US$2.83 million and is set to grow significantly to meet the health aspirations of the consumer. It is the task of the food scientist to provide the hydrocolloids in the most appropriate form for inclusion in the food product. This requires an understanding of their structure and the way in which they act to produce the desired function in the food.

Dietary fibre was first described as the skeletal remains of plant cell walls, which are resistant to hydrolysis, by the digestive enzymes of man. Since this excluded polysaccharide fibres in the diet, the definition was subsequently expanded to include all polysaccharides and lignin, which are not digested by the endogenous secretions of the human digestive tract. Dietary fibre thus mainly comprises non-starch polysaccharides, and indeed has been defined by Englyst and others as the 'polysaccharides which are resistant to the endogenous enzymes of man'. Industrialised countries now generally recognise the health-giving properties of increased consumption of fibre and reduced intakes of total and saturated fat. In this respect 'fibre' is used in a non-specific way, but is generally taken to mean structural components of cereals and vegetables. More recently the concept of 'soluble fibre' has emerged which assists plasma cholesterol reduction and large-bowel fermentation.

The properties of such soluble and insoluble fibre allow them to perform both in a physical role and also to ferment through colonic microflora to give short-chain fatty acids (SCFA), mainly acetate, propionate and butyrate. These have a very beneficial effect on colon health through stimulating blood flow, enhancing electrolyte and fluid absorption, enhancing muscular activity and reducing cholesterol levels. The various hydrocolloids described in this handbook fall into this category.

1.6.1 The physical effect

To be effective dietary fibre must be resistant to the enzymes of the human and animal gastrointestinal tract. If physically suitable it can work effectively as a result of its bulking action. In the stomach and small intestine the fibre can increase digested mass, leading to faecal bulking, which readily explains the

relief of constipation, which is one of fibre's best documented effects. It can increase stool mass and ease laxation very efficiently. This behaviour has considerable human and agricultural importance. The growth of the ruminant animal depends on the fermentable fibre content of the stockfeed.

Soluble as well as non-soluble fibres exert their actions in the upper gut through their physical properties. Those which form gels or viscous solutions can slow down the transit in the upper gut and delay glucose absorption, best explained in terms of 'viscous drag'. Thus the reduction in glycemic response by soluble fibres can be explained.

1.6.2 Fermentation product effects

Large bowel micro-organisms attack the soluble fibres, in fermentation resembling that in the rumen of obligate herbivores such as sheep and cattle. The products too are similar: short-chain fatty acids (SCFA), gases (hydrogen, carbon dioxide and methane) and an increased bacterial mass. The principal SCFAs are the same in humans as in ruminants, and the concentrations are similar too, particularly for omnivorous animals with a similar digestive physiology (for example, the pig). The increased bacterial cell mass also has a positive effect on laxation. Faeces are approximately 25% water and 75% dry matter. The major components are undigested residuals plus bacteria and bacterial cell wall debris. These form a sponge-like, water-holding matrix which conditions faecal bulk and cell debris. The ability of different fibres to increase faecal bulk depends on a complex relationship between chemical and physical properties of the fibre and the bacterial population of the colon.

The production of SCFAs and their beneficial effects in humans and ruminant species has been well established for a considerable time, but the effect was not thought to be relevant to the carnivorous dog and cat. Now this too has been demonstrated.

1.6.3 Health benefits

Whether by physical bulking action or through the production of SCFAs, several health advantages are now established. Increasing fibre (20–30 grams per day in humans) can eliminate constipation through increased faecal bulking and water-holding. The fermentation to produce SCFAs can also assist, since propionate stimulates colonic muscular activity and encourages stool expulsion.

It was at one time thought that fibre lodged in the colon could lead to inflammation and herniation. This has now been disproved, and fibre can now relieve diverticular disease conditions, probably in the same way as it relieves constipation. Applying a solution of SCFA into the colon of ulcerative colitis patients or into the defunctioned portion of surgical patients has given rise to substantial remission in colitis. It could be that the condition arises due to a defect in the fermentation process in these patients or in the products.

SCFAs stimulate water and electrolyte absorption by the mucosa and enhance

their transport through improving colonic blood flow. Fibre fermentation also reduces the population of pathogenic bacteria such as *Clostridia* and can prevent diarrhoea due to bacterial toxins. Epidemiological studies have shown repeatedly that populations with high levels of fibre in their diet have reduced risk of colon cancer. Protection may be through the SCFA butyrate, which inhibits the growth of tumour cells *in vitro*.

When applied to the companion animal, the increased production of SCFAs increases gut acidity marginally, which reduces the activity of putrefaction and pathogenic bacteria and so lowers toxin and thus reduces bad odours and bad smelling faeces. The low level of toxin production reduces the load on the liver and results in better coat and skin quality. Therefore, the ageing animal can look better and produce less offensive faeces.

The behaviour of SCFAs in the intestine can influence the immune system. Thus protection is possible against colonisation by opportunistic bacteria, and the improved colonisation of beneficial indigenous bacteria in the gut gives greater resistance to infectious bacteria.

1.7 Future trends

The introduction of totally new hydrocolloids for food use is restricted by the large financial investment required to obtain the necessary legislative approval. Probably the last hydrocolloid to go through this process was gellan gum. There are certain hydrocolloids, however, that have a long history of use in food in other parts of the world that have potential for use as food additives in the USA and Europe. A typical example is konjac mannan which has been used for hundreds of years in Japan to produce noodles and is eaten as a food in own right. This material has only recently gained approval for use as an additive in the West. When dissolved in water konjac mannan has similar properties to locust bean gum but produces higher viscosity solutions and also has a stronger synergistic interaction with kappa carrageenan and xanthan gum. The search for new synergistic combinations continues and this is becoming more fruitful as our understanding of the interactions and phase behaviour of hydrocolloid mixtures increases at the molecular level. New processing procedures are also being introduced and an area of particular interest at present is the formation of sheared gels to give novel rheological characteristics. This involves applying shear as the hydrocolloid is undergoing gelation and usually results in the formation of micron-size hydrocolloid gel particles. At a sufficiently high concentration, the systems formed can have a very high low-shear viscosity and display strong shear thinning characteristics.

As discussed above, although hydrocolloids have historically been used in foods to control the rheological properties and texture, consumers are being made increasing aware of their nutritional benefits. Many hydrocolloids (e.g., locust bean gum, guar gum, konjac mannan, gum arabic, xanthan gum and pectin) for instance, have been shown to reduce blood cholesterol levels. Others

(e.g., inulin and gum arabic) have been shown to have prebiotic effects. They are resistant to our digestive enzymes and pass through the stomach and small intestine without being metabolised. They are fermented in the large intestine to yield short chain fatty acids and stimulate the specific growth of beneficial intestinal bacteria, notably, bifidobacteria, and reduce the growth of harmful micro-organisms such as clostridia.

All in all the hydrocolloid market is currently very buoyant and the prospects for future growth are excellent.

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2

Hydrocolloids and emulsion stability

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Abstract: This chapter describes the physico-chemical principles underlying the functional role of hydrocolloids in oil-in-water emulsions. Basic terms such as emulsifier and stabilizer are defined, and the origins of electrostatic and steric interaction potentials are explained. The chapter discusses the main factors controlling flocculation, creaming, coalescence and Ostwald ripening, distinguishing between the differing effects of adsorbing and non-adsorbing hydrocolloids. The reader's attention is specifically directed towards new understanding concerning the rheological and microstructural control of emulsion stability by non-adsorbing hydrocolloids, and the great potential of electrostatic protein–polysaccharide interactions at the oil–water interface for enhancing emulsion properties.

Key words: emulsion stability, depletion flocculation, rheology and microstructure, hydrocolloid emulsifiers, protein–polysaccharide complexes.

2.1 Introduction

An emulsion is a dispersion of one liquid (the dispersed phase) as small spherical droplets in another immiscible liquid (the continuous phase). The two main types of emulsions are oil-in-water (O/W) and water-in-oil (W/O). Food emulsions of the oil-in-water type include, for example, milk, cream, salad dressings, sauces and beverages; examples of the water-in-oil type are butter and margarine. The oil phase is either a flavour oil (in soft drinks) or a triglyceride oil (in dairy emulsions). In the latter case the oil phase is semi-solid at ambient temperatures due to the presence of dispersed fat crystals. Some emulsion products also contain other kinds of dispersed phases – gas bubbles (in whipped cream), starch granules (in cake batter) and ice crystals (in ice-cream). Despite the wide diversity of structures, compositions and textures of food emulsions,

there are some basic underlying principles that can be reliably used to understand and predict their general behaviour. One generally important factor influencing their stability properties is the presence of hydrocolloids.

Emulsions are thermodynamically unstable. With time they tend to break down into their constituent oil and aqueous phases. The term 'emulsion stability' therefore refers to the ability of an emulsion to resist this breakdown, as indicated by growth in average size of droplets or change in their spatial distribution within the sample. The more slowly that these properties change, the more stable is the emulsion. In practice, stability is a relative term which depends on the context. For some food emulsions, such as cake batters or cooked sauces, the required time-scale for stability is only a few minutes or hours. But for other products, such as soft drinks and cream liqueurs, emulsion stability must be maintained over a period of several months or years.

A hydrocolloid ingredient may act as an emulsifying agent, as a stabilizing agent, or in both of these roles. An emulsifying agent (emulsifier) is a surface-active ingredient which adsorbs at the newly formed oil–water interface during emulsion preparation, and it protects the newly formed droplets against immediate recoalescence. Given that polysaccharides are predominantly hydrophilic in molecular character, and most hydrocolloids are not surface-active, they cannot act as primary emulsifying agents. There is really only one hydrocolloid – namely, gum arabic – which is commonly employed as an emulsifying agent. The main emulsifying agents used in food processing are the proteins, especially those derived from milk or eggs. A stabilizing agent (stabilizer) is an ingredient that confers long-term stability on an emulsion, possibly by a mechanism involving adsorption, but not necessarily so. In O/W emulsions, the stabilizing action of hydrocolloids such as xanthan, carboxymethycellose, carrageenan, etc., is traditionally attributed to the structuring, thickening and gelation of the aqueous continuous phase.

In the context of this chapter, the expression 'emulsifying agent' is to be preferred over the more concise 'emulsifier'. This is because the latter term normally implies membership of the class of small-molecule surfactants, comprising lipid-based ingredients such as monoglycerides (e.g., GMS), phospholipids (lecithin) and polysorbates (Tweens). The functional role of these small-molecule emulsifiers in food technology is typically not for emulsion making, but for other reasons: controlling fat morphology and crystallization; promoting shelf-life through interaction with starch; and *de*stabilizing emulsions by competitive protein displacement from the oil–water interface (Dickinson, 1992).

Figure 2.1 illustrates the four main kinds of instability processes exhibited by O/W emulsions: creaming, flocculation, coalescence and Ostwald ripening. (For W/O emulsions, the processes are the same, except that sedimentation replaces creaming.) Flocculation is probably the most subtle and complicated phenomenon to control, because it can be triggered by so many different factors, and the resulting emulsion properties can be quite different depending upon whether the flocculation is weak or strong. In dairy-type O/W emulsions, at or below ambient temperature, instead of the full coalescence of liquid droplets, we have

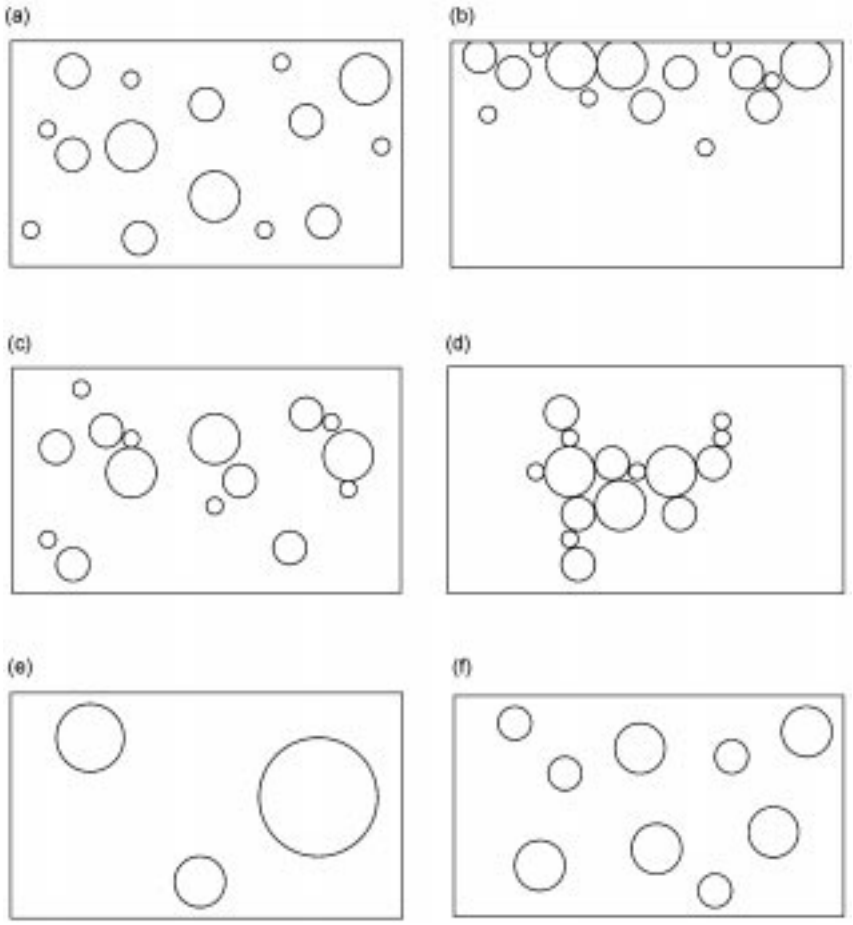

Fig. 2.1 Schematic representation of the key mechanisms of O/W emulsion instability: (a) stable dispersion of droplets; (b) creaming; (c) flocculation (weak); (d) flocculation (strong); (e) coalescence; (f) Ostwald ripening.

so-called 'partial coalescence' ('clumping') of semi-crystalline globules. In practice, two or more of the phenomena shown in Fig. 2.1 may happen at the same time, and the presence of one mechanism (e.g., flocculation) may trigger or enhance another (e.g., creaming or partial coalescence). Emulsion phase inversion, as in the shear-induced transformation of cream (O/W) into butter (W/O), is a multi-mechanism process.

Detailed theoretical analysis of these primary stability mechanisms is beyond the scope of this chapter. Readers looking for such an in-depth treatment are referred to the many standard texts (Dickinson and Stainsby, 1982; Hunter, 1986; Dickinson, 1992; Evans and Wennerstrom, 1994; Hiemenz and Rajagopalan, 1997; Walstra, 2003; Friberg *et al.*, 2004; McClements, 2005a; Leal-Calderon *et al.*, 2007). For any researcher embarking on a new experi-

mental investigation of the stability properties of emulsions, the recent critical overview of techniques and methodologies by McClements (2007) is recommended reading.

2.2 Principles of emulsion stability

A stable emulsion is one where the droplets remain sufficiently small and well separated that Brownian motion alone keeps them evenly dispersed throughout the continuous phase. The physico-chemical principles of O/W emulsion stability are based on the classical colloid theories of electrostatic and steric stabilization (Dickinson, 1992; McClements, 2005a). Electrostatic stabilization arises from the presence of electrical charge on the surface of the droplets, or more usually on the adsorbed stabilizer layer at the surface of the droplets. The greater the charge density at the surface, and the lower the ionic strength (electrolyte concentration) of the continuous phase, the more stable is the emulsion. Steric stabilization arises from the presence of a polymeric (steric) barrier at the droplet surface. To confer long-term stabilization, this polymer must be present at sufficient concentration to cover the oil–water interface completely, and it must remain permanently attached to the surface, with at least part of the molecule projecting away from the surface into the aqueous medium. Steric stabilization is increasingly supplemented by electrostatic stabilization in emulsions containing adsorbed proteins at pH values well away from the protein's isoelectric point (p*I*) (Damodaran, 2005).

Whether emulsion droplets will remain dispersed or will tend to stick together depends on the nature of the interparticle pair potential between the droplet surfaces. Generally speaking, colloidal stability requires that the interparticle repulsion should be of sufficient range and strength to overcome the combined effects of gravity, convection, Brownian motion, and the ubiquitous short-range attractive forces, which together drive the system towards its final and inevitable phase-separated equilibrium condition. Figure 2.2 shows two possible forms of the interaction potential (measured in units of the thermal energy, kT) as a function of the surface-to-surface separation (measured in arbitrary units of the order of nanometres). The shape of the potential $U(d)$ at any separation d tells us about the nature and strength of the interaction force between the surfaces. That is, the force F is equal to the (negative) derivative of the potential, i.e., $F = -\mathrm{d}U(d)/\mathrm{d}d$. Potential A in Fig. 2.2 corresponds to a stable system where the interaction force is repulsive or zero ($F \geq 0$) at all droplet separations. Potential B corresponds to a more complicated situation in which the interaction force is strongly attractive at close separations ($F < 0$, $d < 5$), repulsive at medium separations ($F > 0$), and weakly attractive or zero at larger separations ($F \leq 0$, $d \geq 12$). In this latter case, the system is stable with respect to coagulation (i.e., coalescence of emulsion droplets) but unstable with respect to reversible flocculation. In any particular emulsion, what determines whether the potential $U(d)$ is of type A or type B, or if it has some other

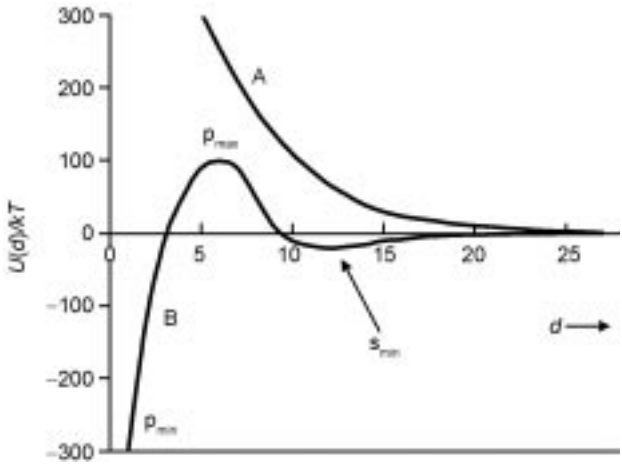

Fig. 2.2 Theoretical interaction potential between a pair of spherical emulsion droplets. The energy $U(d)$ (in units of kT) is plotted as a function of surface-to-surface separation d (arbitrary units). Curve A corresponds to a pure repulsive interaction. Curve B corresponds to a DLVO-type potential having a potential energy barrier more than sufficient to confer colloidal stability (p_{min} = primary minimum, p_{max} = primary maximum, s_{min} = secondary minimum). See text for further details.

functional form, is the delicate balance between the various types of molecular forces contributing to the overall interaction.

2.2.1 Electrostatic interactions

An important contribution to the stabilization of many food O/W emulsions arises from interdroplet electrostatic repulsion. As two identically charged spheres approach, there is an increase in the concentration of small ions of opposite charge (counterions) in the space between the surfaces. The consequent increase in local osmotic pressure in this gap leads to a repulsive force between the surfaces. As this repulsion has its origin in the overlap of the electrical double-layers surrounding the spheres, it is commonly referred to as the (electrical) double-layer repulsion. For a pair of spheres of radius a, the double-layer potential has the form

$$U_R(d) = 2\pi\epsilon_r\epsilon_0 a\psi_0^2 \ln[1 + \exp(-\kappa d)], \qquad 2.1$$

where ϵ_r is the dielectric constant of the aqueous medium, ϵ_0 is the permittivity of free space, ψ_0 is the surface potential, and κ^{-1} is the Debye length (a measure of the thickness of the double-layer). This equation is properly valid for systems with moderate surface potentials ($|\psi_0| \leq 30\,\text{mV}$) which is typically the case for food emulsions. For a simple 1:1 electrolyte (e.g., NaCl), the double-layer thickness is given by

$$(\kappa^{-1}/\text{nm}) = 0.30(I/\text{mol dm}^{-3})^{-1/2}, \qquad 2.2$$

where I is the salt concentration (ionic strength). With increasing salt concentration, the double-layer becomes thinner, and the electrostatic repulsion becomes weaker.

Strong forces of attraction occur between pairs of colloidal particles at close separations. These forces can be considered to arise from a sum of all the van der Waals forces acting between individual molecules in the different particles. For spheres of radius a, the resulting net van der Waals attraction is given by

$$U_{\mathrm{A}}(d) = -A_{\mathrm{H}}a/12d, \qquad 2.3$$

where A_{H} is the Hamaker constant ($\sim 5 \times 10^{-21}$ J for oil droplets in water). For very fine oil droplets, van der Waals attractive forces are important only at very close separations, but for coarse droplets they can be of quite long range (tens of nanometres). In the absence of any opposing repulsive forces (e.g., for uncharged surfaces, or in aqueous media of high ionic strength), dispersed droplets become spontaneously aggregated (coagulated) under the influence of these ubiquitous van der Waals forces. This situation is highly unstable, leading to immediate droplet coalescence.

In the classical theory of electrostatic stabilization, the double-layer energy U_{R} (equation 2.1) and the van der Waals energy U_{A} (equation 2.3) are added together algebraically to produce an overall pair potential:

$$U(d) = U_{\mathrm{R}}(d) + U_{\mathrm{A}}(d). \qquad 2.4$$

Equation 2.4 is commonly referred to as the DLVO potential after the four scientists (Derjaguin, Landau, Verwey and Overbeek) most closely associated with the theory. Due to the different functional forms of equations 2.1 and 2.3, the term $U_{\mathrm{A}}(d)$ tends to be more important at smaller and larger values of d, and the term $U_{\mathrm{R}}(d)$ is relatively more important at intermediate separations ($d \sim 2\kappa^{-1}$).

Potential A in Fig. 2.2 corresponds to the case where the double-layer repulsion is entirely predominant. Such a situation would correspond to a fine dispersion of highly charged droplets in an aqueous medium of low ionic strength. Under such conditions, the term $U_{\mathrm{R}}(d)$ is sufficient to overcome $U_{\mathrm{A}}(d)$ at all separations d, and the emulsion can be considered to be electrostatically stabilized. In estimating $U_{\mathrm{R}}(d)$ from DLVO theory, it is common practice in formulae such as equation 2.1 to replace the surface potential ψ_0 (more correctly, the Stern potential ψ_δ) by the so-called zeta potential (ζ-potential), i.e., the value of the potential at the plane of shear. The ζ-potential is a convenient and popular quantity because it is readily measurable in the laboratory. For most food emulsions, however, this measurement is open to misinterpretation because the classical double-layer model is not properly applicable to polymer-coated surfaces. In particular, the presence of adsorbed polymer tends to shift the plane of shear considerably away from the droplet surface; so we actually have $\zeta \ll \psi_\delta$. Nonetheless, the value of ζ can be quite useful as an indicator of the likely importance of electrostatic contributions to emulsion stability, especially when it is small, or its sign changes from +ve to −ve (or vice versa), as may

occur following adjustment of pH or introduction of a new species (e.g., hydrocolloid) into the aqueous medium. As a general rule, for an emulsion containing droplets with $\zeta < 15\,\text{mV}$, one cannot explain stability entirely in terms of double-layer repulsion. This is true even for aqueous media of very low ionic strength, despite such solution conditions being rarely encountered in food formulations.

Potential B in Fig. 2.2 corresponds to the situation where the electrostatic repulsion is predominant at intermediate separations. Such a situation would correspond to the case of moderately large charged droplets ($a > 1\ \mu\text{m}$) in an aqueous medium of low ionic strength. Curve B is characterized by a primary minimum (p_{min}) at small separations ($d \leq 2\,\text{nm}$), a primary maximum (p_{max}) at $d \approx 6\,\text{nm}$, and a secondary minimum (s_{min}) at $d \approx 12\,\text{nm}$. Here the magnitude of the primary maximum ($U_{max} \approx 100\ kT$) is very much more than sufficient to prevent the droplet pair from aggregating into the primary minimum under the influence of the short-ranged van der Waals forces. As a rough rule of thumb, a barrier height U_{max} of at least 15–20 kT is required to provide long-term colloidal stability with respect to aggregation in the primary minimum (and associated emulsion droplet coalescence). However, a system described by potential B would still be unstable with respect to flocculation in the secondary minimum. This is especially the case for coarse emulsion droplets (a few micrometres in diameter) since U_A increases linearly with the droplet size (see equation 2.3).

2.2.2 Steric interactions

When protein or hydrocolloid is present in an adsorbed layer around the emulsion droplets, there is an additional repulsive contribution to the interparticle pair potential due to the specific polymeric character of the stabilizer layer. There are three main requirements for a polymer to be effective as a steric stabilizer: (i) the adsorbed layer should be sufficiently thick; (ii) the coverage of the interface should be complete; and (iii) the polymer should be strongly (irreversibly) attached to the interface. Theory and experiment have demonstrated (Dickinson, 2006) that an adsorbed layer formed from a biopolymer, or a mixture of biopolymers, should be at least several nanometres in thickness to provide effective steric stabilization. This implies that part of the stabilizing macromolecule should be rather hydrophilic, with a tendency to distribute itself preferentially away from the oil–water interface. In other words, the aqueous continuous phase should be a 'good solvent' for part of the adsorbed polymer in order for it to be able to act effectively as a steric stabilizer. Reduction of this solvent quality (e.g., by change in temperature or pH, or by adding a cosolvent like ethanol) is detrimental to effective steric stabilization.

Steric repulsion arises from the entropically unfavourable overlap and compression of the adsorbed layers when polymer-coated droplets approach close together. The overlap of polymer layers is unfavourable because it leads to a local osmotic pressure gradient associated with the free energy of mixing of

solvent and polymer segments in the interdroplet overlap zone. The compression of layers is also unfavourable because it restricts the volume available to each polymer chain, leading to a statistical bias away from the most-probable (equilibrium) distribution of configurations, in an analogy with the elastic deformation of a rubber-like polymer network. The overall distance-dependent free energy change is given by

$$\Delta G_S(d) = \Delta G_m(d) + \Delta G_{vr}(d), \qquad 2.5$$

where ΔG_m is the 'mixing' (osmotic) free energy arising from polymer layer overlap and ΔG_{vr} is the 'volume restriction' (elastic) free energy arising from polymer layer compression. By analogy with equation 2.4, the overall pair potential is obtained by combining the steric free energy with the van der Waals energy:

$$U(d) = \Delta G_S(d) + U_A(d). \qquad 2.6$$

For sterically stabilized droplets of small size ($\ll 1\ \mu$m) under good solvent conditions, the steric free energy predominates at all separations, and the form of the overall potential $U(d)$ is qualitatively similar to that of curve A in Fig. 2.2. Assuming that the polymer stabilizer stays irreversibly adsorbed, the highly repulsive term ΔG_{vr} always dominates the overall interaction at very close separations, even under poor solvent conditions. Hence, in contrast to the electrostatic stabilization case, the sterically stabilized system has no primary minimum. This means that, as long as the oil–water interface is fully covered by the thick polymer layer, the droplets are extremely stable with respect to coalescence. On the other hand, large sterically stabilized droplets can be susceptible to flocculation due to the presence of an energy minimum in $U(d)$ somewhat analogous the DLVO secondary minimum of curve B in Fig 2.2.

Invariably, in a food O/W emulsion, the droplets carry a significant electrical charge, and the droplet surfaces are also coated with adsorbed biopolymers. Hence most food emulsions are stabilized by a combination of electrostatic and steric mechanisms. In fact, in the same colloidal system there is intimate coupling of factors affecting both mechanisms. That is, for the most effective stabilizing biopolymers, it is the presence of hydrated charged regions of the macromolecules which confers substantial thickness to the adsorbed layer, as well as significant charge density at the plane of shear. At the same time, the adsorbed biopolymer layer affects the DLVO-type potential in two different ways: (i) by changing the value of the effective Hamaker constant in U_A (see equation 2.3), and (ii) by changing the ionization of surface groups and partitioning of mobile ions between bulk phase and interface, causing perturbation of the double-layer repulsion U_R. This complexity makes the identification of the main mechanism of stabilization difficult in specific cases. Nevertheless, generally speaking, for a protein-stabilized emulsion at a moderate electrolyte concentration and a pH not too far away from pI, steric stabilization is likely to be the predominant mechanism.

2.2.3 Flocculation

Flocculation is defined as the coming together of emulsion droplets without rupture of the protective stabilizing layer. It occurs when the overall pair interaction energy at some separation d becomes appreciably negative, i.e., $|U(d)|$ has a value of the order of kT (weak and reversible flocculation) or is much larger (strong and irreversible flocculation). An emulsion may become flocculated in different ways:

- *Lowering of the surface charge density*. Flocculation may be induced by pH change or specific binding of ions (e.g., Ca^{2+}). This is most likely in a system where electrostatic repulsion makes an overriding contribution to $U(d)$, or where the configurations of the stabilizing polymers are especially sensitive to the charge distribution.
- *Increasing the ionic strength*. The addition of simple salts to the aqueous phase reduces the thickness of the electrical double-layer and so diminishes the range of the electrostatic repulsion.
- *Lowering of the solvent quality*. Flocculation is induced under thermodynamic conditions favouring reduced solubility of the adsorbed stabilizer in the aqueous medium, e.g., arising from pH change or ethanol addition.
- *Formation of polymer bridges during emulsification*. Bridging flocculation ('clustering') arises when insufficient emulsifier is readily available during emulsification to saturate the droplet surfaces. Hence adsorbed polymer molecules or particles (e.g., casein micelles) become shared between neighbouring droplets.
- *Formation of polymer bridges after emulsification*. Bridging may arise from cross-linking of adsorbed protein molecules on different droplets as a result of heat treatment. Bridges may also be formed through the development of associative interactions between a hydrocolloid additive and protein adsorbed at the oil–water interface (see Section 2.4.2).
- *Influence of non-adsorbed species*. The presence of various non-adsorbed species (micelles, nanoparticles or polymers) causes depletion flocculation. The effect can be explained in terms of an extra attractive (entropic) contribution to $U(d)$ arising from the exclusion of these species from the narrow gap between closely approaching droplet surfaces (see Section 2.3.2).

2.2.4 Creaming

Creaming is the movement of oil droplets under gravity to form a concentrated cream layer at the top of an O/W emulsion. Stokes' Law tells us that the creaming speed v of an isolated rigid droplet of density ρ and radius a is

$$v = 2a^2(\rho_0 - \rho)g/9\eta_0, \qquad 2.7$$

where ρ_0 and η_0 are respectively the density and (Newtonian) viscosity of the continuous phase, and g is the acceleration due to gravity. Equation 2.7 is strictly

applicable to an emulsion that is dilute, monodisperse and non-flocculated. For such a system, creaming may be slowed down in three main ways:

1. *Reduction of droplet size.* The mean droplet size is mainly determined by the emulsifier concentration and the efficiency of the emulsification equipment. Using a high-pressure homogenizer with an adequate emulsifier/oil ratio under favourable emulsifying conditions (e.g., with milk protein at neutral pH), it is relatively straightforward to prepare fine emulsions ($< 1\ \mu$m) that are non-flocculated and reasonably monodisperse. Nevertheless, some creaming is inevitable because it is impossible in practice to eliminate completely the small fraction of larger droplets.
2. *Reduction of density difference between phases.* The density difference between liquid milk fat and water is $\Delta\rho \sim 1 \times 10^2\,\mathrm{kg\,m^{-3}}$ at ambient temperatures. Apart from triggering the (partial) crystallization of the fat phase in the oil droplets, which reduces the density difference (and hence the creaming speed) by about 20%, there is little that can be done to influence the density difference in dairy emulsions. In flavour oil emulsions prepared for the soft drinks industry, however, it is common practice to incorporate an oil-soluble 'weighting agent' (e.g., ester gum, damar gum) in order to adjust the density of the citrus oil droplets towards that of the aqueous continuous phase (Tan, 2004).
3. *Increase in effective viscosity of aqueous medium.* Rheological control of the aqueous phase is the main function of hydrocolloids in food formulations. To make meaningful predictions of the effect of hydrocolloids on creaming stability, the viscosity η_0 appearing in equation 2.7 should be the apparent viscosity of the solution measured at very low shear stress ($\sim 10^{-2}$ Pa). This corresponds to the stress level exerted on the surrounding medium when gravity acts on micron-sized oil droplets in water.

Away from the dilute Stokes limit, the creaming rate is lower because of hydrodynamic interactions between the droplets. Therefore concentrated emulsions are inherently more stable to creaming than dilute emulsions. In fact, creaming becomes inhibited altogether when the oil volume fraction ϕ is sufficiently high that the droplets become close-packed (as in mayonnaise). A semi-empirical equation describing the creaming of a moderately concentrated emulsion is

$$v = v_S[1 - (\phi/\phi^*)]^{k\phi^*}, \qquad 2.8$$

where v_S is the Stokes speed from equation 2.7, ϕ^* is a parameter (≈ 0.6) approximating the close-packing limit for monodisperse spheres, and k (≈ 8) is an adjustable parameter.

Even though it may not be readily evident to the naked eye, some creaming must inevitably occur during extended quiescent storage of a moderately concentrated emulsion. For instance, Fig. 2.3 shows the oil volume fraction ϕ as a function of height in a sample of surfactant-stabilized emulsion (18 vol%

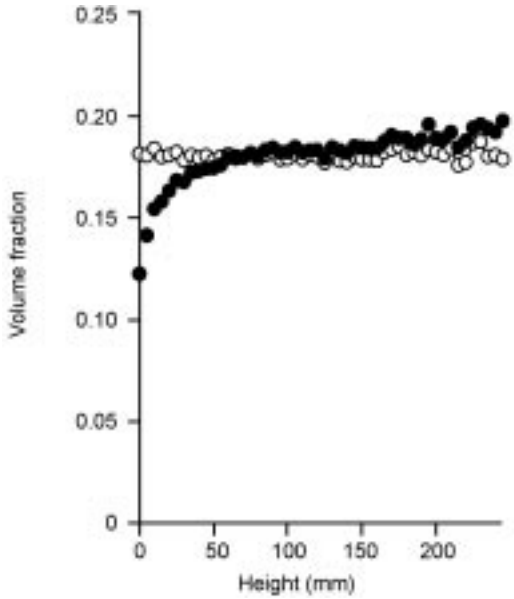

Fig. 2.3 Creaming profile of stable surfactant-stabilized emulsion (18 vol% mineral oil, 2 wt% Tween 20, mean droplet size $d_{32} = 0.65\,\mu$m, height 25 cm) as determined with the Leeds Acoustiscan ultrasonic profiler (Povey, 1998). Oil volume fraction ϕ is plotted against height for an unstirred emulsion sample at 30 ºC: ○, after 3 hours; •, after 188 hours. (Experimental data of Ma *et al.* (1994), as supplied by Prof. Macolm Povey.)

mineral oil, 2 wt% Tween 20) containing small droplets (mean droplet diameter $\ll 1\,\mu$m) stored at 30 ºC (Ma *et al.*, 1994). These data were obtained by ultrasound velocity scanning, which is an automated non-invasive technique allowing the determination of the local oil droplet density as a function of height for emulsion samples that are highly opaque (Povey, 1998). The freshly prepared emulsion has a vertically uniform oil content, but after one week of undisturbed storage there is a decrease in ϕ near the bottom of the sample tube and a corresponding increase in ϕ towards the top. The creaming behaviour shown in Fig. 2.3 is typical of that of a fine, unflocculated, low-viscosity emulsion in the absence of hydrocolloid stabilizer.

Emulsion creaming is strongly affected by the state of flocculation. Provided they do not get in each other's way, flocs move faster than individual droplets because of their greater effective size. Hence flocculation increases the creaming rate at low or medium ϕ. This effect is amplified by increased polydispersity due to the differential creaming speeds of smaller and larger droplets causing them to approach and associate more often than in a more monodisperse system. In a concentrated emulsion, flocculation tends to retard creaming due to the formation of an extensive network of interconnected flocs which are prevented from readily moving past one another under the influence of gravity. Phase separation may then occur only as a result of rearrangements and consolidation of the gel-like structure, with liquid continuous phase being exuded from gaps in the

flocculated droplet network by a process of syneresis rather than by conventional droplet creaming. The cross-over volume fraction from the dilute regime (enhanced creaming) to the concentrated regime (retarded creaming) is sensitively dependent on the microstructure of the flocs.

2.2.5 Coalescence

The merging of two or more emulsion droplets to form a larger single droplet is called coalescence. In the mouth, during eating, droplet coalescence has a positive role in enhancing flavour release. But coalescence perceived during emulsion storage is invariably considered as highly unacceptable in a food emulsion product. Droplet merging during product storage leads to enhanced creaming due to shifting of the overall distribution towards larger droplet sizes. Coalescence is also more probable for larger droplets, and so the process tends to be self-accelerating, leading on to the formation of a separate layer of free oil ('oiling off'). The rate depends on the nature of the interdroplet forces at very close surface–surface separations.

Once prepared without bridging flocculation, fine protein-stabilized emulsions are usually impressively stable towards coalescence. When it does occur, the phenomenon is most commonly found in dense cream layers or flocculated systems, especially when the adsorbed protein layer is weakened by the presence of surfactant (emulsifier), and/or when the emulsion is subjected to aeration or intense stirring.

Partial coalescence ('clumping') is the process whereby two or more partially crystalline oil droplets join together to form an irregularly shaped aggregate (Boode and Walstra, 1993; Walstra, 1996, 2003). It occurs when a solid fat crystal from one droplet penetrates the liquid oil region of another droplet. Each clump is composed of a continuous network of aggregated fat crystals held together by 'necks' of liquid oil. Partial coalescence is influenced by many of the same factors as affect normal coalescence. Two important additional factors are: (i) the temperature cycling of cream layers, which causes growth and melting of fat crystals, and (ii) agitation or stirring, which greatly increases the likelihood of collision-induced crystal penetration between droplets. Partial coalescence is a key mechanism during the churning of cream into butter, and also in the stabilization of air cells during ice-cream manufacture.

2.2.6 Ostwald ripening

Ostwald ripening is the growth of larger droplets at the expense of smaller ones due to mass transport of soluble dispersed material through the continuous phase. Because of the moderately high solubility of flavour oils in water, this is an important instability mechanism in soft drink emulsions. Unlike the other main instability mechanisms, Ostwald ripening is relatively insensitive to oil volume fraction or rheology of the continuous phase.

The thermodynamic driving force for Ostwald ripening is the increasing

chemical potential of a dispersed phase component with decreasing droplet size (Dickinson, 1992; McClements, 2005a). This leads to a greater tendency for dissolution from smaller droplets than from bigger ones. According to the classical Lifshitz–Slezov–Wagner theory, the coarsening rate follows the relationship

$$\mathrm{d}\langle a\rangle^3/\mathrm{d}t \propto K(\gamma)S_\infty D, \qquad 2.9$$

where $\langle a\rangle$ is the mean droplet radius, t is the time, S_∞ is the thermodynamic solubility of the solute in the continuous phase for a droplet with infinite curvature ($a \rightarrow \infty$), D is the diffusion coefficient of the solute in the continuous phase, and K is a characteristic length scale which is proportional to the interfacial tension γ. Equation 2.9 is strictly applicable to a dilute emulsion under steady-state conditions, where the shape of the droplet-size distribution is independent of time. For such a system, Ostwald ripening may be slowed in various ways:

- *Increase of droplet size.* Because the solubility of dispersed phase increases with decreasing droplet radius, the ripening rate becomes greater the smaller the mean droplet size. Hence the preparation a very fine emulsion – with the intention of reducing creaming (and flocculation/coalescence) – is not a successful strategy for ensuring stability if the rate of Ostwald ripening is significant.
- *Increase in monodispersity*. Because the thermodynamic driving force is related to differences in droplet size, the narrower the size distribution, the slower the rate. In theory, a perfectly monodisperse emulsion does not exhibit Ostwald ripening.
- *Reduction in interfacial tension*. Substantial lowering of γ (e.g., by adding surfactant) may be difficult to achieve in practice without adversely affecting the solubility of the dispersed material in the continuous phase. However, growth or shrinkage of individual droplets can be stopped altogether by the presence of an adsorbed elastic layer which mechanically resists the expansion or compression of the droplet surface area.
- *Incorporation of insoluble material into dispersed phase*. In practice, this is the most successful strategy. Entropy of mixing favours a similar composition for all dispersed droplets. So the mixing of another insoluble (or poorly soluble) component into the dispersed phase reduces the coarsening tendency by providing a large counteracting thermodynamic driving force in direct opposition to the Ostwald ripening effect.

2.3 Effect of non-adsorbing hydrocolloids on emulsion stability

A major function of hydrocolloids in food emulsions is to act as a thickening agent of the aqueous medium. The intention is to reduce or inhibit creaming by modifying the rheology of the continuous phase. In addition, the hydrocolloid

may impart a desirable texture and smooth mouthfeel to the product. This type of additive is functionally effective at very low concentrations (< 0.5%) due to its high molecular weight and extended macromolecular structure.

2.3.1 Rheology of hydrocolloid solutions

The effective hydrodynamic size of a non-gelling hydrocolloid in aqueous solution is indicated by its intrinsic viscosity $[\eta]$. Under highly dilute conditions where the solution is Newtonian, the intrinsic viscosity is defined by

$$[\eta] = \lim_{c \to 0}(\eta_{sp}/c), \tag{2.10}$$

where c is the polysaccharide concentration and η_{sp} is the specific viscosity, defined as

$$\eta_{sp} = \eta_r - 1 = (\eta/\eta_s) - 1. \tag{2.11}$$

Here η_r is the relative viscosity, η is the viscosity of the hydrocolloid solution, and η_s is the viscosity of the pure solvent ($c = 0$). The intrinsic viscosity increases with the weight-average molecular weight M_w according to the Mark–Houwink relationship:

$$[\eta] = K' {M_w}^{\alpha}, \tag{2.12}$$

where K' and α are constants (Tanford, 1961; Launay *et al.*, 1986). For linear flexible polymers in a good solvent (e.g., aqueous solutions of dextran, amylose or locust bean gum), the exponent α has a value in the range 0.5–0.8. For highly branched polymers (like amylopectin) the value of α lies below 0.5. Conversely, the value of α is greater than unity for stiff linear polymers and polyelectrolytes (especially at low ionic strength).

Each flexible polymer molecule in a dilute solution does not interact significantly with the other polymers. But as the polymer content increases, a certain concentration is reached ($c = c^*$) beyond which the neighbouring polymer coils begin to overlap. In this *semi-dilute* solution ($c > c^*$) each individual polymer segment (being mainly surrounded by solvent) 'sees' the solution as still dilute, but each whole polymer molecule 'sees' the solution as concentrated, since it is entangled with, and hence is interacting with, other polymer molecules in the solution (de Gennes, 1979). Hydrocolloids differ considerably in their values of c^*. In general, the higher the molecular weight of the polymer, the lower is the overlap concentration. But for the same molecular mass, $[\eta]$ is much smaller for a highly compact branched hydrocolloid like gum arabic than for an extended linear hydrocolloid such as carrageenan. Hence, whereas a 10 wt% gum arabic solution may be regarded as being still dilute ($c < c^*$), a carrageenan solution of 1 wt%, or a xanthan gum solution of only 0.1 wt%, is in the semi-dilute regime ($c > c^*$).

With non-gelling hydrocolloids whose flow properties are determined by non-specific repulsive chain–chain interactions, the relationship between the limiting zero-shear-rate viscosity η^0 and the polymer concentration c changes rather

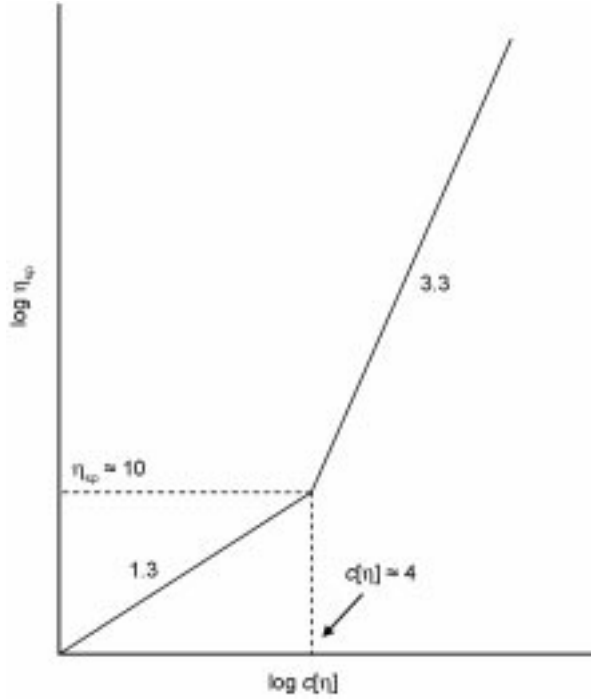

Fig. 2.4 Concentration-dependent viscosity of non-gelling hydrocolloid. The logarithm of the specific viscosity η_{sp} is plotted against the logarithm of $c[\eta]$, where c is the concentration and $[\eta]$ is the intrinsic viscosity defined by equation 2.10. The cross-over point between the dilute regime ($c < c^*$, slope ≈ 1.3) and the semi-dilute regime ($c > c^*$, slope ≈ 3.3) occurs at $\eta_{sp} \approx 10$ and $c^* \approx 4/[\eta]$.

abruptly at the overlap concentration. That is, when represented as a double logarithmic plot of specific viscosity η_{sp} ($\dot{\gamma} \to 0$) against $c[\eta]$, the experimental data fall on a master curve as shown in Fig. 2.4, with straight lines of slopes 1.3 and 3.3 representing the dilute and semi-dilute regimes, respectively (Morris, 1984). The transition point occurs at the concentration $c^* \approx 4/[\eta]$, corresponding to a viscosity of $\eta^0(c^*) \approx 10$ mPa s (i.e., $\eta_{sp} \approx 10$). More complex behaviour than that indicated in Fig. 2.4 is found with some hydrocolloids (e.g., guar gum or locust bean gum) whose molecules have gel-like attractive interactions between specific sequences on different chains. In these cases the onset of the semi-dilute regime occurs at lower c and the slope of the log–log plot of η_{sp} against $c[\eta]$ beyond $c = c^*$ is higher.

Whereas the dilute solution is (nearly) Newtonian in its flow behaviour, the semi-dilute solution is shear-thinning. The dependence of the apparent viscosity η on the shear-rate $\dot{\gamma}$ can be described, for instance, by the semi-empirical Cross equation (van Aken, 2006):

$$\eta = \eta^\infty + [(\eta^0 - \eta^\infty)/(1 + \tau\dot{\gamma}^m)]. \qquad 2.13$$

The quantities η^0 and η^∞ are the limiting viscosities at very low and very high shear rates, respectively. The parameter τ is a characteristic time constant corresponding to the average time of interpenetration/interaction of the deformed coil, and the exponent m has a value of 0.76. The value of τ increases with the hydrocolloid concentration and the molecular weight. For the hydrocolloid thickener of first choice in food emulsions, i.e., xanthan gum, the value of η^0 (as measured at $\dot{\gamma} \ll 1\,\mathrm{s}^{-1}$) is typically several orders of magnitude higher than η^∞ (as measured at $\dot{\gamma} \gg 10^3\,\mathrm{s}^{-1}$). This allows the thickened emulsion to flow readily under the moderately high-shear conditions encountered during product formulation, carton filling/emptying and eating. At the same time, the high value of η^0 provides the emulsion continuous phase with an extremely viscous environment, which resists the movement of individual droplets against the combined influences of gravity, interdroplet forces and Brownian motion.

2.3.2 Depletion flocculation and serum separation

One of the most common types of instability in food O/W emulsions is depletion flocculation caused by the presence of a low concentration of non-adsorbing hydrocolloid. Depletion flocculation is also induced by nanoparticles such as surfactant micelles and casein sub-micelles (Radford and Dickinson, 2004). The depletion contribution to the pair potential $U(d)$ is associated with an osmotic pressure difference between the interdroplet gap region depleted of polymer molecules (or nanoparticles) and the continuous phase beyond the interdroplet gap region. For the idealized case of flocculation of large hard spheres of radius a by small hard spheres of diameter d_s, the Asakura–Oosawa depletion potential has the form

$$U_\mathrm{d}(d) = \begin{cases} (-3kTa\phi_\mathrm{s}/{d_\mathrm{s}}^3)(d_\mathrm{s} - d), & (d < d_\mathrm{s}) \\ \\ 0, & (d \geq d_\mathrm{s}) \end{cases} \qquad 2.14$$

where ϕ_s is the volume fraction of the small spheres. The interaction is relatively weak. For instance, for two emulsion droplets of radius $a = 0.5\,\mu$m in a solution containing nanoparticles of diameter $d_s = 10\,$nm at a concentration of $\phi_s = 0.02$ (i.e., 2 vol%), equation 2.14 gives a maximum depletion attraction at contact ($d = 0$) of $U_d \approx -3kT$.

The depletion interaction arising from flexible non-adsorbed polymers is greater than that from spherical nanoparticles because the configurational entropy of the chains is substantially reduced near the particle surface (Vincent *et al.*, 1986). A useful theoretical expression is

$$U_\mathrm{d}(d) = -2\pi a\Pi(\Delta - \tfrac{1}{2}d)[1 + (2\Delta/3a) + (d/6a)], \qquad 2.15$$

where Π is the osmotic pressure of the polymer solution. The quantity Δ is the depletion layer thickness, which is approximately equal to the radius of gyration of the polymer in the dilute regime. Dilution of the continuous phase with

solvent leads to lowering of Π and hence $U_d(d)$, which means that this type of weak flocculation is completely reversible with respect to dilution by the continuous phase. Therefore its presence is not detectable with the conventional light-scattering equipment (e.g., Malvern Mastersizer) used for routine droplet-size analysis.

Depletion flocculation can have an enormous effect on creaming stability (Dickinson, 1993, 2003, 2004). At very low hydrocolloid concentrations, the entropy loss linked to droplet association outweighs the depletion effect and so the system remains stable. Beyond a certain critical concentration, however, the presence of depletion flocculation is manifest in rapid serum separation. Due to their large excluded volumes, polysaccharides with stiff extended backbones (e.g., xanthan gum) are especially effective at inducing depletion flocculation. Figure 2.5 shows the influence of various concentrations of xanthan on the plot of serum layer thickness against time for samples of sodium caseinate-stabilized emulsions (10 vol% oil, 0.5 wt% protein, pH = 7) stored quiescently at 25 ºC in glass tubes of height 10 cm (Cao *et al.*, 1990). The reference emulsion without added xanthan shows no visible serum separation over the storage period of 72 h, but the presence of just 0.025 or 0.05 wt% xanthan is sufficient to induce rapid serum separation (complete within 1–2 h) of the low-viscosity liquid-like

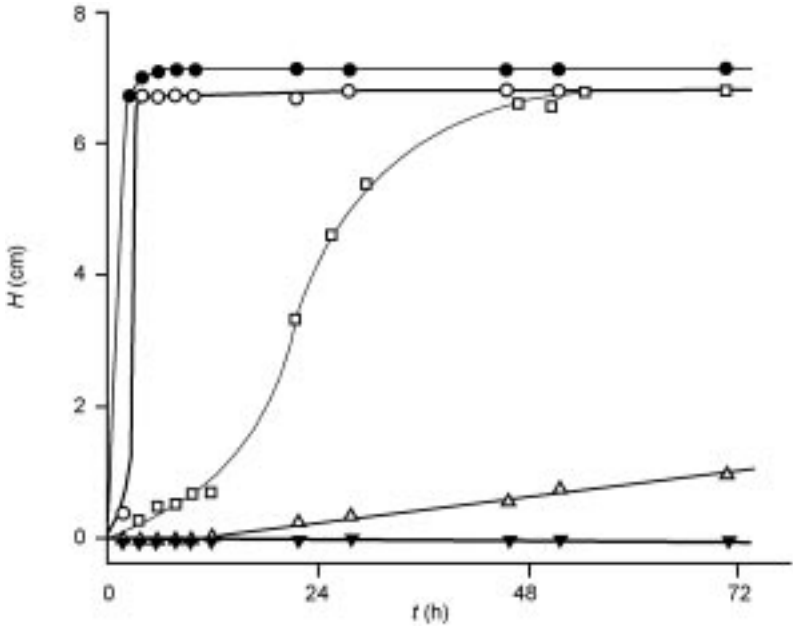

Fig. 2.5 Influence of xanthan gum on creaming of protein-stabilized emulsion (10 vol% oil, 0.5 wt% sodium caseinate, pH = 7, ionic strength = 0.005 M, total sample height = 10 cm). Serum layer height H is plotted against storage time t at 25 ºC for various polysaccharide concentrations: ▼, 0 wt% and 0.125 wt%; ●, 0.025 wt%; ○, 0.05 wt%; □, 0.065 wt%; △, 0.1 wt%. (Reproduced with permission from Cao *et al.*, 1990.)

emulsion, with the serum (non-cream) layer occupying 65–70% of the sample volume. At 0.0625 wt% xanthan, the same degree of phase separation takes 48 h; at 0.1 wt% xanthan, there is only 10% serum separation after 72 h; and, at 0.125 wt% xanthan, the system is as stable as in the absence of xanthan. Inhibition of creaming at high xanthan concentrations is conventionally attributed to the immobilization of the emulsion droplets in a weak gel-like network (very high η^0).

Figure 2.6 shows creaming profiles (volume fraction vs height) for a surfactant-stabilized emulsion (18 vol% oil, 2 wt% Tween 20) containing 0.17 wt% xanthan (Ma *et al.*, 1994). The data were obtained by ultrasound velocity scanning on the same emulsion system (no added salt) as that shown in Fig. 2.3. The sample shows no change in oil content for several hours, but after 1 day there evolves a pronounced continuous gradient in ϕ from the bottom to the top of the sample. After 2 days there is a distinct boundary at height 150 mm between a lower emulsion phase ($\phi \sim 0.05$–0.1) and an upper emulsion phase ($\phi \sim 0.35$–0.4). After 4 days the lower serum has become completely depleted of oil droplets, and after 1 week the upper cream layer has reached an oil content equivalent to close-packed monodisperse hard sphere ($\phi \sim 0.63$).

For more concentrated emulsions (say, $\phi \sim 0.3$), the change in emulsion creaming behaviour caused by addition of non-adsorbing polysaccharide tends to correlate better with the rheology of the emulsion as a whole, rather than with

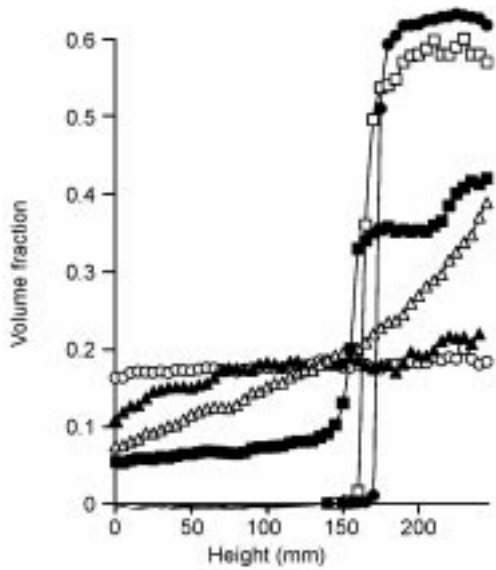

Fig. 2.6 Creaming of surfactant-stabilized emulsion (18 vol% mineral oil, 2 wt% Tween 20, mean droplet size $d_{32} = 0.65\ \mu$m, height 25 cm) containing 0.17 wt% xanthan (low ionic strength). Oil volume fraction ϕ is plotted against height for an unstirred emulsion sample at 30 ºC: ○, after 11 h; ▲, after 20 h; Δ, after 30 h; ■, after 45 h; □, after 99 h; ●, after 188 h. (Experimental data of Ma *et al.* (1994), as supplied by Prof. Macolm Povey.)

the rheology of the hydrocolloid solution making up the continuous phase (Dickinson, 2003, 2004). This implies that stability is controlled by the properties of the long-lasting metastable network of flocculated droplets held together by the short-range depletion interactions induced by the added hydrocolloid (Parker *et al.*, 1995; Dickinson, 2006). A manifestation of the early stages of the system's evolution is phase separation on the *microscopic* scale. This phenomenon can be considered to be somewhat analogous to the phase separation observed in mixed solutions of two polymers exhibiting thermodynamic incompatibility (Tolstoguzov, 1991).

Figure 2.7 shows some microscopic images of a caseinate-based emulsion (30 vol% oil, 1.4 wt% protein, pH = 7, mean droplet size 0.5 μm) containing

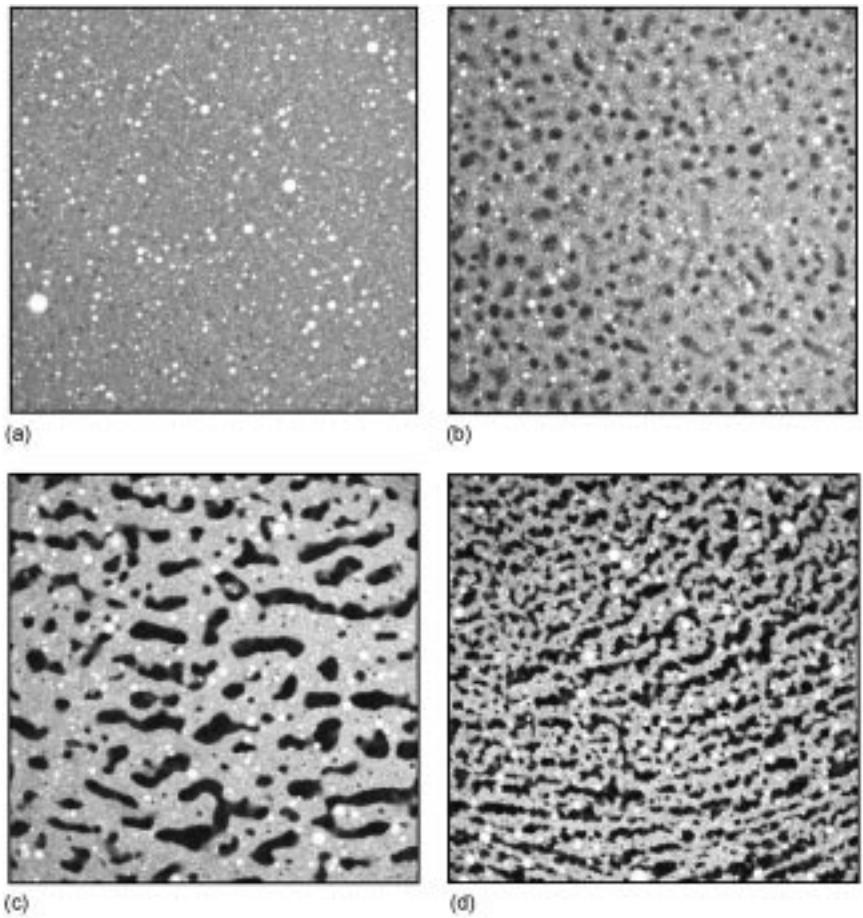

Fig. 2.7 Influence of xanthan gum on microstructure of protein-stabilized emulsion (30 vol% oil, 1.4 wt% sodium caseinate, pH = 7, mean droplet size d_{32} ~ 0.5 μm). Confocal images (250 × 250 μm) were captured 10 minutes after sample stirring had stopped. Polysaccharide concentrations in the aqueous phase: (a) 0.01, (b) 0.02, (c) 0.04 and (d) 0.1 wt%. (Reproduced with permission from Moschakis *et al.*, 2005.)

various concentrations of xanthan in the aqueous phase (Moschakis *et al.*, 2005). The images were recorded 10 minutes after stopping stirring. We can see microscopic phase separation into separate oil-rich and xanthan-rich regions, the latter appearing as dark blobs against the lighter background. In the hydrocolloid concentration range 0.02–0.05 wt%, the samples were observed to exhibit complex dynamic behaviour, including blob shape relaxation processes, microstructural coarsening and microphase coalescence phenomena. The elongated shapes of the xanthan-rich domains arise from the hydrodynamic shear forces applied during sample preparation and mixing. Once stirring ceases, the interfacial tension drives the blob shape towards spherical at a rate which sensitively depends on the viscoelastic properties of the blob and its surrounding local environment. The blob shape relaxation time is a strongly increasing function of xanthan content. For the system illustrated in Fig. 2.7, no microstructural evolution was observed over the experimental time-scale (several hours) for xanthan concentrations above 0.06 wt%. Analysis of the diffusion coefficient of individual probe particles has demonstrated that, in this low polysaccharide concentration range, the effective viscosity of the oil-droplet-rich regions is up to 10^3 times larger than that of the coexisting xanthan-rich regions (Moschakis *et al.*, 2006). Furthermore, the inferred low-stress viscosity of the flocculated oil-droplet-rich region increases dramatically with the xanthan concentration. So, as a result of changes in microstructure and microrheology caused by the hydrocolloid addition, the original liquid-like protein-stabilized emulsion is transformed into a stable, concentrated, viscoelastic emulsion containing 'blobs' of hydrocolloid-structured water.

2.4 Effect of adsorbing hydrocolloids on emulsion stability

2.4.1 Hydrocolloid emulsifying agents

Any hydrocolloid that is substantially surface-active has the potential for acting as both an emulsifying agent and a stabilizing agent. The effectiveness of the emulsifying agent is related to the minimum amount of it that is required to generate and stabilize small droplets during homogenization. A useful quantity in this context is the *surface load*, i.e., the mass of emulsifying agent per unit area of oil–water interface (typically a few mg m^{-2}). The mass adsorbed per unit volume of emulsion (C_s) is equal to the initial concentration of emulsifying agent minus that remaining in the aqueous phase after homogenization, as determined following centrifugation or filtration. The surface load Γ is calculated from

$$\Gamma = (C_s d_{32}/6\phi), \qquad 2.16$$

where d_{32} is the volume–surface mean diameter. Knowledge of the surface load enables the calculation of the minimum amount of emulsifying agent required to prepare an emulsion of known volume fraction and droplet size. For the milk protein β-casein, the theoretical minimum amount lies quite close to the amount

actually needed in practice. But for a less efficient emulsifier (e.g., gum arabic) a substantial excess of emulsifying ingredient is required in order to achieve the desired effect. The reason for this is that the gum is a complex mixture of polymers, and not all of the macromolecular species are capable of adsorbing and stabilizing the interface (Randall *et al.*, 1988). Moreover, even those fractions of the gum that are capable of adsorbing do not necessarily have time to do so during the very rapid process of high-pressure homogenization ($\sim 10^{-4}$ s). Consequently, when employing gum arabic as emulsifying agent, it is necessary to use a rather high gum/oil ratio (1:1) in order to produce fine stable emulsion droplets ($d_{32} \ll 1\ \mu$m) (McNamee *et al.*, 1998).

Even though acacia gums typically contain just a few per cent protein, this fraction seems to play a crucial role in terms of the interfacial functionality (Dickinson *et al.*, 1988; Dickinson, 2003). For acacia gums of variable nitrogen content, there is a good correlation between the protein content and the steady-state interfacial tension at the oil–water interface, and also between the emulsifying capacity and the rate of change of tension with time. However, the nitrogen content alone is not a reliable indicator of the effectiveness of acacia gums for emulsification. Other factors also appear to be important, such as the average molecular weight of the carbohydrate polymers, and the distribution of the proteinaceous fraction amongst the different sized polymer species.

The special emulsifying and stabilizing properties of gum arabic (*Acacia senegal*) is associated with a high-molecular-weight fraction representing less than 30% of the total hydrocolloid (Randall *et al.*, 1988, 1989; Ray *et al.*, 1995). The protein is covalently bound to the carbohydrate in the form of a mixture of arabinogalactan–protein complexes each containing several highly branched polysaccharide units linked to a common protein core. The protein chain firmly anchors the complex to the oil–water interface, and the charged polysaccharide units attached to the protein chain provide a barrier against flocculation and coalescence. Gum arabic is a polyelectrolyte, and so the emulsion droplets carry a net negative charge under beverage emulsion conditions. However, the value of the zeta potential is rather low (10–20 mV), indicating that the main stabilization mechanism is steric in character.

Certain types of pectin have also been shown to be effective for making emulsions. Again the interfacial functionality is mainly attributed to the presence of bound protein. In the case of citrus pectin, the accessibility of the protein fraction for adsorption at the oil–water interface can be substantially enhanced by depolymerization (Akhtar *et al.*, 2002). Whereas for citrus or apple pectin the bound fraction of protein is low (1.5–3%), it is significantly higher (up to 10%) for sugar beet pectin. In fact, due to its higher protein content, sugar beet pectin is even more surface-active than gum arabic, being effective in stabilizing fine emulsions at the relatively low emulsifier/oil ratio of 1:10 (Williams *et al.*, 2005).

2.4.2 Protein–polysaccharide interactions: conjugates and complexes

In searching for a replacement for the expensive and occasionally scarce gum arabic, there has been a move towards preparing composite protein–polysaccharide emulsifiers by alternative methods. One straightforward and practical way to achieve conjugation of protein with polysaccharide is by using the Amadori rearrangement steps in the Maillard reaction carried out under controlled dry-heating conditions (Kato *et al.*, 1990; Dickinson and Galazka, 1991). During this complex sequence of reactions, the terminal and side-chain amine groups on the protein become linked to the reducing end of the polysaccharide. Conjugation of carbohydrate to protein improves solubility under unfavourable solution conditions of low pH and high ionic strength, with consequent benefits for protein emulsification properties. For instance, a whey protein–maltodextrin conjugate made from commercial whey protein isolate and maltodextrin (molecular weight ~ 10^4 Da) can produce fine emulsion droplets ($< 1\ \mu$m) with triglyceride oil or orange oil under neutral or acidic conditions. As a possible replacement for gum arabic, the protein–polysaccharide conjugate emulsifier can be used at relatively low emulsifier/oil ratios to prepare low-pH beverage-type emulsions with a shelf-life of several weeks. Furthermore, in contrast to the corresponding emulsion based on protein alone, the conjugate-based formulation exhibits no precipitation or phase separation on mixing with colouring agents, either before or after extensive dilution (Akhtar and Dickinson, 2007).

Polysaccharides can form various types of physical complexes with proteins depending on the pH, the ionic strength, and the biopolymer charge distributions. The formation of such complexes implies the dominance of short-ranged attractive forces. Attractive protein–polysaccharide interactions may be strong (long-lasting) or weak (reversible). These non-specific protein–polysaccharide interactions arise as a result of averaging in time and space over all the individual specific chemical interactions (ionic, hydrogen bonding, hydrophobic, etc.) on the different macromolecules (Dickinson, 1998). Electrostatic forces are especially important at low ionic strength, and complete dissociation of complexes usually occurs on addition of excess electrolyte. Strong attractive electrostatic interactions are typically found with positively charged proteins (pH < p*I*) and negatively charged polysaccharides. Weaker reversible complexes tend to be formed between uncharged (pH ≈ p*I*) or negatively charged proteins (pH > p*I*) and anionic polysaccharides. On adjustment of pH and/or electrolyte concentration, the net protein–polysaccharide interaction may change substantially, even switching from net attractive to net repulsive.

Some hydrocolloids can achieve interfacial functionality during or after emulsion formation by forming an associative interaction with an adsorbed protein layer. Whilst the charged polysaccharide by itself is not surface-active, the electrostatic complex of protein + polysaccharide has a strong tendency to adsorb at the oil–water (or air–water) interface (Dickinson, 1995, 1998). The overall effect of the interacting polysaccharide on the emulsion stability and microstructure is dependent on the concentration of added polymer, as illu-

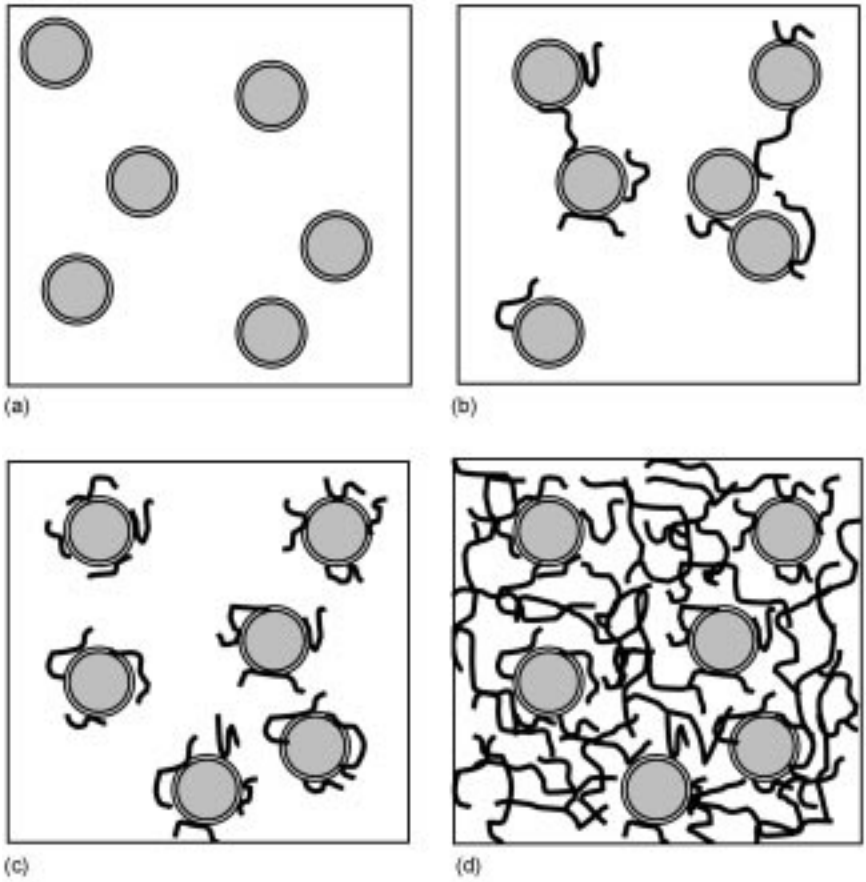

Fig. 2.8 Schematic representation of the effect of attractive protein–polysaccharide interactions on a protein-stabilized emulsion: (a) no added polysaccharide; (b) bridging flocculation at low polysaccharide concentration; (c) steric stabilization of droplets with saturated coverage of polysaccharide; (d) emulsion gel with droplets immobilized in entangled polysaccharide network.

strated schematically in Fig. 2.8. Addition of a small amount of polymer to an initially stable emulsion (a) leads to bridging flocculation (b). When sufficient polymer is present to saturate the droplet interface, the emulsion is stabilized electrostatically by the secondary layer of charged polysaccharide (c). And when the hydrocolloid content in the aqueous phase is sufficiently high ($c > c^*$), the emulsion droplets may be immobilized in a polymer gel network (d). All these stages of (in)stability behaviour have been reported (Dickinson and Pawlowsky, 1997) for emulsions based on bovine serum albumin (BSA) at pH = 6 and low ionic strength in the presence of various concentrations of ι-carrageenan, a sulfated polysaccharide of high charge density. Direct experimental evidence of the presence of protein–polysaccharide interactions at the oil–water interface

can be readily indicated by surface rheological measurements (Dickinson and Galazka, 1992; Dickinson et al., 1998).

The structure of any composite interfacial layer comprising both protein and polysaccharide depends to some extent on the procedure used to make the emulsion. One way is to prepare a mixed solution of the biopolymers, and then use the protein–polysaccharide complex as the emulsifying agent during homogenization. The alternative approach involves first preparing the emulsion with protein as the emulsifying agent, and afterwards adding the interacting polysaccharide to the aqueous phase to produce an emulsion containing droplets coated with a protein–polysaccharide bilayer. The so-called 'layer-by-layer' approach has attracted much attention recently because of its potential for encapsulation technology and its success in protecting emulsions against severe environmental stresses (Guzey and McClements, 2007).

In practice, though, it is not straightforward to use the layer-by-layer approach to make fine stable emulsions containing adsorbing hydrocolloids. A major issue is the tendency for extensive flocculation even under conditions where the protein surface should be saturated with polysaccharide (McClements, 2005b). Bridging flocculation occurs when the polysaccharide content is such that droplet collisions occur faster than the rate of polysaccharide saturation of the protein-coated droplet surface, and depletion flocculation and/or gelation may occur if the system contains too high a concentration of unadsorbed polysaccharide. Typically, with intense mechanical agitation and a low oil content ($\phi \sim 0.05$–0.1), there is a window of polysaccharide concentrations within which fine stable emulsions can be prepared. But for high oil contents, even with vigorous agitation, there is no useful range of polysaccharide content over which unflocculated emulsions are accessible. For this reason, it can be more convenient to prepare emulsions containing protein–polysaccharide complexes by simply mixing together the two biopolymer ingredients in the aqueous phase prior to homogenization (Dickinson *et al.*, 1998; Jourdain *et al.*, 2008).

2.5 Summary

The stability and acceptability of liquid-like oil-in-water emulsions is influenced by a number of mechanistic processes: creaming, flocculation, (partial) coalescence and Ostwald ripening. These processes are closely interrelated. In particular, the creaming stability is very sensitively correlated to the state of flocculation. In physico-chemical terms the properties of an individual emulsion are determined by the structure of the adsorbed layer, which in turn influences the nature of the interaction pair potential between the dispersed droplets. The two main stabilization mechanisms are electrostatic and steric. The basic theory underlying these two mechanisms gives useful practical insight concerning the design of emulsifier properties and the emulsion conditions favouring long-term stability.

Whilst food proteins are typically the primary emulsifying ingredients, a few surface-active hydrocolloids can also act as effective emulsifying agents. More generally, added hydrocolloids affect emulsion stability by modifying the viscosity and non-Newtonian rheology of the continuous phase. The presence of low concentrations of non-adsorbing hydrocolloids leads to depletion flocculation, microscopic phase separation and macroscopic serum separation. Whilst the association of polysaccharides with adsorbed protein can be a source of unwanted bridging flocculation, under optimized conditions these same interactions in electrostatic protein–polysaccharide complexes can also be used to improve emulsion stability.

2.6 References

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3

The health aspects of hydrocolloids

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Abstract: The health effects of hydrocolloids are dependent on how they are incorporated into food. The contribution of each hydrocolloid is small and the health benefits of these compounds cannot be separated from those of other non-digestible carbohydrates. The impact of hydrocolloids on gastric emptying and small intestinal digestion and absorption is well established. More recent work has explored new sources and their mechanisms of action related to their fermentation to short chain fatty acids and their impact on the intestinal microbiota along with potential actions the short chain fatty acids may have on plasma lipids and satiety. This is of increasing importance given the current global epidemic in obesity.

Key words: food hydrocolloids, dietary fibre, health, fermentation, short chain fatty acids, plasma lipids, satiety.

3.1 Introduction

The health effects of food hydrocolloids are dependent on how they are incorporated into foods and in the diet. There are many hydrocolloid carbohydrates naturally present in plant foods as part of the cell wall, such as hemicelluloses and pectin, or with other more specific roles within the plant such as storage polysaccharides like guar gum, exudates like gum acacia, and husk polysaccharides such as ispaghula. There are also alginates and bacterially produced hydrocolloids such as gellan and xanthan. However, the contribution of each individual hydrocolloid in the diet is small and epidemiological studies cannot identify and separate the health benefits of these compounds from those of other non-digestible carbohydrates such as insoluble non-starch polysaccharides, resistant starch and oligosaccharides.

Hydrocolloids can also be incorporated in small amounts into food products

as stabilisers, emulsifiers and fat substitutes. Guar gum levels of <1% are typically added to food products; however, health beneficial effects of guar gum are achieved with higher levels (3–5%). Increasing the amount of dietary fibre within food formulation may result in compromising the product's organoleptic properties. However, hydrocolloids such as partially hydrolysed guar gum have a higher potential to be successfully incorporated into different foods due to their lower viscosity (Ellis *et al.* 1985).

Large doses of hydrocolloids can be prepared as potential therapeutic agents against constipation as with ispaghula or psyllium in medications such as Isogel®, Metamucil® and Regulan®, or for other health benefits. However, many of the feeding studies in animals and humans have used high doses of the hydrocolloids which may not reflect the actions of these polysaccharides in a normal diet or even the effects of palatable supplements.

It would be impossible to cover the health aspects of all of the different dietary hydrocolloids and supplements, so in this chapter the role of hydrocolloids will be discussed as part of the dietary fibre story and then more specific health effects of pure hydrocolloids ingested in significant amounts will be reviewed.

Much of our understanding of the impact of food hydrocolloids on gastric emptying, small intestinal digestion and absorption, postprandial glucose and insulin and stool output is now well established with most of the research being carried at the end of the last century (Schweizer and Edwards 1992). However, more recent work has explored new sources of non-digestible polysaccharides and oligosaccharides including those of algal origin (Goni *et al.* 2001; Panlasigui *et al.* 2003), new fruits (e.g., Chinese Jujuba; Huang *et al.* 2008), seeds (e.g., fenugreek; Hannan *et al.* 2007), and nuts (e.g., almonds; Mandalari *et al.* 2008). The mechanisms of action of hydrocolloids related to their fermentation in the large intestine to short chain fatty acids and their impact on the intestinal microbiota have also been of more recent interest along with the potential actions of the products of their fermentation on plasma lipids and satiety. This is of increasing importance given the current global epidemic in obesity and increase in associated chronic diseases such as cardiovascular disease (CVD). The randomised control trials of soluble fibre and their effects on weight management, appetite and plasma lipids will be reviewed in this chapter.

3.2 Hydrocolloids and non-digestible carbohydrates in food

3.2.1 Definition and properties of hydrocolloids and fibre

Food hydrocolloids are non-digestible carbohydrates that are capable of forming viscous solutions or gels. They could be considered a component of dietary fibre and thus if added to foods should increase the amount of fibre and potentially improve the health effects of the diet. However, there has been a prolonged debate about the definition and classification of non-digestible carbohydrates

including the food hydrocolloids. The problems have centred mainly on an agreement on the classification of dietary fibre, for which there is still no globally accepted definition. This has hampered interpretation of research, especially epidemiology into the health effects of non-digestible carbohydrates, as different definitions and dietary recommendations for dietary fibre exist in different countries. To clarify this, the current proposed definitions are discussed below.

The lack of an agreed definition for dietary fibre is of concern to the food industry where the desire to produce a palatable product with a higher non-digestible carbohydrate content may be facilitated by the use of carbohydrate sources not accepted as dietary fibre in some countries, in particular the UK.

The definition of food fibres and dietary fibre has been a matter for debate for several decades (DeVries *et al.* 1999). This has been mainly a lively discussion on the role of the plant cell wall as the fundamental basis of the dietary fibre concept versus the inclusion of polysaccharides, oligosaccharides and synthetic molecules which have never been part of a plant cell. The original definition of dietary fibre by Trowell (1974) was based on plant cell wall polysaccharides and lignin. This definition developed in the UK to the concept of non-starch polysaccharides (Trowell 1978) which would include most hydrocolloids but would not include resistant or modified starches or non-digestible oligosaccharides. The other major factor in the definition of dietary fibre is the need for a robust analytical method (Champ *et al.* 2003). In the UK, the non-starch polysaccharides need to be separated from starch by a dimethylsulphoxide (DMSO) step in the Englyst method (Englyst and Hudson 1987) followed by colorimetric or GC analysis of sugars derived from the breakdown of the polysaccharides (Englyst *et al.* 1994). In contrast, the AOAC method 985.29 for dietary fibre (McCleary 2003) is a gravimetric method without complete separation of resistant starch. This means that dietary fibre measurements with the AOAC method are higher than non-starch polysaccharides for the same foods. More recent constructive discussions, particularly in the USA, have moved to include more non-plant cell wall carbohydrates including hydrocolloids, oligosaccharides and non-digestible sugars and to link the definitions to demonstrated beneficial physiological effects related either to the action in the upper gastrointestinal tract (slowing gastric emptying, delaying absorption, reduction in postprandial glycaemia, cholesterol and lipidemia, and effects on satiety) or to colonic effects such as fermentation, production of short chain fatty acids and laxation. The American Association of Cereal Chemists (AACC 2000) proposed the definition of dietary fibre to be

> the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibers promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation (DeVries *et al.* 1999).

This would encompass all non-digestible carbohydrates with three or more sugars that had demonstrable beneficial physiological effects.

The US Institute of Medicine (IOM) later proposed a more detailed definition which tried to differentiate between dietary fibre as part of the plant food and added fibre sources which may act differently and be at much higher amounts than in a plant food.

> Dietary fiber consists of non-digestible carbohydrates and lignin that are intrinsic and intact in plants. Added fiber consists of isolated, non-digestible carbohydrates that have beneficial physiological effects in humans. Total fiber is the sum of dietary fiber and added fiber (Panel on the Definition of Dietary Fiber and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes 2001).

The latter definition will have an impact on the concept of functional fibres which are added to foods or taken as supplements as they would no longer be seen as dietary fibre. However, in conjunction with the AACC proposed definition, the new emphasis on physiological benefits places greater emphasis on the need to understand and evaluate the potential health benefits of food hydrocolloids.

There is still much debate on the definitions in the USA, Europe and the UK and no global agreement is yet in sight.

Non-starch polysaccharides

The definition of non-starch polysaccharides is clear: non-digestible polysaccharides which may be insoluble fibres or soluble fibres mostly from plant cell walls. This is the current definition of dietary fibre used in the UK but the definition precludes the inclusion of many non-digestible carbohydrates that may make up a significant part of the material which reaches the large intestine and which may have a significant contribution to the production of short chain fatty acids and impact on the microflora which are associated with the potential health benefits of dietary fibre. A significant amount of ingested starch (resistant starch) reaches the large intestine (Englyst *et al.* 1992) and ingestion of non-digestible oligosaccharides such as the fructo-oligosaccharides from inulin (Roberfroid 2007) and polydextrose (Tiihonen *et al.* 2008), pyrodextrins and other synthesised and modified starch molecules (Laurentin and Edwards 2004) is now greatly increased as food manufacturers include 'prebiotic' carbohydrates (Schrezenmeir and de Vrese 2001) in their products. Many of these non-digestible carbohydrates have very similar effects to traditional dietary fibres and their impact cannot really be separated physiologically or in epidemiology studies. So it is impossible to ignore the other non-digestible carbohydrates when evaluating health effects.

The physiological effects of insoluble dietary fibre have been known for many years. These polysaccharides act mainly on colonic function and act to reduce the risk of constipation, decrease whole gut transit time, and dilute the concentration of potentially harmful compounds such as secondary bile acids,

carcinogens and toxins. However, most insoluble fibres are fermented to some extent, as demonstrated by the increased impact of wheat bran on stool output after antibiotics (Kurpad and Shetty 1986) and may indeed be useful in moving the fermentation of more rapidly fermented polysaccharides to more distal regions of the large intestine where most disease occurs (Govers *et al.* 1999).

The soluble non-starch polysaccharides include most of the food hydrocolloids such as pectin, guar gum, ispaghula, beta glucan, xanthan, locust bean gum and gellan. The physiological action of these will be explored in more detail below. The impact of these polysaccharides is dependent on the dose ingested and whether they are free to form viscous solutions, are bound within intact cell walls, or within a complex food matrix such as in guar bread (Brennan *et al.* 1996).

3.2.2 Physico-chemical properties and physiological effects

The influence of hydrocolloids and other non-starch polysaccharides on events occurring in the gastrointestinal tract are dependent to a great extent on their physico-chemical properties. This includes their ability to hold water (water holding capacity, WHC), the ability to form and maintain high viscosity and their potential for trapping or even binding small molecules such as minerals and bile acids.

If hydrocolloids, such as guar gum, are ingested in high enough amounts to increase the viscosity of gastric and small intestinal contents, then they may delay gastric emptying and inhibit the effects of small intestinal contractions resulting in a slower delivery of food and nutrients to the absorptive epithelium and hence slower absorption and lower postprandial levels of glucose, lipids and other nutrients. This was classically illustrated by David Jenkins and colleagues when they related the viscosity of ingested non-digestible carbohydrates to their effects on postprandial blood glucose (Jenkins *et al.* 1978).

Some charged carbohydrates, such as pectin, may bind minerals or bile acids and thus reduce absorption or interfere with fat digestion, but most binding sites are already occupied in the food and may not be free for binding new molecules. The water holding capacity of a hydrocolloid will be particularly important for its effects in the large intestine. If the WHC is maintained and not lost during fermentation by the colonic microflora, then the hydrocolloid will speed the transit of material through the colon and increase stool output. The preservation of WHC may be due to a low fermentability of the carbohydrate, as in the case of ispaghula and gellan, or may be due to an excessive dose being ingested as in many of the animal experiments which show a stool bulking effect of normally extensively fermented carbohydrates like guar gum and pectin (Armstrong *et al.* 1993; Edwards and Eastwood 1995).

3.2.3 Other non-digestible carbohydrates

There are now known to be many different types of non-digestible carbohydrate which may or may not be classed as dietary fibre but are not hydrocolloids or

non-starch polysaccharides but which may have very similar effects on the gastrointestinal tract and health. These include resistant starch (Asp 1997), modified starches such as pyrodextrins (Laurentin and Edwards 2004), oligosaccharides such as fructo-oligosaccharides (Roberfroid 2007), and other synthetic molecules such as polydextrose (Tiihonen *et al.* 2008) and lactulose. These are generally non-viscous and have most of their effects in the large intestine rather than in the stomach and small intestine and act mostly via the products of their fermentation. It would not be appropriate to discuss their effects in detail but excellent reviews are available and it is important to consider them when trying to understand the overall impact of non-digestible carbohydrates on health.

3.3 Effects on metabolism and health

Epidemiological studies have consistently shown a positive association between high consumption of total dietary fibre and reduction in risk of certain chronic diseases including type 2 diabetes mellitus, CVD and colorectal cancer (Bingham *et al.* 2003; Pereira *et al.* 2004; Schulze *et al.* 2007). High fibre consumption is also associated with reduction of constipation (Dukas *et al.* 2003) and faster gut transit time (Probert *et al.* 1995). The role of fibre in colorectal health has been intensively scrutinised, especially in relation to cancer prevention. The 2007 report from the World Cancer Research Foundation and the American Institute for Cancer Research on Food, Nutrition, Physical Activity and the Prevention of Cancer concluded that a dose relationship was evident between dietary fibre intake and a reduced risk for colorectal cancer. This was supported by evidence of plausible mechanisms; however, confounding by other lifestyle, dietary and environmental factors was not totally excluded. Thus foods containing dietary fibre 'probably' protect against colorectal cancer (World Cancer Research Fund/American Institute for Cancer Research 2007).

The epidemiological evidence on dietary fibre consumption and disease prevention reports positive effects of total dietary fibre or subtypes of fibre (soluble and insoluble fractions) or non-starch polysaccharides derived from certain foods (fruit and vegetables or grains). However, most foods contain a mixture of different types of polysaccharides which vary in their physico-chemical properties, therefore the interpretation and understanding of the role of fibre is a complex issue. Additionally, the impact of fibre intake in healthy subjects is hard to demonstrate, especially when fibre is ingested as a major component of foods. Thus for simplification purposes, this chapter focuses on studies in subjects with high disease risk (e.g. overweight and obese subjects) or subjects already suffering from the relevant disease (e.g. diabetes). This review of the metabolic effects of fibre will be based on randomised control trials (RCTs) using pure (isolated) fibres. The mechanisms of action that may explain the metabolic effects of dietary fibre are discussed below and in Table 3.1.

Table 3.1 Metabolic effect of hydrocolloids – mechanisms of action

Properties	Physiological action	Metabolic effect	Type of DF/ Hydrocolloid	Disease/ Health effect	References
Viscosity/ gel forming properties	• Increase gastric distension • Slow gastric emptying • Slow nutrient absorption • Increase intestinal viscosity (↓ whole gut transit time) • Increase faecal sterol output	• ↓ exposure of upper small intestine to nutrients • delay absorption from small intestine • ↓ postprandial glucose response • ↓ in enzyme production (↓ stimulation of hydroxymethylglutamyl co-enzyme A reductase) • ↓ in total and LDL cholesterol synthesis • ↓ fat absorption • ↑ faecal bile acid excretion • prolonged contact with small intestinal chemoreceptors – enhance production of gut hormones	• Guar gum • Alginates • Inulin • Pectin • β-glucan • Psyllium, ispaghula	• Glycemic control on type 2 diabetes • Reduction in plasma cholesterol • CVD prevention • Weight management	Cummings *et al.* (1992), Dikeman *et al.* (2006), French and Read (1994), Hoad *et al.* (2004)
Bulk/water sequestering ability	• ↓ gut transit time • ↓↑ gut hormones • ↑ stool output • dilution of toxins	• ↑ insulin sensitivity • ↑ satiety (?)	• Insoluble fibre (cellulose, hemicellulose) • RS • Wheat bran • Psyllium, ispaghula	• Weight management • Insulinemic control in type 2 diabetes • Prevention and treatment of constipation	Cummings *et al.* (1992)
Fermentation	• SCFA production: acetate, propionate, butyrate	• Energy sources for colonic epithelial cells • Regulation of colonic cell expression • Modulation of programmed cell death (apoptosis) • ↑ gas (hydrogen, carbon dioxide–methane production, lactate) • Change in microbial biomass/growth prolong contact with small intestinal chemoreceptors • ↓ hepatic glucose production	• Pectin, β-glucan • Non-digestible oligosaccharides (fructooligosaccharides, synthetic polydextrose, inulin) • RS	• Colorectal cancer protection • Lipid modulation • Mucosal healing	Cummings *et al.* (1992), Nofrarias *et al.* (2007), Roberfroid *et al.* (1995), Thorburn *et al.* (1993)

The actions of a polysaccharide on the body can be classified into their effects on the gastrointestinal tract (upper and lower), postprandial plasma levels of glucose and insulin, plasma lipids and cholesterol, their effects on satiety and energy balance, the influence of their colonic bacterial fermentation products and their effect on stool output. The physical/chemical properties of a polysaccharide govern its physiological effect, but it is difficult to predict the specific actions of a carbohydrate without knowledge of how it interacts with other food components, digestive secretions and the colonic bacterial flora. Furthermore, the functionality of non-digestible carbohydrate that forms an integral part of the food matrix may differ significantly from that of isolated gums and is dependent on its physical and chemical structure.

3.3.1 Effects on the gastrointestinal tract and impact on other nutrients

Upper gut effects – gastric emptying

Before a food can be digested and absorbed it must first reach the small intestine where most digestion occurs after the food has mixed with pancreatic enzymes. A major factor in the rate of delivery to the duodenum and hence postprandial plasma levels of blood glucose and other nutrients is the speed of gastric emptying. As gastric distension is also a key signal to prevent overeating, the rate of gastric emptying can also influence satiety and appetite and hence weight control. High-viscosity, gel-forming polysaccharides ingested in high concentrations can change the characteristics of the liquid phase of ingested food in the stomach (Dikeman *et al.* 2006). They increase gastric volume and enhance fullness compared with high- or low-viscosity meals that do not gel and are more homogeneously diluted in the stomach (Hoad *et al.* 2004). The physiological response to this is delayed gastric emptying of liquids (French and Read 1994; Marciani *et al.* 2001; Schwartz *et al.* 1982). In contrast, acceleration of the emptying of discrete solids has also been observed, probably due mainly to an increase in lubrication (Meyer *et al.* 1986). However, not all viscous polysaccharides show the same effects. The effect on gastric emptying is not necessary for their effects on small intestinal digestion and absorption (Edwards *et al.* 1987) and hydrocolloids which do not delay gastric emptying may still delay and reduce postprandial glycaemia and fat absorption.

Small intestine effects

The viscous fibres or hydrocolloids are the most likely dietary fibres to have an impact in the small intestine. If they can raise luminal viscosity they slow the digestion and absorption of nutrients by inhibiting the mixing movements of intestinal contractions (Edwards *et al.* 1988) effectively increasing the unstirred water layer (Blackburn *et al.* 1984; Flourie *et al.* 1984) and reducing enzymatic activity (Isaksson *et al.* 1984). There has been some debate over how much viscous polysaccharides can increase luminal viscosity as the pancreatic and other gastrointestinal secretions dilute the viscous solutions substantially

(Blackburn and Johnson 1981; Cameron-Smith *et al.* 1994; Higham and Read 1992) and it has been very difficult to measure and increase *in vivo* in the small intestine (Higham and Read 1992). It could be that most of the viscosity is developed in the more distal small intestine after much of the water has been absorbed. However, there is substantial evidence that hydrocolloid fibres which have the potential to increase viscosity, if ingested at a high enough dose, reduce the absorption rate of glucose, lipids and some minerals as well as reducing the reabsorption of bile acids. This is probably due to entrapment of the molecules within the matrix of the polysaccharide rather than chemical bonding, for even pectin which has the capacity for cation binding usually has its binding sites filled by cations before it enters the small intestine.

The consequence of trapping bile acids in the small intestine and increasing their delivery to the colon and loss in faeces is to reduce fat absorption and decrease the size of the bile acid pool. The body responds to the loss of bile acids by making more cholesterol in the liver which may result in a significant decrease in plasma cholesterol levels.

Second meal effects

An increase in nutrients in the distal small intestine can stimulate receptors there, which reduce the gastric emptying rate of the next meal (acting as the ileal brake) and hence postprandial levels of the nutrients in the second meal (Nilsson *et al.* 2008) and even the time to reach satiety (Anne *et al.* 2006). This is known as the second meal effect and has been demonstrated in several experiments where a high fibre breakfast has influenced the response to lunch (Burley *et al.* 1987; Holt *et al.* 1999).

Colonic effects

The actions of non-digestible carbohydrates in the large intestine are dependent on whether they are fermented by the colonic bacteria rapidly, slowly or hardly at all. The human colon has a very complex bacterial ecosystem capable of fermenting most dietary non-digestible carbohydrate. There are probably approximately 1000 species, the majority of which are not culturable and are therefore not well characterised. Much research is being directed at improving our knowledge of this microbiota using molecular techniques (Blaut *et al.* 2002) and this will enhance our ability to study the impact of food hydrocolloids on the ecosystem. When a high non-digestible carbohydrate diet is eaten, the majority of the bacterial activity is saccharolytic rather than the less healthy proteolytical activity seen with low fibre intake. As different bacteria can use different hydrocolloids either as a species (Edwards and Rowland 1992) or in combination with other bacterial groups (Morrison *et al.* 2006; Tomlin and Read 1988), it would be expected that a high intake of one type of carbohydrate would increase the activity of the populations of those bacteria that can ferment that carbohydrate. This may then influence the metabolism of other molecules and other bacteria, as many of the bacteria in the gut grow in biofilms (Macfarlane and Dillon 2007) and when they reach a specific population size they change

their metabolism using quorum sensing to produce bacteriocins to inhibit other bacteria and co-ordinate the metabolism of the biofilm.

The ease with which a carbohydrate is fermented is difficult to predict but must depend on the bonds between sugar units, the presence of appropriate bacterial enzyme activity (which may be inducible after several ingested doses) and the context in which the carbohydrate is present. For example, lignin will reduce the fermentability of plant cell wall carbohydrates, and microcrystalline cellulose (Hsu and Penner 1989) is poorly fermented whereas milled cellulose is more easily degraded by colonic bacteria. The solubility of the carbohydrate generally will increase its fermentability and may also influence the proportions of short chain fatty acids (SCFA) produced (Laurentin and Edwards 2004).

Carbohydrates that resist fermentation, such as ispaghula or gellan have a much greater effect on stool output and retain much of their WHC whereas guar gum, pectin and beta glucans are more likely to be completely fermented in the proximal colon having little effect on stool output (Edwards and Eastwood 1995). Slowly fermented carbohydrates such as xanthan may be fermented in the distal colon and combinations of poorly fermented carbohydrates such as ispaghula (psyllium; Morita *et al.* 1999) and wheat bran (Govers *et al.* 1999) with more easily fermented carbohydrates may push the fermentation of the former to more distal areas in the colon which may be of benefit as more disease occurs in the distal colon which usually has a higher pH and more bacterial proteolysis and toxin production than the more acidic proximal colon. Combinations of different carbohydrates may produce different patterns of SCFA than the addition of the SCFA produced by each carbohydrate fermented on its own (Khan and Edwards 2005).

Production and effects of short chain fatty acids

The fermentation of carbohydrate by the colonic microbiota produces SCFAs, mainly acetic, propionic and butyric acids and carbon dioxide, hydrogen and methane gases. If the fermentation is sufficiently fast, pH in the proximal colon can drop as low as 4.5. Acetate is always the main SCFA produced but the exact proportions of each SCFA depends on the composition of the carbohydrate being fermented. Pectin fermentation may increase the proportion of acetate produced, ispaghula and guar gum increase the relative amount of propionic acid and beta glucan, oligofructose and starch fermentation may increase butyrate production (Edwards and Rowland 1992). The amount of propionic acid produced may be related to the presence of beta bonds or an increased solubility as pyrodextrins consistently produced a higher proportion of propionic acid than their parent starch (Laurentin and Edwards 2004). Similar proportions were also produced by cellulose and soluble glucose-based carbohydrates. The production of butyrate may not occur directly from the fermentation of the carbohydrate but may be made instead from intermediates of acetate and lactate (Morrison *et al.* 2006).

The health benefits of SCFAs have been studied extensively (Wong *et al.* 2006). All the SCFAs are potential energy sources and the energy value of

dietary fibre has been estimated as 2 Cal/g to account for this (Livesey 1990). The absorption of the SCFA promotes water absorption and helps prevent diarrhoea (Crump *et al.* 1980). Butyrate is the preferred energy source for the colonic microflora (Roediger 1982) and is thought to be essential for colonic health. Propionic acid is used mainly by the liver. It is gluconeogenic and is thought to reduce denovo lipogenesis from acetate (Wolever *et al.* 1995) and perhaps cholesterol synthesis (Chen *et al.* 1984; Illman *et al.* 1988). Acetate, on the other hand, increases plasma lipids and a lower proportion of acetate to propionate may be important in promoting a healthier profile of plasma lipids (Wolever *et al.* 1991). Propionate given orally has been shown to promote insulin sensitivity (Venter *et al.* 1990). Increased production of SCFAs due to fermentation is a further mechanism that may be involved in improvement of hepatic insulin sensitivity (Thorburn *et al.* 1993). However, it is butyrate production which has received the most research attention due to its potential anti-inflammatory and neoplastic effects. The potential health benefits of butyrate have been extensively reviewed recently (Hamer *et al.* 2008). They include stimulation of proliferation of intestinal cells (Sakata 1987), promotion of colonic healing from surgery (Topcu *et al.* 2002) and after inflammation (Scheppach *et al.* 1992), stimulation of programmed cell death or apoptosis in cancer cells *in vitro* (Hague and Paraskeva 1995) and inhibition of histone deacetylase in the nucleus of cells (Waldecker *et al.* 2008). However, the response to butyrate may depend on the disease state and also genetic background (Bordonaro *et al.* 2008) which may in part explain the difficulties in linking fermentable fibres to reduction in colon cancer risk. Some of these actions are also a function, albeit less prominent, of the other SCFAs; for example, acetate and propionate may also have anti-inflammatory effects in inflammatory bowel disease (Tedelind *et al.* 2007) and can act as fuels for the colonic cells if butyrate is in short supply (Roediger 1982).

However, fermentation can be associated with increased gas production and this may be retained in the colon causing discomfort if a poorly fermented hydrocolloid is also present. Administration of psyllium (30 g/day) for 7 days resulted in prolonged lag time from initiation of gas perfusion to gas expulsion and thus promotion of gas retention (Gonlachanvit *et al.* 2004). Gas production, however, will stimulate stretch receptors in the colonic muscle and this will speed transit through the colon and may also feed back to the stomach and delay gastric emptying.

Prebiotic effects and interactions with probiotics

There has been great interest in the interactions of non-digestible carbohydrates and the 'balance' of the colonic microflora since the emergence of probiotics and prebiotics as potentially therapeutic dietary supplements. Probiotics are usually particular strains of lactic acid bacteria, lactobacilli, bifidobacteria and others, which have been isolated from human sources and have proven health benefits such as inhibiting the survival of pathogens like diarrhoea causing rotovirus, reducing eczema in infants and modulating intestinal motility (Hedin

et al. 2007; Kalliomaki *et al.* 2003; Lemberg *et al.* 2007; Parracho *et al.* 2007). Prebiotics are carbohydrates that selectively promote the growth of beneficial bacteria in the gut and are usually fructo-oligosaccharides from inulin, or galacto- or xylo-oligosaccharides (Gibson and Roberfroid 1995; Roberfroid 2007). Theoretically any non-digestible carbohydrate can influence the growth of the bacteria in the colon by enhancing the growth of those which can utilise the carbohydrate for energy. However, although there are suggestions that resistant starch (Topping *et al.* 2003) and gum arabic (Calame *et al.* 2008) may selectively increase the growth of lactic acid bacteria, studies of food hydrocolloids have not always met with positive results (Hughes *et al.* 2008; Jaskari *et al.* 1998; Kontula *et al.* 1998; Topping *et al.* 2003). However, there is some *in vitro* data to suggest oligosaccharides from pectins may have more prebiotic properties than the parent polysaccharide (Manderson *et al.* 2005; Olano-Martin *et al.* 2002). The impact of a fermentable polysaccharide may be simply to increase the growth of many different bacterial species rather than a select few. However, this does not mean that the fermentation of hydrocolloids has no health benefit. Fermentation that reduces colonic pH and produces SCFAs may still inhibit the growth of pathogens and may benefit the host as explained below as low pH can inhibit cell proliferation in the colon and a low pH has been linked to a lower risk of colon cancer in South African populations (Hughes *et al.* 2008; Jaskari *et al.* 1998; Thornton 1981; Walker *et al.* 1986). Moreover, one benefit of prebiotics is increased absorption of magnesium and calcium from the large intestine (Younes *et al.* 2001) and this has also been shown for some food hydrocolloids (Hara *et al.* 1999).

3.3.2 Metabolic syndrome: obesity, appetite and anti-inflammatory effects

It is now well established that we are suffering a global epidemic in obesity. This is the public health problem of the future. Along with increased body weight and the psychological issues associated with this, obesity can also increase the risk of heart disease, some cancers and many other chronic problems. The metabolic syndrome is a complex of risk factors which predispose to CVD and type 2 diabetes, including high blood pressure, impaired insulin sensitivity, obesity and abnormal plasma lipid profiles. Food hydrocolloids could potentially help prevent the development of these conditions by a variety of mechanisms (e.g., reduction in body weight, plasma cholesterol and lipids, improved appetite regulation and reduced inflammation).

Effects on body weight reduction and weight management – implications for obesity

The primary cause of being overweight and obesity is an imbalance in energy expenditure and energy intake. A surplus in energy intake results in increased body fat deposits and body weight. Energy intake is controlled by cultural, psychological and physiological factors which are influenced greatly by the environment (Rogers 1999). Appetite regulation is a complex process influenced

by individual (physiology and psychology) and external (environment) factors which ultimately determine energy intake. Satiation (the size of an eating occasion) and satiety (eating frequency) are linked to food intake and appetite regulation. Food components (including macronutrients, water, alcohol and non-digestible polysaccharides) modulate satiation and satiety and thus play a major role in appetite regulation (Blundell and Gillett 2001).

There have been studies on the effects of hydrocolloids on satiety and weight control for many years and preparations of non-digestible carbohydrates such as guar gum have been previously sold as slimming agents. However, the success of ingestion of isolated hydrocolloid supplements such as guar gum on body weight reduction is controversial. Although some RCTs support a role for food hydrocolloids in weight management, more robust evidence from meta analysis of RCTs is not supportive. Six well-conducted randomised trials with similar methodological design indicated that overweight and obese patients receiving similar doses of guar gum or placebo (9 to 30 g/d over 3–6 months) with the main aim to reduce body weight but without changing their usual diet, do not differ in mean weight loss (Pittler and Ernst 2001).

Nevertheless, supplementation of guar gum and other hydrocolloids together with energy-controlled/restricted diets has shown promising effects on body weight reduction and maintenance. Obese subjects supplemented with guar gum (40 g/d over a week) and following a fixed normal energy intake based on their usual diet had a significant decrease in energy intake compared with those taking the placebo (Pasman *et al.* 1997). The consumption of a mixture of soluble fibre (3 g plantago ovata husk and 1 g galactomannnan (two or three times a day)) over 16 weeks together with dietary restriction in overweight and obese subjects resulted in higher weight loss than subjects on placebo and energy restricted diet (Salas-Salvado *et al.* 2007). Similar results were observed using doses of 7 g/day during 24 weeks (Rigaud *et al.* 1990). Supplementation with isolated hydrocolloids has also been shown to be useful in maintaining body weight following weight loss (Ryttig *et al.* 1989).

Appetite regulation

Appetite is regulated by complex mechanisms controlled by the central nervous system. Being in the fasted or postprandial state determines the release of signals from peripheral sites such as adipose tissue, muscle, liver, pancreas and the gut to the brain. Specific receptors for these signals are activated in the hypothalamus via corresponding neuronal populations that control appetite. Besides mechanical actions resulting from food intake and digestion (chewing, stomach distension), food composition also plays a role in the regulation of these signals. Fat, protein and carbohydrates have different satiation actions, while certain polysaccharides also elicit effects that may modulate appetite. These effects are related to their physico-chemical properties such as viscosity (Blundell and Stubbs 1999).

Highly viscous or solid foods are expected to elicit stronger satiation/satiety than clear liquids. Increased mastication prolongs metabolic feedback for satiety

signals to the appetite centre in the central nervous system (Fujise *et al.* 1998). Effects on the upper gut that affect satiety and reduce appetite are related to gastric feedback mechanisms, including increased gastric distension and delayed gastric emptying by mediation of gastric mechanoreceptors (Sturm *et al.* 2004), prolonged gastrointestinal transit time and slowed nutrient absorption (Delgado-Aros *et al.* 2004). Slowed absorption results in a reduction in postprandial glucose and insulin responses, which also modulate appetite responses (Jenkins *et al.* 1978). The release of different gut hormones may also play an important role on appetite regulation. These include ghrelin, which is produced in the fasted state in the stomach and is a potent appetite stimulator. Whereas several appetite related hormones, cholecystokinin (CCK), glucagon-like peptide 1 (GLP-1) and peptide YY (PYY) act as appetite inhibitors and are released in the small intestine in response to the presence of food. Prolonged contact of food with these receptors caused by a delayed absorption of nutrients by food hydrocolloids would theoretically induce greater satiety and reduce food intake.

Subjective feelings of appetite and satiety

Studies on appetite regulation and satiety are based mainly on measurements of subjective feelings of hunger and appetite by means of visual analogue scales (Stubbs *et al.* 2000). These studies have serious limitations due to their self-report methodology and also because of behaviour such as voluntary dietary restriction (food restraint) which is common among overweight subjects. Quantitative measurements such as measuring the amount of food eaten directly in the laboratory or by food diaries in more free living conditions can help to confirm results using subjective measurements. Nevertheless, these measurements also have limitations such as changed behaviours due to laboratory settings and/or under-over-reporting of food intake when recorded in a diary.

Guar gum administration to obese subjects resulted in increased mean gastric emptying time, which was correlated with subjective measures of satiety (Wilmshurst and Crawley 1980). Obese subjects supplemented with guar gum (20 g/d over a week) or placebo and following a low energy intake from usual diet reported decreased hunger scores after guar gum supplementation (Pasman *et al.* 1997). Modified guar gum (highly purified galactomannan that forms a gel within 5–7 min after adding water) added to a low-energy diet based on a semi-solid meal plus 7.5 g modified guar gum over 2 weeks vs placebo was effective in reducing weight and prevented increases in appetite, hunger and desire to eat (Kovacs *et al.* 2001).

Early studies on liquid fibre, a substance containing ethyl hydroxyl ethyl cellulose that forms gels after ingestion, showed that ingestion of 300 mL, 3 times a day (2.5 g non-starch polysaccharide) had short-term effects by reducing subjective feelings of hunger, increased fullness and reduced the amount of eaten food in overweight subjects (Tomlin 1995). In a similar fashion, a drink containing alginate-pectin (2.8 g viscous fibre) and calcium, formulated as a two-part beverage to avoid gel forming prior to consumption and to enhance palatability, was tested in non-dieting overweight females to determine the

drink's capacity to reduce energy intake and subjective feelings of satiety. The ingestion of this calcium-gelled alginate-pectin beverage twice a day over 7 days resulted in reduced spontaneous food intake the day after finishing the 7-day trial. This was observed only in women with low eating restraint patterns (Pelkman *et al.* 2007).

In healthy subjects, consumption of a standard enteral formula vs a formula supplemented with pea fibre (50% fermentable and 50% non-fermentable polysaccharides) and fructo-oligosaccharides (5 g/L) as the only nutrition source over 14 days resulted in increased satiety and fullness feelings measured at the end of the supplementation (Whelan *et al.* 2006). Reductions in subjective feelings of satiety during breakfast after daily consumption of 16 g oligofructose were also reported in a small set of normal and overweight subjects (Cani *et al.* 2005b). Changes in subsequent food consumption are, however, not reported in either study.

Modulation of satiety-related hormones

CCK, produced in the duodenum, acts as a strong regulator of gut motility, gall bladder contraction and pancreatic secretion and also acts centrally in the hypothalamus to provoke satiety signals. Guar gum supplementation (20 g/d during 2 weeks) in obese women increased postprandial CCK responses, but not the expected appetite suppression, an increase in feelings of satiety measured by analogue scales (Heini *et al.* 1998). In contrast, supplementation of a low-fat diet containing 20 g fibre from foods (wheat bread, cereal, apple and dried fruit) vs low fat diet without fibre, enhanced and prolonged CCK release after a breakfast meal; increased CCK concentrations were associated with increased feelings of satiety (Burton-Freeman *et al.* 2002). High CCK concentrations were also observed after consumption of 5 g/d of solubilised cellulose with a liquid meal vs placebo in hypercholesterolemic subjects (Geleva *et al.* 2003).

Viscofiber®, a high viscous, water-soluble isolated fibre derived from oats and barley (constituted mainly by β-glucan) supplemented to overweight subjects in doses of 4 g/d over 14 weeks resulted in increased fasting PYY and GLP-1 plasma concentrations after the 14 week supplementation (Greenway *et al.* 2007).

Purified insoluble wheat fibre given to healthy women in the form of bread (10.5 g wheat fibre, 3 times a day) led to blunted postprandial responses of PYY and total ghrelin plasma concentrations if compared to controls. This, however, was not associated to changes in satiety rankings (Weickert *et al.* 2006a).

Effects on blood glucose and insulin sensitivity – implications in diabetes

High glucose and insulin concentrations are the primary indicators for glucose intolerance, insulin resistance and type 2 diabetes. Diet composition plays a major role in the regulation of glucose and insulin responses. Viscous non-starch polysaccharides have been consistently shown to lower postprandial circulating glucose levels in healthy, glucose intolerant or diabetic subjects, improvement in metabolic control in type 2 diabetes is also documented (Jenkins *et al.* 2000).

Early studies in diabetic subjects fed pure xanthan gum (12 g/d) over 6 weeks showed a reduction in fasting and post load serum glucose in the xanthan supplemented group vs controls (Osilesi *et al.* 1985). Several studies have confirmed this effect as described in a recent review of RCT in insulin resistant, glucose intolerant, obese and type 2 diabetic subjects (Galisteo *et al.* 2008). The ingestion of different types and doses of soluble-viscous dietary fibre (including psyllium, ispaghula husk and guar gum), blunted postprandial glucose and insulin responses. In the same line, newly developed viscous-like fibre types, such as modified cellulose fibre (hydroxypropylmethycellulose: HPMC, and methylcellulose: MC) have also been shown to reduce peak blood glucose concentrations in type 2 diabetic and in obese patients when compared to consumption of standard glucose or control meals. Effects are observed after including different doses (4, 8, 10 and 32 g) within a meal; the reductions for peak glucose (incremental area under the curve) ranged from 7–48% with increasing dose. Postprandial insulin excursions were also observed and were consistent with delayed glucose absorption. Reductions in peak insulin concentrations ranged from 21% to 42% with 2 and 8 g of high viscous HPMC supplementation, respectively (Maki *et al.* 2008).

The reduction in glucose and insulin concentrations after ingestion of high viscous non-starch polysaccharides (Leclere *et al.* 1994) is due to retarded digestion and reduced rate of nutrient absorption. As discussed above, this may be related to a slower gastric emptying or reduced mixing and movement of nutrients in the small intestine. Modulation of gut hormone responses that regulate glucose and insulin may also be responsible; however, results are controversial. Reductions in the postprandial secretion of the gastrointestinal hormone glucose-dependent insulinotropic (GIP) following guar gum administration (10 g) in healthy subjects have been reported (Morgan *et al.* 1990). GIP stimulates insulin release. Similar effects are reported in diabetic subjects consuming 7.5 g of guar gum provided within 75 g guar gum bread; reduced postprandial rise in blood glucose and plasma insulin as well as GIP are observed (Gatenby *et al.* 1996).

Insoluble fibre effects on glucose and insulin responses are less conclusive than the effects observed with viscous soluble fibres. However, epidemiological evidence suggest that the consumption of insoluble cereal dietary fibre and whole grains are associated with reduced risk of type 2 diabetes (Schulze *et al.* 2007). Using purified insoluble fibre supplements from cereals (10.4 g three times a day, over 3 days) significantly improved whole body insulin sensitivity in obese women (Weickert *et al.* 2006b). The mechanisms explaining the effect of insoluble fibre on glucose and insulin responses are not related to gastric emptying or macronutrient absorption, thus other factors such as increased fermentation and production of SCFAs may be responsible. Improvement in hepatic insulin sensitivity following fermentation has been reported in humans (Thorburn *et al.* 1993), which may be related to up-regulation of glucagon-like peptide-1 (GLP-1) as reported in diabetic rats supplemented with oligofructose (Cani *et al.* 2005a). GLP-1 is secreted in the small intestine and stimulates

insulin secretion in a glucose-dependent manner; it also suppresses glucagon secretion and also acts as an appetite suppressor.

Effects on blood lipids – implications for cardiovascular disease

Hypocholesterolemic actions are the best documented health effect attributed to certain types of non-digestible polysaccharides. Guidelines from the National Cholesterol Education Program's Adult Treatment Panel III (ATP III) for the treatment of dyslipidemia and for general recommendation on CVD risk reduction recommends the consumption of at least 5–10 g/d of viscous soluble polysaccharides for reduction of plasma LDL-cholesterol while intakes of 10–25 g/d are recommended for additional LDL-lowering effects (Anon. 2001).

Fat digestion and absorption is a complex process that involves pancreatic enzymes (pancreatic lipase), bile acids and micelle formation. The main sites for digestion and absorption are along the small intestine. Bile which also contains cholesterol is a main factor for fat absorption. Following fat digestion and absorption, products of exogenous fat are delivered to the blood as chylomicrons (mainly containing triacylglycerol). Chylomicrons are transported from the enterocyte to the liver (enterohepatic circulation), adipose tissue and muscle. Endogenous cholesterol will be partially absorbed in the enterocyte. In the liver, lipoproteins will be produced where endogenous fat can be incorporated into the metabolic pool and further transported to the blood. Lipoproteins in the liver mainly comprise triacylglycerol, phospolipids and apoproteins. They are defined by their density, which is determined by the ratio of lipid to protein in the lipoprotein particle. Thus, particles with lipid content are lighter and less dense. This property relates directly with the transport function and metabolic inter-relationship in blood. The types of lipoproteins include very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL). High circulation of VLDL- and LDL-cholesterol are associated with quality and quantity of dietary fat and with increased risk of coronary heart disease. Other dietary components also affect circulating blood lipids. Non-digestible polysaccharides such as soluble viscous polysaccharides are capable of binding or competing with binding of bile acids and prevent reabsorption of cholesterol which interrupts the enterohepatic circulation. An increase in faecal bile acid loss due to entrapment of bile acids by viscous polysaccharides results in reduction of fat absorption by inhibition of micelle formation.

Reduction in plasma total cholesterol and LDL-cholesterol has been confirmed in several RCTs using pectin, psyllium, oat bran and guar gum supplements. The lipid lowering effects of hydrocolloids are summarised in Table 3.2.

Based on a meta-analysis of RCTs, Brown *et al.* (1999) reported that the consumption of 2–10 g/d of isolated oat fibre, psyllium, pectin, or guar gum in hypercholesterolemic subjects over a period of 34–66 days produced changes in total cholesterol of 0.045 mmol/l per g of soluble fibre (95% CI − 0.054; −0.035) and LDL-cholesterol of 0.057 mmol/l per g of soluble fibre (95% CI − 0.07; −0.044). The hypocholesterolemic effect of the viscous fibres in these mid-term studies is reported to be modest but significant (Brown *et al.* 1999). Longer-term

Table 3.2 Summary of RCT on the effect of hydrocolloids in blood lipids

Hydrocolloid	Subjects	Dose/Time	Effect	Reference
Pectin, psyllium, isolated oat bran, guar gum	Hypercholesterolemic	2–10 g/d over 34–66 days	↓ Total-cholesterol ↓ LDL-cholesterol	Brown *et al.* (1999)
Mixture of psyllium + galactomannan	Overweight and obese	8–12 g/d over 16 weeks	↓ Total-cholesterol ↓ LDL-cholesterol	Salas-Salvado *et al.* (2007)
Psyllium	Hypertensive-overweight	10.5 g/d over 6 months	↓ LDL-cholesterol	Cicero *et al.* (2007)
β-Glucan from oats	Hypercholesterolemic	6 g/d over 6 weeks	↓ Total-cholesterol ↓ LDL-cholesterol	Queenan *et al.* (2007)
β-Glucan from barley (both high and low molecular fractions)	Hypercholesterolemic	5 and 3 g/d over 6 weeks	↓ Total-cholesterol ↓ LDL-cholesterol ↓ TAG	Keenan *et al.* (2007)
Konjacmannan (glucomannan)	Metabolic syndrome	8–13 g/d over 3 weeks	↓ Total-cholesterol ↓ LDL-cholesterol	Vuksan *et al.* (2000)
Arabinoxylan	Metabolic syndrome	15 g/d over 6 weeks	↓ fasting and postprandial TAG	Garcia *et al.* (2006a,b)

studies (16 weeks) in overweight and obese subjects have also confirmed that the consumption of a mixture of psyllium and galactomannan in smaller doses (8–12 g/d) resulted in reductions in total cholesterol (0.13–0.36 mmol/l) and LDL-cholesterol (0.24–0.38 mmol/l) (Salas-Salvado *et al.* 2007). Similar reductions in LDL-cholesterol were observed in hypertensive-overweight subjects supplemented with 10.5 g of psyllium over 6 months (Cicero *et al.* 2007). Beneficial changes in plasma HDL-cholesterol and triglycerides (TAG) have not been confirmed in any of these studies.

Concentrated β-glucan from oats, a product which is easier to incorporate into meals and shows good acceptability, has also shown hypocholesterolemic effects. The consumption of 6 g/d of β-glucan during 6 weeks in hypercholesterolemic subjects reduced total cholesterol by 0.3 mmol/l and LDL-cholesterol by 0.3 mmol/l from baseline if compared to the control group (Queenan *et al.* 2007). Similar results were reported using concentrated β-glucan from barley. The consumption of high molecular weight barley β-glucan concentrate in doses of 5 and 3 g/d over a 6-week period showed reductions in plasma total cholesterol of 0.74 and 0.48 mmol/l respectively; plasma TAG reductions were 0.27 and 0.14 mmol/l and plasma LDL-cholesterol reductions were 0.57 and 0.35 mmol/l. Low molecular weight barley β-glucan showed similar results; however, the hypocholesterolemic effect of the high molecular weight fractions was more effective (Keenan *et al.* 2007).

Supplementation with isolated glucomannan fibre from the konjac root (konjacmannan) in a dose of 8–13 g/d for a period of 3 weeks in subjects with the metabolic syndrome reduced serum total cholesterol and LDL-cholesterol by 0.49 and 0.74 mmol/l, respectively, when compared to controls (Vuksan *et al.* 2000).

Arabinoxylan, a byproduct of wheat flour processing with viscous type dietary fibre actions, has also been shown to affect lipid metabolism in subjects with the metabolic syndrome. In a cross-over RCT, arabinoxylan consumption (15 g/d) for 6 weeks reduced fasting plasma TAG levels compared with placebo (Garcia *et al.* 2006b). Additionally, TAG postprandial response after a liquid meal challenge test also declined after 6-week arabinoxylan supplementation (Garcia *et al.* 2006a).

The role of insoluble polysaccharides on dyslipidemia is rather controversial. At present, there is no conclusive evidence that the consumption of isolated insoluble dietary fibre has a beneficial effect on blood lipids. The only evidence linking insoluble dietary fibre to beneficial effects on lipids are the studies on carob fibre pods (*Ceratonia siliqua*). Carob fibre contains circa 68% of insoluble fibre and a small amount of soluble fibre (6%). Reductions in total cholesterol (0.36 mmol/l) and LDL-cholesterol (0.43 mmol/l) in carob supplementation (15 g/d during 6 wks) vs placebo were reported in hypercholesterolemic subjects (Zunft *et al.* 2003). However, the hypocholesterolemic effects observed after carob consumption cannot be attributed completely to the insoluble dietary fibre fraction, since carob also contains a high concentration of polyphenols (mostly gallotanins), which may also have contributed to the results.

The effects of other types of dietary fibre (non-digestible oligosaccharides, non-absorbable sugars) such as inulin-type fructans on lipids are inconsistent. In two independent RCTs (blind, crossover design) hypercholesterolemic subjects supplemented with 18 g of inulin during 6 weeks reduced their plasma total cholesterol and LDL-cholesterol (Davidson *et al.* 1998) while supplementation with 20 g inulin/d during 3 weeks resulted in just plasma TAG reductions (Causey *et al.* 2000). Hypocholesterolemic effects of fructo-oligosaccharides have not been reported.

Lactulose, a non-absorbable sugar has detrimental effects on lipids by raising serum cholesterol levels in humans when administered in a dose of 18–25 g/d during 2 weeks (Jenkins *et al.* 1991), which may be related to increased acetate production.

Systemic anti-inflammatory effects

There has been increased interest in the role of dietary fibre on inflammation. Butyric acid produced during fermentation is thought to inhibit colonic inflammation and as inflammation is a major factor in many chronic diseases such as CVD and cancer, the effect of some hydrocolloids on systemic inflammation markers has been studied. However, RCTs on obese subjects have not confirmed any effects on anti-inflammatory markers such as C reactive protein (CRP) (Salas Salvado *et al.* 2007). Studies on the effects of ispaghula (psyllium) on CRP have also shown conflicting results reducing CRP in lean individuals with normal blood pressure but not in hypertensive overweight or obese subjects (King *et al.* 2007, 2008).

3.3.3 Colonic health

As discussed above, the main site of action of dietary fibre is the large intestine and a high fibre diet would be expected to improve colonic health. This may be due to the prevention of constipation by fibres which are poorly fermented and retain water holding capacity such as ispaghula (Ornstein *et al.* 1981; Passmore *et al.* 1993), gellan and wheat bran. These fibres are also thought to dilute and reduce exposure to toxins and carcinogens by reducing transit time through the colon. Fermentable fibres and hydrocolloids lose their structure but may promote colonic health through the production of SCFAs, especially butyrate; see above.

3.4 Clinical nutrition

3.4.1 Supplements

Patients who need nutritional supplements or have reduced capacity to digest complex foods are often given simple foods such as elemental and polymeric diets and other meal replacements. For a long time dietary fibre was not considered necessary or desirable in such feeds which resulted in constipation

and other side effects related to no food reaching the large intestine. If the colon does not receive fibre and its resultant SCFA, its mucosa atrophies and this can result in diarrhoea and prolonged recovery. It is now more recognised that it is important to include some form of easily administered fibre in such feeds.

Partially hydrolysed guar gum (PHGG), the result of enzymatic hydrolysis of native guar gum. has a lower molecular weight and lower viscosity than guar gum. These physico-chemical properties have facilitated its use in the food industry as a source of dietary fibre in several products such as cereals, juices, shakes, yogurt, liquid meal replacements, soups, baked goods and in enteral products.

3.4.2 Enteral feeds

Enteral diets are prescribed when patients are unable or unwilling to eat and their nutritional status is compromised. Enteral diets are liquid to allow transport via tube feeding to the gut to be absorbed. The macronutrient composition of enteral diets includes protein, simple carbohydrates and fat, while non-digestible carbohydrates have been difficult to incorporate due to their physico-chemical properties which results in increased viscosity. The lack of fibre results in poor gastrointestinal function. Thus, fibre supplementation has been recommended to normalise bowel function, improve feeding tolerance and reduce diarrhoea during enteral nutritional support.

A comprehensive meta-analysis of RCTs on the effects of fibre supplementation of enteral feeds in hospitalised patients indicated that the use of fibre supplements significantly reduced incidence of diarrhoea (OR 0.68, 95% CI: 0.48–0.86 from 13 studies) in hospitalised patients. Fibre sources commonly used include soy polysaccharides, a mixture containing soy polysaccharides, alpha-cellulose, arabic gum, inulin, oligofructose and resistant starch, hydrolysed guar gum, pectin and psyllium (Elia *et al.* 2008).

PHGG has been shown to be effective in reducing diarrhoea incidence in septic patients receiving total enteral nutrition (Spapen *et al.* 2001). Reduction in symptoms in patients with irritable bowel syndrome have also been reported when using 5 or 10 g of PHGG over 12 weeks (Parisi *et al.* 2005).

3.5 Future trends

3.5.1 Food matrix effects with phytochemicals in food

Certain foods are naturally rich in non-digestible carbohydrates and phytochemicals such as phenolic compounds. This matrix combination may provide an additive health effect of the bioactive compounds present in the food matrix.

3.5.2 Other applications

Other applications include carbohydrate-gel supplementation and endurance performance during intermittent high-intensity shuttle running. Ingesting a

carbohydrate-gel, along with water, improves performance after prolonged intermittent running in healthy male subjects, possibly by maintaining blood glucose levels during exercise (Patterson and Gray 2007).

3.5.3 New and novel sources

As discussed above, there are always new studies looking at new plant and other sources for food hydrocolloids and the health benefits of these need to be fully explored.

3.6 References

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4

Agar

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Abstract: Agar has its origins in Japan in 1658. It was introduced first in the Far East and later in the rest of agarophyte seaweed producing countries. Its use was introduced in Europe in 1859 and it was being used in bacteriological culture media in 1882. This chapter discusses the seaweeds used the world over as raw material for agar production (agarophyte seaweeds), and presents the industrial processes used for agar production. The chemical structure of agar and its fractions, such as agaroses and agaropectines, are presented, and the relation between its chemical structures and properties are also shown. The gelation and melting of agar and its fractions, such as agaroses and agaropectines, is based only in the formation of hydrogen bridges (physical gels), and thus gelation is extraordinarily reversible. The synergies and antagonisms among agar and other products in the gelation processes are studied. Various applications of agar are presented, in some of which agar is irreplaceable. Use formulations are shown in food preparations. Diverse applications are also presented, in the preparation of food for insects, for plant tissue culture, and in the preparation of culture media for microorganisms, as well as gels for denture moulding, the reproduction of archaeological remains or of fingerprinting in police work. Agar gels are important in food preparations with high content in soluble gross fibre, as agar is the food additive with the highest content in said fibre, superior to that of 94%. Lately the production of more easily soluble agars in water at temperatures below boiling point has been initiated, which proves to have noteworthy advantages for some of its applications. A comparative study is presented among the commercial products existing in the world market.

Key words: agar-agar, kanten, gelose, gulaman, agaroses, agaropectines, gelification, syneresis, agar applications, agar soluble fibre, quick soluble agar.

4.1 Introduction

Agar-agar, also called simply agar, was the first phycocolloid used as a food additive in our civilisation having been employed in the Far East over 300 years ago. Phycocolloids are those gelling products extracted from marine algae that are utilised in several ways solely because of their colloidal properties. The most important ones are agar, alginates and carrageenans that are produced in industrial quantities and presented in the form of clear coloured powders. In the Orient 'natural agars' in the old forms of strips and squares are still being used at home to prepare traditional dishes. Lately such types of agar have reached our dietetic and natural food stores while in Japan they are being substituted by powdered industrial agars prepared as tablets.

Agar is defined as a strong gelling hydrocolloid from marine algae. Its main structure is chemically characterised by repetitive units of D-galactose and 3-6,anhydro-L-galactose, with few variations, and a low content of sulfate esters.

The extraordinary gelling power of agar is based exclusively in the hydrogen bonds formed among its linear galactan chains that provide an excellent reversibility, with gelling and melting temperatures that differ normally by about 45 ºC. Being 'physical gels' they provide agar with interesting and unique properties in many of its applications especially in the preparation of microbiological culture media (bacteria, yeast and moulds) in which such properties become fundamental.

In addition, we can add that agar is also a mixture of polysaccharides made of dextro and levo galactoses united linearly.

4.1.1 Historical background

In Japan, where agar has been used for several hundred years, Tarazaemon Minoya is generally considered its discoverer in 1658. In fact, this phycocolloid had certainly been utilised much earlier than any other phycocolloid, such as alginates or carrageenans, which it predates by 200 years. From Japan its use extended to other oriental countries during the seventeenth and eighteenth centuries.

Agar was introduced in the West by Payen (1859) as a Chinese foodstuff and its microbiological applications were presented by Koch (1882). Hence, it can be said that it was known in the western world towards the end of the nineteenth century. Smith (1905) and Davidson (1906) contributed to its wider application by presenting very clear explanations of the raw materials and manual processes involved in its production in Japan. Further information on the origins of agar production in Japan can be found in Armisén (1999).

Later, in 1946, Professor Tseng published two important monographs (Tseng, 1946a,b) which contributed much information on many aspects of agar related to its raw materials as well as to its maunfacturing processes, although at that time many aspects regarding its chemical structure were unknown and could only be studied years later when spectroscopic techniques such as infrared spectroscopy were developed and even later on when NMR proton and ^{13}C spectroscopy became available.

Other publications that give general information regarding agar include Mantell (1965), who provides historical accounts of international commerce, qualities produced and some manufacturing processes that cover the inter-war periods as well as the post war years until the 1950s. Later, the well-known researcher Dr Martin Glicksman published two works in 1969 and 1983 updating hydrocolloids. These works reflect quite accurately the state of the art in those days regarding chemical structures and applications. The chapter on agar by Norman Stanley published in 1995 puts together quite useful information regarding the structure and gelling properties of agar.

Being the first phycocolloid to be used, it is one of the food ingredients first approved as GRAS (Generally Recognized as Safe) by the US Food and Drug Administration (FDA). This was in 1972, based on the positive as well as lengthy experience acquired by its usage in the Far East for more than three centuries. In addition, it also passed all other controls in its toxicological (FDA 1973a), teratological (FDA 1973b) and mutagenic (FDA 1973c) aspects.

4.1.2 Agarophyte seaweed used for production

In the middle of the seventeenth century, agar was produced in Japan from *Gelidium amansii* exclusively, then China and Korea followed soon after. This 'red' (Rhodophyceae phylum) seaweed type was the one most abundantly available along their respective coasts. When *Gelidium amansii* was in short supply, efforts were made to utilise other Rhodophyceae as substitutes.

When *Gracilaria* seaweeds started to be used, agars with very poor gelling properties were obtained and these were called agaroids. Only in 1938, when Yanagawa discovered the alkaline hydrolysis of sulfates, was it possible to increase the gel strength and produce stronger agars from *Gracilaria*. *Gracilaria* contains in its cells an agar content of a very weak nature which Yanagawa learned to transform into a stronger final product by using this alkaline hydrolysis processing method.

In *Gelidium*, *Gelidiella* and *Pterocladia* seaweed, there occurs a natural internal transformation through an enzymatic process that can be considered a maturing of the polysaccharide in the weed. In *Gracilaria* it is not converted in the needed amount during the weed's lifetime so it becomes necessary to produce it industrially by means of a chemical method before extracting the agar from the weed.

In Fig. 4.1 we can observe the different agarophytes used in the world for agar production. Due to some confusion in taxonomy because of frequent name changes, we have decided to use the classical names applied normally for agarophytes in worldwide commerce. Once DNA determinations are done on each weed, hopefully a permanent description basis will be established.

There are several types of agars available with very different applications due to the distinct characteristics of each one. They have been developed to satisfy diverse applications and they originate from different agarophyte algae that are produced by diverse technologies. Mainly we should distinguish 'natural agars'

Phylum. Rodophyta.
Class. Florideophyceae.
Order. Gelidiales.
Family. Gelidiaceae.
Genus. *Gelidium.*
Species. *G. sesquipedale*, G. amansii*, G. robustum*, G. pristoides, G. canariense, G. rex, G. chilense, etc.*
Genus. *Gelidiella.*
Species. *G. acerosa.*
Genus. *Pterocladia.*
Species. *P. capillacea*, P. lucida**
Order. Gracilariales.
Family. Gracilariaceae.
Genus. *Gracilaria.*
Species. *G. chilense*, G. gigas, G. edulis, G. gracilis, G. tenuistipitata*,*
Genus. Gracilariopsis
Species. *G. lamaneiformis, G. sjostedtii*
Order. Ahnfeltiales.
Family. Ahnfeltiaceae.
Genus. *Ahnfeltia.*
Species. *A. plycata.*

* The most used agarophytes in industry.

Fig. 4.1 The taxonomic classification of agarophytes. *Source*: Armisén (1995).

from 'industrial agars'. The first have been produced by artisans and lack technical controls but are still sanitarily clean and proper for its main use in home cooking. The latter types are manufactured in modern factories, are utilised as industrial food ingredients, and so are subject to all kinds of established controls. Agars utilised for microbiology and biotechnology are also included in this category even though they comprise only 10% of the total volume.

Agar is a polysaccharide that accumulates in the cell walls of agarophyte algae. It is embedded in a structure of fibres of crystallised cellulose, constituting its polysaccharide reserve. For this reason, agar content in weed varies depending on the seasons. Initially an intermediate form of agar with low molecular weight and quite sulfated is secreted by the Golgi apparatus of the cell. Once deposited in the cellular wall, it enzymatically polymerises and desulfates, being converted mostly into agarose that gives the agar its gelling power. The rest remains in the form of agaropectin. Matsuhashi (1990) suggested that agar could be linked to the cellulose fibres by calcium ions. This would explain many phenomena that occur during the extraction process.

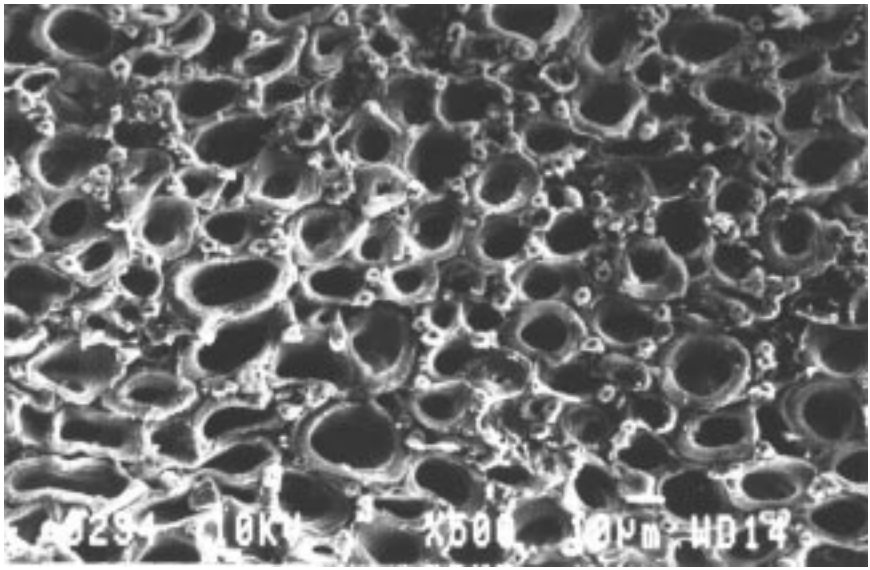

Fig. 4.2 Electronic scanner microphotograph of a section of *Gelidium Sesquipedale*. *Source*: Vignon *et al.* (1994).

In Fig. 4.2 we can observe a section of *Gelidium sesquipedale*, the agarophyte found in Western Europe and Morocco. The image shows the enormous thicknesses of the cellular walls where agar is found. The smaller cells or rhizoids contain agaroses of higher molecular weight. It so happens that the number of rhizoidal cells is higher where the water movements are stronger. Therefore the hydrodynamic conditions of the environment where *Gelidium* grows play an important role in the agar content in the weed as well as its gel strength characteristics.

4.2 Agar manufacture

Agar is called Kanten in Japanese, which means 'frozen sky' referring to the way it was first manufactured by artisans based on freezing and thawing of the extracted agar gel in the open fields. It is derived from the technique recorded by Tarazaemon Minoya in 1658 that has its fundamentals in the insolubility of agar when cooled. The traditional technique adapted by Minoya, developed towards the middle of the seventeenth century, is still in use marginally to produce 'natural agar' in the oriental craft industry in the forms of strip agar (Ito-Kanten) or square agar (Kaku-Kanten). This technique started with careful washings of *Gelidium amansii* employing similar devices to those used to wash tea-leaves.

The washed seaweed was selected by hand to eliminate any foreign body or extraneous seaweed. It was extracted in boiling water adjusting its pH. In olden days the adjustment was made with vinegar or sake but now diluted sulfuric acid

is employed. The liquid extract was filtered while hot through cotton bags, poured into wooden trays and allowed to gel by cooling. The gels were cut into square bars ($4 \times 6 \times 24$ cm) or extruded to produce spaghetti-like strips 25–40 cm long. The gels prepared in these forms were placed on bamboo grills and left to freeze all night in the open, usually in bluffs facing the northern winds. Once totally frozen during one or two nights, the agar was thawed during the daytime by sprinkling water over it. It was then sun dried and kept away from frosts. After the Second World War and up to the 1960s there were approximately 400 artisan plants in operation producing between 4 and 10 kg per day, climate conditions allowing. It can be seen that this basic technique produces low standards and irregular qualities because the process is very dependent on climate. But it is reviewed here, as is a simple way to comprehend the more involved processes that are performed now using more sophisticated mechanical means.

What are known as industrial agars are produced in modern plants in which fully standardised agars are obtained, assuring qualities that comply with physico-chemical and bacteriological specifications in accordance with sanitary codes. The best installed plants comply with ISO-9000 norms, which assure the market that all processes are controlled and traceability is available from raw materials to shipped finished goods. Figure 4.3 shows a general industrial process that can be utilised to produce agar. It is to be noted that two different paths can be followed to dehydrate the gelled agar extracts.

4.2.1 Freezing-thawing method

This traditional method was first used for ‘natural agar’ production, and was not substantially changed until American Agar & Co. (San Diego, USA) started to manufacture agar industrially in 1939, in freezing tanks in the same way as ice bars are made. Immediately after the Second World War the same technique was applied in Japan as well as in the new plants that were built in Spain, Portugal and Morocco. The seaweed extract, which normally contains between 1% and 1.2% of agar during the process, is concentrated after thawing and straining (normally by centrifugation) to contain 10% to 12% of agar which is a tenfold increase. The eluted water carries away oligomers, organic and inorganic salts as well as proteins from the algae including phycoerytrins that produce the red colour of the Rhodophyceae family. This process is one of purification.

4.2.2 Syneresis method

This method is based on the property of gelling colloids by which the absorbed water can be eliminated by means of a properly applied force. The technique was utilised in Japan solely for *Gracilaria* agar on a semi-refined scale. Gelled extracts packed in cloths with a close mesh were pressed under stone blocks to push the water out from the gel. Afterwards, it was pressed by means of small hydraulic presses to eliminate the residual water.

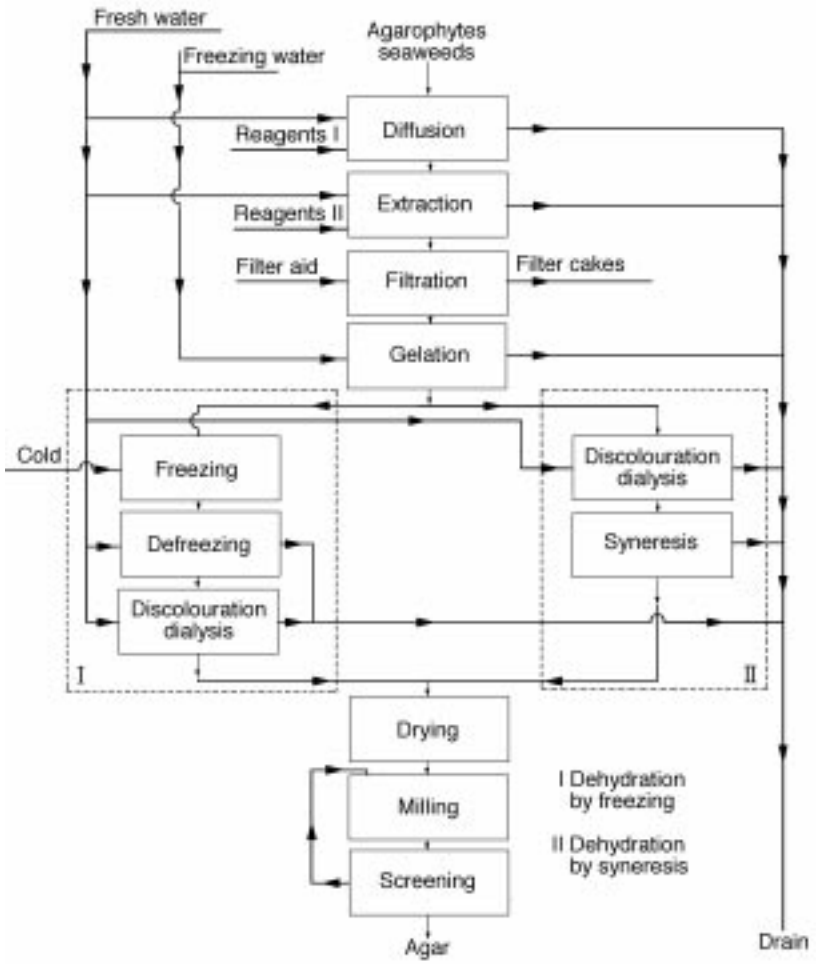

Fig. 4.3 Agar fabrication diagram. *Source*: Armisén and Galatas (1987).

In 1964, Prona, a forerunner of Hispanagar, innovated a modern syneresis technique to concentrate gelled extracts for all agarophytes (not only *Gracilaria*), building new plants in France, Mexico, Chile and South Africa as well as modernising those in Spain, Portugal and Morocco. It gave the company the worldwide leadership in agar production, only shared with Algas Marinas (Chile) in the late 1980s and with INA (Japan) in the last decade. Concurrently Okazaki wrote in 1971 that syneresis was possible only for *Gracilaria*. The method, which allowed a semi-automated process, was later further improved to a totally automated one which is in use nowadays. Vertical mechanical presses have been substituted by horizontal hydraulic ones. This syneresis technique has spread rapidly all over the world due to its reduced energy cost. The freezing method requires an ice production of 80/100 metric

tons to produce one ton of agar while the consumption of energy by the syneresis method is very low. Both methods can be mechanised today with a slight advantage in costs for syneresis. On the other hand, some buyers still prefer the quality of freeze-thaw agar for which they are ready to pay a premium.

Agar purity is increased in syneresis as the dry extract weight after pressing is as high as 20% compared to 11% in freezing which means that the latter has double the water content where the impurities stay while syneresis eliminates more soluble impurities. This is reflected in the lower ash content found in syneresis agars but again we state that many customers do not care about this.

From those operations in Fig. 4.3, we can consider the most important ones in addition to dehydration to be:

- *Treatment*: Usually an alkaline treatment is employed that allows a better extraction of the polysaccharide from the cell walls. For *Gracilaria* a stronger reaction is required to produce an alkaline hydrolysis of the sulfates in order to increase the agar gel strength.
- *Extraction*: The agar contained in the cell wall is detached and dissolved in boiling water and often under pressure. A careful control of the pH is needed to obtain the best yields.
- *Filtration*: Requires special care since clarity and purity will depend on this operation. Most standard filtering techniques can be applied.

4.3 Chemical structure of agar

Agar is a polysaccharide that formerly was considered to be formed by one unitary structure only having sulfate semiester groups linked to a few galactose hydroxyl groups. Choji Araki (1937) showed that agar was formed by a mixture of at least two polysaccharides that he named agarose and agaropectin. Later in 1956 he assigned agarose the structure that can be seen in the upper part of Fig. 4.4. In 1938 Percival, Sommerville and Forbes, and independently Hands and Peat (1938), had discovered the existence of 3-6 anhydro-L-galactose as part of the agar molecule.

When diverse types of agars were carefully studied, the presence of agarobioses which we can observe in the lower part of Fig. 4.4 (Lahaye and Rochas, 1991) was proven. These 11 agarobioses can be produced in many variable forms by the different agarophytes depending on the gender and species which depend on their genetic characteristics. It is influenced by a series of ecological factors such as the nutrient availability, substrate composition on which they grow and the habitat hydrodynamic conditions. But of greater importance for the production of such agarobioses is the harvesting period as agar plants mature gradually through the summer season.

Agaroses are the fractions of agar that essentially gel. They have high molecular weights above 100,000 Daltons and frequently surpass 150,000 Daltons as well as a low sulfate content usually below 0.15%. The rest of the fractions are known as agaropectine. They have a lower molecular weight

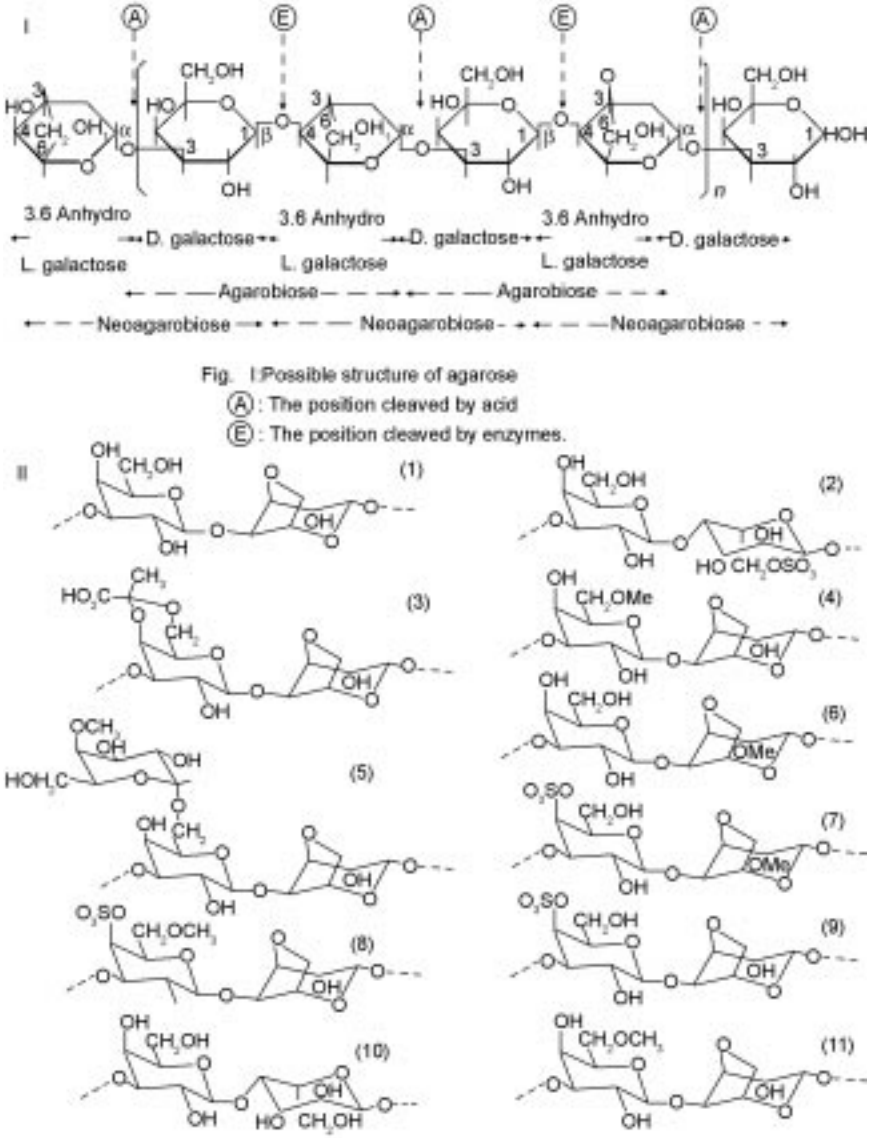

Fig. I Possible structure of agarose
Ⓐ : The position cleaved by acid
Ⓔ : The position cleaved by enzymes.

Fig. 4.4 Agarose chemical structure. *Sources*: Araki (1956) and Lahaye and Rochas (1991).

usually below 20,000 Daltons, typically 14,000 Daltons. Sulfates are in much higher content registering sometimes 5% to 8%. This is far below carrageenans which range from 24% to 53% and even furcellaran which is the least sulfated carrageenan at about 17%.

The determination of molecular weights in these polysaccharides may be obtained through the use of various techniques, but the easiest one is to use non-

specialised laboratories in these particular determinations, where it is carried out applying the 'intrinsic viscosity' determination of its dissolutions (see Hickson and Polson, 1968; Rochas and Lahaye, 1989).

Agaroses and those including agar do not present too much difficulty, but in the case of carragenates (due to their great content of sulphate groups), it is necessary to avoid the formation of ionic bonds with divalent and trivalent cations that bring about molecular artefacts causing false results to be obtained by increasing their molecular weights.

Frequently molecular weights attributed to agaropectins are in many cases higher than the real ones, but this is because generally they have been determined from intrinsic viscosities. In such case those values are much higher than the real ones since the agaropectins bind to earth-alkaline ions such as Ca^{++}, producing molecular artefacts by crosslinking several agaropectins.

Agaropectins have been studied less than agarose due to their lack of practical applications. Their properties do not match those of carrageenans for food applications and any process to produce them would be costly and complicated. As mentioned previously, some agaropectins are precursor polysaccharides for agaroses that are transformed internally by enzymatic polymerisation and desulfation processes.

As will be shown later, the diverse forms of agarobioses determine the physico-chemical characteristics of agar such as gelling and melting temperatures and reactivities or synergies of agars with other products.

4.4 Agar gelation

Agar is a mixture of agarose and agaropectin fractions in variable proportions depending on the original raw material and the manufacturing process employed. Agar gelation occurs only by its agarose content that is produced exclusively by hydrogen bonds. Not needing any other substances to gel, it has an enormous potential in applications such as as a foodstuff ingredient, for biotechnology uses, for cell and tissue culture or as support for electrophoresis or chromatography. Agarose produces 'physical gels' which means that these aqueous gels have all their structure formed only by the polymer molecules which aggregate through hydrogen bonds. Due to this unique gelling property, these gels hold in the interior network a great amount of water which can move more freely through the macroreticulum. In contrast, 'chemical gels' have the polymer molecules linked through covalent bonds.

The most remarkable property of 'physical gels' is their reversibility. They melt just by heating but gel again upon cooling. These transformations can be repeated indefinitely in the absence of aggressive substances that could hydrolyse the agarose molecules or destroy them by oxidation. 'Chemical gels' such as polyacrylamides that have their molecules joined by covalent bonds are irreversible. Another basic property is the gelling mesh size that in the case of agarose is identified by very high exclusion limits. Exclusion limit is defined by

the greatest globular protein size that can traverse the gel in an aqueous solution. In the case of a 2% agarose gel the exclusion limit is 30,000,000 Daltons. Considering that there is no protein of such a size for calibration, it has been necessary to resort to subcellular particles such as ribosomes or viruses.

The agaropectins fractions present in agar narrow the reticulum reducing slightly its exclusion limit. Intermediate between the physical and chemical gels, we find those gels that require the presence of cations to form gel structures as in the case of carrageenans and alginates. In the case of gels formed by alginic acid with di or tri-valent cations we face totally irreversible gels that will not melt by heating. These are gels that have formed ionic bonds that can be broken only by eliminating the bonding cation, which is normally calcium. It is done with the help of a complexing agent such as EDTA (ethylene diamine tetracetate). Hence, these gels can be considered as 'ionic chemical gels' as they form ionic bonds and are irreversible. An important property of agar gels derived from their agarose content is the very high gelling hysteresis, defined as the temperature difference between its gelling (around 38 ºC) and melting temperatures (around 85 ºC).

In Fig. 4.5 we can observe the gelling and melting temperature graphs for regularly available agars. Concentrations are also typical (between 0.5% and 2%) being employed in agar applications. It shows a gelling hysteresis that in every case is above 45 ºC. As a comparison, the strongest gelling carrageenans have a hysteresis of 12 ºC that is 26% less than agar. These temperatures depend on the presence of agarobioses originally in the agarophyte seaweed from where the agar is extracted. Gel temperature is an indicator to identify the agarophyte used to produce an agar. Using Table 4.1 we can determine the origin of an agar by identifying its characteristic gelling temperature.

It has been proven that the gelling temperature is influenced by the methoxylation degree of the C_6 of the agarobioses present in the agar, in such a manner that the more methoxylated correspond to *Gelidiella* agaroses and the least to the *Pterocladia* ones. This is the same as saying that a greater methoxylation in C_6 will correspond to a higher gelling temperature. Curiously, the methoxylation of the rest of the carbons reduces the gelling temperature and its gel strength at the same time. This is due to the inability to establish hydrogen bonds by the hydroxyl groups located in C_6 because of their position in the gelling helices while the rest of the hydroxyl groups are bonding points where hydrogen bridges can be formed. The gelling process of agarose can be seen in Fig. 4.6, obtained from Medin (1995). This is an exothermic process which develops when agarose molecules are dissolved in water. It can be seen on the left-hand side that these molecules are real 'statistical random coils' subjects to Brownian movements. When cooling down close to the gelling temperature, the next structures start to form.

According to Rees and Welsh (1977) we observe in the upper part of Fig. 4.6 the formation of double helices ($\mathbf{B_1}$) which aggregate to form a macroreticulum as pictured in the upper side of **C** and **D**. In the lower part of Fig. 4.6, and according to Foord and Atkins (1989), we can see simple helices $\mathbf{B_2}$ that are joined by hydrogen bridges that produce folded structures (symmetric double

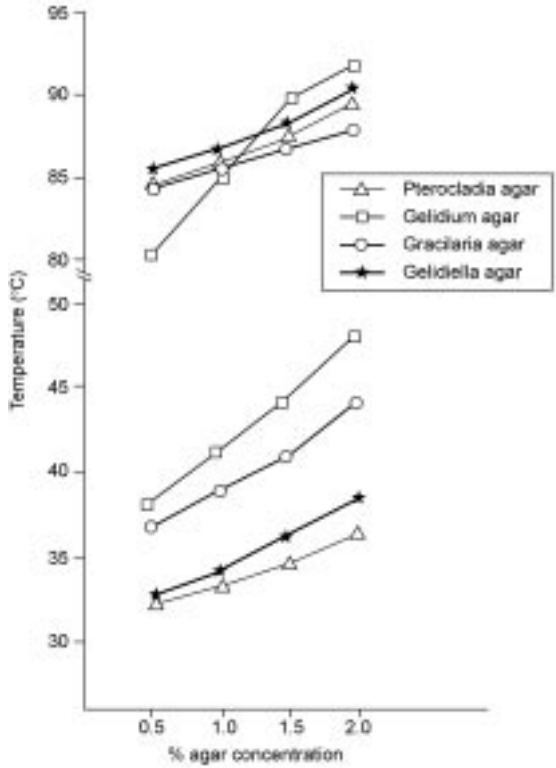

Fig. 4.5 Gelling and melting temperatures of agar gels: gelation hysteresis. *Source*: Armisén (1997).

helices) that will form the macroreticulum as can be seen in the lower side of **C** and **D**. It seems that both gelling processes can coexist and one or the other dominates depending on the cooling speed. A faster rate favors the first process. Both are based in the formation of hydrogen bridges and produce a macroreticular structure.

In Fig. 4.7 we can observe the spongy structure of a 2% agarose gel. The mesh cavities of the gel which can be seen are filled with solvent water that circulates freely through the mesh capillaries.

Table 4.1 Typical gel temperatures of agars extracted from several agarophytes (from Armisén 1993)

Genus	1.5% solution, gel temperature
Gelidiella	42–45ºC
Gracilaria	40–42ºC
Gracilariopsis	38–39ºC
Gelidium	36–38ºC
Pterocladia	33–35ºC

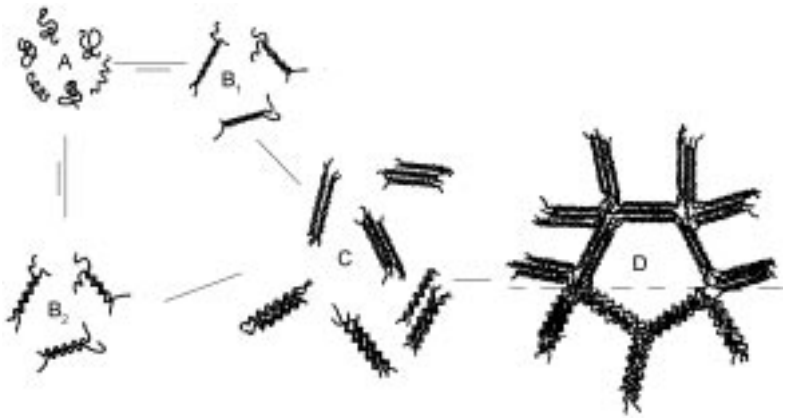

Fig. 4.6 Agarose gelation. *Source*: Medin (1995).

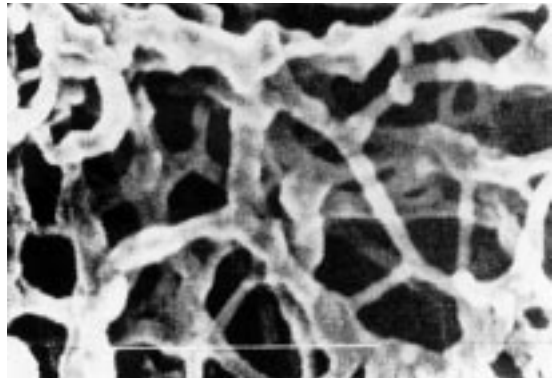

Fig. 4.7 Electronic scanner microphotography of 2% agarose gel. Source: Medin (1995).

The very high exclusion limit of agarose gels allows the passage of soluble macromolecules up to 30,000,000 Daltons of molecular weight. A characteristic property of agarose gels is their syneresis capacity which relates to the capacity to eliminate water contained in the gel mesh. The ejection of aqueous solvents is speeded by pressure conveniently applied on a properly confined gel. In these conditions a 1% agar or agarose gel can eject a great proportion of the water soaked in the capillaries ending the process with a 20–25% dried extract which means that 95% of the water used to dissolve the agar/agarose has been eliminated.

If the undried and synergised gel is submerged in water, it will recover its original size. The gel structure has been maintained during syneresis and upon rehydration it will recover exactly to the previous form. This is known as gelling memory.

4.4.1 Synergies and antagonisms of agar gels

We shall consider in this section the most important cases of blended products which modify agar by increasing its gel strength, modifying its texture or

elasticity and the antagonist products that reduce the gel strength or block in any way the gelling process.

Traditionally food agar gel strengths are controlled following the Japanese Nikan Sui method that measures the force of the plunger in grams that ruptures a gel by means of a cylindrical piston with a surface of 1 cm^2 after applying the force for 20 seconds. This traditional method is used universally even though there are other more precise ones that operate with a growing load that also allow the value of the elasticity of the gel to be obtained. No doubt these controls will be improved in the coming years but nowadays Nikan values provide the basis for commercial transactions worldwide.

Agar-locust bean gum (LBG) synergies

Synergies with this gum are only possible with *Gelidium* and *Pterocladia* agars. It has practical applications because gel strength is increased and the gel texture is modified in a way that rigidity is decreased and elasticity is enhanced, becoming less brittle. As can be seen in Fig. 4.8, a mixture of LBG and *Gelidium* agar dissolved at 1.5% give Nikan gel strengths (g/cm^2) which increase to a 9:1 ratio when its maximum hardness is achieved, returning to the corresponding agar strength when the agar-LBG proportion is 4:1.

Contrary to this, *Gracilaria* agars do not show such synergy and upon substitution the hardness falls in the same proportion as if the agar concentrations were reduced. The agar-LBG synergism is exhibited solely by the agaropectins contained in *Gelidium* and *Pterocladia* but not by those of *Gracilaria*. These differences in the graph are obtained in *Gelidium* agars if in its manufacturing process the alkaline hydrolysis of sulfates has not taken place which is essential in the case of *Gracilaria* when aiming to obtain an agar with acceptable gel strength, as mentioned previously.

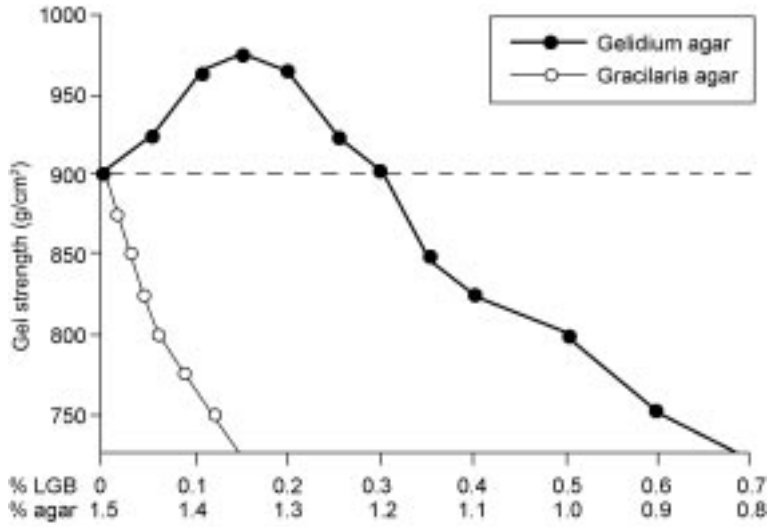

Fig. 4.8 Agar-locust bean gum synergies. *Source*: Armisén and Galatas (1987).

Sugar reactivity
Reactivity with sugar (saccharose) is basically present in *Gracilaria* agars when dissolved in aqueous solutions with a high sugar concentration (around 60%). In agars with high gel strengths and low sulfate content the synergy is caused by the thread pitch of the gelling helices. It always requires the presence of agarose of high molecular weight (around 140,000 Daltons) and is a consequence of the 3-6,anhydro bridge in the L-galactose which is able to assist in the building of hydrogen bridges.

Gelling blockade by tannic acid (TA)
The presence of TA (pentadigaloil glucose) may inhibit agar gelation if the TA quantity is large enough. This acid is found in some fruits such as, squash, apple and prune in variable concentrations. Adding glycerol in small amounts is usually enough to avoid this reaction.

Gelling blockade by chaotropic agents
Chaotropic agents which capture protons can also inhibit agar gelation by avoiding the hydrogen bridges which form between the agarose molecules present. In any case, there is no problem associated with chaotropics for the use of agar in the food industry, as foodstuffs do not contain significant quantities of agents such as urea, guanidine, sodium thiocyanate or potassium iodide.

Acid and alkaline hydrolysis
As with all polysaccharides, agar can suffer hydrolysis reducing its molecular weight and consequently losing its gelling power. Acid hydrolysis in agar appears more readily, as a result of lowered pH and the longer time the agar stays in dissolution at a high temperature. In general, hydrolysis is not an important problem unless the agar undergoes extensive heating at pHs below 5.5. Alkaline hydrolysis is not important at pHs below 8. Enzymatic hydrolysis is not relevant as there are few agarases (enzymes that break down agaroses) which are found only in marine bacteria, in a few bacilli and *Esquizosaccharomycetes* that are not normally found in food products.

4.5 Agar applications

Agar applications are fundamentally based in the enormous gelling power, high hysteresis and perfect gel reversibility which are unique properties conferred by its special ‘physical gel’ structure. Although agar has multiple applications, the traditional one is as a food ingredient that accounts for 80% of its consumption. The remaining 20% is accounted for by biotechnological applications. A list of different uses and the corresponding type of algae required can be found in Table 4.2.

There are various publications in which agar applications are included, and patents regarding those applications are referred to by their dates as we can see in Lawrence (1973, 1976).

Table 4.2 Agar grades depending on their final uses and agarophytes used for their production (from Armisén 1995)

	Agar type applications	Agarophytes used
Natural agar	'Strip' 'Square' Accustomed only on Far East traditional kitchen	Produced mostly with *Gelidium* by traditional methods
Industrial agar	Food grade agar used for industrial food production	*Gelidium, Gracilaria, Gracilariopsis Pterocladia, Ahnfeltia, Gelidiella*
	Pharmacological agar	*Gelidium*
	Clonic plants production grade	*Gelidium* or *Pterocladia*
	Bacteriological grade used for bacteriological culture media formulation	*Gelidium* or *Pterocladia*
	Purified agar used in biochemistry and in culture media for very difficult bacteria	*Gelidium*

4.5.1 Agar in food applications

Agar is a food additive of universal use considered in the USA as GRAS (Generally Recognized as Safe) by the FDA (Food and Drug Administration). In Europe it is considered an E406 additive. In the Register Service of the Chemical Abstracts it is registered as 9002-18-0. The synergies that were described when dealing with agar gelation are important. The synergy between agar-LBG shown by *Gelidium* and *Pterocladia* agars improves hardness as well as texture making the mixture more palatable due to the elasticity conferred and the elimination of brittleness and at the same time can reduce the syneresis of the gels which allows those gels to exude less liquid during handling, transportation and storage. In the same way, *Gracilaria* agars that show reactivity with sugar, experience an increase in gelling properties when used with sugars with a high sugar content (60% or more) such as jams and jellies.

Adding glycerin or sorbitol to aqueous gels prepared with agar, reduces dehydration to such an extent that with sufficient quantities of these humectant products, the drying of gels exposed to air can be avoided. Obviously, the higher the relative humidity of the atmosphere, the added quantity of the products should be lower. The ambient temperature changes during the life of the product also affect this behaviour. Agar is tasteless and cannot be detected in foodstuffs with delicate flavours. In contrast, those gelling agents that need the presence of cations (alginates, calcium or carragenates, potassium) to gel should be blended with foodstuffs with strong flavors to mask the characteristic flavor of the cations. Some agars with an elevated melting point, such as those produced in Portugal by Iberagar, are employed to prepare Mitsumame. This popular Japanese fruit salad

mixes fruits and coloured gel cubes properly flavoured and, after canning, is subjected to heat sterilisation which the agar gel cubes withstand without melting. Normally agar is dissolved by adding it slowly to water with good stirring. In sweet products agar is usually premixed with a part of sugar, then added slowly later to avoid clumps which can form during dispersion.

If acidification is required in a process, it has to take place once all the agar has been dissolved and whenever possible at reduced temperatures to minimise hydrolysis risks. Nevertheless, agar has enough resistance to hydrolysis to the degree that, in meat preserves which require sterilisation in an autoclave at over 121 ºC, it undergoes the treatment successfully without hydrolysis. Aromas, especially volatile ones, should be administered at the final stage of cooling, just prior to moulding and packing of the product. In this way evaporation losses are avoided. In some industries it is customary to also add any colouring ingredients at this stage. Given that agar is utilised in food as a minor ingredient (0.5% to 1.5%) relative to the total weight of the finished product, its nutritional contribution is low because the human digestive system hardly absorbs it. Agar has been consigned a digestibility below 10% of what is ingested. For this reason agar can be used to prepare dietetic formulas and foods for diabetics since, unlike pectins, it does not depend on sugar to gel. Formulations can be made employing sweeteners as substitutes for sugar together with agar which will create very low calorie food products.

For many years agar has been included in the US Pharmacopoeia as a laxative as it has a convenient effect as a bulking agent. In fact it is contained in several formulas for this purpose. It behaves just like natural fibre with the great quality of being totally soluble and resistant to hydrolysis. In addition, the human digestive system produces no agarases, which contributes to the extremely low digestibility of agar. Today agar is included in the US National Formulary to be used as a slow release ingredient for the slow absorption of pharmacological agents.

It is very interesting to realise that the alimentary applications of industrial agar differ depending on the cultural area that is considered. In Table 4.3 we can observe how they vary among geographical areas. We shall follow with some formulations used to prepare these products from our experience in this field and the bibliographical data available. In necessary cases indications will be given to prepare these products.

We wish to state that in no circumstances can this information be considered as an aid to infringe patents or protected processes.

Agar in the preparation of food with a high fibre content

The tendency in modern dietary practice is to increase fibre in human nourishment and since the nineteenth century an increase in the nutrient value of human foodstuff has been an objective which has gradually resulted in diets richer in protein but much poorer in fibre. This has caused a reduction of digested volumes, producing consequently smaller intestinal bolus, which is the reason for the growing constipation experienced by modern humans, especially in the more developed countries.

Table 4.3 Agar applications in several cultural world regions (from a lecture by Modliszewsky 1990)

Applications	Regions		
	Asia	US/Europe	Latin America
Ice cream	+	+	+
Milk shake	+	+	+
Sherbet	+	+	+
Custard pudding	+	+	+
Cakes	+	+	+
Pie filling	–	+	+
Flat icing	+	+	+
Meringue	–	+	–
Cookies	+	+	+
Candy (agar jelly)	+	+	+
Fruit jelly dessert	++	+	+
Jams, Jellies	+	+	+
Processed cheese	–	+	?
Ferm. dairy products	+	++	+
Wine clarification	?	+	+
Gelled meats	++	++	?
Dulce de batata	–	–	++
Mitsumame	++	–	–
Red bean jelly	++	–	–
Agar jelly beverages	++	+	+?

Nowadays, nourishment that is ingested in adequate quantities to balance a diet, being either rich in dietetic fibres such as whole breads and pasta or in pure fibres such as hydrocolloids and gums, should have the capacity to retain other compounds present in the intestine such as water, glucose, protein and fat and also to be able to interact with colonic bacteria.

Edible fibre can be defined as the fraction of the nourishment that is not digested and able to transit through the gastrointestinal tract and upon reaching the colon may partially ferment causing the non-fermented part together with water, intestinal bacteria and other compounds to be excreted. According to this definition agar-agar is the edible substance with the greatest content of soluble fibre in any available food.

Edible fibres are divided into two varieties: those classified as soluble are those which dissolve completely in neutral or acidic aqueous solutions at 100 ºC such as, for example hydrocolloids, while those considered insoluble do not dissolve due to its chemical nature derived from having high cellulose content as in the case of cereal bran. They exhibit the following properties:

- Insoluble fibres (cellulose)
  - have low water retention
  - form high viscosity mixtures in the intestine
  - partially ferment in the intestine because of microflora, which produces flatulence

Table 4.4 Soluble fibre content in foodstuffs (from Galatas 2006)

Foodstuffs	Soluble fibre (%)	Total fibre (%)
Fruits	0.5	2.8
Vegetables	0.6	3.5
Refined cereals	1.0	3.8
Fibre-rich cereals	6.5	30.1
Legumes	0.4	5.2
Bread	2.8	2.8
Meat	0.0	0.0
Fish	0.0	0.0
Carrageenans	75.0	76.0
Locust bean gum	78.0	—
Guar gum	76.5	—
Konjac	75.0	—
Agar agar	94.8	95.0

  - are slightly degraded by coliform bacteria in the colon.
- Soluble dietetic fibres (hydrocolloids)
  - have high water retention
  - form low viscosity mixtures in the intestine
  - do not ferment, therefore do not produce flatulence
  - are degradable by coliform bacteria in the colon.

Phycocolloids have an extremely high content of soluble fibre. Agar is practically composed of soluble fibre since its content is above 94% and carrageens contain from 67 to 80%. Table 4.4 presents soluble fibre contents for diverse foodstuffs as well as for phycocolloids and gums where substantial differences can be noticed.

Some formulations for agar in human diet

Yokan
Matsuhashi, T. (1990)

Yokan is a traditional agar hard gelatin consumed usually in the tea ceremony but also on different occasions. Its ingredients are sugar, azuki bean purée, agar and water. Sometimes chestnuts are used instead of beans for higher priced Yokan. An approximate formula would be:

Agar	50 gm
Water	2000 to 3000 gm
Sugar	1000 to 2000 gm
Inverted sugar	1500 to 3000 gm
Citric acid	2 gm
Flavour and colour	If desired
Azuki beans	Variable according to each case but enough to produce a hard gel.

Agar is dissolved in boiling water with sugar and inverted sugar and maintained at 106 ºC for a few hours to reduce the volume. After a brief cooling, the fruit purée previously prepared and the acid are added together with flavours and colourings. It is left to cool overnight at room temperature. This gel has a dried weight of 70–75%. It is placed in an oven at 55 ºC as long as needed to reach a dry weight of 84–86 ºC and is cut in small pieces that are first folded in a wafer and later in plastic. This wafer is an edible paper made of starch and agar. Inverted sugar is hydrolysed saccharose to avoid crystallisation.

Sweet potato sweet (an Argentinean traditional dessert)
This is a very popular sweet in Argentina and Uruguay and also in some parts of Brazil, being part of its culture.

Components:

Sugar	37%
Glucose	21%, usually syrup of 76º Brix
Agar	0.181%
Locust bean gum (LBG)	0.29%
Fresh sweet potato with skin	80%.

As it is an elaboration process, it is recommended to:

1. Peel the potato.
2. Boil it in water.
3. Sieve it to a state of purée.
4. Mix the purée with sugar and glucose.
5. Concentrate by boiling until 60º Brix.
6. Add the agar that has previously been dissolved at 25%.
7. Bring the mixture to 58/60º Brix.
8. Add to this mixture the LBG that previously has been dry mixed at four times its weight in sugar and dissolved in cold water at 6/7 times of the total weight.
9. Carry all of it to a 60.5º Brix, flavour and can while hot.

Flat cans of 25 cm in diameter and 5 cm in height are usually employed.

Sugar icings
Meer, W. (1980)

Components:

Agar	0.35%
Salt (NaCl)	0.3%
Emulsifier	0.4%
Granulated sugar	15.0%
Coating sugar (Glasee)	73.0%
Water Enough to complete	100%.

Different coatings are prepared with agar using several formulations depending on the solidification speed that is expected for each coating. These icings are

prepared by boiling the agar, used as stabiliser, in a sugar solution followed by the addition of coating sugar. The icing heated up to 50–60 ºC is applied over the products to be coated in 60 seconds. Gelation times can be regulated, increasing or reducing the agar amounts.

4.5.2 Agar in insect culture

Agar is used for the breeding of larvae and other smaller sized animal species. It is applied for fattening silkworms year round in order to lengthen the season that was previously a limited one. The tiny worms could feed only on tender mulberry leaves that were produced at early budding. Agar for this purpose is dissolved and the feed, composed of carbohydrates and proteins, is suspended in the solution. Left to cool, the mixture is extruded in the form of thin spaghetti with adequate sizes for the different worm constitutions from the egg emerging period until the formation of the silk buds. Only agar could be employed for its composition because all other gelling agents have tastes that are rejected by silkworms.

Another classic use of agar is a similar application for feeding larval phases of flies such as *Drosophila melanogaster* used for genetic research. Obviously when technologies for biological insect plague control were developed, analogous methods for larvae feeding were implemented using agar as a base. It dealt with insects that damaged intensive crops and modern methods were required for its control. In this way the Mediterranean fly that damages orchards or *Pectinophora glosipeii* caterpillars that destroy cotton farms are artificially grown in order to be subsequently sexually sterilised by γ radiation. The sterile individuals are kept hibernated and set free at the proper mating period for intercourse. Because these insects are able to copulate only once in their lifetime, their copulation capacity is frustrated rendering void their intercourse with a fertile mate. Without the need for organochlorated insecticides the species is thus controlled but not destroyed as some individuals can mate with fertile partners. This is discussed in Noble (1969).

4.5.3 Vegetable tissue culture formulations

The gelling properties of agar enhance the formulation of solid media for tissue culture growth originated in techniques developed to obtain orchid clones. Media has been formulated to reproduce the most diverse plant specimens to grow identical plants free of viruses. Usually vegetable meristems of plants to be cultivated are cultured in media of the adequate composition, enriched with vegetable hormones such as auxines or cytokines that are applied depending on the rooting desired for the plant and/or its growth velocity. Once the proper plant development is achieved, they are transferred to vegetable ground to continue their growth. Ample information regarding clonic cultivation of vegetables can be found in Pierik (1987).

4.5.4 Culture media for microorganisms

Microbial cultivation began with R. Koch in 1882 and since then its use has been related to the development of microbiology in such a way that it has never been substituted, repeated efforts to the contrary notwithstanding. The special properties of the physical gels that agars form, together with their gelling and melting temperatures and especially due to their enormous gelling hysteresis and reversibility, all contribute to and offer characteristics that are unique for these applications and have found no substitution so far. Likewise their huge resistance to degradation by enzymes that damage other gelling agents and their ability to gel in the absence of cations enable agar to support culture media with very well adjusted osmotic pressures to cell requirements whether red blood cells, bacteria, yeasts or moulds are to be grown. In Armisén (1991, 1993) we can see in more detail agar applications for the preparation of culture media for bacteria, yeast and moulds as well as agarose applications in biochemistry and molecular biology.

4.5.5 Industrial agar application formula

Dental moulds

Meer, W. (1980)

A formula for an industrial use of agar is offered as an example of a general application that is quite common in very diverse uses where high precision moulding is sought. This composition is employed to prepare dental moulds in the USA but in other countries it is utilised to prepare plasters of archeological pieces or moulds for sculptures. Police use it to preserve footprints or similar clues for criminal cases. The enormous reversibility of agar gels that change from solution to gel states by simply cooling them is an incentive due to its convenient use.

Gels can be kept for long periods in tubes similar to toothpaste ones. Before its use, they are melted in boiling water baths and the solution is placed in a tray prepared for the dental imprint. Once an adequate temperature (39 ºC) is reached, the imprint is performed immersing the tooth (or the object to be copied) and maintained this way until the *Gelidium* agar gels at 36 ºC.

Other agars that gel at higher temperatures are not recommended as they can hurt the patient. This is not the case for inanimate objects that may employ other agars. A common formulation follows:

Gelidium agar	13–17%
Borates	0.2–0.5%
Sulfates	1.0–2.0%
Hard wax	0.5–1.0%
Thixotropic materials (Bentonite type products)	0.3–0.5%
Water	Sufficient to arrive at 100%.

This formulation can be modified to achieve gels that will not dry in practice at normal atmospheric conditions. For this purpose sorbitol or glycerin should be added or any other humidifier agent that will absorb the ambient dampness to compensate for the evaporation losses. In normal weather conditions 8–10% of glycerin is required but this percentage must be adjusted for practical uses depending on the maximum and minimum temperatures the product can withstand and on the relative humidities of the environment.

4.6 Future trends

During the last decade efforts have been made to obtain agars with greater dissolving ease, i.e., that dissolve at temperatures below that of boiling water (100 ºC), with lesser heating times while maintaining high gel strengths.

In general, to totally dissolve agar when gel strengths are being determined (for example, using the Nikan Sui method) a 20-minute reflux dissolution is prescribed with which generally all industrial food agars can be placed into solution. It is known that the gel strength of commercial food agars depend basically on their agarose content in relation to their agaropectin contents, which scarcely contributes to the gel strength of the agars. The quick solubility of agars is also conditioned to the agarose molecular weight, being such that when such agaroses have molecular weights of 240,000 Daltons or higher, they need a very long time to dissolve by reflux boiling and if the molecular weight is of an even higher magnitude they may need to be dissolved by applying pressure at temperatures around 121 ºC or even higher. In any case, a faster solution of agar will be obtained if the milled dehydrated gel by syneresis, generally know as xerogel structure, is the most hydrophilic, one which is in the commercial form that food agar is commonly presented. It can be obtained with a thorough milling producing a powder that will swell much more due to a better contact of the hot water and the greater number of agar particles.

The reduction of the apparent viscosity of the agar suspension in water when dissolving contributes to a faster dissolution and it should be taken into account that agaropectins play an important role in these apparent viscosities of agar, especially in the presence of sufficient quantities of calcium ions between various agaropectins present, forming ‘artefacts’ of apparent high molecular weight. These ionic bonds distort many molecular weight statistical measurements of agar made by methods based on the determination of intrinsic viscosities, when the formation of such artefacts has not been blocked by eliminating the ionic bonds by means of an adequate treatment. Such bonds appear when calcium or even other earth-alkaline are present.

Other important factors that have influence in the fast solution are the dehydrated gel by syneresis or xerogel structures, which have been formed during the agar production and principally during the concentration of extracts, be it by syneresis, freezing-thawing or any other process as well as during drying and further treatments. Having the ability to improve agar solubility applying the

Table 4.5 Easily soluble agars comparative analysis

Product	Speed Agar 80	Grand Agar	Quick Soluble Agar
Produced by:	Mitsui Sugar (Japan)	Hispanagar (Spain)	INA (Japan)
Moisture	10.20%	10.20%	15.40%
Ash	1.40%	1.00%	1.60%
Solution clarity[a]	68 NTU	3.75 NTU	10.9 NTU
pH in 1.5% solution	6.61	7.29	6.79
pH in 1.5% gel	4.76	6.07	5.93
Viscosity in 1.5% solution 60 ºC[b]	10 cps	10 cps	19 cps
Gel temperature at 1.0% solution	36.0 ºC	31.9 ºC	38.1 ºC
Melting temperature at 1.0% solution	75.9 ºC	85.7 ºC	82.7 ºC
Gel strength at 1.5%[c] (Dissolved at boiling temperature (100 ºC) for 20 min)	550 g/cm^2	1,200 g/cm^2	1,240 g/cm^2
Gel strength at 1.5%[c] (Dissolved 5 min at 85 ºC)	530 g/cm^2	980 g/cm^2	1,020 g/cm^2
Gel strength at 1.5%[c] (Dissolved 3 min at 90 ºC)	560 g/cm^2	1,100 g/cm^2	1,180 g/cm^2

[a] Measured with Turbidimeter HACH 2100P.
[b] Measured with Viscosimeter Brookfield mod. LVF at 60 ºC.
[c] Measured with Cherri Burrell Gel Tester.

factors considered before requires introducing improvements in production methods that are only possible for industrial corporations with quite advanced technologies.

Lastly, agar solutions can be made faster by adding determined substances before extrusion and drying, but such additives that are used for this purpose can cause problems in the batch applications of such agar.

Table 4.5 gives comparative analytical data of the three quick soluble agars that actually compete in the world market, by Hispanagar (Spain), INA (Japan) and Mitsui Sugar (Japan). The data in the table have been controlled in parallel using the same analytical methods.

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5

Starch

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Abstract: No other single food ingredient compares with starch in terms of sheer versatility of application in the food industry. Speciality starches have been tailored to build textural differentiation; enhance product aesthetics; simplify a label declaration; improve the nutritional profile; reduce recipe/production costs; increase product throughput; eradicate batch rejects; ensure product consistency; and extend shelf-life. This chapter reviews the sources of starch, extraction, structure, advantages and disadvantages as a hydrocolloid, techniques for modification, nutritional profile, uses and applications and regulatory status in the EU.

Key words: starch, amylose, amylopectin, hydrocolloids.

5.1 Introduction

No other single food ingredient compares with starch in terms of sheer versatility of application in the food industry. Second only to cellulose in natural abundance, this polymeric carbohydrate was designed by nature as a plant energy reserve. Man, however, has extended the use of starch far beyond this original design. Modifications – physical, chemical or biochemical – mean that numerous highly functional derivatives have enabled the evolution of new processing technologies and market trends. To this end, speciality starches have been tailored to: create competitive advantage in a new product; enhance product aesthetics; simplify a label declaration; reduce recipe/production costs;

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increase product throughput; eradicate batch rejects; ensure product consistency; and extend shelf-life. Clearly, understanding the capabilities of starch and how to exploit its potential is of relevance to all stages of a food product's lifecycle from development, through production and marketing, to retail. The study of the biosynthesis, structure and modification of starch within the context of food applications is a rapidly developing research area. A good overview of many aspects of starch is given in Eliasson (2004).

5.2 Manufacture

5.2.1 Sources

To 'plug' into the sun is to trap the cleanest and ultimate source of energy for the planet. Photosynthesis does precisely this. Leaves – nature's 'solar panels' – trap light energy and, through a cascade of physico-chemical processes, involving carbon dioxide and water, translate this into sugar molecules, such as glucose. In itself, glucose is too mobile to act as a long-term energy storage system. Nature's solution to this is to immobilise the glucose by forming a polymer; more precisely, a condensation polymer in which glucose chains are linked together by the elimination (condensation) of water. This is starch, an anhydroglucose polymer. Starch is a member of the 'polysaccharide' group of polymers. It is laid down as insoluble, compact and microscopic semi-crystalline granules. Seeds, tubers, roots and stem piths are the repository sites for the starch. When digested, the trapped energy is released as the starch is broken down by hydrolysis back to its constituent glucose molecules and thence further back to the original carbon dioxide and water.

Despite the ubiquity of starch in nature, the number of commercially viable sources is small. The most important origins of starch are maize, potato, wheat, tapioca and rice. 'Corn' and 'maize' starches are American and European terms that actually refer to the same range of starches. The cereal crops maize, waxy maize and wheat are grown in America and Europe whilst potato is largely derived from the cooler climes of northern Europe. Most cassava (the base crop for tapioca starch) is sourced from Brazil, Thailand and Indonesia, while rice originates mainly from Asia. The morphology and size of the ganules of the major commercial sources of starch are shown in Table 5.1.

5.2.2 Extraction

The manufacture of starch employs a variety of processes which isolate purified starch from the other constituents of the raw material. For example, the extraction of maize starch from the maize kernel is a process known as wet milling in which the starch is separated from the fibre, oil and tightly bound protein. Regardless of the extraction processes involved, the objective is to recover the insoluble starch as undamaged or intact granules. In this form it is known as native starch. It can be washed and dried or left as a slurry for future processing into modified starches.

Table 5.1 Starch granule characteristics

Starch	Type	Diameter microns (μm)	Morphology
Maize[a]	Cereal	5–30	Round polygonal
Waxy maize	Cereal	5–30	Round polygonal
Tapioca	Root	4–35	Oval truncated 'kettle drum'
Potato	Tuber	5–100	Oval spherical
Wheat	Cereal	1–45	Round lenticular
Rice	Cereal	3–8	Polygonal spherical compound granules
Sago	Pith	15–65	Oval truncated
High amylose maize	Cereal	5–30	Polygonal irregular elongated

[a] Maize is also often referred to as 'corn', 'dent corn' or 'regular maize'.

5.3 Structure

Structural aspects of the starch granule have been reviewed by several authors (Buleon, Colonna *et al.* 1998; Tester, Karkalas *et al.* 2004; Vandeputte and Delcour 2004; Srichuwong and Jane 2007). For a detailed understanding of this complex macromolecular assembly the reader should refer to these reviews. In this section only sufficient information is given to help the reader understand the basic raw material and some of the reasons for modification of the native material.

5.3.1 Starch polysaccharides

The major components of starch are the two polysaccharides amylose and amylopectin. Amylose is a predominantly linear polysaccharide consisting of (α-1→4 linked D-glucopayranosyl units).

It is recognized that amylose can be separated into a perfectly linear and a branched component. The molecular weight of amylose varies with the source of starch but is of the order of 500,000 Da (weight average). The number of branched points per molecule for the branched component has been estimated as being between 2 and 8 (Hizukuri, Takeda *et al.* 1997). The structure of this branched component is fundamentally different from amylopectin and for all practical application purposes it is appropriate to think of amylose as a linear polymer.

The amylose content of most native starches is in the range of 15–30% (see Table 5.2 on page 114). The predominate polysaccharide is amylopectin. This is a highly branched polymer of glucose, the branch points consisting of (α-1→6) linkages. There is continuing debate on the molecular size, and reported values range from 10^7 to 10^9 Da. One of the reasons for this uncertainty is that to measure molecular size solubilisation of the amylopectin from the granule is required. It is hard to be certain that this procedure does not degrade this giant molecule.

The most generally accepted model for the structure of amylopectin is the cluster model. There is one single C chain in this structure which contains the only reducing end group. The A chains are linked to the main structure at only one position. B chains carry at least one A chain. Information about the fine structure of the amylopectin can be obtained by treating the starch with the debranching enzymes isoamylase and pullananse and measuring the molecular size distribution of the fragments.

5.3.2 Helix formation

The amylose chain readily adopts a helical conformation. There are two types of helices of interest: a double helical structure which is the origin of crystalline order in the native starch granule and a single amylose helix which forms as a result of complexing guest molecule within the core of the helix. There is still some debate about the exact conformation of the amylose double helix melts but the balance of evidence is that it is a left-handed helix with six residues per turn.

The temperature at which the double helical structures 'melt' increases with the length of the participating chains. In high water environments double helices cannot form 'amylose' oligosaccharides with a degree of polymerisation of less than about 10 (Gidley and Bulpin 1987), whereas temperatures of the order of 140 ºC are required to melt out double helices formed from long amylose chains. The amylose double helices can further associate to give rise to crystalline structures. Two types of crystalline organisation can be identified by X-ray diffraction studies. Although the double helical structure in these two polymorphic forms is the same, they differ in the amount of water within the unit cell. In the B type the centre double helical array in the A type structure is replaced by water molecules.

In addition to the formation of coaxial double helices, amylose can form single helical inclusion complexes with guest molecules including iodine, monoacyl lipids and alcohols. The driving force for complex formation is the hydrophobic nature of the helical core. When these associate they give rise to the Vh-amylose X-ray pattern. There has also been substantial interest in encapsulating flavours and other 'actives' within this helical structure (Conde-Petit, Escher *et al.* 2006).

5.3.3 The starch granule

The organisation of the two polysaccharides and the minor components (lipids and proteins) in the granule is still subject to much uncertainty and ongoing research. The traditional view is that the granule is composed of alternating shells of crystalline and amorphous regions. Amylopectin is primarily located in the crystalline region and amylose in the amorphous one. The amorphous region is more susceptible to attack by acids and enzymes and water initially enters this region prior to gelatinisation.

A model for the organisation of the amorphous regions, such as that by Jenkins and Donald (Jenkins, Cameron *et al.* 1993; Jenkins, Cameron *et al.* 1994), with a radial organisation of crystalline arrays of amylopectin double helices, explains why when starch granules are viewed under polarised light they show a characteristic 'Maltese Cross' pattern known as birefringence. When a starch granule is disrupted by, for example, heat gelatinisation this pattern is lost. The degree of crystallinity of the native granule is about 40%. Surprisingly this is the result of the association of double helical regions of amylopectin not the linear amylose component which is believed to be predominantly amorphous, although for cereal starches a proportion will be in the form of a helical complex with internal lipids previously mentioned. X-diffraction shows that the B-polymorph is present in tuber starches whereas cereal starches show the A-polymorph. For legume starches a C type pattern is observed, which is the consequence of the presence of both polymorphs within a single granule. Recent work using atomic force microscopy has provided further evidence that the amylopectin is organised in blocklets. It has been suggested that these are not only in the crystalline growth rings but also in what were previously considered to be amorphous growth rings (Parker, Kirby *et al.* 2008).

5.3.4 Gelatinisation, gelation and retrogradation

When a suspension of starch granules is heated in excess water, the amylopectin double helical structures are lost and the granule swells. This process can be followed in a number of ways, for example observing the loss of the Maltese Cross pattern using hot stage microscopy, following the increase in viscosity or measuring the heat ingress required to 'melt the ordered structure in a differential scanning calorimeter or observing the loss of the characteristic X-ray diffraction pattern. In industry this process is most frequently followed by measuring the change in viscosity with temperature while agitating the starch suspension. In this 'pasting' experiment the dispersion of starch granules is heated held at 95 ºC with continual stirring and then cooled. Although empirical from a rheological perspective, this experiment allows changes in starch to be followed over a wide temperature range and during shearing.

The first indication of gelatinisation is the loss of the diagnostic Maltese Cross or birefringence as viewed under plane polarised light. Once the hydration has reached the critical pasting stage, a rapid onset in the development of viscosity is seen. At this point the structural changes in the granule are irreversible. Pasting temperatures differ with starch origin. Changes in traditional native starch during processing are caused by the varying accessibility of the granule to hydration. The accessibility is determined by: the ratio of amylose to amylopectin; the concentration of lipid material; and other factors such as the presence of phosphate groups. It was believed that the cereal starches, maize and wheat, in contrast to waxy maize, tapioca and potato, reach their pasting temperatures at a slower rate due to an amylose-lipid complex that reinforces the granular structure though interestingly it has been shown recently that the

viscosity curves for maize and wheat starches become almost identical to tuber starches (Debet and Gidley 2006) if the former are washed with sodium dodecyl sulphate suggesting an important role for surface proteins and lipids in controlling the swelling process.

During this heating, holding and cooling regime a number of changes take place in addition to the initial swelling of the granules. When held at high temperatures the swollen granules can break up resulting in a decrease in viscosity on holding at 95 ºC and amylose can be released into the surrounding solution from the granular matrix. The released amylose can associate to produce a three-dimensional network structures. If this occurs in the pasting experiment on cooling there is an increase in viscosity termed setback. If the 'paste' is left to cool without stirring then a firm gel can be formed. It is also possible for the released amylose to complex with lipids which is the origin of a discontinuity that can be seen in the viscosity from the pasting experiments after holding at high temperatures. Waxy starch maize contains next to no amylose thereby allowing the granules to swell more freely to a peak viscosity and ultimately to produce, not a gel, but a thick, colloidal amylopectin dispersion. Potato starch exhibits the greatest swelling due to its larger granule size. In this case, the very rapid viscosity development at low temperatures is due to naturally occurring phosphate groups which are responsible for starch–starch repulsions that weaken the granule and accelerate its rupture. The phosphate groups also impart a strong salt/electrolyte sensitivity. In interpreting viscosity behaviour of potato starch in food formulations, it is always worth remembering this strong electrolyte sensitivity. For example, addition of sodium caseinate can result in a large decrease in viscosity which is simply due to the electrolytes in the caseinate (Kelly, van Wagenberg *et al.* 1995).

Table 5.2 shows the gelatinisation temperature (the range over which order is lost measured by loss birefringence (Maltese Crosses) and the pasting temperature where viscosity shows a significant increase. The gelatinisation temperature will be governed by the temperature required to disrupt the clusters of amylopectin chains and this is dependent on the length of these chains as previously discussed. In the case of high amylose starch where the length of the unsubstituted chains maintaining the ordered structure is much longer, complete gelatinisation is not seen even at temperatures as high as 100 ºC.

In addition to gelation of amylose it is also possible for the branched amylopectin fraction of starch to associate. The distinction between amylose and amylopectin gelation was studied in an excellent early series of paper by Miles, Ring and colleagues (Miles, Morris *et al.* 1985a, 1985b; Ring, Colonna *et al.* 1987). Amylose gels form rapidly at concentrations as low as 2%, amylopectin gels require concentrations as high as 15% to gel and gel formation occurs at lower temperatures. Both phenomena when occurring after gelatinsation of native starch are termed retrogradation. It is important to appreciate that the kinetics are radically different. Amylose retrogradation is a fast process whereas amylopectin retrogradation is much slower. Both the gelatinisation temperature and the kinetics of amylopectin retrogradation are dependent on the fine

Table 5.2 Gelatinisation properties of native starches

Starch	Gelatinisation temp. (°C)	Pasting temp. (°C)[a]	Amylose content (%)	Cooked properties
Maize	62–72	80	25	Opaque gel
Waxy maize	63–72	74	<1	Clear cohesive
Tapioca	62–73	63	17	Clear cohesive, tendency to gel
Potato	59–68	64	20	Clear cohesive, tendency to gel
Wheat	58–64	77	25	Opaque gel
Rice	68–78	81	19	Opaque gel
Sago	69–74	74	26	Opaque gel
High amylose maize	63–92[b]	>90	50–90	Very opaque, very strong gel

[a] Measured for 5% starch suspension.
[b] High amylose maize starches are not completely gelatinised in boiling water.

structure of the amylopectin molecule as revealed by the debranching experiment. This is discussed in some detail in the review by Srichuwong and Jane (2007).

5.3.5 Advantages and disadvantages of starch compared with other hydrocolloids

The major use of hydrocolloids are as thickeners and gelling agents. A suspension of swollen granules is obviously structurally different from a solution of polysaccharides which is the origin of viscosity in solutions thickenend by hydrocolloids such as carboxymethyl cellulose, pectin, guar gum, etc. When a high viscosity suspension of swollen starch granules is mixed with water or saliva, the suspension mixes very rapidly whereas a viscous hydrocolloid solution mixes very inefficiently because of entanglements between polysaccharides.

This difference can be seen by adding an amount of food colouring to a thickened solution and then mixing gently with water. For example, Ferry, Hort *et al.* (2006) demonstrated large differences between the mixing behaviour of gelatinised wheat starch and a solution of high molecular weight hydroxypropyl methyl cellulose both at a viscosity of 350 mPa.s at a shear rate of $50\,s^{-1}$. This efficient mixing of starch compared with hydrocolloids has advantages for both mouthfeel and taste perception. Rapid dilution of the suspension in the mouth rather than remaining as a coherent semi-gel makes the thickened material easier and more pleasant to swallow. In addition, tastants such as salt or sugar reach the taste buds more rapidly. For non-starch hydrocolloids above the c^* concentration (the concentration where polymer coils overlap and entangle) flavour and taste perception decreases with increasing concentration of hydrocolloid due to restricted mixing (Baines and Morris 1987; Morris 1994). Such a restriction does

not occur with starch to anything like the same extent provided the starch is retained in the swollen granule form.

The advantage of the starches at high viscosities is clear. The advantageous short texture and good taste/flavour perception of starch thickened systems at high viscosities is lost if the starch granule is disrupted by overcooking. It has recently been shown that even disruption of the granular structure by amylase action in the mouth may lead to a reduction in the level of salt perception (Ferry, Mitchell *et al.* 2006).

The ability of both amylose and amyloepctin to associate can be an important disadvantage for many applications. For example, heat sterilised products are required to be stable over long storage times. Frozen products stabilised by native starches are not freeze thaw stable. In this case association is aggravated by the increasing concentration of starch in the unfrozen domain during freezing.

5.4 Modifications

One of the major objectives of modifying starches is to maintain the beneficial effect of the short texture and good flavour release of a suspension of non-disrupted swollen granules while avoiding the problems of uncontrolled retrogradation. The selection of the native starch is clearly important. Waxy starches which contain no amylose are frequently used because the gelation/retrogradation of amylose is not an issue. Advances in our understanding of starch biosynthesis promises to develop new starches with useful functional behaviour through genetic modification or accelerated plant breeding (Jobling 2004). Processing and modification of native starches by enzymatic, physical and chemical processes to produce a range of functionalities has become increasingly sophisticated. Starch modification has been reviewed recently by Singh, Kaur *et al.* (2007) and is discussed in Rapaille and Vanhemelrijk (1999). Table 5.3 serves as a ready reference to the type of modification, its effect on the native starch (the objective) and the functionality produced (the benefit).

Starch modifications are a means of altering the structure and affecting the hydrogen bonding in a controllable manner to enhance and extend their application. The alterations take place at the molecular level, with little or no change taking place in the superficial appearance of the granule. Therefore, the botanical origin of the starch may still be identified microscopically. Each chemical and biochemical modification described below is represented schematically in Fig. 5.1.

5.4.1 Cross-linking

Cross-linking is the most important chemical modification in the starch industry. It involves replacement of the hydrogen bonding between starch chains by stronger, more permanent, covalent bonds. In this manner, the swelling of the

Table 5.3 Modifications of starch

Modification*	Objective	Benefit to user	Typical uses
Cross-linking	Strengthen starch granule	Improved process tolerance to heat, acid and shear	Ambient stable products
	Delay viscosity development by retarding granule swelling	Production efficiency: increased heat penetration allowing shorter process time	Bottled sauces
			Sterilised soups and sauces
Stabilisation	Prevent shrinkage of starch granule and provide stability at low temperatures	Excellent chill and freeze/thaw stability to extend shelf-life	Chilled and frozen processed foods
	Lower gelatinisation temperature	Easy to cook in high solids systems	High brix fillings and toppings
Dextrinisation	Break down and rearrange starch molecule providing lower viscosity, increased solubility and a range of viscosity stability from liquid to gel	Easily handled or applied at higher dosage than parent native starch for desired effect	Fat replacers
		Create film-forming properties	Bakery glazes
			Protective coatings in confectionery
			Blistered, crispy coatings in fried snacks
Enzyme conversion (Biochemical modification)	Produce varied viscosity, gel strength, with thermoreversibility and sweetness	Contributes texture and rheology	Fat mimetics
		Economic dispersant	Flavour carriers Dry mix fillers
Acid thinning	Lower viscosity and increase gel strength	Enhances textural properties at higher usage concentrations of starch	Gums, pastilles, jellies

Oxidation	Introduce carbonyl and carboxyl groups which increases clarity and reduces retrogradation of cooked starch pastes	Improves adhesion of coatings	Battered meat, poultry and fish
		Creates soft stable gels at higher dosage than parent native starch	Confectionery
	Provide lower viscosity and low temperature stability		
Lipophilic substitution	Introduce lipophilic groups	Emulsion stabiliser which improves quality of any fat/oil-containing product	Beverage, salad dressings
		Reduces rancidity by preventing oxidation	Flavour-encapsulating agents
Pregelatinisation	Pre-cook starch to give cold water thickening properties	Cold water thickening eliminates need to cook, offers convenience and energy savings	Instant soups, sauces, dressings, desserts, bakery mixes
Thermal treatment	Strengthen starch granule	Unique functional native starch with 'Starch' on label declaration	Ambient stable products
	Delay viscosity development by retarding granule swelling		Bottled sauces
		Improved process tolerance to heat, acid and shear	Sterilised soups and sauces
		Production efficiency: increased heat penetration allowing shorter process time	

* Combination treatments are also commonly used to achieve the desired objective. The most popular are cross-linking/stabilisation or cross-linking/stabilisation/pregelatinisation.

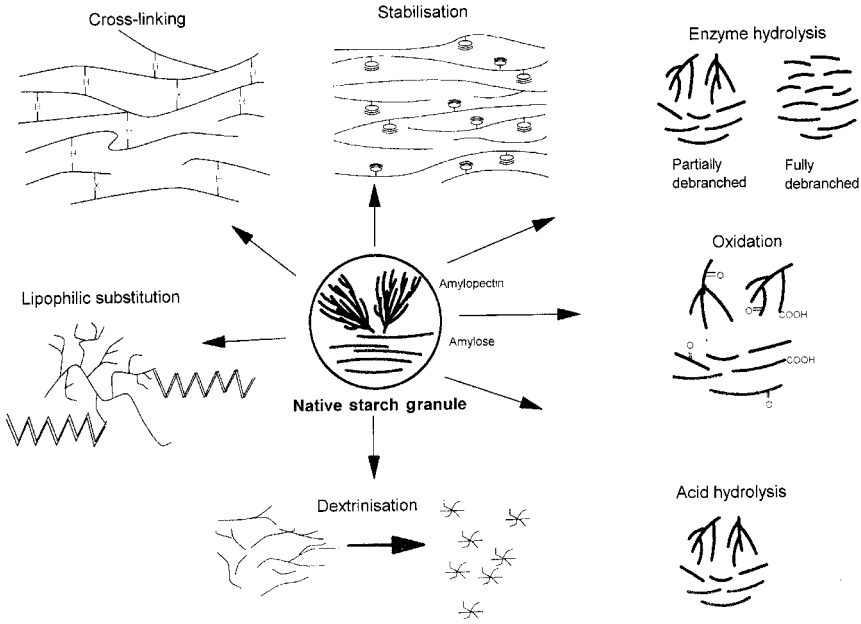


Fig. 5.1 Chemical and biochemical modifications of starch.

starch granule is inhibited, pre-empting disintegration either by chemical attack, mechanical attrition (shear) or cooking. Simply put, the starch granule is, in the molecular dimension, 'spot welded' at random locations to reinforce it. Distarch phosphates and distarch adipates are the most commonly encountered cross-linked starches wherein a phosphate or adipate bridge is present; the latter containing a longer bridge than the former. Because of the more permanent nature of the covalently bonded bridge or cross-link, only a small degree is necessary to produce beneficial effects: typically one cross-link per 100–3000 anhydroglucose units of the starch. As the number of cross-links increases, the starch becomes more resistant to gelatinisation. Consequently, cross-linked starches offer acid, heat and shear stability over their parent native starches.

5.4.2 Stabilisation

Stabilisation, the second most important modification, is usually used in conjunction with cross-linking. The primary objective of stabilisation is to prevent retrogradation and thereby enhance shelf-life through tolerance to temperature fluctuations such as freeze-thaw cycles. In this modification, bulky groups are substituted onto the starch in order to take up space and hinder (steric hindrance) any tendency for dispersed (cooked), linear fragments to re-align and retrograde.

The effectiveness of stabilisation depends upon the number and nature of the substituted group, of which there are primarily two food-approved types:

acetylated and hydroxypropylated. The Degree of Substitution (DS) is a measure of the number of substituents per 100 anhydroglucose units. Low DS starches, those with a DS below 0.2 (i.e., 2 substituents per 10 anhydroglucose units), are the most important commercially. As the DS level is raised, the starch–starch interactions in the granule are weakened and, consequently, hydration and gelatinisation by cooking is achievable at lower temperatures. Such starches benefit from easy cooking and are particularly useful in low-moisture environments and where the moisture level is restricted by competition from co-ingredients.

5.4.3 Conversions

Conversion is a collective term for a range of chain–cleavage reactions of starch.

Acid hydrolysis

Acid-thinned, thin-boiling and fluidity starches are all terms which refer to starches which have been subjected to acid hydrolysis. This form of hydrolysis differs from dextrinisation in that it is conducted in aqueous conditions. The acid predominantly attacks and depolymerises the amorphous regions of the granule such that when the starch is heated beyond its gelatinisation temperature the granules rupture quickly. The effect on cooking is a lower hot viscosity and, due to the increase in the ratio of smaller, linear molecules, a stronger gel develops on cooling (set-back) compared to the native parent starch.

Oxidation

The production of oxidised starches employs alkaline hypochlorite as a reagent. Two important modifications occur: the relatively bulky carboxyl (COOH) and carbonyl (C=O) groups are introduced together with partial depolymerisation of the starch chains. Oxidised starches, like acid-thinned starches, exhibit a significantly reduced hot viscosity due to breakdown of the starch beyond its gelatinisation temperature. Unlike acid-thinned starch however, the steric hindrance of the bulky groups disrupts any tendency towards re-association (set back) of the shorter chains thereby significantly reducing the gel strength. This is an advantage of oxidised over acid-thinned starches. ‘Bleaching’ is, in fact, a milder form of oxidation with less than 0.1% of carboxyl groups.

Dextrinisation

Dextrinisation, also known as pyroconversion, refers to two aspects of the structural modification of starch. The first is a partial depolymerisation achieved through hydrolysis. Hydrolysis is the reverse of condensation. It is the addition of water across a bond resulting in cleavage of that bond. It is usually brought about by dry roasting the starch either alone, making use of its natural 10–20% moisture content, or in the presence of catalytic quantities of acid. This gives rise to a range of polymer fractions of varying chain length (low conversion). The second aspect involves a recombination of these fragments (repolymerisation) but this time in a

branched manner (high conversion). The starches so produced are called dextrins or pyrodextrins. They are typically classified as white dextrins, yellow dextrins or British gums depending on their range of viscosity, cold-water solubility, colour, reducing sugar content and stability.

Enzyme hydrolysis
Selective enzyme hydrolysis is a form of biochemical modification. This reaction can result in a wide range of functionalities. Depending on the extent of enzyme hydrolysis, a range of chain lengths corresponding to glucose (dextrose), maltose, oligosaccharides and polysaccharides can be produced. The exact composition can be determined by measuring the dextrose equivalent (DE), where a DE of 100 corresponds to pure dextrose and a DE of 0 to the native starch. Hydrolysates with a DE below 20 are referred to as maltodextrins whilst those with a DE between 20 and 100 are referred to as glucose syrups. A range of enzymes is employed to produce such a spectrum of hydrolysates. These commonly include amylases for breaking up the straight chains as well as other enzymes to break off the branched segments of starch. α-Amylase selectively and randomly attacks the 1,4-linkages of the starch to produce maltodextrins and low DE syrups. β-Amylase attacks every other 1,4-linkage to release maltose resulting in higher DE syrups. Other commonly used enzymes include iso-amylase and pullulanase which also give high DE syrups through hydrolytic attack at other specific sites on the starch, such as de-branching reactions at the 1,6-linkages.

5.4.4 Lipophilic substitution

The hydrophilicity of starch, its propensity to interact with water, can be transformed into a schizophrenic hydrophilic-hydrophobic duality. This is particularly useful for stabilising interactions between materials such as oil and water. To achieve this the already hydrophilic starch must be given a characteristic that mirrors that of an oil, namely, a long hydrophobic, i.e. lipophilic, hydrocarbon chain. Octenylsuccinate groups containing an eight-carbon chain provide the lipid-mimicking characteristic. Starch octenylsuccinates are attracted to, and stabilise, the oil–water interface of an emulsion. The glucose part of starch binds the water while the lipophilic, octenyl part binds the oil. In this way complete separation of the oil and water phases is prevented.

5.4.5 Pregelatinisation

Pregelatinisation is a physical rather than a chemical modification. Certain starches require cooking to develop their function. These are referred to as ‘cook-up’ starches and the process of pregelatinisation is designed to remove the necessity for cooking. Pregelatinisation may be applied to native or modified cook-up starches to achieve a versatile range of cold thickening starches. The starch is pre-cooked or ‘instantised’ by simultaneously cooking and drying using

one of the following processes:

- drum drying (starch suspensions or starch pastes) – very widely used
- extrusion (semi-dry starch) – rarely used
- spray-drying (starch suspensions) – increasing in use.

Drum-dried starches give a slightly lower viscosity than their base starch because of the damage sustained by the granules when the drum-dried flakes are ground to the desired particle size, of which there are two, fine and coarse. Further, the finer the particle size required, the greater the damage incurred. Consequently drum drying, albeit a traditional and economic method of processing, is not without its drawbacks. Moreover, the fine and coarse grades perform differently in respect of dispersion, smoothness and rate of viscosity development. As a result, spray-dried starches are finding popularity. They offer properties that are closer to their base cook-up starch.

5.4.6 Thermal treatment

As recently as 1997 it was claimed that: 'the search for physical modification techniques that are "greener" and "more natural" alternatives to chemical modification continues ... but today there is no economically viable alternative to chemical modification to meet the many requirements of food processing in the late 1990s and beyond' (Imeson 1997). In the last ten years there has been the advent of processing involving physical rather than chemical modification under proprietary technology particularly to mimic the restricted swelling behaviour of cross-linked starch. These functional native starches are simply labelled as 'starch', thus avoiding the need for the somewhat confusing 'modified starch' label.

5.5 Technical data

5.5.1 Structure–function relationship

The technical attributes of a native or modified starch are brought to the fore through cooking. Depending upon the origin or modification, phases such as gelatinisation (1), pasting (2) and retrogradation (3) can be achieved within temperatures and times that are suited to the food product in which the starch is an ingredient. Further information can be obtained by light microscopy with iodine-staining for further enhancement. Iodine staining gives rise to a characteristic purple colour due to the formation of the inclusion complex previously mentioned. The viscosity profiles illustrate the limitations of native starches. With continued heating the paste viscosity climbs to a peak and then decreases as the granules rupture. This is overcooking and the desirable texture and flavour release properties of starch are lost.

For native starches, the process tolerance is extremely fragile and, in general, native starches suffer from over-cooking. In modified starches that are cross-

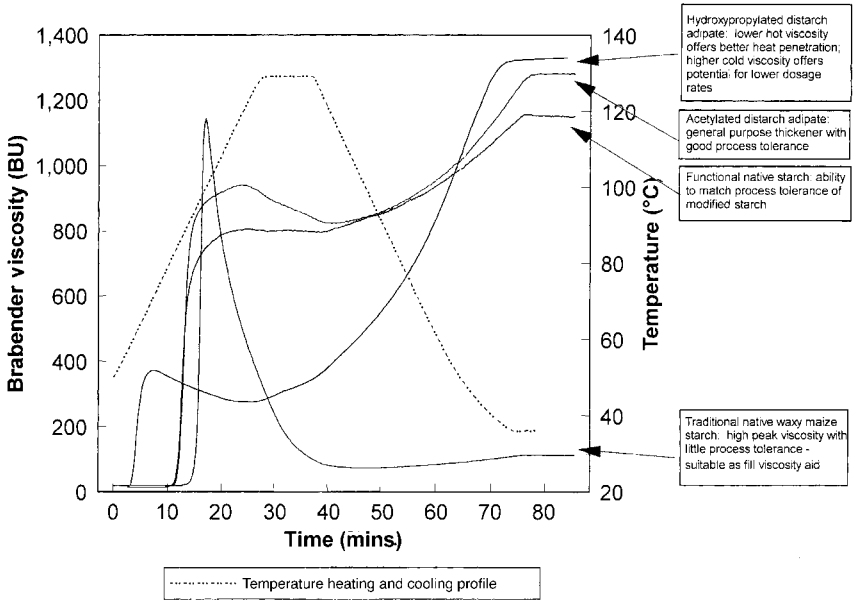


Fig. 5.2 Effect of crosslinking and stabilisation.

linked, the peak viscosity region is drawn out into an extended viscosity plateau. In this region, the modified starch is tolerant of overcooking and retains its high viscosity-building potential (Fig. 5.2). Thermally treated starches can now achieve the same profiles.

Stabilisation is used in conjunction with cross-linking to produce process tolerant starches with freeze-thaw stability. In addition to enhanced process tolerance, stabilisation also lowers the gelatinisation temperature.

5.5.2 Nutrition

Carbohydrates are renowned as the most important dietary energy source – they contribute between 40% (typical of American, Western European and Australian diets) and 75% (Asian and African diets) of total energy intake. The FAO/WHO Expert Consultation on Carbohydrates in Human Nutrition (FAO/WHO 1998) recommended an optimum carbohydrate level of at least 55% of total energy intake, obtained across a range of food categories. ILSI Europe's Concise Monograph on Carbohydrates (Gray 2003) reported that starch provides at least 20–50% of our total energy intake. Further to this key energy providing role, starch has less than half the calories of fat. This feature makes starch an extremely viable option for food formulators in their approach to calorie and fat reduction.

Research in the early 1900s classified starch as a glucose polymer, which could be completely broken down by a combination of digestive enzymes, and was therefore fully digested. Nutritional research has since highlighted that

certain carbohydrates have important functions in metabolism and digestive health due to their slow and non-digestible nature. Terms such as glycaemia index (GI), glycaemia response or glycaemia load are more commonly used today as a means to rate carbohydrate quality based on their rate of digestion and consequently their impact on blood sugar levels. Historically, low GI foods were available for the diabetic market but now that the glycaemia impact of foods is a key part of new weight management strategies, new opportunities for a range of rapid, slow and non-digestible starches has emerged. Another promising area in carbohydrate nutrition is the availability of resistant or non-digestible starch, which has growing popularity and endorsement within fibre fortification and well-being strategies. Recent reviews by ILSI Europe (Gray 2006) and the British Nutrition Foundation (Lunn and Butriss 2007) offer useful insights on the nutritional and health aspects of carbohydrates and dietary fibre.

Resistant starch

The nutritional role of starch was initially identified in the early 1980s when one of the most important discoveries (FAO/WHO 1998) was 'resistant starch (RS)'. Officially, RS was defined in 1992 by EURESTA (European Food-Linked Agro-Industrial Research – Concerted Action on Resistant Starch) as 'the sum of starch and products of starch degradation products not absorbed in the small intestine of healthy individuals' (Asp 1992). Dietary resistant starches are non-digestible starches which have a range of effects including improved digestive and glycaemia health and as such are recognised as a valuable source of dietary fibre. These benefits are now supported by over 25 years of research. Resistant starch mediates its impact via bacterial fermentation in the large intestine producing short chain fatty acids, in particular butyrate which is the favoured energy source for the colon cells (Schwiertz, Lehmann *et al.* 2002). Importantly, RS is one of the best substrates for the production of butyrate, producing levels twice that of wheat bran and four times that of pectin (Champ 2004). The addition of RS as a replacement for digestible carbohydrates will provide a lowering of the glycaemic and insulinemic responses. Resistant starch as 14% or more of the total carbohydrate in the diet was reported to improve insulin sensitivity (Higgins, Higbee *et al.* 2004). Moreover, emerging research has shown RS has the capacity to increase fat oxidation (Higgins, Donahoo *et al.* 2005) which could offer new and exciting opportunities for new product development, although additional research is required especially to provide possible mechanisms.

Resistant starch has been a natural component of our diet for centuries. On average, Europeans ate 50–100 g resistant starch/day through the consumption of intact wholegrains and pulses in the fifteenth to seventeenth centuries. Today, we eat just 3.2–5.7 g/day. The US recommendation of 48 g wholegrains/day would contribute about 8 g resistant starch, 2 g soluble fibre and 8 g of other fermentable fibres according to the latest BNF report on Carbohydrates and Dietary Fibre (Lunn and Butriss 2007). Low intakes of dietary fibre and natural resistant starch within the Western diet continue to provide opportunities for

new product development to meet the demands of increasingly health aware consumers. Natural resistant starch has gained widespread recognition from nutritional authorities globally. The Australian National Health and Medical Research Council (Anon. 2006) specifically identified and included resistant starch in their 2007 dietary fibre advice, as the scientific evidence showing a positive impact of resistant starch on digestive health was so convincing. Progressive health professionals recommend a varied diet containing a combination of soluble, insoluble and resistant starch.

There are four types of resistant starch, of which RS II, III and IV are commercially available. RS I is the physically inaccessible starch found in grain, seeds and legumes. In general, it is not suited as a food ingredient as processing can destroy it. RS II is a granular starch which, in an uncooked state, is naturally resistant to enzyme attack. It is found in green (unripe) bananas, potatoes and very high amylose starch. RS III and IV are formed through thermal modification, e.g. in bread crusts, cornflakes or retrograded high amylose starch; or through chemical modification, e.g. as in repolymerisation to alter the glycosidic linkages such that they are no longer recognised by α-amylase. Commercial sources of RS assay at 30–70% dietary fibre (as measured by the Prosky AOAC method).

RS offers advantages over cellulosic sources of fibre such as bran. It provides low water-holding capacity thereby aiding processing. Furthermore, it enhances the organoleptic qualities of food as a replacement for, or complement to, natural fibre and it can be labelled as 'dietary fibre'. Processing of high-fibre products has traditionally been fraught with problems related to the high water-binding capacity of cellulosic fibres. RS can overcome such processing issues, not only due to its low water-binding capacity, but also due to its negligible impact on dough viscosity and rheology, since it does not compete for water. There is even a textural benefit observed with RS in low moisture systems. Cereals and snacks, for example, which contain RS are more expanded and retain a light, crispy texture. This is in contrast to the texture attributed to oat bran-substituted snacks and cereals which are dense and hard, and consequently have a reputation for limited palatability. Despite government initiatives to increase consumption of wholegrains, it is often their poor palatability and low fibre retention which reduce their attractiveness to consumers. Resistant starch is an excellent complement for wholegrain products or can be used in combination with traditional cellulosic fibres to open up opportunities for dietary enhancement with texturally appetising and nutritionally balanced foods.

Fat mimetics

A third of Western consumers now incorporate lower fat products into their diets (Anon 2008). Effective ways of combating the obesity challenge will continue to be high on the agenda especially with government initiatives promoting 'front-of-pack' symbols for healthier food choices (e.g., Sweden's keyhole labelling scheme or the UK's traffic light scheme). In fat-reduction programmes, the key functions of starches are in achieving a significant calorie reduction coupled to the rebuilding of features previously provided by the fats in the product –

namely, viscosity, body and mouthcoating. Starch will always remain an economical solution provider for fat reduction due to its ease of incorporation, versatility to processing, simple label declaration, availability and sustainability.

The traditional approach is the partial replacement of fat using starches which, when dissolved in water, create stable thermoreversible gels. Soft, fat-like gels can be created by conversion modifications to the degree necessary to produce thermoreversible, spreadable gels. Typically, 25–30% solids, i.e. starch in water, form an optimal stable structure for fat replacement. New generation fat replacers are tailored to mimic more closely the many complex properties of fats or oils in a particular application. These are referred to as fat mimetics. Whilst normal, viscosifying starches require intact, swollen starch granules for maximum viscosity and stability, fat mimetics generally require granule rupture and solubilisation for their functionality. Therefore, the lubricity they are designed to contribute is not generally affected by extended heating, shear or acid. Starch-based fat mimetics are readily available for culinary, dairy and bakery applications. Maximising the synergies of functional ingredients such as hydrocolloids in combination with specific starch fat mimetics can mean that 100% fat reduction is achievable.

5.6 Uses and applications

5.6.1 Starch selection

As a multifunctional and user-friendly ingredient, starches are found widely applied across the food and beverage industry. Matching the right starch within any of these applications requires a multitude of criteria to be considered. The process of starch selection can be rationalised through a knowledge of the range of features in a food product or process that starch can control or facilitate. These include sensory properties, method of manufacture, co-ingredients and shelf-life expectations. All are dealt with in greater detail in the following sections.

Sensory properties

Figure 5.3 displays some key sensory descriptions for appearance, structure, taste and mouthfeel together with examples of how such attributes can be achieved.

Ingredient factors

It is not uncommon, in complex food-product formulations, to encounter effects arising from ingredient interactions. Indeed, on occasions when synergistic interactions are observed, manufacturers will seek to optimise them and, where the opportunity arises, protect their invention through patents. In cooking a starch-based formulation, the effects of three critical co-ingredients should be considered.

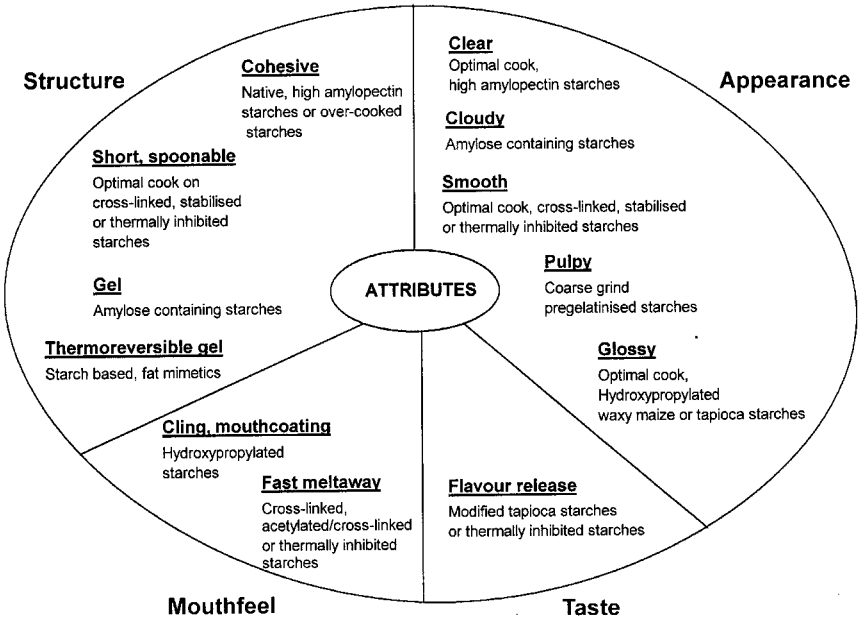


Fig. 5.3 Starch sensory attributes.

Acid

Many foods contain acids either as a means of preservation or for flavour. In such cases, the acidity or pH of the food product is very important to the selection of a suitable starch. Viscosity can vary significantly as a function of pH, not only for native starch but also for modified starch. Acids disrupt naturally occurring hydrogen bonding causing the starch granule to swell more easily and, in extreme pH conditions (e.g., pH 2.5), cooking can lead to premature rupture of the granules with an accompanying breakdown in viscosity. To overcome the effects of acid and provide maximum viscosity at minimum starch usage levels, the starch should be inhibited, i.e., strengthened by chemical cross-linking or thermal treatment, to the degree necessary. This will usually include consideration of the manufacturing process involved for the food product.

Sugars

In 'high-solids' or 'high-Brix' systems, the presence of water-soluble solids, notably sugars, can have a detrimental effect on starch hydration. By competing for, and preferentially tying up, the water necessary for hydration, sugars can cause an increase in the starch gelatinisation temperature which makes it more difficult to cook-out the starch and achieve the desired functionality. A formulation containing 60% sugar raises the starch gelatinisation temperature to above 100 ºC. To achieve such temperatures in water, pressurised cooking would be needed to cook-out the starch. As an alternative in simple operations, the

solution may be to add the sugar after the starch has been cooked; or, to limit the sugar addition to 20% whilst cooking the starch, the remainder of the sugar being added at the end. Complex manufacturing, however, may not be amenable to such alteration. In these circumstances, it is advisable to use a light to moderately inhibited starch which is also highly stabilised or, indeed, a pregelatinised starch. Hydroxypropylated, stabilised starches offer processing advantages in this respect. Their lower gelatinisation temperature allows easy hydration and cooking, even in high-solids formulations. Their low hot viscosity allows excellent heat penetration and affords a shorter process time. This, in combination with their better freeze-thaw stability, provides for a premium quality product with good shelf-life.

Fats and oils

The rate of viscosity development is often lowered in high-fat or oil products. This occurs due to the fat or oil coating the starch and delaying hydration. This feature can be taken advantage of to achieve easier dispersion and rehydration of pregelatinised starches. In food emulsions, lipophilic starches are used as emulsion stabilisers to ensure product quality and shelf-life stability.

Process factors

There are essentially three parameters to consider here, namely, time, temperature and shear during manufacturing and reconstitution. These parameters should not be viewed in isolation since, in most processed foods, at least two of the parameters are involved. Table 5.4 lists examples of processing equipment together with the conditions associated with them. In general, higher temperature, longer hold time and greater shear forces will promote granule swelling and, consequently, the starch will be more susceptible

Table 5.4 Effects of food processing*

Equipment	Time	Temperature	Shear	Pressure
Steam jacketed kettle	Long	Low–medium	Low	
Plate heat exchanger	Short	Medium–high	High	
Scraped-surface heat exchanger	Short	Medium–high	High	
Jet cookers	Short	Medium–high	Medium–high	
Direct steam injection cookers	Short	High	Very high	
Retorts	Very short	High	High	Low–medium
Extruder cookers	Long	High	Low	High
Flash cooling	Short	High	High	High
Piston pump			High	
Mono pump			Low–moderate	
Centrifugal pump			Moderate	
Colloid mill, homogenisers			Moderate–high	Low–High
Microwave			Very high	

* High temperature, shear and pressure are often referred to as 'high stress' processes. For such environments, specific modified starches will be required.

to rupture and breakdown. Tolerance to such processing factors is achieved by strengthening the granule through cross-linking or thermal treatment.

Shelf stability
The conditions that a finished food product has to withstand between production and consumption will also influence the selection of an appropriate starch. Ensuring a processed food reaches the consumer in the same ideal state as it left the factory, is the consideration of shelf stability. The most critical factor is temperature during storage, especially if the temperature fluctuates. Assuming the relevant modified starch in the product is optimally cooked, and the level of cross-linking is matched to the required degree of process tolerance, then a combination modification is required for temperature-storage tolerance. The key combination with cross-linking is stabilisation which prevents freeze-thaw intolerance, i.e., retrogradation, water loss or any textural changes. In this context, the more bulky blocking groups of hydroxypropylated starches, as opposed to those of acetylated starches, offer better freeze-thaw stability and longer term storage stability.

End use
The end use of a product defines how it will be reconstituted or prepared for consumption. The meticulous consideration of factors affecting starch selection during manufacture should be extended to include those factors which will influence the starch cook during reconstitution, and which will consequently affect product quality. If a product is to be reheated, then the method used is important since, not only time and temperature, but shear may also need consideration. Flexibility in reconstitution is a selling point. For example, in a three-way cook using either hob, casserole or microwave, temperature and time are involved in two of them whilst the microwave additionally includes shear. Microwaves have a different mechanism for heating than the conventional oven cooker. Microwave radiation produces heat by 'activating' friction between dipoles such as the hydroxyl groups of molecules (water, starch and sugar have these in abundance). Consequently, microwave processing is a high shear environment and the assessment of the degree of cross-linking needed should therefore include consideration of the means of reconstitution. Fats, compared to water, exhibit little dipolar charge but their much lower specific heat means that they warm considerably faster than water in microwave cooking.

5.6.2 Applications

Baked goods
Wheat flour is the basis of many baked goods and most commercial sources will contain approximately 71–79% carbohydrate, which is essentially starch. It is an economic commodity but is overstretched in respect of modern products such as frozen, chilled, low-fat and gluten-free foods, where aesthetics and processing can be limiting without the addition of more functional starch ingredients.

Whilst formulations will vary significantly depending on the desired final product, typical ingredients, apart from starch and wheat flour, could include fats, sugars, eggs, emulsifiers, milk and/or water. Processing conditions will also vary with the formulations. The effect of these ingredients and processes on the use of starch or modified starch can be exacerbated since baked goods have a limited amount of moisture. Gelatinisation of the starch (in the wheat flour as well as in the added starch) is critical to building structure, texture, moistness and freshness in bakery products.

Since wheat starch will only thicken during baking, pregelatinised starches can be used to bind the limited water available early on. This creates a host of benefits: suspension of particulates (as in muffin mixes); reduction in the stickiness of doughs; improved handling and machinability; increased cake volume; improved water binding for increased moistness; and softer textures. A common problem in bakery products is staling caused by retrogradation. Stabilised starches, in particular hydroxypropylated, pregelatinised starches bind water more effectively thereby providing baked goods with enhanced shelf-life through extension of the perception of freshness. Native maize, waxy maize or tapioca starches are alternatives to wheat flour for gluten-free products for coeliac sufferers who are intolerant to wheat gluten. A further benefit can be realised with the inclusion of resistant starch from high amylose maize or tapioca to increase the dietary fibre. Natural resistant starches from high amylose maize offer a new source of well-tolerated cereal fibre as a complementary fibre source for coelicas.

Glazes and icings are used to enhance aesthetics and add value to baked goods. A glaze is typically a thin wash containing sugar, water and/or milk, whilst an icing may be applied as a thickened layer and usually contains fats. Pregelatinised starches and, especially dextrins, find application here where the main functions are viscosity control, colouring, softening and texturing.

Batters and breadings

The concept of a breaded or battered coating is to create additional value for meat, poultry, seafood and vegetables, including even mushrooms or french fries. Flours are a major component in batters and breadings but a common problem for food manufacturers is fluctuation of the quality of commodity native flours. Speciality starches are used to solve this and extend the features of the native base flour. The wide array of substrates for coatings means that the accompanying wide range of storage and reconstitution methods become key considerations, in addition to textural attributes, in the selection of a starch.

Starches in batters provide viscosity control which, in turn, controls the quantity and thickness of the batter layer; the adhesion efficiency; visual effects (smooth to blistered); texture; storage; and reconstitution stability. Pregelatinised starches are used to control the cold viscosity. These can be cross-linked and stabilised to provide shear and freeze-thaw stability for batter slurries which are to be recycled or used in chilled or frozen products. The textural attributes can be enhanced by high amylose starches for fried or oven-baked

poultry and meat products. High amylose starches need higher temperatures, as in frying, to functionalise them. Stabilisation lowers the gelatinisation temperature and therefore makes the starches suitable for applications with lower cooking temperatures.

Dextrins are used for vegetable coatings where they will enhance colour and, at high dosage rates (~30%), will create blistered effects to transform the surface of a coating. Very high levels of dextrins or incompletely gelatinised, high amylose starches, may cause stickiness. This problem is overcome by reducing the dextrin level or increasing the amylose content to reach the desired crispiness. To achieve a smooth, uniform coating at lower cooking temperatures, a modified high amylose starch is recommended. Since amylose has excellent film-forming properties, the incorporation of high amylase starches in batters can reduce oil pick-up in fried, battered products.

Beverage emulsions and flavour encapsulation

Lipophilic starches have replaced gum arabic, the traditional emulsion stabiliser, in concentrated flavour emulsions for soft drinks and in encapsulated ingredients, e.g., spray-dried flavours and creamers. Indeed, lipophilic starches can be extended beyond gum arabic applications to high-load encapsulation of flavour oils. Generally these starches provide improved oxidation resistance and low temperature emulsion stability. It should be noted that lipophilic starches/starch derivatives act more as emulsion stabilisers rather than as true emulsifiers. As such they must be well dispersed in the water phase to realise their stabilising effect at the oil–water interface and consideration should be given as to whether a viscosifying or non-viscosifying lipophilic starch is advantageous.

Confectionery

Starches are found in a wide range of confectionery products, contributing from soft through to hard gels, and from brittle through to chewy textures. Starch is also active as the structure builder in coatings and even as the moulding medium to support the shaping of confections. Starches are selected primarily on their ease of cooking in high-sugar environments and their ease of handling during production.

The majority of confections are high in sugar, sugar syrups or polyols, with solids in the region of 68–72%. In these products there will be considerable competition for water, with the starch suffering in that gelatinisation temperature is increased and the product more difficult to functionalise under 100 ºC. There are several methods used in confectionery processing to functionalise ingredients: the traditional steam-jacketed kettle (low-to-medium temperature, low shear); heat exchangers (medium-to-high temperature, high shear); or direct steam injection, as in jet cooking (high temperature and very high shear).

Converting amylose and high amylose starches to different degrees of hydrolysis creates a suitable range of acid-thinned or oxidised starches for confections. These starches have a range of low hot viscosities and consequently they allow rapid and efficient cooking of starch solutions in the presence of

concentrated sugar syrups. A further useful modification is stabilisation where the reduced starch gelatinisation temperature allows easier cooking, especially of high amylose starches, so that stronger gels can be achieved with increased clarity and extended shelf-life for the confections. Converted starches are also used in confections on the exterior of products, as in pan-coating. Dextrins, with their good film-forming properties are used with high-sugar solutions to create stable, flexible coatings as in jelly beans or shell-coated chocolates. Texture can be further modified by using starches in combination with other hydrocolloids.

Starches also serve as a process aid rather than an ingredient in confections. The shaping of confectionery pastes normally occurs in starch moulds where the desired shape or design has been imprinted into trays of moulding starch. The moulding starch is also treated with a small percentage of mineral oil which enables it to hold the mould imprint and to minimise dusting during the manufacture process. The moulding starch has two key functions, its shape and the absorption of moisture. The moisture content of the moulding starch is critical in obtaining a high-quality confection. Above 9% moisture, the drying time is extended – this reduces production rates – whilst below 6% moisture, the confection has a crust of hardened exterior as the rate of moisture loss is too fast.

Dairy products

Dairy products have flourished as they are convenient at most daily eating occasions. The latest developments are primarily focused on chilled and frozen products, but there remains a substantial market for ambient and dry mix products. Chilled, frozen and ambient dairy products are processed in order to deliver the required shelf-life. Processing factors which influence the selection of starch are heat and shear. Heating is achieved through direct or indirect processing, and shear is predominantly achieved by the design of the heating equipment or by homogenisation of the product (in this instance before or after heating). Direct processing involves heating the product by steam injection (where steam is forced into product through a nozzle) or by steam infusion (where the steam and product are mixed in a chamber). These systems involve very high temperatures and high shear but short process times. Indirect processing relies on heat exchangers such as the ‘scraped surface’ or ‘tubular’ varieties. More viscous products are best handled through scraped surface heat exchangers where, despite their higher shear intensity, the shear profiles are still lower than those experienced in direct processing. Heat penetration is critical to the production of a high-quality, safe and stable dairy product. Low initial viscosity during processing contributes to a better heat penetration which, in turn, means increased product throughput with less fouling and downtime, together with improved plant efficiency through shorter process times. Hydroxypropylated and cross-linked, waxy maize or tapioca starches offer lower hot viscosities than their acetylated counterparts and yet produce up to 50% greater cold viscosity after 24 hours storage. Hydroxypropylated starches also provide better freeze-thaw stability and produce richer, creamier textures. They are more effective thickening and stabilising agents in low-fat or low-

calorie dairy products due to their greater compatibility with milk protein. Retorted (canned) dairy-based products can bring out the advantages of using hydroxypropylated starches. By comparison, acetylated starches may deacetylate, creating dramatic localised changes in pH which cause the product to curdle or precipitate.

Achieving the desired texture of desserts is dependent upon an optimal cooking of the starch. Understanding the impact of shear on the product is necessary to select a cross-linked or thermally treated starch which provides the level of process tolerance necessary for production. Upstream homogenisation of products at temperatures below the gelatinisation temperature of starch, 60 ºC, will not adversely affect starch functionality. Downstream homogenisation is where the starch is subjected to high shear well above its gelatinisation temperature. In this instance, very highly cross-linked or thermally treated starches are recommended in order to deliver an optimum viscosity.

Starch selection will be influenced not only by the process requirements but also by the product characteristics. Blandly flavoured starches, such as modified tapioca starches or certain thermally treated starch, are particularly important in dairy products due to the high dosage levels required. Starch produces a higher viscosity when cooked in whole milk (3.5% fat) than in either skimmed milk (0.5% fat) or water. Typically, 1–3% starch is used for pouring consistencies, 3–4% for medium viscosity and 4–6% for thick, spoonable textures. Gelled textures, as in cheese analogues, are achievable with some converted starches or high amylose starches. Gelled dairy desserts will also use other hydrocolloids, notably carrageenans, in combination with starch to create the desired textures.

Fruit preparations

Regardless of season, there is now a wide range of fruit bases available throughout the year. For the food manufacturer, this presents a range of conditions, such as pH, to be contended with. This is compounded further by the fact that, even within season, there will be a further natural variation in pH (3–4.5) and pectin within and between fruit types. To achieve consistent fruit preparations, a thickener or stabiliser is required to even out these variations. But the thickener and stabiliser must also be able to tolerate the processing involved to provide the desired texture, freeze-thaw or bake stability. Fruit preparations find application in: yoghurts, layered dairy desserts and ice-creams; as fillings for baked goods, e.g. doughnut; and, as toppings for pastries such as cheesecakes. Formulations will include 20–90% fruit, and sugar solids from 7% (no added sugar) to 70%. Sugar solids (measured as percentage Brix) above 40% increase the gelatinisation temperature beyond 100 ºC. In these circumstances a jet cooker is preferred over the kettle variety to functionalise the starch. Alternatively, adding the sugar at the end of the process, allows the starch to fully gelatinise. In systems where a ‘one-shot’ addition of dry ingredients is only possible, then cross-linked and stabilised, pregelatinised starches or highly stabilised and light-to-moderately cross-linked cook-up starches are suitable as thickeners. The starches must be capable of developing sufficient viscosity to

suspend the fruit, yet be pumpable and mixable with, for example, a yoghurt base. Fruit pieces are particularly delicate during cooking. Therefore, reducing the process time through an increased heat penetration will protect fruit integrity. This is achieved by selecting a cross-linked and stabilised starch which will thicken enough to suspend the fruit pieces; provide a low viscosity for effective heat penetration; provide acid stability; and excellent chill and freeze-thaw stability. Flavour release in fruit preparations is particularly enhanced with process tolerant, thermally treated starches.

Gravies, soups and sauces

This sector includes an enormous range of food products that span the spectrum of shelf stability from immediate consumption to very long ambient shelf-life. Starch selection in this instance depends upon the production process which, in turn, is usually influenced by the pH of the product. Acidic products (especially pH < 4.5) will require a higher degree of cross-linking than neutral products, provided the heat process is the same. Normally acidic products will have a shorter process time or temperature to reach their target sterilisation factor or Fo value. Cross-linking or, more recently, thermal treatment introduces process tolerance by strengthening the starch granule. In 'wet' sauces, processing factors to consider are preheat temperature and hold time; pasteurisation/sterilisation temperature and time; method and rate of cooling; mechanism of pumping; and, indeed any other forms of shear used during cooking or in the introduction of difficult-to-disperse powders. Reference has already been made to the contribution of these factors during food processing and their effect on starch gelatinisation in Section 5.1.3. The shelf-life requirements of this group of products are achieved with stabilised starches which imply freeze-thaw stability. This feature is particularly important in the development of chilled, frozen and ambient-stable products which are recommended for refrigerated storage after opening. Hydroxypropylated starches are the best to use here, especially since their excellent compatibility with milk or milk proteins leads to an enhanced texture and mouthfeel of cream-based sauces.

Besides processing and shelf-life considerations, there are two other important criteria, fill-viscosity and heat penetration. The function of starch as a fill-viscosity aid is to provide sufficient high viscosity during the initial stages of cooking, typically during preheat, that particulates are homogeneously suspended and then evenly distributed into the container. The high initial viscosity is also designed to prevent splashing and reduce unhygienic conditions around the production filling line. Fill-viscosity starches are commonly native starches, primarily waxy maize, which thicken easily and quickly to achieve their peak viscosity. They then breakdown during the sterilisation stage with little or no residual viscosity. The final desired viscosity is achieved by cross-linked and, usually, stabilised starches. This combined, modified starch facilitates heat penetration which supports a higher quality of product with shorter, optimal heat processing. Increasing the level of cross-linking delays viscosity development which is desirable for better heat penetration. However,

there will be an optimum economic level for process tolerance. In general, viscosity-building starches are used at dosages of 2–6%, whilst fill-viscosity starches may be used at 1–3%.

Dry mixes are often required by consumers, food caterers and manufacturers for reasons of convenience, ease of handling and storage stability. Starch selection will depend on the specific requirements of the product and its use. In the case of food manufacturers, time, temperature, shear, pH and storage conditions apply as for wet sauces. Dry mixes for the consumer will demand dispersibility in hot or cold liquid as the critical factor. Whisk-and-serve applications require easy dispersion with fast hydration of the starch to achieve the target viscosity within a convenient time. Pregelatinised or easy-to-cook, 'cook-up' starches are recommended. However, pregelatinised starches may 'lump' as they try to wet-out too quickly, particularly in hot liquids. Lumping restricts viscosity development thereby compounding the problem. Agglomerated, pregelatinised starches can disperse more easily than the traditional drum-dried starches as they are more granulated. These easily dispersed starches are particularly desirable in low-sugar/salt formulations as there is less granulated material to blend. Alternatively, a highly stabilised, hydroxypropylated starch with a lower gelatinisation temperature will overcome the lumping to hydrate quickly and produce a rich, thick and creamy product. In some applications, e.g., microwave reconstitution or in cold pies requiring additional bake stability, a combination of pregelatinised and cook-up starch is used. The pregelatinised starch builds viscosity immediately to suspend the other ingredients, including the cook-up starch. This is essential in microwaveable dry mixes since stirring during cooking is impossible. The cook-up starch will contribute to the final viscosity and stability.

Potato starch has the highest swelling capacity combined with a low gelatinisation temperature. Native or lightly modified potato starches find application in economy-style dry mixes. When products require greater flexibility, e.g., three-way cooking on the hob, casserole or microwave, followed by chilled or frozen storage, as in catering, then cross-linked or stabilised waxy maize or tapioca starches are preferred. Tapioca starches are more suited to delicately flavoured sauces as in fish dishes.

Often dry mixes will require low-moisture starches (2–8%) depending upon the other ingredients and the required water activity for long-term shelf stability. Native starches such as maize or wheat, may be used to increase opacity and change mouthfeel or as bulking agents to aid dispersion. For all varieties of gravy, soup and sauce, when traditional native starches are used, the recommendation for superior product quality is one part native to two parts modified starch. This ratio ensures the beneficial effects of the modified starch compensates for the limitations of the traditional native starches. Thermally treated starch in total replacement of the modified starch, can retain the attributes of the modified whilst dispensing with the 'modified' label declaration (Section 5.7).

Mayonnaise and salad dressings

The main function of starches in this category is to thicken and stabilise. Cross-linked and stabilised starches are commonly found here. More recently, thermally treated starches are finding increased popularity. Two further starch types find specialised application here: lipophilic starches to stabilise emulsions, particularly in egg-free dressings and fat mimetics in low-calorie or fat-free formulations. The processing conditions are very rigorous for mayonnaise and salad dressings. Starches need to be able to tolerate the acidity, where pH can be as low as pH 2.8; a heat process, which generally involves 'hot-fill' as a key sterilisation factor; shear for the emulsification, which will depend on the fat content; and the type of emulsification unit used. Products which are hot-filled and directly stacked onto pallets have a tendency to suffer from 'stack-burn'. This occurs as the core temperature of the stack of product on the pallet remains relatively high for prolonged periods and product viscosity begins to break down as the starch is degraded. A highly cross-linked starch will provide additional heat and acid tolerance to overcome stack-burn. Starches are also used to ensure product quality over a long shelf-life, which could be up to 12 months. While moderate-to-high cross-linked starches are essential for the heat, acid and shear tolerance stabilised, and in particular, hydroxypropylated starches, bring added benefits by introducing freeze-thaw stability. This is a benefit for catering and consumer products which require refrigeration after opening. Low-calorie or low-fat dressings also benefit from hydroxypropylated starches as the main thickeners since they develop rich, creamy textures. Mouthfeel can be further enhanced using starch-based fat mimetics. These are available as replacements for vegetable oils. They bring a unique rheology suitable for pourable dressings, or as thermoreversible gels they are suited to spoonable dressings. A gelled structure can be further enhanced using amylase-containing starches but typically, they are used in combination with cross-linked and stabilised waxy maize starches since these contribute to the process and storage stability.

Salad dressings and mayonnaise will vary in oil content. Here the processing equipment is designed to create the emulsion by dispersing the oil as fine droplets with a diameter of less than 1 μm throughout the aqueous phase. Lecithin, the natural emulsifier in egg yolk and/or any added emulsifier, will stabilise the emulsion by reducing the surface tension of the oil droplets. This makes large droplets unstable and prevents 'creaming' (where oil migrates to the surface). Strictly, starches act as emulsion stabilisers rather than as true emulsifiers. Viscosity-building starches achieve this by structuring the aqueous phase and restricting the movement of the oil droplets. Lipophilic starches are introduced under agitation for optimal dispersion so that they interact with both water and oil to produce a stabilising effect at the oil–water interface. Another advantage of using lipophilic starches is that, as modified starches, they may already be covered on the label declaration if a modified starch has been used as the viscosity builder – this simplifies the list of ingredients on the label.

Meat products

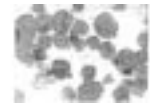
Convenience, quality, health and food ethics are some of the key drivers increasing the diversity of meat products now available. Starches are commercially used not only in formed, tumbled, emulsified and low fat meat products but also in the brines and marinades. In processed meats, starches are used as water binders to increase yields, reduce cooking losses, improve texture, sliceability and succulence, and extend shelf-life. Reformed or comminuted meat products require cooking to a minimum internal temperature of 72–75ºC for product safety. In order to function at such low temperatures with little or no shear or acid, potato starches, which swell easily at low gelatinisation temperatures, are recommended. Waxy maize and tapioca starches, with a high degree of stabilisation to reduce their gelatinisation temperatures, have the additional benefit of excellent water binding during chilled or frozen storage. Lipophilic starches, too, find application in comminuted meats and patés as stabilisers of the emulsion and in the strengthening of the fat- and water-binding properties of the meat protein. Coarse grind, pregelatinised starches bind water quickly and increase the viscosity which assists the moulding of reformed or comminuted meats whilst enhancing a more open, coarse texture. Starch derivates, with excellent film-forming properties, when incorporated into marinades, can provide significant textural and visual appeal. In addition, starch can help control the quality and uniformity of marinade coating. Recent advances in starch technology have produced 'additive-free' starch solutions for formulators attempting to reduce salt and phosphate.

Savoury snacks

Savoury snacks are low-moisture products (15–30% water used in the dough, less than 2% moisture in the final product) and the choice of starch is critical since manipulating the formulation and process equipment will affect gelatinisation and, consequently, will influence the final product characteristics. 'Expansion' is a very important process for snacks. Several processes are available for expanding snacks: extrusion (puffed corn snacks); baking (low fat, sheeted baked snacks); frying (traditional crisps) and microwave (popcorn). Pregelatinised starches are recommended in snacks that are manufactured under low-to-medium shear and medium temperatures (75–120 ºC). Cross-linked or thermally treated 'cook-up' starches are required to withstand the rigours of higher shear, pressure and temperature (160–180 ºC) found in the extrusion process for directly expanded products.

In snacks, texture and appearance are of high priority. The starch polymers of amylase and amylopectin exhibit a very obvious effect on these. In general, the highly branched amylopectin polymer increases dough viscosity and expansion, and a waxy maize starch therefore is the preferred base starch in the production of light, crispy expanded products. Amylose, in contrast, is responsible for strengthening the dough which improves forming, cutting and a harder, more crunchy final texture. In this instance, the linear chains can align very closely.

Table 5.5 Viscosity troubleshooting – evaluation of starch cook*

					
Clarity	Cloudy	Cloudy	Clear	Clear	Clear
Viscosity/ texture	Starch will sediment unless agitated	Thin	Heavy-bodied, short texture	Reduced, cohesive	Reduced, cohesive
Taste	Starchy	Starchy	Neutral	Neutral	Neutral
Stability	Poor	Poor	Good	Poor	Poor

* Light microscope @ 200 magnitude.

Amylose also exhibits excellent film-forming properties which can be advantageous in the reduction of oil pick-up in fried snacks.

5.6.3 Viscosity troubleshooting

When a starch-containing product fails to meet its viscosity specification, it is a common and expensive mistake to add further starch to correct the viscosity. This leads, at worst, to a scenario in which the initial product is overcooked and the additional starch is undercooked. The result will be an unstable product which will taste 'starchy'. Rather than compensating for viscosity it is better to investigate the root cause. Ensuring that the starch is optimally cooked is a good place to start. Table 5.5 illustrates the diagnoses of the various stages of a starch cook.

For example, in the case of undercooking, either further cooking to achieve a 'good cook' or the adoption of a less inhibited starch is required. The converse will apply for overcooking. Sliced and diced vegetables in a product can present viscosity problems due to natural enzyme activity from the high surface area of the vegetable. α-Amylases are the source of the problem. The viscosity loss from enzyme hydrolysis of the starch can be so dramatic as to leave either 'ghost granules' or no granules at all under microscopic examination. The most problematic sources of α-amylase are cabbage, onions and kidney beans. Unheat-treated wheatflour is an important and often over-looked cereal source of α-amylase. Heat treatment provides a straightforward means of inactivating the enzyme.

5.7 Regulatory status: European label declarations

Table 5.6 outlines the current status of native and modified starches; it highlights those that are classified as food ingredients and those classified as food additives.

Table 5.6 List of food starches permitted under European food law

Starch	E Number classification	Classified as	Food product label
Physically modified	–	Ingredient	Starch
Enzymatically modified	–	Ingredient	Starch
Dextrinised	–	Ingredient	Dextrin or modified starch
Acid treated	–	Ingredient	Modified starch
Alkali treated	–	Ingredient	Modified starch
Bleached	–	Ingredient	Modified starch
Oxidised starch	E1404	Additive	Modified starch
Monostarch phosphate	E1410	Additive	Modified starch
Distarch phosphate	E1412	Additive	Modified starch
Phosphated distarch phosphate	E1413	Additive	Modified starch
Acetylated distarch phosphate	E1414	Additive	Modified starch
Starch acetate	E1420	Additive	Modified starch
Acetylated distarch adipate	E1422	Additive	Modified starch
Hydroxypropyl starch	E1440	Additive	Modified starch
Hydroxypropyl distarch phosphate	E1442	Additive	Modified starch
Starch sodium octenyl succinate	E1450	Additive	Modified starch
Acetylated oxidised starch	E1451	Additive	Modified starch

5.7.1 Modified starches classed as food additives

A food additive is defined as any substance not normally consumed as a food by itself and one which is intentionally added to food for a technological purpose in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food. All permitted food additives, which have been approved for use in food within the EU, are assigned E-numbers. E-numbers are unique to each additive; they aid identification and labelling of the additive. Food additives include artificial sweeteners, colours, preservatives, flavour enhancers, emulsifiers, stabilisers, thickeners and modified starch.

Chemically modified starches approved as permitted food additives within the European Union (EU) are also assigned E-numbers. They are regulated under the terms of the EC Miscellaneous Additives Directive 95/2/EC, on food additives other than colours and sweeteners. Under the provisions of this Directive,[1] modified starches are defined as 'substances obtained by one or more chemical treatments of edible starch, which may have undergone a physical or enzymatic treatment and may be acid or alkali thinned or bleached'.

The following modified starches are listed on Annex I of 95/2/EC as generally permitted additives:

E1404 Oxidised starch
E1410 Monostarch phosphate
E1412 Distarch phosphate
E1413 Phosphated distarch phosphate

1. Directive 95/2/EC, as amended, on Food Additives other than Colours & Sweeteners, Art 1, 3, (q).

E1414 Acetylated distarch phosphate
E1420 Acetylated starch
E1422 Acetylated distarch adipate
E1440 Hydroxypropyl starch
E1442 Hydroxypropyl distarch phosphate
E1450 Starch sodium octenyl succinate
E1451 Acetylated oxidised starch.

All of the above are manufactured in accordance with specified purity criteria, defined under Directive 96/77/EC,[2] which list the approved chemical reagents and end-product specifications allowed for each starch modification. As such, the above modified starches have horizontal approval throughout the EU. They are listed on Annex I of the Miscellaneous Additives Directive as generally permitted additives. They may be added to foodstuffs following the *quantum satis* principle (no maximum level indicated) unless otherwise specified and their use is restricted in foodstuffs for which there is existing 'vertical' legislation, e.g. chocolate, fruit juices, jams, jellies, etc., and in baby foods.

5.7.2 Starches and modified starches classed as food ingredients

Under Directive 95/2/EC, the following starches are not considered as food additives:

- White or yellow dextrin, roasted or dextrinised starch
- Starch modified by acid or alkali treatment, bleached starch
- Starch, Physically modified/treated starch, enzyme treated starch.

These starches are categorised as food ingredients. They are not assigned E-numbers.

5.7.3 Labelling of starch and modified starch products

The terms 'starch' and 'modified starch' are listed under EC Directive 2000/13/EEC which relates to the labelling, presentation and advertising of finished food products sold to the consumer. Starch, physically or enzymatically modified starch can be designated the term 'starch'. Chemically modified starches can be labelled simply as 'modified starch' and therefore specific names or E-numbers are not required.

The ingredient declaration 'starch' or 'modified starch' need only be accompanied by an indication of its specific vegetable origin, when the starch or the modified starch may contain gluten.

2. Directive 96/77/EC, as amended by 2000/63/EC.

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6

Gelatin

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Abstract: Gelatin is one of the most versatile biopolymers and has numerous applications in food, confectionery, pharmaceutical/medical, cosmetic, and technical products. This is also reflected by the more than 300,000 metric tonnes of gelatin produced annually worldwide. Gelatin has been investigated and studied by scientists at least since the early twentieth century but was used in foods even before this. Gelatins are derived from the parent protein collagen and the origin of the parent collagen and the severity of the extraction procedures determine the properties of the final gelatin. Today gelatins are mainly produced from bovine and porcine sources, but gelatin may also be extracted from fish and poultry. This chapter focuses on the manufacturing of mammalian gelatin, and the coherence between the chemical compositions and the structure–function relationship of gelatins from mammalian sources, and from cold and warm water fish species.

Key words: gelatin, fish gelatin, extraction, gelation, Bloom, rheology, optical rotation, physical properties, chemical properties.

6.1 Introduction

A large number of uses within a multitude of different application areas make gelatin one of the most versatile biopolymers. This is also reflected by the large worldwide annual production of gelatin (315,000 metric tones in 2006; www.gelatine.org). Making a jelly in your kitchen is not too hard, but when it comes to more advanced uses of gelatine, one really has to put some effort into understanding the extensive possibilities of this hydrocolloid. Decades of research and development lie behind the uses of gelatin in food, confectionery, technical, pharmaceutical/medical and cosmetic products as described in

comprehensive gelatin monographs by Veis (1964), Ward and Courts (1977), and Schrieber and Gareis (2007).

Gelatins are derived from the parent protein collagen by processes that break up the secondary and higher structures with varying degrees of hydrolysis of the polypeptide backbone. The name gelatin is derived from the Latin word '*gelata*' which describes its most characteristic property, i.e., gel formation in water. The relative proportions and sequences of the constituent amino acids in collagen and gelatin are substantially the same, but the physical properties of the two proteins differ markedly. Collagen is the major constituent of all white fibrous connective tissues occurring in animal bodies such as cartilage, sinews, the transparent sheaths surrounding muscles and muscle fibres, skin and ossein (the protein matrix of bone). While collagen is insoluble in water, gelatin is easily dissolved in water upon heating to temperatures above the denaturation temperature of the native collagen. Collagen merely shrinks and loses its ability to hold water at the same conditions. Gelatin can be produced from both mammalian and piscine sources, but the physical properties of these gelatins are different. To obtain a better understanding of the unique properties of gelatin it is important to be familiar with the manufacturing procedures as well as the physical and chemical advantages and disadvantages of both mammalian and piscine gelatins.

6.2 Manufacturing gelatin

6.2.1 Raw material sources

For gelatin production the raw material may be any collagen-containing tissue. Hides, skins and bones from mammalian sources such as porcine and bovine are preferred, but gelatins are also produced from the skins of cold and warm water fish species as well as minor quantities from fowl. The manufacturing process involves cleaning of the source tissues followed by pre-treatment, extraction of gelatin, filtration/purification/sterilization, concentration, drying and finally milling.

At the beginning the raw material is washed to remove impurities. Bones are processed somewhat differently in that, after washing, crushing and rewashing, the degreased, crushed bone chips are exposed to acidic conditions (usually 4–7% hydrochloric acid) for a minimum of two days. This process is also known as maceration and the result is removal of minerals contained in the bone such as hydroxyl apatite ($Ca_5(PO_4)_3(OH)$) and calcium carbonate leaving a sponge-like bone material called ossein behind.

The concentrated raw materials may be processed directly or dried and stored for later use. Following the preliminary treatment described above the raw material is subjected to either acid or alkaline pre-treatment followed by gelatin extractions depending on the source of the collagen and the required quality of the final gelatin. Acid pig skin gelatin is the main product manufactured in Europe and North America, while bovine hide is the main raw material for gelatin production in South America.

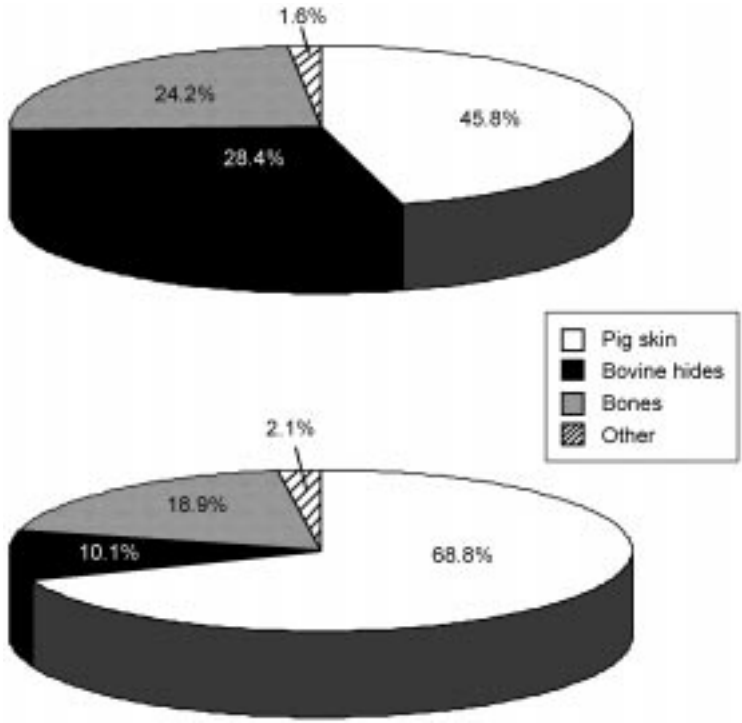

Fig. 6.1 The raw material consumption for the gelatin production in 2006; worldwide (top) and in Europe (bottom). A higher percentage of pig skin gelatin was produced in Europe compared to worldwide (from www.gelatine.org).

Figure 6.1 shows the distribution of raw materials used for gelatin production worldwide and in Europe in 2006. From the figure it can be noticed that the share of pig skin gelatin is much larger in Europe compared to the rest of the world. The percentage of gelatin produced from bovine sources is therefore naturally lower in Europe. The reason for this may be the outbreak of BSE (bovine spongiform encephalopathy) in Europe during the 1990s and because of this porcine sources are still preferred for gelatin manufacturing. Europe does not have numerous Muslim countries and the use of porcine gelatin is therefore also not strongly restricted on ethnical/religious grounds. Such dietary restrictions may in other parts of the world, such as Asia and Africa, give preference to gelatin from bovine sources.

The source labelled 'other' in the two parts of Fig. 6.1 includes fish and poultry gelatins, and these account for only ~2%. Gelatins from cold water fish species have sub-optimal physical properties compared to mammalian gelatins and this limits the application of and demand for these products. Warm water fish gelatins have physical properties more similar to mammalian gelatins and can replace mammalian gelatin directly in many products. The low availability of raw materials for manufacturing fish gelatin limit the amount of gelatin produced.

6.2.2 Acid pre-treatment

The acid pre-treatment gives rise to type A gelatins. In this process the washed hydrated raw material is immersed in cold dilute mineral acid (pH 1.5–3.0) for 8–30 hours (usually 18–24 hours) depending on the thickness and size of the raw material. After treatment the material is washed in running water and neutralized until extraction pH is reached.

6.2.3 Alkaline pre-treatment

Type B gelatins are the final product from the alkaline pre-treatment. A range of alkaline agents may be used for this treatment but saturated lime water ($Ca(OH)_2$, pH 12.0) is generally the most utilized curing liquid. The washed stock is placed in pits or vats along with the liquid and sufficient hydrated lime to maintain saturation. The temperature is kept below 24 ºC and the mixture is agitated at intervals using poles or other mechanical means. The process lasts for at least 20 days up to 6 months (usually 2–3 months) depending on the thickness and type of raw material. When treatment is completed, the limed material is washed with water until approximately neutral conditions before treatment with dilute acid (eg. HCl) to obtain the correct extraction pH.

6.2.4 From extraction to final gelatin product

To extract gelatin the pre-treated raw material is placed in extraction kettles and covered with hot water. A series of extractions are made with consecutive lots of hot water (usually three to five), each extraction performed at increasing temperatures in the range of 55–100 ºC. The combination of pre-treatment and extraction makes the final gelatin product a mixture of polypeptide chains with different compositions and molecular weights, as can be seen from Fig. 6.2. The figure shows the three dominating fragments found in gelatin: free α-chains, β-chains, where two α-chains are covalently linked and γ-chains, where three α-chains are covalently linked. The free α-chains may also be depolymerized into sub-α-chains; polypeptides with lower M_w than one α-chain. This means that gelatin is not a monodisperse protein like, e.g., globular proteins, and that all parameters describing the chemical and physical properties of gelatins are average values.

The high quality gelatins, as judged by average molecular weight and/or gel forming properties, are made at the lower temperature extractions since less hydrolysis of the polypeptide backbone occurs. Each subsequent extraction provides more depolymerized gelatin and a more coloured product. The colour of the gelatin is caused by the Maillard reaction occurring between α-amino groups of the amino acids in gelatin and traces of carbohydrates in the raw material. The ash content of the gelatins at this stage is 2–3% but the ash content may be lowered by ion exchange to remove excess salt.

The aqueous gelatin solutions are continuously concentrated by evaporation until the increased viscosity makes further concentration impractical. This

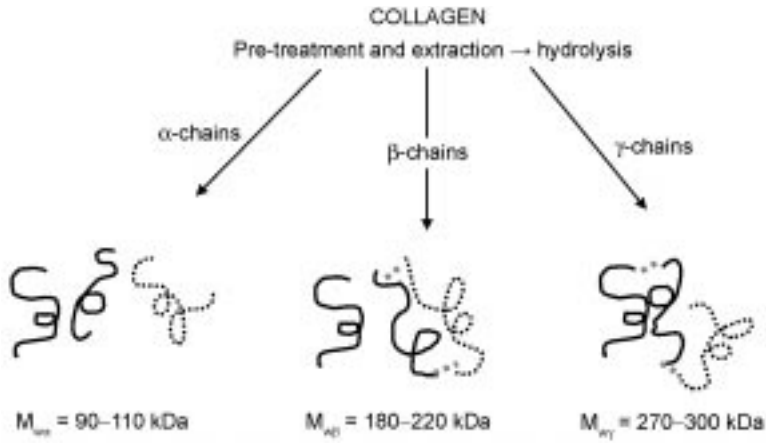

Fig. 6.2 Gelatin is not a monodisperse protein, but rather consists of a mixture of different chain types with varying molecular weights resulting in polydispersity.

usually occurs at a concentration of around 20–25% in high molecular weight gelatins and even above 40% in low molecular weight material. After concentration and filtering the gelatin solutions are sterilized. The sterilization stage involves both indirect sterilization via plate heat exchangers and direct steam sterilization. Sterilization is followed by cooling where the concentrated gelatin solution gels. For powder gelatins the gels are extruded into 'noodles' that are fed onto conveyor belts for drying. The drying process is accomplished using filtered, de-humidified, and microbially clean air where the starting temperature is approximately 30 °C. The temperature of the air is increased according to the dryness of the gelatin. The gelatin noodles are crushed and milled into blends containing particles ranging from 0.1 to 10 mm in diameter (Mesh 140 3/8 inch). The moisture content of commercial gelatins can range from 8 to 12% and determination of the water content is therefore important. For food, pharmaceutical and photographic applications the ash content has to be < 2% to comply with various regulations.

6.2.5 Manufacturers of gelatin

Gelatin manufacturers worldwide are mainly organized in four large associations and information on gelatin suppliers can be found from the web pages of the different associations:

- Gelatin Manufacturers of Europe (GME): www.gelatine.org
- Gelatin Manufacturers Institute of America (GMIA): www.gelatin-gmia.com
- Gelatin Manufacturers Association of Asia Pacific (GMAP): www.gmap-gelatin.com
- South American Gelatin Manufacturers Association (SAGMA): www.sagma-gelatina.org

6.3 Regulations, technical data and standard quality test methods

Strict regulations apply to all steps in the gelatin manufacturing process. Gelatin is produced from natural raw materials which originate from animals that have been examined and accepted for human consumption by veterinary authorities. Hygienic regulations with respect to fresh raw materials are ensured and each batch of raw material delivered to the manufacturing plant is immediately checked and documented.

In addition to the raw material quality, the production process itself is also an effective quality assurance measure. In the production process a comprehensive monitoring system ensures that potential risks are minimized. In USA, the Food and Drug Administration (FDA), with support from the TSE Advisory Committee, has since 1997 been monitoring the potential risk of transmitting animal diseases, especially bovine spongiform encephalopathy (BSE). This study has scientifically proven that the gelatin manufacturing process itself is an effective barrier against the proliferation of possible BSE prions.

The tests were based on a worst case scenario where the raw material came from BSE-infected cattle. No BSE prions could be detected in the gelatin produced by several manufacturing methods. Injections of these gelatins into the brain of experimental animals gave no establishment of TSE diseases. As a result of these experiments the FDA confirmed the safety of bovine bone gelatin. Before this, the Scientific Steering Committee (SSC) of the European Union (EU) in 2003 confirmed that the risk associated with bovine bone gelatin is close to zero. In 2006 the European Food Safety Authority (EFSA) stated that the SSC opinion was confirmed, that the BSE risk of bone-derived gelatin was very small, and that there was no support for the request of excluding the skull and vertebrae of bovine origin older than 12 months from the material used in gelatin manufacturing.

All reputable gelatin manufacturers today follow the Quality Management System according to ISO 9001 to comply with all required physical, chemical, microbiological and technical production and quality standards. In this way all process steps follow international laws and customer-specific quality parameters and are guaranteed and documented. For pharmaceutical grade gelatins strict regulations from the FDA, the European CPMP's regulation and European Pharmacopoeia must be fulfilled. A detailed overview of the regulatory requirements for gelatin production can be found in the *Gelatine Handbook* (Schrieber and Gareis, 2007), pp. 99–101.

6.3.1 Bloom strength – standard method for characterizing gel strength

Bloom strength is essentially the rigidity of a gelatin gel formed and measured under standard conditions. The Bloom gelometer was developed and patented by O.T. Bloom in 1925 (US Patent No. 1 540 979) to measure gelatin gel rigidity by determination of the weight (force in grams) required to depress the surface of a gelatin gel 4 mm using a 12.7 mm diameter flat-bottomed cylindrical

plunger. The Bloom method has been standardized by AOAC (American Organization of Analytical Chemists), GMIA (Gelatin Manufacturers Institute of America, 2006) and GME (Gelatine Manufacturers of Europe, Standardised Methods for the Testing of Edible Gelatine, 2007). It is highly important that the preparation of the gelatin solution and gel, and the measuring of the Bloom strength are fulfilled with high precision and according to the standardized methods to secure reproducible and reliable results. According to the GMIA standard the Bloom test should be performed as described below:

- 6.67% (w/w) gelatin (7.50 ± 0.01 g (dry weight) gelatin + 105.0 ± 0.1 g distilled water in a Bloom jar).
- Complete swelling (1–3 hours at room temperature)
- Dissolution: 65 ºC for 15 min, 61 ºC until total dissolution
- Tempering: 45 ºC for 30–45 min or room temperature for 15–20 min
- Maturing in water bath at 10.0 ± 0.1 ºC for 16–18 hours.

By definition, the Bloom strength represents gelatin gels matured at 10 ºC only. If the gelatin solution does not gel at 10 ºC after 16–18 hours a Bloom value cannot be determined. The Bloom values today are usually determined using a Texture analyzer from Stable Micro Systems, Lloyds or a Stevens LFRA. The test plunger used should be of AOAC standard. The AOAC plunger has the same dimensions as the old BSI plunger (not used since 1998) but has a sharp edge giving a larger surface area and subsequently slightly higher Bloom values. The test parameters on the instruments are also described in the standard methods mentioned above and should be strictly followed. It is important that standard gelatins of known Bloom values are included in the test sequence to account for any other influences on the test procedure such as preparation errors and instrumental failures.

There is a coherence between the gelatin concentration (C) needed to form a gel of a wanted firmness and the Bloom value (B). Using equation 6.1, it is possible to calculate the concentrations needed to achieve comparable gel properties when switching between gelatins of different Bloom values.

$$C_1 \cdot {B_1}^{1/2} = C_2 \cdot {B_2}^{1/2} \qquad 6.1$$

The Bloom values of commercial gelatins are in the range of 50–300 g Bloom, and a higher Bloom value represents among others a gelatin with a higher gelling and melting temperature and a stronger gel. The gelatins are separated as: high-Bloom, 200–300 g; medium-Bloom, 100–200 g; and low-Bloom, 50–100 g.

6.3.2 Viscosity

Viscosity data for a gelatin is usually supplied by the manufacturer as this property is of critical importance in many applications and since the viscosity reflects to a larger extent the average molecular weight of a gelatin sample than the Bloom strength. The viscosity for standard gelatin is primarily measured

using a calibrated pipette, where the flow time for 100 ml 6.67 (w/w)% gelatin solution at 60.0 ± 0.1 ºC is recorded and the viscosity values are expressed in mPas. Other measuring techniques are also used, e.g., for gelatin hydrolysates where the viscosity is determined for 10 or 20% solutions at 25 or 30 ºC. For some applications also rotational viscosimeters, rheometers or other specialized apparatus may be employed at various temperatures and concentrations.

6.3.3 Quality control of characteristics, identification and purity

Although Bloom strength is commercially important, it is not fundamental information regarding characteristic, identification or purity. Additional to Bloom strength and viscosity, gelatin manufacturers also provide information about pH in a 6.67% solution at 55–60 ºC and the water content of the gelatin batch delivered. All suppliers of pharmaceutical gelatin must supply details of their product, including a certificate of analysis to show that the sample meets European Pharmacopoeia (EP) and United States Pharmacopoeia (USP) specifications regarding the characteristics, identification and purity of the gelatin product. A more detailed description of the quality control, the certified product safety, the regulatory requirements and methods for testing can be found in EP (2007) and USP (1995). These topics are also thoroughly described in the *Gelatine Handbook* (Schrieber and Gareis, 2007).

For pharmaceutical gelatin the microbiological tests are fulfilled if 10 g of the gelatin is free of *Escherichia coli* and *Salmonella sp*. It is also a demand that the gelatin must have a total viable aerobic count of less than 10^3 microorganisms per gram, determined by plate count. For food grade gelatin the microbiological tests are fulfilled if *Salmonella* is absent in 25 g gelatin. All these bacteriological requirements, however, may differ according to local legislations.

6.4 Chemical composition and physical properties of collagens and gelatins

6.4.1 Chemical composition of collagen and gelatin

Since all gelatins are derived from collagen it is pertinent to describe the structure of these macromolecules before discussing gelatin. The collagen monomer (tropocollagen) is a triple helical rod made up of three parallel α-chains. The collagen molecule is about 300 nm long, 1.5 nm in diameter and with a molecular weight around 300,000 Da. On gelatin manufacturing, the collagen molecule unfolds into a mixture of chains as illustrated in Fig. 6.2 and described above. The amino acid composition of these chains is quite unique and the three α-chains involved in a collagen molecule may have slightly different amino acid compositions. The general amino acid sequence is Gly–X–Y, where X often is proline and Y often is hydroxyproline. This means that glycine accounts for about one-third of all the residues in collagen and gelatin. Sulphur-containing amino acids are virtually absent and the crosslinks between the

Table 6.1 Amino acid composition of collagen and four different gelatins – amino acid residues per 1000 residues

Amino acid	Type I collagen (bovine)[a]	Type A gelatin[a]	Type B gelatin[a]	Cold water fish gelatin[b]	Warm water fish gelatin[c]
Alanine	114	112	117	112	123
Arginine	51	49	48	49	47
Aspargine	16	16	46	48	48
Aspartic acid	29	29			
Glutamine	48	48	72	72	69
Glutamic acid	25	25			
Glycine	332	330	335	347	347
Histidine	4	4	4	11	6
4-Hydroxyproline	104	91	93	60	79
Hydroxylysine	5	6	4	5	8
Isoleucine	11	10	11	11	8
Leucine	24	24	24	21	23
Lysine	28	27	28	28	25
Methionine	6	4	4	3	9
Phenylalanine	13	14	14	13	13
Proline	115	132	124	96	119
Serine	35	35	33	63	35
Threonine	17	18	18	24	24
Tyrosine	4	3	1	9	2
Valine	22	26	22	18	15

[a] Babel (1996).
[b] Product information from Norland Products Inc. – cod, pollack and haddock.
[c] Sarabia *et al.* (2000).

chains do *per se* not involve these types of residue. The amino acid compositions of different gelatins and collagen are listed in Table 6.1.

Collagen is completely insoluble in water but small fractions are soluble in dilute acid or salt solution. The collagen solubility will, however, decrease as a result of the ageing processes occurring in most mammals. This is due to an increasing number of intra- and intermolecular covalent linkages which are formed in the source tissue upon ageing. It is the stable intra- and intermolecular crosslinks that require the use of severe processing to obtain soluble gelatins, which in turn leads to a polydisperse molecular weight distribution. Type A gelatins have more or less identical amino acid compositions to their parent collagen and the average isoelectric point of type A gelatins are therefore similar to collagen and in the range 7–9.4. One exception to this is type A bovine hide gelatins which have a lower isoelectric point in the range 5.7–7.4 due to specific pre-treatments. Type B gelatins lack many of the non-ionizable glutamine and asparagine residues because these amino acids are converted into their carboxyl forms by alkali deamidation and the gelatins become more acidic. Thus the average isoelectric points of alkali processed gelatins are lower and in the range 4.8–5.5.

A striking difference between gelatins extracted from different sources can be seen by studying Table 6.1. The total proline plus hydroxyproline content of collagens and gelatins vary quite markedly between species. It may vary from 156 residues per thousand for a cold water fish gelatin, to 217–223 residues per thousand for gelatin and collagen extracted from warm-blooded mammals, such as pig and cattle. Warm water fish gelatin, extracted from, e.g., tilapia, has a higher content of proline and hydroxyproline than gelatin from cold water fish species and the total amount of these imino acids were found to be 198 by Sarabia *et al.* (2000).

The gelatin and collagen α-chains consist of polar and non-polar regions. The 'non-polar' regions are made up from the tripeptide Gly-Pro-R, where R is a non-polar amino acid, predominantly hydroxyproline. These 'non-polar' regions are interspersed with polar regions, which are relatively deficient in both proline and hydroxyproline. The presence and distribution of the charged, polar and non-polar amino acids provides gelatin with unique properties. Gelatin is easily dissolved in water at the right conditions due to the presence of charged amino acids and forms colloidal solutions. This means that gelatin by definition is a hydrocolloid. Due to its chemistry, gelatin is a multifunctional hydrocolloid with considerable surface activity.

6.4.2 Molecular weight distribution

It is not common for gelatin suppliers to routinely supply the purchaser with data other than those outlined above. Knowledge of the average molecular weight and the molecular weight distribution is, however, in many cases essential and should always be investigated. This can be achieved by the use of chromatographic methods (GPC/SEC) alone, using a polymer standard (polystyrene sulphonates are most commonly used for gelatin characterization), or in combination with multi-angle laser light scattering (MALLS) which provides absolute molecular weights.

Today, there is no good correlation between Bloom values and the average molecular weight and molecular weight distribution of gelatins. Bloom strength is essentially the rigidity of a gelatin gel formed and measured under standard conditions, as mentioned earlier in the chapter.

Figure 6.3 shows the molecular weight distribution for two gelatins with almost similar Bloom value and it is obvious that these gelatins have very different average molecular weights, which is also reflected by the difference in the viscosity values of the two gelatins.

From Fig. 6.3 it can be seen that the type B gelatin has a small low molecular weight fraction compared to the type A gelatin in which most molecules weigh less than 100 kDa. By taking the viscosity values into account it can easily be seen that there is no clear-cut coherence between the Bloom values and the viscosity values. The viscosity values represent the hydrodynamic volumes of the molecules in solution and it is therefore obvious that high molecular weight fractions will dominate the size of this value.

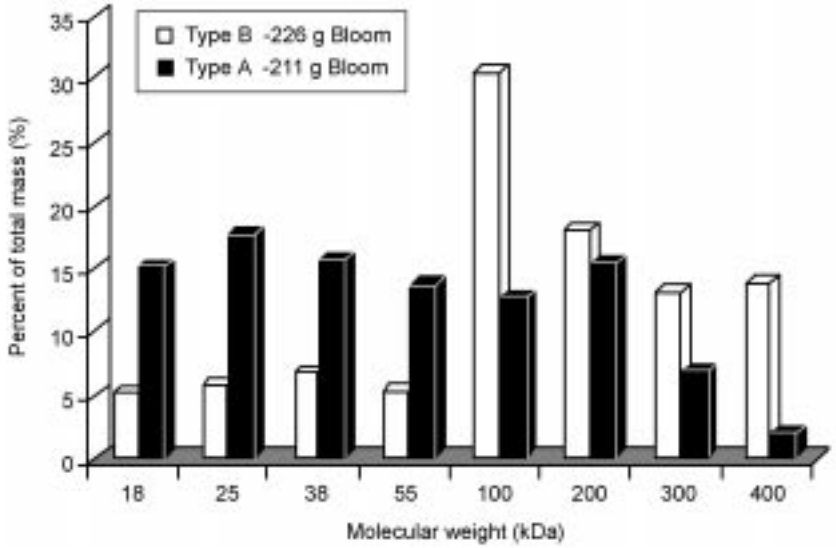

Fig. 6.3 The molecular weight distribution for two gelatins with similar Bloom values (Product information, DGF Stoess, 1999). The type A gelatin has an average molecular weight of 94 kDa and a viscosity of 2.5 mPas (6.67%, 60 °C). The type B gelatin has an average M_w of 171 kDa while the viscosity is 5.9 mPas (6.67%, 60 °C).

The molecular weight distribution is essential in the manufacturing of soft gelatin capsules. A too high content of γ-chains gives a very fast setting, viscous solution which gives rise to misshapen capsules. Conversely if the γ-chains content is too low the gel will set too slowly and fail to peel adequately from the gel-spreading drum. The α and β fractions contribute to the gel strength and viscosity and, if the sample is rich in sub-α chains, it has a relatively low viscosity and a slow-setting, sticky gel which is not suitable for encapsulation. In general a higher content of γ-chains gives a high-setting gelatin while a sample rich in sub-α particles gives a low-setting gelatin.

6.4.3 Physical properties of collagen and gelatin

Several scientists have shown quite clearly that the thermal stability of a collagen is directly related to the pyrrolidine content (Piez and Gross, 1960; Harrington and Rao, 1967; Leuenberger, 1991; Sarabia *et al.*, 2000; Haug *et al.*, 2003) and there is evidence suggesting that hydroxyproline located in the third position of the triplet is the major stabilizer due to its hydrogen bonding ability. Just as the proline plus hydroxyproline content dictates the thermal stability of collagen, the content and distribution of these imino acids are also the major factors determining the physical properties of gelatin. Fish gelatins from cold water fish species have low pyrrolidine contents and thus are far poorer gelling agents than gelatins of similar average molecular weight derived from warm-blooded mammals or warm water fish species. Important properties related to

Table 6.2 Typical physical properties of different gelatins

Property	Mammalian gelatin 220 g Bloom	Cold water fish gelatin	Warm water fish gelatin 220 g Bloom
Gelling temperature[a]	26–27 °C	4–8 °C	21–22 °C
Melting temperature[b]	33–34 °C	14–16 °C	28–29 °C
Solubility	> 40 °C	> 22 °C	> 35 °C
Bloom value	Yes	No	Yes

[a] For 10 (w/v)% solution cooling rate 0.5 °C/min; at $\delta = 45°$.
[b] For 10 (w/v)% gel matured for 2 hours at 4 °C prior to heating at 0.5 °C/min; at $\delta = 45°$.

gelling ability of gelatins are gel formation, texture, thickening and water binding. Some physical properties of mammalian and fish gelatins are summarized in Table 6.2. These values are not absolute and are just examples showing the temperature application ranges for the different gelatins. The gelling and melting temperatures in Table 6.2 are influenced by experimental parameters such as gelatin concentration, cooling and heating rate, and maturing temperature.

A critical parameter for both collagen and gelatin is the helix-to-coil temperature or the denaturation temperature which is dominated mainly by the content of pyrrolidine residues. Above this temperature all triple helical structure is lost since the intra- and intermolecular hydrogen bonds stabilizing the triple helical structures are broken. The result is an unfolding of the triple helices and the collagen and gelatin molecules exist in solution as random coils. The processes of gelatin molecules going from helices to random coils and from random coils to helices can be followed by optical rotation measurements as illustrated in Fig. 6.4 for cold water fish gelatin at a concentration close to, but below, the critical overlap concentration. The experiments and calculations were performed as described by Djabourov *et al.* (1985, 1988).

Below a critical helix-to-coil temperature (similar to the denaturation temperature of the parent collagen), which is between 15 and 20 °C for cold water fish gelatin and ~36 °C for mammalian gelatins (Djabourov and Papon, 1983), the α-chains are organized in helical structures where the helix amount varies with respect to several parameters including gelatin concentration, kinetics and temperature. For gelatin concentrations above the critical overlap concentration (c^*), the gelatin molecules reassemble into a network of triple helices, forming a thermoreversible viscoelastic gel upon cooling. The thermoreversible gelling process for gelatin is illustrated in Fig. 6.5. It is this thermoreversibel gel property that makes mammalian gelatin such a useful and unique ingredient in food and pharmaceuticals since such gels will 'melt in the mouth'.

The critical overlap concentration and gel strength for a gelatin will be dependent on factors such as the average molecular weight, molecular weight distribution, co-solutes, and pH of the solution.

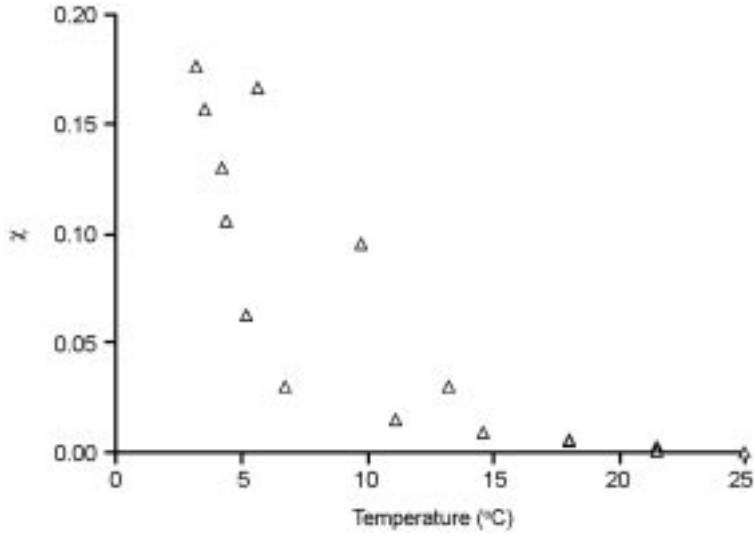

Fig. 6.4 Helix amount (χ) in 2(w/v)% cold water fish gelatin solution cooled and heated at a rate of 0.5 ºC/min in the temperature range 25 º–4 º–25 ºC (Haug, 2003).

The triple helical regions in a gelatin gel have to be stabilized by three pyrrolidine-rich regions coming together, as illustrated in Fig. 6.5. At low gelatin concentrations it is possible that these three regions may originate from one chain to give an intramolecular triple helical structure which will not contribute to a gel network. However, as the concentration is increased, the likelihood of two or even three different chains being involved also increases. Thus, not all reformed triple helical structures will form functional junction zones. In addition to the concentration, the ratio of functional to non-functional junctions will also depend on the molecular weight and the polydispersity index.

For information about the gelling kinetics small-strain oscillatory measurements alone or in combination with optical rotation measurements should be performed. Joly-Duhamel *et al.* (2002a,b) have studied the amount of helices needed to form a gelatin gel for systems with concentrations above c^* and their master curve for gelatin clearly shows that it is the helix concentration

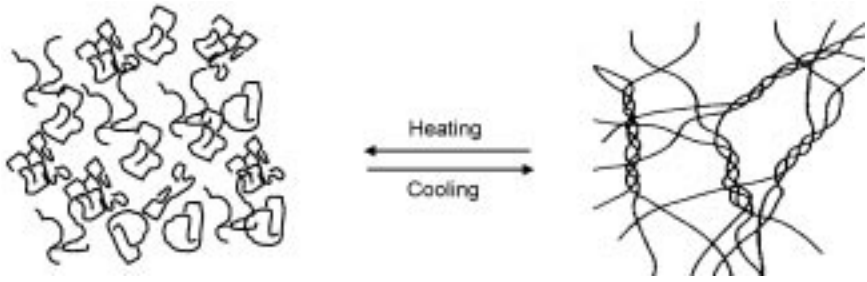

Fig. 6.5 The thermoreversible gelling process for gelatin.

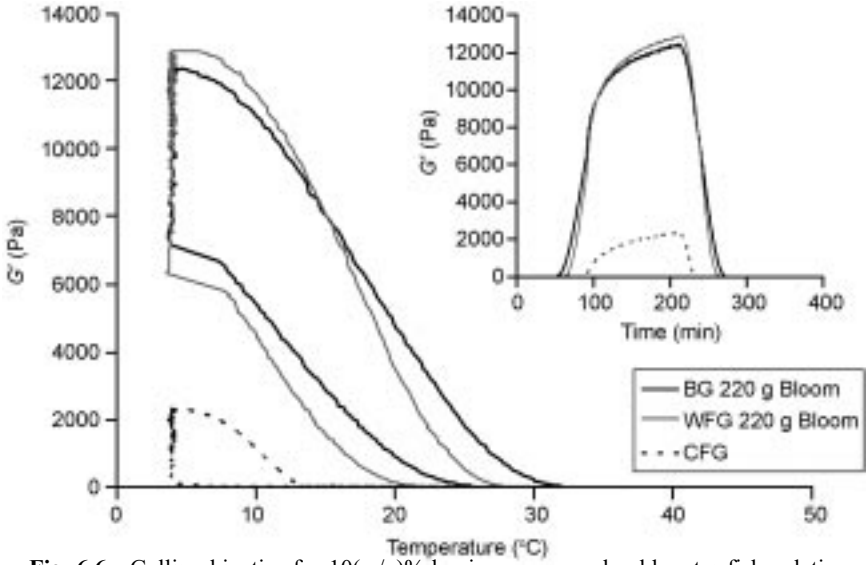

Fig. 6.6 Gelling kinetics for 10(w/v)% bovine, warm and cold water fish gelatin matured for two hours at 4 °C (temperature gradient 0.5 °C/min., $f = 1$ Hz, $g = 5 \times 10^{-3}$).

that determines the final gel strength. Figure 6.6 shows the development in storage modulus for 10% bovine, warm water and cold water fish gelatin determined by small strain oscillatory measurements.

Gelatin gels easily melt before the denaturation temperature of the parent collagen is reached since only a certain fraction of helices are needed to form a gelatin gel network, as shown by Joly-Duhamel *et al.* (2002a,b). By comparing the data for cold water fish gelatin in Figs 6.4 and 6.6, it can be found that the melting temperature for the gel is below the helix-to-coil temperature. The helix-to-coil temperature must not be confused with the gelling or melting temperature of a gelatin system. Both the gelling and melting temperatures for pure gelatin gels are always below the helix-to-coil transition temperature (or the denaturation temperature) of the parent collagen. The hysteresis between the gelling and melting temperature is caused by association of helices in gel state, e.g. the formation and disruption of the total gel network and not only due to conformational changes on the molecular level.

Several studies have shown that the gel network, once formed, is continually being reorganized to include junctions of increasing thermal stability. At all temperatures the strength of a gelatin gel increases with time, albeit only slowly and an 'equilibrium' value for the gel strength is rarely or never reached since the junctions are continually reorganized and new junctions are slowly formed with time (Fig. 6.6).

The gel strength used to characterize gelatins is, however, not the storage modulus, but the Bloom strength. The Bloom strength is the result of a single point measurement and does not reveal information about the gelling kinetics.

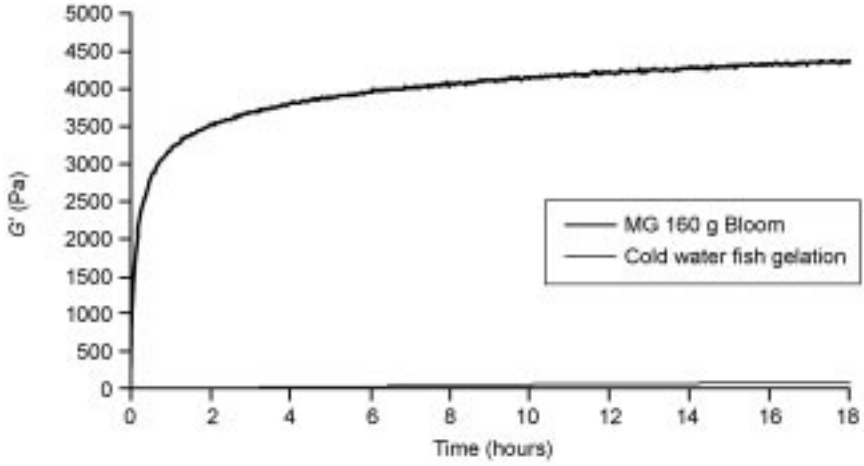

Fig. 6.7 Bloom maturing at 10 ºC for 18 hours measured on a rheometer ($f = 1$ Hz, $g = 5 \times 10^{-3}$) for 6.67(w/w)% mammalian gelatin (160 Bloom, type A/B, Gelita) and cold water fish gelatin (Norland Products Inc).

By following the maturing process (6.67% gelatin, 10 ºC, 18 hours) in the Bloom standard test method by small-strain oscillatory measurements, it is possible to follow the kinetics, as illustrated in Fig. 6.7. The figure also illustrates the difference in the storage moduli for 6.67% 160 Bloom mammalian gelatin type A/B and cold water fish gelatin after maturing at 10 ºC for 18 hours. In fact, the cold water fish gelatin hardly gels at 10 ºC after 18 hours and the gel formed is not strong enough to carry its own weight. It is therefore not possible to measure the Bloom strength for this gelatin by following the Bloom standard test method.

The strengths of gelatin gels, both for mammalian and fish gelatins, are found to be rather independent of pH in the range of 4–10, at least for concentrated systems. The major distinction between gelatins from different sources is the gelling and maturing temperatures needed to achieve gel formation, as can be seen from Table 6.2. Outside this pH range gelation is markedly inhibited and this probably reflects the fact that at these extreme pH values the chains carry a high net positive or negative charge and electrostatic forces inhibit the ability of the chains to enter suitable positions for the formation of junction zones. Gelatin systems also easily form gel networks in the presence of small co-solutes such as salts, sugars and sugar alcohols but both the gelling and melting temperatures of such systems may differ from those of pure gelatin systems. Also the gelling and melting temperatures and the final gel strength of such gelatin systems may be influenced by the presence of the co-solutes, both positively and negatively, especially at high co-solute and/or gelatin concentrations.

The gelatin utilized in almost every application today is of mammalian origin (porcine and bovine). Gelatins from fish are not commonly used in industrial application today due to suboptimal physical and rheological properties (cold water fish gelatin), fear of possible allergic reactions (fish allergy), and due to limited availability and higher prices. Several new patent applications, new

technologies and strict testing to prevent possible allergic reactions may, however, change this in the near future.

6.5 Gelatin derivatives

6.5.1 Cold water soluble (instant) gelatins

Commercial gelatins usually possess both amorphous and crystalline character. However, if the drying process is very carefully controlled it is possible to produce a very finely powdered gelatin which, in contrast to the coarsely granulated conventional gelatin, possesses no crystalline character. The amorphous structure of instant gelatin enables it to swell very rapidly and intensively. Its three-dimensional molecular network is weakly linked; the molecular arrangement is purely coincidental and the physical inter- and intramolecular binding forces are weak. Water can readily be taken up by the structure so that swelling never actually ceases and all the water that is available is absorbed to a gel-like texture.

In rheological terms, the instant gels can be compared with those formed by dissolution in warm water. However, the gel-forming kinetics are different; whilst instant gelatin has achieved 90% of its firmness after ca. 30 minutes, normal gelatin gels require considerably longer time. In addition, normal gels are much firmer, even at comparable concentrations and gelatin quality.

To avoid 'clumping' and to ensure homogeneity it is advisable to mix the cold water soluble gelatin with other fine particle ingredients in any food formulation at a ratio of 1:5–1:7. Such gelatins are extensively used in dessert powders, ready-to-use cake mixes, and whipped cream powders (0.1–3%). Instant gelatins are, however, usually added to products which do not demand transparent gels since instant gelatin gels become turbid. As with conventional gelatins these cold water soluble gelatins can be supplied with a wide range of gelling and viscous properties.

6.5.2 Gelatin hydrolysates

Although gelatin loses its ability to gel when hydrolysed to small peptides, there is an expanding market for such products. Such hydrolysates are manufactured by using chemical, thermal or biochemical degradation – or a combination of these – of gelatin followed by sterilization, concentration and finally spray-drying. Their gross composition is thus very similar to that of the native protein (89–93% protein, 2% ash and 5–9% water). Gelatin hydrolysates typically possess a viscosity of 20–50 mPas in 35% solution at 25 ºC. Unlike many other protein hydrolysates, gelatin hydrolysates do not possess a bitter taste and can thus be used in a wide range of products such as instant teas, beverages, low-fat spreads, low-fat cheese, canned meats, marshmallows, cereal bars and pastilles. They are supplied as light cream coloured water soluble powders for use as nutritional supplements, binding agents, foaming and emulsifying agents and

carriers. A wide range of gelatin hydrolysates are available with molecular weights in the range 3,000–20,000. These zero Bloom gelatins do not gel but are used in confectionery as substitutes for carbohydrates, as a protein source, whipping agent and a binding agent for cereal bars. A typical sugar-free gum may contain about 20% gelatin hydrolysate and 7% conventional gelatin while a muesli bar may contain 23% hydrolysed gelatin. In the dairy industry such hydrolysates are usually used as a whipping agent; 1–3% of the higher molecular weight hydrolysates enable creamy and soft textures to be obtained and provide a final product of high whipping volume. In the meat industry they have been used in finely homogenized canned meats where addition of 1.5–2% can reduce jelly and fat deposits by two-thirds, in cooked sausage at about 2% to reduce cooking losses and improve sliceablility, as edible films on frozen meat to prevent oxidative changes and freezer burn (in conjunction with conventional gelatin). Higher molecular weight hydrolysates have been used in the manufacture of soups, sauces and prepared meals to impart a creamy smooth consistency to the product and in low-fat meat spreads where they act as a binding agent. Hydrolysate gelatin is also used in several energy drinks for athletes.

6.5.3 Chemically modified gelatin

Gelatin contains a number of amino acids which possess side chains with amino-, carboxyl- and hydroxyl groups. These groups can react with numerous mono- and bifunctional reagents, hence altering the chemical and physical properties of gelatin and its derivatives. Chemically modified gelatin is used mainly in the photographic and cosmetic industries; use in the food and medical industries is restricted by law.

6.6 Applications of gelatin

Gelatin is a versatile hydrocolloid and is widely applied in food, pharmaceutical, cosmetic, medical and photographic products. Unless otherwise stated, the gelatins mentioned in this section refer to gelatins of porcine and bovine sources (but warm water fish gelatins can in most cases also be used). The most important properties of gelatin are:

- thermoreversible gel formation
- texturing
- thickening
- high water binding capacity
- emulsion formation and stabilization
- foam formation
- protective colloidal function
- adhesion/cohesion.

6.6.1 Applications in confectionery

The confectionery industry uses gelatin not only for its thermoreversible gelling properties, but also for foam formation and stabilization, binding, emulsification and controlling sugar crystallization. Gelatins with unique properties are developed especially for the confectionary industry. Examples of products containing gelatin are fruit gummies, mallows, meringues, caramels, bar products and sugar-coated candies. Table 6.3 summarizes the Bloom strength, concentration and function of gelatin used in some confectionery products.

6.6.2 Applications in foods

The food industry is still one of the major consumers of gelatins. Gelatin desserts, all types of jellies, are examples of food products that take advantage of the thermoreversible gel formation and the 'melt-in-the-mouth' texture of

Table 6.3 Examples of applications, Bloom values, concentration and function of gelatin in some food and confectionary products. Primary functions are written in boldface (from Schrieber and Gareis, 2007)

Application	Gel strength (g Bloom)	Concentration (%)	Function
Desserts	200–260	1.5–3.0	**Gel formation** Texture Transparency Brilliance
Fruit gummies	200–280	6.0–10.0	**Gel formation** Texture Elasticity Transparency Brilliance
Marshmallows	160–260	1.0–3.0	**Foam formation** Foam stabilization Gel formation
Pastilles	160–220	1.0–2.0	**Binding agent** Texture Melting properties Prevents disintegration
Caramels	140–220	0.5–2.5	**Emulsifier** **Foam stabilization** Chewability
Yogurt	220–260	0.2–1.0	**Stabilisation of syneresis** Texture Creaminess
Meat and sausages	220–260	0.5–2.0	**Emulsion stabilization** Water/juice binding
Broths and canned meats	220–260	0.5–2.0	**Binding agent** Texture Sliceability

gelatin. Gelatin is also essential in some dairy products and pastries to provide the quality of these storable products as required by consumers. Milk is primarily a complex oil-in-water emulsion. The addition of gelatin to dairy products improves the emulsifying capacity as gelatin molecules associate to the surface of the fat droplets and thereby reduce the surface tension towards the aqueous phase. Syneresis is the phenomenon of liquid being exuded from a gel and this is usually undesirable since a product will become less appealing to the consumers. Gelatin can be added to dairy products to bind whey, and in this way hamper secretion of aqueous whey from, e.g., yogurts, curds and cream cheese. By adding gelatin to foamed milk-based desserts like yogurt, curds, ice creams and mousses, gelatin depresses the surface tension of water, enabling formation of foam by mechanical whipping or injection of gas. In ice cream gelatin will also influence the size and distribution of ice crystals formed and thereby influence the texture and mouthfeel of the final ice cream. The meat processing industry also applies gelatin to their products for several reasons, but the ability to bind water and meat juices, and to secure good texture and taste is very important. Gelatin is also widely used in low-fat (fat replacer), low-carb (binding agent) and low-calorie (fat replacer and binding agent) food products. These are just some examples of applications of gelatin in food products and several other applications exist. Table 6.3 summarizes the Bloom strength, concentration and function of gelatin used in some food products.

6.6.3 Pharmaceutical and medical applications

Gelatin is an important and versatile excipient for pharmaceutical and medical applications. The widespread utilization of gelatin in pharmaceuticals and medical devices is due to several excellent properties of gelatin:

- established as a pharmaceutical excipient
- tolerated in food (non-toxic, non-allergenic)
- GRAS status
- excellent biocompatibility
- high quality (purity)
- low immunological activity
- controllable physical parameters.

Gelatin is utilized in plasma substitutes used in emergency medicine and surgery, in vitamin coatings, pastilles, tablets, in the production of globules, paste dressings, sponges and in the formulation of new vaccines. About 90% of all gelatin of pharmaceutical grade is applied in capsule production – soft and hard gelatin capsules.

The hard gelatin capsule is a unit solid dosage form. It consists of two pieces, a cap and a body, which have the form of open-ended cylinders and which fit one over the other. They are produced by dipping stainless steel pins into a warm gelatin solution (high-Bloom, 28–35% gelatin, colour). A gelatin film is formed when the pins are withdrawn from the gelatin solution and the film sets

immediately to form empty gelatin bodies which are dried prior to use. The quantity of gelatin picked up by the pins is dependent on the viscosity of the solution and the speed of the pins. Too high viscosity and pin speed will lead to capsules with wall thickness above normal. The ideal viscosity of the gelatin solution is 750–1000 mPas at 50 ºC. Neither the cap nor the body are uniform in wall thickness, and a hard capsule is usually thinnest on the shoulder (80–120 μm) and thickest at the rounded ends (130–150 μm). The mid part of the capsule usually has a thickness of 100–130 μm. After drying, the water content of the capsules is between 14 and 16% which facilitates removal of the capsule parts from the pins. In the final step, the body and the cap of the hard capsules are assembled in pre-lock position and collected. After filling the capsules with a drug, the two parts of the capsule are finally joined and locked prior to a new drying step down to the final moisture content of ~13%. The hard capsules are manufactured by a small number of specialist companies who supply them to the pharmaceutical industry where they are filled with active compounds to produce the final dosage form.

Soft gelatin capsules are completely closed units. They can be seamless (Globex process) or have a longitudinal seam (rotary die process). Today most of the soft gel capsules are manufactured according to the rotary die process. All capsules are produced, filled and sealed in one operation and are predominantly used for non-aqueous liquids and pastes. The term soft does not necessarily reflect a soft texture of the capsules, but rather that these capsule shells contain softeners or plasticizers (glycerol, sorbitol or a mixture thereof) preventing the capsule material from turning brittle. The concentration and choice of plasticizer, the final moisture content and the thickness of the shell (and seam) are important for the mechanical properties of the capsules. Gelatin with Bloom strengths in the range of 155–210 are used in soft gel capsules and strict control of the viscosity of the gelatin mass used for encapsulation is very important.

6.6.4 Nutritional and health proprieties

Gelatin is a high-quality source of protein, free of cholesterol and sugar and contains practically no fat. Gelatin is easily digested and completely broken down by the human organism. Gelatin is applied in food products to enrich the protein content, to reduce the amount of carbohydrates, the salt concentration, and the amount of fat in low-fat products and as a carrier for vitamins. Some international studies suggest that gelatin may have a preventive and regenerative effect on the skeleton and locomotor system – especially bones, cartilage, tendons and ligaments. It is also suggested that gelatin may help fortifying hair if taken regularly and strengthen the connective tissue, thus ensuring firm skin, shiny hair and strong fingernails.

6.6.5 Cosmetic applications

The health care market is one of the fastest growing markets in the world. In 2004 this market was US$230 billion and the increase has been around 8% per

year during the last five years. Our strive for beauty and wrinkle-free skin has opened up new markets for gelatin and collagen. Collagen and gelatin (from bovine, porcine and fish sources) play important roles in skin and hair products as functional ingredients. Gelatin hydrolysates are, for example, added to skin creams to improve the water-binding capacity, to reduce trans-epidermal water loss and to improve skin feel.

6.6.6 Photographic applications

Modern silver bromide photographic materials are mainly composed of emulsions containing gelatin on a backing material (paper or film). Here gelatin has three functions:

1. It acts as a binding agent for the photosensitive silver bromide.
2. For the fabrication of the emulsion, it is essential that the gelatin swells and forms a solution when heated, which turns into a gel on cooling and, after the water has been extracted, changes into a durable state.
3. The swelling capacity of gelatin guarantees that the photographic baths, necessary for the chemical reactions during the processing of the exposed photographic materials, penetrate into the emulsion and can be easily removed by rinsing.

With the introduction of gelatin more than 100 years ago, films became about 1,000 times more sensitive than their predecessors. However, the spectrum of gelatin applications includes a lot more than just prints, slides, movies and cinema films. Industry processes photographic gelatin to various types of repro-films for the printing trade (intermediate stage for the multi-colour prints of today). To scientific and technical photographic emulsions, such as nuclear trace emulsions for localizing radio isotopes in nuclear medicine, to infrared sensitive emulsions for taking pictures in the 'dark', in astronomy, and in geology and photogrammetry for pictures taken from great heights. Nowadays the greatest demands are made on photographic gelatin for manufacturing X-ray films.

6.7 Acknowledgements

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7

Carrageenan and furcellaran

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Abstract: Red seaweeds contain naturally-occurring polysaccharides which fill the voids within the cellulose structure of the plant. This family of polysaccharides include carrageenan and furcellaran. Gels are produced by heating and cooling solutions of these polysaccharides to give soft, elastic gels with iota carrageenan and firm, brittle gels with kappa carrageenan and furcellaran. Lambda carrageenan gives viscous solutions. Carrageenan is utilised for water dessert gels and glazes, flans, canned meats and pet food. In addition, carrageenan interacts with protein to stabilise, thicken and gel a wide range of products including milk drinks and shakes, ice cream and dairy desserts.

Key words: carrageenan, processed *Eucheuma* seaweed (PES), furcellaran, gelling agent, carrageenan–locust bean gum synergy.

7.1 Introduction

Red seaweeds, *Rhodophyceae*, contain naturally-occurring polysaccharides which fill the voids within the cellulose structure of the plant. This family of polysaccharides include carrageenan, furcellaran and agar. Red seaweeds have a long history of use in foods in the Far East and Europe. Agar has a documented history in food applications since 1658 whereas carrageenan has been used in foods over the past 100 years.

In the past, furcellaran has been called 'Danish agar', a term that describes its firm-gelling properties and the original source of the material. However, this is misleading as furcellaran contains 16–20% sulphate content and is structurally similar to kappa carrageenan. In Europe, furcellaran initially was assigned a

separate E-number but a review and reassessment of carrageenan and furcellaran recognised the structural and functional similarities of the two materials and classified them together as E407. In contrast, agar has a low sulphate content and under food legislation it is recognised as a separate material and assigned E406 (EU, 1995).

Carrageenan, furcellaran and agar have a backbone of galactose but differ in the proportion and location of ester sulphate groups and the proportion of 3,6-anhydrogalactose. The differences in composition and conformation produce a wide range of rheological properties which are utilised in a broad range of foods.

Different types of carrageenan confer a wide spectrum of textures, from a viscous thickener for lambda carrageenan to thermally-reversible gels which range in texture from soft and elastic for iota to firm and brittle for kappa carrageenan and furcellaran. As a consequence of the regular helical conformation adopted when kappa carrageenan solutions are cooled, the chains are able to interact synergistically with other gums, such as locust bean gum and konjac mannan, to further modify the gel texture. A specific interaction between kappa carrageenan and kappa casein is widely used to stabilise dairy products.

7.2 Manufacture

7.2.1 Raw materials

The main species of *Rhodophyceae* used in the commercial production of carrageenan include *Eucheuma cottonii* and *E. spinosum*, now reclassified as *Kappaphycus alverezii* ('Cottonii') and *Eucheuma denticulatum* ('Spinosum') (Blakemore and Harpell, 2008). These are spiny bushy plants, about 50 cm high, which grow on reefs and in shallow lagoons around the Philippines and Indonesia and other island coasts in the Far East. *K. alverezii* yields kappa carrageenan and *E. denticulatum* contains iota carrageenan. Due to its use in traditional food products, *Chondrus crispus* is the most familiar of the red seaweeds. It is found as a small bushy plant, only about 10 cm in height, widely distributed around the coasts of the North Atlantic. Carrageenan extracted from this species comprises both kappa and lambda types, although it has been shown that these do not occur within the same plant but in individual plants which grow together (McCandless *et al.*, 1973). *Gigartina* species are large plants up to 5 m in length which are collected from the cold deep coastal waters off Chile and Peru to give kappa and lambda carrageenans. *Furcellaria* species, found in the cold waters around Northern Europe and Asia, yield kappa and lambda carrageenans.

7.2.2 Manufacturing process

The manufacturing processes for carrageenan are shown in Fig. 7.1. The processes commence with the selection of seaweed to ensure it is harvested at the right time. After the seaweed is collected it is washed to remove sand and

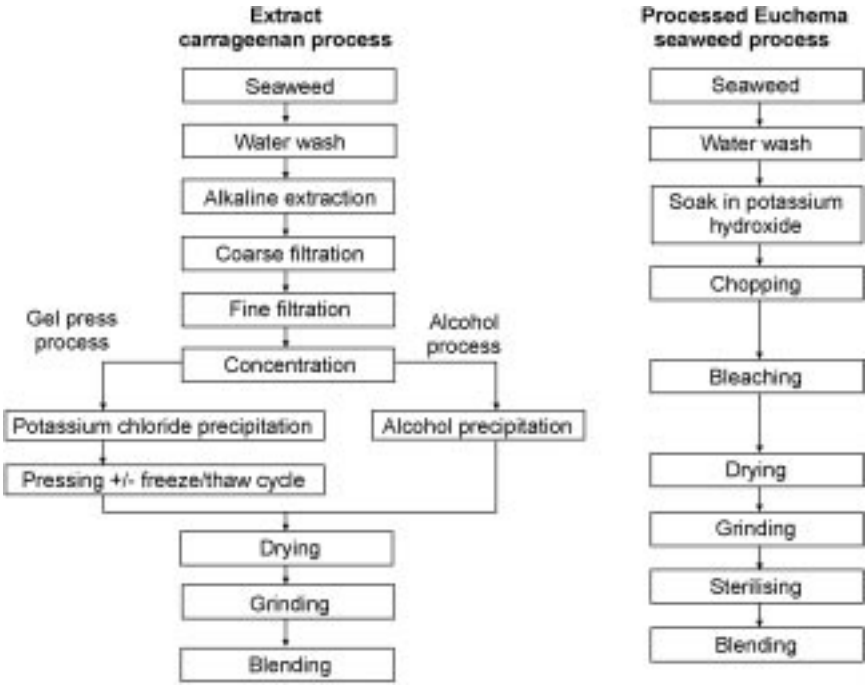

Fig. 7.1 Manufacturing processes for carrageenan and processed *Eucheuma* seaweed (PES).

stones and then dried quickly to prevent microbial degradation and hence preserve the quality of carrageenan. The weed is then baled and shipped to the processing plants and warehoused before use. Manufacturing plants located near the harvesting site may utilise wet seaweed to minimise microbial degradation and to avoid the costs of drying.

At the manufacturing site, the baled seaweed is tested and various lots are selected to produce the desired extract. Proper selection of the raw materials and an understanding of the influence of the process on the properties of the final carrageenan are vital to the production of a high quality, consistent end-product.

After the seaweeds are identified to make a particular extract, they are washed to remove solid impurities before treating with the appropriate type and amount of alkali to swell the seaweed and extract the carrageenan. The alkali may be selected to obtain a particular carrageenan salt which has important consequences for the resultant extract, by determining dispersion, hydration, thickening and gel-forming properties. Prolonged treatment with alkali promotes an internal rearrangement which modifies the polysaccharide backbone. The anhydride bridge which is formed enables the polymer to adopt a regular conformation so that inter-chain associations are stronger. As a consequence, the resulting gels have a higher rupture strength, they deform less before breaking and are more brittle.

After extraction and modification, the dilute carrageenan extracts are filtered and clarified by high speed centrifugation and concentrated by a range of methods. The concentrated solutions are then precipitated with isopropyl alcohol to give a fibrous mass which is pressed to remove impurities and dried.

An alternative recovery process may be utilised with seaweed extracts containing kappa carrageenan or blends with a high proportion of kappa material. This makes use of the specific selectivity of kappa carrageenan for potassium cations to form a gel. As kappa carrageenan solution is extruded into a concentrated solution of potassium chloride, a fibrous mass is formed. The precipitated mass exudes free water, termed syneresis, and is dewatered under pressure to make 'gel press' carrageenan. The precipitated carrageenan may be frozen and thawed to assist this dewatering step. The pressed fibres are then dried and ground to the appropriate particle size.

Each manufacturer carefully controls the raw materials and process parameters to produce a large number of extracts with well-defined properties. Individual extracts are characterised by their thickening and gelling properties. Finished products are made by blending various extracts in order to maintain consistent quality from lot to lot and to provide specific properties needed to meet the particular customer requirements for various applications.

Processed *Eucheuma* seaweed (PES), semi-refined carrageenan (SRC), alternatively-refined carrageenan (ARC), Philippine Natural Grade (PNG) and alkali modified flour (AMF) are the various terms used to describe *Eucheuma* seaweeds which are harvested around the Philippines and Indonesia and directly treated with alkali to modify the carrageenan within the seaweed (Tye, 1994). This is a more economical process as it avoids extracting the carrageenan into dilute solutions that require expensive, energy-intensive separation, filtration, concentration and drying steps to make the dried carrageenan extract.

The process by which processed *Eucheuma* seaweed is converted into commercial gelling grade products is compared with the traditional extraction process in Fig. 7.1. The process commences with selection and washing. Thereafter the process diverges from the manufacture of extract carrageenans. The *Eucheuma* seaweed is soaked in potassium hydroxide solution *in situ* before chopping and bleaching to reduce the colour of the finished powder. After washing, drying and grinding, the powder is sterilised to control the microbiological levels of the finished product.

7.3 Regulatory status

In the EU, carrageenan and processed *Eucheuma* seaweed are listed in Annex 1 of the European Parliament and Council Directive 95/2/EC on Food Additives other than Colours and Sweeteners as amended and assigned E-numbers of E407 and E407A (EU, 1995, 1998).

Carrageenan must be extracted from seaweed and contain less than 2% acid insoluble material to meet the EU definition for E407 ('Extract') although most

commercial grades produced by the traditional process will contain less than 0.5% insoluble material and yield very clear, transparent gels. Initially, it was proposed that furcellaran would have a separate classification of E408 under EEC food legislation. However, a reassessment of carrageenan and furcellaran recognised the structural and functional similarity of the two materials and classified them together as E407.

Processed *Eucheuma* seaweed (PES) contains 8–15% acid insoluble matter compared to 2% maximum for an extract (Phillips, 1996). The acid insoluble matter consists mainly of a network of cellulose which modifies the hydration, appearance and gel characteristics. The heavy metal content of PES is higher than extract carrageenan but still well below limits set for food additives. Under EU regulation, processed *Eucheuma* seaweed is distinguished by a different E-number, E407A (EU, 1998).

Both carrageenan and processed *Eucheuma* seaweed are approved permitted emulsifiers, stabilisers, thickening and gelling agents for use *'quantum satis'*, the level required to achieve a given technological benefit (EU, 1995, 1998).

In the USA, no distinction is made between carrageenan and processed *Eucheuma* seaweed: both are declared as 'carrageenan' on food labels.

In some early toxicological studies, carrageenan was degraded in acidic conditions at high temperature to give a product with a very low molecular weight in the range of 10,000–20,000 Daltons. This low molecular weight material is more accurately called 'degraded carrageenan' or 'poligeenan' and should be distinguished from carrageenan because it is completely non-functional in food applications having no thickening, gelling or stabilising properties. High doses of poligeenan showed some adverse effects, such as ulcerative colitis, in specific animals, such as guinea pigs, when consumed in drinking water rather than in food. Unfortunately, some researchers have not adequately distinguished between carrageenan and poligeenan/degraded carrageenan and have incorrectly attributed the adverse effects of poligeenan to carrageenan, resulting in false criticism of the safety of carrageenan in food.

For use as food additives, carrageenan and PES are clearly distinguished from degraded carrageenan/poligeenan through the application of a viscosity limit of 'not less than 5 cps for 1.5% solution at 75 ºC' (EU, 1998; JECFA, 2002). The limit of 5 cps is approximately equivalent to an average molecular weight of 100,000 Daltons, although commercial carrageenan and PES normally have molecular weights above 200,000 Daltons.

Recently, the possible degradation of carrageenan during food processing and digestion was researched and reviewed. A specific, detailed study on the fate of carrageenan subjected to a range of food processing conditions revealed that normal food processes do not significantly increase the proportion of low molecular weight material (Marrs, 1998). In addition, it was concluded that the presence of associated cations prevents carrageenan hydrolysis during digestion. Confirmation that no degradation occurs when the carrageenan is in the gelled state is demonstrated by the stability of acidic water dessert gels stored for periods of several months at ambient temperatures.

In 2003 the EU Scientific Committee for Food concluded that 'there is no evidence of any adverse effects in humans from exposure to food grade carrageenan, or that exposure to degraded carrageenan from the use of food-grade carrageenan is occurring' (SCF, 2003). To reinforce the distinction between carrageenan and poligeenan they have adopted a molecular weight limit of not more than 5% below 50,000 Daltons for food-grade carrageenan and PES (EU, 2004), although a validated test method has still to be developed (Blakemore and Harpell, 2009).

In the US, carrageenan is generally recognised as safe for use in food when used in accordance with good manufacturing practices (FDA, 2003).

7.4 Structure

Carrageenan is a high molecular weight linear polysaccharide comprising repeating galactose units and 3,6-anhydrogalactose (3,6 AG), both sulphated and non-sulphated, joined by alternating α-(1,3) and β-(1,4) glycosidic links. An almost continuous spectrum of carrageenans exist (Smith and Cook, 1953) but Rees and co-workers (Rees, 1963; Anderson, Dolan and Rees, 1965) were able to distinguish and assign definite chemical structures to a small number of carrageenan disaccharides. The main carrageenan types, lambda, kappa and iota can be prepared in a purified form by selective extraction techniques. Mu and nu carrageenans are considered to be the precursor structures in carrageenan which, as a result of chemical modification by alkali, produce the resulting kappa and iota carrageenans (Fig. 7.2).

The various carrageenan structures differ in 3,6-anhydrogalactose and ester sulphate content. Variations in these components influence the gel strength, texture, solubility, melting and setting temperatures, syneresis and synergy shown by different carrageenans. These variations are controlled and manipulated by seaweed selection, processing and by blending different extracts. The ester sulphate and 3,6-anhydrogalactose contents of carrageenan are approximately 22% and 33% respectively for kappa carrageenan and 32% and 26% respectively for iota carrageenan. Lambda carrageenan contains approximately 37% ester sulphate with little or no 3,6-anhydrogalactose content. Previously, furcellaran has been called 'Danish agar' because this material, originally obtained from Baltic seaweed, forms very firm brittle gels, similar to agar. However, this is a misleading description as furcellaran contains 16–20% ester sulphate, in contrast to agar which typically has a low sulphate content of 1.5–2.5%. Consequently, EU regulations recognise the structural and functional similarities of carrageenan and furcellaran and classify them as E407, together with iota and lambda carrageenan. For food applications, carrageenan is best described as 'extracts from *Rhodophyceae* which contain an ester sulphate content of 20% and above and are alternately α-(1,3) and β-(1,4) glycosidically linked'.

Further differences in carrageenan structure are found in the proportion of sulphate at C2 on the 3,6-anhydrogalactose residue. This amounts to 30-40% in

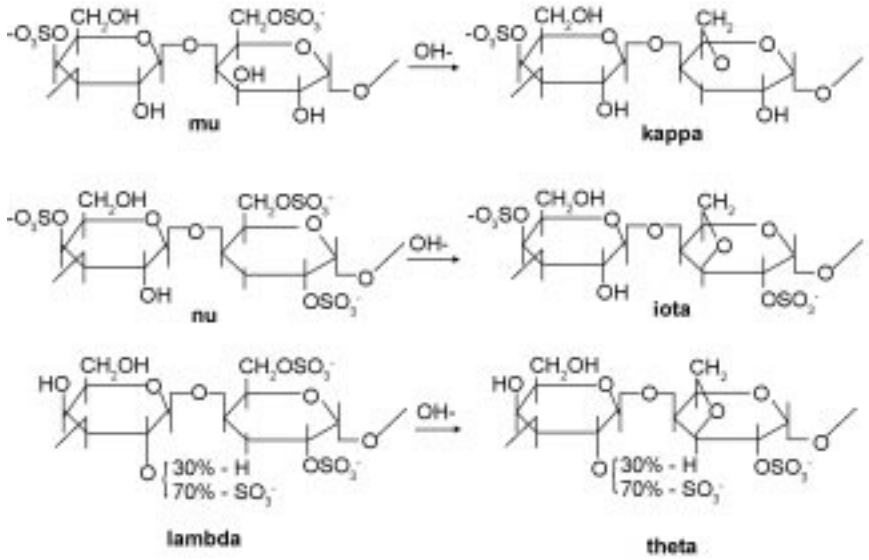

Fig. 7.2 Carrageenan structures and modification by alkali.

Gigartina radula and 40–55% in *G. skottsbergii*. Protein reactivity rises and water gel strength diminishes concomitantly as the ester 2-sulphate level increases, As a consequence of the distinct structure of these materials and their properties in water and milk they are described as 'kappa-2' (Bixler *et al.*, 2001) or 'hybrid' carrageenans (de Vries *et al.*, 2008). The repeating units that comprise the range of commercial carrageenans are shown in Fig. 7.3.

Carrageenan is a high molecular weight, polydisperse material. Commercial kappa carrageenan extracts are between 400 and 560 kDa and processed *Eucheuma* seaweed has a slightly higher molecular weight of 615 kDa (Hoffmann *et al.*, 1996). All carrageenans contain a fraction (<5%) of material below 100 kDa and this low molecular weight material is believed to be inherent in native algal weed.

7.5 Physical properties

The thickening and gelling properties of the different types of carrageenan are quite different. For example, kappa carrageenan forms a firm gel with potassium ions while iota and lambda are only slightly affected. Iota carrageenan interacts with calcium ions to give soft, elastic gels but salts have no effect on the properties of lambda carrageenan. In most cases, lambda is used with kappa in milk systems to obtain a suspension or creamy gel.

The large number of benefits provided by carrageenan and its blends has led to a broad and complex range of commercial products developed to provide the unique combination of properties best suited to a specific application.

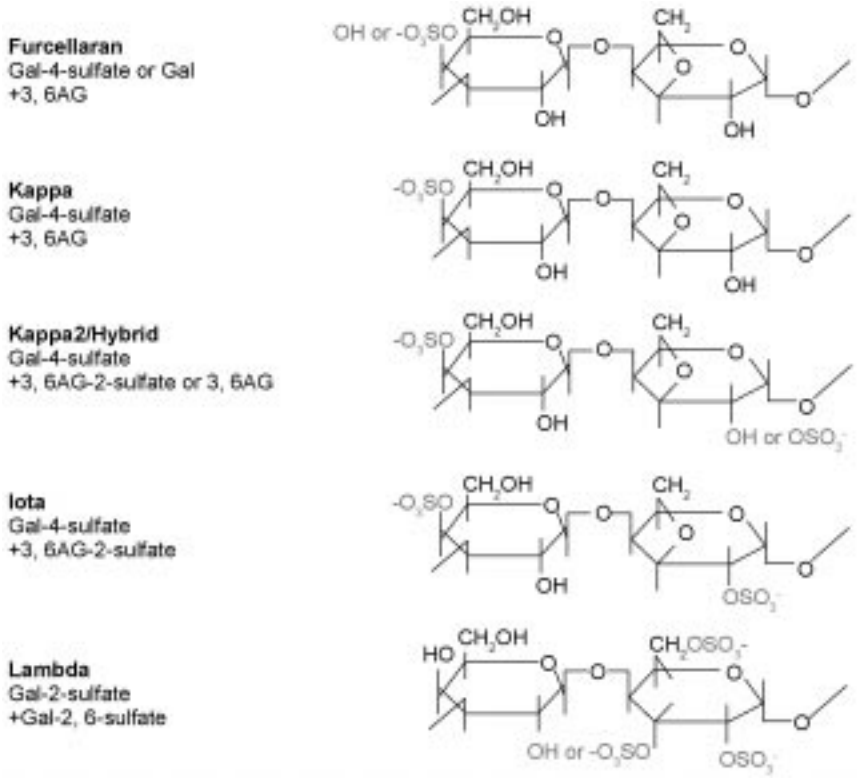

Fig. 7.3 Repeating units of main carrageenan structures.

7.5.1 Solution properties

All carrageenans are soluble in hot water but only the sodium salts of kappa and iota are soluble in cold water. The level of salts normally found in food products has no effect on lambda carrageenan; it develops viscosity in cold water and milk, although higher viscosities are obtained if solutions are heated and cooled. Lambda carrageenan gives viscous solutions which show pseudoplasticity or shear-thinning when pumped or stirred. These solutions are used for thickening, particularly in dairy products, to give a full body with a non-gummy, creamy texture.

The influence of temperature is an important factor in deciding which carrageenan should be used in a food system. All carrageenans hydrate at high temperatures and kappa and iota carrageenans in particular exhibit a low fluid viscosity. On cooling, these carrageenans set between 40 and 70 °C to form a range of gel textures depending on the type and level of cations present.

7.5.2 Acid stability

Carrageenan solutions lose viscosity and gel strength when heated in systems with pH values below about 4.3. This is caused by autohydrolysis which occurs

at low pH values as carrageenan in the acid form cleaves at the 3,6-anhydrogalactose linkage in the molecule (Hoffmann *et al.*, 1996). The rate of autohydrolysis increases at elevated temperatures and at low cation levels. However, once the solution is cooled below the gelling temperature, potassium ions associate with the sulphate groups on the carrageenan and this prevents autohydrolysis proceeding.

To minimise the effects of autohydrolysis, it is recommended that, where possible, carrageenan should be processed under neutral conditions and acid should be added to the food immediately before depositing and filling. In acidic foods carrageenan should be added near the end of the process to avoid excessive polymer breakdown.

Figure 7.4 demonstrates the loss of gel strength over time as solutions are heated at different pH values. Tests on solutions containing 0.5% kappa carrageenan and 0.2% potassium chloride show that the time to decrease the original gel strength by 20–25% is reduced by a factor of three for each drop of 0.5 pH unit and each 10 ºC increase in process temperature. Thus at pH 3 and 120 ºC the carrageenan gel strength is lowered 25% in only a few seconds, but such extreme conditions are not used in food processing. At pH 4.0 and 90 ºC, typical for fruit products, a 10-minute pasteurisation process will reduce carrageenan gel strength by 20–25%. The structure of hybrid/kappa 2 and iota carrageenans do not hydrolyse as rapidly and these materials tolerate acid processing better. The time to affect gel strength will vary depending on carrageenan concentration or system ingredients, such as salts and sugars. In a continuous process the temperature profile can be carefully controlled to give consistent products. In systems above about pH 4.5 the process conditions

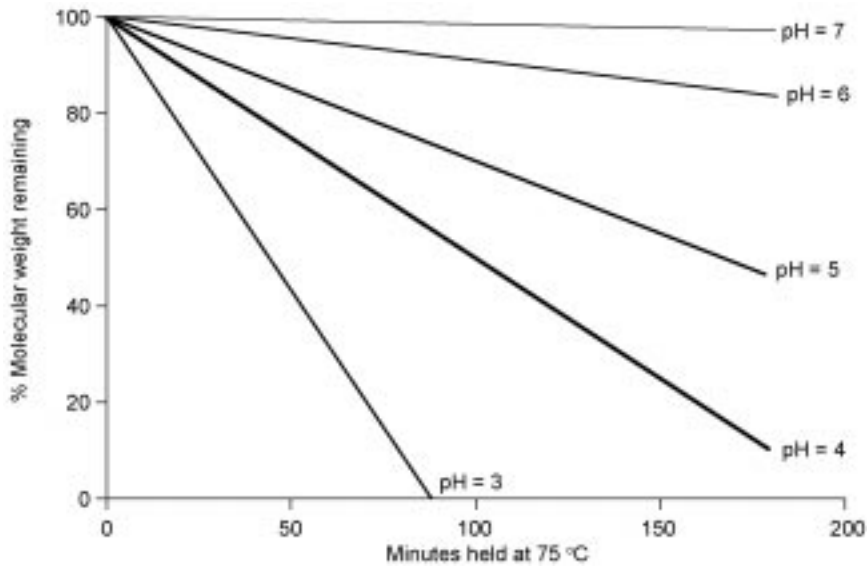

Fig. 7.4 pH stability of kappa carrageenan at 75 ºC.

become irrelevant as the carrageenan solution is stable to most food processing times.

7.5.3 Gel properties

Hot solutions of kappa and iota carrageenans set to give a range of gel textures when cooled to between 40 and 70 ºC depending on the cations present. Carrageenan gels exhibit hysteresis, a difference between setting and melting temperatures. These gels are stable at room temperature but melt when heated 5–20 ºC above the gelling temperature. On cooling, a neutral system will set to give similar gel characteristics. It must be remembered that, in acidic products, the gel strength and texture may be affected by heating and cooling as a result of autohydrolysis.

The ionic composition of a food system is important for effective utilisation of the carrageenan. For example, kappa carrageenan selects for potassium ions to stabilise the junction zones within the characteristically firm, brittle gel. Iota carrageenan selects for calcium ions to bridge between adjacent chains to give typically soft elastic gels.

The presence of these ions also has a dramatic effect on the hydration temperature of the carrageenan and on its setting and melting temperatures. For example, iota carrageenan will hydrate at ambient temperature in water but the addition of salt raises the gel point so that the solution is converted into a reversible gel with distinct yield point, a property that is exploited in the manufacture of cold-prepared salad dressings (Fig. 7.5).

Sodium salts of kappa carrageenan will hydrate at 40 ºC but the same carrageenan in a meat brine will only show full hydration at 55 ºC or above.

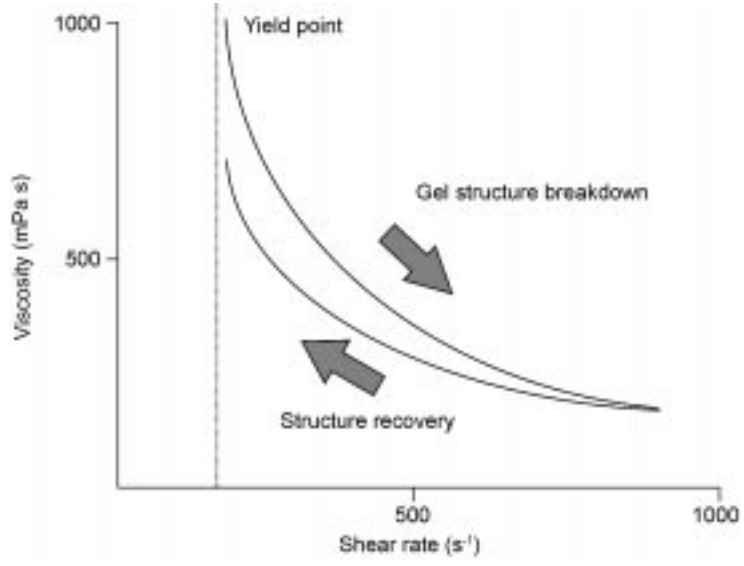

Fig. 7.5 Thixotropic rheology of iota carrageenan.

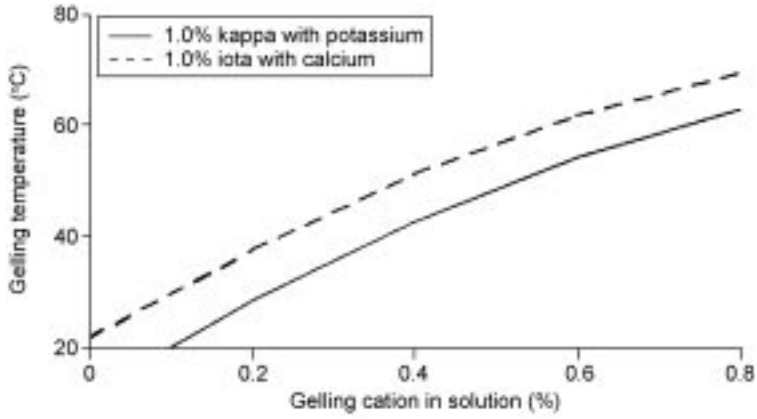

Fig. 7.6 Effect of cation concentration on gelling temperatures for kappa and iota carrageenan.

These effects of salt concentration on gelling temperatures of kappa and iota carrageenan are shown in Fig. 7.6.

As a carrageenan dispersion is heated, particles do not swell or hydrate until the temperature exceeds about 40–60 ºC. As the particles hydrate, the viscosity rises as the swollen particles offer more resistance to flow. Further heating to 75–80 ºC produces a drop in viscosity. On cooling, the solution shows a marked increase in viscosity followed by gelation below temperatures of 40–50 ºC. The hydration and gelation temperatures are strongly dependent on the salts associated with the carrageenan or added separately to the solution. For example, above about 4% sodium chloride can prevent complete hydration of carrageenan in meat brines. In contrast, the very dilute levels of around 200 ppm of carrageenan used to stabilise chocolate milks and other dairy beverages may not form a stabilising gel network until the temperature drops below 20 ºC. In confectionery, the presence of high solids effectively concentrates the carrageenan and cations on the aqueous phase so the gelation may occur at 80–85 ºC or higher, placing limitations on the levels and types of carrageenan suitable for such food applications.

As mentioned previously, kappa carrageenan makes a firm brittle gel which has very poor freeze-thaw stability. In contrast, iota carrageenan forms a thixotropic solution or very elastic gel which has good freeze-thaw stability. Kappa and iota systems may be blended to obtain a range of gel textures with intermediate properties of freeze-thaw stability and moisture binding as illustrated in Fig. 7.7.

Eucheuma seaweeds constitute a limited range of products suited to medium gel strength applications such as cooked meats and some dairy products. The solution and gel properties of PES and kappa carrageenan obtained by traditional extraction processes are similar with some specific differences. The cellulose network in PES reduces the rate of hydration so that solutions develop viscosity after longer heating periods or after heating to higher temperatures. The

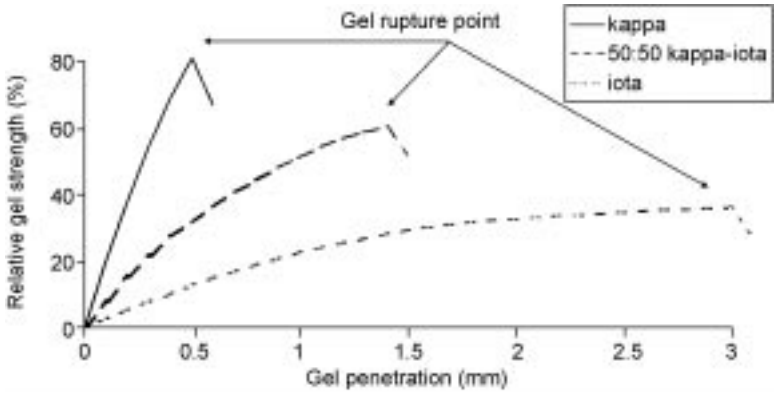

Fig. 7.7 Gel properties of pure and blended kappa and iota carrageenans.

presence of cellulose in the finished gel gives a lower rupture strength with a more brittle and fragile gel. The cellulose particles make the product cloudy and, therefore, unsuited to clear gel applications, such as water dessert gels and cake glazes. The less-refined material may also have slight odour and colour differences compared to carrageenan extracts.

7.5.4 Synergism with other gums

Locust bean gum is a galactomannan with a level of substitution of one part mannose with four parts of galactose. However, there is a non-random distribution of galactose side chains along the mannan backbone and up to ten contiguous units of mannan may be unsubstituted. The mannose-free regions of the locust bean gum are able to associate with the regular helical structures that form within the kappa carrageenan network during cooling. Thus, hot solutions of kappa carrageenan and locust bean gum form strong, elastic gels with low syneresis when they are cooled below 50–60 ºC. The maximum interaction, and hence peak rupture gel strength, occurs at ratios between 60 : 40 and 40 : 60 kappa carrageenan to locust bean gum as shown in Fig. 7.8. These polymer combinations are used in very large quantities in cooked meats and in gelled petfoods.

Kappa carrageenan and clarified locust bean gum mixtures can be used for cake glaze and flan gels or formulated to give clear water dessert gels with an elastic gel texture, similar to gelatin. However, as clarified locust bean gum is expensive, many products have been reformulated to take advantage of recent improvements in formulations of kappa and iota carrageenan blends to give more cost-effective products. These gels have cohesive, elastic textures and are thermally-reversible with setting and melting points between 50 and 60 ºC. In contrast to gelatin, these gels set and reach peak gel strength as the temperature is reduced. The gels set rapidly and are stable when stored at ambient temperature but they do not melt in the mouth. Konjac flour (E425i) interacts even more strongly with kappa carrageenan to form very cohesive, elastic gels

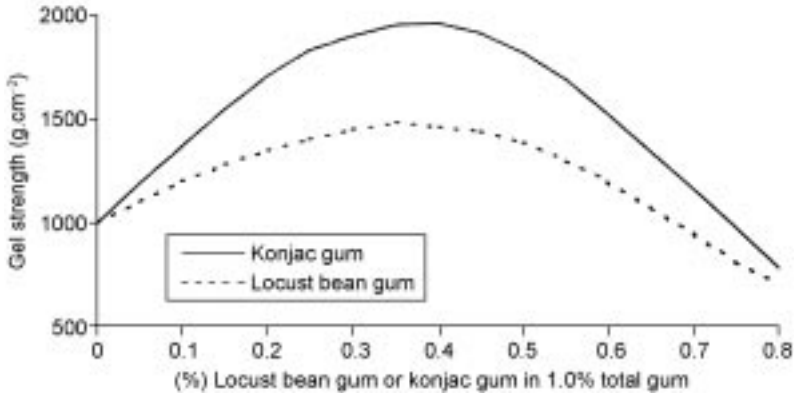

Fig. 7.8 Kappa carrageenan synergy with locust bean gum and konjac glucomannan.

which are several times greater than the rupture strength of kappa carrageenan by itself (Fig. 7.8).

Probably the best known synergy is exhibited between carrageenan and milk proteins. Some of the first uses of carrageenan were in milk gels and flans, and to stabilise evaporated milk and ice cream mixes, where the synergy allows for use levels as low as 0.03%. In these applications the kappa carrageenan not only forms a weak gel in the aqueous phase but it also interacts with positively charged amino acids in the proteins in the surface of the kappa-casein micelles, illustrated in Fig. 7.9. Very low levels of 150–250 ppm of carrageenan are sufficient to prevent whey separation from a range of dairy products during manufacture and storage. These include ice cream and milk shake mixes, cream

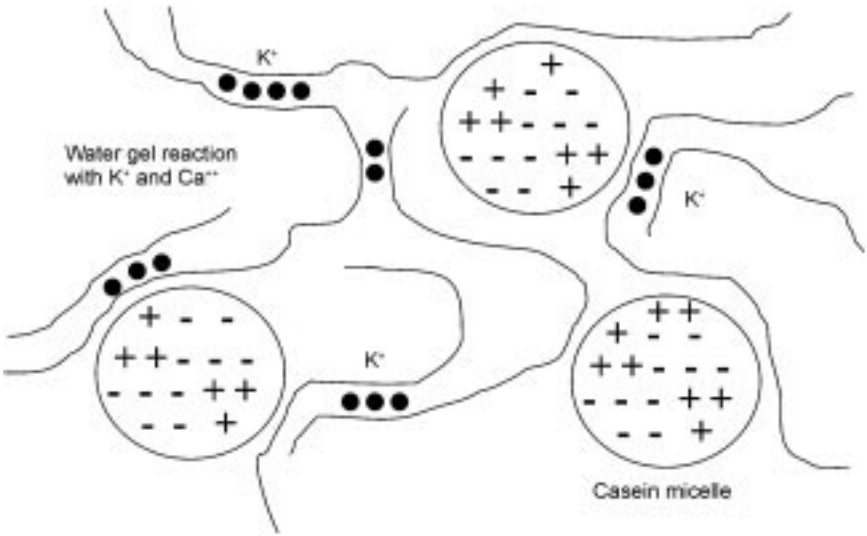

Fig. 7.9 Kappa carrageenan–kappa casein milk protein interaction.

Table 7.1 Summary of carrageenan properties

Carageenan type	Lambda	Iota	Kappa
Solubility			
Hot (80 °C) water	Soluble	Soluble	Soluble
Cold (20 °C) water	All salts soluble	Sodium salt soluble	Sodium salt soluble
Hot (80 °C) milk	Soluble	Soluble	Soluble
Cold (20 °C) milk	Thickens	Insoluble	Insoluble
Cold milk (TSPP added)	Increased thickening or gelling	Thickens or gels	Thickens or gels
50% sugar solutions	Soluble	Insoluble	Soluble hot
10% salt solutions	Soluble hot	Soluble hot	Insoluble
Gelation			
Effect of cations	Non-gelling	Strongest gels with calcium	Strongest gels with potassium
Gel texture	Non-gelling	Soft, elastic	Firm, brittle
Syneresis	Non-gelling	No	Yes
Hysteresis	Non-gelling	5–10 °C	10–20 °C
Freeze-thaw stability	Yes	Yes	No
Synergy with locust bean gum	No	No	Yes
Synergy with konjac glucomannan	No	No	Yes
Synergy with starch	No	Yes	No
Shear reversibility	Yes	Yes	No
Acid stability	Hydrolysis	Hydrolysis in solution, accelerated by heat; gels are stable	
Protein reactivity	Strong protein interaction in acid		Specific reaction with kappa-casein

cheese and dairy desserts. In chocolate milks, this low level of carrageenan is able to prevent separation and also generate a stabilising network which maintains the cocoa particles in suspension.

Iota carrageenan exhibits synergy in combination with starch with which it increases viscosity and mouthfeel and body to four times that of starch alone.

A summary of the solution and gelation properties of carrageenan and synergy with other materials is given in Table 7.1.

7.6 Food applications

Carrageenans which are able to hydrate at low temperatures present problems for their efficient use. Any lumps which are produced when the carrageenan is dispersed in water or milk greatly reduce the rate of hydration and may limit the development of full viscosity or gel strength.

Various methods may be used to ensure the carrageenan particles are fully dispersed before hydration commences. Mixing the carrageenan with 5–10 times

its weight of an inert filler, such as sugar, maltodextrin or salt, physically separates the carrageenan particles. Slurrying the powder in oil gives a hydrophobic barrier around the particles so that the powder may be dispersed before the particles start to hydrate. Alternatively, the powder may be dispersed into a salt solution or into sugar syrup or alcohol or a similar medium which impedes the hydration of the particles or raises the temperature at which the carrageenan particles hydrate.

Carrageenans which have been processed to include a high inherent level of potassium cation and/or blended with additional high levels of salt, such as potassium chloride, do not hydrate at ambient temperatures and they may be readily dispersed in cold water or milk without lumping. High shear mixing may be used to break up any lumps produced during dispersion and vigorous agitation by high speed or high shear mixing will quickly hydrate the dispersed particles. The broad spectrum of thickening and gelling properties of carrageenan result in its use in a wide range of water and milk-based food products, as summarised in Tables 7.2 and 7.3 (Thomas, 1997).

7.6.1 Water gels

Water dessert gels and cake glazes are amongst the most traditional uses for carrageenan. These products are based on the firm, brittle gel properties of kappa carrageenan with the texture modified for elasticity, cohesiveness and syneresis control using kappa 2 and iota carrageenan. Recent improvements in the carrageenan combinations used for these applications have produced vegetarian products which have a similar appearance and texture to traditional gelatin products whilst giving additional benefits of fast-setting and stability at ambient temperatures. An example recipe is shown in Formulation 7.1.

These gels are used for aspics, in canned meats and petfoods, and in cooked, sliced meats. In these latter products, the carrageenan is incorporated to improve moisture retention, cooking yields, slicing properties and mouthfeel and succulence. A typical formulation for a sandwich ham is given in Formulation 7.2.

Formulation 7.1 Fruit-flavoured water dessert jelly

Ingredients	%
Sugar	15.00–20.00
Carrageenan (kappa/kappa2/iota blend)	0.60–0.90
Potassium citrate	0.20–0.35
Citric acid	0.30–0.45
Colour	as required
Flavour	as required
Water	to 100.00
Total	100.00

Formulation 7.2 Cooked ham with 30% added brine

Ingredients	%
Meat, lean ham muscles	62.50
Carrageenan (firm gelling kappa)	0.60
Sodium tripolyphosphate	0.50
Nitrate salt*	1.67
Sodium chloride	0.53
Dextrose	1.20
Water	32.95
Total	100.00

* Sodium chloride containing 0.6% sodium nitrite, giving a 100 ppm sodium nitrite in the finished product. The total brine concentration of sodium chloride is 2.2%.

In this product the carrageenan disperses readily in the meat brine when it is added after phosphate and salt addition. Kappa carrageenan that has been processed with a high level of potassium chloride ('gel press' carrageenan), or blended with additional potassium chloride for enhanced gel strength, may be added to the brine at any stage during the preparation, as the potassium salts will prevent the carrageenan particles hydrating. It is important to ensure that the particles do not swell so that the brine has a very low viscosity and is easily injected and distributed throughout the meat mix. During the cooking cycle, the carrageenan hydrates above about 50–55 ºC to bind moisture. After cooking the ham to a core temperature of 72–74 ºC, the product is cooled and a cohesive gel forms which maintains product integrity during high speed slicing operations and binds moisture throughout the product shelf-life. Other water gel applications for carrageenan include gelled petfoods and meat and fish aspics.

Processed *Eucheuma* seaweed (PES) is frequently used in cooked sliced meats. This material is very cost-effective and it disperses readily without lumping in meat brines. During processing some differences are apparent between this and traditional carrageenan extracts. The small particles do not swell in the brine and may cause less damage when injected into the meat (Philp *et al.*, 1998). The cellulose network in PES reduces the rate of hydration during heating so that solutions develop viscosity after longer heating periods or after heating to higher temperatures. The presence of cellulose in the finished gel gives a lower rupture strength with a more brittle and fragile gel. The dispersed cellulose particles make the product cloudy so that any gel spots in the injected meat will be masked against the background of the meat. As a consequence, meat brines may include both carrageenan and PES to optimise properties and costs. In markets such as the EU, where carrageenan and PES have distinct E-numbers and must be labelled separately, companies often prefer to use carrageenan alone. In the USA and other markets, where no distinction is made between carrageenan and processed *Eucheuma* seaweed, meat brines often contain both materials.

The synergistic interaction between kappa carrageenan and locust bean gum is also used in water dessert gels and glazes, cooked, sliced hams and poultry

products, canned meats and petfoods, air fragrance gels and similar firmly gelled products. The gel produced by this gum combination exhibits benefits of high gel strength, cohesive, elastic texture, reduced syneresis and, depending upon market conditions, it may offer a cost-effective alternative to carrageenan alone. The gums also have an increased hot viscosity to retain meat juices better in cooked meats and to reduce emulsion separation and splashing during filling and cooling in fragrance gels.

Petfood is a very large market for alkali modified flour (AMF), a material that is based on the same seaweed and process as PES but with less strict microbiological limits: AMF is usually employed in non-food applications or products which are sterilised. The AMF is used in combination with locust bean gum for gelled products or with guar gum for gravies.

The interaction between kappa carrageenan and konjac gum is not widely used in Europe and the US as a result of the relatively short time this latter material has been available in a purified form. However, the greater synergy of this gum combination will be utilised in new food developments and initial products have been commercialised in water dessert gels, petfoods, surimi and air freshener gels.

Dilute iota carrageenan forms a reversible gel which is used to suspend herbs and vegetables in vinaigrette dressings. The gel is formed by dispersing the carrageenan into water at ambient temperature to give a viscous solution. Sodium chloride is added and this raises the setting point of the carrageenan gel to form a reversible gel which is very effective for suspending particulates over the long shelf-life of such products (Fig. 7.5). A recipe is given in Formulation 7.3.

Other applications which utilise the suspending properties of this reversible iota carrageenan gel include soy milks and sterilised milk drinks. At higher concentrations, iota carrageenan forms soft, elastic gels suitable for various toothpastes and for gravies for canned meats and petfoods.

Beer and wine fining are applications which rely on the protein reactivity of carrageenan. Coarse particles of kappa carrageenan or processed *Eucheuma*

Formulation 7.3 Vinaigrette-style salad dressing

Ingredients	%
7% spirit vinegar	12.50
Sugar	9.50
Salt	3.20
Carrageenan (iota)	0.30
Xanthan gum	0.15
Chopped spice pieces	1.00
Colour and preservative	as required
Water	to 100
Total	100.00

Table 7.2 Typical applications for carrageenan in water

Application	Function	Carrageenan type	Use level (%)
Dessert gels	Gelation	kappa + kappa2 + iota kappa + iota + locust bean gum	0.5–1.0
Low calorie gels	Gelation	kappa + kappa2 + iota	0.5–1.0
Non-dairy puddings	Emulsion stabilisation	kappa	0.1–0.3
Syrups	Suspension, bodying	kappa, lambda	0.3–0.5
BBQ and pizza sauces	Bodying	kappa	0.2–0.5
Whipped toppings	Emulsion stabilisation	kappa + kappa2	0.1–0.3
Imitation coffee creams	Emulsion stabilisation	lambda	0.1–0.2
Petfoods	Thickening, suspending,	iota + guar gum	0.5–1.0
	Gelation, fat stabilisation	kappa + locust bean gum	0.5–1.0

seaweed are used to interact and aggregate the proteinaceous materials and small protein fragments produced during pasteurisation. These particles can be readily filtered to clarify the beer and reduce chill haze. Applications for carrageenan in water-based products are summarised in Table 7.2.

7.6.2 Dairy applications

Milk-based puddings were one of the traditional uses for carrageenan from *Chondrus crispus* harvested in Ireland. There the seaweed was boiled in milk to give a firm, creamy gel on cooling. Furcellaran was used to give firm textured dairy desserts, such as crème caramel, and the lower level of ester sulphate reduced the effect of milk quality on the consistency of the finished product. This was particularly important for domestic use where milk quality could vary. Now furcellaran tends to be used in combination with other carrageenans to improve the cost and technical performance in dairy desserts.

As shown in Formulation 7.4, the recipe for a basic dairy flan dessert, the kappa-carrageenan–kappa-casein protein interaction gives a firm, gelled texture with much lower levels of carrageenan compared to water-based systems. Typically, use levels will be around 0.25–0.35% in dairy desserts compared to 0.8–1.0% in water. The attributes of carrageenan have been widely adopted worldwide to produce a large range of instant and ready-to-eat flans, crème

Formulation 7.4 Flan dessert

Ingredients	%
Sugar	10.00–11.00
Carrageenan	0.15–0.30
Colour	as required
Vanilla flavour	as required
Milk	to 100.00
Total	100.00

Formulation 7.5 Dairy ice cream mix

Ingredients	%
Butterfat	8.00–10.00
Milk solids non-fat	11.10–10.80
Sugar	10.00
Corn syrup solids	3.50
Carrageenan	0.015–0.025
Other hydrocolloids (guar gum, locust bean gum, xanthan gum, sodium alginate)	0.10–0.20
Emulsifier (glyceryl monostearate)	0.20–0.50
Vanilla flavour	as required
Water	to 100.00
Total	100.00
(Total solids)	33.3–35.0

dessert and mousse utilising the complete range of carrageenan types for thickening and gelling (Bixler *et al.*, 2001, de Vries *et al.*, 2008). Textures may range from firm gels in crème caramel to soft gels in ready-to-eat spoonable desserts and for thickened custards, vla and cream desserts.

Extremely low levels of carrageenan of around 100–200 ppm are used to stabilise and prevent whey separation in a number of dairy products. These include milk shake and ice cream mixes, chocolate milks, and pasteurised and sterilised creams. In these applications, the carrageenan interacts with the dairy proteins to form a stabilising network which is able to suspend particulates, such as cocoa, in chocolate milks. The network controls protein–protein interaction during heat treatment and subsequent aggregation during storage. Thus, carrageenan is used to avoid whey separation and sediment formation in fluid dairy products and reduce shrinkage in ice cream (Formulation 7.5).

The protein reactivity and gelling properties of carrageenan are also utilised for processed cheese. These products are made by incorporating 'melting' or 'emulsifying' salts into a cheese mix to control the melting temperature whilst maintaining the firmness and mouthfeel that will ensure slice integrity and produce a strong block suitable for grating. By using 0.5 to 3% carrageenan, it is possible to reduce the cheese content in such products whilst providing a product with excellent mouthfeel and good grating and slicing properties.

Acidic dairy products, such as soft cheese and yogurt, are generally unsuitable for carrageenan to be an effective stabiliser. The low pH increases the electrostatic interactions between proteins and carrageenan producing unstable aggregates which flocculate and separate. However, blends with equal proportions of a suitable carrageenan and galactomannan are able to control this aggregation to give effective stabilisation and prevent moisture separation whilst conferring a smooth creamy mouthfeel to the finished product. The many dairy products which utilise the properties of carrageenan are shown in Table 7.3.

Table 7.3 Typical applications for carrageenan in dairy products

Application	Function	Carrageenan type	Use level (%)
Milk gels			
Cooked flans	Gelation	kappa, kappa + iota	0.2–0.3
Cold-prepared custards	Thickening, gelation	kappa, iota, lambda	0.2–0.3
Puddings and pie fillings	Reduced starch, improved flavour, less fouling/burn-on	kappa, kappa 2	0.1–0.2
Ready-to-eat desserts	Syneresis control, bodying	kappa 2 + iota	0.1–0.2
Whipped products			
Whipped cream	Stabilise overrun	lambda	0.05–0.15
Aerosol cream	Stabilise emulsion and overrun	kappa	0.02–0.05
Cold-prepared milks			
Shakes	Suspension, mouthfeel, stabilise overrun	lambda	0.1–0.2
Acidified dairy desserts			
Yogurt	Fruit suspension, mouthfeel	kappa 2	0.2–0.5
Frozen desserts			
Ice cream, ice milk	Whey prevention, control meltdown	kappa (+ LBG), kappa 2	0.01–0.02
Pasteurised milks			
Chocolate milks	Suspension, mouthfeel	kappa, kappa 2, lambda	0.015–0.030
Soy milks	Suspension, mouthfeel	iota, kappa	0.03–0.10
Sterilised milks			
Chocolate milks	Suspension, mouthfeel	kappa, kappa 2	0.015–0.025
Evaporated milks	Whey prevention, mouthfeel	kappa	0.005–0.015
Processed cheese			
Cheese slices and blocks	Improve grating, slice integrity, control melting	kappa, kappa 2	0.3–2.0
Cream cheese and spreads	Gelation, moisture binding	kappa + locust bean gum	0.3–0.5

7.7 Conclusion

It is clear that carrageenan has valuable gelling, stabilising and thickening properties which confer a wide spectrum of textural characteristics utilised in a broad range of water- and dairy-based products. Toxicological evidence supports the safety of carrageenan as a food additive and it will continue to be one of the main gelling agents included in future food product developments.

7.8 Glossary

Carrageenan-casein interaction – a specific interaction between kappa-carrageenan and kappa casein which gives a stabilising network in dairy products to prevent syneresis and suspend particulates.

Hysteresis – the difference in temperature between setting and melting points for a gel.

Shear-reversible – a gel which reforms after a solution has been sheared by mixing, pumping, piping or pouring.

Syneresis – the free liquid which exudes from a gel during storage.

Synergy – the increased or enhanced viscosity or gel strength obtained from mixtures of two or more materials compared to the properties obtained from the separate components.

Thermally-reversible – a gel which forms as a result of heating a solution and cooling to form a gel.

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8

Xanthan gum

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Abstract: Xanthan gum is an extracellular polysaccharide secreted by the micro-organism *Xanthomonas campestris*. Commercially it is manufactured by a fermentation process. Xanthan gum is soluble in cold water and solutions exhibit highly pseudoplastic flow and synergistic interaction with galactomannans. An overview of the manufacturing process and chemical structure of xanthan gum is given. Methods for the effective preparation of xanthan gum solutions are described with particular emphasis on dispersion. The effects of food ingredients such as salts, sugars, and acids on solution properties are discussed in relation to the main applications. The synergistic interaction of xanthan gum with galactomannans such as guar gum, locust bean gum and konjac glucomannan is discussed. Finally, the main applications of xanthan gum in food are described with example formulations.

Key words: viscosity, synergy, galactomannan, glucomannan, stability, suspension, shear rate, rheology, fermentation.

8.1 Introduction

Xanthan gum is an extracellular polysaccharide secreted by the micro-organism *Xanthomonas campestris*. Xanthan gum is soluble in cold water and solutions exhibit highly pseudoplastic flow. Its viscosity has excellent stability over a wide pH and temperature range and the polysaccharide is resistant to enzymatic degradation. Xanthan gum exhibits a synergistic interaction with galactomannans such as guar gum and locust bean gum (LBG) and the glucomannan konjac mannan. This results in enhanced viscosity with guar gum and soft, elastic thermally reversible gels with LBG and konjac mannan.

8.2 Manufacture

The bacterium *Xanthomonas campestris* produces the polysaccharide at the cell wall surface during its normal life cycle by a complex enzymatic process.[1] The bacteria are found naturally on the leaves of the *Brasica* vegetables such as cabbage. Commercially, xanthan is produced from a pure culture of the bacterium by an aerobic, submerged fermentation process. The bacteria are cultured in a well-aerated medium containing glucose, a nitrogen source and various trace elements. To provide seed for the final fermentation stage, the process of inoculum build-up is carried out in several stages. When the final fermentation has finished, the broth is pateurised to kill the bacteria and the xanthan gum is recovered by precipitation with isopropyl alcohol or ethanol. Finally, the product is dried, milled and packaged.

8.3 Structure

The primary structure of xanthan gum, shown in Fig. 8.1, is a linear (1→4)-linked β-D-glucose backbone (as in cellulose) with a trisaccharide side chain on every other glucose at C-3, containing a glucuronic acid residue linked (1→4) to a

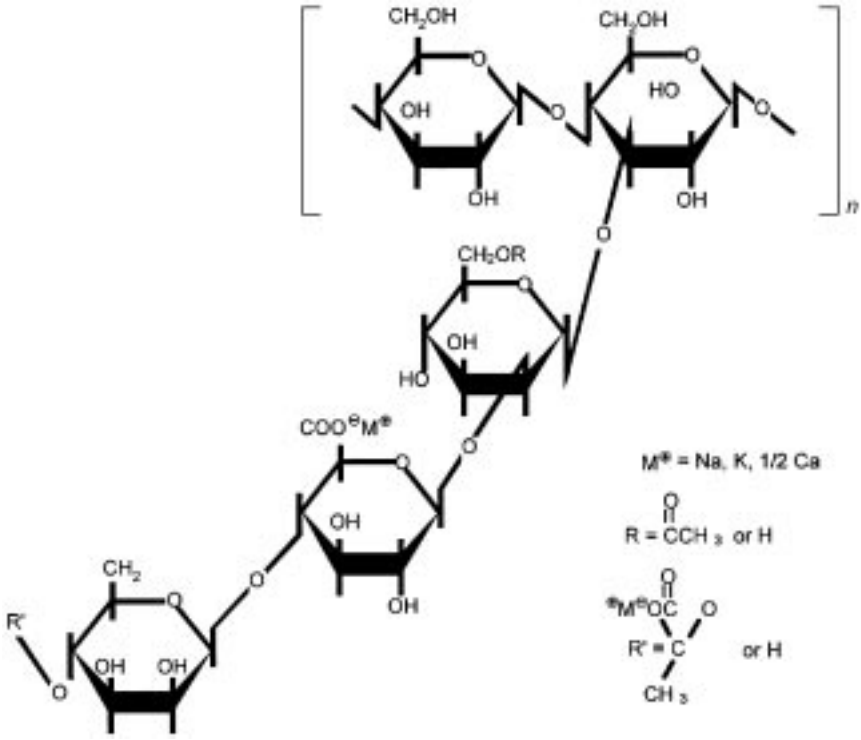

Fig. 8.1 Primary structure of xanthan gum.

terminal mannose unit and (1→2) to a second mannose that connects to the backbone.[2,3] Approximately 50% of the terminal mannose residues are pyruvylated and the non-terminal residue usually carries an acetyl group at C-6. X-ray diffraction studies on the orientated xanthan gum fibres identified the molecular conformation as a right-handed helix with a rise per backbone disaccharide repeat unit of 0.94 nm, i.e. a five-fold helix with a pitch of 4.7 nm.[4] In this conformation the trisaccharide side chains are aligned with the backbone and stabilise the overall conformation by non-covalent interactions, principally hydrogen bonding. In solution the side chains wrap around the backbone thereby protecting the labile β-(1→4) linkages from attack. It is thought that this protection is responsible for the excellent stability of the gum under adverse conditions.

Solutions of xanthan gum undergo a conformational transition during heating which is believed to be associated with the change from a rigid ordered state at low temperature to a more flexible, disordered state at high temperatures. This conformational change was first observed as a sigmoidal change in viscosity.[5] Measurements by optical rotation, calorimetry and circular dichroism have shown that this conformational change coincides with the viscosity change.[6,7] The temperature at which the conformational transition occurs is primarily dependent on the ionic strength of the solution and on structural features such as the pyruvic acid and acetic acid contents of the xanthan molecule.[8,9] The presence of low levels of salts helps to maintain the rigid ordered conformation of xanthan gum and the relative insensitivity of the viscosity to additional salt and elevated temperatures is a result of this molecular rigidity.[8] Below the transition temperature the xanthan helix in solution can be considered as a rigid rod and is also able to form intermolecular associations that result in the formation of a complex network of weakly bound molecules.

8.4 Technical data

The functionality of xanthan gum is dependent upon the correct preparation of the gum solution. Poor solution preparation can lead to poor functionality in the final application. Therefore, before discussing the properties of xanthan gum in more detail, it is important to understand how to prepare a gum solution properly.

8.4.1 Preparing xanthan gum solutions

To obtain the optimum functionality, xanthan gum must be properly hydrated before use. Hydration depends on four factors:

1. dispersion
2. agitation rate of the solvent
3. composition of the solvent
4. particle size.

To hydrate properly the gum particles must be well dispersed. Poor dispersion leads to clumping of particles during mixing which results in formation of

partially swollen lumps of gum (sometimes called 'fish eyes'). Severe lumping prevents complete hydration and reduces functionality. Ideally, the xanthan should be dispersed and hydrated under high shear mixing conditions. Equipment such as a colloid mill, dispersion funnel or high speed paddle mixers are all suitable for preparation of xanthan gum solutions.

Where ideal mixing equipment is not available, other dry ingredients such as sugar, starch or salt can be used as dispersants to aid in the hydration of the xanthan gum. Blending with these materials physically separates the xanthan gum particles and improves the dispersion. An ideal blend ratio is approximately 10 : 1 dispersant:xanthan but ratios of 5 : 1 can also be used. Dispersion can also be improved by separating the xanthan gum particles with non-solvents such as alcohol, glycerol or oils. The xanthan can be slurried in the non-aqueous liquid and poured into the water whilst mixing. Glycerol dispersions should be used within a few minutes of preparation as the xanthan gum tends to swell and partially hydrate.

In some applications use of a stock paste may be desirable. Xanthan gum pastes of at least 6% solids can be prepared and, with the addition of a preservative, stored indefinitely at room temperature. When needed, the appropriate amount of paste can be weighed into a container equipped with an agitator and dilution water added slowly with good mixing. It is recommended to add the water to the stock paste as the reverse results in poor dispersion and hydration.

Several grades of xanthan are specifically processed for ease of dispersion. These can be used when only relatively low shear mixing equipment is available. Examples are GRINDSTED® Xanthan SUPRA, GRINDSTED® Xanthan EASY and GRINDSTED® Xanthan ULTRA. When dispersion and mixing conditions are good, smaller particle size xanthan grades such as GRINDSTED® Xanthan 200 and GRINDSTED® Xanthan CLEAR 200 will hydrate more rapidly than larger particle size grades as shown in Fig. 8.2. When the dry powder particles are dispersed correctly, a solution can be achieved in a few minutes.

Salt concentrations greater than 1–2% in the water slow down the hydration of xanthan gum and so it is therefore recommended to hydrate the gum in the absence of excess salt. Once hydrated, salt can be added up to 10–20% without adverse effects. Special grades of xanthan gum such as GRINDSTED® Xanthan SM are available that can hydrate directly in up to 20% salt solutions. Xanthan gum hydrates in many acidic solutions. For example, it is directly soluble in 5% acetic acid, 5% sulphuric acid, 5% nitric acid and 25% phosphoric acid. Additionally, xanthan gum will hydrate in up to 5% sodium hydroxide. Hydration rate, however, is improved when it is dissolved in water before adding the acid or alkali.

8.4.2 Rheology of xanthan gum solutions

Present knowledge of the structure and conformation of xanthan gum can explain many of the unique solution properties. The relationship between the structure of xanthan gum and its properties is summarised in Table 8.1. Xanthan

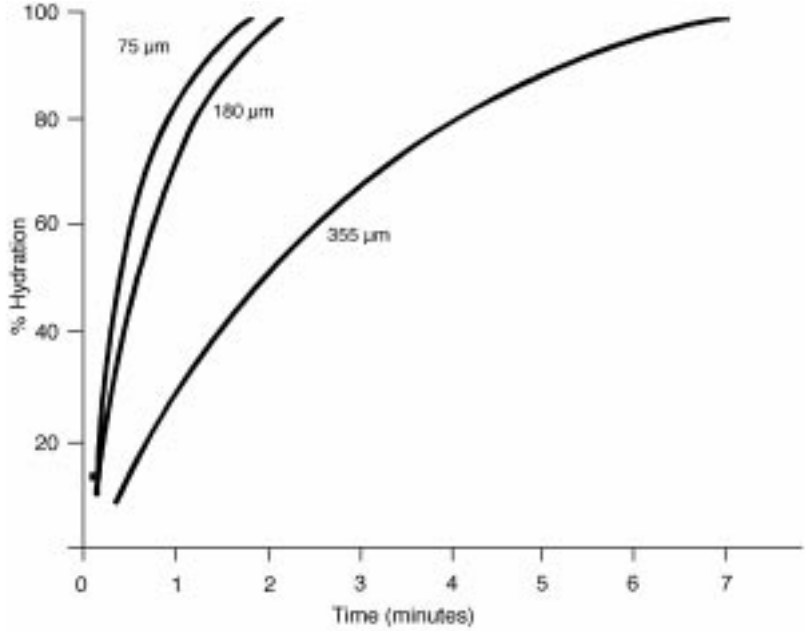

Fig. 8.2 Effect of particle size (μm) on the viscosity development profile of xanthan gum.

gum solutions are highly pseudoplastic. When shear stress is increased, viscosity is progressively reduced but upon removal of the shear, the initial viscosity is recovered almost instantaneously. This behaviour results from the ability of the xanthan molecules, in solution, to form intermolecular aggregates through hydrogen bonding and polymer entanglements. This highly ordered network of entangled, stiff molecules results in high viscosity at low shear rates, and in practical terms, accounts for the outstanding suspension properties of xanthan gum solutions. These aggregates are progressively disrupted under the influence of applied shear, hence the highly pseudoplastic flow characteristics

Table 8.1 Structure/property relationship for xanthan gum

Structural features	Properties
Complex aggregates, with weak intermolecular forces	High viscosity at low shear rates (suspension stabilising properties) High viscosity at low concentrations High elastic modulus Pseudoplastic rheology
Rigid helical conformation, hydrogen bonded complexes, anionic charge on side chains	Temperature insensitivity and salt compatibility
Backbone protected by large overlapping side chains	Stability to acids, alkalis and enzymes

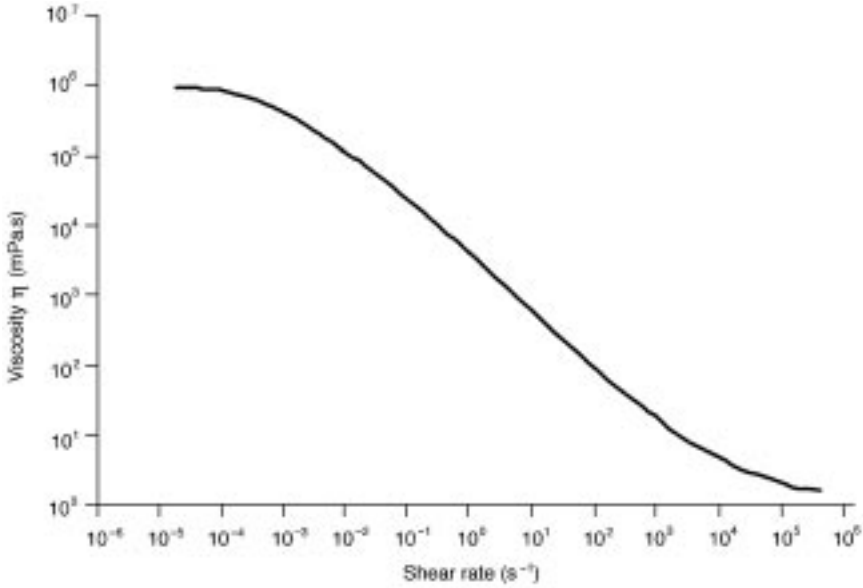

Fig. 8.3 Flow curve of 0.5% xanthan gum solution in standardised tap water. Standardised tap water is prepared by dissolving 1.00g NaCl and 0.15g $CaCl_2.2H_2O$ in 1 litre of deionised water.

of xanthan gum solutions.[8,10] Figure 8.3 shows the effect of shear rate over eleven orders of magnitude on the viscosity of a 0.5% xanthan gum solution. Xanthan gum shows pseudoplastic properties over most of the range, varying in viscosity from 1 million mPa.s at the lowest shear rates to just 1.7 mPa.s (close to water, 1 mPa.s), at the highest rates. At the extremes of shear rate there is evidence of a levelling off of the viscosity indicating an upper and lower Newtonian region.

Solutions of xanthan gum at 1% or higher appear almost gel-like at rest, yet these same solutions pour readily and have low resistance to mixing and pumping. These same qualities are observed at typical use levels of 0.1–0.3%. The high viscosity of xanthan gum solutions at low shear rates accounts for their ability to provide long-term stability to colloidal systems. The reduction in viscosity in response to increasing shear is important to the pouring properties of suspensions and emulsions and to the efficiency of xanthan gum as a processing aid.

In Fig. 8.4 the viscosity of some common gums is compared over a range of shear rates relating to specific functions or processes. At low shear rates, solutions of xanthan gum have approximately 15 times the viscosity of guar gum and significantly more viscosity than carboxymethylcellulose (CMC) or sodium alginate which accounts for its superior performance in stabilising suspensions. At a shear rate of approximately 50–100 s^{-1} all the gums have similar viscosity. Above 100 s^{-1}, however, the viscosity of xanthan gum solutions drops sharply compared to the other gums making it easy to pour, pump or spray.

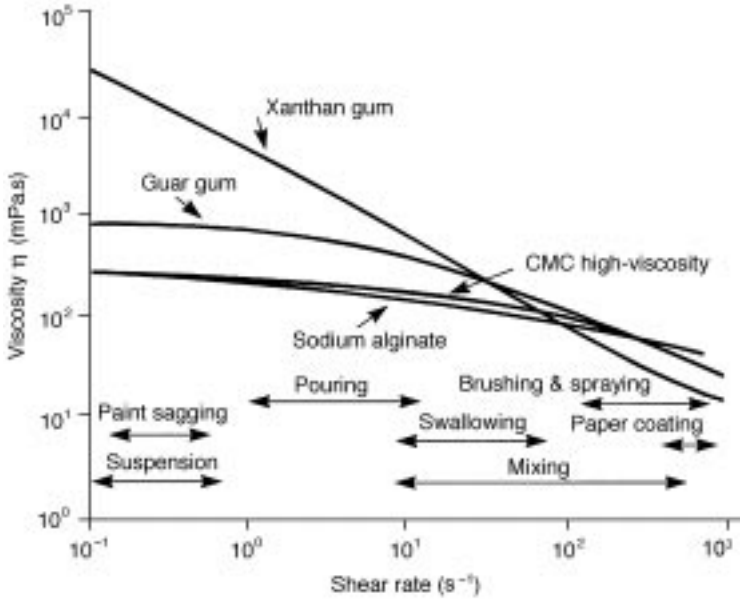

Fig. 8.4 Comparison of the flow behaviour of xanthan gum to other hydrocolloid solutions (0.5% concentration).

Effect of salts on viscosity

How salts affect viscosity depends on the concentration of xanthan gum in solution. Below approximately 0.25% gum concentration, monovalent salts such as sodium chloride cause a slight decrease in viscosity. At higher gum concentrations, viscosity increases when salt is added. At a sodium chloride concentration of approximately 0.1%, a viscosity plateau is reached, regardless of gum concentration, and further addition of salt has little effect on the viscosity. Divalent metal salts such as calcium and magnesium have a similar impact on viscosity. Trivalent ions such as aluminium promote gelation of xanthan gum. To develop a uniform solution with optimal rheological properties, some type of salt should be present; usually the salts found naturally in tap water are sufficient to generate these effects.

Effects of pH on viscosity

There are two questions that need to be answered when considering the effect of pH on a gum solution.

1. How does the viscosity change as a function of pH?
2. How stable is the viscosity as a function of time at any given pH?

The first can be considered a question of sensitivity and the second can be considered a question of stability. Xanthan solution viscosity is sensitive to pH but very stable. Xanthan solutions show a progressive reduction in viscosity below approximately pH 4. This reduction in viscosity is most evident at low

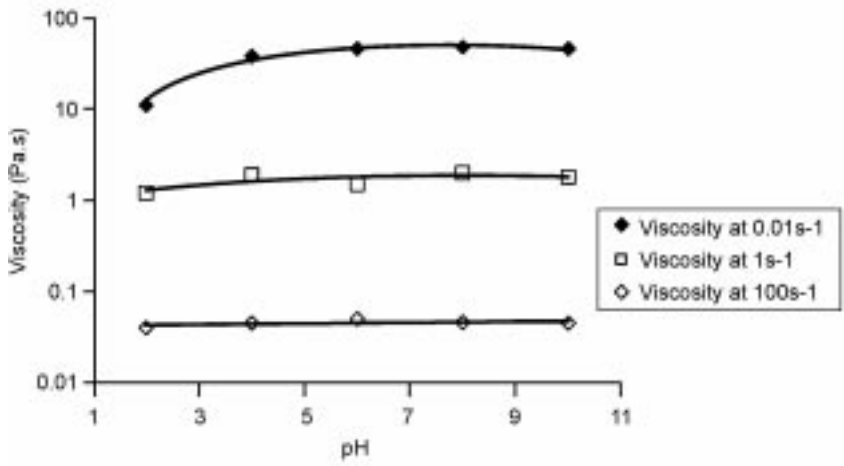

Fig. 8.5 The pH sensitivity of 0.3% xanthan gum in 1% NaCl measured at different shear rates (pH adjusted with a solution of citric acid or NaOH).

shear rates as shown in Fig. 8.5. However, the viscosity is completely recovered when the pH of the solution is neutralised. Xanthan is an anionic polymer and as such the pH sensitivity of the viscosity is expected since changes in the pH will result in changes to the charge density of the xanthan which will in turn influence the molecular associations between xanthan molecules. It has been proposed that reducing pH progressively converts the carboxylate groups from the ionised to the un-ionised form ($COO^- + H^+ = COOH$), with consequent suppression of electrostatic repulsion between xanthan side chains.[11] This in turn could allow a more compact molecular shape which could explain the observed reduction in viscosity. Neutralisation would re-ionise the carboxylate groups and allow the xanthan to return to the original conformation at neutral pH with the associated recovery of the original viscosity.

Although the viscosity is sensitive to changes in pH below pH 4.0, the solution viscosity is extremely stable. For example a virtually constant low shear viscosity is maintained by a 0.3% solution of xanthan gum over 3 months at pH 2.5 (Fig. 8.6). In the presence of most organic acids, stability is excellent as shown in Table 8.2. At elevated temperatures, however, acid hydrolysis of the polysaccharide is accelerated and a reduction in the viscosity will occur.

Effect of temperature on viscosity

Xanthan gum solutions are unique in their ability to retain their viscosity until a definite 'melting temperature' is reached. At this temperature the viscosity drops sharply due to the reversible change in molecular conformation discussed in Section 8.3. At xanthan concentrations up to approximately 0.3% in deionised water the thermal transition occurs at around 40 ºC. However, in the presence of low levels of salt, typical of those used in food products, the thermal transition occurs at temperatures above 90 ºC.

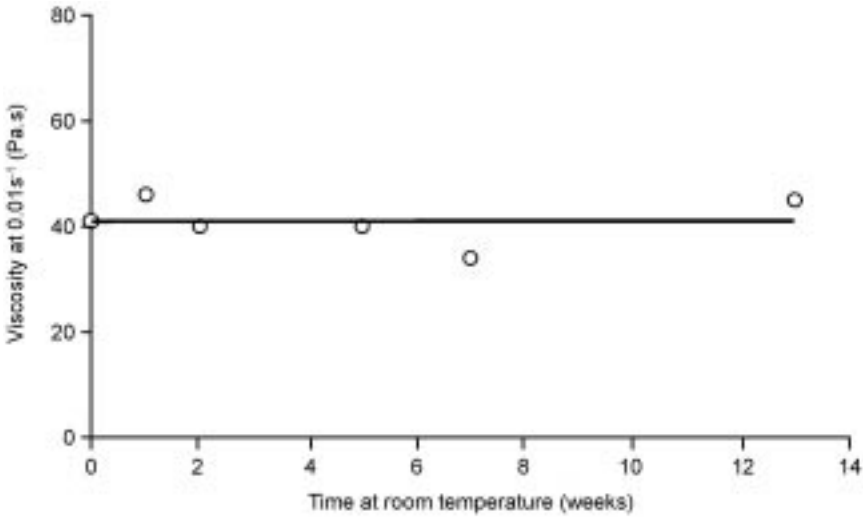

Fig. 8.6 Stability at low pH of the viscosity (measured at 0.01 s^{-1}) of 0.3% xanthan gum in a model vinaigrette stored at room temperature (pH 2.5, 1% NaCl).

8.4.3 Compatibility of xanthan gum

Alcohol

While xanthan gum will not dissolve directly into alcohol, solutions of xanthan gum are compatible with alcohol. Products containing alcohol can be formulated to contain up to 60% water-miscible solvents such as ethanol. This enables its use as a thickener in alcohol-based products such as cocktails and chocolate liqueurs.

Enzymes

Most hydrocolloids are degraded to some extent by enzymes normally present in some foodstuffs. Enzymes commonly encountered in food systems such as proteases, cellulases, pectinases and amylases, however, do not degrade the xanthan gum molecule. It is thought that this resistance to enzymes is due to the

Table 8.2 Stability of xanthan gum solutions to acid at ambient temperature

Acid	Acid concentration (%)	Xanthan gum concentration (%)	Viscosity retained after 90 days (%)
Acetic acid	20	2	100
Citric acid	20	1	75
Hydrochloric acid	5	2	80
Phosphoric acid	40	2	100
Sulfuric acid	10	2	80
Tartaric acid	20	1	75

arrangement of the side chains attached to the backbone which protect the sensitive β-(1→4) linkages. In practice, this resistance is exploited in food systems such as pineapple products, starch-based systems, spice mixes and many other products containing active enzymes in which the xanthan is able to maintain a stable viscosity.

8.4.4 Interactions with galactomannans/glucomannans

A synergistic interaction occurs between xanthan gum and galactomannans such as guar, locust bean, cassia and tara gum and glucomannans such as konjac mannan. This interaction results in enhanced viscosity or gelation. Galactomannans are hydrocolloids in which the mannose backbone is partially substituted by single-unit galactose side chains.[12–14] The degree and pattern of substitution varies between the galactomannans and this strongly influences the extent of interaction with xanthan gum. Galactomannans with fewer galactose side chains and more unsubstituted regions react more strongly. Thus, locust bean gum, which has a mannose to galactose ratio of approximately 3.5:1, reacts more strongly with xanthan than does guar gum, which has a mannose to galactose ratio of slightly less than 2:1. It is generally accepted that the xanthan interacts with the unsubstituted 'smooth' regions of the galactomannan molecules. Xanthan/guar mixtures exhibit a synergistic increase in viscosity as do low concentration mixtures with locust bean gum (< 0.03%). At higher concentrations soft, elastic gels are formed with LBG. Xanthan gum/LBG gels are thermally reversible setting and melting at approximately 55–60 ºC. Solutions of xanthan/guar mixtures can be prepared at room temperature using the guidelines outlined in Section 8.4.1. However, heating of the solutions above 80 ºC does result in a greater synergistic interaction. Mixtures of xanthan and LBG require heating to approximately 90 ºC to fully hydrate the LBG and maximise the synergistic interaction.

The interaction of xanthan gum with galactomannans is dependent on the ratio of the mixture, pH and ionic strength of the solution, molecular weight of the galactomannans and the acetate content of the xanthan gum. Optimum gum ratios are approximately 80 : 20 guar:xanthan (Fig. 8.7) and 60 : 40 for xanthan : LBG (Fig. 8.8). Generally, the synergistic interaction with galactomannans is at its maximum in deionised water at pH 7 and is reduced at high salt concentrations or at low pH (Fig. 8.8). The synergistic interaction also increases with increasing molecular weight of the galactomannans and decreasing acetate content of the xanthan gum.

8.5 Applications in food products

Xanthan gum is used in a wide variety of food products and a summary is provided in Table 8.3. What follows is a brief description of the main application areas.

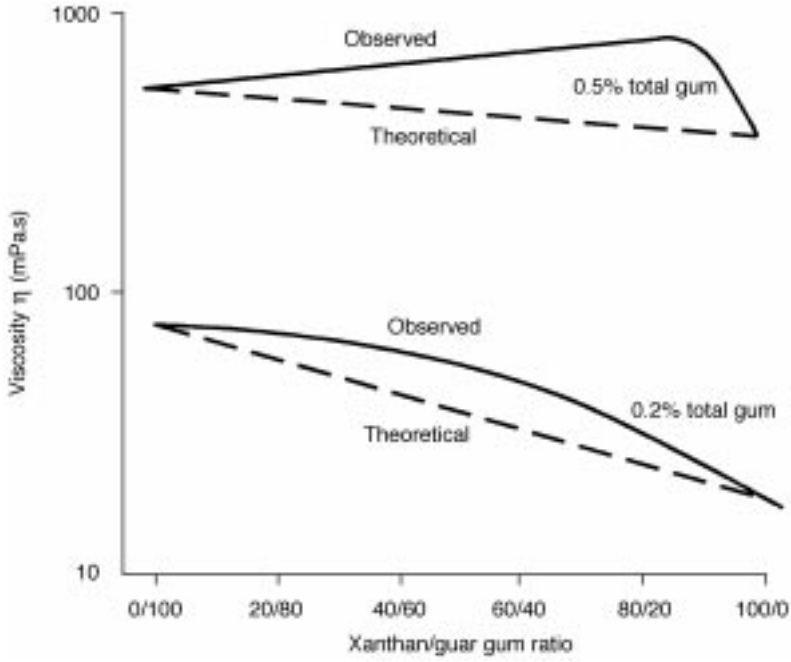

Fig. 8.7 Theoretical (dashed line) and observed (solid line) viscosities for blends of guar and xanthan gum. Data were collected with a Brookfield LVT viscometer at 60 rpm at 25 ºC. The theoretical viscosity line is calculated on the assumption that non-interacting hydrocolloids will obey the log mean blending relationship.

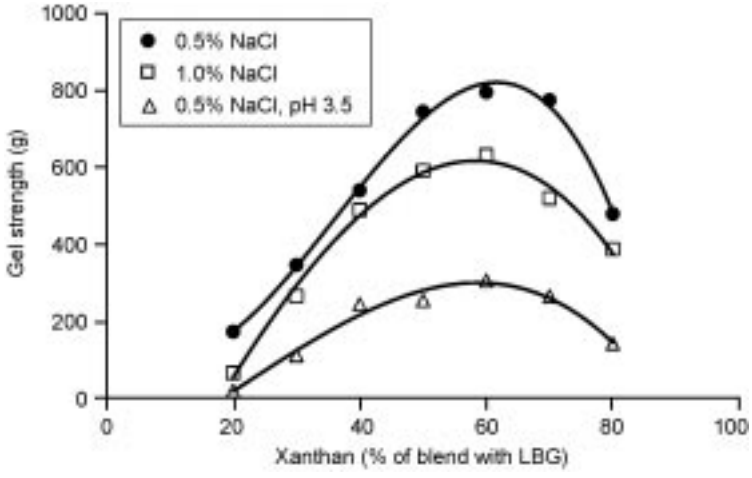

Fig. 8.8 Effect of gum ratio and media on the gel strength of xanthan/locust bean gum gels (1% total gum in 1% KCl).

Table 8.3 A summary of xanthan gum functionality and associated applications

Function / Application		Salad dressings	Gravies, relishes, canned soups and sauces	Instant mix sauces, soups, drinks	Pickles, aspics	Processed meat	Frozen foods	Dairy products, flavoured milk	Whipped cream, mousses	Instant desserts	Ice creams, sherbets, sorbets	Bakery products	Fruit drinks, flavouring concentrates	Jams and jellies, fruit preparations, toppings
Recommended use level (%)		0.1 - 0.5	0.1 - 0.4	0.1 - 0.2*	0.1 - 0.5	0.05 - 0.2	0.2 - 0.5	0.05 - 0.3	0.1 - 0.3	0.1 - 0.3*	0.1 - 0.4	0.05 - 0.2	0.05 - 0.2	0.2 - 0.5
Rheology	Suspension power	♦	♦		♦	♦	♦	♦	♦		♦		♦	♦
	Viscosity	♦	♦	♦			♦		♦	♦				
	Pseudoplasticity	♦		♦				♦	♦			♦	♦	♦
Stability	Salts	♦	♦	♦	♦	♦	♦						♦	
	Acids	♦	♦		♦		♦	♦					♦	♦
	Temperature	♦	♦	♦	♦	♦		♦	♦		♦	♦	♦	♦
Miscellaneous	Synergy	♦				♦		♦		♦	♦	♦	♦	♦
	Cold solubility	♦		♦		♦				♦		♦	♦	
	Syneresis control		♦	♦	♦	♦	♦	♦	♦		♦			♦
	Texture modifier	♦		♦				♦	♦	♦	♦			♦
	Cling	♦	♦	♦	♦		♦							♦
	Crystallisation control		♦				♦				♦			
	Whipping control							♦	♦	♦	♦	♦		

*In final product.

8.5.1 Baked goods, bakery and pie fillings

Xanthan gum contributes to the smoothness, air incorporation and retention and recipe tolerance of batters for cakes, muffins, biscuits and bread mixes. In wet prepared batters, xanthan reduces flour sedimentation; improves gas retention and imparts enzyme, shear and freeze-thaw stability; and provides uniform coating and good cling. In pancake batters, xanthan gum improves spread control, volume and air retention. Baked goods have increased volume and moisture, higher crumb strength, less crumbling and greater resistance to shipping damage. Xanthan improves volume, texture and moisture retention in refrigerated dough, reduced calorie baked goods and gluten-free breads.

Moisture control is essential at all stages of cake production and also when formulating a dry cake mix. Improper moisture control can result in lumpy cake batters and uneven mixing, giving poor structure which results in collapsed cakes during or after baking. The overall quality of the finished cake, particularly after storage, can be affected by poor hydration characteristics of the dry ingredients. For example, volume can be reduced and texture may be non-uniform or fragile when moisture is not evenly distributed throughout the cake. Xanthan gum blended with the other dry ingredients hydrates rapidly and evenly to aid in preventing lumping during the critical initial mixing stage. This aids in the uniform distribution of moisture in the batters, which helps to stabilise the fine air cells formed during the mixing process. The stabilisation of the air cells improves volume and symmetry in the finished cake. In addition to this, the high viscosity at low shear provided by the xanthan gum helps to give even distribution of fruit pieces throughout the cake. Xanthan can be added to the cake batter at 0.05% (of total batter weight) without the need for any other formulation changes. Typically a fine mesh xanthan grade will be used to ensure good mixing with the flour and rapid hydration.

Xanthan can also be used in liquid cake mixes and an example is given in Formulation 8.1. The cake formulation produces a batter which when stored refrigerated will be stable for at least six weeks and bake off to give a cake of good volume and texture.

Adding xanthan gum to either cold or hot processed bakery and fruit pie fillings improves texture and flavour release. The added benefits in cream and fruit fillings are extended shelf stability, freeze-thaw stability and syneresis control.

8.5.2 Dairy products

Blends of xanthan gum, carrageenan and galactomannans are excellent stabilisers for a range of frozen and chilled dairy products such as ice cream, sherbet, sour cream, sterile whipping cream and recombined milk. These economical blends are available pre-prepared to provide optimal viscosity, long-term stability, improved heat transfer during processing, heat shock protection

Formulation 8.1 Chilled liquid cake mix

Formulation	%
Chlorinated cake flour	24.00
Sucrose	19.00
Whole egg	19.00
Vegetable oil	16.00
Polydextrose (LITESSE® II POWDER)	6.44
Invert sugar	4.00
Vegetable shortening	3.00
Glycerol	2.70
Emulsification/antioxidants (GRINSTED®GA 520-M KOSHER)	1.30
Wheat starch	1.25
Whey powder	1.20
95% ethanol	0.60
Coated sodium bicarbonate	0.53
SAPP (sodium acid pyrophosphate)	0.43
Salt	0.35
Flavouring	0.16
GRINSTED® Xanthan 200	0.03
Preservatives (Nisaplin®)	0.02

Process for liquid cake mix

1. Add all dry ingredients to the mixing vessel and blend on low speed until homogeneous.
2. Add invert sugar, glycerol and ethanol to the dry mix, blend until homogeneous.
3. Melt GRINSTED®GA 520-M and vegetable shortening together, then mix well with the vegetable oil.
4. Add oil mix to blend and mix well.
5. Add in the liquid egg and mix on low speed until homogeneous.
6. Check water activity, and if $a_w \leq 0.80$, pack the batter.

Process for cake

Straight from the pack

1. Preheat conventional oven to 1700 ºC.
2. Snip corner of sachet and squeeze content into a greased baking tin.
3. Bake at 1700 ºC until cooked (approx. 30–35 minutes, depending on the dimension of the tin used).

Aerated (to obtain a lighter, larger cake)

1. Preheat conventional oven to 1700 ºC.
2. Snip corner of sachet and squeeze content into a mixing bowl.
3. Mix using electric beater on high speed for approx. 3 minutes to aerate the batter.
4. Pour into a greased baking tin, and baked until cooked (approx. 30–35 minutes, depending on the dimension of the tin used).

and ice crystal control. For example, combinations of carrageenan and xanthan gum can be used to formulate no-fat cold filled flans. The xanthan gum helps to maintain the creaminess often lost when fat is removed. It also helps to avoid shrinkage and syneresis of the carrageenan gel.

8.5.3 Culinary products

This is arguably the largest application area for xanthan gum. Its stability at low pH, salt tolerance, high viscosity at low shear and pseudoplastic rheology make it an ideal thickener and stabiliser for culinary products. For example, dressings formulated with xanthan gum have excellent long-term stability and a relatively constant viscosity over a wide temperature range. Due to the psudoplastic rheology imparted by the xanthan gum they pour easily but cling well to the salad. Use level is typically between 0.2 and 0.4% xanthan depending on the oil content. Generally, as the oil content of the dressing increases, less xanthan gum is required for stabilisation. A guide to use levels for dressings made with combinations of xanthan and starch is given in Table 8.4. Formulation 8.2 provides an example of a xanthan-based dressing formulated with 30% oil.

Low levels of xanthan gum provide high viscosity in sauces and gravies at both acid and neutral pH. Viscosity is also stable to temperature changes and is maintained under a variety of long-term storage conditions. Sauces and gravies containing xanthan gum cling to hot foods. Although xanthan gum provides

Table 8.4 Suggested stabiliser level for salad dressing formulation

% Oil used	10	20	30	40
% Starch	2.0	2.0	1.5	1.5
% Xanthan gum	0.35	0.3	0.25	0.25

Formulation 8.2 30% oil dressing with roasted sesame seeds and Dijon mustard

Ingredients	(%)
Water	To 100.00
Oil	30.0
Salt	1.0
Sugar	2.0
Egg yolk	4.0
GRINDSTED® Xanthan 80	0.5
Vinegar 12%	4.0
Honey	7.0
Dijon mustard	7.0
Roasted sesame seeds	1.0
Sesame oil	1.0

Preparation (cold process. Koruma colloid mill)

1. Mix the water, salt, sugar and antimicrobials.
2. Blend the GRINDSTED® Xanthan 80 with some of the oil and add to the water phase.
3. Add the egg yolk, sesame seeds and honey. Mix in a vacuum for 1.5 minutes.
4. Emulsifying the rest of the oil.
5. Add the vinegar and mustard and mix.
6. Fill.

stable, high viscosity over a range of temperatures, this viscosity is temporarily reduced at retort temperatures, ensuring good thermal penetration in retorted foods. At the same time the ability of xanthan gum to recover its viscosity upon cooling, provides good re-suspension of particulates ensuring a uniform, high quality product. In retort pouch products, xanthan gum improves filling and reduces splashing and fouling of the critical heat-seal area of the pouch. Xanthan gum can be used to partially replace starch in this application to improve heat stability and give a cleaner, less pasty mouthfeel. Typically xanthan is used at a concentration of 0.1–0.2%.

8.5.4 Frozen foods

Stability, syneresis control and consistent viscosity during freeze-thaw cycles and heating are achieved by adding xanthan gum to a variety of frozen products such as whipped toppings, sauces, gravies, batters, ready meals and soufflés. For example, frozen non-dairy whipped toppings and frozen whipped topping concentrates have firm texture, high overrun and excellent freeze-thaw stability.

8.5.5 Dry mixes

In dry mixes, fine particle size xanthan gum provides rapid, high viscosity development in cold or hot systems and yields excellent texture and flavour release. It also permits easy preparation of desserts, salad dressings, dips, soups, milkshakes, sauces, gravies and beverages. In dry mix beverages xanthan gum provides enhanced body to the reconstituted drink. Dry mixes for instant thickening of beverages such as water, fruit juice, green tea or milk are sold in Japan for use by elderly people suffering from dysphagia (swallowing difficulties). For this application xanthan gum is combined with a dispersant such as maltodextrin to produce a dispersible product able to provide very rapid viscosity development with minimal mixing and that has a stable viscosity.[15]

8.6 Regulatory status

Xanthan gum is recognised as a food additive under the provisions of the US Food and Drug Administration regulations (21 CFT 172.695) for use as a stabiliser, thickener or emulsifier. Xanthan gum is designated by the European Union as E415 with a non-specified acceptable daily intake (ADI). GRINDSTED® Xanthan gum grades are available for kosher and halal use.

8.7 Future trends

Xanthan gum has a relatively complex molecular structure that is well characterised from a general point of view. Natural variations from this idealised

structure, however, are known to occur and improving our understanding and control of these could lead to new and improved functionality.

8.8 Sources of further information and advice

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9

Gellan gum

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Abstract: Gellan gum is an extracellular polysaccharide secreted by the micro-organism *Sphingomonas elodea* (ATCC 31461) previously referred to as *Pseudomonas elodea*. Commercially it is manufactured by a fermentation process. It is available in two forms, high acyl (HA) and low acyl (LA). Gellan gum forms gels at low concentrations when hot solutions are cooled in the presence of gel promoting cations. An overview of the manufacturing process and chemical structure of gellan gum is given. The functional differences between the two forms of gellan gum are reviewed in detail with regard to hydration, gelation, stability and texture. Methods for the effective preparation of gellan gum solutions and gels are described for the high and low acyl forms. The effects of food ingredients such as salts, sugars, and acids on gel properties are discussed in relation to the main applications. Finally, the main applications of gellan gum in food are described with example formulations.

Key words: gel, setting temperature, texture, extracellular, fermentation, fluid gel.

9.1 Introduction

Gellan gum is an extracellular polysaccharide secreted by the micro-organism *Sphingomonas elodea* (ATCC 31461) previously referred to as *Pseudomonas elodea*. Gellan gum forms gels at low concentrations when hot solutions are cooled in the presence of gel promoting cations. It is available in a substituted or unsubtituted form. Gel properties depend on the degree of substitution with the substituted form, producing soft, elastic gels whilst the unsubstituted form produces hard, brittle gels.

9.2 Manufacture

Commercially, gellan gum is manufactured by inoculating a fermentation medium with the micro-organism. The medium contains a carbon source, such as glucose, phosphate and nitrogen sources, and appropriate trace elements. The fermentation is carried out under sterile conditions with strict control of aeration, agitation, temperature and pH. After fermentation, the viscous broth is pasteurised to kill viable cells. The polysaccharide can then be recovered in several ways. Direct recovery by alcohol precipitation from the broth yields the substituted native, or high acyl (HA), form. Alternatively, treatment of the broth with alkali prior to alcohol precipitation results in deacylation and yields the unsubstituted, low acyl (LA) form. Gellan gum is currently sold commercially in three basic forms, namely high acyl, unclarified (KELCOGEL® LT100), low acyl, unclarified (KELCOGEL® LT), and low acyl, clarified (KELCOGEL® and KELCOGEL® F). A number of specialty grades are also available.

9.3 Structure

The primary structure of gellan gum, shown in Fig. 9.1, is composed of a linear tetrasaccharide repeat unit: →3)-β-D-Glcp-(1→4)-β-D-GlcpA-(1→4)-β-D-Glcp-(1→4)-α-L-Rhap-(1→.[1,2] The polymer is produced with two acyl substituents

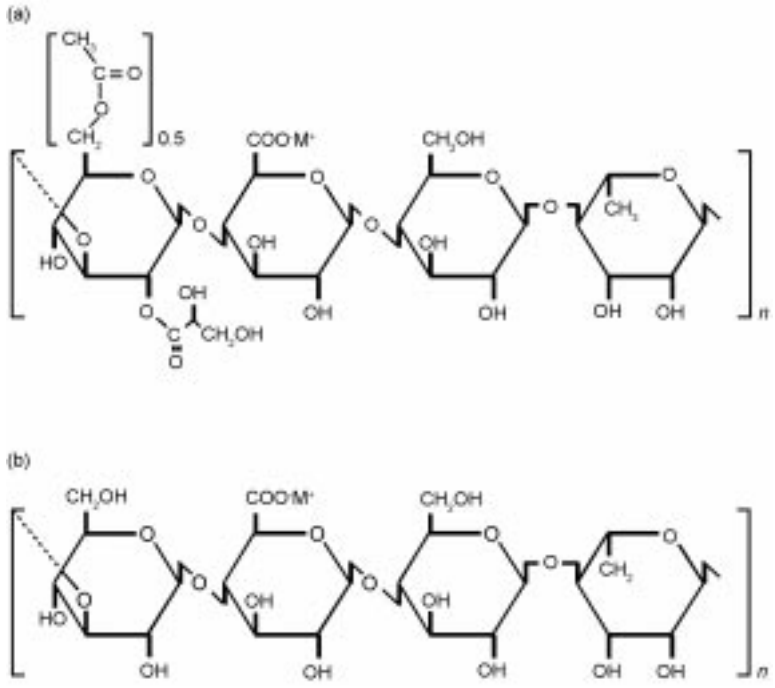

Fig. 9.1 Primary structure of (a) low and (b) high acyl gellan gum.

present on the 3-linked glucose, namely, L-glyceryl, positioned at O(2) and acetyl at O(6). On average there is one glycerate per repeat unit and one acetate every two repeats.[3] X-ray diffraction of low acyl gellan gum has shown that the polymer exists in the solid state as a co-axial three-fold double helix.[4,5]

Computer modelling of the high acyl structure, based on the knowledge of the low acyl form, concluded that the acetate substituents would be positioned on the outside of the double helix.[6] Subsequent X-ray diffraction data revealed that the glycerate substituent was accommodated by an approximately 14º rotation of the carboxyl group about the C(5)-C(6) bond on the adjacent glucuronate residue.[7] This was accompanied by a 17 ± 3º rotation of the glucuronate residue itself to maintain the required spacing between the carboxyl and glycerate groups. It is proposed that this structure results in helices stabilised by interchain associations involving the glycerate groups, with the acetyl substituents positioned on the periphery of the helix.[8]

9.4 Technical data

There are three steps to consider for the successful formulation of gellan gum systems:

1. dispersion
2. hydration
3. gelation.

These will now be considered in turn for both the LA and HA forms of the gum.

9.4.1 Dispersion

The first step in preparing any gum solution is to ensure that the gum particles are properly dispersed in the solvent and do not clump together. Poor dispersion will result in incomplete hydration and loss of gum functionality. Both forms of gellan gum are insoluble in cold water although they will tend to swell in water of low calcium content. The gum can therefore be readily dispersed in deionised water by stirring and adding the powder slowly to the vortex. As the ion concentration in the water increases, dispersion becomes even easier. For example, in moderately hard water (~180 ppm hardness expressed as $CaCO_3$), the gum can be added to the surface of the water and then dispersed with gentle agitation. By blending the gum with dispersants such as sugar (5–10 times weight of gum) or glycerol, alcohol, or oils (3–5 times weight of gum), it is possible to add the gum directly to hot water. Both forms of gellan gum are also readily dispersible in milk and reconstituted milk systems.

9.4.2 Hydration

Low acyl gellan gum

The temperature at which LA gellan gum hydrates is dependent on the type and concentration of ions in solution. The presence of ions such as sodium and, in

Table 9.1 Effect of dissolved salts on the hydration temperature of 0.25% LA gellan gum

Water hardness (ppm $CaCO_3$)	Dissolved NaCl (%)	Hydration temperature (ºC)
0.000	–	75
100	–	88
200	–	> 100
300	–	> 100
–	0.10	50
–	0.25	52
–	0.45	60
–	0.70	70
–	0.90	82
–	1.00	89
–	1.30	> 100

particular, calcium, in solution will inhibit the hydration of LA gellan gum as shown in Table 9.1. It is therefore necessary, in most circumstances, to use a sequestrant to bind the soluble calcium and so aid hydration. Typically, between 0.1 and 0.3% of a sequestrant such as sodium citrate is sufficient to allow complete hydration at 90–95 ºC in water of up to 600 ppm as $CaCO_3$ water hardness. Incomplete hydration will result if the sodium ion concentration exceeds 0.5% (approximately 1.3% sodium chloride). Once the gum is hydrated, additional ions can be added to the hot solution and, provided the temperature is maintained above the gelation temperature, no gel will form. Table 9.2 provides details of the effects of the sequestrant sodium citrate on the hydration temperature of LA gellan gum and shows that hydration can be achieved at temperatures ranging from room temperature to boiling point. Table 9.3 lists the relative sequestering power and effective pH ranges of a number of commonly used sequestrants.

In foods containing sugar, LA gellan gum should be hydrated in water and any sugars can be added to the hot gum solution. However, LA gellan gum can be hydrated directly in sugar solutions up to 80 total soluble solids (tss) by heating to boiling. In some cases a low level of sequestrant such as sodium

Table 9.2 Effect of sodium citrate on the hydration of 0.25% LA gellan gum

Water hardness (ppm $CaCO_3$)	Sodium citrate (%)	Hydration temperature (ºC)
0	0.00	75
100	0.05	25
300	0.10	65
600	0.20	65
900	0.40	68

Table 9.3 Relative sequestering power of sequestrants commonly used in foods

Sequestrant	Parts of sequestrant required to sequester 1 part of available calcium	
	pH6	pH4
Sodium hexametaphosphate	7	7
Tetra sodium diphosphate	10	20
Di sodium orthophosphate	130	180
Tri sodium citrate dihydrate	20	40

citrate (less than 0.3%) is required to bind the free calcium often present in sugar syrups.

LA gellan gum will not fully hydrate below pH 3.9. In this case, the acid should be added, preferably as a concentrated solution, to the hot gum solution. Prolonged heating in acidic conditions should be avoided as this leads to some hydrolytic degradation of the gum resulting in a reduction in the quality of the final gel. However, at pH 3.5, LA gellan gum can be held for up to 1 h at 80 ºC with only a minimal loss in gel quality. In neutral conditions solutions can be held at 80 ºC for several hours. LA gellan gum is readily dispersible in milk and reconstituted milk systems and will hydrate upon heating to approximately 80 ºC without the need for a sequestrant.

High acyl gellan gum

The hydration of HA gellan gum is much less dependent on the concentration of ions in solution than LA gellan gum and generally heating to 85–95 ºC is sufficient to fully hydrate the gum in both water or milk systems. As a dispersion of HA gellan gum is heated, it swells rapidly at approximately 40–50 ºC to form a thick, pasty suspension. With continued heating the suspension loses viscosity suddenly at approximately 80–90 ºC signifying complete hydration. The swelling stage can be avoided by adding the gum directly to hot water (>80 ºC) with the aid of a dispersant such as sugar, oil or glycerol, as described in the previous section.

The hydration of HA gellan gum is inhibited by the presence of sugars; therefore it is recommended to hydrate the gum in less than 40% tss. Additional sugar can then be added to the hot gum solution. As with LA gellan gum, HA gellan gum will not hydrate below pH 4.0. Similar care must also be taken under hot acidic conditions to avoid hydrolytic breakdown and loss of gel quality.

9.4.3 Gelation

The proposed gelation mechanism of gellan gum is based on the domain model which assumes the formation of distinct junction zones and disordered flexible polymer chains connecting adjacent junction zones.[9] As a hot solution cools, gellan gum undergoes a disorder–order transition. This transition has been

attributed to a coil-helix transition.[10] In the case of LA gellan gum, gel promoting cations such as sodium, potassium, calcium and magnesium promote aggregation of the gellan double helices to form a three-dimensional network and the subsequent gels are hard and brittle. Recent studies using atomic force microscopy have challenged this and proposed a fibrous model in which network structures develop through the formation of non-associated fibres or strands via either elongation or branching.[11]

The acyl substituents have a profound effect on the structure and rheological characteristics of gellan gum gels. The gellan gum undergoes a similar disorder to order transition as the solution is cooled but according to the domain model further aggregation of the helices is limited by the presence of the acetyl group.[8] According to the fibrous model the acyl groups inhibit end-to-end type intermolecular associations through a type of steric hindrance, resulting in a decrease in the degree of continuity and homogeneity of the gelled system.[11] The subsequent gels of HA gellan gum are therefore soft and elastic.

Low acyl gellan gum

The easiest and most common method of making LA gellan gum gels is to cool hot solutions. LA gellan gum forms gels with a wide variety of cations, notably calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}), and potassium (K^{+}) as well as acid (H^{+}).[10,12] Divalent cations are more efficient at promoting gelation of LA gellan gum than monovalent ions. Gel strength increases with increasing ion concentration until a maximum is reached. Further addition of ions results in a reduction of gel strength due to the 'over conversion' of the LA gellan gum with excess ions. Ion concentrations for optimum gelation are generally independent of gum concentration but are reduced as the level of sugar is increased. Figure 9.2 compares the effect of both mono- and divalent ions on the modulus of 0.5% LA gellan gum gels in water and in 60% sucrose. Below pH 3.0 LA gellan gum is able to form a gel without the need for mono- or divalent metal ions. Optimum gel modulus occurs for these acid gels at approximately pH 2.8–3.0 regardless of the acid used. This optimum is not affected by the presence of sugars to the same extent as ion requirements. For example, in the presence of 60% sucrose the optimum shifts to approximately pH 2.5–2.7. Acid gels are generally stronger than ion mediated gels in both water and sugar. Addition of other gelling ions, such as sodium or calcium, generally results in a reduction in gel strength of the acid gels. Gel properties at optimum conditions for LA gellan gum gels in water and 60% sucrose are summarised in Tables 9.4 and 9.5 respectively.

In many instances addition of gelling ions is not necessary since there are sufficient ions present in the water or other ingredients to promote gelation of the LA gellan gum. When required, the gel promoting ions can be added to the hot gum solution and, provided the solution is kept above its setting temperature, no gel will form. This allows for the easy preparation of stock solutions (1–2% gum) which can be held at high temperature until required. LA gellan gum is often described as having a 'snap set' since gelation is very rapid once the setting temperature is reached. As with hydration temperature, setting and

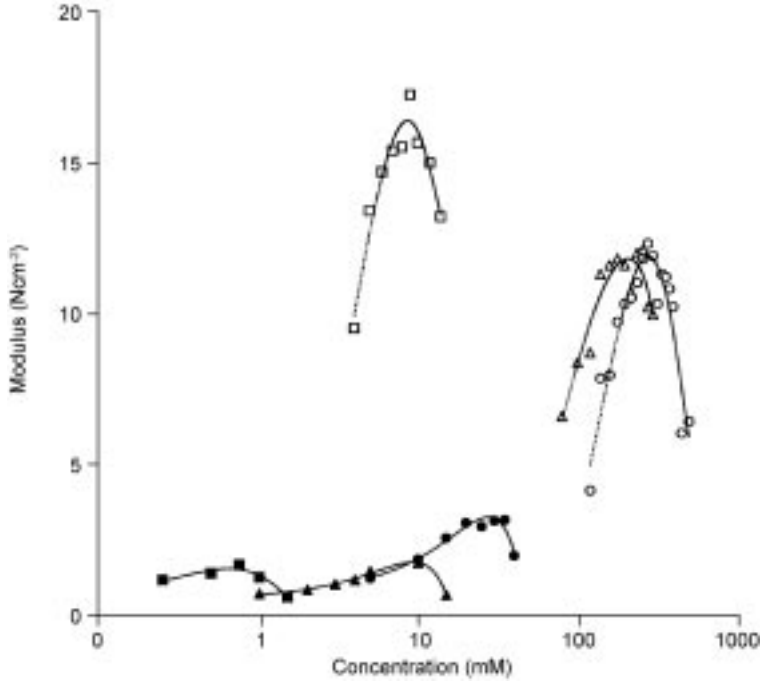

Fig. 9.2 Effect of calcium (squares), sodium (triangles) and potassium (circles) ion concentration on the modulus of 0.5% LA gellan gum gels in water (open symbols) and 60% sucrose (closed symbols).

Table 9.4 Properties of 0.5% LA gellan gum gels in optimum conditions for gel formation in water

Gelling ion	Ion concentration (mM)	Modulus (Ncm^{-2})	Brittleness (%)	Setting temperature (ºC)
Calcium	8–10	19.3	28.5	42
Sodium	260–300	12.3	28.2	54
Potassium	240–260	12.1	30.0	59
Acid	pH2.8–3.0	20.3	27.7	10

Table 9.5 Properties of 0.5% LA gellan gum gels in optimum conditions for gel formation in 60% sucrose

Gelling ion	Ion concentration (mM)	Modulus (Ncm^{-2})	Brittleness (%)	Setting temperature (ºC)
Calcium	0.5–1.0	1.66	61.5	38
Sodium	25–35	3.13	54.1	47
Potassium	8–10	1.71	65.6	43
Acid	pH2.5–2.7	7.74	43.9	64

melting temperatures of the gels depend on the ion concentration in solution. The higher the ion concentration, the higher the setting and melting temperature. Significant thermal hysteresis between the setting and melting temperature is observed in LA gellan gum gels, i.e., the gels melt at a higher temperature than that at which they set.[13] In most conditions LA gellan gum gels are not thermally reversible below 100 ºC. The exceptions are gels formulated with a low level of monovalent ions, particularly potassium, and milk gels. Setting time is governed by the rate at which heat is removed which, in turn, depends on the dimensions of the system being cooled. Thin films on a cold surface, for example, set almost instantaneously. Once set, gel strength does not change markedly over time. LA gellan gum is capable of forming self supporting gels at concentrations as low as 0.05% gum. LA gellan gum gels do not synerise unless cut or broken.

High acyl gellan gum

As with LA gellan gum the easiest way to form HA gellan gum gels is to cool hot solutions. Addition of cations is not necessary for the formation of HA gellan gum gels and their properties are much less dependent on the concentration of ions in solution. Gels typically set and melt between 70 and 80 ºC and show no thermal hysteresis, i.e., they melt at the same temperature at which they set. The setting temperature increases with increasing cation concentration. For example, the temperature increases from approximately 71 ºC to 80 ºC as the calcium increases from 2 to 80 mM. A similar increase is seen when sodium or potassium concentration is increased from 10 to 200 mM.[14] HA gellan gum is capable of forming self supporting gels at concentrations above approximately 0.2% gum. HA gellan gum gels do not synerise.

9.4.4 Texture of gellan gum

Texture is generally regarded as a multifarious property.[15] Texture profile analysis (TPA) is a technique based on compression of free standing gels twice in succession and is capable of providing both fundamental and empirical data on the mechanical properties of gels. It has the advantage of providing data at both low and high strains allowing gels to be characterised by multiple parameters. These include:

- modulus: a measure of gel firmness when lightly squeezed
- hardness: a measure of the force required to rupture the gel
- brittleness: a measure of how far the gel can be squeezed before it ruptures; it is important to note that the higher the brittleness value the less brittle the gel is, i.e., it has to be compressed further to break
- elasticity: a measure of how far the gel springs back after the first compression cycle
- cohesiveness: indicates the degree of difficulty in breaking down the gel in the mouth.

Texture profile analysis has been used to characterise a diversity of foods and

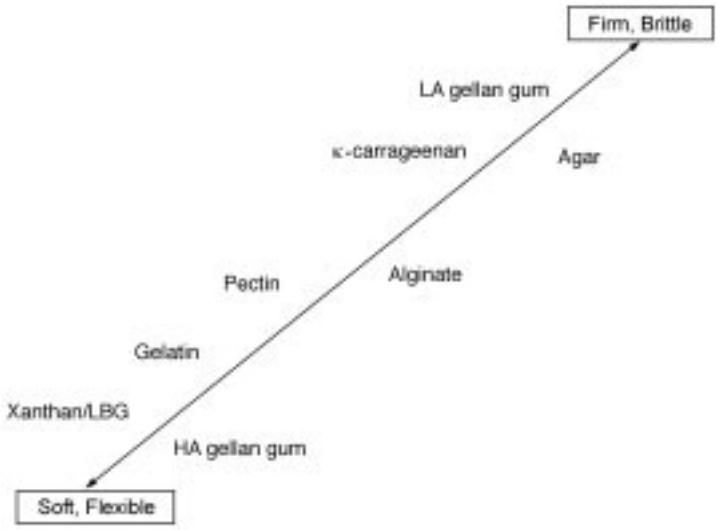

Fig. 9.3 Schematic comparison of the gel texture of HA and LA gellan gum with other common gelling agents.

hydrocolloid gels. A more detailed account of the technique can be found in a review by Pons and Fiszman.[16]

HA and LA gellan gum gels have very different textures that can be considered to be at opposite ends of the textural spectrum of hydrocolloid gels and Fig. 9.3 shows schematically how gellan gum gels compare with other

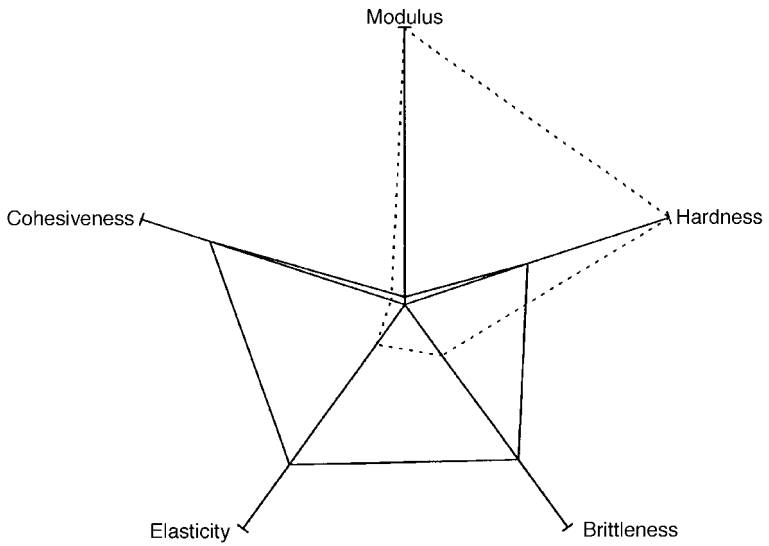

Fig. 9.4 Texture profile of HA (——) and LA (-----) gellan gum gels measured on 1% gels at 70% strain.

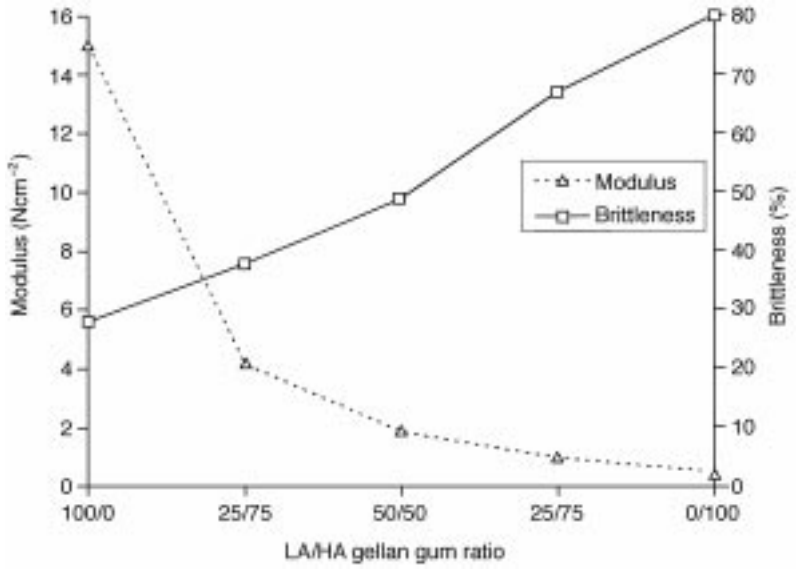

Fig. 9.5 Effect of HA and LA gellan gum blend ratio on the modulus and brittleness of gels prepared at 0.5% total gum concentration.

common gelling systems. LA gellan gum forms hard, non-elastic, brittle gels whereas HA gellan gum gels are soft, elastic and non-brittle. A comparison of the texture of HA and LA gellan gum gels, made using texture profile analysis, is shown in Fig. 9.4. It is immediately apparent that through blending of the two forms, a diverse range of textures can be achieved that encompass many of the textures produced by other hydrocolloids (Fig. 9.5). It has been demonstrated by differential scanning calorimetry (DSC), and rheological measurements that mixtures of the HA and LA forms exhibit two separate conformational transitions at temperatures coincident with the individual components.[8,13] This is important to note since in mixtures consideration for the high setting temperature of the HA gellan gum will need to be made. No evidence for the formation of double helices involving both HA and LA molecules has been found.[17] Properties of the blended system can be varied through control of the blend ratio and level of ions in the mixture.[18,19] At low ionic concentrations the HA form predominates, but as the ionic concentration increases the contribution of the LA form to the texture increases.

9.5 Uses and applications

Before discussing the main applications of gellan gum, an overview of the key properties of both the HA and LA forms is given in Table 9.6. This provides a useful frame of reference from which existing applications can be understood and new opportunities visualised.

Table 9.6 Comparison of the key properties of HA and LA gellan gum

	Low acyl gellan gum	High acyl gellan gum
Hydration	> 80 ºC	> 70 ºC
Sequestrants	Yes	No
Viscosity	Low	High
Gelling ions	Yes (mono or divalent or acid)	Not required
Setting temperature	25–60 ºC	70–80 ºC
Melting	No (except low ionic strength and in milk)	Yes
Clarity	Clear	Opaque
Texture	Firm, brittle	Soft, elastic

9.5.1 Dessert jellies

Water-based dessert jellies are popular throughout the world and have a range of textures. The firm brittle texture of LA gellan gum, for example, complements the flavour of fruit juice jellies. Alternatively, combinations of HA and LA gellan gum can be used to produce jellies with a variety of textures. Products can be 'ready-to-eat' (RTE) or in dry mix form. Example formulations are provided below. Formulation 9.1 is an example of a fruit juice jelly prepared with LA gellan gum. It can be made with apple, orange, grape, pineapple or grapefruit juice and will hydrate as a dry mix in a water hardness of up to 600 ppm (as $CaCO_3$). The pH and solids vary according to the juice used, but are typically pH 3.5–3.7 and 17% total soluble solids. Alternatively, blends of LA and HA gellan gum can be used to give a range of textures. Blend ratio and gum concentration will depend on the final texture required but a 3:1 HA:LA gellan gum blend at approximately 0.3% is recommended as a starting point for textural evaluation.

Formulation 9.1 Recipe for fruit juice jelly using LA gellan gum

Ingredients	Weight (g)	(%)
Water	250.0	42.00
Fruit juice	250.0	42.00
Sugar	90.0	15.15
Citric acid anhydrous	2.4	0.40
Tri sodium citrate dihydrate	1.8	0.30
LA gellan gum	0.9	0.15

Preparation

1. Pre-blend all the dry ingredients.
2. Heat the water to boiling and dissolve the dry ingredients in the hot water.
3. Add the fruit juice, mix and chill.

The gel sets at approximately 40–45 ºC and the use of chilled fruit juice with dry-mix desserts ensures a rapid set.

Formulation 9.2 Recipe for a dessert jelly using LA gellan gum and gelatin

Ingredients	Weight (g)	(%)
Water	500.0	82.6
Sugar	90.0	15.0
Gelatin (type B, 240 Bloom)	10.2	1.7
Citric acid anhydrous	2.3	0.38
Tri sodium citrate dihydrate	1.6	0.26
LA gellan gum	0.35	0.06
Colour and flavour	as required	

Preparation
1. Blend all the dry ingredients.
2. Heat the water to boiling and dissolve blend into the hot water by stirring for 1–2 minutes.
3. Deposit and chill.

LA gellan gum can also be used to modify the properties of traditional gelatin dessert jellies and an example is given in Formulation 9.2. The LA gellan gum in this formulation raises the initial set temperature of the dessert to around 35 ºC, allowing more rapid processing of an RTE product. The formulation can also be used in dry mix composite desserts allowing further layers to be added more quickly than with gelatin alone. The time to consumption is, however, not reduced as the maturation time of the gelatin gel remains unchanged. The gellan gum also raises the melting point of the gel so that desserts maintain their shape for longer, when removed from the fridge. This approach can also be used in savory gelled products such as aspics. Gellan gum is an anionic polysaccharide (−ve charge) whereas gelatin is a protein and as such its overall charge will be dependent on the pH of the system. Below its isoelectric point the gelatin will carry an overall positive charge and will therefore interact with the negatively charged polysaccharide. This can lead to cloudiness in the gel or even precipitation. For this reason it is recommended to use type B gelatins since these have the lowest isoelectic point (pH 4.5–5.5). The extent of the interaction will depend on the pH and the ratio of gelatin to gellan gum.

9.5.2 Suspending agent

Gellan gum is commonly used as a gelling agent; however, it can be used to prepare structured liquids which are extremely efficient suspending agents. These structured liquids are gelling systems which have been subjected to shear either during or after the gelation process. The application of shear disrupts normal gelation and results, under certain conditions, in smooth homogeneous, pourable systems often referred to as ‘fluid gels’.[20]

To produce smooth homogeneous fluid gels with gellan gum, systems must be formulated to give weak gelation, either by manipulating the ion type and concentration or gellan gum concentration. The viscosity and structure of the

system correlates with the gel strength of the unsheared gel. Therefore, the greater the gel strength of the unsheared gel, the greater the viscosity and structure will be when the system is sheared. Systems which gel too strongly, however, can give rise to a grainy appearance in the final fluid.

Gellan gum fluid gels can be prepared using a variety of processes. Three potential processes are outlined schematically in Fig. 9.6. The first step in each case is to hydrate the gellan gum through a combination of heat and sequestrants. Method 1 simply involves continually stirring the solution as it cools to form the fluid gel or allowing the weak gel to form undisturbed, then shearing to form the fluid gel. Alternatively, in method 2 the hot gellan gum solution can be added to cold water whilst mixing. This results in cooling of the solution and formation of a fluid gel. In method 3 it is possible to prepare gellan gum solutions that will not gel on cooling. Addition of ions to these cold solutions results in gelation and formation of a fluid gel.

The application of shear can be achieved by using stirring, homogenisation, filling or even 'shake before use'. Shear can also be applied during or after gelation. UHT, HTST and processes involving scraped surface heat exchangers (i.e. for the production of custards, gravies and ketchup) are an ideal way to shear during gelation as the solution cools. Gellan gum fluid gels can be used to produce shelf stable suspensions in a variety of beverage products.[21]

Generally, HA gellan gum should be used at approximately 0.02–0.05% to give a smooth fluid gel. For LA gellan gum the use level is dependent on the ionic concentration in the system and a guide to the formulation of fluid gels using LA gellan gum is given in Table 9.7. Example formulations for beverages

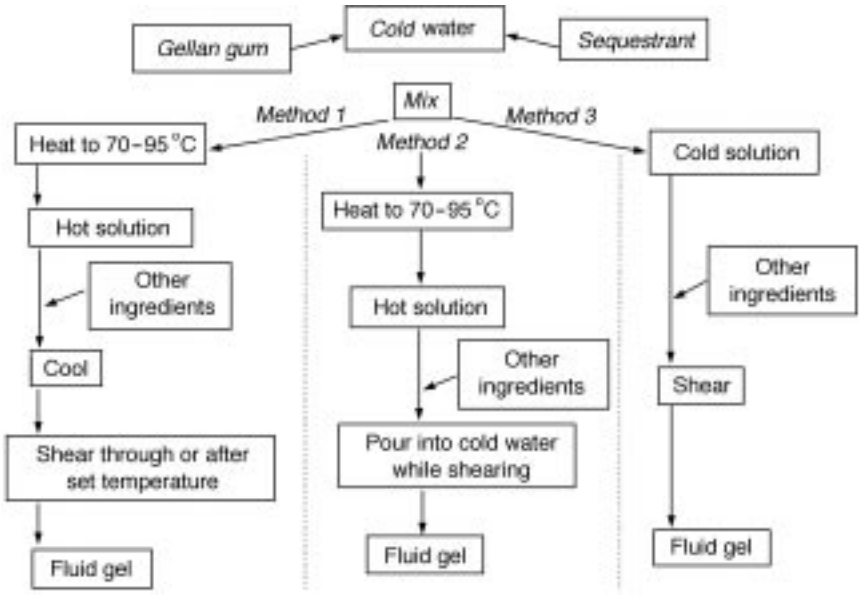

Fig. 9.6 Outline of potential processes for the preparation of gellan gum fluid gels.

Table 9.7 Guidelines for formulation of LA gellan gum fluid gels

Ion	Concentration	LA gellan gum concentration (%)
Calcuim	Low (< 50ppm)	0.05–0.2
	Optimum (100–600ppm)	0.03–0.05
	High (> 600ppm)	0.05–0.2
Sodium	Low (< 0.25%)	0.05–0.2
	Optimum (0.5–2.0%)	0.03–0.05
	High (4.0–10%)	0.05–0.2
Milk		0.05–0.2
Sugars	(40–60%)	0.1–0.3

using LA or HA gellan gum which can be used to suspend gelled beads or fruit pulp are given below.

Formulation 9.3 provides a starting point for a beverage type fluid gel. It has a pH of 2.9 and a setting temperature of approximately 15 ºC. It can be used to suspend jelly beads and is prepared as outlined in method 1 of Fig. 9.6 by shearing after the weak gel has been allowed to form. Formulation 9.4 is an example of a fluid gel formed with HA gellan gum. HA gellan gum fluid gels are less sensitive to the ionic conditions and have longer, more elastic flow properties when compared to LA gellan gum fluid gels.

Formulation 9.3 Recipe for a fluid gel for beverages using LA gellan gum

Ingredients	Weight (g)	(%)
Part 1		
Sucrose	112.0	11.25
Tri sodium citrate dihydrate	0.60	0.06
LA gellan gum	0.28	0.028
Sodium benzoate	0.20	0.02
Deionised water	862.0	86.60
Part 2		
Citric acid	5.00	0.50
Calcium lactate	0.25	0.025
Deionised water	15.00	1.517

Preparation

1. Blend the sucrose, tri sodium citrate dihydrate, LA gellan gum and sodium benzoate and disperse in the deionised water of Part 1.
2. Heat the dispersion to 70–80 ºC to hydrate.
3. Dissolve the citric acid and calcium lactate in the deionised water of Part 2 and add to the hot gum solution.
4. Cool the sample to below 15 ºC undisturbed.
5. Gently agitate the sample to form a fluid gel.

Formulation 9.4 Recipe for a pulp suspension beverage using HA gellan gum

Ingredients	Weight (g)	(%)
Water	338.10	67.62
Fruit juice	100.0	20.0
Sugar	60.0	12.0
HA gellan gum	0.25	0.05
Tri sodium citrate dihydrate	0.25	0.05
Citric acid anhydrous	0.9	0.18
Potassium citrate	0.5	0.1

Preparation

1. Blend the HA gellan gum with the tri sodium citrate dihydrate and disperse in the water.
2. Heat the dispersion to 90 ºC to hydrate the gum.
3. At 90 ºC add the remaining dry ingredients and the fruit juice.
4. Cool to room temperature whilst mixing to form the fluid gel.

9.5.3 Dairy

Unlike water systems, much of the calcium in milk is associated with the milk proteins. During heating any remaining free calcium is also bound by the proteins and therefore does not interfere with the hydration of the gellan gum. Because of this, both HA and LA gellan gum will hydrate in milk above approximately 80 ºC without the need for a sequestrant. Milk also contains sodium and potassium ions and it is therefore not usually necessary to add additional gelling ions to milk systems. Because the LA gellan gum is gelled with a low level of monovalent ions (predominantly K^+), milk gels are thermally reversible, melting at approximately 95 ºC. Thermal stability of LA gellan gum milk gels can be improved by the addition of calcium. Care must be taken when adding calcium to hot milk since it can result in precipitation of the milk proteins if added above 70 ºC. It is recommended to cool the gellan/milk mixture to between 55 and 65 ºC before adding the calcium. This temperature range is above the gelation temperature of the LA gellan gum but below the temperature at which milk protein precipitation occurs.

In many dairy systems milk powders are used. These powders are natural sequesterants and will bind calcium from the water used for reconstitution. Therefore, it is not usually necessary to add a sequestrant when water of hardness up to 400 ppm (as $CaCO_3$) is used for reconstitution. Some sequestrant may be required if less than 2% milk powder is being used or harder water is used to reconstitute the milk powder.

Milk beverages

As described in Section 9.5.2, HA gellan gum at low concentrations is able to form a very weak gel network often referred to as a fluid gel. These fluid gels have extremely good suspension properties and can be used in a range of neutral dairy and soya based products such as chocolate milk. Development of this application was initially limited due to creation of off-flavours as a result of

residual enzyme activity in the native, HA gellan gum acting on the milk. The off-flavour, which is reminiscent of cleaning chemicals, renders the product unpalatable and is linked to the development of para-cresol. Development of a process in which the HA gellan gum is pre-treated with a denaturing agent that is thought to act on the residual enzymes in the gellan gum has led to a new grade of gellan gum for this application.[22]

KELCOGEL® HM-B is a standardised product containing the pre-treated HA gellan gum and can be used at 0.1–0.12% for stabilisation of cocoa in chocolate milk. The HA gellan gum is tolerant to a wide range of UHT conditions and products can be filled at higher temperatures than with carrageenan, the traditional hydrocolloid for this application. It provides excellent suspension of cocoa and long-term stability. It is functional in reconstituted milk, fresh milk, whey substituted beverages and low protein milk beverages.

Yogurt

The standard yogurt process can be followed when using LA gellan gum for both set and stirred yogurt. There are various ways in which yogurt containing LA gellan gum can be made depending on the usual manufacturing process and on the desired properties of the final yogurt. In all cases the initial steps are the same: the LA gellan gum should be blended with skimmed milk powder and other stabilisers (if required) and dispersed in cold milk before heating, homogenising and pasteurising. Generally, the fermentation time is not affected by the presence of LA gellan gum. The important factor to remember is that the setting temperature of LA gellan gum in skimmed milk is about 41 ºC. If shear is applied through the setting temperature a fluid gel will be formed. This acts like a gel under static conditions, but flows like a liquid when shear is applied. If, however, shear is applied below the setting temperature in yogurt systems, a broken gel may result which can lead to unsatisfactory lumps in the final product. Typical use levels for LA gellan gum in yogurt are 0.04%. It can be used in combination with other stabilisers such as starch depending on the final texture required.

9.5.4 Sugar confectionery

One of the fundamental techniques for the manipulation of the texture of sugar confectionery is to use combinations of a variety of sugars such as sucrose, glucose, fructose and various corn syrups. Combinations of sugars produce desirable textures, as well as preventing crystallisation of individual sugars. Another critical ingredient is the hydrocolloid. These impart structure to the product and provide the characteristic jelly texture. Before describing how to make confectionery jellies with gellan gum, it is worth discussing the influence of sugars on the properties of gellan gum.

Effect of sugars on LA gellan gum

The presence of sugars has two major effects on the properties of LA gellan gum gels. Firstly, the ion requirements for optimum gel properties are reduced. The

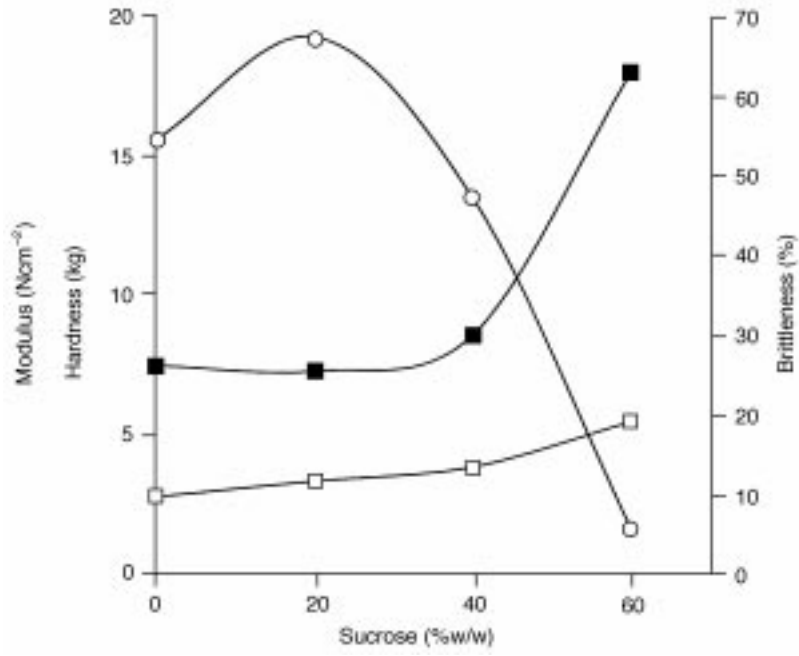

Fig. 9.7 Effect of sucrose concentration on the modulus (○), hardness (□) and brittleness (■) of 0.5% LA gellan gum gels.

presence of 40% w/w sugar approximately halves the calcium required for maximum gel modulus, from 8–10 mM in water gels to 4–5 mM in the sugar gels. Addition of 60% w/w sugar results in an approximately ten-fold reduction in the requirement for calcium, with only 0.5–1.0 mM added calcium required for maximum gel modulus. Similar reduction in the requirements for sodium and potassium are also seen (Fig. 9.2). Secondly above approximately 40% sugar gels become less firm and less brittle, i.e., softer and more elastic (Fig. 9.7). These effects are believed to be the result of the sugars inhibiting the aggregation step of the gelation process.[23,24] These effects are also influenced by the type of sugar. Sucrose has a greater inhibitory effect than glucose, fructose or corn syrups (Table 9.8).[25] The differences observed between sugars mean that texture can, to some degree, be varied by manipulating the sugar composition of the system. For example, partial replacement of sucrose with fructose or corn syrup, a common practice to control crystalisation in confectionery manufacture, results in firmer, more brittle gels.[26]

Effect of sugars on HA gellan gum

Less is known about the specific effects of sugars on HA gellan gum. However, addition of sugars to HA gellan gum gels generally results in an increase in the force required to break the gel. Setting and melting temperature also increase with increasing sugar concentration. In the presence of high levels of sugar (70–

Table 9.8 Effect of sugars at 40% and 60% w/w on the textural properties of 0.5% w/w LA gellan gum gels prepared at ion concentrations giving maximum gel modulus

Sugar	Modulus (Ncm^{-2})		Brittleness (%)	
	40% w/w	60% w/w	40% w/w	60% w/w
Fructose	14.1	3.70	31.3	53.6
Glucose	12.9	2.17	36.7	62.9
Sucrose	13.5	1.60	30.2	63.3
Maltose	15.5	3.83	30.7	51.4
42DE corn syrup	19.0	5.06	27.9	53.0
14DE maltodextrin	16.1	5.88	24.1	43.1

80%), HA gellan gum has very high viscosity even when hot. This can make processes such as mixing and depositing difficult. This is often compounded by the high setting temperature which can result in pre-gelation, i.e., gel formation prior to deposition of the confectionery mix. However, incorporation of low levels of HA gellan gum into confections made with LA gellan gum will increase the chewiness of the jellies.

Preparation of confectionery jellies

LA gellan gum may be used alone or in combination with other gelling agents to produce jelly confectionery by traditional processes. Examples are provided in Formulations 9.5 and 9.6. When prepared with LA gellan gum as the sole gelling agent (Formulation 9.5), the jellies are firm with a short, clean bite and flavour. The jellies can be removed from the starch moulds after about 2 h but are usually stoved for up to 72 h before demoulding. Addition of a thin boiling starch as outlined in Formulation 9.6 results in a chewier texture. Combinations of LA gellan gum with carrageenan can be used to produce gelatin-free confectionery which is suitable for halal.

Pre-gelation is the premature gelation of the confectionery mix prior to, or during, depositing. This makes depositing difficult and results in a weaker gel structure and grainy texture. Table 9.9 provides a guide to preventing pre-gelation in gellan gum confections.

9.5.5 Fruit preparations

This application covers a wide variety of systems from 30 to 75% total soluble solids. Much of the understanding of the effects of sugars described in confectionery applications can be applied to these systems. In addition, the type of fruit used in the formulation is a key consideration when using LA gellan gum since the ion content and pH will vary. Fruit composition may also vary during the season. Table 9.10 shows that the ionic composition varies considerably between different fruits with most fruits containing significant levels of potassium ions.[27] These ionic concentrations become increasingly significant in

Formulation 9.5 Recipe for jelly sweets using LA gellan gum

Ingredients	Weight (g)	(%)
Sucrose	159.0	35.20
Glucose syrup (42DE)	159.0	35.20
Water	120.0	26.51
Citric acid anhydrous	5.00	1.11
Tri sodium citrate dihydrate	5.00	1.11
KELCOGEL® F gellan gum	3.75	0.83
Calcium hydrogen orthophosphate	0.20	0.04
Flavour and colour	as required	

Preparation

1. Blend the LA gellan gum and calcium hydrogen orthophosphate with 1.0 g of tri sodium citrate dihydrate and 40 g of sucrose and disperse in the water.
2. Heat to boiling to hydrate the gellan gum then add the remainder of the sugar while continuing to boil.
3. Add pre-warmed glucose syrup while maintaining the temperature above 90 ºC.
4. Cook the liquor to 80–82% total solids then cool to 90 ºC.
5. Dissolve the citric acid and remainder of the tri sodium citrate dihydrate, colour and flavour in 20 cm^3 of water and stir into the liquor.
6. Deposit at 76–78% total solids into starch moulds.
7. Stove to final solids as required.

Formulation 9.6 Recipe for jelly sweets using LA gellan gum and thin boiling starch

Ingredients	Weight (g)	(%)
Water	220.0	39.0
Glucose syrup (42DE)	159.0	28.2
Sugar	148.5	28.1
Thin boiling starch (FLOGEL 60)	18.8	3.4
LA gellan gum	3.5	0.62
Tri sodium citrate dihydrate	1.8	0.32
Citric acid anhydrous	1.8	0.32
Calcium hydrogen orthophosphate	0.2	0.04
Flavour and colour	as required	

Preparation

1. Slurry the starch in 50 g of water.
2. Blend the LA gellan gum, calcium hydrogen orthophosphate and tri sodium citrate dihydrate with 40 g of sugar and disperse in the remainder of the water.
3. Heat the dispersion to boiling to hydrate the gellan gum then add the remaining sugar and continue to boil.
4. Add pre-warmed glucose syrup and cook to boiling.
5. Add the starch slurry, breaking the boiling point and continue to cook to 78% total solids.
6. Add colour, flavour and citric acid pre-dissolved in a small amount of water.
7. Deposit into starch moulds at 74% total solids and stove to final solids as required.

Table 9.9 A guide to the prevention of pre-gelation in confectionery mixes

Problem	Possible causes	Solution
Pre-gelation when acid added	Hard water	Add sodium hexameta phosphate
	Depositing soluble solids too high	Lower depositing solids
	pH too low	Add sodium citrate with citric acid
Pre-gelation before acid added	Hard water	Increase sequestrant level
	Soluble solids too high	Add water to lower soluble solids

Table 9.10 Ionic composition of raw fruits[27]

Fruit	Ca^{++} (mg/100g)	Mg^{++} (mg/100g)	Na^{+} (mg/100g)	K^{+} (mg/100g)
Apple	4	3	2	88
Blackcurrant	60	17	3	370
Raspberry	25	19	3	170
Strawberry	16	10	6	160
Apricot	15	11	2	270
Peach	7	9	1	160

medium to high solids systems where gellan gum ion requirements are greatly reduced. Therefore, formulations optimised for one fruit will often need modification to accommodate different fruits.

Formulation 9.7 is an example of a low solids (36%) jam which can be formulated to give a range of textures. HA gellan gum provides a soft, spreadable jam with excellent sheen. The addition of a proportion of LA gellan gum may be used where a firmer textured jam is required. LA gellan gum alone can be used to give a more bake stable jam. Various fruits can be used, including strawberries, raspberries and blackcurrants. Formulation 9.8 is slightly higher in solids than Formulation 9.7 and produces a lightly gelled yogfruit with evenly suspended fruit pieces. The gelled structure can be broken down by pumping to give a smooth, viscous yogfruit (pH 3.9, soluble solids 40%).

Finally, Formulation 9.9 with 55% fruit and no added water demonstrates the properties of a gellan gum and starch-based preparation. The LA gellan gum filling has a glossy appearance, good flavour release and excellent bake stability (pH 3.4, tss 56%).

Formulation 9.7 Recipe for a reduced sugar jam using HA or LA gellan gum blend

Ingredients	Weight (g)	(%)
Frozen strawberries	450.0	45.0
Sugar	283.5	28.35
Water	260.0	26.0
Gellan gum*	2.5	0.25
Tri sodium citrate dihydrate	0.5	0.05
Potassium sorbate	1.0	0.10
Citric acid solution (50% w/w)	2.5	0.25

* High acyl and/or low acyl gellan gum can be used depending on desired final texture.

Preparation

1. Dry blend the gellan gum, tri sodium citrate dihydrate and potassium sorbate with the sugar and disperse into the water.
2. Add the fruit and heat to boiling. Cook for 1–2 minutes to ensure hydration of the gellan gum. Check the soluble solids.
3. Remove from the heat and add the citric acid solution. Fill into jars and cap immediately.

Formulation 9.8 Recipe for a peach yogfruit using LA gellan gum

Ingredients	Weight (g)	(%)
Peach purée	200.0	28.50
Diced peach	200.0	28.50
Glucose syrup	300.0	42.65
LA gellan gum	0.35	0.05
Tri sodium citrate dihydrate	1.80	0.26
Sodium benzoate	0.25	0.04

Preparation

1. Combine the fruit and glucose syrup.
2. Add the tri sodium citrate dihydrate, LA gellan gum and sodium benzoate and heat to 90 ºC with constant stirring.
3. Hold for 1 minute then cool, with stirring, to 60 ºC.
4. Deposit and allow to cool undisturbed.

Note: The tri sodium citrate dihydrate in the formulation is added to give a final pH of 3.9. The addition level may be varied depending on the fruit used, and the final pH required.

9.5.6 Other applications

Gellan gum forms films and coatings that can be used in breadings and batters. Films offer several advantages, particularly their ability to reduce oil absorption by providing an effective barrier. Films can be prepared by applying a hot solution of gellan gum on to the surface of the food product, by spraying or dipping, and allowing to cool. Alternatively, in the case of LA gellan gum, the food can be dipped into a cold solution of the gum, allowing ions to diffuse into

Formulation 9.9 Recipe for a bake-stable fruit preparation using LA gellan gum

Ingredients	Weight (g)	(%)
Apple (thawed)	210.0	55.2
Sucrose	160.8	42.2
Modified starch (THERMFLO)	8.00	2.10
LA gellan gum	0.32	0.12
Citric acid solution (50% w/w)	0.80	0.20
Tri sodium citrate dihydrate	0.88	0.18

Preparation
1. Pre-blend the dry ingredients, add to the apple and heat with stirring to boiling.
2. Remove from heat, add the citric acid solution, mix well and deposit.
3. Leave to gel before use. Shear, and use as required.

the solution, resulting in gelation or film formation. LA gellan gum can also be used to produce fat free adhesion systems. Spraying of a cold solution of LA gellan gum onto the surface of products such as nuts, crisps and pretzels forms an instant thin layer of gel when it reacts with the salt thus facilitating adhesion of spice, flavour or sweetener blends.

9.6 Regulatory status

In Japan, gellan gum has been considered a 'natural' food additive since 1988. It is now approved for food use in the USA and the European Union as well as Canada, South Africa, Australia, most of South East Asia and Latin America. Gellan gum appears as E418 in the European Community Directive EC/95/2 in Annex 1. Both the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the European Community Scientific Committee for Food has given gellan gum an Acceptable Daily Intake (ADI) of 'not specified'. Combinations of HA and LA gellan gum have one name. A manufacturer may label a product made with a combination of both types of gellan gum simply, 'E418' or 'gellan gum'.

9.7 Future trends

The current commercial HA and LA gellan gum products can be considered as being at opposing ends of the textural spectrum available with hydrocolloid gelling agents (Fig. 9.3). Blends of the two types enable some intermediate textures to be created but the mixed system retains the high setting temperature of the HA product and the ion sensitivity of the LA product. Far more interesting are gellan gums of intermediate acyl content as these show much more variation in texture and a single homogeneous setting behaviour.[28,29] Methods for creating these partially acetylated products exist but they are yet to be produced on a commercial scale.[30] Realisation of this control over the degree of acylation of gellan gum could lead to a truly universal gelling agent.

9.8 Sources of further information and advice

www.CPKelco.com

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10

Galactomannans

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Abstract: The introduction provides information about the worldwide availability of important seed gums. The following section describes the raw materials and structure of seed galactomannans, including description of the composition of the seeds of the carob tree, tara shrubs and the guar plant, and the amount of recoverable endosperm in fenugreek seeds. For the four types of gum discussed, the amount of overall galactose content is indicated, and the fine structure of the different galactomannans is briefly described, as far as information is available. There is also brief discussion of a possible biosynthesis route for galactomannans, derived from the literature. Attention is also drawn to EC and FAO/WHO specifications for the molecular weights of carob bean gum (E410) and guar gum (E412). The manufacture of carob bean gum/tara gum and guar gum is described and a manufacturing abstract for fenugreek gum is derived from two recent patents.

Brief reference is also made to the development of novel technologies for the purification of guar endosperm. The various galactomannan gums are characterized in the usual way. The relationship of molecular weights of galactomannans of straight and depolymerized carob bean gum and guar gum and the usual Brookfield viscosities are determined. This kind of viscosity is examined in relation to the concentration of aqueous solutions, using regression equations. Uses and applications of galactomannans are very briefly discussed and attention is drawn to other publications in this respect. Potential future developments are also briefly examined.

Key words: water soluble seed gums, carob bean gum, tara gum, guar gum, fenugreek gum, E-numbers, availability, production, galactose-mannose ratios, properties, depolymerized gums, application.

10.1 Introduction

Galactomannans are multifunctional macromolecular carbohydrates found in various albuminous or endospermic seeds. The seed galactomannans from the carob tree (*Ceratonia siliqua* L.) and from the guar plant (*Cyamopsis tetragonoloba* L.) are widely used, while the kernels from the tara shrub (*Cesalpinia spinosa* L.) are used to a much lesser extent. The fenugreek plant (*Trigonella foenum graecum* L.) is another legume that supplies a further kind of galactomannans.

Ground endosperms are the source of trade galactomannan gums. The recovered endosperm halves, of the required purity, of carob, tara and guar, and the endosperm parts of fenugreek seeds, are ground to obtain a fine off-white powder.

World production of carob pods is estimated at about 300,000–350,000 t/year, yields depending on cultivar, region and farming practice. The carob pod is called St. John's bread and has two main constituents:

- 90 wt% of pulp, containing a high amount of sugars (48–56 wt%) with low molecular weight
- 10 wt% of seeds or kernels (undigestible).

The carob kernels are composed of 30–35 wt% of hull, 20–25 wt% of germ and 40–45 wt% of endosperm on an absolute dry basis. The annual consumption of carob bean gum in the food industry is 9,000–10,000 t.

The approximate composition of tara seeds is: ca. 38 wt% of hull, ca. 40 wt% of germ and ca. 22 wt% of endosperm on an absolute dry basis. About 1,500–2,000 t of tara gum per year are available to the food industry.

The average total quantity of guar seeds worldwide is estimated at about 500,000 tons p.a. However, large fluctuations of annual availability of seeds occur, mainly due to weather conditions. The seeds are composed of 20–22 wt% of hull, 44–46 wt% of germ and 32–36 wt% of endosperm on an absolute dry basis. The annual consumption of guar gum in the food industry is about 55,000 t.

For fenugreek, the annual worldwide seed volume is approximately 30,000–50,000 t, mainly used for spice production. This volume could easily be increased on demand (see 'Fenugreek has a role in south-eastern Australian farming systems' by Kate McCormick *et al.* (kmcc@netconnect.com.au). Fenugreek seeds yield about 25 wt% of gum. For ease of comparison, Table 10.1 illustrates the annual consumption in 1990 of different food additives in grams per year per capita in various countries.

10.2 Raw materials and structure

These polysaccharides are strongly hydrophilic, enabling the endosperm to imbibe water to protect the embryo against subsequent drought, during and before germination. They become metabolized after germination.[1] During

Table 10.1 Annual consumption of certain hydrocolloids per capita in grams per year for 1990 (for US citizens and for those in Germany, France, UK and Italy (D, F, GB, I))

Hydrocolloids	US	D, F, GB, I
Starch products	1300	1281
Gelatin	65	122
Agar Agar	2	3
Alginates	10	10
Carrageenans	11	18
Guar gum	34	39
Arabic gum	22	59
Carob bean gum	8	18
CMC	23	6
Other cellulose derivatives	6	1
Xanthan gum	13	3
Pectin	11	19
Total	1505	1576

germination the endosperm absorbs up to 75 wt% of water, based on the dry weight of the seed. This allows the diffusion of enzymes required for the germination process and enables the transport of metabolic low molecular weight end-products, needed for the growth of the plant.

The carob tree bears pods with an average yield of 50–70 kg, which can be collected from orchards with minimum management, producing 2,000–3,000 kg/ha. In irrigated orchards an average of 250–300 kg of pods per tree is possible, yielding 12,300 kg of pods/ha.

The kernels are released in a process called kibbling. The pods are broken between two rollers with special geometry, and the freed seeds are then separated from the rest of the pods using special screens. Due to its high sugar content, the pod pulp is a staple in the diet of farm animals. It is also used to produce alcohol and is sold in fine powder form as a cacao substitute. People ate carob pods in times of famine and children still like them as snacks.

The main interest, however, lies in the kernels. The kernel is oval or oblong in shape, 8–10 mm long, 7–8 mm wide and 3–5 mm thick. The glossy brown testa is hard and smooth. A kernel weighs ca. 0.2 g with small deviations and was used as a weight unit for gold and precious stones (carat is derived from the Latin word *ceratonia*).

The endosperm of *Ceratonia siliqua* seeds or kernels consists of living cells, which can synthesize the enzymes to hydrolyse the galactomannans (α-galactosidase, EC.3.2.1.22, endo-β-mannanase, EC.3.2.1.78 and β-mannosidase, EC.3.2.1.25).

The guar plant is bush-like with a height of 57–105 cm, depending on the variety. It is very drought resistant. Once it has germinated, it requires very little surface water during the main growth period, i.e. for 20–25 weeks. To induce maturation of the seeds, water is needed again. The monsoon season of the

Indian continent, especially in the north-western part of India and north-eastern part of Pakistan usually provides the right amount of rainfall at the right time of year for optimal growth. The growing season starts in July or August in India and Pakistan and the harvest takes place in November or December. The guar pod is 5–8 cm long, almost round in shape and ca. 1 cm wide. It contains 6–9 seeds, amounting to about 60 wt% of the pod.

Green pods are used as cattle fodder and as a vegetable by poor people. Ripe and dry pods can no longer be eaten and empty pods have no commercial value. The yield per ha can be as high as 1,800 kg of pods. On the subcontinent harvesting is done manually and the seeds are recovered by mobile threshing machines. Guar seeds as such are not exported, neither from India nor Pakistan. 100 seeds weigh 2.4 to 3.7 g. The seed yield per plant is a maximum of 50 g.[2] On its convex periphery, the guar endosperm of *Cyamopsis tetragonoloba* seeds has four layers of aleurone cells with a total thickness of 0.1 mm, the only living cell layers of the endosperm (Fig. 10.1), which can also synthesize enzymes similar to those mentioned above for the carob endosperm.[3]

The endosperm of fenugreek (*Trigonella foenum graecum*) seeds also has an aleurone layer as the outer cell layer of the endosperm, which can synthesize the required enzymes for germination. The endosperm of tara (*Cesalpinia spinosa*) seeds is morphologically similar to that of the carob kernel, but the cell structures differ and can be distinguished microscopically.

The hard, compact endosperm of all four seeds contains more than 88 wt% of galactomannans on a dry basis. The endosperm of the guar seed, for instance, develops alongside the embryo or germ and completely envelops it. The endosperm itself is protected by a seed coat (see Fig. 10.2). The germ of the carob kernel is sandwiched between two endosperm halves, but without being completely enclosed by a thin film of endospermic cells. The hard coat of the carob kernel provides the desired protection, and the same is also true for the tara kernel. The seed endosperm contains very little cellulose and no lignin.

The galactomannans derived from these four seed sources are composed entirely of linear (1→4)-β-D-mannan chains with varying amounts of single D-galactose substituents linked to the main backbone by (1→6)-α-glycosidic bonds (Figs 10.3 and 10.4).

These polydisperse galactomannans can easily be distinguished from each other by their overall mannose-galactose ratios between 1.1:1 and ca 3.5:1. The galactomannans of carob bean gum have a galactose content of 17–26 wt%, those of tara gum ca. 25 wt%, guar gum 33–40 wt% and fenugreek 47–48 wt%.

The large amount of galactose side stubs of about 20–48 wt% prevents strong cohesion of the main backbones of different neighbouring macromolecules, so that no extensive crystalline regions can be formed. Water at room or elevated temperatures can thus easily penetrate between the single molecules to hydrate or dissolve the accessible gum. Substitution of the mannan chain by more than 12 wt% of galactose makes the galactomannan water soluble.[4,5]

The fine structure of these galactomannans can be quite irregular with respect to the distribution of the galactose units. As many as five mannose units without

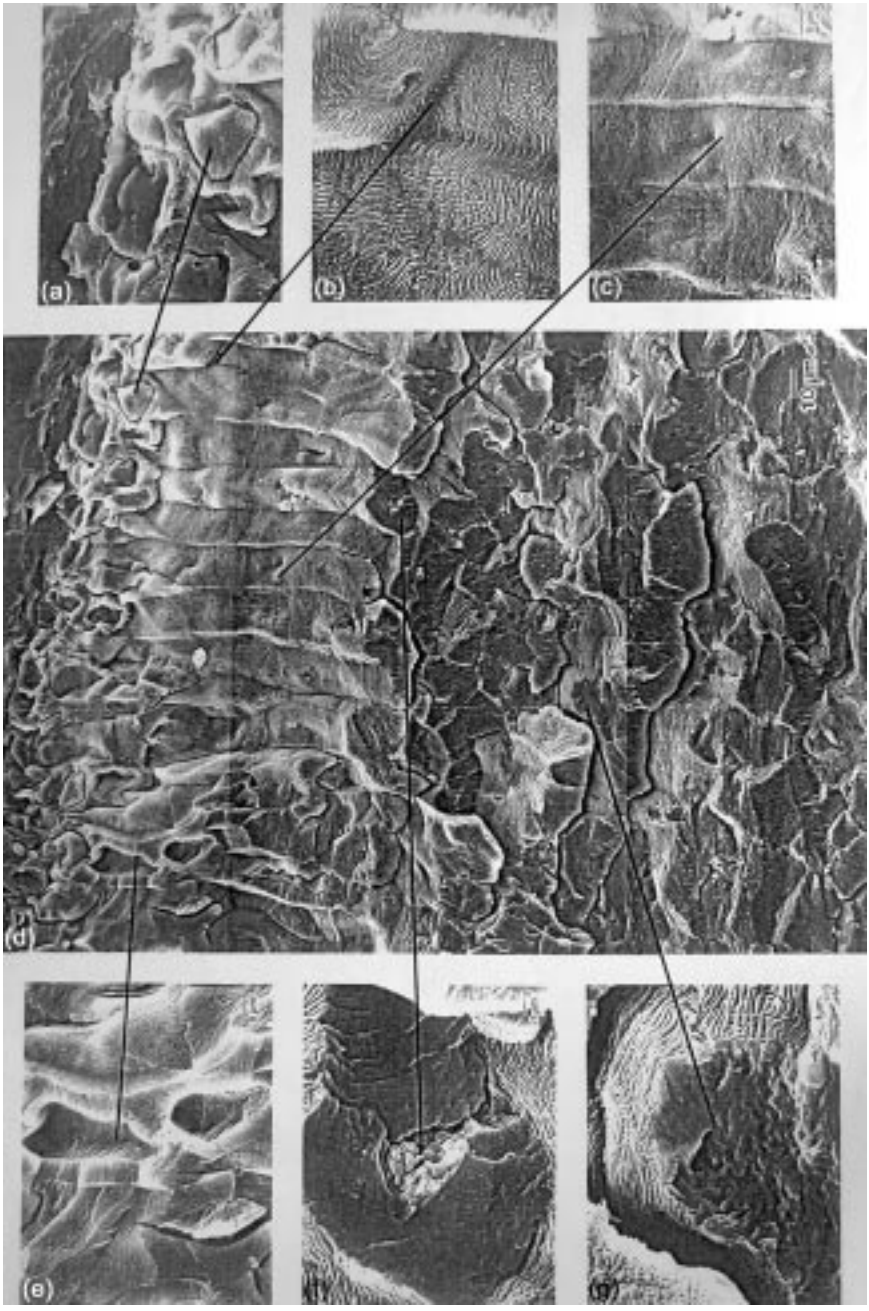

Fig. 10.1 Photos ((a)–(c)) show magnified details of aleurone cell layers (1000×, 2000×, 1000×). The first magnification photos below ((e)), illustrates one detail of the peripheral aleurone layer (1000×); the other two enlargements ((f) and (g)) show inner endosperm cells of a fractured guar split (2000×).

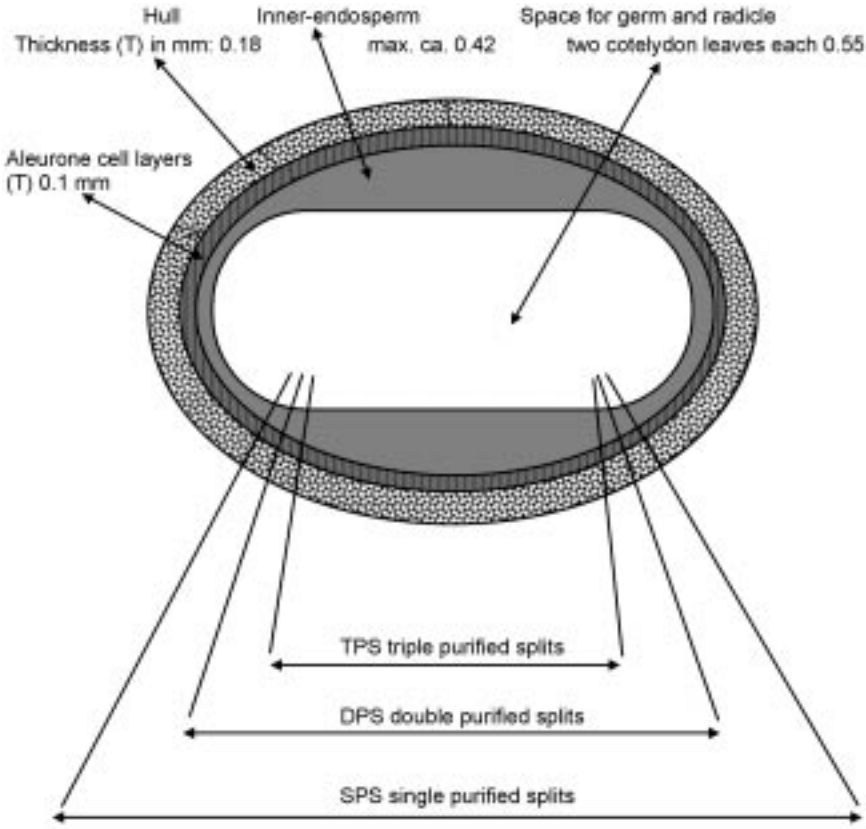

Fig. 10.2 A schematic cross section of guar seed. The endosperm consists of aleuronic cell layers attached to the inner endosperm. The number of mechanical purifications determines the yield of the split quality, each purification abrades more parts of the splits away, i.e. of SPS, DPS and of TPS (as imagined by the hypothetic intersection lines within the lower endosperm half, indicated by the widths between the arrows).

galactose side stubs in a row may occur in certain galactomannans of guar gum, and as many as 10–11 in specific galactomannans of carob bean gum. The fine structure of the polysaccharides of tara gum lies most probably between that of carob bean gum and guar gum. Figure 10.5 shows a possible model for a galactomannan of carob bean gum with about 20 wt% of galactose, with a molecular weight of 68,000 Daltons. (A similar model with a molecular weight of 3 million Daltons would require about 11 pages of this book.) The fine structure of the galactomannans of fenugreek gum does not pose a major problem since almost all mannose residues are substituted by galactose.

The biosynthesis of galactomannans is very complex. The following brief cascading steps describe to some extent the probable route:

- *Step 1*. Galacto- and manno-kinases i.e. phosphotransferases catalyze the phosphorylations of D-galactose to D-galactose-1-phosphate and of D-

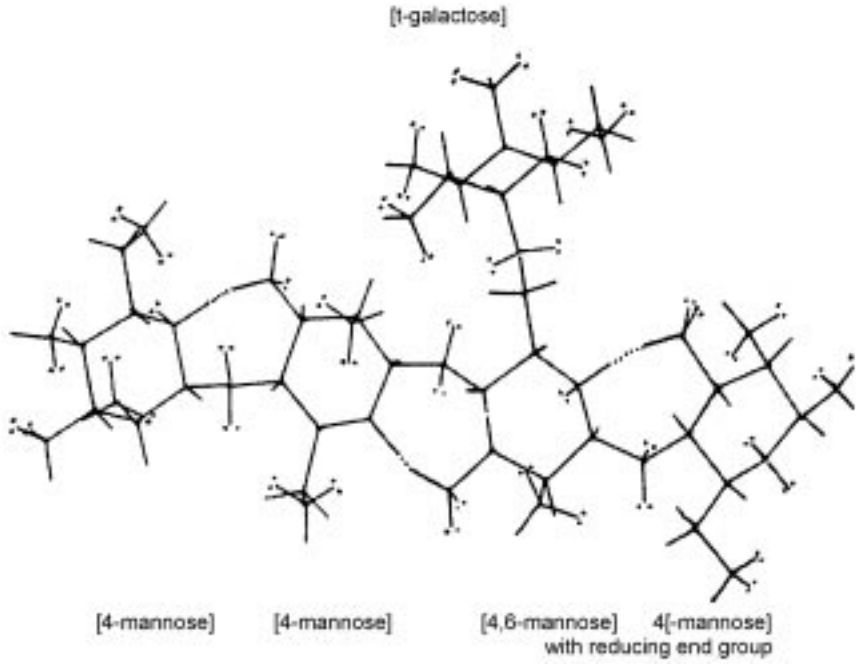

Fig. 10.3 Dreiding model of galactomannan with 20% by weight of D-galactose free electron pairs, indicated by 2 × 2 dots at oxygen atom. Hydrogen bond between OH-group at C3 of D-mannose residue and oxygen of pyranose ring of neighbouring D-mannose unit (on the left side).

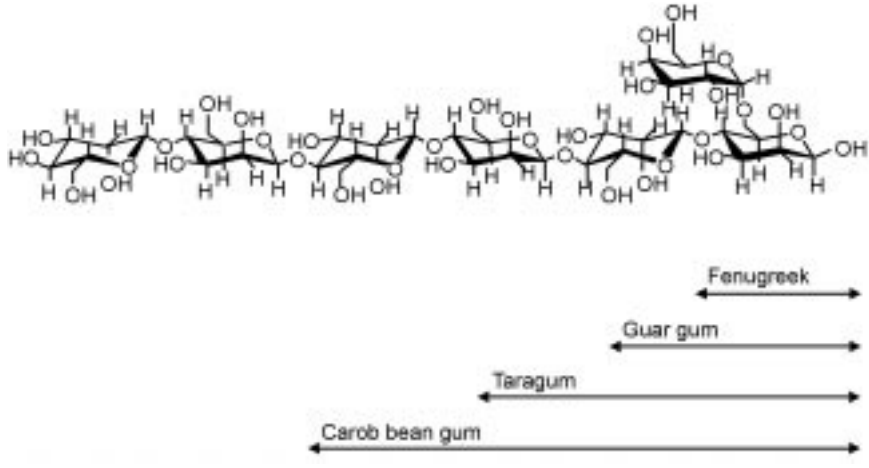

Fig. 10.4 Theoretical repeating units of four different galactomannans.

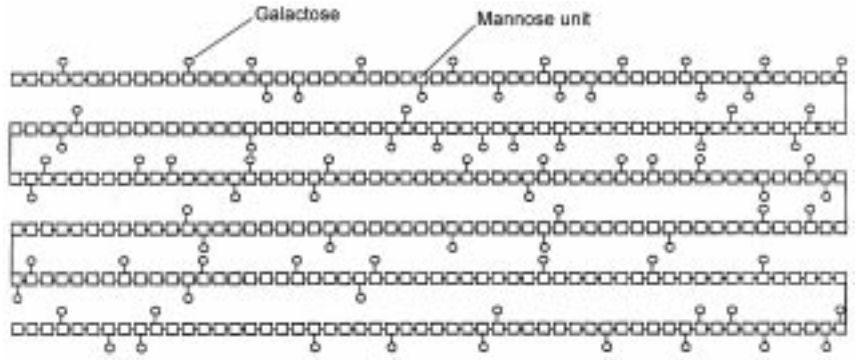

Fig. 10.5 Schematic galatomannan molecule with a molecular weight of 68,000 daltons with about 20% by weight of galactose with a random distribution.

mannose to D-mannose-1-phosphate. These mono-phosphated esters are strongly activated intermediates. The esterified C1 atom of the mannose-1-phosphate is the site to be linked to the growing non-reducing end of the mannan chain and the C1 of galactose-1-phosphate reacts with a primary OH group of the mannose unit, each liberating thereby the phosphate ester group.

- *Step 2*. Uridine triphosphate reacts with D-galactose-1-phosphate to uridine-diphosphate(UDP)-galactose and guanodine triphosphate with D-mannose-1-phosphate to form guanodine-diphosphate (GDP)-mannose. Both esters set pyrophosphate free at this reaction. UDP and GDP are considered to be hexose transferring coenzymes to form glycosides.
- *Step 3*. Now two different Golgi membrane-bound transferase enzymes come into action. One has probably two distinct binding sites for the coenzyme guanodine-diphosphate (GDP)-mannose, which is called the mannan synthase, and together with the co-factor, Mg-ions first elongate the nascent mannan chain each time by mannobiose. The second enzyme, a galactosyl-transferase with docking sites for the coenzyme uridine-diphosphate (UDP)–galactose cooperates with the Mn-ions to introduce the side stub onto this mannan backbone, releasing the UDP and GDP for the process again. Every mannose residue in the growing β-1,4-linked mannan backbone is rotated or inverted 180º with respect to its neighbouring residue, and therefore the sites for two guanosine-diphosphate-mannose molecules, inverted by 180º, must be available at the right position in the mannosyl-transferase. And two sites inverted by 180º for the uridine-diphosphate-galactose units must also be present within the galactosyltransferase to enable the substitution of galactose residues to mannose residues to the growing mannan backbone. The highly specific galactosyltransferase introduces one α- D-galactosyl unit only to one mannose residue, at or close to the non-reducing end of the growing chain, and another possibly to the next mannose unit, or to the following freshly condensed mannose units, inverted by 180º, if required.[6]
- *Step 4*. The galactomannans formed are deposited in layers on the endosperm cell walls of the seeds, according to genetically programmed size and

architecture. It is not yet clear how this deposition should be visualized. Perhaps an electron micrograph of a fractured guar split could offer some insight (Fig. 10.1).

No information has been found in the literature about how the molecular weight distribution of the galactomannans is accomplished during this reproducible synthesis. Many other questions also remain to be answered, including: 'How many different galactomannan chains are synthesized simultaneously in one seed?' and 'Does the time of day and temperature affect the size of the synthesized macromolecules?'.

- *Step 5*. After the complete biosynthesis of the four different beans over several weeks, they are allowed to air dry naturally while still in the fields, before the pods are harvested.

Synthetic and natural high molecular weight substances consist of blends of macromolecules with different degrees of polymerization. The distribution of molecular weights of these substances is therefore very important. Figure 10.6 shows these M_w distributions for carob bean gum, guar gum and xanthan gum. It

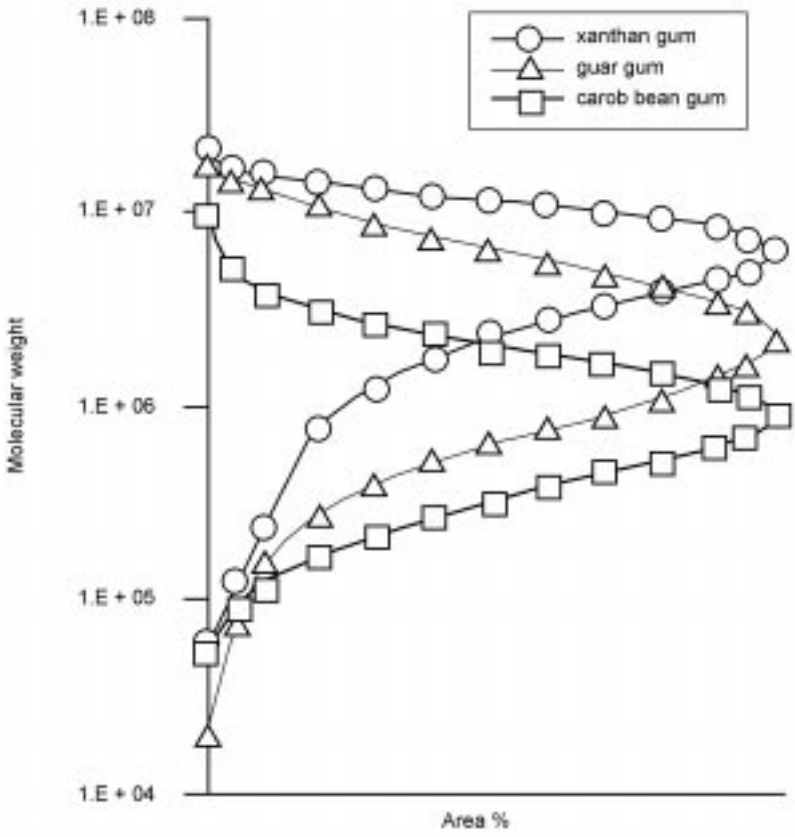

Fig. 10.6 GPC chromatogram of xanthan, guar and carob bean gum.

is not uncommon for polymeric materials to contain macromolecules ranging in molecular weights from a few hundred to several million Daltons.[7,8]

In 1948 Kubal and Gralen published the average molecular weight of carob bean gum as 310,000 Daltons, which was determined at 20 ºC with ultracentrifugation.[8] However, the investigated non-industrial sample of their test substance of carob bean gum in 1948 was prepared on bench scale in their laboratory and had an intrinsic viscosity [η] of 5.0 dl/g. Current industrial carob bean gum products (E410) now on the market have intrinsic viscosities of about 12.0 dl/g, thus significantly higher than that used by Kubal and Gralen.

Hui determined a molecular weight of 1,900,000 Daltons for a selected quality of guar gum, according to the same ultracentrifugation method at 25 ºC.[9] If Hui had used a more realistic value for the partial specific volume, this average molecular weight would have increased to 2,200,000 Daltons.

Industrial guar products (E412) now on the market show intrinsic viscosities of 0.7–15.0 dl/g, which correspond to apparent viscosities of aqueous solutions at 2 wt% concentration (based on 10% of moisture) of about 5 to 100,000 mPa.s, if measured with a Brookfield RVT viscometer, at 20 rpm at 25 ºC after full hydration.

Since 1998 EC and FAO/WHO specifications for the molecular weight of carob bean gum (E410) used as a food additive have required a range of approx. 50,000–3,000,000 Daltons. For guar gum (E412) these specifications call for molecular weights between 50,000 and 8,000,000 Daltons.

10.3 Manufacture

Commercial gum products do not always consist of pure endosperm, but may contain residual hull and germ parts. Trade products are specified mainly according to moisture content, viscosity of aqueous solutions, protein content, acid insoluble residue (an indication of the residual hull content) and particle size distribution.

To extract the endosperms from the seeds, the hull must first be removed as carefully as possible, either by mechanical or physical means. If the seed coat is removed chemically, as might be the case for carob and tara kernels, an efficient washing step with water is needed, followed by a drying process. Then the air-dry peeled seeds are split. In guar seed technology, the seeds are already cracked, after which most of the friable germ and other fines can be screened off. Additional purification cycles are normally needed for the guar split.

Seed varieties, weather conditions during growth and harvesting, geographical influences, different morphological structures of the endosperm, and processing conditions make an exact definition of trade products difficult.

10.3.1 Carob bean gum

The evergreen carob tree can be planted in semi-arid or subtropical zones and grows in calcareous soils. These trees are important for the vegetation of the

entire Mediterranean area, preventing too much erosion of the soil, especially in Morocco and Portugal. The carob tree can grow as high as 10–15 m and its roots can reach a depth of 25 m. It can live for more than 100 years. Grafted carob trees can be interplanted with olives, grapes, almonds and barley in low-intensity farming systems. They are also grown as ornamentals and for landscaping, windbreaks and afforestations.[10]

The carob tree normally yields fruits after 8–10 years and the fruits, i.e. the pods, can be harvested once a year. The pods are 10–30 cm long, 1.5–3.5 cm wide and 1.0 cm thick. They are dark brown in colour, and straight or curved in shape. The pods contain 8–12 seeds or kernels, but exceptionally up to 15 kernels. The fruits are collected when they have a moisture content of 12–18 wt%. They are shaken from the trees with long stakes. The fruits are then dried. The dry pods are leathery.

The kernels are released in a kibbling process, as described in Section 10.2. The hull is without much value. The germ contains about 50 wt% of protein and is used as cattle feed. It is also used as a colouring agent for certain Japanese noodles and in cookies, etc. To obtain the endosperm halves, it is necessary to remove the very hard hull of the seeds in a process called 'peeling' before separating the fragile germ. There are two different peeling processes:

1. *Chemical peeling process*: The hull is carbonized by concentrated sulphuric acid solutions at high temperature. The advantage of this technology is an even peeling effect, allowing the production of white powders. The process also facilitates the separation of the germ from the endosperm halves. Thus, high-grade qualities of carob bean gum with high viscosities can be made. The disadvantage of this technique is the effluent problem.
2. *Thermal mechanical peeling process*: The kernels are roasted at temperatures of up to at least 550 ºC, so that the hull pops off to a large extent. The residual hull fragments are rubbed off mechanically. As this also leads to a simultaneous cracking of some endosperm halves and germ parts, a clean separation of endosperm halves and germ/hull parts becomes more difficult. The advantages of this technology are that it requires only relatively simple production equipment, no special effluent treatment and that the yield is high. The disadvantages are that these carob bean gums show a somewhat lower quality and lower viscosity, all having a very light-yellowish colour due to a small amount of residual germ particles.

The endosperm halves of the carob kernel recovered by both these techniques are then ground to the desired fineness. Special technology produces cold-swelling carob bean gum, attaining at 25 ºC at least 60% of the viscosity of solutions prepared at 86–89 ºC for 10 minutes. Four such cold-swelling products have been developed with viscosities between 20 and 1,800–2,000 mPa.s, dissolved in water at 25 ºC, and with their 'hot' viscosities between 25 and 3,000 mPa.s, dissolved at 86–89 ºC for 10 minutes, at 1% concentration.

The same techniques used for carob kernels can also be used for tara seeds. The yield of high-grade tara gum is only 21–22 wt%, due to stringent food law

specifications with respect to protein content. The EU specification for the protein content of tara gum (E417) is ≤ 3.5 wt% ($N\% \times 5.7$), thus much lower than the specifications for carob bean gum of ≤ 7 wt% and guar gum of ≤ 10 wt% (both $N\% \times 6.25$). Tara gum, therefore, is the most purified of the three sources and one consequence of this is that a lower yield must be accepted. The above-mentioned 22 wt% of endosperm in the composition of the tara kernel can most probably be increased to about 28 wt%, with the hull and germ content adjusted as well. (The analysis of these galactomannan seeds is very difficult when one takes into account the manual separation of the seed components after the swelling of the seeds in water, followed by dehydration in an organic solvent.)

The tara tree or shrub is native to the Cordillera region of Bolivia, Peru and northern Chile and also grows in Ecuador, Colombia, Venezuela and Cuba.

10.3.2 Guar gum

Guar gum is the name of the ground endosperm halves, called guar splits, which are recovered from the seeds of the guar plant *Cyamopsis tetragonoloba* L. This plant is an annual summer legume that grows mainly in arid and semi-arid zones. It has deep, fibrous tap roots. It enriches the soil with nitrogen and is an ideal rotation crop with cotton and grains. Guar has been grown for centuries in the Indian subcontinent and is used as human and animal food. The word guar comes from the Sanskrit word 'Gau-ahar', in which 'gau' means cow and 'ahar' indicates food.

Unlike the seeds of the carob tree, which are called kernels, guar seeds are in fact called seeds. The appearance of guar seeds and the guar pod can be seen in Fig. 10.7. The colour of the seeds varies from light amber to yellowish green to grey olive. Black seeds are the result of the onset of decomposition due to microbiological attack, induced by rain at the wrong time. Black seeds cause problems at the manufacturing stage with regard to specks and the colour of the finished powder.

The average total quantity of guar seeds worldwide is estimated at about 500,000 tonnes p.a. However, large fluctuations of annual availability occur, mainly due to weather conditions. In order to become less dependent on these fluctuations and to meet the constantly increasing demand for guar products, agronomy programmes have been carried out in different parts of the world, especially in the southern hemisphere. Plantings have been made in Malawi, Australia, Colombia, Brazil and Argentina. Guar grows particularly well in parts of Texas, Oklahoma and Arizona. In Texas the harvest is carried out mechanically. The key factor for the success of all agronomy programmes outside the Indian subcontinent is economic conditions.

Guar seeds are composed of 20–22 wt% of hull, 44–46 wt% of germ and 32–36 wt% of endosperm on an absolute dry basis. Table 10.2 provides an analysis of guar seed components with regard to protein content, ash and moisture content, acid insoluble residue (AIR), matter extractable in ether and calculated

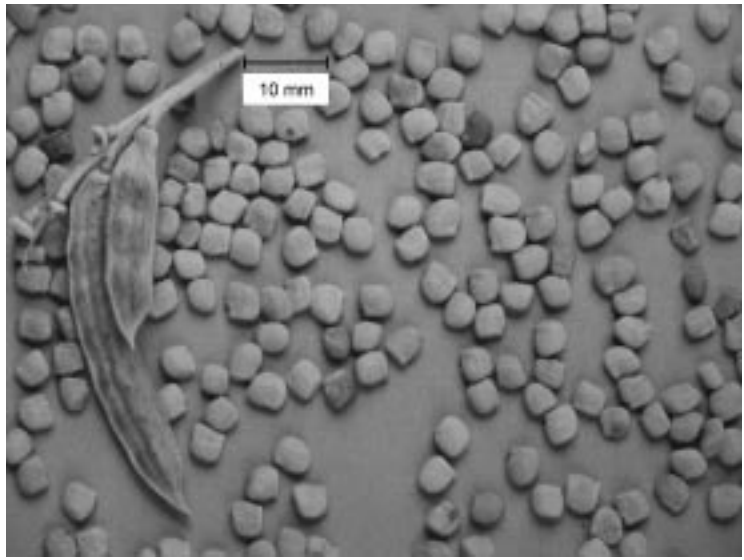

Fig. 10.7 Guar pods and seeds.

gum content, which is defined as [100 wt% − (wt% protein + wt% moisture + wt% AIR)]. The AIR is determined after hydrolysing the products for 6–8 h in 0.4N H_2SO_4 at boiling temperature. The germ contains about 50–55 wt% of protein and is sold as protein-rich cattle fodder, after reduction of the amount of trypsin inhibitor to an acceptable level. Figure 10.8 shows swollen pure guar seed components.

10.3.3 Production of guar splits and guar gum powder

The whole seeds can be fed into an attrition mill or any other type of mill with two grinding surfaces travelling at different speeds. The seed is split into the endosperm halves covered with hull, called the crude crack and fine germ material, which can later be sifted off. The crude crack is heated to soften the

Table 10.2 Composition and chemical analysis of guar seed components

		Composition of each portion					
Seed component	Weight fraction, dry matter	Protein %	Ether soluble %	Ash %	Moisture %	AIR %	Gum content %
Hull	20–22	5.0	0.3	4.0	10.0	36.0	49.0
Endosperm	32–36	5.0	0.6	0.6	10.0	1.5	83.5
Germ	44–46	55.3	5.2	4.6	10.0	18.0	16.7

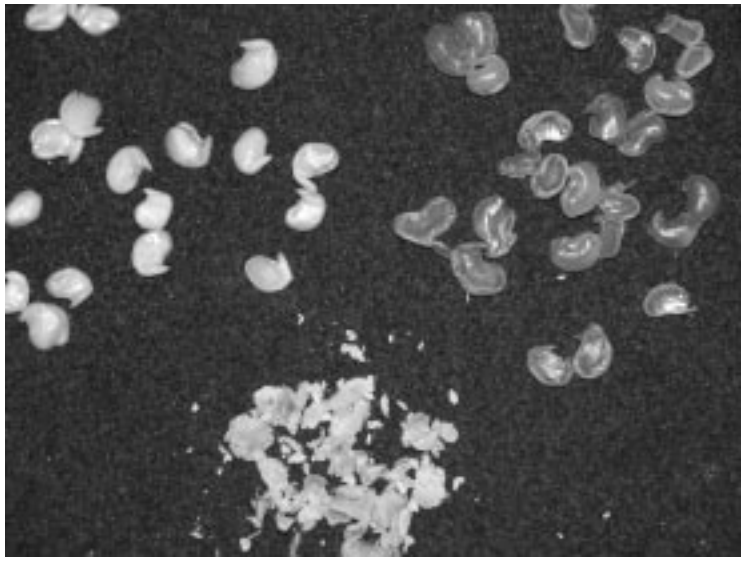

Fig. 10.8 Main seed components of guar seed. Germs are seen in upper left corner and swollen endosperm halves and overlapping edges in upper right corner. Hand-picked hull fragments are shown in the lower middle.

hull, after which it is fed into a mill which can either abrade the hull from the endosperm, or into a hammer mill, where the hull is shattered away. Any remaining germ particles are pulverized during this step and after a further sifting the resulting splits are essentially pure endosperm. The fine material, also containing the thin part of the endosperm which overlaps the small edges of the two cotelydon leaves and the radicles, is called guar meal and is marketed as cattle feed. It should show a minimum protein content of 35 wt% ($N\% \times 6.25$).

The guar endosperm or guar splits are processed to commercial powdered products by hydration, flaking if needed, milling-drying and screening techniques. Single-, double- and triple-purified splits are available on the market.

The endosperm of the guar seed consists of solid cell material and a split for high-grade guar products weighs 4–6 mg, whereas the endosperm half of the carob kernel shows a weight of 50–60 mg. The latter has a dense fibrous cell structure, significantly different from guar splits.

The guar splits are hydrated to different extents and might be flaked and then flash ground. The powdered product becomes sifted and the coarse fraction is recycled to a mill. The finer fractions are blended in order to meet the specifications for particle size distribution, protein, AIR, ash content and viscosities.

Guar is derivatized to supply the appropriate technical industries with hydroxyl-propyl-, hydroxyl-ethyl-guar, carboxy-methyl-hydroxyl-propyl guar, carboxy-methyl guar and cationic guar, as well as cationic hydroxyl-propyl guar, hydrophobic guar and guar phosphates (Fig. 10.9). Guar is depolymerized with heat and acid, with alkaline hydrogen peroxide, enzymatically by electron

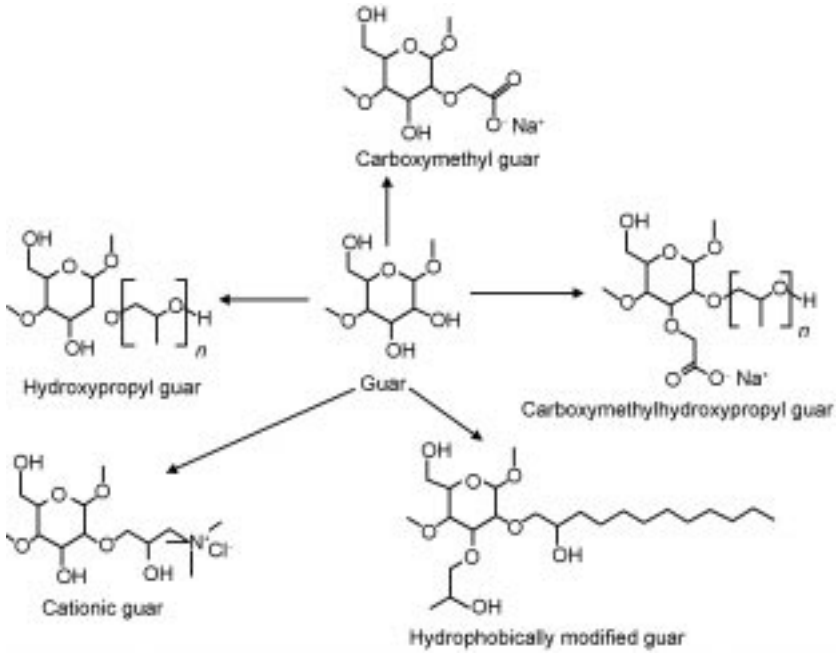

Fig. 10.9 Guar derivatives.

beams, by alkali in the presence of air at elevated temperatures and by irradiation with gamma rays.

Patented economical processes show that the quality of current guar products can be improved considerably. Such products can be used if high clarity aqueous solutions are required.

10.3.4 Production of fenugreek gum

Fenugreek (*Trigonella foenum graecum* L.) is an annual plant species with a height of up to 60 cm, belonging to the *Fabaceae* family. It is a multi-purpose legume that can be grown for the spice and pharmaceutical markets, or for green manure. It produces seeds which contain another kind of water-soluble galactomannans. These polysaccharides consist of mannan chains, of which almost all mannose units are substituted with single galactose residues. The weight of an Australian fenugreek seed is 13–17 mg and that of Canadian seeds 18–22 mg, whereas guar seed weight varies from 24 to 37 mg. Fenugreek seeds are 4–6 mm long and about 2–3 mm wide with a deep groove in the middle, giving them a hooked appearance. The ground seeds have a bitter taste and a special smell. Dry toasting of the seeds can enhance the flavour and reduce the bitterness. Small amounts of toasted ground fenugreek seeds should be found in any good curry powder. The fenugreek plant is native to the Mediterranean region. It has been grown in the Near and Middle East, Africa and India, and today is grown all over the world.

Patent number WO2004048419 filed by Air Green Co. Ltd (JP) in 2004 shows that the recovery of fenugreek endosperms can be achieved exclusively by physical procedures. The seed hull and germ are separated from the endosperms mechanically, presumably in a similar way to the production of the guar split, but also bearing in mind that the germ in the fenugreek seed is much more fragile than its hard endosperm. Morphologically, the embryonic radicle occupies a great deal of space within the seed and is covered by the hull and a thin endospermic film, which most probably becomes cracked away from the rest of the seed. The hull remaining on the main endosperm might also be partially torn off at the first grinding step. The different components of the seed can then be classified by screening. The crude endosperms can be further purified to meet the required specifications and the ground endosperm produces the desired grade of fenugreek.

Another technique, described in Patent No. CA2206157 (1998-11-26) by Emerald Seed Products LTD (CA), uses extraction of the galactomannans from the endosperm in water, then clarifying the extract and precipitating the dissolved gum with an alcohol, like ethanol. The alcohol-wet precipitate can be dehydrated and purified with more alcohol, then dried and ground to the desired fineness. Approximately 25 wt% of Canadian fenugreek seed is recoverable pure endosperm gum.

10.4 Technical data

The functionality of galactomannans is mainly due to their ability to change the rheology of aqueous systems. All four types described above are very efficient thickening agents in aqueous systems and can interact, if present, with agar-agar, Danish agar, carrageenans and xanthan gum to become more efficient with respect to thickening power, or to fortify three-dimensional stabilizing structures. The thickening power of galactomannans depends, of course, upon the size or length of the dissolved substituted mannan chains. Guar gum has the highest thickening power, followed by tara gum. Both carob bean gum and fenugreek gum show a somewhat lower viscosity than tara gum.

Guar gum containing galactomannans with galactose contents of 33–40 wt% is soluble in water at 25 ºC, provided the gum is accessible to the solvent water. The majority of galactomannans of carob bean gum with galactose contents of about 17–21 wt% need heat treatment of 10 minutes at 86–89 ºC, while stirring to dissolve in water. If the galactose content of the galactomannans of guar gum is decreased enzymatically to less than 12 wt%, the final products become insoluble even in hot water.

All natural galactomannans are non-ionic. The hydroxyl groups can be derivatized and thus can provide non-ionic, anionic, cationic and amphoteric derivatives. The primary and secondary hydroxyl groups show practically more or less the same reactivity. Random distribution of substituents is usually obtained. The galactose side stubs each have one primary and three secondary

OH-groups, the substituted mannose unit two secondary OH-groups, and the unsubstituted mannose has two secondary and one primary OH-group. The maximum average degree of substitution (DS) is, therefore, three. The introduction of more than one substituent to one hydroxyl group leads to a molar substitution.

Galactomannans are susceptible to strong acids, organic acids like citric, acetic and ascorbic acid, for instance, to alkali in the presence of air and strong oxidizing agents, especially at elevated temperatures, and also to electron beams and towards irradiation with γ-rays, so that depolymerizations of the galactomannans can be achieved to different extents or may occur randomly.

Both mannose and galactose contain vicinal secondary *cis*-hydroxyl groups, on 2, 3 and 3, 4 respectively. These vicinal *cis*-hydroxyl groups form cyclic complexes with appropriate reagents (such as borate). Semi-dry processes, therefore, allow the production of derivatives and modified products, which can be water-washed if required. The removed impurities and dissolved gums in the effluent can be treated anaerobically to produce methane, which is a welcome additional energy source for the factory. Figure 10.9 illustrates some of the guar derivatives available.

New patented technologies enable the production of ionic, non-ionic and amphoteric guar products, which upon dissolution in water yield water-clear solutions, even at an actual DS of about 0.1. Purified non-derivatized guar products are available for food applications. Dissolved in water, they also yield solutions of excellent clarity. More than 90% by weight of the available carob bean gum and tara gum are no longer derivatized. These products are used as straight gums as food additives in the food and pet-food industries. Table 10.3 summarizes some characteristics of two high-grade carob bean gums.

The powdered guar products are specified for the viscosity of the fully hydrated solutions, according to a specified method (but not an optimal one) and mesh analysis. The other specifications of wt% of H_2O, wt% of protein, wt% of AIR, wt% of ash and pH of 1 wt% aqueous solutions are respectively 12, 5, 3, 1 wt% and 6.5 for guar M100, M175, M200 and M225. Guar CSA 200/50 has slightly less protein and less AIR. The minimum viscosity of these guar products is respectively 3,000 mPa.s for the first three gums, and 3,600 and 5,000 mPa.s for the other two.

The viscosity of the aqueous solutions, prepared at 25 ºC, of the two tara gums M175 and M200 at 1 wt% concentration are both 3,000 mPa.s. Prepared at 86–89 ºC for 10 minutes, the coarser product has a higher viscosity of 4,400 mPa.s compared to the finer M200 type of 4,000 mPa.s.

If 1 wt% solutions of a regular CBG M200 and a cold swelling CBG M200, prepared at 86–89 ºC for 10 minutes while stirring at low shear rates, are spun out at 35,000 g for 30 minutes, respective quantities of insolubles of 17 wt% and 14 wt% are found. The 1 wt% solutions of these two products, prepared at 25 ºC, contain respectively 39 and 70 wt% soluble galactomannans. Additionally, heat treatment at 86–89 ºC solubilizes 44 wt% of galactomannnans for the regular CBG and, as might be expected, much less, i.e. only 16 wt% of galactomannans,

Table 10.3 Typical analysis of two carob bean gum

Specification	High grade CBG M175	High grade CBG M100
% H_2O	10.0–12.0	10.0–12.0
% protein ($N\% \times 6.25$)	6.5	6.5
% AIR	2.0	2.0
1% viscosity, 10 min at 86–89 ºC RVT Brookfield, 20 rpm, 25 ºC mPa.s	min. 3,000	min. 3,000
– M80 (max)	99%	99%
– M200 (max)	25%	10%
Trace elements		
As, ppm	0.2	0.2
Pb, ppm	ca. 0.03	ca. 0.03
Cu, ppm	2.5	2.5
Zn, ppm	5.6	5.6
Cd, ppm	ca. 0.05	ca. 0.05

for the cold swelling product. This means that the latter product can stabilize systems at much lower temperatures than regular CBG, which is very important during a heat process.

Certain galactomannans of carob bean gum in aqueous solutions self-associate under defined conditions, such as at a freezing process when nano-crystalline regions of 3–5 nm can be formed, which alternate with much bigger amorphous regions. Nevertheless, it was found that a test system of this kind yields a weak gel upon thawing, and therefore carob bean gum is used as an excellent ice cream stabilizer.

The company Emerald Seed Products (101 Wood Mountain Trail East, Box 149 Avonlea, SK, Canada, S0H 0C0) produces Canafen Gum, a fenugreek gum from seeds grown in Canada.

Table 10.4 shows the chemical analysis of fenugreek seeds. The applications of this fenugreek gum are considered to be the same as for the other gums described in this chapter. It might be a better candidate as a healthy food ingredient. The galactomannans of fenugreek endosperms are much more resistant towards enzymatic breakdown in the digestive tract, providing the most effective fibre in retaining viscosity. The mannan chains are protected by the side stubs of the galactose units, since the mannose residues in this gum are almost completely substituted. Acid hydrolysis apparently does not reduce the viscosity of the aqueous solutions of fenugreek gum to the same extent as in the other three types: carob bean gum, tara gum and guar gum. Fenugreek gum, therefore, is an ideal ingredient for functional foods with reduced glycemic indices.

The Japanese company Air Green (http://www.airgreen.co.jp/fenugreek/index_e.html) has three grades of fenugreek on the market with different galactomannans content, i.e. one over 86 wt%, the second with 80–86 wt% and the lowest grade containing 60–80 wt%. The specifications of these products are summarized in Table 10.5.

Table 10.4 Chemical analysis of fenugreek seeds (source: November 2006, University of Saskatchewan)

Component	% by weight
Dietary fibre	45.4
Insoluble dietary fibre	32.1
Soluble dietary fibre	13.3
Protein	36.0
Oil	6.0
Ash	3.2
Starch	1.6
Sugar	0.4

Table 10.5 Specifications of three fenugreek products, varying in gum content

Description	Over 86% gum content	80–86% gum content	60–80% gum content
Colour	white or slight yellow	light yellow	light brown
Smell	slight original smell	slight original smell	slight curry smell
AIR %	≤ 3	≤ 3	≤ 5
Water insoluble matter %	≤ 3	≤ 3	≤ 5
Heavy metal as Pb ppm	≤ 20	≤ 20	≤ 20
ppm As	≤ 2	≤ 2	≤ 2
H_2O %	≤ 10	≤ 10	≤ 10
Viscosity, 1%, 25 ºC	$\geq$ 3,400 mPa.s	$\geq$ 3,000 mPa.s	$\geq$ 1,000 mPa.s
Granularity	$\leq$ M70	$\leq$ M70	$\leq$ M100
Protein %	≤ 5	≤ 5	≤ 5
Starch	negative	negative	negative
Fat %	≤ 1	≤ 1	≤ 1
Fibre %	≥ 86	≥ 80	≥ 70
Caloric value kcal/g	≤ 1	≤ 1	≤ 1
Ash %	≤ 1.5	≤ 1.5	≤ 1.5
Galactomannans %	≥ 86	≥ 80	≥ 60
counts per g Bacteria	≤ 300	≤ 300	≤ 300
counts per g Fungi	≤ 300	≤ 300	≤ 300
counts per g *E. Coli*	negative	negative	negative

The fact that guar is freeze-thaw stable had already been shown in 1984, whereas carob bean gum solutions generally form gels after a freezing process. A blend of these two galactomannan gums, 1:1, tends to form gels as well rather than being freeze-thaw stable.[11]

Details of depolymerized guar products can be found in a publication from 1990.[12] The molecular weights, M, of depolymerized guar products in kDa, based on limiting viscosity data and characterized by their Brookfield RVT viscosity of 1 wt% solutions, $\eta_{\mathrm{Br}\,1}$ after full hydration, measured at 20 rpm, at 25 ºC in mPa.s, show a rather good fit with a squared correlation coefficient of 0.9874 with the following power law equation:

$$M = 99.072 \times (\eta_{\mathrm{Br}\,1})^{0.3674} \qquad 10.1$$

The guar products investigated showed Brookfield viscosities, as described above, of ca. 17–5,000 mPa.s. A pocket calculator can be used to calculate the molecular weights in a simple way. This also holds true for the other relationships determined, described below, for the different regression parameters.

Cold swelling CBG with defined Brookfield viscosities of 25–3,000 mPa.s at 1 wt% concentration were characterized by SEC (size exclusion chromatography) to determine the peak, M_w and M_n molecular weights, called M in equation 10.2.[13] These molecular weights in Da (Y-axis) were plotted against the usual Brookfield viscosities $\eta_{\mathrm{Br}\,1}$ (X-axis) and the curves followed an excellent fit with polynomial equations, at least in the above-mentioned viscosity range. The general polynomial regression equation is:

$$M = a \cdot (\eta_{\mathrm{Br}\,1})^2 + b \cdot (\eta_{\mathrm{Br}\,1}) + c \qquad (R^2 = 1) \qquad 10.2$$

Table 10.6 summarizes the different equation coefficients and the squared correlation coefficients for the above-mentioned polynomial equation.

The Brookfield RVT viscosity of high-grade carob bean gum M100 solutions in mPa.s, prepared at 86–89 ºC for 10 minutes, while stirring with concentrations, c wt%, based on 10 wt% moisture, of about 0.5 wt% to about 2.5 wt%, fits the following power law equation:

$$\eta_{\mathrm{Br}} = 2992.1 \cdot (c)^{3.7507} \qquad (R^2 = 0.9979) \qquad 10.3$$

The same potential relationship was also tested for cold swelling carob bean gum in the concentration range of 0.4–2.0 wt%, prepared at 86–89 ºC and at 25 ºC, leading to the following equation values, i.e., the theoretical viscosity at 1 wt% in mPa.s, the exponent n and the squared correlation coefficient summarized in Table 10.7.

Similar data for the different depolymerized and straight guar products are also found in Table 10.8, where the word 'cold' between brackets means dissolved at 25 ºC and 'hot' means 86–89 ºC for 10 minutes.

The curve for P150 (cold), range of concentration 0.3–3.0 wt%, was evaluated by a polynomial regression, giving a better fit, and the following regression equation was obtained:

$$\eta_{\mathrm{Br}} = 1401(c)^3 + 6050(c)^2 - 2896(c) + 313 \qquad (R^2 = 1) \qquad 10.4$$

Table 10.6 Different coefficients of the polynomial equations and the squared correlation coefficients for four solutions of cold swelling cbg of different viscosities at 1% by weight, dissolved at 86–89 ºC for 10 minutes

Type of M_w	Coefficient a	Coefficient b	Coefficient c	Coefficient of correlation R^2
Peak	−0.0262	353.89	146,120	0.9996
M_w	−0.0558	432.41	206,146	0.9999
M_n	−0.110	186.71	97,701	0.9997

Table 10.7 Power law data for solutions of cold swelling carob bean gum at 1% by weight in water

Temperature of dissolution ºC	Calculated viscosity of 1% solution in mPa.s	Exponent (n)	Correlation coefficient R^2
25	1571	3.9202	0.9999
86–89	2790	3.8125	0.9996

Table 10.8 Power law data for different guar products, dissolved in demineralized water

Product type	Calculated viscosity of 1% solution, mPa.s	Exponent n	Squared correlation coefficient R^2	Concentration range in % by weight
P150 (cold)				
P120 (cold)	2237	3.4577	0.9953	0.3–3.0
P90 (cold)	735	3.5225	0.9981	0.3–4.0
P60 (cold)	74	3.8009	0.9992	0.6–5.0
P30 (cold)	16	3.5414	0.9875	0.9–5.0
P150 (hot)	5642	2.6188	0.9995	0.5–3.0
P120 (hot)	4141	2.7484	0.9998	0.5–3.0
P90 (hot)	998	3.4231	0.9996	0.4–4.0
P60 (hot)	137	3.2220	0.9896	0.5–5.0
P30 (hot)	20	3.3844	0.9916	0.5–5.0
P7 (hot)	0.32	3.4268	0.9901	3.0–15.0
P50 (hot)	648	3.6788	0.9980	0.4–2.0
P100 (hot)	1215	3.6865	0.9976	0.4–2.0
P200 (hot)	2115	3.4673	0.9973	0.4–2.0

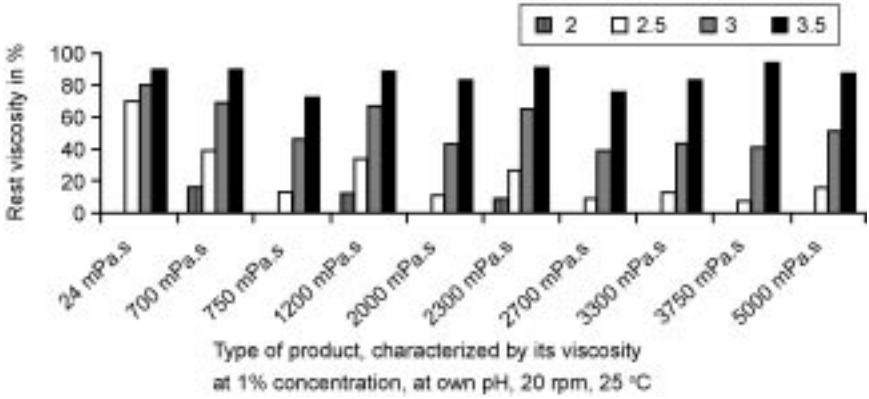

Fig. 10.10 Acid stability of different guar solutions at certain pHs prepared at 86–89 ºC for 10 minutes, while stirring, expressed as rest viscosity in %, based on viscosity at pH 4 times 100%.

The acid stability of depolymerized and straight guar products, specified by the Brookfield viscosity at 25 ºC, 20 rpm at 1 wt% solutions, was tested at different pH values. The products were dissolved at the required pH at 86–89 ºC for 10 minutes and the rest viscosity was expressed by the quotient of the viscosity measured at the defined pH and the viscosity at pH 4 times 100% (see Fig. 10.10).

The guar products which were purposely depolymerized at acidic pH and with heat are more stable towards acidic hydrolysis in the final applications than modified guar products obtained after an alkaline oxidation.

In a liquid food system with a pH below 3.5 one must expect a certain depolymerization of the guar galactomannans, while heating at elevated temperatures. An increase of temperature of a liquid system from 20 to 80 ºC, stabilized with galactomannans, decreases the viscosity at 80 ºC by about 50%, but upon cooling to original temperature, the viscosity returns.

10.5 Uses and applications

These thickening and gelling agents are widely used as additives in food products, mainly to make them appealing and attractive to the consumer. Furthermore, they should also improve shelf-life by binding water, control the texture, influence crystallization, prevent creaming or settling, improve the freeze-thaw behaviour, prevent syneresis and the retrogradation of starch products, maintain turbidity in soft drinks and juices and act as dietary fibres. This means that these food additives find applications mainly in convenience food, dairy products, including frozen products (ice cream), soft drinks and fruit juices, bread and pastry, fruit preserves, baby food, and as household gelling agents in puddings, flans and pudding powder, as dietary fibres, and in pet foods.

They also are used in pharmaceutical and cosmetic products. Other non-food applications of galactomannans are found in the textile industry (carpet dyeing and textile printing), and in the paper, mining, explosive, drilling, construction, oil field and chemical industries.

The functional properties of regular carob bean gum are listed in Table 10.9. Similar tables can also be created for guar gum, tara gum and fenugreek gum. Further examples of applications are to be found in references 11 and 12. The work of P.H. Richardson *et al.* is particularly informative about the behaviour of carob bean gum and guar gum in aqueous sucrose solutions.[13]

10.6 Regulatory status

Apart from fenugreek gum, the other three gums are approved food additives with the following E-numbers: E410 for carob bean gum, E412 for guar gum and E417 for tara gum. Fenugreek gum is on the GRAS list in the USA. It is not quite clear whether fenugreek gum should be considered as a food ingredient.

Table 10.9 Functional properties of carob bean gum

Function	Example	Use level (%)
Adhesion	Glazes, juices	0.2–0.5
Binding agent	Pet foods	0.2–0.5
Body agent	Dietetic beverages	0.2–1.0
Crystallization inhibitor	Ice cream, frozen foods, bread	0.1–0.5
Clouding agent	Fruit drinks, beverages	< 0.1
Dietary fibre	Cereals, bread	0.2–0.5
Foam stabilizer	Whipped toppings, ice cream	0.1–0.5
Gelling agent	Pudding, desserts, confectionery	0.2–1.0
Moulding	Gum drops, jelly candies	0.5–2.0
Protective colloid	Flavour emulsions	0.2–0.5
Suspending agent	Chocolate milk	<0.1
Swelling agent	Processed meat products	0.2–0.5
Synergistic agent	Soft cheeses, frozen products	0.2–0.5
Thickening agent	Jams, pie fillings, sauces, baby food	0.2–0.5

10.7 Future trends

The four gums described, and especially their depolymerized modifications with their distinct overall galactose contents, can all be used either in themselves or in blends to fulfil certain requirements. The dissolution technique used for guar gum products is important. For instance, by applying high shearing forces and heat, 10–15% more gum goes into solution, yielding a higher viscosity. Depolymerized gums could offer major advantages in solving difficult problems, such as improving the creaminess of certain dairy products, to name but one.

As a new potential candidate, fenugreek gum extends the choice for accomplishing the desired hydrocolloidal functionality.

With an improved separation of the three guar seed components, it might, for example, be possible to bleach the hull fraction and use it as insoluble dietary fibre that can absorb a great deal of liquid. The pure germ fraction could be toasted and used as a snack component, since it has a nutty taste. The purer endosperm fraction would lead to an improved quality of guar gum.

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11

Gum arabic

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Abstract: This chapter provides a review of the science and technology of gum arabic. It includes a section concerned with the source and market trends of the material together with how it is collected, graded and processed. The precise definition of gum arabic is discussed. There has been much work undertaken on the chemical and physicochemical characteristics of the gum and this chapter summarises developments in this area and presents the latest views on the molecular structure. In summary the gum is believed to contain three distinct fractions which all consist of a highly branched carbohydrate structure and which differ principally in their molecular mass and protein contents. These are now commonly referred to as the arabinogalactan-protein, arabinogalactan and glycoprotein fractions. The key functional characteristics of the gum are that it forms viscous solutions only at high concentrations and that it is able to stabilise oil-in-water emulsions. Indeed it is the emulsifier of choice in the beverage industry. The relationship between the structural characteristics of the gum and its functional properties is discussed. The recent work on the health benefits of gum arabic is also reviewed.

Key words: gum arabic, *Acacia senegal*, *Acacia seyal*, wattle-blossom structure, arabinogalactan-protein, molecular mass distribution, viscosity, emulsification properties, confectionery, beverages, flavour encapsulation.

11.1 Introduction

Gum arabic or gum *Acacia* is a tree gum exudate and has been an important article of commerce since ancient times. It was used by the Egyptians for embalming mummies and also for paints for hieroglyphic inscriptions. A number of review articles have been published concerning its origin, structure, properties and applications over recent years.[1–6] Traditionally the gum has been

obtained mainly from the *Acacia senegal* species. The trees grow widely across the Sahelian belt of Africa situated north of the equator up to the Sahara desert and from Senegal in the west to Somalia in the east. The gum oozes from the stems and branches of trees (usually five years of age or more) when subjected to stress conditions such as drought, poor soil or wounding. Production is stimulated by 'tapping', which involves removing sections of the bark with an axe taking care not to damage the tree. The sticky gummy substance dries on the branches to form hard nodules which are picked by hand and are sorted according to colour and size (Fig. 11.1). Commercial samples commonly contain *Acacia* species other than *Acacia senegal* notably *Acacia seyal*. In Sudan the gum from *Acacia senegal* and *seyal* are referred to as hashab and talha respectively. The former is a pale to orange-brown solid which breaks with a glassy fracture and the latter is darker, more friable and is rarely found in lumps

Fig. 11.1 Collecting gum arabic from *Acacia senegal* trees.

Table 11.1 Grades of Sudanese gum

Grade	Description
Hand-picked selected	The most expensive grade. Cleanest, lightest colour and in the form of large nodules.
Cleaned and sifted	The material that remains after hand-picked selected and siftings are removed. Comprises whole or broken lumps varying in colour from pale to dark amber.
Cleaned	The standard grade varying from light to dark amber. Contains siftings but dust removed.
Siftings	Fine particles remaining following sorting of the choicer grades. Contains some sand, bark and dirt.
Dust	Very fine particles collected after the cleaning process. Contains sand and dirt.
Red	Dark red particles

in export consignments. Hashab is undoubtedly the premier product but the lower priced talha has found recent uses which have boosted its value. It is not possible to identify precisely the exact balance between these two products in the market-place since it is continually changing. Some typical grades of Sudanese gum available are listed in Table 11.1.

11.2 Supply and market trends

Sudan has traditionally been the main producer of gum arabic and supplies in the late 1960s were in excess of 60,000 tonnes p.a. Drought and political unrest in the 1970s and 1980s resulted in supplies dropping to a low of ~20,000 tonnes p.a. Nigeria and Chad are the other main producers with combined exports of ~10,000 tonnes p.a. The current estimate of the total gum arabic production is 40–50,000 tonnes p.a. Europe is the largest market for the gum and imports averaged ~30,000 tonnes p.a. in the early 1990s. Outside Europe, the biggest market is the USA where imports totalled 10,000 tonnes in 1994. Japanese imports are around 2,000 tonnes p.a.

The variability of supply over the past 20–30 years has led to dramatic fluctuations in price and in turn injected uncertainty into the user market. When supplies almost dried up in the 1970s the price increased from about $1,500 to $5,000 per tonne and then even to $8,000. It is almost impossible to evaluate the equivalence at today's prices since inflation in commodity prices has been uneven and less than in manufactured goods. The price stabilised in 1996 to around $5,000 per tonne but then overnight the Sudanese dropped their price to $2,500 per tonne which led to consternation and extreme problems for the industry's processors, who were left holding stocks at the higher price. The price

reduction was motivated by the inability of the Sudanese to move their stocks which were reported to be in the region of 42,000 tonnes at that time. The price dropped even further in 1998 to $1,800 per tonne. It is now the same in the USA as it was in the 1970s and even cheaper in Europe. Inflation in Sudan has been rampant and hence the price in Sudanese pounds may look attractive locally but hides a dramatic decrease in revenue for the country. The low prices have had a severe effect in Chad and now the value of the trees for energy and other uses approaches the value of the gum. There is currently a great deal of uncertainty in the market-place making long-term planning difficult.

The situation with talha is somewhat different. Hitherto it has been regarded as an inferior gum only to be used for a price advantage or when supplies of hashab were low. The 1996 price of $500 reflected this position. More recently, however, the functionality of talha in 'health beverages' as a fermentable fibre to maintain the well-being of the colon has given it a value in its own right. There has been a rush for talha so much so that it is now difficult to obtain supplies of the required quality. Sudan has depleted its stocks and it is believed it does not intend to increase production. The government policy is to maintain the hashab plantations only and to use the talha trees for charcoal production. There are indications that this policy might change in view of increasing demand. The price is now approaching that of hashab. The traditional product is *Acacia seyal var. seyal* but there is also another material, the so-called 'white talha' from *Acacia seyal var. fistula* which Chad is seeking to exploit. The future supplies of talha, however, are likely to come from Nigeria where it is referred to as 'Nigerian No. 2'.

11.3 Manufacture

Following export to Europe and the US, some grades are processed providing greater quality and convenience to the user but also increasing the price by $1,000 to $1,500 per tone.[7] Processing can involve mechanical grinding (kibbling) which breaks up the nodules into various specific sizes. One of the benefits of kibbled gum is that it dissolves at a much faster rate than lump gum. Spray dried and roller-dried grades are also produced. These processes involve dissolving the gum in water with heating and stirring. The temperature is kept to a minimum in order to ensure that the gum is not denatured since this can have a deleterious effect on its functional properties. After removing insoluble material by decantation or filtering, the solution is pasteurised and then spray or roller dried. Spray drying involves spraying the solution into a stream of hot air. The water quickly evaporates and the dry powder typically 50–100 microns, is separated from the air using a cyclone. During roller drying the solution is passed onto steam-heated rollers and the water is evaporated off by a flow of air. The thickness of the gum film produced is controlled by adjusting the gap between the rollers. The film is scraped off the roll using a knife yielding flake-like particles several hundred microns in size. Spray-dried and roller-dried

samples have an advantage over the raw and kibbled gum in that they are virtually free of microbial contamination and they dissolve much faster.

11.4 Regulatory aspects

Gum arabic has been evaluated by the Joint Expert Committee on Food Additives (JECFA) on several occasions resulting in changes in specifications. Surprisingly, however, none of these specifications were recommended for adoption by the Codex Alimentarius Commission. The specification changes made are summarised below:

- *FAO Food and Nutrition Paper No. 4 1978*: 'A 1 in 10 solution of the sample filtered through diatomaceous earth is slightly laevorotatory'.
- *FAO Food and Nutrition Paper No. 25 1982*: No change
- *FAO Food and Nutrition Paper No. 34 1986*: The requirement of specific rotation is eliminated.
- *FAO Food and Nutrition Paper No. 49 1990*: The specific rotation was re-introduced and 'should be within −26º and −34º. Nitrogen must be between 0.27 and 0.39%'. Gum arabic is defined as 'a dried exudation obtained from the stems and branches of *Acacia senegal* (L) Willdenow or closely related species'. (For the first time nitrogen and specific rotation limits were imposed and the word 'closely' introduced.)
- *FAO Food and Nutrition Paper No. 52 Add. 3 1995*: Both nitrogen and specific rotation requirements were removed but the word 'closely' retained. Additionally tests were introduced to ensure that manose, xylose and galacturonic acid were absent thus eliminating non-*Acacia* gums.
- *FAO Food and Nutrition Paper No. 52 Add. 5 1997*: The acceptance of *Acacia seyal* as a 'closely related' species was acknowledged.

It is evident, therefore, that *Acacia seyal* has always been regarded as a component of commercial gum arabic apart from the time when the radical change was made to the specification in 1990 which was subsequently abandoned in 1995. The full specification that acknowledged this for the first time arose from the 49th JECFA meeting in 1997 and was thought to be the final definition (INS 414). This states as follows:

> Gum Arabic is a dried exudate obtained from the stems and branches of *Acacia senegal* (L) Willdenow or closely related species of Acacia (fam. Leguminosae). *Acacia seyal* is a closely related species. Gum arabic consists mainly of high molecular weight polysaccharides and their calcium, magnesium and potassium salts which on hydrolysis yield, arabinose, galactose, rhamnose and glucuronic acid. Items of commerce may contain extraneous materials such as sand and pieces of bark which must be removed before food use. Gum Arabic from *Acacia seyal* is sometimes referred to as talha.

However, this specification was again changed at the 51st JECFA (1998). There are again important changes, as described in Food and Nutrition Paper 52 Add. 6 (1998). The important changes are summarised here.

Synonyms: Gum arabic (*Acacia senegal*) Gum hashab, kordofan gum
Gum arabic (*Acacia seyal*) Gum talha
Acacia gum, arabic gum INS No 414

The geographical names have not previously been included and related only to the Sudan, and do not represent the other gum arabic producing countries.

Definition: Gum arabic is a dried exudation obtained from the stems and branches of *Acacia senegal* (L) or *Acacia seyal* (fam. *Leguminosae*). Gums from other *Acacia* species are not included in these specifications.

The references to related species as in all other published specifications or closely related species introduced by JECFA in 1990 have been deleted, on the misguided assumption that the producer developing countries could produce one species without any other related species being included unwittingly by the local farmer. For such an eventuality the phrase related species has always been included.

Description: Gums from *A. Senegal* and from *A. seyal* respectively, may be differentiated using immunological methods.

Gum arabic (*A. senegal*) and gum arabic (*A. seyal*) are not necessarily technologically interchangeable.

These new characteristics were presumably introduced to allow the two species that make up gum arabic to be distinguished and to acknowledge their different applications. The revised Gum Arabic Specification prepared at the 51st JECFA (1998) held in Geneva was finally referred for approval to the Codex Committee for Food Additives and Contaminants held in the Hague, in March 1999. A Working Group was convened to screen all the proposals. No definitive conclusion could be taken and the Report of the Working Group reflected the lack of consensus. JECFA were unwilling to take it back for further review because no new scientific information was available to them. Gum arabic, therefore, faced the prospect of being left out in the cold with neither approval of the current JECFA Specification nor its rejection – a most unsatisfactory situation for all concerned.

Eventually in the Plenary Session with all countries present, gum arabic came up for consideration. The old arguments again surfaced, but as the discussion continued, apart from the Sudan, one producing African country after another supported the JECFA Specification. Many trade organisations too added their voices in favour, and then an almost unprecedented situation occurred – the Chairman gave the lead and proposed acceptance of the Specification in Category II (Recommended for Adoption after Editorial Changes, including Technical Revisions). The Editorial changes suggested were the following:

- Under 'Synonyms' delete Gum hashab, kordofan and Gum talha.
- Under 'Definition' delete the last sentence (Gums from other Acacia species are not included in these specifications).
- Under 'Description' delete fourth and fifth indent, i.e. sentences referring to Immunological differentiation and technological interchangeability.

This was the proposal which was accepted and went to the Codex Alimentarius Commission at its 23rd Session in Rome, 28 June–3 July 1999 and adopted. It is thus a historic occasion. For the first time there is an approved Codex Alimentarius Advisory Specification for Gum arabic (Annex 1) which establishes the definition as:

> Gum arabic is a dried exudation obtained from the stems and branches of *Acacia senegal* (L) or *Acacia seyal* (fam. Leguminosae).

There are other regulatory definitions which are adhered to according to specific interests or geographical location.

11.4.1 EU Gum Arabic Specification (E414)

> Acacia gum is a dried exudation obtained from the stems and branches of natural strains of *Acacia senegal* (L) Willdenow or closely related species of Acacia (Fam. Leguminosae). It consists mainly of high molecular weight polysaccharides and their calcium, magnesium and potassium salts, which on hydrolysis yield arabinose, galactose, rhamnose and glucuronic acid.

Thus despite contrary proposals in various drafts, the EU has followed JECFA and not introduced specific rotation limits. However, in an unexpected and quite inexplicable innovation, the Directive has introduced a molecular weight clause indicating that gum arabic should have a molecular weight of ca. 350,000. This appears inappropriate since in the scientific literature molecular weight values of between 200,000 and 800,000 have been reported.

European Pharmacopoeia
Definition: Acacia Gummi (Acacia)

> Acacia is the air-hardened gummy exudate flowing naturally from or obtained by incision of the trunk and branches of *Acacia senegal* (L) Willdenow and other species of Acacia of African origin.

The specification has retained 'A 10% w/v solution is laevorotatory'. Implicitly, therefore, the definition acknowledges the acceptability of *Acacia seyal* within commecial gum arabic.

United States Food Chemical Codex
The Food Chemical Codex is an activity of the Food and Nutrition Board of the Institute of Medicine that is sponsored by the United States Food and Drug Administration. The gum is defined (INS 414) as:

> A dried gummy exudation obtained from the stems and branches of *Acacia senegal* (L) Willdenow or of related species of Acacia (Fam. Leguminosae). Unground Acacia occurs as white or yellowish white spheroidal tears of varying size or in angular fragments. It is also available commercially in the form of white to yellowish white flakes, granules or powder. 1 g dissolves in 2 mL of water forming a solution that flows readily and is acid to litmus. It is insoluble in alcohol.

It should be noted that there is no specific rotation requirement in the current definition.

United States Pharmacopeia and the National Formulary

> Acacia is the dried gummy exudate from the stems and branches of Acacia senegal (linne) or of related African species of Acacia (Fam. Leguminosae).

This definition was reported in the USP 21st Revision, January 1st 1985 NF XVI ed. Official Monographs for USP XXI p. 1538. It remains the same in the current Official Monograph for NF 18 (USP2) 1996. In the 22nd Revision (November 15th 1991) the following was introduced: 'Add the following to Specific rotation: between -25^{o} and -35^{o}, calculated on the anhydrous basis determined on a 1% w/v solution'. However, a subsequent supplement (7USP XXII–NF XVII 1992) removed the specific rotation requirement. USP and NF, therefore, now fall in line with the EU and JECFA.

A new comprehensive regulatory status for gum arabic

As already described, the regulatory approval to use Acacia gum (*Acacia senegal* and *Acacia seyal*) in food has been as a food additive, designated E414 in European Commission Directives and INS 414 in JECFA/WHO and Codex Specifications. The specified uses were as a thickener and stabiliser, neither of which described accurately the many and varied functionalities of gum arabic food products. The industry, through representations via the Association for the International Promotion of Gums, has claimed that this is insufficient since gum arabic is used extensively also as an ingredient in up to 50% or more in some products with functions as a food and in this role forming the structure of the food matrix.

For at least 10 years various EU Working Groups have considered the legal status of additives, such as gum arabic, for which there are dual uses as both ingredient and additive in food. The functions were considered distinct and would require independent approvals.

Two EU bodies and corresponding national authorities in Member States covering Novel Foods and Food Additives were involved in the legal and practical considerations. The advice was that if it could be proved that the use of gum acacia as an ingredient and for special functions as a food dietary fibre was established practice in the food industry before May 1997, then its approval as a food

ingredient could be authorised. This was the date when the Novel Foods Legislation was introduced and would otherwise be the only route to gain regulatory acceptance of the ingredient status. The mechanism was to petition one Member State, which, if the case was accepted after detailed scrutiny of the evidence, the new status could recommend acceptance to the European Commission.

The detailed dossier setting out the use as a food ingredient in products where gum acacia actually formed the matrix of the food, as distinct from imparting a specific technological function was presented to the UK Food Standards Agency (UKFSA). It was accepted on 23 February 2007 when a letter of authorisation was issued by the UKFSA, and this information was passed to relevant EC bodies to achieve uniform European Union acceptance.

This was a historic development in the progress of gum acacia, enabling its wide-ranging role in foods to be officially approved. As a dietary fibre its role is recognised and the only labelling requirement is to record the dietary fibre content as measured by an officially recognised method. This has now been formally recognised by the EU.

11.5 Structure

The gums from *Acacia senegal* and *Acacia seyal* are complex polysaccharides and both contain a small amount of nitrogenous material that cannot be removed by purification.[1–6] Their chemical compositions vary slightly with source, climate, season, age of the tree, etc., but typical analytical data for each are given in Table 11.2. The gums consist of the same sugar residues but *Acacia seyal* gum has lower rhamnose and glucuronic acid contents and higher arabinose and 4-O-methyl glucuronic acid contents than gum from *Acacia senegal*. *Acacia seyal* gum contains a lower proportion of nitrogen and the specific rotations are also very different. Determination of these latter parameters can provide a rapid means of differentiating between the two species. The amino acid compositions are similar (Table 11.3) with hydroxyproline and serine being the major constituents.

Both gums have complex molecular mass distributions that display similar features but the average molecular mass of gum from *Acacia seyal* is higher than that of *Acacia senegal* (Table 11.2). Typical molecular mass profiles of the two gums obtained by gel permeation chromatography (GPC), using refractive index (RI), coupled with light scattering detection and UV absorbance (206 nm) detection are presented in Figs 11.2(a) and (b) respectively. Refractive index is a sensitive measure of gum concentration and the profiles indicate that the gums consist of two components, the main one (peak 1) representing ~90% of the total with a molecular mass of ~ 250,000 for *Acacia senegal* and ~600,000 for *Acacia seyal*. The other (peak 2), which represents about 10% of the total, has a molecular mass of ~1–2 million. The UV absorbance profiles differ considerably and show three peaks. Two correspond to the peaks observed by refractive index but the intensities are different. This has been shown to be due to the presence of

Table 11.2 Characteristics of gum from *Acacia senegal* and *Acacia seyal*

	Acacia senegal	*Acacia seyal*
% galactose	44	38
% arabinose	27	46
% rhamnose	13	4
% glucuronic acid	14.5	6.5
4-O-methyl glucuronic acid	1.5	5.5
% nitrogen	0.36	0.15
Specific rotation/degrees	−30	+51
Average molecular mass (Mw)	380,000	850,000

Table 11.3 Amino acid composition of *Acacia senegal* and *Acacia seyal* gums (residues/1000 residues)

	Acacia senegal	*Acacia seyal*
Hyp	256	240
Asp	91	65
Thr	72	62
Ser	144	170
Glu	36	38
Pro	64	73
Gly	53	51
Ala	28	38
Cys	3	
Val	35	42
Met	2	
Ile	11	16
Leu	70	85
Tyr	13	13
Phe	30	24
His	52	51
Lys	27	18
Arg	15	11

higher concentrations of proteinaceous material in the high molecular mass fraction.[8–10] The third peak corresponds to protein-rich material and represents only about 1% of the total mass. This fraction has a molecular mass of ~200,000.

Most structural studies have been concerned with the gum from *Acacia senegal*. Carbohydrate analysis has indicated that the components of this gum corresponding to the three UV absorbance peaks all have a highly branched structure consisting of a β-1,3-linked D-galactose core with extensive branching through 3- and 6-linked galactose and 3-linked arabinose.[11] Rhamnose and glucuronic acid are positioned at the periphery of the molecules where they terminate some of the branches (Fig. 11.3). The main component (peak 1), commonly referred to as the arabinogalactan component (AG), contains <0.5%

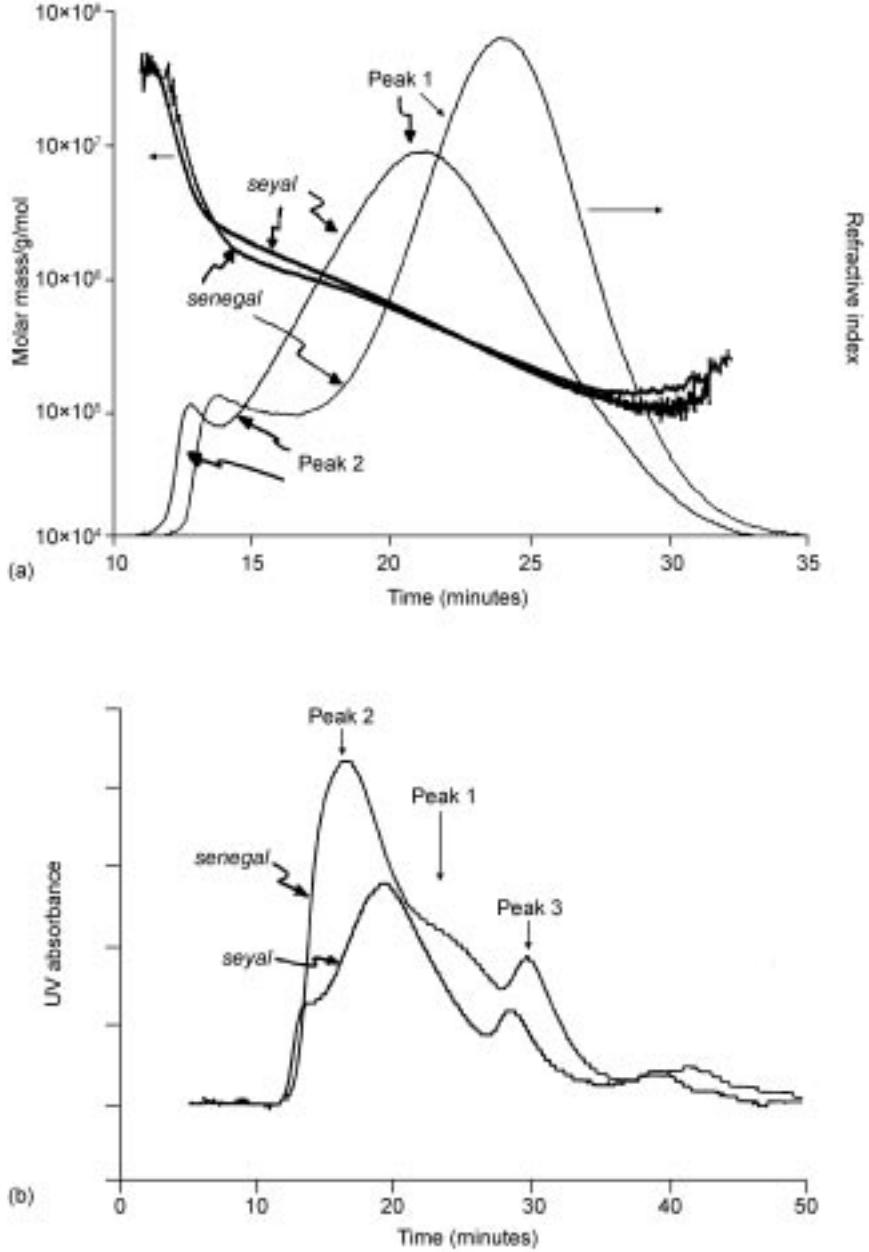

Fig. 11.2 Molecular mass distribution of *Acacia senegal* and *Acacia seyal* gums obtained by gel permeation chromatography using (a) refractive index and multiangle laser light scattering detection; (b) UV absorbance at 206 nm detection.

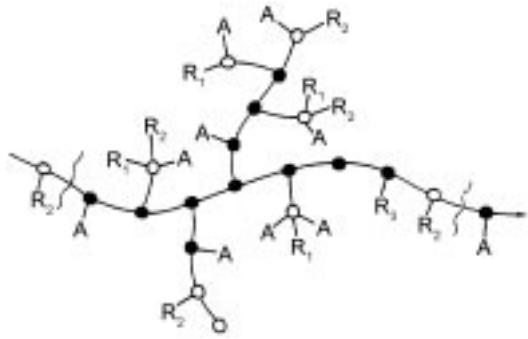

Fig. 11.3 Possible structure of the carbohydrate component of gum from *Acacia senegal*. A = arabinosyl; filled circles = 3-linked galactose (galactose attached); open circle = 6-linked galactose (galactose or glucuronic acid attached or end group); R_1 = rhamnose-glucuronic acid; R_2 = galactose-3arabinose; R_3 = arabinose-3arabinose-3arabinose (from reference 3).

protein, and recent studies using small angle neutron scattering and transmission electron microscopy have indicated that the molecules have a thin disk-like structure with a radius of gyration of ~6.5 nm (Fig. 11.4).[12] Material corresponding to peak 2 has a protein content of ~10% and is referred to as the arabinogalactan protein complex (AGP). Since this fraction is readily degraded by proteolytic enzyme it has been reported to have a 'wattle blossom-type' structure where blocks of carbohydrate are linked to a polypeptide chain through both serine and hydroxyproline residues.[13,14] It has recently been found that the polypeptide chain consists of ~250 amino acids and that the carbohydrate blocks have a molecular mass of ~40,000.[15] It is likely that the

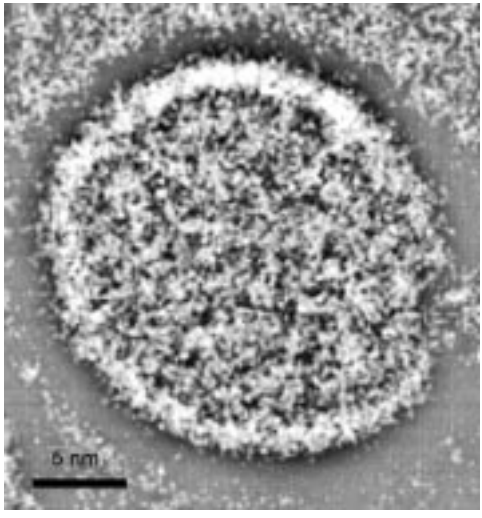

Fig. 11.4 Cryo-transmission electron micrograph of the arabinogalactan component of gum arabic (from reference 12).

carbohydrate blocks are disk-like in shape in keeping with the structure of the AG fraction. The molecules adopt a compact conformation and have a radius of gyration of ~36 nm and a schematic representation of their structure is given in Fig. 11.5.

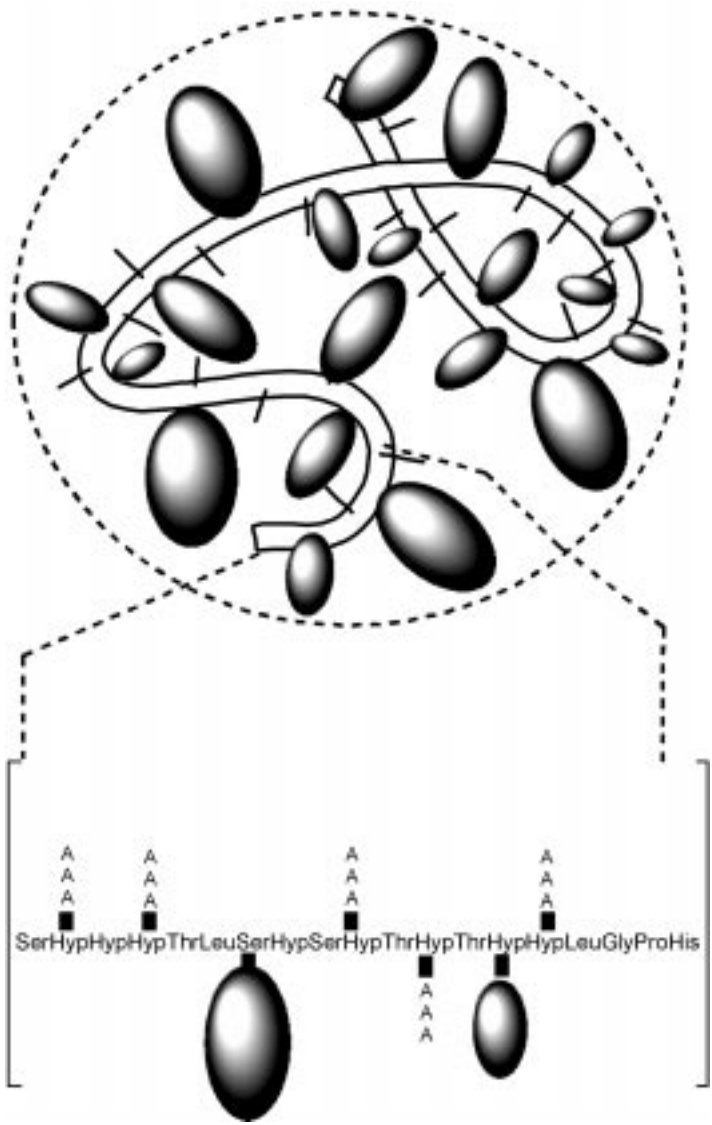

Fig. 11.5 Schematic illustration of the structure of the gum arabic arabinogalactan protein complex. The molecule has a molecular mass of (1–2) $\times$ 10^6 Da and consists of a polypeptide chain possibly containing ~ 250 amino acids with short arabinose side chains and much larger blocks of carbohydrate of molecular mass ~ 4.0 $\times$ 10^4 Da and attached. The molecule adopts a very compact conformation with R_g about 36 nm (from reference 15).

Material corresponding to peak 3 is referred to as the glycoprotein component, (GP), and has a lower glucuronic acid content than the other two fractions with a reported protein content of 20–50%. This fraction is not degraded by proteolytic enzyme. This may be due to the fact that the proteinaceous component is located within the centre of the molecule or it may be as a consequence of its sequence of amino acids since its amino acid composition is different to the AG and AGP components.[8] Whereas the predominant amino acids in the AG and AGP fractions are hydroxyproline and serine, the predominant amino acids in the GP fraction are aspartic, serine, leucine and glycine. All three fractions interact with Yariv's reagent and hence strictly speaking can all be classified as arabinogalactan– protein complexes (AGPs).[14]

11.6 Properties

Gum arabic readily dissolves in water to give clear solutions ranging in colour from very pale yellow to orange-brown and with a pH of ~4.5. The highly branched structure of *Acacia senegal* gum gives rise to compact molecules with a relatively small hydrodynamic volume and as a consequence gum solutions only become viscous at high concentrations as illustrated in Fig. 11.6. A comparison of the viscosity of the gum with xanthan gum and sodium carboxymethylcellulose, which are common thickening agents, is shown in Fig. 11.7. It is seen that even 30% gum arabic solutions have a lower viscosity than 1% xanthan gum and sodium carboxymethylcellulose at low shear rates. In addition, while gum arabic is Newtonian in behaviour with its viscosity being

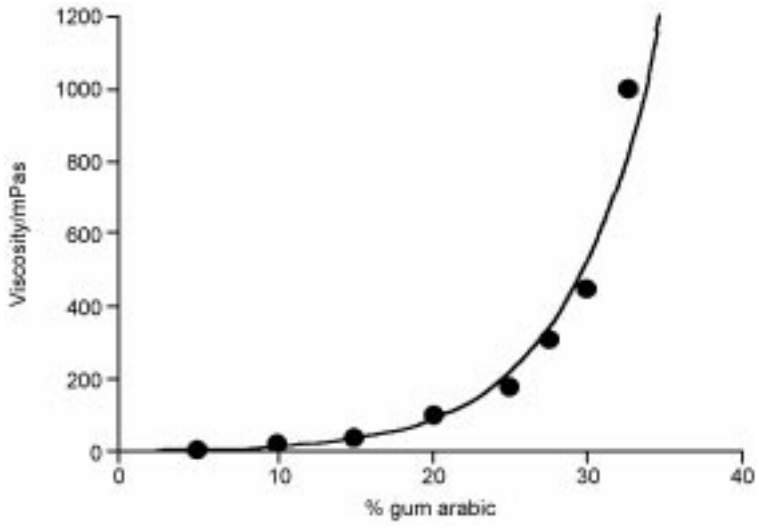

Fig. 11.6 Viscosity of gum arabic as a function of concentration.

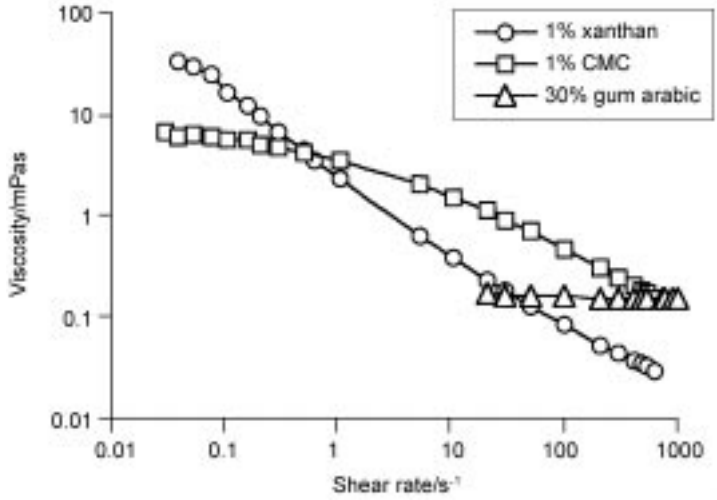

Fig. 11.7 A comparison of the viscosity shear rate profiles of solutions of 1% xanthan gum, 1% sodium carboxymethyl cellulose and 30% gum arabic.

shear rate independent, both xanthan gum and sodium carboxymethyl cellulose display non-Newtonian shear thinning characteristics. This is explained by the fact that the latter are linear molecules and have a much larger hydrodynamic volume such that intermolecular entanglements can occur at much lower concentrations than for the highly compact, branched gum arabic molecules. It has recently been reported that gum arabic solutions do show evidence of shear thinning at very low shear rates even at concentrations in the dilute regime below the critical overlap concentration.[16] Interestingly, it has also been found that the viscosity of gum arabic solutions (3%) increases over time when subjected to a constant shear (Fig. 11.8) and that gum arabic solutions (6%) develop weak gel characteristics on standing for a 2 h period with the storage modulus increasing significantly and becoming larger than the loss modulus (Fig. 11.9).[16] This behaviour has been attributed to intermolecular aggregation.

Since gum arabic is a polyelectrolyte, the solution viscosity decreases in the presence of electrolytes due to charge screening and at low pH when the carboxyl groups become undissociated.

The other major functional characteristic of gum arabic is its ability to act as an emulsifier for essential oils and flavours. It has been found that the protein-rich components adsorb preferentially onto the surface of the oil droplets.[8] This is demonstrated in Fig. 11.10 which shows the RI and UV absorbance (280 nm) GPC elution profiles of gum Arabic before and after preparing a limonene oil-in-water emulsion. The RI profile is sensitive to the concentration of gum arabic eluting while the UV absorbance at 280 nm is sensitive only to the concentration of proteinaceous material eluting. The difference in the intensity of the two RI and two UV profiles before and after emulsification represents the amount of gum arabic and protein adsorbed respectively, and indicates that there is no

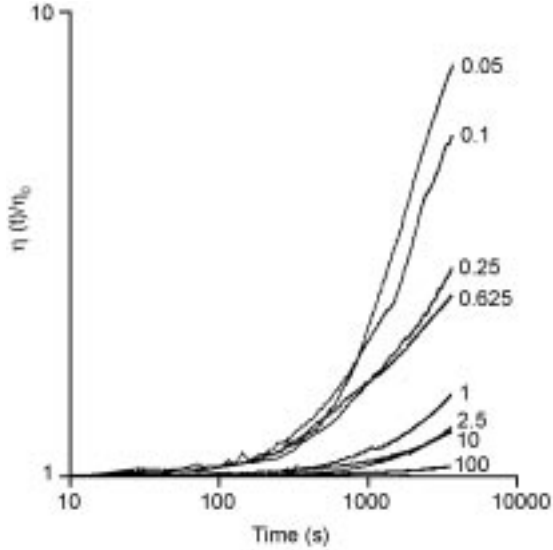

Fig. 11.8 Effect of shearing time on the relative viscosity of 3% gum arabic solutions at pH 4.2 at varying shear rates as indicated on the figure (from reference 16).

molecular mass dependency and that the adsorbed molecules contain protein. It is envisaged that the hydrophobic polypeptide chains adsorb and anchor the molecules to the surface while the carbohydrate blocks inhibit flocculation and coalescence through electrostatic and steric repulsions. This is schematically

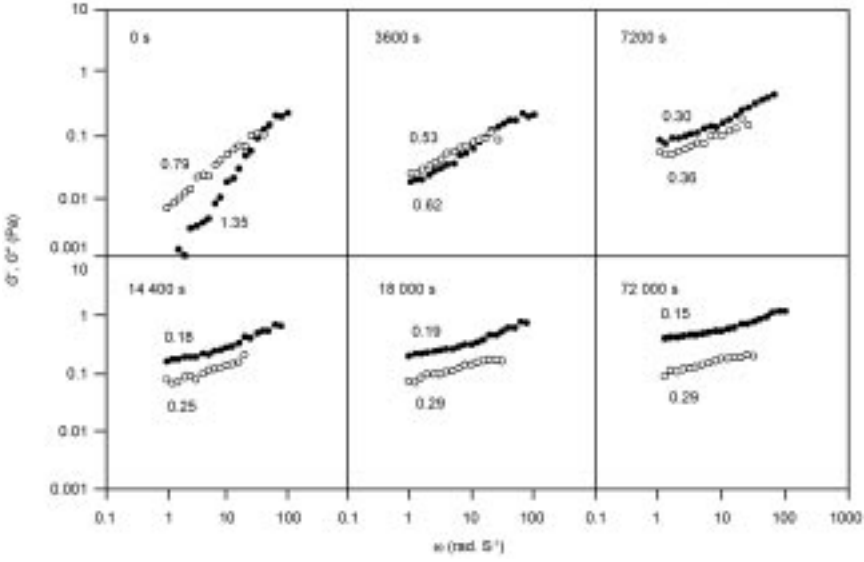

Fig. 11.9 Storage and loss moduli of 6% gum arabic solutions at pH 4.2 after standing for varying times as indicated in the figure (from reference 16).

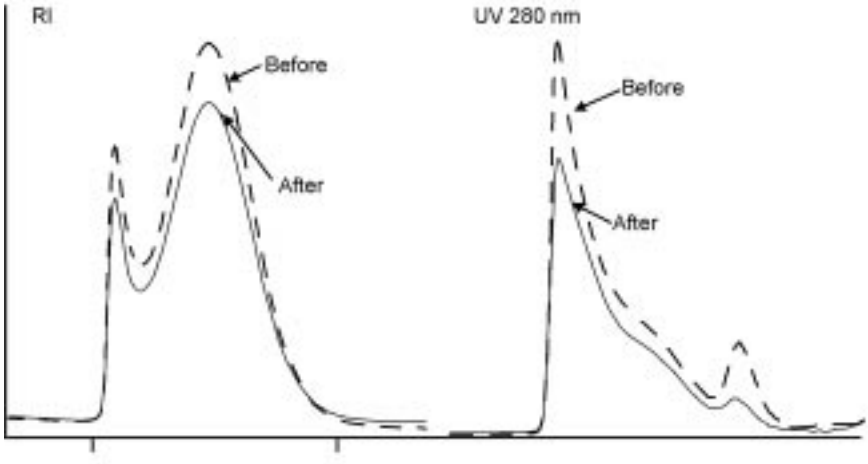

Fig. 11.10 GPC elution profiles monitored by refractive index (left) and UV absorbance at 280 nm (right) of gum arabic solutions before and after emulsification. The difference in the intensity represents the amount of gum arabic adsorbed onto the surface of the oil droplets.

illustrated in Fig. 11.11. Treatment of gum arabic with proteolytic enzyme to remove the protein results in a loss of emulsification efficiency. Since only the proteinaceous components of the gum are involved in the emulsification process, the concentration of gum required to produce an emulsion is much higher than for pure proteins. For example, in order to produce a 20% orange oil emulsion, then gum arabic concentrations of ~12% are required. Once formed the emulsions can remain stable for long periods of time (several months) with no evidence of coalescence occurring. Prolonged heating of gum arabic solutions causes the proteinaceous components to precipitate out of solution thus influencing the gum's emulsification properties.

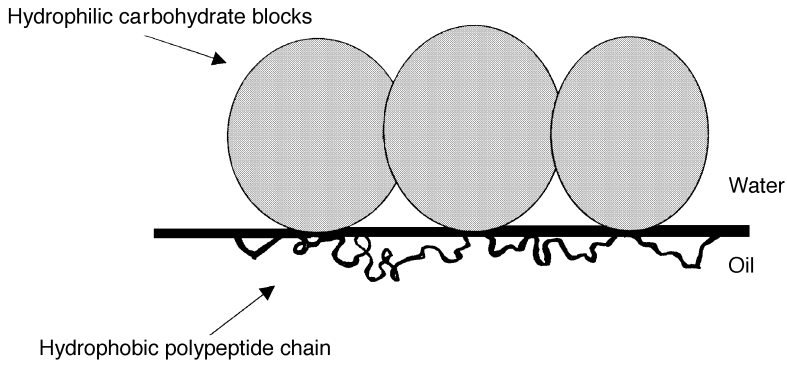

Fig. 11.11 Schematic illustration of the stabilisation of oil droplets by gum arabic molecules.

A new process has been developed to produce a new generation of native gum arabic with greatly improved emulsification properties and is now in commercial use.[17] This new gum arabic family, termed SUPERGUM, has all the natural qualities of the traditional gum arabic with reproducible and improved functionalities. The process is carried out in a dry stainless steel container or on a suitable surface, open to air or in a non-oxidising environment (an atmosphere of nitrogen) under strictly controlled conditions of temperature and humidity. The method promotes the further maturation of gum arabic in a way that emulates and extends that which occurs naturally. As the tree grows in the Sudan the molecular mass of the exuded gum arabic increases from ~250,000 (at 5 years) to ~450,000 (after 15 years) and the amount of protein and high molecular mass AGP fraction also increases with the age of the tree. The maturation process accelerates and enhances this same natural aggregation process, under strictly controlled conditions. The smaller arabinogalactan units containing some protein associate to form larger molecular mass AGP aggregates. In all aspects this specially matured gum is chemically and molecularly identical to the base gum, but because of the difference in distribution of the proteinaceous components, the physical and functional performance is greatly enhanced. The molecular aggregation process is illustrated at the molecular level in Fig. 11.12 which shows the GPC elution profiles of gum arabic subjected to the maturation process and demonstrates an increase in the intensity of the AGP component with ageing time.

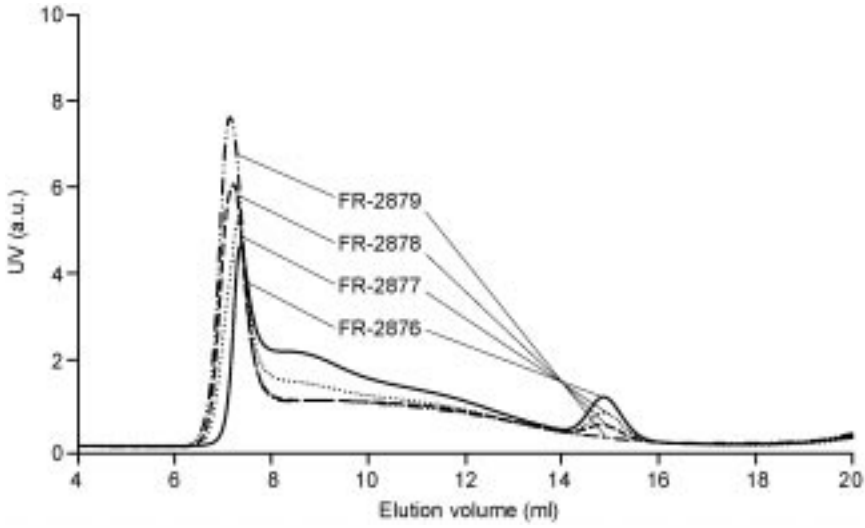

Fig. 11.12 GPC UV absorbance (214 nm) elution profiles of gum arabic after various times of maturation showing the increase in the AGP high molecular mass peak.

11.7 Applications

11.7.1 Confectionery

The major application of gum arabic is in the confectionery industry where it is used in a variety of products including gums, pastilles, marshmallows and toffees. Traditional wine gums incorporated gum arabic at concentrations of 40–55% and wine was used to add flavour. During the preparation the gum is dissolved in water keeping the temperature as low as possible (~60 ºC) in order to avoid precipitation of the proteinaceous components which would give rise to a turbid solution. The gum is then added to a pre-boiled sugar/glucose solution (70%) followed by the flavourings and colours. After standing to allow air bubbles to rise, any surface scum is removed and the liquid deposited into starch trays which are placed in a stoving room for 4–6 days. The gums are then taken from the moulds, brushed to remove starch and often glazed with oil or wax. Softer gums or pastilles can be obtained by reducing the stoving time to 2–3 days. In recent times, because of gum shortages and price fluctuations, considerable effort has been made to find replacements for gum arabic and nowadays pastilles are prepared using gum arabic at much lower concentrations in combination with other hydrocolloids, notably starch, maltodextrin, gelatin, pectin and agar. In these formulations demixing may occur due to incompatibility between the various hydrocolloids. The extent of demixing will depend on the rate of gel formation induced by the other hydrocolloids present and will consequently dictate the final texture obtained.

In marshmallows the gum is used as a foam stabiliser while in toffees it is used to emulsify the fats present. Typical formulations are given in Tables 11.4 and 11.5. Gum arabic is also used to form a glaze on coated nuts and similar products.

Table 11.4 Typical formulation for marshmallows

Water	39.0%
Sugar	37.0%
Dextrose	19.0%
Albumen	1.8%
Gum arabic	2.4%
Gelatin	0.5%
Salt	0.3%

Table 11.5 Typical formulation for caramel-type products

Corn syrup	38.4%
Sweet condensed whole milk	34.4%
Granulated sugar	9.6%
Invert sugar	9.6%
Hydrogenated vegetable oil	3.8%
Salt	0.2%
Gum arabic	4.0%

11.7.2 Beverages

Gum arabic is stable in acid conditions and is widely used as an emulsifier in the production of concentrated citrus and cola flavour oils for application in soft drinks. The gum is able to inhibit flocculation and coalescence of the oil droplets over several months and furthermore the emulsions remain stable for up to a year when diluted up to ~500 times with sweetened carbonated water prior to bottling. In the preparation of the emulsion, a weighting agent is normally added to the oil in order to increase the density to match that of the final beverage and thus inhibit creaming. Typical weighting agents that are used, subject to legislation in various parts of the world, are glycerol ester of wood, gum damar and sucrose acetate isobutyrate (SAIB). SAIB is not normally used by itself but usually in conjunction with rosin or gum damar. The emulsion is prepared by adding the oil to the gum arabic solution under high speed mixing followed by homogenisation yielding a droplet size of ~1 micron. A typical formulation might contain 20% gum arabic, 10% flavour oil and 5% weighting agent while the final beverage might contain 0.1–0.2% concentrated emulsion, 10% sugar and 0.2% citric acid/colouring.

11.7.3 Flavour encapsulation

Microencapsulation is commonly used to transform food flavours from volatile liquids to flowable powders that can be readily incorporated into dry food products such as soups and dessert mixes. The process also renders the flavour stable to oxidation. Encapsulation involves spray-drying an emulsion of the flavour oil which is produced using gum arabic as emulsifier. Nowadays maltodextrin is commonly mixed with the gum in order to reduce costs. The spray-dried particles formed are typically 10–200 microns in size and the retention of the volatile material, which is normally >80%, depends on a number of variables including the inlet temperature of the spray dryer, the emulsion concentration and viscosity and the proportion of gum arabic to maltodextrin. Typical formulations are given in Table 11.6.

Table 11.6 Formulation for flavour encapsulation

Flavour	7%	10%
Gum arabic	28%	15%
Maltodextrin	nil	25%

11.7.4 Gum arabic as a dietary fibre

In regulatory terms there is no agreed international definition of dietary fibre. Codex Alimentarius has been trying for a number of years to get agreement on the following comprehensive definition:

> Dietary fibre means carbohydrate polymers with a degree of polymerisation (DP) not lower than 3, which are neither digested nor absorbed in the small intestine. A degree of polymerisation not lower than 3 is intended to exclude mono- and disaccharides. It is not intended to reflect the average DP of a mixture. Dietary fibre consists of one or more of:
>
> - edible carbohydrate polymers naturally occurring in the food as consumed,
> - carbohydrate polymers, which have been obtained from food raw material by physical, enzymatic or chemical means,
> - synthetic carbohydrate polymers.

A review of carbohydrates in human nutrition agreed to define 'dietary fibre' as 'intrinsic plant cell wall polysaccharides' with gums and mucilages coming into this category.[18] Thus gum arabic qualifies with reference to each of these definitions and it seems most unlikely that there will be substantial changes to these descriptions.

From a scientific point of view the role of gum arabic as a dietary fibre is well established as was confirmed in the confirmation of its regulatory status as an ingredient when evidence of its use for this purpose was presented to the UK Food Safety Agency and the EU. Full details of its beneficial physiological effects have been described.[19]

Gum arabic has been extensively tested for its properties as a non-digestible polysaccharide. It has been shown to retard glucose absorption, increase stool mass, and trap bile acids and has a potential to beneficially modify the physiological status of human subjects. Since gum arabic can reach the large intestine without digestion in the small intestine, it can be categorised as a non-digestible carbohydrate or dietary fibre. It is, fermented by intestinal bacteria to short-chain fatty acids (SCFA), particularly propionic acid, in the large intestine. It has been shown to be a prebiotic dietary fibre and is able to selectively raise the proportions of lactic acid bacteria and bifidobacteria in healthy subjects. It also increases stool output by augmenting the water content of stools. In addition, its intestinal tolerance is excellent and high daily doses can be consumed without any adverse intestinal events. The evidence indicates that gum arabic acts as a prebiotic at a dose of 10 g/day. Several investigations have shown that higher proportions of propionate and butyrate are produced by fermentation *in vitro* and *in vivo* from gum arabic than from other hydrocolloids such as pectin and alginate.[20] There is, therefore, unequivocal evidence that gum arabic is a non-digestible polysaccharide. Its promotive prebiotic effect and propionic acid production in the large intestine have also been established. The health benefits of gum arabic includes treatment for ulcerative colitis because of the trophic effects of gum arabic on gut membrane and a reduction in the incidence of diarrhoea and its duration. Also acacia gum associated with apple pulp in the diet of 25 mild hypercholesterolemic patients was found to lead to a reduction (10%) of serum cholesterol concentration, particularly the LDL fractions. It also exerts a

hypoglycaemic effect because of the absence of glucose in its structure and its low calorific value of not greater than 2 kcal/g. It is also beneficial in renal disease since dietary supplementation with gum, by increasing systemic levels of butyrate, exerts a beneficial effect by suppression of TGF-β1 activity.

In 2009 the US FDA confirmed, in response to a scientific petition, that they would raise no objection to a calorific value of 1–7 kcal/g for gum arabic and not the value of 4 kcal/g used generally for hydrocolloids.

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12

Pectins

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Abstract: Pectins belong to the group of hetero-polysaccharides and are present in all plant primary cell walls. Depending on the molecular composition there are different types of pectin characterising themselves particularly by different gelling mechanisms. In addition to the manufacturing process of pectin, the chapter describes the relation between pectin structure and gelling mechanism. Furthermore, health aspects of pectins are discussed as well as diverse applications in the food sector. Various formulation examples show the different application areas in the food range.

Key words: pectin, food applications, manufacturing process, structure, gelling mechanism.

12.1 Introduction

Commercial pectins used as food additives are hetero-polysaccahrides which contain at least 65% by weight of galacturonic acid-based units, which may be present as free acid, methyl ester or, in amidated pectins, acid amide[1] (Fig. 12.1). They form a part of the wider class of pectic substances which are one of the major cell wall polysaccharides in land plants. The commercial materials normally contain added sugar and may contain added sodium or calcium buffer salts to standardise performance.

This chapter is based on the version from the first edition by Colin D. May.

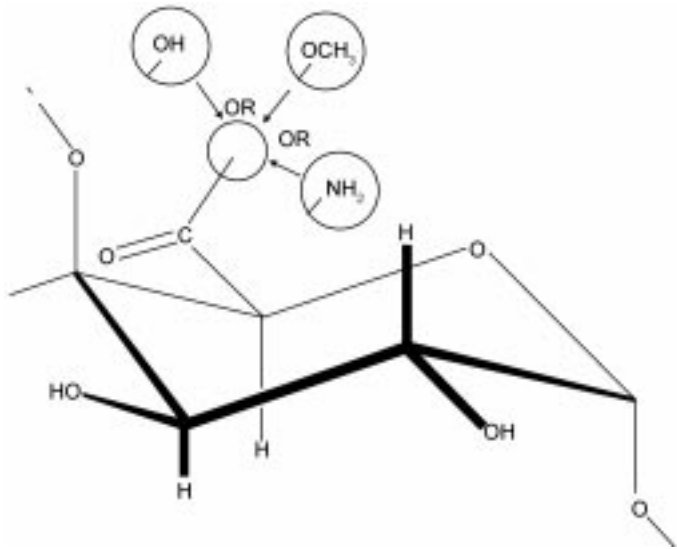

Fig. 12.1 Galacturonic acid, ester, and amide units found in pectins. Arrows indicate the potential for degradation by β-elimination in the ester form.

12.2 Manufacture

Pectins are present in many fruits in variable amounts and qualities. The traditional use of pectin has been as a gelling agent, and this has largely dictated the types of fruits from which commercial grades can be manufactured. A major consideration is the availability of fruit by-products in sufficient quantity and quality throughout the year. Before the development of a distinct pectin industry it was often the practice for jam makers to make a simple pectin extract from by-products of fruit processing such as apple cores or surplus orange pith, but commercial production demands large quantities of available raw material.

The history of the industry up to 1950 is described by Kertesz.[1] Since that date, there has been a geographical shift of the production of pectin. The largest pectin plants today are either in Europe or in Latin America.

12.2.1 Raw materials

Today the major sources are citrus peels, the by-product from the extraction of citrus juice and oil, and apple pomace, the dried residue from the extraction of apple juice. Within the commercially processed types of citrus, the peel from lemons or limes is preferred for most qualities of pectin, although orange peel is available in much larger quantities, and can be used for some applications. Citrus peel may be washed free from acidity and sugar, and carefully dried to preserve the pectin quality, or may be processed directly in the wet state. Wet peel processing requires a large and consistent source of peel very near to the

pectin plant. Pectin is very susceptible to degradation either by enzymes in the wet peel or by heat during drying and subsequent processing, and such loss of quality must always be controlled as far as possible. Pectin producers devote considerable resources to ensuring both the availability and quality of raw materials, and quality has a major effect on the types of pectin which can be economically produced. Scientists always search for new sources of pectin, but not every pectin is suitable for gelation due to its chemical structure.

12.2.2 Production processes

Although various alternative processes have been patented in recent years, most pectin is produced by the extraction of the raw material with hot aqueous mineral acid. Each manufacturer has developed conditions which suit the major type of raw material processed in their plant, but the aim is always to produce a slurry which contains solid residue which can be easily separated by the chosen technology, and a liquid phase containing as high a concentration of high molecular weight pectin as possible, without generating excessive viscosity. The liquid extract may be treated to remove impurities, and clarified mechanically (centrifugation, filtration) by removal of particulate matter, before proceeding to isolate a solid pectin.

In principle, pure pectin may be isolated in various ways. The most commonly used method is to mix the concentrated extract with an organic solvent in which pectin is insoluble, but which will permit many of the impurities to remain in solution. International food standards permit the use of only methanol, ethanol, or isopropanol as the organic solvent. In this process, the clarified pectin extract is concentrated and mixed with sufficient alcohol to give a precipitate firm enough to be handled with the separation technology chosen by the manufacturer concerned, which may be either filtration or centrifugation. The washing liquor may include sufficient food grade alkali to adjust the solution pH of the final pectin to the desired range.

An alternative method, which has been discontinued, is based on the fact that basic aluminium salts form charge-transfer complexes with anionic polymers such as pectin. An advantage was that the pectin extract does not require concentration, and if conditions are right the precipitate flocculates and can be separated easily from the large volume of liquor. The precipitated pectin-aluminium-complex had to be treated with aqueous alcohol acid which leaches out the aluminium leaving purified pectin. The disadvantage was the aluminium salts in the effluents and the negative impact on the environment, which was the reason for closing the US-based pectin factories. Further this process should not be used because of health concerns, as aluminium salts are suspected to induce Alzheimer's disease based on neurotoxic effects.

Once the pectin isolated by either process has been separated from as much alcohol as possible, it is dried and ground to a fine powder. The pectin produced in this way will have a high methyl ester content, viscosity, varying gelling power, depending on the nature and quality of the raw material, and it will make

a rapidly-setting gel under traditional jam-making conditions. The variable gel strength, viscosity, etc., can be adjusted by blending one or more batches with sugar to give a standard performance.

12.3 The chemical nature of pectin

Pectins as defined for use in food are high molecular weight heteropolymers containing a majority (at least 65% by weight) of galacturonic acid units. The acid group may be free (or as a simple salt with sodium, potassium, calcium or ammonium) or naturally esterified with methanol. However, pectins are derived from the breakdown of more complex protopectins which are present in the plant tissue (middle lamella), and also contain a range of neutral sugars, including rhamnose in the main chain, galactose, arabinose and lesser amounts of other sugars as neutral sugar side chains linked to the rhamnose.

Theses sugar units are present in a non-random structure, which consists of blocks of differing character retaining fragments of the original plant cell wall structure. The use of purified enzymes has shown that pectin extracted under very mild conditions contains both linear blocks (smooth regions) consisting of homopolygalacturonic acid, and highly branched blocks (hairy regions) which themselves contain several types of structures (see Fig. 12.2).[2] It is not at present clear exactly how this complex structure and its variations influence the functional performance of commercial pectins, but the practical implications of certain basic aspects of structure are better understood.

The regions of the pectin molecule which contain largely galacturonic acid units consist of a mixture of methyl ester, free acid and salt derivatives of the carboxyl group of the acid. Because commercial pectins are extracted under hot acidic conditions, many of the regions containing a high proportion of neutral sugars are hydrolysed, leaving mostly the more acid-stable galacturonic blocks. In certain pectins, such as those from sugar beet and potato, a proportion of the hydroxyl groups will also be acetylated. It has been known for a long time that the properties of pectin are dependent on pH, and on the percentage of acid group present in the form of ester (degree of esterification).

12.4 Commercial pectin: properties and function

The pectin described above is normally from around 67–73% esterified. Apple pectin can, with great care, be extracted with up to 80% esterification. Pectin is readily degraded by a β-elimination mechanism adjacent to the ester groups at ambient temperature or above at neutral or alkaline pH values. The ester groups can be hydrolysed under either alkaline or acidic conditions, or by pectin esterases. Commercially, acidic treatment is most commonly used, producing pectins with around 60% of ester groups which are ‘slow setting’. Under identical conditions of 65% total sugar solids by refractometry and a pH of say

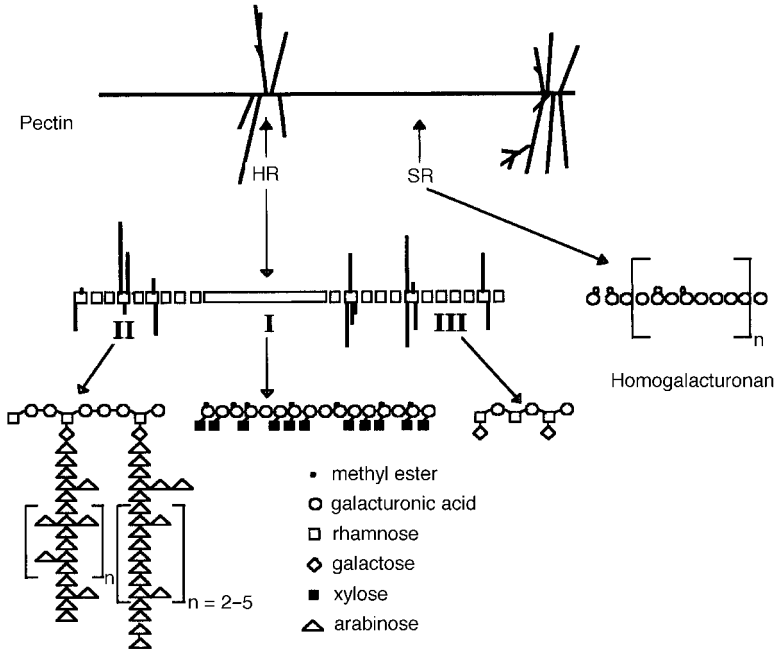

Fig. 12.2 Hypothetical structure of apple pectin based on the work of the Wageningen group, showing **I** xylogalacturonan region, **II** region with arabinan side chains, **III** rhamnogalacturonan region making up the 'hairy region'. (From H. Schols *et al.* 'Structural features of native and commercially extracted pectins', in *Gums and Stabilisers for the Food Industry 9*, ed. P.A. Williams and G.O. Phillips, RSC Cambridge, 1998, by permission of the authors.)

3.1, the gel will take much longer to set. The setting of these gels is both time and temperature dependent, and the setting temperature depends on the rate of cooling. Slow-setting pectins permit gels to be prepared at higher sugar contents, valuable for sugar confectionery, biscuit jams, and so on. Because of the higher charge density on the slow-set pectin molecules, there is also a change in the pH requirements for gelation towards a lower pH in gels of otherwise similar composition.

Further de-esterification to below 50% esterification produces a range of 'low methyl ester' pectins. These show a markedly greater reactivity towards calcium ions, which will cause gelation under suitable conditions of soluble solids and pH. Conditions for effective gelation depend on a balance of several factors, including soluble solids content, pH, calcium and pectin concentrations, and the presence of sequestrants. Amidated pectins (mostly of the low methyl ester type) are produced by reaction of suitable high methyl ester pectins with ammonia. The reaction is normally carried out in an aqueous alcohol slurry of the pectin at ambient or lower temperature. The process requires careful control of the relative rates of de-esterification and amidation, whilst minimising the rate of polymer chain degradation.

12.4.1 Gelation properties of pectins

Because pectin is a charged hydrocolloid, it is sensitive to variations in pH and to a greater or lesser extent to the nature and quantity of cations present in the system. Gelation may be considered as a state between solubility and precipitation of a polymer, and therefore the nature of the solvent is also significant.

Gelation of high methyl ester pectins

High methyl ester pectins will gel only in the presence of sugars or other co-solutes, and at a sufficiently low pH, so that the acid groups in the polymer are not completely ionised. Both gel strength and setting temperature are influenced by these factors. In a system with sucrose as the sweetener, at around 65% soluble solids, typical of high sugar jams and preserves, high methyl ester pectins gel at up to pH 3.4 (rapid set pectin) or 3.2 (slow set pectin). As the pH is reduced, gel strength and setting temperature will increase (Fig. 12.3) up to the point at which the setting temperature approaches the temperature at which the gel is deposited. Below this pH, pectin tends to pre-gel, and the resulting non-homogeneous gel is weaker and more subject to syneresis. However, if the gel mixture is prepared at higher pH, and acidified immediately before or on depositing, the gel strength is maintained to low pH values.

The gelation of high methyl ester pectins is also time-dependent, and setting temperatures will therefore depend on the rate of cooling, being higher with slower cooling. Very rapid cooling under shear can be used to produce a thick heavy texture useful in some industrial applications of fruit products.

Changing the nature of the sugars present has a noticeable effect on the performance of pectin. For example, the replacement of a substantial amount of sugar by glucose syrup leads to increased setting temperature with a corresponding increase in the optimum pH for gelation, but some loss in maximum gel strength. Other sugars such as maltose may show a similar effect.

Increasing the concentration of sugars increases both setting temperature and optimum pH. In making confectionery jellies, at 75–85% soluble solids, a slow

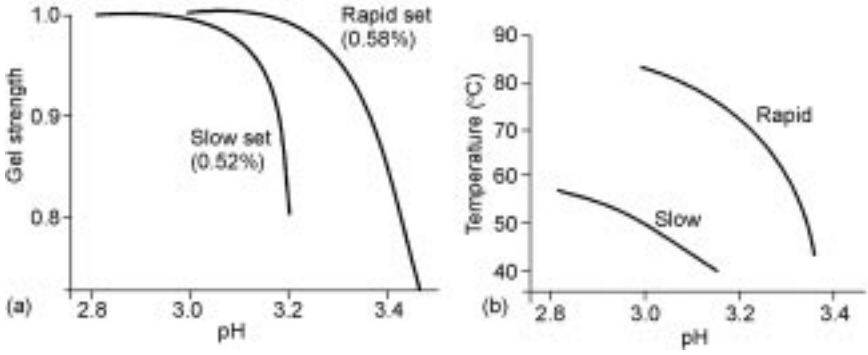

Fig. 12.3 (a) Variation of gel strength of high methyl ester pectins with pH in a 65% sucrose gel (relative values); (b) variation of gel setting temperature in the same system.

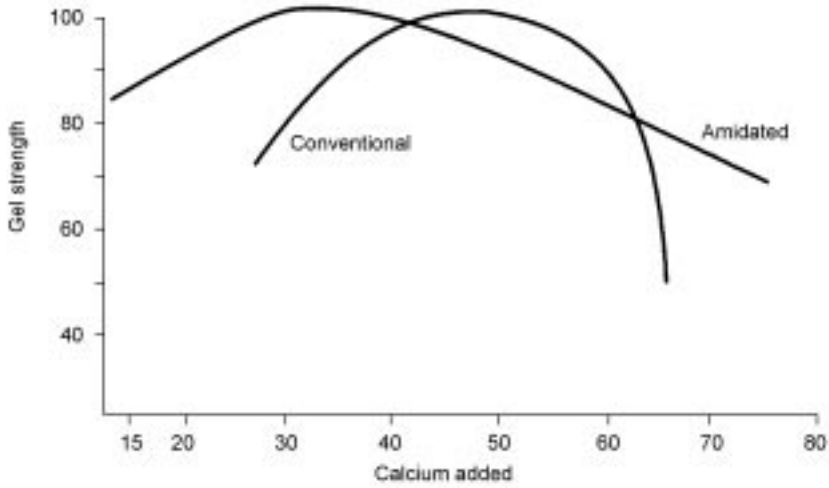

Fig. 12.4 Variation of relative gel strength of low methyl ester pectin gels (conventional and amidated) with added calcium at 30% added sucrose and pH 3.0 with a citric acid/sodium citrate buffer system.

set pectin would be used at a pH of 3.4–3.6 to give a suitable depositing time, enabling a batch to be deposited over 15 to 20 minutes or more.

Gelation of low methyl ester pectins

The gelation of low methyl ester pectins is governed mainly by the interaction between the pectin and calcium ions. For this reason, the availability of calcium ions is important, and this is commonly governed by sequestrants either naturally present (e.g., citrate and other organic acid ions from fruit or milk) or added (commonly food grade citrates, lactates, tartrates or di- or poly-phosphates). Reactivity to calcium is governed by proportion and arrangement of carboxyl groups in the pectin chain. Reactivity increases with decreasing degree of esterification, and is greater but less controllable for gelling purposes if the arrangement of acid groups is less random, with blocks of de-esterified galacturonate units. Amide groups have a moderating influence, and permit gelation over a wider range of calcium concentrations (Fig. 12.4). Gelation is favoured by increased soluble solids, but decreased by increasing pH, or by increasing the level of sequestrant. However, a certain level of a sequestrant such as citrate is essential to produce a practically workable gel system. With correct formulation, low methyl ester pectins can gel over a wide range of soluble solids (10–80%), and in either acidic or less acid-tasting products, at a pH of a little below 3.0 to above 5.0.

12.4.2 Availability of pectin types

Pectin manufacturers generally offer a wide range of pectin types for different applications (Fig. 12.5).

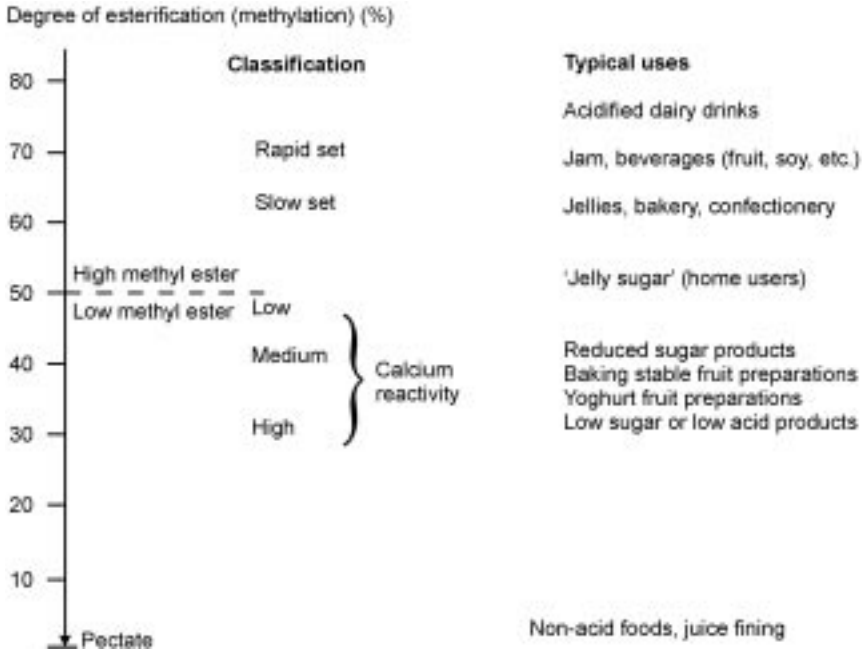

Fig. 12.5 The range of commercial non-amidated pectins with some typical applications.

12.5 Nutritional and health aspects

Manufacturers will provide detailed nutrition data for labelling purposes on request. Pure pectin is essentially a soluble dietary fibre, but the commercial product will contain limited amounts of sodium and calcium and, for technological uses, sugar or dextrose and/or buffer salts for standardisation. The amount of carbohydrate to be declared in nutrition labelling depends on national legislation, as soluble fibre may be either included, or totally excluded. This also affects the declaration of energy value.

Pectins may contain 1–2% of protein, but if legislation requires that protein is declared as $N \times 6.25$ (or other factor) it is arguable whether the amide nitrogen should be included in the calculation. The levels of most other nutrients are not significant when the use level of pectin in the final product is considered, except in the case of blends, with e.g., sodium, potassium or calcium salts.

If fibre is to be declared, the figure will depend on the analytical method accepted locally. Where the AOAC method is specified, the content of pure pectin, after deducting moisture and ash contents, can be taken as fibre. The Englyst method gives lower results, typically around 65% of the AOAC value.

Several studies have attested beneficial effects of pectin on humans such as reduction of liver and blood lipids, total cholesterol, low-density (LD) and/or very low density (VLDL) lipoprotein (L), and the LDL:HDL ratio. Furthermore,

pectic substances lower blood glucose and insulin levels after a carbohydrate meal and have positive influence on some exocrine pancreatic hormones and enzymes. Pectin as a hydrocolloid is able to bind a lot of water in the digestion process. The effect is a feeling of satiety which reduces food consumption and weight. Pectin has been reported to have antiviral activity.[3]

12.6 Uses and applications

In all pectin applications, the action of the pectin is very dependent on the exact conditions in the product, pH, ionic strength and composition, the proportion of sweeteners and their nature, and, where fruit is present, the amount and nature of the pectin provided by the fruit. It is therefore always wise to test any change in formulation, including a new season or source of fruit, on a small (saucepan) scale before embarking on full-scale manufacture.

12.6.1 Dissolving pectin

In most applications it is essential to ensure that the pectin is dissolved before gelling conditions are reached. Pectin will not dissolve when near gelling conditions, and high methyl ester pectins in particular will not dissolve in sugar solutions of more than 20–25%. As with most gums, it is vital to disperse the solid particles before they partially dissolve and stick together. This may be achieved either with a high shear mixing system (batch or in-line) or by mixing the pectin with several times its weight of sugar, and stirring vigorously. Occasionally, the pectin may be dispersed in a sugar syrup and the mixture diluted by stirring to achieve a similar result.

12.6.2 Optimising pectin formulations

Pectin formulations will typically require adjusting to particular production conditions. The factors described above which influence gelation must always be kept in mind. In most practical situations, the soluble solids level and fruit content (if any) are established as part of a product brief, and cannot be modified. There is always an optimum set of conditions between lack of set on the one hand and pre-gelation on the other, but it is possible to mistake some pre-gel conditions for lack of set because processing may result in a smooth syrup rather than a broken gel. If a gel is not obtained, and neither lowering the pH nor increasing the pectin content causes gelation to occur, this should be suspected, especially if the pectin level is already high.

If small changes in pectin content or pH do not result in improved performance, changing the pectin for a slightly different type should be considered. If this is not effective, it is wise to consult the pectin supplier who should be able to advise, and will treat detailed information in confidence if requested. There are many different pectin types, and selection for a particular application requires considerable experience.

12.6.3 High sugar jams, jellies, marmalades and preserves

The earliest application for pectin was in fruit jams with 60–70% total soluble solids and a pH in the range 3.0–3.3. For this application, a high methyl ester pectin can be used. The composition of jams is often closely regulated, and the relevant regulations should be checked. In Europe, there are also specific labelling requirements in addition to the general Food Labelling Regulations.

Depending on the nature of fruit, the fruit content, and the desired soluble solids content, differing amounts and types of pectin will be required. Table 12.1 gives an indication of the proportion of pectin required for a jam at around 65% total soluble solids as measured by refractometer. The type of pectin will be determined by the type of product and the process to be used. Jams made by the traditional open pan cooking method will require a rapid set pectin, whilst those made by vacuum cooking at a lower temperature require a slower setting pectin to avoid pre-setting. In Formulation 12.1 a recipe for a traditional jam with 60–65% soluble solids is given, while Formulation 12.2 shows a recipe for orange marmalade with approximately 63% soluble solids. Clear jellies are usually made with a slow or extra slow set pectin to allow any small bubbles of air to rise before the gel sets in the final container. If there is a problem with floating fruit, it may be useful to use a proportion of low methyl ester pectin to increase hot viscosity.

12.6.4 Low sugar jams and jellies

Low sugar jams and jellies cannot be made with high methyl ester pectins which do not gel when soluble solids are reduced below 60% and are usually best prepared with low methyl ester pectins. Amidated low ester pectin types can most often form a suitable gel texture together with calcium originating from fruit and water in the jam whilst conventional (non-amidated) types normally require the addition of extra calcium. In Europe, only non-amidated pectins may be used if an 'organic' claim is to be made for the product.

With low sugar products, it is particularly important to use good quality fruit in generous quantity to provide an acceptable product. The type of pectin used

Table 12.1 Typical pectin requirement for traditional jam at 65% soluble solids

Fruit type	Fruit content 35%	45%	60%
Low pectin			
e.g. strawberry, peach, raspberry, pineapple	0.30–0.39%	0.22–0.28%	0.11–0.16%
Medium pectin			
e.g. apricot, blackberry, marmalade	0.23–0.29%	0.15–0.22%	0.07–0.11%
High pectin			
e.g. apple, damson, gooseberry, plum, quince, redcurrant	0.14–0.21%	0.08–0.14%	0.0–0.07%

Formulation 12.1 Extra Jam

Ingredients		Weight (g)
A	Fruit	450
	Sucrose	420
	Glucose syrup	200
B	Rapid to slow set HM pectin, DE° ~60–70%	2.5
	Water	50
Final batch weight		1000
pH (as is at 20 °C)		3.0–3.2
Soluble solids (approximately)		60–65%

Preparation
1. Mix fruit, sugar and glucose syrup A and heat to the boil whilst stirring.
2. Dissolve pectin in hot water (approx. 80 °C) using a suitable high shear/speed mixer.
3. Add the pectin solution B to the batch A and boil down to the final soluble solids.
4. Cool to 85 °C and deposit into jars.

Note: The pH should be maintained in the range 3.0–3.2. Depending on the type of fruit it may be necessary to add a dilute solution of citric acid. If this is required it should be added slowly with vigorous mixing at the end of the boiling process to avoid pre-gelation.

Formulation 12.2 Soft set orange marmalade

Ingredients		Weight (g)
A	Orange pulp	150
	Orange peel	80
	Sucrose	580
	Water	200
	Sodium citrate solution (10% w/v)	as required
B	Rapid set HM pectin, DE° ~65–70%	3
	Water	60
C	Citric acid solution (50% w/v)	as required
Final batch weight		1000
pH (as is at 20 °C)		3.0–3.2
Soluble solids (approximately)		63%

Preparation
1. Mix orange pulp, water, orange peel and sucrose (optionally adjust pH with sodium citrate solution to about 3.3 to prevent pre-gelation) and heat to approximately 90 °C whilst stirring.
2. Dissolve pectin in hot water (approx. 80 °C) using a high shear/speed mixer.
3. Add the pectin solution B to the batch A and boil down to final soluble solids.
4. While stirring add the citric acid solution to adjust the pH value.
5. Cool to 85 °C and deposit into jars.

should be matched to the product, and, in general, lower solids require the use of a 'high reactivity' or 'fast setting' low ester pectin. The recipes in Formulations 12.3 and 12.4 illustrate typical reduced sugar jams with 45% and 30% soluble solids. Local regulations and requirements will dictate the preservative to be

Formulation 12.3 Reduced sugar strawberry jam (45% SS)

Ingredients		Weight (g)
A	Strawberries	500
	Sucrose	380
	Water	60
B	Amidated LM pectin (medium reactive)	6
	Water	120
C	Potassium sorbate solution (20% w/v)	5 ml
D	Citric acid solution (50% w/v)	5 ml
Final batch weight		1000
pH (as is at 20 °C)		3.2–3.4
Soluble solids (approximately)		45%

Preparation

1. Mix fruit, sugar and water A and heat to 90 °C until the sugar is dissolved.
2. Dissolve pectin in hot water (approx. 80 °C) using a high shear/speed mixer.
3. Add the pectin solution B to the batch A and adjust soluble solids to 45%.
4. With stirring add the potassium sorbate C, followed by the citric acid D.
5. Cool to 60 °C and fill.

Formulation 12.4 Reduced sugar raspberry jam (30% SS)

Ingredients		Weight (g)
A	Raspberries	500
	Sucrose	240
	Water	100
B	Amidated LM pectin (high reactive)	5
	Conventional LM pectin (high reactive)	3
	Water	160
C	Potassium sorbate solution (20% w/v)	2 ml
	Sodium benzoate solution (20% w/v)	3 ml
D	Citric acid solution (50% w/v)	7 ml
Final batch weight		1000
pH (as is at 20 °C)		3.2–3.4
Soluble solids (approximately)		30%

Preparation

1. Mix fruit, sugar and water A and heat to 90 °C until the sugar is dissolved.
2. Dissolve pectin blend in hot water (approx. 80 °C) using a suitable high shear/speed mixer.
3. Add the pectin solution B to the batch A and adjust soluble solids to 30%.
4. With stirring add the preservative solutions C, followed by the citric acid D.
5. Cool to 80 °C and fill. After closure (and pasteurisation) cool jars to 40 °C or below.

used, if any. Low sugar products without preservatives must be filled and capped hot – or reheated to – and kept at pasteurising conditions for a short time to ensure microbiological shelf stability. To keep fruit pieces evenly distributed at pasteurising conditions it may be necessary to use special pectin types or pectin combinations that will provide hot viscosity. Combinations of amidated and conventional LM pectins or the addition of gums (e.g. locust bean gum) is sometimes used to counteract syneresis tendency at very low soluble solids.

12.6.5 Industrial fruit preparations

These cover a varied and expanding range of products, from traditional bakery and biscuit jams to a range of fruit bases and toppings many of which have to be tailored to a specific end use. For example, fruit bases prepared specifically for fruit yoghurts need to be easy to handle in bulk without fruit separation, but may either be required to mix with the yoghurt, or to be deposited as a layer without forming a skin at the interface. In some cases there may be requirements for the pectin to add extra body to the finished mixed yoghurt.

12.6.6 Fruit preparations for bakery products

For fruit preparations for bakery products various special properties may be required, but bake stability is one of the common requirements. For example, in open jam tarts baked with the filling, the jam must flow to give a smooth glossy surface but must not boil out of the pastry case. In such a complex field it is possible to give more than a few typical recipes, and pectin manufacturers expect to provide technical assistance in formulating recipes for specific requirements. The following recipes provide a few illustrations of the many possibilities for pectins in this area.

Bakers jam (high methyl ester pectin)

A bakers jam that will withstand baking and is suitable as a pie or tart filling can be produced using a high melting high methyl ester pectin. The baking stable fruit preparation in Formulation 12.5 is characterised by an excellent spreadability. The gel network formed by high methyl ester pectins, however, can be destroyed easily under mechanical stress. In destroying this gel structure the bound water is released again. Syneresis emerges and the fruit preparation loses its baking stability because the released water will start to boil during the baking process.

In the traditional manufacture of bakery products, fruit preparations produced with high methyl ester pectins will be exposed to only low mechanical stress during processing (pumping, dosing, etc.). In contrast, in larger scale industrial production the higher mechanical stresses disrupt the gel network and low methyl ester pectins will give better results. When low methyl ester pectins are combined with an appropriate calcium salt, it is possible to initiate a controlled process of pre-gelation and so to obtain a baking stable product (Formulation 12.6) with a stable, easy-to-handle texture.

Formulation 12.5 Bakers jam (high methyl ester pectin)

Ingredients		Weight (g)
A	Fruit pulp	350
	Sucrose	440
	Glucose syrup	200
B	Medium rapid set HM pectin, DE° ~59–64%	6
C	Citric acid solution (50% w/v)	as required
Final batch weight		1000
pH (as is at 20 °C)		3.0–3.1
Soluble solids (approximately)		65%

Preparation

1. Mix fruit pulp, glucose syrup and sucrose and heat to approx. 90 °C whilst stirring.
2. Dissolve pectin in hot water (approx. 80 °C) using a high shear/speed mixer.
3. Add the pectin solution B to the batch A and adjust soluble solids to 65%.
4. If required adjust pH with citric acid solution C.
5. Cool with gentle stirring to filling temperature of approx. 70 °C and deposit as required.

Formulation 12.6 Baking stable fruit preparation (low methyl ester pectin)

Ingredients		Weight (g)
A	LM pectin (high reactive), DE° ~32–36%	12
	Tri-sodium-citrate × $2H_2O$	1.5
	Tri-calcium-dicitrate × $4H_2O$	1
	Sucrose	100
B	Apple pulp	350
	Water	50
C	Glucose syrup	150
	Sucrose	390
D	Citric acid solution (50% w/v)	as required
Final batch weight		1000
pH (as is at 20 °C)		3.5–3.6
Soluble solids (approximately)		65%

Preparation

1. Dry mix ingredients A.
2. Stir mixture A into fruit pulp and water, heat to boil and continue to stir until the pectin is completely dissolved.
3. Add sucrose and glucose syrup C and decoct to final soluble solids.
4. With stirring add citric acid solution D to adjust the pH value.
5. Cool to 40–50 °C whilst stirring and deposit as required.

Glazes and spray glazes

In contrast to the non-melting behaviour of the recipe in Formulation 12.6, it is equally possible to use a pectin system to make fully thermally reversible gels at a range of soluble solids levels, as in Formulations 12.7 and 12.8 for a glazing

Formulation 12.7 Ready-to-use spray glaze

Ingredients		Weight (g)
A	Phosphate buffered amidated pectin	8
	Sucrose	100
B	Water	210
	Citric acid solution (50% w/v)	3 ml
C	Sucrose	180
	Glucose syrup	190
D	Water	330
Final batch weight		1000
pH (as is at 20 °C)		3.4–3.6
Soluble solids (approximately)		44%

Preparation

1. Dry mix ingredients A and stir into water and citric acid solution B. Heat to boil whilst stirring until pectin is completely dissolved.
2. Add sucrose and glucose syrup C and decoct to final soluble solids.
3. Cool to approx. 30 °C whilst stirring.
4. Add rest of cold water D and fill spray glaze cold.

jelly based on an amidated pectin combined with phosphate salts which control both the availability of calcium and the pH of the gel.

Glazes and spray glazes are applied hot to the surface of cakes and pastries and protect the pastries from drying out, prevent discolouring of the fruits and provide the desired glossy surface to the cakes and pastries. If the glaze is to be used in an undiluted form (ready-to-use), gently mash and liquefy the glaze by

Formulation 12.8 Concentrated glaze

Ingredients		Weight (g)
A	Phosphate buffered amidated LM pectin	12
	Sucrose	100
B	Water	330
	Citric acid solution (50% w/v)	5 ml
C	Sucrose	180
	Glucose syrup	190
Final batch weight		1000
pH (as is at 20 °C)		3.4–3.6
Soluble solids (approximately)		65%

Preparation

1. Dry mix ingredients A and dissolve in water and citric acid B and heat to boil whilst stirring until pectin is completely dissolved.
2. Add sucrose and glucose syrup C and decoct to final soluble solids.
3. Cool to 40–50 °C whilst stirring and fill into containers (creamy texture) or fill hot in containers.
4. For preparing the final product, mix the concentrated glaze with 20–50% water and bring to boil.

heating to 90 ºC. The glaze may then be applied at any temperature down to 60 ºC. The thermo-reversible gel structure generated by amidated pectins allows for the complete melting of the semi-finished product.

If the glaze is to be used in a dilute form, gently mash the glaze with up to 60% water and heat to 90 ºC. The diluted glaze may then be applied at any temperature down to 30 ºC. Any unused glaze can be kept and melted back when required without any loss of quality.

12.6.7 Fruit preparations and fruit bases

Fruit preparations for yoghurt are most often cooled from pasteurisation temperatures in closed systems and filled aseptically into large containers. Filling temperatures may vary but are often in the range 40–50 ºC. Accordingly, a certain gel formation is required at this stage to avoid fruit separation. Over time the containers will cool to ambient temperatures but texture of the preparation must remain softly gelled to ensure flowability and pumpability of the product at the end user.

The requirements for absence of fruit separation, pumpability and a degree of restructuring after shear are usually best provided by an (amidated) low ester pectin. To avoid adverse interaction with the yoghurt, pH of the fruit preparation is usually kept in the range 3.5–4.0 and accordingly a relatively reactive LM pectin system has to be used.

The exact properties of these bases need to be tailored to the equipment and systems in use by both the fruit processor and the end user, as well as those demanded by the final product, and recipes can only be a guide to what is possible: fruit contents, soluble solids levels, and flow properties can be adjusted

Formulation 12.9 Fruit preparation for yoghurt

Ingredients		Weight (g)
A	Fruit	500
	Sucrose	350
	Water	50
B	LM pectin (high reactive)	5
	Water	100
C	Sodium citrate dihydrate solution (20% w/v)	as required
Final batch weight		1000
pH (as is at 20 ºC)		3.5–4.0
Soluble solids (approximately)		40%
Filling temperature (approx.)		45 ºC

Preparation

1. Mix fruit, sugar and water A and heat to 90 ºC until the sugar is dissolved.
2. Dissolve pectin in hot water (approx. 80 ºC) using a suitable high shear/speed mixer.
3. Add the pectin solution B to the batch A and adjust soluble solids to 45%.
4. If required adjust pH with sodium citrate solution C.
5. Cool to 45 ºC in a closed system and fill aseptically.

to meet a wide range of requirements. In some cases a custom blend of pectin with other additives (e.g. starches) may be required. A typical fruit preparation for yoghurt is given in Formulation 12.9.

In order to obtain the correct characteristics, a number of factors can be adjusted. The amount of total citrate will control both the acid taste and the binding of the calcium ion present. An increase will increase the perceived acidity, but reduce the body of the base. The pH can be adjusted by altering the balance of these ingredients. If the base is too liquid, either a faster setting, more reactive, pectin can be used, or calcium can be added as a dilute solution of calcium chloride or lactate to the finished product before cooling.

Various modifications to the formulation are possible to produce a base for layer desserts or to add extra thickness to a mixed fruit yoghurt (post-dosage thickening) by interaction between the pectin from the fruit base and the calcium in the yoghurt. Similar formulations may be used as toppings for gateaux and cheesecakes, including also caramel, butterscotch and other non-fruit flavours.

12.6.8 Dairy products

Pectin can have two distinct functions in dairy products and dairy analogues such as those prepared from soya. High ester pectins can act as protein dispersion stabilisers at reduced pH. Typical examples include yoghurt drinks, milk/fruit juice drinks, acidified whey drinks and acidified soy drinks. Low ester pectins behave quite differently, and can be used to gel neutral milk desserts or as texturiser in more acid products (e.g., stirred yoghurt) by interaction with calcium and milk proteins.

Acid dairy drinks

Pectin is an effective protective colloid for casein or similar protein particles at a pH around 4, typical for yoghurt. In yoghurt drinks the pectin is added after fermentation. Yoghurt drinks may be formulated with live bacteria and short shelf-life or they may be pasteurised for long shelf-life. Milk/fruit juice drinks are acidified by means of juice or acid, such as citric acid or lactic acid. Soy drinks and drinks based on other protein sources are most often acidified in a similar manner.

When drinks containing protein are heat treated at acidic pH, the proteins tend to aggregate and form larger clusters that impart a sandy mouthfeel and are able to precipitate. High ester pectin molecules are negatively charged at the actual pH and will bind to the protein particles and protect them from aggregation even during heat treatment. The pectin also creates a weak molecular network throughout the drink that further contributes to stability.

Pectin dosage required depends on protein level in the drink and the size of the protein particles. Smaller particle size generally gives more stable drinks but very small particle size may require increased pectin dosage due to larger total surface area. Protein particle size is generally controlled by optimising fermentation or acidification conditions.

After addition of pectin it is important to homogenise the yoghurt to break up protein aggregates and ensure optimum contact between pectin and protein, before heat treatment is carried out. Surplus pectin over the amount required for stabilisation of the dispersion will increase the viscosity of the final product, which may or may not be desirable. Typically, about 0.4% of a specially produced and standardised pectin will be added either as a solution or dispersed in sugar or a syrup. Heat treatment may, in some cases, be followed by further homogenisation, often at a lower pressure, to ensure maximum stability.

Composition of yoghurt and acidified protein drinks may vary considerably providing products with low or high viscosity and differences in mouthfeel as illustrated in Formulations 12.10 and 12.11.

Set and stirred yoghurt

Suitable low ester pectin types may be used in relatively low concentrations to increase viscosity, creaminess and stability of various yoghurt types. The pectin can be added to the yoghurt milk before fermentation, and accordingly no changes in the production process are required. Both upstream and downstream homogenisation can be used. The example in Formulation 12.12 refers to low fat stirred yoghurt, but the same principles can be used with full-fat or non-fat milk and for products fermented in retail cups (set yoghurt types).

Formulation 12.10 Yoghurt drink

Ingredients		Weight (g)
A	Pectin (dairy stabiliser grade)	2–5
	Water	100–300
B	Yoghurt	100–800
C	Sucrose	0–100
D	Fruit juice, fruit concentrate or flavour	0–100
E	Water	to 1000
Final batch weight		1000
pH (as is at 20 °C)		3.9–4.1
Soluble solids (approximately)		15%

Preparation

1. Dissolve Pectin A in hot water, using a suitable high shear/speed mixer. Cool the solution to ambient temperature.
2. Mix pectin solution and yoghurt B. (Pectin may be added to the yoghurt as a powder using a high speed mixer. In that case heating to 65 °C before homogenisation is required.) Add ingredients under C, D and E and stir well until the mix is homogeneous.
3. Homogenise at 150–200 bar.
4. Pasteurise at 90 °C for 5–15 s.
5. Cool to ambient temperature and fill aseptically.

Note: The pH must be in the range 3.9–4.1 to obtain stability to heat treatment; outside this range the stability will not be as good and different pectin types will be required.

Formulation 12.11 Milk/fruit juice drink

Ingredients		Weight (g)
A	Pectin (dairy stabiliser grade)	2–5
	Water	200–400
	Sugar	25
B	Skimmed milk	100–200
	Water	to 1000
C	Sugar	75
D	Fruit juice	200
	Fruit flavour	optional
E	Citric acid monohydrate solution (50% w/v)	to pH 3.8–4.2
Final batch weight		1000
pH (as is at 20 ºC)		3.8–4.2
Soluble solids (approximately)		15%

Preparation

1. Prepare pectin solution A by dissolving pectin pre-mixed with sugar in hot water using a suitable high shear/speed mixer.
2. Use cold milk mixed with water B or recombined milk.
3. Add the pectin solution to the milk phase. Add the remaining part of the sugar C and mix for a few minutes.
4. While stirring vigorously with high shear/speed mixer, add the fruit juice and acid to obtain the required pH level (3.8–4.2).
5. Homogenise at 150–200 bar.
6. Pasteurise at 90 ºC for 5–15 s.
7. Cool to ambient temperature and fill aseptically.

Note: The principle can be extended to carbonated milk drinks or drinks based on soy or whey protein.

Formulation 12.12 Set and stirred yoghurt

Ingredients		Weight (g)
A	Low fat milk	900–1000
B	Skimmed milk powder	0–40
C	Low ester pectin (special grade)	1.5–2.5
D	Sugar or sweetener	0–50
Final batch weight		1000
pH (as is at 20 ºC)		3.8–4.5

Preparation

1. Dry blend pectin with other dry ingredients and add to the milk using a suitable high shear/speed mixer.
2. Stir until ingredients are dissolved.
3. Homogenise at 60–75 ºC and 150–200 bar.
4. Heat treat at 90 ºC for 12 min.
5. Cool to fermentation temperature and inoculate with culture.
6. Ferment till required pH and stir till homogeneous.
7. Cool to 20–25 ºC and fill. Further cool to 5–6 ºC in closed package.

Note: As over-dosage of pectin may tend to destabilise the system, it is recommended to make a few trials to establish optimum pectin level. Alternatively do not exceed 0.2% low ester pectin in the formula.

Milk gels and desserts

Low methyl ester pectins can be used to gel milk by directly dispersing pectin powder, preferably mixed with several times its weight of sugar, in cold milk, and heating with stirring to boiling to dissolve the pectin. The amount of pectin required will be from 0.6 to 0.9%, and the texture of the gel can range from firm and brittle to very soft and creamy, depending on the pectin type chosen. It is also possible to formulate a fruit syrup which, on adding to cold milk, will produce a lighty gelled fruit dessert (Formulation 12.13).

Formulation 12.13 Syrup for milk dessert

Ingredients		Weight (g)
A	Amidated LM pectin	15
	Sucrose	80
B	Water	400
C	Raspberries	200
	Water	300
	Citric acid solution (50% w/v)	4 ml
	Sodium citrate dihydrate solution (20% w/v)	20 ml
	Sucrose	80
Final batch weight		1000
pH (as is at 20 °C)		4.0–4.2
Soluble solids (approximately)		20%

Preparation

1. Dry mix ingredients A and stir into water B using a high shear/speed mixer. To aid dissolving, place the mixer so as to obtain maximum turbulence. Gradually add the dry mix ensuring addition is complete before thickening slows agitation.
2. Warm ingredients C, add the pectin solution and heat to the boil while stirring.
3. Boil down to 1020 g, remove from the heat, cool and deposit.
4. To prepare the dessert, take equal parts of the syrup and milk. Mix the milk into the fruit syrup for 15–30 s. The dessert may be eaten within 5 min of preparation or may be stored in the fridge until required.

Note: It may be necessary to adjust the amount of citric acid or sodium citrate used.

12.6.9 Other dessert products

Pectin gels can be used as an alternative to gelatine, carrageenan and agar in fruit dessert jellies (Formulation 12.14). Pectin gels can be deposited and set very rapidly so that other components such as sponge or cream can be added after a short cooling stage, enabling the complete dessert to be assembled on line. Compared to other gelling agents, low ester pectin is fully stable at fruit pH and gives an attractive light texture with excellent flavour release.

Formulation 12.14 Fruit flavoured dessert jelly

Ingredients		Weight (g)
A	Low ester pectin (special reactive type)	10
	Sucrose	140
	Sodium citrate dihydrate	2
B	Water (adjust to ~100 ppm calcium)	to 1000
C	Citric acid monohydrate solution (50% w/v)	9 ml
D	Colour and flavour	optional
Final batch weight		1000
pH (as is at 20 °C)		3.4–3.6
Soluble solids (approximately)		15%

Preparation

1. Adjust calcium content of the water B to 100 ppm by a suitable calcium source (e.g. calcium chloride).
2. Dry blend pectin with sugar and citrate A and disperse in the cold water while stirring.
3. Heat to boiling and boil for 5 min.
4. Add citric acid C, colour and flavour D while stirring.
5. Fill hot into cups. Seal and pasteurise at 80 °C for 10 min. Cool to ambient temperature or below.

Note: The texture of the jelly can be altered by changing the pectin/calcium ratio, i.e. a high pectin to a low calcium level will result in an elastic gel, whereas if more calcium is added the resultant gel will become more brittle.

12.6.10 Sugar confectionery

Pectin confectionery jellies can be divided into two types: tender, fruity jellies made with high methyl ester pectin, and elastic jellies based on low methyl ester or amidated pectins blended with sequestering phosphate buffers, suitable for traditional turkish delight flavours, and also mint and other non-acid flavours (Formulation 12.15). High soluble solids contents (approx. 78%), usual in the production of gum and jelly products with high methyl ester pectins, require relatively high pH values to prevent pre-gelation and to achieve long depositing times. For taste reasons, a high product pH is not desired in confectionery jellies, but at the same time long depositing times are required for technological reasons. Therefore, for products with a high soluble solids content, a pectin with a very low setting temperature with simultaneously low viscosity during the boiling process or substances delaying gelation (retarders) is required. Such buffer salts make it possible to work at lower pH values without any risk of pre-gelation. Following there are two recipes with high methyl ester pectins, one recipe with a buffered pectin (Formulation 12.16) and one with a pectin without buffer salts (Formulation 12.17).

Formulation 12.15 Turkish delight jelly

Ingredients		Weight (g)
A	Buffered amidated LM pectin	24
	Sucrose	100
B	Water	220
	Citric acid solution (50% w/v)	10 ml
C	Sucrose	360
	Glucose-fructose syrup	395
D	Colour, flavour	as required
	Citric acid solution (50% w/v)	20 ml
Final batch weight		1000
pH (as is at 20 ºC)		4.0–4.1
Soluble solids (approximately)		78%

Preparation

1. Dry mix ingredients A and stir into water and citric acid solution B. Heat to boil while stirring until the pectin is completely dissolved.
2. Add sucrose and glucose-fructose syrup C and decoct to final soluble solids.
3. Remove from the heat, mix in the colour, flavour and citric acid solution D and deposit into moulds by a depositing temperature of approx. 95 ºC.

Formulation 12.16 Fruit flavoured confectionery jelly with buffered pectin

Ingredients		Weight (g)
A	Medium rapid set HM pectin, buffered, DEº ~56–60%	13
	Sucrose	100
B	Water	220
C	Sucrose	400
	Glucose syrup	330
D	Colour, flavour	as required
	Citric acid solution (50% w/v)	13 ml
Final batch weight		1000
pH (as is at 20 ºC)		3.2–3.4
Soluble solids (approximately)		78%

Preparation

1. Dry mix ingredients A and stir into water B. Heat to boil while stirring until the pectin is completely dissolved.
2. Add sucrose and glucose syrup C and decoct to final soluble solids.
3. Remove from the heat, mix in the colour, flavour and citric acid solution and deposit into moulds by a depositing temperature of approx. 95 ºC.

The lower limit for gelation of high methyl ester pectins is a soluble solids content of approx. 55%. At lower soluble solids high methyl ester pectins do not gel sufficiently, in this range low methyl ester pectins with the addition of calcium salts are used. For confectionery jelly production it is essential to include a proportion of glucose syrup to prevent crystallisation of the sugar.

Formulation 12.17 Fruit flavoured confectionery jelly with unbuffered pectin

Ingredients		Weight (g)
A	Slow set HM pectin, DE° 56–60%	13
	Sucrose	100
	Tri sodium citrate × $2H_2O$	4
B	Water	220
C	Sucrose	400
	Glucose syrup	330
D	Colour, flavour	as required
	Citric acid solution (50% w/v)	18 ml
Final batch weight		1000
pH (as is at 20 °C)		3.2–3.4
Soluble solids (approximately)		78%

Preparation

1. Dry mix ingredients A and stir into water B. Heat to boil while stirring until the pectin is completely dissolved.
2. Add sucrose and glucose syrup C and decoct to final soluble solids.
3. Remove from the heat, mix in the colour, flavour and citric acid solution and deposit into moulds by a depositing temperature of approx. 95 °C.

12.6.11 Other food applications

Pectin is used in various types of dough for bread and pastry, where it improves the volume of the bread and enables moisture and softness retention during storage.

In frozen and in pre-proofed dough, pectin ensures volume and softness when baked. Likewise, it stabilises frozen bread, making properties closer to fresh bread when re-heated.

Special pectin types have been developed to mimic a fat-like mouthfeel as these pectin types form soft smooth gel particles when hydrated. Pectin may further be used to bind water in low fat spreads and thereby improve emulsion stability in these products.

12.7 Legal status

Pectin is generally regarded as one of the safest and most acceptable of food additives, and this is recognised by Acceptable Daily Intake (ADI) levels of 'not specified' by both the Joint Experts Committee on Food Additives (JECFA) for Codex Alimentarius purposes, and the Scientific Committee for Food (SCF) in the European Union, and by Generally Regarded as Safe (GRAS) status in United States legislation. Similar status has been awarded in most jurisdictions. In general, there are few restrictions on the use of pectin in foods, but most authorities prescribe restricted lists or the absence of food additives in a limited number of basic food categories, and pectin may or may not be listed in these cases. The Codex and US specifications include both amidated and non-

amidated pectins. In the EU, there are separate specifications for E440(ii) and E440(i) respectively, but permitted uses are similar except in organic foods, where only E440(i), non-amidated pectin, is permitted.

12.8 References

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13

Milk proteins

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Abstract: This chapter summarises the bovine milk protein system and describes methods for the commercial production of dehydrated bovine milk protein-enriched food ingredients. Selected functional properties and biological activities of these ingredients are presented. In addition, this chapter describes various applications for these milk protein-enriched ingredients in food and nutraceutical products.

Key words: milk proteins, caseins, caseinates, whey proteins, production of milk protein products, functional properties of milk protein products, biological activity of milk protein products, food uses of milk protein products.

13.1 Introduction

Dried milk protein-enriched products produced from bovine (cow) milk are considered to be high esteem food ingredients due to their high nutritional value. In formulated food products these protein enriched ingredients are used to bind and emulsify fat, bind and entrap water and entrap and stabilise air, and these physico-chemical and functional properties are important in the modification of the textural and rheological characteristics of formulated foods and in contributing to product stability and sensory appeal. Milk protein hydrolysates and milk protein derived bioactive peptides have emerged as potential speciality ingredients and nutraceuticals for application in food, and speciality nutraceutical formulations. In this chapter methods for the commercial production of dehydrated bovine milk protein enriched products are outlined and the functional properties of these products and their applications in food and nutraceutical products are described.

13.2 The milk protein system

Normal bovine milk is a highly complex system that contains approximately 3.5% by weight protein (Table 13.1); the protein is traditionally divided into two main fractions based on solubility. The caseins, which comprise about 80% of the total nitrogen in milk, are insoluble at their isoelectric points (~ pH 4.6) at temperatures > 8 ºC and precipitate from milk under these conditions, while 20% of the total nitrogen remains soluble in the serum, about 15% being whey proteins and the remainder being non-protein nitrogenous components. While most of the whey proteins are also insoluble at their isoelectric points (~ pH 5) at very low ionic strength, they are soluble at this pH in the ionic environment of milk.

13.2.1 Caseins

The highly heterogeneous casein fraction comprises four principal primary proteins (gene products), namely α_{s1}-, α_{s2}-, β- and κ-caseins, the γ-caseins (proteolytically derived from β-casein) and several minor proteins and peptides (Fig. 13.1). The α_{s1}- and β-caseins contain no cysteine or cystine while α_{s2}- and κ-casein each possess two half-cystine residues (Table 13.2) that normally exist

Table 13.1 Composition of bovine milk (from Swaisgood, 1992;[11] Zall, 1992[274] and Creamer and MacGibbon, 1996[8])

Component			Concentration g/l
Total protein			30–35.8
	Caseins	α_{s1}-casein	9–15
		β-casein	9–11
		κ-casein	3–4
		α_{s2}-casein	3–4
	Whey proteins	β-lactoglobulin	2–4
		α-lactalbumin	0.7–1.5
		Igs	0.6–1.0
		BSA	0.1–0.4
		Proteose peptones	0.6–1.8
Fat			37–50
Lactose			47–49.6
Ash			6.8–7.4
Water			845–877

Table 13.2 Some physico-chemical characteristics of the caseins

Protein	Number of residues	mol P/ mol protein	Isoionic pH	Mol. wt. (kDa)	Number of prolines	Number of half-cys
α_{s1}-casein	199	8	4.94	23.6	17	0
α_{s2}-casein	207	10–13	5.45–5.23	25.2–25.4	10	2
β-casein	209	5	5.14	24	35	0
κ-casein	169	1	5.61	19	20	2

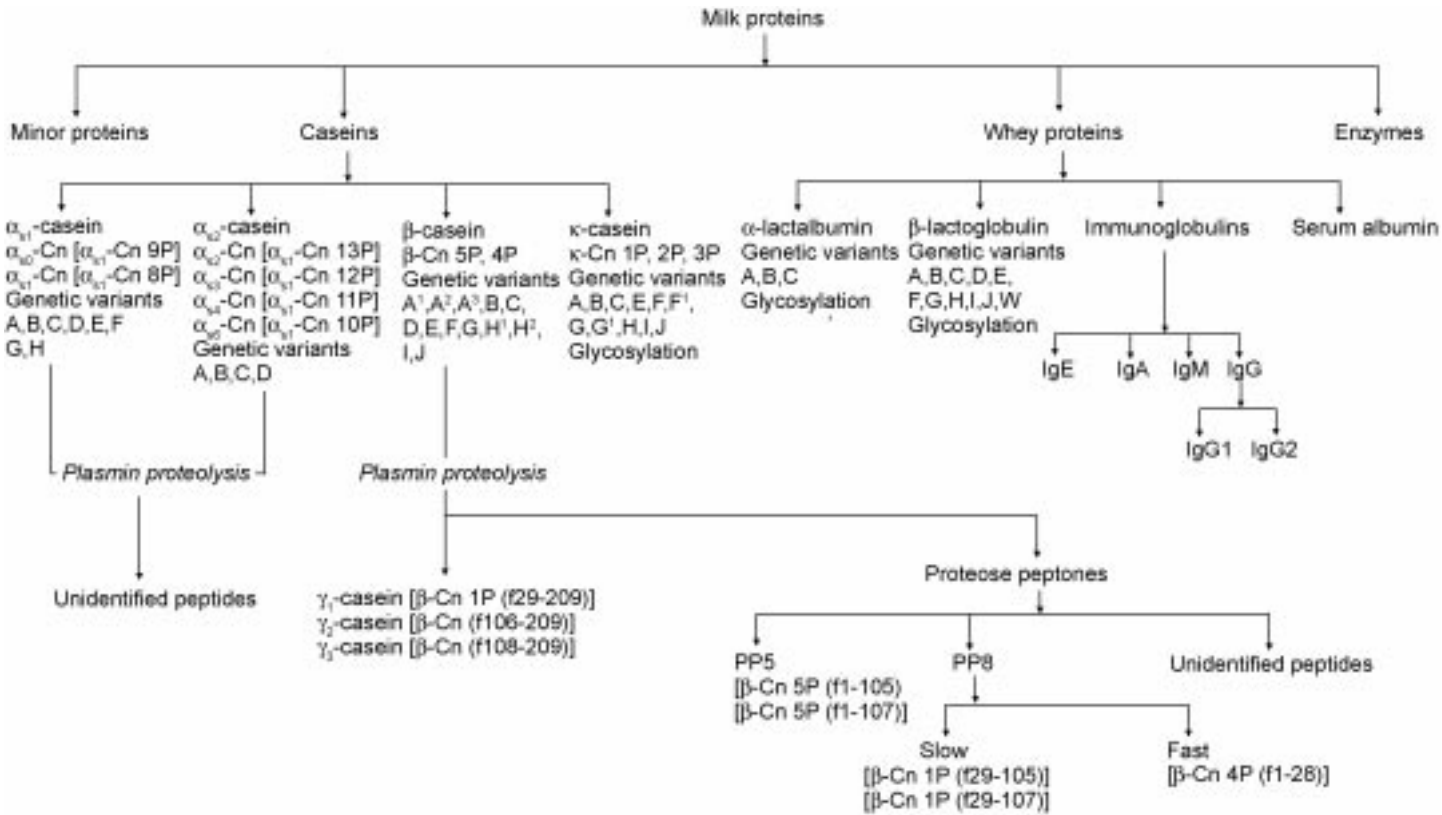

Fig. 13.1 Distribution of proteins in bovine milk.

as homogeneous disulphide bonds. The high prolyl content of caseins tends to prohibit the formation of secondary structure and the protein molecules are small, amphipathic, randomly coiled, relatively open 'rheomorphic' structures and have a tendency to undergo self-association.[1] Genetic polymorphism among the caseins resulting in differences in amino acid contents, different degrees of phosphorylation, and variability in glycosylation of κ-casein (Fig. 13.1) contributes to variability in the protein net charge, hydrophilicity and metal binding and thus influence the behaviour of the milk proteins during manufacturing processes such as renneting and gelation.

The caseins are prone to association due to regions of high hydrophobicity and the charge distribution arising from the amino acid sequence, phosphorylation and glycosylation. In milk the caseins are present in the form of large, roughly spherical colloidal associations, or micelles, ranging in size from ~30 to 600 nm with molecular weights in the region of 10^8 Da, which can be separated from the molecularly dispersed whey proteins by ultracentrifugation. These casein micelles are quiet tightly packed in milk with 10^{14}–10^{16} micelles present per ml of milk, and are roughly two micelle diameter apart from each other.[2] Micelles also contain colloidal calcium phosphate, magnesium, sodium, potassium and citrate. The micelles are believed to be composed of small particles of calcium phosphate referred to as nanoclusters.[3] These nanoclusters interact with serine-phosphate and some glutamate residues in α_{S1}- and α_{S2}-caseins, crosslinking the proteins resulting in the formation of a network structure (micelle). The micelle is stabilised by κ-casein which is largely situated at the micelle surface with the glycosylated C-terminus of κ-casein molecules protruding from the surface of the micelle as 'hairs'. Hydrogen bonding, hydrophobic interactions and electrostatic interactions are all important in maintaining micelle structure. Binding of calcium to charged regions of the proteins modulates hydrophobic interactions between proteins and between submicelles, and colloidal calcium phosphate salt bridges contribute to micelle stability. In native milk the micelles are prevented from aggregating by both electrostatic and steric repulsion due to the κ-casein 'hairy coat' layer. Proteolytic removal of the 'hairy coat' by renneting or ethanol-induced collapse of the coat destabilises the micelles and calcium-mediated aggregation may occur depending on conditions of temperature, pH and ionic environment.

Limited dissociation of casein from micelles may be induced by cooling or heat treatment. Removal of calcium from the micelle by sequestering salts leads to dissociation of caseins and ultimately disintegration of the micelles, depending on the conditions used. Acidification solubilises the colloidal calcium phosphate, disrupts the micelle structure and reduces the charge on the proteins causing aggregation and precipitation of the caseins. Some characteristics of casein micelles are given in Table 13.3.

13.2.2 Whey proteins

The whey protein fraction is also highly heterogeneous (Fig. 13.1) and includes the principal whey protein β-lactoglobulin, α-lactalbumin, blood serum

Table 13.3 Some physico-chemical characteristics of casein micelles (after Fox and Brodkorb, 2008[275])

Characteristic	Average value
Diameter	120 nm (50–500 nm)
Surface area	8.0×10^{-10} cm^2
Volume	2.1×10^{-15} cm^3
Mass	2.2×10^{-10} g
Density (hydrated)	1.0632 gcm^{-3}
Water content (hydrated)	63%
Hydration	3.7 g H_2O/g^{-1} protein
Voluminosity	4.4 cm^3/g^{-1}
Zeta potential (at 25ºC)	-18.7 ± 0.3 mV
Molecular weight (hydrated)	1.3×10^9 Da
Molecular weight (dehydrated)	5×10^8 Da
Number of peptide chains	5×10^3
Number of particles per ml milk	10^{14}–10^{16}
Surface of micelles per ml milk	5×10^4 cm^3
Mean free distance	240 nm

albumin, immunoglobulins, β-casein-derived proteose peptones, numerous minor proteins, including lactoperoxidase and lactotransferrin, and various enzymes. Unlike the caseins, the whey proteins have globular conformations with high proportions of their sequences in ordered structures. These structures display greater hydrophilicity, less amphipathicity and a more limited tendency for self association; they have greater heat sensitivity but are less sensitive to changes in ionic strength and pH than caseins.[4] The monomeric β-lactoglobulin contains five cysteine residues, four of which occur as intramolecular disulfide crosslinks and one as a free SH group. Numerous genetic variants are known, one of which is glycosylated. Monomeric α-lactalbumin is a calcium metalloprotein that has four intramolecular disulphide crosslinks and also exists as several genetic variants that may have minor glycosylated forms. The binding of calcium is essential for proper folding and disulphide bond formation of native α-lactalbumin.[5] The genetic variants present in milks, and the variability in their degree of glycosylation, affects the functional behaviour of the proteins during milk processing. Some characteristics of the major whey proteins are summarised in Table 13.4.

Caseins are extremely heat-stable proteins, whereas the whey proteins denature on heating at ~70 ºC. At its normal pH (~6.7), milk may be heated at 140 ºC for 20 min. before coagulation occurs; however, on heating milk at > 72 ºC, whey proteins denature and interact with casein to form a complex. Some of the differences between caseins and whey proteins form the basis of industrial methods for casein and whey protein isolation; however, caseins and whey proteins can also be isolated together in various high-protein products, referred to as co-precipitates, milk protein concentrates or total milk proteins. Detailed descriptions of the milk protein system are given in Fox,[2,6,7] Holt,[3] Creamer and MacGibbon,[8] Walstra *et al*.,[9,10] Swaisgood,[1,11] and Farrell *et al*.[12]

Table 13.4 Some physico-chemical characteristics of the major whey proteins

Protein	Number of residues	mol P/ mol protein	Isoionic point	Mol. wt. (kDa)	Number of prolines	Number of half-cys
β-lactoglobulin	162	0	5.2	18	8	5
α-lactalbumin	123	0	4.2–4.5	14	2	8
BSA	582	0	5.3	66	28	35
Ig	variable			150–900		
Proteose peptones	variable			4.1–40.8		

13.3 Production of milk protein products

13.3.1 Caseins and caseinates

Dry milk protein powder products are manufactured to convert the protein in perishable liquid milk to products that can be stored for long periods of time without loss of quality, flavour and disperability and that can be cheaply transported for use in food applications. Methods for the manufacture of caseins and caseinates have been reviewed by Muller,[13,14] Mulvihill,[15,16] Fox and Mulvihill,[17] Mulvihill and Fox,[18] Fox and McSweeney,[19] Walstra *et al*.,[9,10] Mulvihill and Ennis[20] and O'Connell and Flynn[21] and are summarised in Fig. 13.2.

Casein is manufactured from skim milk to minimise possible flavour defects arising from deterioration of lipids in the dried casein products. Casein micelles are destabilised by acidifying the milk to pH ~ 4.6 by either (i) chemical acidification by injection of mineral acid (typically 1–2 M HCl) or carbonic acid (CO_2 is added under pressure and combines with H_2O forming carbonic acid) into the milk at ~30 ºC, (ii) mixing milk at ~10 ºC with sufficient cation exchange resin in the hydrogen form to allow exchange of cations in the milk for the H^+ resulting in a milk acidified to pH ~ 2.0; separation of the acidified milk and resin and subsequently mixing this acidified milk with untreated milk to achieve the desired pH, or (iii) inoculation of milk with starter cultures and incubation at 30 ºC to produce lactic acid from lactose naturally present in the milk, whereupon the caseins coagulate as the pI of the proteins is reached. Alternatively, casein micelles are destabilised at the natural pH of milk using any of a number of proteolytic enzyme preparations such as calf rennet or increasingly, genetically engineered chymosin or rennet substitutes such as microbial (aspartic) proteinases. This results in cleavage of the κ-casein to para-κ-casein and soluble glycomacropeptides, consequently the modified casein micelles become susceptible to the calcium present in the serum phase of the milk and coagulate at temperatures above 20 ºC. Continued coagulation of the destabilised micelles to form a curd which may be easily separated from the whey is facilitated by a process referred to as 'cooking' which involves increasing the temperature to 50–60 ºC. The curd is then separated from the whey using vibratory, moving or stationary inclined nylon or stainless steel

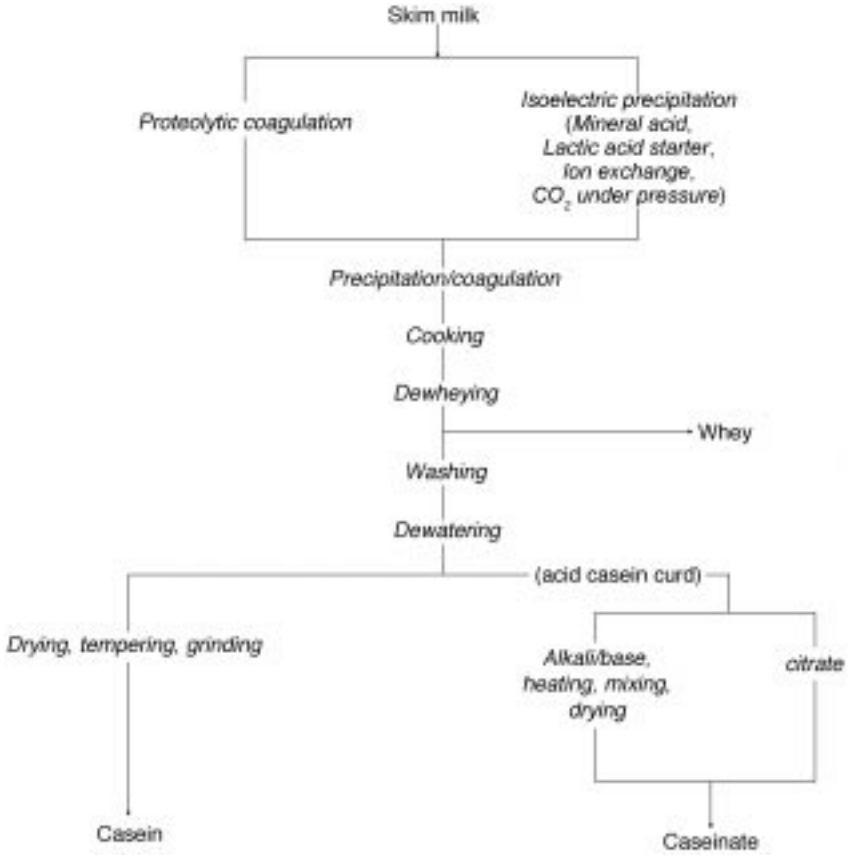

Fig. 13.2 Industrial preparation of casein-based protein products.

mesh screens, or polyester fabric screens in a cascade arrangement, or using mechanical devices such as horizontal bowl centrifuges or roller presses. After separation of the curd from the whey, the curd is washed with potable water to remove residual constituents such as lactose, salts and whey proteins, mechanically dewatered, to minimise the quantity of water to be evaporated during the subsequent drying, and dried using a vibrating fluidised bed, pneumatic ring, a belt drier or an attrition dryer to a moisture content of ~12%. The dried casein is tempered, and blended to achieve a uniform moisture content; this is followed by grinding or milling to the required particle size. Mineral acid casein is produced by method (i), lactic acid casein by method (iii) and rennet casein by the proteolytic coagulation method.

Micellar casein, generally referred to as phosphocaseinate, has similar compositional and physical characteristics to the native casein micelles in milk. Micellar casein is manufactured by microfiltration of fresh skimmed milk at 50 ºC using a suitable membrane of pore size 0.1–1.0 μm which allows permeation of the serum proteins and soluble constituents whilst retaining the casein

micelles.[22,23] Diafiltration with water is used to further remove soluble permeable constituents and the retained solution (retentate) is spray dried.

Acid caseins are not soluble when redispersed in water but can be solubilised by the addition of alkali such as NaOH, KOH, $Ca(OH)_2$ or NH_3OH to produce sodium, potassium, calcium and ammonium caseinate, respectively. Caseinate manufacture has been reviewed by Bergmann,[24] Towler,[25,26] Muller,[13,14] Southward,[27] Mulvihill,[15,16] Fox and McSweeney,[19] Mulvihill and Ennis[20] and O'Connell and Flynn.[21]

Spray-dried sodium caseinate, the water-soluble casein most commonly used in food applications, is prepared as follows. Wet acid casein curd is mixed with water at 40 ºC to a solids content of about 25% (or alternatively, dry acid casein in a slurry with water) is milled in a colloid mill and NaOH (~2.5 M) is mixed with the slurry to give a final pH of 6.6–6.8. The viscous slurry is then vigorously agitated and heated to ~75 ºC in a series of vats to complete solubilisation and then further heated to 95 ºC in a plate heat exchanger. The pH of the solution is adjusted with NaOH, if necessary, to give a caseinate of the required pH; the viscosity of the solution is measured and controlled by addition of hot water, if necessary, ensuring efficient atomisation, and the sodium caseinate solution is then spray dried. Holding time at high temperature is minimised to limit Maillard browning of the casein and the exposure time to high pH during dissolving minimised to prevent lysinoalanine formation and the production of off-flavours.

Other caseinates may be produced by using different manufacturing methods or by employing alternative bases for neutralisation of the acid casein. Roller dried sodium caseinate can be prepared by mixing moist casein curd (50–65% solids) with an alkaline sodium salt (usually Na_2CO_3 or $NaHCO_3$) and feeding this onto the drum of the drier.[26,28] Granular sodium caseinate can be produced by reducing the moisture content of casein curd to below 40%, mixing with sodium carbonate (Na_2CO_3) to ensure a free flowing material and drying in a pneumatic ring or fluidised bed drier to give a high bulk density product with improved dispersability in comparison to spray or roller dried sodium caseinate.[28,29] Potassium, ammonium or citrated caseinates may be produced by replacing NaOH with KOH, NH_4OH or trisodium or tripotassium citrate.[28,30,31] Granular ammonium caseinate may also be prepared by exposing dry acid casein to ammonia gas in a fluidised bed reactor with excess ammonia being removed with a stream of air.[32] Calcium caseinate can be made by mixing milled soft casein curd with a metered volume of 10% aqueous $Ca(OH)_2$ slurry to the required pH, agitating and recirculating until conversion is complete (>10 min) and then heating the dispersion in a tubular heat exchanger to 70 ºC followed by spray drying.[33] In an extrusion process, casein at 50% solids from a dewatering device can be fed into an extruder, and 25% NaOH added to form a viscous sodium caseinate. Caseinate may also be prepared by feeding dry acid casein into the extruder and reacting with 20–40% NaOH at temperatures up to 120 ºC. The caseinates are subsequently roller dried and ground to a powder of the required particle size.[34] Compositional standards for caseins and caseinates are given in Table 13.5.

Table 13.5 Compositions of caseins and casein-derived milk protein products

	EU regulation 2921/90							USDA		Codex Alimentarius Stan A-18-1995			FIL-IDF 72:1974		FIL-IDF 45:1969	
	EU Acid casein		EU Rennet casein		EU Caseinates			Edible dry casein (acid)		Rennet casein	Acid casein	Caseinates	Caseinate		Acid casein	
	Annex I	Annex II	Annex I	Annex II	Annex I	Annex II	Annex III	Extra grade	Standard grade				Extra grade	First grade	Extra grade	Standard grade
Protein (%, min)	–	–	–	–	88.00	88.00	85.00	95 dry basis	90 dry basis	84 dry basis	90 dry basis	88 dry basis	90 dry basis	88 dry basis	95 dry basis	90 dry basis
Moisture (%, max)	12.00	10.00	12.00	8.00	6.00	6.00	6.00	10	12	12	12	8	6.0	8.0	12	12
Fat (%, max)	1.75	1.50	1.00	1.00	–	–	1.50	1.5	2.0	2.0	2.0	2.0	1.5 dry basis	1.5 dry basis	1.7 dry basis	2.25 dry basis
Ash (%, min)	–	–	7.50	7.50	–	–	–	–	–	7.5	-	–	–	–	–	–
Ash (%, max)	–	–	–	–	–	–	6.50	2.2	2.2	–	2.5	–	–	–	–	–
Max fat and ash	–	–	–	–	6.00	6.00	–	–	–	–	–	–	–	–	–	–
Lactose (%, max)	–	–	–	–	–	–	1.00	1.0	1.0	1.0	1.0	1.0	0.5	1.0	0.20	1.0
Max free acid	0.30	0.20	–	–	–	–	–	0.20	0.27	–	0.27	–	–	–	0.20	0.27

Note: Products may also be subject to microbiological testing including standard plate count, coliforms, salmonella, thermophiles, yeasts and moulds.

13.3.2 Fractionation of caseins

Industrial scale fractionation of caseins may be desirable for a number of reasons. Human milk contains β- and κ-caseins but no α-casein, making β-casein an attractive ingredient for applications such as infant formulae. The high surface activity of β-casein makes the protein a desirable emulsifier or foaming agent. As κ-casein is responsible for stabilising micelles; it may find application as a stabiliser in certain milk products. Use of individual casein fractions is also a prerequisite for the generation and recovery of casein derived bioactive peptides of high purity.

Essentially all methods employed for fractionating caseins are based on the association characteristics of the individual caseins. Disruption of the caseins micellar/associated structure in part or fully is necessary for fractionation of the caseins. In a micellar casein system such as milk and in a dispersion of calcium or sodium caseinate, the individual casein molecules associate via a range of forces, including hydrophobic interactions. The strength of hydrophobic interactions is temperature dependent and at low temperatures (< ~5 ºC) when hydrophobic interactions are weaker, the β-casein molecules dissociate from α_s-/κ-casein complexes and exist in solution as monomers.[35] β-Casein can be fractionated by renneting calcium or sodium caseinate at 4 ºC; under which conditions β-casein remains soluble, while α_s- and para-κ-caseins precipitates and can be separated by centrifugation. Precipitation of the β-casein can be achieved by warming the supernatant to 30 ºC.[36] β-Casein may also be fractionated from a slurried casein feedstock by cooling to −10 to 14 ºC at pH 3.5–8.0 for a sufficient time to separate the slurry into a solid phase (β-casein depleted) and a liquid β-casein enriched phase.[37] Terre *et al*.[38] isolated β-casein by treating milk or calcium caseinate with a calcium complexing agent which dissociates all of the casein, prior to microfiltration at 0–7 ºC. The permeate was β-casein enriched and the retentate was β-casein depleted. This procedure has been modified to obtain, on microfiltration at 4 ºC, a β-casein enriched permeate from whole casein adjusted to pH 4.2–4.6, or from sodium caseinate treated with calcium chloride to enhance aggregation of the other caseins.[39,40] Huppertz *et al*.[41] isolated β-casein from renneted skimmed milk; the rennet casein curd was recovered by centrifugation, resuspended in water and the dispersion incubated at 5 ºC to facilitate β-casein dissociation; the dissociated β-casein was recovered by low temperature centrifugation. Murphy and Fox[42] reported a method for fractionation of β-casein from a dilute sodium caseinate solution by ultrafiltration at 4 ºC. The β-casein was recovered from the enriched permeate by heating it to >20 ºC to facilitate polymerisation of the β-casein, following by further ultrafiltration to recover the polymerised β-casein in the retentate, or by precipitation of the β-casein at pH 4.9 at 20 ºC followed by centrifugation.

Law and Leaver[43] described a method of selective precipitation of all four caseins from skimmed milk or caseinate. The skimmed milk or caseinate was adjusting to pH 11 at 30 ºC with NaOH; calcium chloride was added to a final concentration of ~0.06 M, precipitating α_s-/β-casein after which the pH was readjusted to 7 with HCl and the α_s-/β-casein was recovered by low speed

centrifugation or filtration. The κ-casein enriched supernatant was acidified to pH 3.8 to precipitate κ-casein which was recovered by centrifugation/filtration. The precipitated α_s-/β-casein was then redispersed and separated by adjusting the dispersion to pH 4.5 and 2 ºC, which yielded α_s-casein as a precipitate and a supernatant which on warming to 35 ºC yielded a precipitate of β-casein. Chromatographic methods can be used to fractionate caseins and have been shown to be superior to precipitation techniques; [44–47] however, these chromatographic methods are not easily scaled up and require high concentrations of non-food grade dissociating/reducing agent making them unsuitable for producing casein for human consumption. Turhan *et al*.[48] prepared casein fractions by microfiltration of skim milk followed by anion exchange chromatography using L-cysteine as a food grade reducing agent in the eluting buffer, replacing non-food grade reducing agents such as dithiothreitol or β-mercaptoethanol.

13.3.3 Whey protein-enriched products

Whey is the serum or liquid remaining after the removal of fat and casein from milk during the manufacture of cheese or acid and rennet casein. Methods for the recovery of proteins from whey have been reviewed by Marshall,[49] Matthews,[50] IDF,[51] Morr,[52] Mulvihill,[16] Mulvihill and Grufferty,[53] Timmer and van der Horst[54] and Mulvihill and Ennis.[20] These methods are summarised in Fig. 13.3 and are outlined below.

Sweet whey (minimum pH 5.6) is obtained from the manufacture of cheese or rennet casein, while acid whey (maximum pH 5.1) is obtained from the manufacture of acid casein.[55] Acid whey has higher mineral/ash content (increased amounts of calcium and phosphate) than sweet whey, and if starter bacteria produced the acid by fermentation of lactose, the lactose concentration is reduced. Whey and whey protein-enriched solutions are normally pasteurised using minimum temperature and holding times and maintained at low temperature to minimise microbial spoilage and physico-chemical deterioration of the proteins and other whey constituents that would affect the functional and organoleptic properties of the resulting protein-enriched products. Compositions of typical whey-derived milk protein products are given in Table 13.6.

Whey powders and modified whey powders

Whole whey powders containing less than 15% protein are produced by concentrating whey by evaporation alone or in combination with reverse osmosis followed by spray drying. Special methods are used to produce non-hygroscopic, non-caking wettable whey powders. Following concentration to 50–60% solids, concentrates are cooled to 30 ºC, seeded with finely ground α-lactose monohydrate or well-crystallised whey powder, held for several hours and then cooled to 10 ºC to crystallise lactose as α-lactose monohydrate which is less hygroscopic than non-crystalline lactose glass. The concentrate may then be dried by spray-drying in a single stage process or, more commonly, by multistage processes for improved functionality of the powders. In a two-stage

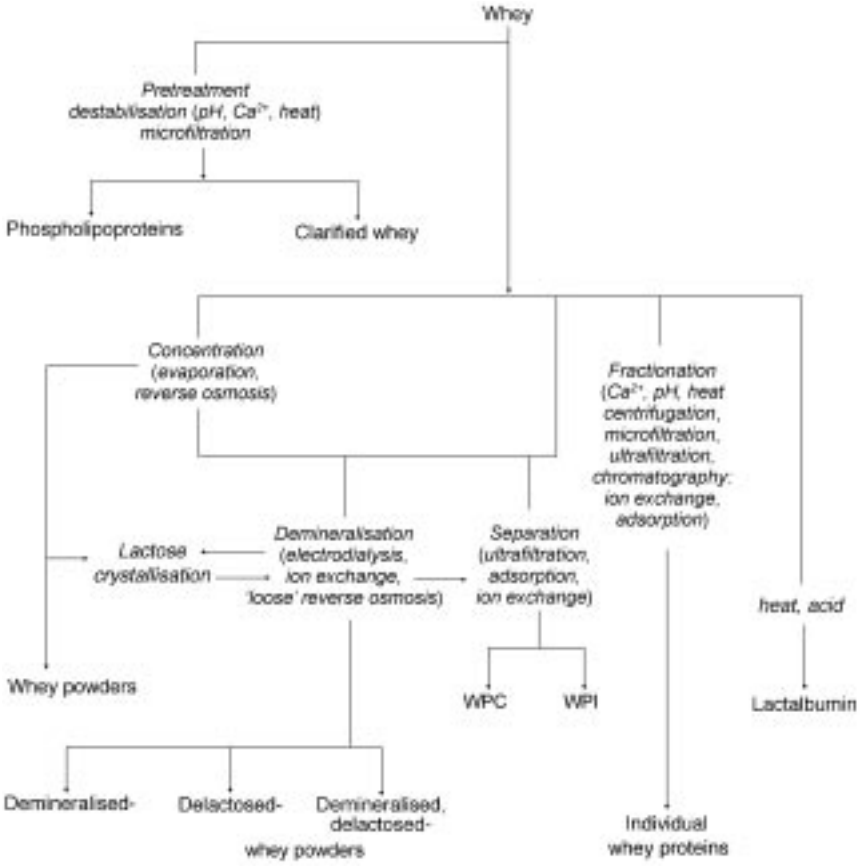

Fig. 13.3 Industrial isolation of protein products from whey.

drying process the pre-crystallised concentrate is spray dried at low temperature and post-crystallisation and final drying takes place in an external vibrating fluidised bed; in a three-stage process the second drying stage is on an integral fluid bed within the spray dry chamber and final drying is in the third stage located outside the chamber. Another three-stage process involves atomisation with primary drying in a low profile chamber, post-crystallisation and two drying stages occur on a conveyor belt system at the base of the chamber. These two- and three-stage processes produce large agglomerated particles of low bulk density that are non-hygroscopic and readily rehydratable.

The mother liquor obtained as a by-product of lactose manufacture may be concentrated and spray dried as a delactosed whey protein concentrate powder containing ~30% protein. Delactosed whey powder has a high mineral content (up to 25%) that may restrict its use in certain food applications, and affect its flavour and nutritional qualities. Demineralisation by reverse osmosis, electrodialysis or ion-exchange and/or lactose crystallisation to reduce the lactose and/or mineral concentration of whey is used to produce modified whey

Table 13.6 Compositions of typical whey-derived milk protein products

	Codex Alimentarius Stan A-18-1995	Codex Alimentarius Stan-A-18-1995	DMV International	DMV International	Davisco Foods International	Davisco Foods International	Glanbia	Glanbia
	Whey powder	Acid whey	WPC	WPC	WPI	WPH	WPC	WPI
			Esprion 300	Textrion PROGEL 800	PiPro	BioZate 3	WPC 30	
Protein (min)	11	10	30	80	95.0	94.0	30.5	90
Moisture (max)	5.0	4.5	5	5	5.0	5.5	4	5
Fat (max)	2	2	3.5	8	1.0	1.0	4	0.5
Ash (max)	9.5	15.0	9.5	6	3.0	5.0	6.5	
Lactose (min)	61.0	61.0	By difference	By difference	1.0	1.0	53.0	1
	Carbery	Carbery	Carbery	Dairygold Food ingredients	Dairygold Food ingredients	Kerry ingredients	Kerry ingredients	Kerry ingredients
	WPC	WPI	WPH	WPC	WPC	Whey powder	90% demineralised whey	Whey permeate
	Carbelac 80	Isolac	Optipep 80	Gold Gel 3503	Gold Gel 4506		W712	
Protein (min)	80	90	80	34.2	45.5	12	12	4
Moisture (max)	6	6	6	3.8	4	<5	<5	<5
Fat (max)	9	1	9	4.1	3.5	<1.5	<1.5	<1
Ash (max)	4	4	8	Typically 6.8	Typically 5.2	7	1	
Lactose (min)	5	<0.1	5	By difference	Typically 40.3			

powders such as demineralised and demineralised-delactosed whey powders which contain 15–35% protein. By using a combination of processes the mineral profile of whey products can be carefully controlled.

Whey protein concentrates (WPC)

Whey protein concentrates (WPC) of varying protein concentrations (35–85% protein) are manufactured under mild conditions of pH and temperature by ultrafiltration (UF), a physico-chemical separation technique in which whey flows under pressure over microporous membranes that facilitate the separation of small molecules such as lactose, salts and water as a permeate from whey proteins, fat globules and suspended solids, which are concentrated relative to other solutes in the retentate. To increase the protein level in the WPC, diafiltration (DF) is used; this involves dilution of the retentate with water to increase the removal of membrane-permeable molecules. Following UF/DF the retentate is normally cooled to 4 ºC and stored until sufficient volume is accumulated for spray drying. To minimise the cost of water removal, the retentate is usually concentrated by evaporation prior to spray drying. Pasteurisation or even UHT treatment of the retentate may also be necessary as bacteria and spores in the whey are also concentrated during UF/DF.

Whey is commonly pre-treated prior to UF/DF processing by methods involving adjustments to temperature and/or pH, addition of calcium or calcium complexing agents and either quiescent standing, centrifugation or micro-filtration to dissolve colloidal calcium phosphate and/or to remove insoluble cheese curd or casein fines, milkfat and calcium lipophosphoprotein complexes.[20] These pre-treatments increase flux during UF, prevent fouling of the membranes, reduce the lipid content and modify the properties of the whey protein concentrates, resulting in low fat products with improved functionality in some applications.

Whey protein isolates (WPI)

Whey protein products referred to as whey protein isolates (WPI), containing 90–95% protein, are produced from whey by ion-exchange chromatographic methods. At pH values lower than their isoelectric points (~ pH 4.6), amphoteric whey proteins have a net positive charge and behave as cations that can be adsorbed onto cation exchangers while at pH values above their isoelectric points, the proteins have a net negative charge and behave as anions allowing fractionation to be achieved by anion exchange chromatographic processes. In a cation exchange process, whey is adjusted to pH < 4.6 with acid, and reacted with a cation exchange resin to allow protein adsorption. Lactose and other non-adsorbed materials are eluted with the whey liquid. The resin is further washed with water and the pH is then adjusted to >5.5 with alkali to desorb the proteins from the ion exchanger. The solution of proteins that elutes from the resin is pH adjusted, concentrated by ultrafiltration and/or evaporation, cooled to 4 ºC and stored until a sufficient volume has been accumulated and then spray dried to produce WPI.[56–59] Whey of pH >5.5 is applied to an anion exchanger to permit

adsorption of negatively charged anionic protein molecules. Following elution of non-protein materials, and resin washing, the proteins are desorbed in an eluate of low pH. The protein eluate solution is pH adjusted, concentrated and spray dried to produce WPI. These WPI products are characterised by high protein and low lactose and lipid concentrations and have good functionality. However, the need to concentrate and purify the dilute protein-containing eluate by UF, evaporation and drying, the time requirement for conducting fractionation, the large volumes of washing and regeneration solutions used and microbial contamination of the reactor are major problems associated with these ion exchange processes and result in high production and final product costs.

Lactalbumin
Globular whey proteins readily denature on heating adopting more random structures with exposed sulphydryl and hydrophobic groups facilitating protein-protein interactions leading to aggregation and precipitation of the denatured proteins to extents dependent on heating temperature and holding time, pH and concentration of calcium. Commercial precipitation conditions depend on whey type and the desired final product characteristics and whey may be pre-concentrated and/or demineralised prior to precipitation. The precipitated protein is recovered by filtration or centrifugation in horizontal solid bowl decanters and washed to reduce mineral and lactose contents before drying using any of several types of drier to give a product referred to as lactalbumin (which should not be confused with the serum protein α-lactalbumin).[60] Lactalbumin containing up to 90% protein on a dry weight basis may be recovered, depending on precipitation pH and degree of washing. As the whey proteins are denatured to facilitate their recovery as lactalbumin the recovered product has poor solubility.

13.3.4 Fractionation of whey proteins

The compositions of bovine milk and human milk differ significantly as human milk does not contain β-lactoglobulin, which is in fact the most allergenic of the bovine milk proteins to the human infant. α-Lactalbumin appears to be more appropriate for use in preparing 'humanised' infant formulae; accordingly, there is considerable commercial incentive for fractionating α-lactalbumin and β-lactoglobulin. Numerous methods with potential for commercial scale fractionation of whey proteins have been reviewed by Maubois *et al.*,[61] Mulvihill,[16] Mulvihill and Grufferty[53] and Mulvihill and Ennis.[20]

Whey protein fractionation processes fall into three main categories: (1) exploiting the physico-chemical properties of the proteins for selective precipitation,[62–65] (2) membrane separation techniques[66–69] and (3) chromatographic techniques.[70–74] Mild heat treatments of whey or a whey concentrate or clarified whey under controlled pH and ionic conditions or demineralisation of whey concentrate under controlled pH conditions can be used to effect selective reversible precipitation of α-lactalbumin or β-lactoglobulin enriched whey protein fractions. Following separation of the precipitate from the β-

lactoglobulin or α-lactalbumin enriched solutions, the precipitate is resolubilised by water addition and pH adjustment and then dried while the soluble protein is further concentrated by ultrafiltration/diafiltration before drying.

Pearce[62,63] developed a heat precipitation method for the fractionation of α-lactalbumin; on acidification of whey to pH ~ 5.0, α-lactalbumin loses its calcium and is transformed into apo-α-lactalbumin; the apo-protein is heat liable and aggregates on heating to ~55 ºC and can be separated from the soluble undenatured proteins by centrifugation, filtration or microfiltration. Various modifications of this heat precipitation method for the fractionation of whey proteins have been reported including those of Maubois *et al*.,[61] de Wit and Bronts,[75] Stack *et al.*[76] and Gésan-Guziou *et al*.[77]

Both α-lactalbumin and β-lactoglobulin are insoluble in pure water at their isoelectric points; β-lactoglobulin requires a higher ionic strength for solubility than α-lactalbumin. This characteristic was exploited by Amundson *et al*.[78] and Slack *et al*.[79]; β-lactoglobulin was successfully precipitated by acidifying UF-concentrated whey to pH 4.65 and demineralising the concentrate to ~0.023% ash by electrodialysis whereupon β-lactoglobulin precipitates and may be recovered by centrifugation. Bazinet *et al*.[80] used a bipolar membrane electro-acidification (BMEA) procedure to fractionate highly purified β-lactoglobulin (97–98% pure) from a whey protein isolate solution. This procedure coupled the effect of demineralisation and acidification using bipolar membranes to dissociate water molecules at their interfaces and cation exchange membranes to demineralise by migration of low molecular weight ionic species. Electroacidification occurred in a batch process at a current of 2.0 A and at 60 V and was stopped when the WPI solution reached pH 4.6; the solution was then centrifuged at 500 g for 10 min at 4 ºC to recover the precipitated β-lactoglobulin fraction which was then washed and resolubilised at pH 7.0 prior to freeze drying.

Bhattacharjee *et al*.[81] prepared a high purity β-lactoglobulin from casein whey which was pre-treated by centrifugation, microfiltration and a four-stage discontinuous diafiltration process to obtain a whey protein concentrate (free of colloidal matters and lipid). This whey protein concentrate underwent a two-stage ultrafiltration process at pH 2.8 or 5.6 using flat disk membranes in a stirred rotating disk module followed by anion ion exchange membrane chromatography using a Vivapure™ Q Mini-H-column at pH 4.6–5.0; the unbound β-lactoglobulin (pI 5.2–5.4) was recovered in the anion exchange flow-through-fraction while α-lactalbumin (pI 4.5–4.8) was bound to the anion exchange membrane and was subsequently eluted by washing with a pH 8.0 buffer containing NaCl. Cheang and Zydney[68] combined two UF membranes of pore size 100 and 30 kDa, to fractionate α-lactalbumin and β-lactoglobulin from whey protein isolate. Mehra and Kelly[82] also used a two-membrane casade filtration process to obtain fractions enriched in α-lactalbumin.

Affinity chromatography,[70] hydrophobic interaction chromatography,[72] gel permeation chromatography,[83] various anion exchange[84–86] and cation exchange[66,87] methods have been successfully used to fractionate whey proteins.

Girardet *et al*.[88] separated all the major whey proteins using a MonoQ anion exchanger and also separated various genetic variants of β-lactoglobulin by varying pH, ionic strength and composition of the buffers used. Ion exchangers used to recover WPI may also be used to fractionate whey proteins. All the whey proteins are initially adsorbed on the resin but on continued passage of whey through the exchanger, β-lactoglobulin, which has a higher affinity for the resin than the other proteins, displaces bound α-lactalbumin and serum albumin which then eluate in an eluant rich in these proteins; a highly purified β-lactoglobulin is obtained by eluting the protein-saturated column using 0.1 M HCl.[84,89]

Lactoperoxidase (LPO), a broad specificity peroxidase present at high levels in bovine milk but at very low levels in human milk, is involved in antibacterial activity and as such is of commercial interest for applications such as cold sterilisation of milk, protection against mastitis and as a supplement for calf or piglet feeds to protect against enteritis.[18] LPO is cationic at neutral pH and can be isolated from either milk or whey by industrial scale cation exchange chromatography.[90] Lactoferrin (Lf) binds iron strongly and is important in iron absorption and protection against enteric infection in neonates.[18] As human milk contains considerably higher levels of Lf than bovine milk, there is interest in supplementing bovine milk-based infant formulae with the protein. Like LPO, Lf is cationic at neutral pH and so adsorbs to the cation exchanger and can be eluted either together with LPO, or separately. LPO and LF have been separated from whey by precipitation using ammonium sulphate followed by separation on carboxymethyl cellulose[91] and also by several gel permeation methods.[92]

Immunoglobulins (Igs), proteins involved in the protection of mammals against infection, are present in mammary secretions. There is considerable interest in the use of Igs to enrich ruminant feeds and in the preparation of formulae for pre-term infants.[18] Milk immunoglobulin concentrates may be prepared by ultrafiltration/diafiltration of acid whey from colostrum and early lactation milk of immunised cows, avoiding temperatures greater than 56 ºC to prevent inactivation of the Igs.[93] Igs may also be isolated from whey using thiophilic gels,[94] or by chelating interaction chromatography.[95] Baick and Yu[96] isolated IgG using gel permeation chromatography, ion exchange chromatography and affinity chromatography, while Xu *et al*.[97] isolated IgG and GMP from whey using an anion exchanger and UF.

The glycomacropeptide (GMP) derived from κ-casein on renneting and present in cheese and rennet casein wheys contains no Phe, Tyr, Trp, Lys or Cys residues, making it suitable for the nutrition of patients with phenylketonuria and it is also reported to have other potential applications as a functional food ingredient. One production method involves passing whey through an ion exchanger; the GMP is in the non-adsorbed fraction which is desalted and concentrated. Another procedure involves selective adsorption of GMP on an ion exchange resin and recovery by desorption using dilute acids or salt solutions. UF combined with heat processing, and heating delactosed whey to induce protein precipitation and subsequent recovery of GMP from the

supernatant by ethanol precipitation have also been used to prepare GMP. Kawasaki *et al*.[98] recovered GMP from WPC by acidification to pH 3.5, where the GMP forms associated non-covalent linked polymers of molecular weight 10–30 kDa which permeate on ultrafiltration using a 50 kDa cut-off UF membrane while the other whey proteins are retained. The GMP rich permeate is re-adjusted to ~ pH 7, where GMP forms polymers of molecular weight 20–50 kDa which are retained by the same ultrafiltration membranes, while the low molecular weight whey components, lactose and minerals pass through the filter. Martín-Daina and Fontecha[99] fractionated GMP from rennet whey or WPC solution by exploiting the high thermal stability of GMP in comparison to the other whey proteins. The whey was heat treated to ~90 ºC for 1 h, causing denaturation and aggregation of the other whey proteins which were recovered by centrifugation at 4 ºC; GMP in the supernatant was then concentrated by ultrafiltration.

13.3.5 Casein-whey protein co-precipitates

Whey proteins can be co-precipitated with casein by first heating milk, at its natural pH, to temperatures that denature the whey proteins and induce their complexation with casein, followed by precipitation of the milk protein complex by acidification to pH 4.6 or by addition of $CaCl_2$ combined with variable levels of acidification. Such products, referred to as high-, medium- or low-calcium casein-whey protein co-precipitates based on calcium content, generally exhibit poor solubility. Insoluble co-precipitates can be dissolved in sodium tripolyphosphate and sodium hydroxide.[14] Methods for preparing similar products with good solubility have been described by Connolly,[100] Lankveldt,[101] and Grufferty and Mulvihill[53]; total milk protein (TMP) may be prepared by heating skim milk to 40–70 ºC, raising the pH to 9.5–10.5 and holding for 3 min, followed by acidification to pH 3.5 and holding for 5 min, before isoelectric precipitation at pH 4.7, washing and drying. Soluble lacto-protein (SLP) may be prepared by adjusting the pH of skim milk to pH 7–7.5, heating to 80–145 ºC for periods of several seconds to 20 min, cooling to 4–45 ºC and isoelectric precipitation of the protein. The curd is separated, washed, dispersed in water, and dissolved at pH 6.7 and spray dried. Hoff *et al*.[102] prepared milk protein concentrate by selectively solubilising lactose from no-fat dry milk using ethanol. The insoluble fraction produced by this one-step process is essentially a casein-whey protein co-precipitate. Ultrafiltration of skim milk to a 4–6 volume concentration ratio (initial volume of milk/volume of retentate) to remove lactose, followed by storage of the retentate at −8 ºC for 1–4 weeks, causing cryo-destabilisation of casein and some of the whey proteins which are sedimentable by centrifugation on thawing.[103]

13.3.6 Milk protein concentrates/isolates

Milk protein concentrates (MPC) is a high protein (up to ~90%) spray dried powder manufactured from skim milk by UF/DF. The casein in MPC is in a

similar, micellar, form to that found in milk while the whey proteins are also largely in their native form.[104,105] The ash contents of these products are relatively high, as the protein-bound minerals are retained. The ash content can be reduced by acidification to dissolve some CCP prior to UF/DF. Klarenbeek[106] prepared a milk protein isolate from skim milk or a mixture of skim milk and whey, by acidification with HCl to pH 3.0 at ~5 ºC prior to a three-stage ultrafiltration, followed by diafiltration of the retentate. The retentate was then diluted, adjusted to pH 6.5, ultrafiltered at 50 ºC and spray dried. A type of MPC may be prepared by mixing skim milk or butter milk with cheese whey, adjusting the pH to 5.5–7.0 and heating to 65–90 ºC and holding, followed by cooling and reverse osmosis to concentrate the proteins.[107]

13.3.7 Modified milk protein products

The utilisation of milk protein products as food ingredients is dependent on their physico-chemical and functional properties. The rising cost of and functional demands on milk protein ingredients have created a need for speciality milk protein ingredients which possess requisite functional properties for specific uses in specific food products. These speciality ingredients may be produced by exploiting the potential to alter the physico-chemical properties of milk proteins by physical, chemical and/or enzymatic modification. Modifications recognised as offering potential for producing speciality milk protein ingredients for food applications include thermal and high pressure processing, protein crosslinking with enzymes such as transglutaminase,[108] protein conjugation by attachment of polysaccharides via a Maillard type[109,110] and specific enzymatic hydrolysis of protein.[111,112]

13.3.8 Milk protein hydrolysates and biologically active peptide fractions

Milk proteins are used in a variety of specific functional and nutritional applications and some milk proteins possess biological activities. Some of these biological activities are associated with the intact proteins themselves while others are associated with amino acid sequence within the proteins that can be generated from the intact protein on hydrolysis by (i) proteolytic enzymes, (ii) microbial proteolytic activity and (iii) some common food processing treatments such as heating, under acid and alkaline condition. Products referred to as milk protein hydrolysates are produced for specific functional and nutritional applications and for generation of these biologically active peptides. The process used for manufacturing protein hydrolysates is highly dependent on the final application of the hydrolysate e.g. protein hydrolysates of low degree of hydrolysis (DH 1–10%) have improved functional properties, mainly foaming and emulsifying properties, and therefore have uses as surface active agents in food applications; extensively hydrolysed milk proteins (DH > 10%) are used as nutritional supplements and in specialised nutritional products.[111,113,114]

The most common way to prepare milk protein hydrolysates for direct use or for recovery of bioactive peptides is by either batch or continuous hydrolysis

using proteolytic enzymes followed by fractionation and enrichment of the peptides produced. Pepsin and trypsin are the gastrointestinal enzymes most commonly used to generate milk protein hydrolysates while other proteolytic enzymes including alcalase, chymotrysin, pancreatin, thermolysin and bacterial and fungal enzymes are also utilised as are combinations of proteinases with carboxy and aminopeptidases.[115,116] Biologically active peptides can be generated in *vivo* during gastrointestinal digestion of milk protein or released in *vitro* on hydrolysis of milk proteins; these biologically active peptides include angiotension-converting enzymes (ACE)-inhibitory, antithrombotic, opioid agonists, opioid antagonists, antimicrobial, immunomodulatory and anxiolytic peptides and casein-phosphopeptides (CCP).[115–118] Gel permeation, ion exchange, hydrophobic interaction and reverse phase chromatography have all been used to fractionate and purify various biologically active peptides from milk protein hydrolysates.[118,119] Ultrafiltration using membranes of appropriate pore size has also been used successful to recover biologically active peptide fractions from casein and whey protein hydrolysates.[116,120] Casein-phosphopeptides were prepared on an industrial scale by enzymatic hydrolysis followed by ion exchange chromatography,[121] by aggregation with bivalent cations in combination with ultrafiltration[122] and by acid precipitation, diafiltration and anion exchange chromatography.[123]

Starter and non-starter bacteria, predominantly lactic acid bacteria, are generally highly proteolytic and are capable of generating bioactive peptides from milk proteins during the fermentation of milk-based products. ACE-inhibitiory, immunomodulatory, anti-oxidative, anti-mutagenic and opioid activities have all been identified in fermented milks and/or cheeses.[124]

13.4 Functional properties of milk protein products

13.4.1 Introduction

The functional properties of proteins are those physical and chemical properties that influence the behaviour of proteins in food systems during processing, storage, preparation and consumption.[125] As the utilisation of many milk protein products in foods is dependent on their physico-chemical and functional properties, a brief overview of their major functional characteristics is included here. Kinsella,[125] Mulvihill and Fox,[126] Mulvihill,[16] Panyam and Kilara,[111] Carr *et al*.,[127] Dickinson,[128] Singh and Havea[129] and Walstra *et al*.[10] provide more extensive reviews on these topics.

13.4.2 Solubility

The solubility of proteins is not only regarded as a primary functional property but as a prerequisite for the proteins' other functional properties.[10] The solubility of a protein denotes the amount of protein that goes into solution or colloidal dispersion under specified conditions (temperature, ionic strength) and is not

sedimented by defined, moderate, centrifugal forces.[130,131] The solubility of a protein is governed by a delicate balance of intermolecular electrostatic and hydrophobic interactions, which are controlled by surface hydrophilic (protein-solvent) and surface hydrophobic (protein-protein) interactions as well as being determined by the native or denatured state of the protein and environmental factors, including pH and temperature.[132]

Acid casein is completely insoluble when dispersed in water as the pH of the dispersion is close to casein's isoelectric pH, i.e. pH 4.6; when the pH of the dispersion is raised to values > 5.5 by addition of a monovalent cation base, the casein is converted to a completely soluble cationic (Na, K, NH_3) salt known as caseinate. Caseinates are soluble up to high concentrations, typically 20–30%, on both the acidic and alkaline side of their isoelectric point.[125] Solutions containing 10–15% caseinate can be easily prepared at pH 6.0–7.0; at higher concentrations high viscosity may become an issue. At pH values < 3.5, casein is also soluble but at this pH the solution is more viscous than at neutral pH values and gel-like systems are formed. Rennet casein is also insoluble in water at pH ~ 7.0, but can be solubilised either by raising the pH above ~ 9.0 or by adding calcium chelators such as food-grade phosphates, polyphosphates and/or citrates.

Sodium salt forms of conventional co-precipitates are also largely insoluble at pH 6.0–7.0, but the sodium salt forms of casein-whey protein co-precipitates prepared from milk heated at alkaline pH values (SLP and TMP) possess solubility characteristics similar to caseinates. Calcium caseinates and medium and high calcium co-precipitates form coarse colloidal dispersions rather than solutions. Traditionally produced co-precipitates generally exhibit poor solubility particularly between pH 6.7 and 7.2. Acid co-precipitates are more soluble than high calcium co-precipitates.

On reconstitution at 5%, w/w, solids in deionised water at 20 ºC, pH 6.9, pilot plant 85% protein MPCs, stored for between 2 days and 2 years at 20 ºC, formed colloidal dispersion. On centrifugation of these dispersions at 700 g for 10 min at 20 ºC, between 32 and 98% of the solids sedimented, with solubility dependent on storage time. The sedimentable protein was found to be predominantly large hydrophobically associated casein particles with some contribution by the minor whey proteins.[133] Commercial MPC samples with different protein contents, produced in a variety of countries also displayed considerable variability in solubility.[134] The 'insoluble material' in MPCs readily dissolves when the MPCs are reconstituted at elevated temperature (50 ºC).[135] The decrease in solubility of MPCs on storage is proposed to be due to crosslinking of the protein at the interface of the powder particles; while the casein in MPCs undergo lactosylation on storage at elevated temperatures, crosslinking by non-covalent interactions may also contribute to the decrease in solubility.[136] The addition of monovalent salt at concentrations of 0.035–0.25 M to the ultrafiltered retentate prior to drying has been shown to improve the solubility of MPCs.[137]

Whey proteins in their native globular conformation are soluble at low ionic strength (> 0.025 M) over the entire pH range encountered in food applications.

Their solubility decreases at high salt concentrations due to salting out. Thermal denaturation of whey proteins occurs at temperatures above ~70 ºC; a major consequence of this denaturation is a reduction in whey protein solubility.[138] The level of denaturation and subsequent insolubility at pH 7.0 and at pH 4.6 (an index of the extent of denaturation caused by processing and storage of protein-rich whey products) depends on heating temperature and time, and whey pH and ionic calcium concentration on heating.

13.4.3 Gelation and coagulation

As already stated, milk undergoes gelation when subjected to a number of treatments (heating, chemical or enzymatic treatments/modifications) and usually casein is the gelling component involved. Protein gelation generally occurs due to an alteration or unfolding of the protein structure which yields polypeptide segments which are capable of specific interactions (protein-protein and protein-water interactions) resulting in the formation of a three-dimensional crosslinked network capable of entrapping large amounts of water.

Limited proteolysis of milk to hydrolyse the micelle-stabilising κ-casein produces para-κ-casein-containing micelles that coagulate at the concentration of Ca^{2+} in the milk serum. Casein micelles may also be destabilised to form gels or precipitates on mixing equal volumes of milk and ~60%, v/v, ethanol.[139] Acid induced gelation or coagulation of milk produces acid gels that may or may not expel whey (synerese), depending on the pre-heat treatment of the milk. Pre-heating to temperatures greater than the denaturation temperature of the whey proteins (usually > 85 ºC) reduces syneresis in fermented milks, while milk used for the production of acid cheeses and caseins is heated as little as possible to promote whey expulsion. Calcium caseinate dispersions also undergo rennet gelation; however, these gels are weak and susceptible to extensive syneresis above refrigeration temperatures.[10] The viscosity of casein is much higher at low pH (2.5–3.5) than at neutral pH and gel-like structures are formed at ~5% protein at temperatures below 40 ºC.[140] Calcium caseinate is the only milk protein system reported to exhibit reversible thermal gelation; concentrated calcium caseinate dispersions (>15% protein) gel on heating to 50–60 ºC, on cooling the gel slowly liquefies but reforms on heating. Gelation temperature increases with protein concentration from 15–20% and with pH in the range 5.2–6.0.[141]

Thermal sensitivity is generally undesirable in the preparation of soluble whey protein-enriched products; however, the property can be exploited for production of thermal gels from whey proteins, which have excellent thermal gelling properties. Characteristics of the whey protein product such as method of production, the extent of whey protein denaturation during manufacture, the contents of protein, total ash, selected minerals and other non-protein components all influence the minimum protein concentration and heating regime required for thermal gelation and characteristics of the gel formed, such as opacity, strength and elasticity or brittleness. Solution conditions such as pH,

ionic species present, other non-protein components added (lipids, salts and sugars) and the presence of reducing agents also influence the gelation characteristics. Depending on these solution conditions during thermal whey protein gelation, different types of gels may be formed (e.g., fine stranded gels, particulate gels) which vary in modulus/hardness, elasticity and turbidity. WPCs and WPIs possessing different gelling properties can be obtained by careful selection of whey type and manipulation of processing conditions during manufacture. Thermal denaturation of whey proteins, in appropriate solution conditions, and subsequent appropriate treatment of the cooled solution can lead to a gelling process called 'cold gelation'.[142] When whey protein isolate solutions of neutral pH and low ionic strength are heated at an appropriate temperature and then cooled, denaturation and possibly minimal aggregation occurs to produce whey protein polymers but gelation does not occur due to strong electrostatic repulsive forces between the polymers formed. Acidification of this cold solution using an acid precursor like glucono-delta-lactone, that slowly reacts with water forming gluconic acid, or infusion of salts into the cold solution, alters the solvent properties reducing electrostatic repulsive forces and thus results in 'cold gelation'.[143] Rapid acidification results in weak brittle gels while slower acidification allows increased exposure of the free thiol groups at the surface of the polymers, increasing disulphide bond formation between aggregates and hence strengthening the gels.[144–146] Enzymatic gelation of milk proteins, other than rennet (chymosin) gelation, may be induced by either protein crosslinking by transglutaminase or by specific hydrolysis using proteolytic enzymes. Transglutaminase induced gelation occurs because the enzyme catalyses the transfer reaction between the amide group of glutamine and an ϵ-amino group of lysine, crosslinking the protein molecules via the formation of covalent intra- and intermolecular isodipeptide bonds.[147] Proteolytic enzyme induced gelation occurs because specific enzymatic hydrolysis of the protein is followed by ordered aggregation of the hydrolysed products to form a gel network.[148] In enzymatically induced gelation of whey protein, rate of gelation and gel strength are dependent on protein concentration, pH, ionic strength and level of protein denaturation.[149]

13.4.4 Hydration properties

The ability to hydrate and thus bind or entrap water is an important functional property of dairy proteins in food applications. The level of hydration is highly dependent on the protein composition, conformation, concentration, pH, salts, the number of exposed polar groups, surface polarity and topography as well as processing conditions, including drying procedure and storage conditions.[125] The level of hydration depends on the particular products; reported hydration values for casein micelles range from 1.4 to 6.4 g H_2O/g, for caseins/caseinates range from 0.7 to 3.8 g H_2O/g and for individual native whey proteins range from 0.32 to 0.60 g H_2O/g. Generally sodium caseinate is capable of binding more water than calcium caseinate or micellar casein particularly at high pH.

Reported values for the water sorption capacity of several milk protein products in a model flour dough system range from 0.96 to 3.45 g H_2O/g of product. Depending on the environmental conditions, thermally denatured whey proteins can have a hydration of over 10 g H_2O/g; this is a result of an increase in exposed protein surface area on denaturation and thus an increased availability of hydrogen bonding sites.[127] When whey protein solutions of sufficient protein content and suitable solution conditions (pH, ions, etc.) are heated, gels are formed and the water holding capacity of such gels make significant contributions to the texture and rheology of a number of processed foods.

13.4.5 Viscosity

Caseinates form highly viscous solutions at concentrations >~15%; this is due to hydration and swelling which increase the hydrodynamic volume, and polymer-polymer interactions, both of which increase resistance to flow and thus viscosity. Below 12% w/w, caseinates are reported to exhibit Newtonian flow while above 12% w/w, flow becomes more pseudoplastic reflecting increasing protein-protein interactions.[150] The viscosity of solutions containing >~20% protein is so high that processing is difficult even at high temperatures.[25] The viscosity of sodium caseinate increases logarithmically with increasing concentration, while there is a linear relationship between log viscosity and the reciprocal of absolute temperature.[42,141,150]

Sodium caseinate viscosity is also very pH-dependent, with minimum viscosity occurring at ~ pH 7.0. By increasing the pH towards 9, an increase in viscosity is observed due to an increase in strong localised inter-molecular interactions. Above pH 9 the casein molecules tend to behave as separate entities and thus viscosity decreases rapidly. The viscosity of casein is also much higher at low pH (~2.5–3.5) than at neutral pH, and gel-like structures are formed at >5% protein at temperatures below 40 ºC. Caseinates exhibit pseudoplastic rheological behaviour and are thixotropic at high shear rates. Calcium caseinate exhibits a lower viscosity than sodium caseinate at the same concentration and ionic strength indicating that the cation present has a significant effect on the viscosity of caseinate ($Na > K > NH_4$).[151] The viscosity of caseinate containing 1% calcium shows unusual temperature dependence; it decreases sharply in a curvilinear fashion with increasing temperature from 30–38 ºC, then remains constant up to ~57 ºC, above which the solution gels at pH 5.4 but not at higher pH values. The relationship between viscosity and temperature depends on protein concentration, pH and $[Ca^{2+}]$.[141] Low levels of added calcium increase the viscosity of sodium caseinate above pH 7.0 but below this pH and at sufficient calcium addition level micelle formation tends to decrease the viscosity.

Manufacturing conditions also affect the viscosity of casein/caseinates. Excessive heating of milk prior to casein manufacture or of casein curd during drying ultimately leads to increased caseinate viscosity. Precipitation at pH values lower than normal (~ pH 3.8) and especially at higher pH values (~ pH 5.05) also increases the viscosity of caseinates. Roller dried caseinates

generally exhibit higher viscosities than spray dried caseinates. Solubilised conventional co-precipitates are more viscous than sodium caseinate and their viscosity increases with increasing calcium concentration, while solutions of total milk protein have viscosities intermediate between those of sodium caseinate and conventional co-precipitates. A logimetric decrease in viscosity of medium calcium co-precipitates containing 1% calcium was reported on increasing temperature; however, the co-precipitate viscosities were far less sensitive to temperature than caseinate viscosities. Increasing the calcium concentration in co-precipitates increases the tendency for gelation at pH 8 and above at ~30 ºC.[152]

Solutions of non-denatured whey proteins are much less viscous than caseinate solutions. They exhibit minimum viscosity around their isoelectric point (pH ~ 4.5); while an increase in pH (6–10) slightly increases viscosity.[150] The viscosity of whey protein solutions is temperature dependent and relative to water, their viscosity decreases between 30–65 ºC, but increases at higher temperatures as a result of thermal denaturation of the globular proteins. WPC solutions containing 4–12% protein are reported to exhibit Newtonian flow while at higher concentrations flow becomes pseudoplastic and at 18–20% yield values are observed. At higher concentrations (>35% w/w), WPC solutions exhibit time-dependent shear thinning behaviour (thixotropic). In general, the addition of salts has little effect on the viscosity of whey protein solutions with the exception of $CaCl_2$ which significantly increases the viscosity.[153]

13.4.6 Surface active properties

Milk proteins are strongly amphipathic and exhibit good surface active properties: they adsorb readily at surfaces/interfaces decreasing surface tension and forming surface/interfacial films via complex intermolecular interactions and thus impart structural rigidity with variable rheological properties. The order of surface activity reported for the individual milk proteins is β-casein > monodispersed casein micelles > serum albumin > α-lactalbumin > α_s-casein = κ-casein > β-lactoglobulin > euglobulins.[154] Sodium caseinate depresses interfacial tension more effectively than whey protein, blood plasma, gelatin or soy protein as it diffuses more quickly to an interface and on reaching the interface adsorbs more quickly than the other proteins.[155] At low protein concentrations sodium caseinate spreads as a thin film with a slightly lower surface coverage than whey protein due to the greater flexibility of the caseins compared to the globular whey proteins, which because of their globular structure tend to absorb as a thicker film on the surface. Aggregated milk protein products such as MPC and calcium caseinate have a limited ability to spread at a surface as the aggregate structures are maintained by calcium bonds and/or colloidal calcium phosphate[156] and this leads to the formation of a multilayer structure which has high surface dilatational modulus/viscosity.

Partially heat denatured whey proteins are capable of binding ionic calcium leading to aggregation; these aggregates are much more readily absorbed at a

surface than native whey proteins and therefore have enhanced surface activity.[157] Limited hydrolysis of milk proteins generates more flexible amphiphilic peptides with a sufficient molecular weight to form stable films at the interface.[111] Hydrolysis of sodium caseinate by plasmin (to produce γ-caseins and proteose peptones) greatly increases its surface activity. The γ_2- and γ_3-caseins are small, very hydrophobic peptides and thus are very surface active. Surface films of sodium caseinate or β-casein are much more flexible and less viscoelastic at both oil/water and air/water interfaces than films of β-lactoglobulin, α-lactalbumin or bovine serum albumin.

13.4.7 Emulsifying and foaming properties

Milk protein products in general, and caseinates especially, are very good fat emulsifiers and are widely used in emulsifying applications in foods. Sodium caseinate stabilised soybean oil emulsions, prepared in a valve homogeniser, exhibited lower creaming stabilities than similar emulsions stabilised by either WPC or soy isolate.[158] Highly dispersed sodium, ammonium and low-calcium caseinates showed higher emulsifying capacities than more aggregated high-calcium caseinate and ultracentrifugal (micellar) caseins, while the emulsions formed using the latter were more stable than those stabilised by the highly dispersed caseinates. For all the proteins studied the fat surface area formed on emulsification increased (i.e. globule size decreased) with increased power input during emulsification and the extent of the increase was inversely related to the degree of aggregation of the emulsifying caseins/caseinates. The emulsions formed using aggregated caseins/caseinates had greater protein loads at the interface (mg/m^2) than for the dispersed caseinates and protein load was directly related to emulsion stability.[159] Heat treatment (>120 ºC) of sodium caseinate solutions has been shown to reduce emulsifying properties while improving foaming properties of the caseinate.[160]

Caseinates generally produce foams with higher overruns but lower stability than foams produced using egg white or WPC and WPI. Whey protein-enriched products are widely used in foaming applications in foods. The foaming properties of WPC increase with solids content, with an optimum of ~10% total solids. Factors such as protein concentration, level of denaturation, ionic environment, protease peptone level, pre-heat treatment and especially the presence of lipids in the WPC or WPI influence whipping properties. The foam-forming capacity is enhanced by partial hydrolysis of the proteins; however, hydrolysis tends to reduce foam stability.[10]

Milk protein ingredients have been specifically modified to improve their foaming and emulsifying properties. Crosslinking of milk proteins with the enzyme transglutaminase produces a polymerised protein; the degree of polymerisation is directly related to an increase in the size of droplet formed on using the modified protein as an emulsifier; however, polymerisation improves emulsion storage stability by increasing the surface shear modulus/viscosity, thus forming a solid-like protein gel around the emulsion droplets.[128]

Attachment of polysaccharide to casein/whey protein via a conjugation reaction improves the emulsifying properties of the protein; the conjugated protein molecules form a more bulky polymeric layer on the droplet surface, with the polysaccharide portion protruding outwards into the emulsion's continuous phase providing better steric stabilization.[161] Limited enzymatic hydrolysis of milk proteins generates peptides that are more amphiphilic, have less secondary structure and are more flexible than the native intact protein; thus a limited degree of hydrolysis confers good foaming and emulsifying properties on protein hydrolysate ingredients. Casein hydrolysates (degree of hydrolysis, DH, 1–10%) and whey protein hydrolysates (DH, 10–20%) diffuse rapidly, and absorb at the interface reducing the droplet size on emulsion formation, but these hydrolysates are less efficient at stabilising emulsions because of their limited ability to form stable surface films.[111] Emulsions prepared with hydrolysates are less stable to reduction in pH or alcohol addition but more stable to calcium ions.[162] Extensive hydrolysis of milk proteins generates low molecular weight peptides and free amino acids, resulting in milk protein ingredients which decreased ability to form and stabilise emulsions in comparison to the intact native proteins.[162–164]

13.5 Biological activity of milk protein products

Milk proteins are a source of nitrogen and amino acids which are essential for the growth of the neonate and for the maintenance of various bodily functions; in addition, intact milk proteins have some important biological activities. Milk proteins have an ability to bind small hydrophobic vitamins such as retinol (vitamin A),[165] folate[166,167] and cyancobalamin (vitamin B_{12}).[168] This binding may improve the absorption of these vitamins in the intestinal tract[169] as it may protect the vitamin against the possibly detrimental environmental conditions during passage through the digestive system thus improving the bioavailability of these vitamins. Milk proteins are a rich source of biologically active peptides which are encrypted in their primary sequence; these biologically active peptides are latent until released on hydrolysis of the intact proteins. These peptides show a wide variety of biological activities including opiate, anti-thrombotic, antihypertensive, immuno-modulating, antibacterial and mineral carrying properties.[170]

13.5.1 Biological activity of intact caseins

In addition to providing nitrogen and essential amino acids for growth, the principle biological function proposed for casein is to transport high levels of calcium to the neonate in a manner that prevents pathological calcification during its transport through the mammary gland.[171] α_{s1}-Casein enhances mitogen-stimulated proliferation of murine splenic T-lymophocytes *in vitro*.[172] β-Casein enhances the mitogen induced proliferation of ovine T and B

lymphocytes in a dose-dependent manner.[173] The glycomacropeptide of κ-casein exhibits an immunosuppressive effect, with those macropeptides containing high levels of N-acetylneuraminic acid showing strong activity against T-lymphocytes.[174]

13.5.2 Biological activity of intact whey proteins

Whole whey protein and intact individual whey proteins have been shown *in vitro* to modulate lymphocyte functions, suppressing T-lymphocyte mitogenesis in cell cultures.[175,176] A whey protein diet has been shown to have a protective role against the development of tumors in the GI tract as well as selectively inhibiting cancer cell growth.[177,178] The principle biological function of α-la is to act as a coenzyme in the synthesis of lactose,[9] while its immunomodulatory and anticarcinogenic properties have also been reported on.[178,179] β-Lg has been shown to act as a carrier for hydrophobic and amphiphilic molecules including retinol; it is proposed that it protects these molecules from oxidation during transportation through the stomach to the small intestine; β-lg may also stimulate lipolysis by binding free fatty acids; it also shows immunomodulatory activities.[180,181] β-Lg chemically modified using 3-hydroxyphthalic anhydride (3-HP) has antiviral activity; it has been shown to be effective in inhibiting HIV-1 infections in humans.[182] BSA functions as a carrier protein for the transport of non-polar molecules in biological fluids.[183] Bovine immunoglobulins (Igs) provide various types of immunity including passive immunity via colostrum to the neonate. Lacteal secretions containing Igs provide an immunological protection against microbial pathogens and toxins, thus protecting the mammary gland from infection.[184] Immunoglobulins include the so-called lactenins, L_1 and L_3, which inhibit gram-positive bacteria; L_1 is particularly efficient against some strains of S*treptococcus pyogenes* and L_3 acts against some strains of *Lactococcus lactis*.[9]

Lactoferrin (LF) is an iron chelating glycoprotein which acts as a natural non-specific defence system for the body; it modulates the immune system by promoting inflammatory, intestinal and peripheral specific antibody response and also by controlling lymphokines production.[184,185] The ability of LF to sequester iron from a relatively iron-free environment is the principle factor governing the antimicrobial, antifungal, antiviral, antioxidative and anticarcinogenic activity of LF. Its ability to create an iron deprived environment and to bind to the membranes of microbes, thus disrupting the structure, function and integrity of the cell membrane allows it to modulate the intestinal microflora.[186–188]

Lactoperoxidase (LP) has biological activity; it provides protection against invading micro-organisms and thus it is regarded as an indigenous antimicrobial agent.[184] It exhibits a broad range of activity against various bacteria, viruses, yeasts and moulds via the LP system. In the presence of H_2O_2, LP oxidises the thiocyanate anion (SCN^-) and certain halides producing numerous intermediary oxidation products, including the hypothiocyanate anion ($OSCN^-$) and hypothiocyanate (HOSCN) which cause structural damage to the cytoplasmic membrane of the micro-organism.[189,190]

13.5.3 Biologically active peptides

Milk proteins are proposed to be the main source of biologically active peptides; the biological activity of these peptides has been reviewed by Meisel,[191] Fitzgerald and Meisel,[117] Mater *et al.*,[192] Kilara and Panyam,[115] Pihlanto-Leppälä and Korhonen,[184] Silva and Xavier Malcata,[193] Gauthier *et al.*,[194] Korhonen and Pihlanto,[116] Nnanna[112] and Gobbetti *et al.*[124]

Opioid peptides

Milk protein derived opioid peptides are referred to as exorphins in order to distinguish them from naturally occurring enkaphalins, endophins and dynorphins.[195,196] Opioid peptides (typically 5–10 amino acids) from casein are termed casomorphins or casoxins, while those from whey proteins are called lactorphins or lactoferroxins. The biological effect of the opioid peptides is dependent on the receptor type to which they bind; μ-receptors are said to control intestinal motility and emotional behaviour, δ-receptors control emotional behaviour and κ-receptors are linked to analgesia and satiety.[117] A common structural trait in opioid peptides is the presence of a Tyr residue at the N-terminal of the amino acid sequence (except for α_{s1}-casein exorphin, casoxin 6 and lactoferroxins B and C), coupled with the presence of another aromatic residue, Phe or Tyr, in the third or fourth position which ensure the binding of the peptide to the opioid receptor.[193] The opioid activity is also dependent on the localised negative potential in the vicinity of the phenolic hydroxyl group of tyrosine, as the removal of tyrosine results in the absence of activity.[197]

The most widely studied of the milk protein opioid peptides are the β-casomorphins which include fragments 60-63, 60-64, 60-65, 60-66 and 60-70 of the β-casein amino acid sequence and act as μ-type ligands.[198–201] α_{s1}-Casein derived opioid peptides, known as exorphins, correspond to fragments 90-96 (Arg-Tyr-Leu-Gly-Tyr-Leu-Glu), 90-95 and 91-96 and are δ-type receptor ligands.[202,203] Whey protein derived peptides from β-lactoglobulin (fragment 102-105 known as β-lactorphin (Tyr-Leu-Leu-Phe) and from α-lactalbumin (fragment 50-53, α-lactorphin (Tyr-Gly-Leu-Phe)) are μ-type ligands but show a weak but consistent affinity for opioid receptors.[198] Perpetuo *et al.*[204] isolated a peptide from β-casein, fragment 114-121 (Tyr-Pro-Val-Glu-Pro-Phe-Thr-Glu), which exhibited opiate activity *in vitro*.

Milk proteins also contain opioid antagonist peptides which suppress the action of opiate agonists and humoral peptides enkephalin. These opioid antagonist peptides, referred to as casoxins, are derived mainly from κ-casein and are μ-type receptor ligands. Casoxins A and B are fragments 35-41 (Tyr-Pro-Ser-Tyr-Gly-Leu-Asn), and 58-61 (Tyr-Pro-Tyr-Tyr) of κ-casein, respectively, and have a low antagonistic potency when compared to naloxone, a synthetic opiate receptor antagonist. Casoxin C is fragment 25-34 of κ-casein (Tyr-Ile-Pro-Ile-Gln-Tyr-Val-Leu-Ser-Arg) and exhibits high opioid antagonistic activity.[205,206]

ACE inhibitory peptides

Angiotensin-I converting enzyme (ACE, peptidyldipeptide hydrolase; EC 3.4.15.1) is a multifunctional, zinc-dependent carboxypeptidase, which plays an important role in the rennin angiotensin system, by influencing the regulation of peripheral blood pressure, the kallikrein-kinin system and the immune system. ACE converts angiotension I to a potent vasoconstrictor, octapeptide, angiotension II. ACE also catalyse the degradation of bradykinin, a vasodilatory peptide, and stimulates the release of aldosterone in the adrenal cortex which decreases the renal output while increasing water retention.[117,184] ACE inhibition primarily results in a hypotensive effect but also affects the immunodefence and nervous systems.[207]

Casein derived peptide inhibitors of ACE are known as casokinins, which have been found in α_{s1}-casein (fragments 23-24, 23-27 and 194-199), β-casein (fragments 74-76, 108-113, 114-121, 177-183, 193-198, 169-174 and 193-202) and κ-casein (fragments 108-110).[117,204] α_{s2}-Casein also contains ACE inhibition peptides (fragments 189-193, 189-197, 190-197, 198-202) but these fragments have a very low activity.[208,209] Whey protein derived inhibitors of ACE are referred to as lactokinins and have been found in β-lactoglobulin (fragment 142-148), α-lactalbumin (fragment 105-110) and bovine serum albumin (fragment 208-216).[210–212]

The binding of ACE is strongly influenced by the C-terminal tripeptide sequence of the substrate. Most of the potent casokinins and lactokinins contain hydrophobic amino acid residues at each of the three C-terminal positions, with proline, lysine or arginine residues being the most efficient in enhancing substrate binding to the negatively charged catalytic site of ACE. The removal or absence of arginine from the C-terminal can result in inactive ACE-inhibitory peptides.[117]

Immunomodulatory peptides

Casein-derived immunopeptides include fragments of α_{s1}-casein (fragment 194-199) and β-casein (fragments 63-68, 191-193 and 193-202), which stimulate phagocytosis activity of murine and human peritoneal macrophages *in vitro* and exert a protective effect against *Klebsiella pneumonaie* infection in mice *in vivo* after intravenous administration.[213] Coste *et al.*[214] showed that a peptide from the C-terminal sequence of β-casein (fragment 193-209) which also contains the ACE-inhibiting peptide (β-casokinin-10) induced a significant proliferation response in rat lymphocytes. Whey protein derived immunopeptides isolated from the N-terminal of α-lactorphins derived from α-lactalbumin (fragments 18-19, 18-20 and 50-51) has *in vitro* enhanced the proliferation of peripheral blood lymhocytes.[215] β-Casein fragment 60-66 displays immunomodulatory activity; it confers a suppressive or stimulatory effect on lymphocyte proliferation of human colonic lamina propria lymphocytes (LPL), which is dependent on concentration. The anti-proliferative effect is reversed by the opiate receptor antagonist, naloxone.[215] Immunomodulatory milk peptides may also alleviate allergic reactions in atopic humans and enhance mucosal immunity in the

gastrointestinal tract.[184] It has been suggested that opioid peptides may affect the immunoreactivity of lymphocytes, because opioid μ-receptors for endorphins are present in lymphocytes.[216]

Caseinophosphopeptide

The caseinophosphopeptides (CPPs) are derived from the highly phosphorylated regions of the caseins; phosphorylated peptides have been isolated from hydrolysates of α_{s1}-casein (fragments 43-58, 45-55, 59-79, 66-74 and 106-119), α_{s2}-casein (fragments 2-21, 46-70, 55-75, 126-136, 138-149) and β-casein (fragments 1-25, 1-28, 2-28).[217] A common motif of CPPs is that their sequence consists of three phosphoseryl residues followed by two glutamic acid residues (SerP-SerP-SerP-Glu-Glu).[218,219] These phosphopeptides are stable under acidic conditions and are very resistant to further proteolysis because of the high negative charge density.[170]

The CPPs are capable of binding and solubilising calcium, magnesium, iron, zinc and copper.[191] Their ability to sequester calcium may be considered beneficial in the prevention of osteoporosis.[220] CPPs also have anticariogenic effects by promoting recalcification of tooth enamel by localising amorphous Ca^{2+} at the surface of the tooth, depressing demineralisation and enhancing remineralisation of the enamel.[220,221] A β-casein derived CPP (fragment 1-25) has been shown *in vitro* and *in vivo* to enhance the bioavailability of iron when compared to whole casein and organic salts and has been used to treat anaemia in rats.[222,223] Dephosphorylated casein peptides do not bind minerals.[224]

Mineral binding peptides have also been found in whey protein hydrolysates (α-lactalbumin, β-lactoglobulin and lactoferrin). These peptides are not phosphorylated; therefore, the minerals bind to the peptides through binding sites other than phosphorylated sites; the ability of these sites to bind minerals may be influenced by peptide conformation. Also both α-lactalbumin and β-lactoglobulin derived mineral binding peptides have shown higher affinity for iron than the native proteins.[225]

Antithrombotic peptides

Antithrombotic peptides derived from the C-terminal, glycomacropeptide portion of κ-casein (fragment 106-169) are known as casoplatelins; fragment 106-116 inhibits the aggregation of ADP-activated platelets and the binding of human fibrinogen γ-chain to platelet surface fibrinogen receptors.[115,126] Smaller peptides (fragments 106-112, 112-116 and 113-116), known as casopiastrin, do not inhibit fibrinogen binding but are capable of inhibiting platelet aggregation.[226,227] Glycomacropeptide mediates the absorption of minerals (calcium, zinc or iron) and modulates the gut microflora; the carbohydrate moities (sialic acid) promotes the growth of bifidobacterium.[228,229] The absence of Phe, Tyr, Trp and Cys residues in GMP makes it a suitable ingredient for the nutrition of phenylketonuria sensitive patients.[19] Non-glycosylated forms of GMP, referred to as caseino-macropeptide (CMP), are reported to inhibit gastric secretions and slow down stomach muscle contractions. CMP also stimulates the

release of cholecystokinin, the satiety hormone involved in controlling food intake and digestion in the duodenum of animals and humans.[230]

Antibacterial peptides

Isracidin, an antibacterial peptide from α_{s1}-casein (fragment 1-23) was isolated by Hill *et al*.[231] Isracidin strongly inhibits the growth of gram-positive bacteria at high concentrations *in vitro* and also confers a protective effect *in vivo* against *S. aureus* and *C. albicans* at low concentrations. McCann *et al*.[232] isolated an antibacterial peptide from α_{s1}-casein (fragment 99-109), which displayed a broad antibacterial activity including activity against gram-positive and gram-negative bacteria. Casocidin-I is an antibacterial peptide isolated from α_{s2}-casein (fragment 165-203) which contains a high proportion of basic amino acid residues (10 of 39) and inhibits gram-positive bacteria (*S. carnosus*) and gram-negative bacteria (*E. coli*).[233] Kappacin is an antibacterial peptide found in non-glycosylated, phosphorylated CMP and has been reported to inhibit gram-positive bacteria (*S. mutens*) and gram-negative bacteria (*P. gingivalis, E. coli*), as well as showing an ability to bind to enterotoxins and inhibiting bacterial adhesion and growth.[234] Whey protein hydrolysates also contain peptides (β-lactoglobulin fragments 15-20, 25-40, 78-83 and 92-100) which exhibit activity against gram-positive bacteria. Modification of the β-lactoglobulin fragment 92-100 via the substitution of Asp with Arg and the addition of a Lys residue at the C-terminus expanded the bactericidal activity to include gram-negative bacteria including *E. coli.*[235] Lactoferricin (fragment 17-41), is an antibacterial peptide produced by enzymatic hydrolysis of lactoferrin. It inhibits a wide variety of bacteria including enterotoxigenic *E. coli* and *L. monocytogenes*, yeast and fungi by disruption of their cytoplasmic membrane.[236–239]

13.6 Food uses of milk protein products

Details of many of the food uses of milk protein products are proprietary to milk protein product producers and food processors and are not reported in the literature. However, reviews on the food uses of milk proteins include Southward and Goldman,[240] International Dairy Federation,[241] Southward and Walker,[242] Otten,[243] Zadow,[244] Hugunin,[245] De Wit,[246,247] Southward,[248,249] Mulvihill,[16] Mangino,[250] Chandan,[251] Mulvihill and Ennis[20] and O'Connell and Flyn.[21] Milk protein products have also had applications as diverse as animal feed ingredients, plastics and industrial glues but these uses are not considered here. The following are brief outlines of some reported food applications of milk protein products.

13.6.1 Bakery products

Milk proteins cannot replace wheat gluten to any great extent in bakery products. Caseins are particularly rich in lysine and so make excellent nutritional supplements for cereals, which are deficient in lysine. Casein/caseinates are added to breakfast cereals, milk biscuits, protein-enriched bread and biscuits,

high-protein bread and cookies as a nutritional supplement and to frozen baked cakes and cookies as an emulsifier and to improve texture. The type of casein/caseinate must be carefully chosen to be compatible with the particular bakery application. Co-precipitates are used in pastry glaze to improve colour, in milk biscuits, cake mixes for diabetics, high-protein biscuits and cookies as a nutritional supplement and in fortified bread to improve dough consistency (due to water binding by the milk proteins), sensoric properties and to increase volume and yield. However, some milk fraction has been described as loaf volume-depressing.[252] Depression of loaf volume by whole whey protein products was associated with proteose peptones since fortification of dough with 1% UF-WPC caused little loaf volume depression. The concentration of whey lipids during UF also contributes to good baking characteristics. Whey protein shows economic and nutritional advantages as a replacement for eggs in cake manufacture. However, simply replacing whole eggs by WPC in madeira-type cakes results in poor quality cakes although better results are obtained if the fat and WPC are pre-emulsified. Various WPCs have been used in products like muffins and croissants to increase their nutritional value. Bakery applications of milk protein products are outlined in Table 13.7.

Table 13.7 Application of milk protein products in baked products

Milk protein product	Application	Effect/property
High-calcium co-precipitate	Pastry glaze, cake mix for diabetics, milk biscuits, cookies	Colour, shine, nutrition, cake volume, texture, appearance
Low-calcium co-precipitate	Cookies	Nutrition, texture, appearance
Casein	Breakfast cereal, high-protein bread	Nutrition
Calcium caseinate	Milk biscuits, biscuits	Nutrition
Sodium caseinate	Frozen baked cake, protein-enriched milk biscuits	Texture, emulsifier, nutrition
	Shortening	Fat encapsulation
	Pie filling	Stabiliser
	Powdered friable fat	Fat stabiliser
Co-precipitate	High-protein biscuit, fortified bread	Dough consistency, sensory, increase volume/yield, fast fermentation
Lactic casein	Cookies	Nutrition, texture, appearance
Acid casein	Non-fat dry milk substitute	Structure building in dough, nutrition, flavour
WPC	Cookies, muffins, croissants	Water retention, colour, emulsifier, nutrition
	Bread	Dough formation
	Cake	Fat binding, heat setting

13.6.2 Dairy products

Milk protein products are used to supplement the protein content in conventionally processed dairy products and in the manufacture of imitation dairy products (Table 13.8). Caseins, vegetable fat, salts and water are used to make imitation cheeses (cheese analogues), which result in significant cost-

Table 13.8 Application of milk protein products in dairy-type products

Milk protein product	Application	Effect/property
Calcium caseinate	Processed cheese spread, imitation Mozzarella cheese, imitation cream cheese	Spreadability, stretch, browning, emulsifier
Sodium caseinate	Coffee creamer, UHT cream, non-dairy creamer, imitation sour cream, cultured cream	Emulsifier, whitener, texture, body, resistance to feathering, sensory characteristics, water binding, viscosity, flavour
	Yoghurt, fruit yoghurt	Reduce syneresis, increase gel firmness, stabiliser, consistency
	Imitation milk, milk shakes, cheese milk	Emulsifier, nutrition, foaming properties, increase yield
	Imitation Mozzarella cheese, imitation cream cheese	Stretch, browning, emulsifier
	Ice cream products	Consistency, flavour, foaming, water binding, aroma
	Dairy-based spreads, butter-type spread, butter powder	Texture, emulsion stabiliser
	Whipping composition for drinking	Foaming
Co-precipitate	Fat-reduced milk	Nutrition
	Spread-type dairy products	Texture
	Cultured milk product	Increase biological value
Potassium caseinate	Fat-reduced milk	Nutrition
Hydrolysed casein	Ice cream	Overrun
	Yoghurt	Growth media for starter
Acid casein	Simulated cheese, cheese analogue, imitation milk	Meltability, stringiness, cost, texture, meltability, nutrition, stability
Rennet casein	Processed cheese, Mozzarella substitute, cheese analogue, cheese-like spread	Emulsifier, meltability, stringiness on melting, texture, flavour
WPC	Soft serve ice cream	Overrun, cost,
	Cheese products	Water and fat binding, cost, emulsification
	Yoghurt	Reduce lactose
	Coffee whiteners	Casein/caseinate replacer
Whey proteins	Yoghurt, Quarg, Ricotta	Yield, nutrition, consistency, curd cohesiveness

saving, compared to the use of natural cheese, when used in pizza, lasagne and sauces and on burgers, grilled sandwiches, macaroni, etc. The important functional properties of casein in this application include fat and water binding, texture enhancing, melting properties, stringiness and shredding ability. Rennet caseins, acid caseins and caseinates are used most commonly for cheese analogues although co-precipitates also have potential in this area. Sodium caseinate in powdered coffee creamers (which also contain vegetable fat, carbohydrate and emulsifiers/stabilisers) acts as an emulsifier/fat encapsulator and whitener, imparts body and flavour and promotes resistance to feathering (i.e. coagulation of cream in hot coffee solutions). These creamers are cheaper, have a longer shelf-life and, requiring no refrigeration, are more convenient to use than fresh coffee creams. Sodium caseinate is used to reduce syneresis and increase gel firmness in yoghurts, and is added to milk shakes for its emulsifying and foaming properties. Caseins/caseinates, vegetable fat and carbohydrate, e.g. corn syrup, are the principal ingredients used in the manufacture of low cost imitation milk products that contain no lactose, to which some people are intolerant. Sodium caseinate is also used as an emulsifying and fat encapsulating agent in the manufacture of high-fat powders for use as shortenings in baking or cooking. Dry whipping fats or whipping creams contain casein products while a number of butter-like dairy spreads are manufactured using milk and/or vegetable fat and various casein products. In these applications casein acts mainly as an emulsifier and in the case of dairy spreads, it also enhances texture and flavour.

Whey protein products are used in yoghurts and cheeses to improve the yield, nutritional value and consistency. Yoghurt viscosity and stability are improved by replacing skim milk solids with WPC. Up to 20% of the casein in Quarg can be replaced by thermally-modified WPC to improve the yield and nutritional value. The use of sweet UF-WPC in Ricotta cheese manufacture increases the cohesiveness of the curd. Emulsions prepared using heat-denatured whey proteins and fat are used as a protein base for formulated cream cheeses and cream cheese spreads. Sliceable and squeezable cheese-type products, based on the emulsifying and gelling properties of whey proteins, are produced by heat treatment of skim milk and WPC solids dispersed in an emulsion of milk fat in WPC. Whey protein concentrates are also used in cheese filling and dips as they complement the cheese flavour and result in a soft product.

13.6.3 Beverages

Casein products are used for their whipping and foaming properties or as stabilisers in drinking chocolate, effervescent drinks and beverages (Table 13.9). Sodium caseinate is used as an emulsifier and stabiliser in cream liqueurs, which typically contain cream, sodium caseinate, added sugar, ethanol, and trisodium citrate to prevent calcium-induced age gelation; it is also used to a lesser extent in other aperitifs. Sodium and calcium caseinates are used in the production of Glucerna™ and Resource™ which are nutritional beverages produced for

Table 13.9 Application of milk protein products in beverages

Milk protein product	Application	Effect/property
Casein	Beer, wine	Clarification, colour removal, stabiliser, palatability, colour stability, removal of phenolic compounds
	Effervescent lemonade ingredient	Stabiliser
Potassium caseinate	White wine	Removal of tannins and phenolic compounds, taste, colour removal
Sodium caseinate	Apple juice	Colour removal
	Cream liqueur, alcoholic cream-containing beverage, wine aperitif	Emulsification
	Soluble tea products	Prevention of 'tea cream'
	Drinking chocolate	Stabilisation
Casein hydrolysate	Non-alcoholic fruit beverage	Whippability, foaming
WPC	Citrus-based beverages, soft drinks	Flavour, nutrition, solubility
	Hot cocoa beverages, chocolate drink	Foamer, cost, colloidal stability

patients with abnormal glucose tolerance. Casein products have also been used as fining agents, to decrease colour and astringency and to aid in clarification in the wine and beer industries. WPCs may be added to fruit juices, soft drinks or milk-based beverages to produce highly nutritious products often marketed as 'sports drinks'. For use in soft drinks, defatted WPCs with a low ash content, good solubility at pH 3.0 and a bland flavour are required. The WPC must also be resistant to physical deterioration or flavour changes during product storage and must not interact with flavour components and thereby mask the typical flavour of the drink. WPCs and WPIs are added to milk-like flavoured drinks to impart viscosity, body and colloidal stability and they are included as protein supplements in high protein powdered athlete-targeted flavoured beverages and in frozen juice concentrates.

13.6.4 Dessert-type products

Sodium caseinate is used in ice cream substitutes and frozen desserts to improve whipping properties, body and texture and to act as a stabiliser and also finds use in mousses, instant puddings and whipped toppings for similar reasons and because it acts as an emulsifier and film-former (Table 13.10). In the manufacture of whipped toppings the basic ingredients of vegetable fat, sugar, protein (sodium caseinate), emulsifier, stabilisers and water are blended at 38–46 ºC and the mixture is pasteurised and homogenised and then either cooled rapidly to below freezing point or spray dried. In ice cream manufacture, part of the skim milk solids can be replaced by whey powder, and even more may be

Table 13.10 Application of milk protein products in dessert-type products

Milk protein product	Application	Effect/property
Sodium caseinate	Whipped dessert, whipping fat, whipped topping, mousse, ice cream frozen dessert, frozen puddings, instant dessert/pudding bases	Whippability, fat encapsulation, overrun, powder flow properties, replace milk solids, emulsifier, stabiliser, flavour, texture
Caseinate	Spongy dessert	Whippability, aeration
Hydrolysed sodium caseinate	Whipped topping	Freeze-thaw stability
WPC	Fruit jellies and jams	Flavour
	Frappes, whipped toppings, frozen desserts/puddings	Overrun, cost, whippability
	Flan-style desserts, custards	Gelling ability

replaced by using delactosed, demineralised whey powder or UF-WPC with no adverse effect on flavour, texture or appearance. WPC has also been used in frozen juice bars and in compound coatings, especially chocolate coatings, for frozen desserts.

13.6.5 Pasta products

Milk protein products may be incorporated into the flour base for pasta manufacture to improve nutritional quality and texture (Table 13.11). Products fortified by addition of sodium or calcium caseinate, low calcium co-precipitate or WPC prior to extrusion include macaroni and pasta. Enrichment of pasta flours with non-denatured whey protein products results in firmer cooked noodles which are also more freeze-thaw stable and suitable for microwave

Table 13.11 Application of milk protein products in pasta products

Milk protein product	Application	Effect/property
Sodium caseinate	Protein enriched pasta	Nutrition, consistency, binder, texture, taste, appearance
Calcium caseinate	Enriched wheat macaroni, high protein pasta	Nutrition, texture
Casein	Enriched, fortified macaroni	Nutrition, texture
Soluble low-calcium co-precipitate	Imitation rice	Nutrition, texture
WPC	Pasta	Flour replacer, nutrition

cooking. Imitation pasta-type products containing substantial proportions of milk protein have also been manufactured.

13.6.6 Confectionery

Whey proteins are suitable for use in aerated candy mixtures and are incorporated as a frappe, a highly aerated sugar syrup containing the whipping protein (Table 13.12). Caseins are used in toffee, caramel, fudge and other confections as they form a firm, resilient, chewy matrix on heating and they contribute water binding and aid emulsification. WPCs are less useful in these products as they produce a softer coagulum and the high lactose content may cause crystallisation during storage. Casein hydrolysates may replace egg albumen as foaming agents in marshmallow and nougat as they confer stability up to high cooking temperatures as well as good flavour and browning properties. Use of WPC or WPI as a replacement for egg white in the manufacture of meringues produces acceptable products only when defatted products are used; in contrast the manufacture of acceptable sponge cakes requires fat-containing WPCs. A milk protein hydrolysate, 'Prodiet F200', has been used in chocolate formulations; it contains a bioactive peptide with relaxing properties.[253] Calcium caseinate, WPC, WPI, MPI, and milk protein hydrolysate ingredients are used in the manufacture of high protein energy bars; they are used to extend bar shelf-life and minimise bar hardening, and nutritionally to optimising muscle performance during exercise. Ensure™, Glucerna™ and Choice dm™ are speciality nutritional bars for people with diabetes; these all contain calcium caseinate.

Table 13.12 Application of milk protein products in confectionery products

Milk protein product	Application	Effect/property
Sodium caseinate	High protein chocolate snack confectionery bars, Aerated cake icing	Nutrition, storage stability, flavour, aeration, body, mouthfeel, texture
Hydrolysed sodium caseinate	Coated confectionery product	Nutrition
Calcium caseinate	Aerated cake icing High protein energy bar	Aeration, body, mouthfeel, texture Nutrition, improve shelf-life stability
Hydrolysed casein	Aerated confection	Whippability
Co-precipitate	Protein-rich chewable bar	Nutrition, texture
Milk protein hydrolysate	Chocolate formulations	Relaxation

13.6.7 Meat products

In comminuted meat products caseins release meat proteins for gel formation and water binding and thus contribute to fat emulsification, water binding and improved consistency. Sodium caseinate is a common additive in meat applications although various co-precipitates have also been used (Table 13.13). Up to 20% of the meat protein in frankfurters and luncheon rolls may be replaced by whey proteins, which are used to prepare pre-emulsions of part of the fat and support network formation, by gelation, during subsequent cooking. Soluble, low viscosity WPCs may be used in injection brines to fortify whole meat products such as cooked hams. Injection of fresh and cured meats with milk protein solution increases yield. In addition, WPI and its hydrolytic products have been used in the preparation of cooked pork patties, where they reduce cook loss as well as inhibiting lipid oxidation during refrigerated storage.[254] Casein phosphopeptides have also been incorporated into ground meat products including ground beef and have been shown to enhance product stability by inhibiting lipid oxidation.[255] Lactoferricin is used in the washing of animal carcasses at refrigeration temperatures to inhibit the growth of food pathogens (e.g., *E. coli* 0157:H7) and spoilage micro-organsims (e.g., *C. viridans*).

13.6.8 Nutritional/medical/pharmaceutical applications

Milk protein products are used extensively in special dietary preparations for the ill or convalescing, for malnourished children and for people on therapeutic or weight-reducing diets (Table 13.14). Modified low mineral whey powders are used to produce 'humanised' infant formulae with a whey protein-to-casein ratio resembling that of human milk. Infant formula is supplemented with α-

Table 13.13 Application of milk protein products in meat products

Milk protein product	Application	Effect/property
Sodium caseinate	Liver sausage, sausage, blood, patties, low fat meat paste	Binder, decolouriser, emulsifier
Potassium caseinate	Low fat meat paste	Emulsifier
Caseinophos-phopeptides	Ground meat products	Enhance product stability, prevent lipid oxidation
Sodium salt of co-precipitate	Paté sausage	Nutrition, sensory, cost
WPC	Meat products	Cost, improved performance, water and fat binding, water solubility at low viscosity

Table 13.14 Application of milk protein products in nutritional, pharmaceutical and medical applications

Milk protein product	Application	Effect/property
Sodium caseinate	Candy for space feeding, Carnation Slender®, enriched dairy drink for infants, meat replacement	Nutrition
Calcium caseinate	Bakery products for diabetics Meat replacement, infant dietary food	Flour substitute Nutrition
Casein	Water dispersible protein, special dietary foodstuffs Toothpaste	Nutrition, texture Prevent caries
Acid casein	Low-sodium infant formula	Nutrition
Co-precipitate	Carbohydrate-free and low-lactose infant food	Nutrition
High-calcium co-precipitate	Cake mix for diabetics	Nutrition, cake volume
WPC	Geriatric/hospital/liquid diets Infant formulae	Nutrition Nutrition, digestability
WPI	Nutritional protein bar/beverages	Improve immuno-defence and nervous system activity
Whey protein hydrolysate	Hypoallergenic infant formulae	Nutrition
Caseinophos-phopeptides	Dental filling material	Mineral binding activity
Lactoferricin	Medication	Treatment of gastritis

lactalbumin, providing a rich source of essential amino acids, in particular tryptophan and its metabolites such as serotonin, which is essential for brain maturation and development of other neurobehavioural regulations of food intake and sleep-wake-rhythm.[256] Whey protein hydrolysates have been used in hypoallergic, peptide-based formulae. Fractionation of whey proteins allows the formulation of infant formulae that possess whey protein compositions more closely resembling that of human milk. β-Casein and α-lactalbumin enriched protein fraction together with lactotransferrin are being used as ingredients for the production of more 'humanised' infant formulae. Lactoferrin is being added to infant formulae for its bacteriostatic and bactericidal activity and CMP is being added to contribute to improved mineral adsorption. Caseinates and co-precipitates are used in low lactose formulae for lactose-intolerant infants, while selected caseinates are used in the production of infant foods where a specific mineral balance is required, e.g., low sodium infant formulae for children with

specific renal problems. Casein hydrolysates are used in specialised foods for premature infants and in formulae for infants suffering from various intestinal disorders; casein hydrolysates, low in phenylalanine, have been proposed uses in formulae for feeding infants with phenylketonuria. The absence of Phe, Tyr, Trp and Cys residues in κ-casein glycomacropeptide make it a possible stable source of nutrition for phenylketonuria-sensitive patients.

Casein products are added to various foods and drinks as a nutritional supplement. Caseins are used in special nutritional preparations for athletes as they enhance athletic performance which is attributed to the high glutamine levels that are important in the maintenance of muscle protein mass. Caseinates, co-precipitates and casein hydrolysates are often used in combination with whey protein concentrate or isolate in protein supplemented powders/beverages for athletes; calcium caseinate and micellar casein are used by athletes as a 'slow release protein' supplement which decreases the rate of muscle degradation while dieting.[21,257] Non-glycosylated caseinomacropeptide is used in weight reduction diet products (low carbohydrate yoghurts), where it stimulates the release of cholecystokinin, resulting in the production of insulin which inhibits gastric secretions that play an important role in controlling food intake and digestion in the duodenum of animals and humans.[230]

A casein-based diet containing high levels of TGF-β (transforming growth factor beta) fed to children with Crohn's disease ameliorated chronic inflammation of the intestine.[258] Whey protein enriched diets have been shown to reduce the growth of tumors in the GI tract, selectively inhibit cancer cell growth in head and neck cancer patients as well as aid the immune defence system against post-operative infections.[177,178] Milk protein hydrolysates are used for intravenous nutrition for patients suffering from protein metabolism disorders, intestinal disorders, and for post-operative patients. Special casein preparations have been used as food for patients suffering from cancer, pancreatic disorders or anaemia. Biologically active peptides have shown potential applications in a wide variety of products; opiate peptides exhibit pharmacological properties similar to morphine while also influencing postprandial metabolism by stimulating pancreatic insulin and gastrointestinal somatisation release, prolonging gastrointestinal transit time and exerting anti-diarrhoeal action.[259–262] 'BioZate', a whey protein isolate hydrolysate which contains an ACE-inhibitory peptide, has been shown to significantly reduce both systolic and diastolic blood pressure, as well as influencing immuno-defence systems and nervous system activity. This product exhibits both emulsifying and foaming properties, enabling this product to have a wide variety of applications including nutritional protein bars and beverages, meal replacers and specialised foods for sport nutrition. 'Calpis'™ is a sour milk drink produced in Japan, and 'Evolus', a calcium-enriched fermented milk drink produced in Finland, both contain antihypertensive peptides and have been shown to reduce blood pressure.[263] Antibacterial peptides including Casocidin-I and Kappacin have a variety of applications in oral care products, due to their strong inhibition of gram-positive bacteria and gram-negative bacteria as well as showing an ability

to bind to enterotoxins and inhibit viral and bacterial adhesions.[234] Lactoferricin, which has antimicrobial and haemolytic activity, has been shown to be useful in the development of peptide antibiotics as therapeutic agents with low toxicity,[191] and preparing medicines for treating acute gastritis, chronic gastritis, peptic ulcer and high stomach acidity.[264]

Casein derived peptides such as phosphopeptides, which have mineral binding ability have uses in the preparation of tablets, toothpaste and dental filling material.[220] Sulphonated glycopeptides prepared from casein have been used for the treatment of gastric ulcers. Several other bioactive peptides may be used in pharmaceutical preparations, for example, casomorphins in the treatment of diarrhoea, casokinins (hypertension), casoplatelins (thrombosis) and immunopeptides (immuno-deficency).[191] In the pharmaceutical industry, the oral route is considered the most convenient way of administering drugs to patients. Hydrophilic drugs cannot readily diffuse across the cells in the intestinal epithelium through the lipid bilayer. Milk proteins possess many of the properties required for effective drug delivery by oral administration as they are readily degraded at the low pH in the stomach and act as good micro-encapsulating agents.

The ability of milk proteins to encapsulate a wide variety of materials suggests several promising applications in the food industry including use in the production of low calorie and reduced fat products, while masking off flavours, preventing oxidation and improving sensory characteristics; in the cosmetic industry to produce easily spreadable creams and lotions with encapsulated ingredients in both the oil and water phases and in the pharmaceutical industry to manufacture drug delivery systems.[265] In the food industry, WPI or WPI/lactose has been used to encapsulate anhydrous milk fat while also conferring good oxidative stability by preventing oxygen uptake.[266] WPC may also be used in many applications as a fat replacer.[267] Whey protein concentrate gels can be used as pH-sensitive hydrogels for controlled delivery of various materials including biologically active substances.[268,269] Casein-dextran conjugates have been used as secondary emulsifiers in the preparation of multiple emulsions with the capability of encapsulating numerous materials (flavours, bioactive peptides and anticancer drugs) depending on their application, while also being safe for use in food, pharmaceutical and medical preparations. Multiple emulsions have been used to produce low caloric and flavoured mayonnaise.[270] Semo *et al*.[271] demonstrated that the casein micelle can be used as a nano-encapsulation vehicle for hydrophobic nutraceutical substances (vitamin D) for enrichment of non-fat or low-fat food products.

13.6.9 Convenience foods

Applications of milk protein products in convenience foods are outlined in Table 13.15. Whey/caseinate blends are used as whitening agents in gravy mixes. Whey solids are included in dehydrated soup mixes and sauces to impart a dairy flavour, to enhance other flavours and to provide emulsifying and stabilising

Table 13.15 Application of not protein products in convenience foods

Milk protein product	Application	Effect/property
Sodium caseinate	Dry cream product for sauces, soups, nut substitute, imitation potato skin shells	Emulsification, nutrition, texture
Caseinate	Nut-like food Gravy mix	Film formation Whitening agent
Casein	Synthetic caviar	Texture
Hydrolysed casein	Whipping mixture	Whippability
Co-precipitate	Potato soup with rice, vegetable cutlets	Emulsification, nutrition, texture
WPC	Salad dressing, egg replacer Quiches, egg replacer Cream-based soups	Viscosity, mouthfeel, emulsification Cost, extender Cost
Whey solids	Gravy mix, dehydrated soup mix	Whitener, flavour, emulsifier, stabiliser

effects. Caseinates are used as emulsifying agents and to control viscosity in canned cream soups and sauces and in the preparation of dry emulsions for use in dehydrated cream soups and sauces. Sauces and gravies containing whey proteins are reportedly less prone to cook-on to utensil walls, require minimum agitation and are stable to freeze-thaw cycling. Caseinate-whey protein blends are used as cheap replacements for skim milk powders in some convenience foods. Whey protein products may replace egg yolk in salad dressing and modified whey protein-based products potentially able to replace lipids in a variety of convenience foods have been developed. Milk protein products have been proposed as texture, stability and flavour enhancers in microwaveable foods.

13.6.10 Textured products

Rewetted acid caseins or acidified rennet casein or co-precipitate, mixed with carbonates or bicarbonates of alkali metals or alkali earth metals, can be extruded to produce puffed snack foods while caseinates can be co-extruded with wheat flour to produce protein-enriched snack-type food products (Table 13.16). Fibrous meat-like structures can be formed from caseins, using fibre spinning techniques, and can be used as extenders in comminuted meats. If whey proteins are co-spun with the casein, fibres stronger than those containing casein alone are produced. Meat-like structure can also be formed from casein or co-precipitates by renneting followed by a combination of heat treatment and extrusion or working. Microwave heating of whey protein solutions results in

Table 13.16 Application of milk protein products in textured products

Milk protein product	Application	Effect/property
Casein	Puffed food	Emulsification, texture, nutrition
Acid casein	Extruded milk protein product Foamed snack bar	Texture
Potassium caseinate	Fine bread, biscuits	Texture
Sodium caseinate	Sucroglyceride for baking	Texture, handling
Rennet casein	Dietary fibre snack	Texture
Co-precipitate	Dietary fibre snack	Texture
Whey protein	Dietary fibre snack	Texture
WPC	Surimi	Texture, cost

simultaneous expansion and gelation to give textured products with potential for use in comminuted meats. WPCs are proposed as cost effective replacements for beef plasma protein or potato starch in the modification of surimi texture.[272]

13.6.11 Films and coatings

Films formed from caseins/caseinates may be water soluble or water insoluble depending on the pH conditions used in their preparation, while the water vapour permeability of the film depends on the type of casein/caseinate used. Thermally induced disulphide crosslinking was found to be necessary when making films using WPCs and WPIs. The WPC-based films were excellent gas barriers, while the water vapour permeability of films could be reduced by incorporating lipids. Tensile strengths of the films were similar to synthetic films, and were enhanced by enzymatic polymerisation, e.g., transglutaminase. The films were generally flavourless, and transparent to translucent depending on protein source. Calcium-caseinate-based emulsions applied to fruit and vegetables were used to reduce moisture loss. The potential for the use of milk proteins in films and coatings in food applications has been discussed by Chen.[273]

13.7 Future trends

A large number of different milk protein products are presently recovered from milk and it is highly likely that this range will be further extended in the future. Cost effective commercial methods for separating individual casein and whey

protein fractions are currently emerging as new technologies are being developed and these fractions will become more widely available in the near future. Tailoring of milk protein products, most likely by enzymatic and physical modifications, will also be further developed to produce new speciality milk protein ingredients which meet the specific physico-chemical and functional demands of specific food applications. Commercial interest in milk protein hydrolysates and biologically active peptides is likely to continue to grow and to focus on procedures for the commercial preparation, isolation and purification of these products. A small number of milk protein derived biologically active peptides are presently commercially available; it is likely that the search for new milk-derived peptides will continue and an area of future focus will be the impact of interactions with other food constituents and technological processing procedures on their biological activity. While the main area of application for milk protein products will continue to be as functional ingredients in the food industry, the expanded range of milk protein-derived products will find increased uses in nutritional and nutraceutical applications. Consumer awareness of food ingredients will grow and consumers will view milk-derived protein and peptide ingredients as natural, wholesome, nutritious and healthy constituents in foods, thus enhancing and expanding their importance.

13.8 Sources of further information and advice

Further information regarding regulatory requirements for milk protein products (and milk products in general) may be obtained from the following:

- American Dairy Products Institute, 300 West Washington Street, Suite 400, Chicago, Illinois, USA, Fax +13127825299.
- USDA/AMS, Dairy Standardisation Branch, Room 2750- South Building, PO Box 96456, Washington, DC, USA. Fax +12027202643.
- Food and Agriculture Organisation of the United Nations (Codex Alimentarius), Viale delle Terme di Caracalla, 00100-Rome, Italy. Fax +390657054593.
- International Dairy Federation, 41, Square Vergote, 1030 Brussels, Belgium. Fax +3227330413.

Information on milk protein products may be obtained from the following manufacturers and suppliers:

- Armor Proteines, Le Pont, 35460 Saint Brice en Cogles, France. Fax + 33 2 99 97 7991.
- Dairygold Food Ingredients, Clonmel Road, Mitchelstown, Co. Cork, Republic of Ireland. Fax + 353 (0) 25 44135.
- Kerry Ingredients Ireland, Tralee Road, Listowel, Co. Kerry, Republic of Ireland. Fax + 353 (0) 68 21562.

- Glanbia (formerly Avonmore-Waterford Group), Ballyragget, Co. Kilkenny Republic of Ireland. Fax + 353 (0)5688 36001.
- Golden Vale Plc., Charleville, Co. Cork, Republic of Ireland. Fax + 353 (0) 63 35001.
- Swiss Milk Company Ltd, 6281 Hochdorf, Switzerland. Fax + 41 41 910 1313.
- DMV International, PO Box 13, 5460 BA, Veghel, The Netherlands. Fax + 31 413 362 656.
- Carbery Food Ingredients, Ballineen, Co. Cork, Republic of Ireland. Fax + 353 (0) 23 47541.
- Arla Food Ingredients, Head Office, Skanderborgvej 277, DK-8260, Viby J., Denmark. Fax + 45 8628 1838.
- New Zealand Dairy Board, PO Box 417, Wellington, New Zealand. Fax + 64 4471 8600.
- Century Foods International, PO Box 257, 919 Hoeschler Drive, Sparta, Wisconsin 54656, USA. Fax + 1 608 269 1910.
- DENA GmbH, Villa Flora, Oberkasseler Street 26, D-40545, Dusseldorf, Germany. Fax +49211555583.

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14

Egg proteins

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Abstract: This chapter deals with the chemical composition and structural characteristics of egg yolk and white in relation to three important functional properties: emulsifying, foaming and gelling properties.

Key words: egg, yolk, white, emulsions, foams, gels, structure, assemblies, interfaces.

14.1 Introduction: technofunctional uses of egg constituents

Hen egg was categorised by Baldwin in 1986 as a polyfunctional ingredient, as it can simultaneously realise several technological functions in the same formulated foodstuff. Its emulsifying, foaming, gelling, thickening, colouring and aromatic properties make it still today a universal basic ingredient for the domestic kitchen and the food processing industry. Whereas egg yolk is well recognised for its emulsifying properties, egg white (or albumen) is a reference in terms of foaming and both parts are used as gelling ingredient in many foods.

Yolk takes part in the formation and the stabilisation of emulsions. In spite of the intensive use of yolk in formulated foodstuffs, and since the invention of mayonnaise three centuries ago, the role of its major constituents is not clear because of its complex structure. Yolk is a mixture of proteins and lipids forming natural assemblies at various scales. These natural assemblies contribute to the nano- and the microstructure of yolk. Thus, an understanding of the emulsifying properties of yolk lies in the comprehension of these various levels of structure.

The exceptional foaming properties of the albumen are also the base of traditional recipes among which meringues act certainly as reference. Indeed, the extreme simplicity of their formula (albumen and sugar, possibly added with flavours) allows albumen to express in an optimal way its foaming properties. However, the technological parameters influence the final quality of foam obtained, and three types of meringues (traditional meringue, Swiss meringue and Italian meringue) can be distinguished, depending on whether whipping is achieved in the presence or absence of sugar, and at ambient or warm temperature. But there is also a great number of other products in which previously foamed albumen is added, which are either fat-free formulas (angel food cake) or lipid-containing formulas (spoon biscuits, 'sponge' cake, blown). In such products, the complexity of the phenomena is extreme, foaming and emulsification taking place simultaneously, which makes the control of the physico-chemical and technological parameters of these operations very delicate.

Concerning the gelling properties of albumen and yolk, they are related to the heat-gelation capacity of egg proteins. Then, these properties imply a cooking step during the food processing. The heat gelation of egg proteins completely conforms to the model of heat gelation of globular proteins. The corresponding mechanisms have been extensively studied, on egg proteins as well as on other ones, and the key technological parameters have now been identified. However, the addition of other ingredients in mixture with egg (polysaccharides, for example) complicates the understanding of the egg gelation behaviour, and developments with more complex models are still needed.

14.2 Physico-chemistry and structure of egg constituents

14.2.1 Egg yolk

Chemical composition

Yolk correspond to 36% of whole hen egg weight. Its dry matter is about 50–52% according to the age of the laying hen and the duration of preservation (Kiosseoglou, 1989; Thapon and Bourgeois, 1994; Li-Chan *et al.*, 1995). The compositions of fresh and dry yolks are presented in Table 14.1: the main components are lipids (about 65% of the dry matter) and the lipid to protein ratio is about 2:1. Yolk lipids are exclusively associated with lipoprotein assemblies. They are made up of 62% triglycerides, 33% phospholipids, and less than 5% cholesterol. Carotenoids represent less than 1% of yolk lipids, and give it its colour. Proteins are present as free proteins or apoproteins (included in lipoprotein assemblies). The interactions between lipids and proteins result in the formation of lipoproteins (low and high density), which represent the main constituents of yolk.

Macrostructure and main constituents

Yolk is a complex system with different structuration levels consisting in aggregates (granules) in suspension in a clear yellow fluid (plasma) that contains

Table 14.1 Composition of hen egg yolk

	Fresh yolk (%)	Dry yolk (%)
Water	51.1	—
Lipids	3.6	62.5
Proteins	16.0	33.0
Carbohydrates	0.6	1.2
Minerals	1.7	3.5

Source: Powrie and Nakai (1986)

lipoproteins and proteins. Granules consist in circular complexes ranging in diameter from 0.3 μm to 2 μm (Chang *et al.*, 1977). Consequently, yolk can be easily separated into two fractions after a dilution (two times) with 0.3 M NaCl and a centrifugation at 10,000 g (30 min) according to the method of McBee and Cotterill (1979): a dark orange supernatant called plasma and a pale pellet called granules (Fig. 14.1).

Granules represent 22% of yolk dry matter, accounting for about 50% of yolk proteins and 7% of yolk lipids. The dry matter content of granules is about 44%, with about 64% proteins, 31% lipids and 5% ash (Dyer-Hurdon and Nnanna,

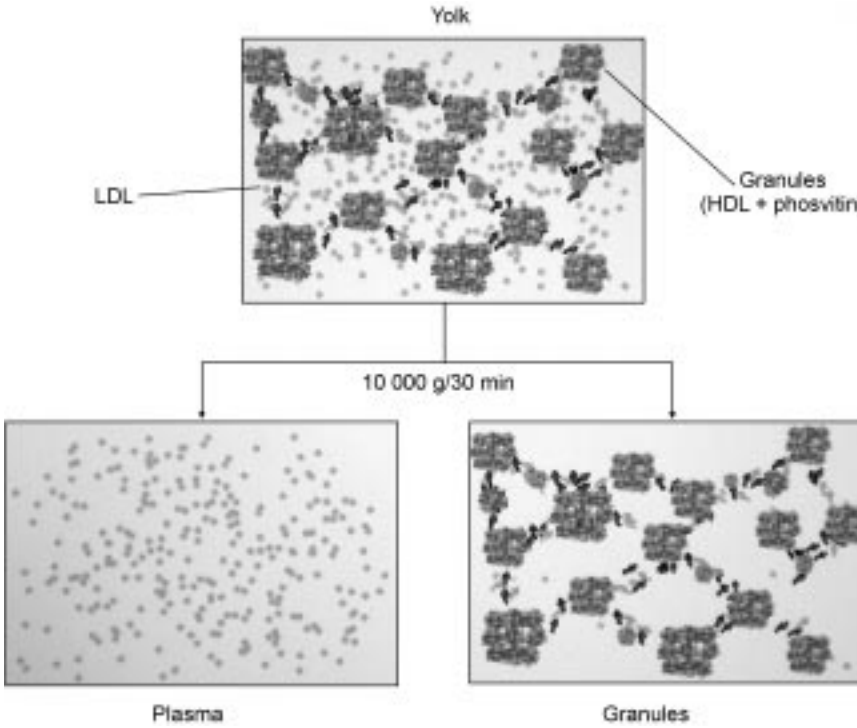

Fig. 14.1 Fractionation of plasma and granules from hen egg yolk.

Table 14.2 Repartition of hen egg yolk constituents

	Yolk D.M. (%)	Yolk lipids (%)	Yolk proteins (%)	Lipids (%)	Proteins (%)
Yolk	100	100	100	64	32
Plasma	78	93	53	73	25
LDL	66	61	22	88	10
Livetins	10	—	30	—	96
Others	2	—	1	—	90
Granules	22	7	47	31	64
HDL	16	6	35	24	75
Phosvitin	4	—	11	—	95
LDLg	2	1	1	88	10

Source: Powrie and Nakai (1986)

1993; Anton and Gandemer, 1997). They are mainly constituted by high density lipoproteins (HDL) (70%) and phosvitin (16%) linked by phosphocalcic bridges between the phosphate groups of their phosphoseryl residues (Burley and Cook, 1961; Saari *et al.*, 1964). Low density lipoproteins (LDL) (12%) are included in the granular structure (Table 14.2).

At low ionic strength, granules mainly form insoluble HDL-phosvitin complexes linked by phosphocalcic bridges as HDL and phosvitin contain a high proportion of phosphoserin amino acids able to bind calcium (Causeret *et al.*, 1991). The numerous phosphocalcic bridges make the granule structure very compact, poorly hydrated, weakly accessible to enzymes, and lead to an efficient protection against thermal denaturation and heat gelation.

At an ionic strength over 0.3 M NaCl, the phosphocalcic bridges are disrupted because monovalent sodium replaces divalent calcium. In such conditions, the solubility of granules reaches 80% because phosvitin is a soluble protein and HDL behave like soluble proteins (Cook and Martin, 1969; Anton and Gandemer, 1997). Complete disruption of granules occurs when ionic strength reaches 1.71 M NaCl. Acidification or alkalinisation similarly cause the disruption of granules and the solubilisation of these constituents by increasing the number of the positive (NH_3^+) or negative (COO^-) charges inducing electrostatic repulsions between granule constituents. Recently, we have established (Sirvente, 2007) a phase diagram drawing the different states of granules as a function of pH and ionic strength (Fig. 14.2).

Plasma comprises 78% of yolk dry matter and is composed of 85% LDL and 15% livetins (Burley and Cook, 1961; Table 14.2). It forms the aqueous phase where yolk particles are in suspension. It accounts for about 90% of yolk lipids (including nearly all the carotenoids), and 50% of yolk proteins. Plasma contains about 73% lipids, 25% proteins and 2% ash. Lipids of plasma are distributed thus: 70% triglycerides, 25% phospholipids and 5% cholesterol.

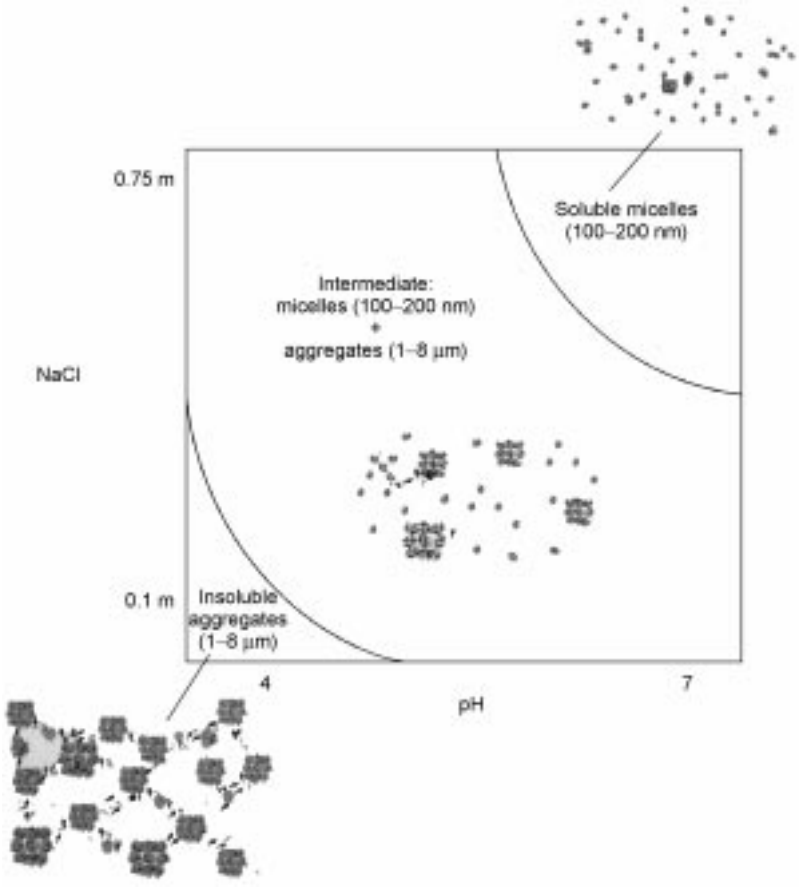

Fig. 14.2 Physical state of granules as function of pH and ionic strength.

LDL are spherical particles (17–60 nm in diameter with a mean of about 35 nm) with a lipid core in a liquid state (triglycerides and cholesterol esters) surrounded by a monofilm of phospholipid and protein (Cook and Martin, 1969; Evans *et al.*, 1973). LDL are soluble in aqueous solution (whatever the pH and ionic conditions) due to their low density (0.982). Phospholipids take an essential part in the stability of the LDL structure because association forces are essentially hydrophobic (Burley, 1975). Some cholesterol is included in the phospholipid film, increasing its rigidity. LDL are composed of 11–17% protein and 83–89% lipid, out of which 74% is neutral lipid and 26% phospholipid (Martin *et al.*, 1964).

14.2.2 Egg white

Egg white represents about 60% of the total egg weight. It consists of an aqueous protein solution, containing few minerals and carbohydrates (Table

Table 14.3 Composition of hen egg white

	% of hen egg white
Water	88.0
Lipids	—
Proteins	10.6
Carbohydrates	0.8
Minerals	0.6

Source: Thapon and Bourgeois (1994)

14.3). During egg storage, different physico-chemical modifications happen, among them the CO_2 departure that induces a pH increase, from 7.5 at the laying moment to 9.5 after a few days. This pH modification should be the cause of the egg white liquefaction, because of the dissociation of a protein complex (ovomucin-lysozyme complex) (Kato *et al.*, 1975). Another evolution observed concerns the ovalbumin modification toward S-ovalbumin, which is a more heat-stable form (Smith and Back, 1965), resulting from isomerisation of three serine residues (Yamasaki *et al.*, 2003).

Proteins

Proteins represent more than 90% of the dry matter of egg white, but until very recently, only the major ones have been identified. However, the recent and powerful techniques for separation and analysis enabled the identification of many minor proteins (Table 14.4) (Guérin-Dubiard *et al.*, 2006; Mann, 2007).

The egg white proteins are predominantly globular proteins, and acidic or neutral, except lysozyme and avidin which are highly alkaline proteins. All are glycosylated, except cystatin and the major form of lysozyme. Some of them are very heat-sensitive and/or sensitive to surface denaturation, explaining their noteworthy functional properties.

The major egg white protein (more than 50% of the total proteins) is ovalbumin, a 45 kDa globular and phosphorylated protein. Half of its amino acids are hydrophobic, and one-third are electrically charged, essentially negatively at physiologic pH. Ovalbumin possess six buried Cys residues, two being involved in a disulfide bridge (Cys^{73}-Cys^{120}). Ovalbumin is then the only egg white protein with free thiol groups, capable of inducing some rearrangements with variations of storage conditions, pH and surface denaturation.

Ovotransferrin (13% of total proteins) molecular weight is around 78 kDa. This protein consists of two lobes, each containing a specific binding site for iron (or copper, zinc, aluminium) (Kurakawa *et al.*, 1995). It is the most heat-sensitive egg white protein, but the complexation of iron or aluminium significantly increases its heat stability (Lin *et al.*, 1994).

Ovomucoïde is a highly glycosylated protein (up to 25% carbohydrates, w/w) of 28 kDa. At pH 7, its denaturation temperature is around 77 ºC, but this protein

Table 14.4 Composition and some physico-chemical and functional properties of egg white proteins

Protein	%	M_w (kDa)	*pI*	Major biological properties
Ovalbumin	54	45	5	Immunogenic phosphoproteine
Ovalbumin Y	5	44	5.2	nd
Ovalbumin X	0.5	56	6.5	nd
Ovotransferrin	13	76	6.7	Iron binding, bacteriostatic activity
Ovomucoïd	11	28	4.8	Trypsin inhibitor
Ovomucin	1.5–3.5	230–8300	4.5–5	Highly glycosylated, viral hemaglutination inhibition
Lysozyme	3.5	14.4	10.7	Lysis of Gram+ bacteria wall
Ovoinhibitor	0.1–1.5	49	5.1	Serine protease inhibitor
Ovoglycoprotein	0.5–1	24.4	3.9	nd
Flavoprotein	0.8	32	4	Riboflavin (vitamin B2) binding
Ovostatin	0.5	760–900	4.6	Serine protease inhibitor
Cystatin	0.05	12.7	5.1	Cysteine protease inhibitor
Avidin	0.05	68.3	10	Biotine binding
Ex-FABP	nd	18	5.5	Lipocaline family
Cal gamma	nd	20.8	6	Lipocaline family
TENP	nd	47.4	5.6	BPI (bactericidal permeability-increasing protein) family
Hep 21	nd	18	6.4	uPar/Ly6/Snake neurotoxin family

Sources: Li-Chan and Nakai (1989), Stevens (1991), Guérin *et al.* (2006).

is much more heat resistant at acidic pH (Lineweaver and Murray, 1947). Ovomucin is also a highly glycosylated protein, with a very high molecular weight (10^4 kDa). Electrostatic interactions can be observed between ovomucin and some of the other egg white proteins. In the freshly laid eggs (pH 7.5), the carboxylic groups of the ovomucin sialic acids especially interacts with the ϵ-NH^{3+} of lysozyme lysine residues to form a lysozyme-ovomucin complex that may be responsible for the gel-like structure of egg white (Kato *et al.*, 1975). Lysozyme is a small (14 kDa) globular, and strongly basic protein. Its structure is very rigid, stabilised by four disulfide bridges.

Glucidic and mineral fractions

The glucidic fraction of egg white consists of free glucose (0.5% w/w) and carbohydrates linked to proteins (0.5% w/w). The mineral fraction is predominantly composed of Na^+, K^+ and Cl^-, as free minerals, whereas P and S are essentially constitutive elements of proteins. Egg white also contains CO_2, in equilibrium with bicarbonate, which plays a major role for pH control (Thapon, 1994).

14.3 Egg yolk emulsions

14.3.1 Basic principles

Emulsifying activity is related to the capacity of surface active molecules to cover the oil–water interface created by mechanical homogenisation, thus

reducing the interfacial tension. Consequently, the more active the emulsifying agent, the more the interfacial tension is lowered. Emulsion stability indicates the capacity to avoid flocculation, creaming, and/or coalescence of oil droplets. Creaming and flocculation are reversible phenomena which can be avoided by a simple agitation of the emulsion. Coalescence is the irreversible fusion of oil droplets due to the rupture of the interfacial film created by emulsifying agents. This phenomenon leads to a complete destruction of the emulsion. This relates the importance of the structure and the viscoelasticity of the interfacial film.

14.3.2 Role of egg yolk constituents

In researching the principal contributor to yolk emulsifying properties, numerous authors have separated yolk into its main fractions: plasma and granules. Large similarities have been observed between emulsifying properties of yolk and plasma, whereas emulsions made with granules behaved very differently (Dyer-Hurdon and Nnanna, 1993; Anton and Gandemer, 1997; Le Denmat *et al.*, 2000). Specifically, emulsions made with granules are more coarse (more important oil droplet size) than emulsions made with yolk and plasma, and notably at acidic pH where granules are not soluble (Le Denmat *et al.*, 2000) (Fig. 14.3).

Concerning the parameters of emulsion stability (creaming), we showed (Le Denmat *et al.*, 2000) that emulsions made with yolk and plasma had the same creaming rate, in function of the medium conditions, whereas emulsions made with granules behaved very differently (Fig. 14.3). Consequently, these studies demonstrated that yolk emulsifying power was situated in plasma.

Among plasma constituents, some authors demonstrated that LDL are better emulsifiers than bovine serum albumin (BSA) (Mizutani and Nakamura, 1984) and casein (Shenton, 1979). Even though some authors suggested that, in certain conditions, HDL were more efficient than LDL to form and stabilise O/W emulsions (Hatta *et al.*, 1997; Mine, 1998), a large number of studies confirm the prevalent role of LDL in yolk emulsions. These findings have been confirmed recently (Aluko *et al.*, 1998; Mine and Keeratiurai, 2000; Anton *et al.*, 2003; Martinet *et al.*, 2003). In particular, it has been established that LDL made emulsions finer than HDL, along different conditions of pH and ionic strength (Martinet *et al.*, 2003). The next question is how to explain the exceptional efficiency of LDL at the interfaces.

14.3.3 Importance of assemblies

Given that any destructurating treatment affects the emulsifying properties of LDL, it appears that the integrity of the structure of LDL seems essential to ensure their interfacial properties (Tsutsui, 1988). Direct adsorption of apoproteins and phospholipids from LDL is not easy because of the non-solubility of these species in water or in aqueous buffer. So the interactions between apoproteins and lipids to assemble the LDL particles are essential to

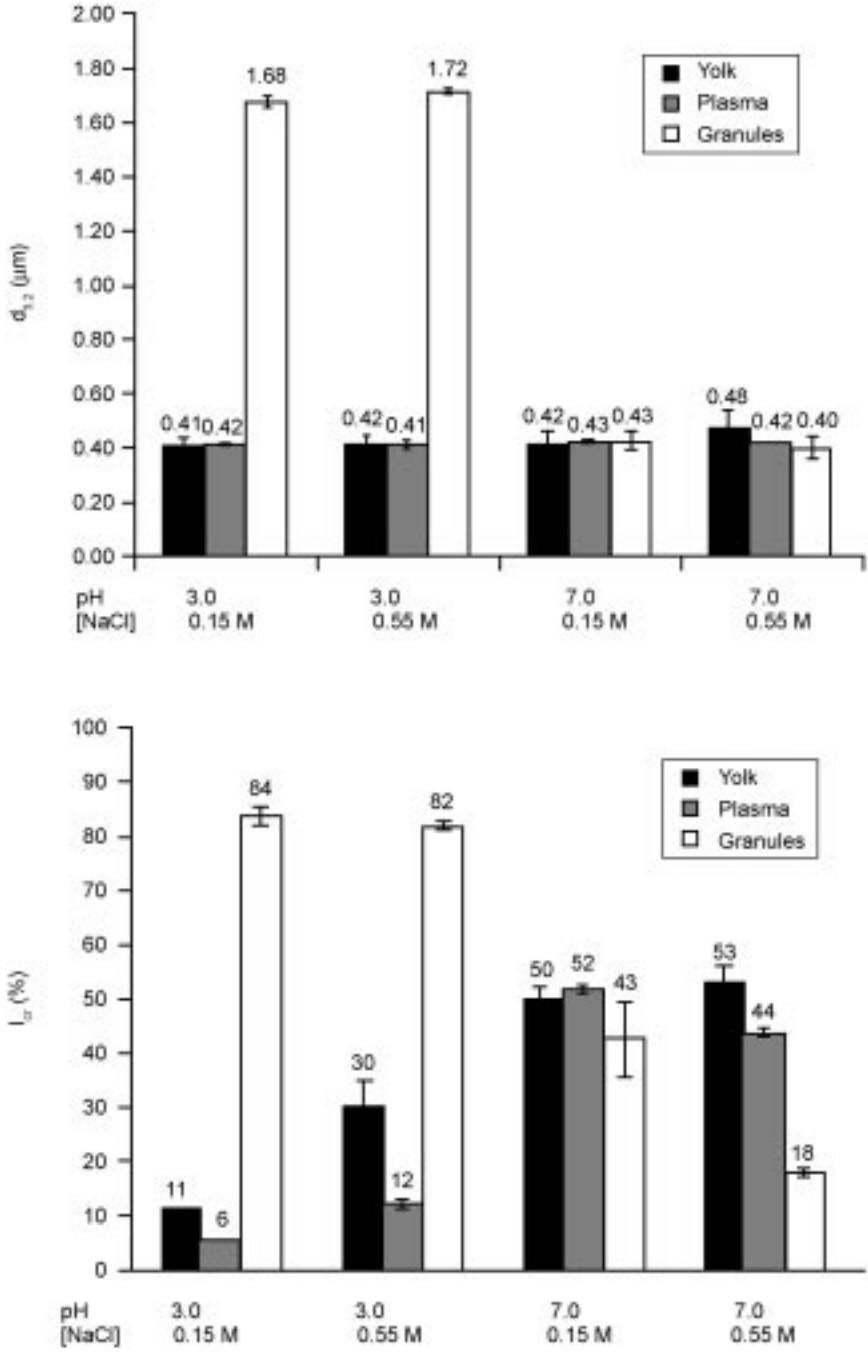

Fig. 14.3 Mean droplet diameter (d3.2) and creaming index (Icr) in oil/water emulsions (30 : 70) prepared with yolk, plasma and granules, protein concentration: 25 mg/ml, homogenisation pressure: 200 bars, $n = 3$.

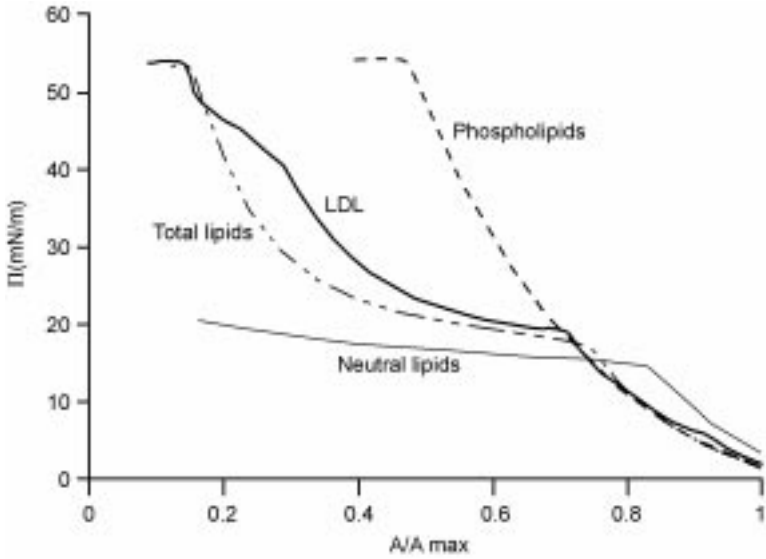

Fig. 14.4 Π/A isotherms of the different lipid constituents extracted from LDL and spread at the air–water interface; neutral lipids = 85 μg, phospholipids = 198 μg, total lipids = 287 μg, compression rate = 100 cm^2/min.

transport the surfactants in a soluble form in the neighbourhood of the interface and then to release them at the interface.

Using Langmuir film balance (air–water interface), three phase transitions have been detected in compression isotherms and these three transitions (19, 41 and 54 mN/m) have been attributed, respectively, to neutral lipids, apoproteins and phospholipids by comparison with films of neutral lipids, phospholipids and total lipids extracted from LDL (Fig. 14.4) (Martinet *et al.*, 2003). The transition observed at 19 mN/m corresponds to the collapse of neutral lipids, and the transition at 54 mN/m corresponds to phospholipid collapse. These different transitions show that LDL actually break down when they come into contact with the interface to release neutral lipids, phospholipids and apoproteins from the lipoprotein core and to allow their spreading. In a recent study made with atomic force microscopy (AFM) after a Langmuir–Blodgett transfer of the layers from the air–water interface to a silica plate, it has been shown that the second transition (previously attributed to apoproteins alone) is not due to apoproteins alone, but to apoprotein–lipids complexes (Dauphas *et al.*, 2006).

So, it has been deduced that LDL serve as vectors of surfactant constituents (apoproteins and phospholipids) that could not be soluble in water, until the interface. At this step the conservation of the LDL structure is essential. Once LDL are near the interface, the structure is then broken up to release surfactant constituents at the interface (Fig. 14.5).

Furthermore, comparing interfacial behaviour of LDL and liposomes (double phospholipid layer not containing proteins), it has been shown that the apoproteins situated on the LDL surface start the LDL disruption mechanism by

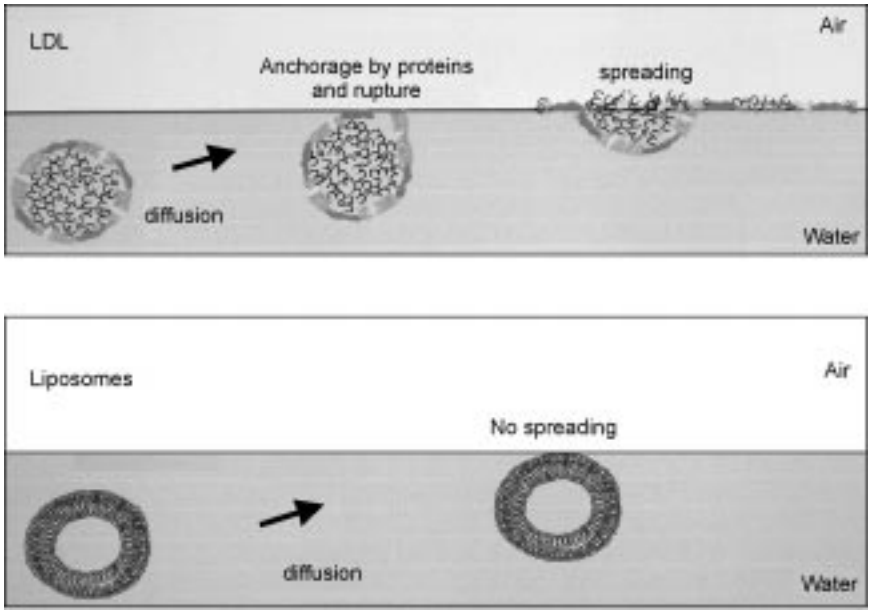

Fig. 14.5 Hypothetical mechanism of LDL adsorption at an oil–water interface as compared with liposome behaviour.

their initial anchorage. This anchorage provokes an unfolding of the protein leading to the destabilisation of the external layer of the LDL. Then this phenomenon could be followed by a deformation of the particle due to the creation of a neutral lipid lens conducive to the spreading of the LDL constituents. In the case of liposomes, without external proteins, the structure remains steady at the interface and then this structure is not able to adsorb efficiently and to decrease interfacial tension (Fig. 14.5).

14.4 Egg white foams

14.4.1 Formation and stabilisation mechanisms

Foam formation is a highly energetic and dynamic process, in which interfacial area is created. The ability of a protein solution, such as egg white, to foam depends on protein structure and conformation, depending themselves on extrinsic factors such as pH, ionic strength, etc. The formation mechanism of globular protein foams can be divided into three phases happening near gas bubbles: protein diffusion towards the air–solution interface, conformation changes of adsorbed proteins, and irreversible rearrangement of the protein film (McRitchie, 1991).

Foams are short-lived states and there is any correlation between foam stability and protein adsorption kinetic (Dickinson, 1996). Foam stability, indeed, depends on protein association at the air–solution interface to form a

continuous intermolecular network. Foam stability is affected by the protein film cohesion, drainage and Ostwald disproportionation.

14.4.2 Interfacial properties of egg white proteins

Interfacial properties of egg white proteins are responsible for egg white's excellent foaming properties. Table 14.5 gathers some data on the kinetics of diffusion towards the air–solution interface of three major egg white proteins. Ovalbumin interfacial behaviour is well known, since a large set of data is available about its tensioactivity, adsorption kinetics, interfacial shear and dilatational rheology (de Feijter *et al.*, 1978; de Feijter and Benjamins, 1987; Benjamins and van Voorst Vader, 1992; Benjamins and Lucassen-Reynders, 1998; Damodaran *et al.*, 1998; Lucassen-Reynders and Benjamins, 1999; Pezennec *et al.*; 2000; Razumovsky and Damodaran, 2001; Croguennec *et al.*, 2007) but also on its structure at the air–water interface (Renault *et al.*, 2002; Lechevalier *et al.*, 2003, 2005; Kudryashova *et al.*, 2003). It is now known that ovalbumin forms a single layer at the air–water interface, whatever its concentration in the bulk (Renault *et al.*, 2002). As for ovalbumin, lysozyme interfacial behaviour has been extensively studied (de Feijter and Benjamins, 1987; Damodaran *et al.*, 1998; Razumovsky and Damodaran, 2001; Kim *et al.*, 2002; Postel *et al.*, 2003; Chang *et al.*, 2005; Roberts *et al.*, 2005; Perriman and White, 2006) as well as its structure at the air–water interface (Lechevalier *et al.*, 2003, 2005). However, its interfacial behaviour differs as lysozyme forms films that are much thicker than a protein monolayer whereas the surface pressure is definitely smaller than the ovalbumin one (Le Floch-Fouéré *et al.*, 2009). These different behaviours observed on planed air–water interface result in different foaming properties. Ovalbumin foaming properties are much better than those of lysozyme, since in native state at pH 7.0, the foaming capacity of lysozyme is very weak (Townsend and Nakai, 1983), probably because of its little surface hydrophobicity and its rigidity due to its four disulfide bonds.

Egg white proteins thus show different behaviour at 2D and 3D air–water interfaces. When they are in mixture, their behaviour is again different. Indeed, Damodaran *et al.* (1998) showed that the adsorption kinetics of egg white proteins are different depending on whether they are in single protein systems or in mixture. They suggested the formation of electrostatic complexes between positively charged lysozyme and other negatively charged egg white proteins. Moreover, the mixture ovalbumin-lysozyme forms films that are much thicker than those of both proteins in single protein systems, suggesting a synergy in interfacial adsorption between the two proteins (Le Floch-Fouéré *et al.*, 2007).

14.4.3 Egg white foams

Egg white is the reference for foaming properties: compared with other protein ingredient of vegetable or animal origin, it still offers the best foaming properties (Vani and Zayas, 1995; Matringe *et al.*, 1999; Pernell *et al.*, 2002;

Table 14.5 Parameters of the kinetic of diffusion towards the air–solution interface of three major egg white proteins

Parameters	Ovalbumin	Ovotransferrine	Lysozyme	Reference
Apparent diffusion coefficient ($10^{-10}\,m^2\,s^{-1}$)	0.5 (C=10^{-4}% prot.) *(in solution: 0.7)*		0.2 (C=10^{-4}% prot.) *(in solution: 1)*	De Feijter and Benjamins (1987)
	0.5 to 1 (C=0.1% prot.)		0.15 (C=$1.5{\cdot}10^{-4}$% prot.)	Xu and Damodaran (1993) Pezennec *et al.* (2000)
Surface concentration ($mg\,m^{-2}$)	1.6 (C=10^{-4}% prot.)		2.4 (C=10^{-4}% prot.)	De Feijter and Benjamins (1987)
	1.5 (C=$5.4{\cdot}10^{-4}$% prot.)	0.8 (C=$1.2{\cdot}10^{-4}$% prot.)	0.5 (C=$0.35{\cdot}10^{-4}$% prot.)	Damodaran *et al.* (1998) Pezennec *et al.* (2000) Croguennec *et al.* (2007)
	2.1 (native protein) to 2.9 (heat-treated protein) (C=0.01% prot.)			
			1.4 (pH 4) to 3 (pH 11) (C=0.1% prot.)	Perriman and White (2006)
Surface pressure ($mN\,m^{-1}$)	1 (C=10^{-4}% prot.)		3.5 (C=10^{-4}% prot.)	De Feijter and Benjamins (1987)
	14 (C=$5.4{\cdot}10^{-4}$% prot.)	2.5 (C=$1.2{\cdot}10^{-4}$% prot.)	2.5 (C=$0.35{\cdot}10^{-4}$% prot.)	Damodaran *et al.* (1998) Pezennec *et al.* (2000)
	24 (C=0.01% prot.)		8 (pH 5.6) to 14 (pH 11) (C=0.012% prot.)	Roberts *et al.* (2005)
			9 (C=$5{\cdot}10^{-4}$% prot.) to 24.5 (C=0.1% prot.)	Chang *et al.* (2005)
Lag phase	YES if C<0.01% *Not enough molecules at the interface to create an increase of* π		YES if C<0.01% *Not enough molecules at the interface to create an increase of* π	De Feijter and Benjamins (1987)
	NO	YES	YES	Damodaran *et al.* (1998)

Table 14.6 Interfacial characteristics of the main egg white proteins

Protein	Surface tension ($mN\,m^{-1}$)	Foamability ($cm^3\,g^{-1}\,min^{-1}$)
Globulins	45.4	4.71
Ovalbumin	51.8	0.59
Ovotransferrin	42.4	0.34
Lysozyme	42.0	0.12
Ovomucoïd	39.0	0
Ovomucin	nd	0
Mixture in the egg white ratio	46.7	3.08

Source: Mine (1995)

Foegeding *et al.*, 2006; Davis and Foegeding, 2007). Egg white can be considered as a solution of efficient surfactants. Its proteins are amphiphilic and show a relatively high surface hydrophobicity, thus diffusing quite rapidly towards the air–water interface where they adsorb efficiently. Their molecular flexibility ensures conformational rearrangement at the interface, resulting in a great decrease in surface tension. Their ability to form a continuous intermolecular network, especially when a certain denaturation level is previously obtained, enable them to form a viscoelastic interfacial film responsible for foam stability. However, egg white proteins do not present at the same level these different characteristics, and thus do not participate in the same way to egg white foaming properties (Table 14.6). A lot of studies have been performed on the different egg white protein foaming properties (Nakamura, 1963; Johnson and Zabik, 1981a; Mine, 1995). However, it is now well known that the complexity and the synergy of the phenomena mean that it is impractical to distinguish the role of the different egg white properties (Lechevalier *et al.*, 2005). It is thus quite difficult to predict foaming properties of any mixture of egg white proteins since phenomena of competition for the interface and possible exchange between proteins at the air–water interface may occur. Nevertheless, foaming properties of isolated egg white proteins are always lower than those of egg white, which tend to confirm the existence and the role of the interactions between proteins. It is thus generally admitted that the natural coexistence in egg white of alkaline protein (lysozyme) and acid ones (most of the other) enables electrostatic interactions, thus explaining the good foaming properties of egg white (Poole *et al.*, 1984; Damodaran *et al.*, 1998).

Egg white foams are an integral component of many foods such as meringue, nougat and angel food cake. A recent study showed that the properties of foams do not predict performance in angel food cake (Foegeding *et al.*, 2006).

14.4.4 Key parameters

Many physico-chemical parameters are susceptible to influence foam formation and stability. In the specific case of egg white proteins, surface hydrophobicity (that conditions the efficiency of protein adsorption at the air–water interface),

the number of disulfide bonds (that conditions protein flexibility/rigidity) and the number of free sulfhydryl groups (that conditions protein reactivity) are decisive in the structural modifications that occur at the air–water interface. Moreover, the number and the nature of inter- and intramolecular interactions determine the rheological properties of the interfacial film and so foam stability. These interactions are favoured by a certain degree of denaturation, however, too much denaturation weakens the interfacial film and the foam collapses (Kinsella, 1976; Trziszka, 1993; Kato *et al.*, 1994; Van der Plancken *et al.*, 2007). Another special feature of egg white foams is their dependence on thick egg white proportion and quality. Foamability increases with egg white natural liquefaction during its storage (Sauveur *et al.*, 1979; Thapon, 1981; Baldwin, 1986), whereas foam stability decreases (Nau *et al.*, 1996). Egg white foaming properties can also be improved by the addition of sucrose (effect on foam stability) and sodium chloride (effect on foamability), as suggested by Raikos *et al.* (2007a).

14.5 Gels

14.5.1 Basic principles

A gel consists of polymers linked through covalent and/or non-covalent interactions, to create a three-dimensional network. In whole egg as well as in white and yolk, proteins are responsible for the gelling properties. Gelation occurs when the protein stability in solution is modified, i.e., when the equilibrium between attractive (Van der Waals) and repulsive (electrostatic, steric) interactions is disrupted. The electrostatic repulsions vary with the net charge of the proteins, that means with the ionisable protein groups and with the physico-chemical characteristics of the solvent (pH, ionic strength). The treatments that decrease the repulsive interactions, such as adjustment of pH at proteins pI or addition of salts, induce destabilisation and thus can result in the formation of aggregates or gels.

Moreover, some treatments can modify the protein structure, with consequences for the repulsive and attractive interactions mentioned above. This is especially the case during heat treatments, which are the major technological treatments used in the food industry for egg white and yolk gelation. Heat-induced gelation of egg conforms completely with the model of heat gelation of globular proteins. It is a two-step phenomenon: in the first stage, unfolding of native proteins occurs, disrupting the well-defined secondary and tertiary structures and producing denatured proteins exposing their inner hydrophobic regions; following unfolding, the denatured proteins interact to form high molecular weight aggregates that can further interact with each other to result in a three-dimensional gel (Clark *et al.*, 2001). The unfolding and aggregation steps depend on many factors (protein concentration, ionic strength, pH, presence of sucrose, etc.) that can modify the number and/or the kind of interactions, with final consequences on the gel rheology. In the heat-induced

gels of egg proteins, the interactions involved are predominantly hydrophobic and electrostatic, but some highly energetic interactions can be observed (disulfide bridges); thiol and amine groups are indeed very reactive, especially in alkaline conditions.

14.5.2 Egg yolk gels

Yolk undergoes a gelation when it is subjected to a freezing-thawing process or a heat treatment. LDL are responsible for yolk gelation, while the other constituents of yolk do not participate directly (Kojima and Nakamura, 1985; Kurisaki *et al.*, 1981; Nakamura *et al.*, 1982; Tsustui, 1988; Wakamatu *et al.*, 1982; Le Denmat *et al.*, 1999).

Freezing-thawing gelation of yolk appears for a temperature below −6 ºC (Lopez *et al.*, 1954). This gelation is undesirable because it makes yolk difficult to handle. Freezing-thawing gelation is influenced by rate and temperature of freezing and thawing, and length and temperature of storage (Kiosseoglou, 1989). Rapid freezing and thawing results in less gelation than slow freezing and thawing. Freezing-thawing gelation is partially reversible and the initial viscosity of yolk is recovered after heating for 1 h at 45–55 ºC.

The mechanism of freezing-thawing gelation of LDL remains hypothetical. Freezing causes the formation of ice crystals which mobilise water (at −6 ºC, about 80% of the water in yolk is in ice crystals) and causes a dehydration of apoproteins of LDL. This dehydration favours a rearrangement of apoproteins of LDL, interactions between their amino acid residues and an aggregation which leads to gelation.

LDL is the constituent of yolk responsible for heat-induced gelation of yolk (Saari *et al.*, 1964). LDL solution (4% w/v) start denaturing at 70 ºC and form gels at 75 ºC (Tsutsui, 1988). LDL solutions, heated at 80 ºC for 5 min, form more stable gels than ovalbumin and BSA (Kojima and Nakamura, 1985). Unlike ovalbumin and BSA, LDL present a heat-induced gelation in a large range of pH (4–9) with a minimal value around their pHi (Nakamura *et al.*, 1982). Between pH 6 and 9, LDL solutions form coagulum gels (opaque) whereas LDL solutions form translucents gels for extreme pH (4–6 and 8–9) (Kojima and Nakamura, 1985; Nakamura *et al.*, 1982).

Heat-induced gelation of yolk is governed by the unfolding of proteins during heating. Then the functional groups are exposed and attracted to one another through hydrophobic bonds resulting in a gel (Nakamura *et al.*, 1982).

The primary stage of the two phenomena (freezing-thawing and heat-induced gelation) is the disruption of the LDL structure (Kurisaki *et al.*, 1981). This disruption is favoured by dehydration in the case of freezing-thawing, or by unfolding under heating. Lipid-protein interactions are disrupted under freezing or heating and interactions between proteins are increased. These interactions are both principally of non-polar nature because a LDL gel is solubilised by SDS which interacts with the hydrophobic residues of apoproteins (Mahadevan *et al.*, 1969). The aggregation product of LDL certainly contains lipids included in the structure

(Tsutsui, 1988). Apoproteins of LDL present a large proportion of hydrophobic amino acids and, consequently, they have a high ability to form such gels.

More recently, Le Denmat *et al.* (2000) have measured the critical concentrations (Cg) for heat gelation of dispersions of yolk, plasma and granules in pH and NaCl ranges of respectively 3–7 and 0.15–0.55 M. In all cases, the domain Cg for plasma is 12–28 mg protein/ml, whereas it is 26–120 mg protein/ml for granules. For yolk solution Cg is comprised between 16 and 39 mg protein/ml. This confirms the preponderant influence of LDL, the major compound of plasma, in the heat gelation of yolk. This underlines the excellent capacity of granules to resist to heat treatments, that could be used for industrial applications.

14.5.3 Egg white gels

The heat gelation of egg white is used in many food applications involving a cooking step. Except ovomucin and ovomucoïd, all the egg white proteins coagulate when heated (Johnson and Zabik, 1981b). But the heat sensitivity of egg white proteins varies significantly: the temperature of denaturation at pH 7 in egg white is 84.5, 74 and 65 ºC for ovalbumin, lysozyme and ovotransferrin, respectively (Donovan *et al.*, 1975). Ovotransferrin is then the more heat-sensitive, which is why it is generally considered as the gelation initiator, and finally as a limiting factor considering the gelling properties. Therefore, ovotransferrin elimination has been suggested to improve egg white gelling properties (Kusama *et al.*, 1990). However, ovotransferrin is more stable at alkaline pH, at high ionic strength, and when metal ions are bound on it. Thus, the gelation temperature of egg white can also be significantly increased by modification of these parameters, and especially by Fe^{3+} or Al^{3+} addition (Cunningham and Lineweaver, 1965).

The extent and the kind of the interactions between the denatured proteins depend on the protein structure, that means on the extent of unfolding at the end of the denaturation step. Indeed, the unfolding governs the more or less important exposure of reactive groups or regions on the protein molecule. The interactions also depend on the physico-chemical conditions that can be either limiting or favouring, resulting in an increase or a decrease of aggregation rate respectively, and then in a decrease or an increase of the denaturation extent before interactions take place (Totosaus *et al.*, 2002).

These mechanisms have been extensively studied for egg white and ovalbumin heat-gelation, with a focus on ionic strength effect on the structure and characteristics of the gels (Holt *et al.*, 1984; Woodward and Cotterill, 1986; Woodward, 1990; Croguennec *et al.*, 2002; Raikos *et al.*, 2007b). Heat denaturation induces an increase of the protein surface hydropbobicity. When heating occurs at high ionic strength, the protein charges are screened, inducing a shielding effect on the repulsive forces between proteins, thus favouring the hydrophobic interactions (Doi, 1993). In these conditions, random aggregates of slightly denatured proteins appear, corresponding to opaque gels, with low rigidity, elasticity and water retention capacity. On the other hand, at low ionic

strength, the electrostatic repulsions are so high that it delays the aggregation (Raikos *et al.*, 2007b), favouring denaturation. Finally, the further aggregation involves some specific area (hydrophobic regions), and induces linear polymeric aggregates. Once placed in higher ionic strength conditions, these aggregates can interact to form performing gels. Thus, a two-step heating process has been proposed to produce translucent gels from egg white, very firm and elastic, and with an exceptional water retention capacity (Kitabatake *et al.*, 1988a). As well as ionic strength influences the net charge of the proteins, pH is another major parameter for egg white gelation control. Close to their pI, the proteins tend to form random aggregates, similar to those obtained at high ionic strength. This phenomenon explains the minimal rheological properties of the egg white gels around pH 5. In contrast, at alkaline pH, egg white offers the best gelling properties (Ma and Holme, 1982; Kitabatake *et al.*, 1988b). The higher reactivity of the thiol groups in these pH conditions probably also contributes to the improvement of the gelling properties, because of disulfide bridges taking place. On the other hand, at acidic pH (2.0), the limited protein solubility would be responsible for the low gelation temperature and the low rheological properties observed (Raikos *et al.*, 2007b).

To improve the gelling properties of egg white, Kato *et al.* (1989) proposed an original approach consisting of an extensive denaturation of proteins while preventing aggregation. Such conditions can be obtained by heating of egg white powder at high temperatures (80 °C) for a long time (up to 10 days). This treatment increase protein flexibility and exposure of reactive groups that can further interact to strengthen the gel formed when previously dry-heated egg white is solubilised and heated in solution. The efficiency of this process can be improved by pH control (Mine, 1996, 1997). The dry-heating process is today the basis for mass production practices of high-gel egg white powders.

14.6 Conclusion

Hen egg contains very high functional proteins, lipids and lipoproteins. These functionalities are due partly to their chemical composition and structure, and partly to the supramolecular assemblies they form naturally or under the action of thermo-mechanical treatments during industrial processes. One of the major challenges for the future is the control of the design of these assemblies and the understanding of their functionalities (interfacial, emulsifying, foaming, phase separation, etc.) to enhance the quality of existing products or to conceive innovative products.

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15

Vegetable protein isolates

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Abstract: This chapter discusses the importance of vegetable proteins as functional ingredients in food formulations. It provides an overview of the main chemical components, world production, history and food applications of the main vegetable protein sources (legumes, cereals, oilseeds, roots and green leaves), with special emphasis on soybean, pea, and wheat. The chapter includes a description of the composition and structure of the main protein fractions, and a general approach to protein extraction, purification and processing technology to prepare protein meals, concentrates and isolates. In addition, the technologically important functional properties of vegetable protein preparations are described. Finally, nutritional and health effects, as well as the regulatory status of vegetable protein products are examined.

Key words: vegetable protein, food applications, meal and isolate, functional properties, protein structure and composition.

15.1 Introduction

The worldwide demand for proteins is increasing and, as a consequence, there is a need for new sources of food proteins. Animal proteins are expensive in terms of market price, land requirement and environmental impact. In addition, consumers' confidence in animal proteins has decreased due to food safety problems related to diseases such as bovine spongiform encephalopathy and the use of animal hormones. The conversion of vegetable into animal protein entails considerable losses in protein, water and energy. Under industrial conditions, the energy consumption per kilogram of animal protein is 8–10 times higher than for vegetable protein. Furthermore, the rising prices for raw materials and energy press market to the production of low-cost high quality protein foods.

Vegetable proteins are economic and versatile alternatives to animal proteins as functional ingredients in food formulations. Nevertheless, an effective replacement of animal proteins requires technological innovations. To achieve these innovations in an effective and efficient way, insight into the relation between protein structure and their functional properties is of prime importance.

The main sources of vegetable proteins currently under scientific and technological study and/or already in the market are the following:

- Legume grains as peas (*Pisum sativum*), soybeans (*Glycine max*), lupins (*Lupinus* spp), chickpeas (*Cicer arietinum*) or peanuts (*Arachis hypogaea*).
- Cereals as wheat (*Triticum* spp), maize (*Zea mays*), rice (*Oryza sativa*), barley (*Hordeum vulgare*), rye (*Secale cereale*), oat (*Avena sativa*) or sorghum (*Sorghum* spp).
- Oilseeds as sunflower (*Helianthus annuus*), rapeseed (*Brassica napus*), sesame (*Sesamum indicum*), cottonseed (*Gossypium* spp), or safflower (*Carthamus tinctorius*).
- Root vegetables as potato (*Solanum tuberosum*), cassava (*Manihot esculenta*) or sweetpotato (*Ipomoea batatas*).
- Leaves from alfalfa (*Medicago sativa*), cassava, amaranth (*Amaranthus* spp) or aquatic plants.
- Fruits as grape seed (*Vitis vinifera*), tomato seed (*Solanum lycopersicum*) or papaya kernel (*Carica papaya*).

15.1.1 Legumes

Legumes have played a vital part in many ancient civilizations. The use of legumes as a basic dietary staple can be traced back more than 20,000 years in some Eastern cultures. Legumes are crop plants from the family *Fabaceae* (or *Leguminosae*). The key characteristic of legume plants is the ability, of nearly all its members, to fix atmospheric nitrogen to produce their own protein compounds thanks to the symbiotic association with nitrogen-fixing bacteria (i.e. rhizobia) found in root nodules.

Farmed legumes can be basically classified in two groups: forage legumes and grain legumes. Forage legumes are grown for grazing or hay, and sometimes for industrial purposes. Grain legumes are cultivated for their grains (Fig. 15.1), which are harvested at maturity and marketed as dry product. Legume foliage and grains have comparatively higher protein content than non-legume material, making them attractive crops in agriculture.

There are about 18,000 legume species. More than 40 species and countless varieties of grain legumes are cultivated throughout the world. Pea is the main cultivated protein crop in Europe. Other major grain legumes grown in Europe are faba beans (*Vicia faba*), lentils (*Lens culinaris*), soybeans, lupins and chickpeas. However, three-quarters of the world production of grain legumes is soybeans, grown primarily in the United States, Brazil, Argentina, Paraguay and Uruguay. The world production of soybeans has increased by 215% over the last 30 years compared with just 50% for other grain legumes.

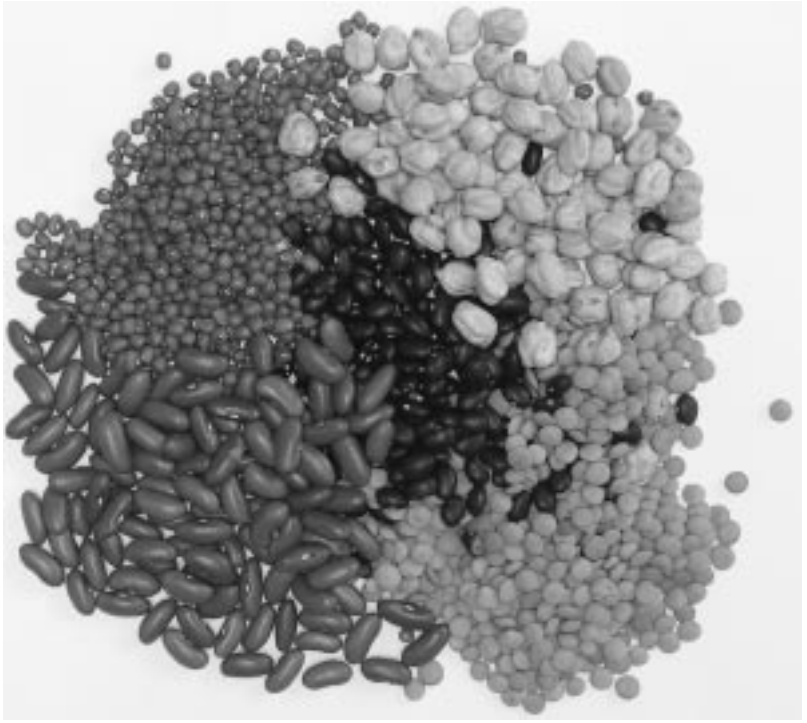

Fig. 15.1 Some popular grain legumes.

Soybean

Soybean is one of humanity's main food crops. It is widely accepted that soybeans originated in China around 5,000 years ago. Cultivation of soybeans, confined mainly to China, gradually extended to other continents. It was first introduced to Europe at the beginning of the eighteenth century. Soybeans were brought to America in the late eighteenth century, but a large-scale official introduction did not take place until the early 1900s. In 1920, combines were first used to harvest soybeans, and in 1922, the first soybean processing plant opened. By 1992, the United States accounted for half of the total world soybean production.

Pea

Native to Southwest Asia, it was among the first crops cultivated by man. Wild field peas of related species can still be found in Afghanistan, Iran, and Ethiopia. Peas were an important staple crop throughout Medieval Europe. It was introduced into America soon after Columbus's expedition. It is a cool season crop and is widely grown in the cooler temperate zones of the world, such as Northern Europe.

15.1.2 Cereals

The first recorded cereal farming communities were found 9,000 BC in the region between the Tigris and Euphrates rivers. Wheat, barley and rice were grown around 2,000 BC in Northern India. Cereal crops or grains are edible seeds of plants belonging to the grass family (*Gramineae*). Cereals are monocotyledonous angiosperms and are cultivated worldwide, from semi-arid conditions to very hot and cold regions. They are a major source of dietary protein for humans, on average representing about 50% of the protein consumed worldwide. The mean annual production of cereals for 2001–2005 surpassed 2,100 million tonnes. The main cereals crops are wheat, rice, maize, barley and sorghum. Wheat, rice and maize account for 85% of the cereal production.

Wheat

Wheat has been used as a food since early human history and it is among the oldest and most extensively grown of all crops. Historical evidence points to the cultivation of wheat in the Nile valley around 5,000 BC, the Indus and Euphrates valleys by 4,000 BC, China by 2,500 BC and England by 2,000 BC. Wheat cultivation expanded from Mediterranean centres of leading agriculture, towards Asia and Europe. Wheat is the most important and widely cultivated cereal crop in the world; 31% of the cereal production is wheat. It is grown in very different climates, from the arctic cold of Russia to the extreme heat of India. Different classes of commercial wheat cultivars are distinguished according to technologically relevant properties such as kernel hardness, bran colour and protein content. Typical distinctions are hard or soft, strong or weak.

15.1.3 Oilseeds

Oilseeds are seeds grown primarily for the production of edible oils. In the broader sense, peanuts and soybeans can be considered oilseeds. The history of oilseeds is closely tied with that of human civilization. Rapeseed and sesame were mentioned in the Indian Sanskrit writings of 2,000 BC, and sunflower was reported to be present in Arizona and New Mexico 3,000 years BC. Vegetable oil is obtained by pressing the seeds and then extracting the oil. The cake or meal obtained in the extraction is a rich source of proteins: sunflower and peanut meals contain 40–50% and 50–60% proteins, respectively. Other oilseed meals contain 35–45% proteins. Soybean, rapeseed, cottonseed, sunflower and peanut represent a 69, 12, 7, 5 and 3% of world protein meal production, respectively. World oilseed production reached 380 million metric tonnes in 2004/2005.

15.1.4 Root vegetables

Root vegetable is a very general definition for a wide cross-section of subterranean storage organs. Root vegetables include both true roots such as tuberous roots and taproots. Roots and tubers were already important in the diet during the early evolution of mankind. With the advent of agriculture, cultivated

root and tuber crops became increasingly critical sources of food, with potato, cassava and sweetpotato representing the 4th, 6th and 7th most important sources of food worldwide. Root vegetables are rich in carbohydrates but poor in protein content.

Potato

Potato belongs to the family of *Solanaceae*. It has been cultivated for thousands of years in South America and it was introduced into Europe in the second half of the sixteenth century. Nowadays, potato is the fourth most major crop for human consumption after wheat, rice and maize. The main industrial outlet of potato is starch production. The by-products that remain after starch manufacture are fibres and the so-called potato juice, wherein proteins are found. Indeed, potato ranks second to soybean in the amount of protein produced per hectare. However, it is chiefly use in animal feed due to its poor solubility after the currently applied industrial process. For this reason the contribution of proteins to the total value of the industrially used potato is limited.

15.1.5 Green leaves and fruits

Protein synthesis is one of the chief activities of the green part of the plant. Some forage crops produce leaf protein in large quantities, and therefore, leaf protein concentrate (LPC) is expected to be a valuable protein supplement for the human diet. LPC was first proposed for human consumption in around 1960, but has not achieved much success due to its bitter, grassy flavour, and dark green colour. Most research has been on alfalfa, amaranth, aquatic plants (e.g. duckweed), and by-product leaves (e.g., faba bean, pea).

The continuous development of new food proteins from secondary and new sources has also found its way into using fruit by-products such as date (*Phoenix dactylifera*) seeds, grape seeds, tomato seeds, papaya, mango (*Mangifera* spp) and apricot (*Prunus armeniaca*) kernels. Tomato seeds are a valuable by-product from the tomato paste manufacturing industry, with a protein content of about 20–30%. Furthermore, the nutritional and functional characteristics of tomato seeds have been found comparable to commonly used vegetable proteins.

15.2 Chemical composition of vegetable proteins

Plants contain a wide range of chemical compounds and display considerable variation in composition. Apart from the obvious interspecies differences, the rate and extent of chemical change depend on the growing conditions, the physiological role of the plant part, the genotype, and the post-harvest environment.

15.2.1 Legumes

In general, legumes are sources of complex carbohydrates, protein and dietary fibre, having significant amounts of vitamins and minerals. Legume seeds are characterized by relatively high protein content, from 17 to 40%.

Soybean

Soybean comprises about 8% seed coat or hull, 90% cotyledons and 2% germ. The proximate composition of soybeans is about 40% proteins, 35% carbohydrates, and 20% lipids. A variation ranging from 13 to 23% in lipids and 32 to 50% in protein has been described among different cultivars.

Pea

The protein content of peas ranges from 20 to 25%. Peas also contain about 33–50% starch, but are low in fat. Pea protein is a good source of essential amino acids, having high lysine content, but is limiting in tryptophan and in the sulphur-containing amino acids.

15.2.2 Cereals

Cereal grains consist of 12–14% water, 55–75% carbohydrates, 7–13% proteins and 1–6% lipids. Oats and maize, however, contain relatively large amounts of lipids. Table 15.1 provides the average proximate composition of some cereal grains. However, the data given should be used only as a guide because of the large differences reported in composition. For instance, the protein content of wheat may range between 8 and 18%.

Wheat

The chemical components of cereals are not homogeneously distributed in the kernel. Hulls and bran are high in cellulose and pentosans. The aleurone layer of wheat contains 25 times more minerals than the endosperm, and lipids are more abundant in the aleurone and germ. The endosperm contains mainly starch and a lesser amount of protein than the germ and the bran.

Table 15.1 Approximate composition (%) of various cereal grains

Grain	Protein	Lipids	Fibre	Carbohydrates
Wheat	11	2	2	70
Rice	7	2	1	64
Maize	10	5	2	63
Barley	11	3	3	56
Sorghum	8	4	4	63
Rye	9	2	2	72
Oats	9	6	2	63

15.2.3 Oilseeds

The proportion of hull and kernel in oilseeds varies considerably and affect the final chemical composition. Oil and proteins are the main components of oilseeds. Approximately 16–40% of the weight of oilseeds is protein and 18–45% is lipids. These values are strongly affected by the oilseed variety.

15.2.4 Potatoes, green leaves and fruits

Potatoes contain 75% water and 25% dry matter. Potatoes are best known for their carbohydrate content, being starch, the major form of carbohydrate. The protein content ranges from 0.7–4.6% and lipid content from 0.02–0.96%.

The composition of vegetables can vary significantly depending on the cultivar and origin. Dry matter comprises about 10–20% in most vegetables, of which 3–20% are carbohydrates, 1–5% proteins, 1% fibre, 0.1–0.3% lipids and close to 1% minerals.

Fruit composition is strongly affected by the variety and ripeness. In general terms the dry matter content varies between 10 and 20%, sugars, polysaccharides, and organic acids being the main constituents. Proteins and lipids are present in much lesser amounts. In nuts, however, the moisture content is below 10%, nitrogen compounds are about 20%, and lipids can be as high as 50%.

15.3 Protein composition and structure

Proteins are complex macromolecules. The linear sequence of amino acids in a protein is known as the primary structure. The primary structure provides valuable information (i.e. molecular weight, ratio hydrophobic/hydrophilic residues, etc.), but far insufficient insight to understand functionality of food products. Besides the primary sequence, conformational folding determines, in a very complex way, the secondary, tertiary, and quaternary structure of proteins, providing information about the actual extent of exposure of hydrophobic regions, apparent net charge, size, shape, etc. Figure 15.2 shows the primary, secondary, tertiary and quaternary structure of soybean glycinin.

Protein content and amino acid composition vary considerable between families, genera, species and cultivars. The parameters are also dependent on the growth stage of the plant, as well as on the part (tissue) under analysis. Most proteins described in this chapter occur in seeds/grains. The usage value of grains from the main crops (cereals, oilseeds and legumes) depends on their composition and on the organization of components (from molecular to macroscopic scale) in the storage organs. Seed proteins can be roughly classified into three groups:

1. Storage proteins.
2. Biological active proteins, such as lectins, enzymes and enzyme inhibitors.
3. Structural proteins, such as ribosomal, chromosomal and membrane proteins.

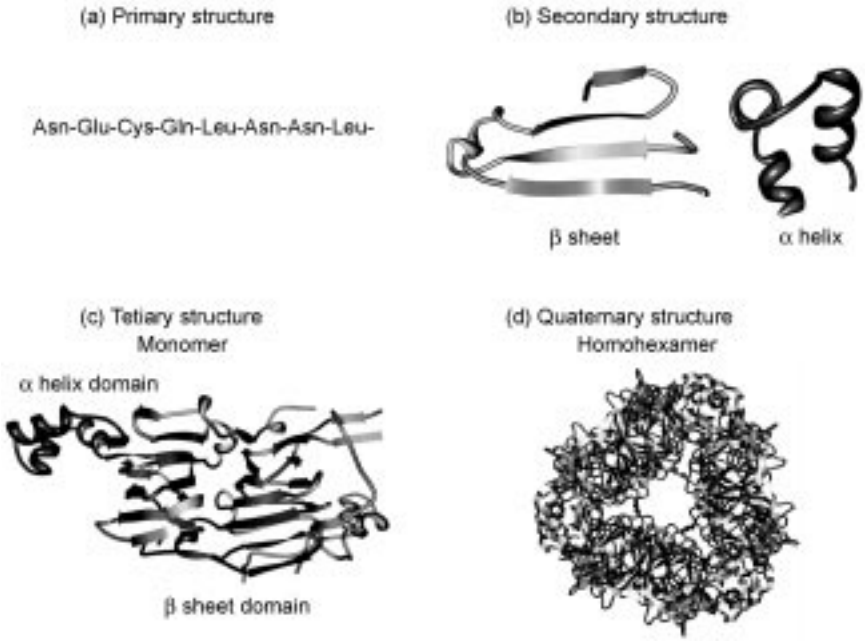

Fig. 15.2 Protein structure of glycinin: Homohexamer of the glycinin A3B4 subunit (PDB: 1OD5).

The storage proteins are the most abundant in seeds and are, therefore, the key components of grain quality. They comprise several protein types with particular structures and (technological/biological) functionalities. There is no clear definition for storage protein, and generally a protein present in an amount of 5% or more of the total protein fraction is considered a storage protein. Storage proteins have also been defined as a group that comprises proteins generated mainly during seed development and stored in the seed to serve as a nitrogen source during germination. Other properties that are generally attributed to storage proteins are the absence of enzymatic activity, the occurrence in a membrane surrounded vesicle (protein bodies or aleurone grain), and the predominance of a multimeric structure. Finally, a further feature of storage proteins is the polymorphism of their polypeptides, both within single genotypes and among genotypes of the same species. This polymorphism is due to the presence of multigene families and to the occurrence of post-translational proteolitic cleavage and glycosylation.

15.3.1 Classification of storage proteins

Seed proteins were empirically classified by Osborne (1924) based on the sequential extraction of crushed and defatted seeds by aqueous and non-aqueous solvents. According to this classification, albumins are soluble in water. Globulins are soluble in diluted salt solutions. Prolamins are alcohol-soluble and

glutelins are the most difficult to solubilize; they are extractable by weak alkaline, weak acidic and dilute detergent solutions. However, it should be emphasized that the classification according to this scheme depends on the conditions during meal preparation, seed pre-treatment, and on the way the fractionation is performed (e.g. time of extraction, liquid to seed ratio, pH, etc.).

Albumins and globulins are the main storage proteins of dicotyledonous plants (e.g., legumes, oilseeds), whereas prolamins and glutelins are major proteins in monocotyledonous plants (e.g., cereals). As expected of a nitrogen source, storage proteins are rich in asparagine (and aspartate), glutamine (and glutamate) and arginine.

Albumins

This group of proteins is characterized by a low molecular weight, sedimentation coefficients of approximately 2S (S stands for the Svedberg unit), high solubility in water and to be compact globular proteins. In addition to high nitrogen content, they are rich in cysteines. Albumins belong to a widely distributed family of seed proteins and their molecular weight ranges from about 10 to 18 kDa. They have been reported to account for 20 to 60% of the total proteins in seed of dicotyledonous plants. Albumins consist of two polypeptide chains (synthesized from a single precursor protein that is proteolytically cleaved) linked by disulfide bonds, except for lupin that lacks the interchain disulfide bonds and sunflower where the 2S protein remains uncleaved.

Globulins

Globulins have been extensively characterized, particularly in nutritionally important legumes and oilseeds. They represent the major storage protein of legumes and oilseeds. The sedimentation coefficients of globulins range from 7S to 12S. The major globulin fractions sediment at approximately 7S (vicilin-like globulins) and 10–12S (legumin-like globulins).

Vicilin-like proteins are regarded as the second major group of seed globulins. The molecular weights are in the 150–190 kDa range and are typically trimeric proteins consisting of subunits of various molecular weights. They lack cysteine residues and consequently cannot form disulfide bonds. Moreover, 7S globulins are generally glycosylated in contrast to 10–12S globulins (with the exception of 12S from lupin and amaranth). As it occurs for the other storage proteins, the vicilin-like proteins are usually assigned descriptive names based on the source as vicilin (pea), β-conglycinin (soybean) or acalin A (cottonseed).

The legumin-like globulins are the major fraction in legumes and oilseeds. Legumin-like globulins are generally designated by names according to their plant origin as glycinin (soybean), arachin (peanut) or helianthinin (sunflower). A high similarity has been found among the legumin-like globulins, with respect to molecular weight (300–370 kDa), size, shape, charge, solubility and other characteristics. They are hexameric proteins consisting of six acidic (α-)

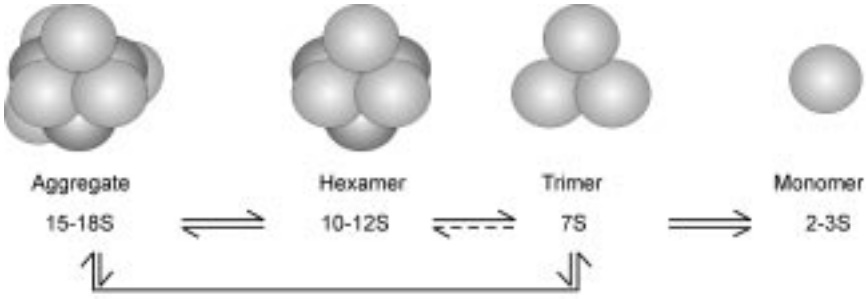

Fig. 15.3 Schematic model for the quaternary structure association–dissociation phenomena of legumin- and vicilin-like globulins.

polypeptide and six basic (β-) polypeptides, generally disulphide linked in pairs (α,β) to form monomers of about 60 kDa. The most accepted model for the quaternary structure of legumin-like globulins is an arrangement of six spherical subunits into a trigonal antiprism with a maximum dimension of 11 nm. This model was determined by electron microscopy and small angle X-ray scattering. In spite of the large diversity in polypeptides and amino acid sequences of the legumin-like globulins from different species, various seed proteins share regions that are conserved in sequence and/or structure. Similarities have been found in quaternary and secondary structure; however, substantial differences have been observed in the tertiary structure of seed globulins.

Although the 7S and 10–12S globulins show no obvious sequence similarities, they do have similar properties, including the ability to form both trimeric and hexameric structures. Another important characteristic shared by globulins is that their quaternary structures are modulated by both ionic strength and pH. They undergo reversible and irreversible association–dissociation phenomena, which have complicated their isolation and characterization (Fig. 15.3). For instance, the majority of 7S globulins from mono- and dicotyledonous seeds associate to form larger molecular weight species (9–12S) at low ionic strength ($I = 0.1$ M). The relatively easy association–dissociation indicates that the subunit interactions are non-covalent in nature (i.e., hydrogen bonds, electrostatic, hydrophobic and van der Waals).

Prolamins and glutelins

Prolamins and glutelins are both hydrophobic and specifically accumulate in small vacuoles or protein bodies. In addition to poor water solubility, they are characterized by high contents of proline and glutamine. Prolamins are the major storage protein in cereals except for rice and oats, where glutelins and globulins are the major proteins. Prolamins are assigned trivial names according to the species of origin, such as gliadin (wheat), hordein (barley), zein (maize), secalin (rye) or avenin (oat). The cereal prolamins are present as monomers or small aggregates, while glutelins form large disulphide-bonded aggregates.

15.3.2 Soybean proteins

Glycinin (11S globulin) and β-conglycinin (7S globulin) are the most important soybean proteins. The 2S fraction is found in a lesser amount and consists of Bowman–Birk and Kunitz trypsin inhibitors, cytochrome *c*, and α-conglycinin. As other legumin-like globulins, glycinin consists of one basic and one acidic polypeptide, which are linked by a single disulphide bond, except for the acidic polypeptide A4. The molecular masses of the basic polypeptides are around 20 kDa, while those of the acidic polypeptides are around 38 kDa. At ambient temperatures and pH 7.6 (I = 0.5 M), glycinin forms hexamers (11S) with molecular masses around 300–360 kDa, while at pH 3.8 glycinin is present as trimers (7S) with a molecular mass of about 180 kDa. Lowering the ionic strength at neutral pH from 0.5 to below 0.1 M induces dissociation of 11S glycinin into 7S glycinin.

β-Conglycinin is a trimeric glycoprotein (7S) consisting of three types of subunits, α' (57–72 kDa), α (57–68 kDa) and β (45–52 kDa) in different combinations. As for other 7S globulins, the subunits are associated via hydrophobic and hydrogen bonded interactions without the contribution of disulphide bonds. At pH 5 and higher, β-conglycinin is found as a trimer (I = 0.5 M), whereas it predominantly exists as a hexamer at ionic strength lower than 0.1 M. At pH values 2–5, β-conglycinin reversibly dissociates into a 2–3S and 5–6S fraction at ionic strength lower than 0.1 M.

15.3.3 Pea proteins

Dry pea seeds contain about 25% proteins, of which 70–80% are globulins: legumin, vicilin and convicilin. Legumin comprises six subunits with a molar mass of about 60 kDa. Each subunit is proteolitically cleaved into disulfide-linked basic and acidic polypeptides. These polypeptides display charge and size heterogeneity, reflecting the production of legumin from a small gene family. Four/five acidic and five/six basic polypeptides have been identified. The sizes of these polypeptides range from 38 to 40 kDa for the acidic ones and from 19 to 22 kDa for the basic polypeptides.

Vicilin, the 7S pea protein, is generally found as a trimer, each subunit having a molar mass of about 50 kDa. Vicilin can be cleaved at one or two sites (called the α-β and β-γ processing sites) as specified by the coding sequence of the vicilin genes. Cleavage at the α-β site generates fragments of 19 and 30 kDa, whereas that at the β-γ site generates fragments of 12.5 or 16 and 33 kDa. Cleavage at both sites generates fragments of 12.5, 13.5 and 16 or 19 kDa. The small fragments of vicilin contribute to the extensive polypeptide heterogeneity of vicilin. Other factors are the large number of vicilin genes in the pea genome, differential glycosylation, and surface charge heterogeneity (around the potential site of cleavage).

Pea convicilin is a 7–8S trimeric protein containing polypeptides with molecular mass of about 70 kDa. Convicilin has an extensive homology with vicilin along the core of its protein, but holds an additional highly charged,

hydrophilic sequence of 120–166 residues close to the polypeptide N-terminus. Convicilin also contains one cysteine residue contrary to the majority of 7S globulins.

15.3.4 Wheat proteins

About 80% of wheat proteins is gluten. Albumins and globulins are minor wheat proteins (20%). Gluten largely consists of two groups of proteins, the sulphur-rich glutenins, able to form a polymeric network by chemical crosslinking and the sulphur-poor gliadins, largely present as monomers that interact with the glutenin in the network. Glutenins affect the elastic properties of dough while gliadins affect the viscous properties of dough. The glutenin fraction consists of high molecular weight subunits, with molecular masses from 80 to 145 kDa, and low molecular weight subunits, having molecular masses from 31 to 48 kDa. There are similarities between both subunits in terms of solubility, sequence and conformation.

15.3.5 Potato and green leaf proteins

Soluble potato tuber proteins have been classified into three groups: (i) patatin, the major protein, (ii) protease inhibitors, and (iii) a third group containing all other proteins. Patatin is a 45 kDa glycoprotein that represents 40–60% of all buffer-extractable tuber proteins. It is considered to be a storage protein because of its high accumulation in the tuber.

Rubisco (Ribulose-1,5-bisphosphate carboxylase/oxygenase) is the most abundant protein in leaves. It accounts up to 30–70% of soluble leaf proteins. It is an enzyme from the Calvin–Benson–Bassham cycle that catalyses the first major step of the photosynthetic CO_2 assimilation. Rubisco is a 550 kDa globular protein with a general structure L_8S_8 consisting of two types of protein subunit, the large chain (L, ~55 kDa) and the small chain (S, ~12.5 kDa).

15.4 Manufacture

In order to design a process to prepare a protein isolate, some considerations should be taken into account. It is important to know the amount of protein required, the degree of final purity, whether loss of activity is acceptable, and the time and cost of the purification. Other factors to consider are the source material, i.e. species, seasonal variation and development stage of the crop, structure of the raw material (seeds, leaves, hulls, etc.), and previous processing.

The first step in protein isolation requires disruption of the tissues in which the proteins are compartmentalized. The next step is the preparation of an extract containing the protein in a soluble form. The efficiency of the method depends largely on the differential solubility of the various components (protein, carbohydrates, pigments, fibres, etc.) as a function of temperature, pH and ionic strength. Extremes pH values may cause protein denaturation, functional and

nutritional adverse reactions. For instance, extraction at extreme basic pH values may cause racemization of amino acids, reduced protein digestibility, and losses of essential amino acids such as cysteine and lysine. For most plant sources, pH values between 4 and 9 should not be harmful for proteins. Proteins from oilseeds and legumes are generally soluble in aqueous media, whereas cereal proteins are much more insoluble. For the latter, the use of surfactants or organic solvents such as ethanol is usually required. Studies of protein solubility usually precede development of large-scale processes, though published information on protein solubility is not always applicable since it refers to high solvent/protein ratios. In large-scale processing the volumes of solvent have a considerable effect on equipment costing and handling.

Once the protein has been extracted, clarification is generally used to separate the protein from the unwanted material. Centrifugation, filtration and membrane processes are the most common techniques for clarification. Nowadays, tangential or cross-flow microfiltration is receiving increased attention, principally for large-scale applications. After the clarification step, the protein is in solution, but its concentration can be very dilute. The next step is to concentrate the clarified protein solution quickly, while keeping it in a stable state, generally by ultrafiltration or precipitation. Protein products concentrated by ultrafiltration are generally of better functional quality than those obtained by precipitation. Precipitation is carried out by isoelectric precipitation, organic solvents, addition of salts (salting-out method), reduction of ionic strength (salting-in method), exclusion polymers or heating.

Isoelectric precipitation is the precipitation method most commonly used. The greatest disadvantage of isoelectric precipitation is that it can lead to extensive protein aggregation and modification of protein solubility, mostly caused by non-covalent interactions. The latter is particularly problematic in large-scale operations since the use of concentrated acids or alkalis, to reduce handling volumes, may result in denaturation by local, transitory extreme pH values. For this reason isoelectric point precipitation is sometimes used to precipitate contaminant proteins, rather than the target protein.

Organic solvents may cause protein precipitation. This method is commonly used by the blood fractionation industry (Cohn process). For instance, for vegetable protein isolation, the use of water/alcohol mixtures is a common concentration step, as soluble carbohydrates, pigments, flavours and saponins are removed. A drawback of the treatment with organic solvents is that it often leads to protein denaturation, so such solvents are generally avoided for recovery of intact protein.

Salting-out methods for protein recovery are scarcely employed commercially because by-product recovery costs are too high. Isolates from salting-in concentration have comparable yield to those produced from isoelectric precipitation, with the advantage that protein denaturation is avoided. Extracted proteins can be precipitated as well by specific cations, e.g., calcium in tofu manufacture, although it renders poor functional isolates because of the difficult resolubilization of the gel formed.

Table 15.2 Approximate composition (%) of the soybean defatted meal, concentrate and isolate

	Defatted meal	Concentrate	Isolate
Protein	55	70	>90
Lipids	1<	1<	1<
Carbohydrates	32	20	3
Fibre	3	4	1<

The exclusion of proteins from high polymer media consists in the addition of neutral polymers (dextroses, PEG, etc.) to protein dispersions resulting in separation of a protein-rich phase. For instance, the addition of PEG to a cold protein solution while stirring forces the protein out of the solution.

Heat coagulation can also be used as a concentration step; however, the protein denatures and subsequent functional use is limited. Thus, heat coagulation is employed when nutrition and digestibility are the only required properties. Heat coagulation can also be used for protein fractionation profiting the differential denaturation temperatures of the various proteins present in the raw material, although this technique is not currently used commercially for protein isolation.

Following these steps, a protein preparation of a various purification degrees (meals, concentrates and isolates; e.g., Table 15.2 for soybean) is achieved. Further purification by chromatographic techniques will render a high purity protein preparation and is mostly used for high value proteins required in relatively small quantities, thus, it is mainly used by the biopharmaceutical industry. Finally, the isolate is dried. This is a critical step in which temperature must be controlled to circumvent protein denaturation. Most vicilin-like globulins denature at temperatures around 65 ºC. Higher temperatures are generally acceptable for legumin-like globulins and cereal proteins. The most commonly used drying methods are drum drying and spray drying (usually in-line with a fluid bed dryer). Freeze drying is only of interest for some special products. In drum drying the protein solution is applied in a thin coating to a hot surface, causing a large amount of water to evaporate within a few seconds. In spray drying the protein solution is atomized and dried with hot air. Spray drying is the most common industrial process to produce thermally undamaged protein preparations, although some irreversible aggregation may occur.

15.4.1 Seed protein extraction

Proteins are not the major component of seeds and they usually appear linked to other components such as cell wall material or starch granules. Many industrial processes exist for the extraction and concentration of proteins from seeds due to their diverse chemical nature and structure. However, certain steps are of common use unless they are demonstrated to be irrelevant for a particular seed source. Figure 15.4 displays an overview of the main steps for seed protein

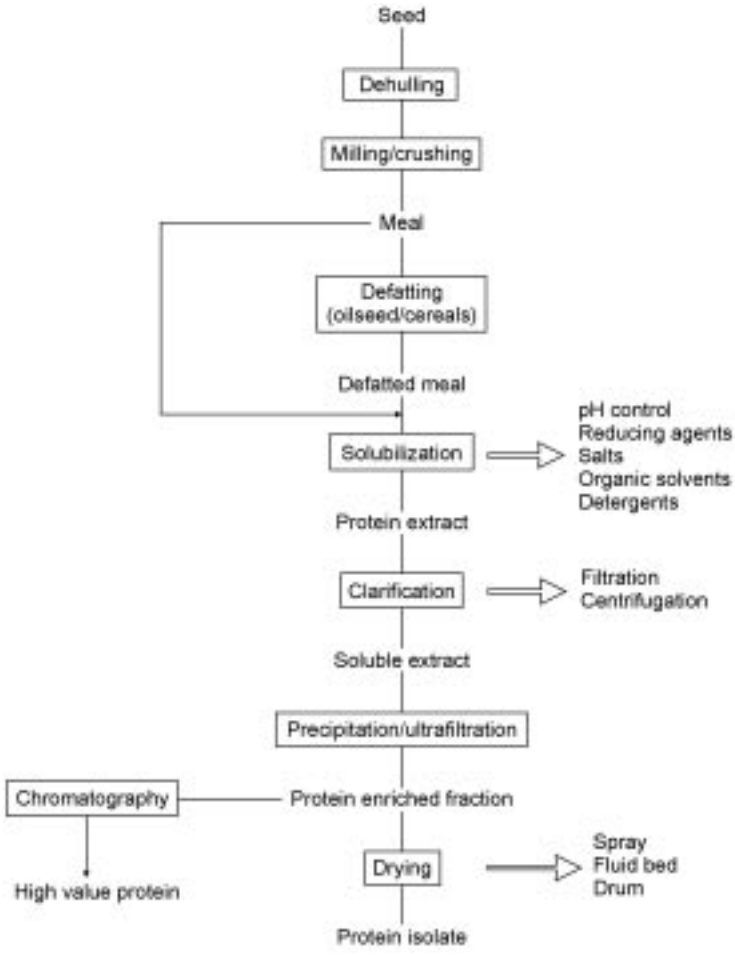

Fig. 15.4 Main processing operations to prepare a vegetable protein isolate.

isolation. Seed coats and hulls are generally removed before extraction as they often contain undesirable compounds such as pigments, phenolic compounds or fibres. For many oilseeds such as soybean, cottonseed and sunflower, hulls can be easily removed, but it is more difficult for smaller seeds, such as rapeseed since the hulls tend to adhere to the endosperm. Seeds are also usually milled prior to liquid extraction. This is an important step as a coarse fragmentation of the seed may impair protein extractability. The regular granulometry of the crushed or milled seeds influences the reproducibility of the protein extraction. The water content of the seed also influences the granulometry and all material should be kept at constant moisture before grinding.

Protein enriched fractions are often obtained by direct air-classification of milled seed flours, mainly for cereals, legumes and defatted meals from oilseeds, as a high lipid content may result in agglomeration. Air-classification is a dry

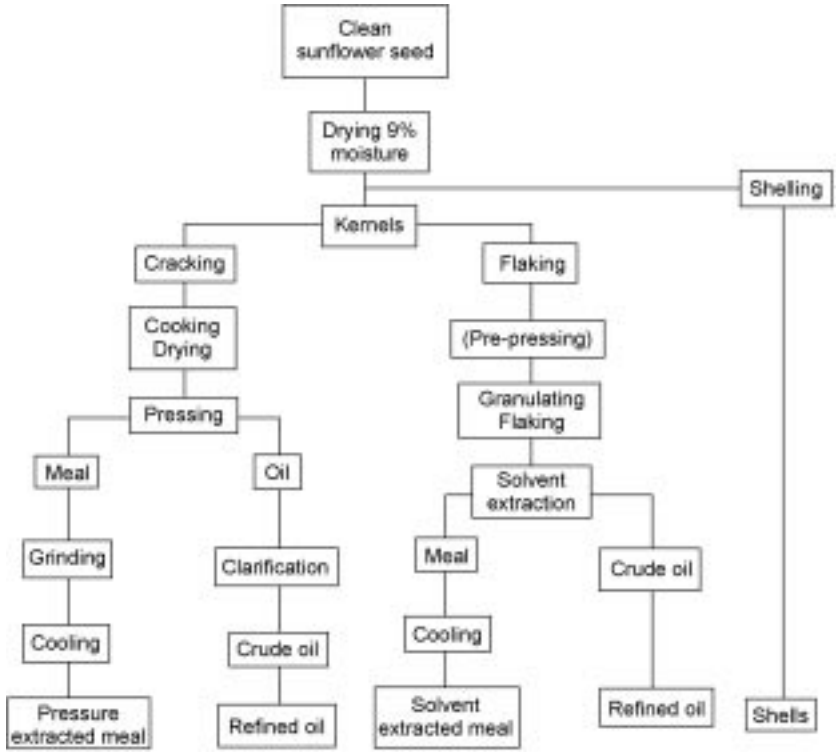

Fig. 15.5 Sunflower oil and defatted meal manufacture scheme.

processing method that blows away the lighter particles (as starch granules), thus removing them from the protein. This technique may render high native protein content flours. Air-classification is commonly used to produce pea protein concentrates (~50% protein content on dry basis) from pea flour. Pea protein isolates (~85% protein content on dry basis), instead, undergo a wet processing in which the extracted proteins are subsequently isolated by selective precipitation at the isoelectric point.

The bulk of oilseeds are still primarily processed for oil. One of the by-products of the oil extraction process is the oilseed meal which has high protein content (35–60%). During oil production protein denaturation occurs due to mechanical pressing and solvent extraction at elevated temperatures, resulting in an insoluble and poorly functional protein fraction. Sunflower seed processing (Fig. 15.5) is a representative example for oilseeds. Two main methods are used. These are the full press method (screw press or expeller method) and the pre-press solvent extraction. Prior to pressing, the seeds are usually partially (70%) dehulled, ground, rolled and heated. Heating facilitates the disruption of tissues, coagulates the protein, inactivates enzymes (such as phospholipases and lipases), increases the fluidity of the oil, eliminates moulds and bacteria, and dries the seeds to a suitable moisture content.

The pre-press solvent extraction is the most common method for sunflower oil extraction. In this method, the seeds are screw-pressed to obtain oil and a cake with an oil content of about 16% (w/w). The cake obtained is subsequently granulated or flaked and the oil extracted with a solvent, usually hexane. In addition to the main methods, the oil can also be obtained by direct solvent extraction. In this method, the kernels are conditioned and flaked, and oil is extracted directly by solvent. This last method is the process commonly used for soybeans. Finally, the solvent is removed from the meal in a desolventizer-toaster (DT) or in a flash desolventizer system (FDS). The heat treatment in a DT is quite intense and results in darker meals, due to Maillard compounds (reaction of sugars with lysine and other amino acids), with a low solubility. Heat treatment in FDS systems is milder and easier to control obtaining whiter meals with higher solubility. The latter meals are preferred for food applications.

Defatting of cereal flours prior to extraction is sometimes advised in order to avoid the formation of lipid–protein complexes, although this may result in some protein denaturation and the loss of some protein components (e.g., lipothionines). Due to the intrinsic poor solubility of cereal proteins, extraction is carried out with aqueous solutions containing alcohols (mainly ethanol and propanol), surfactants, reducing agents (mercaptoethanol, dithiothreitol), weak acids (generally acetic and lactic acid) and urea.

15.4.2 Potato and green leaves processing

Potato tuber proteins are obtained in the potato juice, a by-product of starch manufacture. The process starts washing the potatoes prior to grinding (rasping machine) in the presence of sodium bisulphite to avoid oxidation of phenolic compounds. The rasped potatoes are then passed through rotating sieves that retain the potato pulp (potato fibres). The starch slurry is further processed in continuous centrifugal separators or hydrocyclones and sieves. The resulting potato juice contains approximately 1.5% (w/v) soluble protein.

The basic steps for the production of LPC are plant leave grinding and juice separation by pressing. The soluble protein in the juice is coagulated, usually by heating, and then dried.

15.5 Technical data: functional properties

The first functional protein preparations for food application were dehydrated natural products such as milk or eggs. The first protein replacer in Europe – a milk powder hydrolysate, marketed as egg-white substitute – was produced during the Second World War. Nowadays, protein preparations for food use are extracted from plants, animals and micro-organisms (single cell protein). However, the successful use of proteins for food applications depends on their functional properties rather than on their nutritional quality. Functional properties refer to the overall behaviour of proteins in food and reflect the

various interactions in which proteins take part. Functional properties are related to the physical, chemical and conformational properties of proteins in food systems during processing, storage, cooking and consumption. The primary characteristics that determine the functional properties of proteins in food systems include molecular weight, size, shape, flexibility, amino acid composition, structure, net charge, charge distribution and hydrophobicity. Functionality may vary with the source of protein, its composition, the method of preparation, its thermal history and the prevailing environment (i.e., pH, ionic strength, temperature, presence of salts, etc.).

There is a strong correlation between structure and functional properties of proteins in food systems. The knowledge about this correlation is essential for the optimal use of proteins in food systems. However, establishing the structure–functionality relationship for food proteins is not trivial because of the complexity of vegetable food products. Therefore, of utmost importance is a basic understanding of protein structure, solubility and stability. Protein structure has been described in Section 15.3.

15.5.1 Protein solubility and stability

Solubility of a pure substance is the amount of substance in solution in equilibrium with its crystalline form. For industrial applications, a practical definition of solubility is the amount of protein that goes into solution or colloidal dispersion under specific conditions (pH, ionic strength, etc.) and is not sedimented by moderate centrifugal forces. Proteins vary enormously in their solubility: some vegetable globular proteins (i.e., albumins and globulins) are very soluble while proteins involved in building structural elements in organisms (i.e., membrane proteins) are essentially insoluble. Vegetable protein preparations often contain phenolic compounds that tend to decrease solubility. In general, the more polar its surface is, the more soluble a protein is likely to be, since interactions with solvent molecules principally involve amino acid residues at the protein surface.

Solubility is considered a key functional property since good solubility is a prerequisite for the success of protein preparations in most functional applications. These properties will determine the field of application as well as whether a new protein ingredient will be competitive on the market. If such properties are lacking, the preparations will almost only be used as animal feed and food fortification.

The net stability of the folded (native) state of a protein depends upon a complex balance between the many diverse interactions present in the folded state, the higher conformational disorder of the unfolded state and the interactions with the solvent. The folded state is easily disrupted by environmental conditions often occurring during food processing (e.g., extreme pH values, pressure and temperature, organic solvents, etc.). Denatured proteins are unfolded but do not undergo changes in their covalent structure with the possible exception of breaking and reshuffling of disulphide bonds. Unfolding of proteins

has been reported to improve functionality of vegetable proteins such as those from sunflower, potato and soybean proteins. However, the positive effects of protein unfolding are usually counteracted by protein losses due to precipitation.

15.6 Functional properties for industrial applications

Proteins are expected to contribute to the product consistency and juiciness due to their gelling, texturizing, dough forming, water- and fat-holding capacities. Other desired properties are the formation and stability of foams and emulsions.

15.6.1 Foams and emulsions

Foams and emulsions are colloidal systems in which one phase (air for foams and oil for oil/water emulsions) is dispersed in another phase. For the formation and stabilization of foams and emulsions, the adsorption of proteins and subsequent lowering of the surface tension, onto air/water and oil/water interfaces, respectively, is essential. Molecular properties such as conformational stability/flexibility, surface hydrophobicity, and effective molar mass govern the ability of proteins to lower the interfacial tension during foam and emulsion formation, hence, facilitating the formation of small particles. Although foams and emulsions are both dispersed systems and the processes that occur in the formation and stabilization are similar, gas bubbles are larger and more susceptible to disturbing influences (i.e. temperature gradients, dust, evaporation, etc.) than emulsion droplets.

Emulsions

Proteins are suitable to make oil/water emulsions, but not water/oil emulsions, as described by Bancroft's rule: the phase of an emulsion in which the surfactant (protein) is more soluble becomes the continuous phase upon agitation of the mixture. To make an emulsion, it is necessary to apply a substantial mechanical energy. In most situations the drops are formed by intense agitation, homogenization, rapid blending, stirring or cutting. The smaller the droplet, the more stable the emulsion is. The most important factor during emulsion formation is the effective molar mass of the protein: the larger it is, the higher the amount of protein required to obtain small droplets. However, at high protein concentrations, there are no significant differences among proteins regarding their ability to form small droplets. Once formed, the emulsion is exposed to various physical destabilizing mechanisms, such as creaming, aggregation (flocculation) and coalescence (Fig. 15.6).

Foams

The most common methods to make foams are: (i) via supersaturation under pressure, followed by pressure release, (ii) by gas injection into a liquid; and (iii) by beating in air. The latter is the most commom method used by the food

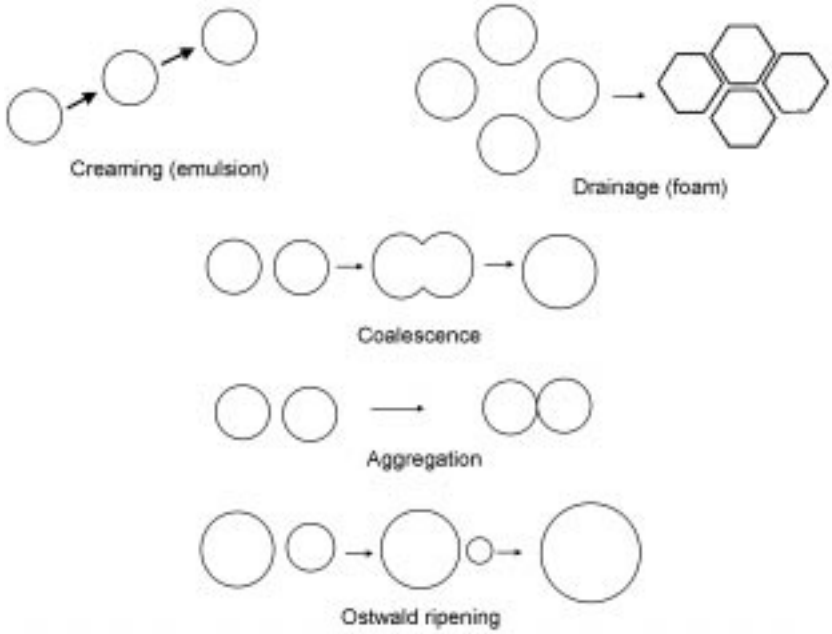

Fig. 15.6 Instability mechanisms of foams and emulsions.

industry. Faster beating often results in higher foam volumes and smaller bubbles.

Proteins are suitable surfactants to make foams, although there are large differences in the foaming ability among proteins, in particular for foams made by beating. This is mainly because at short time scales some proteins (e.g., proteins with high conformational stability) can hardly adsorb to the air/water interface. The main functions of proteins in foams are to decrease interfacial tension, to increase viscous and elastic properties of the liquid and to form strong films. Physical destabilizing mechanisms of foams concern changes in position or size of the bubbles such as drainage, coalescence and Ostwald ripening (Fig. 15.6). Ostwald ripening, the growing of large bubbles at the expense of smaller ones, is the most important type of instability in foams.

15.6.2 Gels and related aspects

Proteins are generally not suitable as thickening agents, due to the high concentration required to achieve an adequate viscosity. However, they can be used to make gels. Protein gelation is used in numerous industrial applications. Gelatine is certainly the most widely used gelling agent; however, as mentioned above, there is an increasing demand for vegetable-based products.

A gel consists of a matrix (three-dimensional network of connected macromolecules or particles) and a continuous liquid phase, mechanically retained by the matrix. A gel is a material with predominant solid-like characteristics but it

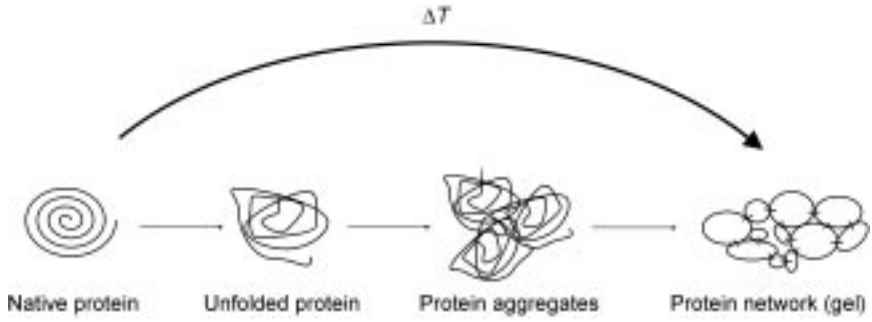

Fig. 15.7 Heat-induced gel formation.

deforms under stress. Gel formation is a complex process that often involves several steps such as denaturation, aggregation and network formation (Fig. 15.7). Protein denaturation preceding gel formation is generally accomplished by heating. Heat-induced gelation is one of the most commonly studied phenomena in food science and responsible for the structure of many food products. Gelation can also be induced by high hydrostatic pressure, cold gelation (salt-induced and acid-induced), and enzyme-induced gelation. During denaturation the protein adopts an unfolded conformation in which functional groups become exposed. Consequently, these exposed groups can interact with each other to form aggregates that may precipitate at low protein concentration and may gel at high protein concentration.

For globular proteins two main types of gel networks can be distinguished according to microscopic observations: fine-stranded and coarse networks. In fine-stranded gels the proteins are attached to each other like a string of beads. This type of gel is generally transparent. Coarse gels are non-transparent and are formed by random aggregation of proteins into clusters with diameters in the range of 100–1,000 times a single protein molecule. The type of gel that is formed depends on conditions during gel formation, generally gels are coarser as the pH value approaches the isoelectric point and when the ionic strength is increased. Network structure affects gel properties, including permeability, the ability to retain water and rheological properties.

15.6.3 Texturization of proteins

Texturization of proteins can be understood as the creation of a three-dimensional structure to substitute partially or totally other proteins (meat analogues) or lipids (fat substitutes) in traditional foodstuffs. Typical structures are fibres, shreds, chunks, bits, slices, films and granules. Protein texturization generally involves a first denaturation step followed by the re-organization and orientation of the partially or totally unfolded proteins by extrusion, rolling/spreading or surface precipitation. The final step is the binding and stiffening of the organized protein structure. Many texturizing methods are available for the food industry such as extrusion cooking, spinning (dry, wet and spinneretless),

freeze texturization, high pressure texturization, and chemical or enzymatic texturization. Because of the numerous processes and products obtained, this point will not be described further.

15.6.4 Water- and fat-holding capacity

The interaction of water with proteins is very important both to the structure of the proteins and to their behaviour in food systems. Water-holding capacity is generally defined as the ability of a protein matrix to absorb and retain bound, hydrodynamic, capillary and physically entrapped water against gravity. Chief factors affecting water binding by food proteins are amino acid composition, protein conformation, surface hydrophobicity, ionic concentration and pH. For instance, proteins that contain large amounts of the charged amino acids tend to bind large amounts of water.

The capacity of a protein to bind lipids is an important characteristic to enhance flavour retention and to improve mouthfeel for such applications as meat substitutes and extenders. Protein isolates can be added to ground meats to promote fat binding and decrease cooking losses since fat separation is a common problem in processed meat products.

15.6.5 Dough formation

Dough is an extensible, viscoelastic protein network formed upon the mixing of water and cereal proteins. Mixing of dough is considered a critical step for bread quality. During wheat dough mixing, gliadins and glutenins unfold exposing hydrophobic groups which cause the proteins to aggregate and to crosslink due to the formation of disulphide bonds. The latter results in a viscoelastic network structure surrounding the starch granules and able to expand when gas is formed by the metabolism of yeast or by the water vapour expansion during heating.

15.7 Chemical and enzymatic modification of protein products

Proteins can be modified to alter specifically their physico-chemical and functional properties. Protein modifications can be defined as irreversible changes in the protein structure by physical, genetic (i.e., GMO), chemical and enzymatic treatments. In this chapter, the last two methods are described briefly.

15.7.1 Enzymatic modification

A broad range of post-translational modifications occur naturally in the cell. Most of them are catalyzed by enzymes. However, only two enzymatic modifications are of general commercial interest: crosslinking of proteins using transglutaminase and proteolysis.

Transglutaminase catalyzes the formation of a covalent bond between gamma-carboxylamide groups of peptide- or protein-bound glutamine residues and primary amino groups, resulting in inter- and intramolecular crosslinks. At functional level this reaction improves viscosity, texture, water-holding capacity and stability (regarding temperature, syneresis and emulsification). However, it may decrease emulsion and foam formation due to the lower protein flexibility. Furthermore, it may hamper gel formation since the gelation temperature is higher. The main current application is in the production of reformed meat and improved sausage texture. Nowadays, many patents have developed regarding transglutaminase crosslinking in food products such as bakery products, noodles and pasta, from soybean and other vegetable proteins.

Proteolysis is conducted in many food industries with various aims such as food flavouring, functionality or pharmaceutical applications. Proteolysis results in decreased molecular weight, increased charge (more terminal residues), and more random structures, including exposed hydrophobic residues. From a functional point of view, there is an obvious improvement of solubility and a higher ability to form foams and emulsions, although generally with decreased stability. Regarding gelation, proteolysis usually results in poor gelling properties. Nutritional properties of hydrolysates reflect their increased digestibility and decreased allergenicity compared to the parental proteins. Besides nutritional aspects, many peptides may exhibit specific physiological properties, such as immunomodulatory effects or antimicrobial activity. Bioactive peptides can be released during protein hydrolysis. Several bioactive peptides have been found in protein hydrolysates, as opioid, anti-hypertensive, and anti-thrombotic peptides. For instance, the peptic hydrolysates of wheat gluten and rice prolamin have been shown to possess an opioid-like activity.

A wide range of commercial proteases are available, being the cheapest the food grade versions of detergent enzymes (e.g., alkaline bacterial proteases). Other sources include plant enzymes such as bromelain, animal enzymes such as chymotrypsin, and fungal (*Aspergillus*) proteases.

15.7.2 Chemical modifications

Chemical modifications are generally carried out under more extreme conditions (pH, temperature, solvent, etc.) and often lead to high heterogeneous products with poor specificity; therefore, legally there is a more restricted allowance for food applications. Furthermore, there are concerns regarding toxicity, loss of nutritional value and worsening of organoleptic properties. Two chemical modifications are commercially used: deamidation and hydrolysis. Hydrolysis is used for preparing hydrolysed vegetable protein mainly as flavour enhancer. Deamidation of carboxamide groups leads to a marked decrease of the isoelectric pH and increased negative charge. Levels of deamidation as low as 2–6% can enhance the functional properties of proteins. Deamidation of gluten proteins have been performed resulting in improved solubility, emulsifying and gelling properties.

Many other chemical modifications have been studied to enhance func-

tionality of vegetable proteins, but the low specificity and complex regulatory issues regarding food applications have limited their use. Most promising are glycosylation, succinylation, phosphorylation, alkylation and redox reactions. For instance, the addition of redox reagents to flour and bakery products has been a common application.

15.8 Vegetable proteins: choosing the best functionality for food application

The large variety of vegetable protein sources provides the food industry with a broad choice of proteins to obtain the desired textural, sensory and nutritional properties. Table 15.3 provides a general overview of the key functional properties of proteins required for various food applications and the most common vegetable protein sources used. However, it should be emphasized that the physico-chemical properties, and thus the functional performance of proteins in foods, vary extensively with the operating conditions and industrial process used for manufacturing the protein preparations. For instance, drum-dried pea isolates have higher fat-holding capacity than spray-dried pea isolates, but lower foaming, colour and flavour properties.

Soybean protein preparations are by far the most common vegetable source of proteins as functional food ingredient. Comparison of other vegetable protein sources with soybean is frequently found throughout the literature because the extensive research performed on soybean protein functionality is a useful base resource to take advantage of. However, the numerous publications on vegetable proteins provide a great variety of results, sometimes conflicting, regarding functional properties. Therefore, they do not often permit general statements on the suitability of specific vegetable proteins for specific applications. The variety of results is likely due to the diversity of tests described in the literature to assess protein functionality, as well as to the array of methods to obtain the protein preparations. The latter results in products that differ in:

- protein content and protein composition (e.g. ratio albumin/globulins)
- presence of non-protein compounds such as pectins, fibres, and phenolic compounds that influence the functionality of the system
- degree of protein denaturation
- the variety and cultivar used.

In conclusion, choosing the most suitable vegetable protein source for an application requires good knowledge of both the preparation and its processing history.

15.9 Applications of vegetable proteins in food products

Vegetable proteins have been used for a number of reasons, besides the obvious nutritional value in foods; to provide specific required properties and structural

Table 15.3 Required functional properties of vegetable proteins for food applications

Food application	Property required	Protein requirement/ mechanism	Main protein sources
Bakery	Viscosity, elasticity, gelation, water binding	Hydrophobicity, disulphide crosslinks, network formation, hydrogen bonding	Cereals
Low-fat bakery products	Fat and flavour binding	Hydrophobic bonding entrapment	Cereals
Desserts, dressings	Solubility, emulsifying/ foaming properties, fat mimetic	Hydrophilicity, molecular flexibility, interfacial adsorption	Soybean, peanut, lupin, other legumes and oilseeds
Dairy substitutes	Solubility, colour-free, tasteless, emulsifying properties, stability to heat	Hydrophilicity, molecular flexibility, interfacial adsorption	Soybean, peanut, lupin, pea, other legumes and oilseeds
Fortification	High nutritional value, solubility	Digestibility, hydrophilicity, absence of allergens	Amino acid balanced vegetable protein mixtures, hydrolysates, nutritional quality improved by fermentation and germination
Infant formula	High nutritional value, solubility, emulsifying properties, stability to heat	High digestibility, fully absence of allergens, hydrophilicity, interfacial adsorption	Soybean, vegetable protein hydrolysates
Meats and sausages substitutes	Texturization, solubility, emulsifying properties, water binding, fat mimetic, gelation	Hydrophilicity, network formation, water entrapment and immobilization, interfacial adsorption, disulphide crosslinks	Soybean, pea, lupin, wheat
Beverages, soups, gravies	Solubility, viscosity, acid stability	Hydrophilicity, protein solvation	Soybean, pea, vegetable protein hydrolysates, fermented cereals and legumes
Cheese-like products	Gelation, solubility, colour-free, tasteless	Hydrophilicity, protein solvation, network formation, water entrapment and immobilization	Soybean

basis to the products in which they are added. With regard to desirable properties provided to food systems, proteins are generally expected to ensure physical stability (avoiding or delaying sedimentation, oil separation, coarsening, etc.) and attractive usage properties such as desirable texture, consistency or viscosity. An example that illustrates the latter is the number of imitations of meat products with vegetable proteins that not only have eating characteristics,

such as texture, colour or flavour, similar to meat, but also an adequate nutritional profile.

The use of vegetable proteins for novel food applications requires an understanding of the complex nature of food systems. Most food systems are heterogeneous regarding:

- protein composition, as it concerns generally mixtures of various proteins,
- water, lipids, carbohydrates, and other minor components such as salts or pigments; some of them can markedly interact with proteins and affect the organoleptic and nutritional characteristics of the food product, and
- structural organization (emulsions, foams, films, etc.).

Moreover, proteins are not often completely native. They can be totally or partially denatured or may have undergone other modifications, such as partial proteolysis.

15.9.1 Food applications of seed protein preparations

Over recent years, vegetable-isolated proteins from seeds have become crucial market commodities for imparting functionality and texture as well as for enhancing nutritional value in a variety of food products. However, existing seed protein containing foods from non-animal protein sources are still considered to have inferior flavour and texture.

The largest market for protein preparation in human foods continues to be soybean (Table 15.4), although protein isolates from other grains, e.g. pea, wheat, lupin, sunflower, cottonseed or rapeseed, are currently being explored with great interest. The consumption patterns of soybean are not homogeneous around the world. In the Far East, soybeans have been consumed for thousand of years in various forms in traditional foods such as tofu, soymilk and fermented products. In the Western world, it is only during the last 40 years that soybean products have been more widely consumed; mostly in the form of refined soybean protein ingredients used by the food industry. However, an estimated 85% of the world's soybeans are converted into oil and defatted meal. The meal is mainly used in animal feed. A small portion is further processed into food ingredients including soybean flour, concentrates, isolates, hydrolysates and textured proteins.

A key limitation of soybean has been the characteristic beany and greeny flavour in soybean products associated with the presence of lipoxygenases. This flavour is particularly problematic to western consumers, who are not familiar with it. Lipoxygenases oxidizes carotenoids and chlorophyll in flour to their colourless form. It can also oxidize fatty acids with the subsequent development of rancidity. A further constraint is the presence of several anti-nutritional factors. However, the latter problems are mostly overcome by applying the proper treatment (e.g., heating).

Nowadays, a broad choice of soybean protein preparation is available for the food market: roasted soybean nuts, full-fat soybean flour and grits (three types:

Table 15.4 Food applications of the main soybean protein preparations

	Bakery	Dairy products/desserts	Meat substitutes	Other products
Flours and grits	Bread, cakes, biscuits, pancakes, pasta, doughnuts	Milk substitutes	Bolognese sauce, meat balls and hamburgers, patties	Snacks, candies, dietary products
Concentrates	Bread, cakes, biscuits, pancakes, pasta, doughnuts	Milk substitutes, cheese, coffee whiteners, frozen desserts, infant formulas	Sausages, Bolognese sauce, meat balls and hamburgers, seafood, patties	Snacks, candies, dietary products, soups, gravies and beverages
Isolates	Bread, cakes, biscuits, pancakes, pasta	Milk substitutes, infant formulas, cheese, coffee whiteners, frozen desserts	Sausages, Bolognese sauce, meat balls and hamburgers, seafood, patties	Snacks, candies, dietary products, soups, gravies and beverages
Texturized protein	Not generally used	Not generally used	Bolognese sauce, meat balls and hamburgers, seafood (e.g. surimi), patties, muscle-like products, dried meat bits, stews	Snacks, soups and gravies

enzyme-active, heat-treated, extruder-processed), enzyme-inactive low-fat soybean flour or grits, enzyme-active defatted flake/soybean flour, lecithinated soybean flour, textured soybean flour, refatted soybean flour, soybean concentrates, textured soybean concentrates, soybean isolates, and organic soybean flour and concentrates. These soybean protein preparations are applied as ingredients in numerous food products as functional and nutritional substitutes for meat, milk and egg protein. There are many textured soybean products, mainly used as meat substitutes, where the chief advantages are reduced lipid content and increased protein levels. Available textured products are marketed in several colours, allowing a variety of applications in chicken, beef, pork or mutton-style dishes. Normally, the meat substitute is dried after extrusion, resulting in a very shelf-stable product. The most important characteristics of the meat substitute are their meat-like appearance, texture and cooking properties. They can be prepared by steaming, barbecuing, frying or stewing, as an original meat product.

Other soybean markets include bread and bakery to enhance the nutritional value of the cereal protein by supplying lysine to the formulation. Soybean proteins also enhance the shelf-life of bakery products thanks to their ability to retain water. Other food applications include dietetic and health foods, beverages, dairy analogues (soymilk, soywhey, tofu, whipped toppings, and ice-creams), breakfast and infant formulae. Figure 15.8 depicts the standard process to prepare soymilk, tofu, soybean whey, and okara (soy pulp).

Peas have low protein content compared to soybeans; pea meal consists mainly of starch and generally is not defatted prior to protein extraction. Therefore, lipid solvent extraction and the subsequent heat treatment to remove the solvent may be avoided. Furthermore, the analogy between globular proteins in pea and soybean means that the extensive functional and technological knowledge already existing for soybean protein products can be exploited. Textured pea protein is finding wide application in vegetarian foods such as burgers and sausages. In recent years, ingredients from pea protein preparations with brand names, such as Arrum (made from yellow peas and wheat gluten in a 1:1 ratio), have been used to formulate new products. Arrum resembles chunks of meat and is suitable for burgers, pies and lasagne. More common uses are in pea snacks, such as fried peas coated with wheat and/or rice flour, or extruded fried green pea flour products flavoured with seasonings.

There is increasing technological and research interest into the development of protocols for preparing legume ingredients and food items accepted by consumers for their sensory and nutritional characteristics. For instance, various protein lupin products are already available in the market for the food industry: toasted and non-toasted flour, grits, granulates, and protein concentrates from non-defatted seed. These lupin protein products have already been developed in different sectors like bakery goods (muffins, biscuits), emulsified foods (drinks, imitation cream, dressings), and extruded foods (pasta, snacks).

Cereals are processed by the food industry to prepare a broad choice of products:

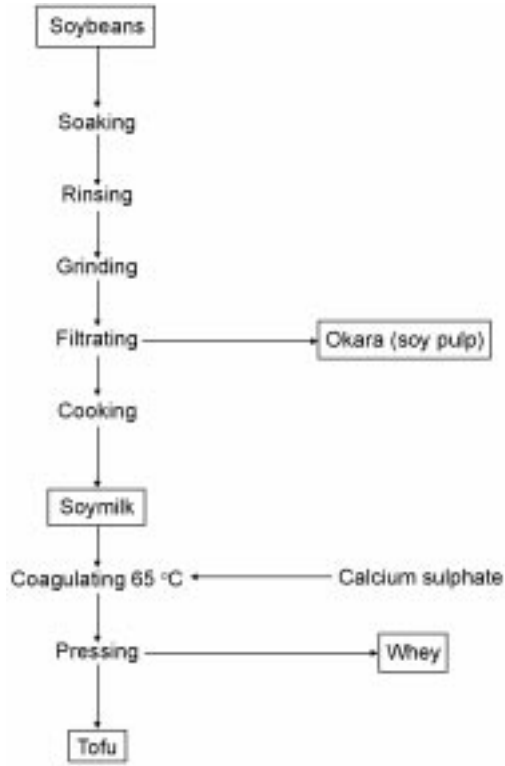

Fig. 15.8 Production of soymilk, soywhey, tofu and okara.

- baked products, from flour or meal, as pastries, pancakes, biscuits, and cakes
- milled grain products, made by removing the bran and usually the germ such as rice, enriched flour, wheat flour, bread flour, durum flour, couscous, grits, hominy, cornmeal, pearled barley, pot barley, semolina, prepared breakfast cereals, soup, gravy, and other thickenings
- on the drinks side, cereals are used for brewing and distilling alcoholic drinks thanks to their high carbohydrate content
- whole-grain products include rolled oats, brown rice, popcorn, shredded and puffed grains, and breakfast foods.

Bread making is the largest market for cereals, employing about 64% of the wheat world production. A relatively recent application of wheat is its use as meat substitute marketed as 'Wheatpro' in the early 1990s. Gluten is processed and extruded to resemble the texture of meat.

Oilseeds can be carefully processed or refined for food use. Some progress has been made for sunflower, peanuts, rapeseed, cottonseed and sesame. However, oilseed protein-based products are still marginally developed. There are some applications to fortify foods (as infant formulae, bakery or pasta products) or milk substitutes (e.g., peanut milk). The use of other oilseed

proteins, such as sunflower or rapeseed, as dairy product analogues has been investigated. Fortified dairy products are prepared by blending pasteurized whole milk, dried skim milk, and oilseed meal or protein isolates. However, the successful production of oilseed protein-based dairy products requires protein isolates with significantly improved flavour, colour and functional properties. The need for such high quality protein products stems from the mild flavour and soft colour of most dairy products.

15.9.2 Food applications of potato and green leaves

Food applications of potato proteins are still constrained by the lack on an efficient non-denaturing large-scale method for their recovery. Furthermore, the presence of protease inhibitors is undesirable in foods. Research efforts are aimed at inactivation procedures that avoid extensive protein insolubilization. Potato proteins can be used for fortifying bakery products. In normal bread dough the possibility of addition of potato protein is limited, while the protein content in crispbread can be doubled without essential changes in its typical characteristics, such as crumb structure, specific volume and hardness.

LPC has been examined as a human food source, because it is potentially the cheapest, most abundant source of available protein. However, so far, due to the extensive denaturation during its preparation, LPC has been used as a nutritionally rich ingredient rather than as a functional ingredient.

15.10 Nutritional and health effects

Meat and fish products are the most readily available sources of complete protein. The protein content, by weight, of cooked animal products ranges from 15 to 40%. The protein content of cooked cereals and legumes is about 3–10%. Potatoes, fruits and leafy green vegetables come in at 3% or lower. An important aspect that greatly influences protein bioavailability and digestibility is the processing history of the protein products, which also has a direct effect on the functional performance of the proteins.

Vegetable protein sources often contain natural occurring anti-nutritional factors that hamper the nutritional potential by interfering with the intake, bioavailability or metabolism of nutrients. Table 15.5 shows the most common anti-nutritional factors that can be found in vegetable protein sources.

The presence of high levels of protease inhibitors from legumes can cause considerable reductions in protein digestibility. Similarly, the presence of high levels of tannins in cereals and legumes can result in significantly reduced protein bioavailability. To improve the nutritional value of the vegetable proteins, several strategies have been developed. Most often used are seed dehulling, soaking, thermal treatments, irradiation and protein fractionation. Other approaches are based on breeding, hydrolysis, germination and fermentation.

Anti-nutritional factors can also be formed during food processing by heating/alkali treatments, organic solvents, oxidizing agents or acids. For

Table 15.5 Antinutritional factors of vegetable protein sources

Antinutritional factor	Health effect	Main vegetable source
Protease inhibitors	Digestion impairment	Legumes, potato, peanut, soybean, tomatoes
Gossypol	Heart and liver toxicity, weight loss, and depressed appetite	Cottonseed
Hemagglutinins and lectins	Interaction with gastrointestinal mucosa, vomiting and diarrhoea	Faba bean, pea, peanut, soybean, castor bean
Cyanogenic glycosides	Headache, muscle weakness, nausea, etc.	Almond, cassava, sorghum, lima beans
Phytic acid	Reduced Ca and Fe absorption	Oilseeds, cereals, soybean
Tannins	Reduced Ca and Fe absorption, gastrointestinal disturbances	Legumes, cereals, faba bean
Oligosaccharides	Flatulence	Legumes
Oxalates	Reduce Ca absorption and encourage kidney stone formation	Sesame, spinach
Oxaldiaminopropionic acid (ODAP)	Neurological lesions (lathyrism)	Grass pea
Goitrogenic factors	Interfere with iodine uptake, goitre	Rapeseed, mustard seed, cassava, sorghum, broccoli
Favism causing compounds (e.g. vicine)	Anemia for people carrying the hereditary disorder	Faba bean, pea

instance Maillard compounds, oxidized forms of sulphur amino acids (such as methionine sulphoxide, methionine sulphone, and cysteic acid), D-amino acids, and crosslinked amino acids such as lysinoalanine and lanthionine.

15.10.1 Legumes

Leguminous species are used as dry grains in appreciable amounts for human nutrition. Because of the balanced proportion of proteins (except for sulphur-containing amino acids), starches, fibres and minerals, legume seeds complement cereals in human diets. They are also good sources of minerals and vitamins. Germinated legumes are rich in vitamin C, riboflavin and niacin. Several studies claim that the consumption of legumes has interesting physiological effects, preventing some common health problems, such as diabetes mellitus, coronary heart disease and colon cancer. Therefore, legume protein preparations are gaining importance in the food industry, representing an attractive alternative in the development of new foods.

15.10.2 Cereals

All cereal grains have high energy value, mainly from the starch fraction but also from the fat and protein. Cereals are rich in fibre, B vitamins and minerals. However, during processing many of the nutrients are lost. In general, the cereals are low in protein content, although oats and millets are exceptions. Cereal proteins are characterized by the high content of glutamic acid and proline, being generally deficient in lysine. Rice and oat proteins are richer in lysine and are often considered to be of better nutritional quality. In contrast, wheat and maize have the lowest lysine content. Tryptophan is the second limiting amino acid in cereals, particularly in maize. Other cereals, such as quinoa, buckwheat and grain amaranth have a well-balanced essential amino acid profile. Quinoa contains up to 18% protein, being an unusual complete vegetable source of protein.

15.10.3 Oilseeds

Oilseeds are energy dense foods, due to their high oil content. They are a source of fibre, vitamins (vitamin E, niacin, and foliate), minerals (phosphorus, iron, and magnesium), monounsaturated (e.g., peanuts) and polyunsaturated (e.g., sunflower) fatty acids. Oilseed proteins are deficient in lysine and sulphur amino acids compared to animal proteins and may contain anti-nutritional factors. Despite these limitations, easily overcome by supplementation with other proteins and physico-chemical treatments respectively, oilseed protein makes a significant contribution to the human dietary protein intake. Furthermore, oilseeds are generally richer in sulphur amino acids than legumes, but poorer in lysine except for rapeseed. The nutritional quality of oilseed meals depends largely on the oil extraction process. Severe heat treatment results in the destruction of several amino acids, particularly lysine, methionine, arginine, tryptophan and cysteine.

15.10.4 Potato and green leaves

Potato, a carbohydrate-rich food, is a good source of vitamins C, B1, B3 and B6, and minerals such as potassium, phosphorus and magnesium. It also contains folic acid, pantothenic acid and riboflavin. As far as the amino acid composition is concerned, the quality of potato protein is fairly good, with relatively high lysine content. In contrast, it is low in methionine. Glycoalkaloids (usually solanine and chaconine) are the main anti-nutritional compounds of potatoes together with protease inhibitors.

Green leafy vegetables are rich in carotenoids as well as in iron, calcium, magnesium, vitamin C, riboflavin and folic acid. Leaf proteins are a good source of amino acids, with methionine being a limiting factor. The main nutritional problems are related to high fibre content and other anti-nutritional factors, such as phytate, cyanogenic glycosides and tannins.

15.10.5 Protein allergenicity

Adverse reactions to foods are becoming an increasingly important health issue. In particular, the incidence and prevalence of food allergies have grown in recent years and are estimated to affect 1–4% of individuals. There are a number of groups of foods that are responsible for most food allergies. These groups include milk, eggs, fish and shellfish, wheat, legumes, nuts, fruits and vegetables.

The majority of known class 1 food allergens (via gastro-intestinal tract) belong to two superfamilies of vegetable proteins: the prolamin and the cupin superfamilies. Proteins belonging to these superfamilies include albumins (e.g. Ara h 2 from peanuts), prolamins and globulins (e.g. vicilin allergens as Ara h 1 from peanuts). Next to these superfamilies, the papain superfamily has also been reported to represent class 1 food allergens.

The allergic reaction may be caused by a peptide of a few amino acids (linear epitopes) or it may be a motif of the protein structure (conformational epitopes). Food processing may help inactivate certain food allergens, but may produce new ones as a result of change in protein conformation. Conformational epitopes are generally more susceptible to protein denaturation, while linear epitopes are more likely to be inactivated by hydrolysis.

15.11 Regulatory status

Vegetable protein products (VPP) are defined as 'food products produced by the reduction or removal from vegetable materials of certain of the major non-protein constituents (water, oil, starch, other carbohydrates) in a manner to achieve a protein content of 40% (dry weight basis) or over'. This definition conforms to the codex general standard 174-1989 developed by the international Codex Alimentarius Commission (Joint FAO/WHO Food Standards Programme). VPP proceed from several vegetable sources and their utilization in foods is highly regulated. The VPP regulation covers important aspects such as the end product labelling, the use of genetically modified organisms (GMOs), the introduction of novel food products on the market, food allergens, and modifications of food products.

The presence of VPP in foods should be clearly indicated on the label. Food products containing VPP should be labelled in accordance with general standards developed by the international Codex Alimentarius Commission (Codex stan 1-1985; Rev. 1-1991) and directives developed by the EU food safety legislation (Directive 2000/13/EC; Directive 90/496). The ingredient statement of VPP should contain the vegetable source (e.g., soy, wheat, pea, groundnut, etc.) and a complete list of ingredients should be declared on the label in descending order of proportion excluding added vitamins and minerals. When VPP are used at low levels for functional purposes, the level of VPP is calculated on a dry weight basis in the final product. The use of VPP as functional ingredients follows the same regulation as other functional

ingredients with no required change in the name of the product. VPP can be used as protein extenders to improve the nutritional value of various food products. The protein nutritional values should be assessed according to the established methods for protein quality measurement. The complementary protein should contain a minimum percentage of certain residues (lysine, methionine, cysteine or tryptophan) if diets are deficient in those residues. Addition of L-amino acids is considered if the complementary or supplementary proteins do not give the desired increase in the nutritional contribution. Fortification of VPP with vitamins and minerals can also be a commercial practice when VPP contain anti-nutritional factors that might interfere with the bioavailability or utilization of nutrients, or when VPP are used as a suitable vehicle for fortification in regions where there is a demonstrated need for intake in certain vitamins or minerals. VPP can also be used to substitute partially or completely animal proteins in foods. The protein quality and quantity of the new product cannot be less than that of the original product; and vitamins and minerals also have to be in equivalent quantities.

The name of the substituted product should describe the true nature of the product. In cases where the VPP substitution results in a product with lower animal protein content than that required to be regarded as belonging to the same category, the name of the standardized animal foods should not be used unless properly qualified. When the animal protein is substituted completely, the name of the food should be the name of the VPP with a brief description. VPP, which are not intended to supplement or substitute animal protein foods, but are used instead as the sole protein source in products, should be safe and produced in accordance with good manufacturing practices. Before the utilization of VPP as human food, VPP need to be tested pursuant to the guidelines developed by the Codex Committee on vegetable proteins (CX-728). VPP which are produced by minor processing variants from sources commonly used as food need not to be tested thoroughly; however, novel VPP, which are processed by new techniques from commonly used sources or produced from sources not previously used as human food, require thorough testing; where a description of the preparative process, nutritional value, microbiological status, and toxicological safety of the novel VPP have to be given and examined.

Some VPP have specific codex standards. For instance, VPP prepared from soybean have different names on the label depending on the protein content (soy protein flour, 50–65%; soy protein concentrate, 65–90%; and soy protein isolate 90% or over). Other descriptions, including the physical form and texture of soybean products, might also be declared on the label (Codex stan 175-1989). Likewise, the name of wheat gluten or wheat protein products to be declared on the label varies depending on the viscoelasticity property of hydrated gluten. The name of wheat protein products shall be ‘vital wheat gluten’ if the viscoelasticity is high; ‘devitalized wheat gluten’ if the viscoelasticity is reduced due to denaturation, or ‘solubilized wheat gluten’ if the viscoelasticity is reduced due to partial hydrolysis (Codex stan 163-1987).

Genetically modified plant varieties have become part of the global food

market and all appropriate measures have to be taken to avoid adverse effects on human health and the environment. Before placing genetically modified material on the market, it has to be approved in the EU under the Directives 2001/18/EC on the deliberate release into the environment of GMOs, under the regulation (EC) No. 1829/2003 on genetically modified food and feed, and under the regulation (EC) No. 1830/2003 concerning the traceability and labelling of GMOs and the traceability of food and feed products produced from GMOs. Genetic modifications have been introduced in several plant varieties, but only seeds of some plant varieties are currently authorized.

For food products where adventitious or technically unavoidable traces of material containing, consisting of, or being produced from authorized GMOs cannot be excluded, a tolerance threshold no higher than 0.9% of the food ingredients is established below which these food products shall not be labelled according to the regulations (EC) No. 1829/2003 and 1830/2003. If the novel food products shall be subject to adequate labelling according to the former regulations, the traceability of the GMOs and the products produced from GMOs at each stage through the production and distribution chains has to be known thoroughly and transmitted in writing. The words 'genetically modified' (name of the ingredient) or 'produced from genetically modified' (name of the organisms) shall appear clearly on the labelling. Authorization of placing on the market novel foods and food ingredients containing, consisting of, or being produced from genetically modified plants is regularly revised and new acts should be consulted in the EU food safety legislation.

Directive 2000/13/EC describes in detail the declaration of the ingredients on food products, but it does not offer satisfactory information to protect persons who react with incompatibilities or allergies to certain food products. In particular protein-containing foods have a potential to cause allergies in humans. A hit-list of major serious food allergens has been created and it includes some present in vegetable products (gluten, soy, peanut, sesame products, etc.). These products should be clearly listed in the labelling of food products and stated on the label if they are in their original or derived form (Directive 2003/89/EC). The list of ingredients that are likely to cause adverse reactions in susceptible consumers is constantly examined and the European Food Safety Authority has considered that certain products of ingredients are not likely, or not very likely, to cause adverse reactions in susceptible individuals. The Commission Directive 2005/26/EC gives a list of food ingredients (including vegetable ingredients) that are provisionally excluded from the list of ingredients that are the cause of allergies or intolerances in consumers. Although there is not a common tolerance threshold for all the allergens in food products, it is proposed to define one for each allergen and to declare it in the list of ingredients of the food product whenever the content exceeds the established threshold.

Contaminants with toxicological properties such as chloropropanols are found in acid hydrolysed vegetable protein. The EU Commission has adopted measures for setting maximum levels for certain contaminants (Regulation EC No. 1881/2006). In particular, chloropropanols are formed during the

hydrochloric acid mediated hydrolysis step of the manufacturing process of vegetable products when the acid reacts with residual lipids present in the defatted meal from oilseeds and proteins from maize, wheat and rice. The maximum level of 3-monochloropropane-1,2-diol in food products has been established to be 0.05 mg/kg in the dry matter. Actions taken to reduce 3-monochloropropane-1,2-diol have an impact on organoleptic quality of acid hydrolysed VPP, and the food industry has adjusted the production process of acid hydrolysed VPP to prevent the occurrence of undesirable flavour components by alkaline treatments or by combination of acid hydrolysed VPP with fermented VPP that contain lower levels of 3-monochloropropane-1,2-diol.

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Protein–polysaccharide complexes and coacervates

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Abstract: Protein–polysaccharide complexes and coacervates are important self-assembling structures occurring naturally in various biological systems. In addition, these biomacromolecular assemblies exhibit a wide range of functional properties. This chapter will begin with an overview of the thermodynamics leading to the formation of protein–polysaccharide complexes and coacervates. Important physico-chemical parameters influencing complex formation and coacervation are then discussed. Recent advances on the multiscale structure and morphology of protein–polysaccharide complexes and coacervates are described. A detailed review of the functional properties of complexes and coacervates, reviewing possible applications in food and non-food products follows. The chapter concludes with hints on the future trends related to research in the field of protein–polysaccharide complex formation and coacervation.

Key words: protein–polysaccharide complexes, electrostatic, biomacromolecules, coacervation, structure, microencapsulation, functional properties.

16.1 Introduction

Proteins and polysaccharides constitute major compounds in biological systems, being responsible for cell structure, energy storage/production or enzymatic reactions (Albertsson 1971; Tolstoguzov 2000). As was discussed in a recent review paper on protein–polyelectrolyte complexes, protein–polysaccharide mixtures can be classified as cognate or non-cognate (Cooper *et al.* 2005). In cognate systems, proteins and polysaccharides can be found in the same

physiological environment and interact specifically as, for example, lysozyme and glycosaminoglycans polysaccharides in mammalian cartilage (Moss *et al.* 1997). By contrast, the non-cognate protein–polysaccharide mixtures encompass all pairs that are not necessarily interacting specifically, but are mixed together for any physiological or technological purpose. Non-cognate protein–polysaccharide mixtures are very common in manufactured food formulations. These two ingredients are firstly fulfilling the nutritional purpose of providing an equilibrated caloric diet to maintain health and well-being for people consuming them. In addition, they are importantly contributing to the structure, texture and stability of the finished goods (Dickinson 2006).

Given the different elementary composition of proteins (covalent assembly of hundreds of amino acids) and polysaccharides (covalent assembly of sugars with possible branching), and further considering that a number of glycoproteins and proteoglycans exist in biological systems, a variety of two- and three-dimensional structures with different physico-chemical properties can be found. One important consequence is that, very similarly to synthetic macromolecules, these two types of biomacromolecules exhibit several phase behaviours upon mixing in an aqueous medium (Tolstoguzov 1986; Piculell and Lindman 1992). These phase behaviours arise from long- or short-range interactions between the biomacromolecules themselves, but possibly because of different affinities between the biomacromolecules and the solvent too (Tolstoguzov 1991, 1993; Ledward 1994). Ideally, when all types of interactions are equivalent within the mixture, proteins and polysaccharides distribute evenly in the solvent, i.e. each macromolecule ignores the presence of the other one. When the system exhibits a macroscopic one-phase behaviour, this is known as *co-solubility* (Tolstoguzov 1997).

When the biomacromolecules exhibit high affinity for the solvent and exert overall repulsive interactions between themselves, the mixture generally separates into two phases, one phase being enriched with the polysaccharide and the other being enriched with the protein. This behaviour is known as *thermodynamic incompatibility* or segregative phase separation and is often the result of strong exclusion volume effects (Bungenberg de Jong 1949a; Burgess 1990; Picullel and Lindman 1992; Syrbe *et al.* 1995; Schmitt *et al.* 1998; Tolstoguzov 2000, 2003; Doublier *et al.* 2000; de Kruif and Tuinier 2001; Turgeon *et al.* 2003, 2007; de Kruif *et al.* 2004; Cooper *et al.* 2005). The last phase behaviour occurs when the protein and the polysaccharide exert strong attractive interactions between themselves. In most of the cases these interactions are electrostatic and the biopolymers associate to form complexes. These complexes generally further aggregate forming a three-dimensional network due to charge neutralization. Because the complexes remain highly hydrated, they form a dispersion of liquid droplets concentrated in biopolymers (so-called coacervates) that tend to coalesce in order to minimize their interfacial free energy. In that case, the mixture phase separates on a macroscopic level, one phase being highly concentrated in the two biopolymers (so-called coacervate phase) and the other being mainly composed by the solvent. This

phase separation phenomenon is known as *complex coacervation* and will constitute the purpose of this book chapter (Bungenberg de Jong, 1949a; Burgess, 1990; Schmitt *et al.* 1998; Turgeon *et al.* 2003, 2007; de Kruif *et al.* 2004; Cooper *et al.* 2005). It is important to note that complex coacervation is a specific case of the more general associative phase separation phenomenon where a concentrated phase can be obtained without interactions between biopolymers in the presence of a poor solvent (Picullel and Lindman 1992).

In Section 16.2, we give a brief overview of the thermodynamics leading to the formation of protein–polysaccharide complexes and coacervates, referring when necessary to protein–polyelectrolyte systems. Important physico-chemical parameters influencing complex formation and coacervation are discussed in Section 16.3. Section 16.4 presents recent advances on the multiscale structure and morphology of protein–polysaccharide complexes and coacervates, and continues in Section 16.5 with the description of the functional properties of complexes and coacervates, reviewing possible applications in food and non-food products in Sections 16.6 and 16.7, respectively. The chapter concludes by giving some hints on the future trends related to research in the field of protein–polysaccharide complex formation and coacervation.

It should be noted here that the specific case of covalent complexes or conjugates between proteins and polysaccharides will not be covered in this chapter as they do not involve similar interactions and are rather irreversible in nature (Kato 1996; Schmitt *et al.* 1998).

16.2 Thermodynamic background, theoretical models and energetics of the formation of protein–polysaccharide complexes and coacervates

16.2.1 Thermodynamic background

The driving force leading to the formation of protein–polysaccharide complexes is the decrease of the total electrostatic free energy of the mixture (Tolstoguzov 1997; de Vries and Cohen-Stuart 2006). The overall decrease in free energy results from a positive enthalpic contribution arising from the electrostatic interactions between the biopolymers. In addition, a negative entropic contribution arises from the release of counterions and water molecules due to the compaction of biopolymers. It should be noticed that the compaction of the protein and the polysaccharide within a complex also results in an unfavourable loss of configurational entropy of the macromolecules. Also, the ordering of water at the complex interface may contribute unfavourably to the entropy of the system (Jelesarov and Bosshard 1999). Nevertheless, it seems that for most of the systems investigated, the large gain of electrostatic entropy due to the release of the counterions is the main driver of the formation of the protein–polysaccharide complexes as discussed in a recent review paper (Turgeon *et al.* 2007). This assumption is supported by the suppression of complex formation above a critical ionic strength leading to the disappearance of the entropic gain arising from the

release of counterions (counterion concentration being more or less equivalent in the solvent phase and in the neighbourhood of the biopolymers; Bungenberg de Jong 1949a; Burgess and Carless 1985; Seyrek *et al.* 2003; Weinbreck *et al.* 2003b). Moreover, it should be noted that the release of counterions hypothesis has been strongly supported by recent experimental evidence of release in the lysozyme/polystyrene sulfonate system using a deuterated tetramethylammonium counterion (Gummel *et al.* 2007a). We will see in the section reporting on the energetics of complex formation, that such behaviour is seemingly strongly dependent on the charge density of macromolecules.

16.2.2 Theoretical models

Several theories describing protein–polysaccharide complexes were developed in the late 1950s. They were based mainly on data obtained for the gelatine-acacia gum or gelatine A-gelatine B systems (Overbeek and Voorn 1957; Veis and Aranyi 1960). Besides the assumption that the polyelectrolytes had a random coil distribution, that the solvent–solute interactions were negligible and that the interactions were not site-specific, Overbeek and Voorn considered that the formation of the coacervate phase was a spontaneous mechanism driven by electrostatic interactions. This theory was slightly modified later by Nakajima and Sato (1972), accounting for the effect of salt addition in the coacervate phase. The theory of Veis and Aranyi takes into account solvent–solute interactions and considers complex coacervation as a two-step process rather than a spontaneous one. Hence, the first step leads to the formation of aggregates resulting from charge neutralization between the polyelectrolytes of low configurational entropy. In a second step, these aggregates (or complexes) rearrange into a coacervate phase in order to increase their configurational entropy. This model assumes also the presence of ion-paired aggregates in the equilibrium dilute phase. An important characteristic of this model is that polyelectrolyte-solvent interactions are weak at low total polyelectrolyte concentration but stronger at high total polyelectrolyte concentration, accounting in part for the observed self-suppression of complex coacervation. Later on, Tainaka (1979) slightly adapted the Veis–Aranyi model by introducing constraints on the charge density of the polyelectrolytes and on their molecular weight. As described in Section 16.4 dealing with the structure formation of protein–polysaccharide complexes and coacervates, experimental evidence tends to support that the Veis and Aranyi model is probably the best at describing the entire phase separation phenomenon.

16.2.3 Energetics

In order to get a clearer understanding of the energetics of complex formation and coacervation in protein–polysaccharide systems and to challenge theoretical models with experimental data, a number of calorimetry-based studies (using differential scanning and isothermal titration calorimetry methods) have been conducted during the last decade (de Kruif *et al.* 2004; Turgeon *et al.* 2007).

Interestingly, the calorimetry results obtained vary markedly depending on the systems considered, as both exothermic and endothermic signals have been reported. For example, exothermic signals have been reported upon titration of β-lactoglobulin (β-lg) with acacia gum (Fig. 16.1), β-lg with pectins, β-lg with sodium alginate or α-lactalbumin (α-la) with lysozyme (Girard *et al.* 2003b; Schmitt *et al.* 2005b; Harnsilawat *et al.* 2006a; Nigen *et al.* 2007).

In contrast, calorimetry data reporting complex formation between proteins and strong polyelectrolytes generally exhibited endothermic signals (Ball *et al.* 2002; Feng *et al.* 2007). These different behaviours can be explained taking into account the recent model based on the Langevin dynamics simulation that described two scenarios for the energetics of complexation of polyelectrolytes depending on whether the polyelectrolyte is weak or strong (Ou and Muthukumar 2006). Hence, in the case of weak polyelectrolytes, it was shown that the complex formation was driven mainly by the enthalpic contribution of electrostatic interactions to the detriment of the counterions release that is entropically driven, leading to an exothermic signal. On the other hand, entropy contribution due to counterions release was predominant in strong polyelectrolyte systems, leading to an overall endothermic signal.

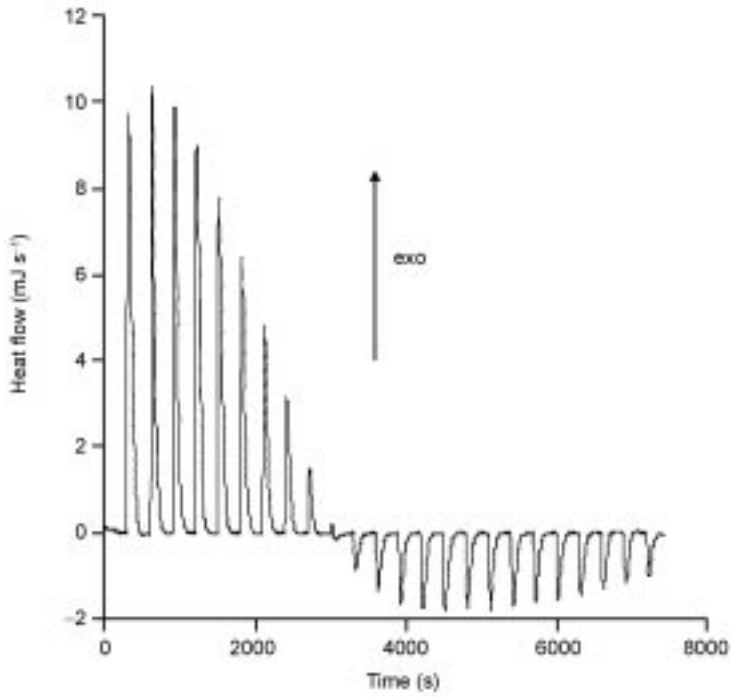

Fig. 16.1 Thermogram obtained during titration of a dispersion containing 1.8 wt% of acacia gum into a dispersion of 0.3 wt% β-lactoglobulin at 25 °C. Both dispersions were prepared in 15 mM citrate-phosphate buffer at pH 4.2. Arrow indicates exothermic reaction. From Schmitt *et al.* (2005b) with permission of ACS.

Since the overall complex coacervation mechanism encompasses both biopolymer complexation and condensation, and considering that biopolymers are weak polyelectrolytes, it is not surprising that different thermodynamic contributions can be observed during one titration experiment. For example, Girard *et al.* (2003b) followed complex formation of β-lg with pectins with high and low esterification degrees at pH 4.0. In both cases, thermograms with negative binding enthalpy exhibited two inflexion points as the protein to polysaccharide ratio (Pr:Ps) increased, revealing two consecutive steps within the complex formation process. The first one was mainly enthalpy driven, and was attributed to the formation of the first electrostatic complexes between the two macromolecules. The second step was mainly attributed to an aggregation of the previously formed complexes, and was therefore mainly entropy driven.

Similar thermal patterns have been reported upon interaction of β-lg with sodium alginate at various pH (Harnsilawat *et al.* 2006a). A strong exothermic signal was obtained at low pH, i.e., when strong electrostatic interactions occurred, whereas an endothermic signal was recorded at high pH values, where possible rearrangement of the biopolymers might occur within the formed complexes. Interestingly, it was also shown that the heat of binding recorded by ITC during complex formation between proteins and polysaccharides could display the successive appearance of an exothermic and an endothermic signal (Ziegler and Seelig 2004; Gonçalves *et al.* 2005; Harnsilawat *et al.* 2006a; Nigen *et al.* 2007) as shown in Fig. 16.1, and even the appearance of a simultaneous exothermic–endothermic signal (Nigen *et al.* 2007). These complex patterns are difficult to interpret since the heat of binding contains all enthalpic and entropic events occurring upon complex formation. However, the occurrence of an endothermic signal following the exothermic one was shown to be induced by aggregation of complexes (Ziegler and Seelig 2004; Gonçalves *et al.* 2005) or coacervation (Harnsilawat *et al.* 2006a).

As we have shown above, complex formation between protein and polysaccharide is driven mainly by electrostatic interactions. In addition to these electrostatic effects, additional short-range interactions such as hydrophobic, van der Waals forces or hydrogen bonds might be secondarily involved in the formation of the protein–polysaccharide complexes (Schmitt *et al.* 1998). As such interactions may contribute significantly to the energetics of the complex formation and coacervation, it is important to review the external and internal parameters affecting these two phenomena.

16.3 Parameters influencing the formation of protein–polysaccharide complexes and coacervates

16.3.1 External parameters

External parameters influencing complex formation and coacervation in protein-polysaccharide mixtures will encompass all parameters not linked to the chemical/molecular composition of the macromolecules. Table 16.1 summarizes

Table 16.1 Summary of external parameters and analytical techniques used for the investigation of complex formation/coacervation in protein–polysaccharide systems

Parameters	Systems	Experimental techniques	References
pH, total biopolymer concentration, ionic strength	11S globulin from *Vicia faba* seeds/CMC	Ultracentrifugation, viscosimetry, electrophoresis, gel permeation chromatography	Antonov *et al.* (2006)
pH, Pr:Ps	Whey protein/chitosan	Turbidimetry	Bough and Landes (1976)
pH, Pr:Ps, ionic strength, types of ions, total biopolymer concentration	Gelatine/acacia gum	Light microscopy, viscosity, turbidity, phase diagrams	Bungenberg de Jong (1949b)
pH, Pr:Ps, pI of gelatin, ionic strength	Gelatine/acacia gum	Microelectrophoretic mobility	Burgess and Carless (1984)
pH, ionic strength, temperature, gelatine concentration, Pr:Ps, time	Gelatine/gelatine	Microelectrophoretic mobility	Burgess and Carless (1985)
pH, ionic strength, temperature, time, treatment of gelatin solutions	Gelatine/gelatine	Microelectrophoretic mobility, coacervate yield determination, photon correlation spectroscopy	Burgess and Carless (1986)
pH, ionic strength, time, total biopolymer concentration	Gelatine, bovine serum albumin (BSA)/ acacia gum	Microelectrophoretic mobility, dry coacervate yield determination	Burgess (1990)
pH, ionic strength, total biopolymer concentration	BSA/acacia gum	Microelectrophoretic mobility, dry coacervate yield determination	Burgess *et al.* (1991)
pH, Pr:Ps, stirring speed, time	Gelatine, BSA/acacia gum	Dynamic light scattering, scanning electron microscopy	Burgess (1994)
pH, ionic strength, temperature, total biopolymer concentration	Pea globulin/gum arabic	Electrophoretic mobility, multi angle light scattering, dynamic light scattering	Ducel *et al.* (2004)
pH, ionic strength	Pea globulin, alpha gliadin/gum arabic, sodium alginate	Electrophoretic mobility, dynamic light scattering, multi angle light scattering	Ducel *et al.* (2005a)

pH, ionic strength, pressure	BSA/ι and κ-carrageenans	Differential scanning calorimetry, capillary electrophoresis, fluorescence spectroscopy, size exclusion chromatography	Galazka *et al.* (1999a)
pH, Pr:Ps, ionic strength	Gelatine/low-methoxy pectin	Differential scanning calorimetry, absorbance measurements	Gilsenan *et al.* (2003)
Pr:Ps, pH, ionic strength, urea, temperature	β-lg/pectin (low- and high-methylated)	Potentiometric titrations, ultrafiltration	Girard *et al.* (2002a)
Pr:Ps, time	β-lg/pectin (low- and high-methylated)	Isothermal titration calorimetry, model curve fitting	Girard *et al.* (2003b)
pH	BSA/dextran sulfate	Differential scanning calorimetry, nephelometry, circular dichoism	Gurov *et al.* (1978)
pH, Pr:Ps	β-lg/chitosan	Isothermal titration calorimetry, light scattering, electrophoretic mobility, solubility	Guzey and McClements (2006a)
pH	β-lg/sodium alginate	Isothermal titration calorimetry, dynamic light scattering, turbidity, zeta potential	Harnsilawat *et al.* (2006a)
pH, ionic strength, Pr:Ps	β-lg/CMC, chondroitin sulphate, κ-carrageenan	Electrophoretic mobility, analytical ultracentrifugation, spectrophotometry	Hidalgo and Hansen (1969)
pH, Pr:Ps	Gelatine/CMC	Viscosimetry, turbidimetry, coacervate phase volume	Koh and Tucker (1988)
pH, ionic strength	BSA, β-lg/furcellaran	Turbidimetry, dynamic light scattering, potentiometry	Laos *et al.* (2007)
pH, total biopolymer concentration, Pr:Ps	Silk fibroin/hyaluronic acid	Turbidimetry, electrophoretic mobility, phase composition, viscosimetry, gravimetric analysis	Malay *et al.* (2007)
pH, Pr:Ps, final total biopolymer concentration	β-lg/acacia gum	Dynamic light scattering, electrophoretic mobility, turbidity, circular dichroism	Mekhloufi *et al.* (2005)

Table 16.1 *Continued*

Parameters	Systems	Experimental techniques	References
pH, ionic strength, temperature	β-lg/chitosan	Solubility, turbidity, viscosity, dynamic light scattering, zeta potential	Mounsey *et al.* (2008)
pH, Pr:Ps	Whey protein/pectin	Light microscopic analysis, electrophoretic mobility, laser light scattering	Neirynck *et al.* (2007)
pH, ionic strength	Egg albumin, human serum albumin/CMC, chondroitin sulphate, hyaluronic acid	Electrophoresis	Noguchi (1959)
Pr:Ps, ionic strength, pH	Faba bean legumin/ chitosan	UV spectroscopy, viscometry, differential scanning calorimetry, turbidimetric titration, surface tension, emulsion stability	Plashchina *et al.* (2001)
Time, Pr:Ps	β-lg/acacia gum	Confocal scanning laser microscopy, small angle static light scattering (SALS), turbidity	Sanchez *et al.* (2002)
pH, time, Pr:Ps	β-lg/acacia gum	SALS	Sanchez *et al.* (2006)
pH, Pr:Ps, total biopolymer concentration	β-lg/acacia gum	Phase composition, electrophoretic mobility, turbidimetry	Schmitt *et al.* (1999)
Pr:Ps, presence of protein aggregates, time, total biopolymer concentration	β-lg/acacia gum	Confocal scanning laser microscopy, diffusing wave spectroscopy	Schmitt *et al.* (2001b)
pH, total biopolymer concentration, Pr:Ps, presence of protein aggregates	β-lg/acacia gum	Fourier transform infrared spectroscopy, circular dichroism, front face fluorescence spectroscopy	Schmitt *et al.* (2001a)

Time	β-lg/acacia gum	Isothermal titration calorimetry, dynamic light scattering, drop tensiometry	Schmitt *et al.* (2005b)
pH, ionic strength, total biopolymer concentration	BSA/alginic acid	Microelectrophoresis, dry coacervate yield	Singh and Burgess (1989)
pH, Pr:Ps	Gelatine/agar	Turbidity, dynamic light scattering, turbidimetric titration	Singh *et al.* (2007)
pH	Collagen/chitosan	Turbidimetry, conductrimetry, infrared spectroscopy, potentiometry, circular dichroism	Taravel and Domard (1994)
Time, GDL concentration, Pr:Ps, percentage of renneting	Casein micelles/pectin	Dynamic light scattering, adsorption measurements, renneting experiments	Tuinier *et al.* (2002)
pH, ionic strength, total biopolymer concentration, Pr:Ps	Whey protein/gum arabic	Turbidimetric titration, static and dynamic light scattering, phase diagrams, conductivity measurement	Weinbreck *et al.* (2003b)
pH, ionic strength, temperature, Pr:Ps	Whey protein/ carrageenan	Turbidimetric titration, conductivity	Weinbeck *et al.* (2004b)
pH, Pr:Ps, ionic strength	Sodium caseinate/gum arabic	Turbidimetry, phase composition, electrophoretic mobility, dynamic light scattering, transmission electron microscopy, zeta potential	Ye *et al.* (2006)

the main external parameters and experimental techniques used for studying complex formation/coacervation in various protein–polysaccharide systems.

Influence of pH

The pH has a paramount importance in controlling the electrostatically mediated interaction between proteins and polysaccharides as it will directly control the degree of ionization of the charged groups carried by these two biopolymers. As was proposed by Bungenberg de Jong (1949a), and later on by Burgess (1990), for every protein–polysaccharide pair considered, there is a pH value for which the number of charges of opposite sign carried by the two macromolecules is maximum and equivalent. This pH is known as the electrical equivalence pH (EEP), where the intensity of the electrostatic attraction between the two biopolymers is the highest. At the EEP, the yield of electrostatic complex formation is maximum as well as the volume of coacervate phase. Practically, the EEP is generally determined by measuring the zeta-potential (ζ-potential) of the two biopolymers on the whole pH range and finding the pH value where the two ζ-potential values are equal but of opposite signs (Noguchi 1956; Burgess and Carless 1984; Daniels and Mittermaier 1995; Schmitt *et al.* 1999; Mekhloufi *et al.* 2005; Guzey and McClements 2006a).

Another analytical technique, presented in Fig. 16.2 for the β-lg/acacia gum system, consists in monitoring the increase of the scattered light in turbidity or light scattering experiments as a function of the pH and to the determine the point of highest turbidity/scattered intensity before dropping (mainly corresponding to macroscopic phase separation) (Xia *et al.* 1993; Weinbreck *et al.* 2003b; Mekhloufi *et al.* 2005).

Several other analytical methods can be used to determine the EEP (Table 16.1). Nevertheless, taking into account the range of natural polysaccharides that could potentially form complexes with proteins, most of them are anionic (with the exception of chitosan and glycosaminoglycans). Consequently, the EEP was generally found below the isoelectrical pH (IEP) of the protein. For example, considering the β-lg/acacia gum system, it was shown that the EEP was around pH 4.2 ($IEP_{\beta\text{-lg}} = 5.2$; Schmitt *et al.* 1999). In contrast, the value of the EEP was pH 6.0 when bovine serum albumin (BSA) was forming electrostatic complexes with chitosan ($IEP_{BSA} = 4.6$; Kayitmazer *et al.* 2007). It is worth mentioning here that subtle variations of pH in the order of 0.5 pH unit, or even less around critical pH values, may lead to substantial reduction of the yield of complex formation and coacervation as reported on the area of the coacervate phase on ternary phase diagram in β-lg/acacia gum mixtures at pH 4.2 and 5.0 (Schmitt *et al.* 1999).

In order to understand how these subtle pH changes may affect complex formation and coacervation, it is advisable to perform a titration of the protein–polysaccharide mixture (for a given ionic strength, protein to polysaccharide ratio and total biopolymer concentration) with a base or an acid and to follow, for example, the variation of the scattered light intensity or the hydrodynamic radius of the particles as a function of the pH. Such an approach was initially

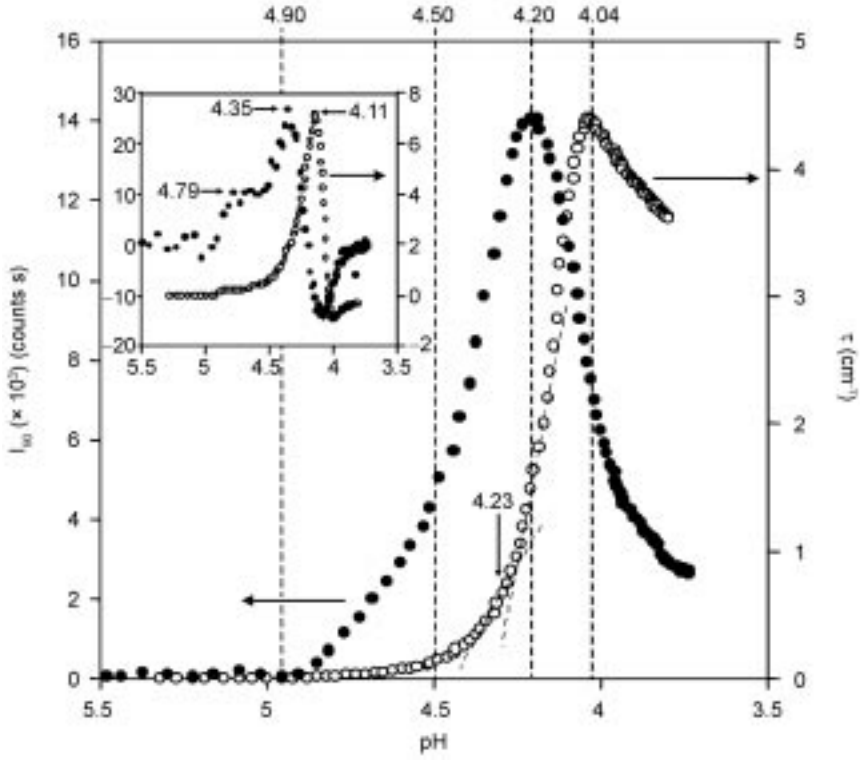

Fig. 16.2 pH-induced evolution of () the scattered light intensity at 90° angle (I_{90}) and (○) the turbidity (τ) as a function of pH for the β-lg/acacia gum mixed dispersions at 0.1 wt% total biopolymer concentration and β-lg/acacia gum weight ratio of 2 : 1. Inset: (●) (dI_{90}/dt) (counts s^{-2}) and (○) ($d\tau/dt$) ($\times 10^{-3}$ cm^{-1} s^{-1}) evolution as a function of pH for the same system. Each point is the average of two independent experiments. Arrows indicate the pH of structural transitions. From Mekhloufi *et al.* (2005) with permission of ACS.

proposed for the study of protein–polyelectrolyte complex formation for protein recovery purposes, but has been successfully extended to the study of the effect of pH on the structural transitions for the whole coacervation process (Strege *et al.* 1990; Mattison *et al.* 1995, 1999; Kaibara *et al.* 2000). Without entering into too much detail of the protein–polysaccharide structure formation, which is described in the next section, the main findings of this type of experiment were that two main pHs of transition could be defined. The first one, called pH_c, or critical pH, characterizes the formation of the first electrostatic complexes that remain soluble because of uneven charge compensation. As a consequence, only a slight increase in the turbidity or scattered light or hydrodynamic radius is measured (Mattison *et al.* 1999). Upon further titration, a second characteristic pH is obtained, pH_ϕ, with reference to macroscopic phase separation. At this pH, all previously measured physical parameters generally exhibit strong divergence, indicating a sharp phase transition within the system. The existence

of these two pHs is important since determining the effect of external or internal parameters on these pH values can provide some insights on the type of interactions occurring between biopolymers. For instance, it was experimentally found that the pH_c of β-lg/high-methoxyl pectin mixtures decreased from 5.0 to 4.5 adding urea to the mixture, indicating that stronger ionic interactions were needed to induce complex formation without hydrogen bonds (Girard *et al.* 2002a).

Such a kind of 'gradual pH change' approach was recently applied to protein–polysaccharide mixtures with additional use of an *in situ* acidifying agent, the glucono-δ-lactone or GDL. Very similar results to the 'classical titration' of protein–polysaccharide mixtures were obtained as described in Fig. 16.2 (Weinbreck *et al.* 2003b; Mekhloufi *et al.* 2005). In the case of β-lg/acacia gum titration, $pH_c = 4.5$ and $pH_\phi = 4.2$, differing by only 0.3 pH unit. It is noteworthy that using this approach and combined complementary experimental methods, more characteristic pHs of structural transitions could be detected (Mekhloufi *et al.* 2005). Keeping the results in mind, it becomes obvious that depending on the pH value at which the protein–polysaccharide mixture is achieved (i.e., above pH_c, between pH_c and pH_ϕ or below pH_ϕ), very different complex formation and coacervation yields can be obtained. Another important external parameter controlling the extent of protein–polysaccharide complex formation and coacervation is the ionic strength of the system.

Influence of ionic strength

As already discussed in Section 16.2, the electrostatic entropy gain induced by the release of the biopolymer counterions upon complex formation is an important driving force for the system. When the ionic strength of the system increases, two major energetically detrimental effects can occur:

1. the screening of the charges of the macromolecules due to small ion-pairing, and
2. the overall equivalence of the concentration in counterions in the bulk phase and in the neighbourhood of the biopolymer chains.

The first effect reduces the number of protein molecules able to interact with the polysaccharide or polyelectrolyte chains (Matsunami *et al.* 2007), whereas the latter one suppresses the energetic advantage of forming a complex as already predicted by the Veis and Aranyi model. In addition, adding salt to the solvent modifies its dielectric constant which is detrimental as far as electrostatic interactions are concerned. The effect of ionic strength on complex coacervation was described by Bungenberg de Jong (1949a), who showed that increased ionic strength was suppressing complex coacervation in the gelatine/acacia gum system. He also reported that when divalent ions were used, suppression of coacervation occurred at lower ionic strength values than for monovalent ions. This can be easily understood in terms of double electrostatic entropy gain when two monovalent ions are released, compared to a single divalent ion. An interesting feature reported by Bungenberg de Jong was that the suppression of

complex coacervation by salt addition could be overcome by dilution of the system with the solvent. The expected effect here was that the bulk ion concentration became lower than that of the counterions close to the polymers, favouring again complex formation.

Later on, Burgess *et al.* (1991) reported a similar effect of ionic strength on the yield of coacervate phase formed in the bovine serum albumin (BSA)/acacia gum system. If coacervation was suppressed by addition of 100 mM NaCl, interestingly, the highest coacervate yield was not obtained for no salt added but after addition of 10 mM NaCl. The likely explanation could be related to the salting-in effect on proteins. This effect could promote exposure of new regions of the protein surface to the solvent, enabling new electrostatic interactions to occur. A deeper understanding of this low salt concentration effect on favouring complex formation was given recently on the system consisting in BSA and a hydrophobically modified polyacrylic acid (Seyrek *et al.* 2003). Here again, a 10–20 mM ionic strength led to maximum complex formation. Based on charge contour modelling on the protein using the Poisson–Boltzmann equation at various ionic strengths, the authors explained that low salt content enables overcoming very short-range repulsions (in the order of 2–4 nm) between the protein and the polyacid. This allowed formation of hydrophobic interactions that were secondarily responsible for the higher yield of complex formation, as already reported by Jönsson *et al.* (2003) for a series of protein–polyelectrolyte systems. Again, suppression of complex formation occurred around 100 mM salt concentration. Very recently, Wang *et al.* (2007c) reported similar low- (10–20 mM) and high-ionic strength (> 20 mM) effects during the electrostatic complexation between BSA and pectin at the surface of a quartz crystal microbalance. Such a salt content dependency of complex formation was also described for purely cognate protein–polysaccharide systems (Moss *et al.* 1997). Hence, it was shown that cartilage lysozyme/chondroitin sulfate complex formation under physiological pH was suppressed in presence of 140 mM NaCl, whereas only 60 mM were sufficient to prevent complex formation between the same protein and hyaluronan. The authors explained that this specific salt dependence of complex formation was at the origin of the ability for mammalian lysozyme to dissociate proteoglycan assemblies in the cartilage.

Some studies have considered the interplay between the ionic strength and the critical pH_c and pH_ϕ. The general trend is that for a given protein to polysaccharide ratio, both pH values are generally shifted towards more acidic values in order to compensate the partial screening of the charges induced by the added microions (Grymonpré *et al.* 2001), i.e., the charge density of proteins needs to be increased so as to reach the same level of charge neutralization between proteins and polysaccharides. For example, pH_ϕ decreased from 9 to 7 upon addition of 200 mM NaCl in the gelatine/κ-carrageenan system at a protein to polysaccharide weight ratio of 1:1 (Fang *et al.* 2006) or from 4.75 to 4.25 in whey proteins/acacia gum mixture at a ratio of 2:1 (Weinbreck *et al.* 2003b).

To conclude this section, it is worth mentioning the very interesting results reported by Weinbreck *et al.* (2004b) on the importance of the type of ions

present in the mixture upon control of ionic strength in the whey proteins/λ-carrageenan system. It was shown that addition of sodium chloride was shifting the pH_ϕ to more acidic pH, as reported for other systems. However, when calcium chloride was used, a marked shift of pH_ϕ towards more basic pH values (up to pH 8 upon addition of 200 mM $CaCl_2$) could be observed. The given explanation was that the two negatively charged biopolymers were indirectly forming complexes via calcium bridges. Thus, ionic strength dependence for complex formation between protein and polysaccharide might not only be related to salt concentration, but sometimes to the type of ion.

After having considered the pH and ionic strength, the two major parameters affecting directly the number of charges on the macromolecules, we will now discuss the effects of mixing ratio that is modifying the balance between the charges at constant pH and ionic strength.

Influence of protein to polysaccharide weight ratio

Electrostatic complex formation and coacervation can be maximized for a given set of pH and ionic strength conditions when the optimum protein to polysaccharide mixing ratio (Pr:Ps) is found. This experimental evidence has been modelled for a globular protein–flexible polyelectrolyte system using Monte Carlo simulation and it was found that these conditions exactly corresponded to full charge compensation between the two macromolecules (Akinchina and Linse 2002). The optimum ratio can be determined from electrophoretic mobility measurements of the two biopolymers at constant weight concentration or by measuring the turbidity and scattered light intensity of mixtures upon titration (Burgess and Singh 1993; Xia and Dubin 1994; Bowman *et al.* 1997; Schmitt *et al.* 1999; Ye *et al.* 2006). At the complete charge neutralization ratio, the electrophoretic mobility of the complexes converges to zero, whereas the turbidity or scattered light intensity passes through a maximum indicating a maximum mass within the electrostatic complexes and/or a maximum number of formed particles. For protein to polysaccharide ratios below the neutralization point, complexes remained soluble because of insufficient charge neutralization. Interestingly, this was generally traduced by a steady state for the size of the complexes, while the scattered light intensity was still increasing as was reported by Weinbreck *et al.* (2003b) during whey proteins–acacia gum complex formation. The latter result clearly indicated that before phase separation occurred, an increasing number of similarly sized complexes were formed. Regarding the interplay between the protein to polysaccharide ratio and the two critical pH values, pH_c and pH_ϕ, it is important to note that pH_c is independent from the mixing ratio (Mattison *et al.* 1995; Kaibara *et al.* 2000). Thus, as soon as protein molecules mix with the polysaccharide ones, soluble complexes start forming, independently from the initial mixing ratio. In contrast, the critical pH value leading to phase separation, pH_ϕ, is strongly ratio dependent as it corresponds to full charge neutralization of the complexes, or in other words, full saturation of the polysaccharide chains by protein molecules (Kaibara *et al.* 2000).

Another important external parameter influencing complex formation and coacervation is the total biopolymer concentration.

Influence of total biopolymer concentration

Veis *et al.* (1967) already took this parameter into account in their model. They assumed that once the biopolymer concentration was higher than a critical value, the energetic entropic advantage of forming electrostatic complexes to release counterions disappeared as counterions would be as concentrated in the dilute and in the coacervate phase. This would be the main reason why experimentally, the two-phase region is defined by a finite area in ternary phase diagrams and why complex coacervation can be induced from a monophasic system upon dilution with the solvent (Phares and Sperandio 1964). From the extensive study of a series of protein–polysaccharide complexes, Tolstoguzov (1986) concluded that suppression of coacervation occurred around a total biopolymer concentration of 4 wt%. In fact, it seems that this critical concentration is highly system dependent as, for example, a critical concentration of 4.5 wt% was needed to observe self-suppression of coacervation in the β-lg/acacia gum system at pH 4.2 (Schmitt *et al.* 2000). Very interestingly, the presence of protein aggregates in the same system induced an increase of the self-suppression concentration from 4.5 wt% up to 20 wt%, suggesting that a control of coacervation self-suppression could be obtained tailoring the size and surface properties of aggregates (Schmitt *et al.* 2000). In a very close system, whey proteins/acacia gum, but a pH 3.5, it was reported that complexes could be observed up to 15 wt% (Weinbreck *et al.* 2003b). The likely reason to explain these experimental differences was that the electrostatic entropy gain at pH 3.5 was still advantageous at 15 wt%, but not more around 4.5 wt% at pH 4.2.

To conclude on the importance of the total biopolymer concentration, it is worth mentioning that it has been reported to have no effect on pH_c or pH_ϕ for total biopolymer concentrations below 0.5 wt% (Mattison *et al.* 1995, 1999; Weinbreck *et al.* 2003b, 2003c). Nevertheless, for higher biopolymer concentration, pH_ϕ has been reported to shift to higher values as was the case in the whey proteins/acacia gum system (Weinbreck *et al.* 2003b). Finally, the size of the coacervates was shown to depend on the total biopolymer concentration up to 1 wt% for the β-lg/acacia gum system at pH 4.2 (Schmitt *et al.* 2000). For higher concentration, the coacervate size remained independent of total biopolymer concentration, showing again the importance of the concentration range tested to observe or not effects.

Apart from the chemical parameters described above, several physical parameters, such as the temperature, the pressure or the shearing, might influence the formation of protein–polysaccharide complexes or coacervates.

Influence of temperature, shearing and pressure

Temperature is known to possibly affect conformation of proteins and polysaccharides, but also to favour several non-electrostatic interactions (Tolstoguzov 1997; Schmitt *et al.* 1998). Low temperature is, in principle,

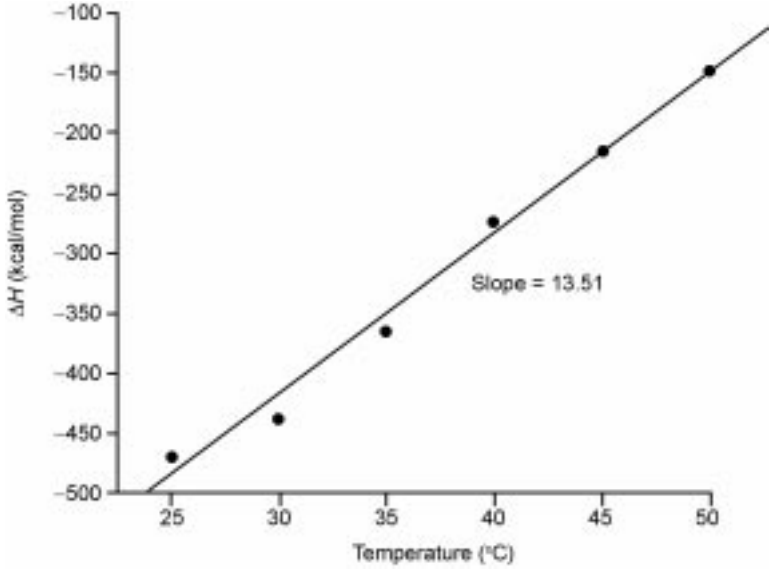

Fig. 16.3 Evolution of the binding enthalpy (ΔH) with temperature during the titration of an aqueous dispersion of total acacia gum (1.8 wt%) into an aqueous dispersion of β-lg (0.6 wt%) at pH 4.2. Line corresponds to a linear fit of data. The slope represents the heat capacity changes (ΔC_p).

favourable to hydrogen bonding whereas higher temperature is favourable to hydrophobic interactions. For example, it can be seen in Fig. 16.3 that the binding enthalpy recorded in the β-lg/acacia gum system decreased (became less negative) with increasing temperature. The molar heat capacity, ΔC_p, calculated from the slope of the ΔH vs. temperature relationship, originated from changes in the degree of surface hydration in the free and complexed molecules, and to a lesser extent from changes in molecular vibrations (Jelesarov and Bosshard 1999). The ΔC_p calculated from Fig. 16.3 was large and positive, a typical signature of ionization/charge neutralization reactions (Ziegler and Seelig 2004; Gonçalves *et al.* 2005; Chung *et al.* 2007). A positive ΔC_p but with a ΔH parameter remaining favourable at all temperatures studied, i.e. $\Delta H < 0$, would be indicative of a significant contribution of hydrogen bonding (Gonçalves *et al.* 2005). The importance of hydrophobic interactions in other protein–polysaccharide systems has been shown. In some cases electrostatic binding between macromolecules was impossible unless strong hydrophobic interactions are generated by heating (Zhang *et al.* 2007).

Other interesting results highlighted the effect of temperature on the conformational changes in BSA and the resulting formation of complexes with alginate (Harding *et al.* 1993). Thus, between 35 and 70 ºC, no complexes were formed at pH 6.8 at an ionic strength of 0.1 M, whereas complexes appeared above 70 ºC. Complex formation was mainly related to the conformational changes of BSA around its denaturation temperature of 55 ºC, so that additional

hydrophobic groups could be exposed, favouring complex formation. Similar effects of temperature have been reported upon complex building between gelatine and κ-carrageenan (Fang *et al.* 2006). Temperature values above the conformational transition of both biopolymers, i.e. > 25 ºC led to complex coacervation, whereas thermodynamic incompatibility occurred below 25 ºC. In a very recent study involving complex formation between two proteins, α-la and lysozyme, it was shown that below 5 ºC, precipitates were obtained whereas coacervates were formed if the mixing temperature was 45 ºC (Nigen *et al.* 2007). This change in the nature of the solubility of the formed complexes was due to a conformational change of the α-la at temperatures higher than 27 ºC, enabling adoption of a molten-globule conformational state that was favouring exposure of hydrophobic regions. This study suggested that electrostatic interactions could be mostly important during the initial biopolymer complex formation but large-scale aggregation or coacervation would be mainly driven by hydrogen bonding or hydrophobic interactions, depending on the temperature.

In a very recent paper, it was shown that temperature could also affect the structure of the polysaccharide (Kayitmazer *et al.* 2007). Hence, the effect of temperature (12 or 25 ºC) on the structure of BSA/chitosan was compared to BSA/poly(diallyldimethylammonium chloride) (PDADMAC). It was shown, that even if the two polyelectrolytes had very similar charge densities, different rheological properties were obtained for the coacervate phase, probably due to the fact that temperature had a stronger effect on the flexibility of chitosan, but not on that of PDADMAC. Several studies were related to the effect of temperature on pH_c and pH_ϕ, leading to the conclusion that these critical pH values were not affected as long as electrostatic interactions were mainly involved in the complex formation mechanism (Kaibara *et al.* 2000; Weinbreck *et al.* 2004b; Singh *et al.* 2007).

Another possibility to control the formation of protein–polysaccharide complexes is to apply pressure to the system in order to partially denature proteins. For example, Galazka *et al.* (1999b) reported the formation of ovalbumin/dextran sulfate electrostatic complexes at low ionic strength and pH 6.5 after high-pressure treatment of the mixture at 600 mPa for 20 minutes. This type of weak electrostatic complex formation upon high pressure treatment was also highlighted when ovalbumin was mixed with ι-carrageenan.

Another important physical parameter influencing more generally the coacervation phenomenon rather than the complexation one is shearing. Most of the studies reported results carried out in non-controlled shearing devices; however, some trends could be discerned. When protein–polysaccharide mixtures were submitted to mixing (below 1000 rpm), the size of the coacervates was generally decreasing with the increase of the shearing rate (Elgindy and Elegakey 1981; Burgess and Carless 1985; Tirkkonen *et al.* 1994; Ovez *et al.* 1997; Sanchez *et al.* 2001). The given explanation was mainly a breakdown of the coavervates due to interfacial destabilization by the shear, as is the case for emulsions, leading to fragmentation of the coacervate phase into smaller

droplets. For higher mixing rates (3500 rpm), an increase of the coacervate size was reported, probably because complex turbulent flow was favouring re-coalescence of the coacervates (Sanchez *et al.* 2001). Interestingly, an additional effect of the time of shearing was noticed for low shearing rates (below 1000 rpm), as an increase of the coacervate size was demonstrated for constant shearing rate in the case of gelatine/gelatine (Burgess and Carless 1985), β-lg/acacia gum coacervation (Schmitt *et al.* 1998) or gelatine/chitosan (Remunan-Lopez and Bodmeier 1996). In these experiments, the shearing applied was likely favouring coalescence of the coacervates or not high enough to prevent it.

Two additional internal parameters have been shown to control protein–polysaccharide complex formation, the charge density and the molecular weight of the biopolymers.

16.3.2 Internal parameters

Influence of biopolymer charge density

The charge density of the biopolymer is defined by the number of charges present for a given distance along the protein or polysaccharide chain. The charge density is of great importance in the type of phase separation obtained in protein–polysaccharide complexes as high charge density (sulphate or phosphate side chains) generally led to precipitates, whereas lower charge densities (carboxylic side chains) led to liquid coacervates (Frugier and Audebert 1994; Weinbreck *et al.* 2003b, 2003c, 2004b). This effect was attributed mainly to the fact that strong electrostatic interactions induced high compaction of the complexes with a high level of local dehydration of the biopolymer chains, leading to insolubilization in the form of precipitates. Interestingly, high charge density allowed the formation of soluble complexes on a larger range of ionic strengths due to the local strong electrostatic interactions that were able to overcome the screening effects induced by microions (Mattison *et al.* 1998; Y. Wang *et al.* 2000).

For a given type of side groups, the charge density could lead or not to the formation of complexes as was shown in mixtures of ovalbumin with ι-carrageenans or dextran sulphate at pH 6.5, i.e., above the IEP of the protein (Galazka *et al.* 1999b). Hence, formation of electrostatic complexes was shown to be possible on the 'wrong' side of the IEP of the protein, i.e. when both biopolymers carry opposite charges (Park *et al.* 1992; Mattison *et al.* 1998). Such local high charge density regions were named 'charges patches'. These patches can be generated on synthetic polyelectrolytes but they can already exist on proteins (de Vries *et al.* 2003). For example, sequence analysis of the charge distribution on the surface of β-lg and α-la led to the conclusion that α-la could bind electrostatically to acacia gum through a single patch, whereas binding of acacia gum to β-lg occurred via several patches (de Vries 2003). This could explain why α-la was able to form electrostatic complexes more than one pH unit higher than its IEP (Weinbreck and de Kruif 2003). Such a type of single electrostatic patch has also been described on the surface of human serum

albumin, allowing complex formation with hyaluronic acid (Grymonpré *et al.* 2001). Another way to describe this phenomenon is that ion–dipole interaction overcomes ion–ion repulsion. However, another possible explanation would be that when the polyacid/polybase is strong enough or the protein has a high enough regulation capacity, a reversal of charge may be induced on the protein (Bisheuvel and Cohen Stuart 2004). The capacitance is an intrinsic property of a protein defining its ability for charge regulation, i.e., to change their charges upon interaction with a polyelectrolyte. It was shown recently by Monte Carlo simulations and perturbation theory that α-la, β-lg and lysozyme displayed very strong capacitance at and near their IEP (da Silva *et al.* 2006). This induces an additional and strong attraction of several kT between proteins and polyelectrolytes through charge-induced charge interactions.

A last internal parameter that is closely related to the biopolymer charge density is the molecular weight, which also plays an important role on the resulting biopolymer flexibility (Kayitmazer *et al.* 2005; Cooper *et al.* 2006).

Influence of biopolymer molecular weight

The increase of the molecular weight of the polyelectrolyte or polysaccharide was shown to favour the formation of electrostatic complexes (Zaitzev *et al.* 1992; Semenova 1996). The understanding of these observations was that the volume occupied by the polyelectrolyte increased with its molecular weight, enabling a higher number of proteins to interact with it and to build complexes. Here, a very interesting effect of the polyelectrolyte and the charge density was shown when polyacrylic acid was mixed with lysozyme or ovalbumin (Shieh and Glatz 1994). No effect of the increase of the molecular weight of the polyacrylic acid was observed with lysozyme, as both polymers had close charge densities. However, a much stronger binding of ovalbumin was detected when increasing the molecular weight of the polyacrylic acid, due to the larger difference in charge density. Y. Wang *et al.* (2000) tested coacervation between anionic surfactant micelles and PDADMAC of various molecular weights. They came to the conclusion that a critical molecular weight of the polycation existed for every total polymer concentration and mixing ratio tested. This critical molecular weight was leading to a critical size of 45 nm for the complexes, enabling coacervation to occur.

Recently, Laneuville *et al.* (2005b) reported the influence of the molecular weight of xanthan gum on the size and compactness (fractal dimension, d_f) of the formed electrostatic complexes. With low molecular weight xanthan gum, smaller complexes with high d_f (2.56, i.e. compact) were obtained, whereas much larger and more linear complexes ($d_f = 2.26$) were obtained with the high molecular weight xanthan gum. It is worth noting that several studies have been undertaken on the modelling of the effect of polyelectrolyte molecular weight on the formation of soluble complexes or coacervates (Skepö and Linse 2003; Akinchina and Linse 2003). The main conclusion was that small molecular weight polyelectrolytes generally led to soluble complexes, whereas larger ones induced further aggregation of the soluble complexes leading ultimately to

complex coacervation. These simulations fitted reasonably with the experiments described above for real systems.

After having reviewed the most important parameters controlling the formation of protein–polysaccharide complexes and coacervates, we now turn to understanding their structure formation and morphology.

16.4 Structure, morphology and coarsening of protein–polysaccharide complexes and coacervates

The understanding of the structure of protein–polysaccharide complexes and coacervate structure from the molecular to the macroscopic level is probably the topic that has received the most scientific interest during the last decade (Schmitt *et al.* 1998; Turgeon *et al.* 2007).

16.4.1 Molecular level

Regarding the molecular structure of the protein and the polysaccharide in the complexes and coacervate, most of the information was gained from the use of calorimetry and spectroscopy. For example, Imeson *et al.* (1977) reported that the denaturation temperature of BSA or myoglobin was increased upon mixing with anionic polyelectrolytes, and concluded that complexes were formed, leading to increased protein heat stability. In another study, the content of α-helix structure of ribulose diphosphate carboxylase was followed by circular dichroism upon complexation with pectins close to the IEP of the protein (Braudo and Antonov 1993). It was shown that complex formation was leading to a loss of α-helix in the protein, enabling identification of which regions of the protein were involved in the interaction. This loss of α-helix structure was also reported upon complexation of β-lg with acacia gum, especially close to the EEP (Schmitt *et al.* 2001a; Mekhloufi *et al.* 2005). The likely explanation for this observation was that this region of the protein was rich in positively charged amino acids at the given pH, but also that these amino acid residues were exposed to the solvent at the surface of the protein. The same α-helical region with high charge density of β-lg was identified in the interaction with pectin (Girard *et al.* 2003a). In addition, a loss of helical structure was also reported upon complex formation between lysozyme and polystyrene sulfonate by means of Fourier transform infrared spectroscopy (Cousin *et al.* 2005). Nevertheless, depending on the structure of the protein, complex formation may also lead to an increase in α-helix as, for example, upon complex formation between poly(L-lysine) and ι-carrageenan (Girod *et al.* 2004). In the same vein, helical regions of α-gliadin proteins were mainly involved in the complex formation with gum arabic, whereas β-sheets were involved when pea globulin protein was tested (Chourpa *et al.* 2006). In another study dealing with complex formation/coacervation processes in β-lg/acacia gum system, a loss in the amount of α-helix protein structure after complexation and a gain in protein secondary

structure after complex formation were shown (Mekhloufi *et al.* 2005). By contrast, Delben and Stefancich (1998) reported no structural changes upon complex formation between ovalbumin and glutamate glucan, which might be due to the compact structure of this protein. It seems obvious that no generalization can be drawn from these different results since both protein conformational changes and unchanged states are described in the literature.

Interestingly, not only could the molecular structure of the protein be modified upon complex formation, but also that of the polysaccharide. For example, the helical structure of ι- and κ-carrageenans was shown to be completely lost upon interaction with β-casein as more protein was bound to the polysaccharide chain (Burova *et al.* 2007). This was an indication that the electrostatic interactions between the two biopolymers were preventing the formation of the secondary hydrogen bonds that are responsible for the helical structure of the bare polysaccharide. Also conformational changes affecting a potassium pectate upon complexing with two enantiomeric forms of poly(lysine) were studied (Paradossi *et al.* 2001). The pectate adopts a super-helical conformation around the α-helix of the poly(L-lysine) but not with poly(D-lysine), indicating a stereo-specificity of the interaction of poly(lysine) with pectate.

16.4.2 Mesoscopic level

This structural level is related to the soluble complexes or soluble complex aggregates arising from the charge neutralization of the two biopolymers. Here, it should be mentioned that a number of experimental data have been reported on protein–polyelectrolyte systems, but that still scarce data are available on protein–polysaccharide complexes (Turgeon *et al.* 2003). However, interesting molecular simulations carried out on model polyelectrolytes/macroions complexes with different chain length, flexibility, charge or mixing ratio led to several possible structures ranging from linear to globular complexes (de Vries and Cohen-Stuart 2006; Ulrich *et al.* 2006). In terms of size, hydrodynamic diameters reported from light scattering experiments on various protein/polysaccharide systems varied between 100 and 300 nm (Grymonpré *et al.* 2001; Weinbreck *et al.* 2003b; Mekhloufi *et al.* 2005; Schmitt *et al.* 2005b). An example of aggregated complexes based on the interaction between β-lg and acacia gum is shown in Fig. 16.4.

An interesting feature related to the structure of the electrostatic complexes close to the EEP was that the polydispersity index measure by light scattering was very low (i.e. below 0.1), indicating a very homogeneous composition of all the complexes before macroscopic phase separation (Mekhloufi *et al.* 2005; Schmitt *et al.* 2005b). The internal structure of protein–polysaccharide complexes has been investigated by means of *in situ* acidification (GDL) coupled with time resolved static light scattering for the β-lg/acacia gum, β-lg/pectin and β-lg/xanthan gum (Girard *et al.* 2004; Mekhloufi *et al.* 2005; Laneuville *et al.* 2006). Results showed that more or less compact fractal aggregates were formed

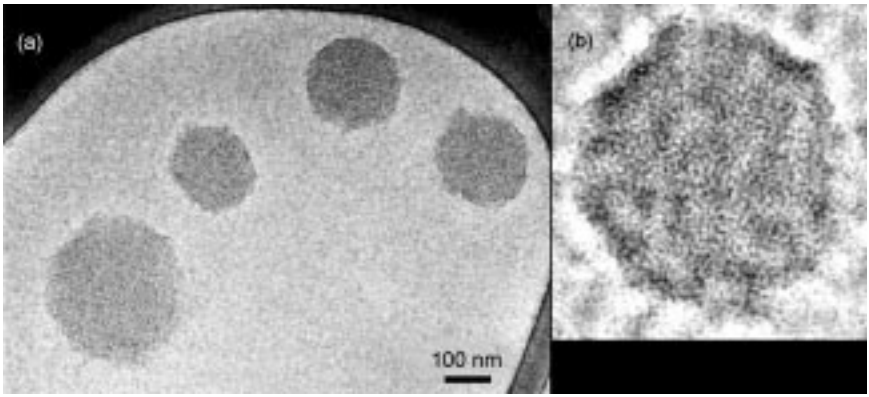

Fig. 16.4 (a) Cryo-TEM micrographs of β-lg/acacia gum aggregated complexes obtained at pH 4.0 and a protein to polysaccharide weight ratio of 2 : 1; (b) structural details of the bottom left particle after FFT filtering.

depending on the type of polysaccharide used (flexible, like acacia gum, or rigid, like xanthan gum). For acacia gum or pectin, fractal dimensions (d_f) ranging between 1.5 and 2.2 were found, indicating linear complexes. In the case of the xanthan gum, diffuse aggregates with $d_f = 1.8$ reorganised in order to form more compact aggregate complexes with $d_f = 2.3$. Interestingly, when the β-lg/xanthan system was submitted to shear, even more compact aggregates with $d_f = 2.53$ were built for the same protein to polysaccharide ratio of 5:1 (Laneuville *et al.* 2005b). In a recent paper dealing with complex formation between lysozyme and polystyrene sulfonate with various chain lengths, small-angle neutron scattering experiments showed that three types of complexes could be formed, leading to gelled or to liquid three-dimensional structures depending on the lysozyme to PSS ratio and the PSS chain length (Cousin *et al.* 2005). The same group showed that when the charge ratio between the lysozyme and the PSS was close to one, the system packed into dense spheres with a radius of about 10 nm and a $d_f = 2.1$ as shown by SANS and freeze-fracture microscopy (Gummel *et al.* 2006, 2007b). Interestingly, Leisner and Imae (2003) reported the aggregation of soluble complexes in a poly(L-glutamate)/synthetic dendrimer system into fractal ones to end up with a three-dimensional network characterizing the coacervate phase. This paper showed that the structure of the coacervate phase was very similar to that of dense aggregated complexes. In a more recent paper considering complexes built between two oppositely charged synthetic polymers, atomic force microscopy investigation allowed getting a very clear insight on the structure of the complexes adsorbed onto a mica surface (Kiriy *et al.* 2006). It was shown that depending on the mixing ratio, complexes were able to rearrange leading to more or less compact morphologies. The more compact ones were obtained for electrostatic neutrality.

The next section considers the structural features of the coacervate phase.

16.4.3 Microscopic level

At this level of structure, liquid droplet of coacervates originating from aggregation of electrostatic complexes with various levels of hydration can be observed using light microscopy (Mekhloufi *et al.* 2005). However, before discussing the morphology and the coarsening of the coacervates, several studies have considered their internal structure. Upon investigation of the diffusion coefficient of protein in the BSA/PDADMAC coacervate using dynamic light scattering, two different regimes were found, indicating that the coacervate phase could behave like a heterogeneous network of polyelectrolyte chains where proteins could diffuse (Bohidar *et al.* 2005). Heterogeneity of structure was also reported in the β-lg/pectin coacervates at various protein to polysaccharide ratios and ionic strengths (Wang *et al.* 2007b). It was concluded that the coacervate could be viewed as a network of pectin chains bound together electrostatically by protein molecules. In excess of proteins, some aggregates tended to form in the network. Similar description of the coacervate as a dense phase (70% of water content) was given for the whey proteins/acacia gum system after 48 hours upon small angle X-ray scattering (Weinbreck *et al.* 2004d). In this case, it was shown that increasing ionic strength or pH led to less homogeneous structure because the strength of the electrostatic interactions between the acacia gum chains and the whey proteins was reduced. Diffusivity measurements of the whey proteins were carried out on the same system using NMR and fluorescence recovery after photo-bleaching (FRAP), leading to the conclusion that whey proteins were free to move within the coacervates, but their diffusion coefficient was reduced in conditions where electrostatic interactions were maximum (Weinbreck *et al.* 2004c). Recently, the meso/microstructure of the BSA/PDADMAC coacervate phase has been probed by FRAP (using interacting and non-interacting fluorescent probes), light scattering and cryo-TEM (Kayitmazer *et al.* 2006). It was shown that the coacervate was composed of dense domains concentrated in protein and polyelectrolyte and more dilute domains, leading to the different diffusion coefficients that were reported previously. It is interesting to observe in Fig. 16.4 that the inner structure of the complexes is heterogeneous with regions having differing affinities for electrons, the structural pattern being close to those observed after polymer spinodal decomposition.

This section dealing with the structure of protein–polysaccharide complexes will finish with some evidence on the coacervate coarsening mechanism and some morphological properties of the coacervates.

16.4.4 Coarsening mechanism and macroscopic level

The charge neutralization and aggregation of protein–polysaccharide complexes leads to the formation of liquid droplets ranging from hundreds of nanometres to several microns in diameter (Kaibara *et al.* 2000; Schmitt *et al.* 2000, 2001b). These coacervates tend to coalesce to form ultimately a dense liquid phase, as mentioned previously. The time coarsening of a β-lg/acacia gum system was

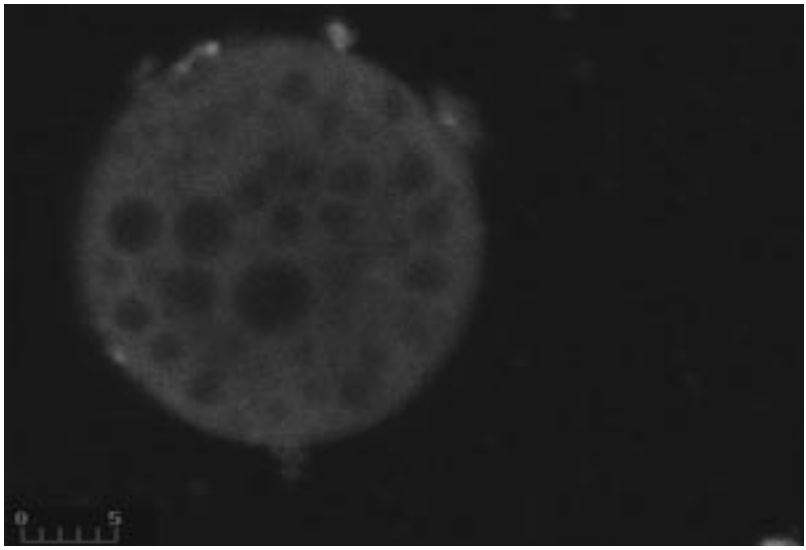

Fig. 16.5 Confocal scanning laser micrograph of aggregate-free β-lg/acacia gum mixture at pH 4.2 and 1 wt% total biopolymer concentration after 3–5 min at a mixing ratio of 1 : 1. Scale bar is 5 μm. From Schmitt *et al.* (2001b) with permission of Elsevier.

followed by static light scattering and confocal scanning laser microscopy (CSLM) after labelling of the two biopolymers (Sanchez *et al.* 2002). It was clearly seen that the initially formed small coacervates tended to coalesce, forming large structures characterized by solvent vacuoles, leading to the so-called sponge structure that can be seen in Fig. 16.5 for the β-lg/acacia gum mixture (Menger *et al.* 2000; Schmitt *et al.* 2001b).

The phase separation kinetics could be scaled with a nucleation and growth model, where initial biopolymer concentration fluctuations induced by electrostatic complex formation lead to hydrodynamically driven coalescence and formation of sharp interfaces delimiting the coacervate phase from the dilute phase (Sanchez *et al.* 2006). The coalescence of the coacervates was already reported by Bungenberg de Jong *et al.* (1940) for the gelatine/acacia gum system. It can be explained by the reduction of the interfacial energy of the system because of the appearance of interfacial viscoelasticity in the phase concentrated in biopolymers. One should recall here that the coacervate phase is mainly liquid in nature as in contains around 70% water (Schmitt *et al.* 2000; Weinbreck *et al.* 2004d). The coalescence phenomenon was shown to occur very quickly (few minutes), especially in optimum conditions of pH and protein to polysaccharide ratio (Schmitt *et al.* 2001b). Over time, the coacervates tend to expel the water in order to increase their free energy as demonstrated by the transition from a turbid system (because of light scattering from the water vacuoles) to a clear viscous and dense phase (Weinbreck *et al.* 2004c).

16.5 Functional properties of protein–polysaccharide complexes and coacervates

The formation of protein–polysaccharide complexes can potentially lead to different functional properties, compared to the two biopolymers taken individually (Schmitt *et al.* 1998; McClements 2006). This is generally due to a synergistic combination of the functional features of both the protein (generally hydrophobic and/or hydrophilic and globular) and the polysaccharide (generally hydrophilic and branched). In this section, the solubility and rheological properties of complexes and coacervates will be discussed, followed by interfacial properties at the oil/water and air/water interfaces.

16.5.1 Solubility and rheological properties

These two functional properties are intrinsically linked to the extent of interaction between the complexes and coacervates with water.

Solubility

The solubility of proteins was shown to be improved upon complex formation with polysaccharide, especially close to their IEP. This property was initially used in systems composed with caseins, in order to induce colloidal stability in acidic dairy products (Payens 1972; Ambjerg Pedersen and Jorgensen 1991; Matia-Merino *et al.* 2004). The physical explanation for this stabilizing effect was given by Tuinier *et al.* (2002), who demonstrated the electrosorption of pectins onto casein micelles upon pH decrease (renneting). The formation of these pectin multilayers at the surface of the casein micelles was able to prevent strong protein aggregation mediated through hydrophobic interactions. This balance between hydrophobic attractions leading to protein low solubility and electrostatic repulsions leading to solubility was also considered upon complex formation between carboxymethylcellulose (CMC) with lysozyme or ovalbumin (Clark and Glatz 1991). Lysozyme, characterized by a high charge density, was able to form a complex with CMC, preventing further aggregation close to IEP. This was not the case for ovalbumin that had a lower charge density and a larger number of hydrophobic regions. Interestingly, several papers have reported that the formation of a complex with a polysaccharide was also preventing protein association upon heating that is known to strongly promote hydrophobic interactions (Shalova *et al.* 2005; Chung *et al.* 2007; Mounsey *et al.* 2008). In this case, the formation of the complex between the polysaccharide and the unfolded proteins prevented the aggregation process leading to the formation of large insoluble protein aggregates.

Obviously, an important parameter controlling the solubility of the protein–polysaccharide complexes is the initial mixing ratio at a given pH and ionic strength. Incomplete charge neutralization led to the formation of soluble complexes, improving protein solubility by electrostatic repulsions or steric effects. In general, these soluble complexes were obtained close to the phase separation boundaries of phase diagrams (Schmitt *et al.* 2000; Weinbreck *et al.*

2003b; Chung *et al.* 2007; Plashchina *et al.* 2007; Feng *et al.* 2007; Jourdain *et al.* 2008). For example, complex formation between β-lg and sodium alginate at pH 5.0 was shown to induce a protein solubility of 70% when 0.75 μM of sodium alginate was added to 0.5 μM β-lg (Harnsilawat *et al.* 2006a). The resulting soluble complexes were characterized by a size of around 80 nm and a ζ-potential of -40 mV, leading to strong electrostatic repulsions. Lastly, it should be noticed that for some protein purification purposes, protein–polysaccharide complex insolubility could be preferred, but this will be discussed in detail in Section 16.6 (Hansen *et al.* 1971; Strege *et al.* 1990). The formation of electrostatic complexes, and further coacervates, may induce changes in the viscosity and rheological properties of the protein–polysaccharide mixtures.

Rheological properties

In order to get a clear picture of the rheological properties of protein–polysaccharide complexes, this section will consider first the flow behaviour of the coacervate phase under shear, then the viscoelastic properties under small deformations and finally the gelling properties induced by temperature.

The flow behaviour of the coacervate phase has been investigated for several systems and the common finding was that the flow could be described as shear thinning, i.e., the viscosity decreased when the shear rate increased to values higher than 20–30 s^{-1} (Burgess and Singh 1993; Weinbreck *et al.* 2004e; Malay *et al.* 2007). This result clearly indicated that the coacervate was a structured phase, whose structure could be modified when submitted to a mechanical stress. Interestingly, most of the flow behaviour of the coacervate could be related to the strength of the electrostatic interactions between the two biopolymers. Weinbreck and co-workers (2004d) reported that the viscosity of a whey protein/acacia gum coacervate phase was the highest at the EEP and was almost linearly increasing with the absolute value of the product of the ζ-potential of the two biopolymers for a given pH. A remarkable flowing feature of the coacervate phase in this case was that the system was able to recover its initial viscosity at low shear rate when submitted to a flow hysteresis. However, the authors reported that the coacervate phase took some time to reorganize after deformation, which might be an indication for some diffusion controlled events, such as alternative formation/disruption of electrostatic interactions between the whey protein molecules and the acacia gum chains.

If the formation of an electrostatic network between protein and polysaccharide within the coacervate phase has already been reported for gelatine-based systems (Muchin *et al.* 1978), it could be proven for globular proteins by submitting the system to small oscillatory deformations (Bohidar *et al.* 2005). It was shown that an elastic (G') and a viscous shear modulus (G'') could be measured in the BSA/PDADMAC system. Similar behaviour has been reported for the β-lg/acacia gum coacervate, where again a frequency dependence for the two shear moduli was reported, indicating that some rearrangements were occurring at various frequencies (Schmitt *et al.* 2005a).

The formation of the weak electrostatic network in the coacervate phase was also demonstrated from the ionic strength dependence of G' in the β-lg/pectin system, as it was shown that a critical value of 200 mM NaCl was screening the charges between the two biopolymers (Wang *et al.* 2007a). In this same study, it was found that the elastic modulus developed by the coacervate phase was also dependent on the protein to polysaccharide ratio, as was the case in β-lg/xanthan gum (Laneuville *et al.* 2006) or pectin/poly-L-lysine systems (Marudova *et al.* 2004).

It should also be mentioned that complex formation between globular proteins and polysaccharide could be used to produce thermogels with various viscoelastic properties (Mann and Malik 1996; Cai and Arntfield 1997). For example, heat treatment of β-lg/CMC coacervate led to a viscoelastic particulate network after heat denaturation of the protein at 90 °C (Capitani *et al.* 2007).

16.5.2 Adsorption at oil/water interface and emulsifying properties

Due to the dual hydrophobic/hydrophilic nature of protein–polysaccharide complexes, their functionality at the oil/water interface and related emulsifying properties has been the purpose of most of the studies reporting on protein–polysaccharide functionality during these last 20 years (Gurov and Nuss 1986; Dickinson 1998; Benichou *et al.* 2007c).

The complex formation between protein and polysaccharide was shown to affect the adsorption behaviour of the protein at the oil/water interface, but also the resulting interfacial tension. Ganzevles *et al.* (2007a) reported that the adsorption rate at the oil/water interface for β-lg/pectin complexes was lower than for the protein alone. This difference was explained by the larger hydrodynamic radius of the complexes compared to the protein, leading to a lower diffusion coefficient in the bulk. It was confirmed by the subsequent increase of the surface tension drop rate close to that of the protein when salt was added to the system. Nevertheless, the equilibrium surface tension value reached at the equilibrium was similar for the β-lg and for its complexes with pectin. Interestingly, it seemed that the lower surface adsorption rate due to diffusion was not a general rule and might be modulated by the intrinsic properties of the biopolymers. For example, a faster adsorption rate of pea globulin/acacia gum coacervates to the vaselin/oil interface was reported compared to pea globulin (Ducel *et al.* 2005b). Such variability in the results could be explained by the lower surface hydrophobicity of β-lg compared to pea globulin, leading to a larger favourable adsorption energy for the latter complexes. These differences in the protein structure on the decrease of surface tension was clearly demonstrated for the same protein, *Faba* pea legumin, comparing the intact protein and a partially hydrolyzed fraction that was more hydrophobic after complex formation with chitosan (Plashchina *et al.* 2001). Both types of complexes were able to lower significantly the interfacial tension depending on the protein to polysaccharide ratio, but complexes with hydrolysed legumin reduced it by more than two times compared to intact protein. The reason

probably resided in an optimal conformation of the protein at the interface, exposing the hydrophobic regions to the interface, while interacting with the chitosan chains on the bulk side, as also proposed previously for the β-lg/pectin system (Ganzevles *et al.* 2007a).

Regarding the interfacial rheology of the adsorbed films composed by protein–polysaccharide complexes, these were generally characterized by an increased surface shear viscosity, indicating formation of a dense and viscous phase at the interface of the oil droplets (Dickinson and Galazka 1992). This interfacial network formation was also highlighted from interfacial dilational measurements, where it was shown that compact layers (high dilational modulus) were formed at protein to polysaccharide ratios close to electro-neutrality of the β-lg/pectin complexes, whereas more diffuse layers were formed (low dilatational modulus) if the complexes were initially soluble (Ganzevles *et al.* 2007a). Similar behaviour was reported for a given ratio, but different pH values, in the pea globulin/acacia gum system, where the higher oil/water interfacial elasticity was obtained for neutral complexes (Ducel *et al.* 2005b).

It should be noticed that several studies proposed not to adsorb complexes at the oil/water interface, but to create the complexes directly at the interface after the protein has been adsorbed, using the so-called layer-by-layer method (McClements 2006). These two methods led to very different physical properties of the emulsions, especially in terms of stability, and we will review some of them in the next paragraph (Garti *et al.* 1999; Jourdain *et al.* 2008).

The use of BSA/dextran sulfate complexes at a 1:3 mixing ratio was shown to improve greatly the stability of a *n*-tetradecane 10 wt% emulsion with no changes in size distribution for more than 3 weeks, compared to the protein stabilized emulsion (changes in size distribution after 24 hours) (Dickinson and Galazka 1992). Similar improvement of emulsion stability was described when complexes of *Portulaca oleracea* gum/casein complexes formed at pH 3 were used (Garti *et al.* 1999). It seems important to note here that the protein to polysaccharide ratio played a major role in the stabilization of emulsions stabilized by protein–polysaccharide complexes formed in the bulk vs. those formed at the interface. Hence, complexes should not be formed in charge neutralization conditions, in order to maintain sufficient electrostatic repulsions between the oil droplets. In addition, the polysaccharide should not be in excess in order to avoid oil droplets bridging flocculation. These conditions of emulsion stability were clearly demonstrated in a study involving whey protein isolate/chitosan complexes (Laplante *et al.* 2006). Hence, for protein/polysaccharide ratios leading to a reduction of the interfacial net electrostatic repulsions, flocculation and syneresis of the emulsion were observed. Very similar results have been reported for whey protein/pectin stabilized emulsions (Neirynck *et al.* 2007). For the study involving chitosan, it should, however, be added that even if the increase of the ionic strength led to a similar reduction of interfacial charge of the droplets at a given ratio, emulsion stability could still be achieved; the likely reason being that in these conditions, more chitosan was adsorbed in the

interfacial layer, inducing steric stabilization of the droplets (Laplante *et al.* 2006; Speiciene *et al.* 2007). Recently, Jourdain *et al.* (2008) showed that emulsions prepared with already formed caseinate/dextran sulfate complexes (mixed emulsions) were more stable regarding pH lowering compared to layer-by-layer emulsions, which could be explained by the different interfacial structures formed at the interface in both cases. In the layer-by-layer case, the interface was probably composed by a protein layer onto which dextran sulfate adsorbed electrostatically, whereas in the case of mixed emulsion, DS formed a more compact layer together with the casein molecules and was therefore less prone to desorption at low pH.

The formation and stability of layer-by-layer emulsion have been extensively studied by the group of Prof. McClements, using several protein–polysaccharide pairs, and even multilayers (Guzey and McClements 2006b). Nevertheless, Dickinson and Pawlowsky (1997) already reported that it was possible to produce a network of polysaccharide stabilized oil droplets emulsified with BSA upon controlling the quantity of ι-carrageenan added to the emulsion. Lower and higher polysaccharide concentrations led to depletion flocculation or aggregation of the oil droplets. Very similar results were reported for whey proteins/κ-carrageenan stabilized emulsions (Singh *et al.* 2003). The creaming stability of layer-by-layer emulsions stabilized by β-lactoglobulin/pectin or β-lactoglobulin/ι-carrageenan towards pH and ionic strength environmental stresses was shown to be improved by secondary emulsification by ultrasound or secondary emulsification in an ι-carrageenan dispersion (Guzey *et al.* 2004; Gu *et al.* 2005a, 2005b). Both techniques allowed creation of protein–polysaccharide multilayers at the oil droplet interface. Interestingly, these results led to the design of multilayered emulsion composed by the superimposition of several protein–polysaccharide layers, such as, for example, β-lactoglobulin/ι-carrageenan/gelatine, each layer being able to respond specifically to an environmental stress, such as, for example, pH change, ionic strength increase or temperature change (Gu *et al.* 2005b). This latest interfacial engineering might be useful for the development of functional capsules to be used in food applications, and will be discussed in the next section.

We now turn to a review of the air/water interfacial and foaming properties of protein/polysaccharide complexes and coacervates.

16.5.3 Adsorption at air/water interface and foaming properties

The ability of protein–polysaccharide electrostatic complexes to stabilize the air/water interface was reported early for the whey protein/carboxymethylcellulose system (Hansen and Black 1972). Thus, these complexes exhibit air/water surface activity due to their amphiphilic nature, as was the case for oil/water interface stabilization. It was shown that complexes obtained between BSA and ι-carrageenan were able to lower the air/water interfacial tension, to the same extent as BSA alone, but at a reduced adsorption rate (Dickinson and Pawlowsky 1997). This delay in interfacial adsorption might be related to the reduced

diffusion coefficient of the complexes compare to the protein alone. This hypothesis was confirmed for the β-lg/pectin system (Ganzevles *et al.* 2006a). It was shown that the rate of surface pressure increase was lower for conditions where more protein–polysaccharide complexes were formed, i.e., for lower pH values or high protein–polysaccharide ratios. An interesting feature of this system was also that pectins with a lower degree of esterification (more negative charges) were delaying more importantly the interface adsorption because of the size of the complexes formed compared to highly esterified pectins (Ganzevles *et al.* 2006a). Similar observations of lower diffusion at the air/water interface were made upon adsorption of ovalbumin/pectin complexes at acidic pH (Kudryashova *et al.* 2007).

Results on adsorption kinetics might be triggered by the molecular characteristics of the polysaccharide. Hence, complexes of β-lg with the globular polysaccharide acacia gum were able to lower interfacial tension as fast as the protein alone (Schmitt *et al.* 2005a). This might be due to the more compact structure of these complexes compared to pectin-based ones, pectin molecules being more linear biopolymers than acacia gum. This hypothesis was tested recently on a system composed by β-lg interacting with pullulan grafted with carboxyl group leading to charge densities ranging from 0.1 to 0.61 (Ganzevles *et al.* 2007b). It was shown that the adsorption kinetics were lowered for the β-lg/pullulan complexes having the highest charge densities, which in turn led to the soluble complexes with the largest hydrodynamic radius.

The interfacial properties of the protein–polysaccharide films were shown to be strongly dependent on the way complexes were formed. Complex formation at the interface led to dilational modulus up to two times higher than for complexes adsorbed from the bulk (Ganzevles *et al.* 2006b). The authors explained that, in the first case, pectin molecules were able to reinforce the existing adsorbed protein layer, whereas in the second case, protein adsorbed less easily at the interface due to its interaction with the pectin chains. Interestingly, it was shown that the surface shear modulus was independent of the protein–polysaccharide ratio for the already adsorbed protein layer, but was maximized when complexes formed in the bulk reached electroneutrality. Neutral β-lg/acacia gum complexes were also shown to increased interfacial dilational viscoelasticity compared to the protein alone (Schmitt *et al.* 2005a). This led also to thicker interfacial films with reduced gas permeability and increased lifetimes (Schmitt *et al.* 2005a; Liz *et al.* 2006). Schmitt *et al.* (2005b) reported the importance of possible rearrangements of β-lg/acacia gum complexes at the interface on the stability of the resulting air/bubble and foams. Adsorption of neutral complexes led to thick viscoelastic hydrated films with low gas permeability, whereas already formed coacervates led to thin elastic films with lower water content and higher gas permeability. Figure 16.6 clearly shows the formation of a thick interfacial layer composed of β-lg/acacia gum complexes having rearranged into a coacervate phase at the interface of air bubble within foam at the EEP. Interestingly, flowing isolated coacervates could be seen in the Plateau border (due to gravitational drainage), but were not

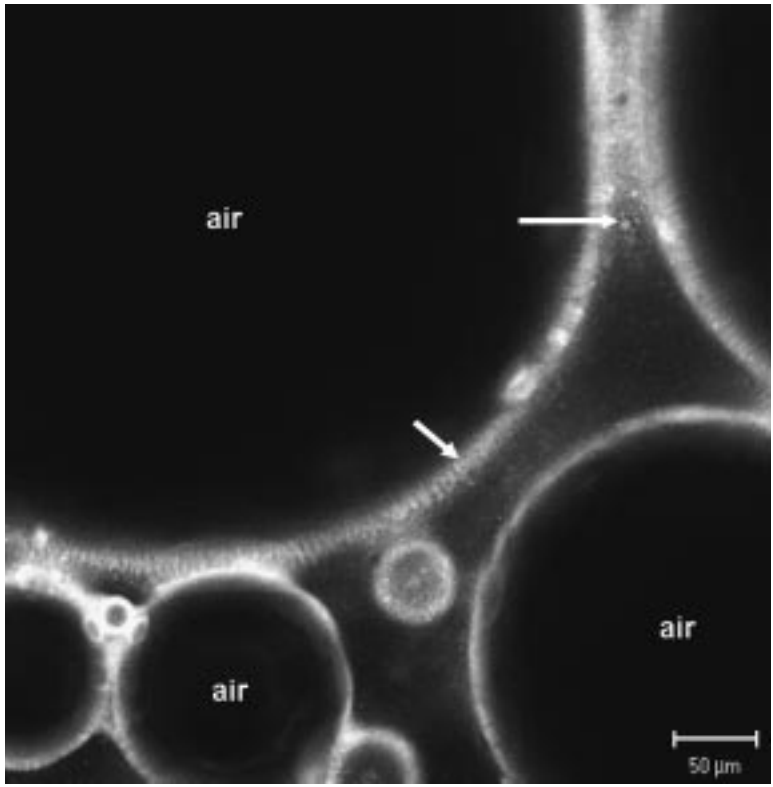

Fig. 16.6 Confocal scanning laser micrograph of aqueous foam stabilized by fluorescently labelled β-lg/acacia gum complexes and coacervates at a total biopolymer concentration of 1 wt% at pH 4.2 and a protein to polysaccharide ratio of 2 : 1. Arrows indicate interfacial layer stabilized by fused coacervates and free coacervates draining in the thin-film. Scale bar is 50 μm. Copyright Nestlé Research Center, Lausanne.

integrated into the interface. Ganzevles *et al.* (2007b) also reported that varying the charge density of the pullulan (by grafting of carboxylic groups) upon complex formation with β-lg led to various interfacial shear moduli at the air/water interface. In this case, the polysaccharide was controlling the protein conformation at the interface through its charge density by the formation of more or less compact complexes.

Several studies reported the improved foam stabilization properties of protein–polysaccharide complexes and related the improvement compared to the single protein mainly to a combined effect of interfacial properties but also from the local increase of the bulk viscosity in the thin-films due to the formation of the complexes (Poole 1989; Ahmed and Dickinson 1991; Glaser *et al.* 2007).

As was shown above, protein–polysaccharide complexes exhibit a variety of functional properties enabling their use in several food and non-food applications (Samant *et al.* 1993; Schmitt *et al.* 1998; Cooper *et al.* 2005), and these are now discussed in Section 16.6 and 16.7, respectively.

16.6 Food applications of protein–polysaccharide complexes and coacervates

Some food and non-food applications of protein–polysaccharide complexes and coacervates will overlap (protein separation, microencapsulation). Nevertheless, in order to give a clearer overview of both fields, these applications will be considered separately.

In this section, we consider the use of complex and coacervate formation for protein purification, microencapsulation, production of fat substitutes and texturization in food products. In the last subsection, some other food applications will be reviewed.

16.6.1 Protein purification

The differences in the IEP of the various food proteins allow recovering them selectively by complex formation with polysaccharides upon proper adjustment of the environmental conditions such as pH, ionic strength and protein to polysaccharide ratio. This purification method offers the advantage of not using organic solvents. In addition, it enables the re-use of the polysaccharide and allows large-scale purification processes. The main drawback is that it is mainly a batch process, unless the polysaccharide can be immobilized on a support. Several studies reported on the extraction of the major whey protein fractions upon complex formation with carboxymethylcellulose (CMC) in acidic conditions (Hansen *et al.* 1971; Hill and Zadow 1974). For example, it was shown that BSA and β-lg could be specifically removed from whey at pH 4.0 using CMC. Further decrease of the pH to 3.2 enabled extraction of α-la, the remaining soluble fraction being composed mainly of proteose peptones (Hidalgo and Hansen 1971). Similar results could be obtained using apple pectin that allowed recovery of 90% of all the whey proteins at pH 3.4, but with no selectivity (Serov *et al.* 1985). Whey protein recovery could also be achieved in basic pH conditions when chitosan was used for complex formation. For example, up to 90% of β-lg could be removed from cheese whey upon complex formation with chitosan at pH ranging from 8 to 10 and ionic strengths ranging from 0.08 to 0.3 M (Montilla *et al.* 2007). Interestingly, a variety of other proteins (e.g., soy or potato proteins) could be fractionated using the same principle (Smith *et al.* 1962; Vikelouda and Kiosseoglou 2004). For example, an efficient and simple technique for isolation of immunoglobulins Y (IgY) from egg yolk by anionic polysaccharides was developed (Chang *et al.* 2000). These proteins can be used as immunological supplements in infant formulae and other foods. Isolation conditions of IgY in egg yolk were optimized by the addition of various levels of Na-alginate, λ-carrageenan, Na-CMC, and pectin to diluted egg yolk. It was found that the pH value of the polysaccharide–yolk mixture affected both the quantity of polysaccharide-lipoprotein precipitates and the immunoactivity recovery of IgY. The IgY recovery was determined to be 33–74% by means of single radial immunodiffusion method when IgY was isolated under optimal conditions. Among the polysaccharides tested in this

study, the use of 0.15% pectin exhibited the best immunoactivity recovery of IgY at pH 5.0.

16.6.2 Microencapsulation

In food applications, microencapsulation can be used in order to protect food bioactive molecules that are sensitive to external stresses such as pH, oxygen content or temperature (Table 16.2). For example, Oliveira *et al.* (2007) evaluated the resistance of microcapsules containing *Bifidobacterium lactis* and *Lactobacillus acidophilus*, produced by complex coacervation using a casein/pectin system, to the spray drying process, a shelf-life of 120 days at 7–37 ºC and the *in vitro* tolerance after being submitted to acid pH (pH 1.0 and 3.0) solutions. The process used and the wall material were efficient in protecting the micro-organisms; however, microencapsulated *Bifidobacterium lactis* lost its viability before the end of the storage time. Other important uses could be to provide a controlled release of aroma or flavours before or upon consumption of the food or even to mask the bad taste of some biologically active material (Gouin 2004).

Any type of triggers can be used to prompt the release of the encapsulated ingredients, such as pH changes, mechanical stress, temperature, enzymatic activity, time, osmotic force, etc. In the case of probiotics, the use of micro-encapsulation for controlled-release applications is a promising alternative to solving the major problems of these organisms that are faced by food industries (Anal and Singh 2007). The probiotics are protected from bacteriophage and harsh environments, such as freezing and gastric solutions and their encapsulated form can be used in many fermented dairy products, such as yoghurt, cheese, cultured cream and frozen dairy desserts, and for biomass production (Anal and Singh 2007). Yeo *et al.* (2005) used temperature as a trigger to controlled release of flavour oil to improve the appeal of frozen baked foods upon heating. They have encapsulated flavour oil in complex coacervate microcapsules using gelatine and gum arabic. The oil remained encapsulated for 4 weeks of storage at 4 and −20 ºC (freezing and thawing) but was released by exposure to 100 mM NaCl at room temperature. When particles were cooled after releasing their oil content, the oil was re-encapsulated.

It is worth mentioning that two methods of encapsulation can be followed for food applications. Interfacial complex coacervation or layer-by-layer deposition around an oil phase can be used. This technique generally leads to microcapsules having diameters ranging from 1 to 50 μm (Lamprecht *et al.* 2001; Weinbreck *et al.* 2004a). Alternatively, complexes can be formed at the surface of a protein or polysaccharide matrix, leading to sub-micrometer particles (Chen and Subirade 2005; Hong and McClements 2007). In both cases, the oil phase and the matrix contain the molecule(s) of interest.

In the case of oil droplet encapsulation, besides the fact that pH, ionic strength, protein to polysaccharide ratio or total biopolymer concentration must be optimized, an important subsequent processing step is to stabilize the

Table 16.2 Examples of protein–polysaccharide systems used for microencapsulation purposes in food and non-food applications

Systems	Technique/Encapsulated material	References
Food applications		
Whey protein isolate/ xanthan gum, /fenugreek gum	Oil in water in oil emulsion/ Medium chain trigycerides/ triacetin, flumethrin®	Benichou *et al.* (2007a)
Whey protein isolate/ xanthan gum, /galactomannans	Water in oil in water emulsion/ Medium chain triglycerides, vitamin B1	Benichou *et al.* (2007b)
2-gliadin/, pea globulin/ acacia gum	Oil in water emulsion/Medium chain triglycerides	Ducel *et al.* (2004)
Gelatine/acacia gum	Oil in water emulsion/ Eicosapentaenoic acid ethyl ester (EPA)	Lamprecht *et al.* (2001)
Whey protein/gum arabic	Oil in water emulsion/Lemon and orange oil flavour	Weinbreck *et al.* (2004a)
Gelatine/gum arabic	Oil in water emulsion/10% natural oils from anise, oregano, garlic, black pepper in 90% vegetable oil	Yeo *et al.* (2005)
Non-food applications		
Gelatine/acacia gum	Oil in water or water in oil emulsion using microchannels/Soybean oil	Nakagawa *et al.* (2004)
Gelatine/gum arabic	Oil in water emulsion/Paraffin oil	Mayya *et al.* (2003)
Alginate/poly-L-lysine	Aqueous coacervation and $CaCl_2$ reticulation/Erythropoietin-secreting cells	Murua *et al.* (2007)
Gelatine/acacia gum	Oil in water emulsion/ Methoxybutropate	Palmieri *et al.* (1999)
Gelatine/sodium alginate	Oil in water emulsion/Dalarelin	Ryszka *et al.* (2002)
Gelatine/acacia gum	Oil in water or water in oil emulsion/Clove oil, sulphamethoxazole	Thimma and Tammishetti (2003)
Gelatine/acacia gum/ tannins	Oil in water emulsion/Capsaicin	Xing *et al.* (2003)
Gelatine/acacia gum	Oil in water microemulsion/ Cypermethrin	Zhu *et al.* (2005)

interface against pH and ionic strength changes to avoid capsule disruption. Hence, it has been clearly shown that unstabilized microcapsules were much more sensitive to leakage or oxidation than crosslinked ones (Weinbreck *et al.* 2004a). If this can be achieved by formation of covalent bonds between protein and polysaccharide by use of glutaraldehyde for non-food applications, other biocompatible means of crosslinking have been developed for the food industry.

Some authors described the formation of multilayers around the oil droplets, each layer being sensitive to different environmental stresses (Harnsilawat *et al.* 2006b). Another approach could be to use food grade solvents (ethanol) or spray drying to dehydrate the interfacial coacervates as has been shown for gelatine/acacia gum capsules loaded with Ω-3 unsaturated fatty acids (Lamprecht *et al.* 2001). Interestingly, it was recently shown that the use of glycerol to crosslink gelatine/acacia gum capsules loaded with sesame oil extract led to similar results to capsules crosslinked with formaldehyde (Huang *et al.* 2007). An alternative solution is to use an oxidizing agent such a dihydroascorbic acid or even 'food-grade' crosslinking enzymes (Weinbreck *et al.* 2003a; Strauss and Gibson, 2004; Littoz and McClements 2008). The obtained microcapsules can thereafter be introduced in a food matrix and release aroma and bioactives, as has been shown for limonene-loaded whey protein/acacia gum capsules in Gouda cheese (Weinbreck *et al.* 2004a).

When a protein or a polysaccharide matrix is used, stabilization can be achieved by subsequent heat treatment of the system. This promotes strong hydrophobic interactions in addition to the initial electrostatic ones as has been shown for β-lactoglobulin/chitosan complexes (Chen and Subirade 2005; Hong and McClements 2007). Nevertheless, it was also shown that non-heat-stabilized chiosan/β-lg core/shell nanoparticles loaded with brilliant blue were less prone to acid and pepsin degradation in simulated gastric conditions, probably because of the different reactivity of native and denatured forms of β-lg (Chen and Subirade 2005).

16.6.3 Food products texturization

Protein–polysaccharide complexes have been shown to be able to control gel-like product texture or replace fat or meat in several food applications (Sanchez and Paquin 1997; Schmitt *et al.* 1998; Norton and Frith 2001).

Owing to the rheological and heat sensitivity of several globular proteins and fibrillar polysaccharides, appropriate processing conditions (heat treatment, homogenization) allowed the production of mixed microparticles matching the textural attributes of fat (spherical aggregates in the range of 1–10 μm) or meat (fibrillar aggregates in the range 1 to 50 μm). For example, Chen *et al.* (1989) reported the use of microfragmented milk and/or egg proteins/xanthan gum complexes obtained upon interaction at pH 3.7 to 4.2 as a fat substitute. The formed complexes had a diameter of around 15 μm, which is close to values reported for electrostatic complexes between whey proteins and xanthan gum (Laneuville *et al.* 2000). These fibrillar complexes were thereafter heat treated to increase their stability by exposure of hydrophobic regions of the protein and microfragmented under high pressure (200–800 bars) and shearing, leading to microparticles of about 2 to 10 μm. Such whey proteins/xanthan gum complexes were shown to efficiently allow reduction of fat in cake frostings or sandwich cookie fillings, provided that the amount of complexes was adapted to the water activity of the sample (Laneuville *et al.* 2005a). Recently, it was shown that

thermogels with a very wide range of texture could be obtained upon complex formation of β-lg or α-la with carboxymethylcellulose (Capitani *et al.* 2007). The production of meat substitutes was achieved in a very similar way, but in this case, alginate and pectins were used to complex milk and soy proteins (Tolstoguzov *et al.* 1974). Interestingly, it was also shown that texturization properties could be obtained without requiring a heat treatment of the complexes as was the case for chitosan/chicken salt-soluble proteins (Kachenechai *et al.* 2007) or amidated pectins and sodium caseinate (Matia-Merino *et al.* 2004). In this respect, polysaccharides played the function of electrostatic crosslinking agents. Recently, similar modulation of protein gel texture was reported upon *in-situ* acidification of β-lg/xanthan gum (Laneuville *et al.* 2006) or skimmed milk/exopolysaccharide mixtures using GDL (Girard and Schaffer-Lequart 2007). In these studies, the importance of the molecular weight of the xanthan gum or the molecular structure of the exopolysaccharides in the rheological properties of the obtained gels was clearly shown.

16.6.4 Interface stabilization

Protein–polysaccharide complexes can be used to stabilize interfaces in several food products. For example, creaming stability of a 30 wt% soya oil emulsion stabilized by whey protein isolate/κ-carrageenan complexes (Singh *et al.* 2003) and 40 wt% rapeseed oil stabilized by whey protein isolate/chitosan complexes (Speiciene *et al.* 2007) could be efficiently improved in acidic conditions. This was due mainly to the formation of a weak electrostatic network between the oil droplets, as it has been previously described for cold-protein gels. Use of spray dried complexes made of whey protein and carboxymethylcellulose (CMC) was tested in order to stabilize 10 or 20 wt% corn oil in comparison to pure whey protein isolate (Girard *et al.* 2002b). It was concluded that the complexes were clearly improving the stability of the oil droplets against coalescence, but were promoting some flocculation due to the presence of a minor fraction of uncomplexed CMC. Similar freeze-dried complexes at a concentration of solids of 4 wt% were able to produce very dense and thick foams that were very close to those obtained with egg white (Hansen and Black 1972).

Poole (1989) reported the very high foaming and foam stabilizing properties of whey protein/chitosan complexes in a model sucrose/corn oil emulsion, likely because complexes were encapsulating the fat, preventing its detrimental spreading at the air/water interface. More recently, the use of whey protein isolate/acacia gum complexes was shown to improve significantly the heat shock stability of air bubbles within a complex acidic ice cream matrix (Kolodziejczyk and Schmitt 2004). Figure 16.7 shows whey protein isolate/acacia gum complexes formed instantaneously in the ice cream mix during whipping/freezing and adsorbed at the interface of the air bubbles. A coacervate layer was formed, conferring very low gas permeability to the bubbles upon several freeze/thawing cycles.

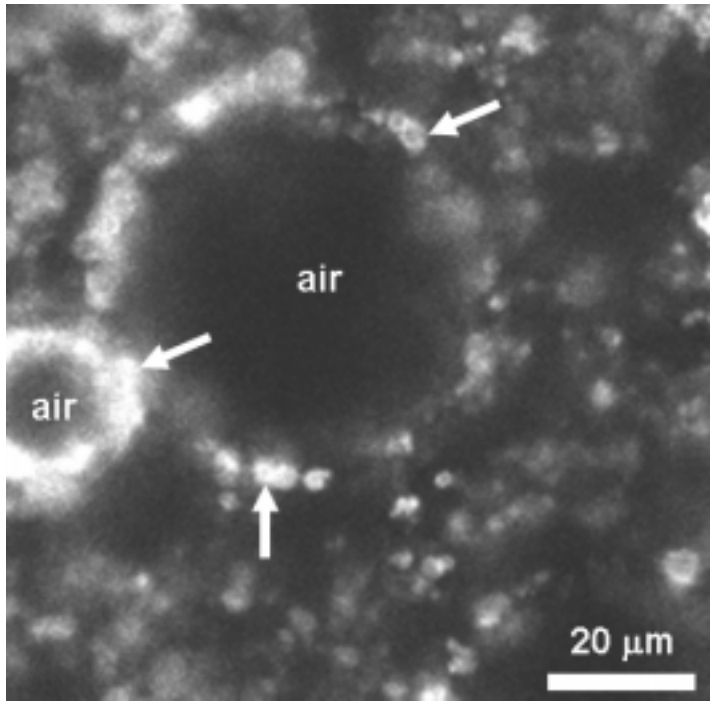

Fig. 16.7 Confocal scanning laser micrograph of a strawberry ice-cream stabilized by whey protein isolate/acacia gum complexes and coacervates at pH 4.2 after 7 days of storage at −40 °C. The protein was coloured by the rhodamine 6G reagent. Arrows indicate the coacervates adsorbed at the interface of the air bubbles. The scale bar is 20 μm. From Kolodziejczyk and Schmitt (2004). Copyright Nestlé Research Center, Lausanne

16.6.5 Other food applications

Some other food applications of protein–polysaccharide complexes might be the design of edible films as has been described for a system composed by soy protein/sodium alginate or /propylene glycol alginate (Shih 1994). Interestingly, it was recently shown that a combination of lysozyme and chitosan with an edible film was able to confer a very high potential for antimicrobial activity (Park *et al.* 2004). In this case an important parameter to control was the protein to polysaccharide ratio that was both controlling the antimicrobial activity, but also the physical properties of the film.

Finally, it has recently been shown that protein–polysaccharide complexes were probably importantly involved in some in-mouth fat perception attributes as the flocculation of lysozyme-stabilized emulsion was due mainly to interaction with the glycated saliva mucin proteins (Silletti *et al.* 2007). Interestingly, such type of electrostatic complex formation has also been discovered between mucins and chitosan (Dedinaite *et al.* 2005).

16.7 Non-food applications of protein–polysaccharide complexes and coacevates

A variety of applications of protein–polysaccharide or protein–polyelectrolyte complexes can be described. They range from hydrogels for protein or enzyme release and entrapment, synthesis of biosensors, use as surfactants in cosmetic applications (T.X. Wang *et al.* 2000) to protein separation (Strege *et al.* 1990) as it was already the case for food applications. Recent examples of these applications can be found in a very detailed review by Cooper *et al.* (2005). In the next two subsections, we consider mainly uses for microencapsulation and synthesis of biomaterials.

16.7.1 Microencapsulation

An important field for non-food applications of protein–polysaccharide complexes and coacervates is microencapsulation for the pharmaceutical industry (Burgess 1994; Table 16.2). Early on, Bungenberg de Jong (1949b) reported that is was possible to entrap carbon particles or ink directly into the dispersed gelatine/acacia gum coacervate phase. This technique was also used to encapsulate micronized Naproxen into gelatine A/gelatine B microcapsules (Burgess and Carless 1985). The drug was dispersed in glycerol and mixed with gelatine B dispersion before coacervation occurred. The yield of drug encapsulation was around 50% in the best conditions of protein to polysaccharide ratio and temperature. In the same vein, Ryszka *et al.* (2002) reported the preparation of microcapsules in the form of a biodegradable coacervate containing dalarelin (analogue of the gonadotrophin-releasing hormone), with a gelatine/alginate coating. The microcapsules were obtained with yield of $66.4 \pm 2.1\%$. It was found that the dalarelin in the form of microcapsules had better bioavailability and was active longer in rats when compared with the dalarelin solution injections.

Ponsart and Burgess (1996) reported the encapsulation of the β-glucuronidase enzyme into three different coacervating systems consisting in gelatine/acacia gum, gelatine/sodium alginate and BSA/acacia gum in the optimum coacervation conditions of pH, ionic strength and protein to polysaccharide ratio. Stabilization of the microparticles was achieved by spray drying, leading to diameters around 5 μm. The enzyme encapsulation yield was shown to be low, 28.8%, but was, however, higher than for non-spray-dried microparticles (11.3%). Interestingly, it was shown that the activity of the enzyme could be maintained upon hydration of the microparticle in phosphate buffer over a period of 8 days, revealing the interesting controlled release properties of these systems.

Recently, a similar approach was used to increase the oral delivery of low molecular weight heparin, tinzaparin, which is not highly negatively charged (Lamprecht *et al.* 2007). In this case, an encapsulation yield of more than 90% was obtained using gelatine A or B in combination with acacia gum. Oral delivery quantification showed that gelatine B particles lead to 4.2% oral

absorption compared to no adsorption with the pure protein or in gelatine A/acacia gum particles, showing the importance of the microparticle charge for promoting adsorption at a given pH.

Xing *et al.* (2006) studied the antimicrobial activities of capsaicin microcapsules prepared by the complex coacervation of gelatine, acacia gum and tannins. The results showed that the optimum pH for the antimicrobial effect was about 5.0, which might be related to the strongest protein-precipitating ability of tannins at this pH value. Another type of sub-micronic pH sensitive particles was obtained upon complex formation between chitosan and ovalbumin (Yu *et al.* 2006). Worth noting was the fact that the hydrophobicity/hydrophilicity of these nanogels could be triggered upon pH adjustment as was demonstrated from fluorescence spectroscopy upon pyrene addition. It was shown that between pH 2 and 5, nanogels were hydrophobic, whereas they became more hydrophilic up to pH 6.8. The likely explanation was the change in the intensity of the electrostatic interactions between the two biopolymers upon pH increase, leading to several molecular conformations of the chitosan chains within the gels.

Another common way of producing microcapsules using complex coacervation is to achieve interfacial coacervation around an oil droplet as already described for food applications. Such technique allows encapsulating hydrophobic molecules that cannot be easily dispersed in the coacervate phase. This was done for example for the anti-inflammatory drug, ketoprofen, using gelatine/acacia gum microcapsules (Palmieri *et al.* 1996). Recently, Lamprecht *et al.* (2000) gave a very interesting insight into the structure of the microcapsules made of gelatine and acacia gum around oil droplets. Using confocal scanning laser microscopy, they were able to localize specifically the two biopolymers into the shell of the microcapsule and demonstrated that the two biopolymers were evenly distributed. Remarkably, the authors showed that if a ternary labelled hydrophobic protein was used (casein in this case), it had the tendency to adsorb preferentially at the oil/water interface, enabling an additional control of the release of the encapsulated material. The same system was used by Mayya *et al.* (2003) to encapsulate paraffin oil in the presence of sodium dodecyl sulfate. They reported that encapsulation yield increases from 35% to 70% in the presence of small quantities of the oppositely charged surfactant.

16.7.2 Biomaterials

Another important field of use of protein–polysaccharide complexes is the synthesis of biomaterials. When used as biomaterials and matrices in tissue engineering, biopolymers offer important options to structure, morphology and chemistry as reasonable substitutes or mimics of extracellular matrix systems (ECM). These features also allow controlling material functions such as mechanical properties in gel, fibre and porous scaffold formats (Velema and Kaplan 2006). Hence, as these materials are supposed to be in direct contact with living organisms, replacing the extracellular matrix, they should be

composed with biopolymers that can be found in the organisms or be as close as possible in terms of structure (Ellis and Yannas 1996). In this respect, chitosan was shown to be relatively close to the structure of glycosaminoglycans (GAGs) occurring in living cells. It was therefore extensively used as a complex with collagen or with its hydrolysate product, gelatine.

Taravel and Domard (1996) studied the interaction of collagen with chitosan and reported on the mechanical and structural properties of the films that were obtained. Such structural properties are very important in order to control the assimilation of the biomaterial by the living body, as, for example, the porosity of the protein–polysaccharide-based ECM was shown to control the speed development and colonization of the graft by the fibroblasts. Porosity could, for example, be modulated by controlling the freezing rate upon drying of collagen/GAG complexes (O'Brien *et al.* 2004). Interestingly, protein–polysaccharide complexes could also serve as a carrier for bioactive molecules in gene therapy as has been shown for the delivery of plasmid DNA to articular chondrocytes using a collagen/GAG matrix (Samuel *et al.* 2002). Here, it was shown that DNA incorporated in the scaffold at pH 7.5 was better released over 28 days upon incubation in Tris-EDTA buffer compared to pH 2.5. In addition, DNA kept a more 'natural' coiled structure in neutral pH conditions.

Erbacher *et al.* (1998) studied pharmacological approaches to gene therapy based on non-viral DNA vectors using chitosan to form polyelectrolyte complexes. They found that chitosan presents some characteristics favourable for gene delivery, such as the ability to condense DNA and to form homogeneous population of complexes (diameter: 50–100 nm). The characterization of physico-chemical properties of this system was carried out by Mao *et al.* (2001). They also confirmed the offered protection for encapsulated DNA from nuclease attack. Recently, the *in vitro* and *in vivo* transfection efficiency of chitosan-pDNA nanoparticles obtained by complex coacervation were studied using quaternized chitosan – 60% trimethylated chitosan oligomer (TMCO-60%) – and two other chitosan molecules differing in molecular weight (Fang *et al.* 2007). It was found that TMCO-60%/pDNA nanoparticles had better *in vitro* and *in vivo* transfection activity than the other two molecules, with especially most prominent delivery in the gastric and upper intestinal mucosa. In the same vein, it was recently shown that 5–10 μm chitosan/gelatine microspheres could be used for controlled and site-specific release of fibroblasts growth factor (Liu *et al.* 2007). It was shown that the growth factor released could be extended for 14 days, with an accumulated release yield of around 72% leading to a significant development of fibroblasts compared to a control with no added microphores.

16.8 Conclusions

In this chapter, we have attempted to give a concise overview of the field of protein–polysaccharide complexes and coacervates. Thanks to the development

of new investigation techniques and the use of computer simulations, increasing knowledge has been acquired on these systems in the last few years. Hence, thermodynamic parameters of the complex formation/coacervation are now accessible, enabling the probing and refining of theories from the late 1950s. However, more refined thermodynamic binding models are needed to take into account the inherent complexity of thermograms obtained with protein–polysaccharide mixtures, especially the existence of both exothermic and endothermic signals. In addition, computer simulations on monodispersed model systems allow prediction of phase behaviour and complex formation in relatively simple real synthetic or semi-synthetic systems. Increasing complexity of the models with experimentally measurable parameters would be required for application to more polydispersed natural systems and for more complex biomolecular architectures. In this respect, one would also expect models enabling prediction of the phase separation kinetics in protein–polysaccharide systems to be developed. The use of mesoscopic and multi-scale modelling techniques can be anticipated in the next few years.

An important contribution to the understanding of the complex and coacervate structure was permitted by the use of novel scattering and microscopy techniques, such as, for example, confocal scanning laser microscopy (CSLM) coupled with fluorescence recovery after photobleaching (FRAP) or particle tracking. Such experiments clearly demonstrated the dynamics of the coacervate phase that can be compared to a heterogeneous electrostatic network of polymers exhibiting capability to diffuse along the polyelectrolyte chains; the diffusion being controlled by the strength of the electrostatic interactions between the protein and the polysaccharide (depending on pH, ionic strength, mixing ratio and other biopolymer intrinsic properties).

In the last few years, emphasis has been given to extend the study of the functional properties of these complexes and coacervates, enabling identification of several interesting fields of applications in food and non-food products. Thus, the rheological and interfacial properties of protein–polysaccharide complexes might be advantageously used for designing food products with various textures, stability and delivery properties. The same conclusions apply to non-food applications, where an increased number of studies reported on the formulation of novel delivery systems sensitive to environmental stresses, enabling specific controlled release of bioactive molecules in the right organs or tissues. In order to expand the different applications, especially at the industrial scale, it would be necessary to better understand the effects of processing conditions such as temperature, shear stresses and pressure on the structure, stability and functional properties of protein–polysaccharide complexes and coacervates. Also, since the formation of protein–polysaccharide complexes and coacervates is mostly totally reversible upon pH changes, stabilization of these systems using physical parameters or by using specific enzymes or chelating biological molecules is expected to be developed in the future.

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17

Gum ghatti

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Abstract: This chapter describes the natural tree exudate gum from India. The exudate gum has a glassy appearance and the colour can vary from dark red to translucent white depending on the shape which can be either as a nodule or spiro. The chapter then reviews the structure and botanical source of gum ghatti. Technical information relating to its solubility, sugar composition, protein content, molecular weight, viscosity and emulsification performance are directly compared with gum arabic. Gum ghatti's current regulatory status together with various food and other applications are also described.

Key words: gum ghatti regulatory status, ghatti molecular weight, ghatti in beverage emulsion, ghatti applications, GATIFOLIA.

17.1 Introduction

Gum ghatti is a natural gum from India. It is exuded from the tree species *Anogeissus Latifolia* which is native to India and Sri Lanka. These trees constitute one of the largest forest coverages in India and are found mostly in dry deciduous forests. This tree can survive in harsh conditions and does not need a lot of water to survive, although if proper nutrients are provided this tree can grow very healthy and large. Gum exudation occurs during times of stress for the tree. The exudation process occurs very slowly over a period of days and depending on the size or age of the tree. The exuded gum nodules can range from 5 g to 50 g in weight. The shape of the exuded nodule depends on environmental conditions such as wind, sunlight, heat and other factors like the mass of the nodule, the pressure within the tree and the shape/size of the fissure from where the gum is exuding. There is no particular shape for a ghatti nodule as found for gum arabic variety *Acacia senegal*. Gum ghatti has a glassy appearance and the

Fig. 17.1 (left) Gum ghatti nodule known in the industry as 'SPIRO'; (right) gum ghatti nodule in a rounded tear shape.

colour of the nodule can vary from dark red to translucent white. The gum is collected in much the same way as most other natural tree exudate gums such as gum arabic and gum karaya. The local community in the forest region (Tribals), who constitute the main population of the forest areas, collect the exudate manually. These Tribals have extensive knowledge about the character of the trees in their local habitats, and provide a very good source of knowledge about the natural gum exudation and tree identification.

Figure 17.1 shows two types of exudates known as a *Spiro* exudation which is clear and tubular in form and a rounded tear shaped nodule from trees in the Central Indian Forest.

The tree grows on a well-drained slope and clay loam soil, and attains considerable height towards maturity in about 70–80 years. On dry rocky slopes, however, it tends to be stunted. It has a peculiar bark, smooth and greenish white in colour, and about 4–5 mm thick. The leaves are shaped somewhat like those of guava. Varying between 5 and 10 cm in length and 3 and 8 cm in width, these are alternate or sub-opposite in arrangement. These are thick and somewhat shining. Old leaves are shed between October and December, when these tend to get a beautiful reddish-brown hue. New leaves appear during February–March. Inflorescence starts appearing during May and stays on up to the end of June. The small flowers are off-white. Fruits start to show in July. When about to mature, these 8–10 mm × 5–6 mm drupes are peculiarly compressed, showing a two-winged form.

The *ghatti* tree grows best in the sunny aspect of peninsula ranges, Shivaliks and outer Himalayas at altitudes ranging from 200 m to 1250 m, and experiencing annual rainfall of 100–200 mm and temperatures varying from 5 to 40 ºC. However, it is quite capable of surviving in adverse climatic and geological conditions like dry rocky slopes. This tree has a moderate rate of growth. It has seven annual rings of growth in an inch of the cross section of its stem. Thus weighing about 28 to 32 kg to a cubic foot, it is one of the best hardwoods on the Indian sub-continent. Though quite difficult to season, it tends to split if left unattended in the sun. The splitting is less if the logs are kept in shade for about six months and sawn pieces are also kept in shade for a long period.

The gum is exuded from the bark of the tree and can be found all over the tree irrespective of the location such as a branch or the trunk, etc. The gum is exuded from the tree in extreme climatic conditions and acts as the buffer stock of nutrients required by the tree for survival. There is no need to make wounds in the bark of this tree to exude the gum as is the common practice with some other natural tree exudate gums. The gum exudation from this species is mostly due to the pressures created within the bark due to extreme natural conditions.

The gum comes naturally from the bark and can be collected by pulling the soft gel-like exude which collects on the tree bark at certain locations. If the gum is not plucked/picked from that location, fresh gum will not exude from the same position again. In order to increase the yield the common practice is to pick the old gum and make way for fresh gum to flow. The gum when freshly exuded has high moisture content and is very soft. When left in the open for days, the gum nodule loses moisture and hardens giving the nodule a rounded tear-like shape which is very hard and does not change. The gum has a faint sweetness to taste and a very faint odour which can only be recognised when the gum is kept in large volumes.

Within the Genus Anogeissus several species have been identified: *A. acuminata (A. pendula)*, *A. bentii*, *A. dhofarica*, *A. latifolia*, *A. leiocarpus (A. leiocarpa)*, *A. rotundifolia*, *A. Schimperi* and *A. sericea*. The specific gum which is being described here is the species *Latifolia* with the following botanical taxonomy:

Kingdom: *Plantae*
Phylum: *Angiosperms*
Class: *Magnoliatae*
Subclass: *Rosidae*
Order: *Myrtales*
Family: *Combretaceae*
Genus: *Anogeissus*

Anogeissus is a genus of trees native to South Asia, the Arabian Peninsula, and Africa, belonging to the family Combretaceae. The genus has eight species, five native to South Asia, two endemic to the southern Arabian Peninsula, and one native to Africa. *Anogeissus latifolia* is one of the most useful trees in India. *A. pendula* is common in the Kathiarbar-Gir dry deciduous forests of western India, where it often forms pure stands in the rocky ridges of the Aravalli Range. *A. leiocarpus* is found in Africa from northeastern Ethiopia to Senegal, and its bark is used to produce Anogelline, a substance used in cosmetics. *A. dhofarica* and *A. bentii* are endemic to the woodlands of the southern Arabian Peninsula.

Gum ghatti is known by many names throughout India and the rest of the world. It is commonly known as *Bakli*, *Tirman*, *Davedi*, *Dindal*, *Dindiga*, *Dhanta*, *Dhao*, *Dhawra*, *Dhokra*, *Dho*, *Kardhai* in different parts of India. Elsewhere in the world it is referred to as *Indian gum*, *Gummi indicum*, *Gummi indici* and the tree is known as *axlewood* in the United States and Australia.

17.2 Manufacture

The gum is usually supplied from the original producer in India to the global industry in lump form without any particular manufacturing process being applied. It is, nevertheless, distinguished on the basis of the amount of extraneous contamination present in the raw material. The main factors that contribute to purity are the colour and extraneous material. White, yellow and red grades are distinguished. There could be further grading according to the quality of the gum in which all the impurities such as bark, wood, sand, and non-ghatti products, etc., have been removed. In the past this mixture of different types was an inherent weakness, since the gum reaching the market in terms of colour, gel content and solubility, was very variable. Now, due to the establishment of a plantation, selection and cleaning before exporting, the quality has greatly improved. Due to this more recent improvement, it has been possible to produce a gum quality by special processing and spray drying which is almost completely soluble and of consistent colour. This special product has been termed GATIFOLIA.

17.3 Structure

Gum ghatti has an extremely complex structure composed of sugars such as L-arabinose, D-galactose, D-mannose, D-xylose, and D-glucuronic acid in a 48 : 29 : 10 : 5 : 10 molar ratio.[1] The gum contains alternating 4-O-subsituted and 2-O-subsituted α-D-mannopyranose units and chains of $1 \rightarrow 6$ linked β-D-galactopyrannose units with side chains which are most frequently single L-arabinofuranose residues. Using ^{13}C NMR spectroscopy suggested that ~6% of rhamnose are linked as side chain to the galactose backbone as α-Rhap-$(1 \rightarrow 4)$-β-galactopyrannose in common with the linkage found in gum arabic.[2]

Most of the information that we have about the structure of the polysaccharide components of gum ghatti has come from the classical work of that doyen of polysaccharide chemistry, Professor Edmund Hirst together with his colleague Gerald Aspinall and their co-workers over the period 1955 to 1969 at the University of Edinburgh.[1,3–5] They showed that the polysaccharide of gum ghatti has an extremely complex structure.

Complex arrays of neutral sugar units (Gal*p*, Ara*f*, Ara*p*) and GluA are attached to a molecular core of alternating β-D-GlucA and D-Man residues, the former linked though O-4 and the latter though O-2. The base structural units were identified using classical carbohydrate techniques.

The aldobiouronic acid:

β-GlcA(1-2) D-*Manp*

was first isolated as a product of the partial hydrolysis of damson and cherry gums and has subsequently been found to be a structural fragment of many plant gum polysaccharides. This unit is frequently found in the interior chains of these complex polysaccharides. It was found by partial hydrolysis of gum ghatti along

with another aldobiuronic acid: 6-O-(β-D-glucopyranosyluronic acid)-D-galactose. In this study by Aspinall[3] the longest oligosacchalride sequences found were:

β-D-Gal*p*-{*(1-6)*-β-D-Gal*p*-}$_n$(1-6)-D-Gal

β-D-Gal*p*-{*(1-6)*-β-D-Gal*p*-}$_n$(1-3)-L-Ara

Smith degradation led to the isolation of a degraded gum from which

3-O-/3-D-galactopyranosyl-D-galactose, 6-O-/3-D-galactopyranosyl-D-galactose (n = 0), 3-O-/3-D-galacto-pyranosyl-L-arabinose (n = 0),

and

3-O-L-arabinopyranosyl-D-mannose

were obtained on partial hydrolysis.

These results imply that D-mannosyl residues are located in the interior of the molecular structure, and the following partial structure was proposed, in which this structural unit was found:

–(1→6)-β-D-Galp-(1→6)- β-D-Galp-(1→3)-L-Arap-(1→3)-D-Manp-l

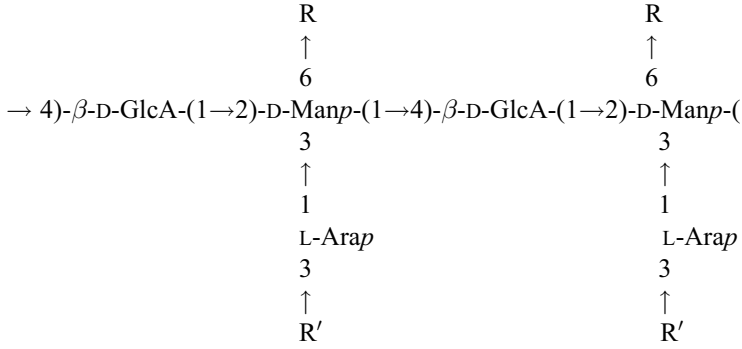
The chains of (1→6)-linked β-D-galactopyranosyl residues are joined through an L-arabinopyranosyl 'link' unit to a D-mannosyl residue in the basal chain. Subsequently, an acidic oligosaccharide (at first erroneously reported to be a trisaccharide) was characterised as the tetrasaccharide:

O-β-D-glucopyranosyluronic acid-(1→2)-O-D-mannopyranosyl-(1→4)-O-β-D-glucopyranosyluronic acid-(1→2)-D-mannose,

and this provided evidence for the following sequence of the sugar residues in the inner chains.

β-D-GpA-(1→2)-D-Man*p*-(1→4)-β-D-GpA-(1→2)-D-Man

Based on these results the overall nature of the assembly was proposed.[6]



R = – L-Ara*f*- or L-Ara*f*-(1→2, 3 or 5)-L-Ara*f*-(1

$$R' = \begin{matrix}\beta\text{-D-Glc}p\text{A-} \\ \text{or} \\ \beta\text{-D-Gal}p\text{-}\end{matrix} \left[\begin{matrix}(1 \rightarrow 6)\text{-D-Gal}p \\ 3 \\ \uparrow \\ R\end{matrix} \right]\text{-(1} \begin{matrix} \\ \\ \\ n\end{matrix}$$

The periodicity of the acidic groups in the main chain is an important feature and there are others scattered though the periphery. Also (1-3)- and (1-6)-linked Gal units make up associated side chains. This structure is further complicated by D-Man residues being present as double branch points. It is this molecular complexity that accounts for the quite spectacular rheological and solution properties of gum ghatti. It is the overall molecular shape arising from the periodicity of the chains which gives it a rod-like shape and accounts also for the chain entanglements which are a feature of systems of this gum in water.[4]

More recently further investigations of this gum using methylation and three successive, controlled Smith degradations confirmed the previous findings of Aspinall, and also showed that the side chains contained mainly 2-*O*- and 3-*O*-substituted Ara*f* units.[2] A second Smith degradation eliminated the remaining α-Ara*f* units, and their β anomers became evident. The proportion of Gal*p* units gradually increased in the form of non-reducing end and Gal*p* units, although 3,6-di-*O*- and 3,4,6-tri-*O*-substituted Gal*p* units diminished. After three degradations groups with consecutive 3-*O*-substituted β-Gal*p* units were formed. The proportion of periodate-resistant 3-*O*- and 2,3-di-*O*-substituted Man*p* units was maintained. These observations support the core nature of the Man*p* units and the linked 1-6 Gal chains. Rhamnose residues were located, which, although present in smaller amounts than the other sugars, are clearly observed in NMR studies. The study observed free α-Ara*f*-(1→2)-Ara*f* and β-Ara*f*-[β-Ara*f*]$_n$-Ara with $n = 4$ and 7, which corresponds with 2-*O*- and 3-*O*-substituted Ara*f* side-chain structures in the polysaccharide, along with:

α-Rha*p*-(1 → 4)-Glc*p*A, α-Rha*p*-(1 → 4)-β-Glc*p*A-(1 → 6)-Gal,

and

α-Rha*p*-(1 → 4)-β-Glc*p*A-(1 → 6)-β-Gal*p*-(1 → 6)-Gal.

On three successive, controlled Smith degradations, a product was formed with consecutive (1 → 3)-linked β-Galp units.

One of the observations in NMR studies is of relevance to the difference in physical forms and heterogeneity which is a feature of this gum as collected from the forests in India. The variations in physical form and colour are not related to a basic difference in chemical composition or specific bonding since the spectra are essentially similar. Nevertheless, it is evident that much more is yet to be learnt about the structure of this remarkable gum.

17.4 Technical data

17.4.1 Solubility

Gum ghatti occurs in nature as a calcium-magnesium salt and contains soluble and insoluble fractions which partially dissolve in boiling water. Gel fractions in ghatti have been reported which can be mostly dissolved by maceration; the gel fractions have a higher proportion of calcium ions.[5] The gel is not primarily due to the presence of calcium ions and forms intermolecular linkages as a result.[6] The viscosity of the gel dispersion was found to be ca. 10–30 times that of the soluble fraction and the gel fraction in commercial batches can vary from 8–23%.[5–7]

17.4.2 Physico-chemical parameters

The quality of gum ghatti, like gum arabic, is also influenced by factors such as location, geographic climate and the form of the exudates (i.e. spiro, white or yellow nodules). The physico-chemical parameters for gum arabic and ghatti are compared in Table 17.1.[8] The sample selected in Table 17.1 is a currently available gum ghatti called Shimla. The percentage loss on drying obtained for gum ghatti is similar to that of gum arabic in the lump gum form. While gum arabic is highly soluble in water, gum ghatti contains varying proportions of

Table 17.1 Physico-chemical and molecular weight parameters of gum ghatti compared with gum arabic

	Gum arabic	Gum ghatti (shimla)
% loss on drying	6.3	11.9
% insoluble matter	0	2.1
Specific optical rotation (deg $dm^{-1}\,cm^3\,g^{-1}$)	−30	−56.5
% galactose	32.3	27.6
% arabinose	30.2	55.7
% rhamonse	13.3	–
% uronic acid	24.2	11.7
% mannose	–	3.3
% xylose	–	1.6
% nitrogen	0.314	0.518
% protein*	2.1	3.4
Intrinsic viscosity (η cm^3/g) in 1 M NaCl	16.8	52.7
Intrinsic viscosity (η cm^3/g) in 0.2 M NaCl	18.3	64.4
Mw (whole gum) $\times 10^5$	6.22	8.7
Rg/nm (whole gum)	29	35
Polydispersity (M_w/M_n) whole gum	2.36	1.35
M_w (1st peak) $\times 10^6$	2.54	1.87
% mass 1st peak	10.6	21.2
Rg/nm 1st peak	41	45
M_w (2nd peak) $\times 10^5$	3.96	5.9
% mass (2nd peak)	89.4	79
Rg/nm (2nd peak)	–	22

*nitrogen conversion factor of 6.6 was used to calculate the protein %.

Table 17.2 Amino acid composition (mol%) of gum arabic and gum ghatti

	Gum arabic	Gum ghatti
Ala	2.6	7.6
Arg	0.9	2.3
Asp	5.1	17.2
Cys	—	—
Glu	3.5	7.9
Gly	5.6	13.8
His	5.8	3.3
Hyp	26.9	nd
Ile	1.3	3.9
Leu	8.0	5.4
Lys	2.9	9.2
Met	0.1	0.3
Phe	3.6	2.3
Pro	7.5	6.9
Ser	14.1	4.7
Thr	7.5	5.1
Tyr	0.8	2.4
Val	3.8	7.7

insoluble gel. The ghatti sample used in this study was specifically supplied as highly soluble gum as demonstrated by the small percentage obtained for the insoluble matter (Table 17.1). One of the major differences between the two gums is the specific optical rotation (gum ghatti −56.5º, gum arabic −30º) and it is directly related to the difference in sugar composition. The presence of xylose and mannose are often used as a means of detecting its presence in formulations. For acacia gums it has been possible to relate the specific optical rotation to the vectorial contributions made by the individual sugars present.[9,10] The second notable difference between the two gums is the protein content. The protein content of gum arabic is ~2% whereas gum ghatti has higher percentage at 3.4%. Gum ghatti contains a considerably higher percentage of Asp, Gly, Lys with lower percentages of Phe, Pro and Ser compared to gum arabic (Table 17.2).

The intrinsic viscosity is a measure of hydrodynamic volume of macromolecules and the value obtained for gum ghatti is at least three times higher than that of gum arabic. This indicates that ghatti molecules are either highly solvated or asymmetric, or both when compared to gum arabic. Both gums contain uronic acid which confers a polyelectrolyte character as demonstrated by the increase in the overall size, due to electrostatic charge repulsion, while decreasing the ionic strength of the solvent (Table 17.1).

17.4.3 Molecular weight

The structure and molecular weight of gum arabic have already been described[11] and are summarised here in order to compare with gum ghatti. Hydrophobic fractionation of gum arabic has shown that it is a highly heterogeneous complex

polysaccharide which consists of three main fractions. These fractions are: arabinogalactan protein complex (AGP), arabinogalactan (AG) and glycoprotein (GP). Each fraction contains an average of different molecular weight components with different protein content. One of the proposed models for the AGP fraction suggests that it is composed of hydrophilic carbohydrate blocks linked to a protein chain and has been reported to have a wattle blossom-type structure due to being readily degraded by proteolytic enzyme.[12,13] Other models to describe the structure of the AGP fraction have been recently reviewed.[14]

Gel permeation chromatography coupled on line to a multi-angle laser scattering, refractive index and UV detectors (GPC-MALLS) is now routinely used to identify the components in gum arabic, as labelled in Fig. 17.2(a).[8,15,16] The same method was applied for the fractionation gum ghatti and the elution profile is shown in Fig. 17.2(b). The light scattering response shows the presence of two peaks with partial separation of the second peak, which appears as a shoulder. The RI response also shows the presence of these two peaks and the first peak coincided with the light scattering response, whereas the second peak

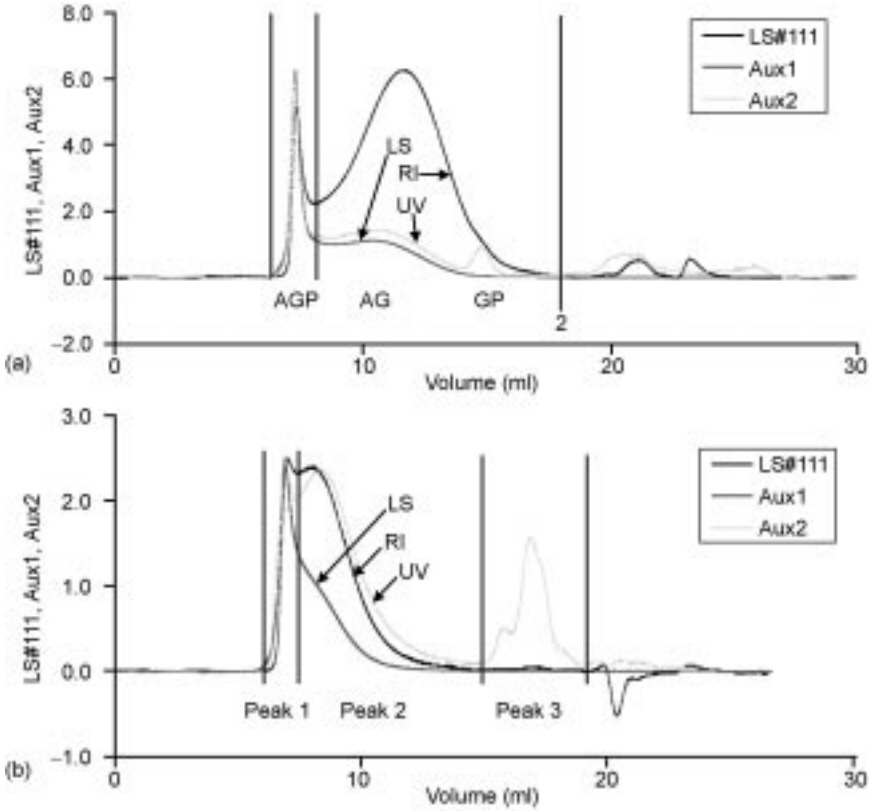


Fig. 17.2 Elution profile of (a) gum arabic and (b) gum ghatti following fractionation by gel permeation chromatography and detection by light scattering, refractive index and UV detectors.

was lower than the first peak and contained the majority of the gum. The UV response showed that there is protein associated with the high molecular weight materials and the second peak also appears as shoulder and follows the same RI response. The UV response shows a third low molecular weight peak which elutes at ~15–18.5 ml before the total volume of the column (~20 ml). The molecular weight parameters of the whole gum and when processed as two peaks are given in Table 17.1 and compared with gum arabic. The weight average molecular weight (M_w) and root mean square radius of gyration (Rg) of gum ghatti is higher than that of standard gum arabic but is less polydisperse.

The M_w and Rg of the first peak in gum ghatti, known as the AGP fraction in gum arabic, is comparable but in ghatti gum with double the concentration. The molecular weight parameters of the second peak are also higher but with slightly lower amounts compared to gum arabic. A third proteinaceous peak with high UV absorption was also identified in gum ghatti (Fig. 17.2(b)) but was difficult to accurately determine its molecular weight due to very low light scattering response. However, integration of the area under the peak for a range of ghatti samples revealed that the proportion of this peak is ~1–2.2% of the whole gum. The proportion of the third for the sample shown in Fig. 17.2(b) was 1.38% of the total injected mass. The total mass recovery for gum ghatti was 102% which indicates a suitability of column used for the fractionation and in agreement with the solubility determined independently using the weight of the materials retained on the filter (Table 17.1).

17.4.4 Rheology

The viscosity of gum ghatti, like other hydrocolloids, increases exponentially with increasing concentration. Gum ghatti makes a viscous solution at 30–35 wt% (Fig. 17.3). Rheological measurements showing the shear flow viscosity of gum arabic and gum ghatti as a function of shear rate are shown in Fig. 17.3 (inset). At relatively diluted concentration (5% ghatti and 10% gum arabic) both show evidence of shear thinning behaviour at low shear rates and were Newtonian at higher shear rates. However, the viscosity of gum ghatti is double that of gum arabic as demonstrated by the almost exact viscosity match between the two gums. With increasing the concentration (45% gum arabic, 30% gum ghatti), both gums show a clear shear thinning behaviour albeit at different shear rates. The viscosity difference between the two gums is largely explained by the higher intrinsic viscosity and proportion of high molecular weight present in gum ghatti compared to gum arabic (Table 17.1 and Fig. 17.2).

17.4.5 Emulsification

The emulsification performance of gum ghatti is greatly influenced by the presence of insoluble components. These insoluble components are usually filtered and removed during the manufacture of beverage emulsion. Gum ghatti, like other hydrocolloid emulsifiers, such as gum arabic and sugar beet pectin, contain a small amount of protein linked to the carbohydrate unit. Enzyme

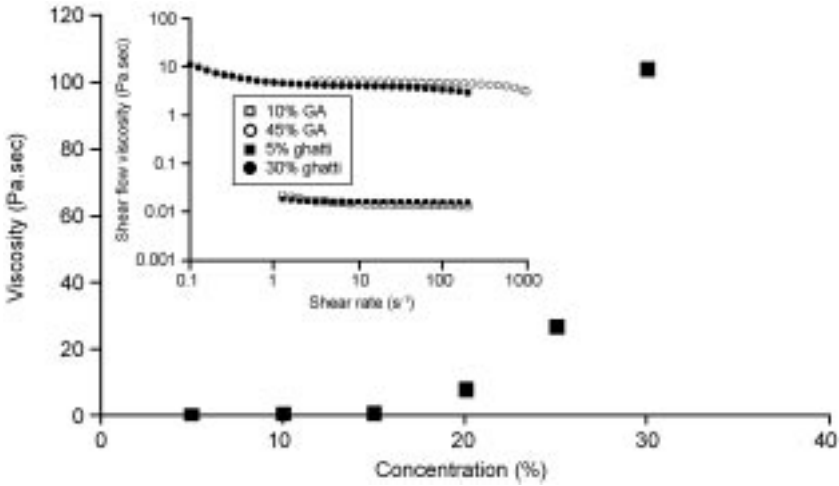


Fig. 17.3 Viscosity of gum ghatti as a function of concentration. (Inset) Shear flow viscosity plotted as a function of shear rate for (□) 10% w/w, (○) 45% w/w gum arabic and (■) 5%, (●) 30% w/w gum ghatti. Solutions were dissolved in distilled water containing 0.005% w/v NaN_3. All measurements were carried out at 25 ºC.

digestion with protease does not greatly reduce the molecular weight. However, when the gum is subjected to processing, such as spray drying, the effect of enzyme digestion can be clearly seen as results of changes in the protein conformation which then become more accessible. Further evidence of protein–carbohydrate linkage is supported by the interaction with Yariv's reagent and hence can be classified as arabinogalactan-protein complex. The emulsification mechanism in gum ghatti is similar to that of gum arabic where the protein is responsible for the surface activity by acting as the strong anchor point at the oil–water interface with the hydrophilic polysaccharide chains providing the protective layer.[17] This is why gum ghatti can emulsify 20% medium chain triglyceride (MCT) oil at much lower concentration (5 wt%) compared to gum arabic as shown in Fig. 17.4. In Fig. 17.4 the emulsification performance and stability of gum ghatti, which is completely soluble, is compared with standard gum arabic at a range of concentrations. The interfacial rheology of gum ghatti is superior to that of gum arabic at the same concentration due to the high protein content and possibly the conformation in solution. Additionally, gum ghatti is more resistant to heat and this is clearly reflected by the emulsion stability following acceleration at 60 ºC for 3 days. Detailed evaluation of emulsification performance of gum ghatti has recently been reported.[18]

17.5 Uses and applications

Gum ghatti has an age-old reputation in India as being a very good medicinal product as well as having very good qualities for food products. This gum has been mentioned in ancient medicinal scriptures like the *Ayurveda* (Indian system

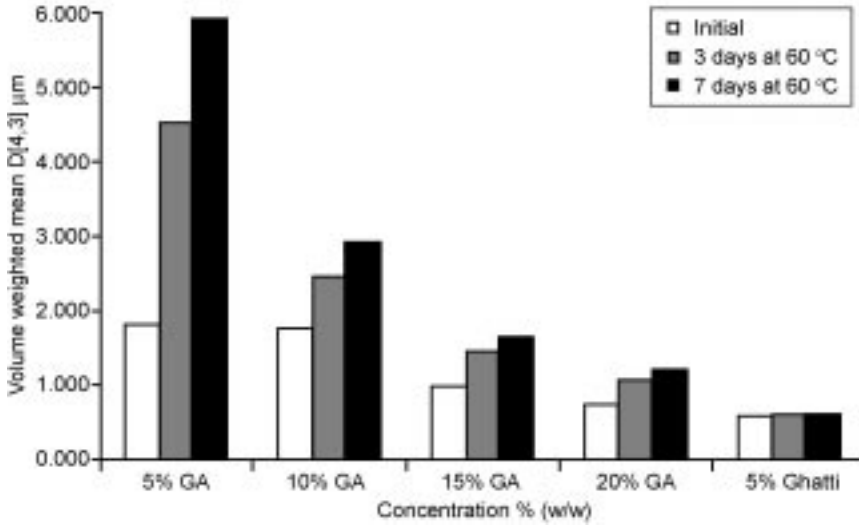


Fig. 17.4 Volume weighted mean (d[4,3]) for initial and accelerated emulsions of gum arabic (5–20 wt%) and gum ghatti at 5 wt%. Oil phase 20% MCT, 0.12% citric acid, 0.13% sodium benzoate.

of medicine) and the *Unani* (Greek system of medicine). In some parts of India, the clean nodules of a variety of ghatti called *Spiro*, are eaten as a luxury product and are considered a sign of wealth and prosperity. It has been used throughout households and industry as a good adhesive. Other uses include hair gel, print industry in India particularly screen printing, confectionary and by the petroleum industry as a drilling mud conditioner.

The newly processed gum ghatti (GATIFOLIA SD) gives a solution of moderate viscosity which falls between gum arabic and gum karaya. By utilising the excellent emulsifying properties and the complete solubility of GATIFOLIA SD it has been possible to show that it can be used in many emulsified products as an emulsifier in formulations which are difficult to stabilise using gum arabic. Examples of various formulations are given below.

17.5.1 Beverage emulsion

Formulation 17.1 MCT

Ingredients	%
Oil (MCT)	20
Gum ghatti	5
Citric acid	0.12
Benzoate	0.13
Ion exchange water	to 100

Preparation
The emulsion is prepared using Nanomiser (Laboratory scale high pressure homogeniser, Yoshida Kikai Co., Ltd) at 50 MPa, 2 passes.

Formulation 17.2 Orange oil–ester gum

Ingredients	%
Oil	14
Orange oil	7
Ester gum	7
Gum ghatti (GHATIFOLIA)	5
Citric acid	0.2
Benzoate	0.1
Ion exchange water	to 100

Preparation
1. The oil phase is prepared by mixing ester gum and orange oil at 1:1 ratio.
2. The oil phase is then added to the aqueous phase, containing citric acid and sodium benzoate.
3. The emulsion is prepared using Nanomiser (Laboratory scale high pressure homogeniser, Yoshida Kikai Co., Ltd) at 50 MPa, 2 passes.

Formulation 17.3 Orange oil, ester gum, β-carotene, MCT

Ingredients	%
Oil	14
Orange oil	4
Ester gum	4
MCT	4
β-carotene	2
Gum ghatti (GHATIFOLIA SD)	5
Citric acid	0.2
Benzoate	0.1
Ion exchange water	to 100

Preparation
1. The oil phase is prepared by mixing ester gum and orange oil at 1:1 ratio.
2. The oil phase is then added to the aqueous phase, containing citric acid and sodium benzoate, followed by the addition of MCT.
3. The emulsion is homogenised using Nanomiser (Laboratory scale high pressure homogeniser, Yoshida Kikai Co., Ltd) at 50 MPa, 2 passes.

17.5.2 Mayonnaise type dressing (70% fat)

Formulation 17.4

Ingredients	%
1. Corn oil	70
2. Vinegar	4.5

3. Apple vinegar	7.0
4. Lemon fruit juice	2.0
5. Gum ghatti (GHATIFOLIA SD)	2.0
6. SAN ACE	0.15
7. Sugar	1.0
8. Salt	2.5
9. Sodium glutaminate	0.1
10. Ion exchange water	to 100

Note: typical emulsifier used in the above formulation is egg white at 10%.

Preparation
1. Slowly add ingredients 5–9 into water while stirring.
2. Add ingredients 2–4 and continue stirring the blend.
3. Add egg yolk at the end of this step.
4. Slowly add oil and stir blend.
5. Emulsify using a colloid mill.

17.5.3 Dressings (30% fat)

Formulation 17.5

Ingredients	%
1. Corn oil	30
2. Vinegar	5.3
3. Gum ghatti (GHATIFOLIA SD)	0.1
4. SAN ACE	0.2
5. Sugar	3.0
6. Salt	5.0
7. Sodium glutaminate	0.4
8. Ion exchange water	to 100

Note: typical emulsifier used in the above formulation is egg white at 0.5%.

Preparation
1. Slowly add ingredients 5–7 into water while stirring.
2. Add ingredients 2–4 and stir blend.
3. Slowly add oil and stir blend.
4. Emulsify using a colloid mill or homo-mixer.

17.5.4 Butter cream

Formulation 17.6

Ingredients	%
Shortening	30
Margarine	25
Sugar syrup	20
GATIFOLIA SD	0.2

Milk flavour No. 71005	0.2
Carotene base No. 80	0.02

Preparation

1. Slowly add GATIFOLIA SD into sugar syrup while stirring the blend.
2. Add flavour and carotene into the syrup and stir blend.
3. Add the syrup into shortening/margarine mixture and stir blend.

17.6 Regulatory status

South America

Argentina, Bolivia, Chile and Peru: Not permitted

Brazil: Permitted as an emulsifier to aid homogenising flavours or to incorporate them in food products. No maximum level specified.

Colombia: Permitted as a natural emulsifier in foodstuffs.

Ecuador: Permitted as a natural emulsifier, stabiliser and thickener.

Guatemala: Permitted in food as emulsifier, stabiliser and thickener. Maximum level in soft drink and non-alcoholic beverages specified at 0.2%.

Mexico: Permitted in all foods as a natural emulsifier, stabiliser, thickener and gelling agent.

Uruguay: Permitted as an emulsifier for flavours with no maximum level specified.

Venezuela: Permitted as a natural emulsifier, stabiliser and thickener. No maximum level specified for carbonated beverages and sweets. Instant drink powder should not exceed 0.1%.

Asia

China: Permitted in food as an emulsifier for flavours. No maximum level specified (an appropriate amount as practically needed).

India: Bureau of Indian Standards (BIS) – IS 7239:1974 (Food Grade)
Permitted in food as a natural emulsifier, stabiliser and thickener as detailed below:

- Stabiliser and emulsifier in sugar-boiled confectionery, processed cheese spread with no maximum level set.
- Emulsifier in chewing gum, bubble gum, malted milk food with no maximum level set.
- Stabiliser in yoghurt, milk ices and milk lollies with maximum level set at 0.5%.
- Emulsifier and/or stabiliser in ice candy, ice cream, kulfi, chocolate ice cream, ice lollies or edible ices with maximum level set at 0.5%.
- Emulsifier, stabiliser and thickener in frozen desserts with no maximum level set.
- Emulsifier, stabiliser and thickener in fat spread table margarine, bakery and industrial margarine, wafer biscuits and flavouring agent with no maximum level set.

South Korea: Permitted in all foods (unless specifically prohibited for use in a compositional standard) as natural food additives with no specific function defined.

Singapore: Permitted in all foods (unless specifically prohibited for use in a compositional standard and in unstandardised foods) as natural emulsifier and stabilizer with no specific function defined.

United States

Permitted in food as an emulsifier, emulsifier salt in

- Beverages and beverage bases, non-alcoholic with a maximum level set at 0.2%.

All other food categories at a maximum level of 0.1%. The following regulations are relevant to the use of gum ghatti in the United States.

- Code of Federal Regulations(CFR), Food and Drug Administration, Department of Health and Human Services CFR – Title 21, Code – 184.1333
- The Flavor and Extract Manufacturers Association FEMA – GRAS status, Limitation – Stabilizer
- Chemical Abstracts Service CAS – 009000-28-6
- Priority Based Assessment of Food Additives PAFA – 2149
- Registry of Toxic Effects of Chemical Substances RTECS – LY8935000

Japan

Permitted in all foods as natural food additives with no specific function defined and subject to the following specification:

- Identification test 1: When 1 g is dispersed in 5 ml of water it forms a viscous, adhesive mucilage.
- Identification test 2: To 5 ml of 1 in 100 solution of the sample (filter through diatomaceous earth if necessary) add 0.2 ml of dilute lead subacetate TS. A small or no precipitate is formed, but an opaque flocculent precipitate is produced upon the further addition of 0.5 ml of ammonia TS.
- Identification test 3: A 1 in 50 solution of the sample filtered through diatomaceous earth is levorotatory.
- Heavy metals: Not more than 4 ppm.
- Lead: Not more than 10 ppm.
- Arsenic (as As_2O_3): Not more than 4 ppm.
- Loss on drying (105 ºC, 5 hours): Not more than 14%.
- Ash content: Not more than 6%.
- Acid insoluble ash: Not more than 1%.
- Viable bacteria: Not more than 10,000/g.
- *Escherichia coli:* Negative.

Europe

Not permitted.

Russia

Permitted as stabiliser, thickener and gelling agent. Not defined for particular oodstuff and no maximum level set.

South Africa

Permitted as an emulsifier in condiments and sauces, soft drinks, breakfast cereals, flour confectionery and cakes mixes with no maximum level set.

Australia

Gum ghatti falls under the list of approved herbs.

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18

Other exudates: tragancanth, karaya, mesquite gum and larchwood arabinogalactan

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Abstract: The collection, processing and trading of plant exudate gums, other than gum arabic, and the production of arabinogalactan from the heartwood of Western larch tree represent an important economic activity in many regions of the world. What this family of materials shares in common is that they are comprised of highly branched heteropolysaccharide structures. This chapter addresses the manufacture, chemical structure, functional properties, main applications and regulatory issues for three well-established hydrocolloids, namely gum tragacanth, gum karaya and larchwood arabinogalactan along with those of mesquite gum, whose full potential utilization is still to be exploited in several fields of application.

Key words: gum tragacanth, gum karaya, mesquite gum, larchwood arabinogalactan, exudates.

18.1 Introduction

Although gum arabic is by far the most important plant exudate hydrocolloid, there are other related gums that have retained their economic and technological importance for centuries despite the availability of several new alternative industrial hydrocolloids. In fact, natural plant gums are the most widely used and traded non-wood forest products other than items consumed directly as food, fodder and medicine (Upadhayay, 2006). Their collection by hand still

represents a source of income for millions of people, dwelling in rural areas mostly in Africa, India, Iran, Turkey, and to a less extent, in Mexico. In northern USA, the extraction of arabinogalactan from larch trees also represents an important economic activity.

Gums are exuded by the bark of trees in the form of tear-like, striated nodules or amorphous lumps, which are vitrified upon drying, thus forming hard, glassy lumps (gum karaya and mesquite gum) or tough opaque thin ribbons (gum tragacanth) of different colours, ranging from red-amber for mesquite gum, pale gray to dark brown for karaya gum, and white to dark brown for tragacanth. In general, the gums are produced by the stem under conditions of heat and drought stress, partly as a natural phenomenon (as part of the normal metabolism of plants) and partly as a result of injury to the bark or stem (due to fungal or bacterial attack) by a process known as gummosis. The other type of polysaccharide gum addressed in this chapter is not strictly an exudate like the others, but it is extracted from the vacuoles of the heartwood of the Western larch tree and related species.

Chemically, these materials are known to be comprised to varying extents either by arabinogalactan (AG) heteropolysaccharides (e.g., larchwood arabinogalactan) or occur as complex mixtures of other acetylated polysaccharides such as rhamnogalacturonan (e.g., gum karaya); mixtures of galacturonan regions and type II AG as gum tragacanth (Verbeken *et al.*, 2003) or macromolecular complexes of type II AG and proteoglycans (arabinogalactan-protein, AGP) comprising ca. 4% of protein such as mesquite gum (Goycoolea *et al.*, 2000). As a consequence of this chemical structural diversity, these polysaccharides exhibit very different functional properties and thus they have found applications in various fields. The individual properties of gum tragacanth, gum karaya, mesquite gum and larch arabinogalactan are discussed throughout the various sections of this chapter.

18.2 Manufacture

18.2.1 Gum tragacanth

Gum tragacanth was first described by Theophrastus several centuries before Christ. The name tragacanth, from the Greek *tragos* (goat) and *akantha* (horn), probably refers to the curved shape of the ribbons, the best grade of commercial gum.

The gum is obtained from small shrubs of the *Astragalus* genus, comprising up to 2000 species indigenous to mountain areas of south west Asia from Pakistan to Greece (Whistler, 1993). *A. gummifer* was considered to be the main tragacanth yielding species, but a field survey established that *A. microcephalus* was the principal source of the gum (Dogan *et al.*, 1985); *A. kurdicus* and *A. gossypinus* have also been documented as botanical sources. The plants are small, low bushy perennial shrubs having a large tap root along with branches. The root and lower stem are tapped for gum.

The main areas of commercial production are the arid and mountainous regions of Iran (accounting for ~70% of the supplies) and the Anatolia region in Turkey (Anderson, 1989), and in lesser amounts in Afghanistan and Syria. In the past, several thousand tonnes of tragacanth were used in food, pharmaceutical and technical applications. However, as a result of very high costs, erratic supply and strong competition from xanthan gum, demand for the gum fell dramatically from several thousand to 200–300 tonnes per year (Anderson, 1989). Iran's recovery in the gum tragacanth export market suggests that, with a correct understanding of the world market and supply of premium product, there is a vast prospect for a bigger and better market for this gum. Trade sources in London have quote prices (mid-1995) at around US$22/kg free on board (FOB) for the top grade (Ribbon no. 1), US$16/kg for Ribbon no. 4 and falling to US$3–4/kg for the lowest grades. Current quoted price for gum from Azerbaijan is US$30/kg.

Plants develop a mass of gum in the centre of the root, which swells in the summer heat. If the stem is slit, soft gum is exuded. The exudate is produced spontaneously on the bark of the shrub, but both the yield and quality are often increased by making incisions in the tap root and lower stem. Abundant rainfall prior to the tapping season, and dry conditions during the harvesting season, constitute optimum climate conditions for gum production. Tapping is carried out in May or June with subsequent collection in August and September (after 6 weeks) for ribbon grades and August to November for Flake grades (Wareing, 1997).

The gum is obtained in two basic physical forms, namely ribbons (superior quality) and flakes (inferior quality). These two forms are obtained from different sub-species of the shrub. Both types of shrubs normally do not grow in the same locality (Robbins, 1988). After collection, the gum is sorted by hand by the natives and carried to sorting centres where it is graded into several grades of ribbons and flakes and exported. The Iranian grading system is more clearly defined than that of Turkey and comprises nine different grades. The most commonly used Iranian qualities are ribbons 1 and 4, mixed ribbon and flakes 27, 28 and 55, while in Turkey there are four grades, namely, Fior Extra and Fior for ribbons and Bianca and Pianto for flakes. The best qualities are regarded as those with higher viscosity, good solution colour and low microbiological limits. Blending is necessary to ensure the desired properties. Processors in the US and Europe purchase material following approval of pre-delivery samples. Quality control inspections of each incoming batch are necessary to ensure powder blends meet well-defined specifications for powder and solution colour and viscosity. Food applications for sauces, dressings, icings, and confectionery normally use mixed ribbon or flake grades. Lower qualities are used where solution colour is less important and where thermal processing, pH and/or the soluble solids level are sufficient to prevent microbial proliferation in the final product.

Limited mechanical treatment to remove foreign matter may be carried out in the exporting countries but no further processing is undertaken. Importers in the US and Western Europe, primarily in the UK and Germany, ensure consistent

quality standards are maintained for the powdered material after milling. The best ribbon grades have low total viable counts of bacteria comprising mainly resistant spores from the soil and airbone contamination. These problems were previously controlled through fumigation with ethylene oxide (ETO). This process was forbidden around 1987 in the treatment of gum destined for food uses, because of carcinogenicity of ETO. The alternative methods of bringing down the microbial counts also cause chemical changes in the gum and accordingly are not acceptable (Anderson and Weiping, 1994). In the US propylene oxide is allowed but its efficacy is limited and permission for its use may be revoked.

18.2.2 Gum karaya

Karaya gum, also known as sterculia gum, is the dry exudate of *Sterculia urens* (Roxburgh), a large and bushy tree. The majority of commercial material is obtained from wild *S. urens* trees, indigenous to central and northern India and more than half of the gum is produced in the state of Andhra Pradesh. Other significant sources are from *S. setigera*, in Senegal and Mali, and minor supplies from *S. villosa* in Sudan, India and Pakistan. The history of gum karaya trading, in contrast to tragacanth, is quite recent. It goes back to the 1920s when the gum used to be sold as an adulterant to tragacanth. World production and usage is currently 1500 tonnes per year. The major users of gum karaya are the US, France and the UK. Minor quantities are imported into Japan, Belgium, Germany and other European countries (Robbins, 1988).

The export of Indian gum karaya declined from 4000 tonnes in 1982 to 1000 tonnes in 1992 and has remained roughly constant up to 2002, mostly due to a sharp decrease in the number of trees available for tapping due to unsustainable harvesting methods (Upadhayay, 2006). Over the past two decades, the prices in India have risen as a consequence of the increase in demand and shortage in production. In turn, exports from Senegal and other countries increased their production in the late 1980s to 1500 tonnes per annum and this has resulted in more competition and more stable prices. Indicative FOB prices quoted by importers in London for Indian karaya (mid-1995) are in the range US$2250–6000/tonne according to grade. Fair average quality (FAQ) gum is about US$3000/tonne.

For production, the trees are incised or tapped and exudation begins immediately and continues for several days forming irregular lumps (or tears) which may weigh more than 1 kg, and large trees can produce up to 4.5 kg (Whistler, 1993). The exudate is allowed to dry on the tree and is later collected, broken, cleaned and sorted. The highest quality of raw gum collected is during the hot months of April, May and June. In September, the gum is again picked. This autumn crop has a greyish colour and is normally less viscous. The gum is cleaned to remove bark and foreign matter (BFM) before sorting. Commercially available quality grades are hand-picked selected (HPS), superior no. 1, no. 2 and no. 3 (FAQ), and siftings (Verbeken *et al.*, 2003). BFM can be found in

white to very light tan HPS and superior no. 1 grades in proportions of 0–0.5% and 1.0–2.0%, respectively; 1.5–3.5% in very light tan superior no. 2; 2.5–4.0% in tan FAQ gum and 5.0–7.0% in the brown colour siftings (Wareing, 1997).

Gum karaya is processed to remove impurities such as bark, stones, fibres and sand. It is then milled, blended and classified according to mesh, viscosity and purity. The gum is offered as granules or in powder form. The granule size ranges from 4–8 mesh and 8–14 mesh and powder size is 160 mesh with viscosity ranging from 500–1200 cps. The powder is light to pinkish gray and has a slight acetic taste and odour. The microbial quality of this gum is similar to that of other exudates, and its use in sauces and dressings is safe, as the low pH of these products and the heat treatments they are regularly subjected to are sufficient to ensure safety.

18.2.3 Mesquite gum

Mesquite trees are leguminous plant trees that are widespread in arid and semi-arid regions of the world and account for one of the major plant species in such places. In fact, the genus *Prosopis* comprises about 44 different species that grow mostly in North and South America, and also in Australia, Africa, and eastern Asia. In Mexico, around ten species are found, of which the most abundant is *P. juliflora* (Vernon-Carter *et al.*, 2000), which has also been suggested to correspond with *P. laevigata*. This species grows from the coastal areas of the Pacific Ocean in the Mexican state of Sinaloa to Panama, in the centre and south of Mexico, reaching all the way to the south-eastern United States under environmental conditions that range from subhumid to areas with an average rainfall of up to 1500 mm.

It is well documented that the bark of *Prosopis spp.* produces an exudate known as mesquite gum as a response to insect attack, wounding or physiological stresses such as severe water and heat. The gum could be defined as 'the dried gummy exudation obtained from the stems and branches of *Prosopis* species'. By contrast with commercial gum arabic, karaya and tragacanth gums, mesquite exudate is not an established hydrocolloid in the world market. However, the gum was widely used by the Indian cultures of the Mexican Northwest (Seri and Yaqui) and southwestern United States (Papago and Pima) (Felger, 1977), mainly as a sweet and as a medicinal aid to prepare eye drops (Felger and Moser, 1974). Presently, mesquite gum, known in Sonora as *chúcata*, is used in few household applications. However, in the past, mesquite gum has been used extensively in food applications and has been traditionally considered as a 'substitute or adulterant of gum arabic, of inferior quality due to its darker colour' (Smith and Montgomery, 1959). In Mexico, there are two main regions where mesquite gum is produced, namely, in the desert plains of the northwestern state of Sonora, where the predominant source is *P. velutina* and in the lowlands of the Northeastern state of San Luis Potosi, where the gum is sourced mostly from *P. laevigata*. The structural and functional properties of mesquite gum have been studied extensively mostly by two independent

Mexican research groups, namely, the group led by Dr J. Vernon-Carter, at Universidad Autónoma Metropolitana, that has worked with the gum from San Luis Potosi, and our group that has worked with the material from Sonora.

The production season of mesquite gum in Sonora begins during the late spring and early summer months (May–July), ending with the summer rains at late July/early August. At present, the collection of mesquite gum is not properly organized and there is not a quality grading system to sort it. Nevertheless, as with other gum exudates, the nodules can be classified by size, colour and by the contents of bark and foreign matter. In some cases dark gum nodules are eliminated by their high tannin content depending on their intended use (Orozco-Villafuerte *et al.*, 2000). Ultrafiltration studies in mesquite gum from Sonora showed that this technology was feasible to reduce the contents of naturally occurring tannin compounds (Goycoolea *et al.*, 1998). Quantitative analysis of the removed tannins indicated that up to ~62% of the original tannin contents can be removed using a hollow fibre membrane of 10 kDa molecular weight cutoff without compromising the emulsification capacity of mesquite gum.

The only information available on production of mesquite gum from wild plantations comes from a few field studies that have tried to estimate the availability of the gum in the two collection regions in Mexico. In San Luis Potosi, it has been estimated that the potential production in an area of 600 km^2 is ~2000 tonnes p.a. (Vernon-Carter *et al.*, 2000), while in Sonora the estimated total annual production was nearly half as much, at ~800 tonnes (Goycoolea *et al.*, 2000). These figures allow us to conclude that the potential production of mesquite gum from wild mesquite forests could fulfil the 2004 demand for gum arabic which was in the order of ~1417 tonnes (Secretaría de Economía, 2004). Unfortunately, to date mesquite gum is neither produced on a large scale, nor are there commercial plantations, extraction methods or efficient collection systems. Besides, the price at which gum arabic is currently imported to Mexico at ~$US3200/tonne (Secretaría de Economía, 2005) renders it economically unfeasible to collect mesquite gum from the wild areas.

In light of the above, alternative production methods have been investigated. *In vitro* studies for culturing of *P. laevigata* and laboratory conditions for the gum production by stem segments (Orozco-Villafuerte *et al.*, 2000) have demonstrated that application of combined environmental conditions (temperature increase) and biotic elicitors, can be utilized for increasing mesquite gum production with similar characteristics to those produced *in situ* by wild trees (Orozco-Villafuerte *et al.*, 2005).

18.2.4 Larchwood arabinogalactan

Arabinogalactan is particularly abundant in larchwood (genus *Larix*) and especially in Western larch (*Larix occidentalis*) from whose heartwood AG can be extracted in high yield (Stephen, 1983). Extraction of water-soluble arabinogalactan from shavings of the butt of the Western larch tree was first described in 1898 (Trimble, 1898), though no quantitative data was reported.

During the twentieth century this material continued to receive economic and scientific interest and later it was found that arabinose and galactose were its main constituents (Wise and Peterson, 1930; Nikitin and Soloviev, 1935). Subsequent efforts at large-scale commercialization were hampered by the economics of extraction and purification (Anderson, 1967). However, technological improvements were made and presently larchwood arabinogalactan is produced on an industrial scale and its market developed as food fibre and for biomedical and healthcare applications (Gallez *et al.*, 1994).

The most practical method of extraction consists of hot water treatment using countercurrent flow of the drilled or chipped heartwood (Adams and Ettling, 1973) from Dahurian larch (*Larix dadurica*), Siberian larch (*Larix siberica*), Eastern larch (*Larix laricina*), European larch (*Larix deciduas*), Japanese larch (*Larix leptolepsis*) and Western larch tree (*Larix occidentalis*), which contains quantities up to 35% of the arabinogalactan in vacuoles and it is the most abundant and available source (Stephen and Churms, 1995). Arabinogalactan is available commercially in ultrafiltered (AG–UF) and in food grades (AG–FG) (Christian *et al.*, 1998). Conditions used to isolate arabinogalactan from *L. occidentalis* include extraction at 70 ºC for several days and use of magnesium oxide (Adams and Ettling, 1973).

The Swiss company Lonza Inc. (which has recently acquired Larex Inc.), is presently the major manufacturer of larch arabinogalactan for commercial applications in the world, including medicinal and food supplements. The company owns patents on composition and extraction processes for a range of AG products of varying qualities, depending on the application fields they are intended for. Their industrial facility has a production capacity for 3.7 million metric tonnes (dry weight) of arabinogalactan. The amount of arabinogalactan that could be obtained from 1% of larch trees each year in the United States is 4.6 million metric tonnes. The intellectual property of the processes to produce this gum from larch trees is covered under various patents (DeWitt, 1989; Adams and Knudson, 1990; Price *et al.*, 1995).

18.3 Structure

18.3.1 Gum tragacanth

The structures of polysaccharides of gum tragacanth were investigated in detail by James and Smith (1945a, 1945b) followed by Aspinall and Baillie (1963a, 1963b). The gum is a slightly acidic salt occurring naturally with calcium, magnesium and sodium cations (Whistler, 1993). Gum tragacanth has a molecular weight of about 840 kDa, calculated by Svedberg's method and formula and an elongated shape of 450 nm by 1.9 nm, providing a high viscosity. *Astragalus* species (*A. gummifer, A. microcephalus* and *A. kurdicus*) have 1–3.6% of protein with the proportions of the major amino acid constituents (Asp, Hyp, Ser, Pro and Val) also varying (Whistler, 1993; Stephen and Churms, 1995).

Gum tragacanth is composed of two major components: tragacanthic acid and a small amount of a water soluble arabinogalactan and the bassorin fraction which is insoluble but swells in water to form a gel. The water soluble tragacanthin, accounts for 30–40% of the gum and is reported as a neutral, highly branched arabinogalactan (of type II) comprising (1→6)- and (1→3)-linked core chain containing galactose and arabinose (both in furanose and pyranose forms) and side groups of (1→2)-, (1→3)- and (1→5)-linked arabinose units occurring as monosccharide or oligosaccharides (Stephen and Churms, 1995; Tischer *et al.*, 2002). Acid hydrolysis revealed that tragacanthin (*Astralagus gummifer*) contains neutral monosaccharides such as L-fucose (L-Fuc), L-(L-Ara), D-xylose (D-Xyl), D-mannose (D-Man), D-galactose (D-Gal) and D-glucose (D-Glc) in a 3:52:29:6:5:5 molar ratio and the arabinogalactan contained L-rhamnose (L-Rha), L-Fuc, L-Ara, D-Xyl, D-Man, D-Gal and D-Glc in a 1:1:68:2:5:22:1 molar ratio (Tisher *et al.*, 2002). This polysaccharide component is soluble in a mixture of ethanol-water (7:3). Recently, intrinsic viscosity [η], molecular weight M_W, and radius of gyration $\langle S^2 \rangle z^{1/2}$ of tragacanthin from *Astragalus gossypinus* were calculated to be, $[\eta] = 9.077 \times 10^{-3} M_W^{0.87}$ (mL g^{-1}), $\langle S^2 \rangle z^{1/2} = 0.021 M_W^{0.59}$ (nm) in the range of M_W from 1.8×10^5 to 1.6×10^6. The conformational parameter of tragacanthin were 1111 g mol for molar mass per unit contour length (M_L), 26 nm for persistence length (q) and 1.87 ratio of $R_G R_H$ (Mohammadifar *et al.*, 2006).

Bassorin, a pectic component (Fig. 18.1), has a chain of (1→4)-linked α-D-galacturonic acid units some of which are substituted at O-3 with β-D-xylopyranosyl units and some of these being terminated with D-Gal or L-Fuc. Bassorin appears to contain some methyl groups. It was reported that for most species of *Astragalus*, the insoluble part has less methoxyl and galacturonic acid than the soluble part. Pectic component is dissolved partly in dilute aqueous sodium hydroxide. Grade precipitation of alkali soluble material gave fractions similar to those isolated from the water-soluble proportion of the gum. Bassorin and tragacanthin have quite different rheological properties: while 1% bassorin solution at 25 ºC shows a high viscosity gel-like structure, tragacanthin solution behaves like semi-dilute to concentrated solution of entangled, random coil polymers (Mohammadifar *et al.*, 2006).

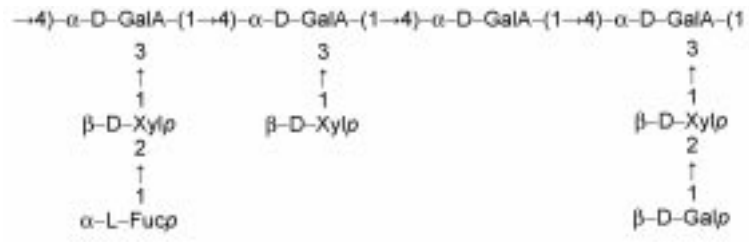

Fig. 18.1 Structure of gum tragacanth pectic component (*Astragalus spp*) (from Stephen and Churms, 1995).

18.3.2 Gum karaya

Chemically, gum karaya is a partially acetylated polysaccharide of the substituted rhamnogalacturonoglycan type. The exudate occurs in the salt form containing calcium and magnesium ions. It has a branched structure and a very high molecular weight (ranging from 9,000 to 16,000 kDa) (Stephen, 1990; Whistler, 1993; Stephen and Churms, 1995). It contains about 37% uronic acid residues and approximately 8% acetyl groups. Due to these acetyl groups gum karaya is insoluble and only swells in water. The native acetylated gum assumes a rather compact and branched conformation in aqueous solution. In contrast, the fully deacetylated karaya gum assumes a more expanded conformation and behaves as a random coil (Le Cerf *et al.*, 1990).

After acid hydrolysis gum karaya produces D-galacturonic acid (D-GalA), D-Gal, L-Rha and small proportions of D-glucuronic acid (D-GlcA). The sugar composition of gum karaya has been given as (in wt%): 37.6% uronic acids; 26.3% D-Gal and 29.2% L-Rha (Aspinall *et al.*, 1986). However, the sugar composition of the gum is dependent on the botanical sources and age of the tree and there is also more than average variability in the proportions of amino acids in the proteinaceous components. It is worth pointing out that gum karaya has a much higher rhamnose content than other commercial exudate gums.

More detailed structural studies after partial acid hydrolysis, acetolysis, methylation analysis and Smith degradation, suggest that the polysaccharide component of karaya corresponds with that shown in Fig. 18.2 (Stephen and Churms, 1995).

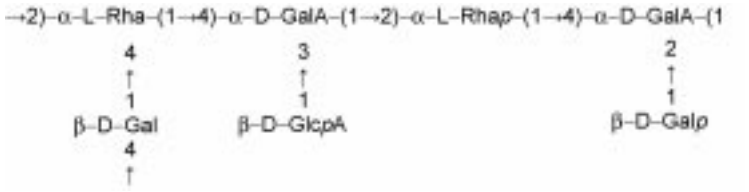

Fig. 18.2 Structure of gum karaya (*Sterculia urens*) (from Stephen and Churms, 1995).

18.3.3 Mesquite gum

Mesquite gum is the neutral salt of a complex acidic branched polysaccharide formed by a core of β-D-Gal residues comprising a (1→-3)-linked backbone with (1→6)-linked branches, bearing L-Ara (pyranose and furanose rings form), D-glucuronic acid and 4-O-methyl-β-D-glucuronic acid (Fig. 18.3) (White, 1946, 1947a, 1947b, 1948; Cuneen and Smith, 1948a, 1948b; Akher *et al.*, 1952; Aspinall and Whitehead, 1970a, 1970b). On acid hydrolysis mesquite gum from *P. velutina* yields L-Ara and D-Gal as main carbohydrate residues with Ara/Gal ratio between 7.32 and 10.61, and traces of D-Glc, D-Man and D-Xyl were also detected (López-Franco *et al.*, 2008). ^{1}H NMR spectroscopy studies have recently been used to analyse the structure of gum from *P. velutina* (Rinaudo *et*

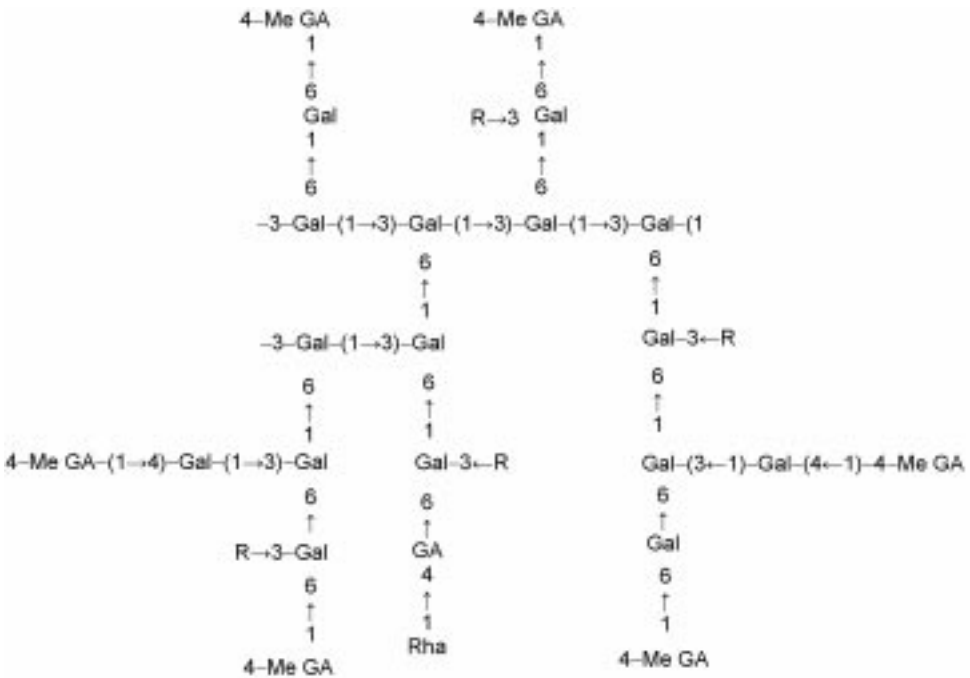

Fig. 18.3 Primary structure for the carbohydrate component of mesquite gum (from Aspinall and Whitehead, 1970a, 1970b); R = Ara-(1→2)-Ara-(1→2)-Ara-(1→2)-Ara-(1→2)-Ara-(1→4)-Ara-(1→3)-Ara-(1 y Ara-(1→6)-Gal-(1→3)-Ara-(1→3)-Ara-(1.

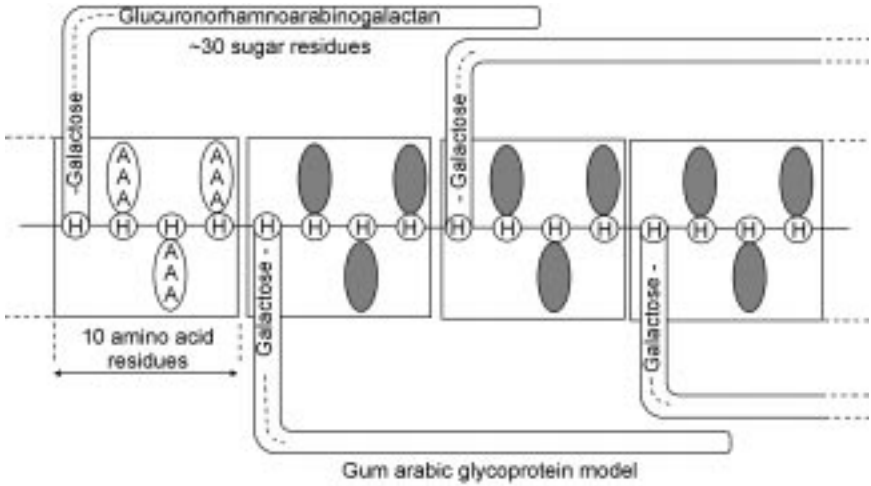

Fig. 18.4 Twisted hairy rope structure proposed to AGPs from gum arabic (*A. senegal*) (from Qi and Lamport, 1991; reproduced with permission of American Society of Plant Biologists).

al., 2008), and it was confirmed that L-Rha is not present in mesquite gum, in contrast with gum arabic whose spectrum shows the corresponding signal for this residue at ~1.32 ppm. By contrast, in the material from *P. laevigata*, a small concentration of L-Rha of ~1.3 mol% has been reported (Orozco-Villafuerte *et al.*, 2003).

In addition to the polysaccharide component, mesquite gum contains a small amount of protein (2–4%) (Fincher *et al.*, 1983; Goycoolea *et al.*, 1998; Orozco-Villafuerte *et al.*, 2003; López-Franco *et al.*, 2004) which plays an important role in its emulsification properties (Vernon-Carter *et al.*, 1996b, 1998; Goycoolea *et al.*, 1995).

The adequacy of models that explain the tertiary structure of mesquite gum has not yet been assessed experimentally. However, light scattering studies have shown that mesquite gum (*P. velutina*) with molecular weight of 386,000 g/mol and radius of gyration (R_G) of 50.47 nm and hydrodynamic radius (R_H) of 9.48 nm (López-Franco *et al.*, 2004), resembles a polydisperse macrocoil in agreement with the 'twisted hairy rope' proposal AGP for gum arabic (Fig. 18.4) (Qi and Lamport, 1991). The intrinsic viscosity of mesquite gum has been recently given as $[\eta] = 1.47 \times 10^{-2}\ M_W{}^{0.50}$ (mL g^{-1}) (Rinaudo *et al.*, 2008). From the absolute values of the constants of the Mark–Houwink relation, it follows that a very low intrinsic viscosity is obtained in consideration of the molecular weight; this is directly related to the highly branched structure.

18.3.4 Larchwood arabinogalactan

Arabinogalactan from larchwood is known to be composed of two main fractions, the more abundant fraction (70–95%) being the high molecular weight

(M_W ~ 37–100 kDa), AG-A, and a proportionally less abundant (5–30%) low molecular weight fraction (M_W ~ 7.5–18 kDa), AG-B (Swenson *et al.*, 1969; Clarke *et al.*, 1979). It is unclear whether a typical A/B ratio exists and differences in reported ratios have been attributed to analytical methodology (Jones and Reid, 1963). The principal material available commercially is the ultrafiltered product, which is known to correspond with AG-A (Ponder and Richards, 1997a). By contrast with other plant arabinogalactans, AG-A is protein free (Clarke *et al.*, 1979; Prescot *et al.*, 1995).

Chemically, arabinogalactans from larchwood have a general structure given by a backbone of (1→3)-linked β-D-galactopyranosyl units that account for about one-third of the molecule, each of which contain a side chain at position C6. Most of these side chains are galactobiosyl units containing a (1→6)-β-D-linkage. Another side chain type that occurs is a single L-Ara unit or 3-O-(β-L-arabinopyranosyl)-α-L-arabinofuranosyl units. Less frequent is a single β-D-Gal*p* or α-L-Ara*f* or a dimer β-L-Ara*p*-(1→3)-α-L-Ara*f* (Ponder and Richards, 1997b). The side group distribution is not uniform and the overall ratio of L-galactose to L-arabinose is ~6:1. Traces of uronic acid units have also been reported as part of the structure of AG-A (Ponder and Richards, 1997a). The representative chemical structure of larchwood AG is shown in Fig. 18.5.

Purified AG-A has been suggested to occur naturally as ordered assemblies of molecules that can be disrupted by alkali to form individual, unassociated molecules, i.e., disordered AG (DAG) (Ponder and Richards, 1997b). This order–disorder transition can be reversed by drying or freezing. Parallel studies have shown that when AG-A (37 kDa) is treated with sodium hydroxide solutions of 0.5 M or greater and 0.1 M sodium borohydride, the average molecular weight of the resulting arabinogalactan falls approximately four-fold to yield fractions of AG (~9 kDa) (Prescot *et al.*, 1995). Based on this evidence it has been proposed that larch AG consists of a series of subunits joined through au unknown type of linkage which is susceptible to cleavage at low alkali concentrations and moderate temperatures. ^{13}C-NMR spectra of AG-A (37 kDa) and AG (9 kDa) are identical except that broader spectral lines are observed in the AG-A spectrum due to its greater molecular weight. Whilst *in vitro* comparison of both materials using isolated asialoglycoprotein receptor shows equivalent bioactivity (Prescot *et al.*, 1995), it has been proposed that the low molecular weight material in the crude AG extract is possibly a biological precursor of the predominant, larger molecular weight form of AG in the extract (Prescot *et al.*, 1997). More recent X-ray fibre diffraction data supports a model for a curdlan-type triple helical structure for the ordered structure of arabinogalactan (Chandrasekaran and Janaswamy, 2002), whereby a galactan triple helix can accommodate disaccharide D-Gal-(1→6)-D-Gal substituents at C6 of every D-Gal unit in the main chain. This side group attachment is not unique and it can be done in several ways while preserving the helix symmetry. Under the proposed model, the arabinogalactan molecule resembles a bottle brush.

AG-B is the form of AG that exists naturally as discrete molecules. It constitutes some 5% or less of a typical AG sample and its average L-Ara

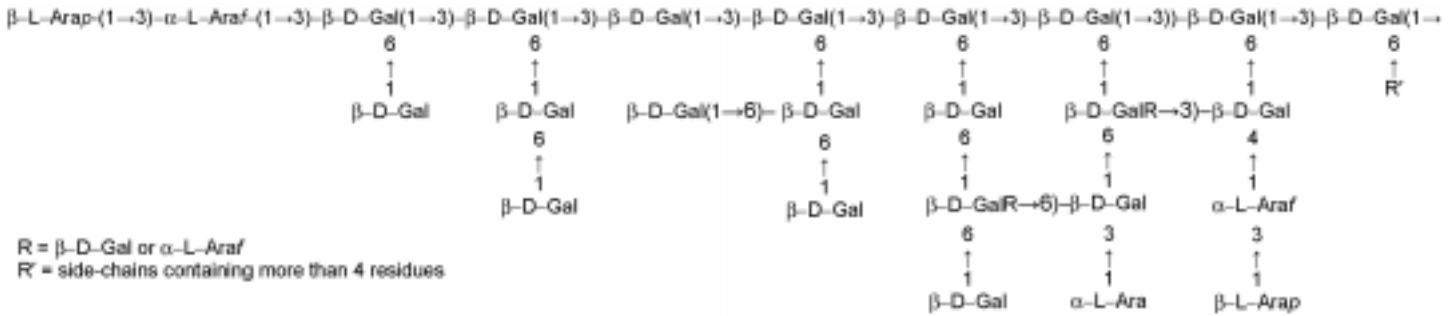

Fig. 18.5 Major structural features of a typical larchwood arabinogalactan molecule (from Ponder and Richards, 1997b).

content is about 38 mol%. It is distinguished from DAG by GPC, having a longer retention time, and its M_w is about 7,000 to 10,000 (Simson *et al.*, 1968; Swenson *et al.*, 1969). Neither drying nor freezing causes it to assume a multimolecular structure, and it contained no uronic acid residues.

18.4 Technical data

18.4.1 Gum tragacanth

Gum powder made from ribbon is white to light yellow in colour, odourless and has an insipid, mucilaginous taste. The flakes vary from yellow to brown to give cream to tan powders in colour. Both ribbon and flake gums are available in a variety of particle sizes and viscosities depending on the end use. A typical product specification of a high grade commercial gum tragacanth powder is:

Appearance: Off white to creamy coloured fine powder
Loss on drying: $\leq$12%
Ash: $\leq$3.0%
Acid insoluble ash: $\leq$0.3%
Viscosity 1% in water: 800 $\pm$ 150 cPs
Particle size: 90% through BSS 150 mesh

Specifications of lower grade gum diverge from top quality gum mostly in that the colour tends to cream and yellow and viscosity values may be as low as ~280 cPs.

Minimum quality and safety standards for gum tragacanth to be used in food and pharmaceutical products have been defined in the United States Pharmacopeia USP31 (USPC, 2007):

Arsenic: $\leq$3 ppm
Heavy metals (as Pb): $\leq$20 ppm
Microbiology: *Salmonella/E. coli* – absent

The main inherent functional properties of tragacanth exudates are briefly discussed next.

Solubility

Gum tragacanth swells rapidly in either cold or hot water to form a viscous colloidal solution, which acts as a protective colloid and stabilizer. While it is insoluble in alcohol and other organic solvents, the gum can tolerate small amounts of alcohol or glycol. The gum solution is fairly stable over a wide pH range down to extremely acidic conditions at about pH 2.

Viscosity

The viscosity is the most important factor in evaluating tragacanth and is regarded as a measure of its quality as well as a guide to its behaviour as a

suspending agent, stabilizer or emulsifier. The viscosity of 1% solutions may range from about 100–3,500 cPs depending on the grade. Ribbon types give a higher viscosity than flake types. The best quality of ribbon type gum tragacanth shows up to 3,500 cPs (1.0%, 25 ºC, 24 h, 20 rpm by Brookfield viscometer). Tragacanth highly viscous colloidal sols or semi-gels can serve as protective colloids and stabilizing agents. The high viscosity of tragacanth solutions results from the molecular characteristics of the gum, and these depend on the grade and physical form, and the manner in which it is taken up in water. For example, the same concentration of solution prepared from whole gum is more viscous than one prepared from powdered gum. Unlike many other gums, solutions of tragacanth have a very long shelf-life without loss of viscosity. The solution viscosity reaches a maximum in 24 h at 25 ºC, 8 h at 40 ºC and 2 h at 50 ºC. Fine powdered gum has shorter hydration time than coarse powder and good dispersion is needed to avoid the formation of aggregates. The maximum initial viscosity of tragacanth solutions is at pH 8, but maximum stable viscosity is at about pH ~ 5 (Stauffer, 1980). The viscosity is quite stable over a wide pH range from 2–10, particularly for the flake type of the gum (Wareing, 1997). The addition of strong mineral and organic acids causes some drop in viscosity. Divalent and trivalent cations can also cause a viscosity drop or may result in precipitation, depending on the metal ion type and concentration.

Rheological properties

The apparent viscosity of tragacanth solutions decreases as the shear rate increases and is reversible, with the original viscosity returning upon the reduction of the shear rate. Such pseudoplastic properties have an effect on the pouring and texture of the finished products.

Acid stability

Tragacanth solutions are naturally slightly acidic. A 1% solution has a pH of 5–6, depending on the grade of gum used. The viscosity is most stable at pH 4–8, but with very good stability at both the higher pH and at the lower end of pH 2. Tragacanth is one of the most acid-resistant gums, and is chosen for this characteristic for use under conditions of high acidity. However, when acids are used in the system, they should not be added until the gum has had time to fully hydrate.

Surface activity

Gum tragacanth has well-defined surface activity properties and produces a rapid lowering of the surface tension of water at low concentration, less than 0.25% (Glicksman, 1982a). Flake types of tragacanth (lower viscosity) are superior to the ribbon types (higher viscosity) for the reduction of surface tension and interfacial tension effects. Stauffer and Andon (1975) reported that at 1% concentration, the ribbon type gave 61.7 dynes/cm surface tension value compared with the value of 52.5 dynes/cm given by the flake type.

Emulsification ability
Gum tragacanth, regarded as a bifunctional emulsifier, is a most efficient natural emulsifier for acidic oil-in-water emulsions. It thickens the aqueous phase and also lowers the interfacial tension between oil and water. It has a reported hydrophilic lipophilic balance (HLB) value of 11.9 (Griffin and Lynch, 1972), but it is believed HLB values run from 11–13.9 depending on the grade of the gum because flake types have lower interfacial tension between oil and water than ribbon types (Anderson and Andon, 1988).

Heat stability
Elevated temperatures may also affect viscosity through a thinning effect on the solution. Upon cooling, however, the solutions tend to revert to nearly their original viscosity. Prolonged heating can degrade the gum and reduce viscosity permanently.

Compatibility
Tragacanth is compatible with other hydrocolloids as well as carbohydrates, most proteins and fats. There is an interesting interaction, however, between gum tragacanth and gum arabic, which results in an unusual viscosity reduction that has been attributed to the molecular association between both gum species (Rabbani *et al.*, 1995). Although the precise mechanism for this interaction is still unclear, it is exploited commercially to produce superior, thin, pourable, smooth emulsions with fish and citrus oils, which also have a long shelf-life.

Preservatives
Tragacanth solutions are less sensitive to microbial attacks and have longer shelf-life without loss of viscosity in comparison with other plant hydrocolloids. When preservatives are needed, glycerol or propylene glycol at 94 mL/litre serve as excellent preservatives in many emulsions. Sorbic acid, benzoic acid or sodium benzoate at less than 0.1% concentration are effective when used below pH 6. A combination 0.17% methyl and 0.03% propyl parahydroxybenzoate is effective at pH 3–9. Benzoic acid esters are also effective for maintaining solution properties throughout product preparation and shelf-life (Wareing, 1997).

18.4.2 Gum karaya
Gum karaya has a slightly acetous odour and taste. The colour of the gum varies from white to tan depending on grade. Cost is based on purity and colour. Powdered karaya contains about 10–14% moisture, but the loss on drying is higher than this due to the presence of volatile substances. A typical specification for top quality commercial gum karaya is shown below (from Importers Service Corporation, NJ, USA):

Appearance: Off white to buff fine powder
Odour: Light acetic acid

Taste: None
Loss on drying: $\leq$20%
Total ash: $\leq$5.0%
Acid insoluble ash: $\leq$1.0%
Particle size: $\geq$99.9% through USS 80 mesh;
$\geq$98% through USS 140 mesh
Viscosity 1% in water: $\geq$400 mPas
Viscosity 2% in water: $\geq$8000 mPas
pH 1% solution: 4.3–5.0
Salmonella: Negative
E. coli: Negative

Specifications for gum karaya from other manufacturers (Arthur Branwell & Co Ltd.) include maximal heavy metal contents, namely:

Heavy metals (as Pb): $\leq$20 ppm
Pb: $\leq$5 ppm
As: $\leq$3 ppm

Solubility

Gum karaya is the least soluble of commercial gums and forms true solutions only at very low concentrations (<0.02% in cold water, 0.06% in hot) (Le Cerf *et al.*, 1990) but highly viscous colloidal dispersions can be produced at concentrations up to 5%, depending on quality. Due to the acetyl group on the gum structure, gum karaya does not fully dissolve in water to give a clear solution; instead it absorbs water rapidly to form viscous colloidal dispersion at low concentration. The fine mesh gum hydrates much more rapidly than coarser gum, and gives a smooth, homogeneous solution. On the other hand, coarse granules yield a grainy dispersion. Up to 4% of gum may be hydrated in cold water to give a viscous gel-like paste of uniform smoothness and texture. Karaya will form viscous solutions in 60% alcohol, but is insoluble at higher concentrations. Deacetylation by using alkali in solution can modify the gum's characteristics from water-swellable to water-soluble (Le Cerf *et al.*, 1990).

Generally, gum karaya of Indian origin (mainly from *S. urens*) has a higher acid value and a more pronounced acetic odour than that of African origin (mainly *S. setigera*), resulting in African karaya having a better solubility than Indian gum karaya; a factor sought by some users.

Viscosity

The viscosity of karaya dispersions ranges from about 120–400 cPs for 0.5% dispersions to about 10,000 cPs for 3% dispersions depending on the grade. At concentrations of 2.0 and 3.0% in water, viscosities approach infinity at low shear rate values and yield stresses of 60 and 100 μN/cm^2 were determined, respectively (Mills and Kokini, 1984). As a consequence, concentrated gum solutions are useful to suspend particles and give soft, spreadable gels with a jam-like consistency (Wareing, 1997). The smoothness of the gum solution is

determined by the particle size and can be modified by prolonged stirring to achieve a smooth texture and reduced viscosity (Nussinovitch, 1997). In comparison, other hydrocolloids, such as guar gum, do not form a network and flow under all shear stresses. Gum karaya, in the dry state, loses viscosity on ageing and builds an acetic odour. The loss of viscosity is related to the loss of acetic acid. The fine powdered gum suffers greater viscosity loss than the granules or the whole exudate. This decrease is most noticeable in the first few weeks after the gum has been ground. High temperature or high humidity storage are harmful to its stability, therefore its recommended storage temperature should not exceed 25 ºC (British Pharmacopoeia, 1998). Climate and time of harvest also affect the viscosity. In solution, karaya is more viscous when hydrated in cold rather than in hot water. Boiling temperatures longer than two minutes particularly reduce the viscosity. The viscosity of karaya solutions may decrease with added electrolytes. The dispersion is not sensitive to weak electrolytes, but when certain strong electrolytes are added, even in small amounts, loss of viscosity occurs (Whistler, 1993). Therefore, salts should be added only after the gum has been fully hydrated. There is no distinct correlation between viscosity and grade. Where viscosity is important, powdered karaya should be used within six months after processing.

Rheological properties

When gum karaya absorbs water, the particles do not dissolve but swell extensively. Gum karaya solutions are thixotropic. The hydrated swollen particles are not stable to mechanical shear and prolonged stirring causes viscosity decrease. Gum karaya does not possess the 'pourability' characteristic of gum tragacanth. Oscillatory small-deformation rheology has been utilized to investigate the rheological properties of karaya gum in the presence of added salt (Silva *et al.*, 2003). Gel-like mechanical spectra were recorded for *S. urens* and *S. striata* gums, with $G' > G''$ moduli and no frequency dependence, thus indicating that a gel network is formed. The presence of acetyl groups in both gums seems to stabilize the gel. In turn, a separate study has shown that karaya gum forms true gels (i.e. $G'/G'' > 3$) only at concentrations greater than 4% and that the addition of NaCl decreases the gel strength (Brito *et al.*, 2005). Karaya gels studied by the latter group did not present any sharp variation in G' or G'' with increasing temperature.

pH stability

The pH of a 1% solution of gum karaya is about 4.5–4.7 for Indian origin and 4.7–5.2 for African origin. The viscosity of solutions decreases upon the addition of acid or alkali. Higher viscosity can be obtained if the gum is fully hydrated prior to pH adjustment (Glicksman, 1982b). Above pH 8, alkali irreversibly transforms the characteristic short-bodied solution into a ropy, stringy mucilage as the molecules lose their acetyl groups through rapid saponification. This has been ascribed to deacetylation of the karaya molecule. Due to high uronic acid content, karaya dispersions withstand acid conditions

quite well and resist hydrolysis in 10% hydrochloric acid solution at room temperature for at least 8 h (Whistler, 1993).

Heat stability
Heating karaya dispersions changes the polymer conformation and increases the solubility, but results in a permanent viscosity loss. Maximum concentrations of 4–5% can be prepared by cold water hydration, but when heating under pressure, smooth, homogeneous, translucent and colloidal solutions at concentrations as high as 18–20% can be obtained (Glicksman, 1982b).

Water-binding properties
Gum karaya has a strong water-binding ability. It can absorb water and swell to more than 60 times its original volume.

Film-forming properties
Gum karaya forms smooth films when plasticized with compounds such as glycols.

Adhesive properties
At high concentrations of 20–50% gum karaya in water gives heavy pastes with strong wet-adhesive properties. This enables karaya gels and pastes to resist loss of strength when diluted (Glicksman, 1982b). These are used in dental adhesives and colostomy bag sealing rings (Wareing, 1997).

Compatibility
Gum karaya is compatible with most gums as well as proteins and carbohydrates. Blending karaya with other gums, such as alginate, can modify the solution characteristics (Le Cerf and Muller, 1994). However, karaya gels are incompatible with pyrilamine maleate, a strong hydrotrope and anti-histaminic agent. Strong electrolytes or excessive acid cause a drop in viscosity, while alkalis make karaya solutions very ropy (Meer, 1980).

Preservative
The viscosity of karaya solution remains constant for several days and decreases gradually with ageing, unless preservatives are used to prevent bacterial attack. Preservatives such as benzoic or sorbic acid, methyl and propyl parahydroxy-benzoate, glycerol, propylene glycol, chlorinated phenols, formaldehyde, and mercuric salts, are suitable.

18.4.3 Mesquite gum

Unprocessed mesquite gum is available as vitrified nodules of varying size and shape and has red amber to tan colour. Dry mesquite gum is dissolved in water to form solutions which are dextrorotatory (ca. +60º). The Mexican Ministry of Health has proposed specifications for gum intended for use in foods (Secretaría de Salud, 1996). These along with the main physico-chemical characteristics

Table 18.1 Analytical parameters for *Prosopis velutina* and specifications for *P. laevigata* gum

	P. velutina (hand sorted)	*P. laevigata*[a]
Appearance	Vitrous nodules	Vitrous nodules
Color	Red amber	Red amber
Loss on drying (%)	9.7 ± 0.1	≤15
Ash (total, %)	2.6 ± 0.01	≤4.0
Ash (acid insoluble (%))	NA	≤0.5
Arsenic (as As)	NA	≤3 ppm
Heavy metal (as Pb)	NA	≤40 ppm
Lead	NA	≤10 ppm
Tannin (%)	0.46 ± 0.03	≤2.0
Starch or dextrin	NA	Passes test
Insoluble matter (%)	0.6 ± 0.1	≤1.0 %
Specific rotation $[\alpha]_D^{20}$	+ 66.7 ± 5.3	+ 77.0
Total nitrogen (%)	0.7 ± 0.1	0.4 ± 0.07
Protein (%)[b]	4.6 ± 0.6	2.6 ± 0.06
Viscosity 20% (cps)[c]	25	NA
Microbiology	Coliform negative	NA
pH	4.5	NA
Acid equivalent weight (g mol)	1282	NA
Glucuronic acid (mol%)	3	16.2 ± 1.3[d]
Arabinose (mol%)	71	40.4 ± 2.04[d]
Galactose (mol%)	26	43.3 ± 1.4[d]
Rhamnose (mol%)	ND	1.3 ± 0.2[d]

[a] Maximum values are taken from specifications from Ministry of Health of Mexico, *Secretaría de Salad* (1996), the rest of the values have been measured experimentally.
[b] Protein = N × 6.53.
[c] At 20 °C in 0.1 M NaCl.
[d] From Orozco-Villafuerte *et al.* (2003).
NA = Not available; ND= not detected

derived from various studies on the gums from *P. velutina* and *P. laevigata* are compiled in Table 18.1.

Solubility

Mesquite gum has extremely high solubility in aqueous medium, which can yield solutions above 50% (w/w) concentration (Goycoolea *et al.*, 1995). It is also soluble in aqueous ethanol up to 70% ethanol, and has limited solubility in glycerol and ethylene glycol but is insoluble in organic solvents and oils (Vernon-Carter *et al.*, 2000). *Prosopis* gum solutions present colours that vary from slight yellow or amber to a dark brown colour depending on the concentrations and botanical origin.

Viscosity

The viscosity of mesquite gum solutions even at high concentrations is very low when compared with that of other polysaccharide gums (Vernon-Carter and

Sherman, 1980). At concentrations below 15% (w/v) the solutions have been reported as being 'shear thickening' as the shear rate increased beyond $100\,s^{-1}$, and attributed this effect to either a change in molecular shape at high shear rates or to an experimental artifact caused by turbulent flow in the coaxial cylinder geometry used. The viscosity of 20% (w/w) solutions in 0.1 M NaCl at 25 ºC was ~25–30 mPas and presented a Newtonian behaviour (Goycoolea *et al.*, 1995), though shear thinning occurred at greater concentrations. At concentrations of 50% (w/v) mesquite gum solutions exhibited a clear non-Newtonian behaviour (Rinaudo *et al.*, 2008).

Effect of pH

The functional properties of mesquite gum are affected by pH. The relative viscosity (η_{rel}) of mesquite gum solutions increases as pH increases from 4.0 to 7.0, due to the substitution of the H^+ ions by Na^+ ions with a greater degree of dissociation, leading to the macroion unfolding and hence to the increase in viscosity. As the pH increases from 7.0 to 9.0 the macroion cannot expand further due to steric constraints, and as the amount of Na^+ counterions increases, they shield the macroion charges and cause it to fold and hence the solution viscosity to decrease (Vernon-Carter *et al.*, 2000). In turn, it has been observed that the effective electrical surface charge (given by the zeta potential) of orange oil-in-water emulsions stabilized with mesquite gum, increases with pH reaching an approximately constant value at pH ~7.0. As the concentration of added NaCl increases from 10^{-3} to 10^{-2} M, the compression of the electrical double layer, due to charge shielding, leads to a comparatively lower zeta potential values at all pHs (Acedo-Carrillo *et al.*, 2006).

Surface activity

Mesquite gum solutions reduce the interfacial tension as a function of the concentration and time (Vernon-Carter and Sherman, 1981). As the gum concentration increases in the range 0.5–25%, the interfacial tension decreases faster. The solution pH also influences the lowering of the interfacial tension with time, an effect that has been directly related to the mesquite gum conformation in solution. The more compact mesquite gum molecular species are, the faster and lower is the decrease in interfacial tension. This has been attributed to the diffusion and/or the conformation of the gum species at the interface. In parallel studies, the absorption of water and oil by mesquite gum at temperatures in the range of 23–45 ºC were greater than those of gum arabic. The activation energy values obtained for water and oil absorption for gum arabic were 21.98 and $39.57\,kJ\,mol^{-1}$, respectively, compared to those of mesquite gum with values of 15.79 and $46.16\,kJ\,mol^{-1}$, respectively (Beristain *et al.*, 1996).

In separate studies, changes in the surface tension of an orange oil–water interface, as probed by a Wilhemly plate, were measured. These measurements showed that the adsorbed surface per molecule for gum arabic was an order of magnitude greater than that of gum mesquite (23.0 and 2.2 nm^2, respectively)

(Goycoolea *et al.*, 2000), revealing that the structural microheterogeneity plays a key role in the functional behavior of these materials. In turn, mesquite gum and its major fractions, separated by hydrophobic affinity chromatography, of varying protein contents (7.18–38.60%) and macromolecular dimensions (M_w ~ 3.89×10^5–8.06×10^5 g mol^{-1}, R_g ~ 48.83–71.11 nm, R_h ~ 9.61–24.06 nm), have been studied in Langmuir monolayers spread at an air–water interface and compared with whole gum arabic and its corresponding fractions (López-Franco *et al.*, 2004). The most active species at the interface were those containing greater amounts of protein. These results have been related with the fine structural differences between the constituent macromolecular species comprising the gum (López-Franco *et al.*, 2004).

Emulsification ability

Like gum arabic, mesquite gum also forms and stabilizes oil-in-water emulsions and has the ability to encapsulate orange citrus oil during spray drying (Goycoolea *et al.*, 1997; Vernon-Carter *et al.*, 1996b; Beristain *et al.*, 1996). Mesquite gum solutions of 15 w/w% are able to form emulsions with *n*-decane, *n*-dodecane, *n*-tetradecane and *n*-hexadecane with mean droplet diameters of 4–4.5 μm; whereas with orange oil the average droplet diameter was found to vary in the range 2.5–3.0 μm (Valdez *et al.*, 2006; Acedo-Carrillo *et al.*, 2006). Moreover, the particle size of emulsions of orange oil-in-water stabilized with 1% mesquite gum remained unchanged for up to 100 h. By contrast, in emulsions with D-limonene and *n*-decane, phase separation starts within the first 24 h (Acedo-Carrillo *et al.*, 2006). This behaviour of mesquite gum on the orange oil emulsions to stop or control Ostwald ripening is attributed, among other causes, to the fact that orange oil is less water soluble than D-limonene. In addition, mesquite gum-stabilized emulsions of orange oil showed the ability to form a gel structure with time, in contrast with similar emulsions stabilized with gum arabic, with those obtained with alkane oils and with D-limonene. These results seem to indicate that the nature of the oil used is a key factor for gel formation and for the prolonged stability of the emulsions formed with mesquite gum (Valdez *et al.*, 2006; Acedo-Carrillo *et al.*, 2006; Rinaudo *et al.*, 2008).

In other studies, it has been found that mesquite gum with a nitrogen content of 0.49% had better emulsifying capacity for chilli oleoresin than gum arabic with nitrogen contents of 0.35% (Vernon-Carter *et al.*, 1996b). The mesquite gum stabilized emulsions had similar initial particle size and exhibited monodisperse particle size distribuition over 8 days, while gum arabic emulsions had a larger initial particle size and polydisperse particle size distribution that broadened with ageing time up to 8 days.

Encapsulation ability

Several materials are commercially available for encapsulation of essential oils, flavours, colorants and vitamins by spray-drying. The most widely used encapsulation agents are gum arabic and modified or hydrolysed starches. Mesquite gum has been reported as having the ability to encapsulate orange peel oil

(Goycoolea *et al.*, 1997) (80.5% of the starting oil) (Beristain and Vernon-Carter, 1994). A blend of 60:40% gum arabic to mesquite gum was able to encapsulate the same amount of orange peel oil as pure gum arabic (Beristain and Vernon-Carter, 1995), whereas a 3:2 ratio of maltodextrin 10 DE to mesquite gum, retained 84.6% of the starting orange peel oil, thus providing a better encapsulating capacity.

Film forming
Mesquite gum-based films have become an important research topic mainly due to their ability to regulate moisture, lipid and gas migration. Such films can be used to extend the shelf-life of foodstuffs. Emulsion films using mesquite gum as structural agent and a blend of candelilla wax with white mineral oil as the lipid phase, prolong the shelf-life of treated guava fruit (*Psidium guajava* L.) by retarding ethylene emission and enhancing the texture of the fruits (Tomás *et al.*, 2005a). On the other hand, blends of mesquite gum (*Prosopis* spp) with whey protein concentrate, have been reported to form edible films with poor moisture barrier properties (Tomás *et al.*, 2005b). A complex of mesquite gum and chitosan complex has also been used to form edible films again with low water vapour permeability (Ruíz-Ramos *et al.*, 2006).

Compatibility
Mesquite gum has been used successfully for various purposes in combination with other gums (e.g., gum arabic), maltodextrins, lipids (candelilla wax), vegetal and animal proteins (e.g., corn zein, soy, whey, peanut proteins, gelatin, casein and milk whey proteins) and with other polysaccharides such as chitosan (Ruíz-Ramos *et al.*, 2006; Pérez-Orozco *et al.*, 2004), sodium alginate and κ-carrageenan (Tomás *et al.*, 2004). Blends of gum arabic and mesquite gum exhibited a synergistic effect that provided greater long-term stability against drop coalescence than either component on its own; however, mesquite gum provided better stability against drop coalescence and deterred pigment degradation better than gum arabic and its blends (Vernon-Carter *et al.*, 1996b). When mixed with gelatin, mesquite gum forms complex coacervates at a 1:1 mass ratio. Microcapsules based on this complex coacervate system have been exploited in encapsulation of corn and orange oil (Vernon-Carter *et al.*, 2000).

Preservatives
Mesquite gum solutions can be preserved with benzoic acid, formic acid and *p*-hydroxybenzoic acid or a combination of sodium benzoate, potassium sorbate and citric acid (Vernon-Carter *et al.*, 2000).

18.4.4 Larchwood arabinogalactan

Commercial larch arabinogalactan (LAG) is a dry slightly yellow free-flowing powder with a very slight pine-like odour and sweetish taste (Kelly, 1999). Food grade LAG ($\geq$98% purity) is free of phenolic, terpenoid or other extraneous chemical and is completely colourless, odourless and tasteless. This material is

used for clinical applications. Low purity arabinogalactan has significant levels of polyphenolic lignin impurities which impart a light yellow colour and strong odour.

Typical specifications for food grade LAG are given below (from Lonza Ltd):

Appearance: Fine, off-white to white free-flowing powder
Carbohydrates > 90%
Physical state: Texture – free-flowing powder; flavor – minimal; odor – minimal
Color (CWF): L > 85; a – Record; b <15; whiteness > 60
Dissolution: Sink – wet pass; lumps – None
Heavy metals < 5 ppm
Lead <0.1 ppm
Arsenic <0.4 ppm
Cadmium <4.1 ppm
Mercury <0.3 ppm
Bulk density 0.30–0.40 g/ml
Particle size (+40 mesh) < 20%
Viscosity (30%) < 15 cPs
Moisture $\leq$ 6%
Microbiological: S.P.C. <1000 cfu/g; yeast <10 cfu/g; mold <100 cfu/g; Salmonella – negative; coliform <10 cfu/g; *E.coli* – negative; *Staphylococcus aureus* – negative

The main inherent properties of larchwood arabinogalactan are discussed.

Solubility

AG is highly soluble in water and stable over a wide range of concentrations, pH and temperatures (Fitzpatrick *et al.*, 2004). Aqueous solutions of arabinogalactan are fluid up to 60% concentration; above this concentration, they form a thick paste and finally a glass that is friable when the moisture content is below about 10%.

Stability to pH

Arabinogalactan is stable at a wide temperature and pH range which provides for instant, trouble-free application in various systems. In beverages it does not degrade or lose functionality and will not hydrolyse.

Osmolality

UF grade AG has an osmolality of 75 mOsm kg^{-1} (at 30%, w/w) with AG contributing about 25 mOsm kg^{-1}, whereas, food grade AG contains a 1–10 mM concentration of non-AG components, primarily salts.

Viscosity

The viscosity of LAG solutions in water shows a linear dependence on concentration in the range 0–6% and beyond this concentration deviations from

linearity start to occur (Owens, 1940). Even at very high concentrations (30%) the viscosity of LAG solutions remains very low (~15 cPs). This is in line with the behaviour of mesquite gum and gum arabic highly branched arabinogalactans.

Mouthfeel
Along with its high solubility, LAG provides very little sensory impact, offering minimal 'mouthfeel' and viscosity (Nazareth *et al.*, 1961). Testing has also shown that it has very little off-taste or unpleasant aftertaste.

Moisture retention and shelf-life
LAG can be easily formulated into food and beverage systems. It retains moisture in baked goods and has improved dough-handling characteristics. It helps to contribute to a finer, more uniform grain and has improved taste and aroma in tortillas. Also, LAG is effective in lowering water activity in sweetener compositions. It provides film-forming properties for extended shelf-life and tack-on aid.

18.5 Uses and applications

18.5.1 Gum tragacanth

Gum tragacanth, like gum arabic, has been in commercial use for well over 2,000 years. It has many industrial uses (e.g., arts, foods, pharmacy) because of its bland flavour and mucilaginous qualities and stability to heat and acids. Another important characteristic of tragacanth is its bifunctional action as an emulsifier that increases the viscosity of the aqueous phase and lowers the interfacial tension between oil–water emulsions (Whistler, 1993). Gum tragacanth is available in grades of varying quality and refinement with 1% viscosities of about 300 cPs to 3,000 cPs. Solutions are pseudoplastic, show a reversible decrease in viscosity at elevated temperatures and possess good yield value.

Food applications
Gum tragacanth is used in food in accordance with the FDA Code of Federal Regulations (CFR section 184.1351); (FDA, 2006). Its superior water absorbing qualities make it an excellent thickening agent. Gum tragacanth is used in many everyday commercial products of low viscosity, such as jellies and pourable dressings. It is also used in syrups, mayonnaise, sauces, liqueurs, candy, ice cream, desserts and popsicles.

Also, it is the traditional binder used to make a paste used in floral sugarcraft to create life-like flowers on wires used as decorations for cakes. It makes a paste which dries brittle in the air and can take colourings.

By virtue of its surface activity combined with its effect on viscosity, gum tragacanth is used widely in ice cream to optimize texture to the product, and it

also prevents the formation of ice crystals during storage. The water-swellable tragacanthic acid component has been used in frozen desserts, such as water ices, sorbets, and ice pops, to hold the free water, thus preventing the migration of flavour and colour components during storage and consumption (Weiping, 2000). Tragacanth is also used to some extent as a binder and adhesive in confectionery, especially in candies that contain natural fruit acids, to which it is stable (Whistler, 1993; Stephen and Churms, 1995).

Xanthan gum may replace gum tragacanth in many of its more traditional food applications, at a more cost-effective and stable price. It also has the added advantages of constant quality and virtual sterility as a result of its manufacturing process (Anderson and Weiping, 1994). However, due to certain outstanding and unique properties, there are certain applications in which gum tragacanth cannot be replaced successfully by xanthan or any other gums.

Non-food applications

Gum tragacanth is used as thickening agent in the preparation of dyes for calico printing, textile dyes and for dressing fabrics. It is also a thickener in making glues, water colours and ink (where it supplies a gloss). It is a binding agent in paper making, a culture medium in laboratories and a water-proofing agent of fabrics, etc. Gum tragacanth can be used in a variety of polishes such as furniture, floor and auto polishes (Whistler, 1993; Verbeken *et al.*, 2003). Furthermore, it is used as a binder to make incense cones, sticks and pellets (Genders, 1994).

Pharmaceutical applications

Gum tragacanth has been used medicinally for thousands of years. It is an effective suspending agent for many pharmaceutical products. In folk medicine it has been used as a laxative, and for the treatment of persistent cough, diarrhea, and as an aphrodisiac. Modern pharmaceutical applications include its use (0.4–0.8% w/w) as an adhesive agent for pills and tablets, and for emulsifying oil droplets in pastes, hand creams and lotions. Gum tragacanth can act as the suspending agent in several types of toothpaste with a humectant such as glycerol or propylene glycol. An important use of gum tragacanth is in spermicidal jellies, where it acts as a spermatozoa chemical immobilizing agents upon contact (Whistler, 1993).

Historically, tragacanth has been taken by mouth to treat digestive complaints and coughing. It has also been used in small amounts as a laxative because it swells up and becomes slick as it is exposed to fluids in the stomach and intestines. The resulting soft, slippery mass may help to relieve constipation by triggering intestinal muscle contractions, which assist in expelling intestinal contents. Although it would seem contradictory, in larger doses, tragacanth's ability to absorb excess water and add bulk to intestinal contents may be moderately effective for treating diarrhea. In the past, tragacanth was added to cough syrups and lozenges because of its soothing effect on irritated mouth and throat tissue.

An Asian species (*Astragalus membranaceus*) has been used for centuries in traditional Chinese herbal medicine. Known as Huang Ch'i, radix astragali and astragalus root, the boiled root strips are taken in a tea to increase one's ch'i or 'wind energy'. The ground root is also available in capsules and as a liquid extract. This remedy is used to overcome fatigue, lower blood pressure, and to treat colds, nephritis and hypoglycemia. There are a number of published medicinal uses for this species, either by itself or decocted with other herbs, for the treatment of diabetes mellitus, cancers and malaria. Because of its anti-bacterial properties, it has been used in traditional Chinese medicinal tonics for upper respiratory infections. Other remedies include the treatment of coronary heart disease and anemia. It has also been effective in the treatment of chronic hepatitis by increasing cellular immunity. Complex glucoarabinan poly-saccharides isolated from a related Asian species *A. mongholicus* have been shown to stimulate the production of T-cells and antibody-producing plasma cells (Hikino, 1985; Wagner and Proksch, 1985).

In powder, gum tragacanth is used as a vehicle for active and heavy medicines, for the purpose of giving cohesion and firmness to lozenges, and to form paste, which chemists use to label their prescriptions. Furthermore, tragacanth was used as a vehicle for a novel non-nucleoside reverse transcriptase inhibitor of human immunodeficiency virus type 1 (HIV-1) known as Emivirine (6-benzyl-1-(ethoxymethyl)-5-isopropyl-uracil). Emivirine was suspended in 0.5% gum tragacanth and was administered orally to male Sprague–Dawley rats, beagle dogs, and monkeys (*Macaca fascicularis*) at different concentrations to the compound to study its absorption, distribution into the brain, effects on hepatic drug metabolizing enzymes, and biliary excretion (Szczech *et al.*, 2000).

Other application of gum tragacanth is the formation of stable emulsions containing 50% insect repellant. They are effective as pure repellant compounds against mosquitoes, mites, chiggers, ants and some fleas (Whistler, 1993).

18.5.2 Gum karaya

Gum karaya (*Sterculia urens*) has been used commercially for about 100 years. Its use became widespread during the early twentieth century, when it was used as an adulterant or alternative for gum tragacanth (Verbeken *et al.*, 2003). However, investigation indicated that karaya possessed certain physico-chemical properties that made it more useful than tragacanth; furthermore, gum karaya is less expensive. Traditionally, India is the largest producer and exporter of gum karaya. Increasing amounts are exported by African countries (Verbeken *et al.*, 2003). The quality of gum karaya depends on the thoroughness of impurity removal. Food-grade gum is usually a white to pinkish gray powder with a slight vinegar odour. Pharmaceutical grades of karaya may be almost clear or translucent.

Food applications

Gum karaya is generally recognized as safe by the FDA and has a number of applications in the food industry. This is supported by the observation that

dietary gum karaya is neither digested nor degraded by gut microflora or absorbed to any significant extent in human beings. Taking into account these properties, gum karaya is used in concentrations from 0.2–0.4% as a stabilizer for aerated dairy products and frozen desserts, controlling the formation of ice crystals. It is used as an acid resistant stabilizer for sherbets, fruit ices and similar low pH products, in stabilizing packaged whipped cream products and meringue toppings. It is also used to prevent syneresis and improve the spreadability characteristics of cheese spreads when used in concentrations up to 0.8%. It is a good emulsion stabilizer for French-style salad dressings because it increases the viscosity of the aqueous phase of the oil–water emulsion. It is used as a binder for making low calorie dough-based products such as pasta, bread and other bakery products. In addition, is very effective in preparation of special quick-cooking farina cereals and ground meat products as it provides good water-holding and -binding properties to yield good quality finished products.

Industry

In the paper industry, gum karaya is used in the manufacture of long fibred, lightweight papers. It is used in textile printing operations as a thickening agent for the dye in direct colour printing on cotton fabrics (Verbeken *et al.*, 2003).

Pharmaceutical uses

Medicinally, gum karaya is an effective bulk laxative as gum particles absorb water and swell 60 to 100 times their original volume. The mechanism of action is an increase in the volume of the gut contents. Gum karaya should be taken with abundance of fluid and it may take a few days for effects to be noticeable. On the other hand, karaya has also been used as an adhesive for dental fixtures and ostomy equipment, and as a base for salicylic acid patches (Bart *et al.*, 1989). *In vitro* experiments showed that use of karaya coating may be an effective means of preventing the accumulation of denture plaque and the associated problems such as denture induced stomatitis, staining and unpleasant odours (Wilson and Harvey, 1989). The demulcent properties of the gum make it useful as an ingredient in lozenges to relieve sore throat. Gum karaya matrices have also been used as drug carriers such as caffeine and diclofenac-sodium in ratios of gum:drug of 3:1 and 1:1 (Munday and Cox, 2000). In addition, Murali and coworkers (2002) showed that modified gum karaya could be used as a potential carrier in the dissolution rate enhancement of nimodipine.

18.5.3 Mesquite gum

Mesquite gum has been used in Mexico for centuries mostly in folk medicine and more recently as a substitute for gum arabic in food and drinks. However, the fact that it does not have approval from the FDA has limited its more widespread use in the world. Many studies account for the novel and beneficial uses of mesquite gum in food and other systems, and the main applications have been reviewed (Vernon-Carter *et al.*, 2000).

Flavour and colour emulsification

As mentioned in the previous section, mesquite gum solutions are effective in the preparation and stabilization of oil-in-water emulsions. This has been exploited mostly for the stabilization of orange peel essential oil and oleoresins. Independent studies have demonstrated that mesquite gum exhibits smaller average oil droplet size and better stability than identical emulsions made with gum arabic (Beristain, 1996; Acedo-Carrillo *et al.*, 2006). Mesquite gum has been used in trials by the food industry. Soft drinks made from concentrated orange essential oil-in-water emulsions were found to require 70% less mesquite gum than gum arabic for achieving similar initial particle size and stability, while no significant differences in flavour were detetected among both formulations (Vernon-Carter *et al.*, 2000). In other studies, Aztec marigold (*Tegetes erecta*) (the source of an FDA-approved xanthophyll – a carotenoid – pigment) oleoresin-in-water emulsions have been stabilized against drop coalescence and loss of colour. Also, in chilli oleoresin-in-water emulsions, mesquite gum has been found to confer smaller and more uniform initial particle size and greater stability against droplet coalescence and colour degradation than gum arabic (Vernon-Carter *et al.*, 1998).

Flavour and colour microencapsulation

Flavour and colour encapsulation by spray-drying are among the chief industrial applications of gum arabic, hence, the utilization of mesquite gum for this purpose has been sought. To this end, mesquite gum has been used as the sole encapsulation agent (Beristain and Vernon-Carter, 1994) or mixed with maltodextrin (Goycoolea *et al.*, 1997, 1998; Beristain and Vernon-Carter, 1994) to encapsulate orange essential oil during spray-drying. In both series of studies, it was found that mesquite gum has the ability to retain more than 80% of the oil load, though to a slightly lesser extent than gum arabic. In low-tannin ultrafiltrated mesquite gum, it was found that the removal of glycoproteic fractions of 50 kDa affected its capacity to retain orange oil; however, when only species of 10 kDa are removed, the oil encapsulation capacity is not significantly affected when compared with that of gum arabic (Goycoolea *et al.*, 1997, 1998). Other potential applications of mesquite gum in food systems include its use in bread making, where it was found that the sensory properties of baked bread containing mesquite gum in the range 0.8–1.2% (w/w) increased by 9 days (Vernon-Carter *et al.*, 2000).

Non-food applications

Among the earliest documented uses of mesquite gum in the chemical industry was as a source of L-arabinose (Anderson and Otis, 1930), which is an important ingredient in culture media. The use of mesquite gum for the microencapsulation of shrimp diets and larvae feedstuff was found to improve the survival rates and overall quality of the microcapsules (cited in Vernon-Carter *et al.*, 2000). Mesquite gum has also been used as a binder in tablet dosage forms and as a suspending agent, where it compared well with gum arabic and was superior to

tragacanth gum (Khanna *et al.*, 1997). In Sonora, household applications of mesquite gum include hardening of hats and as paper glue (Balderrama, 1998).

18.5.4 Larchwood arabinogalactan

Common applications for the arabinogalactan are their use as emulsifiers, stabilizers and binders in the food, pharmaceutical and cosmetic industries. More recently interest has focused on the use of this polysaccharide as a low viscosity dietary fibre and as a prebiotic (Robinson *et al.*, 2001). Arabinogalactan from *Larix laricina* and *Larix deciduas* have many characteristics such as complete miscibility with water and low viscosity at high dissolved solids contents (Adams and Ettling, 1973).

Industry

Larch arabinogalactan can be used in the printing and ink market for litho plate protection and ink colour transfer. In addition, arabinogalactan can be used in the mining industry for the reserve flotation of iron ores. On the other hand, arabinogalactan has been tested as a novel protecting agent for maintaining precious metal nanoparticles in colloidal suspension (Mucalo *et al.*, 2002).

Biomedicine

The high solubility in water, biocompatibility, biodegradability and ease of drug conjugation in an aqueous medium, make arabinogalactan an attractive drug carrier (Jung *et al.*, 1997). The degree of oxidation of the polymer can be varied to a large extent, thus leading to various amounts of bound drug.

Arabinogalactans from Larix are used to obtain a conjugate with a water insoluble antifungal agent (amphotericin B) via an imine or amine bond. The conjugates are highly water soluble and could be appropriately formulated for injection. In addition, the conjugate shows comparable MIC values against *C. albicans* and is about 40 times less toxic than free AmB in mice (Ehrenfreund-Kleinman *et al.*, 2002a).

Another application is the formation of insoluble three-dimensional sponges a basis of dextran and AG covalent crosslinking to oxidized polysaccharides with diamines or polyamines such as alkanamines or chitosan. AG-chitosan sponges have an inflammatory response confined to the implant site which decrease with time (Ehrenfreund-Kleinman *et al.*, 2002b).

The composites of average particle size in the range 1.7–2.5 μm on the basis of available natural polysaccharide arabinogalactan isolated from the Siberian Larch (*Larix sibirica*) and metal (oxides of iron, cobalt, copper, nickel as well as metals gold, palladium, platinum) in ranges 0.1–21.0%, possess high antimicrobial activity against gram-negative enterobacteria such as *Escherichia coli*, *Salmonella typhimurium*, *Candida albigans*, *Bacillus subtilis* and *Staphylococcus aureus* (Aleksandrova *et al.*, 2004) and immunomodulating activity (Borisov *et al.*, 2004). The derivatives containing from 1 up to 21% of silver can be used as bactericidal additives to lacquer coating in medicine.

Larch arabinogalactan from *Larix occidentalis* showed an increase in the circulation of peripheral blood monocytes. Tumor cells pretreated with larch arabinogalactan enhanced NK cell cytotoxicity and phagocytic capacities of macrophages and lymphocytes, and increased release of various cytokines, such as IFN-γ, TNF-α, IL-1β, and IL-6 (Kim *et al.*, 2002).

For targeted drug delivery purposes, microscopic evidence has revealed that fluorescein-labelled arabinogalactan accumulates preferentially in the liver where it is taken up by parenchymal cells. This process is mediated by the high affinity of AG to the asialoglycoprotein receptors (Kaneo *et al.*, 2000). This has been attributed to the presence of numerous terminal galactose residues in AG structure (Fig. 18.5) (Groman *et al.*, 1994). Moreover, since arabinogalactan is not immunogenic and is a very safe compound (Naim and van Oss, 1992; Groman *et al.*, 1994) it is recognized as an excellent hepatotropic carrier candidate for the receptor-mediated delivery of enzymes and drugs to the liver parenchymal cells (Enriquez *et al.*, 1995; Cui *et al.*, 1997; Kaneo *et al.*, 2000).

Food

In food, arabinogalactan is neutral in taste, odour and colour. It is used as an emulsifier, stabilizer, binder or bonding agent in essential oils, humectant, non-nutritive sweetener, flavour base (Fitzpatrick *et al.*, 2004). It enhances the shelf-life of many types of products by up to 10%, it retains moisture and enhances mouthfeel and texture. The texture of baked products is improved by reducing the stickiness of the dough and improving the external symmetry and internal grain scores.

In confectionery foods, arabinogalactan lowers water activity and aids in flavour and oil retention. In addition, it has also been used to increase the stability of oils that are sensitive to degradation. On the other hand, LAG can be used in browning compositions for uncooked foods, in seasoning powders to improve flow and reduce hygrocopicity and starch containing foods to inhibit swelling.

Arabinogalactan is highly water soluble, and thus readily disperses in hot beverages within 30 seconds and does not cause turbidity or changes in viscosity. The only disadvantage is its elevated cost in relation to other food gums (e.g., arabic and guar). Other important food uses of LAG are in the formulation of sports bars and meal replacements. It adds slight water binding activity, which is useful in keeping the bars moist over time.

18.6 Regulatory status

18.6.1 Gum tragacanth

Gum tragacanth is generally recognized as safe (GRAS) by the FDA when used in accordance with good manufacturing or feeding practice (FDA, 2006). Gum tragacanth can be regarded as natural forms of soluble dietary fibre, with long histories of safe use in food and pharmaceutical formulations (Anderson, 1988).

Several investigations of the toxicity, immunogenicity and teratogenicity of gum tragacanth support its use as a food additive (Strobel *et al.*, 1982; Eastwood *et al.*, 1984; Anderson, 1989). In Europe, tragacanth is a permitted food additive under code E413 without restrictions on use.

18.6.2 Gum karaya

Gum karaya also has GRAS status from the FDA after toxicological, teratological and mutagenic tests proved its safety (FDA, 2006). Several studies have shown that gum karaya is safe for food due to the fact that it is neither digested nor degraded by enteric microflora or adsorbed to any significant extent in human beings (Eastwood *et al.*, 1983; Anderson, 1985, 1989). In Europe, karaya gum is a permitted food additive, with code E416. However, due to changes in legislation, the use of gum karaya has been limited in the European Union to a specific range of products with maximum allowed limits (*Official Journal of the European Communities*, 1995). The list includes: cereal and potato-based snacks (5 g/kg); nut coatings (10 g/kg); fillings, toppings and coatings for fine bakery wares (5 g/kg); desserts (6 g/kg); emulsified sauces (10 g/kg); egg-based liqueurs (10 g/kg); chewing gum (5 g/kg) and dietary food supplements (*quantum satis*).

18.6.3 Mesquite gum

As it was mentioned above, mesquite gum is not a permitted food additive neither by the FDA in the United States nor by the Codex Committee on Food Additives in Europe. Despite this, the Secretariat of Health in Mexico has granted authorization for its use in food and beverages (Secretaría de Salud, 1996) pending further tests confirming non-toxic effects, and with a recommendation to seek for authorization of use in food by the welfare agencies around the world. The authorization was given after a toxicological study that included three generations of Wistar rats and a mutagenicity test (Vernon-Carter *et al.*, 1996a). The mesquite gum for these studies was sourced from *P. laevigata* and had a tannin content of 1.92% and was compared with a diet based on commercial gum arabic and a cellulose diet. No statistical differences for the various blood assays (e.g., haemoglobin, haematocrite, glucose, etc.) were found between the three diet treatments ($p < 0.01$). The study concluded that the rats' growth, development and survival rate were not affected by the inclusion of mesquite gum in the diet. In addition, the results from the mutagenicity assays concluded that mesquite gun did not induce any kind of mutagenicity, thus it did not present any carcinogenic activity. This evidence along with the documented history of use of mesquite gum in folk medicine by the Seri Indians in Sonora (Felger and Moser, 1974), speak in favour of its safety for human consumption, towards an eventual application for a GRAS Notice to the FDA and other international agencies.

18.6.4 Larchwood arabinogalactan

Western larch arabinogalactan was approved in 1964 for use as a food additive in the United States (GRAS Notice No. GRN 000047) and eastern larch arabinogalactan in 1997 (GRAS Notice No. GRN 000084). Both can be used as a film-former, foam adhesive, additive, thickener, bulking agent, emulsifier, and as a therapeutic agent. Based on food grade status and numerous studies supporting the safety of larch arabinogalactan, it is considered to be extremely safe with minimum to no toxicity. There are numerous patents identified in product development using larchwood arabinogalactan. FDA also approved its use as an adjuvant component in the making of microcapsules for flavouring substances (FDA, 2008). In the European Union larch arabinogalactan is admitted as a food additive under code E409.

18.7 References

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19

Xyloglucan

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Abstract: The origin, production, properties and application of tamarind seed xyloglucan (TSX) are described. TSX has been used as a thickener and stabilizer, gelling agent, ice-crystal stabilizer, starch modifier in food and related industries. Interaction of TSX with sugars, polyols, polyphenols, and other polysaccharides has been described because it is important both for scientific and industrial applications.

Key words: xyloglucan, tamarind seed, synergistic interaction.

19.1 Introduction

Xyloglucan is a major structural polysaccharide in the primary cell walls of higher plants (Fry *et al.*, 1993; Hayashi, 1989; Takeda *et al.*, 2002). Seeds of tamarind tree (*Tamarindus indica*) contain xyloglucan as a storage polysaccharide, tamarind seed xyloglucan (TSX), which has been widely used in the food industry (Glicksman, 1986; Yamatoya, 1997). Aqueous solution of TSX is stable against heat, pH and shear and new applications are anticipated, since it can act as a thickener and stabilizer, gelling agent, ice-crystal stabilizer and starch modifier. TSX is used as a sauce thickener and in ice cream, dressing, processed vegetables and flour products.

19.2 Origin, distribution and preparation

The presence and mobilization of xyloglucans have been reported in the stage of seed germination (Reiss, 1989; Kooiman, 1960). The botanical distribution of xyloglucans has been reviewed (Fry, 1992). They have been found in

dicotyledons (e.g., hypocotyls of *Phaseolus*, fruits of Olea, Malus and *Phaseolus*, fibers of *Lactuca*, and cell cultures of Acer, Rosa, Glycine and Populus), monocotyledons (e.g., bulbs of Allium, endosperm of Oryzae, seedlings of Oryza and Hordeum, shoots of bamboos, and cell cultures of oryza), and gymnosperms (e.g., cell cultures of *Pseudotsuga*) (Moore and Staehelin, 1988). Xyloglucan may make up 20–30% of the dry weight of the primary wall, with lower amounts (e.g., 5–10%) in the *Gramineae*. The seeds of a few plants (ex. *Tamarindus indica*, Nasturtium (*Tropaeolum majus L.*), Impatients, *Hymenaea* courbaril L., *Detarium senegalense G.*) have abundant deposits of xyloglucan surrounding their cotyledonary cells. Some xyloglucans can be extracted from tissue with cold water. However, alkali is needed to extract most of the xyloglucan from the cell wall.

Cell growth and enlargement in higher plants are controlled by the looseness of a thin net of microfibrils made of cellulose. Xyloglucan crosslinks these cellulose microfibrils and provides the flexibility necessary for the microfibrils to slide. Figure 19.1 shows a potential linkage between xyloglucan and cellulose (Hayashi, 1989). It has been suggested that the cleavage of crosslinking xyloglucan by endolytic enzymes is necessary for cell enlargement during growth (Hayashi, 1989). Some of the xyloglucan oligosaccharides released by β-glucanase have biological functions and are capable of promoting plant cell growth (Hayashi, 1989).

The localization of xyloglucan was determined using a polyclonal antibody against sycamore xyloglucans with ovalbumin as a protein carrier (Misaki *et al.*,

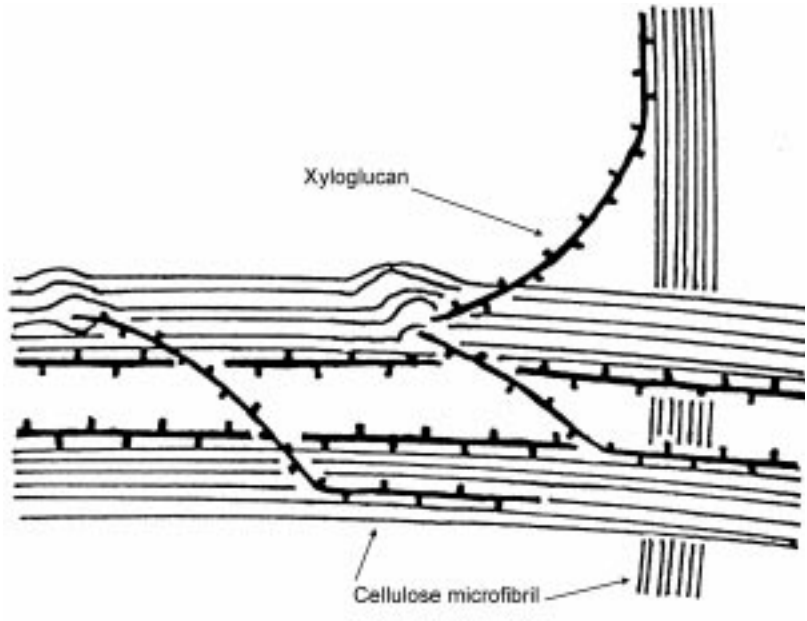

Fig. 19.1 Potential linkages between xyloglucan and cellulose (Hayashi, 1989).

1995) and also using antibody IgG against xyloglucan oligosaccharide with BSA (bovine serum albumin) as a protein carrier (Hayashi and Maclachlan, 1984). Xyloglucans were found both on and between cellulose microfibrils (Hayashi *et al.*, 1984). Xyloglucan levels per fresh weight of tissue steadily decline during development. As a result, the xyloglucan:cellulose weight ratio decreases from about 0.7 in growing regions to 0.11 in mature regions of pea stems (Edwards *et al.*, 1985). Nasturtium xyloglucan and the mobilization of xyloglucan after germination have been studied biochemically (Reid and Edwards, 1995; Buckeridge *et al.*, 2000). It has been reported that edible vegetables and fruits contain xyloglucan and the composition of xyloglucan can vary (Kato and Matsukura, 1994). Therefore, various edible foods may contain xyloglucan polysaccharides and oligosaccharides (Dea, 1993; Rao and Krishna, 1947).

Tamarind seeds have been consumed as food and feed for their sugars, protein and oils for thousands of years. In India, Southeast Asia, the West Indies and Brazil, numerous tamarind trees are grown for food (fruit) and ornamental use. Tamarind trees grow rapidly: the mature evergreen tree can reach a height of 25–30 m. The trees start to blossom and bear fruit 12–13 years after planting. They continue to bear large amounts of fruit for more than 60 years, and are said to survive over 120 years. Tamarind trees are popular for their splendid appearance and long life span, and for the many benefits they provide. In the northern hemisphere, tamarind trees blossom from April through May, and bear fruit from autumn through winter. A mature tamarind tree produces 200–250 kg of fruit annually.

Regarding the preparation of TSX, the first step is basically washing the seeds with water and heating to make the testa or seed-coating brittle and friable (Glicksman, 1986). The seeds are decorticated to leave the heavier crushed endosperm, which is then ground to yield tamarind kernel powder (TKP), a form of unpurified xyloglucan. This TKP or tamarind flour usually contains at least 50% TSX. For further purification, TKP is boiled and agitated with about 30 to 40 times its weight of water for about 30 to 40 min and allowed to sit overnight in a settling tank to allow the protein and fibre to precipitate and settle out. The supernatant liquid is concentrated to about half its volume, mixed with filter-aid and filtered through a filter press. The purified liquid is then dried and ground to yield a purified seed extract. Additional purification can be achieved by precipitation with salts and washing with alcohol.

Purified tamarind seed gum started to be sold commercially in large scale in Japan in 1964, and the only commercially available xyloglucan was Glyloid from Dainippon Sumitomo Pharma Co., Ltd. This is manufactured by separating and purifying the major component of tamarind seeds using water. Three types of TSX are available: Glyloid 2A is insoluble in cold water and requires heating at 75 ºC for at least 15 min for solubilization; Glyloid 3S forms viscous solutions in cold water without heating; and Glyloid 6C is also cold water soluble, but a highly transparent type.

It has been shown that the polysaccharide obtained from Hymenaea seeds is xyloglucan, similar to tamarind (Uno *et al.*, 1996; Buckeridge *et al.*, 1997).

Seeds of Hymenaea, which grows in the Amazon valley, have not been used much, except that native children sometimes eat a powder of its fruit. It is also useful as a thickener, a gelling agent, and as a stabilizer for suspensions or emulsions in foods, chemicals and medicines, but it is not yet commercially available.

19.3 Structure and fundamental properties

19.3.1 Chemical structure

The chemical structure of the repeating units of TSX is shown in Fig. 19.2 (Fry *et al.*,1993; Hayashi, 1989). TSX has a β-(1,4)-linked D-glucan backbone that is partially substituted at the O-6 position of its glucopyranosyl residues with α-D-xylopyranose. The detailed structure depends on the source of plant. Some of the xylose residues are β-D-galactosylated at O-2. Tamarind seed xyloglucan consists mainly of four types of oligosaccharides as repeating unit, confirmed by using endo-1,4-β-D-glucanase digestion, and the complete structures and relative proportions of the xyloglucan oligosaccharides were determined using ^{1}H-NMR (nuclear magnetic resonance) and FAB-MS (fast atom bombardment mass spectrometry) (York *et al.*, 1990).

The unit structures of tamarind xyloglucan, heptasaccharide (referred to as XXXG, Glu_4Xyl_3), octasaccharide (XXLG or XLXG, Glu_4Xyl_3Gal) and nonasaccharide (XLLG, $Glu_4Xyl_3Gal_2$) are shown in Fig. 19.2. The galactose: xylose: glucose ratio was 1:2.25:2.8 (York *et al.*, 1990) and the xyloglucan heptamer:octamer:nonamer ratio was 13:39:48 (Yamatoya *et al.*, 1996) in TSX. The ratios of XXXG, XXLG, XLXG, and XLLG can be determined basically by HPLC (high performance liquid chromatography) (Mitsuishi and Kosugi, 1991), but it is difficult to separate XXLG and XLXG only by HPLC. Combined use of

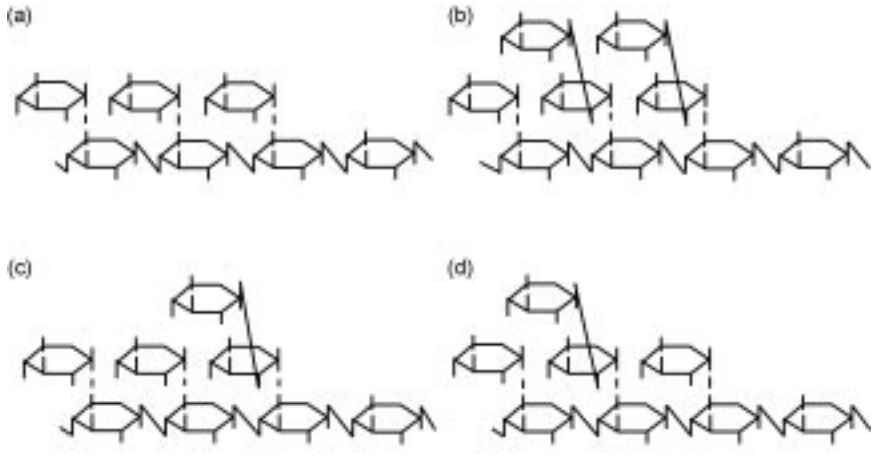

Fig. 19.2 Chemical structure of repeating unit of xyloglucan: (a) heptasaccharide, (b) octasaccharide I, (c) octasaccharide II, (d) nonasaccharide.

a specific enzyme of β-galactosidase and spectroscopic methods makes the estimation more precise (York *et al.*, 1993). It has been reported that isoprimeverose (IP)-producing oligoxyloglucan hydrolase, which is highly specific for xyloglucan oligosaccharides and splits off IP units from the non-reducing end of the backbone of the oligosaccharide, is a powerful tool for identifying the fine structures of xyloglucan oligosaccharides (York *et al.*, 1993; Kato *et al.*, 1985; Konishi *et al.*, 1998).

19.3.2 Molecular weight

The molecular weight of TSX has been reported to be 115,000 (Glicksman, 1986) or 650,000 (GPC calculation based on pullulan standard) (Nishinari *et al.*, 2000), 880,000 (light scattering) (Gidley *et al.*, 1991), 2,500,000 (light scattering) (Lang and Kajiwara, 1993), 1,160,000 (light scattering) (Dentini *et al.*, 2001) or 470,000 (light scattering) (Kato, 2000). It has been reported that light-scattering data from tamarind seed xyloglucan often give different (averaged) values and distributions of molar masses (Lang *et al.*, 1993), depending on sample preparation procedure, such as temperature, pressure, and stirring speed, even for samples prepared with the same extraction procedure. This effect is due to a strong tendency for the self-association of xyloglucan, and weight averaged molecular weight M_w measured by light scattering is strongly affected by high molecular weight portions even if the amount of aggregation is small.

Cell wall xyloglucan has a lower molecular weight compared with tamarind seed xyloglucan as a storage polysaccharide. The average molecular weight of xyloglucan after auxin treatment for 48 h is 50,000, and much lower than the value (330,000) measured before auxin treatment (Hayashi *et al.*, 1984). A continuous decrease of molecular weight was observed from 330,000 in growing cells to 230,000 in elongated control cells (Hayashi *et al.*, 1984). This decrease may have been due to the partial degradation of xyloglucan molecules by endo-1,4-β-glucanase.

19.3.3 Dilute solution properties

The structure and conformation of TSX have been investigated by light scattering (LS) and small-angle X-ray scattering (SAXS) (Gidley *et al.*, 1991; Lang *et al.*, 1993), which has enabled the structural information to be obtained within the range from 1 to 2000 nm.

Ross-Murphy and co-workers investigated the dilute solution properties, isolated chains, of xyloglucan using pressure cell, and reported some physico-chemical properties, such as intrinsic viscosity, steady shear viscosity, shear moduli, and characteristic molecular parameters, such as the radius of gyration R_g and persistence length L_p, of xyloglucan from three different sources: tamarind seed, *detarium* and *afzelia africana* (Picout *et al.*, 2001; Picout and Ross-Murphy, 2002).

Figure 19.3 shows the relation between molecular weight and intrinsic viscosity (Mark–Houwink–Sakurada plots) of xyloglucans from different

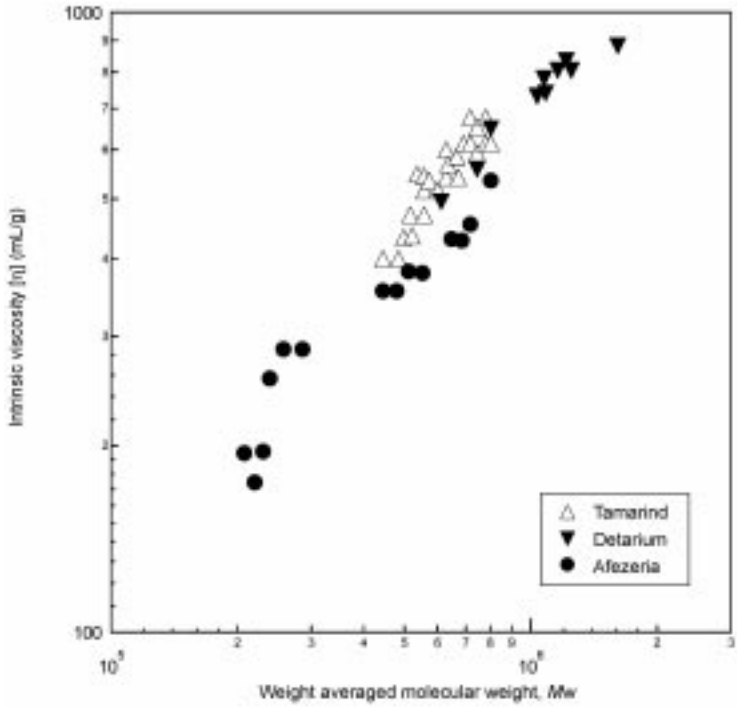

Fig. 19.3 Mark–Houwink–Sakurada plots of xyloglucans from different sources (based on Picout *et al.*, 2003).

sources (Picout *et al.*, 2003). This slope reflects the conformation or flexibility and homogeneity of the polymer, for example, the averaged molecular weight dependence of degree of branching. It is known that the slope is 1.8 for rigid rod, 0.5–0.8 for flexible random polymer, and 0 for hard sphere. The obtained slope is 0.62 ± 0.04 for tamarind seed, 0.76 ± 0.07 for *detarium*, and 0.66 ± 0.05 for *afzelia* xyloglucan, as also listed in Table 19.1. The overall slope for all three samples is about 0.7, which is within the range of 0.5–0.8 for a linear flexible polymer chain with excluded volume. Linear conformation is also supported by the Flory plot of R_g against M_w (Ren *et al.*, 2004).

These values are almost the same, and do not depend remarkably on the type of xyloglucan, tamarind, detarium, and afzelia. Ross-Murphy and co-workers argued (Picout *et al.*, 2003; Ren *et al.*, 2004) that these results reflect only the stiffness of the backbone of the xyloglucan, β-(1,4)-linked-D-glucose, because the values of the Flory exponent of xyloglucan are consequently closer to that of cellulose in non-aqueous solutions. Compared with galactomannans which are relatively unsubstituted polysaccharides (L_p = 3–5 nm), the L_p of xyloglucans are a little bit longer, but much shorter than those of the stiff polysaccharides in the helix state, such as xanthan (L_p =120 nm (Lee and Brant, 2002)) and schizophyllan ($L_p \approx$ 180 nm) (Norisuye *et al.*, 1980). In summary, from their results, in aqueous solutions xyloglucan molecules behave almost as flexible random coil polymers,

Table 19.1 Estimated molecular characteristic parameters, MHS exponent a, ($[\eta] = kM^a$), Flory exponent, n, ($R_g = AM^n$), characteristic ratio C_∞, persistence length L_p (Picout *et al.*, 2006)

Type of xyloglucan	MHS exponent a	Flory exponent	C_∞	L_p (nm)
Tamarind	0.76 ± 0.07	0.54 ± 0.07	19 ± 4	4–6
Detarium	0.62 ± 0.04	0.49 ± 0.09	25 ± 4	5–8
Afzelia	0.66 ± 0.05	0.56 ± 0.1	18 ± 2	4–6

and do not show any conformational change such as coil-helix transition, as seen in some other polysaccharides, such as carrageenan, gellan and xanthan gum. Relatively higher viscosity can be expected due to the self-aggregation of xyloglucan compared to polymers with the same contour length.

The solution properties of the polymer especially the characteristics of the isolated chains do not largely depend on the source of xyloglucan, such as tamarind seed, detarium, and *afzelia africana*, indicating that the sequence of saccharides (galactose substitution pattern) and the ratio of each repeating unit seem to be less important for relatively large-scale, macroscopic solution properties. However, it was reported that the degree of self-association of xyloglucan depends on the source, and tamarind seed xyloglucan has a strong tendency to aggregate. This suggests that the ability to interact with other xyloglucans or other molecules depends on the ratio of each repeating unit or galactose substitution patterns. The former factor can be investigated by SAXS for each repeating unit of xyloglucan, as will be mentioned later. Investigation of the latter is not easy, because analysis of the polysaccharide sequence has not yet been developed.

Lang and Kajiwara (1993) reported that the statistical Kuhn length L_k is 150 nm and R_g (radius of gyration) $= 148 \pm 5$ nm, cross-sectional radius of gyration $R_{g,c}$ 0.29 ± 0.02 nm for TSX. This value can be compared with values of 0.20 nm for linear dextran, 0.25 nm for iota-carrageenan, 0.26 nm for agarose, 0.26 nm for guar, and 0.30 nm for hyaluronic acid (Gidley *et al.*, 1991). The $R_{g,c}$ value of 0.29 nm for TSX is at the high end of this range presumably due to the high degree of substitution by side chains. This value is also not compatible with the values of single polysaccharide chains, which lie within the range of 0.25–0.3 nm, and the observed value for TSX is rather typical for multistranded polysaccharides, like xanthan gum and the triple helix of schizophyllan, which has an $R_{g,c} = 0.55$ nm (Gidley *et al.*, 1992). Static LS data show that the parameter C_∞ (characteristic ratio), which is an estimate of the rigidity of the polymer, has a value of 110 (Gidley *et al.*, 1991). C_∞ can be compared with those for galactomannan and carboxymethylcellulose, which have similar backbone structures but characteristic ratios that are lower by a factor of ~10 (Robinson *et al.*, 1982). The side chains significantly impair the mobility of the internal chains, particularly at high levels of substitution. They argued that the C_∞ value for TSX is higher than that for other neutral polysaccharides and is comparable to those for alginates that have ribbon-like structures stiffened by mutual electrostatic repulsion between adjacent residues. The greater stiffness of

the chain of TSX might be due in part to the high proportion of side chains (Gidley *et al.*, 1991). It is possible that the stiffness of the chain is not due solely to hindered rotation but also to specific aggregation in solution and 'hyper-entanglements' may have arisen through a tenuous alignment of stiffened chains (Gidley *et al.*, 1991). The value 110 for C_∞ obtained by Lang *et al.* is much larger than the value obtained by Ross-Murphy *et al.*, shown in Table 19.1. This large difference probably arises from aggregated molecules.

SAXS revealed that in xyloglucan from another plant source, Detarium, the chains are stiff and the cross-sectional radius of gyration is around 0.5 nm (Dentini *et al.*, 2001; Wang *et al.*, 1996), probably due to lateral aggregation since the cross-sectional radius of gyration of xyloglucan polymers is almost identical to that evaluated from its oligomers. The ρ value, which is the ratio of the radius of gyration, R_g, and the hydrodynamic radius, R_h, obtained from static LS (or SAXS) and dynamic LS, respectively, ($\rho = R_g/R_h$), is sensitive to the shape of the particle (e.g. $\rho = 0.78$ for a sphere, $\rho = 1.3$ for branched, $\rho = 1.50$ for a random coil, $\rho \geq 2.0$ for linear shape).

The repeating unit of TSX (heptamer, octamer and nonamer) in solution was produced by enzymatic degradation, fractionated, and investigated by SAXS (Yamanaka *et al.*, 1999a,b; Yuguchi *et al.*, 1997). The SAXS results indicate the presence of aggregates in aqueous solutions of heptamer and octamer, while no such aggregation was observed in the aqueous solution of nonamer. The lack of a galactose side-chain seems to promote the aggregation. The corresponding radius of gyration was evaluated from the linear portion in the respective Guinier plots.

The radius of gyration does not seem to vary with the molecular weight in the range examined, although the cross-sectional radius of gyration increased slightly with the number of residues in side chains. The removal of galactose side chains causes thinning of the cross-section of xyloglucan. The cross-sectional radius of gyration of xyloglucan monomers is considerably larger than that of a 1,4-β-glucan backbone (0.21 nm for cellulose) (Yamanaka *et al.*, 1999a,b; Yuguchi *et al.*, 1997), indicating that xylose or galacto-xylose branches protrude from the backbone. Another characteristic of the xyloglucan cross-section is its flatness. In the elliptic models that are fit to each monomer, the length of the shortest semi-axis is constant at 0.22 nm for all three types of monomers. The width of the cross-section increases from 0.62 nm (heptamer) to 0.71 nm (octamer) and 0.75 nm (nonamer) as the number of residues in the side chains increases. The results indicate that the side chains protrude horizontally from the flat 1,4-β-glucan backbone surface. The longest axis may represent the backbone length, which should be the same among the three types of monomer if the backbone conformation is identical. However, a shorter length was found for a larger monomer, suggesting a more arched structure.

19.3.4 Rheological properties at higher concentrations

The concentration dependence of viscosity of TSX is shown in Fig. 19.4 together with commercially available polysaccharides. TSX is stable under high

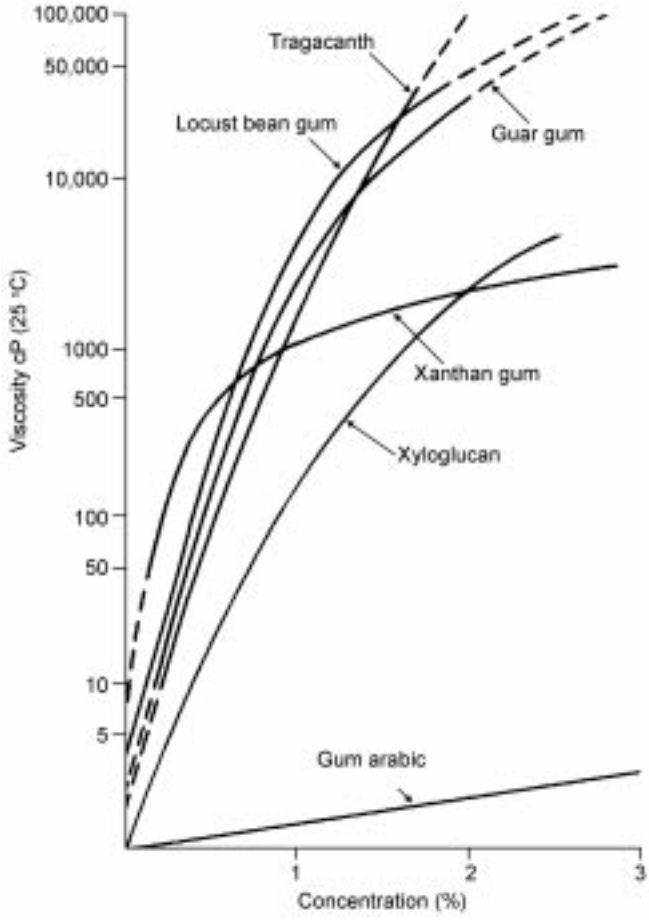

Fig. 19.4 Concentration dependence of viscosity of TSX at a shear rate of $8.6\,s^{-1}$.

temperature, and against acid and salts over extended ranges. For TSX, salting-out and viscosity decrease do not occur even at high saline concentrations (20%). TSX itself is stable against mechanical shear. TSX also imparts stability to starch when used in combination. For example, the viscosity of corn starch alone decreased to about half value by high mechanical shear treatment with a colloid mill (clearance 0.3 m/m, 5000 rpm, recycle 3 times), while there is almost no change in viscosity with TSX, either alone or in combination with corn starch, using the same treatment.

Aqueous solutions of TSX exhibit a common flow behavior of polymer solutions, as shown in Fig. 19.5. In the case of polymers including polysaccharides, at low shear rates, the steady shear viscosity is constant (Newtonian viscosity region), and at high shear rates, the steady shear viscosity decreases with increasing shear rate (shear thinning). It is known that this shear thinning arises from the shear-induced orientation of the polymer against rotational

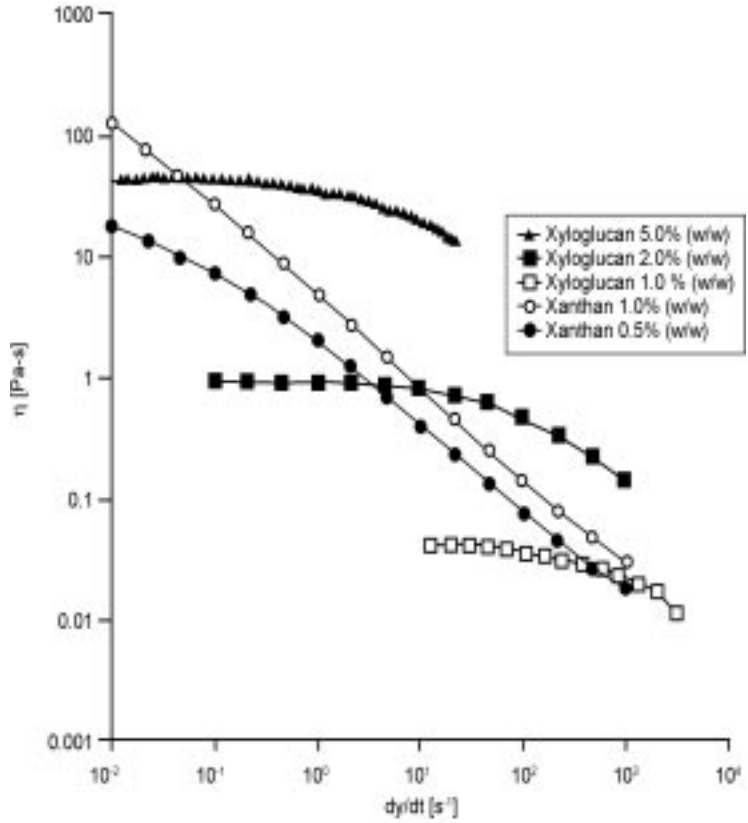

Fig. 19.5 Steady shear viscosity of xyloglucan and xanthan solutions as a function of shear rate at 25 °C.

diffusion. The onset shear rate at which η starts to decrease, depends on molar mass, because the size of molecules affect the rotational behaviour of molecules. Except xanthan gum, which usually has a high molecular weight (apparent molecular weight is about 1.0×10^7g/mol) and rigid, rod-like polymer, most of the other polysaccharides, such as pullulan, galactomannan, glucomannans, and cellulose derivatives, exhibit similar flow behaviour to that shown in Fig. 19.5.

The rheological behaviour of TSX was discussed by Gidley *et al.* (1991). For TSX, the average intrinsic viscosity was $[\eta] = 600 \pm 0.05$ ml/g. Based on the radius of gyration and the molecular weight from light-scattering experiments, the intrinsic viscosity can be predicted using the Fox–Flory theory. This approach predicts the intrinsic viscosity of 5040 ml/g, which is about eight times higher than the corresponding experimental value. This discrepancy was attributed to polydispersity, excluded volume and draining effects. Information concerning the hydrodynamic behaviour of finite concentrations can be obtained from a double logarithmic plot of zero-shear specific viscosity ($\eta_{sp,0}$) versus the product of concentration (c) and intrinsic viscosity ($[\eta]$). Since the intrinsic

viscosity is related to the volume occupied by an isolated polymer molecule, $c[\eta]$ is a measure of the total volume occupied by polymer molecules at a particular concentration. By using a range of viscometers, extrapolated values of the zero-shear viscosity were obtained for 0.03–3.0% tamarind xyloglucan (Gidley *et al.*, 1991). Two limited slopes were obtained that corresponded to dilute and semi-dilute solution regions. The transition between these two states occurs when individual polymer coils begin to interpenetrate, and this has been represented as a single discontinuity, or as two discontinuities, but most likely the transition is gradual and reflects the gradual nature of viscosity-detected coil interpenetration with increasing concentration.

For random-coil polysaccharides, the slopes of log $\eta_{sp,0}$ vs. log ($c[\eta]$) in dilute and semi-dilute regimes were 1.3–1.4 and 3.3–3.4, respectively (Lapasin and Pricl, 1999). Extrapolated break points between the two regimes were found at $c[\eta]$ and η_{sp} values of ~4 and 10, respectively (Morris *et al.*, 1981). For tamarind xyloglucan, slopes of 1.13 and 4.7 were found for dilute and semi-dilute regimes, respectively, with an extrapolated break point at $c[\eta] = 3.6$ and $\eta_{sp} = 4.5$ (Gidley *et al.*, 1991). This behaviour showed a much greater percentage increase with concentration than that of random-coil polysaccharides such as guar. This effect has been ascribed to hyperentanglements, which survive longer than non-specific entanglements. This hyperentanglement of the chains may be due to the tenuous alignment of natural and stiff segments in solution. Like many other polysaccharides, tamarind xyloglucan is not molecularly dispersed in aqueous solution, but rather shows a high degree of aggregation (Lang *et al.*, 1993).

Figure 19.6 shows the frequency and temperature dependence of the storage shear modulus G' and loss shear modulus G'' of xyloglucan solutions of various concentrations (Yoshimura *et al.*, 1999). G'' was larger than G' at all the

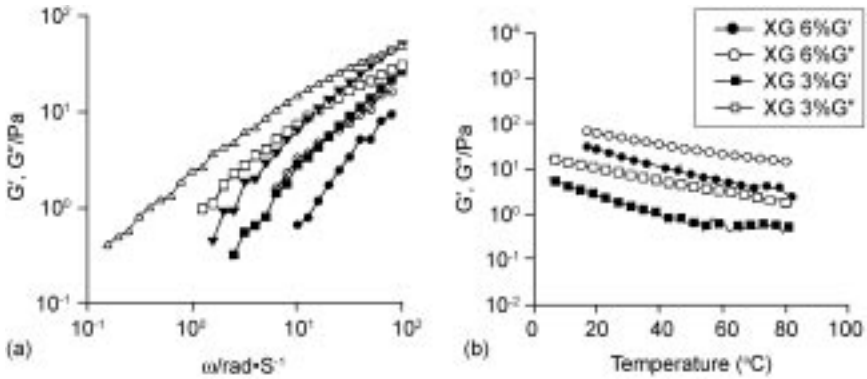

Fig. 19.6 (a) Frequency dependence of G' and G'' for dispersions of xyloglucan with various concentrations at 5 ºC. Strain, 0.05. Concentration of xyloglucan, ●, 1.05 wt% G'; ○, 1.05wt% G''; ■, 1.40 wt% G'; □, 1.40 wt% G''; ▼, 1.75 wt% G'; Δ, 1.75 wt% G''. (b) Temperature dependence of G' and G'' for 3.0 and 6.0 wt% dispersions of xyloglucan. Heating rate, 1 ºC/min. Angular freq, 1 rad/s, strain, 0.05.

frequencies from 10^{-1} to 10^{2} rad/s for a 1.05% TSX solution, while G' and G'' showed a crossover at an angular frequency of about 10^{2} rad/s for TSX solutions with higher concentrations. At a fixed frequency of 1 rad/s, both G' and G'' decreased monotonically with increasing temperature and G'' was always larger than G', as usually observed for most liquids.

19.4 Interactions with tamarind seed xyloglucan

19.4.1 Gelation by change of solvent

Tamarind seed xyloglucan alone does not form a gel in aqueous solution even at high concentrations, and just makes a viscous solution. By adding the other types of polysaccharides, such as gellan and xanthan gum, or small molecules acting as crosslinker, such as congo red, catechin, or by changing the solvent qualities by addition of large amounts of sugar or alcohol, the aqueous solution of xyloglucan forms a thermoreversible gel (Glicksman, 1986; Yuguchi *et al.*, 2004; Yamanaka *et al.*, 2000).

TSX increases viscosity when used in combination with large amount of sugar such as sucrose, glucose or starch syrup. The solution of TSX forms a gel in the presence of 40–70% sugar as shown in Fig. 19.7. Glicksman (1986) reported that the TSX aqueous solution forms a gel by the addition of ethanol. This gel shows high elasticity and low water release. The gel with alcohol is harder and has a lower melting point than the gel with sugar. A freeze-thaw process makes TSX-sugar gel harder and more elastic. Preliminary X-ray fibre and powder diffraction studies and a simulation of molecular conformations have indicated that the xyloglucan backbone adopts a cellulose-like conformation (Levy *et al.*, 1991; Narasaiah and Millane, 1990; Ogawa *et al.*, 1990). Thus, in the condensed state, the molecular conformation is very similar to that of cellulose. Therefore, gelation under a high sucrose concentration is likely to involve chain–chain association by the aggregation of a regular, ribbon-like, doubled, cellulose-like conformation (Levy *et al.*, 1991). The sol-gel transition takes place at 47 ºC on heating a system containing TSX in 15% ethanol aqueous solution. Time-resolved SAXS was observed in this system with heating at a rate of 0.5 ºC/min (Yamanaka *et al.*, 2000). The structures of gels formed in the presence of monohydric or polyhydric alcohol were found to be different (Reid and Edwards, 1995). Yuguchi *et al.* (2001) concluded that a gel formed in the presence of monohydric alcohol, such as methanol, ethanol, and propanol, consists of a random network, while the gelation in the presence of polyhydric alcohol is caused by weaker side-by-side associations of a few chains, which is similar to gelation by addition of a large amount of sugar alcohol. If alcohol (up to 20%) is added to the TSX solution in the presence of sugar, the amount of sugar needed to form a gel can be substantially reduced to get all of the gum into solution and even eliminated.

As described above, the degree of self-association of xyloglucan depends on the source of xyloglucan and the repeating unit of xyloglucan. Since the side

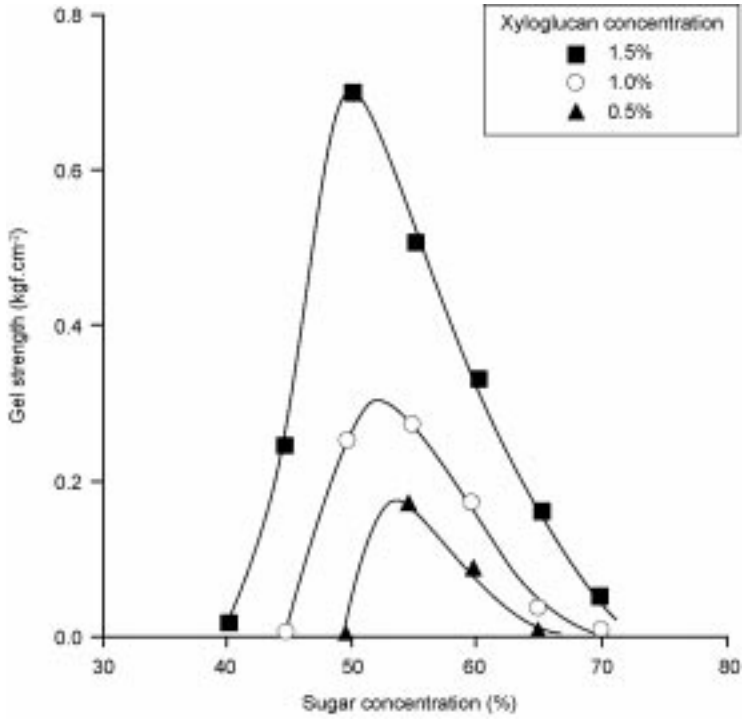

Fig. 19.7 Gel strength of TSX gel of various concentrations as a function of sugar concentration at 25 ºC.

chain of xyloglucan, especially the galactose, has an important role in its water solubility, the removal of galactose is expected to affect the solubility of xyloglucan, which in turn leads to the association of xyloglucans and induces the gelation without addition of other chemicals.

Reid *et al.* (1988) found that xyloglucan forms a gel by the removal of a part of galactose using β-galactosidase. Shirakawa *et al.* (1998a,b) reported a rare feature of this kind of gel. In the case of removal of 35% galactose residues from xyloglucan, the solution forms a thermoreversible gel only in the limited temperature range from 40 to 80 ºC, and the solution becomes sol not only at high temperatures, but also at low temperatures. The phase diagram is shown in Fig. 19.8. It formed a gel on heating and reverted to a sol on cooling below the low temperature transition point. Xyloglucan with a galactose removal ratio of 58% transformed from sol to gel at 5 ºC. Although the sample remained a gel at 100 ºC, it transformed to sol again on heating at 110 ºC. These sol–gel transitions were completely reversible.

Most polysaccharides forming thermoreversible gels, such as agarose, carrageenan, and gellan gum, etc., form a gel at low temperatures. Some other polysaccharides do at high temperatures, such as curdlan, methylcellulose and some other cellulose derivatives. As far as we know, only tamarind seed

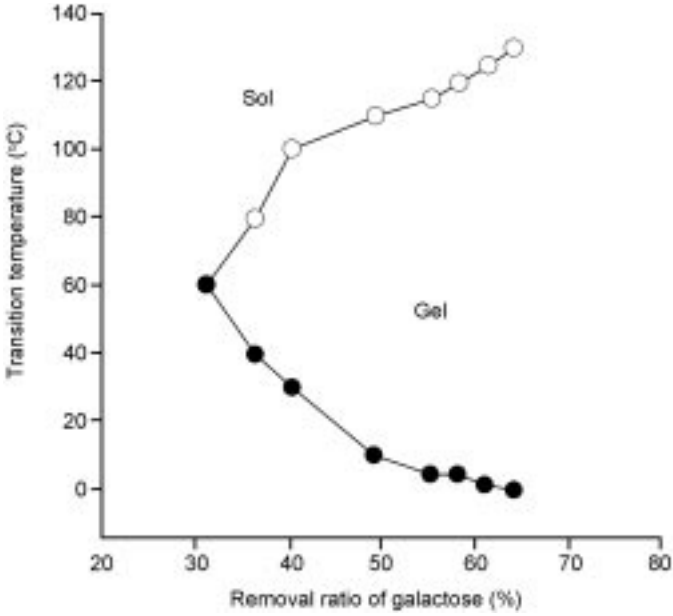

Fig. 19.8 Sol-gel transition temperature diagram of TSX as a function of the removal of ratio of galactose from xyloglucan. ○, high-temperature transition point; ●, low-temperature transition point.

xyloglucan after partial removal of galactose has unusual characteristics except a block copolymer of 2-(2-ethoxy)ethoxyethyl vinyl ether and 2-methoxyethyl vinyl ether (Aoshima and Sugihara, 2000).

Figure 19.9 shows a typical result of the rheology and DSC on heating of xyloglucan with a 43% galactose removal ratio. An endothermic DSC peak appeared around 32 ºC at which the storage modulus G' significantly increased.

As described above, xyloglucan chains consisting of nonasaccharides do not aggregate, and those consisting of heptasaccharides do. Therefore, the fact that the removal of galactose promotes the gelation is consistent with the characteristics of the repeating unit, because the removal of galactose increases the ratio of hepta- and octa-sacchardes, and decreases the ratio of nona-saccharides. It is believed that this gelation is caused by the association of main chains by hydrophobic interaction (Shirakawa *et al.*, 1998b). Small angle X-ray scattering investigations (Yamanaka *et al.*, 1999a,b) suggested that a flat, plate-like structure of thickness about 3 nm was formed in the gel state. Four or five xyloglucan main chains might stack laterally, and constituted the crosslinking domain.

19.4.2 Gelation by addition of polyphenols

It was reported that xyloglucan aqueous solution can form a thermoreversible gel by mixing with green tea-extract polyphenols such as epigallocatechin

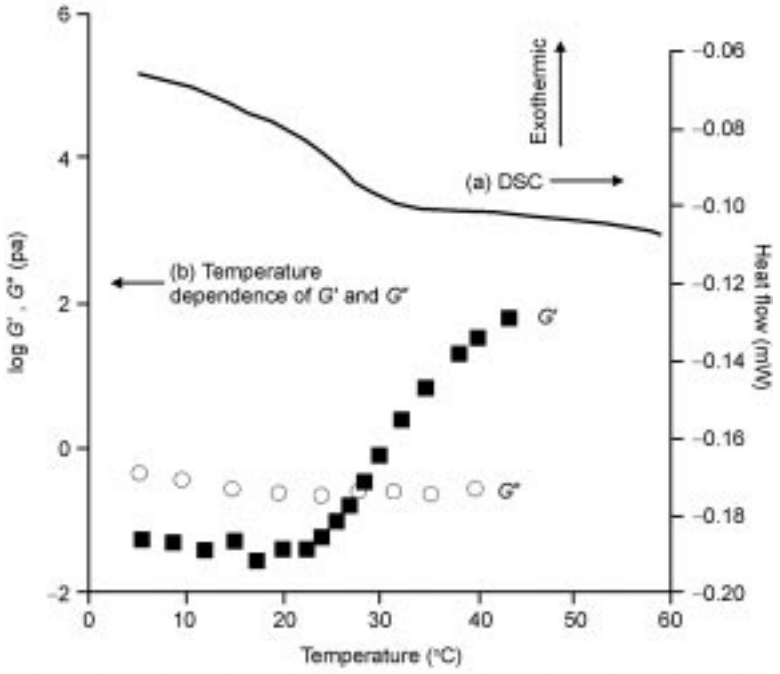

Fig. 19.9 Comparison of DSC (a) and rheological measurements (b) at low-temperature gel formation for a 1.0 wt% TSX solution with a 43% galactose removal ratio. Angular frequency, 1.0rad/s; heating rate, 0.5 ºC/min.

gallate (Yuguchi *et al.*, 2001). SAXS suggested that the gel was formed by random aggregation, which may be similar to the gelation mechanism for the mixture with alcohol (Yuguchi *et al.*, 2001). Rheological and DSC measurements (Nitta *et al.*, 2004) showed that the solution forms a gel even at low xyloglucan concentrations, or precipitates depending on the EGCG and xyloglucan concentrations.

Figure 19.10 shows the rheological and DSC curves on cooling and subsequent heating of 2.0 wt% TSX aqueous solution in the presence of 0.1 wt% EGCG. On cooling, both G' and G'' increased significantly at temperatures below 20 ºC, and around 18 ºC G' exceeded G''. On subsequent heating, G' and G'' decreased with increasing temperature, and above 30 ºC both G' and G'' showed similar values observed on cooling, which means that the solution formed a thermoreversible gel on cooling and melted on subsequent heating. A large thermal hysteresis was observed (Nitta *et al.*, 2004). An exothermic DSC peak appeared at about 17 ºC where G' and G'' steeply increased on cooling, and an endothermic peak appeared when G' and G'' steeply decreased on subsequent heating. As for xyloglucan or EGCG alone, no such characteristic changes were observed in this temperature range. Therefore, the appearance of DSC peaks and the drastic changes of shear moduli can be attributed to the synergistic interaction between xyloglucan and EGCG, and probably caused by intermolecular association.

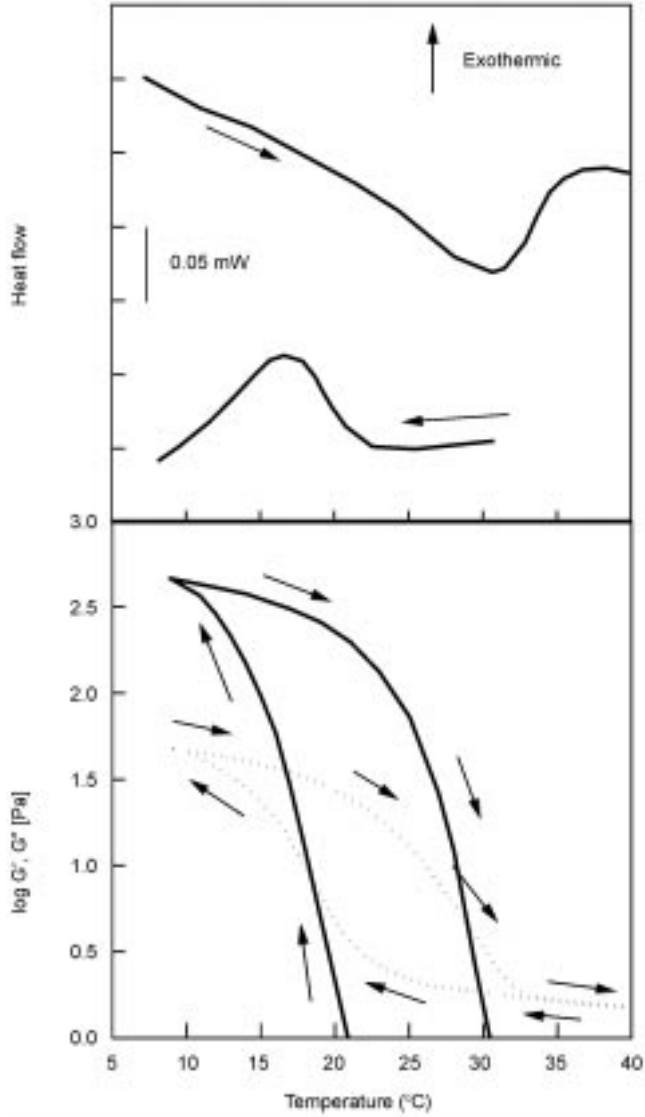

Fig. 19.10 (top) Cooling and subsequent DSC curves, (bottom) Temperature dependence of G' and G'', for 2.0 wt% TSX dispersions with 0.1 wt% EGCG. Scan rate, 0.5 ºC/min. Angular freq, 1 rad/s.

Direct evidence for the intermolecular association between xyloglucan and EGCG was obtained from NMR measurements. Figure 19.11 shows the NOESY spectrum of a mixture of xyloglucan and EGCG. Only at low temperatures in the gel state, the NOE (nuclear Overhauser effect) crosspeaks appeared between xyloglucan and EGCG. NOE is a magnetization transfer through the space, and the NOE is inversely proportional to r^{-6} where r is the distance between two

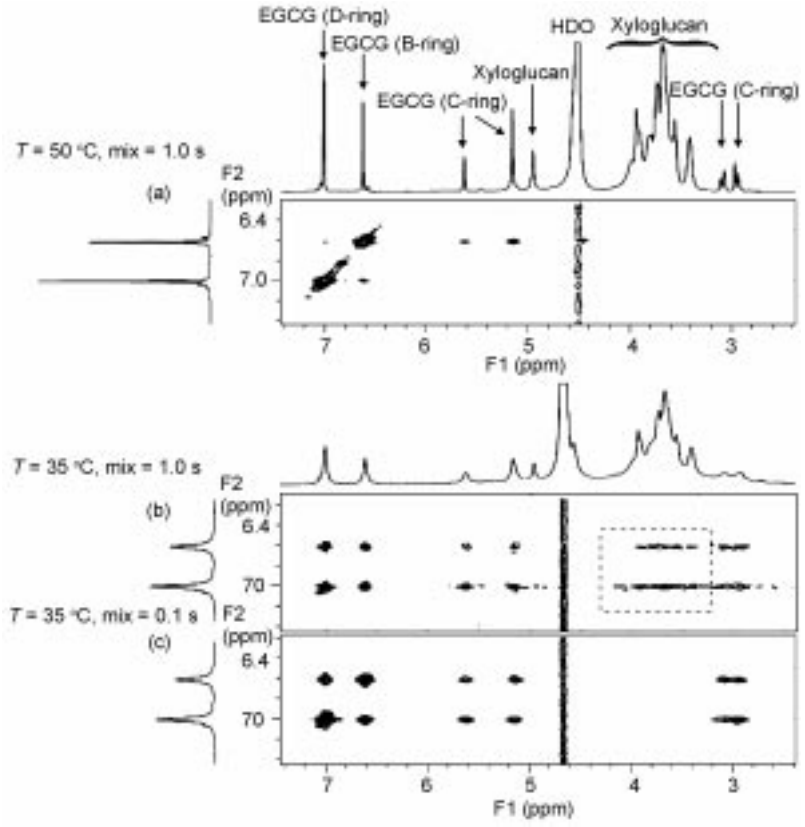

Fig. 19.11 ^{1}H 1D spectra and ^{1}H-^{1}H NOESY spectra of 0.5 wt% TSX with 0.2 wt% EGCG in D_2O at 35 and 50 °C. Intermolecular cross-peaks observed in (b) are highlighted in a box. The sign of all the peaks in (a) except the diagonal peaks (minus) is plus, and that in (b) and (c) is minus. An artifact was observed near the peak assigned to HDO (plus), and is caused by the large intensity of the HDO signal.

protons. Normally, NOE peaks can be observed for the protons whose distance is less than 5Å due to sensitivity limitations. Hence, the observation of NOE crosspeaks between different kinds of molecules, xyloglucan and EGCG, indicates that there is an heterotypic association between them.

The shear modulus of 1.0% (w/w) xyloglucan-EGCG gels as a function of EGCG concentration showed a maximum at around 0.6% and beyond this EGCG concentration the gel became opaque and the modulus decreased.

19.4.3 Gelation of tamarind seed xyloglucan by mixing with helix forming polysaccharides

It had been believed that TSX does not show any remarkable synergism with other hydrocolloids, as seen between xanthan gum and galactomannan. However, it was reported that two helix-forming polysaccharides, gellan gum and

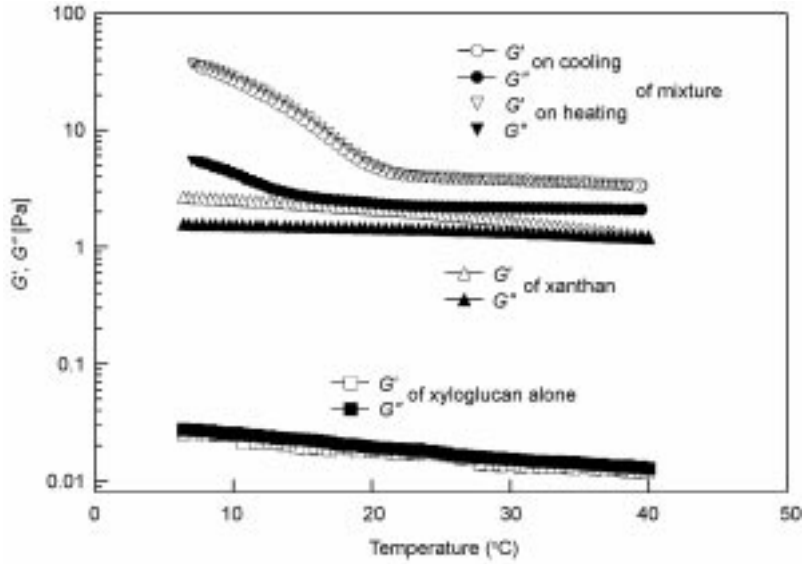

Fig. 19.12 Temperature dependence of G' (□) and G'' (■) for a 0.5 wt% TSX, G' (△) and G'' (▲) for a 0.5 wt% xanthan, G' (○) and G'' (●) (cooling), G' (▽) and G'' (▼) (heating) for a 1.0 wt% mixture of TSX and xanthan (weight ratio 1:1) solution.

xanthan gum can form a complex with TSX resulting in a gel and/or an increase of viscosity under the condition that each polysaccharide solution cannot form a gel (Nitta *et al.*, 2003; Kim *et al.*, 2006). Temperature dependence of G' and G'' of 1.0% (w/w) mixtures of TSX and Na-form gellan gum showed that gelation occurs by mixing TSX and Na-form gellan gum. (Nitta *et al.*, 2003).

Figure 19.12 shows the temperature dependence of G' and G'' of 1 wt% mixtures of TSX and native xanthan carrying both acetyl and pyruvate groups (Kim *et al.*, 2006). Although both solutions of xyloglucan and xanthan alone showed only a gradual increase in G' and G'' with decreasing temperature, the mixture of xyloglucan and native xanthan showed a step-like increase at about 20 ºC. This means the gelation occurs at this temperature. Both mixtures of TSX with gellan gum or xanthan gum clearly exhibit a synergistic interaction, although there is a difference from the viewpoint of thermal hysteresis. Xyloglucan/gellan mixture showed a thermal hysteresis, but no detectable thermal hysteresis was observed for xyloglucan/xanthan mixture.

Morris (1995) pointed out that there are two common features for the synergistic interaction of polysaccharide mixtures, in xanthan/galactomannan (GM), xanthan/konjac glucomannan (KGM), agarose/GM or KGM, carrageenan/GM or KGM.

1. One polysaccharide undergoes coil-helix conformational transition, such as agarose, carrageenan, furcellaran, gellan and xanthan gum.
2. The backbone linkage of the other polysaccharide in the mixture is β(1,4)-linkage, such as in konjac glucomannan or galactomannan, or xyloglucan.

The backbone of xyloglucan is also β(1,4)-linked glucose, and both gellan and xanthan gum also exhibits coil-helix transition. The gellan (or xanthan) and xyloglucan mixture systems also have the same common features. This supports the idea that these common features may play an essential role, because their chemical structures and structure of helix, such as pitch, related directly to the stability of the complex structure, are different from each other. Although the origin of this synergistic interaction has been in dispute for the past 30 years or more, Chandrasekaran *et al.* (2003) proposed a model from X-ray fibre diffraction of a stretched film of mixed polysaccharides: the crosslinking mechanism is the hybrid helix of two polysaccharides.

19.4.4 Iodine colour reaction

When a solution of potassium iodide (0.1 M) is added to a xyloglucan solution to make the final concentration of potassium iodide 0.2 mM, a purple or light blue colour appears. Thus, TSX and other xyloglucans, such as starch, have been called 'amyloids' (Kooiman, 1960) because of the characteristic blue stain that is produced with iodine/potassium iodide solution. The name amyloid may be derived from amylose or amylopectin.

The addition of iodine and an appropriate salt makes xyloglucan aqueous solution promote weak gel formation. The SAXS profile in the gel state cannot be explained by random aggregation. It is speculated that this may involve a complex of iodine molecules and xyloglucan (Yuguchi *et al.*, 2001). In this complex, two rigid xyloglucan molecules encapsulate iodine molecules with side chains. This conformation allows iodine molecules to be in an environment similar to the inside pocket of an amylose helix.

19.5 Applications in the food industry

Tamarind seed xyloglucan has been widely used as a food additive in Japan. TSX is principally used in sauces, dressings and mayonnaise, ice cream and some flour products (Table 19.2) (Glicksman, 1986; Yamatoya, 1997).

19.5.1 Thickener or stabilizer

TSX is used as a thickener without a sticky texture or trailing threads which are sometimes shortcomings for starches. TSX also provides body and a denser feeling in the mouth. TSX can be used to replace or in combination with starch.

TSX has been used in sauces for pork cutlet and meat, since they require low pH, high viscosity and high stability over a long period. Only a very small level (0.05%) of TSX is needed to provide body or improve texture. In low-fat milk, fruit beverages and cocoa, TSX can have an excellent effect. Since TSX stabilizes small particles in suspension, it can be used in fruit-pulp beverages or *shiruko* (sweet red bean soup with rice cake). In batter mixes for fried products, it is important to maintain a stable viscosity during the battering process. Even

Table 19.2 Food applications of tamarind seed xyloglucan

Action	Effects	Example foods
1. Thickener and stabilizer	❍ Provides good viscosity free of a pasty and trailing threads. ❍ Provides density and body. ❍ Provides stable emulsification. ❍ Suspends particles for stable dispersion ❍ Retains water	Pork cutlet sauces, meat sauces, fruit sauces, batters, pickles, low-fat milk, coffee milk, dressings, mayonnaise, fruit juice beverage, cocoa, seasoning solutions, seaweed *tsukudani*
2. Gelling	❍ Forms water release-free gel by synergism with sugar ❍ Forms gel by synergism with alcohol. ❍ Forms gel resistant to freeze-thawing ❍ Water retention by gel.	Fruit jellies, cocktail jellies, low-sugar jams, *youkan, kuzu mochi*, frozen jellies.
3. Ice crystal stabilization	❍ Forms fine ice crystals. ❍ Excellent shape retention ❍ Excellent acid-resistance ensures stable overrun. ❍ Thread-free viscosity offers good dissolution in the mouth. ❍ Good compatibility with other stabilizers offers versatile performance.	Ice cream, frozen dessert, glazing
4. Starch modification	❍ Suppresses starch ageing. ❍ Confers heat-resistance upon starch for protection. ❍ Improves wheat flour product texture. ❍ Confers mechanical strength upon starch for protection	Custard creams, flour pastes, curry, stew, noodles, rice cake, Japanese traditional confections, baked goods

though enzymes in wheat flour reduce the viscosity of batter mix, TSX can stabilize the viscosity throughout the battering process.

19.5.2 Emulsion stabilizer

Since TSX helps to stabilize emulsions, it has been used in salad dressings and mayonnaise. TSX gives smaller oil droplets in the emulsion than xanthan gum and carrageenan, although it has a weaker stabilizing effect than xanthan gum. The emulsion-stabilizing effect of TSX increases depending on the concentration and pH (Yamatoya, 1997). Maillard-type protein-xyloglucan conjugates prepared by a naturally occurring reaction exhibited dramatically enhanced emulsifying properties (Kato, 2000). The heat-stability of the protein was also dramatically increased by conjugation. Figure 19.13 shows the emulsifying properties of soy

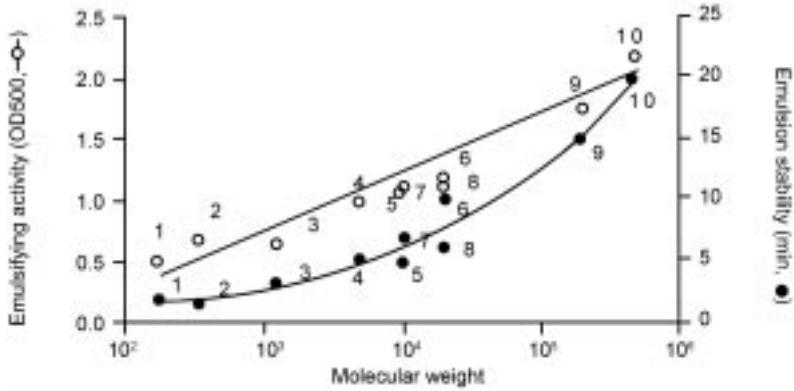

Fig. 19.13 Effect of molecular weight of saccharides on emulsifying properties of soy protein – saccharide conjugates in the conjugates. 1, glucose; 2, lactose; 3, xyloglucan ($M_w = 1{,}400$); 4, galactomannan ($M_w = 3{,}500$–$6{,}000$); 5, galactomannan ($M_w = 10{,}000$); 6, galactomannan ($M_w = 20{,}000$); 7, dextran ($M_w = 9{,}300$); 8, dextran ($M_w = 19{,}600$); 9, dextran (M_w = 200,000–300,000); 10, xyloglucan ($M_w = 470{,}000$) (Kato, 2000).

protein-polysaccharide conjugates. The xyloglucan conjugate has a greater emulsifying effect than conjugates with other polysaccharides (Kato, 2000). The attached polysaccharide may function as a molecular chaperone that prevents the aggregation of unfolded protein molecules (Kato, 2000).

19.5.3 Ice crystal stabilization

TSX alone or in combination with other polysaccharides (e.g., guar gum, locust bean gum or carrageenan) is a very effective stabilizer in frozen desserts such as ice cream and sherbet. TSX gives a good overrun, no whey-off, excellent heat-shock stability and good water-holding properties without the separation of ice crystals and sugar after prolonged storage.

19.5.4 Gelling agent

TSX provides an elastic gel with concentrated sugar solutions over a wide range of pH. TSX is not affected by boiling in neutral aqueous solutions due to its heat stability. The gel is water-release free and resistant to freeze-thawing. It can be used as a substitute for pectin fruit jelly or jam. Some Japanese traditional desserts such as *youkan* and *kuzu mochi* can also be produced using TSX. Recently, a dessert jelly of xyloglucan with green tea has been proposed.

19.5.5 Starch modification

TSX can suppress the ageing of starch under certain conditions. It can also protect starch by conferring heat stability and mechanical strength. TSX improves the texture of starch, especially in wheat flour. In combination with starch or a starch substitute, TSX has been used in custard cream, flour paste, stew, noodles and traditional Japanese confections (e.g., *dango* or rice cake).

Based on the effects of TSX on the gelatinization and retrogradation of corn starch, it has been suggested that xyloglucan molecules might entangle corn starch and prevent reordering of the structure, and hence retard retrogradation during long-term storage (Yoshimura *et al.*, 1999).

19.5.6 Fat replacement

With recent consumer demand for healthier foods, low-calorie and low-fat foods have been needed. A polysaccharide solution has been shown to exhibit the desired emulsion-like properties. As a fat replacement or fat mimetic, TSX solution has excellent mouthfeel and fat/oil-like properties (except for frying and nutritional considerations) (Yamatoya, 1997). Fat-reduced dressing and mayonnaise products are now an important application of TSX. In many cases, xanthan gum or other polysaccharides and starch or dextrin are used in combination with TSX (Yamatoya, 1997).

19.5.7 Application of low-viscosity tamarind seed gum (LVTSX)

The viscosity of TSX was reduced to 10% of the value of native TSX to prepare a low-viscosity tamarind seed gum (LVTSX), which is useful in creating a smooth texture (Fig. 19.14). Its functions, such as texture modification, stabilization, gelling with sugar, ice crystal stabilization and starch modification, remain undamaged after the modification (Table 19.3).

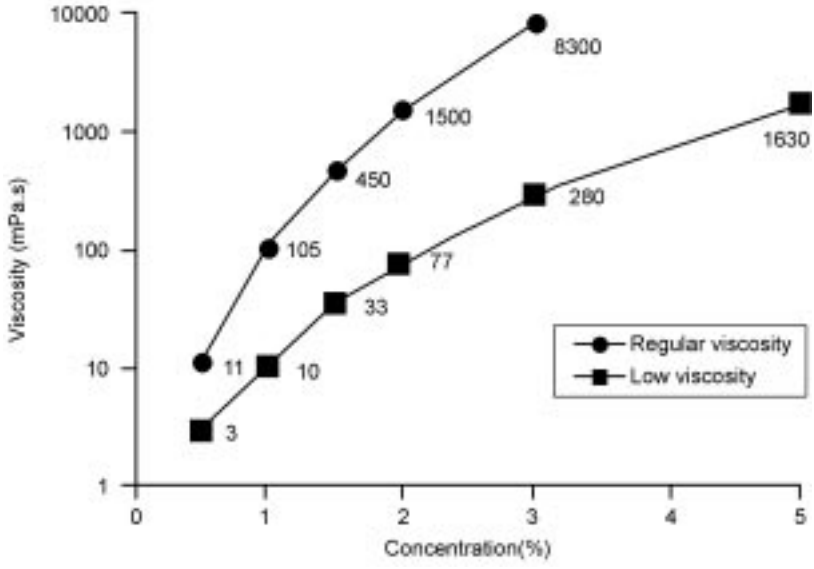

Fig. 19.14 Concentration dependence of steady shear viscosity of TSX and LVTSX at 25 ºC and at a shear rate of 8.6 s^{-1}.

Table 19.3 Food applications of low viscosity tamarind seed xyloglucan

Effects	Example foods
Prevents sticking	Noodles, boiled rice, pasta
Retains water	Boiled rice, bakery products
Provides body	Beverage, yoghurt
Prevents water release	Jelly, salad dressing
Forms fine ice crystals and offers excellent shape retention	Ice cream, frozen dessert
Forms gel with sugar	Jam, sheet jelly, good flow honey
Inhibits starch ageing	Sponge cake, bakery

LVTSX keeps cooked noodles without sticking and good texture for several hours. It is only necessary to dip noodles in LVTSX solution or to spray the solution on the noodles. The same effects can be achieved in cooked rice with LVTSX. LVTSX keeps rice not sticky and makes mixing other ingredients with rice easier. It is possible to add more water to rice boiling by the addition of LVTSX, and the yield of boiled rice increases. LVTSX prevents water release from vegetables and dressing when adding 0.5% of LVTSX powder to vegetables. Also the dressing sticks well to vegetables. The volume of sponge cake increased about 10% with the addition of LVTSX (Nagashima, 2005). It is reported that an addition of LVTSX improved mixing properties of dough, firmness and volume of bread (Nagashima, 2005).

19.6 Regulatory status in the food industry

As a food additive, purified TSX has been permitted in Japan and countries in Southeast Asia (Taiwan, South Korea and China). TSX is not yet permitted as a food additive in the US or EU.

19.6.1 Safety data

Since all higher plants contain xyloglucan polysaccharides, xyloglucans are necessarily consumed as part of vegetables and fruits. TSX has undergone a battery of rigorous safety studies, including acute toxicity, subacute toxicity, mutagenicity and chronic toxicity studies. There were no TSX-related abnormal findings. TSX was incorporated at a level of 4, 8, or 12% in a standard commercial diet and fed *ad libitum* to male and female rats for 2 years (Iida *et al.*, 1978). No significant changes were noted in the behaviour, mortality, body weight, food intake, biochemical analysis of urine and blood, haematological tests, organ weight or histopathological findings of rats receiving TSX. The carcinogenic potential of TSX was examined in both sexes of $B6C3F_1$ mice (Sano *et al.*, 1996). Groups of 50 male and 50 female animals were given diets containing 0, 1.25 and 5% of tamarind seed polysaccharide for 78 weeks. Body

weight reduction or retardation of the increase in body weight was seen in females in the 1.25 and 5% groups from the 34th week to termination. However, there were no treatment-related clinical signs or adverse effects on the survival rate, food and water consumption, haematological findings or organ weights. Detailed histopathological examination also revealed that there was no treatment-related increase in the incidence of any non-neoplastic or neoplastic lesions. These results demonstrated that TSX is not carcinogenic in $B6C3F_1$ mice of either sex.

19.7 Physiological effects of xyloglucan

19.7.1 Improved lipid metabolism

Xyloglucan is an indigestible polysaccharide that acts as a dietary fibre. Dietary fibre has been recognized as a nutritive component that is important for human health. Although dietary fibre is thought to be involved in various physiological functions, its daily intake has tended to decline. Dietary fibre has been shown to control blood glucose and reduce cholesterol (Lairon, 1996; Jenkins *et al.*, 1978; Roehring, 1988). These effects are important for the treatment and prevention of chronic diseases, including diabetes and cardiac disorders. The partial hydrolysis of polysaccharides reduces their viscosity to allow for easy intake while retaining their physiological activities and without changing the texture of the food (Yamatoya, 1989, 1994, 1997; Yamatoya *et al.*, 2000).

The improvement effect of xyloglucan and hydrolysed xyloglucan on lipid metabolism was examined (Yamatoya *et al.*, 1996, 1997, 2000). In a high-cholesterol diet, the addition of xyloglucan and hydrolysed xyloglucan did not affect body weight gain, food intake, feeding efficiency or liver weight. Instead, caecum weight was significantly increased and the weight of adipose tissue was significantly reduced. Table 19.4 shows plasma and hepatic lipid levels. Xyloglucan (intact) significantly reduced plasma total cholesterol, plasma β-lipoprotein, liver total lipid, liver cholesterol and liver triglyceride. Hydrolysed xyloglucan significantly reduced plasma triglyceride and liver triglyceride levels. The addition of hydrolysed xyloglucan to a high-fat diet did not affect body weight gain, food intake, feeding efficiency or liver weight. Hydrolysed xyloglucan significantly increased the caecum weight and reduced the weight of adipose tissue. Table 19.5 shows plasma and hepatic lipid levels. Among the plasma lipids, total lipid, cholesterol, triglyceride and β-lipoprotein were reduced 14–17% by hydrolysed xyloglucan. Among the hepatic lipids, total lipid, cholesterol, triglyceride and phospholipid were all significantly reduced by hydrolysed xyloglucan.

These findings were the first to demonstrate the biological activity of hydrolysed TSX in animals. TSX and hydrolysed TSX can be easily applied to food because of their water solubility, low viscosity and good mouthfeel.

Table 19.4 Effects of hydrolysed xyloglucan on plasma lipids (mg/dl) and liver lipids (mg/g) in rats fed a high-fat diet

	Control	Hydrolysed xyloglucan 5% diet
Plasma lipids (mg/dl)		
Total lipid	1,646 ± 105	1,367 ± 258*
Total cholesterol	168 ± 17	144 ± 25
Triglyceride	774 ± 65	656 ± 189
β-lipoprotein	1,020 ± 111	848 ± 219
Liver lipids (mg/g)		
Total lipid	107.7 ± 7.0	87.6 ± 3.2**
Total cholesterol	17.1 ± 2.3	11.6 ± 2.4**
Triglyceride	49.4 ± 3.6	41.9 ± 4.4*
Phospholipid	20.6 ± 3.9	15.3 ± 1.3*

Values are means ± SD for 6 rats.
* $p < 0.05$, ** $p < 0.01$: significantly different from the corresponding value in rats fed a control diet.

Table 19.5 Effects of xyloglucan and hydrolysed xyloglucan on plasma lipids (mg/dl) and liver lipids (mg/g) in rats fed high cholesterol diet

	Control	Xyloglucan 5%diet	Hydrolyzed xyloglucan 5% diet
Plasma lipids (mg/dl)			
Total cholesterol	131 ± 27	86 ± 11**	123 ± 22
Triglyceride	19.1 ± 4.8	16.3 ± 9.7	10.0 ± 3.0**
β-lipoprotein	277 ± 147	113 ± 31**	281 ± 110
Free fatty acid (mEq/l)	1.10 ± 0.22	0.95 ± 0.15	0.99 ± 0.11
Liver lipids (mg/g)			
Total lipid	248 ± 19	165 ± 17**	235 ± 17*
Total cholesterol	133 ± 21	70 ± 11**	122 ± 14
Triglyceride	49.5 ± 6.1	41.5 ± 5.84	40.7 ± 5.7*

Values are means ± SD for 7 rats.
* $p < 0.05$, ** $p < 0.01$: significantly different from the corresponding value in rats fed a control diet.

19.7.2 Carbohydrate metabolism

It has been reported that among tamarind xyloglucan oligomers, octasaccharide and nonasaccharide, which contain D-galactose residues, effectively inhibited the absorption of 3-*O*-methyl-D-glucose, using everted sacs from rat small intestine, and these inhibitory effects were concentration-dependent (Sone *et al.*, 1992). On the other hand, heptasaccharide, which does not contain galactose, did not inhibit the absorption of 3-*O*-methyl-D-glucose. The ratios of the serosal and

mucosal 3-*O*-methyl-D-glucose concentrations when heptamer, octamer and nonamer were present in the mucosal fluid were 1.31 ± 0.03, 1.18 ± 0.03 and 1.10 ± 0.04, compared with a control value of 1.33 ± 0.08. This indicated that xyloglucan octamer and heptamer, which have galactose residues on their side chains, inhibited the absorption of D-glucose by the same mechanism as phlorizin, which binds to the carrier protein in a brush border membrane by means of D-galactose residues located at the non-reducing ends of side chains. This controls blood glucose and insulin levels, which increases cholesterol synthesis and is one of the mechanisms by which xyloglucan improves lipid metabolism.

19.7.3 Biological functions

Different types of polysaccharides were investigated with regard to their antimutagenic activity against different standard mutagens. In the screening, pronounced antimutagenic effects were found for xyloglucan and different pectins against 1-nitropyrene-induced mutagenicity (Hensel and Meier, 1999). The inhibition rates were dose-dependent and it was proposed that the polymers directly interacted with cells to protect organisms from mutagenic attack. It seems that only comb-like structures exhibit antimutagenic effects: xyloglucan as well as pectins are characterized by a linear backbone branched with short side chains. It seems that a comb-like structure with a linear backbone and linear side chains which is not too voluminous may be a prerequisite for activity.

C3H mice were exposed to 5 kJ/m^2 UVB from unfiltered FS40 sunlamps and treated with between 1 pg and 10 μg tamarind xyloglucans or control polysaccharides methylcellulose or dextran in saline (Stickland *et al.*, 1999). Three days later the mice were sensitized with *Candida albicans*. Tamarind xyloglucans and purified aloe poly/oligosaccharides prevented the suppression of DTH (delayed-type hypersensitivity) responses *in vivo* and reduced the amount of interleukin (IL)-10 observed in UV-irradiated murine epidermis. Tamarind xyloglucan was immunoprotective at low picogram doses. Tamarind xyloglucans and Aloe poly/oligosaccharides also prevented the suppression of immune responses to alloantigen in mice exposed to 30 kJ/m^2 UVB radiation. Tamarind xyloglucan blocked the UV-activated phosphorylation of SAPK/JNK (stress-activated protein kinase/c-Jun *N*-terminal kinase) protein, but had no effect on p38 phosphorylation. These results indicate that animals, like plants, may use carbohydrates to regulate responses to environmental stimuli (Stickland *et al.*, 1999). The mechanism by which oligosaccharins regulate gene expression in plant cells is currently unknown. The finding that tamarind xyloglucans were active at low picogram quantities in intact mice suggests that the carbohydrate may act on a pathway in mammalian cells that is analogous to that in plants. Tamarind xyloglucans reduced the phosphorylation of the stress-activated SAPK/JNK, a member of the MARK (mitogen-activated protein kinase) family of proteins, in UV-injured murine keratinocytes. This regulation led to the reduction of IL-10 protein in keratinocytes and skin exposed to UV radiation.

The influence on the health of endocrine disrupting chemicals (so-called environmental hormone) and the influence on the reproductive function have been investigated. Acceleration of metabolism and excretion of endocrine disrupting chemicals by xyloglucan have been reported (Ikegami and Yamatoya, 2004). The accumulative amount of HCB (hexachlorobenzene) in the inside of the body has greatly decreased by taking xyloglucan instead of cellulose. The decrease in HCB accumulation correlated with a decrease in the amount of adipose tissue.

19.8 Other aspects and applications

19.8.1 Plant growth and plant defence systems

Xyloglucan-oligosaccharide, which is obtained by the degradation of a xyloglucan, i.e., a polysaccharide that is found in the cell walls of higher plants, participates in plant growth. It has been shown that 1) a pentasaccharide, heptasaccharide and nonasaccharide of xyloglucan-oligosaccharide each activate endo-1,4-β-glucanase in plants (Farkas and Maclachlan, 1988), 2) a heptasaccharide, octasaccharide, and nonasaccharide of a xyloglucan-oligosaccharide each promote the elongation of a slice of pea-hypocotyl (McDougall and Fry, 1998), and 3) fucose-containing xyloglucan-oligosaccharides inhibit the auxin-induced elongation of pea stems (York *et al.*, 1984; McDougall and Fry, 1988). Xyloglucan endotransglycosylase (XET) has been proposed to participate in dynamic changes in xyloglucan crosslinking (Fry *et al.*, 1992; Nishitani and Tominaga, 1992). Xyloglucan, which has a 1,4-β-backbone, can bind specifically to cellulose microfibrils by hydrogen bonds (Hayashi *et al.*, 1994). Microfibrils seem to be coated with xyloglucan, which is located both on and between microfibrils throughout cell elongation (Hayashi and Maclachlan, 1984).

The role of xyloglucan in plant defence systems is not yet clear. Large quantities of agricultural chemicals are now used to prevent microbial infections in crop cultivation. However, there are various problems with this practice, such as the effects of chemicals remaining on the crops on human health, and environmental pollution. Plants have various defence systems against infections, and when a plant is infected by a micro-organism, an anti-microbial substance which is not present in normal tissue is induced to resist the infection. This substance is referred to as a phytoalexin. A phytoalexin is induced by some cell component of a micro-organism, a fragment of a polysaccharide which comprises the cell wall of the plant infected by a micro-organism, or something similar. This substance which induces a phytoalexin is referred to as an elicitor. A fragment of β-glucan derived from the cell wall was the first substance to be shown to have elicitor activity (Albersheim and Darvill, 1985). Moreover, it is thought that elicitor activity not only induces a phytoalexin but also participates in inducing an enzyme-inhibitory substance such as a protease inhibitor, β-glucanase, and in producing a plant hormone, e.g., ethylene. A fragment of β-

glucan derived from the cell wall of *Phytophthora megasperma* (Sharp *et al.*, 1984) and oligogalacturonic acid, which is a fragment of a pectin (Albersheim and Darvill, 1985; Komae *et al.*, 1990), are known to possess elicitor activity. It has been proposed that a substance with elicitor activity may be useful as a biotic pesticide.

Xyloglucan oligosaccharide has also been shown to have elicitor activity and is useful for inducing a phytoalexin (Misaki *et al.*, 1997). In this experiment (Misaki *et al.*, 1997) using soybean cotyledons, hydrolysed TSX showed a 7.8-fold induction of the phytoalexin glyceollin. Elicitor activity was determined as described in the literature (Ayers *et al.*, 1976). Both TSX oligosaccharides (hydrolysed TSX) and oligosaccharide fragments prepared from pea xyloglucan inhibited the development of necrotic lesions induced by tobacco necrosis virus (TNV) on cotyledons of *Cucumis* L. cv. *'Laura'*. Virus infection was inhibited by 44% to 84% when xyloglucan fragments were applied in amounts ranging from 10 to 100 μg per cotyledon 24 h prior to virus inoculation. These results suggest that xyloglucan may play a role in the mechanism of induced resistance against viruses (Subikova *et al.*, 1994). The activities of chitinase and β-1,3-glucanase increased more rapidly 24 h after the inoculation of treated cotyledons with tobacco necrosis virus (Slovakova *et al.*,1994). The stimulation of the release and accumulation of these enzymes by xyloglucan oligosaccharides is related to the hypersensitive reaction to TNV. However, contrary to previous assumptions, purified TSX fragments did not elicit anti-TNV resistance in cucumber cotyledons (Subikova *et al.*, 2001).

19.8.2 Drug delivery systems

It has been suggested that TSX may be useful in an ophthalmic delivery system for the topical administration of antibiotics (Ghelandi *et al.*, 2000), may have beneficial effects on conjunctival cell adhesion, a primary event before cell differentiation (Burgalassi *et al.*, 2000), and may be a useful adjuvant in an ophthalmic delivery system (Mannucci *et al.*, 2000).

Enzymatic modification of TSX gel has been used in various types of drug delivery systems. It can be used for rectal, intraperitoneal and oral drug delivery. Plasma levels of indomethacin after the rectal administration of TSX gels (partially degraded by β-galactosidase) to rabbits indicated a broader absorption peak following administration of the gels, and the residence time was longer than that in a control system (Suisha *et al.*, 1998).

Photomicrographs of rectal mucosa show no detectable tissue damage resulting from exposure to a single dose of TSX partially degraded by β-galactosidase (Suisha *et al.*, 1998). The enzymatic modification of TSX gel offers many advantages for use in drug delivery systems (Yamamoto and Horii, 1994; Miyazaki *et al.*, 1998).

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20

Curdlan

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Abstract: Because of its unique gelling behaviour, curdlan has been the subject of much investigation especially for applications in the food industry. Recently rheological and thermal properties and conformational, morphological analysis as well as the physiological functions such as anti-tumour and anti-HIV activities have been studied. In the present chapter, physico-chemical properties of curdlan are reviewed and the structural characterization of curdlan is also emphasized.

Key words: curdlan, gel, food, biomedical application.

20.1 Introduction

Curdlan is an extracellular microbial polysaccharide and was first discovered and investigated by Harada *et al.* in 1964 who coined the name curdlan, derived from 'curdle' to describe its gelling behaviour at high temperatures.[1,2] Curdlan is composed entirely of 1,3-β-D-glycosidic linkages, which occur widely in nature involved in cell structure and food storage in bacteria, fungi, algae and high plants.[3] Cotton fibres are known to comprise ca. 10% 1,3-β-D-glycosidic linkages 20 days after anthesis.[4] 1,3-β-D-glycosidic linkages are present in pollen, root hair and stomata of plants. Mushrooms and yeasts are also known to contain 1,3-β-D-glucosidic linkages.[5]

Curdlan has utility as a food additive in its ability to form an elastic gel. Curdlan forms a heat-set gel at both relatively high and low temperatures or on neutralization or dialysis of alkaline solution of curdlan.[6–8] The unique gelling

characteristics of curdlan are not only opposite to cold-set gels such as agarose,[9] gelatin, carrageenan[10] and gellan,[11] but also different from other heat-set gels, e.g., konjac glucomannan,[12] methylcellulose[13] and hydroxypropyl-methyl-cellulose.[14] In addition, curdlan is believed to show strong bioactivities.[15–18] These conspicuously unusual properties of curdlan have not been observed in other synthetic and natural compounds.

For more than 30 years, quite a few works have been published since the discovery of curdlan. Because of its unique properties, curdlan has been the subject of remarkable investigation especially for use in the food industry. Recently rheological and thermal properties and conformational, morphological analysis as well as the physiological functions such as anti-tumour and anti-HIV activities have been studied. In the present work, physico-chemical properties of curdlan are reviewed and the structural characterization of curdlan is also emphasized.

20.2 Production

Curdlan is produced in a fermentation process (Fig. 20.1) from the mutant strain of the bacteria *Alcaligenes faecalis var. myxogenes* 10C3 which can be isolated from soil.[1,2] Commercial curdlan may contain cellular debris, proteins and nucleic acids[19] and other organic acids.[20] More than 100 tonnes/year of curdlan is now produced by Takeda Chemical Industries Co. Ltd., Osaka, Japan.

20.3 Chemical structure

The chemical structure of curdlan is shown in Fig. 20.2. Curdlan is one of the biopolymeric molecules known as 1, 3-β-D-glucans.[21,22] Such a polysaccharide is characterized by repeating glucose subunits joined by a β-linkage between the first and third carbons of the glucose ring, which differs only in the linkage manner of repeating units from cellulose, a well-known natural biopolymer consisting of cellulose residues glycosidically linked in the 1, 4-β-D-configuration. With respect to the 1, 3-β-D-linkage, the structure of curdlan is similar to that of carrageenan, agarose and gellan gum. Unlike gellan and κ- and ι-carrageenan, curdlan is a neutral polysaccharide without acidic components. The average degree of polymerization of curdlan determined using Manners' method[23] is about 450.[6,7] However, the molecular weight of currently commercially available curdlan can be higher than two million Dalton.

20.4 Native curdlan

In the solid state, curdlan may exist in a triple helical structure. Curdlan has been shown to have a triple helical structure by ^{13}C NMR analysis. In its natural state, curdlan is poorly crystalline[24] and is found as a granule in the form of doughnut

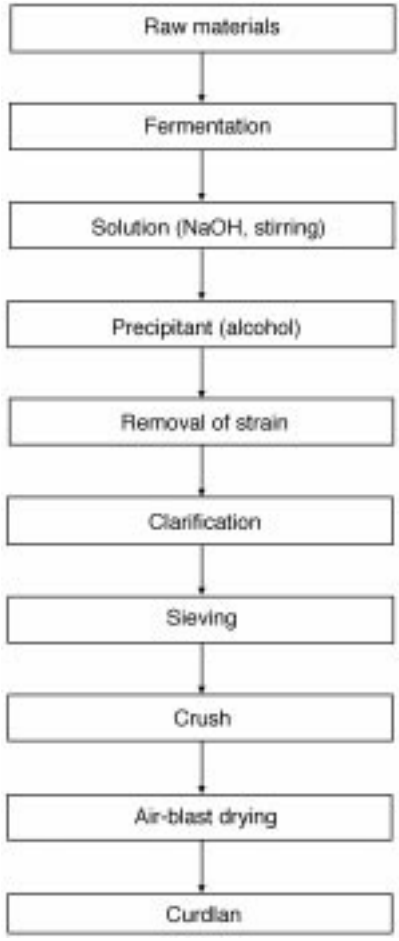

Fig. 20.1 Typical process for the manufacture of curdlan.

shaped structure[25] as shown in Fig. 20.3, much like that of starch. The granule is insoluble in distilled water but readily dissolves in an aqueous NaOH due to the ionization of hydrogen bonds. There is extensive hydrogen bonding that is holding the granule together, most likely by strongly binding the helices to form

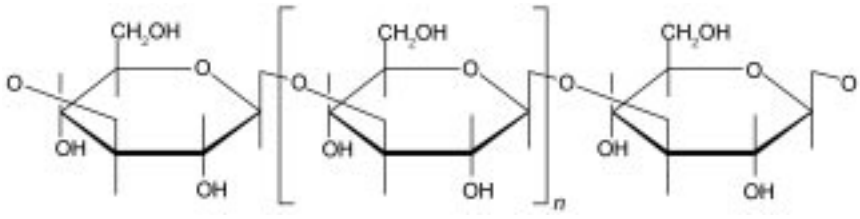

Fig. 20.2 Chemical structure of curdlan.

Fig. 20.3 Electron micrograph of curdlan granule.[25]

microfibrils and then binding together the microfibrils. When these bonds are broken through swelling, the granule loses its structure, i.e., the microfibrils have dissociated from each other during hydrolysis.

20.5 Functional properties

20.5.1 Solution properties and conformations

While the primary structure is a long linear chain, curdlan forms more complex tertiary structures due to intra- and intermolecular hydrogen bonding. Curdlan is not soluble in water at room temperature but dissolves in an alkaline aqueous solution, cadoxen [Tri(ethylene diamine) hydroxide] aqueous solution and DMSO (dimethyl sulfoxide). The water insolubility of curdlan may be attributed to the existence of extensive intra- and intermolecular hydrogen-bonded crystalline domains like that of cellulose.[26] It has been reported that 1,3-β-D-glucans with a very low degree of polymerization of below 25 DP is soluble in water[27] and it was suggested that curdlan is soluble in water at elevated temperatures.[25,28] At present, however, there is no direct evidence to clarify the water solubility of curdlan though an aqueous suspension of curdlan becomes clear when heated at above 55 ºC.

Hirano *et al.*[29] carried out light scattering and viscosity measurements on nine fractionated samples of curdlan in the 1:1 (v/v) water-diluted cadoxen in the range of molecular weight M_W from 6.6×10^4 to 6.8×10^5 at 25 ºC. The experimental results of z-average mean-square radius of gyration $\langle s^2 \rangle_z^{1/2}$ and intrinsic viscosity $[\eta]$ of curdlan were concluded as

$$\langle s^2 \rangle_z^{1/2} = 3.2 \times 10^{-2} M_w{}^{0.53} \text{ nm} \qquad 20.1$$

$$[\eta] = 2.5 \times 10^{-4} M_w{}^{0.65} \text{ cm}^3 \text{ g}^{-1}. \qquad 20.2$$

Nakata *et al.*[30] made light scattering and viscosity measurements on solutions of eighteen fractions of curdlan in 0.3 M NaOH (Table 20.1). The relationship

Table 20.1 Light scattering and viscosity data obtained for fractionated samples of curdlan at 25 °C[30]

Sample	$10^{-6}M_W$	$10^{11}<s^2>/cm^2$	$10^4A^2/g^{-2}\,cm^3\,mol$	$[\eta]/cm^3\,g^{-1}$
1	2.02	6.39	4.2	665
2	1.91	6.70	4.1	643
3	1.81	5.54	3.6	590
4	1.45	4.64	4.5	520
5	1.28	3.99	5.0	468
6	1.26	3.33	3.6	443
7	1.12	3.41	3.6	387
8	0.95	2.56	3.9	343
9	0.91	2.58	2.7	295
10	0.56	1.85	6.1	238
11	0.50	1.62	6.0	218
12	0.45	1.18	5.6	200
13	0.39	1.16	6.4	165
14	0.28	0.74	6.1	140
15	0.23	0.57	5.4	129
16	0.17	0.36	7.4	96
17	0.092	0.29	6.0	60
18	0.053	0.11	10.9	38

between weight-average molecular weight M_W and $\langle s^2\rangle_z^{1/2}$ and $[\eta]$ and second virial coefficient A_2 were summarized as

$$\langle s^2\rangle_z^{1/2} = 3.6 \times 10^{-2}\, {M_w}^{0.53}\,\text{nm} \tag{20.3}$$

$$[\eta] = 7.9 \times 10^{-4}\, {M_w}^{0.78}\,\text{cm}^3\,\text{g}^{-1} \tag{20.4}$$

$$A_2 = 2.8 \times 10^{-2}\, {M_w}^{0.3}\,\text{g}^{-2}\,\text{cm}^3\,\text{mol}. \tag{20.5}$$

They concluded that the behaviour of curdlan solution cannot be explained by the conventional two-parameter theory of flexible polymer chains by evaluating the Flory universal viscosity constant Φ ($= [\eta]M/(6\langle s^2\rangle)^{3/2}$), which is much smaller than the usual value of about 2.5×10^{21} dl/mol cm^3. Compared with cellulose chain, the values of $\langle s^2\rangle_z^{1/2}$ for curdlan are only one half of those for cellulose at fixed M_W in 1:1 water-diluted cadoxen, indicating that curdlan chain has a more contracted conformation and both curdlan and cellulose molecules are not so flexible as shown in Fig. 20.4.[31]

As for the conformation transformation of curdlan in aqueous NaOH solution, there are several changes that occur from a triple helix to random coil depending on the concentration of sodium hydroxide used. Ogawa *et al.*[32] reported that the transition occurred at a NaOH concentration of 0.19–0.24 M in studies on the optical rotatory dispersion, viscosity and flow birefringence. This transition was confirmed by Saito *et al.*[33] by ^{13}C NMR analysis. Stipanovic and Giammatteo[34] measured the steady shear viscosity of curdlan solution at different NaOH concentrations. A significant increase in viscosity was observed in the range of

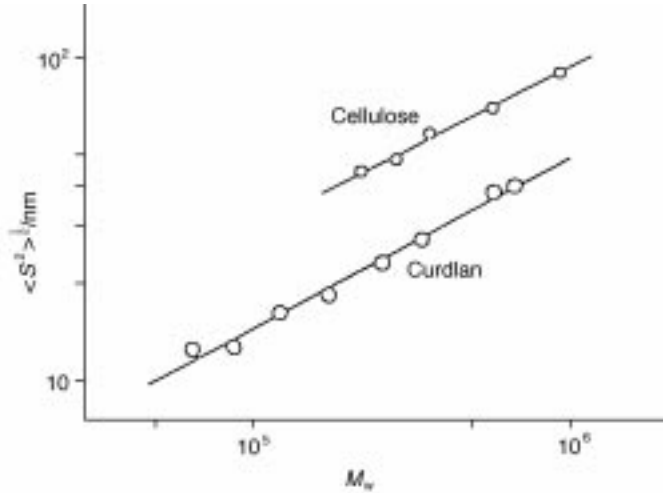

Fig. 20.4 Molecular weight M_w dependence of mean-square radius of gyration $\langle s^2 \rangle_z^{1/2}$ for curdlan and cellulose in 1:1 (v/v) water-diluted cadoxen at 25 ºC.[31]

0.05–0.1 M NaOH corresponding to the solvation of triple helices followed by a viscosity reduction above 0.25 M NaOH as the triple molecules are dissociated into single chains of lower molecular weight, which were supported by their ^{13}C NMR experiments. Figure 20.5 shows a plot of intrinsic viscosity of curdlan vs. NaOH concentration at 25 ºC measured by Nakata *et al.*[30] They believed that the sharp depression of [η] at 0.22 M NaOH in Fig. 20.5 might be due to the conformational transition of curdlan chain from helix to coil.

Tada *et al.*[35–37] studied the structure of molecular association of curdlan at diluted regime in alkaline aqueous systems by rheological, static light scattering

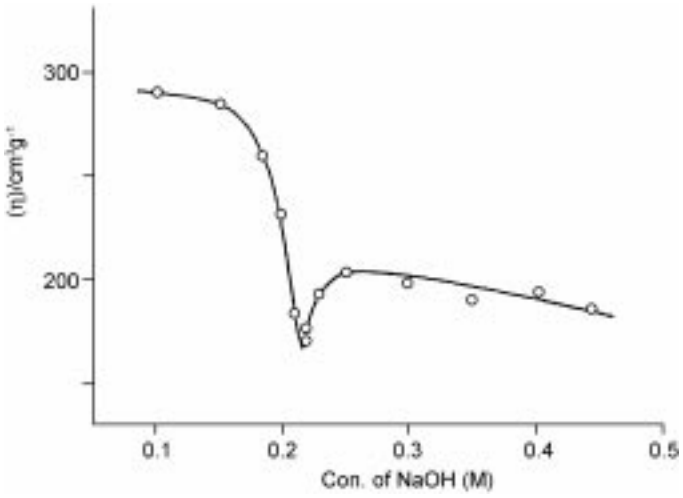

Fig. 20.5 NaOH concentration dependence of intrinsic viscosity [η] for curdlan at 25 ºC.[30]

and small-angle X-ray scattering measurements. They found that the degree of association of curdlan molecules increased with decreasing alkaline concentration and the viscoelastic properties depend strongly on the alkaline concentration, i.e., concentrated curdlan solutions show almost a Newtonian flow at high alkaline concentrations whereas a solid-like behaviour at low alkaline concentrations. Curdlan in DMSO at above 1% behaves like a concentrated polymer solution,[38] similar to in high concentrations of NaOH aqueous solutions of above 0.05 M.[35]

20.5.2 Aqueous suspension properties

Although there have been extensive studies on curdlan, the majority in the literature have concentrated on curdlan gels and curdlan solutions, and only a few have investigated curdlan suspensions. Curdlan can only be suspended in water due to insolubility. Using a homogenizer can make curdlan into well-dispersed suspension without separation when left standing. When curdlan is dispersed in water, it gives a paste-like suspension unlike konjac glucomannan which forms a suspension with very high viscosity.[39] Tako and Hanashiro[40] reported that curdlan preparations at 38 ºC showed a Newtonian behaviour at 0.1% but plastic behaviour at above 0.2%. Plastic behaviours of curdlan suspensions were also observed in other reports.[41,42] The values of yield stress were estimated to be 0.10, 0.12 and 1.2 Pa for 2%, 3% and 4% suspensions, respectively. The frequency dependence of storage modulus G' and loss modulus G'' of curdlan suspensions showed a solid-like behaviour at 40 ºC (Fig. 20.6).

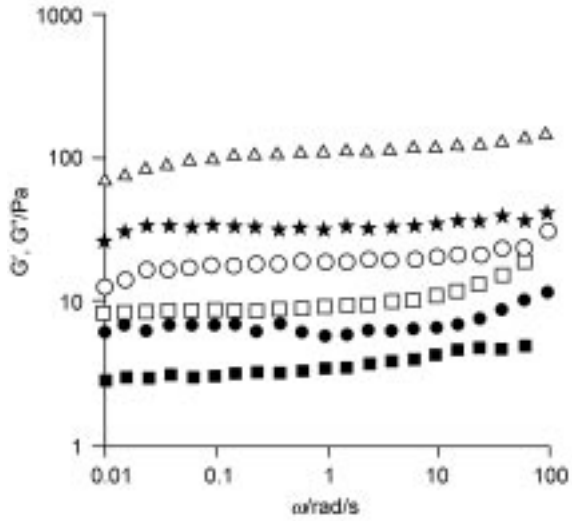

Fig. 20.6 Frequency dependence of storage modulus G' and loss modulus G'' of curdlan suspensions at various concentrations at 40 ºC[41] (□ G', ■ G'' for 2%; ○ G', ● G'' for 3%; Δ $*G'$, ★ G'' for 4%).

20.5.3 Gelation

Gel formation

Curdlan can form a gel through the heating process alone, rather than relying on accompanying conditions such as pH, sugar concentration, or the presence of cations. Curdlan hydrogels can be formed by various methods, some of which are heating a curdlan aqueous suspension,[43,44] curdlan/DMSO/H_2O suspension[38], neutralizing or dialysing against water an alkaline solution of curdlan[25,44] or a solution of curdlan in DMSO[25] in a stationary state at ambient temperature. Furthermore, by heat treatment a curdlan aqueous suspension is capable of forming two types of gels depending on heating temperature, one of which is a thermo-reversible gel termed as a low-set gel formed by heating up to about 55 ºC then cooling, and the other thermo-irreversible gel termed as a high-set gel formed by heating at above 80 ºC. This change is explained by the hypothesis that microfibrils dissociate at 60 ºC as the hydrogen bonds are broken, but then reassociate at higher temperatures as hydrophobic interactions between the curdlan molecules occur. An additional change to an even more ordered form is suggested in some sources as the temperature is raised above 120 ºC.[45] Besides temperature, many other factors, such as molecular weight, concentration, heating rate, dispersing method and the addition of inorganic salts, influence the formation and the mechanical properties of curdlan gels.[46–50] For example, the transition temperature from thermo-reversible to irreversible gel is dependent on molecular weight[49] and the increase in concentration also lowers the transition temperature from the generally reported 80 ºC.[48,50]

As for the heating rate,[47] thermo-reversible gelation upon cooling is inhibited more with decreasing heating rate, which is pronounced at higher concentrations of curdlan. Also, the longer the incubation at the swelling temperature range (50–60 ºC), the smaller the dynamic storage modulus of the final gels (formed after heating and subsequent cooling processes). These can be attributed to the intermolecular associations during swelling though hydrophobic interactions, creating heterogeneous network structures and inhibiting the rearrangement of the molecules upon cooling. Another hypothesis which is quite prominent at present, however, is that curdlan gels have network structures composed of energetic junction zones with entropic disordered polymer chains, in which particles that are not completely dissolved are embedded, playing as a filler to fortify the gel matrix because curdlan has highly ordered crystalline structures within the molecules as found in starch. Both slower heating rate and longer incubation within the temperature range for swelling weaken the crystalline structures, losing the unmelted crystalline structures composed of triple helices which contribute to the mechanical strength. As for the effects of inorganic salts, their addition decreases the mechanical strength of curdlan gels. This is attributed to the inhibition of swelling or hydration and thus disordering of curdlan molecules through breakage of intra- or intermolecular hydrogen bonds, resulting in decreased number of molecules that participate in associations via new or re-natured hydrogen bonds to form junction zones in the network structures of curdlan gels.

Interestingly, Dobashi and his co-workers[51–54] found that the dialysis of curdlan dissolved in alkaline solution into aqueous solutions of metal salts yielded multifold gel structures. Aqueous calcium salts induced liquid crystalline gel with refractive index gradient/amorphous gel alternative structure,[52] which is affected by molecular weight of curdlan.[53] Aqueous salts of trivalent aluminum and ferric cations induced a rigid liquid crystalline gel, which shrank above a threshold concentration of each salt.[51] These new gel materials could be used as an optical component such as a polarizer or an indicator.[54]

Gel properties

The physical properties of aqueous curdlan gels were studied extensively by Harada and co-workers. The gel strength increases with concentration of curdlan[2] as do many other polysaccharide gels (Fig. 20.7). The relationship between the heating temperature and gel strength[2] is shown in Fig. 20.8. Gel was formed at about 55 ºC, the gel strength stays almost constant at 60–80 ºC, and thereafter increases with heating temperature until 100 ºC. The gel strength is strongly dependent on heating temperature and it is found that the strength of a gel formed by heating for 3 min at 90 ºC is much greater than that obtained by heating for 4 h at 70 ºC. The gel strength does not change between pH 3 and 10, and can be enhanced greatly by adding borate, but the presence of urea, a reagent which breaks hydrogen bonds, caused a decrease in the gelling temperature and a marked decrease in gel strength as the concentration of urea increased above 2 M (Table 20.2)[2] and elastic modulus of curdlan gel.[55]

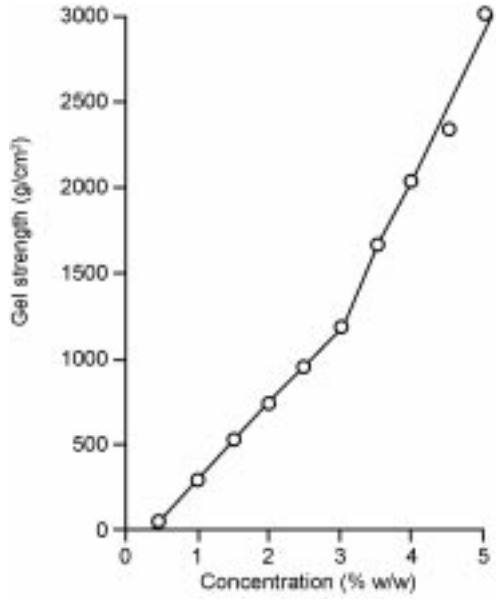

Fig. 20.7 Concentration dependence of gel strength for curdlan at 30 ºC[2] (curdlan gel was obtained by heating at 90 ºC for 10 min).

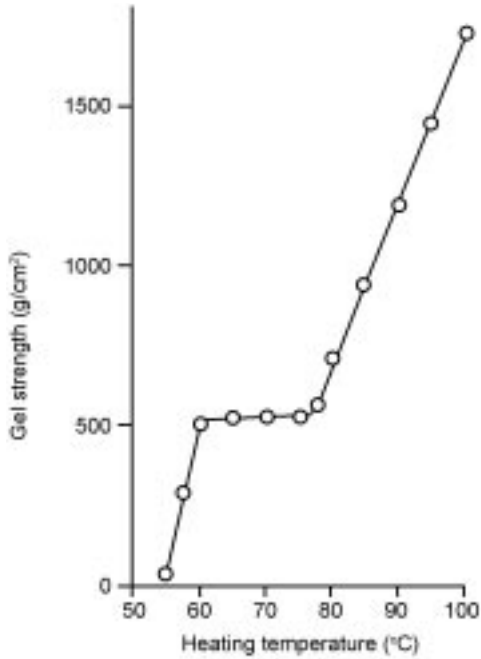

Fig. 20.8 Effect of heating temperature on gel strength of curdlan at a concentration of 3%.[2]

However, the reason why the gel strength shows a maximum (Table 20.2) is unknown.

The high-set gel[7] has the properties of being much stronger and more resilient and shows less syneresis than the low-set gel (Table 20.3) and neutralized gel (Table 20.4) and is not broken when frozen and thawed.[56] The high-set gel was resistant to enzymatic and acidic hydrolyses, but the neutralized gel was not

Table 20.2 Effect of urea on gel strength of curdlan (2%) obtained after heating at 90 °C for 10 min[2]

Molar concentration of urea (M)	Gel strength ($g\,cm^{-2}$)	Gel-forming temperature (°C)
0	1190	54
1	1660	47
2	1910	45
3	1380	44
4	1170	42
5	980	38
6	860	36
7	64	35
8	—	—

Table 20.3 Gel strength and syneresis at 30 °C of gels of curdlan (3%) obtained after heating at various temperatures in water for 20 min[7]

Heating temperature (°C)	Gel strength ($g\,cm^{-2}$)	Syneresis (%)
55	310	2
60	530	4
70	540	8
80	630	10
100	940	16
120	1100	18
145	1200	20
160	1800	29
170	2200	34

Table 20.4 Gel strength and syneresis at 32 °C of gels of curdlan formed by neutralizing alkaline solutions of curdlan with different concentrations[56]

			After freeze-thawing	
Concentration of curdlan (%)	Gel strength ($g\,cm^{-2}$)	Syneresis (%)	Gel strength ($g\,cm^{-2}$)	Syneresis (%)
1	220	2	0	100
2	460	2	170	42
3	620	2	430	36
4	870	2	720	24
5	920	2	780	18

resistant at all.[56] Gels set at above 90 °C are soluble only in concentrations of NaOH above 1 M whereas neutralized and 60 °C-set gels are soluble in 0.01 M NaOH.[56] It has been reported that tannin, sugar and starch are capable of reducing the syneresis of curdlan gel.[5,42]

Molecular conformations

It is difficult to decipher the exact molecular conformation of curdlan because of its polymorphism and dependency on many factors such as molecular weight, concentration, degree of hydration, heating history and dispersing method. Marchessault *et al.*[3,24,57,58] determined the molecular and crystal structure of the anhydrous form of curdlan by X-ray diffraction analysis (Fig. 20.9) and proposed that curdlan is composed of a triple stranded helix as shown in Fig. 20.10(a). The three strands of the glucan single helix are parallel, right-handed and in phase along the helix axis, three secondary OH groups form an inequilateral hexagonal shape perpendicular to the helix axis, and the crystal structure is extensively hydrogen-bonded. However, Shinkai *et al.*[59] put forward another intermolecular hydrogen-bonding model based on semi-empirical molecular-orbital package and *ab initio* calculations for the curdlan helix in

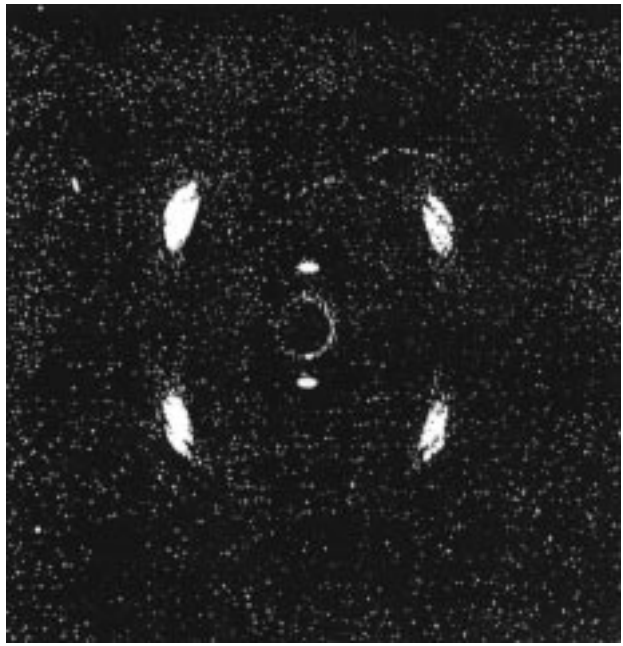

Fig. 20.9 X-ray fibre diffraction pattern of the anhydrous form of curdlan. The fibre axis is vertical.[3]

which the hydrogen bonds are formed between the O(2)-atoms on different *x-y* planes along the curdlan helix, traversing three curdlan chains to make a left-handed helix, hence the hydrogen bonds are not perpendicular to the helix axis as shown in Fig. 20.10(b).

Fulton and Atkins[60] investigated the molecular structure and gelling mechanism of curdlan using X-ray diffraction and infra-red spectroscopy. They proposed that triple helices are dominant for most curdlan molecular chains. The triple stranded molecules are bound by hydrogen bonding to the interstitial water of crystallization to form a micellar domain, in other words, interstitial water forms a hydrogen-bonded network with the triple helices, binding them into a micellar structure (Fig. 20.11(a)). The gelling mechanism of curdlan involves the interactions between these micelles and not the untwining and retwining of single helices into triple stranded junction zones. It is the association of these micelles which form the junction zones of the gel network (Fig. 20.11(b)).

Stipanovic and Giammatteo[34] demonstrated that three crystalline conformations, anhydrous, hydrate and swollen, differing in the degree of lattice hydration and chain extension, are distinguishable by solid-state ^{13}C NMR spectra. The anhydrous and hydrate forms adopt a sixfold triple-helical conformation, whereas the swollen polymorph is a sevenfold triplex. In the gel state, the conformation of curdlan is similar to that of the swollen sevenfold helical form for a low-set gel prepared at 60 ºC, whereas a partial or dominant transformation from the swollen sevenfold form to the anhydrous or hydrate sixfold

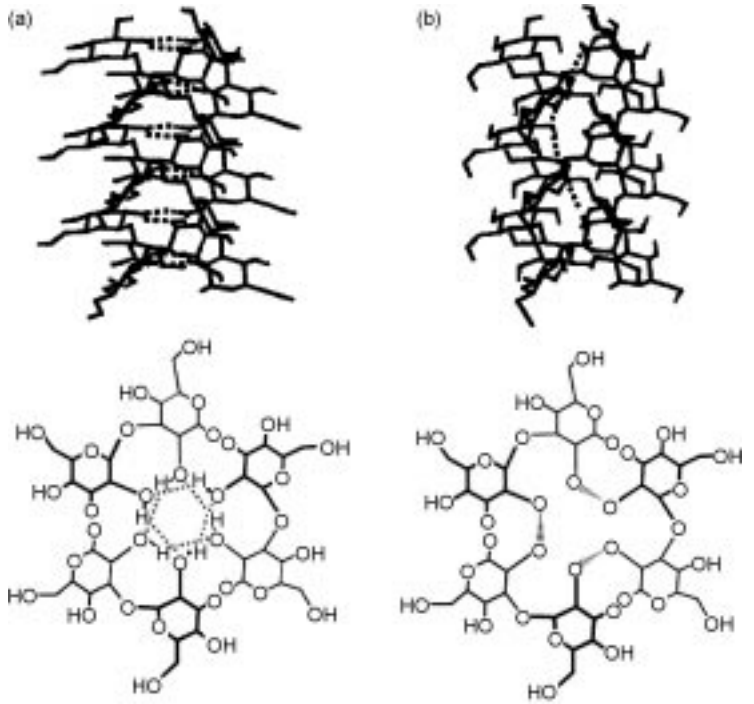

Fig. 20.10 Proposed two types of hydrogen bonds in curdlan triple helix. (a) The hexagonal intermolecular hydrogen bonds. The upper model is a side view of the full turn triple helix, here the dotted lines indicate the hydrogen bonds, and the lower one is a cross-sectional view of the helix (perpendicular to the helix). (b) The left-handed helical hydrogen bonds. The side view and cross-sectional view are presented in the same manner as in panel (a). The hydrogen bonds are connected along the helix, traversing three curdlan chains to make a left-handed helix. Therefore, the hydrogen bond array makes a reverse helix of the main chain.

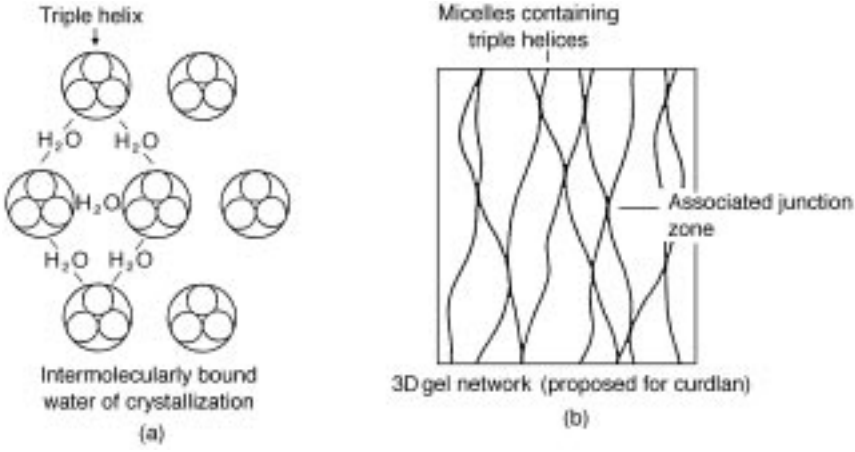

Fig. 20.11 Schematic gel network of curdlan.[60]

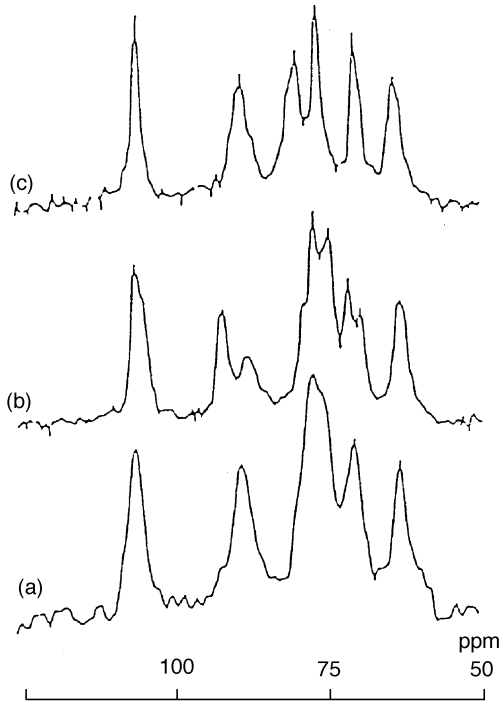

Fig. 20.12 ^{13}C NMR spectra of curdlan gels:[34] (a) 60 °C, (b) 95 °C; (c) 120 °C.

triplex was determined for a high-set gel formed at 95 or 120 °C respectively (Fig. 20.12). The increase in gel strength is due to the additional degree of crosslinking resulting from such a transformation.

However, Saito *et al.*[61–63] suggested that the anhydrous form is ascribed to a single chain form and swollen curdlan adopted a conformation of single helix, whereas annealed (hydrate) curdlan is readily identified as the triple-helix form. They suggested that, in any kind of heat-set curdlan gels, the conformations of curdlan exhibit an identical single helix with a low proportion of a triple helix, i.e., the single helix is present in any gel preparations. The constant gel strength between 60–80 °C (Fig. 20.8) corresponds to the formation of pseudo-crosslinks arising from hydrophobic association of the single helical chains while the increased gel strength in the high-set gel is due to increased proportion of triple helical conformation.[63]

Harada and co-workers[7,31,64] also indicated that the helix structure was transformed from single strand to triple strands at higher temperatures. An elaborate interpretation of structural change was proposed as shown in Fig. 20.13. The gelation mechanism of the low-set gel is different from that of the high-set gel. For a low-set gel, curdlan micelle interior is packed mostly by 7/1 single helical molecules which are hydrogen bonded to one another by water molecules and some parts of the micelle are occupied by triple helical molecules

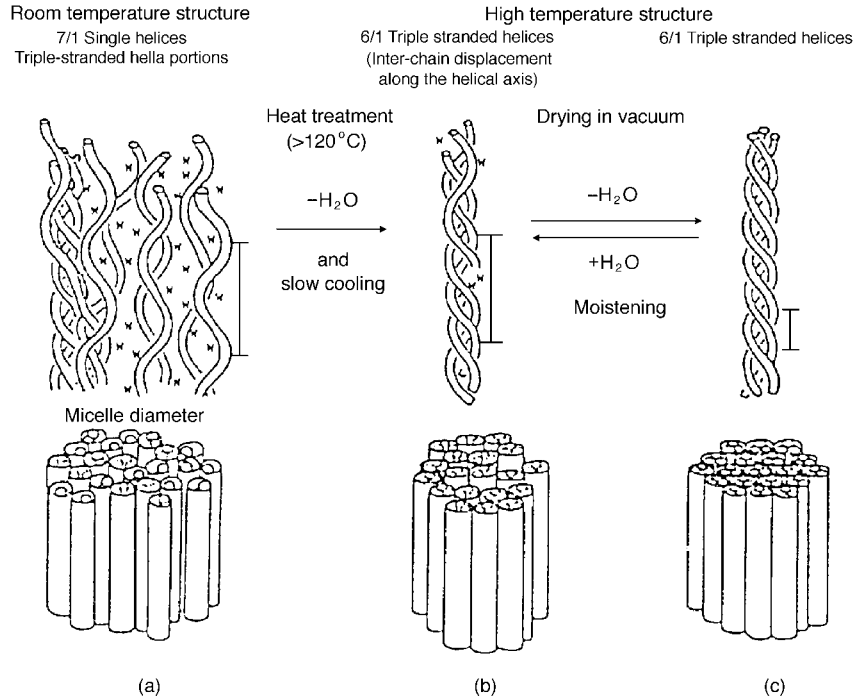

Fig. 20.13 Schematic representation of structural change between three forms of curdlan: (a) room temperature structure; (b) high temperature structure at high humidity; (c) high temperature structure at low humidity.[31]

which are also hydrated, whereas for a high-set gel, curdlan molecules change their conformation to 6/1 triple helices and curdlan micelle is occupied by molecules of triple-stranded helix in which hydrophobic interactions between curdlan molecules play a predominant role in the formation of the gel.

Our recent studies[65] on the conformations and conformational transitions of curdlan molecules by tapping mode atomic force microscopy (TMAFM) and on the intermolecular interactions by cyclic voltammetric method support the proposition given by Saito[63] and Harada.[64] Single helix chains were extensively observed on mica surface at the condition parallel with the formation of low-set gel which underwent a heat treatment in the temperature range of 60–70 ºC. The single helix chains readily associate into microfibrils with increasing concentration possibly driven by cooperative interaction between predominant hydrogen bonding and hydrophobic interaction. At elevated temperature above 80 ºC, the single helix chains associate in a different pattern in which hydrophobic interaction may play a more important role, and the proportion of triple helix chains also increases. Both of these two aspects may contribute to the formation of high-set gel. Some factors such as higher temperature, slower heating rate, longer time hydration and dialysis process were found to facilitate the formation of triple helix conformation. Therefore, triple helix conformation

should be more thermodynamically stable than single helix, because single helix chains tended to convert to triple helix when enough relaxation time and necessary temperature were available under the above-mentioned conditions.

20.5.4 Thermal and morphological analysis

According to thermal analysis, the DSC curves of original curdlan in aqueous suspension show a sharp endothermic peak at 50–64 ºC,[6,7,38,41,42,44] a shallow endothermic peak at 70–100 ºC,[6,7,38] and another endothermic peak at ca. 150 ºC (Fig. 20.14).[38] The first endothermic peak is ascribed to swelling of curdlan due to the breakup of some hydrogen bonds and the second the occurrence of hydrophobic interaction between curdlan molecules. As for the endothermic peak at ca. 150 ºC, it might be caused by the structural change of curdlan gels by further heating, corresponding to some molecular conformation transformation mentioned in Section 20.5.3. It is noteworthy that no exothermic peaks were observed when heat-treated silver pans were used.[38,65] One may find a marked difference in the shape of DSC curves obtained by using non-heated silver pans.[6,7,44] Therefore, the calibration of silver pans by heat treatment is vital to obtain reliable DSC results through eliminating the unfavourable interactions between DSC pan and water, which might be a cause of misreading of experimental results.[66]

When curdlan dispersions were heated to different temperatures then cooled, double exothermic peaks appeared at about 38 and 31 ºC, which were attributed to the structure ordering due to the formation of hydrogen bonds.[41] When the cooled gel was heated again, an endothermic peak appeared at around 60 ºC.[7,41] Figure 20.15[41] shows the second run heating DSC curves for 5% dispersions of curdlan which had been heated at various specified temperatures, *T*, for 60 min and then quenched to 10 ºC. Dispersions heated at temperatures higher than

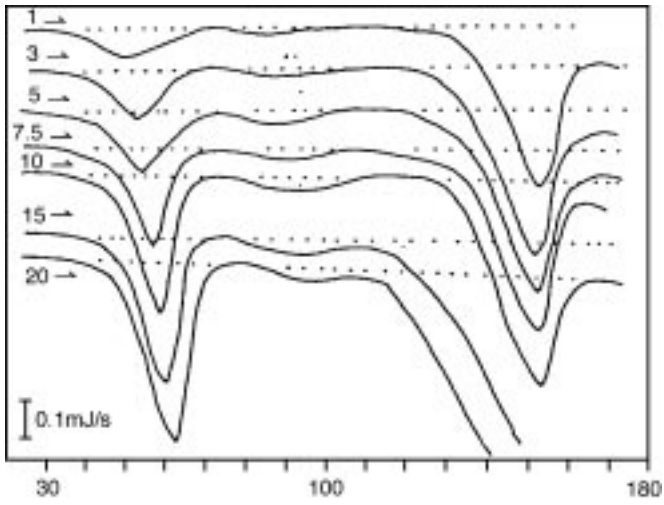

Fig. 20.14 DSC heating curves of curdlan aqueous dispersions at various concentrations. Figures beside each curve represent the curdlan concentration in wt%.[38]

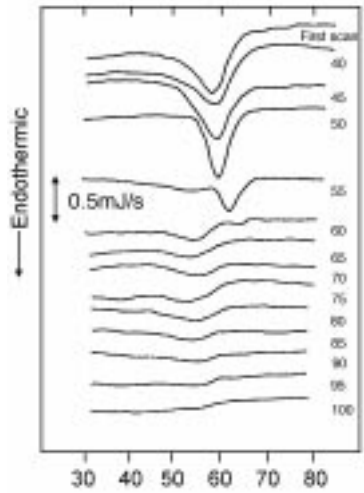

Fig. 20.15 DSC heating curves of 5% aqueous dispersions of curdlan after heating at various temperatures for 60 min. Heating rate: 1 ºC min^{-1}. The number beside each curve represent the temperature in ºC at which the dispersion was kept.[41]

60 ºC showed an endothermic peak much broader than those at lower temperatures below 60 ºC. Figure 20.16 shows the degree of thermo-irreversible gelation dG which is defined as $dG = 1 - \Delta H_{2T}/\Delta H_1$ for a 10% aqueous dispersion of curdlan, where ΔH_1 and ΔH_{2T} are the endothermic enthalpies determined from the endothermic peaks in the first and second run DSC heating curves, respectively. If gels formed by heating are completely thermo-irreversible, no endothermic peak should appear in the second run DSC heating curve. As is seen clearly from Fig. 20.16, the degree of thermo-irreversible gelation increases steeply in the temperature range from 50 to 70 ºC (a partially irreversible gel), and tend to become almost an irreversible gel when heated above 120 ºC. How fast dG increases with increasing heating temperature T

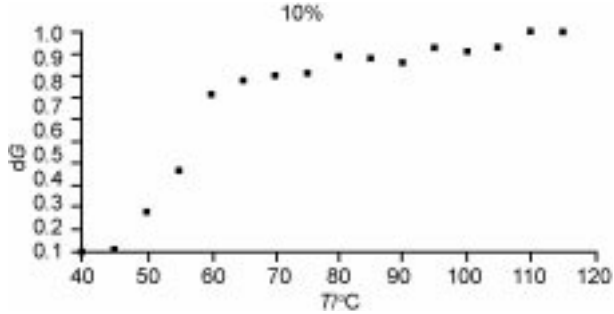

Fig. 20.16 The degree of thermo-irreversible gelation $dG = 1 - \Delta H_{2T}/\Delta H_1$ for a 10% aqueous dispersion of curdlan as a function of heating temperature T, where ΔH_1 and ΔH_{2T} are the endothermic enthalpies determined from the endothermic peaks in the first and second run DSC heating curves, respectively.[41]

should depend not only on the concentration but also on the molecular weight of curdlan, and this should be explored in the future.

Supramolecular structures of curdlan molecules and molecular assemblies have been investigated mainly using electron microscopy,[67–71] transmission electron microscopy (TEM)[68] and atomic force microscopy (AFM).[72,73]

Figure 20.17 shows the morphology[7] of ultrastructure of curdlan obtained after heating a neutralized gel at various temperatures by TEM. Electron

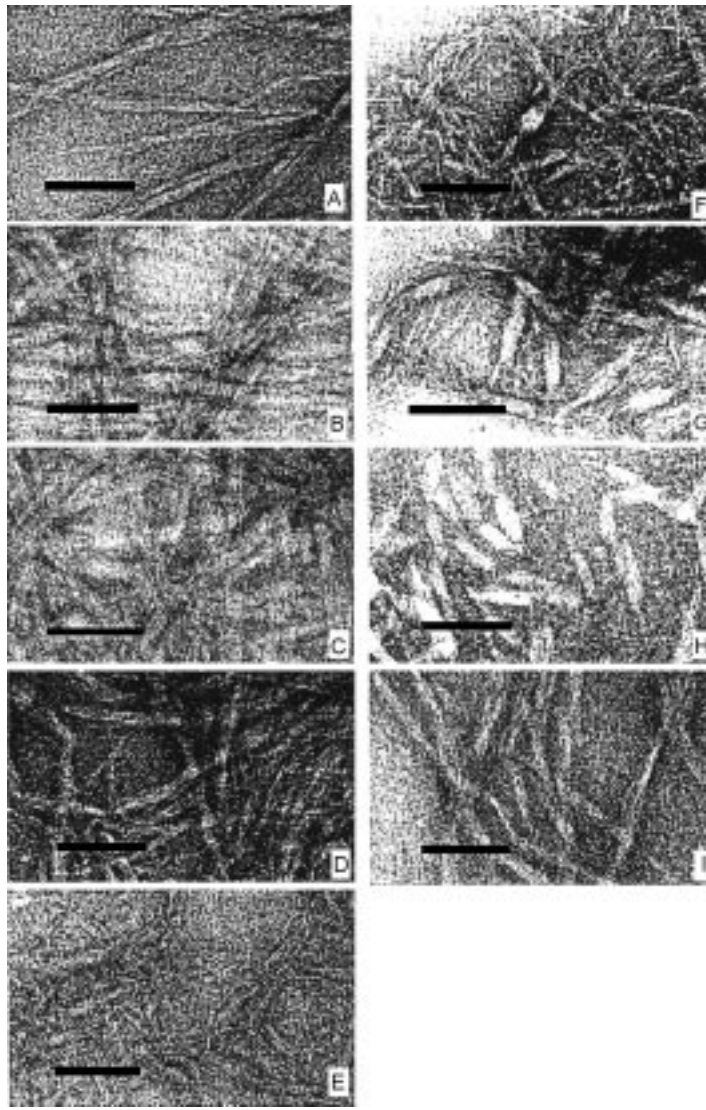

Fig. 20.17 Morphologies of neutralized curdlan gels heated at different temperatures[7] (heating temperature: A, without heating; B, 55 °C; C, 60 °C; D, 70 °C; E, 80 °C; F, 100 °C; G, 120 °C; H, 145 °C; I, 170 °C). Bars represent 0.1 μm.

microscopy displayed microfibrils of endless length consisting of fibril units of about 100 nm length and 10–25 nm width. The fibril units may be formed by several connected subunits which are composed of several molecules of single stranded helix. Microfibrils in non-heating curdlan dispersions are similar to those in the dispesions heated at 55 ºC and 60 ºC. Above 70 ºC hydrophobic interactions between microfibrils occur and microfibrils with released fine stubs were observed. On heating above 120 ºC, triple-stranded helices were formed from single-stranded helices resulting in formation of pseudo-crystalline forms of 100 nm length and 30 nm width.

Flexible single chains of curdlan with an average diameter of 0.65 ± 0.05 nm from dilute DMSO solutions were imaged by AFM.[72] These chains became rigid and readily associated into network structures on mica surface with increasing concentration of curdlan. The heterogeneous supramolecular assemblies of curdlan were also observed on mica surface prepared from dilute NaOH solution.[72,73] The assemblies were composed of microfibrils, the lengths of which were in the order of micrometers and the cross-sectional heights of which were about 2–3 nm.

Though curdlan may be considered as the best polymer to clarify the mechanism of gel formation due to its neutral characteristics, several conflicting gelation mechanisms of curdlan have been proposed. There is still a great deal of confusion concerning the exact structure of curdlan as well as the results of DSC analysis, because curdlan takes various different conformations. It has been suggested that curdlan can exist as a triple helix, single helix, single chain, or a random coil depending mainly on crystallinity of curdlan, heating temperature and type and concentration of solvents used. Obviously, Harada's and Saito's groups prefer that most molecular conformation of curdlan is a single helix, whereas Marchessault's, Atkin's and Stipanovic and Giammatteo's groups hold that triple-stranded helices are predominant. In view of the heterogeneous nature of the curdlan gel, the conflicting views mentioned above might originate from the dissimilarity in the sample measured and its preparation and techniques used to determine the conformation of curdlan chains.

20.6 Applications

20.6.1 Food applications

Curdlan is tasteless, odourless, colourless and possesses excellent gel forming ability and water-retaining properties, and therefore has been widely used in the food industry. Curdlan produces a retortable, freezable food gel, making possible the development of previously impossible food products, such as tofu noodles. It can form a gel even while incorporating large amounts of fats and oils. Curdlan used in noodle doughs reduces the leaching out of soluble ingredients and softening of the noodles, resulting in clearer soup broths. Frozen tofu containing curdlan keeps the smooth texture after thawing whilst when usual tofu is frozen, the texture becomes rough, which has been used as Koori

tofu in traditional Japanese dishes. Adding to surimi products improves the elasticity. In frozen sweet products, it can improve the texture of cakes and the shape retention of ice creams. Funami *et al.*[74–77] studied the effects of addition of curdlan to meat products and found that curdlan can modify the texture and improve the water-holding capacity of processed meat products. Curdlan is also used as an effective main ingredient in fat mimetics for processed meat products.[75] Non-fat sausages are formulated by incorporating a curdlan-based fat mimetic system comprising curdlan and other hydrocolloids to realize the rheological and textural profiles of its full-fat counterpart. Alternatively, low-fat sausages are formulated by replacing animal pork fat with curdlan gels containing vegetable oil because curdlan is capable of incorporating oil within the gel matrix as mentioned above. The coarse-cut sausages thus prepared have a similar appearance and texture to their full-type counterpart, achieving ca. 40% fat reduction and ca. 30% calorie reduction.[78]

As for the applications to confectioneries, curdlan lowers oil uptake and moisture loss of doughnuts during deep-fat frying more effectively than methylcellulose.[79] This relates to the thermal gelation of curdlan, and the heat-induced gel during frying probably serves as an oil and moisture barrier. Jellies can be prepared by flavouring and colouring curdlan gels to taste, which is storable not only refrigerated but also frozen, warmed in a microwave, and served hot.[80] Also, jellies with high palatability can be made by optimizing the heating conditions, forming double-layered structures, where the gelation of curdlan occurs partially at the periphery and the centre part still remains in thickened liquid, exuding in biting.

The applications of curdlan used as a food additive or an ingredient in new procedures for food production[78,81] are summarized in Table 20.5.

20.6.2 Other applications

Hirata *et al.*[82] reported that addition of 3% curdlan into starch is efficient to increase the water resistance of the extrudates which may have a potential use as an environmentally degradable material. Curdlan is effective as an agent in making superworkable concrete to prevent cement and small stones from segregation and also used as an organic binding agent for ceramics and active carbon.[5]

Although curdlan is known to activate innate immune systems of invertebrate[83] as well as mammalian cells[84] as a member of a class of compounds known as biological response modifier (BRM), the insolubility in water limited its practical applications. An alternative solution is to chemically modify it to increase its solubility. It is found that carboxymethylated,[85] sulphated and phosphated curdlan[86] have a good water solubility and also have an enhanced biological activity.[87] More recently, regioselective curdlan derivatives with various functional appendages were successfully synthesized through 'click chemistry',[88,89] which opened up a new avenue to access various curdlan-based advanced functional materials. However, most curdlan derivatives lost their

Table 20.5 Food applications of curdlan[79]

Application	Function	Use level (%)
Noodle	Texture modifier	0.2–1
Kamaboko (boiled fish paste)	Texture modifier	0.2–1
Sausages, hams	Texture modifier, water holding	0.2–1
Processed cooked foods	Binding agent, improvement in moisture retention and product yield	0.2–2
Processed rice cake	Retention of shape	4–6
Cakes	Retention of moisture	0.1–0.3
Ice cream	Retention of shape	0.1–0.3
Jellies	Gelling agent (stable against heating and freezing-thawing)	1.5
Fabricated foods Noodle-shaped tofu Processed tofu (frozen, retorted, freeze-dried) Thin-layered gel food (frozen) Konjac-like gel food (frozen) Heat-resistant cheese food	Gelling agent (stable against heating and freezing-thawing)	1–5
Edible films	Film formation	1–10
Dietetic foods	Low-energy ingredient	30–100

gelling ability at elevated temperature possibly due to the unavailability of hydrogen bonds after modification and the replacement of methylene groups at C-6 position by hydrophilic groups which impede the hydrophobic interactions between curdlan chains above 55 °C.[85] Among various curdlan derivatives, curdlan sulfates are conspicuous for their anti-tumour and anti-HIV activities.[15–18] The bioactivities of curdlan and curdlan derivatives are believed to depend on their chemical structure, molecular mass and conformations. Nevertheless, the relationship between solution conformation and bioactivity of curdlan is not very clear yet. Young *et al.*[90] proposed that the partially opened triple helix was the biologically active form of curdlan inducing inflammatory responses in rats. However, the effectiveness of curdlan to stimulate nuclear factor-κB in mouse macrophages was reported to be dramatically enhanced by the presence of single helix chains produced by pretreatment with dilute sodium hydroxide or dimethyl sulfoxide.[84] Other studies also suggested that the curdlan single helix is more potent than the triple helix as an anti-tumour agent.[91] When curdlan assumes a random-coil conformation or is composed of shorter chains, such anti-tumour activity will disappear.[52] Based on these immunomodulating responses, curdlan was proposed for use as a protective agent for farmed fish.[92] In addition, curdlan was reported to be used to eliminate mycotoxin contamination from the food and the feed chain through the formation of a complex with them at acidic or neutral conditions, for directly protecting animals fed on contaminated feeds, thereby protecting the consumers of edible animal products.[93] It has also been found applications in radical scavenging and inhibition of autoxidation in some emulsion systems[94] as well as decreasing serum and hepatic cholesterol

concentrations in rats fed with a diet containing 5% partially hydrolysed curdlan.[95]

It is noteworthy that curdlan has recently been found to exhibit excellent helix-forming ability with DNA/RNA,[96,97] some conjugate polymers[96] and nanoparticles[97] which can be used as carriers of genes and 1D host in the fabrication of unique nanostructures, therefore, showing potential in the fields of biotechnology and nanotechnology.[98]

As a relatively new object of study, curdlan has exhibited unique gelling properties and molecular conformations. The use of curdlan in foods as well as in other applications will increase, providing new opportunities for novel products.

20.7 Regulatory status

As an inert dietary fibre, curdlan has been approved for food use in Korea, China and Japan. In 1997 Takeda Pharmaceutical Company Ltd, Japan, received approval for curdlan from the Food and Drug Administration of the United States, which is the first direct food additive completely developed and petitioned by a Japanese company to be approved by the US government.

20.8 References

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21

Other microbial polysaccharides: pullulan, scleroglucan, elsinan, levan, alternant, dextran

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Abstract: Other microbial polysaccharides (pullulan, scleroglucan, elsinan, levan, alternan, dextran) deals with the miscellaneous polysaccharides possessing important and potential applications in the food industry. It provides a comprehensive but concise description of all these microbial polysaccharides. The chapter begins with an introductory section which gives a brief overview of the polysaccharides. This section is followed by respective sections for each of the individual polysaccharides. Each of these sections discuss the origin and manufacture, chemical structure, functional properties, technical data, uses and applications and regulatory status of each polysaccharide. The chapter concludes with the future prospects of these polysaccharides.

Key words: pullulan, scleroglucan, elsinan, levan, alternan, dextran.

21.1 Introduction

The major microbial polysaccharides including xanthan gum, curdlan, gellan gum, bacterial cellulose, etc., have been detailed in other chapters of this book. There are several other microbial polysaccharides which possess important and potential applications in the food and pharmaceutical industries. Therefore, this chapter is aimed to provide a comprehensive but concise description of such polysaccharides from microbial origin. These microbial biopolymers will be specifically discussed with reference to their structure, manufacturing, functional properties, applications and current regulatory status. This chapter will

cover microbial biopolymers including pullulan, scleroglucan, elsinan, levan, alternan and dextran.

21.2 Pullulan

21.2.1 Introduction

Pullulan is an extracellular polysaccharide which was first reported from *Aureobasidium pullulans* (syn. *Pullularia pullulans*) (Bender *et al.*, 1959). It is a natural water-soluble polysaccharide produced from starch by fermentation. It is an odorless, flavorless, and highly stable white powder (Khan *et al.*, 2007). The commercially available pullulan (Pullulan PI-20) has more than 90% purity. The major impurities present are mono-, di- and oligosaccharides. The film-forming properties of pullulan make it a suitable substitute for gelatin in the production of capsule shells, tablet coatings, and edible flavored films. It has been used as a food ingredient and as an additive in Japan since 1976 (Joint FAO/WHO Expert Committee on Food Additives, 2005).

21.2.2 Manufacture

Pullulan is produced commercially by a batch-wise cultivation of *A. pullulans* on a medium containing 10–15% of starch hydrolysates (Leathers, 2003). The medium also contains peptone, phosphate, and basal salts. Cultures are stirred and aerated at 30 ºC. The initial pH of the culture is adjusted to 6.5, which is decreased, particularly in the first 24 h, to a final pH value of about 3.5. The maximum culture growth occurs within 75 h while the optimum yields of pullulan are obtained within about 100 h. After completion of fermentation, the fungal cells are removed by microfiltration. The filtrate is sterilized with heat and then treated with activated charcoal for the removal of melanin and other impurities. The decolorized filtrate is cooled and deionized using cation and anion exchange resins. The deionized solution is concentrated to a solid content of about 12% and again treated with activated carbon. Then, it is filtered using diatomaceous earth. The filtrate is concentrated to a solid content of about 30% and dried in a drum dryer. The dried pullulan is pulverized to a desired particle size and finally packed in sterilized polyethylene bags. More than 70% yields of pullulan with respect to initial substrate have been reported. The production and molecular weight of pullulan is dependent on various culture conditions including pH, temperature, substrate, and strain (Leathers, 2003).

21.2.3 Structure

Pullulan is a linear homopolysaccharide, which consists of maltotriose and maltotetraose units with both α-(1→6) and α-(1→4) linkages in regular alternation (Fig. 21.1). It may also contain a small proportion of α-(1→3) linkages depending on the culture conditions and strain differences (Khan *et al.*, 2007).

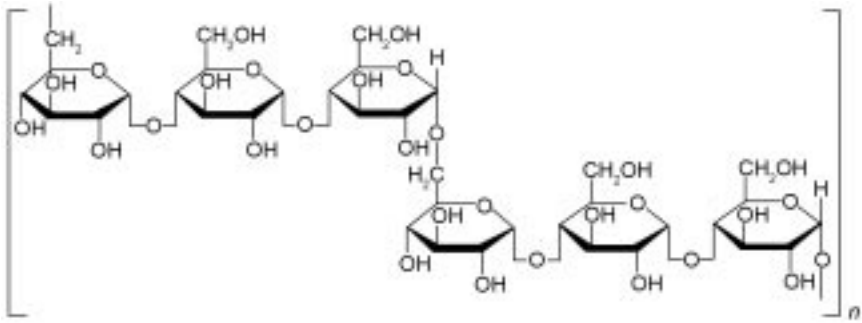

Fig. 21.1 Structure of a segment of pullulan.

The molecular weight of pullulan varies from about 10 to 3000 kDa which depends on the production conditions including duration, pH and phosphate concentration (Joint FAO/WHO Expert Committee on Food Additives, 2005).

Pullulan has close structural similarities to starch amylopectin and maltodextrin which, like pullulan, also consist of glucose units with α-(1→4) and α-(1→6)-glucosidic linkages. The major dissimilarity is the difference in the proportion of these linkages. Maltodextrin contains approximately 20% α-(1→6)-glucosidic bonds while pullulan approximately 30%. Similarly, corn starch contains 95% α-(1→4)-glucosidic bonds and 5% α-(1→6)-glucosidic bonds. Moreover, the tertiary structure of the molecule and the extent and mechanism of degradation of the materials in the human gut is also different in pullulan and these glucans (Joint FAO/WHO Expert Committee on Food Additives, 2005).

21.2.4 Properties

The unique linkage pattern (regular alternation of glycosidic linkages) provides structural flexibility and enhanced solubility characteristics to the pullulan molecule. Pullulan yields high-viscosity solutions at relatively low concentrations. Due to these properties, pullulan possesses distinctive physical traits including adhesive properties, moldability into fibers and capability of formation of strong, oxygen-impermeable films (Khan *et al.*, 2007; Leathers, 2003). Pullulan is a non-toxic, non-mutagenic, odorless, tasteless, edible white colored powder. It is non-hygroscopic and contains less than 6% water. Pullulan is readily soluble in cold or hot water but is insoluble in organic solvents except for in dimethylformamide and dimethylsulfoxide. It gives clear and viscous solution in water. Although, pullulan forms viscous aqueous solutions, it does not form gels (Khan *et al.*, 2007; Leathers, 2003). The viscosity (10% w/w, 30 ºC) of pullulan PI-20 is 132–179 mm^2/s. The viscosity of pullulan solutions is less than other water-soluble polysaccharides, such as guar gum. The viscosity of pullulan solutions is not significantly affected by heating, changes in pH, and most metal ions, including sodium chloride. The viscosity of an aqueous solution of pullulan PI-20 decreases upon incubation with pullulanase

(EC 3.2.1.41). An aqueous solution of pullulan PI-20 (10% w/w) has a pH of 5.0–7.0. Pullulan PI-20 has a number-average molecular weight (*Mn*) of 96,900–101,000 Da and a weight-average molecular weight (M_w) of 433,000–479,000 Da (Khan *et al.*, 2007; Joint FAO/WHO Expert Committee on Food Additives, 2005; Leathers, 2003).

21.2.5 Applications

Pullulan is a unique biopolymer that has numerous uses in the manufacturing, pharmaceuticals, electronics, and food industries (Bender *et al.*, 1959; Khan *et al.*, 2007; Joint FAO/WHO Expert Committee on Food Additives, 2005; Leathers, 2003). Pullulan provides few calories due to its resistance to mammalian amylases and thus can be used as dietary fiber. It also functions as a prebiotic and promotes the growth of beneficial bifidobacteria. It can be used as a substitute for starch in pastas or baked goods (Leathers, 2003).

Pullulan yields relatively low viscosity solutions and thus can be used as a low-viscosity filler in beverages and sauces. Pullulan is used in cosmetics, lotions, and shampoos. Pullulan and its derivatives can be used in wound-healing compositions due to their adhesive properties. Pullulan can also be used as a denture adhesive, a binder and stabilizer in food pastes, and to adhere nuts to cookies due to its adhesive properties (Leathers, 2003).

Pullulan forms transparent, water-soluble, fat-resistant, antistatic films of low oxygen and moisture permeability. The resulting films have excellent mechanical properties. Because of these properties, pullulan is used as a substitute for gelatin in the production of capsule shells for dietary supplements and medicinal products. Pullulan is used as a glazing agent, as a film-forming agent, as a thickener or as a carrier in the production of capsules for dietary supplements (substitute for gelatin), tablet coatings (dietary supplements), for production of edible flavored films (breath fresheners), jams and jellies, confectionery and some meat and fruit products. It is also used as a texturizer in chewing gum and as a foaming agent in milk-based desserts. Pullulan-based edible films can also serve as a matrix to hold flavors. Pullulan films with, for example, menthol dissolve quickly on consumption, releasing the bound flavor and thus acting as an instant breath freshener. As pullulan is edible, the removal of its coating before eating or cooking is not mandatory. Tobacco packing with pullulan film prolongs the shelf-life of the product and also retains its aroma. Moreover, it protects the product from oxidation and mold attack. Water insoluble coatings of pullulan can also be prepared by using its esterified or etherified forms. In Japan, pullulan film is used for decorations of candies and bakery goods. It is also used in beverages. It is applied as a binder for seasoning, as a sheet for wrapping various food items, and as edible packaging material for instant noodles or packages of tabletop sweeteners. Currently in the US, the commercial interests are focused on pullulan film as a flavor substrate for a mouth-refreshing item (Bender *et al.*, 1959; Khan *et al.*, 2007; Joint FAO/WHO Expert Committee on Food Additives, 2005; Leathers, 2003).

Pullulan can also be used as a food additive, providing bulk and texture. It is used as a component of flour, as a texturizer for tofu, ham and sausage, and as a substrate for flavors and as a mean of protecting flavors through microencapsulation. It can be applied as an additive in low-calorie foods and drinks, in place of starch or other fillers due to its resistance to mammalian amylases and thus provides only few calories. In addition, pullulan inhibits fungal growth and has good moisture retention, and thus can be used as a preservative (Khan *et al.*, 2007; Leathers, 2003).

Pullulan and its derivatives have many potential pharmaceutical, clinical, and health care applications. It can be used in pharmaceutical coatings, including sustained-release formulations. Oral care products based on pullulan films have been commercialized. In addition, pullulan and its derivatives have photographic, lithographic, and electronic applications (Leathers, 2003).

Derivatization of pullulan is carried out mostly to reduce its water solubility or to introduce charged or reactive groups for functionality. Esterification or etherification of pullulan is performed in order to reduce its water solubility. Similarly, hydrogenation increases the heat stability, and carboxylation enhances its solubility in cold water. Crosslinked pullulan beads are used in gel permeation chromatography. Cyanoethylated pullulan has potential applications in electronic devices. Pullulan has been sulfated, chlorinated, sulfinethylated, and chloroalkylated. Azidopullulan and siloxane derivatives have been prepared. Cholesterol or fatty acids substituted pullulan are used to stabilize fatty emulsions. Pullulan derivatives are useful as non-toxic conjugates for vaccines and interferon and can facilitate liposome delivery. Pullulan-based hydrogels and nanoparticles may have a variety of uses (Leathers, 2003).

21.2.6 Regulatory status

The Food and Drug Administration (FDA) in the United States is of the view that pullulan is Generally Recognized As Safe (GRAS) (Spears *et al.*, 2005), through scientific procedures, for use in food in general, including meat products, for multiple technical effects.

21.3 Scleroglucan

21.3.1 Introduction

Scleroglucan, an extracellular polysaccharide, was first reported by Halleck from fungus *Sclerotium glucanicum* (Survase *et al.*, 2007). Today, the term scleroglucan is used to designate a class of glucans of similar structure produced by fungi, especially those of the genus Sclerotium. The commercial product is known as Scleroglucan, although, it is also known as actigum, clearogel, polytetran, polytran FS, sclerogum, etc. (Coviello *et al.*, 2005). Scleroglucan is a non-ionic, water-soluble homopolysaccharide consisting of a linear chain of β-D-(1-3)-glucopyranosyl and β-D-(1-6)-glucopyranosyl groups (Survase *et al.*, 2007). Stability at higher temperature and a wide range of pH and resistance to a

variety of electrolytes makes scleroglucan suitable for use in food, pharmaceutical and other industrial applications.

21.3.2 Manufacture

Scleroglucan is synthesized by several species of the genus *Sclerotium*, but *S. glucanicum* and *S. rolfsii* are the most commonly used. The production of scleroglucan depends on the inoculum preparation, growth medium composition, environmental conditions, and byproducts formation. Production is usually higher in media with high carbon to limiting nutrient ratio. Yields up to 60–80% with respect to the utilized carbon source have been reported. Generally, the media contain carbon, oxygen, nitrogen, phosphorus, sulphur, potassium and magnesium. Glucose and sucrose are the most commonly used carbon sources. High nitrogen contents retard while addition of phosphorus, sugar nucleotides and amino acids improves the scleroglucan production. The optimum temperature is 28 ºC while the production is improved with a two-stage pH control, first at 3.5 for optimal growth and then at 4.5 for the maximum production. Limited oxygen supply can limit the growth of *Sclerotium* but increases the production of the biopolymer (Survase *et al.*, 2007).

For the recovery of scleroglucan, the culture broths are neutralized with NaOH or HCl (as required), diluted three- to four-fold with distilled water, heated at 80 ºC for 30 min, homogenized and then centrifuged (10,000 × *g*, 30 min). Scleroglucan is then recovered from the supernatant by three different methods (Survase *et al.*, 2007). In the first method, the supernatant is cooled at 5 ºC followed by precipitation with an equal volume of ethanol (96%) or isopropanol. After incubation of the mixture for 8 h at 5 ºC, the precipitates are recovered with a fine sieve and then re-dissolved in distilled water. This crude product is purified twice by re-precipitation with ethanol (96%) followed by either drying at 55 ºC for 8 h or freeze-drying and milling (Survase *et al.*, 2007; Farina *et al.*, 2001). In the second method, insoluble precipitate of calcium oxalate are obtained by addition of divalent cations preferably calcium chloride (0.5–2.0%). The precipitate is removed by centrifugation or filtration and then re-dissolved in distilled water. The scleroglucan is precipitated from the solution with isopropyl alcohol or ethanol and followed by separation of the precipitate by centrifugation or filtration. Further purification of the product is performed by re-dissolution and re-precipitation (Survase *et al.*, 2007; Johal and Coleman, 1990). In the third method, scleroglucan is recovered by using 0.5–2.0% calcium chloride followed by adjusting the pH of the supernatant to about 10–12. This precipitate is obtained by centrifugation or filtration and which can be further purified by repeated precipitation and varying the pH (Survase *et al.*, 2007; Johal and Coleman, 1990).

21.3.3 Structure

Scleroglucan is a neutral branched homopolysaccharide of glucose. It is composed of a main chain of (1→3)-linked β-D-glucopyranosyl units with a

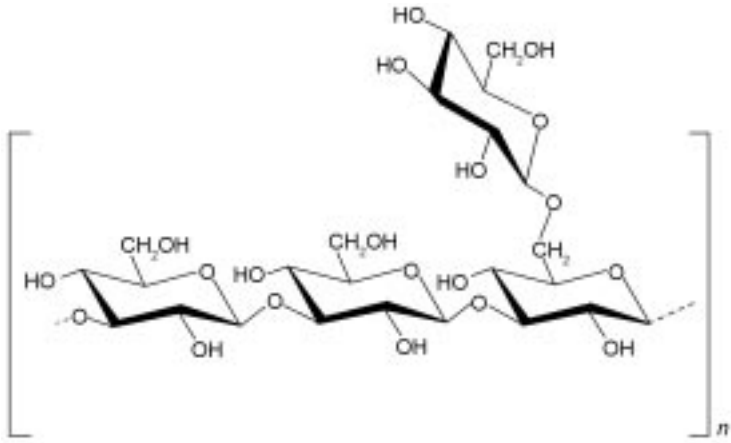

Fig. 21.2 Repeating unit of scleroglucan.

single β-D-glucopyranosyl unit linked (1→6) at every third unit (Survase *et al.*, 2007; Coviello *et al.*, 2005; Wang and McNeil, 1996) (Fig. 21.2).

Scleroglucan forms linear triple helices when dissolved in water, where the side D-glucosidic groups protrude and prevent the helices from coming close to each other and aggregating. The triple helix structure is thermostable, yet monodispersed in a single-chain random coil in dimethyl sulphoxide (DMSO) or at pH over 12.5. Scleroglucan has structure similarities to schizophyllan, produced by *Schizophyllum commune*, although the latter has slightly higher molecular mass than scleroglucan (Survase *et al.*, 2007; Coviello *et al.*, 2005). The molecular mass of scleroglucan varies with the microbial strain with an average ranging from (1.3–3.2) $\times$ 10^5 to (0.3–6.0) $\times$ 10^6 Da (Survase *et al.*, 2007; Wang and McNeil, 1996).

21.3.4 Properties

The properties of scleroglucan may vary with the molecular mass, recovery methods and the extent of purity (Survase *et al.*, 2007). It is readily soluble in water at room temperature. The pure grades of scleroglucan lead to pseudoplastic solutions in hot and cold water while the crude products yield low-viscosity solutions. The viscosity of scleroglucan solutions is slightly influenced by changes in temperature but remains unaffected in the pH range of 1 to 11. The viscosity of 0.5 and 2.0% scleroglucan solutions remains constant between 10 and 90 °C; however, thermoreversible gels are formed at low temperatures, close to 7 °C. The viscosity, specific rotation, and sedimentation coefficient of scleroglucan solutions is reduced in dimethyl sulphoxide, in aqueous solutions of pH 12.5 or higher, or at temperatures above 90 °C (Survase *et al.*, 2007).

Although, scleroglucan is compatible with different electrolytes, gel formation or flocculation of solution may occur at higher electrolyte concentrations.

Moreover, scleroglucan is also compatible with the majority of thickeners such as locust bean gum, alginates, xanthan, and carrageenan and cellulose derivatives (Survase *et al.*, 2007).

Pseudoplasticity can be observed in scleroglucan solutions of 0.2% or lower, but the flow becomes more Newtonian as the concentration decreases below 0.2%. Scleroglucan solutions of lower than 0.8% concentration do not exhibit significantly thixotropic behavior, except when the temperature drops to or below 10 ºC. Gel states are not always clearly defined due to high degree of pseudoplasticity. Solutions of 1.2–1.5% scleroglucan form self-supporting sliceable gels at about 25 ºC, even very diluted solutions form diffusely structured gels at temperatures below 10 ºC that tend to shrink and undergo syneresis when left undisturbed for long periods of time. Such diffused gels disperse quickly with mild agitation (Survase *et al.*, 2007).

Scleroglucan can act as a suspending agent for very fine particles due to its pseudoplastic behavior. The resulting suspensions have good pourability. For example, the purified scleroglucan at 0.1–0.2% is an effective suspending agent for the stabilization of 5–10% aqueous suspensions of fine powders such as zinc oxide, reprecipitated calcium carbonate, and sulphamerazine. Moreover, the viscosity of scleroglucan in combinations with bentonite suspensions has synergistic effects. Scleroglucan results in stable oil-in-water emulsions due to its ability to form very low energy dispersion. This stabilizing effect may be due to the combination of its suspending effect and ability to prevent coalescence (Survase *et al.*, 2007).

21.3.5 Applications

Scleroglucan finds various industrial applications due to its unusual rheological properties and its resistance to hydrolysis, temperature, and electrolytes (Coviello *et al.*, 2005). It is used in food as a thickener, gelling or stabilizing agent. If higher productivity of scleroglucan could be obtained at lower cost then it would replace xanthan in many foods including jams and marmalades, soups, confectionery products and water-based gels, frozen foods, dairy products, low calorie or non-fat products, or in fabricated structural foods. Scleroglucan could also be useful especially in those food formulations which involve a heating process due to its high thermal stability. It can also inhibit syneresis that occurs during refrigeration of cooked starch pastes (Survase *et al.*, 2007).

Scleroglucan is mainly used in the recovery of oil and has a higher stability than xanthan over a wide range of temperatures and pH. It increases the hydraulic pressure of sea water or brine used to extract oil by increasing the viscosity. Furthermore, scleroglucan lubricates the drill and controls the back-pressures produced during drilling (Survase *et al.*, 2007; Coviello *et al.*, 2005).

Scleroglucan is an effective biological response modifier (agent which stimulates immunity and increase resistance to neoplastic and/or microbial disease). It also possesses antitumor and antiviral effect. It also has a higher immune stimulatory, antineoplastic, and antimicrobial activity than any other β-

D-glucan. Scleroglucan can lower the cholesterol levels and increase the excretion of lipids. In the pharmaceutical industry, scleroglucan is used in tablet coatings, ophthalmic solutions, injectable antibiotic suspensions and calamine lotion. Scleroglucan has the advantages of controlled release properties as well as compatibility, biodegradability, and bioadhesiveness and thermal and chemical stability. Carboxylated derivative of scleroglucan is used as a matrix for drug delivery in the form of tablets or films. The native scleroglucan can be used for the preparation of sustained release tablets and ocular formulations. Oxidized and crosslinked scleroglucan can be used as a matrix for dosage forms sensitive to environmental conditions. Co-crosslinked scleroglucan/gellan is also applied for the drug delivery. Furthermore, a novel hydrogel obtained with this polysaccharide and borate ions is used for controlled drug delivery (Survase *et al.*, 2007; Coviello *et al.*, 2005).

Scleroglucan is used in the cosmetic industry in the formulations for hair sprays and in various skin care preparations such as creams, protective lotions, emollients, demulcents and antisoilants. In agriculture, it is used as an antisettling agent for phytosanitary products. It is also used in preparation of spraying mixtures particularly improving the contact of the sprayed droplets onto leaves. It may also be used in pesticides, defoliant sprays and seed coatings. It also has potential applications in porcelain and ceramic glazes, extruded refractory products, integrated circuit chips, water-based paints, printing inks, liquid animal feed concentrates, source of gentiobiose, and as a ceramic binder (Survase *et al.*, 2007; Coviello *et al.*, 2005).

21.3.6 Regulatory status

Beta glucans are Generally Recognized As Safe (GRAS) by the FDA. No significant toxic effects, adverse reactions or sensitization of scleroglucan have been observed in rats and dogs, guinea pigs, rabbits, and humans (Survase *et al.*, 2007).

21.4 Elsinan

21.4.1 Introduction

Elsinan is a novel water-soluble extracellular polysaccharide produced by *Elsinoe leucospila* and other Elsinoe species, i.e., *E. fawcetti* and *E. ampelina*. It is tasteless, odorless, edible and non-toxic, biodegradable and hydrolysable by α-amylase. It has a number of applications including those as various types of films, in the food, pharmaceutical and other industries (Misaki, 2004; Misaki *et al.*, 1980a).

21.4.2 Manufacture

Elsinan is extracellularly produced by *Elsinoe leucospila* and other Elsinoe species. The micro-organisms are grown in a culture medium (solid or liquid

form) that is composed of a suitable carbon and nitrogen source, minerals and other nutrients. Elsinan can be produced in static cultivation and the shaking or submerged cultures with the latter resulting in better yields. Yields of 23–25 g/L have been reported using 5% sucrose medium. One or more sugars like sucrose, glucose, maltose, fructose and starch hydrolysates are the suitable carbon sources for the production of elsinan, while synthetic compounds such as nitrates, ammonium salts, urea and natural organic substances such as polypeptone, corn steep liquor, yeast extract, defatted soybean extract, peptides and amino acids may be used as nitrogen sources. Similarly, the suitable minerals include phosphates, potassium salts, sulfates, and magnesium salts. Fermentations are carried out with an initial pH 5–8, 20–30 ºC and for 3–7 days. The cells and mycelia are removed from the culture broth by filtration or centrifugation while the elsinan is precipitated with methanol, ethanol, and acetone. The precipitated elsinan is recovered with filtration or centrifugation. The obtained crude elsinan may be further purified by re-dissolution in water and re-precipitation with a suitable solvent followed by the recovery and drying of the product (Misaki *et al.*, 1980a; Sugimoto and Yokobayashi, 1981; Misaki, 2004).

21.4.3 Structure

Elsinan is an essentially linear polymer containing (1→4)- and (1→3)-α-D-glucosidic linkages (ratio, 2.6:1). Chemical analysis indicated that elsinan consists of maltotriose and maltotetraose units in the sequence: →3)G(α 1→4)G(α 1→4[4]G)$_{1-2}$ (1→ (Fig. 21.3). It has a five-fold helical structure, as shown by X-ray diffraction study. The molecular weight of elsinan ranges from 5,000 to 10,000,000 Da (Misaki *et al.*, 1980a; Sugimoto and Yokobayashi, 1981; Misaki, 2004).

21.4.4 Properties

Elsinan possesses interesting rheological properties due to its unique structure. The aqueous solution of elsinan exhibits high viscosity. Elsinan is stable over a broad range of pH (3–11), temperature (30–70 ºC), and salt concentrations (Misaki, 2004).

Elsinan is a white powder and is odorless and tasteless. It easily dissolves in water, 0.1 N NaOH, formic acid (90%), formamide, or dimethyl sulfoxide while it is insoluble in methanol, ethanol, acetone, chloroform or ethyl acetate. It turns

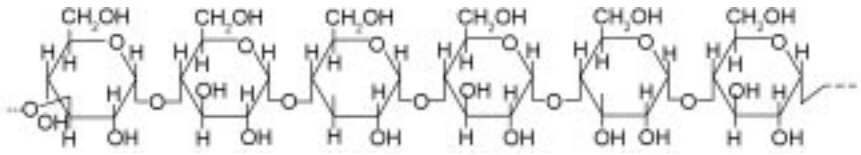

Fig. 21.3 Structure of a segment of elsinan.

green by the anthrone-sulfuric reaction, yellow by the cystein-sulfuric acid reaction, but remains colorless by the Morgan–Elson reaction. Elsinan is negative to iodine stain (Misaki *et al.*, 1980a; Sugimoto and Yokobayashi, 1981).

Elsinan has the ability to form film. Such a film is freely soluble in hot water with a temperature of about 80 ºC and above, while below 40 ºC in water, it only swells maintaining its initial shape. High humidity or wet conditions at room temperature do not lead to blocking of the film. The elongation properties of elsinan films can be easily modified by varying the type and the amount of plasticizer such as water and glycerin. The film can also be prepared by casting and winding. As the film does not retrograde, the desirable initial properties, transparency, flexibility, and folding characteristics of the film, can be maintained for a long period.

Elsinan film can be easily and firmly heat-sealed below 20%w/w. The elsinan film is extremely tough and flexible and is highly resistant to fats, oils, oily foods and oil soluble vitamin and also has high gas impermeability. The film is non-conductive and electric chargeable. It is glossy, colorless, transparent, tasteless, odorless, edible and non-toxic and is hydrolysable by α-amylase. The film is a non-environmental pollutant and is biodegradable (Misaki *et al.*, 1980a; Sugimoto and Yokobayashi, 1981).

The average molecular weight of elsinan can be easily adjusted in the range of about 5,000 to 10,000,000 Da because it can be produced by either chemical or biochemical procedure and can be hydrolyzed with hydrochloric acid, sulfuric acid, etc. Elsinan can be easily formed into various shaped bodies such as granule, pellet, filament, fiber, thread, stick, rod, rope, net, cloth, gauze, film, sheet, paper, tube, capsule, tablet, sponge, laminate and coating (Sugimoto and Yokobayashi, 1981).

21.4.5 Applications

Elsinan has a number of industrial applications including those of film formation. It can be used as a pharmaceutical ingredient. It exhibits remarkable dietary fiber effect and thus reduces serum cholesterol level in hypercholesterolemic conditions. Elsinan also possesses antitumor activities (Misaki *et al.*, 1980a; Sugimoto and Yokobayashi, 1981; Shirasugi and Misaki, 1992; Misaki, 2004).

As mentioned above, elsinan can easily be shaped into various body shapes. These shaped bodies are moisture and water resistant, hot water soluble, transparent, non-toxic, edible, and can be stored for a long time without losing their desirable properties. Such shaped bodies can be used for various purposes including food, clothing, house-building and many industrial applications as well as agriculture, forestry, fisheries, and stock raising, and also as industrial materials for chemicals, cosmetics and pharmaceutical products. The film has extremely low oxygen or air permeability and thus can be used as an oxidation barrier for products that are easily oxidizable by atmospheric oxygen, for example, perishable foods, oily foods, processed foods, pharmaceuticals such as

vitamin, enzyme, hormone and biomedicals, and other materials such as botanical seeds and metals, when it is used as a film to seal, package or wrap the products, or to apply a coating thereon. The film or coating is highly effective in retaining the original flavors of the contents because volatilization of flavor ingredients can be prevented. Moreover, the film is non-conductive and electric chargeable and thus can be used as an insulator for electric machinery and tools. The film is glossy, colorless, transparent, tasteless, odorless, edible and non-toxic and, if necessary, it can be freely colored, made opaque, seasoned, and flavored. The film is hydrolyzable by α-amylase and thus decomposed readily by α-amylase in the digestive tract. Thus it can be used for orally administered medicines to display their efficacy at the proper time and site and for the required period in digestive tract by sealing, tableting or encapsulating the medicines in elsinan-shaped bodies of adequate shape, structure or composition (Sugimoto and Yokobayashi, 1981).

21.4.6 Regulatory status

Although elsinan has no clear approval as a food additive in the USA or in Europe, several patents have been granted related to its use in food and pharmaceutical products.

21.5 Levan

21.5.1 Introduction

Levan is a non-toxic, biologically active, extracellular polysaccharide that can be produced by both plants and micro-organisms. It is a sugar polymer composed of fructose with 2,6-linkages (Melo *et al.*, 2007). Microbial levans are produced by a variety of microorganisms including *Bacillus subtilis*, *Bacillus polymyxa*, *Aerobacter levanicum*, *Streptococcus sp.*, *Pseudomonas sp.*, and *Corynebacterium laevaniformans*. Levans have potential industrial applications as thickeners, encapsulating agents, for medical uses and as a substitute for petrochemicals in some applications (Hangit and Clarke, 1990; Han, 1990, Combie, 2006).

21.5.2 Manufacture

Microbial levans are produced from sucrose-based substrates by different micro-organisms including *B. subtilis*, *B. polymyxa*, *Aerobacter levanicum*, *Streptococcus sp.*, *Pseudomonas sp.*, and *Corynebacterium laevaniformans* (Hangit and Clarke, 1990). In the case of production of levan by *B. polymyxa* (NRRL B-18475) (Hangit and Clarke, 1990), the organism is grown on a medium composed of sucrose, peptone, yeast extract, K_2HPO_4, $(NH_4)_2SO_4$, and $MgSO_4$. Using lactose, maltose, and raffinose as the carbon source can result in the production of a small amount of levan. However, no levan can be produced from

glucose or fructose. Moreover, sugarcane juice without addition of any other ingredients can also be used for the production of levan, but the yield is lower compared to the basal medium. A minimum of 10 days cultivation time is required for a maximum yield. The pH of the medium falls from the initial pH 7.0 to 4.7 due to acid production during cultivation. Optimum temperature for growth of organism and production of levan production is about 30 ºC. Yield of about 50% with respect to the available fructose has been reported after 10 days of cultivation. The polysaccharide production is improved by gentle shaking of the culture broth during the cultivation period, however, vigorous agitation and aeration hinders efficient production of levan. For the recovery of levan, the bacterial cells are removed by centrifugation of the culture broth. Levan is harvested by precipitation from the supernatant by addition of 1.5 volumes of ethanol or isopropanol. The final product is a brownish white, gummy material that could be freeze-dried or vacuum-dried. It can be further purified by dialysis or ultrafiltration of a second precipitation with ethanol (Han and Clarke, 1990).

21.5.3 Structure

Levan is a naturally occurring fructan, the homopolymer of fructose. Its main chain is composed of repeating five-member fructofuranosyl rings connected by β-(2→6) links (Fig. 21.4). The main chain is branched through β-(2→1) linkage of the fructofuranosyl rings. The molecular weight and degree of branching of levans differ with the producer organisms. Moreover, the molecular weights of bacterial levans are much higher than the plant levans due to the multiple branching. The molecular weights of bacterial levans range from 2 to 100 million Da while plant levans have molecular weights ranging from about 2000 to 33,000 Da (Arvidson *et al.*, 2006; Li *et al.*, 2006; Barone and Medynets, 2007; Rairakhwada *et al.*, 2007).

21.5.4 Properties

Levan is an unusual polysaccharide that does not swell in water and has an extremely low intrinsic viscosity (Combie, 2006; Arvidson *et al.*, 2006). Its intrinsic viscosity is quite low compared to other molecules of similar molecular

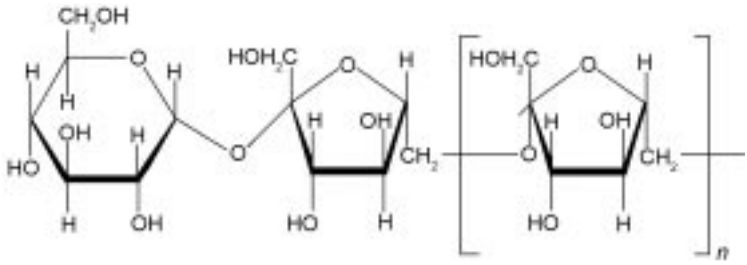

Fig. 21.4 Chemical structure of levan.

weight range. For example, the intrinsic viscosities of most commercial polysaccharides such as cellulose, carrageenan, xanthan, and guar gum range from 5 to 50 dL/g, while flexible chain polysaccharides such as dextrose and compact coil polysaccharides such as amylose have intrinsic viscosities of about 1 dL/g. On the other hand, the intrinsic viscosities for levans in water range from 0.07 to 0.18 dL/g with molecular weights ranging from 16 to 24 million Da. The intrinsic viscosities of levans are comparable to those of spherical and dendritic polymers which have intrinsic viscosities in the range of 0.05–0.10 dL/g (Arvidson *et al.*, 2006).

Levan has an ellipsoidal molecular shape which indicates a radial extension of the branches and formed at almost the same rate (Arvidson *et al.*, 2006). Levan in combination with 20–30 wt% glycerol form cohesive and flexible films. However, films with less than 10 wt% glycerol are too brittle while films obtained with greater than 30 wt% glycerol are not cohesive and are 'liquid'-like. Efficient extrusion of levan/glycerol blends requires at least 35% glycerol. Levan can be thermally processed through traditional molding and extrusion techniques (Barone and Medynets, 2007). The levan produced by *B. licheniformis* is non-viscous and water insoluble. Even at a concentration of 18 g/L, the viscosity of the culture broth is similar to that of water. It forms a non-transparent suspension which deflects visible light where the concentration can be correlated with turbidity (Ghaly *et al.*, 2007).

Levan undergoes partial hydrolysis by treating with 1 N HCl at 70 ºC for 1 h. It can produce films of diverse characteristics in combination with various agents like cloisite Na^+, sorbital, PEG, glycerol, montmorillonite, laponite, clay, etc. The films prepared with levan, PEG and montmorillonite has oxygen transmission under 0.05 cc/m^2-day and water vapor transmission 123 g/m^2 day. Because of the extremely low intrinsic viscosity, levan requires less energy to handle. Levan is released naturally from producing cells which makes it a cost-effective raw material. The intrinsic viscosities for levan are significantly higher in dimethyl sulfoxide than in water, reflecting the higher hydrodynamic volume in the former (Bahary *et al.*, 1975). Levan may have an important role during early stages of infection and may mask and protect the pathogen in the host tissue (Li *et al.*, 2006).

21.5.5 Applications

There are a variety of potential applications for levan. It may be used as an emulsifying, stabilizing, or sweetening agent in foods. It is used in edible food coatings. It also finds applications in medicine as a blood plasma extender and as an encapsulating agent for pharmaceuticals. The polymers of various levan derivatives can be used in medicine and food processing. In addition, levan acetate and levan crosslinked with epichlorohydrin can be used to form thin plastic films. Levan can be used as an adhesive. Films of levan can be produced by blending it with ethylcellulose (Barone and Medynets, 2007). Levans are possible substitutes for dextrans in cases where low viscosity, high water

solubility and susceptibility to acid hydrolysis is required. Levan polymer can be used as a plugging agent to plug the pores of high permeability soils (Ghaly *et al.*, 2007).

In pharmaceutical and biomedical fields, levan is used as a blood plasma extender, hypocholestrolemic agent, cell membrane (tumor cells) modifier, immunomodulatory agent and tablet binder (Rairakhwada *et al.*, 2007). It has antitumoral effect while high molecular weight levan is an immunomodulator, affecting macrophages as well as humoral and cell-mediated immunity (Leibovici, 1984). As a strong adhesive and a water soluble film former, levan has the potential to make a temporary coating or bandage (Combie, 2006). Levans have potential industrial applications as thickeners and encapsulating agents (Han, 1990).

It has potential applications as a cosmoceutical ingredient. Levan has moisturizing effects, cell proliferation effect in human fibroblast and keratinocyte cell lines. Levan possess an anti-inflammatory effect against inflammatory reactions to skin irritants, and also it has a cell-proliferative effect in bioartificial skin (Kim *et al.*, 2005). Levan is a raw material to replace petrochemicals in certain personal care products and in the products of many other industries. Levan can be derivatized to make powerful surfactants.

21.6 Alternan

21.6.1 Introduction

The polysaccharide alternan was first described by Jeanes *et al.* in 1954 (Jeanes *et al.*, 1954; Leathers *et al.*, 2006) as one of two extracellular α-D-glucans, referred to as fraction S, produced by *Leuconostoc mesenteroides* NRRL B-1355. The structure of this fraction was later determined by Misaki *et al.* in 1980 (Misaki *et al.*, 1980b). The name 'alternan' was used for this polysaccharide by Cote and Robyt in 1982 (Cote and Robyt, 1982). The enzyme known as alternansucrase was found to be responsible for the synthesis of this polysaccharide. Alternan has potential applications as a low viscosity bulking agent and extender in foods and cosmetics (Monsan *et al.*, 2001).

21.6.2 Manufacture

Alternan is produced from sucrose by rare strains of the bacterium *Leuconostoc mesenteroides*. The glucose units of this biopolymer are derived from the hydrolysis of sucrose in a reaction catalyzed by the enzyme alternansucrase. Three *Leuc. mesenteroides* strains are known to produce alternansucrase: *Leuc. mesenteroides* NRRL B-1355, NRRL B-1501 and NRRL B-1498. Jeanes *et al.* (1954) were the first to report the production of two different glucansucrases by *Leuc. mesenteroides* NRRL B-1355. The native polymer can be produced *in vivo* by the intact bacterial culture or *in vitro* with cell-free supernatant containing alternansucrase (Leathers *et al.*, 1996; Wyckoff *et al.*, 1996; Monsan *et al.*, 2001).

Fermentative production of a product having a high proportion of alternan to dextran can be accomplished by growing the producer organism in a suitable nutrient medium containing sucrose as a carbon source. It also contains assimilable sources of nitrogen and inorganic salts. Components such as liver, beef and yeast extract, and peptone are usually required as nitrogen source. The need for these complex components as a nitrogen source is the main limiting factor in commercial production of this biopolymer. The fermentation for the production of alternan is generally carried out under aerobic conditions or static culture at 25–30 ºC for 2–3 days. The polysaccharide can be obtained from the fermentation broth by removal of bacterial cells by centrifugation or filtration. Alternan is recovered from the resulting supernatant by precipitation with ethanol, methanol or isopropanol. The crude product is further purified by dissolving it in water and re-precipitating it with ethanol or other suitable solvent. Finally, the product is dried by spray drying, oven drying or lyophilization (Leathers *et al.*, 1996; Wyckoff *et al.*, 1996; Monsan *et al.*, 2001).

Alternan can also be produced enzymatically from sucrose by using alternansucrase from a suitable strain. For this purpose, the enzyme preparation is added to a sucrose solution and incubated until alternan is formed. Alternan is recovered by precipitation with a suitable organic solvent as mentioned above. The product can be further purified and finally dried as discussed above (Leathers *et al.*, 1996; Wyckoff *et al.*, 1996; Monsan *et al.*, 2001).

21.6.3 Structure

Alternan is a branched homopolymer which has a unique structure with alternating with α-(1→3) and α-(1→6)-linked D-glucose residues and present for 46% and 54%, respectively. Alternan is approximately 7–11% branched through 3,6-di-substituted D-glucosyl residues (Fig. 21.5). This structure imparts properties of high solubility and low viscosity as well as resistance to most known microbial and mammalian enzymes. Native alternan has been estimated to have an apparent weight average molecular weight of 10^6–10^7 Da (Leathers *et al.*, 1996, 2003).

21.6.4 Properties

Because of its unusual structure, alternan and its derivatives have properties of high solubility and low viscosity. The polysaccharide alternan is resistant to most known microbial and mammalian enzymes (Leathers *et al.*, 2003). It is resistant to hydrolysis by known endoglucanases and is a poor substrate for most exoglucanases (Leathers *et al.*, 1997). The only enzymes that can hydrolyze alternan to any significant extent are isomaltodextranases and alternase. Its resistance to microbial and mammalian enzymes makes it suitable for the production of ingredients for functional foods such as prebiotics. It has unique properties that resemble certain functional characteristics of gum arabic, maltodextrins or polydextrose. Alternan solutions are extremely stable at 4–37 ºC and

Fig. 21.5 Structure of a portion of alternan.

at pH 3–9. Dry preparations of alternan are bright white, tasteless powders that are not highly hygroscopic (Leathers *et al.*, 2003). Likewise, they should be better at resisting mold and bacterial growth. Alternan is a stable, dry white powder, producing clear, colorless solutions. While alternan does lack emulsifying properties similar to gum arabic, researchers are working to find a way to mix alternans with emulsifiers to keep food components from separating (Meinhold, 1992).

21.6.5 Applications

Alternan has potential commercial applications in foods, cosmetics, and other industrial fields due to its unusual structure and properties including high solubility and low viscosity. It can be applied as a low-viscosity bulking agent and extender in food and cosmetics. It has potential value as a non-caloric, carbohydrate-based soluble food additive in artificially sweetened foods. Its resistance to microbial and mammalian enzymes makes it suitable for the

production of ingredients for functional foods such as prebiotics. In addition, fructose is a by-product of the enzymatic synthesis of alternan. The hydrolysis of native alternan polysaccharide with isolates of Penicillium bacterial strains leads to the production of potential replacers of commercial gum arabic (Leathers *et al.*, 1997).

Alternan could also possibly replace polydextrose, maltodextrins, and gum arabic, for use as soluble fibers, binders and bulking agents in foods and as components of inks, adhesives, cosmetic creams, and ointments (Cote, 1992).

21.6.6 Regulatory status

L. mesenteroides is a GRAS micro-organism and known to be non-pathogenic. All the strains are widely used for the preparation of various common food or food ingredients and have a safe history for use in the EU (European Commission, 2000; Colar, 1996).

21.7 Dextran

21.7.1 Introduction

The name dextran was first used by Scheibler in 1874. He observed that a carbohydrate having an empirical formula ($C_6H_{10}O_6$) and a positive optical rotation caused the thickening of cane and beet sugar juices. This sugar was formed by *Leuconostoc mesenteroides* as the fermentation product in sugar cane or beet syrups. Today, the term 'dextran' collectively refers to a class of glucans that are produced extracellularly by members of the genus *Lactobacillus*, *Leuconostoc*, and *Streptococcus*. Dextran is a linear polysaccharide of D-glycopyranose containing α-(1→4) and α-(1→6) linkages. It is highly soluble in water and has various applications in the food, packaging, film and pharmaceutical industries (Naessens *et al.*, 2005; Khan *et al.*, 2007).

21.7.2 Manufacture

The commercial production of dextran is mainly carried out by growing cultures of *L. mesenteroides* in media containing sucrose, an organic source of nitrogen such as peptone, growth factors, certain trace minerals, and phosphate. The fermentations are carried out anaerobically because *L. mesenteroides* is facultatively anaerobic or microaerophilic. The other fermentation conditions include an initial pH 6.7–7.2, temperature 25 ºC, initial sucrose concentration 2% and culture time 24–48 h. The pH decreases to ~5.0 during the first 20 h of fermentation due to the formation of organic acids. The branching of dextran increases at higher temperatures. Dextran is harvested from the fermentation medium by alcohol precipitation and purified by repeated precipitation after re-dissolution in water. Cell debris is removed by centrifugation (Naessens *et al.*, 2005).

Dextran can also be produced enzymatically using cell-free culture supernatants that contain dextransucrase. This allows dextran synthesis under controlled conditions and yields a purer polymer. Conditions in the enzyme method are more constant and easier to control than in the whole-culture method. Similarly, the product is more uniform and easier to purify. The enzyme-producing fermentations are optimized by improving the different culture characteristics. The optimal pH for enzyme production is 6.5–7.0, whereas the optimal pH for enzyme activity is 5.0–5.2. Fermentations are carried out at 23 ºC. Control of the sucrose level in the fermentation broth around 0.5–1.0% leads to higher yields of dextransucrase. Enzymatic synthesis is advantageous due to product molecular weight and quality control, and also obtaining fructose as a co-product. However, this method is not routinely used for commercial production of dextran probably for economic reasons (Naessens *et al.*, 2005).

21.7.3 Structure

Dextrans are the homologous polymers of glucose polymerized predominantly (50–97% of the total linkages) in α-1→6 linkage. The presence of a smaller proportion of α-1→2, α-1→3, or α-1→4 linkages results in a highly branched molecule (Fig. 21.6). However, the exact structure of dextran differs with microbial strain and thus depends on the specific type of dextransucrase(s) involved. Dextrans are synthesized outside the cell by dextransucrase, which catalyzes sucrose to produce D-fructose and D-glucose, and transfers the latter to an acceptor to form dextran. Lower molecular weight dextrans have less branching and narrower range of molecular weight distribution, while those with molecular weight greater than 10,000 Da are highly branched. Dextran molecules attain greater symmetry as the molecular weight increases; those with molecular weight lower than 2,000 Da are more rod-like, while those from 2,000 to 10,000 Da exhibit the properties of expandable coils (Naessens *et al.*, 2005; Khan *et al.*, 2007).

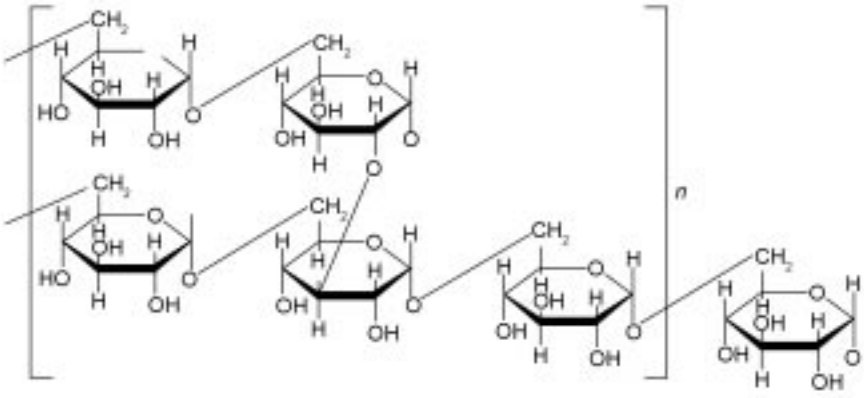

Fig. 21.6 Chemical structure of dextran.

21.7.4 Properties

The properties of microbially produced dextran vary greatly due to their structural differences. They have different molecular weight, degree of branching, relative quantity of particular type of glycosidic links, solubility, viscosity, specific rotation, physiological action and content of nitrogen, phosphorus, and ash. The molecular weight of native dextran ranges between 10^7 to 10^8 Da which can be decreased by acid hydrolysis irrespective of the nature of acid used. Some dextrans are water-soluble while others are insoluble but still most have greater than 30 mg/ml solubility in water. They are soluble in formamide and dimethylsulfoxide and insoluble in alcohol and acetone. Native dextran also possesses a high degree of polydispersibility. The terminal reducing end groups of dextrans can be oxidized in alkaline solutions. Generally, dextran has high solubility characteristics and promotes low solution viscosities. The intrinsic viscosity is affected by the nature and pH of solvent, degree of branching, number of intermolecular bonds and temperature. It (M_w = 500,000) exhibits Newtonian behavior at concentrations <30% w/w. However, higher molecular weight dextran solutions possess slight pseudoplasticity at concentrations >1.5% w/w. The optical rotation of aqueous solutions of various dextrans varies from +199º to +235º (Naessens *et al.*, 2005; Khan *et al.*, 2007).

21.7.5 Applications

Dextrans, partially degraded dextrans and their derivatives have various important commercial applications. Initially, dextrans (relatively low molecular weight) were commercially produced for use as a therapeutic agent in restoring blood volume for mass casualties. Dextran can retain ~20 ml of water/g in the circulation. The molecular weight of dextran used as blood-plasma expander (also called clinical dextran) ranges from 40,000 to 100,000 Da. Dextran produced by *L. mesenteroides* B-512F has low antigenicity, high water solubility and high biological stability in the human blood stream and thus is the material of choice for clinical use as a suitable blood-plasma substitute. Dextrans of higher molecular weights and/or with greater proportions of non-α(1,6)-linkages can cause allergic reactions which is the limiting factor in the routine use of clinical dextran as a blood-plasma substitute (Naessens *et al.*, 2005). Moreover, dextrans also find applications in microsurgery for its antithrombotic effect (Jallali 2003). Parenteral iron dextran has been used for the rapid treatment of iron deficiency anaemia. Moreover, special iron dextran preparations have also been developed for the improvement of magnetic resonance imaging techniques (Naessens *et al.*, 2005). The sulfate ester of dextran has anticoagulant properties and is a potent ribonuclease inhibitor. It has antiviral activity especially against the human immunodeficiency virus (HIV) and has been used to treat arteriosclerosis. Mercaptodextran has potential to be used in acute heavy metal poisoning and in environmental cleanup of such metals. The incorporation of dextrans into X-ray and other photographic emulsions can improve their effects. Dextrans are used in several ophthalmic and skin care and other cosmetics formulations (Naessens *et al.*, 2005).

Dextrans have great potential for applications in various food products as conditioners, stabilizers, bodying agents or related uses. They are used in confectionery for improvement of moisture retention, viscosity, and inhibition of sugar crystallization. In gum and jelly candies they are used as gelling agents. In ice cream they are applied as crystallization inhibitors, while in pudding mixes they provide the desirable body and mouthfeel (Whistler and Daniel, 1990; Day and Kim, 1993; Jallali, 2003; Khan *et al.*, 2007). Different ester and ether derivatives of dextran furnish macromolecules with diverse properties and negative, positive, or neutral charges. The most widely used dextran derivative is known as Sephadex® (the name is derived from SEparation PHArmacia DEXtran) which was commercialized in 1959 by Pharmacia Fine Chemicals (Uppsala, Sweden). It is a gel and can be obtained by reacting an alkaline solution of dextran with epichlorohydrin. Sephadex® is used as a molecular sieve which brought revolution to the purification and separation technology of various biological macromolecules such as proteins, nucleic acids, and polysaccharides. Dextrans are also an essential component of various Sephadex derivatives (Jallali, 2003). Dextrans have many other potential applications such as emulsifying and thickening agents, high-viscosity gums, explosives, deflocculants in paper products, secondary recovery of petroleum, oil drilling muds, soil conditioners and surgical sutures (Whistler and Daniel, 1990).

21.7.6 Regulatory status

Dextrans have no clear approval as a food additive in the USA or in Europe, but *L. mesenteroides* is a GRAS-status organism and is present in many fermented foods. Moreover, patents have been granted related to the use of dextran preparations in baked products (Jallali, 2003).

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22

Cereal β-glucans

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Abstract: Mixed linked (1→3), (1→4) β-D-glucans are hydrocolloid polysaccharides found in cereals, but are particularly found in higher concentrations in oats and barley. This chapter will focus on recent advances in cereal β-glucan research. It reviews research on the important nutritional and health benefits of cereal β-glucans together with the challenges faced by agricultural refineries and food manufacturers in producing highly concentrated β-glucan ingredients that then need to be formulated into commonly consumed foods without altering the sensory characteristics. The chapter discusses the range of innovative developments in methods for producing β-glucan-enriched ingredients and their potential application in many food products.

Key words: mixed linked (1→3), (1→4) β-D-glucans, cereal β-glucan-enriched ingredients, oats, barley, β-glucans.

22.1 Introduction

Cereals are an economically important group of grass crops that are cultivated in greater quantities and provide more energy worldwide than any other crop. While maize, rice and wheat are by far the dominant three cereals grown globally, other cereal grains contain unique metabolites of economical and nutritional benefit, such as beta-glucans (β-glucans). Mixed linked (1→3),

Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable. All programmes and services of the US Department of Agriculture are offered on a non-discriminatory basis without regard to race, colour, national origin, religion, sex, age, marital status, or handicap.

(1→4) β-D-glucans are hydrocolloid polysaccharides found in cereals, but are particularly found in higher concentrations in oats and barley. This chapter will focus primarily on advancements made in cereal β-glucan research over the last decade.

There are many human health benefits attributed to a diet rich in β-glucans from oats or barley. Nutritionists are increasingly stressing diets of Westernised consumers consist of inadequate fibre consumption. Consumers have been educated that a high-fibre diet is important for good health and demand for fibre-rich foods is steadily increasing. Because the human digestive system cannot digest the mixed-linkage of cereal β-glucans, there is strong interest from food manufacturers to incorporate oats, barley or isolated, highly-concentrated β-glucan ingredients derived from these grains. Cereal β-glucans have been implemented in lowering blood cholesterol levels,[1,2] reduced coronary heart disease,[3] reduced diabetic symptoms,[4–6] lowered blood pressure,[7] cancer prevention[8] and improved gastric emptying and nutrient absorption.[9] The Food and Drug Administration (FDA) recognised the importance of oat soluble fibre, generally called β-glucan, from oat flour and bran by allowing a health claim on food labels.[10] Incidence of coronary heart disease, diabetes, cancer, high blood pressure and obesity are all on the rise in industrialised nations, which has necessitated a great upsurge of research on the health benefits of cereal β-glucans, which will be discussed in this chapter.

In order for consumers to receive health benefit from cereal β-glucans, agricultural refineries and food manufacturers face challenges of producing highly concentrated β-glucan ingredients that then need to be formulated into commonly consumed foods without altering the sensory characteristics. There have been many innovative approaches to producing β-glucan-enriched ingredients, and their potential application in many food products has been explored, and both will be discussed in this chapter.

Advancements in molecular biology have allowed scientists to gain greater understanding of how β-glucans are biosynthesised in plants. This is important, as β-glucan structure influences its functionality, so therefore genetically engineering cereal β-glucans could increase yield, expand the potential applications and thereby improve overall economics of β-glucan products. Yeast and fungal β-glucans differ in solubility to cereal β-glucans due to a (1→3), (1→6)-β-D-glucan molecular structure that is capable of enhancing immune system function in humans.[11] Genetic modification of cereal β-glucan structure may expand its potential human health benefits. Additionally, with an uncertain future about consumer acceptability of genetically modified food products, plant breeding can also play an important role in producing cereal β-glucans with higher yields, novel structure and broader functionality. Selection of cereal lines with greater β-glucan yields has already been achieved[12] and is further improving.

22.2 Botanical distribution

β-glucans are predominant cell-wall components of cereal grains, particularly oats and barley.[13–15] However β-glucans can also be found at lower levels in other cereals such as spring-wheat, winter-wheat, sorghum, rye, rice, millet and spelt.[16–20] β-glucan levels in cereal grains usually range from 2 to 6% (dry weight), but can vary considerably.[21-27] Waxy barley varieties, that contain 100% of starch molecules in the form of amylopectin, have been reported to have grain with higher β-glucan content.[28,29] Mutant barley lines have shown that there is high β-glucan content in lines that have low starch content, in which alleles produce β-glucan to compensate for low starch.[30] Heat stress during grain fill growth stage can reduce β-glucan content.[31] High rainfall or irrigation also will reduce β-glucan content of cereal grains.[32,33] Conflicting findings have been reported on whether oat grain protein content is related to β-glucan content.[34,35]

22.3 Structure and analysis

The primary structure of mixed-linkage cereal β-glucans is a linear chain of glucopyranosyl monomers linked by a mixture of single β-(1→3) linkages and consecutive β-(1→4) linkages (Fig. 22.1).[18,36,37] Oligosaccharides obtained by action of (1→3), (1→4)-β-D-glucan-4-glucanohydrolase, commonly known as lichenase, on (1→3), (1→4)-β-D-glucan from cereal grains can be characterised using methylation analysis. Using this analysis for oat β-glucans, composition was predominantly β-(1→3)-linked cellotriosyl (3-*O*-β-cellobiosyl-D-glucose, DP3) and cellotetraosyl (3-*O*-cellotriosyl-D-glucose, DP4) units with a small number of regions containing 4–8 consecutive (1→4)-linked units.[38] Endo-1→4-β-glucanases and one non-cellulolytic β-glucanase isolated from *Trichoderma reesei* were found to specifically cleave cellotetraosyl units and higher homologues, allowing for isolating cellotriosyl units that can be characterised by lichenase hydrolysis.[39] Structural features of lichenase-hydrolysed products can also be confirmed using ^{13}C-NMR.[38]

Lichenase hydrolysis of cereal β-glucans enables determination of percentage cellotriosyl and cellotetraosyl units in entire β-glucan molecule (Fig. 22.2). A typical chromatogram from lichenase hydrolysis of cereal β-glucan, showing predominance of DP3 and DP4 is shown in Fig. 22.3. Water-soluble and -insoluble β-glucans from oat and barley were studied by digesting with lichenase and analysing oligosaccharides using HPAEC-PAD. This analysis

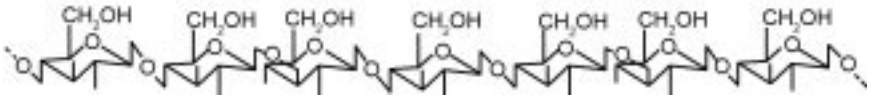

Fig. 22.1 General structure of cereal β-glucans. Frequency of (1→4) and (1→3) linkages will vary.[49]

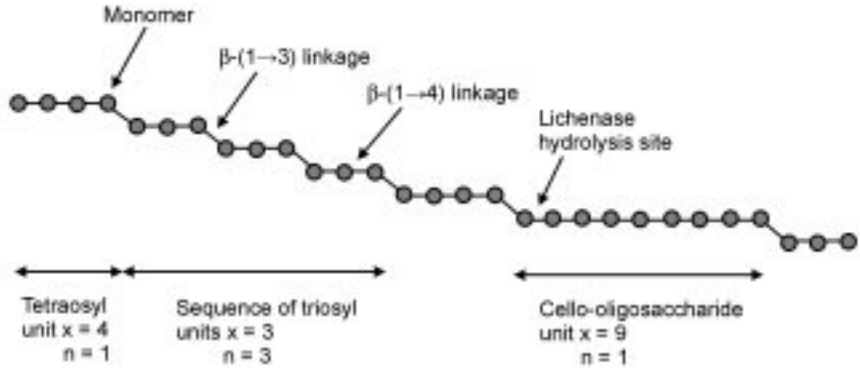

Fig. 22.2 Representation of general cereal β-glucan linear chain, showing variation in sequence of triosyl units, the infrequent cello-oligosaccharide length, and potential sites of lichenase hydrolysis.[212,216]

revealed that the trisaccharide and tetrasaccharide cellulosic units in oat β-glucan accounted for 95% of molecule.[40] In other studies, cellulosic oligomers released by lichenase showed cellotriosyl and cellotetraosyl units accounted for 91–92% for barley and 92–94% for oats for one study[23] and in another study barley β-glucans hydrolysed by lichenase had 93% of residues as tri- or tetrasaccharide cellulosic units.[41]

HPLC can be used to determine the molar ratio of tri- to tetrasaccharide (DP3:DP4) in cereal β-glucans. This molar ratio varies, but is typically about 2.1

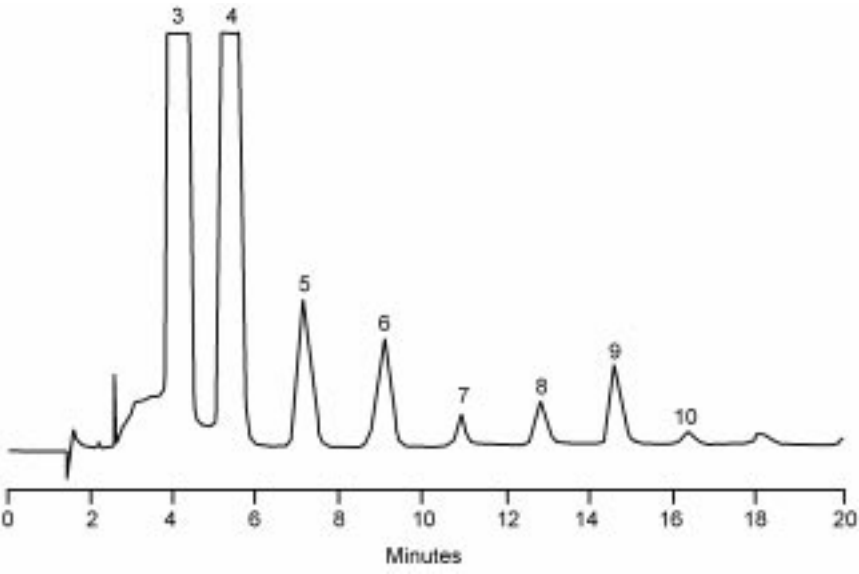

Fig. 22.3 High-performance anion-exchange chromatograms of oligosaccharides released by lichenase from barley β-glucan.[29]

for oats, 3.2 for barley and 3.5 for wheat,[42] and the ratio always is higher for barley than oats.[18,37,43] HPAEC-assisted lichenase treatment was used to characterise barley β-glucan structure, and molar ratio of 1→4 to 1→3 linkages for barley varieties ranged from 2.26 to 2.35.[44] In other studies the DP3:DP4 ratio ranged from 2.73 to 3.05 for barley and 2.16 to 2.42 for oat[23] varieties, another study reported barley β-glucan has a molar ratio of 2.19,[41] and sorghum β-glucan has a 1→4 to 1→3 glucosidic linkage ratio of 2.3:2 measured using GC-MS and ^{13}C-NMR.[17] Hull-less barley DP3:DP4 ratio was 1.5 to 1.8, substantially lower than reported for barley with hulls.[45]

Molar mass of β-glucans can be determined by high-performance size-exclusion chromatography (HPSEC) with the β-glucan detection method used resulting in considerable variation in molecular weight (Fig. 22.4), partly because β-glucanase activity interferes with molecular characterisation of β-glucans. HPSEC analysis equipped with MALLS and RI detectors can be successfully utilised to determine β-glucan molecular weight providing samples are prepared by extraction of β-glucans at pH 9.0 at 100 ºC. Higher temperatures (140 ºC) reduce extraction and molecular weight of β-glucans, while autoclaving, ethanol and calcium chloride treatments reduce β-glucanase activity, resulting in higher molecular weight.[46] β-glucan molecular weight varies greatly with oats ($0.065–3 \times 10^6$) typically higher than barley ($0.15–2.5 \times 10^6$) or wheat ($0.25–0.7 \times 10^6$).[24,38,45,47–49] Molecular weight is important in influencing β-glucan solubility as 500,000 g/mol molecular weight has been reported for soluble β-glucans of both oat and barley and < 200,000 g/mol for insoluble β-glucans.[50] A strong linear relationship has been well demonstrated between β-glucan molecular weight and apparent viscosity (Fig. 22.5). While

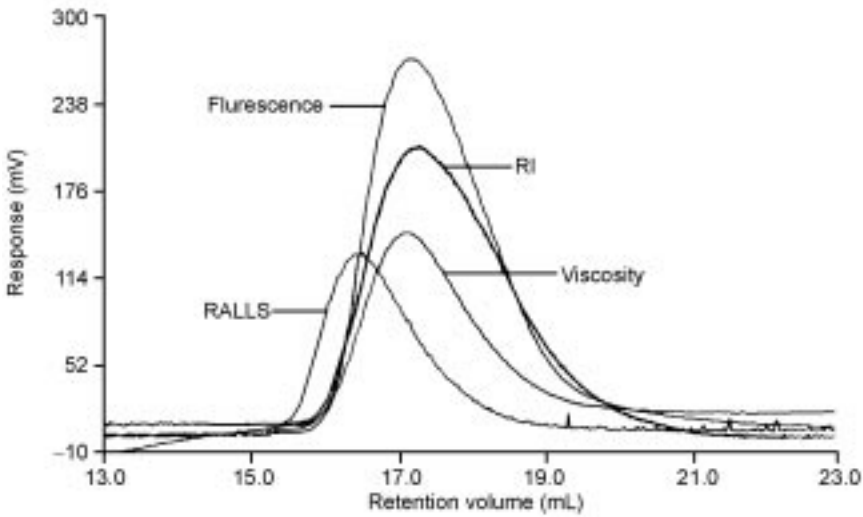

Fig. 22.4 High-performance size-exclusion chromatogram of oat β-glucan standard using refractive index (RI), viscosity, right-angle laser-light scattering (RALLS) and fluorescence detectors.[47]

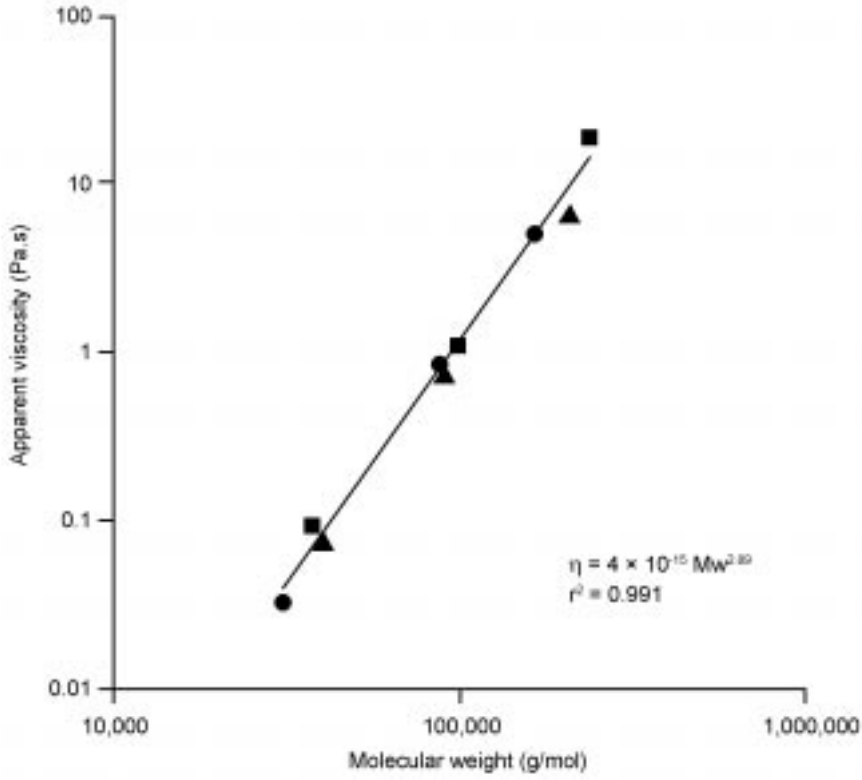

Fig. 22.5 Apparent viscosity of 6% oat β-glucan partial hydrolysate solutions at 10/s. ● acid hydrolysed, ▲ cellulose hydrolysed, and ■ lichenase hydrolysed.[216]

MALDI-MS cannot be used to determine large molecular weight of unmodified β-glucans, it can successfully characterise the structure of lichenase-hydrolysed products.[51]

Quantification of β-glucan content in cereal grains is routinely measured using commercially available diagnostic kit from Megazyme International (Ireland). A collaborative study involving eight laboratories streamlined a β-glucan quantification assay for oats and barley in which ground flour samples are cooked to hydrate and disperse β-glucans, which are subsequently hydrolysed to soluble fragments by lichenase. After volume and pH adjustments, and filtration, β-glucosidase is added to a portion of the solution to hydrolyse β-gluco-oligosaccharides to D-glucose, and the monosaccharide is measured using glucose oxidase-peroxidase (GOPOD) reagent. The remaining portion of lichenase-hydrolysed solution is treated directly with GOPOD reagent to measure free D-glucose in test sample.[52] For higher accuracy measurements, murine monoclonal antibodies have been developed that strongly react to β-glucans, but not other polysaccharides, and an ELISA method has been established that can detect nanogram levels of soluble β-glucan extracted from cereal flour.[53]

Depolymerisation kinetics of oat β-glucan by (1→4)-β-D-glucan 4-glucanohydrolase (lichenase) suggest faster hydrolysis at sites comprising longer sequences of consecutive (1→4)-linkages, and the presence of a relatively higher proportion of (1→3)-linkages restricts affinity of enzyme. Hydrolysis products after extensive degradation were glucose, cellobiose, laminaribiose, 4-*O*-β-laminaribiosyl D-glucose, 4-*O*-β-laminaribiosyl D-cellobiose and 3-*O*-β-cellobiosyl D-cellobiose, which indicates other enzyme activities are present.[54]

22.4 Biosynthesis

The biosynthesis of β-glucans in cereal grains is not well understood. The reason grasses synthesise β-glucans remains unknown. The ability for rapid accumulation and hydrolysis of β-glucans may impart a greater flexible architecture enabling rapid response to physiological cues involving initiation of cell-wall extension during growth, especially during germination. There are few studies that attempted to investigate the stages during grain development in which β-glucan biosynthesis occurs. Barley endosperm accumulates β-glucans during early developmental stages.[55] Barley and corn grain cellulose microfibrils are coated with β-glucans and may act as structural elements of walls in growing cells at early development stages.[56,57] β-glucans are the main cell-wall component of wheat endosperm at early grain development stages, whereas arabinoxylans accumulate at the beginning of cell differentiation.[58,59] In mature wheat, β-glucans are not present as a major cell-wall component.[60]

Only recently has there been considerable advancement in the understanding of the genes and enzymes associated with β-glucan biosynthesis. Synthases of β-glucans and other polymers containing (1→4)-β-linked glucosyl, mannosyl and xylosyl units have to overcome a substrate orientation problem in catalysis because the (1→4)-β-linkage requires that each sugar unit is inverted nearly 180º with respect to adjacent monomers. β-glucan synthase, located in the Golgi apparatus, requires Mg^{2+} or Mn^{2+}, high external pH in Golgi lumen, and has high affinity for uridine diphosphate (UDP)-glucose. A mechanism with two modes of glycosyl transfer within a single catalytic subunit that generates disaccharide units has been proposed, but this mechanism does not work for β-glucans that have cellotriosyl and cellotetraosyl units linked by (1→3)-β-linkages. A three-mode model of glycosyl transfer is proposed to link predominantly cellotriosyl and cellotetraosyl units together.[61,62]

In 1996, *CesA* gene encoding cellulose synthase catalytic subunit was discovered.[63] Deduced amino acid sequences of *CesA* share regions of similarity with bacterial *CesA* genes, but there are two plant-specific regions.[63] The function of plant *CesA* gene in cellulose synthesis was confirmed by cloning *Arabidopsis AtCesA* gene in mutants that restored synthesis of cellulose microfibrils.[64] Numerous *CesA*-like genes (*CSL*), each lacking several key sequences of *CesA*, have been reported, with eight distinct classes found.[65–67] However, two CSL classes encode polypeptides unique to grasses[67] and it is

possible that one of these classes could encode a β-glucan synthase. Two sites or modes of glycosyl transfer residing with a single catalytic unit is proposed where disaccharide units are added iteratively.[62,68–72] Trisaccharide units of β-glucans can be formed if modification of an ancestral cellulose synthase complex occurs allowing a third mode of glucosyl transfer in addition to cellobiose generating activity.[69]

22.5 Extraction and purification

Solubility, extractability and yield of oat β-glucan are influenced by particle size, pre-treatment of cereal material, and extraction conditions such as temperature, pH, solvents and ionic strength.[73] The most simplified procedure for enriching β-glucan content is dry milling of cereals. Several methods have been reported on dry milling with sieving or air classification to enrich β-glucan content that set the platform for present day commercial production.[38,74–76] Increased β-glucan content can be achieved during dry milling of oats when a greater yield of bran is obtained, a higher proportion of broken oat groats are milled, or groats milled are harder.[77] Roller-milling or impact-milling followed by sieving of oat groats successfully retains larger particles that enriched β-glucan content by 1.7-fold. Tempering oat groats at 12% moisture for 20 min before roller-milling further improves bran yield by two-fold. Pearl-milling only isolates outer groat layers, resulting in low β-glucan yields as the majority of β-glucan is located in the exterior endosperm region.[78] Impact-milled and air-classified barley flour has enriched β-glucan content in the coarse and largest-sized fine particles, however, β-glucan extractability decreases with increasing particle size probably because lower endogenous β-glucanase activity and poorer availability of substrate in larger particles.[79]

Distribution of β-glucan within cereal grains varies depending on variety and therefore the corresponding milling fractions rich in β-glucan may differ. Pearled fractions of hull-less barley showed β-glucan content was very low in the outermost 20% of kernel. For hull-less barley, low β-glucan-containing cultivars had β-glucans predominantly present in the subaleurone kernel region, whereas greater than 80% of β-glucans were distributed evenly throughout endosperm for high β-glucan-containing cultivars. Growing conditions, location and season all had significant effects on β-glucan content of hull-less barley cultivars, but did not affect β-glucan distribution within the kernel.[25]

There are many enzymatic methods developed to produce functional oat β-glucan-enriched products. Among the earliest was Oatrim, developed and patented for oats[80] and other cereals[81] by the US Department of Agriculture (USDA). Oatrim production involves thermostable α-amylase at 95 ºC for 10–60 min, converting gelatinised starch from oat flour or bran to maltooligosaccharides. After enzymatic hydrolysis, α-amylase is inactivated by passing the mixture through a jet cooker at 140 ºC. Soluble fibre (β-glucan) and maltooligosaccharides, recovered by centrifugation, is called hydrolysed oat flour, or Oatrim.

Several enzymatic procedures combined with solvent extraction have been developed since the production of Oatrim to enrich oat fibre components. Enrichment of β-glucan has been developed where milled oat flour has β-glucananse and pentosanase enzymes inactivated.[82] Inactivated oat flour is formulated with water and heated to 60–85 ºC to extract β-glucan. Polar organic solvents are used to extract lipids, and precipitate soluble fibre which is dried and later hydrated with insufficient amount of water to allow starch gelatinisation to occur. Before heating, proteolytic enzymes are added, resulting in enriched β-glucan material due to protein degradation, although proteolytic enzymes have been shown to lower both molecular weight and viscosity of barley and oat β-glucans.[49,83-89] Trypsin is the protease enzyme typically used because it lacks β-glucanase activity and is relatively affordable compared with other proteases.[82] In another recent method, ground barley is boiled in 70% ethanol, washed in 96% ethanol, extracted in hexane and then hot-water treated with thermostable α-amylase, producing a highly concentrated β-glucan solution.[90]

Enrichment of cereal β-glucans can also be achieved utilising acidic[91] or alkaline[92] conditions in combination with elevated temperature, dry-milling, enzymes and solvents. Using a slightly alkaline solution of pH 7.0 or 8.0 at 55 ºC, 87% of β-glucan is extracted from barley flour, with 89% purity. β-glucan extraction is more effective at 55 ºC than at 40 ºC, but increasing pH has no effect.[93] Subsequent studies found highest yields of barley β-glucan are achieved with boiling at pH 7 for non-waxy barley and refluxing at pH 8 with α-amylase for waxy barley (79–81% yield). Barley β-glucan extracted without subsequent heat-treatment or prior refluxing has a high level of protein and starch impurities.[94]

Increased β-glucan solubilisation is achieved with increasing water temperature from 20 ºC to 80 ºC. Untreated and enzyme-deactivated oat bran concentrate can be extracted with aqueous sodium carbonate at pH 10 at 40 ºC, and oat β-glucan is subsequently isolated by dialysis, ultrafiltration or alcoholic precipitation. All methods produce an oat product with 60–65% β-glucan; however, viscosity and molecular weights vary depending on processing conditions used. Dialysis results in highly viscous β-glucan solution, but lower yields, whereas ultrafiltration and alcoholic precipitation extract higher yields of β-glucans exhibiting lower viscosity. Enzyme-deactivated oat bran concentrate extracts give oat products with higher β-glucan content and solution viscosity but yields are lower.[95]

Other components of cereal grains can restrict β-glucan extraction, and enzymatic degradation of these components can facilitate β-glucan enrichment. Dehusking barley using sulphuric acid substantially decreases extractable β-glucan amount, indicating enzymes capable of solubilising β-glucans are present. Enzymes that promote solubilisation of β-glucans in barley are endo-β-(1→3), (1→4)-glucanase, endo-xylanases, arabinofuranosidase, xyloacetyl-transferase and feruloyl esterase. Arabinoxylan in cell walls is most likely ester-linked to ferulic acid and acetyl groups, rather than β-glucan. Therefore, the ability of esterases, xylanases and arabinofuranosidase to solublise β-glucan indicates cell wall pentosan units restrict β-glucan extraction.[96]

22.6 Health benefits

Cereal β-glucans have long been implicated in promoting many health benefits. The best known health benefit that the majority of consumers are aware of is the cholesterol-lowering effect of cereals, especially oats, due to β-glucans. There have been extensive studies demonstrating the ability of cereal β-glucans to lower cholesterol levels in humans or laboratory animals, including a large number of studies in the last decade (Fig. 22.6).[97–108] In one study, adults (156) with low-density lipoprotein (LDL) cholesterol levels above 180 mg/dL or between 130 to 180 mg/dL with multiple risk factors were randomised into seven groups, with six groups receiving various doses of oatmeal or oat-bran, and one group receiving farina as a β-glucan control. After 6 weeks, all six groups fed oat products had significantly lower total- and LDL-cholesterol compared with farina control. Fifty-six grams of oat-bran resulted in significantly greater reductions in LDL-cholesterol than same the amount of oatmeal in diets. Nutrient analysis showed no differences in fat among treatment groups and therefore higher β-glucan content most likely accounts for observed differences.[98]

However, some studies have found that the cholesterol-lowering effects of cereal β-glucans are reduced when the hydrocolloid is incorporated into foods. In a randomised, controlled, crossover diet study investigating effects of oat β-glucan in bread, cookies and orange juice, only the beverage decreased LDL-cholesterol in 48 subjects. This suggests that the food matrix, food processing, or their combination, could have adverse effects on the hypocholesterolaemic function of oat β-glucans.[102] A recent study involving 47 subjects found adding 5 g of oat β-glucan into fruit drinks did lower serum LDL-cholesterol levels.[101] To further complicate the role of β-glucans in cholesterol-lowering, beverages enriched with 5 or 10 g of β-glucan from barley or oats were fed to 89 subjects. Compared with controls, 5 g of oat β-glucan significantly lowered total cholesterol and postprandial glucose and insulin, but no reduction in serum lipids was observed for subjects consuming 10 g of oat β-glucan. Barley β-glucan had no significant effect on cholesterol, glucose or insulin levels.[109] Rats fed diets containing barley β-glucan or cellulose found the former diet had significantly lower plasma LDL-cholesterol and higher faecal fat excretion, suggesting impairment of intestinal fat absorption.[103] Further studies involving 16 subjects fed a control or diet containing 5 g of oat β-glucan daily, found serum cholesterol and LDL-cholesterol were reduced by 6% and 9%, respectively, for the latter diet.[104] The cholesterol-lowering mechanism of oat β-glucan is due to increased synthesis of bile acids as subjects fed oat bran showed increase in serum 7 α-hydroxy-4-cholesten-3-one within 8 h of consuming a meal.[110] Cholesterol-fed rat studies have shown barley β-glucan lowers serum cholesterol by up-regulating cholesterol 7 α-hydroxylase.[111]

Hypocholesterolaemic effects of cereal β-glucans may also be limited for non-hypercholesterolaemic individuals as a study of 18 mildly hyperlipidemic men fed a highly-enriched barley β-glucan diet in a randomised controlled

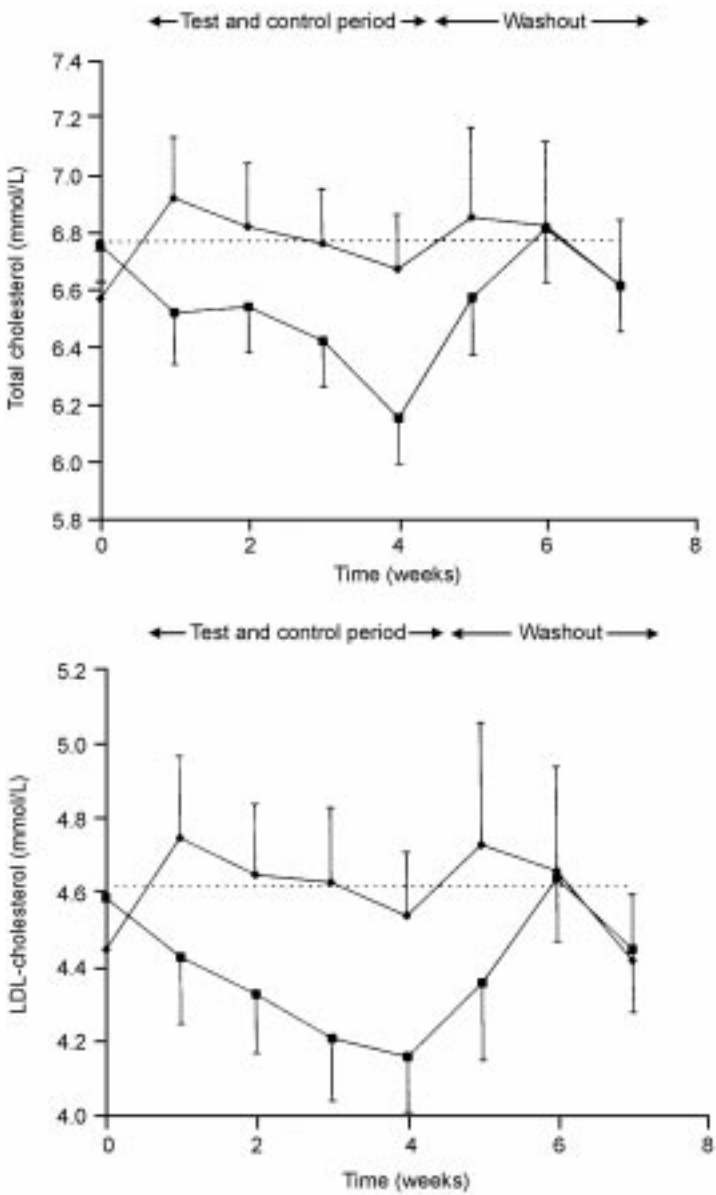

Fig. 22.6 Changes in serum total and LDL-cholesterol during a 4-week crossover trial and washout in hypercholesterolaemic subjects consuming 5.8 g β-glucan per day. Weeks 4–7 represent washout between phases, or a washout at the study end when β-glucan was fed last. ◆ maltodextrin control diet; ■ oat β-glucan isolate diet. The dashed line is baseline, mean of pre-treatment values and control values, including washout following placebo.[99]

crossover study found no change in LDL and HDL cholesterol, triacylglycerol levels or fasting and postprandial glucose among the diet treatments, revealing that effect of barley β-glucan on lipid profile was highly variable among subjects.[105] In contrast to these findings, barley β-glucan incorporated at levels of 0.4, 3 or 6 g into the diet of 18 moderately hypercholesterolaemic men, found total- and LDL-cholesterol decreased significantly by 14–24%, depending on the β-glucan level in the diet.[106] Nutritional studies predominantly indicate that cereal β-glucans can provide physiological health benefits to a wide spectrum of demographics, irregardless of gender, ethnicity or age.[107,112]

Cereal β-glucans also have the ability to alleviate diabetes by decreasing the glycaemic index of foods. Despite previous findings where beverages but not bread or cookies reduced blood cholesterol levels,[102] a recent study found breakfast barley β-glucan supplementation (using Cerogen™ containing 6.3 g β-glucan per meal) effectively improved glycaemic response when incorporated into food, but no beneficial effect was observed for beverages.[113] Twelve type-2 diabetic patients were fed random-order diets containing oat-bran flour, oat-bran crisp or glucose, where oat-bran flour, but not oat-bran crisp, lowered glucose response 0 to 120 min after start of meal.[114] Type-2 diabetic patients have also been given a control diet recommended by the American Diabetic Association (ADA) or a low-calorie diet containing oat β-glucans as a fat replacer, and sweetened with sucralose and fructose. After patients walked for 1 h, all patients had reduced body weight and body mass index (BMI), lipid profile and basal glucose but the oat-based diet had greater increase in beneficial HDL-cholesterol and larger decreases in haemoglobin, body weight and BMI.[6] Carbohydrate digestion is reduced in humans consuming a diet enriched with barley β-glucan.[115] Sixteen type-2 diabetes patients consumed diets consisting of white bread, a commercial oat-bran cereal and a prototype breakfast cereal or bar containing twice the β-glucan content of oat-bran cereal. Glycaemic indices of patients consuming prototype β-glucan-enriched cereal products were significantly lower than commercial oat-bran cereal.[116] A low glycaemic index diet containing 3 g of oat β-glucan consumed by type-2 diabetic patients significantly lowered fasting plasma cholesterol, but not fasting glucose compared with a high glycaemic index diet.[117] High prevalence of low-nocturnal blood glucose levels is a major problem for children with diabetes. A bedtime β-glucan-enriched snack was consumed by 38 children, with blood glucose curve flattened before midnight but could not prevent low glucose levels at 2 a.m.[118] Beneficial low glycaemic response of oat β-glucan has also been demonstrated for non-insulin dependent diabetic men.[5] Ten subjects consuming oat-bran products had lowered glycaemic index compared with controls, with higher β-glucan-containing foods resulting in lower blood peak glucose levels.[119]

Metabolic control of diabetes by cereal β-glucans is most likely due to their innate high viscosity absorption delay of nutrients.[120] To maintain health benefit, food manufacturers must select the right β-glucan concentration in the raw material that will have sufficient solubility and resistance to mechanical and enzymatic degradation during processing.[121] A recent study feeding 22

volunteers oat breads found no significant difference in blood concentrations of lipids, glucose or insulin between breads with high- or low-molecular weight β-glucans.[122]

Oat β-glucans have been shown to delay gastric emptying, diminish nutrient absorption, affect motility in the small bowel and prolong postprandial satiety. In the large bowel, β-glucans increase fermentation, especially short-chain fatty acids such as butyric acid that enhance growth and colonisation of probiotic bacteria that may help prevent colon cancer.[9] A recent study investigated hypercholesterolaemic effects of oat β-glucan and compared the fermentation products of the cereal hydrocolloid with inulin and guar gum. Seventy-five hypercholesterolaemic subjects had significant reduction in LDL-cholesterol levels in blood after consuming an oat β-glucan diet compared with control. Oat β-glucan fermentation products were short-chain fatty acids and acetate, at largely similar concentrations to inulin or guar gum, but oat β-glucan produced butyrate at higher concentrations.[123] Rats fed on oat β-glucan diet have also been shown to have reduced serum lipids, higher counts of beneficial bifidobacteria and lower counts of harmful coliforms, and short-chain fatty acid fermentation products were detected.[124]

Chemical modification of cereal β-glucans can improve the physiological action of their health benefits. Amination of oat β-glucans, thereby producing a cationic derivative, has stronger *in vitro* bile acid binding activity, enhanced antimicrobial action against *Escherichia coli* and *Bacillus subtilis*, inhibition of angiotensin-converting enzyme (which is important in high blood pressure, heart failure, diabetic nephropathy and type-2 diabetes) and improved lung function compared with native β-glucan.[125]

Oat β-glucan can also alleviate blood pressure. A study involving 97 patients with resting systolic (SBP) and/or diastolic blood pressure (DBP) consumed foods containing oat β-glucan for 12 weeks. Compared with a control diet, patients consuming an oat β-glucan diet had lower insulin levels, but overall blood pressure response was not altered. However, in patients with BMI above median (31.5 kg m^2), both systolic and diastolic blood pressure were lowered.[126] In another study, 18 hypertensive and hyperinsulinaemic patients consumed a control or oat cereal diet, with the latter resulting in 7.5 mm Hg reduction in SBP and 5.5 mm Hg reduction in DBP, whereas the control diet had no change.[127]

Some new medical advances are finding applications for cereal β-glucans in preventing cancer. Mice with disseminated lymphoma were treated with rituximab, a chimeric monoclonal antibody that treats B-cell non-Hodgkin's lymphoma, or a combination of rituximab and barley β-glucan. Mice survival was greater and lymphoma growth suppressed with the combination, compared with either rituximab or β-glucan alone.[128]

Finally, some miscellaneous nutritional information; there is some suggestion that oat-based products may have negative effects on mineral absorption, due to high phytate content in grain,[129] so phytate levels in oat β-glucan-enriched ingredients needs to be considered when developing nutritionally beneficial foods. Although almost all focus on cereal β-glucans is on hydrocolloids

isolated from the grain, even non-grain plant tissues of cereal crops can contain nutritionally beneficial β-glucans. Hypoglycaemic effect in rats has been found from leaf blades, sheaths, stems and young leaves of the largely unknown African grass, *Rhynchelytrum repens*.[130]

22.7 Commercial products

Many methods of β-glucan enrichment proved impractical for commercial manufacturing processes because of high costs and/or low yields. Oatrim was one of the earliest commercial oat β-glucan products to overcome these challenges and was developed and patented by the US Department of Agriculture (USDA) in the early 1990s.[80,81] Oatrim is currently marketed as Beta Trim® and distributed by Skidmore Sales and Distributing Inc., West Chester, OH.

Oatrim is an excellent fat replacer because β-glucan and maltooligosaccharide combination produces a fat-like texture,[131] has one calorie per gram compared with nine for fat,[132] and mimics the appearance and taste of traditional higher-fat foods. A 25% Oatrim gel is heat-stable for baking and pasteurising operations, substituting shortening on equal weight basis.

Oatrim powder or gel can be used in food applications such as meats, dairy products, frozen desserts, salad dressings, mayonnaise, sauces, soups, margarine, baked foods and beverages.[133–141] Oatrim's blood cholesterol lowering properties were found in chick[133] and hamster[142] studies, and also lowers glycaemic index in humans.[6] Clinical studies found 5 g of oat β-glucan from Oatrim, consumed daily, gave a wide variety of health benefits including hypocholesterolaemic effect of lowering total blood cholesterol, weight control and decrease in urine oxidation products.[1,143]

The rheological properties of Oatrim suspensions have been characterised.[141,144,145] Depending upon β-glucan content, Oatrim suspensions can exhibit shear-thinning, or shear-thickening behaviours.[145] High-sugar ratio cake batters formulated with Oatrim result in high shear-thinning at low shear rates and fewer bubbles that have uniform diameter.

There have been many innovative methods developed to produce cereal β-glucan-enriched products. The USDA has improved on Oatrim, developing β-glucan-enriched products, Nutrim-OB, which has about 12–13% β-glucan, and C-trim which ranges from 20–50% β-glucan content. Nutrim-OB is prepared by heat-shearing oat flour or bran aqueous slurry in a series of treatments that solubilises oat β-glucans but maintains basic bran composition. Slurry viscosity is reduced by >90%, rendering it flowable through sieve pores (40–400 μm) or allowing separation of insoluble crude fibre particles by centrifugation and subsequent drum-drying.[146] Nutrim-OB is currently manufactured and marketed by Van Drunen Farms, Momence, IL.

Nutrim-OB can substitute for fat, imparting moistness, softness and cohesiveness to baked foods,[147] cheese[148,149] and Asian noodles.[150] Nutrim-

OB has lower molecular weight β-glucans than unprocessed material, but processing engenders β-glucans more biologically active.[142] Nutrim-OB has high viscosity at 5–15% solids and ambient temperatures and exhibits shear-thinning throughout shear rate range studied during a thixotropic loop experiment.[151] High-sugar ratio cake batters formulated with Nutrim-OB have higher viscosity and unaltered shear-thinning compared with cake batters without hydrocolloids.[147] The pasting profile of Nutrim-OB has high peak viscosity during heating with little setback upon cooling when analysed on a Rapid ViscoAnalyser using a typical starch test procedure.[152] Pasting properties can be attributed to β-glucans since starch was already gelatinised during the jet-cooking and drum-drying processing. A scanning electron micrograph of Nutrim-OB revealed large porous particles (250–400 μm in length) with ragged sides, that may act as a sponge, allowing high water-holding capacity (Fig. 22.7).[152]

Three new oat β-glucan hydrocolloids (designated as C-trim20, C-trim30, and C-trim50, corresponding to 20–50% β-glucan) obtained through thermal-shearing processing have recently been developed at USDA that have potential use as functional food ingredients.[153] C-trim products differ from Nutrim-OB by having an additional centrifugation step prior to jet-cooking that eliminates a higher proportion of starch, and centrifugation and sieving steps after jet-cooking determine the final β-glucan concentration (20–50%). C-trim oat products are currently produced and marketed by Van Drunen Farms, Momence, IL.

Using steady and dynamic shear measurements of cookie dough, C-trim20 exhibits shear-thinning and increased dynamic viscoelastic moduli with increasing concentration.[154,155] Cookies containing C-trim20 spread less than

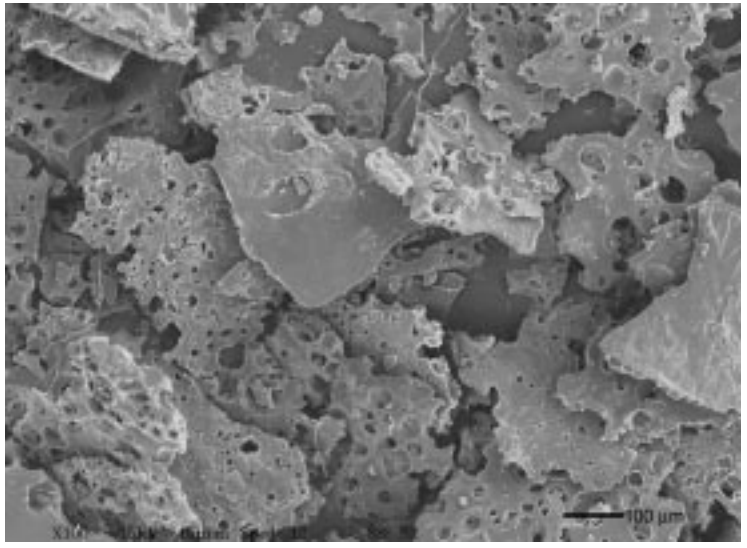

Fig. 22.7 Scanning electron micrograph of Nutrim-OB at 100× magnification, scale bar represents 100 μm.[152]

controls due to increased springiness, and have higher water content, but up to 10% flour replacement with C-trim20 did not alter sensory properties.[155] C-trim20 incorporated into cake formulations increased elasticity of cake batter but did not alter cake volume and texture.[156]

A dry milling procedure followed by air-classification to remove some starch was developed by Swedish Oat Fibre AB, Väröbacka, Sweden.[157] Fat stabilisation technique during the process produces oat-bran ingredient resistant to lipid oxidation, with 14% β-glucan content, marketed as OatWell® 14% Oat Bran. Further air-classification is used to produce oat products enriched with 16% and 22% β-glucan, marketed as OatWell® 16% and OatWell® 22% Oat Bran. OatWell® is very light yellow-brown, with neutral taste, fluffy texture and small particles (35–300 μm). The primary benefits of incorporating OatWell® in foods are increased delivery of soluble and insoluble fibre, protein, fat replacement and shelf-life extension. OatWell® has good applications in cereal bars, snacks, breakfast cereals, breads, cookies, powdered drinks and pasta. The health benefits demonstrated by incorporating OatWell® in diet are cholesterol-lowering[100,104,158] and diabetes control.[5,116,117,159,160]

Two other defatted and fractionated commercial oat β-glucan-enriched products are Nuture® 1500 with 15% β-glucan content, manufactured by GTC Nutrition (Missoula, MT), and Natureal® which has 20% β-glucan and is manufactured and marketed by Finn Cereal, Vantaa, Finland.[161] Nuture® 1500 has applications in fruit juices, skim-milk, shakes, powdered beverages, breads, cakes and pasta. Natureal® is produced by dry milling and air-classification. Natureal® forms highly viscous solutions that contain naturally occurring vitamin E and is well suited for health-promoting foods such as beverage powders, bakery, confectionery, meal replacement products and cereals.

An enzymatically-produced concentrated oat or barley β-glucan product with high viscosity, called Viscofiber® has recently been developed by Cevena Bioproducts Inc., Edmonton, Canada. Viscofiber® is produced by forming flour slurry in 40–50% ethanol with flour to alcohol ratio of 1:2 to 1:10, filtered using 40–75 μm screen and fibre residue is subjected to a combination of sonication, protease and α-amylase treatment.[162] Enzymes and ethanol washes extract starch and protein while leaving soluble β-glucans intact within cereal cell walls, preserving high viscosity of native β-glucan which has shown to be important in eliciting functional effects.[163] Viscofiber® has light colour, bland flavour and high viscosity that make it suitable for food applications such as nutritional bars, beverages, soups, breakfast cereals, baked products, pasta, ice cream, yoghurt and dietary supplements. Viscofiber® can impart high viscosity to foods, a textural attribute with potential to combat the global rise in obesity.[164] Viscofiber® β-glucans can be retained within grain cell walls, forming a honeycomb structure that is preserved using an encapsulant.[165]

An innovative procedure to produce 'organic natural oat fibre' that avoids using alkaline hydrogen peroxide method has been developed by Grain Millers Inc., Eugene, OR, that reduces processing costs and environmental impact. Avoiding processing aids, such as additives or chemical catalysts, produces an

oat fibre source resembling the original that does not occur with the alkaline hydrogen peroxide method. The green process involves only mechanical and hydrothermal treatments, achieving similar functionality to chemically-catalysed products. Extraction is enhanced by changing cellulose crystalline configuration to an amorphous form that increases water absorption, and eliminates 'sand-like' texture. Naturally occurring short-chain fatty acids are stabilised by denaturing hydrolytic or lipophilic enzymes through hydrothermal treatment, thereby improving fibre shelf-life.[166] Additionally, naturally occurring phytochemicals and chemoprotectants such as lignin, vanillin, protocatechualdehyde, and ferulic, caffeic and *p*-coumaric acids are maintained. Nutritional benefits of maintaining lignin include treatment or prevention of colonic disorders,[167] reducing cholesterol by binding bile acids[168,169] and lowering cancer risk[170] by scavenging free-radicals.[171] Natural oat fibre is light cream to tan, with a slightly sweet flavour and odour compared with white and bland for hydrogen peroxide-bleached fibre.

A β-glucan enrichment process that uses a freeze/thaw step and does not inactivate endogenous enzymes has been developed.[172] Commercial focus has been on barley, but the technology is patented for all β-glucan-containing cereals. Cereal flour is mixed with water at 50 ºC for 1 h. Enzymes associated with cereal grain partially hydrolyse β-glucans, lowering average molecular weight. Enzymes responsible for hydrolysis are most likely cellulases.[173] Solids from extract are removed by centrifugation and supernatant is frozen. On thawing a precipitate is recovered and the resulting product, known as Glucagel®, is undergoing commercial production by Gracelinc Ltd, New Zealand. Glucagel® has a β-glucan purity of 75–80%, with smaller molecular weight β-glucan product (< 80 kDa) dissolving readily in water up to 20% at temperatures > 80 ºC, while larger molecular weight product is less soluble.[174] ^{13}C CP/MAS NMR measurements of Glucagel® reveal the hydrocolloid product contains regions with two distinct conformations. β-glucan chains can associate to form unique A-conformation, whereas other regions are in amorphous B-conformation. After several days, individual fibres of Glucagel® solutions can be observed using AFM, suggesting that junction zones that enable gelation occur due to interactions among β-glucan chains in A-conformation.[175] Glucagel® forms soft thermoreversible, translucent gels that melt and set at about 60 ºC.[174] Applications of Glucagel® include bakery, dairy, dressings and edible films.

Recently, a warm-water extraction method has been developed by Roxdale Foods Ltd, Auckland, New Zealand, that can use either barley or oats for β-glucan enrichment. The product, marketed as Cerogen™, contains 70–90% β-glucan. Cerogen™ is a unique patented form of β-glucan, that is cold-water-soluble and on standing will form soft translucent gels. In a recent study, Cerogen™ was shown to lower glycaemic response in healthy adult males.[113]

Nurture Inc. developed a method that enriches β-glucan in an aqueous environment, utilising alkaline conditions.[176] The procedure involves an alkaline aqueous extract of β-glucan from oats or barley with a preferred pH of 10,

neutralised with acid, heating extract between 60 ºC and 100 ºC, subsequent cooling between 25 ºC and 45 ºC, resulting in flocculation. Centrifugation is utilised to remove flocculate and β-glucan solution is further purified by ultrafiltration using a 0.2 μm pore size or evaporated using drum or spray dryers. From this alkaline extraction method, Nurture Inc. has produced at Missoula, MT, the commercially available product, OatVantage™ that has greater than 54% β-glucan content, is beige powder with light oat odour, neutral taste and GMO free. Applications for OatVantage™ include encapsulation, tableting, beverages, functional foods and nutraceutical and dietary supplements.

Ceapro Inc., Edmonton, Canada, has recently developed a highly concentrated, clear and viscous oat β-glucan extract in which a nanofiltration step (< 1 μm screen) collects submicron β-glucan particles.[177] The nanoparticle β-glucan can be used in food applications but is also suitable for cosmetics due to its ability to penetrate skin, via lipid bilayers, stimulating collagen production and can substitute for anti-wrinkle medicines such as Botox®.[178]

22.8 Food applications

Cereal β-glucans can be incorportated into a wide range of food formulations, imparting new functionality and improved nutrition. Detailed food recipes providing examples of cereal β-glucan applications have already been well presented in the first edition of this book.[179] One of the most common food applications of enriching β-glucan content of foods is in bread baking and other wheat-based baked foods. Glucagel® incorporated into white wheat bread at 2.5 or 5% significantly decreased loaf volume and height, which may be due to dilution of gluten or β-glucans may tightly bind appreciable amounts of water, thereby reducing available water for gluten network development.[180] Additionally, water-binding by β-glucans may limit steam production, resulting in reduced loaf volume and height (Fig. 22.8).[181] Glucagel® decreased rate of reducing sugar release,[180] most likely because β-glucans reduced availability of starch for degradation.[182,183] When comparing high and low molecular weight β-glucan incorporation into wheat bread, both fractions resulted in stiffer dough, reduced loaf volume and height, and attenuated reducing sugar release, compared with controls. High molecular weight β-glucans were degraded during bread baking and resulted in greatest loss of dough and bread quality. No degradation was observed for low molecular weight β-glucans during bread baking.[184] Freeze-thaw cycling of baked goods, such as oat bran muffins, reduces oat β-glucan solubility and attenuates hypoglycaemic effect.[185] Cookies formulated with 10% C-trim 20 or C-trim 30 had reduced spreading, increased elasticity and water content, and similar textural attributes compared with cookies without hydrocolloid.[154]

Incorporating barley β-glucan into durum wheat pasta attenuated reducing sugar release during *in vitro* digestion and resulted in pasta with greater solid loss during cooking, increased swelling and decreased hardness. Physico-

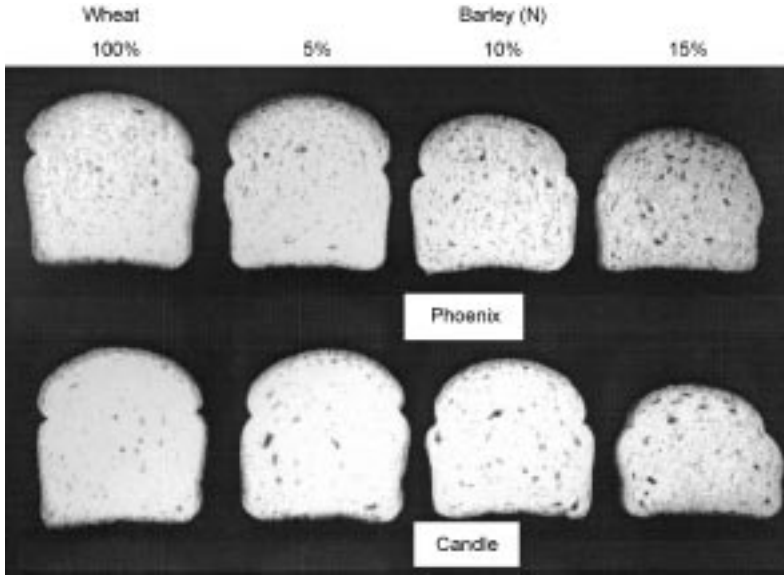

Fig. 22.8 Comparison of breads baked with 100% wheat (left) or barley flour (Phoenix or Candle variety) substituted for wheat flour at 5, 10 or 15% (last three from right) prior to dough mixing, demonstrating effect of increased β-glucan on loaf volume.[181]

chemical properties of cooked durum wheat pasta formulated with β-glucan were altered due to a combination of interference with starch-protein matrix and high water-binding capacity of β-glucans.[186] Functional pastas were produced by substituting 50% of durum wheat semolina with β-glucan-enriched barley flour fraction (9–11% β-glucan) obtained from abrasion products of fourth and fifth hullers during pearling and subsequent sieving. Barley β-glucan-enriched pasta was darker compared with durum wheat pasta, but had excellent cooking attributes with regards to stickiness, bulkiness and firmness.[187] Oat β-glucan, in form of Nutrim-5, has successfully been incorporated into Asian noodles, allowing for 50% reduction in use of rice flour, with similar noodle firmness, taste and cooking loss.[150]

Cereal β-glucans have been successfully incorporated into some dairy products, but others still face challenges. β-glucan from oats was found to perform better than barley in improving probiotic viability and stability, and enhancing lactic and propionic acid production in yoghurt. Casein gels formed among the β-glucan network; however, syneresis occurred at higher β-glucan concentrations.[188] A similar probiotic yoghurt-like fat-free β-glucan-enriched milk product has been developed utilising rolled oats.[189] Nutrim, an oat β-glucan-enriched ingredient, has been used as a fat replacer in cheeses. Compared with low-fat control Cheddar cheese, formulations with Nutrim had less firmness, fracturability, desirable flavour and melt-flow index time, and similar elasticity and cohesiveness.[148] Utilising cereal β-glucans as fat replacers in cheese clearly still faces some challenges to produce a final product with unaltered sensory attributes.

Use of oats and barley in animal feed has traditionally been limited due to the negative effect of β-glucans on feed digestibility[190] but this can be overcome by inclusion of β-glucan hydrolysing enzymes in the diet.[191]

Sensory characteristics of soups with high molecular weight β-glucan from oat bran or low molecular weight β-glucan from processed barley or oat β-glucan were studied before and after freezing. Freezing had no effect on sensory qualities. Soups made with oat bran were more viscous, higher perceived thickness and flavour intensity, indicating feasibility of cereal β-glucans as thickening agents in soups, especially in high molecular weight form.[192]

Barley β-glucan has been shown to form a gelled network that stabilises whey protein concentrate foams, decreases emulsion droplet size and substantially reduces phase separation.[193] Whippability and foam stability of barley β-glucan gums were optimised at pH 8.0 and 45 ºC, demonstrating their thickening and stabilising potential.[93]

Sausages made with 0.3% β-glucan and 12% reduction in fat had similar firmness to full-fat sausages, but formulations with 0.8% β-glucan were perceived by sensory panellists to be less firm.[194] Cereal β-glucans have also been shown to substitute 50 to 90% of fat in hamburger beef and still maintain similar cooking yield, patty diameter reduction during cooking, and retain higher moisture.[195]

Beverages formulated with 0.3–0.7% barley β-glucan, adjudged by sensory panel, were not perceived to be different for peely- and fruity-orange aroma compared with pectin ingredients. Beverages containing 0.7% β-glucan were perceived to have less desirable sourness than beverages containing 0.3% pectin. Sensory panellists perceived beverage viscosity of both hydrocolloids to be higher at 0.5 and 0.7% concentration compared with 0.3%. β-glucan beverages became lighter and cloudier with increased concentration, whereas pectin beverages were unaffected.[196]

Films for food applications have been developed using barley or oat 75–80% β-glucan extracts that also contained proteins, lipids and ash. Glycerol is used as plasticiser, producing translucent films with smooth, homogeneous structure. Film water vapour permeability prepared from 4% β-glucan extracts is higher than 2% solutions, despite similar water vapour transmission rates. Oat β-glucan films have higher tensile strength and water solubility, but a lighter colour, are less opaque and deformable compared with films made from barley. Barley films remain intact after 24 h immersion in water.[197]

Although from a nutritionist respect, β-glucans are highly desirable, in the brewing industry, barley β-glucan is a hindrance, greatly influencing malting potential by regulating rate of endosperm modification and wort viscosity during brewing.[198,199]

Complete hydrolysis of cell wall bound β-glucans to glucose requires several enzyme categories. 1→3,1→4-β-D-glucan endohydrolases (EC 3.2.1.73) are very important in complete degradation of germinated grain cell walls and hydrolyse 1→4-β-glucosidic linkages where these linkages are adjacent to a 1→3-β-D-glucosyl residue, releasing cellotriosyl and cellotetraosyl units as main

hydrolysis products.[200] An unusual 1→3,1→4-β-D-glucan endohydrolase releases larger 1→3,1→4-β-D-glucan molecules with 60 to 100 degrees of polymerisation.[200–202] Oligoglucosides released by endohydrolases can be further hydrolysed by β-D-glucan exohydrolases, which are difficult to classify because some exohydrolases also have preference for β-D-oligoglucosides with 1→2,1→4 and 1→6 linkages.[203,204]

22.9 Processing

The desire to incorporate cereal β-glucans into foods to improve human nutrition has led to a considerable increase in studies investigating the effects of processing on β-glucan structure and functionality. In particular, molecular weight ideally should not be reduced unless biological function, viscosity and gelation properties are unaltered. To produce the vast array of food products available formulated with cereal β-glucans, the hydrocolloids must have resistance to a wide range of processing conditions such as high or low temperatures, acidic or alkaline conditions, high pressure, shear and other mechanical forces, chemical modification, food additives and possibly enzymatic degradation. One advantage cereal β-glucans have compared with many other dietary fibres is their relatively high solubility that facilitates many food processing operations. Because (1→3)-β-linkages occur at irregular intervals, β-glucans tend to have an irregular shape overall which reduces tendency to pack into stable regular aggregates. This property allows β-glucans to remain relatively soluble in water, but solubility can be reduced at low temperature, particularly in presence of ethanol.[205,206]

One of the most common food processing conditions that causes cereal β-glucans potentially to be degraded or have altered functionality during food manufacture is high temperatures. Heating barley grains by autoclaving (121 ºC) or steaming substantially increases slurry viscosity, whereas roasting has been found to have no effect.[207] Cooking barley flour at even higher temperatures (195 ºC) increases β-glucan extractability.[208] Alternatively, cereal β-glucan ingredients may be used in frozen food applications such as fat replacement in ice cream, and therefore need to have excellent freeze-thaw stability. However, aqueous solutions of β-glucan undergo physical changes when exposed to freezing, frozen storage and subsequent thawing, with formation of physical crosslinked networks occurring due to cryogenic conditions. Compression tests of cereal β-glucan cryogels reveal increased strength can be obtained with increasing β-glucan molecular weight and decreasing cellotriosyl units.[209]

A high proportion of food systems are acidic and therefore cereal β-glucans must have high degradation to hydrolysis by acids. Under mild acid conditions (pH 1 at 37 ºC), no degradation of β-glucan occurs in HCl for 12 h, but total hydrolysis to glucose is observed for β-glucan in highly-concentrated acid at 120 ºC.[210] Since most food systems have pH > 2, there should not be problems with stability of cereal β-glucans under acidic conditions. Cereal β-glucans have

also been shown to be stable in moderately alkaline conditions (pH 8)[93] and few food systems have pH above this level.

Gelation is an important property of cereal β-glucans that can be influenced by food processing conditions. Despite the ability to form gels, barley β-glucan was found to demonstrate random coil-type, viscoelastic behaviour[23,24,211] in aqueous solutions. Steady shear did not induce sol-gel transition but repeated cycles of short duration steady-shear accelerated gelation.[212] Low molecular weight β-glucans have shorter gelation time than higher molecular weight β-glucans.[23,24,211] Mechanical stability of β-glucan gels increases with higher molecular weight, and oat β-glucan has more junction zones than barley, allowing faster gelation rate of the former.[23,212] Sections of consecutive cellotriose units most likely form crosslinks, enabling gelation to occur.[213] Incorporation of barley β-glucans into bread dough results in decreased molecular weight during dough mixing and fermentation. Endogenous β-glucanases in flour degraded β-glucans rather than yeast enzymes.[45] To retain nutritional benefit of high molecular weight β-glucans, duration of dough mixing and fermentation should be minimised. β-glucanases either do not act randomly on bread dough or are physically impeded from hydrolysing certain regions of polysaccharides in dough. Bread-baking does not reduce β-glucan molecular weight. β-glucan solutions at concentrations < 1% exhibit perfect viscous behaviour, but viscoelastic properties are observed at 2%.[214] Storage and loss modulus of oat meal and bran increased with increasing frequency, exhibiting viscous behaviour. Elasticity improved with increasing frequency.[215] Hydrolysed cereal β-glucans can also be used as ingredients, and effects of processing need to be considered. One study partially hydrolysed oat β-glucans using concentrated HCl acid, cellulase or lichenase, producing hydrolysates ranging in molecular weight from 0.3 to 2.3 $\times$ 10^5 g/mol. After 4 d storage at 4 ºC of 6% solutions, low molecular weight β-glucans formed elastic gels whereas large molecular weight β-glucans remained as viscous liquids after 7 d. Gels melted at 62 ºC. Cellulase, which preferentially cleaves regions of β-glucan molecules with longer contiguous β-(1→4) linked D-glucopyranosyl units, produces hydrolysate products with greater tendency to produce elastic gels with stronger junction zones compared with lichenase hydrolysis, which preferentially cleaves β-(1→4) glycosidic 3-*O*-substituted glucose links. This suggested that β-(1→3)-linked cellotriose sections of β-glucans form junction zones in gel network rather than cellulose-like segments (Fig. 22.9).[216] Highly significant correlations were found between total and extractable β-glucan content and viscosity after dispersion.[27] Viscosity increased with addition of amyloglucosidase, with no β-glucanase activity present, suggesting increased β-glucan solubilisation.[27]

Puffing oat or barley flour, forming puffed cakes, results in decreased β-glucan molecular weight, with greatest changes occurring with higher temperatures and longer durations. Both puffing temperature and time increased β-glucan solubility. Jet-cooking also lowers β-glucan molecular weight.[217] Extruded barley flours have β-glucans with higher solubility than flour without

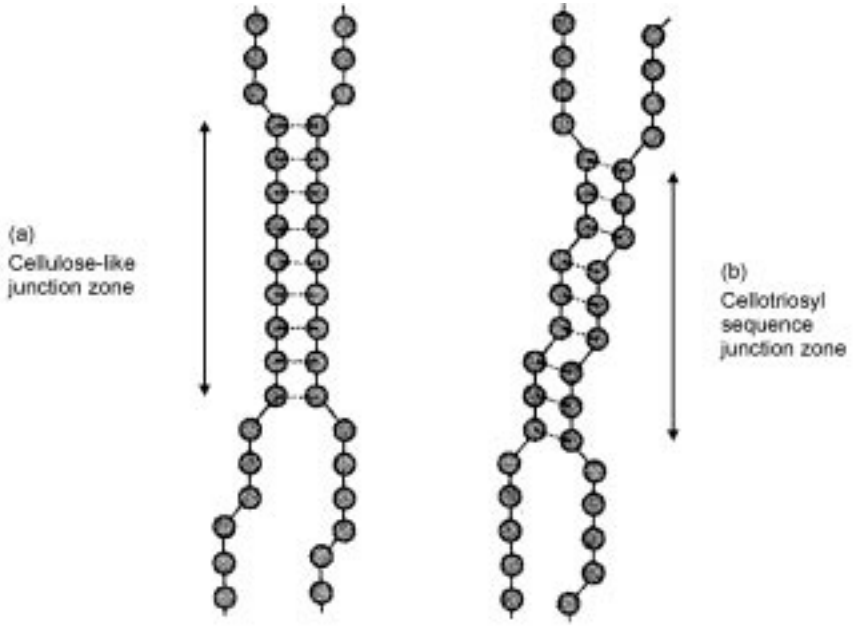

Fig. 22.9 Schematic diagram of interactions that may form junction zones between (1→3), (1→4)-β-D-glucan molecules: (a) interaction between cellulose-like sections of β-glucan and (b) interaction between sequential cellotriosyl units.[216]

extrusion, largely due to an increase in terminal glucose as extrusion cooking fragment molecules.[218]

Sucrose and salt at high concentrations reduce viscosity of β-glucan suspensions due to preferential solvation by the co-solute rather than formation of polymer-solvent interactions.[219]

Sulphated-derivatived oat β-glucan, produced using formamide and chlorosulphonic acid, has molecular weight reduced by half, while viscosity and solubility doubled in magnitude compared with native β-glucan. Sulphation did cause reduction in *in vitro* bile acid binding capacity due to anionic charge and lower molecular weight, but increased anticoagulant activity, showing potential to prevent blood clotting.[220]

22.10 Regulatory status

All cereal β-glucan-enriched products have GRAS (generally regarded as safe) status, since they are simply concentrating a naturally occurring cereal grain component. Oatrim is an enzyme-hydrolysed oat product and did not qualify for the FDA health benefit claim until 2003.[221] Nutrim-OB, all C-trim products, Oatwell®, Nuture® 1500, OatVantage®, Viscofiber®, 'organic natural oat fibre' (Grain Millers Inc.), Glucagel®, Cerogen™ and β-glucan produced by Ceapro

Inc., all qualify for the FDA health benefit claim. Viscofiber® also has kosher status and 'organic natural oat fibre', as its name implies is organically produced.

22.11 Future trends

The future looks prosperous for commercialisation of cereal β-glucans. Although growth is unlikely to be spectacular, steady growth of β-glucan ingredients is practically assured due to degeneration in nutritional standards by populations in industrialised nations. While there will be a never-ending influx of companies promoting fad diets that attempt to confuse people about recommended nutritional guidelines, studies find consumers still consistently identify fibre as an extremely important component of a healthy diet.[222–226] Coronary heart disease continues to be a major cause of mortality in industrialised nations and diabetes is sharply increasing, especially in the two most populous nations, China and India.[227,228] Obesity incidence in adults and children is globally rising at alarming rates, with 80% of coronary heart disease and diabetes disorders related to obesity.[229] Health-care costs will rise from the increasing cases of nutritional disorders, forcing governments to place greater emphasis on educating and encouraging societies to consume diets rich in cereal grains, fruits and vegetables. An outcome of this is consumers will increase their demand for cereal grains high in β-glucans, such as oats and barley, requiring food manufacturers to include more oat and barley ingredients in foods. Consumers will not purchase foods if sensory properties are undesirably altered when cereal β-glucans are included, and there is little achieved effectively incorporating cereal β-glucan ingredients into foods without changing taste and appearance, but degrading β-glucans in a manner than diminishes their human biological functions. Therefore further research needs to be continued in developing processing methods to produce cereal β-glucan-enriched ingredients that either retain structure or human biological function. Research also needs to be continued to expand the number of food products that consumers can purchase that include health-benefiting cereal β-glucans, thereby ensuring adequate fibre is consumed in diets. Developing new technologies of producing cereal β-glucan-enriched ingredients at lower cost will also assist food manufacturers in producing foods that deliver sufficient fibre to consumers.

Because various processing conditions degrade cereal β-glucans, but conflicting reports exist on whether human biological function is retained upon structural modification, greater understanding needs to be gained on the relationship between cereal β-glucan molecular weight and health benefits. Clinical studies must continue to gain better understanding on how food matrix that cereal β-glucans are incorporated into can influence received health benefits. Optimising cereal β-glucan daily intake from processed foods to receive health benefits still needs some determination. Nanofiltration, producing submicron oat fibre particles, is an emerging technology. To improve overall oat

fibre production economics, oat processors may need to continue to explore beyond food uses to further expand industrial uses such as cosmetics and pharmaceuticals.

Plant breeding needs to continue identifying genetic traits associated with higher β-glucan yield in cereal grains, thereby improving the economics of producing β-glucan-enriched ingredients and the likelihood of foods containing cereal β-glucans being affordable to consumers. Understanding the molecular biology and biochemistry involved in β-glucan biosynthesis may play an important future role in producing β-glucans with novel structure, functionality and health benefits.

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23

Arabinoxylans

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Abstract: Arabinoxylans are predominant non-cellulosic polysaccharides of primary and secondary cell walls in cereals and grasses. They are of interest to cereal chemists and technologists because of their technological importance. They also have important applications in bakery and dairy products, in packaging films, and pharmaceutical products. Arabinoxylans display functional properties including viscosity enhancement, gel formation, emulsion or foam stabilizations, water absorption, fat replacement, and prebiotic activity. They affect the use of cereal grains in milling, brewing, breadmaking, and animal feed production. As constituents of dietary fiber, arabinoxylans also have nutritional and health-promoting benefits. This chapter reviews their extraction, isolation and purification. It also discusses their structure and properties, technological functionality and applications, as well as nutritional benefits.

Key words: arabinoxylans, wheat, rye, cell walls, xylo-oligosaccharides.

23.1 Introduction

Xylan-type polysaccharides are known to occur in various terrestrial plants and algae; they may even constitute different tissues within one plant. The structural diversity and complexity of xylans are likely related to their functionality and the evolution scheme of plants. Homoxylans with β-(1→3) or β-(1→3,1→4) glycosidic linkages are known to substitute cellulose in the cell wall architecture of green algae (*Caulerpa* sp.) or red seaweeds (*Palmariales* and *Nemaliales*), respectively. Xylans of all higher plants possess β-(1→4) linked D-xylopyranosyl (Xyl*p*) residues as the backbone, substituted to various degrees with sugar units, uronic acids, and *O*-acetyl groups. 4-*O*-methyl-glucuronoxylans can be found in the wood of dicots, and contain xylan backbone with

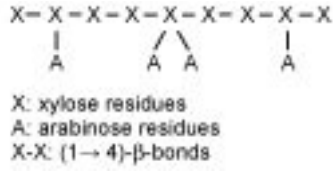

Fig. 23.1 General structure of neutral arabinoxylans.

single chains of 2-linked 4-*O*-methyl-α-D-glucopyranosyl uronic acid units. Neutral arabinoxylans are the pentose-containing carbohydrate polymers and hence referred to as pentosans. They consist of a linear xylan backbone chain to which mostly single α-L-arabinofuranosyl (Ara*f*) residues are attached at position 2 and/or 3 of Xyl*p* (Fig. 23.1). Arabinoxylans are predominant non-cellulosic polysaccharides of primary and secondary cell walls in cereals and grasses. In walls of starchy endosperm and aleurone cells they function mainly as matrix phase polysaccharides maintaining the wall integrity by forming a gel reinforced by cellulosic microfibrils. In the lignified walls of pericarp tissues, arabinoxylans might be covalently crosslinked with one another through diferulate bridges as well as covalently linked to lignin and, therefore, contribute to strength and rigidity of the walls (Carpita, 1996). Arabinoxylans in the secondary walls of grasses and cereals may have a more complex structure and carry single chains of 2-*O*-linked α-D-glucopyranosyl uronic acid units and/or its 4-*O*-methyl derivative, in addition to 3-linked Ara*f* residues; they are referred to as arabino(glucurono)xylans.

Cereal arabinoxylans were first described by Hoffmann and Gortner in 1927 as a viscous gum present in wheat flour. Ever since, they have been of interest to cereal chemists and technologists because of their technological importance. Arabinoxylans display several interesting functional properties including viscosity enhancement, gel formation, emulsion or foam stabilizations, water absorption, fat replacement, and prebiotic activity. They affect the use of cereal grains in milling, brewing, breadmaking, and animal feed production. As constituents of dietary fiber, arabinoxylans have an impact on the nutritional quality of cereal foods; they offer nutritional benefits of soluble and insoluble fiber and, because of the presence of phenolic moieties in their molecular structures, they may also have some antioxidant properties (Katapodis *et al.*, 2003). Arabinoxylans might constitute a significant portion of human dietary fiber intake because they occur in a wide variety of cereal crops. The availability of arabinoxylans and other xylan-type polysaccharides is immense since some agricultural by-products with little current economic value, such as corn stalks and cobs, brewer's and distiller's grain, flax seed cake, straw, hulls and husk of various grains, or banana peels, are potential sources of these polysaccharides. Several important technological properties as well as physiological and immunological activities of arabinoxylans promise valuable applications of these polymers in both the food and non-food industries and include possible use in bakery and dairy products, in packaging films, and pharmaceutical products.

23.2 Occurrence and content of arabinoxylans

Arabinoxylans have been found in all major cereal grains, including rye, wheat, barley, oats, rice, sorghum, maize, millet (Izydorczyk and Biliaderis, 1995), as well as in other plants, such as psyllium (Fischer *et al.*, 2004), flax seed, pangola grass, bamboo shoots, and rye grass. Among the common cereal grains, the highest content of arabinoxylans is found in rye followed by wheat, barley, oats, rice, and sorghum (Table 23.1). In cereal grains, arabinoxylans are localized in the cell walls of starchy endosperm, aleurone, in the bran tissues, as well as in the husk of some cereals. However, depending on the genus and species, the amount and molecular structure of arabinoxylans in a particular tissue may vary. For example, in barley the aleurone CW are composed primarily of arabinoxylans (Table 23.2) with smaller amounts of arabinoxylans in the endosperm CW (Bacic and Stone, 1981a,b). In wheat, there is more arabinoxylan in walls surrounding the cells of starchy endosperm (70%) than those of aleurone (65%).

Considerable genotypic and environmental variations in arabinoxylan content have been reported for wheat, rye and barley (Lempereur *et al.*, 1997; Henry, 1986). Hansen *et al.* (2003) examined the effect of genotype and harvest year on the content and composition of dietary fiber in seven rye varieties grown in Denmark, and found that yearly variations in the content of total arabinoxylans (8.7–11.5%) were higher (38% of total variance) than those associated with genotypes effects (20% of total variance). Yearly differences in the content of total arabinoxylans were also found among five rye varieties grown in Finland (Saastamoinen *et al.*, 1989). It was also reported that a cold and wet season resulted in small rye kernels with high arabinoxylan contents. In contrast to rye, limited variation in total arabinoxylan levels (5.8–7.6%) were found in 14 wheat varieties grown in three successive growing periods in Belgium, with 18% of the variance explained by the genotype and none by the harvest year (Dornez *et al.*, 2008). The levels of water extractable arabinoxylans in wheat, on the other hand, varied from 0.42–0.99%, and the genotype accounted for 57% of the variance, while harvest year explained 37% (Dornez *et al.*, 2008). Coles *et al.* (1997) found a positive relationship between the amount of arabinoxylan accumulated in wheat and the dry growing conditions. Also, the exposure of plants to UV light increased the degree of crosslinking in arabinoxylans, but the effect on the total content of these polymers was not clearly established. The content of arabinoxylans in barley also depends on genetic and environmental factors (Fleury *et al.*, 1997; Izydorczyk *et al.*, 2000; Holtekjølen *et al.*, 2007) but appears to be less variable than that of β-glucans. Fleury *et al.* (1997) and Izydorczyk and Dexter (2008) reported that the amount of arabinoxylans in the western Canadian hull-less barley was significantly lower than in the two- or six-rowed covered barley, and linked the differences to the absence of hulls in the former (Table 23.1). Six-rowed barley cultivars generally contain slightly higher levels of arabinoxylans than two-rowed cultivars (Fleury *et al.*, 1997).

Table 23.1 Content of total and water soluble arabinoxylans in whole grains and grain tissues

Grain/tissue	Total arabinoxylan (%)	Water-soluble arabinoxylans (%)	References
Rye			
Whole grain	7.6	—	Bengtsson and Åman (1990)
Whole grain	8–12.1	2.6–4.1	Hansen *et al.* (2003)
Bran	—	1.7	Figueroa-Espinoza *et al.* (2004)
Flour	3.2–3.64	2.2	Cyran *et al.* (2003)
Barley			
Whole grain, hulled	5.98–8.05	—	Knutsen and Holtekjølen (2007)
Whole grain, hulless	3.36–4.94	—	Knutsen and Holtekjølen (2007)
Whole grain, de-hulled	3.98–5.44	0.5–0.7	Izydorczyk and Dexter (2008)
Whole grain, hulless	3.50–6.05	0.4–0.8	Izydorczyk and Dexter (2008)
Whole grain, hulled	5.41–6.42	—	Fleury *et al.* (1997)
Whole grain, hulless	3.37–4.40	—	Fleury *et al.* (1997)
Pearlings	11–12	—	Izydorczyk *et al.* (2003a)
Pearled flour	—	0.3–1.08	Dervilly-Pinel *et al.* (2001)
Wheat			
Whole grain	4.79–6.92	—	Saulnier *et al.* (2007)
Whole grain	—	0.38–0.83	Saulnier *et al.* (1995a)
Flour	1.65–3.14	0.3–0.8	Saulnier *et al.* (2007)
Flour	1.37–2.06	0.54–0.68	Izydorczyk *et al.* (1991)
Durum wheat	4.06–6.02	0.37–0.56	Lempereur *et al.* (1997)
Oats			
Whole grain	2.73	0.17	Hashimoto *et al.* (1987)
Whole grain	2.0–2.4	0.1–0.2	Author's unpublished results
Bran	3.50	0.33	Hashimoto *et al.* (1987)
Bran	3.0–4.1	0.08–0.16	Author's unpublished results
Flour	0.7–1.2	0.06–0.13	Author's unpublished results
Hulls	8.8	0.4	Hashimoto *et al.* (1987)
Rice			
Whole grain	2.64	0.06	Hashimoto *et al.* (1987)
Hulls	8.4–9.2	0.11–0.12	Hashimoto *et al.* (1987)
Bran	4.8–5.1	0.35–0.77	Hashimoto *et al.* (1987)
Sorghum			
Whole grain	1.8	0.08	Hashimoto *et al.* (1987)
Corn			
Bran	29.86	0.28	Hashimoto *et al.* (1987)
Bran	42	—	Saulnier *et al.* (1995a)
Soybean			
Hulls	13.1	—	Hashimoto *et al.* (1987)

Table 23.2 Neutral monosaccharides and phenolic acids in various tissues of barley grain.[a] Reproduced from Izydorczyk and Dexter (2008) with permission

Tissue/cultivar	Neutral monosaccharide composition of total polysaccharides (%)					Ara/Xyl ratio	Phenolic acids (μg/mg)			Major polysaccharide components
	Gal	Man	Ara	Xyl	Glc		Ferulic	DDFA[b]	p-Coumaric	
Pericarp[c]										
AC Metcalfe	3.0	2.8	33.7	30.1	30.4	1.12	4.42	3.81	0.32	25.8% cellulose 4% β-glucans 63.8% arabinoxylans
CDC Fibar	3.0	3.4	31.9	27.7	34.0	1.15	3.21	2.62	0.12	28% cellulose 5.8% β-glucans 59.6% arabinoxylans
Aleurone[c]										
AC Metcalfe	1.1	—	24.9	43.1	30.8	0.57	4.97	1.16	0.03	2% cellulose 28.8% β-glucans 68% arabninoxylans
CDC Fibar	2.1	—	28.2	42.7	27.0	0.66	3.99	0.96	0.03	2% cellulose 25.5% β-glucans 70.9% arabinoxylans
Starchy endosperm[c]										
AC Metcalfe	10.9	13	5.62	6.2	64.1	0.92	0.024	0.0033	—	2% cellulose 62.1% β-glucans 11.8% arabinoxylans
CDC Fibar	8.8	7.8	3.9	4.9	74.4	0.81	0.065	0.0044	—	2% cellulose 72.2% β-glucans 8.8% arabinoxylans

[a] Izydorczyk (unpublished results).
[b] Dehydro diferulic acid.
[c] All tissues were obtained by hand dissection of the grain immersed in 50% ethanol followed by microscopic examination to ensure tissue purity.
Reproduced from Izydorczyk and Dexter (2008) with permission.

23.3 Extraction, isolation and purification of arabinoxylans

23.3.1 Aqueous extraction from endospermic tissues

The most common approach to isolating arabinoxylans from various plant materials involves aqueous extraction of these polymers either from whole grains or from specific plant tissues. If water is used as an extraction medium, the resultant extract contains the water-soluble population of arabinoxylans along with water-soluble proteins, sugars, other polysaccharides, and minerals that need to be removed during purifications steps. Depending on genotypic or cellular origin of arabinoxylans, a considerable portion of these polymers may not be water extractable. In the intact wall, arabinoxylans are crosslinked with other wall constituents to form a structural framework that is not soluble in an aqueous environment (Fry, 2004). Some of the crosslinks involved are non-covalent (e.g., hydrogen bonds, van der Waals), and while individually weak, they confer strength and insolubility if present in large numbers. Arabinoxylan chains can also be covalently crosslinked to each other or to other cell wall constituents and require harsher treatments with alkali solutions to liberate them from the networks of covalent and non-covalent bonds as well as physical entanglements. Once isolated from the cell wall matrix, arabinoxylans are water soluble. Following aqueous or alkali extraction, the major hurdles involved in obtaining high purity arabinoxylans are removal of contaminating starch, proteins, and β-glucans. Purification procedures usually involve inactivation of endogenous enzymes in aqueous extracts and the use of hydrolytic enzymes to eliminate the contaminants. Crowe and Rasper (1988) and Izydorczyk *et al.* (1990) removed protein from wheat flour extracts by adsorption of contaminating proteins on various clays.

A large-scale procedure for isolation of water-soluble and insoluble pentosans from wheat flour, developed by Faurot *et al.* (1995), was based on mixing flour and water (50 kg/150 l), separating the soluble arabinoxylans from the insoluble residue by centrifugation, and heat treatment of the supernatant (Fig. 23.2). The insoluble residue was then treated with Alcalase and Thermamyl to solubilize the initially water-unextractable polymers. The yield of water-soluble and -insoluble arabinoxylans ranged from 100 to 200 g and from 250 to 350 g respectively. However, the preparation was contaminated with either soluble proteins or starch, with the enrichment factor from 98 to 68 for water-soluble and 27 to 19 for water-insoluble arabinoxylans compared to their initial content in wheat flour. A pilot-scale isolation of water-extractable arabinoxylans from rye (Delcour *et al.*, 1999) involved a heat-treatment of the ground wholemeal (130 ºC, 90 min) to inactivate the endogenous enzymes, followed by stirring of the rye wholemeal (10 kg) and deionized water (100 l) at room temperature (90 min) in a pilot-scale brewing vessel. After decanting of the supernatant, the water-extractable arabinoxylans were purified by treatments with a heat stable α-amylase, protein coagulation, and partial concentration by heat evaporation. Subsequently, arabinoxylans were precipitated by addition of ethanol. The precipitated material contained about 54% arabinoxylans, 22%

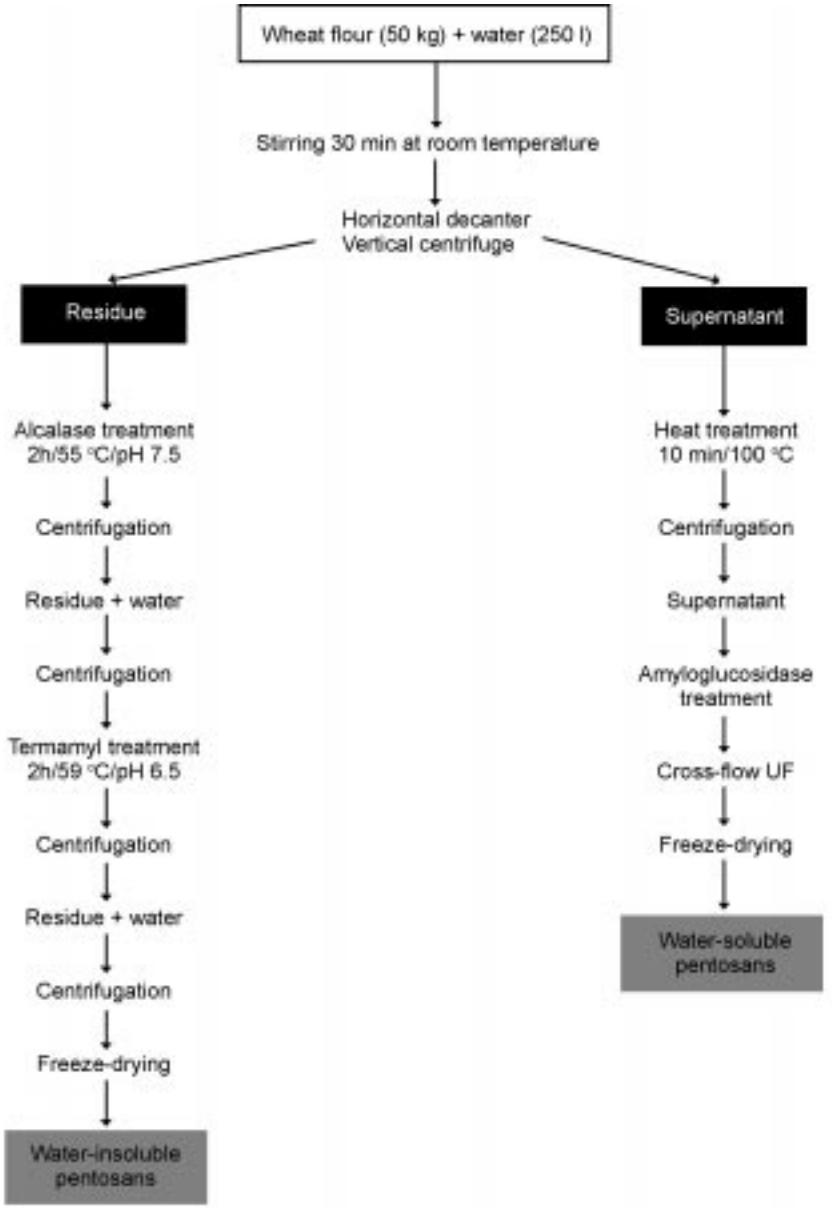

Fig. 23.2 Procedure for a large-scale isolation and purification of water-soluble and -insoluble arabinoxylans from wheat flour. Adapted from Faurot *et al.* (1995).

proteins, 4.4% arabinogalactan peptide and 4.7% β-glucans. An additional purification step, based on a treatment of the dissolved material with montmorillonite clay, increased the arabinoxylan content to about 93%, leaving almost no protein in the extract (Delcour *et al.*, 1999).

Gruppen *et al.* (1989) reported a large-scale isolation of highly purified arabinoxylans-enriched cell wall material from wheat endosperm based on dough kneading in combination with wet sieving. Mares and Stone (1973) developed a method for preparation of cell wall material enriched in arabinoxylans based on wet-sieving of wheat flour in aqueous ethanol to remove starch granules, followed by sonication and/or removal of starch and intracellular proteins by organic solvents to improve the purity of preparations (Selvedran and DuPont, 1980).

23.3.2 Strategies to extract arabinoxylans from agricultural by-products

Considerable interest has recently evolved towards extraction and purification of arabinoxylans from agricultural by-products. Brewer's (spent) grain, straw, stalks, wheat or rye bran, hulls, husks, sugar beet pulp, corn cobs, or banana peels constitute abundant low-value by-products of the food industry. These materials are potential sources of arabinoxylans as they are rich in non-cellulosic polysaccharides, with arabinoxylans being a primary component. The liberation of xylan polysaccharides from the lignified grass cell walls is restricted by the presence of lignin network as well as ester and ether lignin–carbohydrate linkages. Isolation of arabinoxylans from non-endospermic tissues usually involves delignification before the alkaline extraction of xylans. Bataillon *et al.* (1998) combined delignification (with 37% sodium chlorite) and alkali extraction (with 43% sodium hydroxide) to obtain arabinoxylan preparations from de-starched wheat bran. The extracted arabinoxylans were purified by a microfiltration and dried by an atomization system; the yield and purity of this preparation were 13% and 75%, respectively (Bataillon *et al.*, 1998). Processes involving an alkaline and H_2O_2/NaOH delignification step and subsequent alkaline extraction step have been successfully used to substitute the hazardous and expensive $NaClO_2$ delignification.

Various hydrolyzing enzymes, such as β-glucanases, arabinofuranosidases, endoxylanases, and ferulic acid esterases have been proven to facilitate the liberation of arabinoxylans from the cell wall materials. Figueroa-Espinoza *et al.* (2004) combined various physical treatments (extrusion and high shear) with the use of different endoxylanases (from *Aspergillus niger, Talaromyces emersonii*, and *Bacillus subtilis*) to investigate the release of high molecular weight arabinoxylans from rye bran. The best results were obtained when the rye bran was extruded at high temperature (~140 ºC) and extracted in the presence of endoxylanase from *Bacillus subtilis*. The amount of hydrolytic enzymes, however, needs to be controlled to avoid hydrolysis of arabinoxylans and a consequent reduction of their molecular weight and ability to form gels. Further improvement in solubilization of arabinoxylans was achieved when β-glucanase was added to the extraction buffer. The high shear treatments, achieved by pumping of rye bran slurry through a reactor used for production of micro-emulsions, did not improve the extractability of arabinoxylans. The use of hydrolytic enzymes to assist the extraction of arabinoxylans from brewer's grain

and wheat bran has also been explored by Faulds *et al.* (2004). An enzyme preparation from the thermophilic fungus *Humicola insolens*, containing endoxylanases and feruloyl esterases, proved efficient in releasing ferulic and diferulic acid residues and solubilizing arabinoxylans present in these by-products. Lu and co-workers (2000) utilized a by-product generated during processing of wheat flour into starch and gluten to produce arabinoxylan-rich fiber. The arabinoxylan-enriched residue left after flour processing was simply collected on a sieve (75 μm), washed with water and spray-dried to a powder. Another product, containing 60% of arabinoxylan-enriched dietary fiber, was obtained from waste water, remaining after starch extraction, after a series of enzymatic and fermentative treatments followed by cross-flow ultrafiltration and spray-drying (Zunft *et al.*, 2004).

Most recently, considerable efforts have been directed towards developing integrated processes for value added production of arabinoxylans as co-product of biorefineries (Broekaert *et al.*, 2008; Mustafa *et al.*, 2007; Sadhukhan *et al.*, 2008). It is recognized that bioethanol production from wheat and other grain requires co-production in order to achieve viable commercial operation, and arabinoxylans are considered promising co-products for extraction from wheat bran. Sadhukhan *et al.* (2008) designed integrated operations involving arabinoxylan extraction from bran obtained by debranning of wheat with ethanol production from the remaining grain (Fig. 23.3). The pilot-scale extraction and purification processes, as recommended by Sadhukhan *et al.* (2008), employ low toxicity chemicals, omit steps that are inappropriate at the industrial scale, such as acetone washing, dialysis, freeze drying, combine decantation and centrifugation steps, and use rotary drum dryers to allow ethanol recovery. Some 96% of ethanol, from ethanol recovery stage is fed into the Precipitation and washing (2) steps (Fig. 23.3). The ethanol is then recovered from Centrifugation and washing (2) steps and topped with ethanol from the Recovery stage to meet the requirements for Treatment (1) and Sieving and washing (1); the waste streams from those units are then sent back to Ethanol recovery.

A novel process for isolation of arabinoxylans from barley husk by steam explosion, ultrafiltration, and diafiltration has been described by Krawczyk *et al.* (2008). Hydrolysis of glycosidic bonds in hemicellulose and cleavage of hemicellulose-lignin bonds during steam treatment make arabinoxylans soluble. The low molecular weight products are subsequently removed by ultrafiltration and diafiltration, resulting in a preparation with the average molecular mass of polysaccharides of 54,000 Da and arabinoxylans making up 45% of the polysaccharide fraction. Hromádková *et al.* (2008) presented evidence that the application of ultrasound during extraction of xylan polysaccharides from wheat bran can significantly shorten the total extraction time and reduce the consumption of NaOH. Furthermore, ultrasound can be utilized in preparation of water-soluble arabinoxylans with different Ara/Xyl ratio and phenolic-rich heteroxylans potentially applicable in food, industry, pharmaceuticals, and cosmetics.

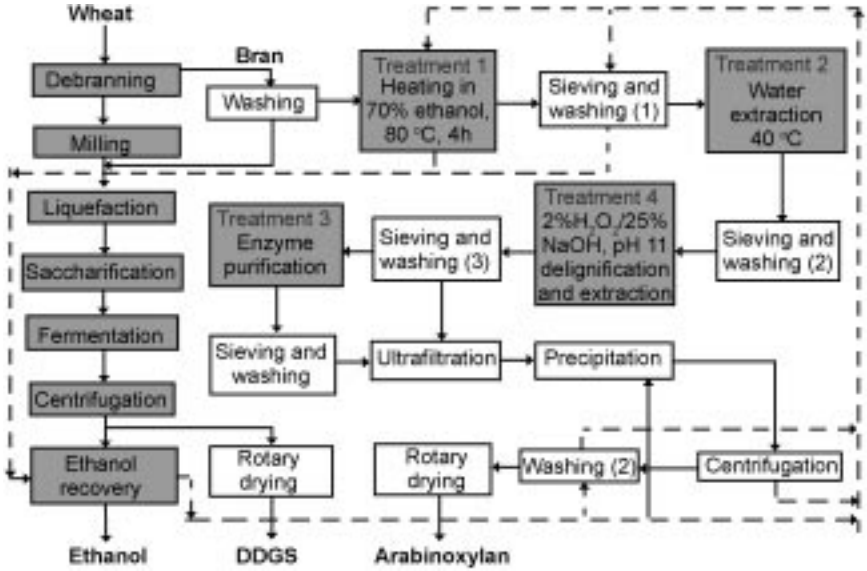

Fig. 23.3 Integrated processes for ethanol and arabinoxylan co-production from wheat. Adapted from Sadhukhan *et al.* (2008).

23.3.3 Arabinoxylan-enriched fractions obtained by physical grain fractionation

Substantial enrichment of arabinoxylans can be obtained by physical fractionation of grain due to variable concentration of arabinoxylan in various grain tissues. The advantages to this approach include chemical-free processing and the presence of other dietary fiber ingredients and phytochemicals (polyphenols, phytosterols, vitamins, etc.) naturally present in grain tissues.

In the study by Glitsø and Knudsen (1999) the outer grain layers, containing the pericarp/testa enriched fractions, were obtained by de-hulling. The remaining grain was passed through three corrugated roller mills and sieved to obtain particles smaller than 1200 μm, thus producing the fraction enriched in aleurone cells. Another part of the rye material, remaining after dehulling, was passed through one smooth roller mill retrieving particles smaller than 1200 μm and through another smooth roller mill retrieving particles smaller than 250 μm, thus producing the endosperm enriched fraction (Glitsø and Knudsen, 1999).

Fractionation of barley grain via pearling and roller milling was also shown to afford fractions enriched in specific components of dietary fiber (Fig. 23.4) (Izydorczyk *et al.*, 2003a). The roller milling of whole or pearled barley involved several break and sizing passages using corrugated rolls, and reduction passages with smooth frosted or corrugated rolls. It was shown that addition of impact milling between grinding passages enabled better separation of the bran and endosperm cell walls and resulted in fiber-rich fractions containing between 15 and 25% of β-glucans and 8–12% arabinoxylans, depending on the barley

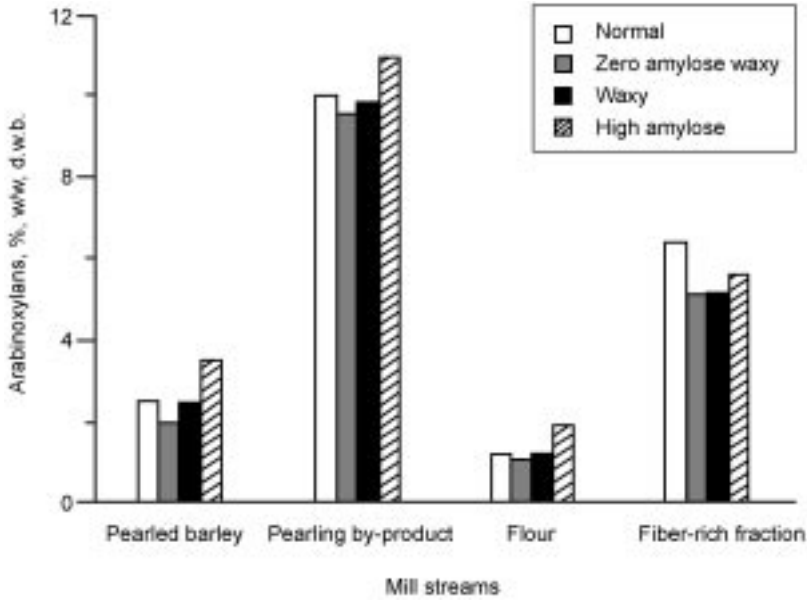

Fig. 23.4 Arabinoxylan content in various fractions of barley obtained by pearling and roller milling. Adapted from Izydorczyk *et al.* (2003a).

genotype and certain pre-processing practices, such as pearling and tempering of grain (Izydorczyk *et al.*, 2003a).

Recently, Harris *et al.* (2005) described a large-scale procedure for the production of aleurone-rich and pericarp-rich fractions from hard, Australian wheat. The pericarp fraction was obtained after wheat grain, conditioned to 16% moisture, was abraded using a debranning machine to remove 2.5% of bran by weight. The debranned wheat was put onto the first break of the pilot mill. The bran and pollard produced by this milling was mixed to form the aleurone-rich fraction, which included the seed coat with its cuticle. Both fractions were enriched in arabinoxylans, but the concentration of ester-linked ferulic acid and β-glucans was greater in the aleurone than in the pericarp-rich fraction. Consistent with the results of Glitsø and Knudsen (1999), it was found that the arabinoxylans in the walls of the pericarp-rich fraction were more highly substituted (Ara/Xyl ratio of 0.93) than those in the walls of the aleurone-rich fraction (Ara/Xyl ratio of 0.59) (Harris *et al.*, 2005).

Bohm and co-workers (2002) obtained a patent for the mechanical method for extraction of aleurone from wheat bran. The aleurone was separated from the pericarp layer by a special mill and then isolated by several purification steps including micro-milling on a friction roller mill. The preparations contained mostly arabinoxylans with smaller amounts of β-glucans and cellulose and were enriched in phenolic acids and phytosterols compared to whole bran.

23.3.4 Production of xylooligosaccharides

Recent advances in the area of prebiotic activity of arabinoxylo-oligosaccharides (Grootaert *et al.*, 2007) and increasing pressures to ensure total utilization of agro-industrial by-products have prompted interest in obtaining partially degraded arabinoxylans from agricultural biomass. It has been shown that xylooligosaccharides have prebiotic properties and improve the intestinal function by enhancing the growth of healthy colonic microflora while suppressing the growth of pathogenic organisms (Jaskari *et al.*, 1998; Crittenden *et al.*, 2002; Izumi and Kojo, 2003). The breakdown of arabinoxylans into xylo-oligosaccharides can be carried out by direct enzymatic or acid conversion of raw materials or by hydrolysis of isolated polysaccharides. Endo-β(1→4)-xylanases, that cut internal β-(1→4) linkages in the arabinoxylans backbone, belong to glycoside hydrolase families (GHF) 10 and 11 (Biely *et al.*, 1997). Endoxylanases of GHF 10 (from *Cryptococcus albidus* or *Aspergillus aculeatus*) and of GHF 11 (from *Trichoderma reesei* or *Bacillus subtilis*) have been reported to have different substrate specificities and therefore may produce different oligosaccharides (Biely *et al.*, 1997; Trogh *et al.*, 2004). Purification of xylooligosaccharides from the hydrolysis liquors involves selective adsorption and/or chromatographic separation (Pellerin *et al.*, 1991; Sun *et al.*, 2002). The barley residue remaining after the brewing process may contain up to 40% of arabinoxylans that upon digestion with feruloyl esterases and glucosyl hydrolases from *Humicola insolens* are converted to various arabinoxylo-oligosaccharides, feruloylated oligosaccharides and free ferulic acid (Faulds *et al.*, 2004, 2006; Mandalari *et al.*, 2005). The presence of phenolic moieties in arabinoxylans and arabinoxylo-oligosaccharides contributes to their antioxidant and antimicrobial properties (Katapodis *et al.*, 2003).

Vegas *et al.* (2004) treated rice husk with hot, compressed water to cause hydrolytic degradation of arabinoxylans. Another method to produce arabinoxylo-oligosaccharides involves hydrothermal autohydrolysis of hardwood or brewery spent grain (Kabel *et al.*, 2002). In this method a suspension of plant material is heated in a special reactor at 150–190 ºC for 20–60 min. However, due to the high reaction temperature, there is a possibility of formation of undesirable side products (Carvalheiro *et al.*, 2004).

23.4 Molecular structure

23.4.1 General molecular features

Arabinoxylans consist of a linear chain backbone of β-D-xylopyranosyl (Xyl*p*) residues linked through (1→4) glycosidic linkages. α-L-Arabinofuranosyl (Ara*f*) residues are attached to some of the Xyl*p* residues at *O*-2, *O*-3, and/or at both *O*-2,3 positions, resulting in four structural elements in the molecular structure of arabinoxylans: monosubstituted Xyl*p* at *O*-2 or *O*-3, disubstituted Xyl*p* at *O*-2,3, and unsubstituted Xyl*p* (Fig. 23.5). The majority of Ara*f* residues in arabinoxylans is present as monomeric substituents; however, a small proportion

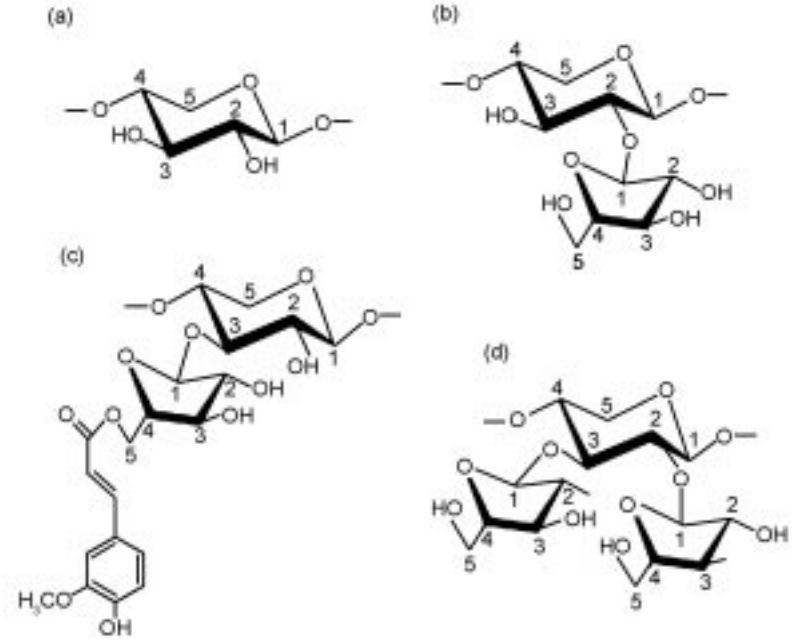

Fig. 23.5 Structural elements present in arabinoxylans: (a) unsubstituted Xyl*p*; (b) monosubstituted Xyl*p* at O-2; (c) monosubstituted Xyl*p* at O-3 with ferulic acid residue esterified at Araf; (d) disubstituted Xyl*p* at O-2,3.

of oligomeric side-chains, consisting of two or more Ara*f* residues linked via 1→2, 1→3, and 1→5 linkages, has been reported. The arabino(glucurono)-xylans from the secondary walls in the pericarp and testa tissues of bran carry 4-*O*-methyl glucuronic acid residues.

Arabinoxylans from rice, sorghum, finger millet, and maize bran are more complex than those from common cereals (Subba Rao and Muralikrishna, 2004; Ebringerova and Heinze, 2000). They may contain considerable amounts of 2-*O*-linked glucuronic acid residues and are referred as glucurono(arabino)xylans. Sorghum glucurono(arabino)xylans comprise structural features of neutral arabinoxylans but also contain disaccharides composed of two Ara*f* residues linked at position *O*-3 to the xylan backbone. Corn cob arabino(glucurono)-xylans carry side branches that in addition to arabinose residues contain small amounts of xylopyranose, galactopyranose, and α-D-glucuronic acid or 4-*O*-methyl-α-D-glucuronic residues; their peculiarity is also the presence of single terminating Xyl*p* and disaccharide side chains composed of β-D-Xyl*p*(1→2)α-L-Ara*f* (Fig. 23.6) (Saulnier *et al.*, 1995a).

The molecular structure of the physiologically active polysaccharides of psyllium husk (*Plantago* species) is quite unique. The major fraction obtained by aqueous alkali extraction of psyllium husk followed by acidification of the extract yields gel-forming polysaccharides that consist of neutral arabinoxylans,

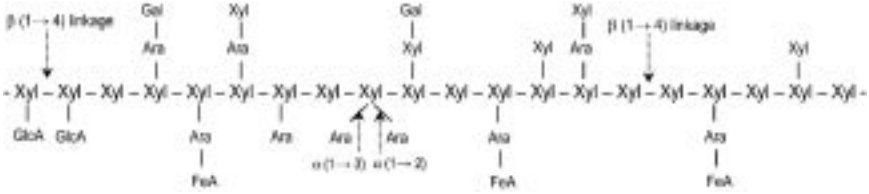

Fig. 23.6 General structure of arabino(glucurono)xylans.

containing mainly arabinose (22.6%) and xylose (74.6%) residues, with only traces of other sugars. Psyllium arabinoxylans, despite their low Ara/Xyl ratio (0.30), are highly branched polymers with a xylan backbone carrying single xylose units at position *O*-2 and trisaccharide branches (Ara*f*-α-(1→3)-Xyl*p*-β-(1→3)-Ara*f*) at position *O*-3 (Fischer *et al.*, 2004). Some controversies still exist with regard to the linkage composition in the main chain of arabinoxylans in various *Plantago* species. While Edwards *et al.* (2003) and Fischer *et al.* (2004) reported that the xylan chain in psyllium polysaccharides consist only of densely substituted (1→4)-linked xylopyranosyl residues, others presented plausible evidence that both (1→3)- and (1→4)-linked β-D-Xyl*p* could be randomly distributed along the backbone (Samuelsen *et al.*, 1999; Guo *et al.*, 2008). The unique molecular structure of psyllium arabinoxylans is associated with their unusual physico-chemical and physiological properties, such as strong gelling potential and low fermentability by the intestinal microflora.

A unique feature of arabinoxylans is the presence of hydroxycinnamic acids, ferulic and *p*-coumaric, esterified to *O*-5 of Ara*f* linked to *O*-3 of the xylose residues (Fig. 23.7) (Smith and Hartley, 1983). Ferulate esters can dimerize via phenoxy radicals into dehydrodiferulate esters, which are responsible for covalent crosslinking between arabinoxylans chains. Three phenoxy radicals of ferulic acid give rise to at least five known diferulate esters with 8-5′, 8-*O*-4′, 8-8′ being the most common (Fig. 23.7). Higher oligomers of ferulic acid such as 5-5′/8′-*O*-4″, 8-*O*-4′/8′-*O*-4″, and 8-8′/4′-*O*-4″ coupled dehydrotriferulic acid were isolated from pericarp cell wall of maize (Bunzel *et al.*, 2003; Funk *et al.*, 2005). Covalent crosslinking via diferulate bridges is partially responsible for insolubility of arabinoxylans; the degree of crosslinking in insoluble arabinoxylans was reported to be 8 to 39 times higher than in their water-soluble counterparts (Bunzel *et al.*, 2001). Ferulic acid residues are also involved in crosslinking between arabinoxylans and lignin or arabinoxylans and protein (via tyrosine), thus promoting cohesion, restricting cell expansion, and controlling the mechanical properties of mature tissues (Piber and Koehler, 2005). Thermal stability of cell adhesion and maintenance of crispness of plant-based foods (e.g., water chestnut after cooking), gelling properties of cereal arabinoxylans, and limited cell wall degradability by ruminants are related to the formation of ferulate crosslinks (Parker *et al.*, 2003; Grabber *et al.*, 1998; Izydorczyk *et al.*, 1990).

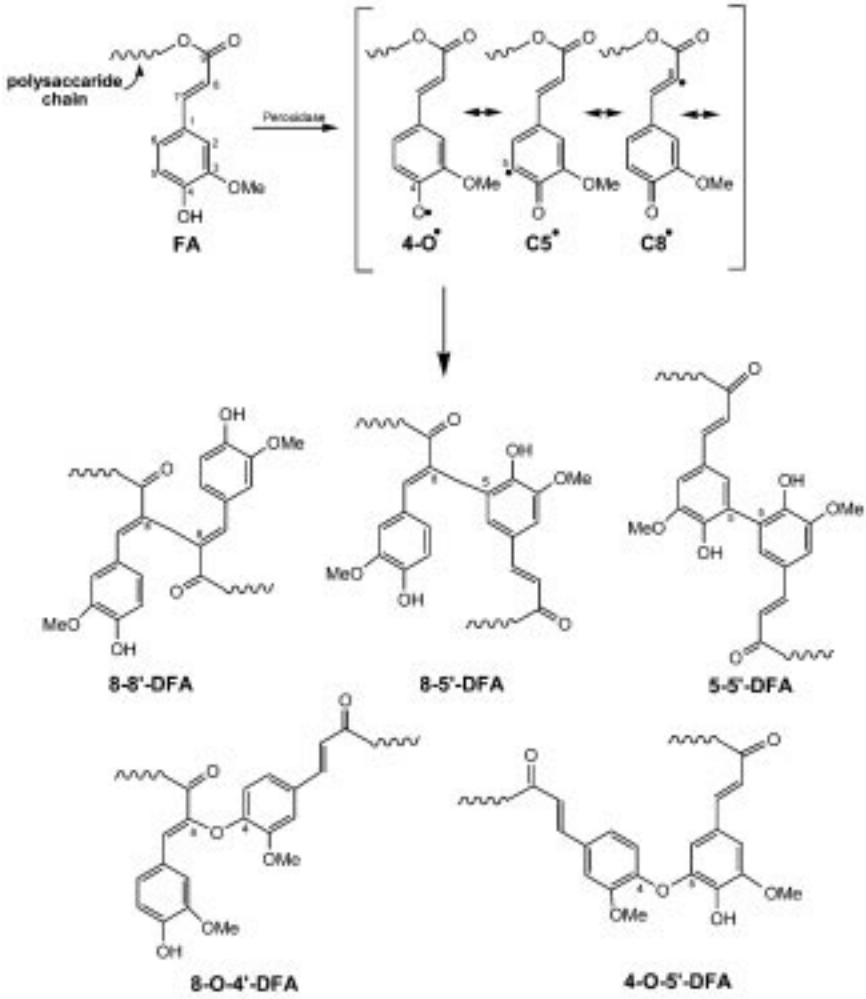

Fig. 23.7 Three electron-delocalized phenoxy radicals of ferulic acid generated by horseradish peroxidase/H_2O_2, and molecular structures of five known dehydrodiferulic acids.

23.4.2 Structural heterogeneity

The molecular features of arabinoxylans that can be at variance depending on their genotypic or cellular origin include the ratio of arabinose to xylose residues (Ara/Xyl), the substitution pattern and the average degree of polymerization (DP) of the xylan backbone, and the presence of other substituents. The ratio of Ara/Xyl residues indicates a degree of branching in the polymer chain. In cereals, the Ara/Xyl ratio may generally vary from 0.3 to 1.1 depending on the CW type. In wheat, arabinoxylans in outer pericarp, scutelum, and embryonic axis are relatively highly substituted with arabinose (Ara/Xyl > 1), whereas

those of aleurone (Ara/Xyl < 0.5), and especially hyaline layer are poorly substituted (Ara/Xyl < 0.3) (Antoine *et al.*, 2003; Barron *et al.*, 2007; Saulnier *et al.*, 2007). The ratio of Ara/Xyl in wheat endosperm generally varies from 0.50 to 0.71. Similar differences in the degree of substitution of arabinoxylans due to their histological origin have also been demonstrated in barley (Izydorczyk and Dexter, 2008). The ratios of Ara/Xyl in arabinoxylans from pericarp tissues were almost twice as high as in arabinoxylans from aleurone of both hulled and hull-less barley cultivars; whereas arabinoxylans from the endosperm CW exhibited intermediate values (Table 23.2).

The extent of feruloylation of arabinoxylans in barley and wheat is also tissue dependent (Table 23.2). Arabinoxylans in the CW of starchy endosperm are very lightly esterified with ferulic acid, whereas the arabinoxylans of aleurone and pericarp tissues are highly esterified. *p*-Coumaric acid, although in lower abundance than ferulic acid, is associated with arabinoxylans from pericarp. Notably, the arabinoxylans in the CW of starchy endosperm and aleurone have low amounts of esterified dehydrodiferulic acid (DDFA), whereas those in pericarp are extensively crosslinked by DDFA (Table 23.2).

The cellular variations are masked when arabinoxylans are extracted from whole grains. Storsley *et al.* (2003) reported the ratio of Ara/Xyl in the range of 0.59–0.77 for water-extractable and in the range of 0.51–0.64 for water-unextractable arabinoxylans from the whole grain of eight Canadian hull-less barley genotypes. Similar values of Ara/Xyl (0.64–0.78) were reported by Oscarsson *et al.* (1996) for arabinoxylans from six Swedish genotypes of hull-less barley. In rye whole grain, the ratio of Ara/Xyl may vary from 0.50–0.82 (Vinkx and Delcour, 1996). The Ara/Xyl ratios of wheat arabinoxylans were also cultivar dependent, as shown by the range of Ara/Xyl in water-extractable (0.47–0.58) and water-unextractable arabinoxylans (0.51–0.67) obtained from 20 French wheat flours (Ordaz-Ortiz and Saulnier, 2005).

The fine molecular structure of arabinoxylans is better described by the substitution pattern of the xylan backbone with Ara*f* residues. All four structural elements, unsubstituted, monosubstituted (at *O*-2 or *O*-3), and disubstituted (*O*-2,3) Xyl*p* residues have been identified in cereal arabinoxylans, but their relative amount vary with genotypic and cellular origin of these polysaccharides. For example, in wheat, the monosubstituted Xyl*p* units at *O*-2 are rare (0.3–1.9%) (Cleemput *et al.*, 1995; Saulnier *et al.*, 2007) whereas in barley such substituted Xyl*p* residues are relatively abundant (Oscarsson *et al.*, 1996; Izydorczyk *et al.*, 2003b). The content of *O*-2 monosubstituted Xyl*p* ranged from 6% to 16% in water-extractable arabinoxylans from whole meal of hull-less and hulled Swedish genotypes (Oscarsson *et al.*, 1996), and from 6% to 14% in $Ba(OH)_2$-extractable arabinoxylans from Canadian hull-less genotypes (Storsley *et al.*, 2003).

The water-soluble arabinoxylans from barley grain generally contain 47–65% of unsubstituted, 20–25% monosubstituted and 19–26% disubstituted xylose residues (Oscarsson *et al.*, 1996; Izydorczyk *et al.*, 1998a, 1998b; Dervilly *et al.*, 2002; Storsley *et al.*, 2003; Trogh *et al.*, 2004). Some genotypic differences in

the patterns of substitution were observed when the alkali-extractable arabinoxylans from eight Canadian hull-less barleys were investigated; it was noted, for example, that the amount of unsubstituted Xyl*p* was higher in high amylose and normal genotypes than in waxy lines, but some variations were also observed between lines within the same type of barley (Storsley *et al.*, 2003). The differences in substitution patterns in water-extractable arabinoxylans from pearling by products and fiber-rich fractions were more pronounced; in general, the water-extractable arabinoxylans from PBPs were more substituted, contained a lower amount of unsubstituted Xyl*p*, and higher amount of monosubstituted Xyl*p* at *O*-2 than their counterparts in the fiber-rich fraction.

It is generally agreed that cereal arabinoxylans are highly polydisperse and consist of range of structures with different degree and pattern of distribution. However, the distribution of Ara*f* along the xylan backbone is non-random in all cereal arabinoxylans. The tentative structural models for arabinoxylans suggest that highly substituted regions, abundant in disubstituted Xyl*p* and sequences of contiguously substituted Xyl*p*, are separated by less densely substituted regions, with blocks of contiguously unsubstituted Xyl*p* and Xyl*p* residues more frequently mono- than disubstituted (Vietor *et al.*, 1992; Gruppen *et al.*, 1993; Izydorczyk and Biliaderis, 1995; Vinkx *et al.*, 1995). For the water extractable arabinoxylans from wheat endosperm, it was shown that the less substituted fractions of wheat arabinoxylans, obtainable by precipitation at a relatively low concentration of ammonium sulphate or ethanol, may contain sequences of at least up to six but possibly more contiguously unsubstituted xylose residues. In the highly substituted regions (or entire chains), on the other hand, blocks of up to six contiguously substituted xylosyl residues were found (Dervilly-Pinel *et al.*, 2004). It appears that during biosynthesis of arabinoxylans, the arabinosyl tranferases favour di-substitution and contiguous substitution of xylosyl residues and that arabinoxylans initially deposited in CW are heavily substituted with Ara*f* residues (Gibeaut *et al.*, 2005). The de-arabinosylation, probably mediated by the action of arabifuranohydrolases, is occurring in response to external stresses during the plant development, such as changes in temperature, availability of metabolites or the presence of pathogens. The removal of Ara*f* from the initially heavily substituted arabinoxylans is in agreement with the non-random pattern of Ara*f* substitution along the xylan backbone (Dervilly-Pinel *et al.*, 2004).

23.5 Analysis and detection

The content of arabinoxylan in grain and grain fractions can be determined by the colorimetric phloroglucinol-HCl or orcinol-HCl reactions which show high but not absolute specificity for pentoses (Douglas, 1981). Alternatively, the arabinoxylans content can be derived from the amount of Ara and Xyl residues following complete hydrolysis of grain/tissues or preparations containing these polymers, followed by chromatographic analyses (HPLC or GC) (Izydorczyk

and Biliaderis, 1995). The water soluble arabinoxylans can be quantified after extraction with water and determination of pentose sugars by colorimetric or chromatographic methods. Viscosity measurements of the water extracts from ground grain provide an indirect way of estimating the content of water-soluble arabinoxylans; this method, however, is affected by the presence of endogenous or microbial endoxylanases that would affect the results (Saulnier *et al.*, 1995b). NIR spectroscopy can also measure arabinoxylans content (Hong *et al.*, 1989; Kacuráková *et al.*, 1994).

^{1}H-NMR and ^{13}C-NMR spectroscopy and methylation analysis are considered reference methods to elucidate the fine molecular features of arabinoxylan's structure such as linkage composition and the presence and amount of sugar residues and various substitutions (Izydorczyk and Biliaderis, 1995). These methods, however, require relatively large amount of isolated and purified material and are tedious to use on a large number of samples. Ferulic acid monomers and dimers may be analyzed directly by HPLC after saponification (Waldron *et al.*, 1996) or by GC as their trimethylsilyl derivatives (Lam *et al.*, 1990).

Partial hydrolysis of arabinoxylans with endoxylanases and subsequent chromatographic analysis of the hydrolysis products, constitute an alternative method to study the molecular structure of these polymers. Arabinoxylooligomers and xylooligomers can be separated and quantified by high performance anion exchange chromatography, whereas the structure elucidation of the oligomers can be conducted by ^{1}H-NMR spectroscopy or matrix-assisted laser desorption/ionization time-of-flight/time-of-flight (MALDI-TOF/TOF) tandem mass spectrometry (Maslen *et al.*, 2007). The xylanase action is influenced by the structure of arabinoxylans, and the type and amount of specific degradation products give information about their molecular features. Xylanase hydrolysis can be carried out on various grain tissues, flour, and whole grain (Ordaz-Ortiz *et al.*, 2004).

Fourier transform infrared (FT-IR) and FT-Raman spectroscopy can be applied in complementary ways to determine structural features of arabinoxylans (Kacuráková *et al.*, 1999; Philippe *et al.*, 2006a). Both FT-IR and Raman microspectroscopy have been shown to be useful in investigation of *in-situ* cell wall composition. FT-IR microspectroscopy with principal component analysis discriminates between AX with different degrees of arabinosylation in walls of starchy endosperm and aleurone (Barron *et al.*, 2005; Robert *et al.*, 2005; Toole *et al.*, 2007). Raman spectroscopy (Piot *et al.*, 2001; Barron *et al.*, 2006; Philippe *et al.*, 2006b) also allows determination of structural heterogeneity and relative concentrations of arabinoxylans and additionally of ferulic acid content and protein ratios in walls of grain sections. Philippe *et al.* (2006a) used confocal Raman microspectroscopy to determine spatial and temporal changes in the structure of arabinoxylans during grain development.

A number of polyclonal and monoclonal antibodies, raised against AX with both high and low degrees of substitution, allows their location and

differentiation in cereal tissues with light and electron microscopy (Migne *et al.*, 1999; Ordaz-Ortiz *et al.*, 2004; McCartney *et al.*, 2005). Using gold-labeled monoclonal antibodies to arabinoxylans and β-glucans and autofluorescence, Philippe *et al.* (2007) tracked the changes in the composition of cell walls and the deposition of ferulates in the developing wheat grains.

23.6 Physico-chemical properties

23.6.1 Molecular weight

The reported molecular weight values for arabinoxylans have shown considerable variations influenced by extraction procedures, purity of preparations, and methods of determination (Izydorczyk and Biliaderis, 1995). Specific chain conformation, polydispersity with regard to molecular mass, and possible molecular aggregation pose some difficulties associated with accurate determination of the molecular weight of arabinoxylans by size exclusion chromatography using dextran or pullulan standards for calibration. More recently, the use of light scattering detectors and application of suitable techniques (e.g., alkali solvents, sonication) to aid solubilization and prevent chain aggregation alleviated some of the problems. The weight average molecular weight (M_w) of water extractable arabinoxylans of wheat of approximately 300,000 Da was determined by Dervilly *et al.* (2001) using HPSEC with light scattering detector. As shown in Fig. 23.8, a range of M_w varying from 220,000 to 700,000 Da was determined for arabinoxylans fractions obtained by sequential fractionation of polymers isolated from wheat flour with water. Dervilly *et al.* (2001) also reported several populations with M_w varying from 70,000 to 655,000 Da and Ara/Xyl ratio from 0.4 to 1.2 obtained from water extractable wheat arabinoxylans with M_w of 280,000 Da and polydispersity index (M_w/M_n) of 1.8. The water-insoluble arabinoxylans require extraction

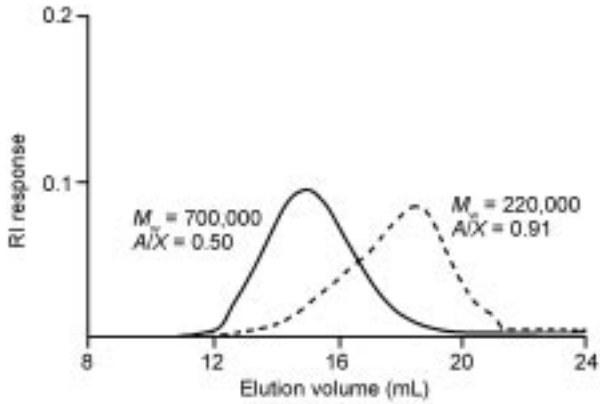

Fig. 23.8 High performance size exclusion chromatographs (HPSEC) of two arabinoxylan preparations from wheat flour. The average M_w were determined by HPSEC-multiangle light scattering detector (unpublished results).

procedures that may cause degradation of the polymeric chains. Nevertheless, higher values (850,000 Da) were reported for alkali extractable wheat endosperm arabinoxylans than for their water soluble counterparts (Gruppen *et al.*, 1992). Also high M_w values were reported for $Ba(OH)_2$-extractable arabinoxylans from hull-less barley (850,000–2,430,000 Da) (Izydorczyk *et al.*, 2003b). Lowly substituted arabinoxylans (corn bran, rye bran) have a strong tendency to aggregate that precludes them from proper characterization in aqueous systems without chemical derivatization (e.g., xylan sulphates or carbanilates) (Ebringerova and Heinze, 2000).

23.6.2 Solution properties

The solubility of arabinoxylans in aqueous solutions is closely related to the presence of the arabinose substituents along the xylan backbone. Andrewartha and co-workers (1979) demonstrated that with removal of Ara*f* side units the solubility of arabinoxylans can drastically decline, especially below the Ara/Xyl ratio of 0.43. It is postulated that the debranched chains aggregate, and eventually precipitate out of solution, by forming non-covalent associations between unsubstituted regions of the polymer chains. The solubility of arabinoxylans is also affected by the pattern of distribution of Ara*f* along the xylan backbone as well as by the chain length. Izydorczyk and MacGregor (2000) provided empirical evidence of non-covalent interactions between sparsely substituted arabinoxylan chains (Ara/Xyl 0.18–0.32) and cellulose-like fragments of β-glucans. The initial solubility/extractability of arabinoxylans from the CW networks is directly related to the extent of non-covalent self-association and intermolecular interactions with other CW constituents as well as to covalent crosslinking of arabinoxylans (through ferulic acid residues) among themselves or to lignins and proteins.

Recent studies, based on light scattering measurements, indicate that arabinoxylan molecules may have extended lengths, and in solution behave as locally stiff, semi-flexible random coils whose flexibility is largely unaffected by the Ara/Xyl ratio in the range of 0.39–0.82 (Dervilly-Pinel *et al.*, 2001; Picout and Ross-Murphy, 2002). The conformational parameters such as the exponent 'a' of 0.74, derived from the Mark–Houwink equation ($[\eta] = KM_w^a$) and the hydrodymanic parameter 'v' of 0.47, calculated from the radius of gyration and molecular weight relationship ($R_g \approx M_w^v$), determined for water-soluble fractions of wheat arabinoxylans, indicated a random coil conformation for these polymers. However, the persistent length (L_p) parameter, which is a direct measure of polymer chain rigidity, was reported to be 7.8 ± 1.4 nm (Dervilly-Pinel *et al.*, 2001) or 3.1 ± 0.3 (Picout and Ross-Murphy, 2002), and indicated that arabinoxylans chains are semi-flexible in comparison with very flexible polymers such as pullulans ($L_p = 1.7$ nm) or very stiff polymers represented by xanthan gum ($L_p = 100$–150 nm).

Due to the high M_w and locally stiff semi-flexible coil conformation, arabinoxylan chains occupy large hydrodynamic volumes (Izydorczyk and Biladeris,

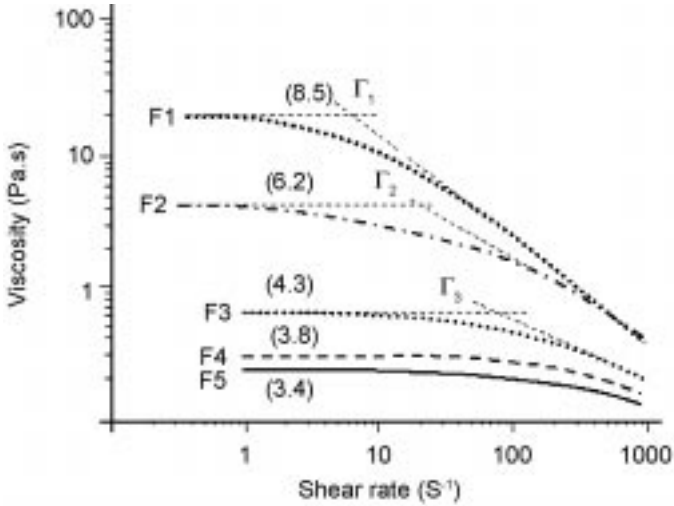

Fig. 23.9 Effect of shear rate on the apparent viscosity of aqueous solutions of arabinoxylan fractions with variable intrinsic viscosity values (indicated in brackets). Adapted from Izydorczyk and Biladeris (1992a).

1992b; Vinkx and Delcour, 1996), and, therefore, display viscosity building properties in solutions. The viscosity of arabinoxylan solutions is dependent on polymer concentrations and M_w (Figs 23.9 and 23.10). In dilute solutions, the zero shear rate specific viscosity $(\eta_{sp})_0$ increases linearly (with a slope ~ 1) with increasing $c[\eta]$, where c is the concentration and $[\eta]$ the intrinsic viscosity (Table 23.3). Above the critical polymer concentration, marking the onset of coil overlap, the viscosity dependence on polymer concentration is much greater (slope ~3.7–3.9 for wheat arabinoxylans) (Table 23.3) (Izydorczyk and Biliaderis, 1992b). The apparent viscosity of concentrated arabinoxylan solutions is also strongly dependent on the rate of shear; at low shear rates arabinoxylan solutions behave like Newtonian fluids, whereas at high shear rates they exhibit shear thinning characteristic for pseudoplastic materials (Figs 23.9 and 23.10). Izydorczyk and Biliaderis (1992b) showed that wheat arabinoxylans with relatively high molecular weights may form weak pseudo-gels as evidenced by changes of the viscoelastic properties of arabinoxylan fractions from that of viscous solution ($G'' > G'$ at all frequencies) to weakly elastic ($G' > G''$ at higher frequencies) (Fig. 23.10). Warrand *et al.* (2005) emphasized the importance of hydrogen bonds in stabilizing arabinoxylan macrostructures in aqueous solutions. These weak gel properties of arabinoxylans can be diminished in the presence of chaotropic salts, known for weakening the hydrogen bonds between solute molecules. The viscosity building properties are probably the most important characteristics responsible for many technological properties of arabinoxylans in food systems and functions in the digestive tract of humans (Lu *et al.*, 2000; Zunft *et al.*, 2004).

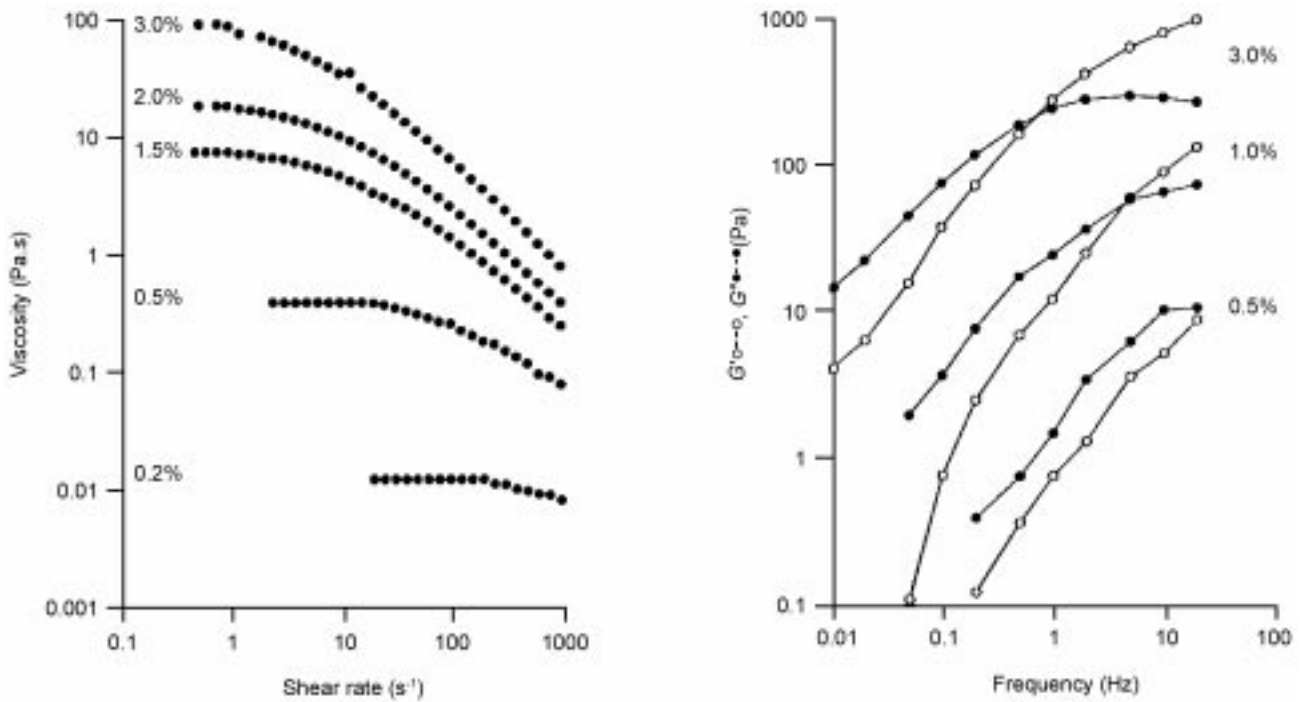

Fig. 23.10 Effect of shear rate and polymer concentration on the apparent viscosity of arabinoxylan solutions (left); and frequency dependence of elastic (G') and viscous (G'') moduli at various polymer concentration. Adapted from Izydorczyk and Biladeris (1992a).

Table 23.3 Molecular and physico-chemical characteristics of arabinoxylan fractions obtained by stepwise precipitation with ammonium sulphate and gel filtration chromatography

Arabinoxylan fraction	Ara/Xyl	$[\eta]$ (dl/g)	c^* (g/100 ml)	Slope		Elastic modulus[c], G' (Pa)
				Dilute region	Concentrated region	
F60[a]	0.58	4.70	0.26	1.13	2.19	35.0
F70	0.71	4.20	0.31	1.12	2.04	0.33
F80	0.85	3.16	0.38	1.07	2.00	0.05
F95	0.88	1.90	nd	nd	nd	0.02
F1[b]	0.69	8.5	0.17	1.1	3.9	61.4
F2	0.68	6.2	0.20	1.1	3.7	48.0
F3	0.66	4.3	0.28	1.1	3.8	15.5
F4	0.65	3.8	0.29	1.1	3.8	6.8
F5	0.63	3.4	0.30	1.1	3.9	4.9

[a] Fractions obtained by stepwise precipitation of isolated and purified water-soluble wheat flour arabinoxylans with ammonium sulphate; the numbers 60–95 indicate the saturation level of ammonium sulphate.
[b] Isolated and purified water-soluble wheat flour arabinoxylans were fractionated by size exclusion chromatography.
[c] Solutions of arabinoxylan fractions were treated with horseradish peroxidase (0.22 PU/ml) and H_2O_2 (1.5 ppm); the reported values are for gels/viscous solutions at polymer concentration of 1.0% w/w.
Adapted from Izydorczyk and Biliaderis (1992a,b).

23.6.3 Gelation

Cereal arabinoxylans containing ferulic acid residues are capable of forming gel networks stabilized by covalent crosslinks between feruloyl groups of neighboring chains. Gelation of arabinoxylans is induced by free radical generating agents such as H_2O_2/peroxidase, laccase/oxygen, linoleic acid/lipoxygenase, ferric chloride. Several dehydrodiferulates 8-5′, 8-*O*-4′, 8-8′, and 5-5′ in the proportion 5:3:1:1 are formed by H_2O_2/peroxidase catalyzed oxidative coupling indicating that both the aromatic ring and the propenoic chain are involved in dimerization. As gelation of arabinoxylans proceeds, the peaks at λ_{max} 320 and 375 nm in the UV spectra due to ferulic acid residue diminish, since the newly formed dehydrodiferulates have lower absorption (Izydorczyk *et al.*, 1990). The gelation progress can also be followed by small oscillatory measurements. Usually, a rapid rise of the elastic modulus (G') upon addition of the free radical agent is followed by slower increases; this behavior is attributed to formation of crosslinks between neighboring chains followed by limited restructuring due to impediment of chain movements associated with dramatic increases of viscosity (Fig. 23.11(a)). The kinetics of gelation depend on temperature, pH, and the type and concentration of the oxidizing agent.

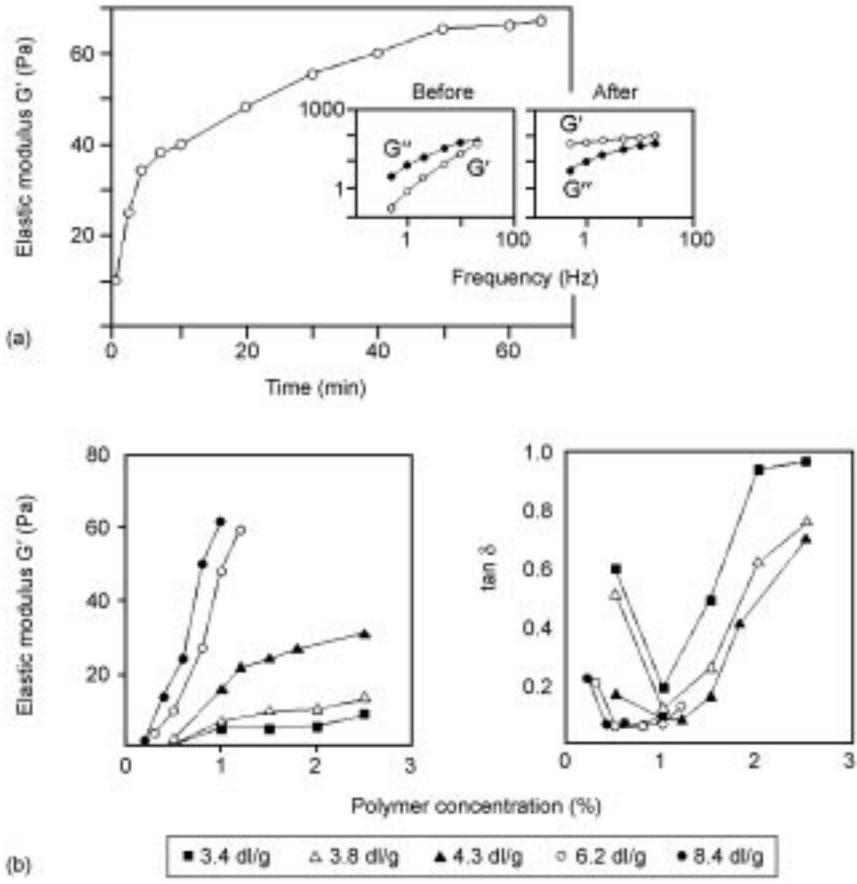

Fig. 23.11 (a) Development of elastic modulus (G') during gelation of arabinoxylans. Insets show the mechanical spectra of arabinoxylans before and after gelation. (b) Concentration and molecular size dependence of elastic modulus (G') for arabinoxylan fractions with various intrinsic viscosities after 1 h of treatment with horseradish peroxidase (0.22 PU/ml/H_2O_2 (1.5 ppm) at 15 ºC. Adapted from Izydorczyk and Biladeris (1992a,b).

Molecular features of arabinoxylans affect the gelling potential and rigidity of gels. Generally, strong gel networks are obtained with arabinoxylans having high content of ferulic acid residues, high molecular weight (Fig. 23.11(b)) and a low degree of substitution (Table 23.3) (Rattan *et al.*, 1994; Izydorczyk and Biliaderis, 1995; Dervilly-Pinel *et al.*, 2001; Carvajal-Millan *et al.*, 2005). The concentration of polymers and oxidizing agents also affect the rate and extent of gelation. Simultaneous formation of non-covalent bonds (H-bonding) between arabinoxylans chains (governed by their molecular features) contributes to the overall strength of arabinoxylans gels (Carvajal-Millan *et al.*, 2005).

23.7 Technological functionality and potential applications of arabinoxylans

23.7.1 Role of arabinoxylans in bread-making

Arabinoxylans play a significant role in various stages of the bread-making process as well as affecting the storage, organoleptic, and nutritional properties of the final product. Clear distinction, however, has to be made between water-soluble arabinoxylans and the population of water-unextractable polymers. The influence of arabinoxylans is already evident during the first steps of dough preparation, involving water addition and ingredient mixing. According to Bushuk (1966), arabinoxylans in wheat flour have disproportionally high water absorption properties (22%) in relation to their absolute content. When added to wheat flour, both water-soluble and water-unextractable arabinoxylans absorb large amounts of water, thus depleting the pool available for proper gluten development and film formation. Biliaderis *et al.* (1995) used two preparations of arabinoxylans to demonstrate the effect of molecular size of these polymers on baking absorption and showed that the high molecular weight arabinoxylans increased the farinograph water absorption to a greater extent than their low molecular weight counterparts. The addition of water soluble AX to dough negatively affects gluten yield and results in an increased resistance of dough to extension and a decrease in dough extensibility (Michniewicz *et al.*, 1991; Wang *et al.*, 2004). These effects, however, can be corrected by adding more water or adding xylanase containing enzyme preparations which release arabinoxylan-bound water.

The involvement of arabinoxylans in moderating dough behavior and improving loaf volume was clearly demonstrated by the action of endogenous and added xylanases (McCleary, 1986; Rouau *et al.*, 1994; Petit-Benvegnen *et al.*, 1998). Water-soluble arabinoxylans are believed to increase the viscosity of the dough aqueous phase and, therefore, to have a positive effect on the dough structure and its stability, especially during the early baking processes when a relatively high pressure is generated inside the gas cells. The increased stability of the film surrounding the gas cells is useful in prolonging the oven rise and preventing coalescence. Santos and co-workers (2005) reported that the presence of water-soluble pentosans reinforced the gluten network and decreased the irreversible changes occurring during heating the gluten. As a consequence, loaf volume and crumb structure are enhanced by the addition of water soluble arabinoxylans to doughs (Fig. 23.12) (Delcour *et al.*, 1991; Michniewicz *et al.*, 1991; Biliaderis *et al.*, 1995). The overall effect of arabinoxylans on the bread-making process is, however, dependent on the concentration and molecular structure (size) of these polymers in the dough system. Higher than optimum amount of arabinoxylans may cause viscosity buildup and hinder their beneficial effects.

Water-insoluble arabinoxylans that remain in a dough system as discrete particles (i.e., cell wall fragments) can form physical barriers for the gluten network during dough development. The resulting gluten has lower extensibility

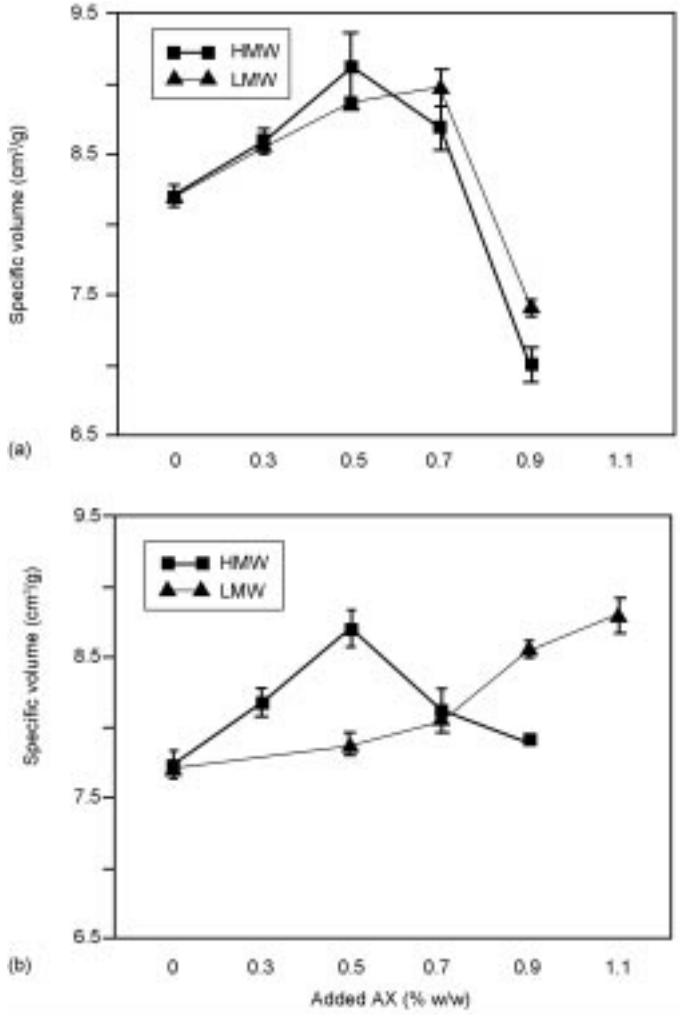

Fig. 23.12 Effect of concentration of high and low molecular weight arabinoxylan preparations on specific volume of bread baked from good (a) and poor (b) quality wheat flours. Reproduced with permission from Biliaderis *et al.* (1995).

and a lower rate of aggregation (Wang *et al.*, 2003). During fermentation, water insoluble arabinoxylans decrease the film stability. Water-unextractable arabinoxylans depress loaf volume and create coarser and firmer crumbs. However, their negative effects can be reversed by using endoxylanases with specificity towards the water-insoluble arabinoxylans (Courtin and Delcour, 2002). These enzymes catalyze the conversion of detrimental water-insoluble arabinoxylans to the high molecular weight water-soluble counterparts. The addition of optimal doses of xylanases has been reported to improve dough consistency, fermentation stability, oven rise, loaf volume, and crumb structure

and softness (Hilhorst *et al.*, 1999; Courtin and Delcour, 2002; Jiang *et al.*, 2005).

Biliaderis *et al.* (1995) demonstrated that over a 7-day storage period, the arabinoxylan-fortified breads exhibited lower crumb firmness than the controls. The effects were attributed to the increased moisture content of breads substituted with arabinoxylans and to the plasticizing effects of the additional water on the starch–gluten matrix. Contrary to the observed decrease in crumb firmness, bread supplemented with arabinoxylans showed higher starch retrogradation rates, as measured by differential scanning calorimetry and x-ray diffraction. The enhanced kinetic of chain re-ordering in amylopectin was attributed to greater mobility of the starch molecules in the presence of additional water. As previously established (Zeleznak and Hoseney, 1986) starch retrogradation increases with increasing moisture content, especially between 20 and 45% moisture in the system. Gudmudson *et al.* (1991) showed that the rate of amylopectin crystallization in starch gels containing arabinoxylans is either increased or decreased, depending on the final water content of starch; retrogradation was minimal in starch gels with moisture content below 20% and increased significantly in gels with moisture content between 20 and 30%. Breadcrumb supplemented with arabinoxylans had a coarse alveolar structure compared with control breads and breads baked with added soluble protein; the results were interpreted in terms of the different effects of added polysaccharides and protein on the properties of the liquid film over the alveoli (Fessas and Schiraldi, 1998).

23.7.2 Applications of arabinoxylans

Aqueous extraction of arabinoxylans from grain and agricultural by-products provide potentially suitable material for use of arabinoxylans as gelling agents, cryostabilizers, as a source of prebiotics, and various non-food applications such as film formation and cosmetics.

Arabinoxylans gels exhibit tremendous water-holding capacity of up to 100 g of water per gram of dry crosslinked polymer. The hydration properties of crosslinked arabinoxylans are not sensitive to electrolytes contrary to many synthetic hydrogels. The capacity of arabinoxylans to imbibe water increases with increasing number of effective crosslinks up to the optimum concentration, beyond which water penetration into the network and swelling are impeded due to a high density of crosslinks. Vansteenkiste *et al.* (2004) showed that proteins embedded in the arabinoxylan gel network resist enzymic hydrolysis. Since the rheological properties of arabinoxylan gels can be controlled by either altering the initial ferulic acid content or the concentration of polymers before gelation, their capacity to load and release proteins can also be manipulated (Carvajal-Millan *et al.*, (2005). The possibility to modulate protein release from arabinoxylan gels makes them useful for controlled delivery of therapeutic proteins and potentially for controlled releases of active agents in the food, cosmetic and pharmaceutical industries.

Film formation

Carbohydrate films and coatings can effectively control moisture, oxygen, lipid, aroma, and flavor of food systems, thus improving quality, safety, and shelf-life of products. Arabinoxylans from various grains and by-products have been shown to form films of high strength and lubricity with and without addition of plasticizers. Arabinoxylans from maize bran have been cast into edible films which are soluble, form a continuous and cohesive matrix, with neutral taste and odor, and have mechanical barrier properties similar to films of gluten or starch (Chanliaud, 1995). Arabinoxylans from barley husk have been shown to form films without addition of plasticizers (Sternemalm *et al.*, 2008).

The material properties and performance of arabinoxylan films can be further enhanced. Hydrophobic films have been produced by covalent binding of lauryamine chains to maize bran arabinoxylans (Fredon *et al.*, 2002). Composite hydrogenated palm oil-arabinoxylan emulsion films were shown to have improved water barrier efficiency and surface hydrophobicity, but lower tensile strength and elasticity (Péroval *et al.*, 2002). Edible arabinoxylans films with decreased water vapor permeability have been created by grafting ω-3 fatty acids onto arabinoxylans chains using cold oxygen plasma and electron beam irradiation; the targeted application of these films was in dry biscuit coatings to delay moisture absorption from stuffing (Péroval *et al.*, 2003). Partial removal of arabinose residues from the xylan backbone of rye arabinoxylans resulted in formation of semicrystalline films with lower oxygen permeability (Höije *et al.*, 2008). The moderately debranched films with the Ara/Xyl ratio of 0.37 exhibited yielding and had stress/strain behavior similar to synthetic semicrystalline polymers, which in combination with oxygen barrier properties make it attractive for packaging material.

Cryostabilizers

Water-soluble arabinoxylans are potential candidates as cryostabilizing agents in food and drug preparation. Their ability to inhibit ice formation can be explained by enhancement of bulk viscosity and mechanical interference with growth of ice crystals on cooling. The state diagrams of wheat arabinoxylans-H_2O binary mixtures have been determined (Fessas and Schiraldi, 2001) using two preparations with Ara/Xyl ratios of 0.66 and 0.51, molecular weight 235,000 and 650,000 Da and dispersity values of 4.2 and 1.6, respectively. The glass transition temperature, T'_g, the lowest temperature at which liquid is still present, was higher for the higher M_w polymers ($-17\,^{\circ}C$) than for the low M_w ($-35\,^{\circ}C$) and the X'_g, which is related to the solute composition at T'_g, was one order of magnitude lower for the low M_w than the high M_w arabinoxylans.

Surface active agents

Some arabinoxylans exhibit surface active properties that make them suitable for stabilizing emulsions and protein foam. The small amounts of phenolic compounds and proteins may give arabinoxylans some capabilities for hydrophobic interactions. The abilities of arabinoxylans to retain gas in dough

and protect protein foam against thermal disruption were attributed to their viscosity and film-forming properties (Hoseney, 1984; Izydorczyk *et al.*, 1991).

23.8 Physiological effects of arabinoxylans

Since humans do not produce any autochthonous enzymes that degrade the cell wall polysaccharides, arabinoxylans contribute very little to the digestible energy. Depending on the source and methods of preparations (isolates vs. grain fractions vs. hydrolyzates), arabinoxylans pose properties of soluble and/or insoluble fiber. Due to their thickening properties, water-soluble arabinoxylans are thought to enhance viscosity of the digesta in the small intestine and to impair dispersion and mixing of the food mass with the fluid layer adjacent to the mucosal surface. Arabinoxylans have been implicated in a slowing of the rate of absorption of glucose and reduction of the glycemic response as well as having beneficial effects on long-term weight management. Indeed, consumption of water soluble arabinoxylan-supplemented diets was shown to reduce the postprandial glucose, insulin, and triglyceride in healthy subjects as well as those with impaired glucose tolerances (Lu *et al.*, 2000, 2004; Zunft *et al.*, 2004; Garcia *et al.*, 2006, 2007). However, the exact mechanisms of these metabolic responses have not yet been fully explained (Möhlig *et al.*, 2005; Garcia *et al.*, 2007).

Arabinoxylans are not digested in the small intestine but potentially provide a fermentable carbon source for micro-organisms that inhabit the large bowel. Numerous studies provided evidence that both arabinoxylans and arabino-xylooligosaccharides may enhance the growth of potentially health-promoting bacteria. Intestinal bacteria that can be successfully proliferated by poly- and oligosaccharides include many *Bifidobacterium (Bi.)*, *Bacteroides (B.), Lactobacillia*, and *Clostridia* species. Jaskari *et al.* (1998) and Crittenden *et al.* (2002) reported that xylo-oligosaccharide preparations (DP 2-5) supported the growth of many *Bifidobacterium* and *Bacteroides* species, as well as *Lactobacillus brevis*, but were not fermented by *E. coli*, enterococci, *Clostridium* sp. and the majority of *Lactobacillus* sp. Van Laere *et al.* (2000) generated arabino-xylooligosaccharides with degree of polymerization (DP) 5-10 and doubly-branched xylose residues and observed that they were completely fermented by *Bi. adolescentis, Bi. longum*, and *B. vulgatus*. *Bi. longum*, *Bi. adolescentis* and *B. ovatus* were grown on intact arabinoxylans from wheat, but only *B. ovatus* was supported by glucuronoarabinoxylans from sorghum (Van Laere *et al.*, 2000; Grootaert *et al.*, 2007).

Fermentation of arabinoxylans by human intestinal bacteria is related to the production of short chain fatty acids (SCFA): butyrate, propionate, and acetate, which may have cholesterol-reducing properties and other benefits. Butyrate is a preferential source of energy for colonic mucosal cells and causes differentiation of tumor cells and suppression of cell division *in vitro*, whereas propionate and acetate are metabolized systemically. Hopkins *et al.* (2003) investigated *in vitro*

breakdown of arabinoxylans by the intestinal microbiota, collected from fecal samples from children, and found a relatively high production of propionate together with acetate. Gråsten *et al.* (2003) reported that in healthy humans, the pentosan bread diet increased the concentration of butyrate, whereas the inulin bread diet increased the concentration of acetate and propionate in feces. Fecal levels of SCFA only partly reflect the SCFA production in the colon, because they can be absorbed rapidly in the proximal colon. However, the authors claimed that a high fecal concentration of SCFA indicates also their high level in the distal colon, the site of most colon tumors. In addition, it was postulated that butyrate has beneficial effects on maintaining normal functions in colonocytes, and implied that pentosans are beneficial in the human large intestine (Gråsten *et al.*, 2003).

The molecular structure of arabinoxylans as well as composition and physical form of arabinoxylan preparations seem to affect their fermentability. Water-insoluble arabinoxylans or unsubstituted xylans are not likely to be digested by intestinal microflora. Glistø *et al.* (1999, 2000) and Harris *et al.* (2005) reported that pericarp-rich fractions obtained from rye and wheat exhibited relatively low digestibility by animals compared to endospermic or aleurone tissues. Alkali/xylanase treatments, extrusion or other pre-processing of grain fractions improve the rate and extent of fermentation due to partial solubilization of arabinoxylans (Glistø *et al.*, 2000; Aura *et al.*, 2005).

Water-insoluble and unfermentable portions of arabinoxylans, like other insoluble dietary fibers, increase fecal bulk and decrease the transit time through the colon, thus potentially reducing the residence time of irritants and carcinogens in the colon and alleviating constipation. The water-soluble psyllium arabinoxylans, known for their laxative and cholesterol-lowering properties (Uehleke *et al.*, 2008), possess unique molecular features that obstruct their fermentation by the colonic bacteria and distinguish them from the extensively fermented cereal arabinoxylans (Fischer *et al.*, 2004).

Another beneficial role of arabinoxylans in the human diet might be associated with their chemo-protective and antioxidant properties due to the presence of hydroxycinnamic acids bound to these polymers. Several studies showed that feruloylated arabinoxylans in bran have strong anti-inflammatory properties, inhibit chemically induced carcinogenesis in rats and plays a role in inhibiting lipid peroxidation and low density lipoprotein (LDL) oxidation, and scavenging oxygen radicals (Adam *et al.*, 2002; Rondini *et al.*, 2004). The bioavailability and efficacy of the active phenolic moieties in arabinoxylans could be improved when feruloylated oligosaccharides are released from grain tissues by partial enzymic hydrolysis. Katapodis *et al.* (2003) demonstrated that feruloyl arabinoxylotrisaccharide (FAX_3) from wheat flour had profound antioxidant activity in 2,2-diphenyl-1-picrylhydrazyl reduction assay and inhibited the copper-mediated oxidation of human LDL, whereas Yuan *et al.* (2005) showed that feruloylated oligosaccharides from wheat bran efficiently protected normal rat erythrocytes against hemolysis induced by free radicals under *in vitro* conditions.

23.9 References

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24

Soluble soybean polysaccharide

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Abstract: Soluble soybean polysaccharide (SSPS) is a water-soluble polysaccharide extracted and refined from soybean. SSPS consists mainly of the dietary fibre of the soybean cotyledon and has relatively low viscosity and high stability in an aqueous solution. SSPS has various functions such as dispersion, stabilization, emulsification, and adhesion. Therefore, SSPS can be used not only as a dietary raw material for fibre-fortified food, but also for pharmaceutical and industrial applications as well as for many other food applications. The chemical structure, properties and applications of SSPS as a functional food ingredient are summarized.

Key words: soybean, polysaccharides, structure, function, stabilizer, emulsifier.

24.1 Introduction

Soluble soybean polysaccharide (SSPS) is a water-soluble polysaccharide extracted and refined from soybean. Fuji Oil has been marketing SSPS under the brand name 'SOYAFIBE-S' since 1993. SOYAFIBE-S consists mainly of the dietary fibre of soybean cotyledon and has relatively low viscosity and high stability in aqueous solution.

During the manufacture of soy protein isolate, soymilk and tofu (soybean curd), 'okara', which is the insoluble residue from protein extraction, is produced.[1,2] Okara is a rich source of dietary fibre with reported health benefits and bowel-conditioning effects.[3,4] Therefore, okara can be used as an ingredient in various types of food, including salads, soups, sauces, baked goods, desserts, sausages and okara burgers.[5,6] However, most okara is still used ineffectively and is treated as industrial waste. Its difficult handling is caused mostly by its

high moisture content (about 80%). We started to study the high utilization of okara about 18 years ago and from the research developed the SSPS, SOYAFIBE-S.

SSPS has been shown to be functional as a dispersing agent, stabilizer, emulsifier, and for its adhesion properties.[7,8] Therefore, SSPS can be used not only as a source of raw materials for fibre-fortified foods, but also for pharmaceutical and industrial applications as well as for many food applications. In this chapter, the material structure, properties and applications of SSPS are introduced.

24.2 Manufacture

The water-soluble soybean polysaccharide, SSPS, derives from the cotyledon in the seed of *Glycine max* MERRILL. SSPS is extracted from okara, which is the insoluble material resulting from the manufacture of soy protein isolate, by heating in weak acidic conditions. The patented extraction technique is very efficient to make varieties of SSPS which differ in their physical properties and functionality, such as emulsifying or protein stabilizing. Extraction is followed by refining, pasteurizing, and spray-drying as shown in Fig. 24.1. Reports on the differences in SSPS composition depending on changes in the extraction process have already been published.[9–11] No hazardous chemical material whose residue may cause any safety problems is used in the production of SOYAFIBE-S.

24.3 Structure

24.3.1 General composition

Table 24.1 shows the typical composition of SOYAFIBE-S-DA100 (the most common type of SSPS). The predominant sugar components are galactose, arabinose, and galacturonic acid, but many other sugars such as rhamnose, fucose, xylose and glucose are also present. The composition is similar to that of pectins, acidic polysaccharides found in abundance in the peel of various fruits (e.g., citrus, apple). However, the amount of neutral monosaccharides in SSPS is much higher than in pectins. SOYAFIBE-S is supplied in different types, each

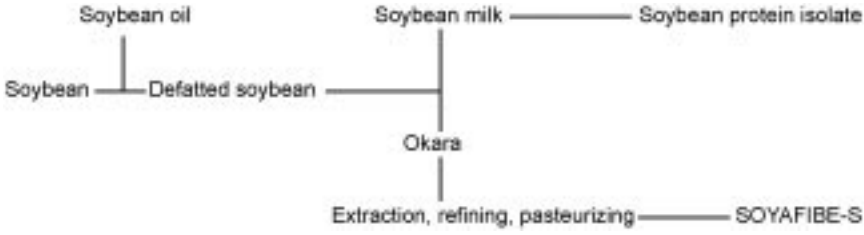

Fig. 24.1 The manufacturing process of SOYAFIBE-S.

Table 24.1 Chemical composition of SOYAFIBE-S-DA100

Moisture (%)	Crude protein (%)	Crude ash (%)	Dietary fibre content[a] (%)	Sugar composition[b] (%)						
				Rha	Fuc	Ara	Xyl	Gal	Glc	GalA
5.8	9.2	8.6	66.2	5.0	3.2	22.6	3.7	46.1	1.2	18.2

[a] The analysis was conducted by AOAC official method (Prosky method).[12]
[b] Neutral saccharides were analysed by GLC after being converted to alditol acetate, and galacturonic acid were by Blumenklantz method.[13]

modified to have specific functionality. For example, a type containing less than 5% residual protein is useful as a protein stabilizer under acidic conditions and also useful as an edible film or coating.

24.3.2 SSPS structure

Soybean okara, which is the residue after oil and protein extraction from soybean, contains soluble and non-soluble dietary fibre from soybean cotyledon. In soluble dietary fibre from soybean cotyledon, the sugar compositions and sequences have been reported,[14–17] but the full aspects of their structure are not fully known.

The gel-filtration chromatographic analysis of SSPS by HPLC shows three components having approximate molecular mass of 550 kDa, 25 kDa, and 5 kDa, respectively, and the mean value is estimated to be several hundred kDa (Fig. 24.2). The chemical structure of the main component of SSPS, having the molecular mass of 550 kDa, has been clarified.[18] This major component of SSPS has three types of units in the main backbone consisting of homogalacturonan and rhamnogalacturonan, composed of the diglycosyl repeating unit, -4)-α-D-

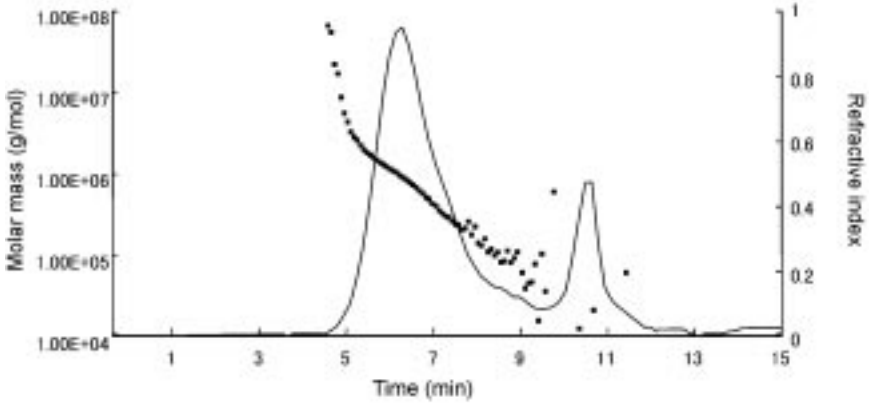

Fig. 24.2 Molecular mass distribution of SSPS obtained by gel permeation chromatography using refractive index and multiangle laser light scattering detection.

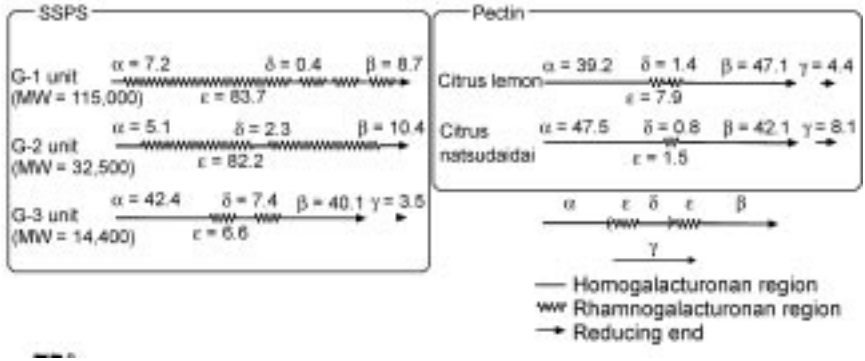

Fig. 24.3 Distribution of galacturonan region in SSPS and citrus pectin.

GalpA-(1→2)-α-L-Rhap-(1-. The SSPS backbone consists of long-chain rhamnogalacturonan and short-chain homogalacturonan, while citrus pectin, for example, consists of short-chain rhamnogalacturonan and long-chain homogalacturonan (Fig. 24.3). The neutral sugar side chains of β-1,4-galactans (degree of polymerization = 43–47), branched with fucose and arabinose residues, and α-1,3- or 1,5-arabinans are linked to the C-4 side of rhamnose residues in the rhamnogalacturonan (Fig. 24.4).[19–21] The physical properties of SSPS have also been studied using static and dynamic light scattering, and a radius of gyration of the polysaccharide of about 23.5 ± 2.8 nm was measured.[22] In addition SSPS is characterized by a compact, globular structure.[22]

24.4 Basic material properties and characteristics

24.4.1 High dietary fibre content

As shown in Table 24.1, the content of dietary fibre as measured by the AOCS official method is more than 60%. Therefore, SSPS has the same physiological functions as those of other dietary fibres. We have found that SSPS is partially metabolized and changed into organic acid by enteric bacteria and effectively shortens the gastrointestinal transit time in rats.[23]

24.4.2 High solubility and stable viscosity against heat, acid and salts

SSPS is a non-gelling polysaccharide soluble in both cold and hot water, and shows a relatively low viscosity compared to the viscosity of other gums or stabilizers such as guar gum, allowing a highly concentrated (more than 30%) solution to be produced (Fig. 24.5).[24] Furthermore, the viscosity of the solution is not significantly affected by heating, addition of acid or salts (Figs 24.6 and 24.7). These physical properties can be explained by the globular structure of SSPS in aqueous solution.

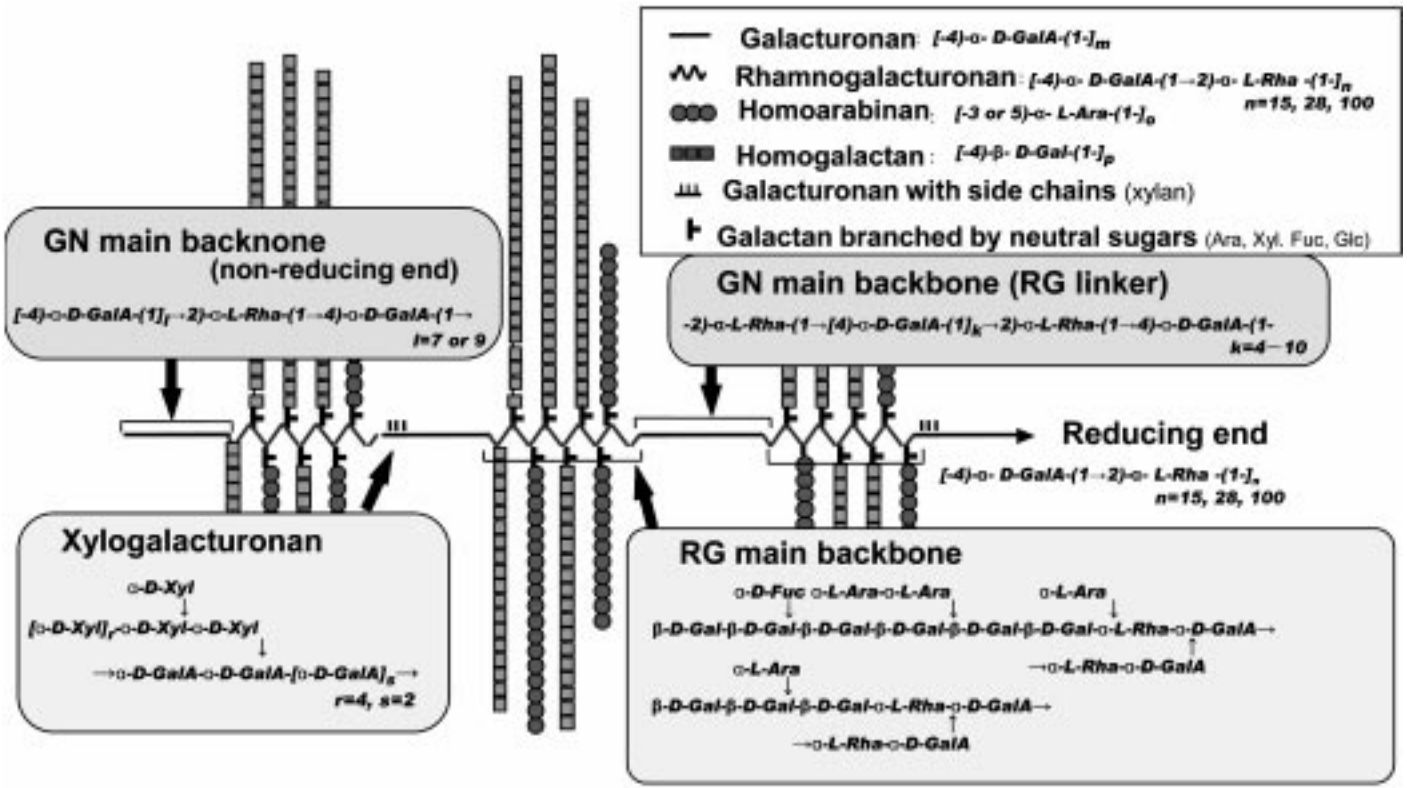

Fig. 24.4 Structure of main fraction of SSPS.

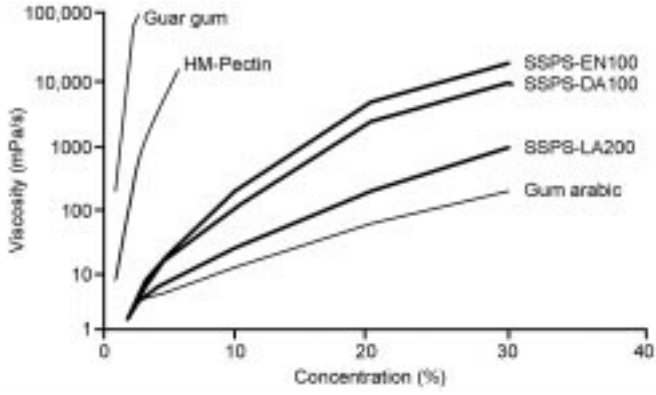

Fig. 24.5 Viscosity comparison of various polysaccharide solutions at 25 °C.

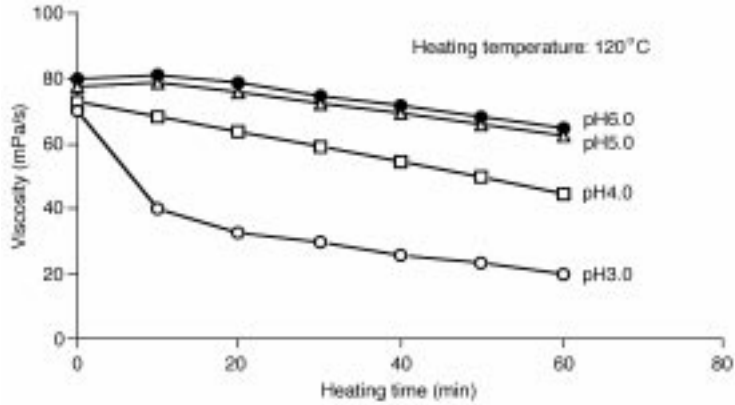

Fig. 24.6 Viscosity change by heating at various pH ranges (10% SSPS aqueous solution).

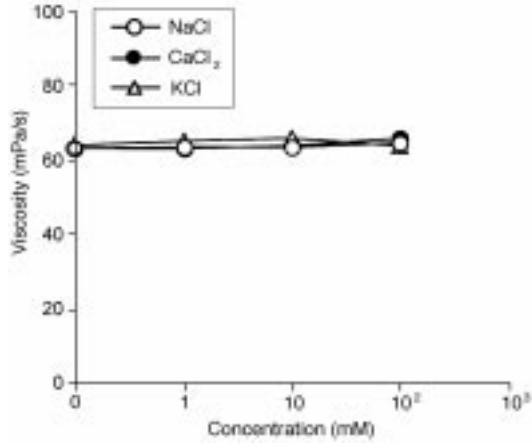

Fig. 24.7 Effect of various salts on viscosity of a 10% SSPS solution at 20 °C.

24.4.3 Excellent adhesive and film-forming property

SSPS has a strong adhesive property. The adhesive strength was tested according to the JIS standard methods, K6848-1987 and K6851-1976. As shown in Table 24.2, the adhesive strength was as strong as or better than that of pullulan (which comes from *Aureobasidium pullulans*), a polysaccharide generally regarded as having very high adhesive strength.[25] With this property, SSPS can be employed as a binder not only in dried food applications like snacks and cereals, but also in paper, wood or glass applications (Table 24.2). Its film-forming properties allow us to make colourless, transparent, water soluble, and edible films. This filmability can also be used to coat the surface of foods such as tablets and other materials.

Table 24.2 Adhesive strength and material property of film

Material	Adhesive strength (kgf/cm^2)	Tensile strength (kgf/cm^2)	Young ratio (kgf/cm^2)
SOYAFIBE-S	46.6	540	9,730
Pullulan	40.5	509	12,800
Gum arabic	30.7	N/D*	N/D*

* The film was easily cracked not allowing the tension to be measured.

24.4.4 Antioxidative property

It has been confirmed that SSPS prevents oxidation of oils and could be employed in the manufacturing of flavour oils.[26] This is presumed to be due to the stabilization of free radicals by the pectic polysaccharides constituting the main component of SSPS. [27] Figure 24.8 shows the antioxidant properties of

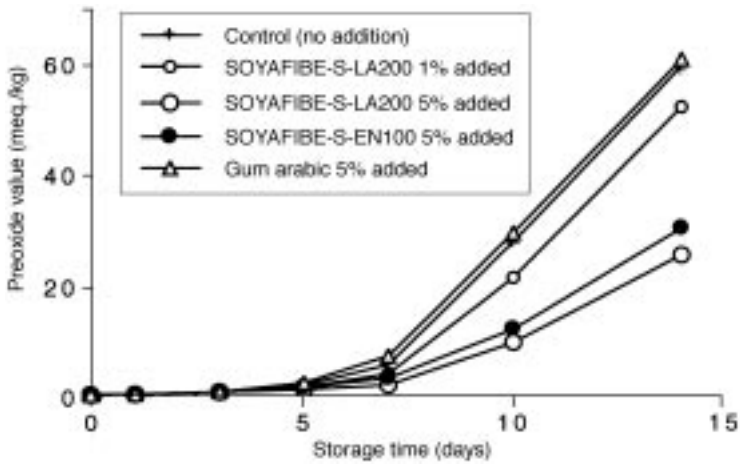

Fig. 24.8 Antioxidative effect of SSPS on soybean oil (stored at 60 ºC).

SOYAFIBE-S-LA200, in a soy oil-based powdered flavour application. When compared with gum arabic (polysaccharide commonly used in the stabilization of flavour emulsions), gum arabic did not show an antioxidative effect.

24.5 Functional properties and reported use of soluble soybean polysaccharide in foods and pharmaceutical applications

Table 24.3 summarizes the functional properties and applications of SOYAFIBE-S, among which dispersion stabilizing, emulsifying, and anti-sticking effects are outstanding. SOYAFIBE-S is supplied in a variety of types tailored for specific applications (Table 24.4).

Table 24.3 Functions and applications of SOYAFIBE-S

Functions	Applications	Type of SOYAFIBE-S
Soluble dietary fibre	General dietary fibre-fortified foods	All types
Stabilizing effects under acidic conditions	Drinkable yoghurt, ice cream, acidic dessert, sour cream	DN DA100
Emulsifying Emulsion stabilizing	Flavour emulsion, powdered flavour, coffee cream, dressing, cleaner	EN100 LN LA200
Adhesion Film-forming property	Edible film, coating agent, particle forming agent, snack, bakery, coating for printing rolls	DN RA100
Dispersing	Various types of paint, agricultural chemicals, ceramics, cement	DN LA200 LA200
Foam stability	Meringue, surfactant	DN DA100 LA200
Anti-sticking effect	Various types of cooked rice and noodles	DN DA100 LA200
Softening effect	Bread, cake, ham, sausage, Kamaboko (boiled fish paste), cream sauce	All types

24.5.1 Stabilization of protein particles under acidic conditions

Under acidic conditions, SSPS prevents protein particles from aggregating and therefore prevents settling. Compared to high methoxyl pectin (HM-pectin), the polymer most commonly used to stabilize drinkable yoghurt,[28,29] what differentiates SSPS is its ability to stabilize protein particles at low pH conditions without raising viscosity. CMC (sodium carboxymethylcellulose)[30] and PGA (propylene glycol alginate)[31] may also be used as stabilizers in these applications. Because of their structural characteristics, these two hydrocolloids tend to give a high viscosity to the final products. By using

Table 24.4 Varieties of SOYAFIBE-S

Type	Crude protein (%)	Crude ash (%)	Viscosity (mPa/s)	Characteristics
SOYAFIBE-S-DN	9.2	8.6	56	Developed as a stabilizer for protein particles under acidic conditions
SOYAFIBE-S-DA100	6.2	8.4	62	Improved flavour over DN For a stabilizer of protein particles under acidic conditions
SOYAFIBE-S-LN	10.4	6.6	24	Developed as an emulsifier
SOYAFIBE-S-LA200	7.5	6.5	16	Improved flavour of SOYAFIBE-S-LN For powdering bases
SOYAFIBE-S-EN100	8.7	7.2	71	Improved stability of LN in suspensions Developed for flavour emulsions
SOYAFIBE-S-RA100	5.0	5.2	120	Excellent colour and high viscosity For cooked rice, edible films and coatings
SOYAFIBE-S-HR	12.0	7.0	15	Developed as a low viscous emulsifier

SSPS acidic milk drinks with low viscosity, light taste and no sticky mouthfeel can be produced.[32]

Figure 24.9 shows the viscosity, rate of precipitation, and particle size of drinkable yoghurts made with SSPS or HM-pectin as a stabilizer. Acidic milk drinks prepared with SSPS show a lower viscosity than those prepared with HM-pectin. In lower pH products (pH < 4.0), SSPS shows excellent stabilizing properties, while at higher pH (pH 4.4), it is less effective than HM-pectin. In other words, SSPS is suitable for acidic milk drink with lower pH and less non-fat milk solids. This function of SSPS can be applied especially to many other products, such as beverages, ice creams, desserts, etc., and provides an excellent stability and refreshing taste under acidic conditions. Furthermore, SSPS has the merit of having low reactivity with calcium, thus ensuring its full performance even if applied at an early stage of processing before fermentation. This beneficial feature allows the manufacturing process to be improved.

Figure 24.10 suggests a possible stabilizing mechanism for SSPS. This mechanism is rather different from that of HM-pectin. As shown in Fig. 24.10, SSPS contains about 20% of galacturonic acid, which is located in the main backbone of a SSPS molecule. It is possible to hypothesize that the anion groups in the backbone bind to the surface of cationic protein particles, and then hydrophilic polysaccharide layers coated on the protein particles prevent aggregation by steric repulsion. This thick layer is estimated to be about 40 nm corresponding to the hydrodynamic dimensions of SSPS in water.[33,34]

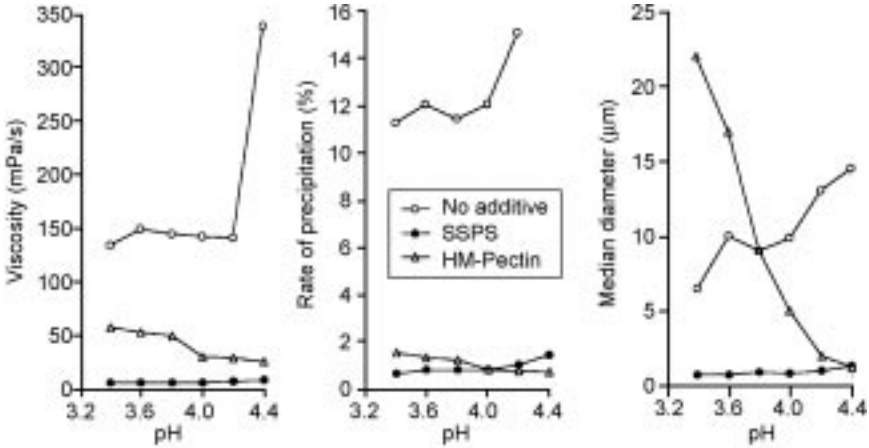

Fig. 24.9 Effects of SSPS and HM-pectin on dispersion of acidic milk protein.[1]
1. Formulation of acidic milk drink: non-fat milk 8.0%, stabiliser 0.4%, sugar 7.0%.
2. Measured with a B type Viscometer Model BM at 10ºC (Rotor no. 1, 60rpm).
3. After each 50 gram solution has been centrifuged at 3,000G for 20 min., the supernatant was decanted and the residue was allowed to stand for 20 min. in order to remove the remaining supernatant before measurement of the extent of precipitation.

$$\text{Rate of precipitation} = \{\text{Precipitate (g)}/50\ \text{(g)}\} \times 100\ (\%)$$

4. Measured with Laser Diffraction Particle Size Distribution Analyzer (SALD-2000A, Shimadzu Corp.)

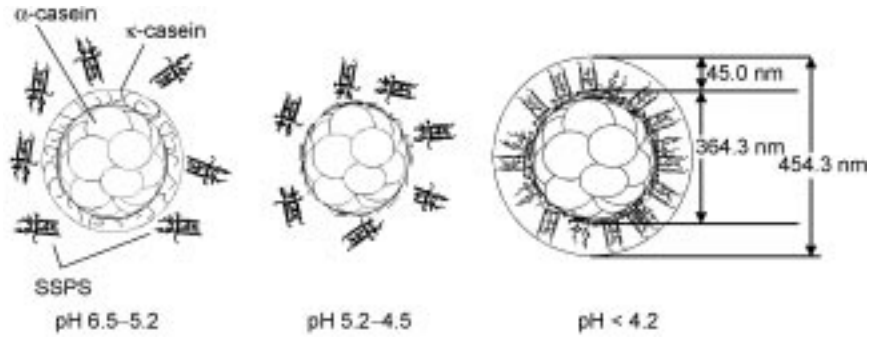

Fig. 24.10 Stabilizing mechanism of protein particles under acidic conditions.

24.5.2 Emulsifying stability

Flavour emulsions are often used as a suspension at low concentrations (i.e. 0.1%) so that a colourless transparent drink can be obtained, with a pleasant taste, scent and colour. As mentioned above, gum arabic from *Acacia senegal* is used widely in the field of flavour emulsions by virtue of its excellent emulsifying properties.[35] SSPS has an emulsifying function comparable with gum arabic, and can be used as an emulsifier and stabilizer for any emulsified foods including flavour emulsions and powdered flavours. Table 24.5 and Fig.

Table 24.5 Formulation of flavour emulsions

	SOYAFIBE-S-EN100			Gum arabic		
Test no.	1	2	3	4	5	6
Oil phase[a]	20	20	20	20	20	20
Gum arabic	–	–	–	30	20	15
SOYAFIBE-S-EN100	20	15	10	–	–	–
Glycerol	20	20	20	20	20	20
Water	60	65	70	50	60	65
Citric acid[b]	sufficient	sufficient	sufficient	sufficient	sufficient	sufficient
Total	120	120	120	120	120	120

[a] Lemon oil:MCT:SAIB = 5:55:40 (Specific Gravity 1.010)
[b] To adjust pH at 4.0

24.11 show the formulation and processing steps in the manufacturing of a flavour emulsion when SSPS is used instead of gum arabic, including the changes in the ratio of the oil phase to the emulsifying agent. From the results shown in Table 24.6, it can be seen that compared with gum arabic, a smaller amount of SSPS can be employed to formulate a flavour emulsion with good suspension stability.

SSPS contains glycoproteins, whose structures we presume similar to that of the Wattle Blossom Model suggested for gum arabic.[36,37] The protein fraction associated with SSPS seems to be responsible for anchoring the carbohydrate moieties of the polysaccharide onto the oil/water interface.[38] SSPS stabilizes the oil droplets by steric repulsion, as its hydrophilic portion creates a thick, hydrated layer of about 30 nm, which prevents the droplets from coalescing (Fig. 24.12). It is also important to mention that SSPS suppresses deterioration of flavours with its antioxidative effects on oils, as shown in Fig. 24.8.

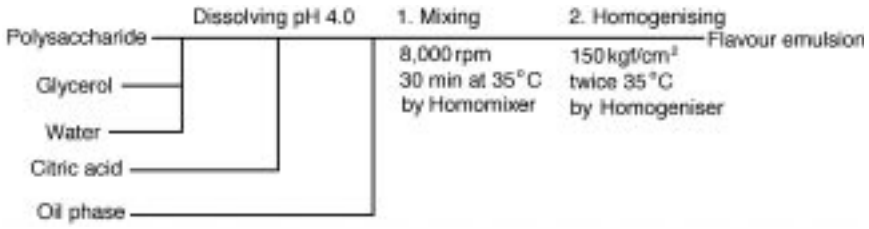

Fig. 24.11 Preparation of flavour emulsions.
1. Dissolve polysaccharide and glycerol in water and mix completely.
2. Adjust the solution's pH at 4.0 with citric acid.
3. Pour oil phase into the solution, and mix it by homomixer at 8000 rpm for 30 min. at 35 ºC.
4. Homogenise twice at 150 kg/cm^2, 35 ºC.

Table 24.6 Results of emulsification test

	SOYAFIBE-S-EN100			Gum arabic		
Test no.	1	2	3	4	5	6
Water phase viscosity (mPa/sec)[a]	1600	413	95	485	105	45
Particle size (μm)	0.62	0.73	0.83	0.50	0.58	0.77
Heat stability[b]	0.0%	2.7%	14.5%	6.0%	10.3%	10.4%
Clouding stability[c]	A	B	C	B	C	D

[a] Measured by BM Type Viscometer at 20 °C.
[b] Shown in the increased rate of the oil particle size in the flavour emulsions after four-week storage at 35 °C.
[c] Dispersed 0.1% of flavour emulsion in water containing 8.7% sugar and 0.3% citric acid, and observed clouding stability after four-week storage at 50 °C.
A; Superior, B; Good, C; Acceptable, D; Not acceptable.

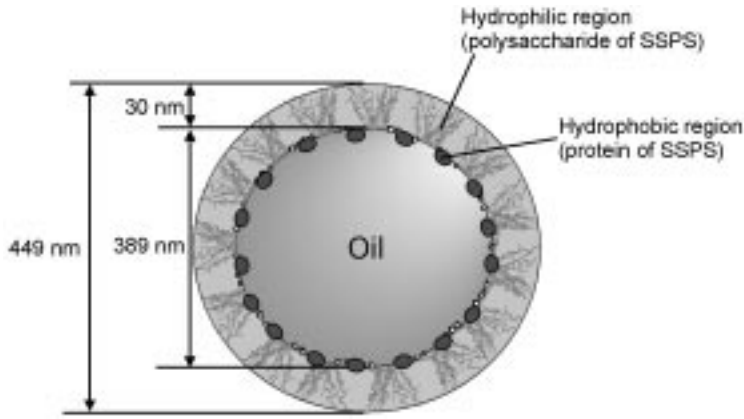

Fig. 24.12 Stabilizing mechanism of emulsion oil droplet prepared with SSPS.

24.5.3 Foam stabilizing function

SSPS also has foam stabilizing properties. The stability of foams when 2% of SSPS was added to 2% aqueous solution of hydrolysed soy protein, a common foaming agent, is shown in Table 24.7, from which we can see that SSPS is more effective than λ-carrageenan. This property can be used for stabilizing meringue foam.

24.5.4 Anti-sticking effect of cooked rice and noodles

SSPS keeps rice, such as plain rice or rice with other ingredients like pilaf, not sticky for many hours after being cooked. This means addition of SSPS can be of benefit when mixing other ingredients with cooked rice in the food-making process.[39] This property is also useful for making frozen rice. Furthermore, as the rice boiled with SSPS shows a hard texture, more water can be added to the

Table 24.7 Results of foam stability test[a]

Sample solution	Quantity added (%)	Viscosity (mPa/sec)	Foaming ratio[b]	
			After 10 min.	After 3 hrs
SOYAFIBE-S	0.2	4.9	2.0	1.8
λ-Carrageenan	0.2	11.3	2.6	1.4
No additive	–	4.4	1.6	0.1

[a] 50 ml of the hydrolysed soybean protein solution was placed in a 100 ml measuring cylinder and a change was observed on standing after shaking strongly for one minute and allowing to stand.
[b] Foaming ratio = volume of foam (ml)/volume of liquid layer (ml).

rice on boiling, increasing the yield of cooked rice. The rice processed with SSPS maintains good texture and does not harden during cold storage, as shown in Fig. 24.13.

The same property can be applied to cooked noodles. SSPS maintains the texture of cooked noodles for many hours. Just dipping the boiled noodles in SSPS aqueous solution or spraying the solution on the noodles prevents the noodles from sticking to each other for many hours. The same effect is obtained when the noodles are boiled in SSPS aqueous solution. SSPS also keeps spaghetti and chow mein not sticky for a long time without using oil. SSPS can be added directly to the sauce, causing the noodles to remain unsticky for many hours.

SSPS is adsorbed on the surface of cooked rice or noodles and coats the surface. It is assumed that the driving force of adsorption is the galacturonan of the main backbone in SSPS and the thickness of the coating layer of SSPS is the cause of the anti-sticking effect (Fig. 24.14).

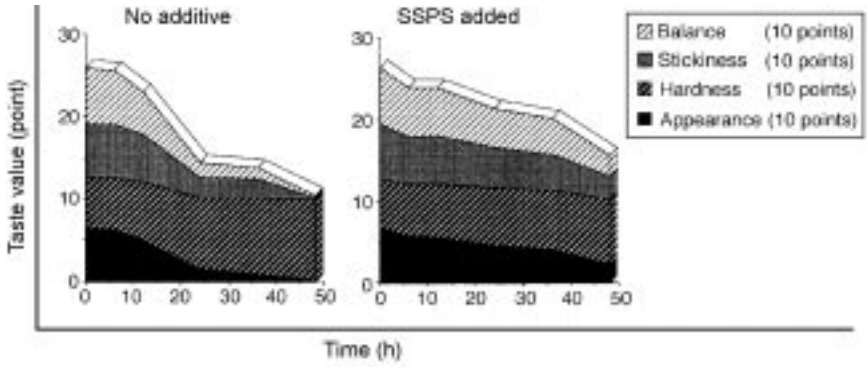

Fig. 24.13 Change in the taste value of boiled rice, stored at 10 ºC. Rice was boiled with 1.2 times of water in no additive case. On the other hand, where SOYAFIBE-S was added, rice was boiled with 1.44 times of water containing 1.0% of SOYAFIBE-S for the rice. The boiled rice was stored at 10 ºC for 48 hours and the change in the taste value was analysed by the Rice Taste Analyzer STA-1A (Satake Corporation).[40]

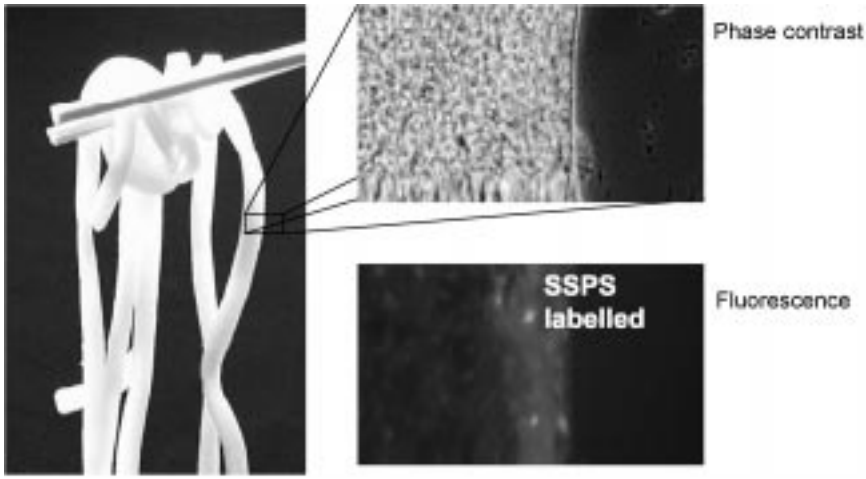

Fig. 24.14 Coating phase of SSPS on the surface of cooked noodles observed by a fluorescence microscope. The noodle was immersed in the solution of SSPS-DA 100 pyridylamidated previously, and stored at 4 ºC for 24 h. The noodle was then observed by fluorescence microscope.

24.5.5 Other applications

SSPS is a dietary fibre and can be used for a fibre enrichment in various foods. Many other applications are possible, such as a film coating agent for tablets and softener of baked foods. Thus, it may be useful not only in various foods, but also in various industrial applications. SSPS can stabilize dispersions of inorganic particles in a similar way to protein particles under acidic conditions. Therefore, applications as a dispersion agent for water colour, ceramics, and cement are possible.

24.6 Regulatory status

In Japan, SSPS is classified both as a food ingredient and food additive with no limitation of application. It must be labelled as 'soybean polysaccharide' or 'soybean hemicellulose' as a food additive, according to supplement 3 of the Food Sanitation Law 1996. On the other hand, it can be labelled as 'soybean fibre' for use as a food ingredient.

In the USA, SSPS has the status of self-affirmed GRAS.

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25

Cellulosics

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Abstract: The range of cellulosics approved for food applications is reviewed. These are methyl cellulose (mc), hydroxypropyl cellulose, hydroxypropyl methyl cellulose, methyl ethyl cellulose and carboxymethyl cellulose (cmc). Manufacture, structure, properties and regulatory status are summarised. A range of applications is discussed for each cellulosic, including mc applications using thermal gelation properties, and cmc applications involving thickening and involving protein stabilisation at reduced pH levels.

Key words: cellulosics, methylcellulose, carboxymethyl cellulose, applications.

25.1 Introduction

Cellulose is probably the most abundant organic substance existing in nature and is the major constituent of most land plants. It is the starting material for a wide range of modifications with uses both in the food industry, and an even greater variety of uses outside this sector. Cellulosics, as used in this chapter, covers the range of modified celluloses generally approved as food additives. These are methyl cellulose E461, hydroxypropyl cellulose E463, hydroxypropyl methyl cellulose E464, methyl ethyl cellulose E465, and sodium carboxymethyl cellulose E466 which is frequently called simply carboxymethyl cellulose and also known as cellulose gum. The respective abbreviations mc, hpc, hpmc, mec and cmc are widely used. Properties of modified celluloses such as hydroxyethyl cellulose, which do not have approval for food additive use, are not included in this chapter.

The common feature of all of these additives is that they are hydrocolloids derived from cellulose raw material by chemical modification. Since there are

many points which are common to this range of additives, and to avoid repetition, where appropriate topics will be covered as a class rather than as the individual additives.

25.2 Manufacture

25.2.1 Raw material

The raw material for modified celluloses is cellulose pulp, which in turn is produced from wood pulp from specified species or from cotton linters. Cotton linters are the short fibres from the cotton ball, which are too short to be suitable for use in thread and weaving. The polymer chain length of cellulose varies with the different raw materials and hence the polymer length and the resultant viscosity required in the final product will govern the selection of the raw material.

25.2.2 Manufacturing process

In general terms, cellulose pulp is dispersed in alkali solution to form alkali cellulose and is then treated with appropriate reagents, under tightly controlled conditions, to substitute the anhydroglucose monomers of the cellulose chain. The substitution is at the hydroxyl groups and the substitution reagents are as follows:

- methyl cellulose – chloromethane
- hydroxypropyl cellulose – propylene oxide
- methyl hydroxypropyl cellulose – mixed substituents as above
- methylethyl cellulose – chloromethane and chloroethane mixed substituents
- carboxymethyl cellulose – monochloracetic acid.

The two stages of the reactions can be summarised as follows:

1. Cellulose + Alkali + Water $\rightarrow$ Alkali cellulose
2. Alkali cellulose + R–X $\rightarrow$ Alkyl cellulose
   Alkali cellulose + R–CH(O)CH_2 $\rightarrow$ Hydroxyalkyl cellulose
   Alkali cellulose + X–R–COOH $\rightarrow$ Carboxyalkyl cellulose

The substitution reaction is followed by purification and washing stages to remove by-products and to achieve the purity levels specified for food additives.

25.3 Structure

The structure of the cellulose molecule is shown in Fig. 25.1. It is shown as a polymer chain composed of two repeating anhydroglucose units (β-glucopyranose residues) joined through 1,4 glucosidic linkages. In this structure, n is the number of anhydroglucose units or the degree of polymerisation.

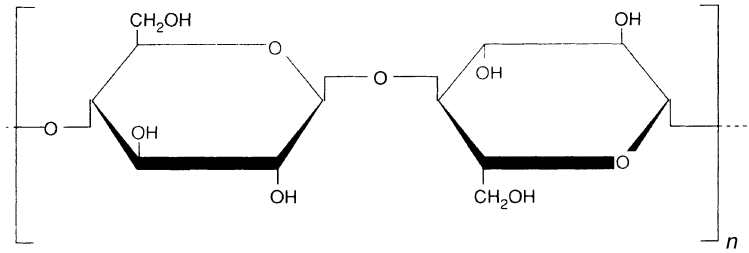

Fig. 25.1 Structure of cellulose.

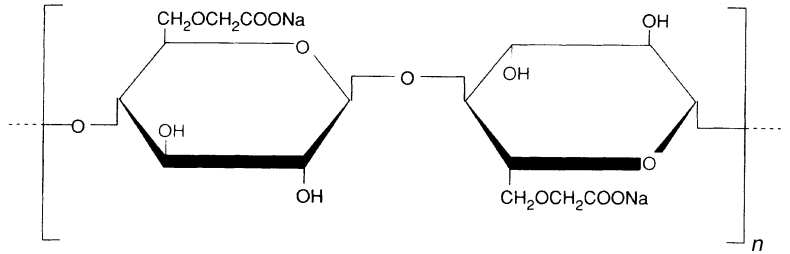

Fig. 25.2 Idealised unit structure of cellulose gum, with a ds of 1.0.

Each anhydroglucose unit contains three hydroxyl groups, which in theory can be substituted. The average number of hydroxyl groups substituted per anhydroglucose unit is known as the degree of substitution (ds). Without exception, the ds required to produce desirable properties is much below the theoretical maximum. In the example in Fig. 25.2, carboxymethyl cellulose with ds 1.0 is shown.

25.4 Properties

25.4.1 General

There are three main factors, which influence the properties of modified celluloses. These are first, and most importantly, the type of substitution of the cellulose, secondly, the average chain length or degree of polymerisation of the cellulose molecules (dp) and thirdly, the degree of substitution of the chain. Additionally, the particle size of the hydrocolloid may be varied. Particle size and powder bulk density affect the dissolving characteristics of the product. Granular material is less prone to clumping or balling but takes longer to dissolve. Fine powdered material can give very rapid hydration, but does not disperse so easily and good stirring or blending techniques are necessary. Degree of polymerisation is a measure of the chain length of the polymer. Increasing dp very rapidly increases the viscosity of the modified cellulose in solution, although the viscosities of two differently substituted modified celluloses of comparable dp will not necessarily be comparable.

In general the modified celluloses give neutral-flavoured, odourless and colourless clear solutions. It should be noted that all modified celluloses, in powder or even granular form, are capable of absorbing water from the atmosphere. It is therefore desirable to store these products in airtight packs.

25.4.2 Methyl cellulose and hydroxypropyl methyl cellulose

The properties of these two hydrocolloids are very similar and will be covered together. Mc and hpmc are both soluble in cold water to give solutions with a wide range of viscosity, which is dependent on both dp and ds. These solutions show reasonable viscosity stability over the range pH 3–11. More important, however, is the behaviour of solutions on heating since the solution will change into a gel once the temperature of the solution has been raised above a point known as the incipient gel temperature (igt). The igt varies from 52 ºC for mc, to a range of 63–80 ºC for hpmc types with increasing degree of hydroxypropyl substitution increasing the igt. These gels are reversible on cooling although there is a pronounced hysteresis between heating and cooling. Both polymers are good film formers and also exhibit some surface activity. Commercially mc and hpmc are distinguished by viscosity in 2% aqueous solutions, and also by the ds.

25.4.3 Hydroxypropyl cellulose

Hpc is also soluble in cold water and again a range of viscosity can be obtained dependent on dp. Hpc becomes insoluble at temperatures above approximately 45 ºC but unlike mc and hpmc, no gel is formed. Hpc is unusual in food hydrocolloids in that it is soluble in ethanol and mixtures of ethanol and water. However, the most interesting properties of hpc are probably its good film formation and its high surface activity compared to most other hydrocolloids. Commercially, the various grades of hpc for food use are differentiated by viscosity.

25.4.4 Methylethyl cellulose

In common with mc or hpmc, mec is also soluble in cold water and forms gels on heating, albeit weak gels, above the igp. These properties alone would not justify great interest in mec as a food additive, rather it is its surface activity and consequent excellent performance as a whipping aid, particularly in the presence of protein, which are of technical use.

25.4.5 Carboxymethyl cellulose

General

Cmc is soluble in both hot and cold water to give clear and colourless solutions with neutral flavour. As with other modified celluloses, the solution viscosity depends on dp, but it is possible to produce 1% aqueous solutions with viscosity

of 5,000 mPas at ambient temperatures. These solutions do show a reversible reduction of viscosity on heating but in food systems do not gel either alone or with other hydrocolloids. The rate of viscosity build-up is obviously dependent on dp, particle size and to some extent on ds. With suitable fine grind powders an extremely rapid viscosity development can be obtained.

A maximum degree of substitution of 1.5 is permitted in a recent amendment to EU legislation, but more typically ds is in the range 0.6–0.95 for food applications. ds, together with the uniformity of substitution, affects the rheology of the solution. Solutions of lower ds are thixotropic, whereas higher ds tends to pseudoplasticity. Uniformity of substitution favours pseudoplastic rheology, and solutions of such types give a particularly 'smooth' mouthfeel.

Commercially, cmc types are distinguished by viscosity, by particle size and to a more limited extent by ds and special solution characteristics. It is necessary to check the concentration of the solutions for which viscosities are specified, as there is not a single standard value for concentration.

Interaction of cmc with proteins

Cmc is an ionic polymer and this allows the formation of complexes with soluble proteins such as casein and soy at, or around, the isoelectric region of the protein. Although the effect on the system is primarily dependent on pH, it is also dependent on the composition and concentration of the protein, temperature, and the concentration and type of the cmc. At pH less than 3.0 or higher than 6.0, cmc reacts in the cold with the proteins in milk to form a complex, which can be removed as a precipitate. In the pH range approximately 3.0–5.5, a stable complex is formed. At the maximum of stability the viscosity is abnormally high compared to the individual components. A representation of the effects of pH on the viscosity of a solution of cmc and casein is shown in Fig. 25.3.

The system containing the cmc and casein complex is relatively shear sensitive, and the viscosity decreases under agitation. The complex is heat stable and little viscosity decrease is observed on heating. The casein is denatured to a much smaller extent than would be the case in the absence of cmc.

25.5 Applications

25.5.1 Methyl cellulose and hydroxypropyl methyl cellulose

The major applications of these two hydrocolloids are in the fields of binding and shape retention, film formation and barrier properties, and avoidance of boil-out and bursting at higher temperatures.

The thermogellation properties of mc and hpmc can be used to bind and to give shape retention to products where the ingredients themselves do not have particularly good biding properties. This includes such categories as reformed vegetable products such as potato croquettes and waffles, onion rings and the whole range of shaped soya protein and similar vegetarian products. These have

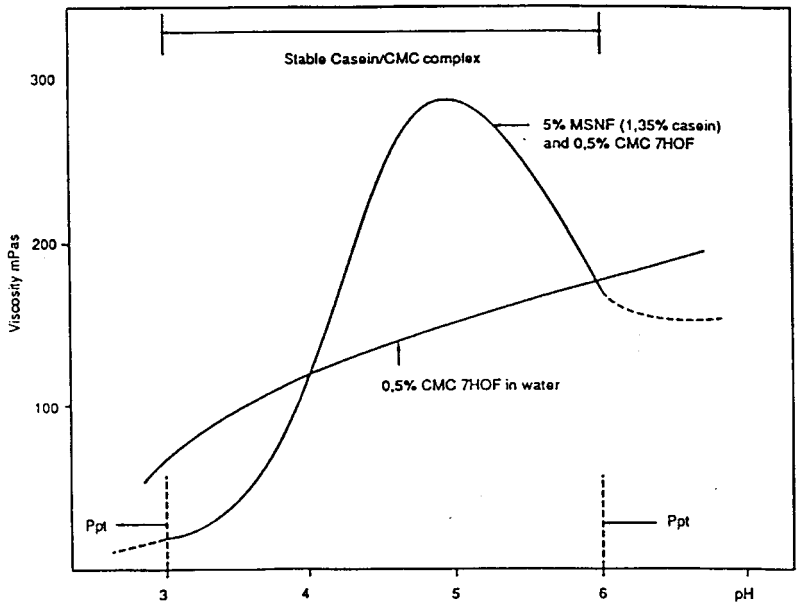

Fig. 25.3 Viscosity effect of CMC-casein complex at varying pH.

poor binding properties and a tendency to disintegrate on heating due to the disruptive effects of steam formed during heating. Inclusion of hpmc, or better, mc means that on heating above the igp the hydrocolloid will gel and bind the ingredients of the product. Because the gelation is thermoreversible and the gel has reverted to solution form at temperatures above normal eating temperatures, no alteration in texture from gelation is observed by the consumer. Two examples of applications of this type are given in Formulations 25.1 and 25.2.

In each of these cases the hydrocolloid is added to the cold mix in order that it may hydrate. Neither mc nor hpmc will hydrate in hot water above the igp, a property that can be used to ensure good dispersion of the gum if it is necessary to produce solutions and good stirring is not available.

The use of thermogelation properties to inhibit boil-out in is shown in Formulation 25.3. This is a bakery filling, but the principle is valid for sauces and other fillings where boil-out needs to be avoided. In this case the dry

Formulation 25.1 Potato croquettes

Ingredients	Composition (%)
Mashed potato	79
Potato flake	11
Salt	1
Benecel® hpmc type MP852	0.5
Water	to 100

Formulation 25.2 Soya burgers

Ingredients	Composition (%)
Soya protein	21
Vegetable fat	15
Starch	2
Potato flour	2
Benecel type M043 methyl cellulose	2
Dried onion	1.5
Salt	1
Seasonings and flavours	0.5
Water	to 100

Formulation 25.3 Bake-stable filling

Ingredients	Composition (%)
Sugar	13
Pregelatinised modified starch	5
Skimmed milk powder	4
Whole milk powder	3.5
Benecel type M043 methyl cellulose	0.5
Disodium phosphate	0.15
Flavour and colour	to taste
Water	to 100

ingredients are blended and mixed cold with the water to permit satisfactory solution of the methyl cellulose, and after standing 60 minutes the filling is baked. In cases where the sauce or filling is heat-treated prior to storage, care must be taken in the choice of addition point of the mc or hpmc, otherwise the dissolved hydrocolloid in the product will gel in the heat-processing equipment. A solution to this problem is to disperse the hydrocolloid in the hot product, where it will not dissolve until the heating stages are complete and the product is cooled below the igp. The thermogellation properties will then be exhibited at the next heating cycle.

The same thermogelling binding properties are utilised in batter and coating mixes. Low or medium-low viscosity grades are used at levels of up to 1% to improve adhesion and also to reduce oil or fat absorption. This application could also be said to utilise film formation properties, and film formation also contributes to the reported use of these additives in providing reduced oil uptake when deep frying such products as seafood and potatoes. Coating is normally effected by dipping or spraying a solution of the cellulosic, followed by drying.

25.5.2 Hydroxypropyl cellulose

To an extent, hpc is still a product waiting for applications in the food industry. The good surface activity of hpc is exploited in use of lower viscosity grades of

Formulation 25.4 Topping for whipping

Ingredients	Composition (%)
Vegetable oils or fats	20–35
Milk protein	1–4
Klucel® hydroxypropyl cellulose type GF	0.2–0.3
Emulsifiers	0.4–1.0
Sugar or glucose syrup	6–20
Salt, flavour and colour	to taste
Water	to 100

hpc in toppings for whipping or dispensing from aerosol cans. Toppings stabilised with hpc retain the whipped structure at high ambient temperatures and in this respect are considered superior to other hydrocolloids. Use levels are typically 0.2–0.3% of the topping. A recipe example is given in Formulation 25.4.

Hpc is soluble in ethanol and gives clear solutions in aqueous ethanol when the ethanol concentration is under approximately 50%. This gives potential for variations in viscosity or mouthfeel in a wide range of alcoholic beverages. It also has good film-forming properties and these films exhibit good flexibility, good oil and air barrier properties and a lack of tackiness. These properties have been examined in the pharmaceutical industry and may have potential in the speciality confectionery sector.

25.5.3 Methyl ethyl cellulose

The major use of mec has been in foam formation and stabilisation. Solutions of mec can be whipped to produce a fine foam with an over-run comparable to egg white. The solutions can be re-whipped even if the foam is allowed to return to liquid after standing. Importantly, mec foams are compatible to many common food ingredients including egg white and fat. This has made it suitable for toppings, mousses, batters and the like.

25.5.4 Carboxymethyl cellulose

General

Since viscosity production is the primary property of cmc, this review will start with applications where viscosity is the major property required. In such cases it is normal to use high viscosity grades of cmc, partly for economic reasons as cmc prices are based more on the quantity of the gum than on the amount of viscosity developed. Additionally, the lower concentrations of gums required with high viscosity grades, compared to medium or lower viscosity grades, produce more acceptable and less gummy mouthfeel.

A range of granulometries is available. Although the standard particle size will be suitable in many applications, the coarser particles will be preferred when it is necessary to make up solutions with poor mixing equipment, and the

thirty minutes stirring necessary to dissolve this particle is not a problem. On the other hand, extra fine powders will be necessary for vending mixes and other instant applications. In these applications cmc will normally give a more rapid viscosity build up than guar gum.

Instant products

With instant products such as vending drinks and powdered drink mixes fine grind cmc is used to provide the required rapid build-up of viscosity. If high viscosities are required and consequently high cmc concentrations are used, there may be a perception of a 'gummy' mouthfeel. This can be reduced or eliminated by use of evenly substituted types with smooth flow properties, such as Blanose® cmc type 7H3SXF.

Some examples of cmc use in both cold and hot instant products are given in Formulations 25.5 and 25.6, illustrating that cmc has both hot and cold viscosity. The first formulation shows use of the high viscosity type Blanose cmc 7HXF, but this could be substituted by the Blanose cmc type 9M31XF at approximately twice the use level.

Frozen products

The viscosity produced by cmc contributes to the stabilisation of frozen products such as ice cream, water ices and ripples. Benefits observed include the

Formulation 25.5 Instant fruit drink powder

Ingredients	% of powder	% of drink
Fine sugar	89.45	10.55
Citric acid	6.50	0.75
Sodium citrate	2.45	0.30
Clouding agent	0.80	0.10
Blanose® cmc type 7HXF	0.80	0.10
Flavour and colour	to taste	
Powder	100	
Water		88.2
Drink		100

Formulation 25.6 Instant chocolate drink

Ingredients	% of powder	% of drink
Skimmed milk powder	53.5	8.9
Sugar	36.0	6.0
Cocoa powder	9.75	1.6
Blanose cmc type 7H3SXF	0.75	0.13
Total powder	100.0	16.6
Water for 5:1 dilution		83.4

Formulation 25.7 Water ice or ripple

Ingredients	Water ice (%)	Ripple syrup (%)
Sucrose	25	30
Dextrose	7	
Blanose cmc type 7HOF	0.25	0.75
Fruit juice		40
Citric acid	0.4	0.3
Flavour and colour	to taste	to taste
Water	to 100	to 100

Formulation 25.8 Ice cream

Ingredients	Composition (%)
Milk fats	12
Milk solids non fat	11
Sucrose	12.5
Glucose syrup	4
Emulsifier	0.1
Blanose cmc type 7HXFMA	0.17
Genulacta® carrageenan type L-100	0.03

conservation of texture and inhibition of ice crystal formation, a slow meltdown and an improved resistance to dripping. Where products such as water ices have a low pH it may be desirable to use a cmc which tolerates acidic conditions without loss of viscosity, such as Blanose cmc type 7HOF (Formulation 25.7).

Cmc can also be used as the primary stabiliser in ice cream to control ice crystal size and growth during freeing and storage, to provide a smooth eating texture and to provide heat shock resistance (Formulation 25.8). When compared to locust bean gum or guar gum, there is a higher over-run which means that cmc is particularly suitable for use in soft serve products. Use of cmc tends to produce whey separation, but this can be avoided by use of carrageenan together with cmc in a ratio of about 1:6.

Table sauces and dressings

The thickening effects of cmc can be used in products such as salad dressings and tomato sauces. Properties of cmc which make it suitable for these applications include its rapid solubility in both hot or cold water, its good water binding and good tolerance to low pH levels. In salad dressings, typical use levels would be 1.0% Blanose cmc type 7HOF when oil contents are 30% and 0.75% when oil contents are increased to 50%.

Tomato sauce or tomato ketchup is a further application for cmc, and is particularly interesting because variation of the cmc type can be used to vary the structure of the product. In Formulation 25.9, type 7HOF can be used for a long-

Formulation 25.9 Ketchup

Ingredients	Compositions (%)	
Tomato concentrate (28%)	40.0	40.0
Sugar	20.0	20.0
Salt	1.5	1.5
Citric acid and vinegar (to pH 3.7–3.9)	7.5	7.5
Blanose cmc type 7HXFMA	1.1	
Blanose cmc type 7HOF		0.9
Preservative	0.2	0.2
Water	44.7	44.9

Note: If tomato concentrate levels are increased to 40%, then the cmc levels can be halved to around 0.5%.

structured ketchup, while type 7HXFMA will provide a much pulpier and shorter textured product.

Soft drinks

The viscosifying properties of cmc are widely used in ready-to-consume soft drinks. Additional body or mouthfeel can be provided to low calorie drinks sweetened with intense sweeteners, to more closely match the mouthfeel obtained with sucrose or glucose syrup. Use levels are typically 0.025–0.5% of the drink. Cmc is widely used to suspend fruit pulp and to inhibit 'neck ringing' by flavour oils in fruit squashes and other dilutable soft drinks and in ready-to-consume drinks containing fruit pulp. This has become more necessary as manufacturers have substituted intense sweeteners for a part of the bulk sweeteners. Generally, the concentration of Blanose cmc type 7HOF will be 0.05–0.1% of the fruit squash or ready-to-consume drink depending on the exact composition and density of the drink. Medium viscosity cmc types such as Blanose type 9M31F are used at approximately twice the addition level. Since cmc develops its viscosity more effectively if dissolved in water before addition of either acids or bulk sweeteners, it is good practice to produce a 1% stock solution of high viscosity types which can then be added as required.

Bakery products

A further application where viscosity development is required is in cake mixes and batter mixes. Incorporation of cmc can improve the volume yield of certain doughs as a result of its viscosity drop during baking and improves the suspension and distribution of ingredients such as dried fruit. Additional water is required in comparison to recipes without cmc, and so results in increased yields and improved moistness, particularly after storage. The fine grind types will normally be used in bakery mix concepts. In this case not only is the granulometry more compatible with flour, but also the fine grind allows a better water absorption in competition with other components such flour and sugar (Formulation 25.10).

Formulation 25.10 Fruit cake mix

Ingredients	Composition (%)
Flour	37
Sugar	31
Fat	31
Baking powder	0.6
Blanose cmc type 7H4XF	0.3
Eggs	31
Water (additional to control)	5

Some speciality cmc types have particularly good water binding properties, although their solution properties may be comparatively poor. This excellent water binding is utilised in baked products, including breads and morning goods, to either increase yield or to retard staling and hence improve consumer acceptability and prolong shelf life. The effect is compatible with other bread additives and improvers. Use of Aquasorb® cmc type A-500 at levels of 0.5–1.5%, and normally 0.5–1.0%, of flour weight is recommended in this type of application.

The water binding effect of these cmc types can also be used to reduce the fat absorption of doughnuts during cooking. The improved water binding is believed to inhibit fat absorption. Use levels of up to 0.3% are quoted.

Low pH milk products

Due to its ionic nature, cmc can react with soluble proteins to form a complex at or around the isoelectric point of the protein. In a sour milk medium at pH3.8–5.0, cmc reacts with casein to form a soluble complex, stable to heat treatment and to storage. In practice this effect is best utilised in the higher part of this pH range since at pH below about 4.2 there tends to be too great variation in viscosity with even small changes in pH. At pH levels 4.3–3.8 pectin is frequently the preferred stabiliser, although even at these levels cmc is sometimes used if economy is of prime importance. Examples of low pH milk preparations are given in Formulations 25.11 and 25.12.

Formulation 25.11 Buttermilk drink pH4.5–4.6

Ingredients	Composition (%)
Buttermilk	89.6
Sugar	10
Blanose cmc type 7HOF	0.3–0.4

Preparation

1. Blend the sugar and cmc and add this blend gradually into the buttermilk while stirring rapidly. Continue stirring for 15 minutes.
2. Heat up to 70 °C in a heat exchanger.
3. Homogenise to 150 to 200 bar.
4. Pasteurise at 85 °C for 15 seconds.
5. Cool to 20 °C and fill aseptically if extended shelf life is required.

Formulation 25.12 Milk orange juice beverage at pH4.5–4.6

Ingredients	Composition (%)
Whole milk	53.8
Orange juice (11° Brix)	41.3
Sugar	4.7
Blanose cmc type 7HOF	0.15–0.20

Preparation

1. Blend the cmc and sugar and add the blend to the cold milk with stirring.
2. Stir until the cmc is fully dissolved (15 minutes with good agitation).
3. Slowly add the orange juice, cooled to 10 °C or lower, to the milk while stirring well.
4. Continue to stir for at least five minutes after the end of juice addition.
5. Heat to 70 °C in a heat exchanger.
6. Homogenise at 150–200 bar.
7. Pasteurise at 85 °C for 15 seconds.
8. Cool to 20 °C and fill.

25.6 Regulatory status

25.6.1 Names and serial numbers

In the member states of the European Union, use of the modified celluloses described in this chapter is permitted by Directive 95/2/EC on food additives other than colours and sweeteners. National legislation in the individual member states implements this directive. The EU directive has assigned names and serial numbers (E numbers) to these additives as follows:

- E461 methyl cellulose
- E463 hydroxypropyl cellulose
- E464 hydroxypropyl methyl cellulose
- E465 methyl ethyl cellulose
- E466 carboxymethyl cellulose or sodium carboxymethyl cellulose.

It is hoped that in the future the style ‘modified cellulose’, which is already permitted in other jurisdictions, will also be permitted in EU member states.

The directive sets specifications for these additives when they are to be used in food applications. These specifications cover such matters as purity, permitted degree of substitution, molecular weights and moisture levels for each individual additive, but detailed discussion of specifications is outside the scope of this chapter. Food industry users of modified celluloses should be aware that in general there is a greater use of these products in other classes of industry, where specifications are to lower standards of purity, and checks should always be made that food specification material is being used. For example, cmc for food use has a minimum purity level of 99.5% cmc, whereas technical grades are commonly sold at only 98.0% purity or lower.

25.6.2 Permitted use levels

All these additives are placed in Annex I of the directive, which permits their use at 'quantum satis' level in products other than those where there are specific regulations on composition. These specific exceptions are listed in Annexes VI, VII and VIII, but these limited exceptions will not be detailed. The term 'quantum satis' means that no maximum level of the additive in or on a food is specified but in or on a food the additive may be used in accordance with good manufacturing practice at a level not higher than is necessary to achieve the intended purpose and provided that such use does not mislead the consumer.

Outside Europe, use of modified celluloses is also permitted under purity criteria set by the Food and Agriculture Organisation and the Wold Health Organisation (FAO/WHO) and the US Food Chemicals Codex. In the United States use of modified celluloses is allowed in a wide range of foods, and this is true in many other countries. However, space does not permit a detailed review of the legislative situation in every country, and specialised advice should always be sought in case of doubt. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) assigned in 1990 an Acceptable Daily Intake (ADI) 'not specified' to the modified celluloses.

25.6.3 Ingredient declarations

In EU member states there is an approved manner to label foodstuffs where an additive is used and has a technological effect in the foodstuff. The additive should be described on the foodstuff package ingredient label as 'thickener' or 'stabiliser' or 'gelling agent' as appropriate, followed by the serial number (E-number) or the approved name as given in Section 25.6.1. There is no legal requirement to include processing agents in the ingredient declaration of a foodstuff.

26

Bacterial cellulose

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Abstract: Bacterial cellulose consists of an ultra-fine network of cellulose nanofibers (3–8 nm) which are highly uniaxially oriented. This type of 3D structure results in a higher crystallinity (60–80%) of bacterial cellulose and tremendous physico-chemical and mechanical properties. This chapter deals with a comprehensive description of the cellulose synthesized by microbial cells. The introduction gives a brief account of the various aspects of bacterial cellulose. Sections 26.2 and 26.3 present a concise historical outline of the evolution of bacterial cellulose from its discovery to its use in the modern day advanced functional materials. Section 26.4 provides a description of the process(s) currently used in the manufacture of bacterial cellulose. Section 26.5 provides a discussion of the physico-chemical structure of bacterial cellulose. Section 26.6 describes the physico-mechanical properties of bacterial cellulose with special attention to those distinguishing it from plant cellulose. Section 26.7 focuses on the detailed description of the uses and applications of bacterial cellulose in various fields. Special emphasis will be given to its applications in the food industry. Section 26.8 includes the various regulations related to the use of bacterial cellulose as a drug, a food or formulations thereof.

Key words: bacterial cellulose, *Acetobacter*, *Rhizobium*, *Agrobacterium*, *Gluconacetobacter*, *Sarcina*, cellulose-negative (*Cel*$^-$) mutant, shear stress, cellulose synthase operon, static cultivation, agitated culture, membrane, glucuronic acid, crystalline structures, thickener, stabilizer, texture modifier, audio component, wound dressing.

26.1 Introduction

Cellulose, a homopolymer composed of β-(1,4) glucose, is the most abundant biopolymer in nature. It is the major component of the cell walls of nearly all plants, fungi and some algae. Cellulose can also be produced by some bacterial strain belonging to the genera *Acetobacter*, *Rhizobium*, *Agrobacterium*, and *Sarcina* (Delmer, 1999; Brown 1886; Embuscado *et al.*, 1994). Plant-derived cellulose contains both hemicellulose and lignin but cellulose produced by bacteria, also called bacterial cellulose (BC), is the pure form of cellulose. Although the chemical structure of BC is the same as that of plant cellulose, it possesses different physical and chemical properties. For example, the fiber diameter of BC is about one hundredth that of plant cellulose while its Young's modulus is almost equivalent to that of aluminum (Khan *et al.*, 2007). Due to its purity and unusual physico-chemical properties, a wide range of specialty applications of BC can be envisaged in the food and medical field, while it could be used in bulk in the paper and textile industries. However, the current price of BC remains too high to make it commercially attractive.

26.2 Historical overview

In 1886, Brown discovered that *Bacterium aceti* (now known as *Acetobacter xylinum* or *Gluconoacetobacter xylinum*) produces a tough gelatinous film of pure cellulose. This bacterium was then known as 'the vinegar plant' because it was the major source of acetic acid. BC attracted more attention in the mid-twentieth century. In 1954, Hestrin and Schramm reported the production of microbial cellulose in a glucose-rich medium using the bacterium *A. xylinum*. Next, Colvin (1957) detected cellulose synthesis in samples containing cell-free extract of *A. xylinum*, glucose, and adenosine triphosphate (ATP).

Schramm and Hestrin (1954) also reported the occurrence of non-cellulose producing (cellulose-negative) mutants of *A. xylium*. When cellulose-producing strains were grown and transferred repeatedly in a shaken culture, spontaneously forming cellulose-negative (Cel^{-}) mutants accumulated, causing the lower production yield of BC. Further, Steel and Walker (1957) found that isolates incapable of forming cellulose were present when *A. xylinum* was grown under agitated and aerated conditions. Coucheron (1991) noted that the insertion sequence element of genes was associated with the inactivation of cellulose production by an *A. xylinum* strain. The cellulose deficiency in mutants might be due to insertions (i) in the region of the operon outside the 9.9 kb HindIII fragment, (ii) in genes representing known functions in the production of cellulose but which are not part of the cellulose synthase operon, or (iii) in genes whose role in this formation has not yet been identified. For large-scale production of BC, isolation of a strain and finding culture conditions suitable for producing BC in a shear stress field are required.

In 1995, Bio Polymer Research (BPR) Co. Ltd. isolated a high cellulose producing strain, *A. xylinum* subsp. *sucrofermentans* BPR2001 from agitated culture (Toyosaki *et al.*, 1995) and also isolated sulfaguanidine-resistant

mutants, BPR3001E, from BPR2001 (Ishikawa *et al.*, 1995). Cellulose production by BPR3001E was 40% higher than that by BPR2001. More recently, it was found that the cellular activity of cellulose production can be preserved without the spontaneous occurrence of Cel^- mutants in consecutive shake-cultures using a medium containing ethanol (Park *et al.*, 2003a,b). It is generally known that the shear stress generated in shaking cultivation causes the *Acetobacter* strains to be converted into Cel^- mutants.

Since 1996, the effects of the tricarboxylic acid (TCA) cycle related organic acid on BC production has been reported (Matsuoka *et al.*, 1996; Naritomi *et al.*, 1998; Premjet *et al.*, 1999). Supplementing organic acid such as pyruvate, succinate, acetate and lactate to the medium increase the BC yield. BC was produced by *A. xylinum* BPR2001 in a 50 liter air-lift reactor, whose power consumption is significantly lower than that of the stirred tank reactor, using fructose as the main carbon source (Chao *et al.*, 1997). At present, this 50 liter air-lift reactor is the largest-scale BC production system.

26.3 Biosynthesis

The biosynthesis of BC is a precisely and specifically regulated multi-step process, involving a large number of both individual enzymes and complexes of catalytic and regulatory proteins, whose supramolecular structure has not yet been well defined. The process includes the synthesis of uridine diphosphoglucose (UDPGlc), which is the cellulose precursor, followed by glucose polymerization into the β-1,4-glucan chain, and nascent chain association into characteristic ribbon-like structure, formed by hundreds or even thousands of individual cellulose chains.

26.3.1 Biosynthetic pathway

As known from the literature (Yoshinaga *et al.*, 1997; De Wulf *et al.*, 1996), the cellulose formation includes five fundamental enzyme mediated steps: the transformation of glucose to UDP-glucose via glucose-6-phosphate and glucose-1-phosphate, and finally the addition of UDP-glucose to the end of a growing polymer chain by cellulose synthase. Cellulose synthase (UDP-glucose: 1,4-β-D-glycosyltransferase; EC 2.4.1.12) is regarded as the essential enzyme in the synthesis process. It is subjected to a complicated regulation mechanism, which controls activation and inactivation of the enzyme (Vandamme *et al.*, 1998).

The cellulose synthase from *A. xylinum* is subjected to a complex form of regulatory control which has not been encountered previously in a living system. The basis of this regulation appears to lie in the intracellular concentration of an unusual cyclic guanyl nucleotide dimer (originally isolated as an incubation product of guanosine triphosphate (GTP) with cell extracts) (Ross *et al.*, 1985, 1986), which functions as an allosteric effector of enzyme activity. *In vitro*, cellulose synthase activity displays nearly absolute dependence on the presence

of nanomolar concentrations of this compound, which was identified (Ross *et al.*, 1987) as cyclic bis(3′→5′)-diguanylic acid(c-di-GMP or cGpGp). Currently, it is unknown whether c-di-GMP affects other cellular processes in addition to cellulose synthesis.

The pathways of synthesis and degradation of c-di-GMP are catalyzed by enzymes which occur in both soluble and membrane-associated form in cell-free extracts. The formation of the nucleotide activator is attributed to diguanylate cyclase, a predominantly soluble enzyme, which condenses two molecules of GTP in a two-step reaction, via the intermediate linear triphosphate pppGpG. A membrane-bound phosphodiesterase, termed PDE-A, degrades the activator, cleaving a single phosphodiester bond in the cyclic structure to form the linear 5′-phosphoryl dimer pGpG (Ross *et al.*, 1987; Mayer, 1987).

This initial degradation product, which is devoid of stimulatory effect, is then further hydrolyzed to yield two molecules of 5′-GMP in a reaction attributed to a second phosphodiesterase, termed PDE-B. This latter phosphodiesterase has been deemed distinct from PDE-A on the basis that the ratio of PDE-A to PDE-B activity in membrane preparations is approximately 10:1, whereas in soluble extracts this ratio is reversed (Mayer, 1987). Furthermore, in contrast to the PDE-B reaction, PDE-A activity is inhibited at low concentrations of Ca^{2+} ions, suggesting that this phosphodiesterase serves as an additional locus of regulatory control, maintained by this cation. A regulation model of cellulose synthesis in *A. xylinum* was proposed by Ross *et al.* (1987).

26.3.2 Biosynthetic mechanism

Electron microscopy studies have demonstrated that cellulose-synthesizing complex or terminal complex (TC) is linearly arranged in the longitudinal axis of the bacterial rods, and in association with pores at the surface of the bacterium (Zaar, 1979). The diameter of pore in the middle of each TC probably corresponds with that of the cellulose molecule.

In the first step of cellulose formation glucan chain aggregates consisting of approximately 6–8 glucan chains are elongated from the TC which is located between the outer and the cytoplasma membrane. These sub-elementary fibrils are assembled in the second step to form microfibrils followed by their tight

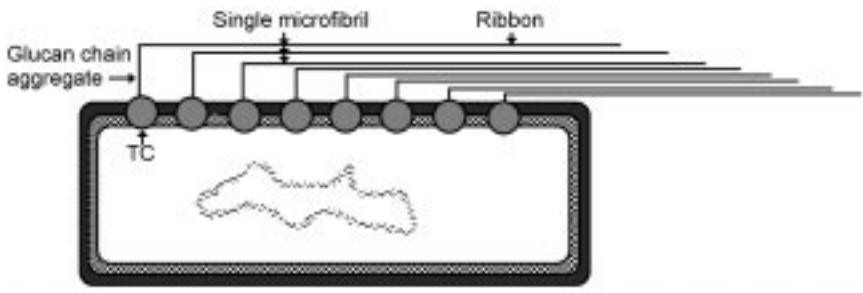

Fig. 26.1 Generalized model of ribbon assembly in *A. xylinum* (Brown, 1992).

assembly to form a ribbon as the third step (Fig. 26.1). The matrix of interwoven ribbons constitutes the BC membrane or pellicle (Brown, 1992).

The ribbon consists of approximately 46 microfibrils which average 1.6×5.8 nm in cross section and elongates at a rate of 2 μm/min (Brown *et al.*, 1976).

26.4 Manufacture

Cellulose-producing bacteria include members of the genera *Acetobacter*, *Rhizobium*, *Agrobacterium*, and *Sarcina* (Jonas and Farah, 1998). *Acetobacter xylinum* is the most efficient synthesizer of bacterial cellulose and thus has been applied as a model microorganism for basic and applied studies on bacterial cellulose (Cannon and Anderson, 1991). The production of bacterial cellulose could be carried out in either solid-phase cultivation or submerged culture (El-Saied *et al.*, 2004). Two methods are generally employed for the production of bacterial cellulose; namely stationary culture and agitated culture (Watanabe *et al.*, 1998). In the static cultivation, the bacterial cellulose is produced as a gelatinous membrane on the surface of the medium, while in the agitated culture, the bacterial cellulose is accumulated in dispersed suspension as irregular masses, such as granule, stellate, and fibrous strand. The agitated culture method is mainly applied for industrial production of bacterial cellulose (El-Saied *et al.*, 2004), although most of the biomedical and cosmoceutical applications require bacterial cellulose to be in a proper shape (e.g., film, membrane or sheet), which is produced in static cultivation mode. Until now, the stationary culture conditions have been successfully reported, however, agitated culture for the production of bacterial cellulose faces many problems, including genetic instability of the producer organism and their subsequent conversion to cellulose non-producing mutants, non-Newtonian behavior during mixing of bacterial cellulose, or proper oxygen supply (Czaja *et al.*, 2004). These problems associated with agitated cultures have been thoroughly investigated with some degree of success. For example, Park *et al.* (2003a,b) reported the possibility to preserve the cellulose production ability of the cells without the spontaneous occurrence of cellulose non-producing mutants.

Whatever the cultivation mode for the production of bacterial cellulose, the main carbon source by *Acetobacter* or *Gluconacetobacter xylinus* is glucose or sucrose because the precursor in cellulose synthesis is uridine diphosphoglucose. The biosynthesis of cellulose from other carbon sources, such as 5- or 6-carbon monosaccharides, oligossaccharides, starch, alcohol, and organic acid has also been reported. Moreover, fructose and glycerol are also used as carbon sources and these result in almost similar yields of bacterial cellulose as that that from glucose while galactose and xylose yields are smaller. The bacterial cellulose yield from sucrose is half the yield from glucose. The use of D-arabitol as a carbon source results in six times as much cellulose as that from D-glucose. A specific complex nitrogen source is also required for the cellulose-producing

strain. Most of the media used for the production of bacterial cellulose utilizes yeast extract and peptone as the nitrogen sources. A few amino acids, e.g., methionine and glutamate, have also been recommended for this purpose. The vitamins pyridoxine, nicotinic acid, p-aminobenzoic acid, and biotin stimulate cell growth and cellulose production (El-Saied *et al.*, 2004).

The cell growth and cellulose production is significantly affected by the pH of the culture broth and therefore, pH control around an optimum value is desirable. The conversion of glucose to gluconic acid leads to a significant drop in pH of the medium in the batch culture. The optimal pH range for cellulose production by *A. xylinum* is 4–6 while some other researchers (Oikawa *et al.*, 1995; Delmer and Amor, 1995) demonstrated pH 4–7 as optimum. In addition to the pH of the nutrient broth, the yield of bacterial cellulose is also temperature dependent. The optimal growth temperature for cellulose production is 25–30 ºC. The cellulose synthesis occurs at the air/cellulose pellicle interface, and thus oxygen is an important factor for cellulose production. The production rate and the yield of bacterial cellulose are proportional to the oxygen transfer rate (OTR) and oxygen transfer coefficient (KLa). Excessive oxygen supply is reported to result in a decrease in bacterial cellulose productivity due to a loss of substrate by direct oxygen (El-Saied *et al.*, 2004).

After fermentation, the bacterial cellulose is generally harvested from the culture medium by centrifugation or filtration followed by washing with distilled water and re-centrifugation or filtration. The microbial cells are removed from the bacterial cellulose by destroying them during a hot caustic treatment. The suspension is then filtered and the filter cake is washed thoroughly with distilled water in order to remove the traces of sodium hydroxide. The bacterial cellulose is finally freeze-dried.

26.5 Structure

Cellulose is an unbranched polymer of β-1→4-linked glucopyranose residues. The chemical structure of plant and bacterial cellulose is the same except the degree of polymerization, which is about 13,000 to 14,000 for plant and 2,000 to 6,000 for bacterial cellulose (Jonas and Farah, 1998). Moreover, the macromolecular structure and properties of bacterial cellulose also differ from the latter (Bielecki *et al.*, 2002).

During biosynthesis, the bacteria utilize various carbon compounds of the nutrition medium which are then polymerized into single, linear β-1,4-glucan chains and finally secreted outside the cells through a linear row of pores located on their outer membrane. The subsequent assembly of the β-1,4-glucan chains outside the cell leads to the formation of subfibrils (consisting of 10–15 nascent β-1,4-glucan chains). The subfibril having a width of approximately 1.5 nm is one of the thinnest naturally occurring fibers. These subfibrils are crystallized into microfibrils, in turn into bundles, and the latter into ribbons. The ribbon is composed of about 1000 individual glucan chains (Bielecki *et al.*, 2002). This

series of processes results in the formation of a thick, gelatinous membrane in static culture conditions. The 3D structure of such a membrane consists of an ultrafine network of cellulose nanofibres (3–8 nm) which are highly uniaxially oriented. This type of 3D structure results in a higher crystallinity (60–80%) of bacterial cellulose and tremendous mechanical strength. Vascular plant cellulose, however, lacks such a 3D structure (Bielecki *et al.*, 2002).

Native bacterial cellulose occurs in two different crystalline structures, namely cellulose Iα and cellulose Iβ (Yoshinaga *et al.*, 1997). A triclinic unit cell consisting of one cellulose chain for cellulose Iα and a monoclinic unit cell consisting of two cellulose chains for cellulose Iβ. These two types of crystalline structures appear to be separately distributed in the microfibril of cellulose with exception of tunicin (sea squirt cellulose) which is pure cellulose Iβ. Cellulose Iα is dominant in bacterial cellulose while cellulose Iβ is dominated in plant cellulose (Sugiyama *et al.*, 1991).

26.6 Functional properties

Bacterial cellulose has the same chemical structure as that of plant cellulose, yet it possesses different physical and chemical properties. The characteristic physical properties of bacterial cellulose are summarized in Table 26.1 and are discussed as follows.

26.6.1 Macromolecular structure

As mentioned earlier, the macromolecular structure and properties of bacterial cellulose differ from those of the plant cellulose. Bacterial cellulose is composed of unique ribbon-shaped fibrils (El-Saied *et al.*, 2004). The width of the fibril is approximately 1.5 nm and belong to the thinnest naturally occurring fibers. It is worth noting that the fiber diameter of bacterial cellulose is about one hundredth that of plant cellulose (Khan *et al.*, 2007). The ribbons are made up from several smaller microfibrils. Iguchi *et al.* (2000) stated that these were 20–40 Å^2 in diameter, Brown *et al.* (1976) stated that the cross-sections averaged $16 \times 58\ \text{Å}^2$, with ~46 microfibrils making up a ribbon, and Fink *et al.* (1997) found evidence for crystallite dimensions of $130 \times 70\ \text{Å}^2$.

26.6.2 Crystallinity and degree of polymerization

Bacterial cellulose is also distinguished from its plant counterpart by a high crystallinity index (above 60%). The native bacterial cellulose exist in two different crystalline structures, i.e., cellulose Iα and cellulose Iβ (Yoshinaga *et al.*, 1997). The content of cellulose Iα is approximately 60% in bacterial cellulose while it is only approximately 30% in the higher plant cellulose, cotton and ramie. In contrast, cellulose Iβ is the major component in plant cellulose (Sugiyama *et al.*, 1991). Crystallinity is considered to be a key determinant of the properties of cellulose.

Table 26.1 Functional properties of bacterial cellulose

Property	Description
Purity	Chemically pure form of cellulose and is free from hemicelluloses, lignin and pectin
Biodegradability	Biodegradable, recyclable and renewable
Mechanical strength	High tensile strength, consistent dimensional strength, light weight and durable
Water-holding capacity	Remarkable high water-holding capacity, selective porosity, high mechanical strength in wet state, high surface-to-volume carrier capacity
Cellulose orientation	Bacterial cellulose has dynamic capabilities of fiber forming Uniaxially strengthened membranes can be produced
Direct membrane formation	Direct assembly of extremely thin, submicron, optical clear membrane during biosynthesis and thus avoiding the need for the intermediate of paper or textile formation from pulp
Moldability	Bacterial cellulose has a very high moldability and can be produced in the form of a gelatinous membrane which can be molded into any shape and size during its synthesis
Direct modification	The crystallization of bacterial cellulose can be delayed by introduction of dyes into culture medium. The physical properties such as molecular weight and crystallinity can be controlled during biosynthesis
Direct synthesis of cellulose product	Various cellulose derivatives (such as cellulose acetate, carboxymethylcellulose, methyl cellulose, etc.) can be directly synthesized. The desired cellulose crystalline allomorph (cellulose I or cellulose II) can be directly controlled

Moreover, the degree of polymerization (DP) of cellulose from both these natural sources is also different from each other. The degree of polymerization of bacterial cellulose is usually between 2,000 and 6,000 (Jonas and Farah, 1998) but, in some cases, reaching even 16,000 or 20,000 (El-Saied *et al.*, 2004). On the other hand the average degree of polymerization of plant cellulose varies from 13,000 to 14,000 (Teeri, 1997).

The structural features of bacterial cellulose vary according to culture conditions in which it has been produced and the strain used. The crystallinity and cellulose Iα content of cellulose are lower in an agitated culture than that in a static culture. Similarly, the degree of polymerization of cellulose molecule is also lower in agitated culture conditions (El-Saied *et al.*, 2004).

26.6.3 Mechanical strength

It has been reported that the dried sheet of purified gelatinous membrane of bacterial cellulose has extremely high Young's modulus, of more than 15 GPa. This value is the highest of planar-oriented sheets or non-oriented sheets made

Table 26.2 Mechanical properties of bacterial cellulose and other organic layer materials (adapted from Klemm *et al.*, 2005)

Material	Young's modulus (GPa)	Tensile strength (MPa)	Elongation (%)
Bacterial cellulose	15–35	200–300	1.5–2.0
Polypropylene	1.0–1.5	30–40	100–600
Polyethylene terephthalate	3–4	50–70	50–300
Celluphane	2–3	20–100	15–40

from various organic polymers, which have a Young's modulus of less than 5 GPa. Furthermore, the specific Young's modulus, which is the quotient of Young's modulus by density, corresponds to that of aluminum. This high Young's modulus of the sheet may be due to the strong interfibril binding of the ultrafine fibrils of the bacterial cellulose and may be dependent on the crystallinity of the bacterial cellulose. The sheet prepared from the bacterial cellulose produced in an agitated culture has a slightly lower Young's modulus than that of statically-produced bacterial cellulose. This is because the cellulose produced with agitation has a substantially disordered structure compared with that in static culture (Yoshinaga *et al.*, 1997). Nishi *et al.* (1990) reported a tremendously high Young's modulus which was close to 30 GPa, for sheets obtained from BC pellicles when adequately processed. Table 26.2 describes the mechanical properties of bacterial cellulose and other organic layer materials (Klemm *et al.*, 2005). Due to this remarkable modulus, bacterial cellulose sheets seem to be an ideal candidate as raw material to further enhance the Young's modulus of high-strength composites.

26.6.4 Water-holding capacity

Bacterial cellulose is water-insoluble and due to its large network of fibers it has a very large surface area. These fibers have approximately 200 times the surface area of fibers from plant cellulose. Due to the unique nano-morphology coupled with its ability to form hydrogen bonds which accounts for their unique interactions with water, bacterial cellulose can absorb up to 200 times of its dry mass of water. When bacterial cellulose is used in suspension, it exhibits pseudoplastic thickening properties. Moreover, bacterial cellulose displays great elasticity, high wet strength, and conformability (Czaja *et al.*, 2006; US Congress, 1993). The small size of bacterial cellulose fibrils and thus the high water-holding capacity seems to be a key factor that determines its remarkable performance as a wound-healing system. Furthermore, the never dried cellulose membrane is a highly nano-porous material that allows for the potential transfer of antibiotics or other medicines into the wound, while at the same time serving as an efficient physical barrier against any external infection (Czaja *et al.*, 2006).

26.6.5 Miscellaneous properties

Bacterial cellulose has a very high moldability. It can be produced in the form of a gelatinous membrane which can be molded into any shape and size during its synthesis, depending on the fermentation technique and conditions used (Czaja *et al.*, 2006). The BC is the chemically pure form of cellulose and is free from hemicellulose, pectin, and lignin that are associated with plant cellulose and are difficult to eliminate (Khan *et al.*, 2007). A vigorous treatment with strong bases at high temperatures allows the removal of cells embedded in the cellulose net, and it is possible to achieve a non-pyrogenic, non-toxic, and fully biocompatible biomaterial (Czaja *et al.*, 2006). The various desired properties of bacterial cellulose can be directly controlled during biosynthesis. For example, the crystallization of bacterial cellulose can be delayed by introduction of dyes into culture medium. The physical properties such as molecular weight and crystallinity can be controlled during biosynthesis. Moreover, various derivatives of cellulose (such as cellulose acetate, carboxymethylcellulose, methyl cellulose, etc.) can be directly synthesized during production of bacterial cellulose. The desired cellulose crystalline allomorph (cellulose I or cellulose II) can also be directly controlled (El-Saied *et al.*, 2004).

26.7 Uses and applications

Bacterial cellulose is a new functional material for a wide range of applications even in those areas where the use of plant cellulose is limited. This may be largely attributed to a high purity with a crystallinity structure and high water absorption capacity and mechanical strength in the wet state. The major current and potential applications of bacterial cellulose can be summarized as follows.

26.7.1 Food applications

Bacterial cellulose has important applications in a variety of food formulations due of its unique properties and structure. Its use is particularly recommended in situations where low use levels, lack of flavor interactions, foam stabilization, and stability over wide pH range, temperature, and freeze-thaw conditions are required. Potential uses include pourable and spoonable dressings, sauces, and gravies; frostings and icings; sour cream and cultured dairy products; whipped toppings and aerated desserts, and frozen dairy products. The use of bacterial cellulose in combination with other agents such as sucrose and carboxymethyl-cellulose improves the dispersion of the product. It is also a low-calorie additive, thickener, stabilizer, texture modifier, and can be used in pasty condiments and in ice cream (Khan *et al.*, 2007).

Nata de Coco, produced by *G. xylinus* using coconut milk as the carbon source, is a popular food or dessert in the Philippines. It is also imported into Japan. Nata de Coco has the plasma cholesterol-lowering effect and is believed to protect against bowel cancer, artheriosclerosis, and coronary thrombosis, and prevents a sudden rise of glucose in the urine. Chinese Kombucha or Manchurian

Tea, obtained by growing yeast and *Acetobacter* in a medium containing tea extract and sugar, is also a popular bacterial cellulose containing food product. Kombucha is believed to protect against certain cancers (El-Saied *et al.*, 2004).

26.7.2 Applications in paper and paper products

Bacterial cellulose is composed of very small clusters of cellulose microfibrils. Therefore, it increases tremendously the strength and durability of pulp when included into paper. The Ajinomoto Co. and Mitsubishi Paper Mills in Japan are currently producing bacterial cellulose for paper products. The disintegrated bacterial cellulose functions as a high retention aid for paper making. Bacterial cellulose is also a valuable component of synthetic paper since nonpolar polypropylene and polyethylene fibers, providing insulation, heat resistance, and fire-retarding properties, cannot form hydrogen bonds. The amount of wood pulp in this type of paper is usually from 20 to 50% to achieve good quality (El-Saied *et al.*, 2004). Recently, several efforts have been directed towards making electronic paper which consists of bacterial cellulose with an electronic dye between transparent electrodes. The device at first looks like fine white paper but when a voltage is applied, the dye turns dark and remains dark, even when the power is turned off. When an opposite voltage is applied, the dye lightens and the device again appears paper-white. This technology could be a basis for electronic books, wallpaper that changes patterns, flexible electronic newspapers, and dynamic paper (Shah and Brown, 2005).

26.7.3 Development of audio components

The development of an acoustic transducer (audio speaker diaphragms) by Sony Corporation and Ajinomoto from bacterial cellulose was the first example of the commercial applications of this valuable product. This application exploits the mechanical properties, especially the higher Young's modulus, of bacterial cellulose. A high sonic velocity and high internal loss is required for an acoustic transducer. The sonic velocity is equal to the square root of the specific Young's modulus, which is equivalent to the quotient of Young's modulus by density. The sonic velocity for bacterial cellulose sheet is approximately 5000 m/sec due to an extremely high Young's modulus and substantially low density. The bacterial cellulose sheet has a high internal loss of 0.03 despite its high sonic velocity. These properties make the bacterial cellulose an ideal material for such diaphragms. The bacterial cellulose is already used extensively for the production of various types of speakers units and headsets (Yoshinaga *et al.*, 1997; El-Saied *et al.*, 2004).

26.7.4 Biomedical applications

Bacterial cellulose is a very versatile material that finds a wide array of biomedical applications, from topical wound dressings to the durable scaffolds required for tissue engineering.

The Johnson & Johnson Company initiated efforts in the early 1980s to commercialize the application of bacterial cellulose in the treatment of different wounds. A Brazilian company, BioFill Produtos Bioetecnologicos (Curitiba, PR Brazil) developed a new wound healing system based on bacterial cellulose. The various products from this company includes Biofills and Bioprocesss (used in the treatment of burns, and ulcer therapy as temporary artificial skin), and Gengiflexs (applied in treatment of periodontal diseases). These products differ from each other with regard to the variable initial concentrations of carbon sources, surface/volume ratios, and extended times of fermentation. Biofills is produced within 2 days, whereas Gengiflexs takes 8 days of fermentation. A gelatinous membrane of bacterial cellulose was commercialized in Brazil as an artificial skin (wound dressing) which was superior to conventional gauze due to a high mechanical strength in the wet state, substantial permeability for liquids and gases, and low irritation of skin. A US-based corporation, Xylos, introduced the XCells family of wound care products which includes XCells Cellulose Wound Dressing and XCells Antimicrobial Wound Dressing. These products have been marketed in the US since 2003. Another bacterial cellulose preparation, Prima Cel, from Xylos Corp. has been applied as a wound dressing in clinical tests to heal ulcers (El-Saied *et al.*, 2004; Czaja *et al.*, 2006, 2007; Klemm *et al.*, 2005).

It has been demonstrated that bacterial cellulose can be suitably shaped for application during biosynthesis. Bacterial cellulose can be synthesized in the shape of formed hollow bodies directly in the culture medium without subsequent treatment. The hollow fibers (bacterial synthesized cellulose (BASYC) tubes) can be used as artificial blood vessels and ureters. Bacterial cellulose is used to protect immobilized glucose analyzers in biosensors for assays of glucose levels due to its high tensile strength, durability, and permeability to liquid and gases. This bacterial cellulose membrane introduced the electrode stability. Cellulose gels containing immobilized animal cell were used for the synthesis of interferon, interleukin-1, cytostatic, and monoclonal antibodies (Klemm *et al.*, 2001, 2005; El-Saied *et al.*, 2004; Czaja *et al.*, 2006, 2007).

BC has potential to be used as a substrate for tissue engineering of cartilage due to its high strength in the wet state as well as its moldability *in situ*, biocompatibility and relatively simple, cost-efficient production (Svensson *et al.*, 2005). Moreover, bacterial cellulose was successfully used in experiments on dogs as a substitute for the duramater in the brain (El-Saied *et al.*, 2004). Bacterial cellulose has potential to be used for veterinary and cosmetic applications (Klemm *et al.*, 2005).

26.7.5 Other applications of bacterial cellulose

Weyerhaeuser Co. (Tacoma, Washington, USA) and Cetus Co. (Emeryville, California, USA) has developed a bacterial cellulose product 'Cellulon', a bulking agent with a broad spectrum of applications, e.g., in the mining sector, in binding and coating applications. Bacterial cellulose can also be applied as a

carrier for immobilization of biocatalysts. It can also be used as a raw material for preparation of cellulose derivatives, e.g., CMC, hydroxymethyl cellulose, cellulose acetate, and methyl cellulose. Bacterial cellulose is also a good binder in nonwoven fabric-like products which is applied in surgical drapes and gowns, and containing various hydrophilic and hydrophobic, natural, and synthetic fibers, such as cellulose esters, polyolefin, nylon, acrylic glass, or metal fibers. The tensile and tear strength of the fabric can be improved by incorporation of a small amount of bacterial cellulose, e.g., 10% of the bacterial polymer is equivalent to 20–30% of latex binder (El-Saied *et al.*, 2004). Philip Morris Inc. developed a paper wrapper for tobacco using bacterial cellulose while The Goodyear Tire and Rubber Co. achieved reinforcement of tire by application of bacterial cellulose (Baldwin *et al.*, 1993; Tung *et al.*, 1994).

26.8 Regulatory status

Bacterial cellulose has been determined to be 'generally recognized as safe' (GRAS) as a food ingredient through the self-determination process under 21 CFR 182.1 using scientific procedures in accordance with 201 (s) (21 USC Section 321 (s)) of the Federal Food, Drug and Cosmetic Act. A GRAS affirmation petition was filed on the basis of the GRAS determination of bacterial cellulose on December 11, 1991, which was accepted by the Food and Drug Administration (FDA) for filing on April 13, 1992. Amendment of the GRAS affirmation petition to GRAS notification was requested under the Interim Policy provision of the FDA's April 17, 1997, GRAS notification proposal (Sec. 21 CFR 170.36 (g) 2) (Omoto *et al.*, 2000).

26.9 References and further reading

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27

Microcrystalline cellulose

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Abstract: This chapter describes the technology and functional properties of microcrystalline cellulose (MCC) in food applications. A number of the functional properties comprise texture modification, suspension of solids, heat stability, fat replacement, ice crystal control, bulking agent, emulsion stabilization, and foam stability. New line extensions of colloidal MCC-based products with improved physical properties have been developed to address certain requirements in a range of food systems. Today a variety of MCC products are used extensively for food, pharmaceutical, and industrial applications. Formulations and procedures are included to assist the formulator in addressing their specific needs.

Key words: microcrystalline cellulose, Avicel®, colloidal, texture, functional properties.

27.1 Introduction

Microcrystalline cellulose (MCC) has been used for over 40 years to provide physical stability and texture modification in a wide variety of food applications. MCC can be used in virtually all food segments such as dairy, convenience, and low moisture applications that require unique stabilization solutions or bulk filler properties to develop stable and palatable products. Properly utilized, MCC can provide heat stability in bakery applications and suspension of insoluble particulates in beverages. Other examples include fat and solids substitution in salad dressings and heat shock stability and texture enhancement in frozen desserts. In addition to stabilization, food scientists encounter other hurdles when creating new products. Generally, stabilizers can have a tendency to disrupt flavor release and interfere with processing efficiencies. Microcrystalline cellulose is known to impart clean flavor without masking desirable flavor

profiles. Manufacturers also rely on the unique rheological properties of microcrystalline cellulose to assist in processing. The combined attributes of texture modification, physical stability, and processing advantages associated with MCC make it a versatile stabilization ingredient.

As food distribution channels become more advanced, new markets emerge, and manufacturers consolidate production facilities, new challenges will be placed on food scientists. As a result, research and development continues in the exploration of new MCC-based products that will expand the spectrum of its functionalities. FMC Corporation is offering newly developed alloys of MCC that will overcome some of the limitations of the more traditional grades of MCC. Several of these newly developed products will be discussed in light of the emerging trends which are driving their development.

A detailed description of microcrystalline cellulose and the functional attributes within each food segment will be discussed in detail in this chapter. The organization of information will be based on the physical properties inherent in MCC and on a description of practical applications that utilize those properties. Formulations and procedures will be included to assist the reader in addressing their specific needs.

27.2 Raw materials and manufacturing process

Microcrystalline cellulose (MCC) is a purified form of cellulose, which is the key structuring agent in all plant material. It is the most abundant and naturally occurring polysaccharide found in nature. Cellulose is a polysaccharide of glucose where the recurring unit is actually two consecutive glucose anhydride units (cellobiose). It is of sufficient chain length to be insoluble in water or dilute acids and alkalis at ordinary temperatures.[1] The 1,4-β-glycosidic linkages of glucose units is responsible for the non-digestive nature of the polymer. The beta linkage also allows an extension of the molecular chain giving rise to tight linear arrangements between individual polymers. Figure 27.1 illustrates the molecular structure of cellulose where the value of n (degree of polymerization) may range from about 50 to 3500 depending on the native source and/or chemical treatments.[2]

The purification process utilized to manufacture microcrystalline cellulose renders the polymer into a highly functional food ingredient. Highly refined pulpwood is most commonly used as the starting raw material for manufacturing

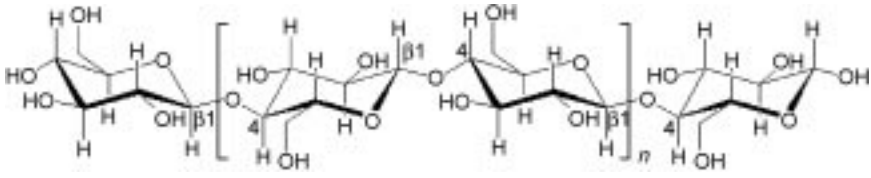

Fig. 27.1 Molecular structure of cellulose.

MCC, although cotton linters and various agricultural by-products can also be sourced. The pulping process is utilized to remove lignin, polysaccharides, low molecular weight cellulosic material, and extractives. The product of dissolving grade wood pulp processes is a high molecular weight cellulose fiber (α-cellulose). These fibers are composed of millions of microfibrils. The individual microfibril is composed of two regions, amorphous and crystalline. Amorphous regions are characterized by the nonlinear arrangement and flexible mass of polymeric linkages. Crystalline regions are composed of tight bundles of cellulose polymers assembled in a rigid and linear arrangement. Crystalline alignments give rise to very powerful associative forces responsible for the great strength of cellulosic materials.[2]

Strong mineral acid hydrolysis is employed to remove all amorphous cellulose portions of the fiber. The product of hydrolysis is a mass of cellulose crystals processed to a level off degree of polymerization (LODP). Following the steps of neutralization, washing, and filtration, the purified microcrystalline cellulose wetcake is diluted in water, and spray-dried to provide large particulate (non-colloidal) MCC. Non-colloidal MCC products are useful in food as a source of fiber and bulk and may also be used as anti-caking agents for oily substances such as shredded cheese. The wetcake can also be subjected to a wet mechanical disintegration step prior to drying. In this process, MCC particles are separated to submicron size and co-dried with carboxymethylcellulose (CMC) or other functional hydrocolloids, e.g., alginate and pectin. These are often referred to as colloidal grades of microcrystalline cellulose and represent the category of MCC products most commonly used in food applications as stabilizers and texture modifiers.

Colloidal MCC products are marketed as dry ingredients that hydrate in the presence of water to form dispersions of insoluble microcrystals capable of providing numerous functional attributes. Properly hydrated sols of MCC form a three-dimensional network based on the electrostatic repulsion of negatively charged cellulose crystals, a charge that is imparted on the surface by the strong association of the functional group inherent in the specific soluble hydrocolloid component. Water management based on this type of network is the basis for all the unique properties imparted to food systems containing microcrystalline cellulose.

Colloidal MCC/CMC (Avicel® CL-611, RC-591, RC-581, and RC-501) products were historically the first colloidal MCC products that were developed to provide special functional properties for specific end uses.[3] These functional properties include ice crystal control, texture modification, emulsion stabilization, heat stability, foam stability, suspension of solids, and fat replacement.

More recently, modification of the functional properties of MCC, by novel coprocessing concepts, has resulted in the discovery of several potentially useful MCC alloys, each offering special properties for specific uses. By modifying the degree of the MCC/CMC interaction through special processing techniques, a colloidal MCC/CMC product (Avicel® RT 1133), is made which has the ability to meet and exceed the thermal F_o death time requirements of retort sterilization while minimizing total process time. An ultra fine MCC/CMC/$CaCO_3$

composition, which is an effective suspending agent in calcium fortified milk, has been made by adding $CaCO_3$ during coprocessing. The $CaCO_3$ was effective in further attriting MCC aggregates during the process to prepare ultra fine MCC particles and thus create a uniquely effective colloidal ingredient.[4]

Another colloidal MCC/CMC product, denoted as Microquick WC-595, had been developed for use in dry mix food systems, where only a minimal amount of shear was available to achieve full dispersion. This readily dispersible ingredient was produced by incorporating dried, sweet dairy whey with the colloidal material prior to spray-drying.[5] Besides keeping all of the traditional functionalities of colloidal RC/CL products, the colloidal MCC/alginate (Avicel® AC-4125) exhibits an additional range of properties which extend the already broad field of its applications even further.[6] These properties include colloidal MCC dispersibility in milk systems, milk gelling properties, dry ingredient blending, and low pH stability (pH 3.5–4.0).

MCC has also been coprocessed with high methoxyl (HM) pectin to provide colloidal MCC stabilization in low pH protein-based beverage systems. The MCC/HM pectin (Avicel® BV-2815) provides new and improved colloidal cellulose properties such as low pH stability, fruit pulp suspension, protein stability, emulsion stability, bake stability and colloidal MCC dispersibility in both dairy and non-dairy systems. Potential areas of application include specialty low pH protein-based beverages, drinkable yogurts and cultured products, fruit sherbets, low pH sauces, baked goods (fruit fillings), and products labeled all natural.

The MCC functional properties may also be modified by the proper choice of CMC. Recently, a colloidal grade of MCC coprocessed with a special type of CMC was developed. This product exhibits unique structural properties, i.e., it has a high degree of elasticity indicative of a well-dispersed and stable system. Because of this property, the MCC/CMC (Avicel® BV-1518) provides effective low viscosity suspension in neutral beverages (calcium fortified milk, chocolate beverages). In addition to neutral pH beverages, other market opportunities include non-dairy and dairy desserts (texture modification), aerated food systems (foam stability), low pH sauces and dressings (emulsion stability), squeezable mayonnaise (viscosity control), and UHT cooking cream (uniform shelf-life consistency without gelation).

27.3 Nutritional and regulatory information

Nutritionally, microcrystalline cellulose is a carbohydrate and is categorized as an insoluble fiber source. It is non-digestible by humans and hence provides no caloric value. In the US, microcrystalline cellulose has GRAS status and has been used safely in foods for over 30 years. Specifications are provided in the Food Chemicals Codex. In the US it is labeled as cellulose gel.

In Europe, microcrystalline cellulose is listed in Annex I of the European Parliament and Council Directive 95/2/EC of March 18, 1995 on Food Additives other than Colours and Sweeteners. It is approved as E460(i) in the list of

permitted emulsifiers, stabilizers, thickening and gelling agents for use 'quantum satis', the level required to achieve a given technological benefit. Purity standards of food grade materials are listed in the EC Directive 98/86/EC. In European markets, MCC is labeled as Cellulose, Microcrystalline Cellulose, or E460. Microcrystalline cellulose has been evaluated by both the EC Scientific Committee for Food (SCF) and the Joint FAO/WHO Expert Committee for Food Additives (JECFA). Both committees determined the maximum Acceptable Daily Intake (ADI) to be 'not specified'.[7] Microcrystalline cellulose is assigned INS 460 (i) and there are published JECFA specifications.

Carboxymethyl cellulose, calcium alginate, high methoxy (HM) pectin, and guar gum are also listed in Annex I of the directive 95/2/EC and are approved for use under numbers E466, E404, E440, and E412 respectively.

27.4 Physical properties

Most stabilizers, viscosifiers, gelling agents, fat mimetics, and textural agents are carbohydrate ingredients based on a starch or gum technology. The utilization of MCC technology to solve food product or processing problems is uniquely different from most hydrocolloids and possesses multi-dimensional properties to provide effective structure, texture, and physical stability in a vast number of food systems. The multi-dimensional nature of MCC ingredients are based on the ability to control viscosity, gelling, surface area, thixotropy, or water binding through an interaction of microcrystalline cellulose with other hydrocolloids and process parameters. The functional properties of MCC based ingredients include suspension of solids, high temperature stabilization, low pH stability in certain instances, emulsion stability, ice crystal control, foam stability, texture modifications, and fat replacement.[8]

When MCC/hydrocolloid grades are properly dispersed, the cellulose particulates and soluble hydrocolloid set up a network. It is the formation of this insoluble cellulose structural network that provides the functionality. Rheology characterization reveals that the formed gel possesses certain solid-like properties of elasticity which exhibits relatively high yield stresses and a time dependent type of flow behavior. Figure 27.2 illustrates the strain sweep curves (G' and G'' versus % strain) for a traditional MCC/CMC product. At 2.6% concentration, the strain sweep indicates that the MCC/CMC has a strong gel structure.

Figure 27.3 shows that this same MCC/CMC gel is shear thinning and the thixotropic loop shows a high degree of thixotropy. Thixotropic properties impart a variety of desirable characteristics suitable for oil and water emulsions or dispersion type products.

Most MCC/hydrocolloid systems are heat stable. Temperature changes have little or no effect on the functionality and viscosity of MCC/CMC dispersions. It is not until temperatures are above 80 ºC, that a slight decrease in viscosity may be detected. This property is extremely important in the preparation of heat stable food products, especially when acids are present. The MCC/hydrocolloid

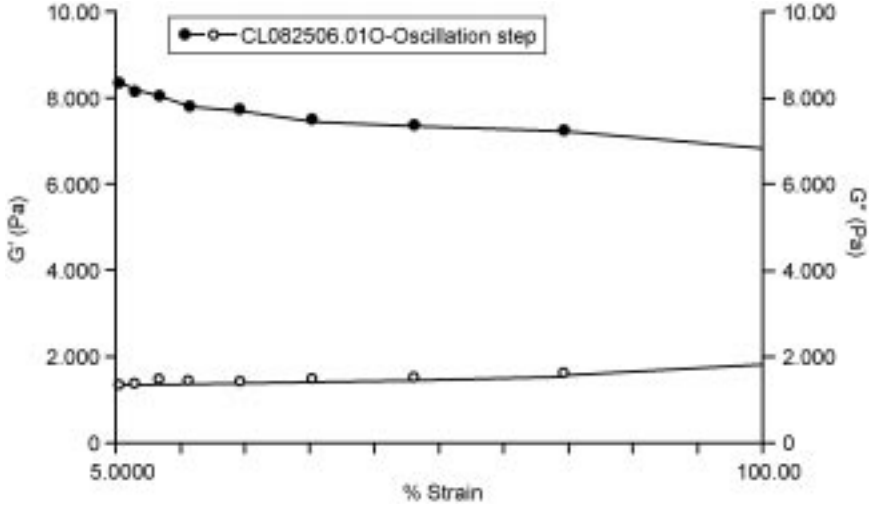

Fig. 27.2 Strain sweep curve for traditional MCC/CMC.

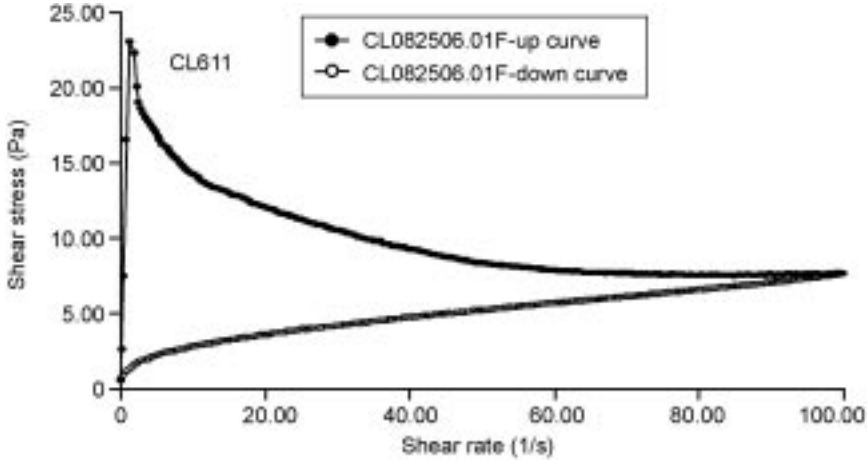

Fig. 27.3 Thixotropy of traditional MCC/CMC.

products will hold up under extremely high temperatures including those used during baking, retorting, UHT processing, and microwave heating, with minimal loss in viscosity, consistency, or color.[9]

27.5 Food applications and functionality

27.5.1 Beverages (suspension of solids)

With proper dispersion, coprocessed MCC/hydrocolloids form a unique 'cellulose gel' network. This network imparts the functional properties

Formulation 27.1 An indulgent healthy chocolate beverage

Ingredients	% by weight
Sugar	6
Cocoa	1.5
Avicel-plus® CM 2159	0.45
$CaCO_3$	0.3
Omega-3 nutritional ingredient	0.24
Vitamin mix	0.2
Vanilla flavor	0.06
Milk flavor	0.06
Partially skimmed milk	to 100

Procedure
1. Prepare a blend of all dry ingredients.
2. Disperse the blend into milk while stirring until homogeneous.
3. Apply UHT treatment (e.g. preheat 75 ºC, heat: 142 ºC for 4 sec); homogenize 170/30 bar. Downstream or upstream homogenization can be alternatively used.
4. Cool down to approximately 10 ºC.
5. Fill aseptically and store (at refrigerated or ambient temperature).

necessary to effectively suspend solids in beverages and other food systems without a significant increase in the viscosity of the product. In an indulgent healthy chocolate beverage (Formulation 27.1), Avicel-plus® CM 2159 provides a uniform suspension of cocoa, calcium, and vitamins; it maintains that stability even at ambient temperatures; and it imparts a creamy 'mouthfeel'.

Formulation 27.2 Calcium fortified, recombined soy beverage

Ingredients	% by weight
Soy protein isolate	3.75
Sugar	2.00
Maltodextrin 15 DE	2.00
Soy bean oil	1.50
Avicel® BV 1518	0.40
Tri-calcium phosphate	0.15
Soy masking flavor	0.10
Flavor	to suit
Demineralized (or soft) water	to 100

Procedure
1. Add the sucrose/Avicel blend to process water and mix at high speed for 5 min.
2. Heat mixture to 50 ºC.
3. Add protein and carbohydrate; continue mixing while slowly raising the temperature to 70 ºC.
4. Turn off heat and continue mixing at low–moderate speed for another 15 min.
5. Add fat and calcium, mix at high speed for 2 min.
6. Add flavors and mix for 2 min.
7. UHT: upstream processing; homogenization 200/20 bar, pre-heating 75 ºC, sterilization 140 ºC.
8. Cool product down to 10–15 ºC.

Avicel® BV 1518, coprocessed with a special type of CMC, creates a uniquely different structure which has added surface charge. It has demonstrated significant improvements in its functional properties, e.g., low viscosity suspension, low pH stability, salt stability, and a high degree of elasticity. In a calcium fortified, recombined soy beverage (Formulation 27.2), Avicel® BV 1518 will provide excellent long-term suspension and shelf stability at ambient temperatures at very low viscosity, similar to that of plain soymilk. In certain regional target applications, the ingredient makes it easy to re-suspend large volumes of insoluble particulates.

27.5.2 Beverages (high temperature stability)

A specialized coprocessed Avicel® RT 1133 was developed to provide rheology stability under retort processing. Use of this ingredient allows the manufacturer to meet and exceed the thermal F_o death time requirements of retort sterilization

Formulation 27.3 Retorted adult nutritional beverage

Ingredients	% by weight
Corn syrup solids 24 DE	8.600
Sucrose, dry granular	6.600
Skim milk powder	3.300
Corn oil	2.500
Soy protein isolate	0.710
Cocoa, red dutched	0.600
Cocoa, natural	0.600
Avicel® RT 1133	0.5–0.8
Potassium citrate, monohydrate	0.300
Soy lecithin	0.280
Natural and artificial vanilla flavor	0.250
Potassium chloride	0.230
Dipotassium phosphate	0.200
Vitamin/mineral premix	0.103
Calcium carbonate	0.100
Sodium chloride	0.070
Viscarin® GP 209 carrageenan	0.01–0.02
Water	to 100

Procedure

1. Disperse Avicel® RT 1133 stabilizer in water using medium shear (a propeller-type mixer) for 10 min.
2. Dry blend vitamins, minerals, proteins and Viscarin® GP 209F carrageenan. Add to above dispersion and continue mixing for 30 min.
3. Add oil and all remaining ingredients. Mix for 5 min.
4. HTST process at 80 °C (175 °F) for 3 s.
5. Homogenize beverage at 3500 psi (3000 1st and 500 2nd).
6. Bottle and rotary retort to the appropriate F_0 value. Cool to 32 °C (90 °F).
7. Alternatively, static retort at 118 °C (245 °F) and 15 psi for 20–30 min. On removal from retort, shake bottles to disrupt any localized gel structure. Continue to shake several times while cooling.

while minimizing total process time. This is especially important in static retort processing where overall product 'thickening' may occur; the net result of which may reduce heat penetration and thereby increase the process time required. This product provides a low viscosity-suspending network, particularly useful in retorted/canned, and UHT/retort processed beverages and foods. See Formulation 27.3 for an example.

27.5.3 Beverages (low pH stability)

For low pH protein beverages the stability requirements for suspension are more complicated. Avicel® BV 2815 consists of MCC coprocessed with high methoxyl pectin. It provides superior stability and viscosity control when compared to other stabilizers, such as pectin, xanthan gum, and propylene glycol alginate, in many of today's high growth markets, including soy-based drinks, drinkable yogurts, functional drinks, energy drinks, and fluid milk products.

Formulation 27.4 Acidified milk-juice beverage

Ingredients	% by weight
Skim milk	20.00
Sugar	8.00
Orange juice concentrate	4.21
Nonfat dry milk	1.73–5.03
Avicel® BV 2815	0.40
HM pectin	0.35
Citric acid	0.25–0.40
Water	to 100
(The pH of the finished product ranges between 4.0 and 4.3)	

Procedure

Phase I (Juice/stabilizer)

1. Blend orange juice concentrate and water.
2. Disperse Avicel® BV 2815 in orange juice/water mixture; heat to 145 ºF (63 ºC) and continue mixing for 15 min.
3. Add pectin and continue mixing for 10 min.
4. Add citric acid; mix until hydrated – approximately 5 min.

Phase II (Milk protein)

1. Dry blend non-fat dry milk and sugar.
2. Add to skim milk at ambient temperature; heat slowly to 145 ºF (63 ºC); mix for 20 min.
3. Maintain temperature throughout formula assembly at 145 ºF (63 ºC).

Cool both phases to ~ 110 ºF (43 ºC), then

Add Phase II (Milk protein) into Phase I (Juice/stabilizer)

Reduce foaming by adding antifoam.

Process:

1. Ultra-pasteurize to 195 ºF (90 ºC) for 15 s; cool to 165 ºF (74 ºC).
2. Homogenize at 2500 psi or 172 bar (two-stage: 2000 psi/500 psi; 138 bar/34.5 bar).
3. Cool to 45 ºF (7 ºC); fill into desired packaging.

This MCC/HM pectin can be activated with low shear in the protein phase, the make-up water, or in the low pH phase including juice and juice concentrates. Order of addition and the process conditions must be chosen carefully in order to provide protein protection and full shelf-life stability.

At protein levels ranging from 3–7 g/8 oz serving in milk-juice, acidified soy-juice, or whey-juice beverages, this product provides long-term stability at lower stabilizer use levels to achieve suspension. Pectin by itself may appear stable as it has minimal serum separation. However, higher levels may be needed to prevent sedimentation over time, especially at higher protein levels, which may result in higher viscosity beverages. Pectin as a sole stabilizer may not provide complete stability in low pH, high protein beverages, and can result in sedimentation. Avicel® BV 2815 consists of MCC and pectin, which may both be considered natural. Additional HM pectin is most often added in combination with MCC/HM pectin to provide complete and effective stability over the entire expected product shelf-life. See Formulation 27.4 for an example of an acidified milk-juice beverage.

27.5.4 Dressings, sauces, and cooking cream (emulsion stability)

Effective stabilization against the coalescence of oil globules in an emulsion system can be obtained utilizing the 'cellulose gel' network. MCC functions as an emulsion stabilizer and thickener because of the strong affinity cellulose has for both oil and water. This results in an orientation of solid particulates at the oil-in-water interface.[10] In addition, the viscosity developed by the MCC/hydrocolloid products acts to thicken the water phase between the oil globules preventing their close approach and subsequent coalescence. This combined functional effect can be utilized to stabilize emulsions and dispersions in food products under adverse process and storage conditions.

Avicel-plus® CM 2159 and Avicel-plus® SD 4422 prevent separation of cream during shelf-life at temperatures up to 40 °C and improve cooking stability under difficult conditions, e.g., wine sauce. Creams with different viscosities can be created by using the appropriate Avicel-plus® stabilizer, e.g. Avicel-plus® SD 4422 provides high hot viscosity and improved cling to pasta dishes. The ingredients enable the possibility to create fat reduced recipes with health benefits for the consumer and offer a cost reduction for the manufacturer. Formulation 27.5 provides an example.

27.5.5 Vegetable fat whipping cream (foam stability, syneresis control, fat replacement)

In aerated food systems, foam stability depends primarily on the types of additives present and their ability to produce the necessary structural strength in preventing the coalescence and subsequent collapse of the air bubbles. MCC is not a whipping aid or film-forming material, but it does provide effective foam stabilization in a variety of whipped and/or aerated food systems. MCC disper-

Formulation 27.5 UHT dairy cooking cream

Ingredients	5% fat formula, wt%	20% fat formula, wt%
Cream, 40% fat	12.5	50
Functional native tapioca starch*	2.0–2.5	1.0–2.5
Emulsifier E 471	0.2	0.2
Avicel-plus® Stabilizer	see Table 27.1	see Table 27.1
Flavor	to suit	to suit
Skimmed milk	to 100	to 100

*for UHT processing

Procedure

1. Blend all dry ingredients and add to a mix of cream and milk.
2. Pre-heat to 75 ºC (167 ºF).
3. Homogenize at 100/20 bar (approx. 1500/250 psi).
4. UHT treatment at 140 ºC (284 ºF) for 3 to 5 s.
5. Cool to 15 ºC (59 ºF) and fill aseptically.

Table 27.1 Viscosities obtained at different fat levels

Stabilizer with 2% starch	Viscosity*, cP 5% fat	12% fat	18 to 20% fat	30% fat
0.35% Avicel-plus® CM 2159	1,500–2,500	5,000–6,000	12,000–14,000	25,000–30,000
0.70% Avicel-plus® SD 4422	4,000–5,000	9,000–11,000	16,000–18,000	NA

* Viscosity measurement at 4 ºC: Brookfield equipped with spindle#2, speed 12 rpm, measured after 1 minute.

sions act to thicken the water phase between air cells and provide added structural integrity to the protein film surrounding the air cells. The cellulose gel network created improves the body and texture, foam stiffness, and stability of both dairy and non-dairy type whipped topping products. In addition, MCC is effectively used to stabilize mousse products, marshmallow toppings, confectionery products, and controls overrun in frozen desserts.[8]

The inherent rheological properties of cellulose gel dispersions allow the MCC/hydrocolloid products to be utilized in many reduced fat or nonfat food applications. The consistency of oil-in-water emulsions can vary from a thin fluid material at low oil levels, to a thick viscous paste at very high oil levels. The water-holding capacity of the cellulose gel network and the thixotropic nature of the gels allows the majority of oil/fat to be removed while maintaining the required rheological and textural properties found in the full fat counterpart of these same food products.[11]

Avicel® can be blended with other dry ingredients and added directly to the water phase, allowing a straightforward use, requiring no pre-hydration. The MCC can stabilize the liquid cream emulsion, preventing phase separation and maintaining a constant viscosity during shelf-life. It further insures a short whipping time and will generate a smooth whipped cream with excellent foam stiffness and stability. MCC provides perfect syneresis control of whipped cream, prevents foam collapse, and improves freeze thaw stability. An example of a vegetable fat whipping cream is given in Formulation 27.6.

Formulation 27.6 Vegetable fat whipping cream

Ingredients	27% Fat, wt %	22% Fat, wt %
Fat phase		
Hydrogenated vegetable oil	27.00	22.00
Lactic acid ester of mono-diglycerides of fatty acids	0.50	0.70
Tartaric acid ester of mono and diglycerides of fatty acids	0.20	0.20
De-oiled soybean lecithin	0.10	0.10
Aqueous phase		
Sugar	11.00	11.00
Sorbitol (70% solids in water)	0.80	0.80
Sodium caseinate	0.70	0.70
Avicel® BV 1518	0.45	
Avicel-plus® GP 3419		0.45
Di-potassium phosphate	0.10	0.10
Flavor	0.05	0.05
Water	to 100	to 100

Procedure

1. Gently and completely melt the vegetable fat at 70 ºC then add the emulsifiers and ensure a clear and homogeneous solution is obtained.
2. Dry blend the Avicel, Na-caseinate and phosphate. Sugar can be blended with these ingredients or added separately to the water phase.
3. Disperse this blend in hot water (about 70 ºC) for 10 min with intensive mixing (e.g. with a high speed propeller mixer or a rotor stator mixer). Water hardness should be as low as possible. If hard water is used, a sequestrant should be included in the water phase, e.g. 0.2% tri-sodium citrate (E331).
4. Add sorbitol and flavors to the water phase and mix intensively for 5 min.
5. Make a pre-emulsion by slowly adding the oil phase to the water phase while stirring continuously for 5 min.
6. UHT process by pre-heating to 70–80 ºC, sterilizing at 142 ºC for 4 s, cooling to 70–80 ºC, homogenizing at 180/20 bar then cooling product rapidly to 5 ºC.
7. Allow product to maturate (aging) for 24 hours at 4 ºC.

Note: As with all vegetable fat whipping creams, storage should be between 4 and 7 ºC in a dry place and should never exceed 20 ºC.

27.5.6 Whipping process

Creams should be whipped at 4–7 ºC using a kitchen type mixer (e.g. Hobart®, Kitchenaid®, Kenwood®). During whipping, overrun and foam stiffness will increase in parallel up to a maximum level. Thereafter further whipping will reduce the overrun and lead to phase inversion. With some stabilizers the structure is developed during whipping causing the cream to form a consistent mass on the whisk. This requires continuous visual inspection of the whipping process to determine the optimum whipping time. Creams with Avicel develop their texture immediately after whipping due to its unique thixotropy. Therefore visual inspection is not necessary and the whipping process should be stopped after the designated whipping time, e.g. 2 to 3 minutes (see Table 27.2).

Table 27.2

(a) Avicel® charcteristics

	Purpose	Declaration
Avicel® BV 1518	Stabilization of regular (27% fat) whipping creams	Cellulose (E460), Cellulose gum (E466) Sugar added for standardization Calcium chloride added as a carrier
Avicel-plus® GP 3419	Stabilization of reduced fat (22% fat) whipping creams	Cellulose (E460), Cellulose gum (E466) Sugar added for standardization

(b) Typical product characteristics

Characteristic	Comments
Cream pH	6.5–6.7
Cream viscosity stability	No significant increase after 8 weeks at 4 ºC
Emulsion stability at 40 ºC, 1 week	Good (no phase separation)
Whipping time	2–2.5 min, Kitchenaid® at max speed
Foam stiffness (SMS Texture Analyzer)	120–150 g
Foam stiffness stability	No decrease after 24 h at 20 ºC
Overrun	300–400%
Syneresis (water drainage)	None after 24 h at 20 ºC
Freeze thaw stability	Good

27.5.7 Ice cream (heat shock stability, ice crystal control)

Microcrystalline cellulose has been used for many years in frozen desserts. It was first formulated into fat-free ice cream in the early 1960s to replace the lost eating qualities resulting from the removal of butterfat. Since these earlier applications, the quality of MCC colloidal products has advanced providing broader stability solutions to ice cream. Manufacturers are constantly confronted with the need to minimize shelf-life defects (mainly ice crystal development)

and transportation concerns (altitude protection). Process improvements, cost reduction, and improved eating quality also require constant consideration.

Sufficient shear forces are required in order to properly disperse colloidal grades of MCC in ice cream mix. Homogenization pressures upwards of 2,500 psi are typically recommended. Recent developments by FMC Corporation have significantly reduced the homogenization requirements to less than 1,500 psi. Properly hydrated, MCC imparts a unique stabilization mechanism into the ice cream mix. Dairy proteins and MCC interact to form stable matrices capable of eliminating whey separation in resale mix and melted ice cream. This physical complex also adds considerable integrity to the air cells translating to improved protection against altitude abuse and to a better and more uniform melt down behavior.

Newly developed forms of microcrystalline cellulose provide resistance to the coalescence of air cells in ice cream during heat shock. The value is reflected in smoother and creamier textures, controlled melt, and altitude protection. The light photomicrographs (Figs 27.4 and 27.5) reveal the improvements in air cell integrity. Figure 27.4 represents the newest form of colloidal MCC compared to the standard grades of MCC in Fig. 27.5.

Heat shock protection is a major concern for manufacturers. MCC is the only stabilization system that relies on insolubility to assist in the inhibition of ice crystal growth.[12] As a result of freeze concentration, the serum phase of the frozen ice cream is highly concentrated with MCC. The concentration of MCC sols produce strong elastic gels capable of restricting water migration and setting up physical barriers to the onset of larger ice crystals during abusive temperature

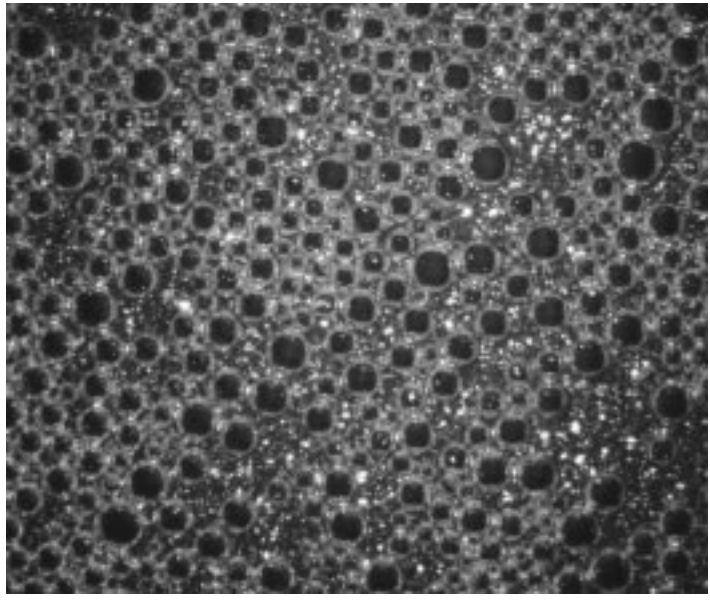

Fig. 27.4 Low-fat ice cream air cell integrity with new colloidal MCC.

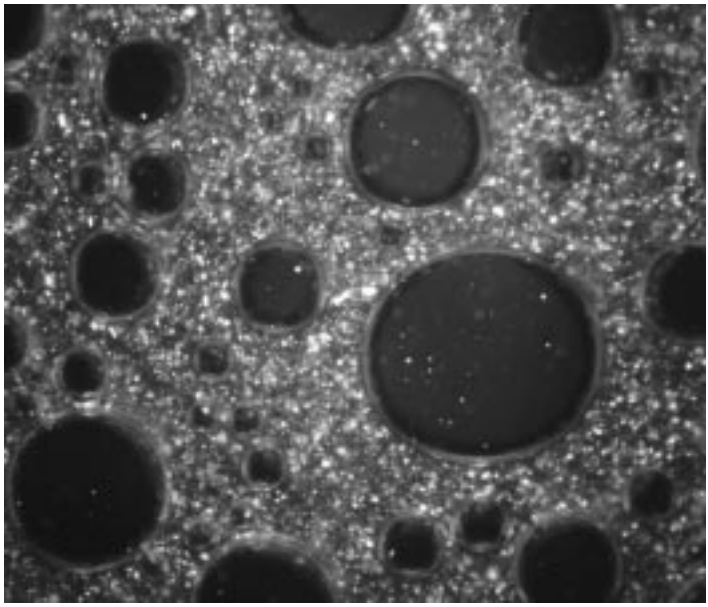

Fig. 27.5 Low-fat ice cream air cell structure with standard MCC.

conditions. Highly structured serum phases stabilized with MCC are also responsible for rich mouthfeel and melt resistance. Quality improvements such as these are often the focus of formulations low in fat, low in solids, and/or low in sugar.

Historically, consumer acceptance of lean ice cream formulations such as reduced/low fat, reduced sugar, and reduced milk solids not fat (MSNF) has been poor. The removal of solids from ice cream formulations can partly be replaced with sugar alcohols, and other bulking agents. These alone will not impart the needed solids replacement to match the mouthfeel and texture of the finished product. Unlike many other hydrocolloids typically used in frozen desserts, MCC imparts the rheological properties of a higher solids formula. This translates into the perception of a more full bodied texture even at lower total solids. The solids-like properties of microcrystalline cellulose also make it a useful additive in novelty applications where shape retention is lacking. For example, ice cream sandwiches and extruded bars can be produced with short textures providing clean cuts and well defined impressions. A formula for a low-fat frozen dessert is given in Formulation 27.7.

27.5.8 Non-colloidal MCC (bulking agent, fat replacer, flow aid, tablet excipient)

The powdered non-colloidal grades of microcrystalline cellulose (Avicel® PH) are white, odorless, tasteless, relatively free-flowing powders that were originally used as sources of fiber in low calorie foods or as a flow aid for

Formulation 27.7 Low fat ice cream

Ingredients	% by weight
Sugar	12.0
Milk solids nonfat	9.0
Corn syrup solids 36 DE	8.0
Butterfat	4.0
Whey solids	3.0
Gelstar® IC 3542 Stabilizer System: [Colloidal MCC/CMC, mono, diglycerides polysorbate 80, carrageenan]	0.5–0.6
Total solids	36.55

Procedure

1. Disperse the Gelstar® IC 3542 stabilizer into the mix at the point of greatest agitation to prevent clumping and promote better incorporation. To minimize processing viscosity, add Gelstar® IC 3542 into the milk and cream.
   When preparing concentrated dispersion of Gelstar® stabilizer in milk, do not exceed 2% concentration in order to minimize the dispersion viscosity.
   Note: When preparing reconstituted formulations, add milk powder prior to adding Gelstar® IC 3542. For best results, add the stabilizer slowly into the milk while mixing with a high speed blender to avoid clumping and ensure optimum functionality of the stabilizer.
2. Pasteurize and homogenize according to established guidelines: 2,000/500 psi and HTST at 80 ºC or 175 ºF for 25 s. Good homogenization is important to activate the stabilizer.
3. Cool mix to 40 ºF (8 ºC) and age for a minimum of 4 hours for more effective freezing and crystallization of the butterfat.
4. Freeze and package.
5. Rapid hardening assures minimal ice crystal formation.

grated cheese. These later found wide acceptance within the pharmaceutical industry for use as a binder/excipient in direct compression tablets. The PH grades are insoluble, chemically inert, and crystalline in nature with a very high degree of porosity.

More recently, small particle sized MCC (Avicel® LM-310), less than about 30 microns, have been used in foods as bulking agents and as fat substitutes. The particles are considerably smooth, having a high absolute density, a high loose bulk density (>0.40 g/cc), a low degree of oil absorptivity (<1.0), and a rather spheroid shape, as seen at 150 times magnification. The spray dried, wet-milled microcrystalline cellulose has found application in low moisture cookie fillings and coatings.[13]

The non-colloidal Novagel® RCN grades consist primarily of colloidal MCC particles coprocessed with guar gum to produce a bulking agent with fat mimetic properties. The cellulose-guar hydrogen bonding interaction was utilized to function as a fat-like substance in aqueous-based food systems.[14] The development of a guar layer adsorbed onto hydrated cellulosic surfaces provides the MCC/guar aggregates with the textural smoothness and palatability characteristics resembling the physical and organoleptic properties of fat/oil emulsified

in water. These products have been used as a bulking agent in nonfat frozen desserts, nonfat processed cheese, nonfat salad dressings/mayonnaise, low fat spreads, and other non-aqueous food systems, e.g., peanut butter, chocolate coatings.[15–18] (These have also been used in pharmaceutical applications as a chewable tablet excipient for taste masking vitamins and drug actives such as APAP, aspirin, etc.).

In reduced fat/calorie and low moisture products, such as confectionery products and biscuit fillings, due to water activity and texture limitations, structured water cannot be used as a direct replacement for fat, as is the practice with emulsion systems. These applications, in contrast, require high levels of non-caloric or low calorie bulking agents to achieve a sufficient calorie/fat reduction. The new grades of cellulose microcrystals were manufactured to reduce the porosity and surface area of the particle so that absorption properties are minimized. These grades can be used up to 15%, often in conjunction with sugar syrups, to function as a high quality replacement for fat in low moisture foods with water activities restricted to the range 0.45–0.6. An example of a nougat-style confection is given in Formulation 27.8.

Formulation 27.8 Nougat style confections

Ingredients	% by weight
Part I	
Corn syrup #1 42 DE	10.0
Water #1	8.7
Powdered sugar 10X	5.0
Egg white solids	2.0
Avicel® LM 310	1.0
Part II	
Corn syrup #2 42 DE	32.5
Sugar	31.6
Water #2	8.4
Cocoa powder 10–12% fat	0.8
Total	100

Procedure

Part I

1. Dry blend powdered sugar and egg white solids in Hobart® mixer.
2. Add water to the dry blend and mix for 1 min at low speed. Hydrate for 5 min.
3. Add corn syrup and Avicel® LM 310 cellulose gel; then beat to a stiff peak at speed #3 using the whipping attachment.

Part II

1. Combine corn syrup, sugar and water. Heat to 135 °C (275 °F).
2. Slowly add this hot solution into the Hobart® mixer containing the Part I mixture and stir with whip attachment until a uniform mix is achieved. Whip at high speed for 1 min.
3. Add cocoa and mix at low speed for 2 min, or until uniform.
4. Transfer to cooling slab and cut or extrude into desired size or shape. Nougat can be enrobed with chocolate or other coatings, if desired.

27.6 Future trends

Health and well-being are among the most important drivers of growth in applications of MCCs. A brief listing of upcoming developments in the field of coprocessed MCC-based stabilizing ingredients follows.

27.6.1 MCC/lipids

Colloidal MCC-based/lipid compositions comprising MCC and one or more lipids, in which the lipid is adsorbed onto the surface of the cellulose, are envisioned to function in vitamin fortified milk systems, nutraceutical enhanced foods, and other low moisture and non-aqueous food systems. In recent years, MCC/surfactant composites have been developed to impart improved surfactant dispersibility and to provide low calorie bulking properties, low viscosity emulsion stability, and enhanced textural attributes in low-moisture or in non-aqueous reduced calorie food systems.[19] These food systems include convenience cooking sauces, high fat nut butters; confectionery spreads including cookie and wafer spreads (such as in cream-filled cookies); chocolate confections and other compound confectionery coatings; confectionery fillings such as nougat, caramel, truffle, fudge; icings and glazes, and bakery cream fillings.[20,21] Pharmaceutical uses include tabletting excipient/lubricant for controlled release drug delivery systems.

27.6.2 MCC/food approved hydrocolloids

This product concept involves coprocessing colloidal MCC with food approved natural hydrocolloids to produce unique, novel properties for special food applications. For example, a coprocessed MCC/carrageenan product has been developed to provide acid and salt stability and thus offers new growth opportunities.[22] Some of the potential areas of application include cultured dairy products (yogurt, sour cream, etc.), low pH dairy systems (yogurt drinks, stabilized frozen yogurt, whipping creams, puddings), specialty sauces/dressings (fermented sauces, vinaigrettes, etc.), beverages (citrus-flavored drinks, nectars, etc.), dry mixes, and products labeled ‘all natural’.

27.6.3 MCC-based bulking agent for fat and sugar calorie reduction

Several new coprocessed MCC-based bulking agents involve using polydextrose, maltodextrin, inulin, lactose, sugar alcohol derivatives, such as mannitol, sorbitol, etc., as a barrier-dispersant for the MCC particles during drying. For example, maltodextrin offers advantages in terms of its low degree of hygroscopicity, stability, and high degree of solubility without a marked effect on the viscosity of the systems. The coprocessed MCC/maltodextrin can potentially be used where colloidal grades of MCC are used including frozen desserts, salad dressings, beverages, and bakery products. Other potential uses are as a bulking agent/fat replacer in non-aqueous food systems, e.g., peanut butter and confectionery fillings and coatings.[23]

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28

Hydrocolloids for coatings and adhesives

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Abstract: Much progress has recently been made in the fields of coatings and adhesives. In foods, coatings serve as inhibitors of moisture, gas, aroma and lipid migration. They can include antioxidants, preservatives, nanoparticles or other additives to improve foods' mechanical integrity, handling and quality, and to change surface gloss. Hydrocolloid glues are distinguished from most organic-based adhesives by their hydrophilic, non-toxic nature, and are used at many different concentrations, viscosities, and molecular weights. The chapter describes properties of adhesive hydrocolloid preparations, their adhesion mechanisms, their utilization in foods and other areas, how to measure their adhesiveness, as well as novel and possible developments.

Key words: coatings, glues, composite, barrier, nanoparticles, adhesion.

28.1 Introduction

Water-soluble gums are beneficial in many fields, including adhesives, agriculture, biotechnology, ceramics, cosmetics, explosives, food, paper, textiles and texturization, among many others. Two fields in which many novel utilizations of gums have been developed and much progress has been recently made are coatings and adhesives. In foods, hydrocolloids are used to produce thin layers of edible materials on surfaces or between food components. Such films serve as inhibitors of moisture, gas, aroma and lipid migration. They can include antioxidants, preservatives or other additives to improve food mechanical integrity, handling and quality, and to change surface gloss.[1] In addition to foods, gums are also used in coatings for seeds, fiberglass, fluorescent lamps, glass, metals, optical products, paper products, latex and textiles.[2] Hydrocolloid glues are distinguished from most organic-based adhesives by

their hydrophilic, non-toxic nature, and are used at many different concentrations, viscosities, and molecular weights. Gums are inflammable and possess good wettability properties which enhance their penetration into porous substrates. The development of synthetic hydrocolloids throughout this century has broadened their uses with such commodities as paper, wood, textiles, leather, food, cosmetics, and medicine.[3]

28.2 Today's edible protective films

28.2.1 Edible packaging materials – a general approach

Hydrocolloids (gums) are high molecular weight molecules that can be used to produce solutions or highly viscous suspensions or gels with low dry-substance content.[4] Many gums, such as alginate, carrageenan, cellulose and its derivatives, pectin, starch and starch derivatives, among others, are hydrophilic, and the coatings they produce are therefore naturally limited in terms of moisture-barrier properties. However, if they are used in gel form (with no drying), they can retard moisture loss, for example from meats and soft white-brined cheeses, during short-term storage when the gel acts as a sacrificing agent rather than a barrier to moisture transmission.[5] Reviews on food-packaging materials based on natural polymers can be found elsewhere.[6–10] Macromolecules found in edible films are mainly polysaccharides, proteins, and lipids.[5] Polysaccharide and protein films are good gas barriers, but poor moisture barriers. Conversely, pure lipid films are good moisture barriers, but poor gas barriers. Considerable attention has been focused on the development of composite edible films that utilize the favorable functional characteristics of each class of macromolecule.[8] Edible coatings should be tailored to suit the food product they are designed to enrobe. Organoleptic properties of coatings (usually tasteless) are very important for consumable food products. Another option is to deliberately change the taste of the coating, allowing its sensorial properties to create a nice blend with those of the product itself. Besides being tasty or tasteless, the coatings should have the required mechanical properties and tailor-made barrier stability. Other relevant benefits are simple application, biodegradability and non-toxicity, safety for the manufacturer and consumer, and low cost.[7]

The use of plastic for packaging has greatly expanded in recent years. In this context, biodegradable films are energy-saving and environmentally friendly.[11] Biopolymers are alternative materials used in the production of edible and/or biodegradable films. A very important restriction with these films is their sensitivity to environmental conditions, such as temperature and relative humidity. The production of films based on gelatin and polyvinyl alcohol blends can provide an alternative solution, reducing the sensitivity to humidity.[12] Biodegradable films obtained from chitosan and methylcellulose can also reduce environmental problems associated with synthetic packaging. However, the cost of biodegradable films is still prohibitive; the use of chitosan, a waste by-

product of the fishing industry, could provide a good alternative.[13] The literature contains many suggestions for the production of biodegradable films. Examples include films prepared from kafirin, the prolamin protein of sorghum, which could be an environmentally friendly alternative to synthetic-plastic packaging films. Their inferior functional properties can be improved by adding up to 20% (w/w) tannic acid or sorghum-condensed tannins as modifying agents during casting.[14] Biodegradable films and hard capsules for pharmaceutical applications could be developed from vegetable starches with physical and mechanical properties similar to those of synthetic and gelatin products.[15] Another inexpensive source for biodegradable films might be fish-muscle-protein films, prepared from blue marlin meat that is stored at 30 °C to intentionally lower its meat's quality. It was revealed that biodegradable films can even be produced from very low quality fish meat, and that the bacterial population in those films could be drastically reduced by the addition of e-polylysine.[16]

Summing up, natural biopolymers could serve as a choice material for the construction of different types of wrappings and films. Edible and/or biodegradable packagings produced from macromolecules of agricultural origin provide a supplementary and sometimes essential means of controlling physiological, microbiological, and physico-chemical changes in food products. This is accomplished by controlling mass transfer between the food product and the ambient atmosphere or between components in heterogeneous food products, and by modifying and controlling the food's surface conditions (pH, level of specific functional agents, and slow release of flavor compounds).[17] The material's characteristics (polysaccharide, protein, or lipid, plasticized or not, chemically modified or not, used alone or in combination) and fabrication procedures (casting of a film-forming solution, thermoforming) must be adapted to each specific food product and usage conditions (relative humidity, temperature). Some potential uses of these materials could be the wrapping of various fabricated foods, protection of fruits and vegetables by controlling maturation, protection of meat and fish, control of internal moisture transfer in pizzas, all of which hinge on the organoleptic, mechanical, and gas- and solute-barrier properties of the films.[17]

28.2.2 Meat, seafood and fish coatings

The use of edible protective films has risen rapidly due to the search for improved food protection to prevent moisture loss, retard bacterial growth, eliminate oxidative rancidity, and improve appearance and food handling. Many of today's food coatings are similar to those used in the past, and some of the so-called novel or innovative approaches have already been tried. A good example is the inclusion of antibiotics or other preservatives in meat coatings. While the literature contains numerous examples of different coatings and their compositions, properties, success or limitations, this section deals only with a limited number of applications.

Meat is a main source of dietary protein in the United States. In its broadest definition, it is animal tissue used as food. Most often it refers to the skeletal

muscle and associated fat, but it may also refer to non-muscle organs, including the lungs, liver, skin, brains, bone marrow, and kidneys. Lean meat trimmed of all visible fat is composed of ~75% water, 19% protein, and ~2.5% fat. Its components are muscle fiber, connective tissue, fat tissue, bone, and pigment. The proportions of these ingredients affect the eating quality of the meat. Worldwide, commonly eaten animal meats include beef, veal, lamb, pork, fowl, and less often, game animals.[18] After slaughter, as much blood as possible is removed from the animal. Since there is no oxygen supply to the muscles, glycogen (stored form of carbohydrates in living animals) can only be oxidized to lactic acid and its accumulation decreases the muscles' pH. This in turn affects their water-holding capacity and thus the eating quality of the meat.[18] After slaughter, the supply of adenosine triphosphate (ATP) to the muscles is exhausted and stiffening of the carcass (i.e. rigor mortis) sets in.[18] Aging is recommended in order to soften the muscle, a phenomenon that occurs as a result of a decrease in the number of chemical bonds between actin and myosin in the myofibrils, and the activity of inherently present proteolytic enzymes. Ideally, aging should be practiced for 3 to 6 weeks at 1–2 ºC, under 70% humidity and a carbon dioxide atmosphere. However, the more commonly practiced, less expensive aging protocol consists of 2 days at 21 ºC in 80–90% humidity. Meat coatings are designed to treat fresh or processed meats.[18]

More than 240 different species of seafood are consumed in the United States. Seafood may be divided into two general groups: fish and shellfish. Fish as a food describes the edible parts of water-dwelling, cold-blooded vertebrates with gills. Fish are generally sold after they have been 'dressed', i.e. scaled, eviscerated and their head, tail and fins removed. They can be purchased whole or in cuts, i.e. as steaks, chunks, single fillets or butterfly fillets (the two sides of the fish held together by the belly's skin). They can be sold after freezing but are also widely preserved through drying, salting, and canning. Other edible water-dwelling animals, such as mollusks and crustaceans, are often collectively referred to as shellfish. Crustaceans include crab, lobster, and shrimp. Mollusks include abalone, clams, oysters, and scallops. Crabs and lobsters are purchased fresh or cooked. Meat from cooked crustaceans is available chilled, frozen, and canned.[18] Alginate-based coatings have been used to coat meats,[19–23] and are good oxygen barriers.[24] Calcium-alginate coating of lamb carcasses helped reduce surface microbial growth and achieve a faster chill rate.[25] A number of hydrocolloids, namely alginates, carrageenans, cellulose ethers, pectin and starch derivatives, have been used to improve stored meat quality. Such coatings serve as sacrificing agents, i.e. moisture loss is delayed until it evaporates from the film.[26] Meat, poultry, seafood, dough products, vegetables, extruded foods and cheeses coated with calcium-alginate exhibit reduced shrinkage, and decreased oxidative rancidity, moisture migration and oil absorption during processing.[27] Alginates, carrageenans, cellulose ethers, pectin, and starch derivatives have been reported to improve stored meat quality.[26] Alginate coatings have been used to retard the development of oxidative off-flavours in precooked meat patties. The shelf-lives of frozen shrimp, fish, and sausages were extended by use of alginate coatings.[28,29]

In Japan, a polysaccharide edible film (Soafil) has found wide use in the meat industry as a casing for processed smoked meat. The meat can be wrapped in edible film before soaking and steaming. The smoke flavor permeates the film during the smoke cycle, and the film dissolves during steaming. The finished ham has a smooth lustrous surface and a good-textured bite on its surface. Carrageenan coatings have also been used to reduce off-odor development in chicken carcasses.[4] Carrageenan coatings with soluble antibiotics have been found to be effective spoilage-retardation agents.[3,30] Similarly, agar coatings with water-soluble antibiotics were found to effectively extend the shelf-life of poultry parts.[31] Agar coatings have also been used as a vehicle for the addition of bacteriocin (nisin) to fresh poultry stored at 4 ºC in order to reduce contamination by *Salmonella typhimurium*.[32] The antimicrobial activity and film-forming property of chitosan make it a potential natural food preservative or coating material. In this review, we focus on the applications of chitosan for the quality improvement of various foods of agricultural, poultry, and seafood origin.[33]

Chitosan has been found useful for the preparation of safe and stably-coated mutton kebabs and streaky bacon.[34] Coating of pink salmon fillets with chitosan was effective at reducing relative moisture loss by about 50% compared to control non-coated fillets. Chitosan delayed lipid oxidation, and there were no significant effects of coating on the color parameters or whiteness values of the cooked fillets after 3 months of frozen storage.[35] Chitosan is also beneficial in the development of coatings for the shelf-life extension of fresh fillets of Atlantic cod and herring. A significant reduction in relative moisture loss was observed for cod samples coated with chitosan after up to 12 days of storage. Chitosan coating also significantly reduced chemical spoilage due to lipid oxidation and growth of microorganisms.[36]

It can be concluded that edible films have a great number of potential applications in meat and fish processing, and that their functional characteristics depend on their constituents. Edible films prepared from lipids and hydrophobic compounds (waxes and resins) show good water vapor barrier properties. A variety of polysaccharides and their derivatives (alginate, pectin, carrageenan, starch, cellulose, and cellulose derivatives) reduce fat absorption. Oxygen and carbon dioxide permeability is reduced for films containing proteins.[37] An edible coating composed of whey proteins and acetylated monoglycerides protected smoked salmon fillets against dehydration and lipid oxidation. A multicomponent coating comprised of collagen, casein and cellulose derivatives reduced oil absorption during frying. Microbial proliferation, in particular *Listeria monocytogenes* growth, was reduced by coating fish products with a formulation of hydrocolloids, organic acids (lactic and acetic acids) or antimicrobial compounds.[37]

28.2.3 Gum coatings for fruits and vegetables – past and present

A fruit is the ripened ovary – together with the seeds – of the flowering plant. Fruits are the means by which flowering plants disseminate their seeds. Many

foods are botanically classified as fruits but are treated as vegetables in cooking. Examples include squash, pumpkin, cucumber, tomato, peas, beans, corn, eggplant, and sweet pepper. The definition of a vegetable is traditional rather than scientific and is somewhat arbitrary and subjective. Fresh fruits include 75–90% water. Except for a few fruits (e.g. avocados, coconuts, and olives), their fat content is low. Fruits also include carbohydrates, minerals, acids, enzymes, pigments, aromatic compounds, and vitamins.[18] An acceptable estimate is that 25%, and up to 80%, of freshly harvested fruits and vegetables are lost through spoilage.[38] Fruits and vegetables continue their metabolic activity after harvest and will either rapidly ripen if climacteric, or senesce if non-climacteric, unless special procedures are adopted to slow down these processes.[4] Post-harvest shelf-life extension can be achieved by applying edible coatings, which are semi-permeable to water vapor and gases. Such coatings can enhance or replace other techniques used for the same purpose, such as modified atmosphere (MA) or controlled atmosphere storage.[39,40] Other achievements of coating applications include improvement of mechanical handling properties, and retention of volatile flavor compounds.[41,42]

Coatings are designed to affect permeability to oxygen and carbon dioxide: thus, the coated fruit or vegetable becomes an individual package with a MA.[4] Respiration of the commodity causes oxygen depletion and carbon dioxide build-up, and care must be taken in designing the coating: if oxygen levels drop too low, anaerobic reactions will proceed, resulting in off-flavors and abnormal ripening.[43] Ethanol and acetaldehyde concentrations in the tissue can be used as monitors for final and next-to-final products of anaerobic respiration. Levels of oxygen below 8% decrease ethylene production, and carbon dioxide levels above 5% delay or prevent many responses to ethylene in the fruit tissue, including ripening.[43] Therefore, if the coating can create moderate MA conditions, a climacteric fruit will exhibit decreased respiration, lower ethylene production, slower ripening, and extended shelf-life.[4]

Other sodium-alginate and gellan-based coatings have been investigated.[44–47] As a barrier to moisture loss, these hydrocolloid coatings postponed the drying of mushroom tissue, thereby preventing changes in its texture during short periods of storage. Coated mushrooms had a better appearance and gloss, as did garlic.[48] Incorporation of ingredients found naturally in garlic skin or which are chemically similar to these into the gum solution before coating improves film adhesion to the surface of the coated commodity (Fig. 28.1).

Water loss and browning of cut apple slices were inhibited by coatings of chitosan and lauric acid.[49] The chitosan derivative NOCC (N, O-carboxymethyl chitosan) selectively forms permeable nontoxic films which are used as a post-harvest edible coating for fresh foods.[50–52] Synthetic polymers can modify the permeation response of a chitosan membrane to O_2 and CO_2.[53] Extracellular microbial polysaccharides, such as pullulan, levan, and elsinan, can be used to produce edible and biodegradable films. Pullulan films are clear, odorless and tasteless and act as efficient oxygen barriers.[54] Hydroxypropylated amylomaize starch has been used to produce transparent water-soluble films, with low

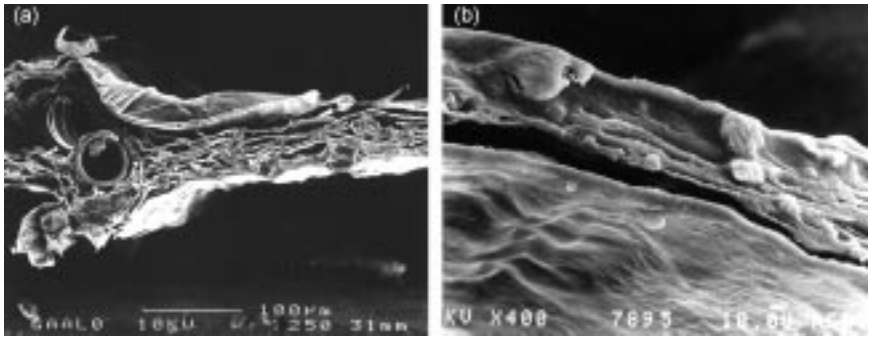

Fig. 28.1 (a) Coated garlic in cross section. The coating glued onto the garlic epidermis is strong and transparent (adapted from Nussinovitch and Hershko[48]).
(b) Gellan-sitosterol-coated garlic. The distance between the film and the garlic is equal to or smaller than 10 μm.

permeability to oxygen, increased bursting strength and elongation, and reduced tensile strength.[55] Other components helped to develop coatings of this nature for prunes, raisins, dates, figs, nuts, and beans. The National Starch and Chemical Co. (West Bridgewater, NJ) has developed starch formulations for a variety of different products, for example starch hydrolysates to coat dried apricots, almonds and apple slices.[56]

A few water-soluble composite coatings composed of carboxymethyl cellulose (CMC) with fatty acid ester emulsifiers are used for fruits, such as pears and bananas.[57–62] In the latter, delayed ripening and changes in internal gas levels are observed.[58,59,63] This type of commercial coating was first called Tal Pro-long (Courtaulds Group, London) and later, Pro-long. It increases resistance to some types of fungal rot in apples, pears, and plums but is not effective in decreasing respiration rate or water loss in tomato or sweet pepper.[64,65] Valencia oranges coated with Tal Pro-long have a better flavor and lower ethanol levels than controls.[66] Another coating with a similar composition, Semperfresh (United Agriproducts, Greely, CO), contains a higher proportion of short-chain unsaturated fatty acid esters in its formulation.[57] These coatings retarded color development and retained acids and firmness as compared to controls when tested on apples.[60] Semperfresh also extended the storage life of citrus.[67] The addition of waxes to Semperfresh gives fruit with a better shine and higher turgidity, less decay and good flavor. On the other hand, Semperfresh is not effective at retarding water loss in melons.[68]

28.2.4 Coatings for fried products as oil resisters

Frying is a unit operation used to alter the edible quality of a food. A secondary consideration is the preservative effect that results from thermal destruction of microorganisms and enzymes and from the reduction in water activity at the surface of the food.[69] Fats and oils are cooking mediums. Frying cooks foods rapidly because of the high temperatures involved. Moreover, many people enjoy

the flavor and texture of fried foods. However, these foods are higher in calories than foods cooked with water or by other methods. Thus, fried foods with lower calorie contents are in demand and can be achieved, at least to a certain extent, by the use of appropriate coatings.[18] Gellan-based films can be used to reduce oil absorption. Such coatings have been used for several years in Japan and other Asian countries with tempura-type fried foods.[70] LMP (low-methoxyl pectin), which resembles alginate in its crosslinking mechanism, can be used for coating purposes. This film, coated with lipids, increases resistance to water vapor transmission.[71] An edible coating system called Fry Shield, developed and patented by Kerry Ingredients (Beloit, WI) and Hercules (Wilmington, DE), is based on calcium-reactive pectin and reduces fat intake during frying. French fries treated with pectin took up half the usual amount of oil.[70]

28.2.5 Miscellaneous coatings

Edible films can be utilized to coat nuts and peanuts, confectionary products and lightly processed agricultural products. In addition, they can be used to control the migration of preservatives and dough additives, in composite bilayers, blends and biopolymer films, and for many biotechnological uses.[4] A very interesting type of edible coating was developed more than 100 years ago in Japan (Fig. 28.2). 'Oblate' is a registered trade name for edible paper which is prepared from starch and agar. The first Japanese patent for its production appeared in 1902 and an improved version was patented in 1912. The paper is 10 to 15 μm thick and is generally sold as 93 mm diameter circles in drug stores in Japan for dosing powdered medicines. However, Oblate is a convenient and useful material for wrapping condensed, sticky, sweet agar jelly. Oblate is

Fig. 28.2 (a) Oblate is a registered trade name for edible paper which is prepared from starch. The product is generally sold in drug stores in Japan for dosing powdered medicines. (b) Toffee coated by an edible film composed of starch, lecithin (from soybean), and flavoring and coloring agents (caramel). (Courtesy of Madoka Hirashima (Mie University, Japan).)

prepared by adding 100 parts of a 5% aqueous starch solution to 200 parts of 2.5% aqueous agar solution and brushing the hot mixed solution onto the glossy surface of a metal or glass body in order to produce a dry, rigid film which is then removed and cut into the required shape.[2]

Gum arabic with or without gelatin has been used to produce protective films for chocolates, nuts, cheeses, and pharmaceutical tablets,[72] and has also been reported to inhibit darkening of cooked potatoes.[73] A composite film of gum acacia and glycerolmonostearate was reported to have good water vapor barrier properties.[74] Chitosan produces films which are clear, tough, and flexible, and are good oxygen barriers.[54,75] Edible coatings based on modified food starches serve as adhesives for seasoning and are applied to the surface of oil-roasted and dry-roasted peanuts. Investigations of edible film properties have progressed appreciably during the last century; the results are expected to be applied to a wide variety of food and other applications.[76,77]

The carrageenan-based coating Soageena (edible polysaccharide) was developed by Mitsubishi International Corp. (Tokyo, Japan).[78] Aside from its designation for fresh produce, no information is available.[79] Other carrageenan coatings have been used to retard moisture loss from coated foods.[80] A carrageenan-based coating applied to cut grapefruit halves was successful in decreasing shrinkage and taste deterioration in storage at 4.5 ºC for 2 weeks.[81] Carrageenan, gellan, and alginate have been used to coat fresh, soft-brined cheeses.[82] Cellulose can be used following chemical modifications to produce edible and biodegradable films. Methyl cellulose (MC), hydroxypropylmethyl cellulose (HPMC), hydroxypropyl cellulose (HPC), and CMC are examples of raw materials for the production of films with moderate strength, resistance to oils and fats, flexibility and transparency. Other desired properties include lack of odor and taste, and the ability to serve as a moderate barrier to moisture and oxygen.[83–85] Information on MC and HPMC can be found elsewhere.[86] MC films are good barriers to oil and fat migration.[87] MC and HPMC can be used to create composite films with solid lipids.[88–91] Such films can be produced from ethanol solutions of fatty acids and cellulose. Bilayer films consisting of a solid lipid and a layer of MC or HPMC have been used to reduce the migration of water in model foods.[92] Non-ionic cellulose ethers are capable of producing tough, transparent and flexible films that are both water-soluble and fat- and oil-resistant.[93] MC and HPMC films are effective in reducing oil absorption by French fries, onion rings, and other fried, processed products.[94] In addition to decreasing oil penetration and absorption by dry foods, they can reduce weight loss and improve the adhesion of batter to products.[95] MC films prevent lipid migration.[45] HPMC and a bilayer film consisting of stearic-palmitic acid slowed moisture transfer from tomato paste to crackers.[96] Formulations involving MC, HPMC and HPC delayed browning and increased volatile flavor components.[97] HPC films retard oxidative rancidity and moisture absorption in nut meats, coated nuts, and candies.[93,98]

Whey is the watery, fluid part of the milk that remains after the curd, or casein precipitate, has been removed. Whey contains proteins which are not

precipitated by either acid or rennin, along with most of the lactose and water-soluble vitamins and minerals. It is used primarily as animal feed, but some is also used to make special cheeses. The use of whey in the production of alcohol and beer has been investigated. It can also be used as a liquid for gelatin desserts and salads. Whey protein fractions (α-lactalbumin and β-lactoglobulin) and whey protein isolate have been studied for their potential to form edible films because of their beneficial functional properties and industrial surplus.[99] Whey proteins produce transparent, flexible, colorless and odorless edible films which are moderately good moisture barriers and excellent oxygen barriers.[100,101] Such films generally need the incorporation of plasticizers (e.g. polyols and mono-, di- and oligosaccharides) to reduce their brittleness. Whey protein isolate films have limited barrier properties which can be enhanced by the inclusion of other film-forming polysaccharides.[102] Scanning electron microscopy has demonstrated these films' favorable structure and the addition of pullulan at low concentrations was seen to be sufficient to significantly modify their barrier properties and improve their potential characteristics for food applications.[103]

28.3 Novel products

The use of edible films is becoming more and more popular and novel products in the fields of dental care, multi-symptom cough and cold medicines, soluble pouches and sachets of liquid and powdered products, drugs on film, detergents and fabric conditioners for home laundry, dishwasher additives, garden products such as weed killers and plant food, cake decorations, and vegetable/fruit alternatives for seaweed sheets (nori) used for sushi and other Asian cuisine can be bought everywhere.

Listerine PocketPaks® are small patches of edible film that melt instantly on the tongue, releasing bursts of breath freshener strong enough to wipe out all signs of spicy or smelly consumed food. Developed by Warner-Lambert, Listerine PocketPaks was nearing its launch when Pfizer bought the company. Launched in 2001, the innovative film-strip delivery system for Listerine PocketPaks propelled the dental-care category into bold new territory by being the first on-the-go product for active consumers. PocketPaks are sold in Cool mint, Cinnamon, Fresh citrus and Fresh burst (mild spearmint flavor) varieties. Cool mint, for example, in addition to many other ingredients such as flavors, menthol, aspartame, potassium acesulfame, copper gluconate, polysorbate 80, glyceryl oleate, eucalyptol, methyl salicylate, thymol, propylene glycol, and FD&C Green No. 3, contains at least four hydrocolloids: pullulan, carrageenan, xanthan gum and locust bean gum (LBG). This product is sugar-free, alcohol-free, and contains no calories. The manufacturer's claims for this product are instant dissolution and release of the antiseptic ingredients as well as killing over 99% of bad-breath germs in just 30 s in lab tests. The success of Listerine PocketPaks paved the way for many other products.

Strips could prove appealing as a vehicle for drug delivery. Film strips dissolve quickly without requiring water. This is good news for elderly patients who have trouble swallowing and even better news for children who hate syrupy medicine. In 2004, the Swiss pharmaceutical giant Novartis introduced Triaminic® and Theraflu® Thin Strips™. A mere 3 weeks after their official launch, they were named 'Best Product of 2004' by *Convenience Industry News* (CIN) magazine. Thin Strips were the first multi-symptom cough and cold medicines to deliver an accurate dose in thin-film format and were selected from more than 100 applicants for their taste and quality ingredients, packaging, consumer acceptance, innovation, differentiation, and convenience. In 2006, Thin Strips were followed up by Gas-X Thin Strips, a new twist on a nearly 30-year-old brand. The Triaminic franchise now claims a 20% share of the pediatric cough and cold medicines market. In 2006, retail sales of Theraflu and Triaminic strips were up 28% and 26% respectively, bringing in more than US$19 million.[104,105]

Clarifoil, the producers of films for print and packaging uses, launched Clarisol, an innovative new range of water-soluble films. Clarisol is a cast polyvinyl alcohol (PVA/PVOH) water-soluble film aimed at the growing market for soluble pouches and sachets of liquid and powdered products. Household consumer examples include detergents and fabric conditioners for home laundry, dishwasher additives, and garden products such as weed killers and plant food. Clarisol also has applications in the global commercial and industrial market for soluble sachets of products such as cleaning fluids and pre-measured doses of chemical and pharmaceutical products used on farms and in professional horticulture. Clarisol is water-soluble at low temperatures and is being offered as a consistent-quality product suitable for a wide range of functional uses where requirements are convenience, delivery of measured doses, and easy handling for consumers and industrial operatives. It could also potentially have uses in specialty products.

Pets may be the next target for 'drugs-on-film'. Edible films could serve as an alternative to stuffing pills down the throats of pets (mainly dogs and cats) that hide them under their tongues or inside their jaws and then spit them out when the owner is not looking. It could be that drugs can also be given at lower doses on films because they are better absorbed through the tongue. It is estimated that pet pharmaceuticals represent a US$1 billion market. In the meantime, the food industry has found many new uses for the edible-film technology. Some meat manufacturers are using films to cure and glaze hams. There are electrolyte strips that athletes can consume in lieu of sports drinks to fight dehydration. Films may someday be used as a moisture barrier, separating the tomato sauce from the crust on a frozen pizza, for example, so that the crust stays crisp. Thus, these films can improve the quality and shelf-life of foods. Origami Foods, in cooperation with the United States Department of Agriculture (USDA)'s Agricultural Research Service, has developed a new, unique food product that could help in the development of novel, healthy, convenience foods that appeal to both parents and children indulging in today's 'dashboard-dining' lifestyles.

The manufacture of flavorful, visually appealing vegetable and fruit alternatives for seaweed sheets (nori) used for sushi and other Asian cuisine is aimed at satisfying the desire among consumers for increased variety and additional flavors. Almost any fruit, vegetable or their combination can be used to create such edible films. This allows the continued introduction of new product concepts, flavor combinations and seasonal promotions utilizing edible-film products. Moreover, these products are low-fat, low-calorie, great tasting and healthy, and were developed for people who have an aversion to seaweed, or are interested in an alternative.[106,107]

Another development in the area of edible films relates to decorations. It is possible today to decorate cakes with computer-designed images. These images (sometimes clip-art) are printed on edible rice paper, using a standard inkjet printer. The attractive printouts can be placed on any cake or bakery product – the sky's the limit! Of course, all ingredients are food-grade materials, carefully prepared under strict conditions in accordance with international standards and approvals. These developments initiated the invention of new high-quality food-grade inks basically composed of deionized water, color combinations and flavors. The same process can be used to produce designed candy wrappers, rolls of paper for pies or quiches, or decorative protective covers for condiments and tortes, and in hundreds of other applications where a cosmetic or personal element is to be added to a dessert product.

28.4 Inclusion of food additives in edible films

Edible coatings may serve as carriers for a variety of food additives, such as antimicrobial and antibrowning agents, colorants, flavors, nutrients, and spices.[108–111] These additives could serve, for example, as a potential treatment for reducing the deleterious effects imposed by minimal processing of fresh-cut fruit.[110–115] Antibrowning agents are aimed at minimizing the browning of vegetative tissue on cut and exposed surfaces. Different processing operations, such as peeling, cutting, and shredding, induce enzymatic browning, which influences quality but can be successfully controlled by sulfites.[116] However, ever since the ban on the use of sulfites for fresh fruits and vegetables,[117] a replacement has been urgently sought. Several chemical compounds other than sulfites were suggested to be able to inhibit enzymatic browning.[118] Subsequently, the incorporation of antibrowning agents into edible films in minimally processed fruits was studied by several groups.[119,120] Most of these agents are hydrophilic compounds and may increase water vapor permeability and water loss when incorporated into films and coatings.[121] Edible coatings based on alginate (2%, w/v) and gellan (0.5%, w/v) proved to be good carriers for antibrowning agents (N-acetylcysteine and glutathione) in a study on fresh-cut Fuji apples.[122] In that particular case, the edible coating was generally applied before the antibrowning agents such that the gel coating adhered to the fruit and the antibrowning agents were then incorporated in the dipping solution, which

contained calcium for crosslinking and instant gelling of the coating. In general, an increase in a^* (a^* and b^* are colorimetric coordinates) or decrease in h^* ($h^* = \arctan b^*/a^*$) values were indicative of browning in fresh-cut apples. It was observed that the antibrowning agents only had a significant effect on the a^* value. An increase in the concentration of *N*-acetylcysteine in both the alginate and the gellan coatings led to a decrease in a^* values (absence of browning), demonstrating that *N*-acetylcysteine is an effective antibrowning agent for incorporation into the formulation of edible coatings. In both cases, concentrations of around 1% (w/v) of each antibrowning agent were necessary to maintain a^* values close to the initial values, and this was independent of the type of coating employed.[122]

In minimally processed fruits, preparation steps such as peeling, cutting or slicing increase the product's functionality but induce wounding. Microorganisms that are present on the food surface may be involved in spoilage.[123,124] Incorporating antimicrobial compounds into edible films provides a novel way of improving the safety and shelf-life of ready-to-eat foods.[115] Commonly used antimicrobials include traditional preservatives such as benzoic and sorbic acids, and other potent ingredients that include bacteriocins (nisin and pediocin), lysozyme, lactoferrin, and plant-derived secondary metabolites, such as essential oils and phytoalexins. An essential oil is any concentrated hydrophobic liquid containing volatile aroma compounds from plants. Essential oils are generally extracted by distillation, expression or via the use of solvents. They are designated as GRAS (generally regarded as safe),[125] and used as flavoring agents in baked goods, sweets, ice cream, beverages, and chewing gum.[126] They have also been evaluated for their ability to protect food against pathogenic bacteria in, among others, contaminated apple juice.[125,127,128] Essential oils can be added to edible films and coatings to modify flavor, aroma, and/or odor, as well as to introduce antimicrobial properties.[115] Eleven years ago, the first edible films made from fruit purées were developed and were shown to be a promising tool for improving quality and extending the shelf-life of minimally processed fruit.[129–131] Another group took this concept a bit further when they investigated the effect of plant essential oils on the antimicrobial and physical properties of edible apple-purée films.[132] The effect of lemon grass, oregano oil and vanillin incorporated into the apple purée-alginate edible coatings on the shelf-life of fresh-cut 'Fuji' apples was investigated.[133,134] All antimicrobial coatings significantly inhibited the growth of psychrophilic aerobes, yeasts, and molds. The antimicrobial effect of essential oils against *Listeria innocua* inoculated into apple pieces before coating was also examined. Coatings containing lemon grass (1.0 and 1.5%, w/w) and oregano oil (0.5%, w/w) exhibited the strongest antimicrobial activity against *L. innocua* (fourfold log reduction). Vanillin-containing coatings (0.3%, w/w) were the most effective in terms of sensory quality after 2 weeks of storage.[135]

When antimicrobial agents such as benzoic acid, sorbic acid, propionic acid, lactic acid, nisin, and lysozyme were incorporated into edible films, the films retarded surface growth of bacteria, yeasts, and molds on a wide range of

products, including meats and cheeses. Various antimicrobial edible films have been developed to minimize the spread of spoilage and growth of pathogenic micro-organisms, including *L. monocytogenes*, which may contaminate the surface of cooked ready-to-eat foods after processing.[115] Benzoic acid ($C_7H_6O_2$) is a colorless crystalline solid and the simplest aromatic carboxylic acid. This weak acid and its salts are used as a food preservative and can be included in edible coatings. Benzoic acid inhibits the growth of molds, yeasts, and some bacteria. It is either added directly or created from reactions with its sodium, potassium, or calcium salt. Skinless tilapia was coated with a gelatin coating containing benzoic acid as an antimicrobial agent. Tilapia flesh includes ~20% protein, ~5% crude lipid, 0.2% carbohydrate and 1.2% ash and is an important source of protein for its consumers. After coating, aerobic and anaerobic microbial loads, volatile basic nitrogen contents, and sensory evaluation were used as indicators to survey the potential of gelatin coatings for prolonging the shelf-life of tilapia fillets under refrigeration.[136] The initial aerobic microbial counts in fresh tilapia fillets were about 5×10^5 CFU/g. The aerobic microbial counts increased to 10^9–10^{10} CFU/g for fillets without coating and fillets coated with gelatin containing no benzoic acid after 7 days of storage. In contrast, the aerobic microbial counts only increased to 6.5×10^6 CFU/g for fillets coated with gelatin containing benzoic acid. The application of an antimicrobial gelatin coating could extend the shelf-life by at least 4 days, a doubling of the original storage period. Moreover, after 7 days of storage under refrigeration, tilapia fillets coated with gelatin containing benzoic acid had acceptable volatile basic nitrogen contents and showed no significant sensory difference as compared to fresh fillets. The results indicated that an antimicrobial gelatin coating is suitable for the preservation of tilapia fillets.[136]

Lipid materials such as wax, fatty acids, neutral lipids, and resins, are generally used to improve the water barrier properties of biopolymer-based edible films and coatings. Water vapor barrier properties of lipid-based biopolymer films and coatings are affected by many factors, depending upon the nature of the lipid materials used, the film structure, and factors such as temperature, vapor pressure or physical state of the water contacting the films.[137] In laminated methylcellulose/corn zein-fatty acid films, water vapor permeability was reported to decrease as chain length and concentration of the fatty acids increased. The elongation values for films containing fatty acids varied in inverse proportion to their tensile strength.[138] Another report on composite films composed of soy protein isolate and fatty acids demonstrated that their physical properties were greatly influenced by the type of fatty acid added and its concentration. Tensile strength decreased with the addition of fatty acids up to a concentration of 20%, then increased with the addition of over 20% fatty acids. Independent of fatty-acid type, elongation decreased as the concentration of fatty acids increased. Water vapor permeability of the soy protein isolate film decreased significantly with the incorporation of fatty acids, with a reduction of about 260% for films with 40% palmitic acid and stearic acid, respectively. Water solubility of films with lauric acid, unlike

those with palmitic acid, stearic acid, or oleic acid, was adversely affected and the effect became more severe as the concentration of the fatty acid increased.[139] Many other references reached the conclusion that lipid-based edible films and coatings can play an important role in the food industry by controlling the moisture barrier properties of biopolymer-based edible films and coatings.

The addition of probiotics to obtain functional edible films and coatings is in its infancy. Potential health benefits and biological functions of bifidobacteria in humans include the intestinal production of lactic and acetic acids, inhibition of pathogens, reduction of colon cancer risks, reduction of serum cholesterol, improved calcium absorption, and activation of the immune system, among others.[140–142] A viable bifidobacterial population of 10^5 CFU/g in the final product has been indicated as the therapeutic minimum needed to gain the aforementioned health benefits.[143] A recent study described the formulation of alginate- and gellan-based edible films containing viable bifidobacteria for coating fresh-cut fruits. Values above 10^6 CFU/g *Bifidobacterium lactis* Bb-12 were maintained for 10 days during refrigerated storage of these fresh-cut fruits, demonstrating the ability of alginate- and gellan-based edible coatings to carry and support viable probiotics on fresh-cut fruit.[144]

Emulsifiers are widely used in the food industry to improve texture, stability and other features of food products. Sucrose esters are obtained by esterifying sucrose with edible fatty acids from palm oil. By varying the degree of esterification of the sucrose molecule, it is possible to obtain emulsifiers with HLB (hydrophilic lipophilic balance) values ranging from 1 up to 16 for the high mono-esters. This enables them to be used as emulsifiers in virtually all food products. All sucrose esters can be declared as 'emulsifier E473' or 'sucrose esters'. Aside from their wide HLB spectrum, sucrose esters are neutral in taste, odor and color, stable at high temperatures for short times, soluble in cold water, kosher, non-GMO (genetically modified organisms), and suitable for vegetarians. When included within the composition of gelatin films they decreased their tensile strength and water vapor permeability.[145] Films containing fatty acids and their sucrose esters exhibited superior water vapor permeability relative to those containing only the fatty acids (palmitic and stearic). Films that included fatty acids and their sucrose esters were generally more transparent than those with only the fatty acids. Chain lengths of the fatty acids and their sucrose esters affected the properties of the films differently; depending upon the gelatin source.[145] Another manuscript demonstrated that sucrose esters have a great effect on the stabilization of emulsified edible-film structures containing arabinoxylans and hydrogenated palm kernel oil.[146] Sucrose esters also improved the moisture-barrier properties of these coatings. Both lipophilic (90% di- and tri-ester) and hydrophilic (70% mono-ester) sucrose esters can ensure the stability of the emulsion used to form the film, especially during preparation and drying.[147]

28.5 Parameters to be considered before, during and after food coating

The coating process involves wetting the food surface with the coating gum solution, possible penetration of the solution through the food surface[148] and possible adhesion between these two commodities. The wetting stage (spreadability) is the shortest and most important; if the compound used for spreading is ideally suited to the food to be coated, spreadability is spontaneous.[149] However, it is almost impossible to find hydrocolloid solutions or their combinations that are ideally suited to the surface properties of the object, i.e., surface tension and polarity. Thus the closest possible combination should be determined to achieve compatibility. Effecting a successful, tailored coating that adheres to a food surface requires estimation of the interfacial tension between the coating solution and the surface. It depends on the surface tension of the surface, the surface tension of the coating solution and the contact angle between the two.[150] To gauge a solid's surface tension, the critical surface tension first needs to be calculated. Prior to this, the critical surface tension of the surface to be coated should be derived from Zisman plots followed by extrapolation. These plots are obtained by calculating the cosine of each measured contact angle of the pre-chosen liquids on this surface and plotting it against already-known surface tension values of the solvents being used.[151,152]

The detection of a suitable coating gum solution can be very complicated because coating solutions are water based, i.e., they contain mostly water with a surface tension of 72.8 dyne/cm, and lower surface tension values for many solid surfaces must be taken into account. In general, hydrocolloids have the potential to lower the surface tension of solutions designated for use as coating agents.[153] The lower the surface or interfacial tensions of a gum solution, the higher its surface or interfacial activity.[153] Considering that efficient coating involves compatibility between liquid and solid surface tensions,[154] it is logical to reduce the surface tension of the coating solutions to conform to the lower surface tension of the food surface, and to lower the interfacial tension to improve adhesion.[155] In our previous studies,[44] we observed that the addition of sterols effectively yields better adhesion between fruit and vegetable surfaces and coating films due to better compatibility with respect to the hydrophobicity of the two adhered surfaces.[48]

Another aspect to consider is spreading which is enhanced by a food's rough surface for coating solutions having contact angles smaller than 90°[76] and inhibited by rough surfaces for solutions with contact angles greater than 90º. Surface roughness has been defined as the ratio of the true area of the solid to the apparent area. Wenzel[156] was the first to propose a relationship between the contact angle of a liquid on a rough surface to its contact angle on an ideally smooth surface. Surface roughness can be evaluated with a roughness tester[157] or from image processing of atomic force microscopy (AFM) micrographs.[148] Roughness decreases the interfacial tension as a result of improved spreadability.[155] The importance of the magnitude of the interfacial tension is

well recognized by the polymer coating industry. Better compatibility between the coated object and the coating films can be achieved by incorporating surface-active agents within the coating gum solution. It can be concluded from the compatibility requirements that tailor-made hydrocolloid coatings for different food materials can be achieved only by further determination of the chemical and physical properties of the coating solutions and the coated objects.

28.6 Hydrocolloid non-food coatings

Seed coating is an important application of polymers, whereby the seed surface is coated with a hydrophilic polymer. After planting, the polymer absorbs water and the rate, as well as the chances of germination, increase. Because coating composition and manufacture can be controlled, they can be designed to delay germination, inhibit rot, control pests, fertilize or bind the seed to the soil. Seed coatings have been composed of agar, alginates, various water-soluble cellulose ethers and hydrolysed starch-polyacrylonitrile copolymers (e.g., HSPAN). Many seeds have been coated, among them soybean, cotton, corn, sorghum, sugar beet and those of a number of vegetables. As a result, cotton and soybean yields increased 20–30%. The poorer the growing conditions, the more pronounced the advantage of the coated seeds vs. controls.[1,158] Although most of the reports on seed coatings deal with alginate coatings, reports on other gels can also be found.[159]

Many hydrocolloids are used to obtain effective paper coatings. Agar has been found suitable for use in photographic papers when esterified with succinic or phthalic anhydride and after enzymic hydrolysis. Agar can also be used as an adhesive in the gloss-finishing of paper products. HPC is used for coating, due to its solvent solubility and thermoplasticity. HPC serves as an oil and fat barrier and is responsible for thermoplastic coating. Polyethylene oxide (PEO) is used for paper coating and sizing. As a processing additive, it is used as a fiber-formation aid.[1] The associative behavior of CMCs, hydroxy ethyl celluloses (HECs) and hydrophobically modified cellulosic thickeners (HMCTs) was determined in clay-based coatings and their effects on coating rheology and coated-paper properties identified. The anionic CMCs were less adsorbed to kaolin clay than the non-ionic HECs and HMCTs. Coatings containing less clay-adsorbent CMCs were less resistant to flow under high shear than those containing the clay-adsorbent HECs, but they appeared to have a weaker after-blade structure. Coatings thickened with HMCT had both low flow resistance under high shear and a strong afterblade structure.[160] Polyvinyl alcohol (PVA; a synthetic resin) is used in paper sizings and coatings. PVP (polyvinyl-pyrrolidone) is used in all types of paper manufacture, mostly as an economical fluidizer and anti-blocking agent in paper coating. The starches used in coating colors are enzyme-converted starches, thermochemically converted starches, oxidized starches, dextrins, hydroxyethyl starch ethers, and starch acetates.[2]

The use of polyacrylic acid (PAA) as a film-former has many applications.

One example is the suggested use of the sodium salt of PAA as the major component of non-glare coatings for headlights. Anti-fogging coatings of glass and transparent plastics for optical use have been made by crosslinking PAA with aminoplast resins to produce scratch- and water-resistant coatings. These polymer acids have been used as release coatings for polymerization molds, as parting agents, and as soil-release coatings for hard surfaces subject to spattering by printing ink.[2] In the manufacture of fluorescent lights, phosphor bonding to the glass tube is enhanced by using PEO resin as a temporary binder in combination with barium nitrate as a bonding agent. The coating is usually applied by preparing a suspension of the phosphor, binder, and dispersant. The suspension is used to flush the glass tube, leaving a thin coating of phosphor on the inside. Subsequent drying and lehring removes water, solvents, and organic additives and leaves the phosphor coating, bonded to the inside of the tube. The advent of water-based latex paints raised the need for speciality CMC types to meet the rheology requirements of the flow point under the roller or brush. The availability of property variations during the manufacture of CMC to meet the specific coating system requirements has been greeted with acceptance by the coating industry. For the manufacture of coatings, paints, foams, or adhesives, xanthan gum is compatible with the common types of latex emulsions, making it effective as a stabilizer, thickener, and modifier of rheological properties.

28.7 Film-application techniques and stages

Films can be applied by either dipping or spraying, the latter being better suited to planar surfaces.[161] Brushes, falling-film enrobing technique, panning or rollers can also be used to apply films to the surfaces of the coated objects.[162] Foods to be coated are dipped in a hydrocolloid solution followed by draining and drying. The coating of a surface with a gelling agent that requires subsequent cation crosslinking involves a process that can be theoretically divided into four successive steps and time stages: immersion of fresh produce in a hydrocolloid solution (not including the cationic crosslinking agent) can take between 15 and 120 s for a complete coating. Duration depends on wettability, the concentration and viscosity of the hydrocolloid solution, as well as the surface roughness, and possible penetration of the coating solution into the biological specimen.[163] At this point, the coating solution can be dried to achieve a dry film adhering to the food surface; thus a film that did not pass through a gel state is formed. A second immersion of the hydrocolloid-coated food in a crosslinking bath to induce gel formation (i.e., in alginate, LMP, κ- or λ-carrageenan or gellan) takes between 30 s and ~2 min depending on the concentration and temperature of the crosslinking agent, the thickness of the coating gum solution, and the geometric complexity of the coated object. (It is important to note that a food coated with a thin layer of gel that will be further dried into a coating film can be produced in one step if gelling agents that gel directly on the food surface such as agar are used.) The third stage of producing

a crosslinked coating is the continuous strengthening of the gel coating layer at high relative humidity. The fourth stage, namely drying, can result in different dry-film textures and structures, depending on the duration of drying. When the coated object is dried, after a short or long gelation process or none at all, texture and structure of the dried film will vary according to the time required for the gelled film layer to lose its inherent moisture. The properties of the dried films also depend on properties of the drying equipment and thickness and composition of the coating film. A casting technique is used in many laboratories to produce flat films of nearly constant thickness for an easy determination of their mechanical properties (by puncture and tensile tests) and permeabilities. Thus, the peeling of films produced on foods is avoided. The gel or hydrocolloid solution is poured into a 'sandwich' prepared from glass plates. After the gels are cast, they are left to equilibrate under high humidity at room temperature. Next, the films are dried with warm air until a predetermined amount of moisture remains within them. They are then ready for testing.

28.8 Methods for testing coatings

Gas permeability of packaging films can be measured in several ways.[164–166] Many devices for measuring film permeability to oxygen are on the market.[167,168] Water vapor transmission rates through dried coatings can be determined by ASTM E96-93 ('Gums for coatings and adhesives test methods for water-vapor transmission of materials'). Peel testing, i.e. the force necessary to peel the coating, is used to estimate the film's degree of adhesion to a surface. The coating is peeled at 90º from the substrate, and the adhesion strength is estimated by the force per unit width necessary to peel the coating. It is important to study surface wetting and adhesion properties of coated commodities to obtain 'good' coatings. As discussed, the roughness of products and of film surfaces can be studied using a roughness tester, and electron and AFM (the latter for finer mapping of surface roughness). Parameters which can be studied include *Rt*, the distance between the highest peak and the deepest valley of the roughness profile within an evaluation length of the tested surface. Other important parameters, such as *Ra*, the arithmetic mean of the absolute values of the roughness profile's deviations from the center line within the evaluation length, can also be determined. The surface tension of gelling and inducing solutions, and their contact angles on fruit and vegetable surfaces, can be studied with surface tension instruments (maximum adhesion requires a contact angle of 0º). It is important to note that 'if the coating does not spread spontaneously over the substrate surface, so that there is intermolecular contact between the substrate surface and the coating, there cannot be interactions and hence no contribution to adhesion'.[169]

28.9 Market estimates for edible films

Over the past five years, there has been a tremendous growth in both the development and marketing of edible films. In 1999, sales were just US$1 million, mostly from niche products such as edible underwear – a popular gag gift at bachelor parties. In 2005, market revenues of more than US$100 million were recorded. Retail sales of edible films are expected to hit at least US$350 million by 2008.[170] The demand for edible packaging films is also on the rise and could reach US$25 million in sales revenues over the next three to four years.[171]

28.10 The next generation of edible films and possible research directions

Research and development of bio-nanocomposite materials for food applications, such as packaging and coating of other food contact surfaces, are expected to grow in the next decade with the advent of new polymeric materials and composites with inorganic nanoparticles. Their inclusion in edible and nanocomposite films is expected to improve mechanical and oxidative stability, barrier properties, and eventually, the biodegradability of conventional polymeric matrices.[172]

A nanometer (symbol nm) is a unit of length in the metric system, equal to one billionth of a meter. It can be written in scientific notation as 1×10^{-9} m (engineering notation) meaning 1/1,000,000,000 meters. The science of nanotechnology is progressing rapidly and is expected to radically alter the business world over the next few years – and to continue to do so for the foreseeable future. Within a decade, nanotechnology is expected to constitute the basis of US$1 trillion worth of products in the United States alone and to create anywhere from 800,000 to 2 million new jobs.[173] Nanotechnology has already entered the field of coatings. A few examples include a coating process to make sponge-like silica latch onto toxic metals in water and the development of antimicrobial clothing covered with nanocrystalline silver. Stain-repellent khaki dress shirts and ties have also been developed, where each fiber of the fabric is coated with 'nano-whiskers', i.e., surface fibers of 10–100 nm. In a different area, double-core tennis balls have a nanocomposite coating (a mix of butyl rubber intermingled with nanoclay particles) that keeps them bouncing twice as long as the old-style balls and also gives the ball substantially longer shelf-life.[173]

Even though edible films have been extensively studied and applied, to date only a few research studies have indicated the possibility of incorporating nanoparticles in order to improve the physical properties of these materials. It has been reported that adding clay montmorillonite to pectins could lower the diffusion of oxygen.[174] Similarly, a considerable improvement in physical properties was recorded for nanocomposites prepared with gelatin and montmorillonite.[175] An appreciable increase in stability in chitosan-layered

nanocomposites was also reported.[176] A recent publication describes the inclusion of different-sized fillers, all less than 1.0 μm, in HPMC-based films. The water vapor permeability of the HPMC film did not change significantly with the addition of the fillers; on the other hand, the tensile strength of the control film increased from 29.7 ± 1.6 MPa to 70.1 ± 7.9 MPa with the addition of 500 nm particles, while it increased to only 37.4 ± 5.5 MPa with the addition of 3 μm particles. Elongation percentage of the control HPMC film did not decrease significantly with the addition of submicron-sized fillers. This study demonstrated that the increased surface area per weight of the smaller-size microcrystalline cellulose fillers compared to their larger counterparts is highly beneficial in terms of improving the film's mechanical properties.[177] These results will enable food scientists to envision a new generation of composite edible films and barriers; they will no longer be restricted to emulsified and bilayer films for current and novel applications.[177] The application of nanocomposites promises to expand the use of edible and biodegradable films.[178,179] It will also help to reduce the packaging waste associated with processed foods and will support the preservation of fresh foods, extending their shelf-lives.[180,181] In addition, inorganic particles may be used to introduce multiple functionalities, such as color and odor, but also to act as reservoirs for the controlled release functions of drugs or fungicides.[182–185] Numerous food researchers are working on improving the delivery of medicines or fragile micronutrients into everyday foods by creating tiny edible capsules, or nanoparticles, that release their contents on demand at targeted spots in the body. It is thought that this will provide significant health benefits, such as reduced risk of heart attack, stroke, neurodegenerative diseases, and cancer.[186,187] Although promising results have been obtained, the road to successful bio-nanocomposites is still mostly untravelled. Nanotechnologies promise many stimulating changes that will enhance health, wealth and quality of life, while reducing environmental impact.

As stated, edible coatings and films constitute a viable means of incorporating food additives and other substances in order to enhance product color, flavor, and texture and to control microbial growth.[188] To this aim, nanoparticles can be used as carriers of antimicrobials and additives (see also Section 28.4). Nanotechnology provides food scientists with a number of ways to create novel laminate films suitable for use in the food industry. A nanolaminate consists of two or more layers of material with nanometer dimensions that are physically or chemically bonded to each other. A deposition technique can be utilized to coat a charged surface with interfacial films consisting of multiple nanolayers of different materials.[189] This technology provides food scientists with some advantages for the preparation of edible coatings and films over conventional technologies and may thus have a number of important applications within the food industry. Nanolaminates will be used as coatings that are attached to food surfaces, rather than as self-standing films, because their extremely thin nature makes them very fragile.[190,191] To produce these layers, a variety of different adsorbing substances could be used, including

natural polyelectrolytes (proteins, polysaccharides), charged lipids (phospholipids, surfactants), and colloidal particles (micelles, vesicles, droplets). The functionality of the final film will be dependent on many factors, including type of materials, number of layers, their sequences, preparation conditions, etc.

To summarize, future research directions will make use of the tools of nanotechnology to improve film quality (tensile, barrier, and delivery properties), to develop hydrophobic nanoparticles for improving water barrier properties and to use nanoparticles to deliver health-promoting compounds, antimicrobials, sweetness, flavors, and other active agents. It is estimated that the next generation of edible films will include, but not be limited to, invisible edible films, added-value fruit and vegetable films, antimicrobial edible films, inclusion of options from nanotechnology, and use of kosher fish gelatin, and will benefit from changes or developments in film-production technologies and packaging opportunities.

28.11 Non-food uses and applications of adhesives

The adhesive properties of many hydrocolloids (gums) have been known for centuries. The word 'gum' means a sticky substance, and was previously defined as such by the Egyptian term qemai or kami, referring to the exudate of the acanthus plant and its adhesive capacity.[192] A large number of hydrocolloids have been mentioned in the literature as adhesive agents.[193,194] They include gum talha (similar to gum arabic), gum ghatti, gum karaya, gum tragacanth, arabinogalactan, dextran, pectin, tapioca-dextrin, CMC, MC, HPC, HPMC, carbopol, PEO, PVP, PVA, retene, pullulan, and chitosan. Some lesser-known water-soluble gums which possess wet-adhesive bonding properties include: gum angaco, brea gum and psylium seed gum,[195] gum cashew,[196] gum damson, jeol, myrrh[197] and scleroglucan.[192] Bioadhesive applications of hydrocolloids in medicine and cosmetics have been widely explored. A few examples are adhesive biodelivery systems,[198–203] adhesive bioelectrodes,[204] cosmetic preparations,[205] pressure-sensitive adhesives,[206,207] ostomy rings,[192] adhesive ointments,[208] and dental adhesives.[209]

The use of hydrocolloids in the paper industry began in ancient Egypt when starch was used to adhere papyrus sheets.[210] Now starch, as well as dextrin, gelatin, PVA, gum arabic and cellulose derivatives make up the major portion of the glue market for the paper industry. Synthetic glues are based on polyvinyl acetate, ethylene-vinyl acetate, acrylic acid polyurethane and latex, among many others.[211] Hydrocolloid glues are used for paper lamination, to prepare cardboard.[212] Special methods (e.g., the 'Stain Hall' procedure) are used to prepare starch adhesives for paperboards.[193,213] For envelopes, stamps, wallpapers and similar products, special dry hydrocolloid glues that regain their adhesive properties upon wetting are used. They are based on gum arabic, PVA, dextrins and other gums.[214–215] For paper bags, mostly starch or modified starch and dextrin are used.[193] Glue's resistance to water is increased by including PVA and/or polyvinyl acetate in its composition, as well as urea

formaldehyde at 5–15% of the gum's weight in the formulation.[216] Paper glues can be used to attach paperboard to gypsum sheets. Such glue can be composed of calcium carbonate, mica, attapulgite clay, HPMC, ethylene glycol, preservative, anti-foaming agent, and latex. Tobacco is sometimes packaged in paper cylinders that are glued together with starch or gum tragacanth.[211]

In ancient times, gum arabic and gelatin were used by the Egyptians in furniture manufacturing.[217] The use of hydrocolloid glues in the modern wood industry began at the turn of the century. Until 1912, gelatin and starch were the most commonly used adhesives. Today, many other glues are also in use. They include polyamide, neoprene, polyvinyl acetate, urea formaldehyde, phenol formaldehyde, melamine urea formaldehyde, recorcinol phenol formaldehyde, epoxy ethylene vinyl acetate, and others. Gelatin is still used, especially for gluing cloth to wood.[218] CMC, at ~30% of the dry solids in the glue, is used to retain moisture better in the recipe, without influencing adhesive strength. Synthetic PVA is used as an additive in urea formaldehyde glues to improve the strength of the wet glue and its stability over time. Chitosan has been used to glue pine-tree plates, in a mixture with water and acetic acid.[219] As part of a glue composed of bentonite, asbestos, coquina, and glycerol, HEC was used to fill cracks in wood boats. Another hydrocolloid used in the preparation of elastic and strong glues for woods is fonuran, combined with carbon disulfide. The preparation involves mixing with sulfuric acid and later adding sodium hydroxide, heating to 50 ºC for 10 min followed by heating for 3 h at the same temperature after adding the carbon disulfide.[220]

Hydrocolloid glues are used as a tool in drying leather. The leather is smeared with a polymeric glue and attached to glass, aluminum, stainless steel or porcelain plates. The plates move perpendicularly through a drying tunnel at ~93 ºC for several hours. The better the adherence of the leather to the plate, the less shrinkage occurs. The glues are composed mainly of MC, CMC and PAA. MC is the most efficient hydrocolloidal ingredient due to its tendency to gel when heated and to maintain its elasticity during drying, resulting in less leather shrinkage during the process. In addition, the gel can be easily removed at the end of drying without damaging the leather's fiber structure. CMC can be substituted for MC.[221,222] PAA can also be used in combination with starch or CMC.[223] In ancient times, mummies were wrapped in bandages glued with gum arabic.[210] Starch and dextrins are used for fiber strengthening, as are HEC and CMC. A typical formula for such glues includes cornstarch, sulfonated oil, kerosene, and water. Special starches, following acetate modification, are also applicable. Oxidized starch is also used for printing and finishing.[224]

28.12 Adhesive hydrocolloid preparations: pastes and hydrogels

From a practical point of view, it is generally accepted that gelling hydrocolloids cannot be used as thickeners or adhesives and vice versa, with the exception of

numerous thickeners which can also be categorized as adhesives. Such thickeners may produce sticky hydrocolloid wet pastes (glues) at concentrations ranging from ~15–75% (w/w) (see Section 28.18). Many types of hydrocolloid pastes are mentioned in the patent literature, most of them for use in topical preparations or ointments, some for mucosal applications.[225–228] Medical pastes generally leave adhesive residue on the skin or other biological tissues upon removal and are thus non-repositionable. They cannot be applied in tape form and are commonly inconvenient for the patient. To prevail over the many disadvantages of sticky hydrocolloid pastes, several types of adhesive hydrocolloid gels (hydrogels) can be found in the patent literature.[229–234] The use of hydrocolloids to produce adhesive gels is, however, unique and requires specialized skills. Adhesive hydrogels for medical applications have advantages over many conventional products, namely, acrylate or silicone PSAs (pressure-sensitive adhesives). The latter, containing no water, are limited in terms of being able to accommodate specific drugs for transdermal delivery, they exhibit limited electrical conductivity in bioelectrodes and they generally cause physiological irritation, manifested as inflammation of the sweat glands, keratin peeling, tissue injury after adhesive removal, and contact dermatitis, to name a few.[235–237]

28.13 Adhesion mechanisms of hydrogels

Seeking novel biocompatible adhesives for medical applications in general and topical uses in particular is a major task for pharmaceutical formulators.[238] Due to the advantages of adhesive hydrogels over the conventional PSAs, the former hold great potential for topical applications such as transdermal drug delivery.[235,236,239] Studying hydrogel adhesion mechanisms is a key step in developing future PSA products for medical or cosmetic applications. However, while scientists have successfully studied the chemical and rheological behaviors of many hydrocolloid systems, their adhesion properties and mechanisms remain unclear. Adhesion is a very complex field, beyond the reach of any single global model theory. Given the large number of phenomena involved in the process, the variety of substances to be bonded, and a plethora of bonding conditions, the search for a universal theory capable of elucidating all of the experimental facts is a lost cause. In practice, several adhesion mechanisms can be involved simultaneously.[240] Six main theories have been proposed to explain the interactions between adhesive and adherend which, taken together, can be seen as both complementary and contradictory.

The theory of mechanical interlocking perceives mechanical keying (anchoring) of the adhesive to the cavities, pores, and superficial asperities of the solid adherend as the major factor controlling adhesive strength.[241] One of the most important criticisms of this theory, as suggested in various studies, is that better adhesion does not necessarily result from the mechanical-interlocking mechanism; instead, surface roughness can raise the viscoelastic (or plastic)

dissipated energy due to stress concentrations.[242] In the electrical model theory, the adhesive-adherend system is treated as a plate capacitor whose plates consist of the electrical double layer which occurs when two materials of differing nature are brought into contact.[243] During interfacial failure of this system, separation of the capacitor plates leads to an increasing potential difference until discharge occurs. The diffusion theory of adhesion has been suggested for identical compatible polymers. Accordingly, the adhesion strength of two macromolecules in intimate contact is due to mutual diffusion (interdiffusion) of the molecules across the interface, thus creating an interphase.[244] Such a mechanism implies that the macromolecular chains or chain segments are sufficiently mobile and mutually soluble.[245] At the theoretical level, diffusion has been described to occur predominantly at the ends of the macromolecular chains.[246]

The thermodynamic adsorption theory claims that adhesion occurs (upon intimate contact between materials owing to the short acting distance of intermolecular forces) under conditions of sufficient wetting.[247] The rheological model claims that the failure energy of an adhered assembly can be expressed as the product of two terms, the first being the reversible energy of adhesion (depending only on the surface properties of the adherends), and the second, a parameter that depends upon the separation rate and the temperature.[248] The latter term accounts for the dissipation of energy resulting from the irreversible deformation of the viscoelastic solid adhesive during failure propagation and depends on the bulk properties of the adhesive. Adhesion can also be greatly improved by chemical bonds formed across the adhesive–adherend interface.[245] These bonds are generally considered primary bonds (covalent, ionic) as opposed to secondary-force interactions (van der Waals, hydrogen bonds). Primary bonds correspond to high interaction energies of ~60–700 $kJ\,mol^{-1}$, whereas secondary bonds do not exceed 50 $kJ\,mol^{-1}$.[249] The formation of chemical bonds depends on the reactivities of both the adhesive and the adherend.

28.14 Food uses and applications of adhesives

Adhesion is governed by mechanical interlocking, wetting, electrostatic and chemical forces, and diffusion. The different methods of determining food adhesion that are discussed and detailed in the literature include: direct observation, evaluation (weighing, UV absorbance and adhesive loss), adhesion strength measurements, and indirect measurements via the wetting theory (tilted plane method, contact angle, and surface tension).[250] The article also discusses the importance of proteins, a product's rheological properties, the solid's surface properties and wetting phenomena in many adhesion cases.[250]

A simple way of achieving different textures and tastes in the same bite is to build a food product composed of layers with different properties. A few multilayered food products are already available, e.g., crunchy wafers, which include a sweet vegetable fat-based chocolate- or vanilla-flavored filling

between brittle wafers. For children, a multilayered, sweetened agar- or starch-based three-color confection can also be found, in which the texture of the layers is similar, but the tastes and colors differ. In the Orient, where the awareness of different gel textures is much more developed than in the West, a curdlan-based, sweetened, multilayered gel has been developed.[251–253] In this case, all layers are built from the same hydrocolloid, because two types of curdlan gel can easily be prepared from its powder by heating the suspension to different temperatures. Multilayered foods based on hydrocolloids are important in the framework of foods of the future.

Recently, multilayered gels (composed of different combinations of agar, four galactomannans, xanthan, carrageenan and konjac mannan), and gelled texturized fruits (based on banana, apple, kiwi and strawberry pulps and agar-LBG combinations), glued together by three different adhesion techniques, were studied.[254] The gluing techniques consisted of pouring the hot hydrocolloid solution onto the gelled layer, using melted agar as a glue between already gelled layers, or simultaneously pouring pre-gelled (gum solution before setting) hydrocolloid solutions. The compressive deformabilities of these gels were predicted. Two assumptions were made: that the normal force in the layers is the same, and that the deformations are additive. The effects of lateral stresses were considered negligible. The calculation was performed using a mathematical model previously developed for double-layered curdlan gels.[255] The model constants were determined from the behavior of the individual layers. Good agreement was found between experimental and fitted results over a considerable range of strains. Thus the model's applicability to a given gel system was demonstrated, suggesting a very convenient tool for analyzing and predicting the compressive behavior of any number of arrays with different layer combinations.[254]

Additional information regarding other food uses of hydrocolloids (as adhesives), such as crumb and batter adhesives, adhesion of breading to shrimp and fish, adhesives for snack foods, poultry patties and nuggets, can be found elsewhere.[4]

28.15 Uses and applications of bioadhesives

Hydrocolloids are used in biodelivery adhesive systems to carry fluoride,[200] benzylamine-HCl or other drugs to cure gingivitis. The hydrocolloids are part of both the biodelivery and protective layers. The first is prepared, for example, from HPC, HPMC, gum karaya, propylene glycol alginate 400, and the drug. The second is composed of ethyl cellulose, sodium CMC, and HPC, and is not adhesive. The whole preparation is dried and compressed to a thickness of ~100 μm.[256] Many other adhesive biodelivery systems have been described.[254,257] Other additional gums can be used in these preparations, such as PVP, PVA, and carbopol. Agar is frequently used for the intermediate, non-adhesive layer. Many hydrocolloids have been used as biological glues to mucus, with PC and HPC

being preferred. One of the earliest discussions on hydrocolloids' ability to serve as glues in biological systems was published 30 years ago.[194] These authors also presented results of *in vitro* tests comparing adhesion characteristics. Nearly 15 years later, Smart *et al.*[203] examined the mean adhesive forces of many hydrocolloids to mucus. They found that sodium CMC produces the strongest adhesive force, followed by carbopol and gum tragacanth. Similar results were obtained by Robinson *et al.*[201] Other research[257] estimated the minimal amount of polymer needed for the glue to stay in position for about the same duration. The following order of efficiency was found: HEC > HPC > PVP and PVA.

Other scientific approaches can be found in the literature calculating adhesion energy of different formulations attached to biological membranes.[198] It is interesting to note that although LBG-xanthan systems were evaluated as mucosal glues and found unsatisfactory, they are nevertheless used because of their good mouthfeel and for health safety reasons.[256] Biological hydrocolloid glues for the vaginal area and eyes, and in sprays for the nose and other mucosal areas, have been developed.[200,256] Chitosan, pullulan, gum tragacanth, HPC, starch, and carbopol have been suggested for these preparations. Adhesive dressings contain many hydrocolloids in their compositions, such as PVP, PEO, and PVA. Adhesive bioelectrodes use PVA, hydroxyethyl methacrylate, gum karaya, agar, MC, or CMC in their formulations.[204] Other biological applications of hydrocolloids are the use of dextran in cosmetic preparations, pullulan and CMC as part of medicinal adhesive tapes, gum karaya for ostomy rings, and a wide range of hydrocolloids such as gum karaya, gum arabic, cellulose derivatives, polyox, and others for dentistry.

28.16 Structure-function and hydrogel-adherend relationships

Hydrocolloids can be classified into adhesive and non-adhesive. Upon hydration, adhesive hydrocolloids are generally sticky, whereas non-adhesive hydrocolloids form a 'slippery' mucilage.[258,259] It is well known that the linear structure of many polysaccharides (e.g., pectin, CMC, etc.) hampers their ability to form water-based adhesives. In contrast, branched molecules (e.g., gum arabic, larch arabinogalactan, synthetic polymers) are inherently tacky.[260] Many of these branched molecules are gum exudates and among them, numerous form adhesive gels with PSA properties. The branched structure of *Sterculia urens* polysaccharide is a prerequisite for wet adhesion. Nevertheless its uniqueness lies mainly in its inherently high molecular weight and high degree of acetylation, which are fine starting points for the production of a wide range of depolymerized and deacetylated products.[260]

Adhesive hydrogels formulated from PVA were evaluated and despite its commercial availability in a wide range of molecular weights and degrees of hydrolysis, they were found to be inferior to the *S. urens*-based hydrogels as a model, due mainly to the complex and time-consuming preparation involved.[261]

The unique chemical structure of the *S. urens* polysaccharide is a gift from nature as it would most likely be impossible, from a practical point of view, to synthesize such a complex heteropolysaccharide. Its outstanding adhesive features and unique structure have not only raised it to commercial success, but also make it an exceptional tool for studying the adhesive mechanism of hydrogels in a reliable model system.[261]

Acetyl-free *S. urens* polysaccharide-based hydrogels (in typical composition including water and polyols) have a more compact macromolecular structure than systems in which acetyl is present. This is characterized by multiple molecular self-associations, a considerable decrease in molecular mobility and reduced bound water. Pressure-sensitive tack behavior of acetyl-free hydrogels follows the Dahlquist criterion developed for the loss of pressure-sensitive tack in PSAs (tack ceases completely when creep compliance approaches $10^{-6}\,Pa^{-1}$).[262] In contrast, native *S. urens* polysaccharide-based hydrogels containing acetyl showed evidence of an expanded conformation, increased molecular mobility, increased bound water and improved bonding-debonding characteristics. Acetylation was assumed to influence the interactions between the polysaccharide chains by simple steric hindrance, preventing the close molecular associations that would allow the formation of hydrogen bonds, or by altering the hydrophilicity of the polysaccharide, thus affecting its bonding with water molecules (hydrophobic hydration). As a result, bonding and debonding were, respectively, characterized by increased wettability and elongation, and hence a higher measured tack energy. An excess increase of water in the hydrogel accounted for the network's low rigidity, which in turn increased wettability on the one hand, but reduced the energy dissipated during debonding on the other, resulting in a decrease in tack energy.[262] Likewise, relative to acetyl-free hydrogels, any change in the degree of acetylation or in the proportion of acetyl and water, or the addition of a plasticizing auxiliary, could account for changes in adhesion performance presumably owing to conformational shifts.

Relations between hydrogels and adherends were considered in terms of some basic adhesion theories (see also Section 28.13). The effect of ionic (electrostatic) primary bonds on adhesion using versatile chitosan probes and hydrogels, which varied in their degrees of cationic and anionic charge, was studied. It was clearly shown that the quantity of carboxylate groups has a direct effect on the adhesion and the interface between the hydrogels and the protonated chitosan probes. The presence of polyols was found to increase the free carboxyl, presumably mainly by increased molecular pseudoplasticity of the polysaccharide-polyol aqueous mixture. It turned out that this ionic bonding is a time-dependent kinetic process which relies, concurrently, on the extent of anionic-cationic charges, rheological characteristics of the hydrogel and possible interdiffusion.[262] The surface energy of adherends has a prominent effect on dissipation energy (during debonding), compared with only a modest rheological effect of the hydrogel. The bonding process of typical *S. urens* polysaccharide-based hydrogels to low- and high-energy surfaces (18.5 and 39.2 $mJ\,m^{-2}$,

respectively) is on the order of 10^{-4} s, typical of industrial PSAs and a prerequisite for providing instantaneous tackiness. To complete bond formation, a value of ~100 s is likely to be a common rule for many hydrogels, regardless of their rheology or the surface energy of the probe.[262] *Ra* (arithmetic mean of the absolute values of the roughness profile's deviation from the centerline within the evaluation length) values in the range of 0 to 0.5 μm could potentially serve as a general tack criterion for viscoelastic materials, particularly in terms of achieving improved hydrogel adhesion. Increasing *Ra* values in the range of 0.5 to 25 μm had a negative effect on tack, because roughness induces a delay in the establishment of intimate contact between the adhesive and the probe. Adhesion of polysaccharide-based hydrogels is derived from the structure of their three-dimensional network, and from their physico-chemical properties, dictated by the extent of hydration and solubility. Possible influences of degree of acetylation on hydration or of molecular weight on solubility could be important factors affecting hydrocolloid adhesion. In addition, hydrogel-adherend interfacial phenomena are liable to exist via polyanion-polycation electrostatic interactions, intrinsic surface energy, adherend surface roughness, contact pressure and contact dwell time.[262]

28.17 Hydrocolloid adhesion tests

A relatively large number of tests have been proposed to evaluate adhesive-bonding strength. They include peeling at 90º (Fig. 28.3(a)), and tensile-bond and lap-shear tests. In tensile tests, tension is used to examine adhesive joints, bonds, or design via the application of perpendicular forces on the adhesive layer. Different modes of failure have been recognized: failure within the adhesive layer – cohesive failure; failure at the interface between the adherent and the adhesive – adhesive failure, and failure of the adherent itself. After testing, the ratio between adhesive and cohesive failure should be estimated. Samples in which the failure occurs within the substrates should be discarded, since they do not constitute a test of the adhesive material.[263,264] Peel tests are used for quality control or for comparing different adhesives, and are important in quantifying stripping or peeling forces. In peel tests, a flexible adherend that is adhesively bonded to another rigid or flexible adherent is stripped. During this operation, the distribution of the stress within the peel joints is complex and influenced by the properties of the adherent and the joint geometry. The width of the peeled adherend is a major factor in calculating the peel strength, usually in pounds per inch. The average load needed to maintain the peeling after initiation is used to estimate the peeling force.[1] In shear tests, the structural adhesive within bonded structures is usually designed so that it will sustain shear loads most of the time, the reason being the strength of adhesives in shear, as compared to that under peel or tensile loads. Applied loads act in the plane of the adhesive layer when the adhesively bonded structure is considered under shear. As a result of the loads, the adherents can slide, which in turn causes shearing or

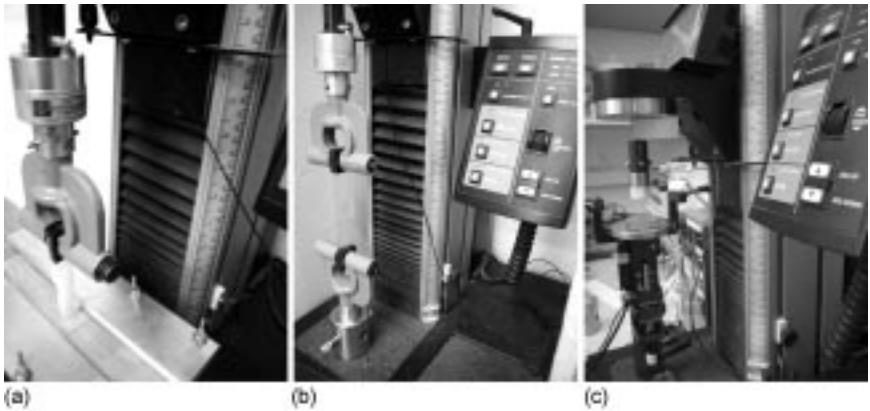

Fig. 28.3 (a) Specimen mounted on the Instron Universal Testing Machine (UTM) during the 90º peel test. (b) Specimen mounted on the Instron UTM prior to application of the lap-shear test. (c) A modified probe-tack test in which a special device is capable of detecting the first contact between the probe and a pressure-sensitive adhesive (PSA) and determining this contact as the initial dwell time.

sliding of the adhesive. A certain length of overlap between adherents is typical when lap-shear tests are applied (Fig. 28.3(b)). The layer between the overlapping area consists of the structural adhesive, and tension-to-failure is used during the lap-shear test. The critical shear strength is calculated by dividing the load at failure by the area of the overlap.[263] The bond-strength value measured by a specific test is not just an inherently fundamental property of the type of adhesive; it also depends on other factors. Many experimental procedures, using biological or other hydrocolloid adhesives, have been conducted to test different important variables such as crosshead speed at debonding,[202] adhesive-layer thickness,[203] water-holding capacity of a specimen,[194,208] length of contact,[194,203] the effect of molecular weight[203] and the type of adhesive.[194]

Recently, a modified probe-tack test (Fig. 28.3(c)) was described in which a special device is capable of detecting the first contact between the probe and a PSA and of determining this contact as the initial dwell time.[265] In tests using viscoelastic PSA materials (hydrogels), this modified device gives results which differ significantly from those obtained with conventional probe-tack testers. In particular, when tack values are plotted against dwell time, the modified tester reveals a regime of sigmoid low-energy tack and then a transition to the normal power-law high-energy tack. This transition behavior was studied as a function of crosshead velocity, compression pressure, adhesive rheology, and adherend surface roughness. The practical implication is that far more information can be obtained from this modified device than from conventional probe-tack testers. The modified machine and methodology should prove particularly valuable as an experimental tool and for quality control tests in the manufacture of PSA products, particularly soft and tacky viscoelastic substances.[265]

28.18 Hydrocolloids as wet glues

Twenty-six hydrocolloids were tested for their ability to produce wet glues, i.e., they were studied for their ability to create very thick suspensions with 'good' adhesive properties at predetermined gum concentrations ranging from 10 to 75% by weight. The hydrocolloids were: gum talha, gum ghatti, gum arabino-galactan (AG), gum karaya, gum tragacanth, dextran, apple pectin, CMC, HPMC, tapioca-dextrin, carbopol-934, HPC, MC, gelatin, casein, starch, LBG, guar gum, alginate, κ-carrageenan, tara gum, fenugreek gum, konjac mannan, xanthan gum, gellan, and curdlan. The hydrocolloids (at different concentrations) were added in powdered form to double-distilled water and mixed with a standard dough mixer for at least 15 min until a thick, uniform, and smooth paste wet glue was obtained. Preliminary tests revealed that only 13 of these, namely: gum talha, gum ghatti, gum karaya, gum tragacanth, AG, dextran, pectin, tapioca-dextrin, CMC, MC, HPC, HPMC, and carbopol, can serve as bioadhesives in hydrophilic systems. Wet glues were produced from these hydrocolloids and tested over a wide range of concentrations, i.e., 10–75% by weight, and the color and pH of each glue was determined. All preparations were tested immediately following their production. Paste temperature was taken at the end of mixing, and 5 min later had risen by 0.97 ± 0.14 ºC. The pH of the wet glues ranged from 1.2 with carbopol to ~9.6 with AG. pH may be an important factor in the utilization of bioadhesive materials. Studies have shown[108] that gum karaya (pH ~ 3.6) may cause allergic reactions such as hives and angioneurotic edema. Paste color may be a factor in choosing an ointment or bioadhesive for a particular application. A variety of different colors could be found among the wet glues, ranging from off-white to yellowish or dark-brown.[266]

Once in very thick paste form, the 13 hydrocolloids were smeared homogeneously onto two different substrates: (a) cellulose-acetate film, normally used for dialysis, and (b) a skin-surface model (SSM), proposed[266] for testing the adhesion of medical adhesives. The water content in the SSM was approximately 1%. The three aforementioned mechanical tests, i.e. peel, tensile and lap-shear tests, were performed to check the properties of the wet glues.[266] Seven typical hydrocolloids (gum ghatti, AG, pectin, tapioca-dextrin, dextran, HPMC, and carbopol-934) were chosen for an evaluation of their physical properties as representatives of tree and shrub exudates, tree extracts, fruit extracts, grains, exocellular polysaccharides, cellulose derivatives, and petrochemicals, respectively.[266] Typical curves for the 90º peel, tensile load and lap-shear tests are presented in Fig. 28.4. Two common types of curves were obtained from the peel test. When a sample is pulled apart at a constant crosshead speed, the measured force should ideally be constant after reaching a steady-state condition. In practice, however, this is not always the case. There is evidence in the literature that when such results are reported, the mean values (averages) of the curve's ruggedness (deviations from a smooth line after reaching a steady-state condition) can be calculated and observed. In some

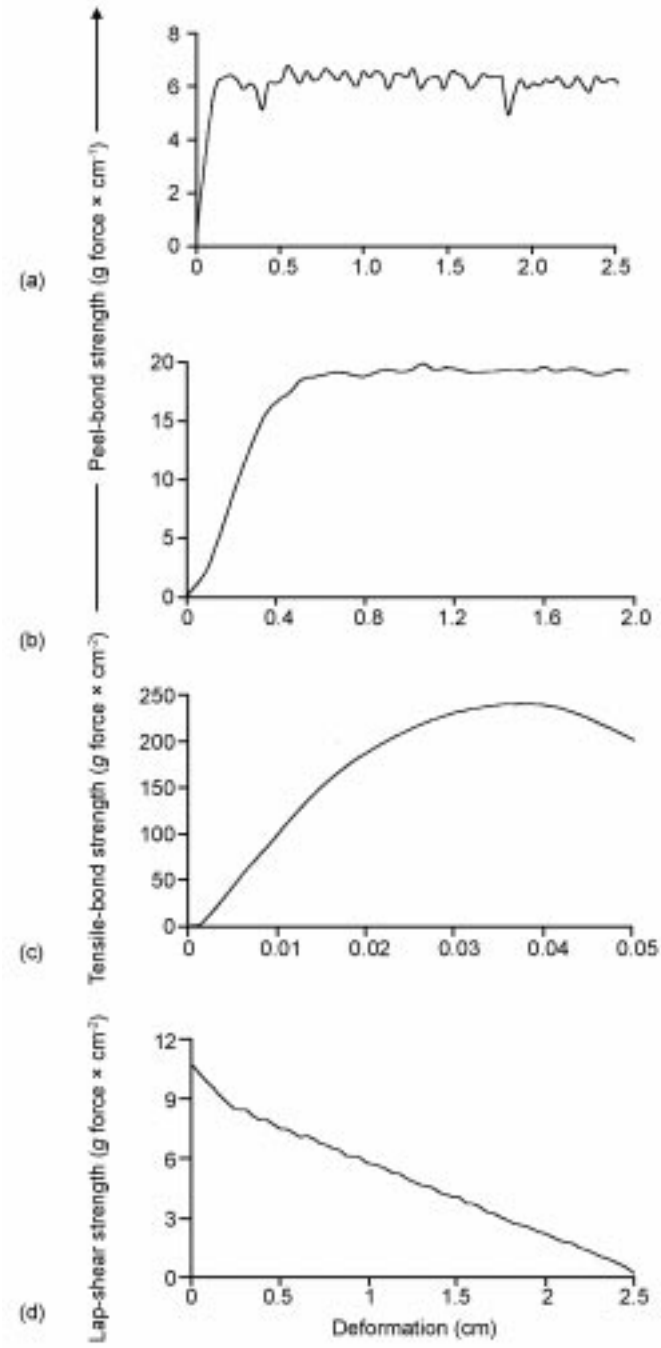

Fig. 28.4 Typical curves for the 90º peel test: (a) pectin, 25%; (b) gum talha; (c) typical curve for the tensile-bond test; (d) typical curve for the lap-shear test (HPMC, 20%). (Courtesy of O. Ben-Zion.)

instances, as observed macroscopically during testing, the rupture process occurs abruptly, sample failure propagates faster than the rate at which the sample is pulled apart, and failure is initiated periodically. The force has been claimed to go through well-defined maxima and minima, and the distance between two minima or maxima is independent of testing rate.[267] This author also mentions that the variability of the force is due to sample imperfections, that all the points on such a curve can be considered a statistical population, and that their frequency distribution is Gaussian.[267] Tensile load (Fig. 28.4(c)) and lap shear (Fig. 28.4(d)) were applied and plotted as load ($g \times cm^{-2}$) vs. displacement (cm) curves.[266] Tensile-bond strength increased in parallel to increases in deformation, until the beginning of failure. Tensile-bond tests are commonly used to analyse various adhesives, ranging from those for wood to those for metal (ASTM D-897 and ASTM D-2094, respectively[268]).

Lap-shear strength decreased linearly as deformation increased. Lap-shear tests are used to examine adhesion when the samples are relatively easy to construct and closely resemble the geometry of many practical joints (ASTM D-1002, ASTM D-3528). Data were collected continuously as the specimens were stretched uniaxially to rupture. The maximal force was recorded in both cases.[266] It was difficult to run comparative, economic, or practical analyses of the various hydrocolloids tested in this study, since the concentrations and viscosities differed, and formation of the desired suspension did not necessarily require the same time or energy. Therefore, in choosing a hydrocolloid for a particular application, such as wet biological adhesives or ointments, one needs to consider, in addition to the aforementioned factors, clinical or medicinal aspects such as the ability of the gum to perform as a medium for different electrolytes that could cause changes in viscosity and thus changes in adhesion potential (e.g., bioelectrodes). Also important are the wet glue's potential to cause dermatological irritation or allergic reactions, and its cost.[266] The previous reference gave experimental values of tensile bond and lap-shear strengths for seven hydrocolloids (representing seven different origins of natural and synthetic gums), tensile bond vs. 90º peel adhesive strengths, the influence of deformation rate on the peel bond strength values, the dependence of peel bond strength on adhesive layer thickness, the influence of water absorbance on peel bond strength and the influence of molecular weight on peel bond strength. In general, adhesion and bioadhesion studies on hydrocolloids reveal that compounds capable of inducing good wet adhesion have high molecular weights. Thus, chain length may contribute heavily to adhesive strength.

28.19 Future trends

An adhesive is basically defined by its function, which is to hold two surfaces together. To fulfill this function, the properties of an adhesive must include easy positioning at the interface, rapid and complete bond formation and subsequent hardening, and a bond strength that is adapted to the specific application

(structural, permanent, removable; rigid, or soft).[269] A variety of solutions exist in practice, and their application requires an understanding of both polymer chemistry and materials science.[269] Conventional PSAs are soft viscoelastic polymers that degrade, leave residue behind, self-adhere, and attach accidentally to inappropriate surfaces. Engineered adhesive nanostructures inspired by geckos (geckos attach and detach their adhesive toes in milliseconds while running with reckless abandon on nearly any surface and the adhesive on gecko toes differs dramatically from that of conventional adhesives) may become the glue of the future but it is too early and therefore difficult to estimate whether hydrocolloids will be part of this development.[270] On the other hand, future trends and research directions for PSAs, from the point of view of end-use properties alone, are moving towards heterogeneous polymer structures and the incorporation of additional functions, such as thermal or electrical conductivity or controlled drug release, as in nicotine patches, into a PSA matrix without altering its self-adhesive properties.[271] It is also evident that the nano revolution, as was discussed for edible coatings, will also be a part of the adhesives' future. Evidence for this can be found in a study that included nano-organo-clays within PSAs. It was observed that during drug release, the initial burst is reduced and can be controlled. Moreover, by optimizing the level of the organo-silicate additive to the polymer matrix, superior control over drug-release kinetics and simultaneous improvements in adhesive properties could be attained for a transdermal PSA formulation.[272] Another future research direction may be related to cell adhesion and changing surface modifications,[273,274] or the involvement of adhesive hydrocolloids, or those that change adhesiveness, in tissue engineering and regenerative medicine.[275]

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29

Alginates

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Abstract: Being essentially independent from temperature with respect to their gelling properties, alginates from brown marine algae differ from most other gelling polysaccharides and proteins. The present chapter focuses mainly on the major structure/function correlations in alginates and how they can be used to control gel properties. Furthermore, their application limitations linked to their inherent stability and solubility properties are also discussed. Finally, some aspects of food and nutrition, as well as their regulatory status, are also addressed.

Key words: structure/function, gels, gelling technologies, stability, food and nutrition.

29.1 Introduction

As structural components in marine brown algae (*Phaeophyceae*) and as capsular polysaccharides in soil bacteria, alginates are quite abundant in nature. The industrial production is roughly 30,000 metric tons annually, being probably less than 10% of the annually bio-synthesised material in the standing macroalgae crops. The sources for industrial production of alginate may be regarded as unlimited even for a steadily growing industry since macroalgae may also be cultivated and since production by fermentation is technically possible (although not economically feasible at the moment).

The biological function of alginate in brown algae is as a structure-forming component. The intercellular alginate gel matrix gives the plants both mechanical strength and flexibility (Andresen *et al.*, 1977). This relation between structure and function is reflected in the compositional difference of

alginates in different algae or even between different tissues from the same plant (see Section 29.3.2). In *Laminaria hyperborea*, an algae growing in very exposed coastal areas, the stipe and holdfast have a very high content of guluronic acid, giving high mechanical rigidity (see Section 29.5.1). The leaves of the same algae which float in the streaming water have an alginate characterised by a lower G-content giving a more flexible texture. In bacteria, like *Azotobacter vinelandii* (Sadoff, 1975) and *Pseudomonades* (Linker and Jones, 1966), the biological function is not fully understood but in the case of *A. vinelandii* it has been shown that alginate production is required for dormant cyst formation.

Pharmaceutical, food and technical applications (such as in printing paste for the textile industry) are the quantitative main market areas for alginates, but there is also a large and growing potential for alginate in biotechnological applications. The latter is mostly connected to high value applications such as encapsulation of living cells for *in vitro* or *in vivo* use (Smidsrød and Skjåk-Bræk, 1990; Soon-Shiong *et al*., 1992). This type of application has been a driving force for research aimed at understanding structure-function relationships in alginates at an increasingly detailed level. Basic knowledge gained from biotechnological research has made alginate one of the best characterised and well understood gelling polysaccharides.

The focus in this chapter will be on giving an overview of the understanding of structure-function relationships of the alginate system with emphasis on existent and potential gelling methodology and how to control these. As any application will have to fit within a framework with boundaries given by alginate functionality, some focus has also been put on chemical and physical limitations of alginates such as solubility and stability.

29.2 Manufacture

Alginate was first described by the British chemist E.C.C. Stanford in 1881, and exists as the most abundant polysaccharide in the brown algae comprising up to 40% of the dry matter. It is located in the intercellular matrix as a gel containing sodium, calcium, magnesium, strontium and barium ions (Haug, 1964). Alginate is widely used in industry because of its ability to retain water, and its gelling, viscosifying and stabilising properties. Several bacteria also produce alginate exocellularly (Linker and Jones, 1966; Gorin and Spencer, 1966; Sutherland, 1977), and *Azotobacter vinelandii* has been evaluated as a source for industrial production. But at present, all commercial alginates stem from algal sources.

The extraction of alginate from algal material is schematically illustrated in Fig. 29.1. Because alginate is insoluble within the algae with a counterion composition determined by the ion exchange equilibrium with seawater, the first step in alginate production is an ion-exchange with protons by treating the milled algal tissue with 0.1–0.2 M mineral acid. In the second step, the alginic acid is brought into solution by neutralisation with alkali such as sodium

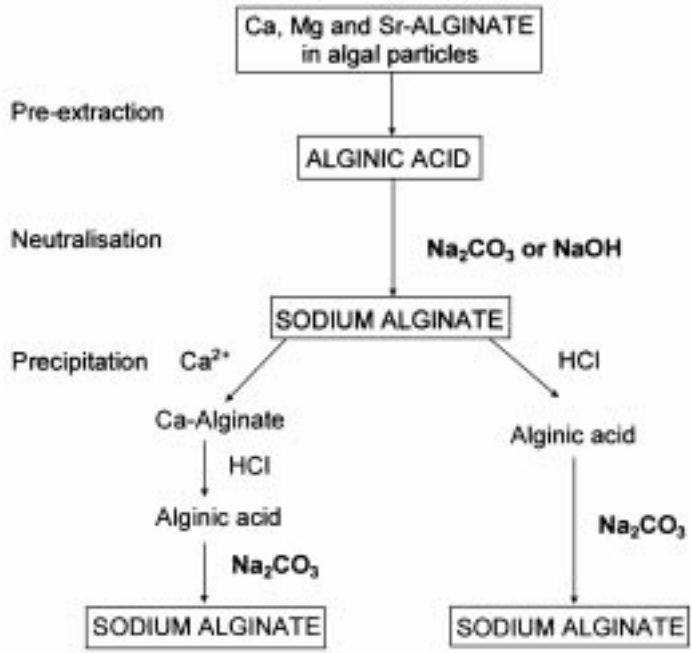

Fig. 29.1 Principal scheme for the isolation of alginate from seaweeds.

carbonate or sodium hydroxide to form the water-soluble sodium alginate. After extensive separation procedures such as sifting, flotation, centrifugation and filtration to remove algal particles, the soluble sodium alginate is precipitated directly by alcohol, by calcium chloride or by mineral acid, converted to the sodium form if required and finally dried and milled. In addition to Na-alginate, other soluble alginates are produced such as the potassium and ammonium salts. The only derivative of alginates today having a commercial value is the propylene glycol alginate (PGA; see also Section 29.4.1).

Following the increased popularity of alginate as an immobilisation matrix, FMC BioPolymer/Novamatrix A/S commercially manufactures ultrapure alginates highly compatible with mammalian biological systems. These qualities are low in pyrogens, and a low content of aggregates facilitate sterilisation of the alginate solution by filtration.

29.3 Structure and physical properties

29.3.1 Chemical composition and sequence

Alginate is a family of linear binary copolymers of (1→4)-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues (see Fig. 29.2(a) and (b)) of widely varying composition and sequence. The first information about the sequential structure of alginates came from the work by Haug and co-

workers (Haug, 1964; Haug and Smidsrød, 1965; Haug and Larsen, 1966; Haug *et al.*, 1966, 1967a) where alginate was separated into three fractions of widely differing composition. Two fractions contained almost homopolymeric molecules of guluronic and mannuronic acid, whereas a third fraction consisted of nearly equal proportions of both monomers containing a large number of MG dimer residues. It was concluded that alginate was a true block copolymer composed of homopolymeric regions of M and G, termed M- and G-blocks, respectively, interspersed with regions of alternating structure (MG-blocks; see Fig. 29.2(c)).

In a series of papers (Painter *et al.*, 1968; Larsen *et al.*, 1970; Smidsrød and Whittington, 1969) it was shown that alginates have no regular repeating unit and, furthermore, that the distribution of the monomers along the polymer chain cannot be described by Bernoullian statistics. Hence, knowledge of the monomeric composition is not sufficient to determine the sequential structure of alginates. Modelling of the alginate molecule suggests that a second order Markov approach seems to be required for a general description of monomer sequence in alginates.

More detailed information about the structure became available following the introduction of high resolution ^{1}H , and ^{13}C NMR spectroscopy (Grasdalen, 1983; Grasdalen *et al.*, 1977, 1979; Penman and Sanderson, 1972) in the sequential analysis of alginate. NMR has made it possible to determine the monad frequencies F_M and F_G, the four nearest neighbouring (diad) frequencies

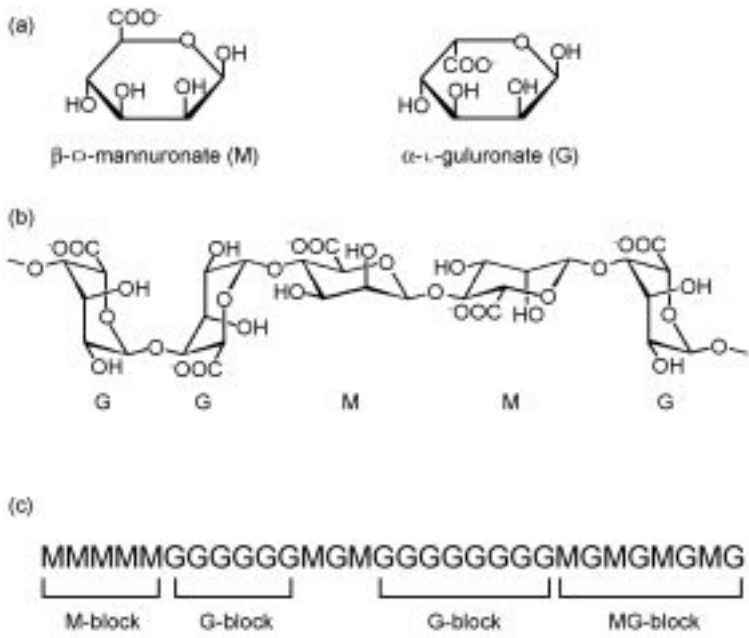

Fig. 29.2 Structural characteristics of alginates: (a) alginate monomers, (b) chain conformation, (c) block distribution.

F_{GG}, F_{MG}, F_{GM}, F_{MM}, and the eight next nearest neighbouring (triad) frequencies. Knowledge of these frequencies enables calculation of the average G-block length larger than 1: $N_{G>1} = (F_G - F_{MGM})/F_{GGM}$, which has shown to correlate well with gelling properties (see Section 29.5.1). It is important to realize that in a population of alginate molecules, neither the composition nor the sequence of each chain will be alike. This results in a compositional distribution of a certain width.

29.3.2 Source dependence

Commercial alginates are produced mainly from *Laminaria hyperborea*, *Macrocystis pyrifera*, *Laminaria digitata, Ascophyllum nodosum*, *Laminaria japonica*, *Eclonia maxima*, *Lessonia nigrescens*, *Durvillea antarctica* and *Sargassum* spp. Table 29.1 gives some sequential parameters (determined by high field NMR spectroscopy) for samples of these alginates. The composition and sequential structure may, however, vary according to seasonal and growth conditions (Haug, 1964; Indergaard and Skjåk-Bræk, 1987). Generally, a high content of α-L-guluronic acid is found in alginate prepared from stipes of old *Laminaria hyperborea* plants. Alginates from *A. nodosum*, *L. japonica* and *Macrocystis pyrifera* are characterised by a low content of G-blocks and a low gel strength.

Alginates with more extreme compositions can be isolated from bacteria (Skjåk-Bræk *et al.*, 1986) which can contain up to 100% mannuronate. Bacterial alginates are also commonly acetylated. Alginate with a very high content of guluronic acid can be prepared from special algal tissues such as the outer cortex of old stipes of *L. hyperborea* (see Table 29.1), by chemical fractionation (Haug and Smidsrød, 1965; Skjåk-Bræk *et al.*, 1986) or by enzymatic modification *in vitro* using mannuronan C-5 epimerases from *A. vinelandii* (Ertesvåg *et al.*, 1999). This family of enzymes is able to epimerise M-units into G-units in different patterns from almost strictly alternating to very long G-blocks. The

Table 29.1 Composition and some sequential parameters of algal alginates

Source	F_G	F_M	F_{GG}	F_{MM}	$F_{GM,MG}$
Laminaria japonica	0.35	0.65	0.18	0.48	0.17
L. digitata	0.41	0.59	0.25	0.43	0.16
L. hyperborea, leaf	0.55	0.45	0.38	0.28	0.17
L. hyperborea, stipe	0.68	0.32	0.56	0.20	0.12
L. hyperborea, outer cortex	0.75	0.25	0.66	0.16	0.09
Lessonia nigrescens	0.38	0.62	0.19	0.43	0.19
Ecklonia maxima	0.45	0.55	0.22	0.32	0.32
Macrocystis pyrifera	0.39	0.61	0.16	0.38	0.23
Durvillea antarctica	0.29	0.71	0.15	0.57	0.14
Ascophyllum nodosum, fruiting body	0.10	0.90	0.04	0.84	0.06
Ascophyllum nodosum, old tissue	0.36	0.64	0.16	0.44	0.20

epimerases from *A. vinelandii* have been cloned and expressed, and they represent at present a powerful new tool for the tailoring of alginates. Commercial alginates with less molecular heterogeneity, with respect to chemical composition and sequence, can also be obtained by a treatment with one of the C-5 epimerases.

29.3.3 Molecular weight

Alginates, like polysaccharides in general, are polydisperse with respect to molecular weight. This may arise for two different reasons: (i) polysaccharides are not directly coded for by the DNA, but are synthesised by polymerase enzymes, and (ii) a depolymerisation occurs during extraction. Due to this polydispersity, the 'molecular weight' of an alginate becomes an average over the total distribution of molecular weights.

There are several methods for averaging the molecular weight, the two most common are the number-average, $\bar{M}_n$ (which weighs the polymer molecules according to the *number* of molecules in a population having a specific molecular weight), and the weight-average, $\bar{M}_w$ (which weighs the polymer molecules in a population according to the *weight* of molecules having a specific molecular weight). The fraction $\bar{M}_w/\bar{M}_n$ is called the *polydispersity index* (PI). A PI of less than 2.0 suggests that some fractionation has occurred during the production process. Precipitation, solubilisation, filtration, washing or other separating procedures may have caused loss of the high or the low molecular weight tail of the distribution. A PI of more than 2.0 indicates a wider distribution, and suggests that blending of different batches with different molecular weights to obtain a sample of a certain average molecular weight (viscosity) or that a non-random degradation of the polymer has taken place. The molecular weight distribution can have implications for the uses of alginates, as low molecular weight fragments containing only short G-blocks may not take part in gel network formation and consequently not contribute to the gel strength. Also, in some high-tech applications, the leakage of mannuronate-rich fragments from alginate gels may cause problems (Stokke *et al.*, 1991; Otterlei *et al.*, 1991).

29.3.4 Selective binding of ions

The ion-binding characteristics of alginates represent the basis for their gelling properties. Alginates show characteristic ion-binding properties in that their affinity for multivalent cations depends on their composition (Haug, 1964). These characteristic affinities are exclusive to polyguluronate; polymannuronate is almost without selectivity. The affinity of alginates for alkaline earth metals increases in the order Mg << Ca < Sr < Ba; a property unique for alginates compared to other polyanions. The only polyanion resembling alginates in this fashion is pectic acid. From experiments involving equilibrium dialysis of alginate (Smidsrød, 1973, 1974), it was found that the selectivity for alkaline

earth metals and transition elements increased markedly with increasing content of α-L-guluronate residues in the chains, and that polymannuronate blocks and alternating blocks were almost without selectivity.

The high selectivity between as similar ions as the alkaline earth metals indicate that the mode of binding cannot be by non-specific electrostatic interaction only, but that some chelation caused by structural features in the G-blocks must be responsible. This characteristic property was eventually explained by the so-called 'egg-box' model (Grant *et al.*, 1973), based upon the linkage conformation (di-axial) between the guluronate residues. Although other, more accurate steric arrangements have been suggested, supported by X-ray diffraction (Mackie *et al.*, 1983) and NMR spectroscopy (Steginsky *et al.*, 1992), the simple 'egg-box' model still persists, as it may be regarded as giving an intuitive understanding of the characteristic chelate-type ion-binding properties of alginates. The simple dimerisation in the 'egg-box' model is, however, at present under scrutiny. This is partly based on data from small-angle X-ray scattering on alginate gels suggesting lateral association far beyond a pure dimerisation with increasing [Ca^{2+}] and G-content of the alginate (Stokke *et al.*, 2000), and partly from the fact that isolated and purified G-blocks (totally lacking elastic segments; typically DP = 20) are able to act as gelation modulators when mixed with a gelling alginate (see Section 29.5.1).

29.4 Technical data

29.4.1 Limitations for the use of alginates

Solubility

In general, there are three essential parameters which will determine and limit the solubility of alginates in water; the pH, the total ionic strength and the content of gelling ions. In the latter case, the 'hardness' of the water (i.e., the content of Ca^{2+} ions) is most likely to be the main problem.

By potentiometric titration (Haug, 1964) it was found that the dissociation constants for mannuronic and guluronic acid monomers were 3.38 and 3.65, respectively. The pK_a value of the alginate polymer differs only slightly from those of the monomeric residues. Alginate solutions may behave in two different ways when the pH in the solution is lowered. An abrupt decrease in pH causes a precipitation of alginic acid molecules, while a slow and controlled release of protons may result in formation of an 'alginic acid gel' (see Section 29.5.2). Precipitation of alginic acid molecules has been extensively studied (Haug and Larsen, 1963; Haug *et al.*, 1967c), and addition of acid to an alginate solution leads to a precipitation within a relative narrow pH range. This pH range depends on the molecular weight of the alginate but definitely also on the chemical composition and sequence; alginates containing more of the 'alternating' structure (MG-blocks) will precipitate at lower pH values compared to the alginates containing more of the homogeneous block structures (poly-M and poly-G). The presence of homopolymeric blocks thus seems to favour

precipitation by formation of crystalline regions stabilised by hydrogen bonds. By increasing the degree of 'disorder' in the alginate chain, like in alginates isolated from *Ascophyllum nodosum* (see Table 29.1), these crystalline regions are not as easily formed. A certain alginate fraction from *A. nodosum* is soluble at all pH values down to pH 1.4.

The only alginate derivative of some commercial value that is used in food is propylene glycol alginate (PGA). Steiner (1947) first prepared PGA, and the process was improved by Steiner and McNeely (1950). PGA is produced by a partial esterification of the carboxylic groups on the uronic acid residues by reaction with propylene oxide. The main product gives stable solutions under acidic conditions where the unmodified alginate would precipitate. This is mainly due to the introduction of a bulky group preventing inter-molecular interactions. At high esterification rates, the alginate molecule loses its gelling potential. PGA is used in acidic food products, such as to stabilise acid emulsions (e.g., French dressings), acid fruit drinks and juices. PGA is also used to stabilise beer foam, especially in the US.

Generally, any change of ionic strength in an alginate solution has a profound effect on polymer behaviour, especially on polymer chain extension and hence also the solution viscosity. At high ionic strengths, the solubility is also affected. Ionic strength effects generally exhibit large hysteresis; less than 0.1 M salt is necessary to slow down the kinetics of the dissolution process and hence limit the solubility (Haug, 1959), whereas considerably higher concentrations are required to salt-out alginates from a solution. The drive of the dissolution process of alginate in water is the gradient in the chemical potential of water between the bulk solvent and the solvent in the swelled alginate particle due to a very high counter-ion concentration in the particle. This drive becomes severely reduced when alginate is attempted to be dissolved in an aqueous solvent already containing ions. If alginates are to be applied with salt, it is recommended that the polymer should first be fully hydrated in pure water followed by addition of salt under shear.

Swelling of dry alginate powder in aqueous media containing Ca^{2+} seems to show a profound limit at approximately 3 mM free calcium ions (unpublished results). Below 3 mM Ca^{2+}, almost all of the alginate is found within the supernatant, whereas almost no alginate (1–3%) is found in solution above 3 mM free calcium ions. This limit is not likely to depend on alginate concentration since the system under consideration is swelling of dry alginate powder. Alginate can be solubilised at $[Ca^{2+}]$ above 3 mM by adding complexing agents, like polyphosphates or citrate, prior to adding the alginate powder.

Stability

Dry sodium alginate powder may have a shelf-life of several months provided it is stored in a dry, cool place without exposure to light. In the deep freezer, sodium alginate may be kept for several years without any significant reduction of molecular weight. In contrast, dried alginic acid has a very limited stability at

ordinary temperatures due to an intramolecular acid catalysed degradation (Smidsrød *et al.*, 1966). For many uses, it is important to be aware of the factors which determine and limit the stability of aqueous alginate solutions and the chemical reactions being responsible for the degradation. The relative viscosity of an alginate solution may be severely reduced over a short period of time under conditions favouring degradation. The glycosidic linkages are susceptible to both acid and alkaline degradation and oxidation by free radicals. And because alginates are derived from natural sources, many micro-organisms digest alginates. Extracellular alginate lyases have been isolated from different marine and soil bacteria (Gacesa *et al.*, 1989).

As a function of pH, the degradation is at its minimum around neutrality and increases in either direction. The increased instability at pH values less than 5 can be attributed to a proton catalysed hydrolysis, whereas the reaction responsible for the degradation at pH 10 and above is the β-alkoxy-elimination (Haug *et al.*, 1963, 1967b). An esterified alginate (like propylene glycol alginate) will have degradation rates 10^4–10^5 times that of the unsubstituted alginate because an esterification increases the electron-attracting effect of the carbonyl group at C-6 which in turn increases the rate of removal of H-5, the first step of the β-elimination reaction. Any physical property depending directly on molecular weight, e.g., viscosity and gel strength, will be more severely affected by chain breaks the higher the molecular weight of the starting material. The alginate producers are aware of this connection and will recommend higher concentrations of medium to low viscosity (molecular weight) products if an application involves the risk of polymer degradation.

Degradation can also be significant even at pH values around neutral. Most brown algae contain varying amounts of phenolic compounds which are extracted together with the alginate and represent a contamination in most commercial alginates, and the amount will vary with different algal species. The degradation of alginate in solutions can be diminished by reducing the content of phenols; this is normally achieved by crosslinking the phenols in the algae with formaldehyde prior to extraction (Smidsrød, 1980). The degradation effect of phenolic compounds belongs to the class of ORD reactions (oxidative-reductive depolymerisation) resulting from an auto-oxidation of reducing compounds with a subsequent formation of peroxides. This auto-oxidation increases with the amount of dissolved oxygen and with pH in the solution. Degradation is caused by free radicals, probably hydroxyl radicals, formed in reactions between the reducing compound and peroxides (Smidsrød *et al.*, 1963, 1967). It is important to be aware of the potential impact reducing compounds may have at physiological conditions where polysaccharides and other natural polymers are regarded as being completely stable. For alginates, it is important to know that the phenolic content of a commercial alginate varies with the algal source from which the alginate is isolated and the isolation procedure. It is also possible to remove most of the phenolics with simple purification procedures (Skjåk-Bræk *et al.*, 1989b).

Sterilisation of alginate solutions and powder almost always causes depolymerisation, loss of viscosity and possibly also reduced gel strength. It

has been shown (Leo *et al.*, 1990) that sterilisation processes as diverse as heating (autoclaving), ethylene oxide treatment and γ-irradiation all lead to polymer breakdown. Heating of an alginate solution will inevitably enhance polymer breakdown because this process increases the reaction rate of all the depolymerisation processes outlined above. In addition, thermal degradation (homolysis) may take place at high temperatures. For immobilisation purposes, sterile-filtering has been recommended rather than autoclaving to preserve the alginate solution's original viscosity (Draget *et al.*, 1988).

The effect of γ-irradiation on both solutions and dry powder of alginate is often disastrous. It has been observed that an applied standard sterilisation dose of 2.32 Mrad from a Co-60 source on dry alginate powder reduces the relative viscosity of the resulting solution by 95% with an almost complete loss of gelling capacity (Leo *et al*., 1990), most probably caused by the formation of the very reactive ·OH free radical (Parsons *et al.*, 1985). The O_2 level gets depleted very quickly under irradiation, and when the period of irradiation extends up to 1.5 hours, additional O_2 may diffuse into the sample and increase the amount of free radicals. An electron accelerator could be an alternative to Co-60 as irradiation source because the dose is applied very quickly (typically within 1 min) compared to 1.5 hours for the Co-60 source.

29.4.2 Properties and applications of alginate in the liquid phase

Due to the unique cold setting property of alginate gels, one generally tends to look upon applications of alginate as being mainly in the gel state. This is only partly true; there is, for example, a quantitatively most important market for alginate as a shear-thinning viscosifier in printing paste for the textile industry. There are also abundant uses of alginate in liquid foods where properties like viscosity enhancement and stabilisation of aqueous mixtures, dispersions and emulsions are exploited. PGA is also used solely in liquids as a stabiliser on the acidic side.

Furthermore, it has been established that the alginate molecule itself, with its high variety of possible chemical compositions and molecular weights, has different effects on, e.g., biological systems and gives different technological properties in the liquid phase. A biological effect of alginate was observed in the first animal transplantation trials of encapsulated Langerhans islets for diabetes control. Here, overgrowth of alginate capsules by phagocytes and fibroblasts, resembling a foreign body/inflammatory reaction, was reported (Soon-Shiong *et al.*, 1991, 1993) and this induced response depended to a large extent on the content of mannuronate residues. Alginate fragments, rich in mannuronate, which would not take part in the gel network, leached out of the capsules and directly triggered an immune response (Stokke *et al.*, 1993). The presence of immunologic response can, at least partly, be linked to $\beta(1\rightarrow4)$ glycosidic linkages since also other homopolymeric di-equatorial polyuronates, like D-glucuronic acid (C6-oxidised cellose), also exhibit this feature (Espevik and Skjåk-Bræk, 1996). *In vivo* animal models have now revealed the immunologic

potential of polymannuronate in such diverse areas as for protection against lethal bacterial infections and irradiation, and for increasing non-specific immunity (Espevik and Skjåk-Bræk, 1996).

29.5 Gels and gelling technologies

In contrast to most gelling polysaccharides, alginate gels have the particular feature of being cold setting. In principle, this implies that alginate gels set more or less independent of temperature. The kinetics of the gelling process may, however, be strongly modified by a change in temperature. The properties of the final gel will also change if gelling occurs at different temperatures, due to the non-equilibrium property of the alginate gels (Smidsrød, 1973). Another implication of thermo-irreversibility is that alginate gels are heat stable. Practically, this means that alginate gels can be heat treated without melting. This is the reason why alginates are used in baking creams.

One should generally look on the application of alginate gels as consisting of two stages with different optimisation criteria. First, the gelling kinetics should be considered and optimised against the manufacturing process. Since the gelling of alginate cannot be controlled by temperature, the importance of other parameters must be evaluated. The most important factors would be alginate concentration and chemical composition and sequence, the ratio between gelling and non-gelling ions and the presence of complexing agents such as phosphates or citrate as illustrated in Fig. 29.3. It is also important to keep in mind that alginate is a poly-electrolyte, implying that under favourable conditions, alginates may interact electrostatically with other charged polymers (e.g. proteins) in mixed systems resulting in a phase transition or an increase in viscosity. These types of interactions can be utilised to stabilise and increase the

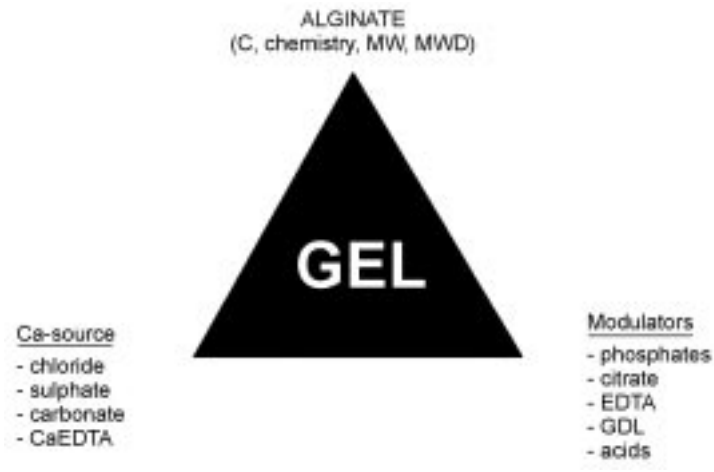

Fig. 29.3 Parameters controlling the kinetics and final properties of alginate gels.

mechanical properties of, e.g., some restructured foods and even feed (Bækken *et al.*, 2001). In studies involving gelling of bovine serum albumin (BSA) and alginate on both sodium and calcium form, a considerable increase in Young's modulus was found within some specific range of pH and ionic strength (Neiser *et al.*, 1997, 1998) suggesting electrostatic interactions to be the main driving force for the observed strengthening effects. On the other hand, if the purpose is to avoid such electrostatic interactions, the mixing of alginate and protein should take place at relatively high pH where most proteins have a net negative charge.

Secondly, the properties of the resulting gel are to be optimised against the desired specifications of the product. This includes parameters such as modulus, elasticity, brittleness and syneresis ('ageing' of the gel).

29.5.1 Ionic crosslinking

Due to the very rapid and irreversible binding reaction between multivalent cations and alginates, a direct mixing of these two components rarely produces homogeneous gels. The most likely result of such mixing is a dispersion of gel lumps ('fish-eyes'). The only exception is if a low molecular weight alginate is mixed with low amounts of crosslinking ion under high shear. This produces a weak gel which may be applied in some food products.

For most gelling purposes, ability to control the introduction of the crosslinking ions is essential. This control is possible by the two fundamentally different methods of preparing an alginate gel: the diffusion method and the internal setting method. The diffusion method is characterised by letting a crosslinking ion (e.g. Ca^{2+}) diffuse from an outer reservoir into an alginate solution (Fig. 29.4(a)). Internal setting (sometimes also referred to as *in situ* gelation) differs from the former in that the Ca^{2+} ions are released in a controlled fashion from an inert calcium source within the alginate solution (Fig. 29.4(b)). Controlled release is usually obtained by a change of pH and/or by a limited solubility of the calcium salt source.

Ionic crosslinking always implies an ion exchange process where the water soluble alginate (e.g., sodium or potassium form) has to exchange its counter-ions with Ca^{2+} to obtain a sol/gel transition. Gelling kinetics and the final gel properties may depend on the type of counter-ion present. It has been shown that at identical conditions (Draget *et al.*, 1998), K-alginates undergo a faster sol/gel transition compared to the Na-alginate, and that at apparent equilibrium and at low Ca^{2+} concentration, the Na-alginate gels exhibited reduced elastic moduli compared to the K-alginates, these differences increasing with increased content of guluronic acid residues in the alginate sample.

It has also been shown (Simensen *et al.*, 1998; Draget and Smidsrød, 2006) that if highly purified G-blocks are removed from a 'normal' alginate molecule by acid hydrolysis and subsequently refined by fractionation methods, these molecules (typical $DP_n = 20$) are able to modify both gelling kinetics and apparent equilibrium properties when mixed with a high M_w gelling alginate. The observed effects were higher gel strengths at high Ca^{2+} concentrations,

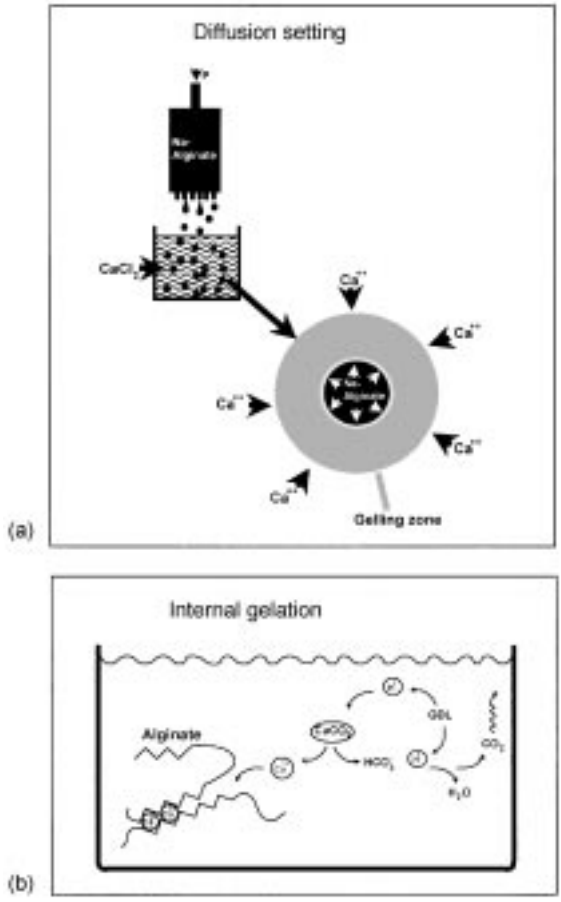

Fig. 29.4 The two principal methods of the manufacturing of alginate gels: (a) diffusion setting, (b) internal gelling.

reduced sol/gel transitional kinetics and a considerably reduced degree of syneresis. These modifications are obtained without rendering the viscosity of the solution prior to gelling since these low DP molecules will not contribute to viscosity. Higher gel strengths are rather unexpected since these molecules do not possess elastic segments. A model has been proposed that the pure G-blocks act as some kind of glue between topologically restricted G-blocks within the gelling alginate. Applying G-oligomers suggests control of alginate-based gelling systems on a new level.

Diffusion setting

This method has gained popularity as an immobilisation technique, but is also used in several methods for the restructuring of foods such as artificial berries, pimiento strips and onion rings (Onsøyen, 1996). Diffusion setting is characterised by rapid gelling kinetics, and this high speed setting is indeed

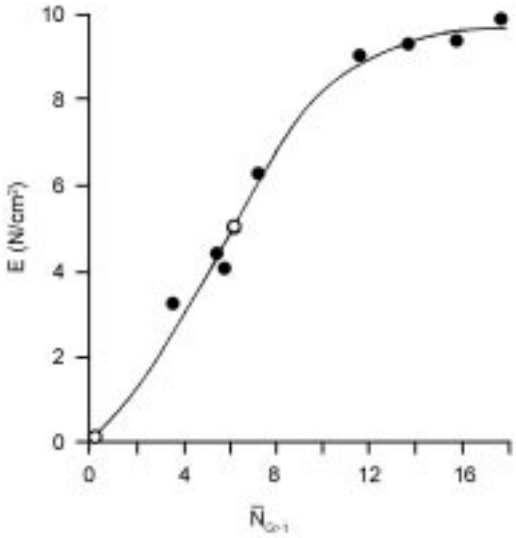

Fig. 29.5 Elastic properties of alginate gels as function of average G-block length.

utilised for immobilisation purposes where each droplet of alginate solution makes one single gel bead entrapping the active agent. Rapid gelling is also beneficial in the restructuring of foods when a given size or shape is to be achieved. It has been shown that as long as alginates with a weight average molecular weight above 100 kDa are used, the molecular weight dependence in this system is negligible (Smidsrød, 1974). The resulting gel strength is, however, strongly dependent on chemical composition and sequence. Figure 29.5 shows that the gel strength correlates with the average G-block length larger than 1 ($N_{G>1}$).

An important feature in the diffusion setting method is that the final gel can exhibit an inhomogeneous distribution of alginate, with the highest concentration at the surface and gradually decreasing towards the centre of the gel. Extreme alginate distributions have been reported (Skjåk-Bræk *et al.*, 1989a) with a five-fold increase at the surface (from the concentration in the alginate solution prior to gelation) and virtually zero concentration in the centre. This result has been explained by the fact that diffusion setting will create a sharp gelling zone moving from the surface towards the centre of the gel. The activity of alginate (and of the gelling ion) will equal zero in this zone, and alginate molecules will diffuse from the internal, non-gelled part of the gelling body towards the zero activity region. It is important to know that the degree of homogeneity can be controlled, and how different parameters can govern the final alginate distribution. Maximum inhomogeneity is obtained by letting a low molecular weight alginate gel at low concentration of the gelling ion and in the absence of non-gelling ions. Maximum homogeneity is reached by gelling a high molecular weight alginate in the presence of high concentrations of both gelling and non-gelling ions (Skjåk-Bræk *et al.* 1989a). Non-gelling ions are

also important with respect to the stability of the gels. It has been shown that alginate gels start to swell markedly when the ratio between non-gelling and gelling ions becomes too high, and that the observed destabilisation increases with increasing F_M (Martinsen *et al.*, 1989).

Internal setting

As outlined earlier, this technique is based on addition of an inactive form of the crosslinking ion into an alginate solution. In the case of calcium the insoluble $CaCO_3$ or the slightly soluble $CaSO_4$ may be used, or the Ca^{2+} ions may be complexed in a chelating agent (EDTA, citrate, etc.). The activation of the crosslinking ions is usually linked to a change in pH caused by the addition of organic acids or lactones. Lowering of the pH readily releases Ca^{2+} from $CaCO_3$ and complexing compounds. Chelating agents do, however, have discrete pH ranges where the complexed ions are released; in the case of EDTA, pH has to be lowered to around 4.0 to obtain a release of calcium ions. By using salts like $CaCO_3$ and $CaSO_4$, gels can be prepared over a much wider pH range (Draget *et al.*, 1991).

The main difference between internal and diffusion setting is the gelling kinetics. Internal setting allows for the tailoring of an alginate gelling systems towards a given manufacturing process. For example, in the alginate/$CaCO_3$/D-glucono-δ-lactone (GDL)-system, reducing the average particle size of the carbonate, and thereby increasing the total surface available to the acid, reduces the transition time (Draget *et al.*, 1991). The modulus of the final gel, however, approaches the same value independent of gelling kinetics. Other mediators can be necessary for the control of gelling kinetics. In the case of $CaSO_4$, the solubility is so high that gelation would occur spontaneously if complexing agents such as polyphosphates were not present. Internal setting almost always gives homogeneous gels, except where a low viscosity solution allows particle sedimentation.

The gel strength of internally set alginate gels depends more on molecular weight compared to gels set by diffusion (Draget *et al.*, 1993). Whereas gels made by the latter method describe almost a step function where gel strength becomes independent of molecular weight at around 100 kDa (Smidsrød, 1974) (weight average degree of polymerisation, $DP_w \sim 500$), the internally set gels still depend on molecular weight even at 300 kDa ($DP_w \sim 1{,}500$). This is, at least partly, due to the fact that the internally set gels are calcium limited compared to the gels made by diffusion, implying that the non-elastic fraction (sol and loose ends) will be higher in the internally set gels at a given molecular weight.

Observations have shown that the internally set gels are more exposed to syneresis than gels set by diffusion. As a rule of the thumb, $[Ca^{2+}] \cong 0.5[G]$ represents the limit at which syneresis becomes prominent in internally set gels (Draget *et al*., 1991). There is no detailed understanding of this difference at the moment, but part of the explanation is certainly the different modes of gelation. Diffusion setting gives a gelling zone, moving towards the centre of the gelling

body, where the alginate molecules become saturated with Ca^{2+} and their activity drops towards zero. Internal setting implies a process where gelling starts simultaneously at a large number of locations. This puts some topological strains on the alginate molecules, but their activity and translational mobility does not equal zero. One can therefore imagine that after the primary gel network has been formed, elastic segments with free G-blocks will still be present that could create new junction zones given the proximity of another free G-block and the presence of calcium ions. Thus, if the concentration of Ca^{2+} increases, a second class of junction zones may therefore be formed which will contract the gel network resulting in volume reduction.

29.5.2 Alginic acid gels

It has been known and utilised for several decades that alginates precipitate at pH below the pK_a value (Haug, 1964). In fact, the discovery that alginates were block co-polymers originated from the discovery that the different types of blocks had different solubility at low pH. It is also well known that, under controlled conditions, alginates may form acid gels at these low pH. These acid gels are, however, far less studied than the ionically crosslinked alginate gels, and with the exception of some pharmaceutical recipes (e.g. the anti-reflux agent Gaviscon), the number of applications is rather limited. The preparation of an alginic acid gel has to be performed with care. Direct addition of a mineral acid to, e.g., a Na-alginate solution leads to an instantaneous precipitation rather than a gel. pH must therefore be lowered in a controlled fashion, and this is most conveniently carried out by the addition of slowly hydrolysing lactones like D-glucono-δ-lactone (GDL). It has been shown (Draget *et al.*, 1994) that gel strength of acid gels prepared by this method becomes independent of pH below 2.5. GDL is added as dry powder, and a sol/gel transition is observed within 30–60 minutes, depending on the chemical composition and molecular weight of the alginate. The strength of the acid gels is more or less independent on the method by which they are made; acid gels thus seem to be independent of the history of formation. An important difference between the acid gels and the ionically crosslinked gels seems, therefore, to be that the former behave more as being of an equilibrium nature.

If acid gels are made from alginates with different chemical composition, it has been found that these gels resemble ionic gels in the sense that a high content of guluronate (high values of $N_{G>1}$) give the highest moduli. But in contrast to ionic gels, also poly-mannuronate sequences support acid gel formation. Poly-alternating sequences seem to perturb gel formation in both cases. The obvious demand for homopolymeric sequences in acid gel formation suggests cooperative processes to be involved just as in the case of ionic gels. A broad molecular weight dependence has been observed, and this dependence becomes more pronounced with increasing content of guluronic acid residues.

29.6 Foods, nutrition and health

29.6.1 Applications in food products

Some examples of the use of alginates in food products have already been given in the previous chapters. Their ability to improve, modify and stabilise the texture of foods represents the basis for applying alginates as food additives, e.g. as a viscosity enhancer, gel former and in the stabilisation of aqueous mixtures, dispersions and emulsions in general. Alginates are also used to control the melting behaviour of ice cream. Most applications are based on the physical properties of alginates themselves, but may also result from interactions with other components of the food product, e.g. proteins or fibres. For detailed descriptions and formulation, see Cottrell and Kovacs (1980), Sime (1990) and Littlecott (1982).

Restructured food based on Ca-alginate gels is simple (gelling being independent of temperature), and it is a steadily growing alginate application. Such processes are based on binding together a flaked, sectioned, chunked or milled foodstuff to make it resemble the original. Examples of such products already on the market are meat products (both for human consumption and as pet-food and feed), onion rings, pimento olive fillings, crabsticks and cocktail berries.

The synergetic gelling between alginates high in guluronate and highly esterified pectins may be utilised for the use in jams, jellies, fruit fillings, etc. (Toft *et al.*, 1986). These mixed alginate/pectin systems may give thermo-reversible gels in contrast to the purely ionically crosslinked alginate gels. Such gels are also almost independent of sugar content, in contrast to pectin gels, and may therefore be used in low calorie products. As already mentioned, propylene glycol alginate is the only alginate derivative used in food products. The main advantage of PGA is that it may be used to stabilise liquids under acidic conditions where the unmodified alginate would precipitate.

29.6.2 Nutritional aspects and health benefits

Alginates as such are regarded as essentially non-digestible in the human gastrointestinal (GI) tract and are classified as so-called poorly fermentable soluble (viscous) fibres (Brownlee *et al*., 2005). Hence, they can not act as a direct source of energy but may provide other health-related benefits. Such soluble dietary fibres do in general reduce the rate of small intestinal absorption of nutrients (Jenkins *et al.*, 2000), which in turn may reduce the likelihood of cardiovascular diseases (by reducing the overall cholesterol levels) as well as the onset of diabetes type II (by reducing the glycaemic load). Specific to alginates, it has been shown (Sunderland *et al.*, 2000) that, to a large extent, they can inhibit the activity of proteases. As outlined in Section 29.4.2 it has been shown that alginates high in mannuronate stimulate the production of cytokines *in vitro*. It has not been proven that an oral administration of such alginates would result in a general immuno-response in humans, but it has been suggested that they could enhance the repair of mucosal damage in the GI tract (Brownlee *et al.* 2005).

The fact that alginate also may undergo a sol-gel transition may be exploited to reduce the overall energy intake by stimulating endogeneous satiety signalling (Pelkman *et al.*, 2007) and that the products for this type of application can be formulated as a calcium-gelled fibre beverage. It has also been shown that microencapsulation (see Section 29.5.1) of probiotic bacteria has a profound effect on improved survival rates when such bacteria are exposed to acids and bile salts (Ding and Shah, 2007). Finally, it has also been proposed that the highly specific ion binding properties of the alginate molecule (see Section 29.3.4) could represent a way to reduce the damage of ingested radioactive strontium isotopes following a nuclear accident. One study (Beresford *et al.*, 1999) shows that when 5% calcium alginate is incorporated into the diet of dairy goats, the transfer of radioactive Sr into the milk was reduced by approximately 50%.

29.7 Regulatory status

The safety of alginic acid and its ammonium, calcium, potassium, and sodium salts were last evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) at its 39th meeting in 1992. An ADI 'not specified' was allocated. JECFA allocated an ADI of 0–70 mg/kg bw to propylene glycol alginate at its 41st meeting in 1993.

In the US, ammonium, calcium, potassium, and sodium alginate are included in a list of stabilisers that are generally recognised as safe (GRAS). Propylene glycol alginate is approved as a food additive (used as an emulsifier, stabiliser or thickener) and in several industrial applications (coating of fresh citrus fruit, as an inert pesticide adjuvant, and as component of paper and paperboard in contact with aqueous and fatty foods). In Europe, alginic acid and its salts and propylene glycol are all listed as EC approved additives other than colours and sweeteners.

Alginates are inscribed in Annex I of the Directive 95/2 of 1995 and as such can be used in all foodstuffs under the quantum satis principle in the EU (with the exception of those cited in Annex II and those described in article II of the Directive). A general prohibition exists on the use of alginate (as well as against several other gelling biopolymers) in so-called jelly mini-cups (amendment of 2005). Propylene glycol alginate (PGA) is inscribed in Annex IV ('other permitted additives') of the directive with a maximum level ranging from 0.1 mg/l–10 g/l (1.2–8 g/kg) depending on the food product.

29.8 Future trends

Containing only the two monomer units M and G linked by the same 1,4 linkages, the alginate molecule may look very simple. This may have lead potential commercial users of alginate to treat alginate as a commodity in much the same way as many of the cellulose derivatives. But as already outlined,

alginates exhibit a very high diversity with respect to chemical composition and monomer sequence resulting in a large variety of physical and biological properties. So, contrary to what may be expected by just looking at the monomer composition, the family of alginate molecules represents a challenge to the unskilled users of alginate, but an advantage for those end-users of alginate aiming at research-based, high value applications. The possibility of the tailoring of alginates to fit new and demanding applications is increased even further if epimerase-modified alginates are taken into consideration. It is therefore possible to foresee a future trend, which has already started, where the exploitation of alginate gradually shifts from low-tech commodity applications with increasing competition from low-cost alternatives, to more advanced knowledge-based applications in the food, pharmaceutical and biomedical areas.

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Inulin

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Abstract: This chapter describes inulin and some of its application aspects in food. First the structure and industrial production are described followed by the technical properties relevant for food applications (e.g., solubility, viscosity, stability, interaction with hydrocolloids and gelling properties). Secondly, the nutritional and health benefits such as the low caloric value and the prebiotic properties are outlined, followed by some examples of the use of inulin in food applications. Emphasis is on those applications in which inulin is used as a fat and sugar replacer, to improve mouthfeel, etc. Finally, the regulatory status and some future developments are discussed.

Key words: inulin, gelling properties, fat replacement, sugar replacement, interaction.

30.1 Introduction

Inulin has been well known for a long time. In 1804 Rose isolated a substance from *Inula helenium* (elecampane) that was later (in 1811) called inulin by Thomas (see Suzuki, 1993). These carbohydrates are synthesised in at least ten families of higher plants. They can be found in many economically important plants such as chicory, Jerusalem artichoke, onion, garlic, barley, rye and wheat. The data compiled in Table 30.1 show that inulins are an intrinsic ingredient of many of our common foodstuffs and as such these ingredients have been consumed for longer than we can remember. Inulin-containing crops were and are consumed not only from the crops mentioned in Table 30.1, which belong to a western type diet, but also in other countries, such as yacon tubers in South America and Japan, and murnong by Australian aboriginals. Jerusalem artichoke was consumed in Europe in the sixteenth century and in the early twentieth

Table 30.1 Inulin content of different crops

Source	Inulin content (% of fresh weight)	Range of DP
Banana (*Musa cavendishii*)	0.3–0.7	2–5
Barley (*Hordeum vulgare*)	0.5–1.5	No data
Chicory (*Cichorium intybus*)	15–20	2–60
Garlic (*Allium sativum*)	9–16	2–50
Globe artichoke (*Cynara scolymus*)	2–3	2–250
Jerusalem artichoke (*Helianthus tuberosus*)	3–10	2–50
Leek (*Allium ampeloprasum*)	16–20	No data
Onion (*Allium cepa*)	1–8	2–12
Wheat (*Triticum aestivum*)	1–4	2–8

Based on data of van Loo *et al.* (1995) and Sensus (unpublished). DP: degree of polymerisation (see Fig. 30.1)

century inulin was already known as a carbohydrate suitable for diabetics, as it did not give rise to a glycemic response.

Inulin is built up of 2–60 fructose units with one terminal glucose molecule (Fig. 30.1). Inulin is the generic name covering all β-(2,1) fructans. In most cases inulins are a polydisperse mixture of fructan chains with a chain length (DP: degree of polymerisation) distribution dependent on the source (Table 30.1) and moment of harvesting. The term oligofructose or fructo-oligoaccharides is used for β-(2,1) fructans with a DP up to about 10, and an

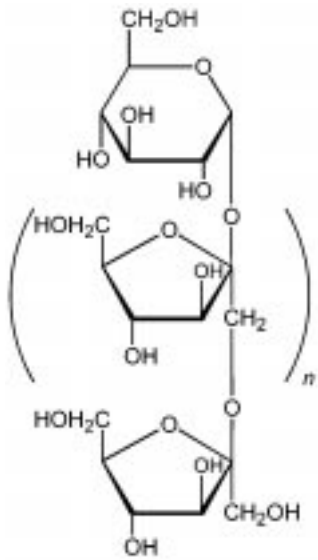

Fig. 30.1 Basic chemical structure of inulin GFn with terminal glucose residue (G: glucose; F: fructose; DP $= n + 2$; with $n = 1$–60 for inulin from chicory).

average DP of about 4. The topic of this chapter is mainly (long-chain) inulin with an average DP of at least 20.

30.2 Production process of inulin

In the early 1990s production of inulin as a food ingredient started in Europe. Industrial production of inulin almost exclusively uses chicory roots as raw material and is concentrated in the Netherlands and Belgium. Processing of chicory roots to produce inulin takes place under more or less the same conditions and with the same equipment as for the production of sucrose from sugar beet. As with sugar beet, processing also takes place in a campaign period from autumn to winter.

After washing and slicing the roots, inulins are extracted with hot water. The so called diffusion juice can be transformed into a clear juice with liming and carbonation, similar to the purification of the diffusion juice in sugar beet processing. Alternatively, proteins and cell components can be precipitated and flocculated under slightly acidic conditions and separated off by filtration. Further refining by crystallisation at this stage as for white sugar production is not suitable for native inulin: the low solubility of long-chain inulin allows separation of this fraction by crystallisation, but the short-chain inulin fraction will remain in solution. For this reason separation techniques common for production of liquid sugars such as glucose are used in the refining of native inulin solutions. Removal of salts and most of the colour takes place by ion exchange. To prevent hydrolysis of inulin at low pH the whole process of ion exchange is carried out at low temperatures. After this step a slightly acidic, nearly fully demineralised inulin thin juice is obtained which is also low in colour. Finally the characteristic bitter taste of chicory is removed by activated carbon, which also removes any residual colour. The refined inulin juice is concentrated by evaporation and finally spray dried to obtain a powdered product. Spray drying takes place from completely dissolved inulin solutions resulting in amorphous powders with good solubility.

Native inulin can be separated in short- and long-chain fractions by crystallisation. After crystallisation both long- and short-chain fractions can be spray dried to amorphous powders. This delivers types of inulin with a chain length distribution different from native inulin, which offer possibilities for different applications. The long-chain inulin with a DP ranging from about 10 to 60 is the main type of inulin discussed in this chapter.

30.3 Technical properties

30.3.1 Solubility

Solubility in water is mostly dependent on the inulin chain length distribution. In Fig. 30.2, the maximum or direct solubility of native and long-chain inulin types is shown. As can be seen, the solubility increases with decreasing chain length.

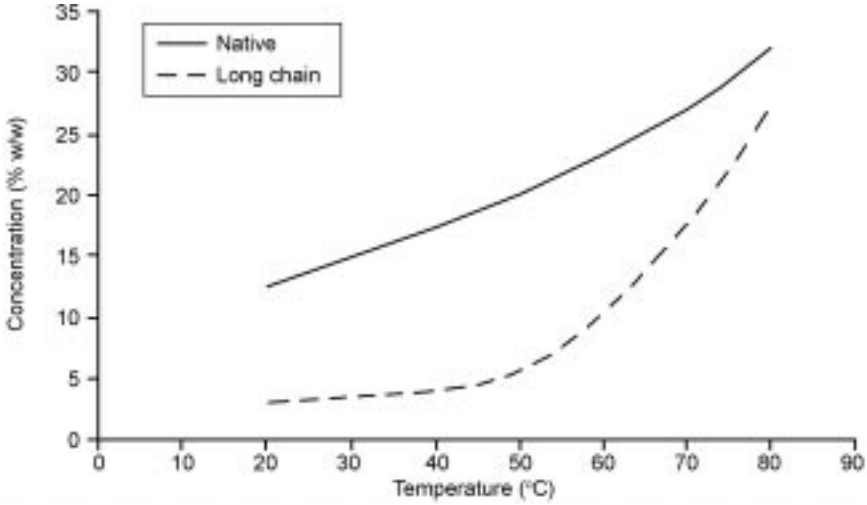

Fig. 30.2 Maximum solubility of native and long-chain inulin as a function of temperature. Data from Sensus (2003a).

Oligofructose is commercially available as syrup with a dry matter content of 75% which stays clear even at 5 ºC.

Storage time and temperature are important factors for transparency of inulin solutions. Longer storage time might result in development of haze due to precipitation of long-chain inulins. In Table 30.2 the concentrations are given for obtaining a stable clear inulin solution in water for a long period of time. This is important, e.g., for the development of beverages which should stay clear for a longer period, or in products where precipitation may lead to a rough texture by the precipitated inulin particles.

30.3.2 Viscosity

Solutions of native inulin in water are very low in viscosity, and also long-chain inulin exhibits only a slightly higher viscosity in solution compared to native inulin: e.g., a 20% solution in water has a viscosity of about 4.5 mPa s at 20 ºC.

Table 30.2 Long-term solubility of inulin at different temperatures

Type of inulin	Storage temperature	
	5 ºC	20 ºC
Native inulin	< 2.0%	< 2.5%
Long-chain inulin	< 1.0%	< 1.5%

Inulin concentration in relation to inulin type and storage temperature resulting in a clear solution after 13 weeks of storage (Sensus, 2003a)

30.3.3 Heat stability

Inulin is stable to temperatures up to 140 ºC (when dissolved at near neutral pH) and can therefore be processed easily in most if not all food applications. It will break down at temperatures above 140 ºC. In powder form the heat stability is much higher.

30.3.4 Acid stability

The β-(2,1) glycosidic bond in inulin is susceptible to acid hydrolysis. The degree of hydrolysis depends on pH, food product composition (dry matter content) and time-temperature combination (during heat treatment or shelf-life). In general, hydrolysis will not occur at pH levels above 4.0. At pH levels below 4.0, hydrolysis may occur with the rate dependent on actual pH and temperature. For example, hydrolysis is not observed at temperature levels below 10 ºC at any pH between 3.0 and 7.5, but at pH 3 at 25 ºC about 10% hydrolysis takes place in 1 week. An example of inulin loss in acid solution with different inulin types is given in Fig. 30.3.

30.3.5 Inulin and hydrocolloids

The function of hydrocolloids, which are also known as gums, thickeners, gelling agents, stabilisers, texturisers, is to thicken or gel aqueous systems. In doing so, they provide texture, body and mouthfeel to food products. Most of the hydrocolloids are high molecular weight polysaccharides. The water-binding property of these polymers is basically due to the fact that these macromolecules can form junction zones and so enclose large amounts of water. Because of this

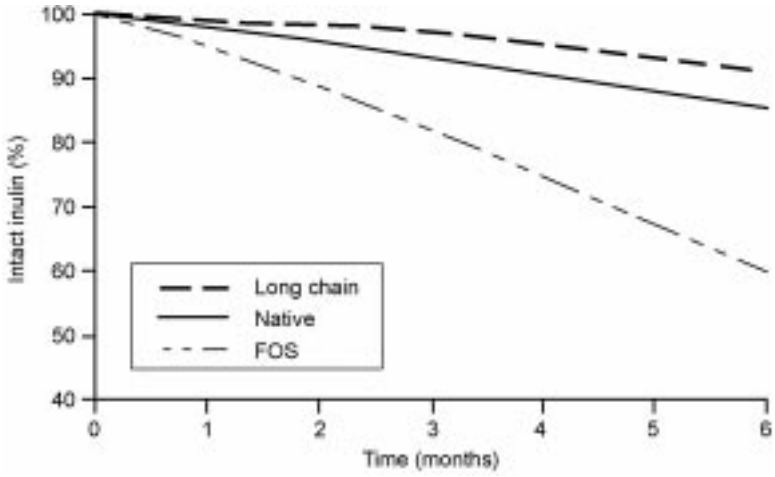

Fig. 30.3 Inulin hydrolysis of different inulin types at pH 3.5 and 20 ºC. Inulin or FOS solutions (1%) were prepared in buffer and stored at pH 3.5 and 20 ºC. The inulin content (defined as inulin with DP > 2) was determined using Shodex GPC measurements.

high water binding effect, they can be used at low dosage levels. Proteins like gelatin also have the ability to form gels.

Hydrocolloids can be divided in three groups: emulsifying/stabilising agents, thickening agents and gelling agents. Thickeners are used to thicken and increase viscosity of aqueous systems while gelling agents are able to convert water to a demouldable solid or gel.

Inulin has different thickening and stabilising properties from hydrocolloids. The inulin molecule is much smaller and the water-binding ability is low compared to hydrocolloids. When concentrations exceed about 15%, inulin has the ability to form a gel or cream. Below this concentration, low viscous aqueous solutions are obtained, as mentioned above. Gel formation of inulin is also different compared to hydrocolloids. Inulin forms particle gels, similar to those formed by some starches (Harris and Day, 1993). Gel formation by hydrocolloids or the increase of viscosity with most hydrocolloids is due to interaction between the polymer chains.

Due to the high number of hydroxyl groups present in inulin, it can be expected that inulin plays a role in hydrogen bonding in food systems, and that it may influence the solubility of other water-binding ingredients such as guar or xanthan gum, carrageenan, alginate, pectin, maltodextrin and starch. In other words, inulin takes part in the competition for water as solvent. Development of viscosity or gel formation will be changed as a result of this interference of inulin with the hydration of other hydrocolloids. This might result in a retardation of viscosity development, a higher viscosity, a more brittle gel (see Fig. 30.4 for carrageenan and inulin interaction) or a smoother product flow, and also syneresis can be reduced (Sensus, 2002).

Research in model systems has shown that inulin in combination with hydrocolloids can influence the rheological behaviour of these ingredients and

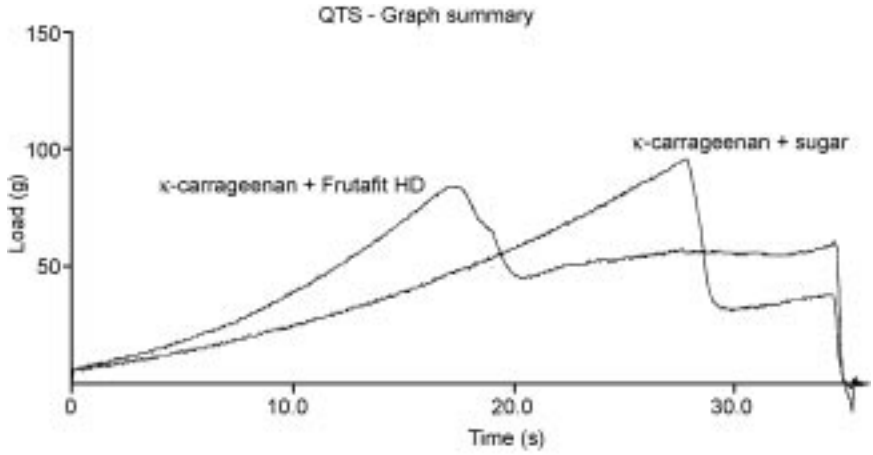

Fig. 30.4 Effect of inulin on rheology of a carrageenan gel. Gel strength of a κ-carrageenan gel with inulin or sucrose was measured in a Carrimed Rheometer. Based on data from Sensus (2002).

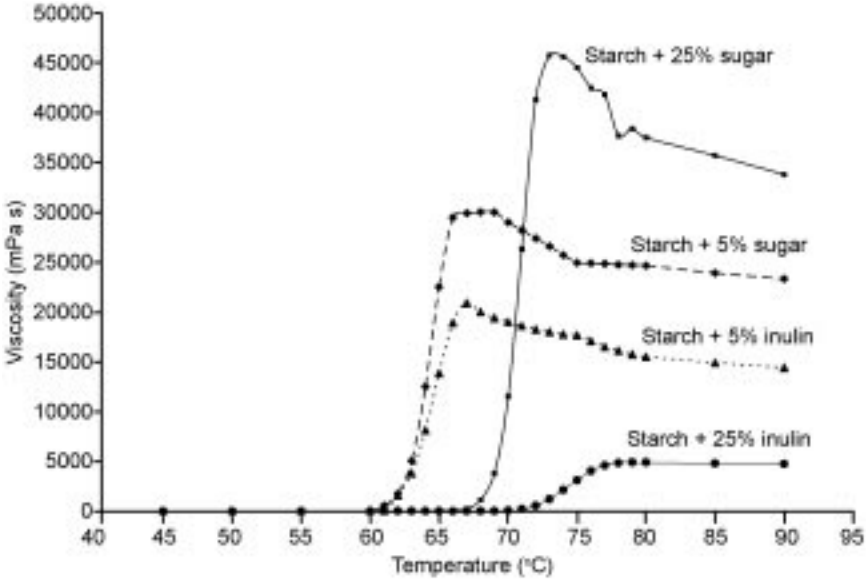

Fig. 30.5 Effect of inulin on starch viscosity in relation with temperature.

additives. Inulin can provide a useful tool to affect rheology and textural perception of a food product.

Although inulin does not act not like a thickener or gelling agent, in combination with hydrocolloids it influences the behaviour of these polysaccharides. In this way, inulin can optimise the rheological properties of products. The inulin product is highly polydisperse. It has significant influence (with its hydrogen bonding efficiency and consequent solubility (gel formation)) on various high water-binding ingredients such as guar gum, xanthan gum, carrageenans, alginates, pectins, locust bean gum, maltodextrin and starch. By adding inulin to thickeners the viscosity (decrease or increase) and flow characteristics of the aqueous solution can be affected. In Fig. 30.5, an example is shown how inulin may affect the viscosity of a starch solution at different temperatures. With high inulin concentrations viscosity is much lower than with lower concentrations. This may be due to the effect that the inulin network interferes with the starch network in such a way that viscosity is lowered.

30.3.6 Inulin as a gelling agent

Preparation of an inulin gel is straightforward: inulin is dissolved in water and upon cooling the molecules start to precipitate and crystallise, and a gel is formed (Table 30.3). The rate of precipitation or crystallisation and consequently the size of the inulin particles depend on temperature, concentration and cooling process.

The submicron-sized crystals go on to form aggregates which become interlinked to form a network. Within a few hours the free water is captured in

Table 30.3 Typical procedure for making an inulin gel

Preparation of a 25% (w/w) inulin gel
1. Dissolve 50 g inulin with DP about 25* in 150 g tap water.
2. Place the dispersion in a water bath at 72 ºC.
3. Heat the dispersion for 15 min while stirring.
4. Remove the solution from the water bath.
5. Place in a cold room, 5 ºC for at least 6 h.

* E.g., Frutafit®TEX as supplied by Sensus (the Netherlands)

the network of crystallised inulin particles, resulting in a gel structure. This gel has rheological properties very similar to those of fat and therefore inulin has been identified as an interesting ingredient for structuring in low- or zero-fat food products (Silva, 1996; Bot *et al.*, 2004).

30.3.7 Parameters affecting gel characteristics

The rheological features of an inulin gel can be influenced by the following parameters:

- chain length distribution
- concentration
- preparation temperature
- amount of shear (Kim *et al.*, 2001)
- use of seeding (Sensus, 2003b; Kim and Wang, 2001).

In Fig. 30.6, an example is given of the influence of concentration and inulin type on the firmness of a gel as measured with a Texture Analyser: long-chain inulins give a higher gel strength and for gel formation to occur with native inulin at least 20% of inulin is needed. With oligofructose no gels can be prepared at any concentration.

As has been mentioned before, the formation of an inulin gel is based on the precipitation and crystallisation of inulin molecules. Research has shown that only the longer inulin molecules (DP > 10) participate in the gel structure and the smaller molecules remain dissolved (Hebette *et al.*, 1998). It can be seen from Fig. 30.6, that inulin types with a higher average chain length, containing more long-chain inulin molecules, will form firmer inulin gels. An inulin gel belongs to the group of particle gels, and firmness increases with increasing concentration as shown in Fig. 30.6. Firmness of an inulin gel reaches its maximum when, during the manufacturing process, nearly all the inulin molecules become fully hydrated, with the exception of some seed crystals. These seed crystals are necessary to initiate the gelling process. Seed material other than inulin, such as maltodextrin or β-(2,6)-linked fructans are not effective (Sensus, unpublished observations). The degree of hydration depends on the inulin concentration and temperature.

The application of a shear treatment immediately after pasteurisation or during cooling will have a positive effect on increasing the firmness of an inulin

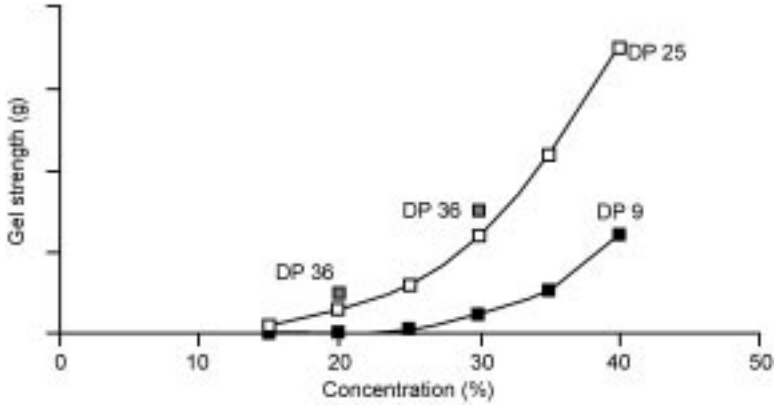

Fig. 30.6 Gel strength in relation to concentration of native inulin (DP 9), long-chain inulin (DP 25) from chicory roots and inulin (DP 36) from *Cynara scolymus*. Inulin gels were prepared at the concentrations given on the X-axis by dissolving inulin at 85 ºC and cooling down overnight (see Table 30.3 for the procedure). Gel strength was measured with a Stevens Texture Analyser (Sensus, 2003b).

gel (Fig. 30.7). Maximum firmness can be achieved by using the so-called seeding process: an inulin solution is heated to a high temperature, so that all the inulin molecules are completely hydrated. Addition of inulin seed crystals during cooling, in combination with a shear treatment will result in a firm inulin gel with perfect sensory properties (Fig. 30.7).

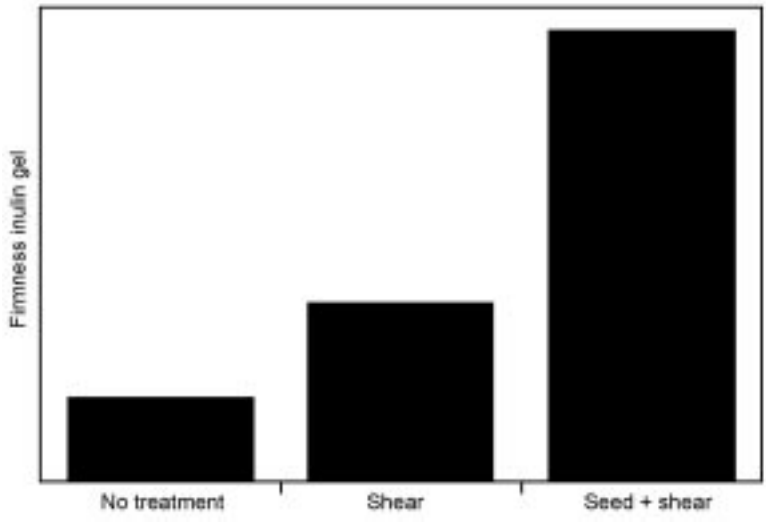

Fig. 30.7 Effect of shear treatment and seeding on firmness of inulin gels.

30.4 Nutritional and health benefits

As the glycosidic bonds between the fructosyl residues are resistant to gastric acid and since humans lack the digestive enzymes for splitting these bonds, inulins and fructo oligosaccharides (FOS) pass unharmed from mouth to small intestine. After reaching the colon they are completely fermented by the bacteria present in that organ. This behaviour explains why inulins are dietary fibres. The products from this fermentation process (short chain fatty acids, SCFA; acetic, propionic and butyric acid) will be absorbed by the host and subsequently metabolised. This salvages part of the energy, but only about a third of the caloric content compared with digestible carbohydrates is liberated by this process. This explains why inulins and oligofructose are low caloric food ingredients with a caloric value of about 1.5 kcal/g (Roberfroid, 1999).

The fermentation in the large intestine leads to a change in the composition of the colonic microbiota: specifically bifidobacteria (and lactobacilli) are favoured in their growth which leads to an increase in the number of these genera. This effect is observed in children (Kim *et al.*, 2007) and in adults (e.g., Kolida *et al.*, 2007). Concomitantly, often a decrease in *Clostridium* and *Bacteroides* is found. This effect on the bacterial composition is called the prebiotic effect of inulins. Gibson and Roberfroid (1995) coined this term and described this effect as follows: a prebiotic modulates the colonic bacterial flora beneficially by stimulating the growth of health bacteria (*Bifidobacterium* and *Lactobacillus*) and decreasing the number of potentially harmful species (e.g. clostridia).

This fermentation of inulins may lead to a whole range of other physiological effects, some of them related to the fibre functions and others more specifically to the increase of health associated bacteria as described. It is beyond the scope of this overview to discuss all effects in detail. It suffices here to describe the different effects briefly, with a focus on the results from studies with human volunteers.

The fibre effects include the contribution to an improved defecation pattern (Kleessen *et al.*, 1997; den Hond *et al.*, 2000) and a stool bulking effect (den Hond *et al.*, 2000), but the bifidogenic effect may also play a role here.

The fibre properties (the non-digestibility) are also relevant for the low glycemic response elicited by inulins and FOS. The glycemic response (GR) of inulins is very low: the purest commercially available inulin has a GR of 5, and native inulin a GR of about 14 (all relative to glucose set at 100; Meyer, 2007). This makes inulin a suitable ingredient for food for diabetics, and for the development of low GR food products (Meyer, 2007).

The fermentation of inulins may lead to a locally lowered pH thereby increasing the solubility of various salts. This improves their availability for absorption and it has been found that calcium and magnesium absorption in humans increases following inulin or FOS consumption (Meyer and Stasse-Wolthuis, 2006). This may have a positive effect on bone density as has been found in various animal trials (e.g., Scholz-Ahrens *et al.*, 2002) and in humans volunteers (Abrams *et al.*, 2005).

Another effect attributed to inulins is the lowering of serum lipids, thereby possibly contributing positively to heart health by lowering the risk for cardiovascular disease. Some studies showed a lowering of triglycerides (e.g., Causey *et al.*, 2000) or cholesterol (e.g., Davidson *et al.*, 1998), but others showed no effect (e.g., Pedersen *et al.*, 1997). It appears that especially in dyslipidemic volunteers inulin consumption may lower serum lipids (Beylot, 2005).

Recent data show that inulin can also have a positive effect on satiety feeling and energy intake. In people consuming inulin as a fat replacer in sausages, energy intake over the day is diminished (Archer *et al.*, 2004) and similar data were reported by Cani *et al.* (2006) for volunteers consuming oligofructose.

Inulins can also affect the immune system, possibly directly, but also through the increase in bifidobacteria and lactobacilli. Most of the effects described so far originate from experimental animal trials, and only a few examples from human studies have been published. These include a non-significant lowering of the incurrence of traveller's diarrhoea with FOS consumption (Cummings *et al.*, 2001) and a reduced inflammation of the mucosa of the ileal reservoir in ileal pouch-anal anastomosis patients (Welters *et al.*, 2002).

There is also evidence to suggest that inulin may lower the risk for colon cancer. Especially in experimental animal trials with chemically induced colon cancer, positive results have been obtained showing that with inulin consumption, and especially in combination with probiotics, the occurrence of colon cancer can be prevented (e.g., Femia *et al.*, 2002). It has also been reported that certain biomarkers for colon cancer risk in humans can be affected such that this risk may be lowered by such inulin-based synbiotics (Rafter *et al.*, 2007).

30.5 Applications

The rheological and sensory characteristics of inulin gels make them an excellent fat replacer in a wide range of foods (Silva, 1996). An overview is presented in Table 30.4, with emphasis on those applications for long-chain and native inulin in which the features discussed above play a role. For an overview of applications of all types of inulin, including oligofructose, we refer to Meyer *et al.* (2007). Generally speaking inulins are applied in the following markets: dairy, bakery, beverages, cereals and cereal bars, infant nutrition, confectionery, ice cream, but also in feed and pet food (see Verdonk *et al.*, 2005 for a review of the latter applications).

A positive influence is observed in the following applications by adding inulin. With thickeners (xanthan, guar gum and pectins) stabilised beverage systems become more homogeneous when inulin is added. Due to H-binding influences, inulin provides the necessary flow properties and physiological effects. This is also particularly effective in gum-based fat-free dressings and sauce applications where fat-like flow and mouthfeel are of primary importance.

Table 30.4 Overview of applications of native and long-chain inulins

Application	Chain length[a] L/N/S	Dosage (%)	Replacement		Technological functionality
			Fat	Sugar	
Dairy products					
Yoghurt	L/N	2–4	+		Mouthfeel
Milk drink	N	3–5	+		Mouthfeel
Breakfast drink	N	2–5	+		Mouthfeel
Mousse	L/N	3–20	+	+	Foam stabilisation, mouthfeel
Dairy spread	L	4–10	+		Texturiser, syneresis prevention
Bakery products					
Bread	L/N	3–10			Flour replacement
Hamburger bun	L/N	3–7			Flour replacement
Short dough biscuit	N/S	2–8	+	+	Crispiness, gluten softening, sweetness
Maria biscuit	N/S	3–10		+	Crispiness, gluten softening, sweetness
Aerated cake filling	N	5–15	+		Texturiser, foam stabiliser, mouthfeel
Biscuit filling	N	5–20	+		Bulking agent
Microwave pizza	L/N	1–3			Crispiness enhancement
Wafer	N	1–3			Crispiness enhancement
Miscellaneous					
Fat spread	L	4–20	+		Texturiser
Extruded cereals	L/N	3–20		+	Crispiness; bowl life
Dressing	L/N	2–10	+		Mouthfeel
Chocolate	L/N	3–20	+	+	Bulking agent
Meat products	L/N	2–7	+		Texturiser, mouthfeel
Tablets	N	5–40			Direct compressible excipient
Pasta filling	L/N	2–7			Texturiser, mouthfeel

[a] Chain length: L: long-chain inulin (average DP 20–25); N: native inulin (average DP 9–11); S; oligofructose with average DP about 4. Based on data from Sensus (2005) and Franck (2000).

The viscosity of starch is influenced because of the greater affinity for water of inulin compared to starch. A smoother flow is obtained which can be a benefit in starch-based sauces.

A creamier mouthfeel is also obtained when inulin is added to dairy products due to interactions with the dairy components, whey proteins and caseinate (Kip *et al.*, 2006). This effect is even stronger when combined with κ-carrageenan as seen in flans and instant mousses. In dairy- and low-fat spreads, stabilised by gelatin or maltodextrin, the spreadability and mouthfeel is improved when inulin is added and a more fat-like structure is obtained.

30.5.1 Fat spreads and dairy spreads

Addition of 7.5% native inulin to fat spreads with 20–40% fat (w/o emulsions) results in a product with a good structure and a perfect taste. The excellent

spreadability and mouthfeel make it a perfect, low-fat substitute for sandwich margarines with 80% fat. An inulin gel based on 20% long-chain inulin is the base for a very low-fat spread (o/w emulsions) with no more than 5% fat and for dairy spreads like cream and cheese spreads.

30.5.2 Low-fat mayonnaise/dressing

Traditional problems with low-fat or no fat mayonnaise and dressings are a lumpy texture with an acidic taste and a dry and sour aftertaste. With the addition of inulin (5%), a stable low-fat mayonnaise or dressing with good flow properties is achieved. Furthermore, inulin is capable of masking the acidic/sour taste.

30.5.3 Low-fat yoghurt

The preparation of low-fat, no fat yoghurt is done by starting with skimmed milk. To obtain the same mouthfeel as traditional yoghurt, inulin is added. By adding inulin the viscosity of the yoghurt is increased and a similar mouthfeel to normal yoghurt is obtained. Research has shown that especially airiness in low-fat yoghurts is increased by the addition of inulin. The increase of this texture attribute contributes significantly to the increased creaminess (Fig. 30.8; Kip *et al.*, 2006). A problem which occurs in other fat-free yoghurts is that the taste is more acidic. Inulin is capable of masking this effect.

30.5.4 Low-fat and low-sugar ice cream

Fat in a premium quality ice cream contributes to sensory properties (such as mouthfeel, melting behaviour and creaminess) and physical properties (such as hardness and stability). The role of sugar in ice cream is very much related to

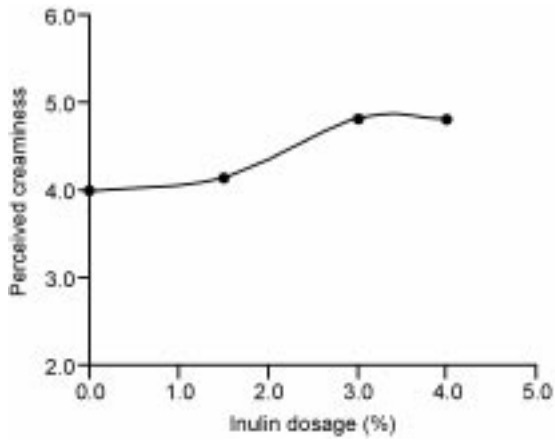

Fig. 30.8 Effect of long-chain inulin on creaminess of skimmed yoghurt. Data from Kip *et al.* (2003).

texture, hardness and stability, as well as taste and sweetness. Removing fat and sugar from ice cream will thus affect taste, texture and mouthfeel of the final product.

Inulin and oligofructose can return the desired sensory attributes to low-fat and low-sugar ice cream products (Schaller-Povolny and Smith, 1999) with inulin contributing to a creamy mouthfeel, homogeneous melting and improved heat shock stability, and oligofructose providing a sweet taste, scoopability, and synergy with high intensity sweeteners, helping mask the aftertaste. Both ingredients help to maintain small ice cream crystals after heat shock, which is necessary for good mouthfeel and improves the shelf-life.

Inulin and oligofructose offer excellent opportunities for sugar and fat replacement in ice creams. High quality products can be obtained in terms of the microstructure, sensory characteristics, melting properties and heat shock stability (McDevitt-Pugh and Peters, 2005).

30.5.5 Low-fat cake

Partial replacement of the pastry margarine by an inulin gel results in a fat-reduced cake with perfect sensory properties and a long shelf-life. Research has also shown that the combination of inulin with maltodextrin results in cakes with more volume.

30.5.6 Fillings

The creamy structure of an inulin gel makes it a perfect base for fillings which allows biscuits to be made with a filling in which 25% of the fat was replaced.

30.5.7 Wafers

The most important characteristic of wafers is their crispness, because this influences the perception of freshness. They should not lose their crispness during storage and preferably they should also stay crisp when they are filled with ice cream and fruit or as a sandwich wafer. The final wafer product becomes crisper when inulin is used. The crispness of a product can be characterised by the sound during breaking of the product.

The degree of crispness depends on several aspects. The density of the product has a major influence; the lower the density the easier the wafer breaks. At higher moisture contents the wafer will also not be crisp. Another aspect is the absorption profile of the starch used. The absorption profile is characterised by the relation between the water content and the water activity (A_w). A product stays crisp until a water activity of 0.1–0.2.

When the absorption profile line is steep (see Fig. 30.9, curve 1) the product is crisp only until a water content of approximately 3%. With a less steep absorption profile the product stays crisp until a higher water content (Fig. 30.9, curve 2). Inulin is able to bind water, so there will be less free water in the

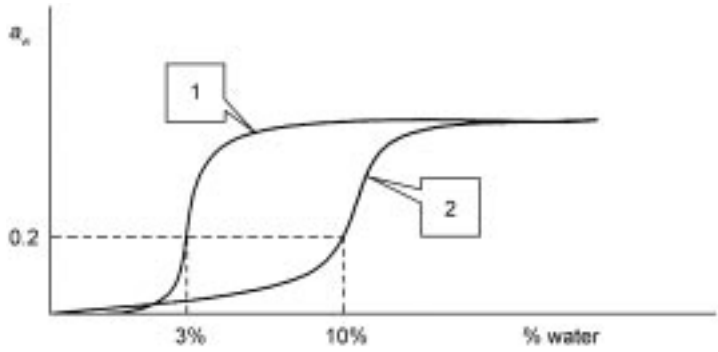

Fig. 30.9 Absorption profiles.

product (see also Schaller-Povolny *et al.*, 2000). This means that a wafer with inulin can contain more moisture and stay crisp.

30.5.8 Low-fat hazelnut spread

Commercial hazelnut spreads are fat-based products. Dry ingredients are milled and finely dispersed in the fat phase to achieve the right mouthfeel. A structure based on 12% native inulin with addition of sugars, cocoa powder, milk proteins and hazelnut paste makes it possible to produce a delicious low-fat (water continuous) hazelnut spread. Compared with the conventional product a calorie reduction of 45% can be achieved.

30.6 Regulatory status

Regulations differ from country to country. Because inulin is part of our daily diet (Table 30.1), it is considered as a food ingredient, so it has no E-number in the EU (it is not an additive) and it can be used *ad libitum* in all food categories. Inulin has the status of dietary fibre in almost every country.

As described above inulin can be used as a fat replacer and this creates the possibility to make nutrition claims based on the reduced fat or energy content of the food product. Table 30.5 shows such claims and conditions applying to them from EU 1924/2006, the latest legislation in the 27 member states of the EU (European Commission, 2006). As inulins are also a dietary fibre for labelling purposes, the fibre claims as mentioned in the Table 30.5 are equally applicable.

Also in the USA nutrition claims are possible, but in contrast to the EU where the percentage in the food is the basis, the concentration of inulin per serving size is the basis in the USA for making claims. As an example, a ‘low fat’ claim is allowed when the fat content is less than 3 g, whereas a ‘fat free’ claim requires less than 0.5 per serving (see FDA, 1999). Dietary fibre claims, such as ‘excellent source of fibre’, are based on a certain percentage of the reference daily intake of 25 g/d (FDA, 1999).

Table 30.5 Overview of nutrition claims from EU 1924/2006

Claim	Conditions
Low energy	Less than 40 cal (170 kJ)/100 g for solids, or 20 kcal (80 kJ)/100 ml for liquids. For table-top sweeteners a limit of 4 kcal (17 kJ)/portion applies
Energy-reduced	Energy value reduced by at least 30% (with an indication of the characteristic(s) which make(s) the food reduced in energy)
Energy-free	Less than 4 kcal or 17 kJ per 100 ml, for table-top sweeteners less than 0.4 kcal (1.7 kJ) per portion
Low fat	Less than 3 g of fat per 100 g for solids, or 1.5 g of fat per 100 ml for liquids (1.8 g per 100 ml for semi-skimmed milk)
Fat-free	Less than 0.5 g per 100 g or 100 ml (*X*% fat-free is prohibited)
Source of fibre	At least 3 g of fibre per 100 g (or 1.5 g per 100 kcal)
High fibre	At least 6 g of fibre per 100 g (or 3 g per 100 kcal)
Reduced [ingredient]	Reduction of at least 30% compared to a similar product
Increased [ingredient]	Increase of at least 30% compared to a similar product
Light/lite	Conditions as for 'reduced' and an indication of the charcteristic(s) which make(s) the food light/lite

For further details, see European Commission (2006).

Health claims based on inulin, such as based on the prebiotic or other effects described above, are also regulated in EU 1924/2006. A list of approved health claims describing the effect of an ingredient on a physiological function will be published at the latest on 31 January 2010. These Article 13 claims will be based on generally accepted scientific evidence. It seems very likely that (some of) the health effects of inulin will be on this list; for instance, a bifidogenic gut health claim is already approved in France (AFSSA, 2005) and the Netherlands (Rombouts *et al.*, 2002).

For so-called Article 14 claims (risk of disease reduction claims or claims aimed at growth and development of children) pre-market approval is required. The procedure to obtain such approval is described in EU 1924/2006 (European Commission, 2006).

In the USA, so-called structure-function claims, which describe the effect of an ingredient on the structure or physiological function, do not require pre-market approval. Care should be taken to avoid mentioning a disease in this type of claim, and that the necessary evidence is available, for instance to claim the effect of inulin on calcium absorption.

30.7 Future trends

In view of the oncoming obesity pandemic, it is to be anticipated that development of low caloric food products with an optimal taste and texture will continue at an increasing speed. Inulin on its own or in combination may be an important ingredient for these developments as it is low caloric and provides numerous health benefits, some of which are clearly relevant for the trends described, in particular the ability to improve satiety.

Also the interactions with hydrocolloids are important as these may contribute to proper food texture and flavour release in a variety of products. In connection with this, it might be interesting to look also for new tools to investigate these aspects; instead of focusing on the bulk rheological properties of the product, the interaction of the product in the mouth, with palate and tongue, might provide more clues to the assessment of texture and its sensory attributes, such as creaminess (e.g., Dresselhuis *et al.*, 2007).

This means that future developments are to be aimed at providing more insight into the mechanisms behind the interactions, which will contribute to building better food products. By including inulin the health properties of such products might be further enhanced. Further research to provide the scientific evidence for the health benefits of inulin will create the possibility of making health claims on inulin-containing foods to convey a credible message to the consumer.

30.8 Acknowledgements

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31

Chitin and chitosan hydrogels

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Abstract: A large number of hydrogels of modified chitins and chitosans have become available during the last few years for a variety of applications in research and technology. The simplest example of hydrogel formation is the treatment of a chitosan acetate solution with carbodiimide to restore acetamido groups. A large variety of substituted amides have been obtained by reacting chitosan with anhydrides and acyl chlorides. Hydrogels are formed by polyelectrolyte complexation as in the case of chitosan salts treated with pentasodium tripolyphosphate or other polymeric anionic substances including DNA, polysaccharides, proteins, inorganic or synthetic polymers, but also according to more sophisticated approaches such as the use of tyrosinase, laccase and other enzymes, or crosslinking agents like glycidoxypropyltrimethoxysilane. While the controlled and targeted delivery of drugs is obtained today with chitosan-based vectors capable of forming safe and bioresorbable hydrogels, on the other hand intelligent articles and sensors are currently manufactured from chitosan hydrogels as well. The biochemical significance of chitin and chitosan in the food area is based on the role that chitosan gels play when interacting with bile salts (cholesterol lowering, overweight control), and with intestinal bacteria (control over bacterial flora, and enhanced absorption). The preservation of fruit and vegetables from microbial spoilage with the aid of chitosan gels and films is becoming more and more important, because today chitosan is recognised as safe and it is approved for human consumption in many countries.

Key words: chitin, chitosan, drug delivery, nutritional value, cholesterol lowering, antimicrobial activity, polyelectrolytes.

31.1 Introduction

Hydrogels, composed of water-soluble or swelling macromolecules, exhibit high water content, biocompatibility and desirable mechanical properties; the xerogels resulting after dehydration exhibit large specific surface area, and a network suitable for the incorporation of biomolecules. Present and future microgel applications require control over properties that include novel functionality, controlled particle size and tunable biodegradability. Major advances in the area of microgel/nanogel particles deal with drug delivery carriers for biological and biomedical applications (Oh *et al.*, 2008), but food applications have long testified to a great deal of interest in natural and semi-synthetic hydrogels. This chapter provides information on some chitin- and chitosan-based hydrogels suitable for applications in the food and welfare areas.

Among polysaccharides, chitin is known for a large variety of hydrogels that can be obtained through chemical or enzymatic modification. Chitin, (1-4)-linked 2-acetamido-2-deoxy-β-D-glucan, is widely distributed among invertebrates. It is found as α-chitin in the calyces of hydrozoa, the egg shells of nematodes and rotifers, the radulae of chitons and limpets (Shaw *et al.*, 2008) and the cuticles of arthropods; sponges possess chitin as a component of their skeletons (Ehrlich *et al.*, 2007). β-chitin is present in the shells of brachiopods and molluscs, the cuttlefish bone, the squid pen, and pogonophora tubes. Chitin is found in exoskeletons, peritrophic membranes and cocoons of insects. The chitin in the fungal walls varies in crystallinity, degree of covalent bonding to other wall components, mainly glucans, and its degree of acetylation. Basic information on chitin and chitosan is available in books, encyclopedias and review articles (Enescu and Olteanu, 2008; Goosen, 1996; Kumar *et al.*, 2004; Jollès and Muzzarelli, 1999; Kurita, 2006; Mano *et al.*, 2007; Morimoto *et al.*, 2002; Mourya and Inamdar, 2008; Muzzarelli, 1977; 1985a,b; 1993; 1996a,b; 2000; 2001; 2005; 2009a,b; Muzzarelli *et al.*, 1986; Rinaudo, 2006a,b; Ruel-Gariepy and Leroux, 2006; Terbojevich and Muzzarelli, 2000; Varghese and Elisseeff, 2006; Vinsova and Vavrikova, 2008; Yalpani, 1988).

Today, chitins and chitosans of various grades are commercially available in large quantities from producers all over the world. Chitin isolates differ from each other in many respects, including degree of acetylation, defined as the molar fraction of GlcNAc, typically close to 0.90; elemental analysis, with nitrogen content typically close to 7%, and N/C ratio 0.146 for fully acetylated chitin; molecular size and polydispersity. The average molecular weight of chitin *in vivo* is probably in the order of one million Da, but chitin isolates have lower values due to partial random depolymerisation occurring during the chemical treatments and depigmentation steps. The presence of a substantial quantity of nitrogen is a point of difference from other abundant polysaccharides: the fact that chitin contains four elements instead of three is often ignored when chitin is compared to cellulose.

Chitosans are a family of deacetylated chitins. In general, chitosans have nitrogen content higher than 7% and degree of acetylation lower than 0.40. The

removal of the acetyl group from chitin is a harsh treatment usually performed with concentrated NaOH. Protection from oxygen, with a nitrogen purge or by addition of sodium borohydride to the alkali solution, is necessary in order to avoid undesirable reactions such as depolymerisation and generation of reactive species. Commercial chitosans may contain insoluble highly acetylated fractions that come from the core of the granules submitted to heterogeneous deacetylation. The acetyl groups in the acid-soluble fractions are randomly distributed, whilst the insoluble fractions contain relatively long sequences of acetylated units.

Chitin and chitosan are not present in the human tissues, but acetyl-glucosamine and chitobiose are found in glycoproteins and glycosaminoglycans. Since chitosan is biodegradable, non-toxic, non-immunogenic and biocompatible in animal tissues, much research has been directed toward its use in medical applications such as drug delivery, artificial skin and blood anticoagulants.

31.2 Chitosan chemistry

31.2.1 Chemical structure and molecular characterisation

The degree of acetylation for commercial samples is about 0.20. Experimental chitosan samples can be deacetylated almost completely, giving poly-glucosamine. The evaluation of the degree of acetylation can be carried out by a number of techniques, depending on the amount of acetylated units. A total of 22 analytical methods were proposed for the determination of the degree of acetylation (Hein *et al.*, 2008), including one based on UV spectrophotometry (Muzzarelli and Rocchetti, 1985; Wu and Zivanovic, 2008).

High-resolution ^{1}H and ^{13}C NMR spectroscopy can provide information not only on the degree of acetylation in solution and solid state, but also on the sequence structure: in particular, random and block arrangements of GlcNAc and GlcN units are detected. In order to evaluate the average molecular weight of chitosan, viscometric and gel permeation chromatographic techniques are used. In contrast to the static light scattering method, which gives absolute values for molecular weight, the above techniques are empirically related to chain length and require calibration curves. On the other hand, light scattering measurements are difficult to perform and sometimes the data are difficult to interpret, in the presence of aggregation and association. In summary, the molecular weight characterisation of chitosan remain problematic: probably the viscometric method, easy and rapid to perform, and not greatly affected by the presence of negligible amounts of very high molecular weight polymer, is the best choice, provided that the Mark–Houwink–Sakurada equation is correctly used with sets of values as reported in the literature (Kasaai, 2007), for various aqueous salt systems in which the viscometric measurements are performed. Average molecular weight for chitosans can reach values of 500 kDa or more.

Gel permeation chromatography methods are available to evaluate the molecular weight distribution of chitosan samples: calibration is performed by

means of commercial standards (pullulan, for instance) or chitosan samples (Terbojevich *et al.*, 1993). A good improvement is obtained by using a low-angle laser light scattering instrument as an on-line detector (Beri *et al.*, 1993). Samples of chitosan with polydispersity index ca. 1.2–1.5 were obtained by preparative gel permeation chromatography; no variation of the degree of acetylation related to molecular weight was found. Attempts to perform molecular weight fractionation of commercial chitosans by selective precipitation with ethanol or acetone from aqueous solutions or by ion exchange were only partially successful. The reasons for this are depolymerisation of chitosan chains during the experiments, and incomplete recovery of the polymeric material from the chromatographic column.

Depolymerisation of chitosan

Chitosan chains can be chemically depolymerised by cleavage of the glycosidic bond catalysed by acids and, to a lesser extent, by bases. The rate of depolymerisation depends on the type and the concentration of the acid and on the temperature: the extent of depolymerisation can be calculated following the equations reported in the literature (Terbojevich *et al.*, 1992).

In the presence of nitrous acid, nitrosating species attack the glucosamine, but not the *N*-acetylglucosamine moieties and a 2,5-anhydro-D-mannose unit is formed at the reducing end of the cleaved polymer. As a consequence, after the depolymerisation, the samples have not only lower molecular weight than starting chitosans, but also different composition and sequence arrangement. Hydrogen peroxide can also be conveniently used to depolymerise chitosan.

As for enzymatic depolymerisation, many commercial enzyme preparations exert hydrolytic activity on chitosans, that appear to be unexpectedly vulnerable to a range of hydrolases: several proteases such as pepsin, bromelain, ficin and pancreatin display lytic activities towards chitosans that surpass those of chitinases and lysozyme. Cellulases, hemicellulases, lipases, proteases and pectinases are also effective (Yalpani and Pantaleone, 1994). This unspecific activity is possibly due to the simplicity of the enzyme–substrate complex formation.

Almost all the cellulases produced by different kinds of micro-organisms can degrade chitosan to chitooligomers, thus they advantageously replace chitinases that are very expensive. The existence of bifunctional enzymes with cellulase and chitosanase activity is one of the reasons for cellulase efficacy on chitosan hydrolysis (Xia *et al.*, 2008).

In order to prevent the browning of the chitooligomers prepared via enzymatic hydrolysis, the chitooligomers were reduced with potassium borohydride to improve their chemical stability. The reduced chitooligomers had no oral acute toxicity, and they had almost the same biological significance in mice when injected intraperitoneally as the fresh chitooligomers (Qin *et al.*, 2008).

31.2.2 Chitosan production

The chemical composition and the distribution of different units along the chitosan chains are strictly related to the polymer preparation; therefore there is an important relationship between production and properties of chitosans, both in solution and in the solid state.

The deacetylation of chitin is studied as a function of NaOH concentration, reaction time and temperature as determined by alkalimetric titration. Deacetylation is performed by traditional heating or under microwave irradiation. The maximum degree of deacetylation (98%) can be obtained after 30 min of thermal heating at 135 ºC in 50 wt% NaOH solution (Leonhardt *et al.*, 2008).

The shells of the brine shrimp *Artemia urmiana* are a rich source of chitin (29.3–34.5% of the shell dry weight). *Artemia* chitosan exhibited a medium molecular weight (450–570 kDa), regular degree of deacetylation (67–74%) and relatively low viscosity (29–91 centiposes). The physico-chemical characteristics (e.g., ash, nitrogen and molecular weight) and functional properties (e.g., water-binding capacity and antibacterial activity) of the prepared *Artemia* chitosans were enhanced, compared to crab control and commercial samples (Tajik *et al.*, 2008).

Chitosan of fungal origin in the molecular range 5–10 kDa was firstly prepared directly from the *Absidia coerulea* mycelia. To improve the low molecular weight chitosan production, the solid-state fermentation media were optimised to investigate the influence of substrate and supplemental medium components on low molecular weight chitosan production. The low molecular weight chitosan was obtained after treatments with 2% NaOH and 10% acetic acid. Maximal low molecular weight chitosan production was 6.12 g/kg substrate. Gel permeation chromatography combined with laser light scattering gave a molecular weight of 6.4 kDa with M_w/M_n of 1.09. Fourier transform infrared (FTIR), X-ray diffraction and ^{13}C NMR spectra of the product showed typical peak distributions the same as those of standard chitosan which confirmed the extracted product to be chitosan-like. The method provided a new, simple and green technology to produce low molecular weight chitosan directly (Wang *et al.*, 2008).

The existence of chitosan in nature was discovered when it was isolated from the yeast *Phycomyces blakesleeanus* (Kreger, 1954). Chitosan occurs as the major structural component of the cell walls of certain fungi, mainly of the *Zygomycetes* species (Davies and Bartnicki-Garcia, 1984). However, to date, chitosans have been produced commercially by alkaline deacetylation of crustacean chitins, but experimental samples are available from other animals, such as houseflies and honey bees (Draczynski, 2008).

31.3 Properties of chitosans and derivatives

31.3.1 Solubility

The solubility of chitin is remarkably poorer than that of cellulose, because of the high crystallinity of chitin, supported by hydrogen bonds mainly involving

the acetamido and alcoholic groups. The insolubility of chitin is also pointed out as a serious drawback, nevertheless all chitins become soluble with the aid of hypochlorite as 6-oxychitins (Muzzarelli *et al.*, 1999), and beta-chitin becomes soluble in a number of chemicals such as calcium chloride saturated methanol, among others (Nagahama *et al.*, 2008).

The presence of a prevailing number of 2-amino-2-deoxyglucose units in a chitosan allows the polymer to be brought into solution by salt formation. Chitosan is a primary aliphatic amine that can be protonated by selected acids, the *p*K of the chitosan amine being 6.3. The following salts, among others, are water soluble: formate, acetate, lactate, malate, citrate, glyoxylate, pyruvate, glycolate; the solubility of chitosan ascorbate depends on the preparation conditions.

Under particular conditions chitin and chitosan can give hydrophilic water swellable hydrogels: gel formation is also promoted by crosslinking agents or organic solvents, particularly for chitosan derivatives. Chemical and physical gels are produced, thermally reversible and not reversible.

Chitosan is insoluble in organic solvents, in acids at high concentrations and in alkali; it is also insoluble in aqueous solution at pH $\leq$ 6, except for low molecular weight samples. Chitosan is soluble in aqueous acidic media, following protonation of amino groups in the repeating unit; this polycationic structure is unique, other polysaccharides usually being neutral or anionic. Chitosan is also soluble in alkaline solutions (ammonium bicarbonate, pH 10) where it forms glycosylamine functions (Muzzarelli *et al.*, 2003).

The selective introduction of saccharide residues at the chitosan amino group facilitates the conversion of the water-insoluble chitosan into various branched derivatives soluble in both neutral and slightly acidic (pH 5–6) aqueous media, with solubility being attained for some products with degree of substitution as low as 0.14. Chitosan can be reacetylated with acetic anhydride to obtain also water-soluble partially reacetylated chitin (Hirano and Horiuchi, 1989). The formation of derivatives suitable for industrial applications with good solubility in various organic solvents can be effected through the introduction of hydrophobic substituents by acylation with long chain fatty acyl halides or anhydrides.

As reported for polyelectrolyte chains, the intrinsic viscosity of chitosan samples in aqueous solutions depends upon the ionic strength of the medium: plots of intrinsic viscosity vs. the reciprocal of the square root of the intrinsic viscosity are linear, and the intrinsic viscosity decreases with increasing salt concentration in the system is due to the shielding effect of the counter-ions.

The chitosan molecule is rather stiff, less than DNA but more than polyacrylate; increasing acetylation leads to a more extended conformation and an even stiffer chain. It was confirmed that this is due to intra-residue hydrogen-bonding between the carbonyl oxygen of the *N*-acetyl group and H6 in the next unit. A persistence length of ca. 22 nm was found, which is lower than that of chitin (ca. 35 nm) and the existence of a cholesteric mesophase was demonstrated (Terbojevich *et al.*, 1991).

Hydrophobic derivatives of chitosan, containing a small number of hydrophobic side chains, can be obtained from long chain acyl chlorides or

anhydrides. A series of *N*-alkylchitosans (C3–C12) was obtained by reacting chitosan acetate in an ethanol–water mixture with the desired aldehyde and reducing the Schiff base with sodium cyanoborohydride (3 moles per mole of amine, 24 h). In aqueous solution, above a certain polymer concentration, intermolecular hydrophobic interactions lead to the formation of associations. As a consequence, these copolymers exhibit thickening properties equivalent to those observed for higher molecular weight homopolymers and play important roles as viscosity modifiers in a variety of waterborne technologies.

N-Alkylated chitosans were synthesised by Michael addition reaction of chitosan and hydroxyethylacryl. The ^{1}H NMR results indicated that the degree of substitution was from 0.18 to 1.2. The amorphous chitosan derivatives exhibited an excellent solubility in water. The thermal stability of the derivatives was lower than that of chitosan and hydroxyethylacryl-chitosan with degree of substitution higher than 1 decomposed at 226 ºC. In the presence of lysozyme, the initial degradation rate of the chitosan derivatives was dependent on the molecular weight. The antimicrobial activity of the chitosan derivatives was lower than that of chitosan (Ma *et al.*, 2008).

31.3.2 Gelation

Gel materials are utilised in a variety of technological applications and are currently investigated for advanced exploitations such as the formulation of 'intelligent gels' and the synthesis of 'molecularly imprinted polymers'. A typical simple example of gel formation was provided with chitosan tripolyphosphate and chitosan polyphosphate gel beads. pH-responsive swelling ability, drug-release characteristics, and morphology of the chitosan gel bead depend on polyelectrolyte complexation mechanism and molecular weight of the enzymatically hydrolysed chitosan (Mi *et al.*, 1999). The complexation mechanism of chitosan beads gelled in pentasodium tripolyphosphate or polyphosphoric acid solution was ionotropic crosslinking or interpolymer complex, respectively. The chitosan-polyphosphoric acid gel bead is a better polymer carrier for the sustained release of anticancer drugs in simulated intestinal and gastric juice media than the chitosan-tripolyphosphate gel beads.

Acylation

Chitosan can be efficiently *N*-acylated without concomitant hydroxyl modifications, using anhydrides (2–3-fold excess) in organic media; quantitative or near quantitative acylations are accomplished at room temperature within a few minutes. Further improvements in chitosan reaction rate are obtained using a number of pre-treatment methods. Mixed *N*- and *O*-acylated products are prepared under similar conditions, using 10-fold excess anhydride (Yalpani, 1988).

One of the simplest ways to prepare a chitin gel is to treat chitosan acetate salt solution with carbodiimide to restore acetamido groups. Thermally irreversible gels are obtained by *N*-acylation of chitosans: *N*-acetyl-, *N*-propionyl- and

N-butyryl chitosan gels are prepared using 10% aqueous acetic, propionic and butyric acid as solvents for treatment with appropriate acyl anhydride. Both *N*- and *O*-acylation are found, but the gelation also occurs by selective *N*-acylation in the presence of organic solvents, as methanol, formamide and ethylene glycol. The importance of many variables has been studied by determining their effects on the gelation, such as chitosan and acyl anhydride concentrations, temperature, molecular weight of acylating compound, and extent of *N*-acylation. Probably the gelation is due to the aggregation of chitosan chains through hydrophobic bonding.

Chitosan gels can also be prepared by using large organic counter-ions. The process involves mixing heated solutions of chitosan acetate and of the sodium salt of either 1-naphthol-4-sulphonic acid or 1-naphthylamine-4-sulphonic acid, the mixture gelling on cooling: the chitosan concentration required for gel formation is low, about 2–5 g/l, and similar to the concentrations used for gel formation with other polysaccharides such as the carrageenans.

Gel-like properties were found in *N*-carboxymethyl chitosan: this behaviour was explained in terms of association of ordered chains into a cohesive network, analogous to that in normal gels but with weaker interactions between associating chains, i.e., a weak gel (Lapasin *et al.*, 1996).

Chitosan gel beads could be prepared in amino acid solutions of about pH 9, despite the requirement for a pH above 12 for gelation in water (Kofuji *et al.*, 1999). This phenomenon was observed not only in amino acid solutions but also in solutions of compounds having amino groups. A solute concentration of more than 10% was required for preparation of gel beads at pH 9. Gelation of the chitosan beads required about 25–40 minutes depending on the species of amino acid.

pH-Sensitive hydrogels were synthesised (Qu *et al.*, 1999a,b) by grafting D,L-lactic acid onto the amino groups in chitosan without a catalyst; polyester substituents provide the basis for hydrophobic interactions that contribute to the formation of hydrogels. The swelling mechanisms in enzyme-free simulated gastric fluid (pH 2.2) or simulated intestinal fluid (pH 7.4) at 37 ºC were investigated. The crystallinity of chitosan gradually decreased after grafting, since the side chains substitute the $-NH_2$ groups of chitosan randomly along the chain and destroy the regularity of packing between chitosan chains.

Enzymatic reactions leading to gels

Stable and self-sustaining gels were obtained from tyrosine glucan (a modified chitosan synthesised by reaction of chitosan with 4-hydroxyphenylpyruvic acid) in the presence of tyrosinase that oxidises the phenol to quinone, thus starting crosslinking with residual free amino groups. Gels were also obtained with 3-hydroxybenzaldehyde, 4-hydroxybenzaldehyde and 3,4-dihydroxybenzaldehyde (Muzzarelli and Ilari, 1994; Muzzarelli *et al.*, 1994; Chen *et al.*, 2003).

As an extension of these works, a mushroom tyrosinase was observed to catalyse the oxidation of phenolic moieties of the synthetic polymer poly(4-hydroxystyrene) in water-methanol. Although oxidation was rapid, in the order

of minutes, only a small number of phenolic moieties (1–2%) underwent oxidation. Enzymatically oxidised poly(4-hydroxystyrene) was observed to undergo a subsequent non-enzymatic reaction with chitosan, based on UV spectra (Shao *et al.*, 1999; Kumar *et al.*, 1999).

Further progress (Edwards *et al.*, 1999a,b) led to internally skinned polysulphone capillary membranes coated with a viscous chitosan gel and useful as an immobilisation matrix for polyphenol oxidase. Bench-scale, single-capillary membrane bioreactors were then used to determine the influence of the chitosan coating on product removal after substrate conversion by immobilised polyphenol oxidase during the treatment of industrial phenolic effluents. The results indicate that greater efficiency was achieved in the removal of polyphenol oxidase-generated products by the chitosan membrane coating, as compared with chitosan flakes.

Dilute solutions of chitosan with 3,4-dihydroxyphenylethylamine (dopamine) become hydrogels within a short time by virtue of the tyrosinase-catalysed oxidation of dopamine to quinone: the viscosity of the chitosan solutions greatly increases as a result of the immediate reaction of quinone with the chitosan amino groups that confers water-resistant adhesive properties to the gel (Yamada *et al.*, 2008).

NaCl particles (porogen) and gamma-glycidoxypropyltrimethoxysilane (crosslinking agent) were used to make chitosan-silica porous hybrid membranes with optimal chitosan/crosslinker weight ratio 1. Macropores (ca. 10–202 μm) in the membrane matrix, and micropores (8–10 nm) within the macropores were observed. Histidine, glutamic acid, tyrosine, L-DOPA, and p-aminobenzoic acid were individually linked onto said material with the aid of genipin: the p-aminobenzoate chitosan membrane exhibited the best affinity adsorption of tyrosinase from a crude *Agaricus bisporus* solution (Chao, 2008). Hydrogels of chitosan crosslinked with genipin appear to be most biocompatible and safe as indicated in a recent review article (Muzzarelli, 2009c).

A biosensor was fabricated by immobilising tyrosinase on the surface of multiwalled carbon nanotubes–chitosan composite modified glassy carbon electrode. The composite film provided a biocompatible platform for the tyrosinase to retain the bioactivity and the nanotubes possessed excellent inherent conductivity to enhance the electron transfer rate. The biosensor showed high sensitivity (412 mA/M), broad linear response, low detection limit (5.0 nM) and good stability for determination of phenol. The biosensor was further applied to rapid detection of the coliforms, represented by *Escherichia coli* that could be detected as low as 10 colony-forming units (CFU) per ml (Cheng *et al.*, 2008).

Reactions with aldehydes

The Schiff reaction between chitosan and aldehydes and ketones gives the corresponding aldimines and ketimines, which can be converted to *N*-alkyl derivatives on hydrogenation with cyanoborohydride. A wide range of aliphatic and aromatic carbonyl compounds have been used, including formaldehyde, unsaturated alkyl aldehydes, aldehydo and keto acids, carbonyl-containing

carbohydrates and dialdehydes, such as glutaraldehyde. In particular, *N*-carboxymethyl chitosan was obtained in a water-soluble form by a proper selection of the reactant ratios, i.e., using equimolecular quantities of glyoxylic acid and amino groups (Muzzarelli, 1988).

Fully substituted *N*,*N*′-dicarboxymethylchitosan was obtained by the alkylation of chitosan at pH 8–8.5 and 90 ºC using a monochloroacetic acid : chitosan weight ratio of 4:1. The water-soluble derivative is suitable for applications involving chelation (An *et al.*, 2008). The reaction with 2-oxoglutaric acid under reducing conditions gave glutamate glucan. The attachment of various reducing mono-, oligo- and polysaccharides to chitosan, under mild conditions and in the presence of sodium cyanoborohydride, affords products with degree of substitution 0.54–0.97 in high yields (70–100%, depending on the residue length). Reductive alkylations were also performed with other types of saccharides, such as fructose, streptomycin sulfate and selectively oxidised beta-cyclodextrin (Yalpani, 1988).

Studies on intestinal permeation enhancers were also reviewed as they provide information on the mechanism of action and safety of polymers. The active polymers are classified into: polycations (chitosan and its quaternary ammonium derivatives, poly-L-arginine, aminated gelatin), polyanions [*N*-carboxymethyl chitosan, poly(acrylic acid)], and thiolated polymers (carboxymethyl cellulose-cysteine, polycarbophil-cysteine, chitosan-thiobutylamidine, chitosan-thioglycolic acid, chitosan-glutathione conjugates) (Di Colo *et al.*, 2008).

Glutaraldehyde is a very popular crosslinking agent for chitosan (Muzzarelli *et al.,* 1976). Chitosan networks were obtained by reaction with glutaraldehyde in lactic acid solution (pH 4–5). The rheology of the chitosan-glutaraldehyde gel system was studied by Arguelles-Monal *et al.* (1998). By reaction of chitosan with aldehydes, *N*-alkylidene or *N*-aryldene chitosan gels are produced; the extent of modification of the amino groups is about 80% and the minimum amount of aldehyde required for gel formation increases by increasing the aldehyde molecular weight.

In the search for biocompatible hydrogels based exclusively on polysaccharide chains, chitosan and the dialdehyde obtainable from scleroglucan by controlled periodate oxidation were linked together (Crescenzi *et al.*, 1995); the reaction takes place at pH 10 and the reduction of the resulting Schiff base is performed with $NaBH_3CN$. The swelling capacity of the hydrogel is remarkable, considering the highly hydrophilic character of both polysaccharides, and strongly depends on the pH of the bathing solutions. Likewise, β-cyclodextrin-modified chitosan was synthesised via the Schiff base reaction with 6-*O*-(4-formylphenyl)-β-cyclodextrin (Liu *et al.*, 2008), as well as a variety of chitosans containing aromatic groups (Sajomsang *et al.*, 2008); the use of glutaraldehyde in most diverse applications does not decline at all.

A semi-interpenetrating network was synthesised with poly(ethylene oxide) and chitosan, and crosslinked with glyoxal (Khalid *et al.*, 1999). Swelling studies were performed on the chitosan/poly(ethylene oxide) semi-

interpenetrating network and on the reference hydrogel (crosslinked chitosan) at pH 1.2 and 7.2. The semi-interpenetrating network displayed a high capacity to swell, adjustable by pH. Rheological studies performed in simple shearing and in oscillation showed that the semi-interpenetrating network had elastic properties. Poly(ethylene glycol) dialdehyde diethyl acetals of different molecular sizes were synthesised and used to generate *in situ* PEG dialdehydes for the crosslinking of partially reacetylated chitosan via Schiff reaction and hydrogenation of the aldimines. The water-soluble products obtained were instrumentally characterised. Upon freeze-drying, they aggregated to yield insoluble soft and spongy biomaterials that swelled immediately upon contact with water. When exposed to papain and lipase, at physiological pH values, progressive dissolution of the biomaterials was observed, but no dissolution took place with lysozyme, collagenase and amylase. They were found to be biocompatible (DalPozzo *et al.*, 2000). A continuation of this work was provided by Deng *et al.* (2007) who reported on the reaction with the counter-ion tripolyphosphate, and formation of nanoparticles.

31.3.3 Polyelectrolyte complex formation

At high degree of protonation of the amino groups, chitosan spontaneously forms macromolecular complexes by reaction with anionic polyelectrolytes. These complexes are generally water insoluble and make hydrogels. Several reviews on polyelectrolytes have been published (Tsuchida and Abe, 1982; Kubota and Kikuchi, 1999; VanTomme *et al.*, 2008). A variety of polyelectrolytes can be obtained by changing the chemical structure of component polymers, such as molecular weight, flexibility, functional group structure, charge density, hydrophilicity and hydrophobicity, stereoregularity, and compatibility, as well as changing reaction conditions, such as pH, ionic strength, polymer concentration, mixing ratio and temperature. This, therefore, may lead to diverse physical and chemical properties of the complexes.

Polyelectrolytes of chitosan with other polysaccharides, proteins, DNA and synthetic and inorganic polymers were investigated. A hydrogel with high sensitivity to the change in external pH was prepared between chitosan (DA = 0.18) and dextran sulfate (Sakiyama *et al.*, 1999): the maximum volume of the complex gel was observed in a dilute NaOH solution at pH 10.5, and was about 300 times as large as the volume at pH values below 9 (Jiang *et al.*, 1999).

Nanoparticle made of two bioadhesive polysaccharides, hyaluronan and chitosan, were obtained by a very mild ionotropic gelation technique. The nanoparticles had a size in the range of 100 to 235 nm and a zeta-potential of -30 to $+28$ mV. Toxicological studies were conducted in human corneal epithelial and conjunctival cell lines. The confocal images indicated that the nanoparticles were internalised by fluid endocytosis and that this endocytic process was mediated by the hyaluronan receptor CD44 (DeLaFuente *et al.*, 2008a,b).

Hyaluronan-chitosan nanoparticles made of low molecular weight chitosan (10–12 kDa) led to high levels of expression of secreted alkaline phosphatase in

the human corneal epithelium model. Following topical administration to rabbits, these nanoparticles entered the corneal and conjunctival epithelial cells and were assimilated by the cells. More importantly, the nanoparticles provided an efficient delivery of the associated plasmid DNA inside the cells, reaching significant transfection levels (DeLaFuente *et al.*, 2008a,b).

Hydrogels composed of poly(*N*-isopropylacrylamide) with chitosan (Fang *et al.*, 2008) or α,β-glycerophosphate with chitosan (Zhou *et al.*, 2008) can be mentioned as examples of temperature-sensitive hydrogels with transition at 37 ºC.

31.4 Chitin as a food component

Crustaceans, insects, rodents, frogs, snails, earthworms, spiders, scorpions, centipedes, and millipedes have been used as food sources for millennia. A close examination of the world insect consumption shows that more than 2,000 edible species have been utilised as a food source, although this is deemed to be under-reported and research will increase this number (Paoletti, 2005; Backwell and D'Errico, 2001; DeFoliart, 1992, 1999). Currently preferred insects are listed in Table 31.1. On the other hand, of the 15 million plants, animals and microbes on Earth, more than 90% of the world's food supply comes from just 15 crop species and 8 livestock species.

Chitin digestion requires chitinolytic enzymes: chitinases are present in plant foods, and human and bacterial chitinolytic enzymes occur in the human digestive apparatus. The bibliographic data confirm the presence of chitinolytic enzymes in plant organs, and thus vegetables and fruits that are consumed by humans could help in the digestion of chitin. Although the level of plant chitinases is often correlated to the presence of stress, such as infections, these enzymes also occur in healthy plant tissues and above all in fruits under maturation, which are most often used in human nutrition (Taira *et al.*, 2004; Truong *et al.*, 2003). For examaple, the phyla Leguminosae have lectins, chitinases and glycohydrolases as major defence proteins in their seeds. Electrophoresis of the proteins of tamarind (*Tamarindus indica* L.) indicated the presence of a 34 kDa class III endochitinase (Rao and Gowda, 2008).

Table 31.1 Consumption of insects as food in the world

Continent	Species	Most popular insects cooked and eaten
Africa	524	*Gonimbrasia belina* (grub)
Central and South America	679	*Rhynchophorus* spp (snout beetle) *Aegiale hesperiaris* (butterfly) *Atta* spp (ant)
Asia	349	*Locusta* spp *Bombyx mori* (silk worm)
Australia	152	*Rhychophorus ferrugineus papuanus*

Chitin digestion by humans has generally been questioned or denied. Only recently have chitinases been found in several human tissues and their role has been associated with defence against parasite infections and to some allergic conditions. The gastric juices of 25 Italian subjects were tested on the artificial substrates 4-methylumbelliferyl-β-D-*N*,*N*′-diacetylchitobiose and fluorescein isothiocyanate chitin to demonstrate the presence of a chitinase activity currently called acidic mammalian chitinase. It was found to be present in gastric juices of 20 out of 25 Italian patients in a range of activity from 0.21–36.27 nmol/ml.h^{-1}. In the remaining five gastric juices, the chitinase activity was almost absent. The allosamidine inhibition test and the measurement at different pH values confirmed that this activity was characteristic of a chitinase. The absence of activity in 20% of the gastric juices may be a consequence of virtual absence of chitinous food in the western diet (Paoletti *et al.*, 2007).

In the excreta of healthy people there is at least a bacterial species able to hydrolyse chitin to *N*-acetyl glucosamine. *Clostridium paraputrificum* in the human colon is able to synthesise and secrete chitinases and β-*N*-acetyl glucosaminidase, that could take part in the digestion of chitin. Similarly, chitotriosidase, a chitinase produced by activated macrophages is able to hydrolyse chitin (Malaguarnera, 2006; Piras *et al.*, 2007; Gianfrancesco and Musumeci, 2004; Renkema *et al.*, 1995, 1998).

An inherited deficiency in chitotriosidase activity is frequently reported in the plasma of Caucasian subjects, whereas this deficiency is rare in the African population. The study by Musumeci *et al.* (2005) compares chitotriosidase activity in the colostrum of 53 African women and 50 Caucasian women. Elevated chitotriosidase was found in the colostrum of African women on the first day after delivery (1230 ± 662 nmol/ml.h^{-1}) which decreased with time. The chitotriosidase activity on the first day after delivery in the colostrum of Caucasian women, however, was significantly lower (293 ± 74 nmol/ml.h^{-1}) and decreased to 25 ± 20 and 22 ± 19 nmol/ml.h^{-1} on the second and third day, respectively. The chitotriosidase activity in the plasma of African women was also higher (101 ± 80 nmol/ml.h^{-1}) than that of Caucasian women (46 ± 16 nmol/ml.h^{-1}), but no correlation was found between the plasma and colostrum activities. The elevated chitotriosidase activity in the colostrum of African women is indicative of the presence of activated macrophages in human milk, consistent with the genetic characteristics of the African population, and their chitin eating habits. In fact, a chitin-rich diet induces the secretion of human chitinases.

31.5 Nutritional and health effects

A number of European countries as well as the United States and some oriental countries (Japan, South Korea) have approved the sale of chitosan-based nutraceuticals as over-the-counter products for the control of obesity, hypercholesterolemia and hypertension. Chitosan, in this context, is generally

regarded as safe, being a partially deacetylated chitin, i.e. a polysaccharide widely present in nature, in particular in human food such as certain cheeses and marine animals including crustaceans and squids.

The chitosans used to prepare dietary supplements come, in fact, from crustaceans, mainly shrimps. The freshly caught crustaceans are peeled in the canning and freezing factories, and while the meat is canned or packed, the shells are collected and treated by chemical or microbiological means, in order to extract chitin. It has been verified that seasonal fluctuations and varieties of shrimps and squids caught during fishing activities introduce negligible differences in the chitosans resulting from the chitin extraction (Lavall *et al.*, 2007); novel technologies have been experimented with, but with limited success (Kjartansson *et al.*, 2006).

The exposure of chitin to acids, alkali, surfactants, solvents for the extraction of carotenoids, and the submission of the chitosan flakes to drying, milling, sieving and other operations, introduce more diversity among various lots of chitosan. Thermal drying, for instance, may introduce a limited degree of crosslinking while the degree of deacetylation of the final chitosan may depend on the grain size of the chitin powder exposed to hot alkali during the deacetylation process.

To date, there is no chitosan standard for any application. In other words, even though various grades are available such as technical, food and medical grades, no clear recommendation has been made and accepted for adopting a chitosan standard in food applications. As a consequence the chitosan currently used to manufacture chitosan tablets for human consumption are chosen mainly on the basis of their constant supply and economical convenience. In the eyes of today's producers, their characteristics are a secondary aspect and in general only the degree of acetylation, the viscosity and the microbiological contamination are certified. The producers do not declare the degree of crystallinity, the polydispersity of the molecular weight, the presence of amino acids and metals. Therefore it is difficult to predict the performances of the dietary supplements.

A further aspect of uncertainty is introduced by the preparation of the tablets: the tendency is to adopt the most usual tabletting process regardless of the consequences that certain excipients have on chitosan activity *in vivo*. For example, tabletting involves the use of a binder in order to hold together the poorly compressible chitosan powder. Magnesium stearate, a slightly soluble compound, once coated on the chitosan powder, deeply alters the capacity of chitosan to react or effectively contact other compounds. Site-specific controlled release systems have been extensively investigated during the last decade. Chitosan salts with succinic acid, adipic acid, and suberic acid were prepared by spray-drying and were coated with stearic acid by the same technique. In a study the carriers were characterised in terms of morphology, size, swelling, mucoadhesive properties and drug loading, and focused on the *in vitro* influence of chitosan salts on the release behaviour of vancomycin hydrochloride from the uncoated and coated systems at pH levels of 2.0, 5.5 and 7.6 (Bigucci *et al.*,

2008). The results of the study supports the view that chitosan tablets containing stearate salts might lack full anti-hypercholesterolhaemic activity simply because the stearate excipient prevents hydrogel formation in the stomach and effective contact with bile salts.

31.5.1 Cholesterol lowering in humans

Chitosan was first shown to reduce serum cholesterol in humans in 1993, when adult males fed chitosan-containing meals for two weeks (3 g/d for week 1, 6 g/d for week 2) experienced 6% decrease in total cholesterol (Maezaki *et al.*, 1993). The subjects also exhibited a 10% increase in high density lipoprotein cholesterol. Two studies have reported serum cholesterol reductions with chitosan treatment. Obese women consuming 1.2 g of microcrystalline chitosan for 8 weeks demonstrated significant reductions of low density lipoprotein, although not total serum cholesterol. A total of 84 female subjects with mild to moderate hypercholesterolemia receiving 1.2 g of chitosan per day experienced a significant decrease in total serum cholesterol (Bokura and Kobayashi, 2003).

Metso *et al.* (2003) observed 83 middle-aged men and women without severe disease and with a total cholesterol of 4.8–6.8 mmol/l and triglycerides below 3.0 mmol/l. Their conclusion was that treatment with microcrystalline chitosan had no effect on the concentrations of plasma lipids or glucose in healthy middle-aged men and women with moderately increased plasma cholesterol concentrations.

For oral administration to humans, chitosan is generally recognised as safe (Harrison, 2002; Kumar *et al.*, 2004). Some 21 overweight normo-cholesterolemic subjects were fed a supplement containing equal amounts of glucomannan and chitosan for 28 days: the observed serum cholesterol reduction was mediated by increased faecal steroid excretion and was not linked to fat excretion. Greater faecal excretion of neutral sterols and bile salts was observed. The topic has been reviewed by Muzzarelli and Muzzarelli (2006) and by Pittler and Ernst (2004).

In Sprague–Dawley rats, chitosan did not affect food intake but decreased body weight gain and significantly increased faecal fat and cholesterol excretion, reduced the lipid level in plasma and liver, increased liver hepatic and lipoprotein lipase activities (Zhang *et al.*, 2008).

To understand the mechanism of cholesterol lowering by chitosan, one should consider first that bile salts are formed from cholesterol in the liver and are secreted into the duodenum by the enterohepatic circulation: the bile salt pool is maintained stable, the newly ingested cholesterol compensating the excreted quantities. If, however, bile salts are sequestered by any suitable compound, some cholesterol is oxidised to produce more bile salts. Bile is produced at the rate of 700–1200 ml/day, bile salts accounting for 1.24–1.72%, cholesterol for 0.86–1.76 g/l; the average pH is 7.3. For the various intestinal tracts, the pH values are as follows: duodenum 4.7–6.5, upper jejunum 6.2–6.7, lower jejunum 6.2–7.3, ileum 6.1–7.3, colon > 7.3. It is worth noting that these

values tend to keep the bile salts in solution, while depressing the chitosan solubility (chitosan $pK = 6.3$).

The uptake of bile salts into chitosan-alginate gel beads was observed by Murata *et al.* (1999): the presence of weak acids (orotic, citric, folic and ascorbic) does not hinder the uptake; rather, chitosan orotate salt was found to enhance it. Various chitosans and heavily modified Chitopearl® chitosans were studied by Murata *et al.* (2003) and found to have capacities in the range 0.53–1.20 mmol taurocholate per gram of chitosan (various degrees of acetylation) and 0.2–0.9 mmol taurocholate for Chitopearl® products; the capacity of Questran® was ca. 1.0 mmol taurocholate/g. Higher capacities were observed for taurodeoxycholate (1.8 mmol taurodeoxycholate/g chitosan). Of course, these capacities depend on the initial bile salt concentration, grain size and other parameters. One millimole of taurocholate corresponds to 515 mg, thus the weight ratio taurocholate/chitosan orotate is 0.515 : 1.000, which appears to be a high ratio, notwithstanding the uncertainties related to the description of the experimental conditions.

By using chitosan cinnamate and analogue compounds such as those of vanillic acid, hydroxycinnamic acids, cumaric acids and rosmarinic acid, the bile acid adsorption and the release of cinnamate were investigated *in vitro*. When chitosan cinnamate was soaked in a taurocholate solution, it adsorbed the bile acid while simultaneously releasing cinnamate. The amount of cinnamate analogue released was 0.286 ± 0.001 mmol/g, practically coincident with the amount of taurocholate adsorbed. The amount of released cinnamate analogue was altered extensively by the species of analogue used for gel-bead preparation. Results were indicative of high affinity of taurocholate for chitosan, and suggest that those compounds may be of interest for complementary medicine to prevent lifestyle-related diseases (Murata *et al.*, 2006).

Eleven chitosan samples were evaluated for their fat- and bile acid-binding capacities, physico-chemical properties, and the correlations between each binding capacity and individual physico-chemical properties. The bile acid- and fat-binding capacities were estimated using *in vitro* assays, whereas the measured physico-chemical properties were deacetylation degree, swelling capacity, and solution viscosity. Chitosan samples might differ in their binding capacities against fat and/or individual bile acids. The bile acid-binding capacities were 0.20–0.61, 0.43–1.63 and 0.61–1.61 micromol/g chitosan for cholic, deoxycholic, and chenodeoxycholic acids, respectively. Stronger binding capacity of chitosan against a selected bile acid did not imply greater binding capacity for other bile acids (Zhou *et al.*, 2006).

Thongngam and McClements (2005 a,b) provided thermodynamic data by isothermal titration calorimetry on the binding of Na taurocholate to chitosan. At 30 ºC, Na taurocholate binds strongly to chitosan to form an insoluble complex containing ca. 4 mmol Na taurocholate/g chitosan at saturation, that means taurocholate : chitosan molar ratio ca. 2 : 3 and weight ratio ca. 2 : 1. Ionic strength had scarce influence; the enthalpy changes went from endothermic (at 10 ºC) to exothermic (at 40 ºC) indicating the importance of changes of

hydrophobic interactions leading to the formation of micelle-like clusters within the chitosan structure. The binding capacities of sodium glycocholate to chitosan, diethylaminoethyl chitosan, quaternised diethylaminoethyl chitosan, and cholestyramine were 1.42, 3.12, 4.06 and 2.78 mmol/g, respectively. The capacity of dialkylaminoalkyl chitosans increased with the number of carbons in the alkyl groups, indicating that hydrophobic interaction plays a major role in the sequestration of bile acids (Lee *et al.*, 1999); similarly, the capacity of 6-oxychitosan for cholic acid decreases with increasing degree of oxidation, i.e., loss of cationicity (Yoo *et al.*, 2005).

Among the chitosan salts that instantly form from chitosan and bile acids *in vitro*, chitosan taurocholate appears to possess infrared spectral characteristics that qualify it as a real ionic, water-insoluble, poorly crystalline, hydrophobic chitosan salt. In chitosan taurocholate and homologues, chitosan accounts for no more than one half by weight, therefore the hydrophobic nature prevails when contacting lipids. Butter and oils are collected to high extents with no discrimination of their components, including cholesterol and tocopherols. For these chitosan salts the lipid uptake is much higher than for plain chitosan. The salts studied in the present work are scarcely hydrolysed by a variety of hydrolases, and it is presumed that their complexes with lipids would be even more resistant to enzymatic attack.

If the above findings are extrapolated from the *in vitro* model to the physiological environment, it seems reasonable to speculate the following:

- Chitosan glycocholate and chitosan taurocholate insoluble salts subtract bile salts from the circulation thus forcing the organism to replete the bile pool at the expenses of cholesterol.
- The activity of lipases on triglycerides is depressed as a consequence of the poor emulsification of lipids due to the lowered availability of taurocholate, the emulsifier. It is known that the pancreatic lipases require a certain dimension of the oil droplets in the emulsion in order to hydrolyse triglycerides: now, when the bile salts become scarce, inadequate emulsions are formed and then limited hydrolysis of triglycerides takes place. Ample information on digestive lipases supports these views (Mukherjee, 2003) that have been experimentally confirmed (Helgason *et al.*, 2008). Lipases work thanks to the presence of bile salts that in one case activate the bile salts-dependent lipases, and in the other case provide the emulsion necessary to the pancreatic lipases for enzymatic activity. Moreover, as soon as the bile salt availability decreases due to chitosan ingestion, the bile salts-dependent lipases are poorly activated, and assimilation of lipids by the organism decreases sharply.
- While pectinase is one of the most representative bacterial enzymes in the intestine, the resistance of the chitosan taurocholate, glycocholate and taurodeoxycholate salts to hydrolysis by this enzyme, as well as by other hydrolases, would certainly be an indication of the capacity of these hydrophobic salts to be excreted, presumably with accompanying adsorbed lipids.

Table 31.2 Time (min) necessary for total digestion of freeze-dried chitosan salts suspended in aqueous solutions of animal, fungal and plant enzymes at 20 ºC and optimum pH

Enzyme	CTC		CGC		CTDC	
	pH	min	pH	min	pH	min
Cellulase, *Trichoderma reesei*	6.8	60	6.6	90	6.5	no
Alpha-amylase, barley malt	7.1	1440	7.0	1440	4.5	no
Alpha-amylase, porcine pancreas	6.8	no	6.8	no	7.0	no
Pectinase, *Aspergillus niger*	7.1	no	6.9	no	6.4	no
Lysozyme, hen egg white	7.3	30	7.0	4.5	7.0	no
Lipase, wheat germ	6.7	no	6.6	no	6.7	no
Lipase, porcine pancreas	7.3	no	7.0	no	6.9	no

CTC = chitosan taurocholate; CGC = chitosan glycocholate; CTDC = chitosan taurodeoxycholate.
No = No appreciable digestion after 48 hours.
Source: Muzzarelli *et al.* (2006).

Upon mixing a solution of Na taurocholate with a solution of chitosan acetate, both at pH values close to 4, immediate precipitation of chitosan taurocholate salt is observed. The salt obtained with nearly stoichiometric amounts of both reagents was characterised by infrared spectrometry, and found to exhibit new bands indicative of its ionic nature. Similar compounds were obtained from Na taurodeoxycholate and Na glycocholate. When exposed to a number of hydrolases at pH values close to neutrality and 20 and 37 ºC, they were found to be poorly susceptible to enzymatic degradation: only *Trichoderma reesei* cellulase, egg white lysozyme and barley malt α-amylase were effective on chitosan taurocholate and glycocholate, as shown in Table 31.2. The observed capacities of the freeze-dried salts for olive oil were 22 g oil/g of chitosan taurocholate, 60 g oil/g of chitosan glycocholate, and 27 g oil/g of chitosan taurodeoxycholate. The capacities were 22.1 g butter oil/g of chitosan taurocholate and 22.1 g of corn oil/g chitosan taurocholate. These data, substantially much higher than similar data published for plain chitosan and various oils, mean that the lipid uptake takes place mainly by hydrophobic interactions with the insoluble salts formed by chitosan upon contact with bile (Muzzarelli *et al.*, 2006).

31.5.2 Overweight control

Fourteen published trials including a total of 1,131 participants met the inclusion criteria in a study by NiMhurchu *et al.* (2005a,b) published in review format by Jull *et al.* (2008). Analyses including all trials indicated that chitosan preparations result in a significantly greater weight loss (weighted mean difference −1.7 kg; 95% confidence interval (c.i.) −2.1 to −1.3 kg), decrease in total cholesterol (−0.2 mmol/L; 95% c.i. −0.3 to −0.1), decrease in systolic (−5.9 mmHg; 95% c.i. -7.3 to -4.6) and diastolic (−3.4 mmHg; 95% c.i. −4.4 to

−2.4) blood pressure compared with placebo. If recalculated in terms of mg/dl, the loss of cholesterol is 3-4%, i.e., clinically significant. It was remarked that the quality of certain studies was sub-optimal. The conclusions were that evidence exists that chitosan is more effective than placebo in the short-term treatment of overweight and obesity.

NiMhurchu *et al.* (2004) also conducted a 24-week randomised, double-blind, placebo-controlled trial, with a total of 250 participants (82% women; mean (s.d.) body mass index, 35.5 (5.1) kg/m^2; mean age, 48 (12) y). The chitosan group lost more body weight than the placebo group (mean (s.e.), −0.4 (0.2) kg (0.4% loss) vs +0.2 (0.2) kg (0.2% gain), $P = 0.03$) during the 24-week intervention.

Studies of the effect of chitosan on human adiposity suggest that results may differ depending on whether the subjects are eating *ad libitum* or are on a weight loss diet. Overweight subjects consuming 2.4 g/day of chitosan for 28 days, also consuming their normal diet, showed no change in body weight during the trial (Gallaher *et al.*, 2002).

A six-week study was conducted on obese adults who lost more body weight (-2.3 vs 0.0 kg), body fat (-1.1 vs 0.2%) and absolute fat mass (-2.0 vs 0.2 kg). The combination of glucomannan, chitosan, fenugreek *Gymnema sylvestre* and vitamin C were therefore effective (Woodgate and Conquer, 2003). Gades and Stern (2005) tested the fat-trapping capacity of a chitosan product in 12 men and 12 women. Faecal fat excretion increased with chitosan by 1.8 ± 2.4 g/day in males, but did not increase with chitosan in females: therefore fat-trapping claims should not be generalised.

Chitosan greatly increases faecal fat excretion when consumed in sufficient amounts and can accelerate weight loss when subjects follow a low calorie diet, but will be ineffective in those consuming their habitual diets.

31.6 Food industry applications

Chitosan, whose applications in the food industry have been reviewed by Rudrapatnam and Farooqahmed (2003) exhibits antimicrobial activity against a range of food-borne micro-organisms and consequently has attracted attention as a potential natural food preservative (Chen *et al.*, 1998; El Ghaouth *et al.*, 1992; Shahidi *et al.*, 1999). Binding of trace metals and effect on membrane permeability have been postulated to be the main mechanisms for its antibacterial action (Helander, 2001; Zheng and Zhu, 2003). Muscle foods have low oxidative stability and are very susceptible to rancidity during production and storage. Numerous studies have indicated that lipid oxidation in meat and meat products may be controlled or minimised through the use of antioxidants (Gray *et al.*, 1996; Nissen *et al.*, 2004; Rhee, 1987). However, chitosan has a bitter taste, which may limit the use of artificial antioxidants in meat and meat products, when flavour is an important consideration.

The antimicrobial and antioxidant potential of spices and herbs, such as basil, thyme, rosemary, garlic, clove, coriander, ginger, mustard and pepper are well

known (Georgantelis *et al.*, 2007a,b; Sebranek *et al.*, 2005; Tipsrisukond *et al.*, 1998). Mint (*Mentha spicata*) is used extensively (Choudhury *et al.*, 2006). The mint extract has very good antioxidant potential, comparable to that of the synthetic antioxidant butylated hydroxy toluene (Kanatt *et al.*, 2007, 2008). Mint extract did not show any antibacterial activity, though essential oils of some *Mentha* species have been reported to have antibacterial activity (Marino *et al.*, 2001; Moreira *et al.*, 2005). Mixed mint and chitosan efficiently scavenged superoxide and hydroxyl radicals and was particularly effective against Gram-positive bacteria. The shelf-life of pork cocktail salami, as determined by total bacterial count and oxidative rancidity, was enhanced in mint + chitosan-treated samples stored at 0–3 ºC. Chitosan films prepared with oregano essential oil were applied on bologna slices. Release of the essential oil compounds during film preparation and application on the meat product and consumer acceptability of bologna enriched with oregano essential oil were found satisfactory (Chi *et al.*, 2007). Antimicrobial and physico-chemical properties of chitosan films and chitosan films enriched with essential oils were determined *in vitro* and on processed meat. Antimicrobial effects of pure essential oils of anise, basil, coriander and oregano, and of chitosan-essential oil films against *Listeria monocytogenes* and *Escherichia coli* were investigated. The films have the potential to be used as active biodegradable films with strong antimicrobial effects.

Edible films can provide supplementary and sometimes essential means of controlling physiological, morphological and physico-chemical changes in food products. High density polyethylene film, a common packaging material used to protect foods, has disadvantages like fermentation due to the depletion of oxygen and condensation of water, which promotes fungal growth. Due to their filmogenicity, chitin and chitosan are satisfactorily used as food wraps. Semi-permeable chitosan films modify the internal atmosphere, decrease the transpiration and delay the ripening of fruits. For the preparation of chitosan/pectin laminated films and chitosan/methylcellulose films several approaches have been used, including simple coacervation. Chitosan films are tough, flexible and tear-resistant; moreover, they have favourable permeation characteristics for gases and water vapour.

Chitosan is also suitable as a texturising agent for perishable foods: for instance, high viscosity chitosan solutions were used to prepare tofu, a widely consumed oriental food, for which the organoleptic properties did not vary appreciably while shelf-life was extended (Kim and Han, 2002; No *et al.*, 2002).

Processing of clarified fruit juices commonly involves the use of clarifying agents, including gelatin, bentonite, tannins, potassium caseinate and polyvinyl pyrrolidone. Chitosan is a dehazing agent used to control acidity in fruit juices, as well as being a good clarifying agent for grapefruit juice with or without pectinase treatment, besides apple, lemon and orange juices, and a fining agent for apple juice, which can afford zero turbidity products with as little as 0.8 kg/m^3 of chitosan. No impact on the biochemical parameters of the juices was found (Chatterjee *et al.*, 2004). Apple juice can be protected from fungal spoilage with the aid of modest additions of chitosan glutamate (Roller and Covill, 1999).

Chitosan has a good affinity for polyphenolic compounds such as catechins, proanthocyanidins, cinnamic acid and their derivatives that can change the colour of white wines due to their oxidative products. By adding chitosan to grapefruit juice (15 g/l), the total acid content (citric, tartaric, malic, oxalic and ascorbic acid) was sharply reduced.

Jang and Lee (2008) demonstrated the stability of tripolyphosphate-chitosan nanoparticles for ascorbic acid during heat processing and suggested the use of ascorbate-loaded chitosan nanoparticles to enhance antioxidant effects because of the continuous release of ascorbate from the nanoparticles in food processing.

31.7 Applications in drug delivery

The usefulness and versatility of chitosan hydrogels is particularly evident when the field of drug delivery is considered. Plain chitosan, a safe biopolymer, has been extensively applied to the human body by all possible means and for a variety of purposes: chitosan is a functional ingredient of cosmetics, a biocompatible coating for prosthetic materials, a drug delivery item, a gene vector, a dietary supplement, and a wound healing dressing capable of restoring the native histoarchitecture.

In a large group of materials used to enhance absorption of drugs, chitosan ranked first in terms of maximum drug bioavailability (enhanced absorption) and minimum damage to the membranes (Illum and Davies, 2005). Of course, the limitations of plain chitosan, mainly its scarce solubility at neutral and alkaline pH values, can be overcome by selecting adequate chemical or enzymatic modifications free from adverse effects on the living tissues: for example, the derivatisation of chitosan with the aid of glyoxylic acid (the simplest aldehydoacid) leads to *N*-carboxymethyl chitosan that can be interpreted as a glycine derivative as well. Because glycine is one of the most abundant amino acids in the human body, in collagen for instance, *N*-carboxymethyl chitosan is safe in principle, as confirmed experimentally; indeed it is a functional ingredient of hydrating creams present on a niche market for more than a decade. Pyruvic acid exemplifies the ketoacids from which aminoacid glucans can be derived via ketimine formation with chitosan. Such modified chitosans were developed in the 1980s (Muzzarelli *et al.*, 1985; Muzzarelli and Zattoni, 1986) and have found applications in various areas (Sun *et al.*, 2007). The safety of carboxymethylated chitosans has been demonstrated by Janvikul *et al.* (2007).

According to Sun and Wan (2007), to qualify as a drug delivery aid and absorption enhancer, a modified chitosan should exhibit hydrogel characteristics, particularly because it should remain in contact with the mucosae long enough to achieve maximum effect, and any consequence on the mucosae should be completely reversible. Other requirements are also satisfied by chitosan hydrogels, because they are generally compatible with drugs, have no odour, are non-irritating, non-toxic and non-allergenic. From the pharmaco-

logical standpoint, chitosans are inert at the concentrations required in practical use: they exhibit mild anti-inflammatory action.

In fact, when chitosans contact the mucosal tissue a reaction takes place between the chitosan's glucosamine units and the sialic acid units of the mucin and epithelial cell membrane (Deacon *et al.*, 1999). Therefore a decreased clearance of administered formulations and an increased absorption are observed. In a sheep model, chitosan was far superior to diethylaminoethyl (DEAE) dextran in terms of absorption enhancing effect. A reason for the difference between the two polymers was that the DEAE dextran is randomly and scarcely substituted, is a branched polysaccharide as opposed to linear chitosan that carries one primary amino group on each anhydroglucose ring. Some of the nitrogen atoms are hidden in the DEAE dextran network and are therefore unavailable for interaction with the mucosae (Harding *et al.*, 1999). Experimental studies have led to the development of gastroretentive bioadhesive microsphere formulations and to devices for nasal delivery as well as for various mucosal routes. For example, chitosan-tripolyphosphate and chitosan–tripolyphosphate-chondroitin sulfate core-shell type microspheres were prepared by a continuous method using a multi-loop reactor under sterile conditions. All the types of microspheres produced were spherical in shape and had a porous structure; their mechanical resistance increased in the presence of chondroitin sulfate, which toughened the microsphere shell structure. For a drug release application, the preparation included the dissolution of ofloxacin, an antibiotic, in the chitosan solution before complex formation (Vodna *et al.*, 2007).

The study of the bioadhesive characteristics of chitosan led to the important discovery that this polysaccharide is not only bioadhesive so that it can be used to alter the intestinal transit of formulations and nasociliary clearance, but also has exceptional absorption effects when given nasally to rat and sheep models together with highly polar molecules such as insulin, calcitonin and morphine.

The impressive increase in the bioavailability of the drugs administered together with chitosan could not be explained simply by a prolonged period of residence: it is now generally accepted that the mechanism of action of chitosan in enhancing the transport of drugs across the mucosal membranes is due to the combination of bioadhesion and the transient opening of the tight junctions between the cells of the mucosal membrane (Illum, 2003). Chitosan gels releasing insulin in response to glucose concentration have also been developed (Kashyap *et al.*, 2007).

For these purposes, chitosans can be formulated either as viscous solutions, as spray-dried powders, or as microspheres or nanoparticles. In most cases the effects of chitosan powders and microspheres are superior in providing enhancement of the nasal absorption of polar drugs as compared to chitosan solutions (nearly fivefold bioavailability of insulin, for instance). This may be justified by the formation of more effective hydrogels by spray-dried chitosans (see below).

As a polar compound, morphine is not readily absorbed via the nose when administered in simple formulations (bioavailability in humans ca. 10%). A nasal chitosan morphine solution formulation can give rapid absorption of the

drug with the peak plasma concentration within 10 min and 60% bioavailability that can be optimised at 80%, offering patients rapid and efficient pain relief by a non-injectable route (Illum *et al.*, 2002).

Chitosan microparticles produced by emulsification, precipitation, complex coacervation or solvent evaporation are optionally stabilised by crosslinking or by polyelectrolyte complex formation. The drug is either encapsulated during production or absorbed into the particles after production. The freeze-drying techniques are generally preferred (Kas, 1997). For the production of chitosan nanoparticles, one may rely on the ability of chitosan to gel in contact with the negatively charged counterparts, for example tripolyphosphate ions. The definition of the experimental conditions for the formation of the chitosan-tripolyphosphate nanoparticles has been made by Pan *et al.* (2002).

31.7.1 Trans-dermal drug delivery

The use of chitosan has great potential as an aid to trans-dermal drug delivery and has been shown to mediate trans-dermal drug availability. The reported widening of tight junctions by chitosan observed in Caco-2 cells *in vitro* may be partially responsible for the ability of chitosan to improve trans-dermal drug delivery.

Tight junctions are dynamically regulated protein complexes that link adjacent epithelial cells in such a manner as to prevent the passage of molecules through the paracellular space. They are composed of known trans-membrane proteins (occludin and claudin) and several associated intracellular proteins (Tsukita *et al.*, 2001). Tight junction integrity can be investigated in numerous ways including measuring the trans-epithelial electrical resistance across confluent Caco-2 monolayers.

The quaternary derivatives of chitosan have been shown to have penetration enhancement properties because they open the tight junctions of the intestinal epithelia at neutral and alkaline pH values. The use of nanoparticulate systems has the advantage of protecting the peptidic drugs from the harsh environment of the gastrointestinal tract. Trimethyl chitosan and diethylmethyl chitosan, both with quaternisation degree of ca. 50%, were then used to prepare insulin nanoparticles with two different methods: ionotropic gelation and polyelectrolyte complexation. The latter nanoparticles had higher insulin loading efficiency and zeta potential than those made by ionotropic gelation. Trimethyl chitosan was also loaded with antigens for intranasal vaccination (Amidi *et al.*, 2007). *In vivo* studies performed with trimethyl chitosans in rabbits confirmed the trans-dermal permeation enhancement, which increased with increasing degree of quaternisation (He *et al.*, 2008). The polymers in free form had higher antibacterial activity against Gram-positive bacteria than in the nanoparticulate form (Sadeghi *et al.*, 2008).

Previous studies have shown chitosan-mediated decreases in electrical resistance of up to 80% across Caco-2 monolayers. This is accompanied by an increase in the permeability of the monolayers to inert hydrophilic protein

markers such as inulin or mannitol (Borchard *et al.*, 1996; Dodane *et al.,* 1999; Kotze *et al.*, 1998; Ranaldi *et al.*, 2002; DeBritto and Assis, 2007). A chitosan-mediated increase in permeability to inert markers points to passive transport that is possible only through the paracellular space (Artursson *et al.*, 1996). As the chitosan-mediated changes in resistance do not appear to be a result of cell toxicity, it is supposed to act through an effect on the barrier properties of the cellular tight junctions. Also quaternised chitosan has been studied extensively as an absorption enhancer and proved to be non-toxic, mucoadhesive and capable of opening the tight junctions between epithelial cells.

Changing the chitosan concentration or increasing the number of crosslinks by using chitosan derivatives with branching side chains can modify the rate of release of drug from these formulations. Once applied, the chitosan present in this formulation mediates prolonged contact with the epithelium via electrostatic interaction of the positively charged chitosan and the negatively charged glycoprotein residues on the cell surface. Passive diffusion of the drug down its concentration gradient onto the underlying epithelium over a prolonged period results in absorption of drug (Park *et al.*, 2000; Ramanathan and Block, 2001).

There are several advantages of trans-dermal drug delivery as opposed to oral delivery. For example, trans-dermally delivered drugs avoid the hostile environment of the gastrointestinal tract (hydrochloric acid and enzymes), and avoid first pass metabolism (the skin empties into the venous circulation and the drug is not routed directly through the liver where much drug metabolism occurs as with orally applied drugs). In addition, drugs delivered by the trans-dermal route show sustained plasma profiles over long periods of time, thus minimising the risk of fluctuations of drug plasma levels at night or between oral doses. Other attractive features lead to better patient compliance. The most popular and commercially available trans-dermal drug delivery system is the nicotine patch for nicotine replacement during smoking cessation.

Chitosan gel-mediated trans-dermal drug permeation may be enhanced by the simultaneous application of other technologies known to aid trans-dermal drug delivery. The application of an electric current to aid the passage of drugs across the epithelium, along with dermal application of chitosan-drug hydrogels, resulted in a greater drug flux across the skin barrier compared to either method individually. This represents an enormous increase in the amount of permeated drug across the usually impermeable skin.

31.7.2 Spray-drying

As a drug carrier, chitosan offers certain advantages over other polymers, such as cationicity, bioadhesion via interaction with mucin, degradability by lysozyme and lipases, generation of safe oligomers and monomers (glucosamine and *N*-acetylglucosamine), absence of allergic reactions, and immuno-stimulating activity. Chitosan helps overcome insolubility and hydrophobicity of drugs, but the semi-crystalline powder does not lend itself to direct compression; however, amorphous chitosan microspheres obtained by spray-drying

are free-flowing and compressible powders, most suitable for drug delivery (He *et al.*, 1999; Rege *et al.*, 1999, 2003; DeLaTorre *et al.*, 2003; Huang *et al.*, 2003; Muzzarelli *et al.*, 2004). Spray-drying of chitosan salt solutions provides chitosan microspheres having diameters close to 2–5 micron and improved binding functionality. While chitosan was often spray-dried, the following are significant examples.

Betamethasone disodium phosphate-loaded microspheres demonstrated good drug stability (less 1% hydrolysis product), high entrapped efficiency (95%) and positive surface charge (37.5 mV). Formulation factors were correlated to particulate characteristics for optimising the pulmonary delivery. The betamethasone release rates were influenced by the drug/polymer ratio in the manner that an increase in the release percentage and burst release percentage was observed when the drug loading was decreased. The *in vitro* release of betamethasone showed a dose-dependent burst followed by a slower release phase that was proportional to the drug concentration in the range 14–44% w/w (Huang *et al.*, 2002).

Chitosan microspheres containing chlorhexidine diacetate, an antiseptic, were prepared by spray-drying. Chlorhexidine from the chitosan microspheres dissolved more quickly *in vitro* than chlorhexidine powder. The minimum inhibitory concentration, minimum bacterial concentration and killing time showed that the loading of chlorhexidine into chitosan could maintain or improve the anti-microbial activity of the drug, the improvement being particularly high against *Candida albicans*. It should be noted that the drug did not decompose despite its thermal lability above 70 ºC.

An inclusion complex composed of progesterone and hydroxypropyl-β-cyclodextrin was prepared by spray-drying and freeze-drying methods. Progesterone alone and its inclusion complex with hydroxypropyl-β-cyclodextrin were incorporated into chitosan by spray-drying and freeze-drying. Release data showed significant improvement of the dissolution rate of progesterone and controlled release was obtained in the presence of chitosan (Cerchiara *et al.*, 2003).

31.7.3 Pulmonary drug delivery

Chitosan is able to enhance the surface activity of 0.5 mg/ml of bovine lipid extract surfactant and to resist albumin-induced inactivation at an extremely low concentration of 0.05 mg/ml, one thousand times smaller than the usual concentration of poly(etylene glycol) and 20 times smaller than for hyaluronan (Zuo *et al.*, 2006).

Learoyd *et al.* (2008) described the preparation of highly dispersible dry powders for pulmonary delivery that display sustained drug release characteristics; they were prepared by spray-drying 30% aqueous ethanol formulations containing terbutaline sulfate as a model drug, chitosan as a drug release modifier, and leucine as an aerosolisation enhancer. The aerosol properties of the spray-dried powders were investigated. By selecting the average molecular

weight of chitosan it was possible to exert some control over the rate of drug release: upon increasing the molecular weight of chitosan a more sustained release profile was obtained. For example, the low molecular weight chitosan released the totality of terbutaline after 30 min, whereas the high molecular weight chitosan required 2 h.

Of course, the *in vitro* dissolution tests of this nature do not take into consideration the environment that would be encountered following inhalation, such as the relatively low quantity of lung secretions, the large surface area, the lungs' clearing mechanism such as the mucociliary escalator and the mucus layer and the presence of lung surfactant. The powders are expected to deposit predominantly in the central and peripheral regions of the lung following inhalation, with minimal oropharyngeal concurrent deposition. By virtue of their hydrogel behaviour in the lung, these powders would display delayed rather than instantaneous drug release thus offering the opportunity to reduce dosing frequency.

Ventura *et al.* (2008) incorporated moxifloxacin, a wide spectrum antimicrobial active against common respiratory pathogens, in spray-dried chitosan microspheres, and observed that its intrapulmonary concentration was superior to that generated in plasma. By this means, they could overcome the common adverse events associated with the oral administration of this drug such as nausea and diarrhoea due to the impact on the human intestinal microflora. Uncrosslinked microspheres rapidly swelled in the lungs and released some chitosan that altered the biomembrane permeability to the drug. Glutaraldehyde-crosslinked microspheres, on the other hand, did not exhibit this property. Therefore the microspheres retarded the absorption of moxifloxacin, and within 6 h the cumulative amount of permeated drug was ca. 18, 11 and 7% w/w for free drug, loaded crosslinked microspheres, and loaded uncrosslinked microspheres, respectively.

Microspheres of methylpyrrolidinone chitosan, a modified chitosan amply recognised as one of the least cytotoxic materials, was found suitable for the intranasal delivery of metoclopramide HCl (Giunchedi *et al.*, 2002). In their further study (Gavini *et al.*, 2008) attention was devoted to the hydrogel formation from methylpyrrolidinone chitosan. The microspheres obtained by spray-drying were contacted with buffer solutions: when HCl/KCl or acetate buffers are used, gel forms and then dissolves upon dilution with water. However, when phosphate buffers are used, a hydrogel forms which does not dissolve in water regardless of the amount used, but dissolves in diluted HCl solutions. Hydrogel formation is therefore dependent on the medium, but not on the presence of a drug. The phosphate anion at pH values 5.5–7.4 seems to bridge the chitosan chains leading to the formation of an ionically interconnected hydrogel. Because methylpyrrolidinone chitosan microspheres retain adhesion properties and lend themselves to incorporation of a variety of drugs, it appears that they are most suitable for biomedical applications involving drug delivery.

31.7.4 Drug delivery to the intestine

Neutron activation-based gamma scintigraphy was used to evaluate the gastro-retentive properties of formulations containing chitosan (M_w = 150 kDa) in humans. At the same time, the transit of the formulations (40 or 95% of chitosan) was monitored in the small intestine: although the chitosan studied had exhibited marked mucoadhesive capacities *in vitro*, retention of the chitosan formulations in the upper gastrointestinal tract was not sufficiently reproducible and the duration of retention was relatively short (Sakkinen *et al.*, 2006). Therefore, chitosan tablets should be protected with an enteric coating or similar, if they are expected to reach the lower intestine without damage.

In this light, there is interest in preparing chitosan-Ca-alginate microparticles that can effectively deliver 5-aminosalicylic acid (5ASA) to the colon after *per os* administration. A solution containing 5ASA and sodium alginate was spray-dried to obtain microspheres smaller than 10 μm, that were crosslinked and coated into a solution of $CaCl_2$ and chitosan. Of importance, ^{1}H NMR and UV-vis spectra of 5ASA showed no degradation when working under light protection with freshly prepared solution, and using nitrogen to prevent the oxidative self-coupling of 5ASA moieties. The microspheres showed acceptable morphology, and prevailing localisation of chitosan in the particle wall, while alginate was homogeneously distributed throughout the particle imparting anionic character. The IR spectra of 5ASA-loaded Ca alginate microparticles indicated absence of covalent bonds between the polymer and the drug that was molecularly dispersed within the chitosan alginate microspheres during the production process (Mladenovska *et al.*, 2007).

Chitosan alginate nanoparticles were investigated as mucoadhesive vehicle for the prolonged topical ophthalmic delivery of an antibiotic, gatifloxacin. A modified coacervation or ionotropic gelation method was used to produce gatifloxacin-loaded nanoparticles with average particle size 205–572 nm and zeta potential 17.6–47.8 mV (Motwani *et al.*, 2008).

A combination of spray-dried chitosan acetate and hydroxymethylpropyl cellulose was tested as a new compression coat for 5ASA tablets. In a simulated system, such tablets were able to pass through the stomach, pH 1.0, and the small intestine, pH 6.8. The delayed release was controlled owing to the swelling with gradual dissolution of chitosan and modified cellulose at low pH and depressed solubility at neutral pH. After reaching the colon, the dissolution of chitosan triggered the 90% drug release within 14 hours. Moreover, the chitosan was found to undergo degradation by unspecific activity of β-glucosidase in the colonic fluid thus enhancing the drug release (Nunthanid *et al.*, 2008).

Because the chitosan powder can be easily compressed into a tablet, it was demonstrated that 5ASA can be admixed with the plain chitosan powder and the resulting tablet can be coated with convenient enteric coatings: the anti-inflammatory action of chitosan enhances the curative properties of 5ASA while being degraded by enzymes provided by the colonic flora (Muzzarelli, 2005).

Following a more traditional approach, chitosan hydrogel beads were prepared by the crosslinking method followed by enteric coating with

Eudragit®S100. The size of the beads was ca. 1.04 ± 0.82 mm. The amount of the drug released after 24 h from the formulation was ca. 97% in the presence of extracellular enzymes as compared with ca. 64% and 96% release of drug after 3 and 6 days of enzyme induction, respectively, in the presence of 4% cecal content (Jain *et al.*, 2007). Uncoated and Eudragit®-coated chitosan-Ca alginate microparticles efficiently loaded with budesonide, with bioadhesive and controlled release properties in the gastrointestinal tract, were prepared by spray-drying. Microspheres had mean size 4.05–5.36 μm, narrow unimodal distribution and positive surface charge. $CaCl_2$ limited the swelling ratio, while the swelling behaviour of coated beads was mainly determined by the Eudragit® enteric coating. The controlled release properties were suitable for local treatment of inflammatory bowel diseases (Crcarevska *et al.*, 2008).

The water-soluble *N*-(2-carboxybenzyl)chitosan (Muzzarelli *et al.*, 1982) was crosslinked with glutaraldehyde and its swelling characteristics were studied in different pH buffer solutions. The swelling ratio decreased with an increase in the amount of glutaraldehyde, and the swelling was more evident in alkaline solutions than in acidic media, showing the lowest swelling ratio at pH 5.0. The latter increased in alkaline solutions with the raising of the degree of substitution, but no significant change was observed in an acidic environment. The crosslinked carboxybenzyl chitosan showed swelling reversibility when alternately soaked in pH 1.0 and 7.4 buffer solutions. Results qualified the crosslinked carboxybenzyl chitosan as a potential pH-sensitive carrier for colon-specific drug delivery system (Lin *et al.*, 2007).

31.8 Conclusion

The good performance of chitosan itself and its derivatives in the dietary food area and in the pharmaceutical area, accompanied by the more thorough understanding of the chemistry of chitosan-based gels, support the expectation that chitosan gels will expand their current range of applications in the near future. This versatile biopolymer, remarkable for its cationicity, has attained a unique position among the hydrocolloids.

31.9 References

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32

Konjac mannan

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Abstract: Konjac mannan is a main component of tubers of konjac which is a perennial plant of *Araceae*. It is a heteropolysaccharide consisting of β-D-glucose (G) and β-D-mannose (M), with a G/M ratio of 1 to 1.6. Konjac mannan contains very small amounts of acetyl groups and the viscosity of its aqueous solution is quite high. Deacetylation occurs with alkali treatment and a chewy irreversible gel is prepared. The gel has been used as a traditional dietary food in Japan for a long time. Konjac mannan interacts synergistically with other polysaccharides and forms thermoreversible gels. In this chapter, the following subjects are explained: cultivation of the konjac tuber, the production process and purification of konjac flour, the chemical structure and molecular weight of konjac mannan, the component analysis of commercial konjac flour, the properties of mixture gel synergistically prepared by konjac mannan and other polysaccharides, the uses and applications of konjac flour, and regulatory status.

Key words: konjac mannan, glucomannan, konjac tuber, cultivation, production process, purification, structure, gel, viscosity.

32.1 Introduction

Konjac (*Lasioideae Amorphophallus*) is a perennial plant and a member of the family of *Araceae*. The original home of the konjac plant is not certain, but is considered to be in Southeast Asia. There are many species of konjac plants in the Far East and Southeast Asia that belong to the *Amorphophallus*,[1] for example, *A. konjac* C. Koch (Japan, China, Indonesia), *A. bulbifer* Bl. (Indonesia), *A. oncophyllus* Prain ex Hook. f. (Indonesia), *A. variabilis* Blume (the Philippines, Indonesia, Malaysia), etc. Only *Amorphophallus konjac* C. Koch grows in Japan. They contain konjac mannan in their tubers. Konjac

Fig. 32.1 Konjac plants.

mannan is a heteropolysaccharide consisting of β-D-glucose (G) and β-D-mannose (M), with a G/M ratio of 1 to 1.6. The konjac tuber grows in size year by year and three- to five-year-old plants bloom with purplish-red flowers in the spring. Konjac is an allogamous plant and plant breeding is performed by cross-fertilisation. Figures 32.1 and 32.2 show konjac plants and tubers.

The main component of the konjac tuber is konjac mannan (KM), which varies in composition from 8–10% of a raw tuber. Starch, lipid and minerals are also present in the tuber. KM is accumulated in egg-shaped cells covered with scale-like cell walls[2,3] and the KM cells are observed within the parenchyma of the tuber. The size and number of the KM cells increase with distance from the epidermis, reaching ~650 μm at the central part of the tuber. Other types of organelles in the parenchyma surround the KM cells. Starch exists in spherical organelles as small particles. Bunches of needle-like crystals are also observed in the tuber and the size of a crystal is ca. 150 μm $\times$ 5 μm. Since a high content of calcium was detected in the crystal by energy dispersive X-ray (EDX) analysis, the needle-like crystal is considered to be calcium oxalate. The konjac

Fig. 32.2 Two-year-old konjac tubers.

tuber, unprocessed, has a harsh taste. This can be removed from the konjac flour by processing.

Irreversible konjac mannan gel is prepared by alkali treatment of grated konjac tuber or konjac flour aqueous solution. KM has very small amount of acetyl groups and deacetylation occurs with the alkali treatment.[4] It is considered that the gelation of konjac mannan is induced by deacetylation. The lowest critical concentration of konjac flour aqueous solution necessary for gel formation is about 0.5%.

The konjac gel (Kon-nyaku in Japanese) is classified as a dietary fibre and it has a chewy texture. The first description of konjac gel and its preparation process are found in an old Chinese poem composed by Zuo Shi and its annotation written in the third century.[5] It is thought in Japan that the production method of konjac gel was introduced from Korea with Buddhism in the sixth century as a medicine. However, it took a long time before konjac gel became a popular food and this was due to two important investigations for the production process of konjac flour.

T. Nakajima (1745–1826) developed a manufacturing technique to produce konjac flour by pulverising dried chips of konjac tuber (Arako). K. Mashiko (1745–1854) improved on this technique to obtain cleaner konjac flour (Seiko). He polished Arako using a mortar worked by a water wheel and separated impurities from the konjac flour by wind sifting. Nowadays, konjac flour is produced in very modern factories controlled by computer systems. However, the principle of the production manufacturing process is the same.

It is well known that konjac mannan interacts synergistically with kappa carrageenan[6] and xanthan gum[7,8] and forms elastic thermoreversible gels. These synergistic gels are major products in the food industry as new healthy gel foods,

particularly in Japan. In the United States, the US Department of Agriculture recently accepted the use of konjac flour as a binder in meat and poultry products. Konjac flour is suitable for thickening, gelling, texturing, and water binding. It may be used to provide fat-replacement properties in fat-free and low-fat meat products.

32.2 Manufacture

32.2.1 Cultivation

Only *Amorphophallus konjac* C. Koch grows in Japan and selective breeding of konjac plants has been carried out. Recently, five species of the *A. konjac* have been cultivated, namely, Zairai, Shina, Haruna-kuro, Akagi-ohdama and Miyogi-yutaka. The latter three species are improved breeds and Haruna-kuro and Akagi-ohdama account for more than 90% of the total tuber output.

The cultivation process of the konjac tuber in Japan is as follows. Seed tubers (Kigo) and/or one-year-old tubers are planted in the spring. The tubers push out new shoots and are consumed completely. The konjac plants grow during the summer and have new tubers. In the late autumn, the plants die and new tubers are dug from the ground. The new tuber has seed tubers at the top of its suckers. The two-year-old tubers are used to produce konjac flour. One-year-old tubers and the seed tubers are kept in a storehouse with heating during the winter to avoid freezing. This cycle is repeated in the following spring.

In China, there are six kinds of konjac plants containing konjac mannan and two species can be cultivated, namely, *A. rivieri* Duieu and *A. aldus* Lie et Chen. The selective breeding of konjac plants is also actively carried out.

32.2.2 Production process of konjac flour

The two-year-old konjac tubers are brought to a storehouse in containers from farmhouses. The tubers are transported to a washing apparatus using conveyer belts and are washed with water, brushing away mud and epidermis and then distributed to each line. The washed konjac tubers are sliced into thin chips, and the chips are dried in a hot-air drier equipped with a heavy oil burner. This is because konjac flour contains a small amount of sulphur dioxide as an impurity. Sulphur dioxide bleaches konjac chips and for this reason the colour of lower-quality konjac flour is extremely white. The dried konjac chips are called Arako in Japanese. The dried chips are pulverised and konjac mannan (KM) particles (i.e., konjac flour) are obtained. Since the KM particles are very tough, they are polished after being produced to remove impurities surrounding the KM cells. Then konjac flour is separated by wind shifting. The polished konjac flour is called Seiko. Micro-fine powder obtained as a by-product is collected using a dust collector. The by-product is called Tobiko in Japanese, which literally means flying powder. The main components of Tobiko are starch and fine KM powder. Protein (ca. 24%) and ash (ca. 10%) are also included in Tobiko.

The viscosity of konjac flour is dependent on the raw tubers and is controlled by the mixing of flours produced from different types of tubers. Then the konjac flour thus prepared is packed into the bags and is kept in a cool storehouse to avoid a change in quality.

32.2.3 Purification of konjac flour

Commercial konjac flour (Seiko) is a light-coloured powder with fish-like smell and a slightly harsh taste. The current practice of several companies is to wash konjac flour with ethanol aqueous solution to remove the micro-fine powders remaining on the surface and the impurities trapped inside the konjac particles.

The konjac flour is whitened by washing. Figures 32.3 and 32.4 show the SEM images of commercial konjac flour, with Fig. 32.4 being the one which has been highly purified. The surface of konjac flour shows scale-like patterns and seems to have been worn smooth (Fig. 32.3). After purification, the scale-like patterns are more clearly observed.

Table 32.1 shows the composition of the various components in konjac flour before and after purification. Since the protein content was determined by nitrogen analysis, the value represents not only protein but also all nitrogen-containing substances. The carbohydrate content increased with washing but the concentration of the other components decreased by washing. The carbohydrate value parallels that of KM.

The fish-like smell decreases remarkably by washing. It has been reported that alkali treated konjac gel contains trimethylamine and that the fish-like smell of the flour is caused by the amine.[9,10] Konjac flour with and without

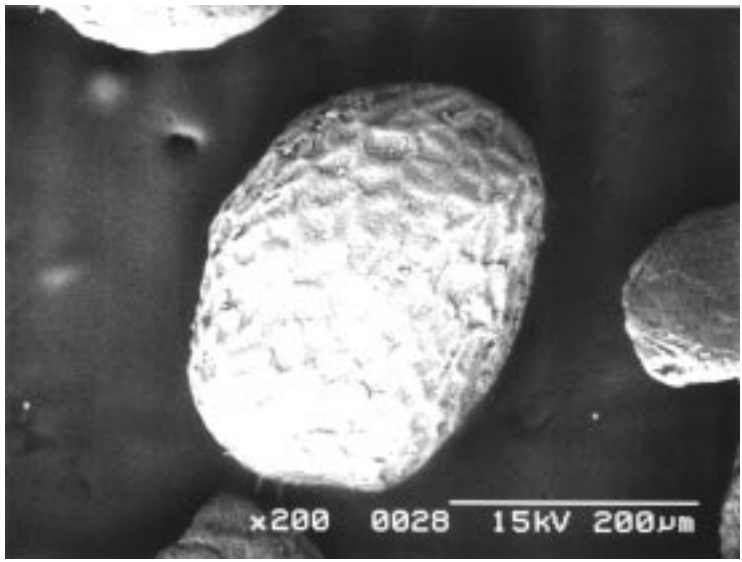

Fig. 32.3 Scanning electron micrograph of konjac flour (Seiko).

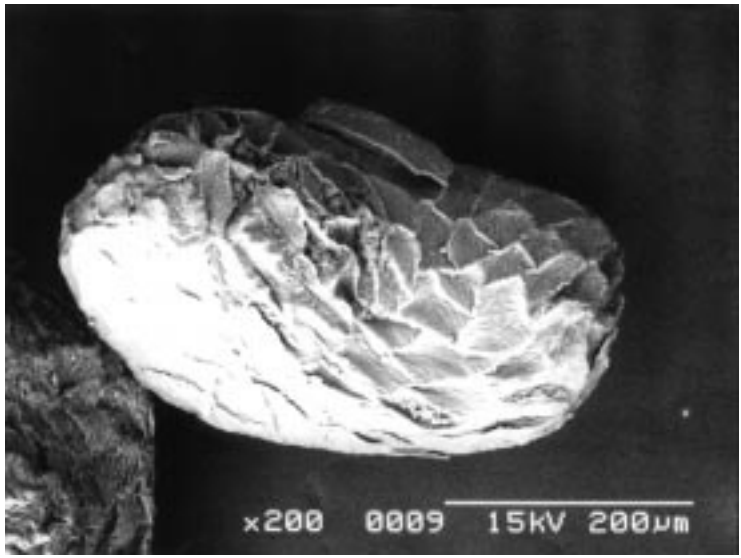

Fig. 32.4 Scanning electron micrograph of purified konjac flour.

Table 32.1 Analytical results of components in konjac flour before and after purification

	Contents (g/100g of sample)					
	Water	Protein	Lipid	Carbohydrate	Fibre	Ash
Konjac flour	7.2	2.2	2.3	82.6	0.5	5.2
Purified konjac flour	7.5	0.8	0.9	88.6	0.5	1.7

purification showed mass spectra attributable to nitrogen-containing substances, but they were not identical to trimethylamine.[2] This demonstrates that konjac flour does not contain trimethylamine as an impurity. Trimethylamine should be separated from other nitrogen-containing substances by the alkali treatment.

The purification of konjac flour is very effective in preventing the putrefaction of konjac gel prepared by alkali treatment and the syneresis of the mixed gels prepared by konjac mannan and other gums.

32.3 Structure

The main component of konjac flour is a glucomannan called konjac mannan (KM), whose main chain consists of D-glucose and D-mannose linked by β-D-1,4 bonds. The ratio of glucose (G) to mannose (M) is reported to be 1 to 1.6[11–13] or 2 to 3.[14,15] Although the repeating structural unit of the main chain is still uncertain, typical proposals for the unit by research scientists are as follows:

1. G-G-M-M-M-M-G-M or G-G-M-G-M-M-M-M[11]
2. M-M-M-G-G[13]
3. G-G-M-M-G-M-M-M-M-M-G-G-M.[13, 16]

It is also reported that KM has side chains and the branching position is considered to be the C3 position of mannose residues[11, 17] or C3 positions on both glucose and mannose[13] in the main chain. The degree of branching is estimated at approximately three for every 32 sugar units[14] or at one for 80 sugar residues.[11] The length of the branched chain was also evaluated as 11 to 16 hexose residues[17] or as several hexose units.[11]

KM contains acetyl groups in the main chain. Figure 32.5 shows a Fourier transformation infra-red (FT-IR) spectrum of purified KM. An absorption due to stretching vibration of C=O group in acetyl group is observed at 1730 cm^{-1}. The acetyl group content was estimated at one for 19 sugar residues.[4] Figure 32.6 shows the chemical structure of KM proposed by Okimasu.[1, 18]

The crystalline form of KM was studied by the X-ray diffraction method.[19] KM shows a different X-ray diffraction powder pattern from both crystalline polymorphs of other glucomannans (mannan I and mannan II) which have been studied. The fibre pattern of the annealed KM indicated that it exists in an extended two-fold helical structure.

Since konjac flour forms very viscous solutions, measurement of the weight average molecular weight (Mw) and the mean square radius of gyration ($<S^2>^{1/2}$) of KM was carried out using partially methylated KM samples.[20] The average values of Mw and $<S^2>^{1/2}$ were determined to be 10×10^5 and 110nm. It was also reported that both Mw and $<S^2>^{1/2}$ were found to be dependent on species of konjac plant, cultivation districts and preparation method. The authors[21] measured molecular weight (M_w), molecular dispersity and root mean square (RMS) of KM (Akagi ohdama species obtained in Gunma prefecture, Japan) using the Dawn multi-angle laser light scattering method, associated with a gel permeation

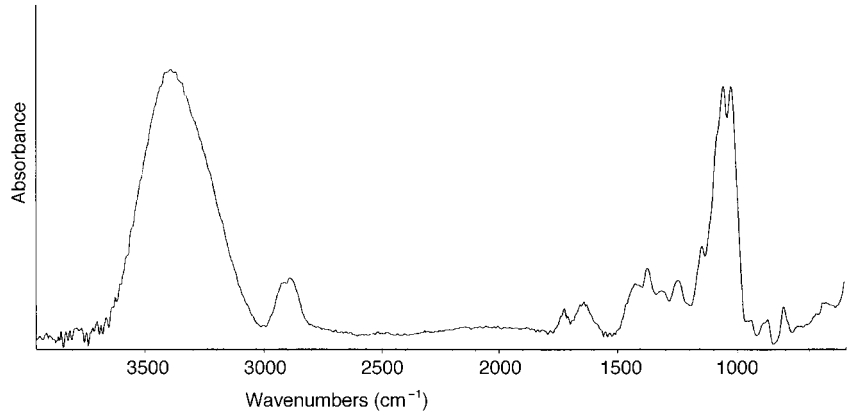

Fig. 32.5 FT-IR spectrum of konjac mannan analysed by the attenuated total reflection (ATR) method.

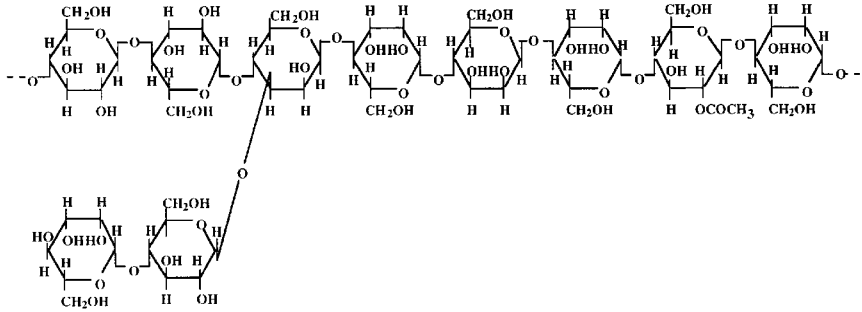

Fig. 32.6 Chemical structure of konjac mannan.

chromatographic (GPC) fractionation. The M_w, molecular dispersity and RMS were 13.2×10^5, 2.1 and 130nm, respectively.

32.4 Technical data

The quality of commercial konjac flour is appraised by the size of KM particles, viscosity, whiteness, moisture and admixing of impurities such as pieces of scorched epidermis and denatured KM particles during the hot-air drying. Some kinds of bacteria are observed in konjac flour but these are not *colon bacilli*.[22] They cause putrefaction of konjac gel and degradation of molecular weight of konjac mannan. The most important criterion of the quality of konjac flour is its high viscosity in aqueous solution, which in turn depends on the molecular weight of the polysaccharide.

Table 32.2 shows typical technical data of two types of commercial konjac flours and purified flour of them. The data is kindly given by Ogino Shoten Co. Ltd. in Gunma Prefecture, Japan. The Chinese konjac flour is a bonded one and was pulverised by Ogino Shoten Co. Ltd.

Konjac mannan is a water-soluble polymer but it needs a special technique to dissolve it in water completely. To dissolve at room temperature, konjac flour must be added to water with stirring until the powder is completely dissolved. It is important to stir the solution continuously so that the powder does not lump. Hot water is not effective to dissolve konjac flour.

The relationship between viscosity of purified commercial konjac flour and stirring time is shown in Fig. 32.7. The konjac flour, Rheolex RS, was characterised by a very fine mesh size (80 mesh sieve) and the measurements were carried out at 25 ºC using a viscometer. The data was kindly provided by the Shimizu Chemical Co. Ltd. in Hiroshima Prefecture, Japan. The viscosity of KM aqueous solution increases with stirring time and reaches a constant value after two hours. The viscosity of KM aqueous solution increases gradually with increasing concentration until 1% and then increases remarkably. As seen in Fig. 32.7, the viscosity of a 2% aqueous solution is more than 12 times higher than that of 1% solution. The viscosity of KM aqueous solution is not affected by salt

Table 32.2 Analytical results of components in commercial konjac flours

	Japanese konjac tuber		Chinese konjac tuber (bonded)	
	Ordinary flour	Purified flour	Ordinary flour	Purified flour
Viscosity (mPas)[+]	15.0–15.2	17.0–18.0	13.5–13.6	18.0
Whiteness	66–68	73	69.9	68.9
Water*	6.5	6.6	8.4	5.3
Protein*	2.1	1.1	3.0	1.5
Lipid*	1.3	0.3	0.9	0.3
Carbohydrate*	84.6	89.2	82.2	90.0
Fibre*	0.5	0.6	0.6	0.8
Ash*	5.0	2.2	4.9	2.1
Sulphur dioxide	0.65 g/kg	0.17 g/kg	2.1 g/kg	0.64 g/kg
Arsenic*	Not detected	Not detected	Not detected	Not detected
Lead*	Not detected	Not detected	Not detected	Not detected
Trimethyl amine**	490 ppm	85 ppm	760 ppm	170 ppm
Number of germ	Less than 300/g	Less than 300/g	420/g	Less than 300/g
Coliform bacteria	negative	negative	negative	negative

[+] 1% konjac aqueous solution at 35 °C after 4 h stirring at 90 rpm.
* g/100 g of konjac flour.
** nitrogen-containing substances.

concentration, but is affected by pH of the solution. The effect of pH on viscosity change for 1% and 2% KM solutions is listed in Table 32.3. The viscosity of KM solution decreases with decreasing pH value. At a high pH, KM solution changes to gel.

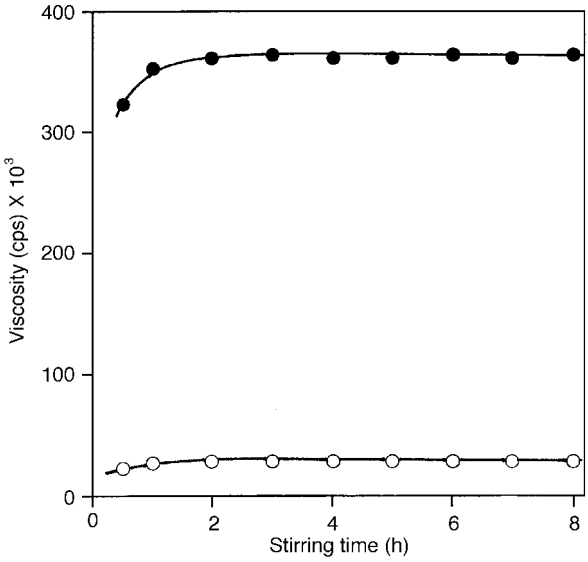

Fig. 32.7 Relationships between viscosity of purified commercial konjac flour, Rheolex RS, and stirring time: (○) 1%, (●) 2%.

Table 32.3 Effect of pH on viscosity change for 1% and 2% KM solutions

KM* concentration (%)	Viscosity (cps)			
	Water (no pH adjust.)	pH 4	pH 3	pH 2.5
1	31,600	31,800	29,900	18,600
2	341,000	340,000	301,000	251,000

* – Rheolex RS.

Table 32.4 Viscosity of mixtures of KM and other gums with various composition. Total concentration of the mixtures is 1%

KM* concentration (%)	Other gums concentration (%)	Viscosity (cps)							
		Xan	LBG	Gel	Pec	Car	Aga	GG	CMC
0.0	1.0	8,250	225	0	0	300	0	4,250	75
0.2	0.8	8,800	650	125	75	12,750	60	6,800	225
0.4	0.6	12,000	2,700	1,525	600	17,500	725	10,000	1,065
0.6	0.4	13,250	7,500	5,860	3,750	51,000	3,740	14,750	4,075
0.8	0.2	161,000	15,750	14,700	11,640	113,600	12,500	20,750	12,200
1.0	0.0	29,500	29,500	29,500	29,500	29,500	29,500	29,500	29,500

* – Rheolex RS; Xan – xanthan gum; LBG – Locust bean gum; Gel – Gelatin; Pec – Pectin; Car – κ-carrageenan; Aga – Agar; GG – Guar gum; CMC – Carboxymethyl cellulose.

Table 32.5 Gel strength of mixtures of KM and other gums with various compositions. Total concentration of the mixtures is 1%

KM* concentration (%)	Other gums concentration (%)	Gel strength (g)							
		Xan	LBG	Gel	Pec	Car	Aga	GG	CMC
0.0	1.0	–	–	–	–	24.1	21.4	–	–
0.2	0.8	7.8	–	–	–	118.7	25.7	–	–
0.4	0.6	161.7	–	–	–	185.3	20.3	–	–
0.6	0.4	84.3	–	–	–	129.0	11.7	–	–
0.8	0.2	34.7	–	–	–	–	4.0	–	–
1.0	0.0	–	–	–	–	–	–	–	—

* – Rheolex RS; Xan – xanthan gum; LBG – Locust bean gum; Gel – Gelatin; Pec – Pectin; Car – κ-carrageenan; Aga – Agar; GG – Guar gum; CMC – Carboxymethyl cellulose.

Konjac mannan interacts synergistically with other polysaccharides and forms thermoreversible gels. The viscosity of the mixtures and the gel strength are listed in Tables 32.4 and 32.5, respectively. The synergism is observed for the combination of KM and xanthan gum, KM and carrageenan, and KM and agar.

Table 32.6 shows the effect of sugar concentration on the gel strength for 1% mixed gel with various ratios of KM to κ-carrageenan. The addition of sugar

Table 32.6 Effect of sugar concentration on gel strength of mixed gel of KM and κ-carrageenan with various compositions. Total concentration of the mixtures is 1%

KM*/Car ratio	Gel strength (g)			
	Sugar concentration (%)			
	0	5	10	15
8:2	–	–	–	–
7:3	–	141.6	134.9	145.3
6:4	121.5	205.4	195.8	220.7
5:5	331.0	275.3	299.1	260.9
4:6	299.7	285.2	297.4	222.4
3:7	216.8	213.8	187.8	101.1
2:8	137.5	100.1	100.3	109.7

* – Rheolex RS; Car – κ-carrageenan.

Table 32.7 Effect of salt concentration on gel strength of mixed gel of KM and κ-carrageenan with various compositions. Total concentration of the mixtures is 1%

KM*/Car ratio	Gel strength (g)			
	Salt concentration (%)			
	0	1	3	5
8:2	–	41.0	–	–
7:3	–	72.0	–	–
6:4	121.5	120.7	–	–
5:5	331.0	247.7	–	–
4:6	299.7	342.2	27.9	–
3:7	216.8	529.0	87.5	–
2:8	137.5	265.4	126.5	–

* – Rheolex RS; Car – κ-carrageenan.

enhances the gel strength slightly for the gel with higher composition of KM but reduces the strength for the gel with lower composition of KM.

Table 32.7 shows the influence of the addition of salt on the gel formation for a 1% of mixture of KM and κ-carrageenan. The synergistic gel formation is inhibited by addition of salt.

32.5 Uses and applications

Konjac flour has been used as an important food ingredient for more than a thousand years. With the addition of a mild alkali such as calcium hydroxide, konjac flour aqueous solution (ca. 3% of concentration) changes to a strong, elastic and irreversible gel. The alkali treated konjac gel is quite a popular traditional Japanese food and is called Kon-nyaku in Japanese. Recently,

Table 32.8 Applications and functional uses of konjac mannan

Application	Function
Confectionery	Viscosity, texture improver, moisture enhancer
Jelly	Gel strength, texture improver
Yoghurt	Fruit suspension, viscosity, gelation
Pudding	Thickening, mouthfeel
Pasta	Water-holding capacity
Beverage	Fibre content, mouthfeel
Meat	Bulking, fat replacer, moisture enhancer
Edible film	Water soluble, water insoluble

synergistic gels prepared by mixing of other hydrocolloids are major products in the food industry as new types of healthy jellies.

Clinical studies indicate that konjac mannan solution has the ability to reduce serum cholesterol and serum triglyceride. Konjac mannan also has an influence on glucose tolerance and glucose absorption. However, the alkali treated gel food does not have such effects.

Konjac flour is suitable for thickening, gelling, texturing, and water binding. It may be used to provide fat replacement properties in fat-free and low-fat meat products. Applications and functional uses of konjac mannan are listed in Table 32.8.

32.6 Regulatory status

In Japan, konjac flour is accepted as a food ingredient and a food additive for thickening and as a stabiliser according to the provisions of the Food Sanitation Act. For regulatory purposes, a distinction must be drawn between konjac flour and konjac mannan, the separated polysaccharide. The Food Chemical Codex lists the current uses of konjac flour in the United States as gelling agent, thickener, film former, emulsifier, and stabiliser. Konjac flour is also used as a binder in meat and poultry products.

Konjac mannan has been recognised as GRAS (generally recognised as safe) by the Food and Drug Administration (FDA) since 1994 and the US Department of Agriculture (USDA) accepted the use of konjac flour as a binder in meat and poultry products in 1996. In Sweden, it was recognised that konjac mannan has the ability to reduce serum cholesterol and indication of the effect was officially accepted enabling claims to be made for its use as a functional food.

Konjac flour imported into Europe for diet food and pet food is rarely of consistent quality and does not meet EU standards. However, konjac mannan received a provisional European classification number as a food additive (E425) in 1998. Konjac mannan can thus be imported into Europe because it has achieved an E number.

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