7
Proteins from Plant Materials

Lei Xu and Levente L. Diosady
University of Toronto
Toronto, Ontario, Canada

I. INTRODUCTION

As the world population continues to grow exponentially, the demand for food proteins will inevitably increase substantially. It has been forecast that within the next decade, in order to just maintain the present level of human nutrition, the supply of vegetable proteins must be doubled together with a fourfold increase in the production of animal-based proteins (1). Since such a dramatic growth in meat production is hard to achieve, human diet will likely rely more on vegetables and cereals as protein sources. In addition to continuing to sustain of human life as they have done for thousands of years, plant-based proteins (or vegetable proteins) offer great potential as functional food ingredients. In the past decades, many processes have been developed to produce proteins to be used as meat extenders in processed meat products or as protein-enriching agents in beverages. In the processing of vegetable proteins, much attention is paid to the preservation or improvement of their nutritional value as well as organoleptic and functional properties.

Storage proteins in oilseeds, legumes, and cereals have been the focus of process and product development for food and industrial protein products. Protein products can be produced by the preferential extraction of nonprotein seed components, as is the case with soy protein concentrate, or by the dissolution, purification, and recovery of proteins in the form of protein isolates.

For protein isolate production a number of aqueous and some nonaqueous organic solvents have been investigated (1). The storage proteins include a number of albumins, globulins, prolamines, and glutelins with a variety of molecular weights and a range of solubility characteristics. Albumins are typically soluble

in water (pH 6–8), globulins in dilute salt solutions, prolamines in ethanol, and glutelins in dilute acid or alkali. Most of these proteins are highly soluble in strong alkali, although their functional properties may be altered by partial or complete denaturation at these extreme conditions.

Soy proteins have been used as traditional foods in the orient. Accordingly, they have been studied extensively, and there are numerous commercial processes for the production of a wide range of commercial soy protein products. Many other protein sources were investigated in attempts to find alternatives to soy proteins. This was driven by the functional, nutritional, and allergenic problems of soy proteins, as well as the desire to develop value-added products from a number of underutilized plants or plant products, such as oilseed meals after oil extraction. Significant research advances were made in the production of wheat gluten, corn zein, and a number of oilseed proteins from peanuts, cottonseed, canola, and others.

In cereal grains the main storage proteins are usually the alcohol soluble prolamines, while the salt-soluble globulins are abundant in oilseeds and legumes (2, 3). Regardless of their origins, these globulins all belong to two classes of different molecular sizes, usually denoted in the literature by their sedimentation coefficients as 7S and 11S (1).

Most protein extraction processes are based on aqueous extraction using single- or multistage batch operation. The latest advances made in the methods for protein extraction feature the combinations of solvents with optimized extraction parameters and advanced separation technologies such as membrane processing to increase both the yield and quality of the protein products.

In addition to their main use as functional ingredients in many food systems, proteins from vegetable sources are also used in some industrial processes as size, adhesive, or dispersive agents.

II. SOY PROTEINS

The processing of soybean proteins has evolved since ancient times. For nearly 15 centuries people in Asia have combined the use of rice and the products of soybeans to produce an inexpensive diet that is reasonably well balanced in terms of essential amino acids. They learned that the greatest nutritional value from soybeans is obtained through a water extraction followed by coagulation of the resulting milky liquid into a curd. Crude as this ancient method is, it has provided the basis for the development of many modern processing methods.

Nutritional studies show that soy proteins are an excellent source of food proteins. The amino acid composition of soy protein isolate nearly meets the essential amino acid pattern set by FAO/WHO/UNU as shown in Table 1 (3, 4). In combination with rice, the low methionine level of soy and the low lysine

Table 1 Comparison of Essential Amino Acid Content of Soy Protein Isolates to WHO/FAO/UNU Requirements

Essential amino acid	WHO/FAO/UNU suggested pattern (mg/g protein)			Essential amino acid content of isolated soy protein (mg/g protein)
	2–5 years	10–12 years	Adult	
Histidine	19	19	16	26
Isoleucine	28	28	13	49
Leucine	66	44	19	82
Lysine	58	44	16	63
Methionine and cysteine	25	22	17	26
Phenylalanine and tyrosine	63	22	19	90
Threonine	34	28	9	38
Tryptophan	11	9	5	14
Valine	35	25	13	50

Source: From Ref. 3.

level of rice are both overcome. The high digestibility of soy proteins has been demonstrated by extensive nutritional studies on both animals and humans. Currently, four major classes of soy protein products are in commercial production: flour, protein concentrates, protein isolates, and textured products. Their total production exceeds one million tonnes.

Soy flour can be made from either full-fat dehulled soybeans or dehulled defatted flakes. Full-fat soy flour contains fat in excess of 18%, while the oil content of defatted flour is usually less than 1%; thus, the protein content is much higher than that of full-fat flour. To process full-fat flour, high-quality, sound, clean, yellow soybeans are selected. Conventionally, these beans are first conditioned to a desirable moisture level and then cracked in roll mills. The cracked beans are dehulled by aspiration and screening. The meats are cooked at temperatures above 93°C by steam in another conditioner to inactivate enzymes such as lipoxygenase. After being dried, they are ground in two steps using a hammer mill to particle sizes small enough to pass through a 0.149-mm screen (No. 100 U.S. Standard Screen) (5).

The production of defatted soy flour is directly linked to oil extraction from soybeans, which involves solvent extraction and desolventizing. The defatted and desolventized soy flakes with an appropriate moisture content are then ground in a hammer mill until 97% passes through a 0.149-mm screen (No. 100 U.S. Standard Screen).

The term *protein concentrate* refers to products containing at least 70% protein produced by alcoholic extraction of carbohydrates, leaving behind the

insoluble proteins, whereas *protein isolate* is usually used to describe products with more than 90% protein produced by the extraction, purification, and recovery of soy proteins. Although both protein concentrates and isolates can be made from full-fat soybeans, the products find limited use due to their appreciable fat content. Therefore, manufacturing of these protein products usually start with defatted soy flour after the grinding of the defatted flakes to facilitate subsequent extraction. The dehulled flour has a protein content of about 50%.

Protein concentrates are produced by washing the meal to remove soluble carbohydrates with dilute acid, aqueous ethanol, or hot water, as none of these dissolves significant amounts of soy protein. With dilute acid, the pH of the extraction solution is adjusted to 4.5, the observed isoelectric point of soy proteins, where the solubility of soy proteins is minimal (6). The insoluble material containing most protein is thus separated from the soluble impurities in a batch operation that is repeated several times. The effect of ethanol is similar as most proteins are insoluble in 60–80% aqueous ethanol; thus, soluble oligosaccharides are extracted and separated (7). Aqueous alcoholic extraction also causes denaturation of the trypsin inhibitors present in the seed and removes some of the undesirable phenolic components, thus improving the quality of the product. The ethanol is removed from the protein concentrate and recovered by flash desolventizing. For hot water extraction, the starting material is first toasted to denature the proteins and decrease their nitrogen solubility index (NSI), and thus decrease protein solubility, while maximizing carbohydrate dissolution (8). In the treatment, the soy flakes are extracted with hot water at 66–93°C in either a batch or a continuous countercurrent manner, and pH is maintained in the range of 5.3–7.5.

The isolation of soy proteins takes an approach opposite to protein concentration (5, 9–11), wherein the protein is dissolved while most of the impurities are left behind in the solids. A generic process is outlined in Fig. 1. The extraction is carried out with dilute alkali, pH 8–10, usually at elevated temperatures of 50–55°C. Although more protein could be extracted in more alkaline solutions, protein tends to deteriorate faster at high pH values. Extraction conditions, including pH, temperature, liquid-to-solids ratio, and additional reagents, vary among different manufacturers, and information about most of the detailed operating conditions remains proprietary. After extraction, separation of the aqueous extract from the solids is achieved by screening, centrifugation or decanting, and polish-filtration. Optionally, the extract may be further clarified by chemical purification by adsorbents, depending on the process adopted by the manufacturer. A food grade acid is then used to acidify the extract containing the soluble protein portion to pH 4–5. The most commonly used acids are acetic, hydrochloric, phosphoric, and sulfuric. Upon acidification a protein precipitate is formed, collected by centrifugation or filtration, and washed. The precipitate is usually neutralized with dilute food grade alkali to form sodium proteinates, which can

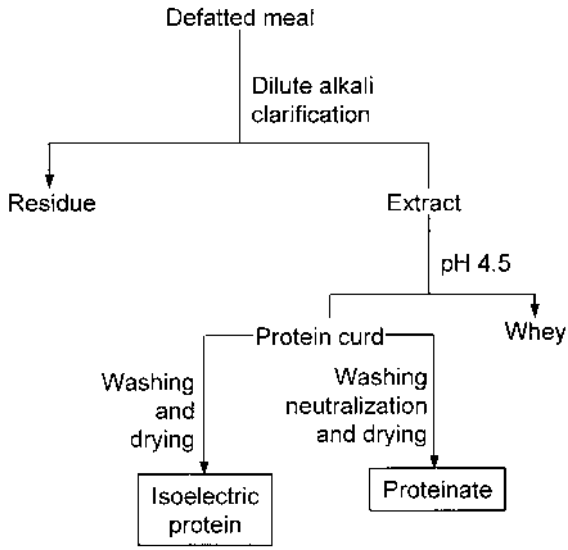

Figure 1 Commercial production of soy protein isolates (42).

be modified by jet cooking at 141–160°C, followed by treatment with a proteolytic enzyme for 1–30 min to improve the wettability and dispersibility of the product (10). Although spray drying is the preferred drying method, the product may be dried by other means such as a forced draft oven or a range drier. In the end, about one-third of the starting mass is recovered as a protein isolate containing more than 90% protein on a moisture-free basis ($N \times 6.25$), one-third remaining as the insoluble residue, and one-third in the whey solution. After drum drying, the insoluble residue is used as a feed ingredient. The whey solution contains several undesired flavor components and trypsin inhibitors that must be heat inactivated before it can be used even for feed.

Despite the generally desirable nutritional properties of soy protein concentrates and isolates, these products are typically powdery and do not exhibit the consistency required for palatable foods. In order to expand their use, novel processes have been developed to obtain soy protein products with the more desirable meat-like textures. Technologies involved in these processes include extrusion and spinning. In the 1960s, an extrusion cooking process was developed for the production of a fully toasted, full-fat, and texturized soy flour (12). The extrusion of soy protein is similar to other food extrusion processes. The extruders for texturizing are designed to handle high-moisture materials. In the process, the dehulled flakes are first cooked with steam at 93–100°C and preconditioned to a moisture content of 18%. The cooked material is then passed

through a high-speed mixer where more steam is mixed with the flakes before entering a Wenger extruder, wherein the material is pushed along inside a barrel by a rotating screw while being heated by friction or indirect steam. High temperatures of 121–143°C are reached for 1–1.5 min within the barrel. At these high temperatures some hydrogen bonds break, unfolding the structured proteins, which are then elongated and aligned by shear forces. When the melt is forced through the die at the end of the barrel, it is rapidly expanded by the release of steam upon leaving the extruder, producing a porous matrix. As the proteins cool, new hydrogen bonds form to give the product a fibrous, meatlike texture. The cooled product is ground in a pin mill to desirable particle sizes and used in foods such as "bacon bits."

The spinning process was originally developed in the synthetic textile industry for the production of nylon fibers. It has been adapted to the commercial production of soy protein fibers (10, 11). A typical process for soy protein spinning is shown in Fig. 2. A soy protein isolate is first dissolved in strong alkali to make a "dope," which is a solution with a protein concentration of about 20% at a pH of 12–13. After filtration, the dope is pumped through a spinneret made of a platinum plate with thousands of small holes less than 1 mm in diameter into a coagulating bath. As the dope contacts the acid and salt in the coagulating bath, the protein precipitates, forming protein fibers. Phosphoric acid and NaCl are usually used as the precipitating agents in the bath. To continue the texture-forming process, the fibers are combined to make tows about 0.5 cm in diameter to be stretched. The stretched tows are then washed to remove the acid and salt, and heated to be hardened. Binders such as egg albumin are added to improve the cohesiveness of the product, followed by the addition of fat, flavor, color, and supplementary nutrients in order to obtain a palatable food. Because the final product has a texture similar to that of processed meat, it is used as a meat analogue.

The functional properties of these soy protein products play a critical role in determining their use in food systems. These include solubility, hydration and water absorption, viscosity, gelation, emulsification, foaming properties, and organoleptic properties such as color and flavor. Commercially available soy proteins exhibit a wide range of solubilities at pH 7 from 25% to 95% (13), and the solubility is dependent on pH and salt concentration. While higher pH results in high solubility, the effect of elevated salt level is to reduce solubility. Commercial soy proteins also have a wide range of water binding ability (14). Insoluble protein granules bind much more water than soluble proteins. Slurries of soy proteins have relatively low apparent viscosity and do not form strong gel networks; however, heat or alkali treatments can increase viscosity and improve gelation functionality (15). As with other functional properties, commercial soy proteins display a range of emulsion and foam properties. Kolar et al. (10) reported a range from 10 to 40 mL oil/100 mg protein for oil binding. Unlike

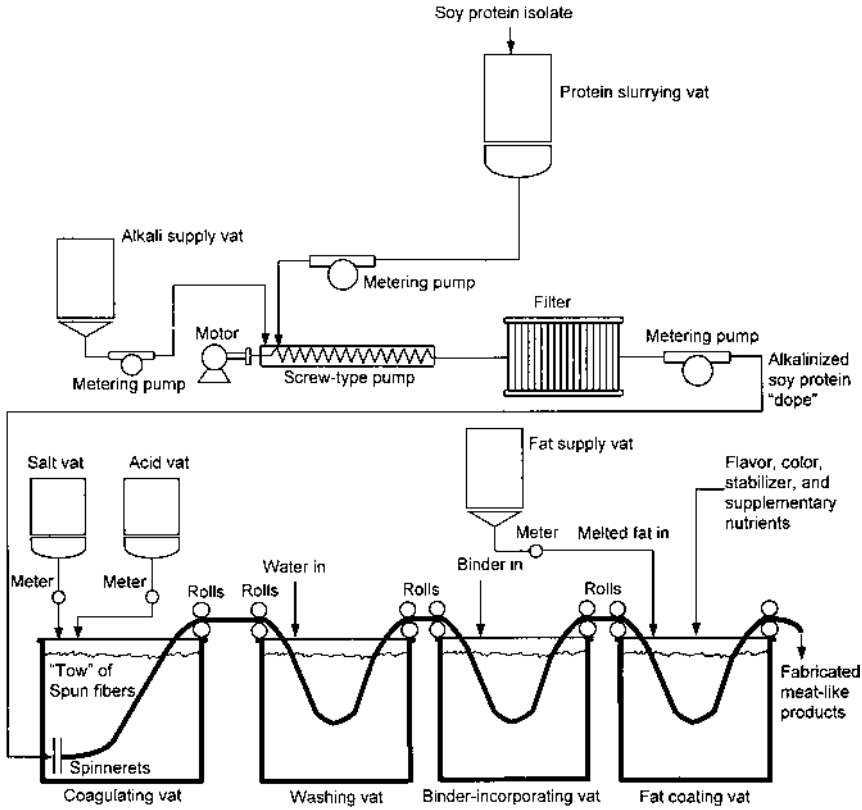

Figure 2 Spinning process for production of soy protein fibers (41).

other functional properties, color and flavor are primarily due to small nonprotein compounds and their interactions with proteins. A number of phenolic compounds and their oxidization products have been linked to the yellowish brown color of soy protein products (16). The products of lipid oxidation catalyzed by lipoxygenase are responsible for the undesirable beany flavor of most commercial soy proteins (17). Once formed, these compounds bind to proteins and thus are hard to remove. However, for commercial food applications these organoleptic problems can be avoided through careful control of food processing conditions.

Presently a large variety of soy protein products are manufactured commercially worldwide. These are used in processed meat products, imitation cheese, whipped toppings, soy milk, nutritional beverages, and baked products. There is also a significant market for the industrial use of soy proteins in paper products, adhesives, and polymers.

III. GLUTEN

"Gluten" refers to the insoluble portion of proteins in cereal grains, first identified more than 200 years ago in dough from wheat flour, and isolated by simply washing the dough with water. Much research has been done on gluten and the flour proteins ever since.

The wet impure gluten carries 65–70% water, has a creamy color, and feels like rubber. When dried, the crude gluten contains 75–80% protein and 5–15% carbohydrates, the remaining being lipids. Gluten can be divided into two fractions. One fraction is soluble in 60% aqueous alcohol, known as gliadin or prolamine. The other fraction, although insoluble in neutral water, is soluble in acidic or alkaline solutions, and is known as glutelin (18).

The nutritional and functional properties of gluten have been well documented. Gluten has acceptable levels of most essential amino acids but is low in lysine. Its functional properties can be changed by reducing and oxidizing agents, and heat treatment. Oxidizing agents make gluten lose its extensibility and become brittle, whereas reducing agents increase its extensibility. Gluten may lose some or all of its "vitality" when subjected to heat treatment.

In early industrial processes gluten was only a byproduct of wheat starch processing. The Alsatian method (19) is among the first methods to recover gluten, wherein whole wheat is steeped in water for 1–2 days. The softened wheat grain is then placed in bags of mesh to permit dissolution of starch while retaining the gluten. The bags are passed between a series of rolls with progressively smaller gaps, thus squeezing out starch but leaving gluten in the bags. The further separation of gluten from the hulls is long, and the yield of gluten is usually low. The Martin process uses wheat flour to make both starch and gluten (20). It starts with making a stiff dough containing about 40% water. After hydration for 1 h, the dough is rolled between fluted rolls under a spray of water to wash away the starch and leave the gluten in a single, coherent mass. This method produces good-quality gluten with high yield. Another process, called the batter process, involves making a batter, which is broken up while water is added to wash away the starch from the gluten (21). The gluten in this method is recovered as fine curds with more than 80% protein. Although developed decades ago, both the Martin and the batter processes are still used in separating starch and gluten from wheat flour. Figure 3 shows the major steps involved in either method. The gluten is separated from starch milk by a series of centrifuges. In the refining step, the lighter low-grade starch is separated from the heavier prime starch. The gluten is either flash or spray dried. The mass distribution among products is 59% prime starch, 8% tailing starch, and 15% gluten with a protein content of 80%. Based on the batter process, a continuous process has been developed (22). First a slack dough is made by mixing flour with water at a ratio of 0.7 : 1. Then the dough is heated to 48–57°C, followed by breaking up of the dough in the presence of additional water. The starch is

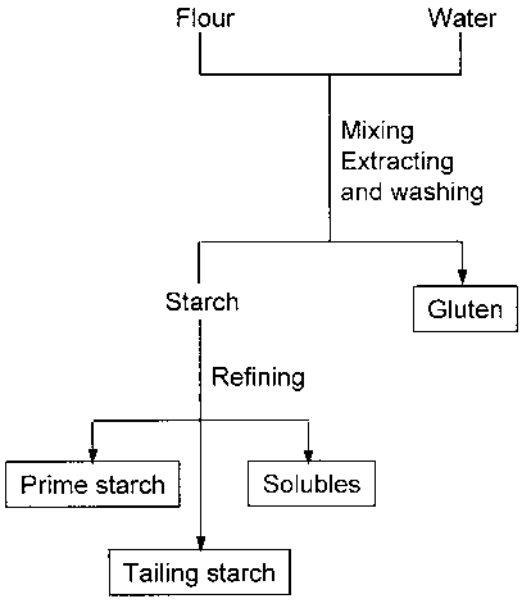

Figure 3 Manufacture of wheat starch (41).

thus largely washed out. The gluten is recovered as curds and separated from the starch by screening the slurry.

Other processes include the alkali process (23), in which the wheat protein is dissolved in 0.03 N NaOH solution by mixing 1 part of flour with 18 parts of the alkaline solution, and after centrifugation to separate starch, the supernatant is acidified with H_2SO_4 to produce a precipitate containing 70–90% protein, which has no vitality. This method was modified with NH_4OH to replace NaOH (24). As a result, the gluten is recovered with vitality.

The vital gluten is used extensively as an ingredient in yeast-raised baked goods, particularly bread. Besides increasing the final protein content in the bread, it also results in a greater volume of better crumb texture and a longer shelf life of the bread. Canada has been a major producer and exporter of wheat gluten and contributed significantly to the commercial development of gluten extraction processes.

IV. RAPESEED AND CANOLA PROTEIN PREPARATION

Although its oil has been consumed as food by humans for thousands of years, the idea of using rapeseed protein in food is relatively recent. One obvious

reason for the delay is directly related to the organoleptic characteristics of rapeseed meal after oil removal. It has an unappealing dark green color and an unpleasant taste that may be characterized by both bitterness and astringency. According to modern studies, both problems are caused by phenolic compounds in rapeseed (16). Nevertheless, due to increasing demand for vegetable oils, the production of rapeseed has grown drastically in the last three decades. It is now ranked second in world production, exceeded only by soybeans (24a). The meal is used in animal feed but commands a significantly lower price than soy meal. Nutritional studies have shown that rapeseed proteins have well-balanced amino acid composition (25, 26), and are particularly high in lysine (27), which is a limiting amino acid in most cereal and oilseed proteins. Due to its abundance and high-quality proteins, rapeseed should play an important role in meeting the need of the world's fast increasing population for food proteins. Consequently, there has been continued research interest in extracting rapeseed proteins for food.

Rapeseed meal has a protein content of up to 40% after oil extraction; however, its direct use as a food protein source is prevented by its undesirable components. In addition to phenolic compounds, it contains high levels of glucosinolates (more than 100 μmol/g defatted meal), which upon hydrolysis release toxic compounds such as isothiocyanates and oxazolidinethiones (27a). Although the genetically improved "canola" varieties have much lower amounts of glucosinolates (20–30 μmol/g defatted meal), their meals are still unacceptable for incorporation into any food formulations. Canola meal also has a much higher phytate content of 3–5% than many other cereals and oilseeds (28, 29). Phytates are undesirable in foods as they tend to strongly bind minerals such as Fe and Zn, and make them unavailable for metabolism (30, 31). It is obvious that before rapeseed or canola protein can be used for food, much or all of these compounds must be removed.

Although much process research has been directed to treating the meal, a variety of methods have been developed to isolate high-quality canola proteins. Most of the published methods were based on soy protein extraction technology: aqueous extraction followed by protein precipitation. Unlike soybeans, canola contains a wide range of proteins, with a broad band of isoelectric points and a wide range of molecular weights.

For rapeseed protein extraction, alkaline aqueous solutions have been suggested as solvent. While over 80% extractabilities with dilute NaOH solutions have been reported by several researchers (32–34), these values cannot be attained using commercial meal as starting meal. To improve extraction, researchers used consecutive extractions at differing pH values. Blaicher et al. (35) extracted rapeseed protein at pH 9.5 and 12.0 in two consecutive stages, respectively, and achieved a 92% extractability. Although higher pH results in increased protein extractability, it could cause chemical modification of the proteins such as

the formation of harmful lysinoalanine (36) under extremely alkaline conditions (pH > 12). Since rapeseed proteins are predominantly globulins (37), dilute sodium chloride solution has been explored as a protein solvent. NaCl concentrations from 0.2 N to 2 N have been reported (25, 38–40). Extractabilities obtained with NaCl solutions were typically lower than those obtained with NaOH media. The recovery of salt-extracted protein is complicated by the need to remove the salt by dialysis.

Following the example of soy technology, rapeseed proteins are usually recovered by isoelectric precipitation. The precipitate, which is usually washed and dried, constitutes the protein isolate. A wide range of isoelectric points from pH 2.6 to 10.0 are observed with rapeseed proteins (32, 34, 41, 42), due to their complex compositions as well as varietal difference among rapeseed strains. Consequently, protein recovery of single-step isoelectric precipitation is low, and the highest ever reported is 65.7% of the amount of protein extracted at pH 11, achieved at pH 3.6 by El Nockrashy et al. (43). Multistage precipitation at different pH values does not significantly increase the yield of protein precipitate (34, 44).

Despite extensive studies in the past, no process has been commercialized due to low protein recovery, poor product quality, and high cost of the processing methods. To reduce the product losses and improve product quality, a membrane-based process was developed for rapeseed and canola protein isolation starting with defatted meals (45, 46). It consists of five main steps: alkaline extraction, isoelectric precipitation, ultrafiltration followed by diafiltration, and drying (Fig. 4). Two protein isolates are produced: soluble and precipitated, with a combined protein recovery of more than 70% of the protein in the meal. Both products are high in protein (>90%), low in phytates (<1%), essentially free of glucosinolates (<2 μmol/g), and have desirable functional properties comparable to those of soy protein. This process is simple, economical, and seems commercially viable. Recently, it has been modified with additional membrane processing to remove phenolic compounds (47). As a result, both color and taste of the products have been significantly improved, thus making the process more attractive. The high quality of both canola protein isolates generated much commercial interest, but commercial production has not yet been initiated.

V. OTHER EDIBLE PROTEIN SOURCES AND PROCESSES

A. Cottonseed

Cottonseed kernels contain up to 50% protein. The presence of toxic gossypol in the pigment glands of cottonseeds makes the protein unacceptable for food use. Hence, all of the processes for production of cottonseed protein for human

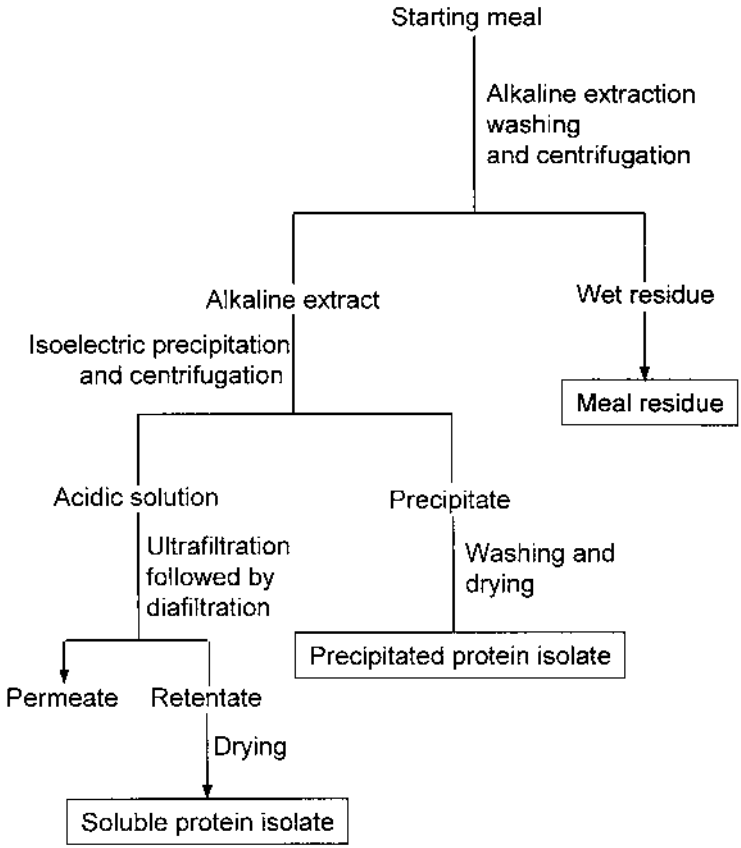

Figure 4 Membrane-based canola protein isolation process (66).

consumption involve removal of the pigment glands. A mixed solvent extraction process was developed for the simultaneous removal of the oil and pigment (48). In this process, cottonseeds are dehulled and flaked to a thickness of 0.07–0.24 mm in the conventional manner. The moisture content is adjusted to 7–15% by drying of the flakes at 60°C. The pigment glands in the meats remain intact under these conditions, so the gossypol generally does not mix with the protein of the meats. The flakes are then extracted and washed with a solvent mixture made of 53% acetone, 44% hexane or petroleum ether, and 3% water (v/v) at ambient temperature. Both oil and pigments are dissolved in the solvent mixture, and the extracted meats could have a protein content up to 90% or higher, and are essentially free of gossypol.

High protein concentrates for human consumption are also produced by a liquid cyclone process (49). Preparation of the seeds entails drying, flaking, disintegrating, and separating by screen and gravity. The cottonseed flakes are mixed with hexane to form a slurry fed to a liquid cyclone for classification and separation. The underflow from the cyclone contains essentially all of the intact pigment glands whereas the overflow carries the fine solids with the desirable protein. After filtration and drying, a protein concentrate with a protein content of 73% is obtained. There has been limited commercial development of this process.

B. Sunflower Seed

Defatted sunflower meal can also be a valuable source of protein for incorporation into food products such as breakfast cereals, processed meat products, and snack foods, as both a protein supplement and a functional ingredient. Processes for the isolation of sunflower protein have also been based on the soy protein isolation process, involving alkaline extraction of the meal to extract protein at pH 9–11, followed by acidic precipitation of protein from the extract at pH 3.5–6 (50). The recovered precipitates contain more than 90% protein. However, the products thus obtained readily turn to an undesirable green color. This is caused by chlorogenic acid, a phenolic compound in sunflower meal. Once it appears, the green color cannot be removed from the product by any conventional technique. It is, therefore, necessary to remove the color-causing compound prior to protein isolation. This can be achieved by multistage acid washing of the meal with water adjusted to acidic pH close to that for precipitation (51). Another method is ultrafiltration of the alkaline extract of sunflower meal to remove chlorogenic acid before acid precipitation of protein (52). Both treatments lead to a much lighter colored protein isolate, but the complexity and expense of these processes have thus far prevented commercialization of sunflower protein isolates.

C. Sesame Seed

Sesame seed is an excellent ancient food source. Its oil content is greater than 50%. After oil removal the meal contains about 60% protein, and its dehulled flour is even richer in protein. In commercial processing, sesame oil is extracted by expelling and/or solvent extraction before the recovery of the protein materials. However, in a patented aqueous process both sesame oil and protein were recovered simultaneously (53). The dehulled cracked seed was extracted with calcium hydroxide solution. The mixture of oil and protein was then either (a) sent to a precipitation tank where the pH was brought down to 4–5, and the resulting precipitate was separated by centrifuge and spray dried; or (b) passed

to an oil separator where the oil was removed by centrifuge, and the dissolved protein was then recovered as calcium proteinate by spray drying.

D. Peanuts

In peanut processing, it is essential to use the dehulled kernels in good condition in order to avoid aflatoxin contamination. Skins may also be removed by blanching followed by air aspiration to produce clean cotyledons. Prepress solvent extraction is commercially used for oil removal (54). Peanut kernels are ground in a hammer mill, and conditioned to desirable moisture content in a stack cooker. The cooked meats are expelled to prepress the oil. The prepressed meal is ground again and reconditioned before being extracted with hexane. The defatted flakes are desolventized in steam-jacketed tubes at temperatures increasing from 65°C to 107°C during the process. The desolventized flakes are cooled and ground to a flour that contains about 60% protein.

Processes for making full-fat and partially defatted flakes from peanut kernels involve first grinding the low-moisture kernels to a flour consistency and mixing the flour with water to form an emulsion-suspension, which is heated to high temperatures between 93°C and 116°C to inactivate lipoxygenase. The solids are drum dried as flakes. Full-fat flakes contain 50% oil and 30% protein, whereas partially defatted flakes have 30% oil and 40% protein (55–57).

E. Lupin

Lupin is a legume with a high protein content ranging from 25% to 44%, depending on the variety. In the past its use has been limited to the feed for ruminant animals due to the presence of toxic alkaloids. The emergence of genetically improved "sweet" varieties low in alkaloids are now making it possible to use lupin as a protein source in food. Lupin is attractive particularly in areas where soybeans do not grow well whereas lupin is abundant, such as in northern Europe. Like soy and rapeseed proteins, lupin protein can also be isolated by a process based on alkaline extraction followed by acidic precipitation (58, 59). The protein in defatted lupin flour is approximately 70% soluble under slightly alkaline conditions such as pH 8.6. Lowering the pH to 4–5 permits precipitation of about 80% of the soluble protein. The obtained precipitates contain more than 90% protein. Although studies show that the protein efficiency of lupin protein alone is low, it is significantly improved when used in combination with additional proteins or amino acids (58). Due to their high solubility, lupin protein isolates can be used in protein-enriched drink formulations. In Chile, lupin protein products have been incorporated into milk-based formulas for school breakfast and lunch programs since the mid-1980s (58). Extensive development work is proceeding in Denmark and Germany. However, there are several hurdles due to the high toxicity of the untreated seed.

REFERENCES

1 . MF Marcone. Biochemical and biophysical properties of plant storage proteins: a current understanding with emphasis on 11S seed globulins. Food Res Int 32: 79–92, 1999.
2. VA Portyanko, NR Sharopova, AA Sozinov. Genetic control and polymorphism of some subunits of storage globulin in cultivated oat. Cytol Gene 31:20–23, 1997.
3. DH Waggle, FH Steinke, JL Shen. Isolated soy proteins. In: RH Matthews, ed. Legumes: Chemistry, Technology and Human Nutrition. New York: Marcel Dekker, 1989, pp. 99–138.
4. WHO/FAO/UNU. Energy and protein requirements. Report of a joint WHO/FAO/UNU meeting. Technical Report Series 724. Geneva: WHO, 1985.
5. SJ Circle, AK Smith. Processing soy flours, protein concentrates, and protein isolates. In: SJ Circle, AK Smith, eds. Soybeans: Chemistry and Technology, Vol. 1, Proteins. Westport, CT: AVI, 1972, pp. 294–338.
6. L Sair. Proteinaceous soy composition and method of preparing. US Patent 2,881,076 (1959).
7. GC Mustakas, LD Kirk, EL Griffin Jr. Flash desolventizing defatted soybean meals washed with aqueous alcohols to yield a high protein product. J Am Oil Chem Soc 39:222–226, 1962.
8. FA Norris. Protein recovery from seed meal. Canadian Patent 697,264 (1964).
9. CW Kolar, SH Richert, CD Decker, FH Steinke, RJ Vander Zanden. Isolated soy protein. In: AM Altschul, HL Wilcke, eds. New Protein Foods, Vol. 5, Seed Storage Proteins. New York: Academic Press, 1985, pp. 260–299.
10. WJ Wolf, JC Cowan. Soybeans as A Food Source. Boca Raton, FL: CRC Press, 1975.
11. HE Snyder, TW Kwon. Soybean Utilization. New York: Van Nostrand Reinhold, 1987, pp. 74–144.
12. GC Mustakas, EL Griffin Jr, VE Sohns. Full fat soybean flours by continuous extrusion cooking. World Protein Resources. Am Chem Soc Adv Chem Ser 57: 101–108, 1966.
13. AM Nash, WJ Wolf. Solubility and ultracentrifugal studies on soybean globulins. Cereal Chem 44:183–192, 1967.
14. CW Hutton, AM Campell. Water and fat absorption. In: JP Cherry, ed. Protein Functionality in Foods. Washington, DC: American Chemical Society, 1972.
15. N Catsimpoolas, EW Meyer. Gelation phenomena of soybean globulins. I. Protein–protein interactions. Cereal Chem 47:559–570, 1970.
16. FW Sosulski. Organoleptic and nutritional effects of phenolic compounds on oilseeds protein products: a review. J Am Oil Chem Soc 56:711–714, 1979.
17. JE Kinsella. Functional properties of soy proteins. J Am Oil Chem Soc 56:242–258, 1979.
18. JW Knight. Wheat Starch and Gluten. London: Leonard Hill, 1965, pp. 84–105.
19. DA Fellers. Fractionation of wheat into major components. In: Y. Pomeranz, ed. Industrial Uses of Cereals. St. Paul, MN: American Association of Cereal Chemists, 1973, pp. 207–228.
20. RA Anderson. Manufacture of wheat starch. In: RL Whistler, EF Paschall, eds. Starch: Chemistry and Technology. New York: Academic Press, 1967, pp. 53–63.

21. CE Rist. Batter process makes wheat flour a source of gluten and starch. Sugar J 11(9):26–27, 1949.
22. RA Anderson, VF Pfeifer, EB Lancaster, C Vojnovich, EL Griffin, Jr. Pilot-plant studies on the continuous batter process to recover gluten from wheat flour. Cereal Chem 37:180–188, 1960.
23. RJ Dimler, HA Davis, CE Rist, GE Hilbert. Production of starch from wheat and other cereal flours. Cereal Chem 21:430–446, 1944.
24. KL Phillips, HR Sallans. Ammonia process for separating starch and gluten from wheat flour. Cereal Sci Today 11(2):61–64, 1966.
24a. ISTA. Oil World Statistics Update. Hamburg: Mielke, 1998.
25. MT Lo, DC Hill. Evaluation of protein concentrates prepared from rapeseed meal. J Sci Food Agric 22:128–130, 1971.
26. FW Solsulski, G Sarwar. Amino acid composition of oilseed meals and protein isolates. Can Inst Food Sci Technol J 6:1–5, 1973.
27. R Ohlson, K Anjou. Rapeseed protein products. J Am Oil Chem Soc 56:431–437, 1979.
27a. CH VanEtten, ME Daxenbichler, IA Wolff. Natural glucosinolates in foods and feeds. J Agric Food Chem 17:483–491, 1969.
28. B Uppström, R Svensson. Determination of phytic acid in rapeseed meal. J Sci Food Agric 31:651–656, 1980.
29. M Naczk, LL Diosady, LJ Rubin. The phytate and complex phenol content of meals produced by alkanol- ammonia extraction of canola. Lebensm Wiss Technol 19:13–16, 1986.
30. A Rutkowski, H Kozlowska. Chemical constituents and protein food processing of rapeseed. J Am Oil Chem Soc 56:475–477, 1979.
31. M Cheryan. Phytic acid interaction in food systems. CRC Crit Rev Food Sci Nutr 15:297–335, 1980.
32. A Girault. The study of some properties of rapeseed protein with a view to protein concentrate production. J Sci Food Agric 24:509–518, 1973.
33. AJ Finlayson, RS Bhatty, SL McKenzie. The effects of extraction solvents on the yields and structures of Brassica sp. meal proteins. Can Inst Food Sci Technol J 9:212–215, 1976.
34. L Gillberg, B Törnell. Preparation of rapeseed protein isolates: Dissolution and precipitation behavior of rapeseed proteins. J Food Sci 41:1063–1069, 1976.
35. FM Blaicher, F Elstner, W Stein, KD Mukherjee. Rapeseed protein isolates: effect of processing on yield and composition of protein. J Agric Food Chem 31:358–362, 1983.
36. P Pfaender. Lysinoalanine—a toxic compound in processed proteinaceous food. World Rev Nutr Diet 41:97–109, 1983.
37. RS Bhatty, SL McKenzie, AJ Finlayson. The proteins of rapeseed (Brassica napus L) soluble in salt solutions. Can J Biochem 46:1191–1197, 1968.
38. DF Owen. Detoxification and isolation of rapeseed protein by aqueous saline extraction and isoelectric protein precipitation. US Patent 3,758,452 (1973).
39. RD Siy, FDF Talbot. Preparation of low-phytate rapeseed protein by ultrafiltration: II. Membrane development and testing. J Am Oil Chem Soc 58:1021–1023, 1981.

40. TJA Finnigan, MJ Lewis. Nitrogen extraction from defatted rapeseed, with particular reference to United Kingdom commercial rapeseed meal. J Sci Food Agric 36:520–530, 1985.
41. FW Sosulski, A Bakal. Isolated proteins from rapeseed, flax and sunflower meals. Can Inst Food Sci Technol J 2:28–32, 1969.
42. E Keshavarz, RK Cheung, RCM Lui, S Nakai. Adaptation of the three stage extraction process to rapeseed meal for preparation of colorless protein extracts. Can Inst Food Sci Technol J 10:73–77, 1977.
43. AS El Nochrashy, KD Mukherjee, HK Mangold. Rapeseed protein isolates by countercurrent extraction and isoelectric precipitation. J Agric Food Chem 25: 193–197, 1977.
44. LP Kodagoda, S Nakia, WD Powrie. Preparation of protein isolates from rapeseed flour. Can Inst Food Sci Technol J 6:135–141, 1973.
45. YM Tzeng, LL Diosady, LJ Rubin. Production of canola protein materials by alkaline extraction, precipitation, and membrane processing. J Food Sci 55:1147–1151, 1990.
46. L Xu, LL Diosady. The production of Chinese rapeseed protein isolates by membrane processing. J Am Oil Chem Soc 71:935–939, 1994.
47. L Xu, LL Diosady. Removal of phenolic compounds in the production of high-quality canola protein isolates. Food Res Int 35:23–30, 2002.
48. WH King, VL Frampton. Cottonseed meal of improved quality. US Patent 2,950,198 (1960).
49. EA Gastrock, EL D'Aquin, PH Eaves. High-protein oilseed, particularly cottonseed, concentrate production by a continuous process. US Patent 3,615,657 (1971).
50. LP Hanson. Vegetable Protein Processing. Park Ridge, NJ: Noyes Data, 1974, pp. 186–214.
51. DE O'Connor. Light-colored protein isolate from sunflower meal by acid washing prior to alkaline extraction. US Patent 3,586,662 (1971).
52. DE O'Connor. Isolating light-colored protein from sunflower meal by alkali extraction under an inert gas blanket followed by membrane ultrafiltration. US Patent 3,622,556 (1971).
53. JJ Liggett. Recovery of protein and oil from oilseeds with a saturated calcium hydroxide solution. US Patent 3,476,739 (1969).
54. JG Endres. Legume protein flour and concentrates. In: RH Matthews, ed. Legumes: Chemistry, Technology, and Human Nutrition. New York: Marcel Dekker, 1989, pp. 139–160.
55. JH Mitchell. Process for making peanut flakes. US Patent 3,689,287 (1972).
56. JH Mitchell. Peanut flakes. US Patent 3,800,056 (1974).
57. JH Mitchell. Process for making flavorless food extenders derived from peanuts, and a method of recovering peanut oil. US Patent 3,947,599 (1976).
58. J King, C Aguirre, S de Pablo. Functional properties of lupin protein isolates (Lupinu albus cv Multolupa). J Food Sci 50:82–87, 1985.
59. FS Taha, SS Mohamed, AS, El-Nockrashy. The use of soya bean, sunflower and lupin seeds in the preparation of protein bases for nutritious beverages. J Sci Food Agric 37:1209–1216, 1986.