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Removal of Cholesterol from Food Products Using Supercritical Fluids

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I. INTRODUCTION

The necessity of some consumers to reduce or control weight due to health problems or for private reasons, as well as the general concerns of the population as a whole of the effects of diet on health are the major driving forces to produce foods with low fat and cholesterol contents (1). When consumers in the United States were asked in a recent survey about their concerns of food products, 61% were worried about fat and 35% about cholesterol (2, 3). The recommendation is that total fat calories in a daily diet should be limited to 30% with the saturated fatty acids contribution not to exceed 10% (4). A high cholesterol and fat concentration in the blood can lead to their precipitation in the circulatory system and cause arteriosclerosis.

Worries and concerns about cholesterol and fat on the part of the worldwide population can be appreciated when observing the decrease in the consumption of whole milk (5) and egg yolk–containing food products (6), and the resulting increase in the production of low-fat, skim milk, and cholesterol-free egg yolk products to satisfy growing consumer demands for healthier products. Butter and butter oil have, traditionally, been the main derivatives of milk processing, with skim milk being a subproduct. The shift in consumer behavior in search for light products has generated a large surplus of butter and butter oil. The surplus in butter oil reached 155×10^6 kg in 1990 and was expected to reach the 550×10^6 kg mark in 2000 (7).

Cholesterol removal and fractionation of fat can be an attractive solution to this surplus problem and a viable alternative in the production of new fat products with characteristics that meet new consumer concerns and demands. Cholesterol, a byproduct, can be used for a variety of applications, including the production of sterols, emollients, skin creams, and so forth (8).

Various technologies have been suggested for the removal of cholesterol from food products, including enzymatic conversion of cholesterol to a nonabsorbable steroid (2), steam stripping (2, 9), distillation (10), complex formations with or without adsorption (11), molecular distillation (12), melt crystallization (13, 14), complexation with β-cyclodextrins (15, 16), use of cholesterol-degrading bacteria (17), and supercritical extraction using CO_2 (18–21) and ethane as solvent (22). Rizvi and Bhaskar (19) made a comparison of the methods used for the extraction of cholesterol from milk fat and concluded that fat fractions produced with supercritical CO_2 had favorably distinct and different physical and chemical properties than those obtained by other methods. Fractionation techniques have been reviewed by Hamm (23), who divided these processes into dry crystallization, solvent fractionation, and detergent fractionation, together with the new, attractive, and promising supercritical fluid fractionation technology as an alternative.

The technique to complex cholesterol with β-cyclodextrin is applied on an industrial scale to produce low-cholesterol milk with over 90% reduction in cholesterol content (16). A low cholesterol liquid egg product (80% less in cholesterol content) has also been introduced in North America but with little success due to the high cost of the β-cyclodextrin complexation process (24).

Other processes have also been suggested for the reduction of cholesterol content in food products, but they seem to be somewhat inconvenient because they introduce significant chemical changes in the protein and triglyceride contents in the raw material (24).

Consumer concern over chemical residues in foods and increasing demand for high-quality healthy food products have rendered supercritical fluid extraction processes with solvents that are nontoxic, inexpensive, and having a relatively low critical temperature, such as carbon dioxide, one of the most promising alternatives to the use of chemical solvents in the removal of cholesterol from food products.

The solubility of cholesterol in supercritical fluids has been widely investigated (25–31). Furthermore, experimental data on the removal of cholesterol from dehydrated meat (32), chicken (33), pork (34), fish (35), eggs and derivatives (36–39), and butter oil (2, 8, 14, 40, 41) using supercritical carbon dioxide have also been reported. Cully et al. (18) registered a patent on the process for the removal of cholesterol or cholesterol esters from egg yolk powder and butter fat by extraction with compressed CO_2. The possibility of producing milk fat with 90% less cholesterol while maintaining the original color and flavor was

described by Bradley (8). The removal of cholesterol and fractionation of butter oil with supercritical fluids have been reported by Shishikura et al. (42), Rizvi et al. (19), and Mohamed et al. (22). The use of cosolvents to improve the removal efficiency in the extraction of cholesterol with supercritical CO_2 was evaluated by Saldaña et al. (21) and Singh et al. (30).

In any extraction process, it is often difficult to reconcile conditions that lead to high recovery yields with those that result in high selectivity. A possible approach that could allow this reconciliation is through the coupling of the extraction process to a highly selective separation step. Shishikura (42) obtained butter oil fractions with only 5% of the cholesterol in the initial butter using supercritical extraction followed by adsorption on silica gel. However, in this process it was only possible to obtain a maximal extracted oil yield of 50% of the original oil. Huber et al. (43) investigated selective removal of cholesterol from anhydrous milk fat using supercritical carbon dioxide in a multiseparator process in which selective removal was only possible with the use of silica gel as an adsorbent. Mohamed et al. (22) recently presented data on the simultaneous removal of cholesterol and fractionation of butter oil through a coupled extraction/adsorption process that uses carbon dioxide or ethane as the supercritical solvent and alumina as the adsorbent.

The extraction of cholesterol from animal fats with supercritical CO_2 using a descending pressure profile with fractions collected in several separators connected in series was described by Chao et al. (44).

There seems to be a substantial lack of detailed study in the literature, however, on the changes in the sensory, nutritional, and physiological properties of foodstuffs with the loss of important lipophilic components other from cholesterol during the extraction.

In what follows, we present a brief summary of the physiological effects of cholesterol on health; cholesterol analysis and solubilities in supercritical fluids; a description of extraction and fractionation of cholesterol from different food products, followed by some experimental laboratory data on the potential use of supercritical fluids for the removal of cholesterol, and future prospects in the reduction of cholesterol levels from important meals.

II. PHYSIOLOGICAL EFFECTS OF CHOLESTEROL AND IMPLICATIONS ON HUMAN HEALTH

Cholesterol (Fig. 1) is a sterol, differentiated from the main steroid ring structure by an aliphatic side chain, methyl groups, and a hydroxyl (OH) group. The presence of the OH group makes cholesterol a steroid alcohol or sterol (45).

Cholesterol is an extremely important biological molecule with a crucial role in membrane structures as well as being a precursor for the synthesis of

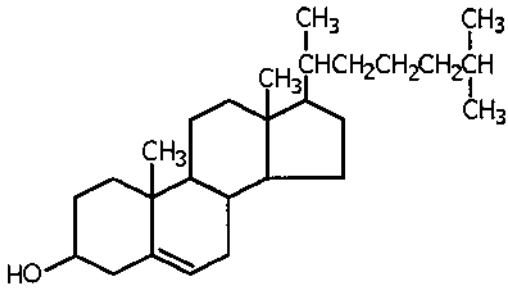

Figure 1 Cholesterol molecular structure.

steroid hormones, bile acids, and sexual hormones (46). The cholesterol molecule and the challenges faced by researchers to elucidate its role is illustrated in the work of Vance and Van den Bosch (47). In a general manner, it is well established that the human body not only synthesizes cholesterol but also absorbs it through the intestine, as cholesterol is eventually transported to the liver (48, 49).

Some of the body cholesterol is produced by hepatocytes in the liver; therefore, every cell in the body is capable of producing cholesterol (50). In the cell, cholesterol is made available through synthesis in the endoplasm reticulum and by endocytosis of cholesterol-containing ligands (46). The complex mechanisms that govern the synthesis and the distribution of cholesterol in the cells are well described in the work of Blanchette-Mackie (46). The accumulation of cholesterol in the body depends on several factors such as nutrition, stress, and obesity (51).

It is well established that a high cholesterol concentration in the blood can lead to its precipitation in the circulatory system and arteriosclerosis (52). The low-density lipoprotein (LDL) cholesterol, called "bad cholesterol," circulating in the blood can slowly precipitate and accumulate in the walls of the arteries that feed the heart and brain. Together with other substances it can form plaque, a thick, hard deposit that clogs the arteries. This condition is known as arteriosclerosis. On the other hand, one-third to one-fourth of total blood cholesterol is what is known as high-density lipoprotein (HDL), also called "good cholesterol." A high level of HDL cholesterol is believed to prevent the precipitation of the LDL part and therefore arteriosclerosis that could lead to serious health problems such as heart attacks and strokes. The association of high blood cholesterol level with heart diseases or cancer is the motivating factor in recent research on the reduction of cholesterol levels in consumed meals (53, 54).

The American Heart Association recommends that there be no more than 300 mg/d of cholesterol in daily diet. The cholesterol contents in some foods of

animal origin are presented in Table 1. The removal of cholesterol from food products without altering the composition or properties of the other constituents would be necessary for these products to be accepted by consumers in the form of low-cholesterol butter, cheese, ice cream, and others (2).

III. DETERMINATION OF CHOLESTEROL CONTENT IN FOOD PRODUCTS AND EVALUATION OF ITS SOLUBILITY IN SUPERCRITICAL FLUIDS

A. Cholesterol Compositional Analysis

Several different techniques are applied for the compositional analysis of cholesterol (56). Jiménez-Carmona and Luque de Castro (57) reported some cholesterol and cholesterol oxides analysis using gas chromatography (GC) and high performance liquid chromatography (HPLC). When using GC, an appropriate capillary column and a flame ionization detector are normally used, and before the analysis samples must be pretreated according to the standard AOAC 933.08 method (58). Recent HPLC techniques use a nonaqueous reversed-phase system with saponified or esterified derivatives (59–62). The AOAC also reported different methods for cholesterol analysis in food components such as eggs or oils and fats. The method 941.09 (63) is a titrimetric method for determination of cholesterol in eggs as a whole, while the method 43.290 (64) is recommended for determination in egg yolks. For oils and animal fats with low levels of unsaponifiable matter the AOCS official method Ca 6a-40 (65) is recommended.

As reported by Pasin et al. (66), most of the methods used to determine the cholesterol content in foods were developed with serum cholesterol procedures as the starting point. These methods can be divided into three groups: colorimetric, chromatographic, and enzymatic. The colorimetric methods were based on the Liebermann-Burchard color reaction or the Zlatkis procedures (67, 68). Some researchers have questioned the accuracy and the limitations of these

Table 1 Cholesterol Contents in Foods (mg/100 g)

Foods	Raw	Boiled
Pork	49–54	56–97
Chicken	58–80	75–124
Chicken with skin	104	139
Beef	51–52	66–67
Eggs	33–190	1000–1019

Source: Adapted from Ref. 55.

methods (66). The chromatographic methods, which include gas chromatography (69), gas–liquid chromatography (70), HPLC (71), or capillary supercritical fluid chromatography (37), require a pretreatment saponification step of samples prior to analysis. These pretreatment steps are time consuming, cumbersome, and seldom environmentally friendly, all of which place strong limitations on sample throughput (66). Pasin et al. (66) also reported on an enzymatic method for determination of total cholesterol in fresh, frozen, and dried egg yolk using a diagnostic cholesterol reagent. The results revealed that this enzymatic method could be used for the quantitative determination of cholesterol without the need for saponification. Cholesterol determination is made by dye absorbance analysis. The intensity of the color produced is directly proportional to the total cholesterol in the sample (66).

B. Cholesterol Solubility in Supercritical Fluids

In order to explore the ability of supercritical fluid extraction in the reduction of cholesterol content in foods and products of animal origin (fats, meats, egg yolk, etc.), the solubility behavior of cholesterol in supercritical solvents must be determined. Some experimental data on the solubility of cholesterol in supercritical fluids, particularly CO_2, have been presented in the literature. Some of these data, when compared with each other, show some disagreements and inconsistencies that are believed to be associated with the method used and the purity of substances employed.

Yun et al. (29) used a continuous high-pressure flow apparatus to obtain solubility data of cholesterol and triglycerides in supercritical CO_2. A similar apparatus was used by Neves (20) for the same measurements, with the data obtained found in good agreement with those of Yun et al. (29). When Yun et al. (29) compared their data with those of Chrastil (25) (who used a batch technique) and also with those of Wong and Johnston (27) (who used a micro sampling apparatus), some discrepancies were observed; these were attributed to differences in the experimental techniques used.

Chrastil (25) studied the cholesterol solubility in carbon dioxide at pressures ranging from 10 to 25 MPa and temperatures ranging from 40°C to 80°C, with 20° intervals. An increase in solubility with increase in pressure at constant temperature was observed, as expected by the resultant increase in the fluid density and consequent increase in the solvent power of the supercritical fluid. A decrease in solubility with increase in temperature at constant pressure was also observed. This is the commonly encountered phenomenon known as retrograde condensation, a characteristic of all supercritical extraction systems (72). A similar result was observed by Neves (20) at the same temperature and pressure ranges. This phenomenon is often attributed to the fact that the increase in vapor pressure of the solute with increase in temperature cannot compensate the loss of solvent power caused by the resultant decrease in solvent density (72).

Neves (20) reported that the change from retrograde to nonretrograde behavior occurred at a pressure of 16 MPa. This pressure is known as the upper crossover pressure below which the effect of vapor pressure prevails over the effect of solvent density. This behavior can be explored among the many other possibilities in determining the optimal conditions for the extraction, separation, and fractionation of solutes present in a mixture.

To correlate the solubility data, Yun et al. (29) tested several models, including a model proposed by Chrastil based on association between solute and solvent molecules to form a solvation complex. This model had successfully correlated the solubility of lipids (25). Another model proposed by Kumar and Johnston (73) provides a more realistic description of the solvent–solute molecular interactions.

The data available in the literature for the system of cholesterol-carbon dioxide (25, 28, 29, 31) revealed low solubility values at relatively low pressures, with substantial increase in solubility at high pressures and relatively high temperatures. Therefore, a viable extraction process of cholesterol with supercritical CO_2 implies high energy costs. An increase in solubility without the use of substantially high pressure and temperatures could be brought about with the use cosolvents or solvents with better affinities for cholesterol. The cholesterol molecule has a large nonpolar tail (similar to a hydrocarbon). For this reason, the use of a hydrocarbon as a solvent or a cosolvent could be an attractive alternative. The use of hydrocarbon solvents such as supercritical ethane, which has a critical temperature very close to that of carbon dioxide, a critical pressure that is lower than CO_2, and is an acceptable solvent in food processing (30), has been explored. Singh et al. (30) presented solubility data of cholesterol in supercritical ethane and in the binary solvent systems: ethane-propane (3.5 and 14 mol % propane) and ethane-carbon dioxide (3.5 and 14.5 mol % ethane) at pressures ranging from 7 to 29 MPa and temperatures ranging from 308 to 338 K. Saldaña et al. (21) presented solubility data of cholesterol in CO_2/ethane mixed solvent systems at 55°C and pressures from 12 to 20 MPa and concentrations of ethane ranging from 8 to 96 mol %. These investigations revealed the same effects of pressure and temperature on cholesterol solubility obtained earlier with CO_2 but with cholesterol solubilities that are 3 to 15 times higher than in supercritical CO_2 (25, 27, 28, 31). Results reported by Mohamed et al. (22) and Saldaña et al. (74) are presented in Figs. 2 and 3, respectively. This higher solubility of cholesterol in ethane was attributed to the larger ethane-cholesterol dispersion forces in comparison with the weaker polar interaction forces between CO_2 and cholesterol molecule. Mendes (75) and Mohamed et al. (22) reported similar observations for the β-carotene-ethane and cholesterol-ethane-butter oil systems, respectively.

As observed in Figs. 2 and 3, solubilities in mixed solvents are intermediate to those obtained with pure solvents but not linear in behavior, as the increase in cholesterol solubility is not proportional to the amount of ethane added

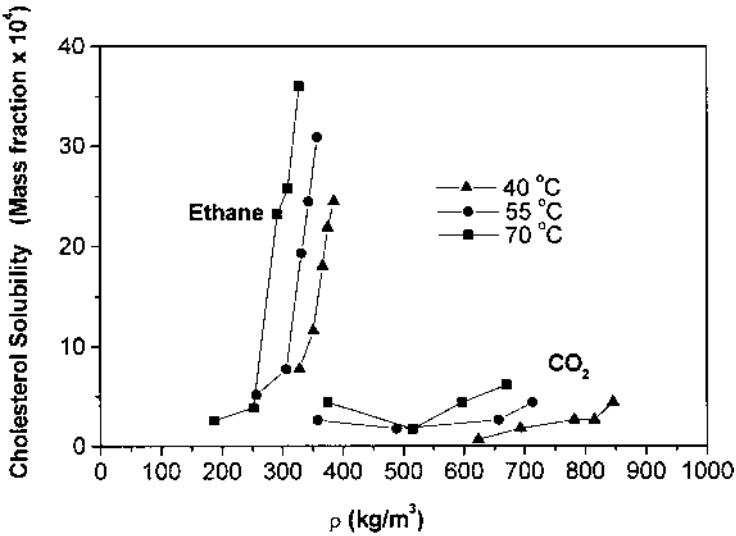

Figure 2 Cholesterol solubility in CO_2 and ethane.

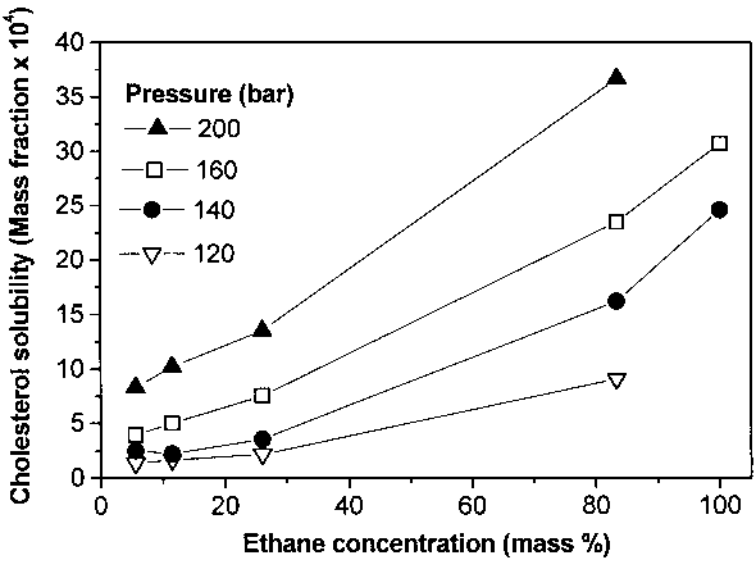

Figure 3 Solubility of cholesterol in mixtures of CO_2 and ethane at 328.15 K.

to CO_2 (21). A similar increase in solubility was observed when using propane as a cosolvent (30).

Moreover, Foster et al. (76) also observed the increase of cholesterol solubility with the increase in the concentration of acetone and hexane when added as cosolvents to supercritical CO_2 and supercritical ethane.

IV. POTENTIAL APPLICATIONS OF CHOLESTEROL REMOVAL FROM FOOD PRODUCTS

The removal of cholesterol from milk fat (77), eggs (38, 78, 79), chicken (33), pork (34), fish (35), beef (80), and others using supercritical carbon dioxide is among the extraction and fractionation operations tested in laboratory with several patents reported in the literature (9, 12, 17). While the removal of cholesterol from these products can employ organic, nonorganic, or supercritical solvents, extraction with supercritical carbon dioxide eliminates the risks of potential toxic residues due to the inert nature of CO_2 (19, 72), and thermal degradation of extracted products due to mild operating temperatures (near the CO_2 critical temperature of 31°C).

In what follows, a more detailed description of cholesterol extraction of food products using conventional and supercritical fluid solvents is presented.

A. From Milk Fat

Milk fat, a major product in the dairy industry, has been primarily used as butter. It is a good source of essential fatty acids and contains a high proportion of short-chain fatty acids, which contributes to its easy digestibility. Milk fat contains a mixture of triglycerides with a wide range of molecular weights. The most desirable property of milk fat is its pleasant flavor not found in other fats. However, the relatively high saturated fatty acids content and the presence of cholesterol in milk fat has raised nutritional and health concerns that have resulted in a substantial decrease in the direct consumption and utilization of milk fat as an ingredient in many industrial products. In order to reduce its cholesterol and saturated fatty acids contents, Arul et al. (14), Bradley (2, 8), and others suggested the fractionation of milk fat to obtain fractions with desirable physical, functional, and nutritional properties.

Milk fat fractionation is a potential technology for the development of novel ingredients with varied functional properties for use in numerous food formulations. Hamm (23) reports the possibility of three types of fractionation: (a) dry fractionation, also known as melt crystallization; (b) solvent fractionation using such solvents as acetone or hexane; and (c) detergent fractionation, wherein a surfactant solution is used to transfer the crystallized material from

the oil phase to the aqueous phase and facilitate its subsequent separation. Dry fractionation of milk fat is performed using the Tirtiaux process (81). Norris et al. (13) described the fractionation by crystallization at different temperatures (melt crystallization) with or without the use of solvents. Although the use of solvents or surfactants produces a good separation of triglycerides, these techniques are not environmentally friendly due to problems related to solvent removal and disposal. The commercial value of the secondary fraction produced in any fractionation process plays an important part in determining the viability of the overall process, and upgrading of this secondary fraction is an important part of the total production and marketing process (23).

Modification of milk fat can also be carried out using chemical methods such as interesterification (82) and hydrogenation (83), but these methods cause losses of many desirable characteristics and destroy the natural flavor of it (13).

The fact that none of the conventional methods could provide an adequate flavor concentrate has motivated the urgent search for another method for the removal of cholesterol from milk fat. Several research groups have suggested the use of supercritical CO_2 to fractionate and modify milk fat (2, 8, 14, 42, 84–87) and to remove milk cholesterol (88).

Using supercritical carbon dioxide at 20 MPa and 80°C, Kaufmann et al. (40) fractionated butter oil into a liquid fraction and a solid fraction with different cholesterol contents. Arul et al. (14) also studied the distribution of cholesterol in milk fat fractions obtained with supercritical carbon dioxide at temperatures of 50°C and 70°C and pressures varying from 10 to 35 MPa, and compared the cholesterol contents in these fractions with those in fractions obtained by distillation and crystallization. Cholesterol removal efficiency was highest with distillation followed by supercritical fluid fractionation. Bradley (2) reported that 90% cholesterol removal efficiency from milk fat at 80°C and pressures from 15.8 to 41.4 MPa is quite feasible for a broad range of dairy foods. A redistribution of cholesterol in butter oil was also presented by Chen et al. (41) in which cholesterol concentrations were increased in fractions extracted at 40°C and 10.3, 13.8, 24.1, and 27.6 MPa. A similar cholesterol redistribution in butter oil fractions extracted with supercritical CO_2 was reported by Bradley (8). Hammam et al. (89) characterized the supercritical carbon dioxide fractionation products, including the redistribution of cholesterol in such products according to their physical properties. Bhaskar et al. (90) fractionated milk fat into fractions of short chain (C_4–C_8), medium chain (C_{10}–C_{12}) and long chain (C_{14}–C_{18}) fatty acids and concluded that fractions collected at higher pressures were richer in the higher molecular weight triglycerides and that carbon dioxide displays low selectivity for cholesterol in relation to triglycerides. Shukla et al. (91) presented physicochemical and rheological properties of butter fractions obtained when using supercritical fluids for the fractionation of milk fat. The butter obtained with supercritical fluids showed a potential use at ambient and higher tempera-

tures in contrast with the oiling off and leakage problems exhibited by normally encountered market butter. Furthermore, the extracted butter was reported to be richer in unsaturated fatty acids and lower in cholesterol content (117.6 mg/100 g) than those found in commercial butter (240.6 mg cholesterol/100 g). Rizvi and Bhaskar (19) also separated milk fat into saturated and unsaturated fatty acids using supercritical CO_2, identified the physical properties, and quantified the cholesterol content. They concluded that the fractions obtained with supercritical CO_2 were unique exhibiting different characteristic physicochemical properties. They also presented a summary of all recent research on the fractionation of butter oil with supercritical carbon dioxide and a basic study for scale-up of the process.

Solubility and fractionation data of cholesterol in supercritical carbon dioxide and ethane were obtained by Mohamed et al. (22) using a high-pressure experimental extraction apparatus (92). The amounts of cholesterol in butter oil extracted using supercritical ethane were found to be much higher than when using supercritical CO_2 (Fig. 4).

Both supercritical CO_2 and melt crystallization processes produce fractions with varying physical properties (93). The advantage of using supercritical CO_2 is that the flavor components are simultaneously concentrated during the fractionation. Furthermore, solid fractions obtained with supercritical CO_2 have

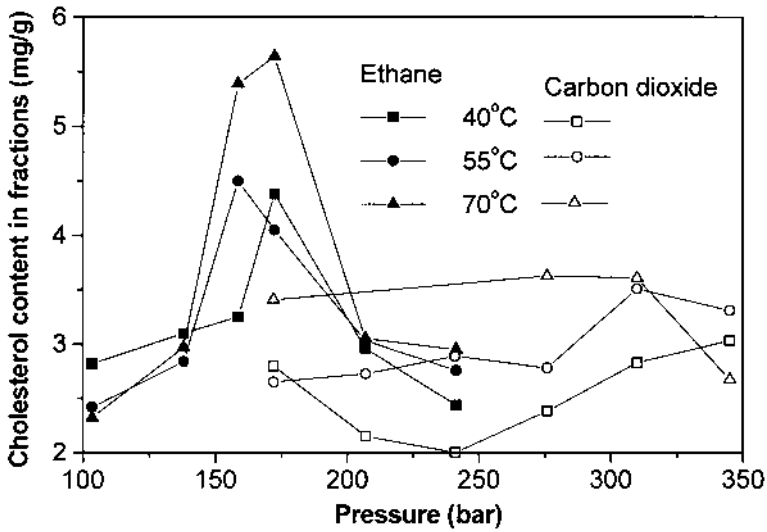

Figure 4 Cholesterol contents in fractions obtained with the extraction of butter oil using supercritical CO_2 and ethane at different pressures and temperatures.

lower cholesterol content than those obtained by melt crystallization. However, process cost favors melt crystallization (0.02–0.05 $/kg) over supercritical CO_2 extraction (0.10–0.15 $/kg) as reported in a detailed study by Singh and Rizvi (7). The lower cholesterol content and consequently higher quality of milk fat fractions obtained with supercritical CO_2 could still make this process a very viable option.

Shishikura et al. (42) concluded that the preparation of a low-cholesterol butter oil by simple extraction with supercritical CO_2 is not practical due to the observed relatively low butter oil capacity and cholesterol selectivity. They proposed the use of supercritical extraction in conjunction with an added adsorbent. Cholesterol levels were substantially reduced when passing the extract through a silica or alumina adsorbent (22, 42). Adsorption and desorption of cholesterol in continuous supercritical fluid processing of anhydrous milk fat were also reported by Lim and Rizvi (87), with magnesium silicate as the adsorbent.

The extraction/fractionation operation was also coupled with an adsorption step that uses alumina as the adsorbent (22). The combined extraction/adsorption operation resulted in the removal of more than 97% of the cholesterol in the original butter oil (Fig. 5). The increase in cholesterol content in fractions obtained in late stages is attributed to the desorption of cholesterol from the alumina bed to the supercritical fluid stream. The operation has also resulted in

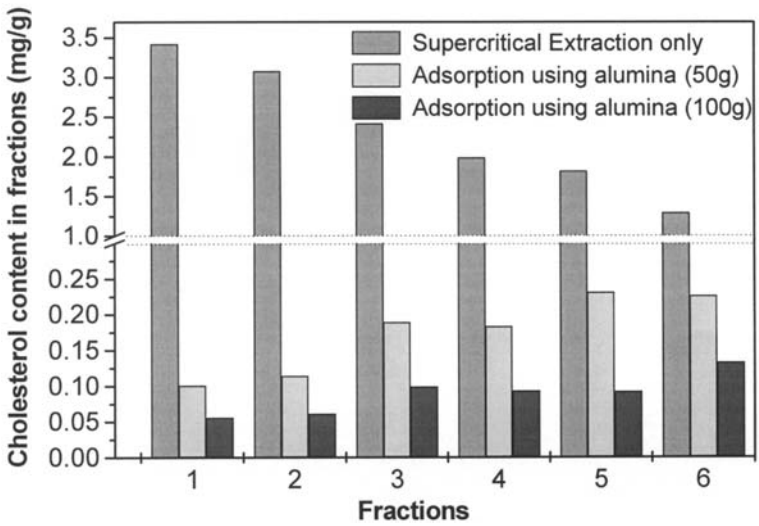

Figure 5 Cholesterol extraction curves of butter oil for the fractionation/adsorption process with supercritical ethane.

the generation of butter oil fractions with characteristic properties that are distinctly different from those of the original oil.

The detailed economic analysis for the continuous supercritical CO_2 processing of milk fat performed by Singh and Rizvi (7) and referred to earlier shows this process to be economically viable. The final processing cost can be offset by the value of the byproduct, cholesterol. Some economic analysis for continuous countercurrent processing of milk fat fractionation was also carried out by König-Schreer (93). Assuming a plant capacity ranging from 800 to 10,000 t/a for a continuous milk fat fractionation by supercritical CO_2, a payback period of about 5 years was estimated. These calculations confirm that an optimized milk fat fractionation process could be carried out on an industrial scale. The addition of an adsorption step for cholesterol would improve the economics of this process. Semicontinuous systems are not as economically favorable as continuous large-scale systems.

B. From Eggs

Eggs are one of the most complete foods consumed by humans, being an important source of high-quality protein, vitamins, and minerals (38). However, egg yolk has a high concentration of cholesterol, and concerns about cholesterol concentration in blood serums and potential health problems are the principal causes of the worldwide decrease in egg consumption. Products containing white eggs, low or no egg yolk are already available in the market, resulting in a surplus of egg yolk (78). Removal of the cholesterol would be a promising solution for this surplus problem and the return to normal whole-egg consumption.

Extraction of lipids with organic solvents for the purpose of reducing the cholesterol content in egg yolks is no longer acceptable because organic solvents are nonselective, and such extractions result in the loss of other valuable components such as phospholipids which carry out important functional properties including emulsion stabilization (38). In these processes, proteins are denatured, which impedes the use of the resultant protein concentrate (38), and toxic residues could potentially contaminate both final product and environment (79). Supercritical fluid extraction using carbon dioxide as a solvent has presented good results in cholesterol selective extraction, resulting in the extraction of approximately 70% of the cholesterol content of dried egg yolk (38) and the total removal of cholesterol from egg yolk powder (79). Therefore, the works of Paraskevopoulou et al. (94) extracted about 45% of the lipids and 75% of the cholesterol content in dried egg yolk using a continuous supercritical fluid apparatus at 31.4 MPa and 35–45°C. Phospholipids were not coextracted with cholesterol and other lipids, which is highly desirable due to their important functional and organoleptic properties. Egg yolk, both dry and degreased, exhibited

similar stability, and the concentrate had a satisfactory performance in the cake preparation.

Wu and Hou (79) extracted egg yolk powder using supercritical carbon dioxide as a solvent in a pilot plant at pressures and temperatures ranging from 25 to 36 MPa and 35 to 70°C, respectively. A complete extraction of all egg yolk oil was obtained. The egg yolk powder particle size had no influence on the extraction rate, and increasing the flow rate resulted in a faster extraction. A kinetic model developed to describe the extraction egg yolk presented results in good agreement with experimental pilot plant data.

C. From Beef, Chicken, Fish and Pork

Meats, important sources of protein in the human diet, generally have high contents of fat and cholesterol. Reduction of the fat and cholesterol contents in meat products is one of the challenges of the food industries to attend to consumer needs for a healthy diet and important for those consumers who desire to lose weight or those with a heart problem.

Supercritical fluid extraction of cholesterol and fat from chicken (33), pork (34), fish (35), and beef (80), among others, has been studied. Results have revealed the process to be efficient, in some cases resulting in the removal reducing of more than two-thirds of the original cholesterol and fat content.

Wehling (95) extracted fat and cholesterol in dehydrated beef using supercritical CO_2 at pressures and temperatures ranging from 23.4 to 38.6 MPa and 45 to 55°C, respectively. At a solvent density of 0.9 g/cm^3, about 87% of the total cholesterol and fat content from dehydrated beef powder was removed, and high temperatures favored the extraction of lipids, which were in the solid state at 45°C but were completely melted at 55°C. The authors also reported on the effects of particle size on the extraction efficiency and on the color alteration of the dehydrated beef powder due to removal of pigments, which could be very desirable as it allows the product to be a source of protein in various prepared foods. Lin et al. (34) observed this same loss in pigments during the removal of cholesterol from fried shredded pork using continuous supercritical carbon dioxide extraction. Operating at pressures and temperatures ranging from 7.3 to 34.4 MPa and 50 to 150°C, respectively, about 50–70% of the cholesterol in the meat sample was removed. The increase in temperature from 50°C to 150°C resulted in higher pigment removal, which in this case is not desirable as whiteness could be perceived to indicate a poor-quality product. Sensory analysis indicated that products obtained with these extraction conditions could not be differentiated from those bought in a local supermarket, with 34 MPa and 150°C being the optimal extraction conditions. Froning et al. (33) also demonstrated the efficiency of the supercritical fluid process in the extraction of cholesterol

and fat from dehydrated chicken meat powder and chunks, along with recovery of important flavor components from the residues (extracted lipids).

A summary of the main products containing cholesterol and their extraction with supercritical fluids is presented in Table 2. These results indicate the great potential of supercritical fluid extraction in the recovery of meat products with acceptable cholesterol and fat contents.

V. FUTURE PROSPECTS

During the past 20 years, the use of supercritical fluid technology has advanced quite rapidly. Supercritical fluids have been applied in diverse areas, including the food and pharmaceutical industries, biotechnology, new materials technologies, petroleum, among others. Removal of cholesterol contents from different food products, including fish oil capsules, foodstuffs sausages, mayonnaise, noodles, and cheese (71, 96), have been recently reported, and the determination of cholesterol contents in food products and blood serum using capillary supercritical fluid chromatography is believed to be a potential and fast analysis technique for programmed control of the optimal consumption diets.

The analytical determination of cholesterol in food, as described by Jiménez-Carmona (57) using a reverse micelle formation to accelerate the extraction of cholesterol and in which a surfactant is added to the sample from which cholesterol will be extracted, is also a potential new application.

Table 2 Supercritical Fluid Extraction of Cholesterol with CO_2 from Products of Animal Origin

				Cholesterol (mg/g)		
Product	Ref.	*P* (Mpa)	*T* (°C)	Before	After	Yield (%)
Dried egg yolk	39	16.5–37.8	40–55	18.52	6.34	65.8
Dried egg yolk	68	24.1–37.8	45–55	18.94	0.38	98.0
Dehydrated beef	86	23.4–38.6	45–55	1.56	0.19	87.8
Beef patties (cooked)	70	17.2–55.1	40–50	1.94	0.12	93.8
Pork (cooked)	34	7.3–34.4	50–150	0.80	0.22	70.1
Dried chicken meat	33	30.6–37.6	45–55	4.96	0.54	90.0
Milk fat	20	10.1–36.4	40–70	2.50	0.21	91.5
Milk fat[a]	22	8.0–24.0	40–70	2.50	0.20	93.4

[a]Using supercritical ethane as solvent.

In general, alternative processes using supercritical fluids have been claimed to be highly versatile techniques, which could be applicable in a number of situations where conventional processes could not be applied or have serious limitations. Some new applications of supercritical fluid processes for the reduction of cholesterol content include the enzymatic conversion of cholesterol to sterols that are unabsorbed by humans. In this application, a supercritical fluid as a reaction medium in enzymatic catalytic processes is considered as a means to increase the solubility of hydrophobic components and revealed to be highly successful as shown in the recent literature (97, 98). King et al. (99) reported the use of supercritical carbon dioxide as a reaction medium with lipase as a catalyst in a natural process, applicable to produce additives that can be incorporated directly in food formulations.

Some sterol esters can be added to foods acting as cholesterol-lowering agents, with important implications for the food and nutraceutical industries. Enzyme-catalyzed esterification of cholesterol using vinyl acetate as a cosolvent in supercritical ethane and pressurized hexane was studied by Sarkari et al. (97), who concluded that the enhancement in the esterification rate is a strong function of the pressure and cosolvent concentration in the system when using supercritical ethane as a reaction medium,. When using hexane, the effect of pressure was less pronounced. These facts are much important as they point to the possibility of controlling the reaction rate by choosing the appropriate solvent and pressure system.

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