Dynamic supercritical CO_2 extraction for removal of cholesterol from anhydrous milk fat

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Summary The feasibility of different extraction techniques for the selective removal of cholesterol from anhydrous milk fat (AMF) by supercritical carbon dioxide has been studied. A dynamic extraction system was used to determine both the experimental solubility of anhydrous milk fat in the supercritical solvent and the selectivity of cholesterol over anhydrous milk fat at 40–70°C and 8–40 MPa at various stages during extraction. In addition, adsorbents were used for the selective removal of cholesterol from anhydrous milk fat. The results indicate that a direct extraction alone or with several separators in series are not practical, but a selective removal of 97% of the cholesterol from the extracted anhydrous milk fat is possible by using an adsorbent with recovery of solvent and cholesterol. A schematic industrial countercurrent process for the removal of cholesterol and the fractionation of milk fat is proposed.

Keywords Countercurrent extraction, flow rate, pressure, selective adsorbtion, silica gel, temperature.

Introduction

It is generally accepted that an elevated level of serum cholesterol is an important risk factor in coronary heart disease. The European Society for Atherosclerosis has considered hypertension, diabetes mellitus, smoking and a diet rich in cholesterol and saturated fatty acids as further risk factors (International Task Force, 1992). The American Heart Association has recommended reducing the daily intake of fat, especially saturated fat and cholesterol, and modifying the composition of the fat to increase the proportion of polyunsaturated fat (Hegsted, 1991). Several National Institutes of Health have suggested a maximum intake of 300 mg cholesterol per day (Sieber, 1993; Sidhu & Oakenful, 1992).

For all these reasons, some food industries (especially those involved in dairy, egg and meat products) have been interested in reducing the cholesterol content to produce low-cholesterol products. Several processes have been studied and developed for removing cholesterol from foods: Vacuum steam (Marschner & Fine, 1989), short-path distillation (Pfaudler, 1982), molecular distillation (Bracco, 1980), melt crystallization (Norris *et al*., 1971; Arul *et al*., 1988), complexation with ß-cyclodextrins (Courrelongue & Maffrand, 1988; Roderbourg *et al*., 1990), use of cholesterol-degrading bacteria (Chosson *et al*., 1988), and supercritical fluid extraction.

The technique to complex cholesterol with ß-cyclodextrin is used industrially to produce low-cholesterol milk fat; a reduction of over 90% could be achieved (Roderbourg *et al*., 1990). The American company Michael Foods has introduced a low cholesterol egg product in the American market (Kohlrausch, 1993), but with low success. The cholesterol content of this liquid egg product was reduced by 80% using ß-cyclodextrin, but the complexation process is quite expensive with production costs of 25% (related to the raw material) calculated (Sidhu & Oakenful, 1992).

As an alternative, extraction with supercritical carbon dioxide (eventually with modifiers) could

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be considered. This technique already has industrial applications in the field of natural products, e.g. decaffeination of coffee, extraction of hops (Krukonis *et al.*, 1993; Stahl *et al.*, 1988) and spices (RAPS & Co, 1993). Carbon dioxide has many advantages for processing of natural products intended for human food, because it is easily removable, non-toxic, non-flammable, non-corrosive, non-oxidizing, cheap and available in large quantities and high purity. It has a critical pressure of 7.38 MPa and a very low critical temperature of 31.1°C, which make it suitable to extract food ingredients.

The extraction of cholesterol from anhydrous milk fat (AMF) using supercritical carbon dioxide has been widely investigated (Arul *et al.*, 1988; Kankare & Antila, 1989; Chen *et al.*, 1992). Anhydrous milk fat is a complex mixture of triglycerides with a wide range of molecular weights and degree of unsaturation. The solubility of cholesterol and AMF in supercritical carbon dioxide has also been intensively studied (Wong & Johnston, 1986; Yeh *et al.*, 1991; Kosal *et al.*, 1992; Yu *et al.*, 1992) and the selectivity of cholesterol over AMF has been calculated (Yu *et al.*, 1992).

To selectively extract cholesterol from AMF, the AMF was dissolved in a supercritical fluid and this supercritical mixture (AMF, CO_2) was passed through a suitable adsorbent (Shishikura *et al.*, 1986; Cully *et al.*, 1989; McLachlan *et al.*, 1990; Lenhard-Lubeseder *et al.*, 1992). The extraction of cholesterol from a supercritical mixture (animal fat, CO_2) using a descending pressure profile in several separators, connected in series, was suggested (Chao *et al.*, 1991), but the results were unsatisfactory (Chao *et al.*1993).

Earlier studies have largely concentrated on the solubility of AMF (and cholesterol) in supercritical CO_2, as well as on the selectivity of cholesterol both at the final stage of the extraction using static or dynamic systems. The present work relates to dynamic systems which investigate the solubility of AMF and the selectivity of cholesterol both at various stages during extraction; the effects of temperature, pressure and flow rates are investigated. The use of selective adsorbents with different particle sizes is also investigated. The findings are used as the basis for a schematic proposal for a countercurrent process for milk fat fractionation and in-line cholesterol removal from AMF.

Materials and methods

Anhydrous milk fat (AMF)

AMF was prepared by melting cream (fat content: 80%) and removing the water by centrifugation. The water content of the AMF was 1% (Karl–Fischer titration) and the cholesterol content was 0.24%. The AMF was stored at −18°C for subsequent experiments.

Extraction equipment and operating conditions

A stainless steel high pressure extraction plant (Nova Werke AG, Effretikon, Switzerland) was used throughout the course of this investigation (Fig. 1). The apparatus is composed of a diaphragm-type compressor (C), a 0.2 L extractor for liquids (i.d. 55 mm), a 0.2 L extractor for solids (i.d. 40 mm) and two 0.2 L separators (i.d. 40 mm) connected in series. Magnetic stirring (MS) in the liquid-extractor vessel is possible. The pressures in the extractors and in the separators were regulated to ±1% by pressure regulators (PCV), and were measured with pressure gauges (PI). The vessel temperatures were set and maintained at ±1°C by connecting the heat exchangers (HE) with the thermostatic bath (TB-1–3) and measured with thermocouples (TI). The solvent flow rate was regulated by the micrometering valve (FMV). The flow rate of CO_2 and the total flow were measured with a combined mass flow meter/flow totalizer (FI).

Operating conditions varied from 8 MPa to 40 MPa and from 40°C to 70°C. The CO_2 flow rate varied from 8.5–17.0 g CO_2 min^{-1}. Magnetic stirring in the liquid-extractor vessel was used for all experiments. For each run 88 g of AMF was loaded into the liquid extractor.

The experimental studies were carried out in a CO_2 atmosphere which prevents oxidation.

Direct extraction of cholesterol

To investigate the feasibility of a direct extraction of cholesterol, the solubility of AMF in super-

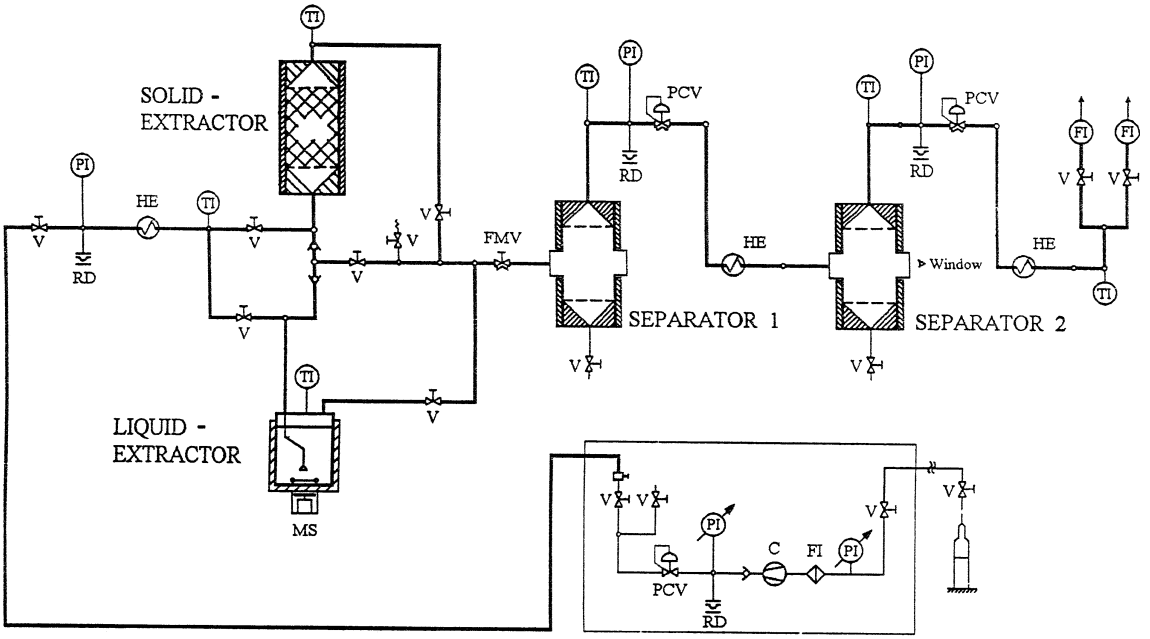

Figure 1 Flow Diagram of the High Pressure Extraction Plant. PI: pressure gauges, C: Compressor, PCV: pressure regulator, V: shut-off valve, HE: heat exchanger, MS: magnetical stirring, FMV: micrometering valve, FI: flow meter/flow totalizer, TI: temperature controller.

critical CO_2 and the selectivity of cholesterol over AMF were determined as a function of the extracted milk fat (cumulative amount of milk fat removed during extraction). The extraction pressure varied from 20 to 40 MPa and the temperature from 40 to 70°C. No floating of the AMF was possible, because, in these conditions, the density of CO_2 was always lower than the density of AMF. The CO_2 flow rate through the extractor was 8.5 or 17.0 g CO_2 min^{-1}. The first separator was at ambient pressure and 40°C.

The solubility of AMF Sol_{AMF} in supercritical CO_2 was defined as:

$$Sol_{AMF} = W_{AMF}/W_{CO2}$$

where W_{AMF} is the weight of the extracted AMF (cumulative amount of milk fat removed during extraction) and W_{CO2} is the weight of the used CO_2.

The selectivity of cholesterol over the AMF ($S_{C/AMF}$) was defined as:

$$S_{C/AMF} = Chol_S/Chol_{AMF0}$$

where $Chol_S$ is the cholesterol content in the separated milk fat fraction and $Chol_{AMF0}$ the cholesterol content in the used AMF sample ($Chol_{AMF0}$.: 0.24%)

Extraction of cholesterol using separators in series

In a second experiment, the solubility of AMF and the selectivity of cholesterol as a function of the pressure were determined by dissolving the AMF at 40 MPa and 70°C in CO_2 (solvent flow rate: 12 g CO_2 min^{-1}). Then, the dissolved AMF was fractionated by two separators connected in series. The pressure profile in the first separator varied from 8 to 25 MPa and from 40 to 70°C, respectively, while the second separator was maintained at ambient pressure at 40°C. As defined above, selectivity of cholesterol and solubility of AMF were calculated from the milk fat fraction obtained in the second separator. No raffinate was obtained in the liquid-extractor.

Removal of cholesterol by adsorbents

The separation of cholesterol from AMF with different adsorbents ($CaCO_3$, silica gel) was inves-

tigated by dissolving the AMF in the liquid-extractor (40 MPa, 70°C, solvent flow rate: 12 g min^{-1}). The dissolved AMF was then passed isobarically and isothermally through an adsorbent in the solid-extractor. The AMF was separated in the first separator maintained at ambient pressure and 40°C. No raffinate was obtained in the liquid-extractor.

Cholesterol analysis

The cholesterol content in milk fat (AMF) was determined by Supercritical Fluid Chromatography (SFC). Both the sample preparation (including saponification and extraction) and the SFE procedures were previously developed and validated by the authors (Huber *et al.*, 1995). A Lee Scientific Supercritical Fluid Chromatograph (model 602, Dionex) equipped with a timed-split injector, a flame ionization detector and a 20 m × 50 μm i.d. capillary column (SB Phenyl 5; 0.25 μm film thickness) was used with carbon dioxide (UN 1013 SFC Grade, Scott Speciality Gases, USA) as carrier. Ninety-eight per cent purity cholestane (Aldrich) was used as internal standard. The accuracy and precision of the analytical method was determined by analysing reference material (CRM 164) from the Community Bureau of Reference in Brussels, Belgium. The relative error related to the indicated value of the reference material (2.73 mg g^{-1}, ±0.39 mg g^{-1}) was only 1%. The reference material was analysed at the beginning of our experimental work and after 6 months. We did not find any significant changes ($P > 0.05$, Analysis of Variance) in the results of the analysed reference material (Huber *et al.*, 1995).

Results and discussion

In Fig. 2 the solubility of AMF in supercritical CO_2 is plotted against the extracted milk fat (cumulative amount of milk fat removed during extraction) at different pressures, temperatures and solvent flow rates. Under these operating conditions the solubility of AMF decreases as the amount of the extracted milk fat increases. The solubility of AMF increases with increasing pres-

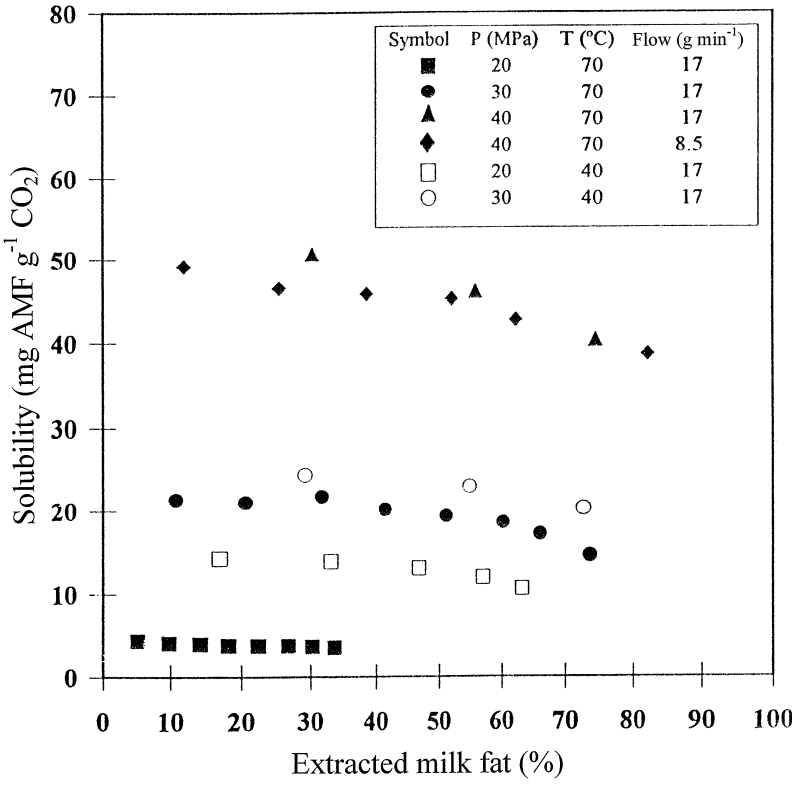

Figure 2 Solubility of milk fat (AMF) in supercritical carbon dioxide at different pressures, temperatures and flow rates as a function of the extracted milk fat (cumulative amount of milk fat removed during extraction).

sure at constant temperature, but decreases as temperature increases at constant pressures for operating conditions. This decrease of the solubility of AMF with the temperature is much less at higher pressure zones (30 MPa) than at lower pressure zones (20 MPa), because of the balance of the density of the gas and the effect of the vapour pressure of triglycerides on solubility. This solubility behaviour of AMF was also reported by other authors (Chen *et al.*, 1992).

Figure 3 shows the selectivity of cholesterol over AMF as a function of the extracted milk fat (cumulative amount of milk fat removed during extraction) at a constant solvent flow rate (17 g CO_2 min^{-1}). Selectivity of cholesterol increases as the amount of the extracted milk fat increases, decreases with increasing pressure at constant temperature, and increases as temperature increases at constant pressure. However, these results indicate that the selectivity of cholesterol is too poor for a direct extraction of cholesterol from AMF. In addition, the solubility of AMF is very poor (<0.5%) at highest selectivity conditions (20 MPa, 70°C). Therefore, a direct extraction is not feasible.

To study the feasibility of the extraction of cholesterol using separators in series, the solubility of AMF and the selectivity of cholesterol over AMF for pressures below 25 MPa and 40–70°C has been investigated. To get a high solubility of AMF and therefore rapid extraction, the AMF was dissolved at 40 MPa and 70°C in supercritical CO_2. The dissolved milk fat was then fractionated into two fractions. In Figs 4 and 5 the solubility of AMF and the corresponding selectivity of cholesterol are plotted against the pressure in the first separator, at different temperatures. The solvent flow rate was constant at 12.0 g CO_2 min^{-1}.

Figure 4 shows that solubility data are consistent with the results reported in Fig. 2. The solubility of AMF increases with increasing pressure at constant temperature, but decreases as temperature increases at constant pressures. Also, selectivity data of cholesterol reported in Fig. 5 are consistent with selectivity data in Fig. 3. The

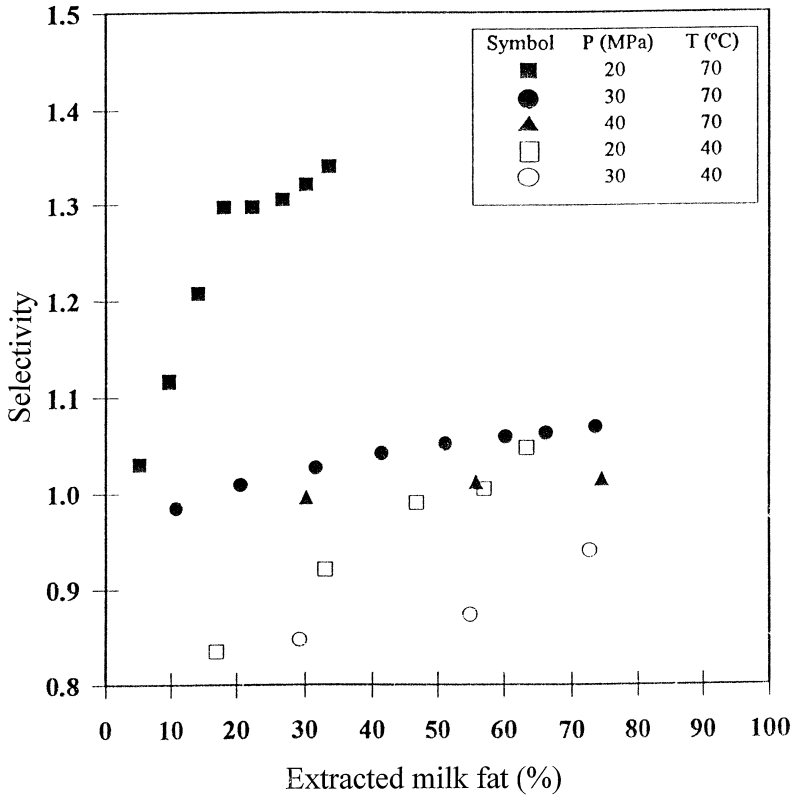

Figure 3 Selectivity of cholesterol over milk fat (AMF) as a function of the extracted milk fat (cumulative amount of milk fat removed during extraction) at different pressures, temperatures, but at a constant flow rate of 17 g CO_2 min^{-1}.

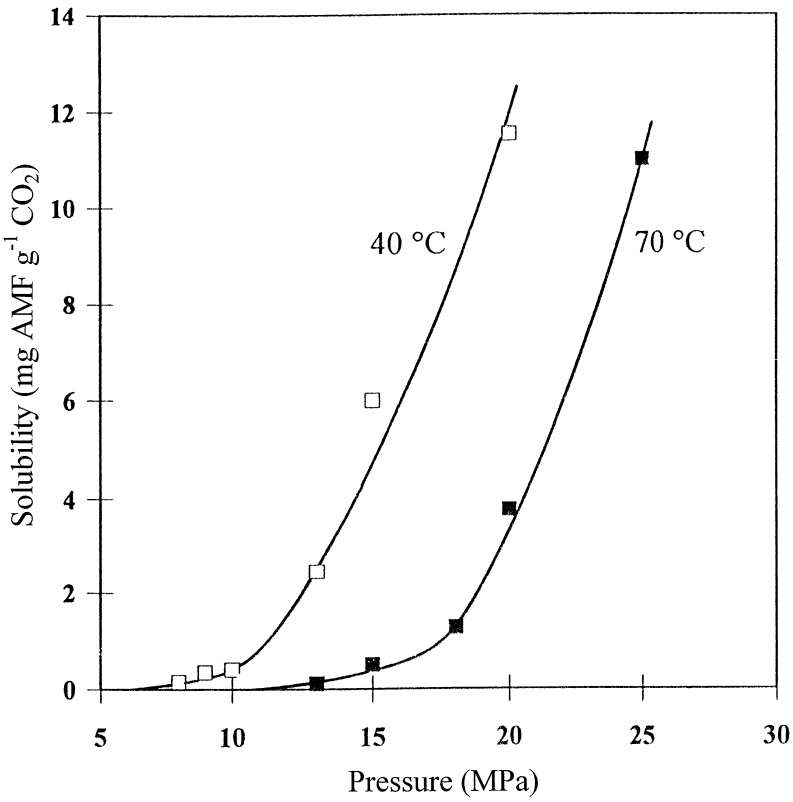

Figure 4 Solubility of milk fat (AMF) as a function of the pressure at different temperatures and at a constant flow rate of 12 g CO_2 min^{-1}.

selectivity of cholesterol decreases as pressure increases at constant temperatures and increases with increasing temperature at constant pressures. Table 1 shows the selectivity of cholesterol and the solubility of AMF at different flow rates. The results indicate that the experiments were carried out in equilibrium conditions because neither the solubility nor the selectivity were different at different solvent flow rates. There is good agreement between the solubility data obtained by using our dynamic flow apparatus and those obtained by using a static recirculation apparatus (Yu *et al.*, 1992). In their work the optimum selectivity for cholesterol over the triglycerides was achieved between 8 and 12 MPa at 40–60°C.

Table 2 shows the removal of cholesterol from AMF by using different types and sizes of adsorbents. Silica gel with a particle size of 5–40 μm is a suitable adsorbent to achieve a selective removal of 97% of cholesterol from the extracted AMF at 40 MPa, 70°C and at a flow rate of 12.0 g min^{-1}. The use of silica gel with a particle size of 1–3 mm removes much less cholesterol (about 18%). Obviously, small particle sizes are favourable at high solvent flow rates. Calcium carbonate (particle size: 50–100 μm) has little effect under these conditions.

Table 1 Selectivity of cholesterol and solubility of AMF at different CO_2 flow rates (operating conditions: 10 MPa, 40°C)

Flow rate (g CO_2 min^{-1})	Selectivity	Solubility (mg AMF g^{-1} CO_2)
6	1.45	0.41
12	1.40	0.40
18	1.41	0.43

Table 2 Removal of cholesterol from AMF using different sizes and types of adsorbents (40 MPa, 70°C; flow rate: 12 g CO_2 min^{-1})

Adsorbent	Particle Size	Cholesterol Removal
Silica gel	5–40 μm	97.3%
Silica gel	1–3 mm	18.2%
$CaCO_3$	50–100 μm	9.8%

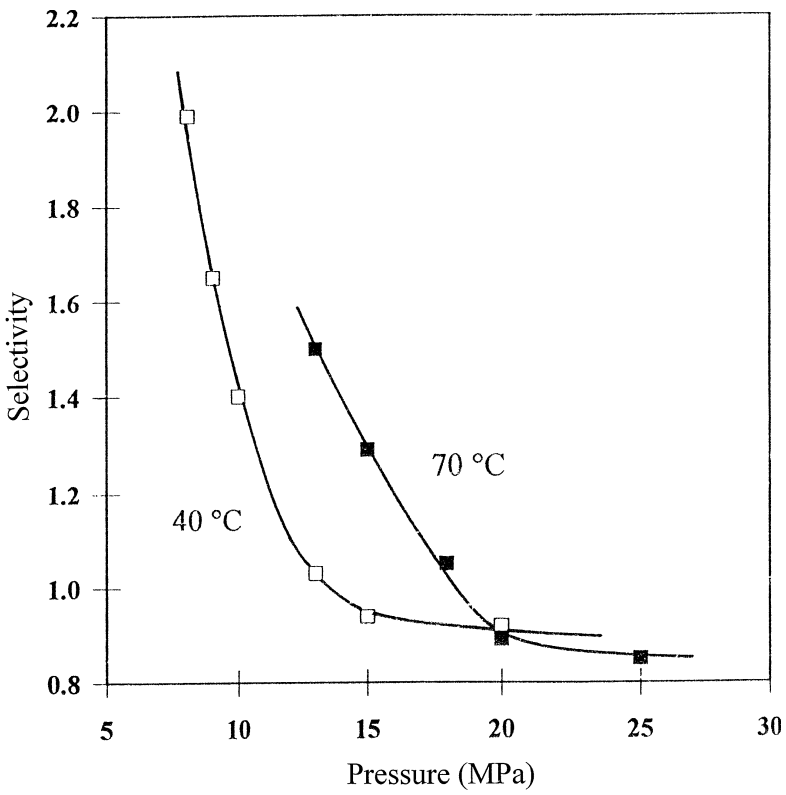

Figure 5 Selectivity of cholesterol over milk fat (AMF) as a function of the pressure at different temperatures and at a constant flow rate of 12 g CO_2 min^{-1}.

In conclusion, we propose a schematic process which could be used to produce different fractions of triglycerides low in cholesterol, low-cholesterol milk fat, and cholesterol (Fig. 6). In the proposed process, the AMF would be extracted by supercritical carbon dioxide under high solubility conditions for AMF (e.g. 40 MPa, 70°C) to achieve rapid extraction. The dissolved AMF would be passed isobarically and isothermally through a high pressure column, filled with a suitable adsorbent (e.g. silica gel), to eliminate cholesterol. The supercritical mixture (low-cholesterol milk fat, CO_2) could then be fractionated by a descending pressure profile (and/or by an ascending temperature profile) in separators connected in series (Bhaskar *et al.*, 1993). The adsorbent could then be regenerated by pumping ethanol at 50°C through the adsorption column. The ethanol could be evaporated to recover both ethanol and cholesterol (Kankare & Alkio, 1993) and silica gel could be regenerated by heating at 105°C for about 12 h. The gaseous carbon dioxide can be liquefied by using a refrigeration system (in combination with a high pressure pump) or reused directly (in combination with a compressor), but a production plant must use liquid CO_2 for energy efficiency.

Some economic analysis for milk fat fractionation was carried out. Assuming a plant capacity of 800–10000 tons $year^{-1}$ for a continuous milk fat fractionation, a payback period for the fixed capital of 5 years was calculated (Rizvi *et al.*, 1994). Another author has calculated manufacturing expenses between \$0.7 and \$4.0 per kg milk fat fraction (plus costs for AMF) in a batch process assuming a plant capacity of 10000 tons per year (König-Schreer, 1994). These calculations confirm that an optimized milk fat fractionation process could be carried out on an industrial scale. The addition of an in-line adsorption step for cholesterol would improve the economics of this process.

A low-cholesterol milk fat could be obtained by homogenizing the raffinate (insoluble milk fat) with the very low-cholesterol milk fat obtained after the adsorption step, but before fractionation. To operate an industrial process economically, only about 80% of the AMF should be dissolved in the supercritical fluid (less time and energy consumption). Therefore, a part of the

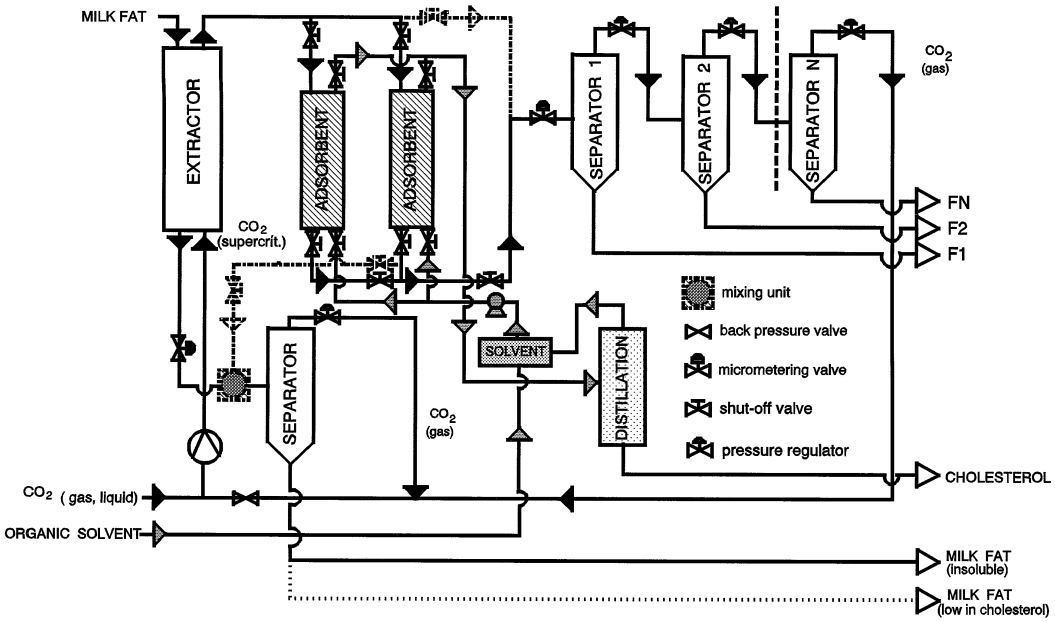

Figure 6 Schematic diagram of a possible industrial plant for producing different fractions of triglycerides (F1,F2,FN) very low in cholesterol, low-cholesterol milk fat, and cholesterol (by-product).

AMF (yield: about 20%) will not be dissolved in the supercritical solvent (raffinate). This raffinate is low in cholesterol. A cholesterol reduction of 51% for the raffinate at 24 MPa and 40°C was reported (Bhaskar *et al.*, 1993).

However, it is more interesting to use the proposed process to produce milk fat fractions (low in cholesterol) rather than low-cholesterol milk fat, because of following reasons: (i) the revenues from sales are higher, because milk fat fractions have a high value as additives for pastry or dairy products; (ii) it is technically difficult (and expensive) to homogenize the raffinate (insoluble milk fat) with the very low-cholesterol milk fat to get a low-cholesterol milk fat.

Conclusions

A direct supercritical extraction of cholesterol from AMF is not feasible, because (i) the selectivity of cholesterol over AMF is relatively low over a wide range of operating conditions and; (ii) even at the best selectivity conditions (8–10 MPa, 40°C) the solubility of AMF is poor. Moreover, under these conditions, important milk flavours were separated with the cholesterol (de Haan *et al.*, 1990; Haring, 1988).

For the same reasons, the separation of cholesterol from AMF dissolved in supercritical carbon dioxide using a descending pressure profile in separators in series is not feasible.

By contrast, a process for removal of cholesterol from AMF dissolved in supercritical CO_2 using suitable adsorbents (e.g. silica gel) shows good results and is technically possible. To get commercially acceptable flow rates, small particle sizes of silica gel (e.g. 5–40 μm) are required.

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Received 6 June 1995, revised and accepted 13 March 1996