Removal of Cholesterol from Milk Fat Using Supercritical Carbon Dioxide[1]

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ABSTRACT

The ability of supercritical fluids to dissolve materials of low volatility was reported 109 yr ago. This technology has received increased attention lately because of increased energy costs, questioned use of organic solvents, and separation problems that defy solution. Supercritical fluids have enhanced solvent power as a liquid but also behave as gases with no surface tension. Selectivity of supercritical CO_2 for cholesterol is a temperature-dependent and pressure-dependent phenomenon.

In studies using ascending pressure profile extraction, milk fat contained in the extraction chamber was stripped of 90% of its cholesterol. Efficiency of separation was measured on each fraction collected at each pressure interval. Cholesterol was assayed using an AOAC procedure and gas chromatography. Distribution coefficients and selectivities calculated for two processes that were ideal in separation efficiency indicated that cholesterol can be effectively separated. Fatty acid composition plays a key role in supercritical extraction; short-chained acids complicate extraction.

DISCUSSION

The record of the dairy industry is indelibly clear in its responsive actions to consumer demands. One has to reflect on the content of a dairy product display case to find products that are results of consumer influence. For example, whole milk was all that could be found many years ago, but this has been supplanted by 2%, 1%, and skim milk. Light cream gave way to half-and-half and sour cream to sour half-and-half. Finally, lower fat products have been developed in the cottage cheese, cream cheese, and recently, the ice cream areas. Survival might have been the rationale behind the move to lower fat and to broaden the scope of products in the display case, but regardless, the consumer was served. Other evidence of consumer demand and the responsiveness of the dairy industry is in the area of tamper-evident packaging.

One should also consider the impact on the consumer of "cholesterol-free" advertising on sales of animal-based products. One hard hit product is butter with a loss of 50% of its market in 25 yr. Another contributing factor is that Americans consume on an average of 500 to 600 mg/d of cholesterol. This is diametrically opposed to the dietary requirements set forth by the American Heart Association, which recommends no more than 300 mg/d of dietary cholesterol. Emphasis and awareness concerning dietary intake of cholesterol is continuing with advertising and with mass cholesterol screening programs scheduled for shopping malls even through there is insufficient evidence to link dietary intake of cholesterol and blood serum levels in the general public.

Perhaps a valid picture of the thinking of the consumer can be expressed by a survey reported in the *Wall Street Journal* (1). The survey asked consumers how much they were concerned about various items in food. The survey suggests consumers have a basic distrust of the food industry and a marked fear of food fats and cholesterol. Sixty-one percent of consumers surveyed judged fats to be a serious health hazard and 35% thought fats in the diet were somewhat of a health hazard. For cholesterol, 59% thought cholesterol in the diet was a serious health hazard and 35% thought it was

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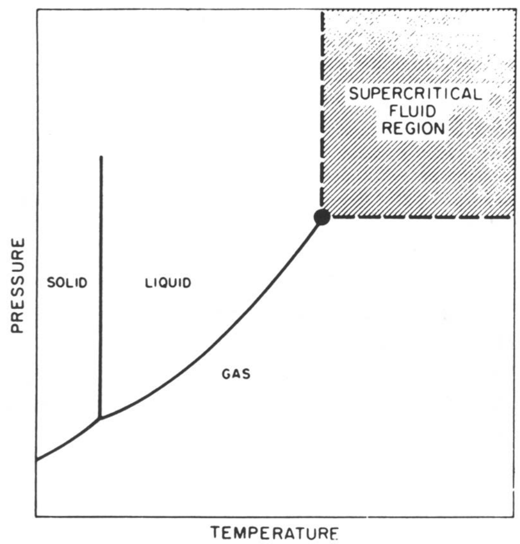

Figure 1. Pressure-temperature phase diagram for a pure component (4). Reproduced, with permission, from M. A. McHugh and V. J. Krukonis, *Supercritical Fluid Extraction: Principles and Practice* (Stoneham, MA: Butterworth Publishers, 1986).

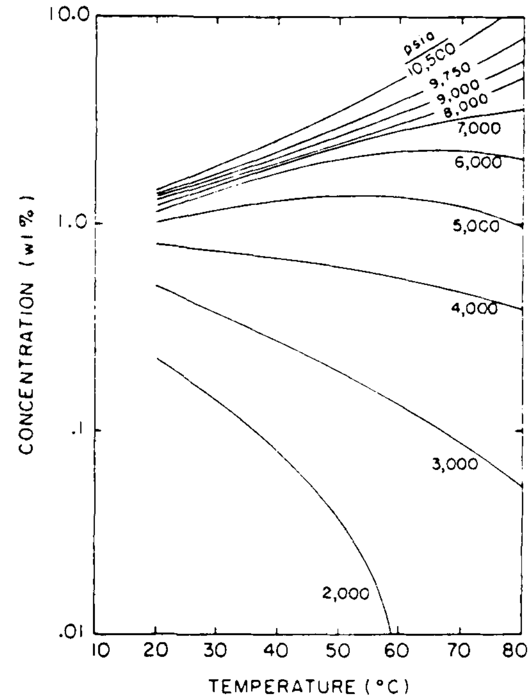

Figure 3. Solubility behavior of soybean triglycerides at various pressures and temperatures (4). Reproduced, with permission, from M. A. McHugh and V. J. Krukonis, *Supercritical Fluid Extraction: Principles and Practice* (Stoneham, MA: Butterworth Publishers, 1986).

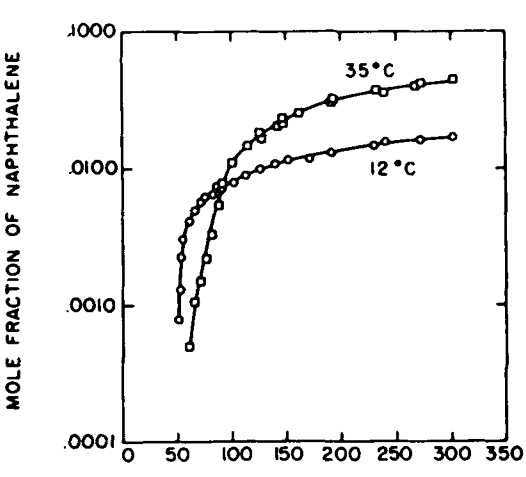

Figure 2. Solubility behavior of naphthalene in supercritical ethylene (2, 4). Reproduced, with permission, from M. A. McHugh and V. J. Krukonis, *Supercritical Fluid Extraction: Principles and Practice* (Stoneham, MA: Butterworth Publishers, 1986).

somewhat of a hazard. Thus, removal of cholesterol from products like milk fat without altering the composition or properties of the fat would appear to place milk fat in an enviable position in the marketplace.

The process used for this extraction is a simple distillation conducted under pressures of up to 492 kg/cm^2. Carbon dioxide in the supercritical state, thus a liquid, is the extractive solvent. The theory underlying this practice is not new; it is only the practical application that is the emerging technology. In fact, the subject of the solubility of solids in gases was reported in 1879 by Hannay and Hogarth (3).

The motivation behind this new separation process is because of projected increases in energy costs, increased regulatory scrutiny of common industrial solvents like chlorinated hydrocarbons, more stringent pollution controls, and increased performance demands on materials.

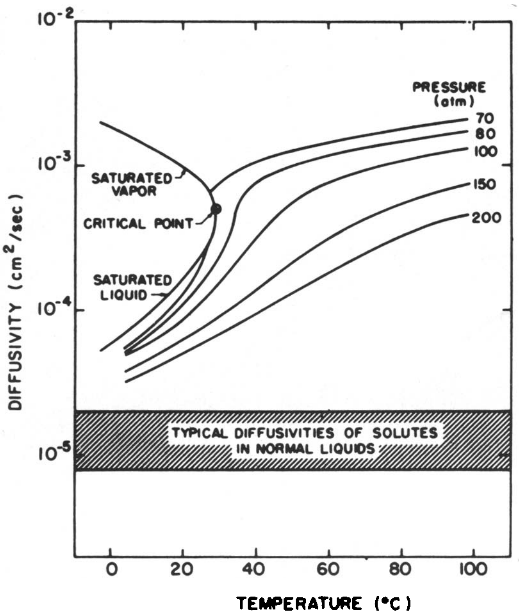

Figure 4. Diffusivity behavior of carbon dioxide (4). Reproduced, with permission, from M. A. McHugh and V. J. Krukonis, *Supercritical Fluid Extraction: Principles and Practice* (Stoneham, MA: Butterworth Publishers, 1986).

Supercritical extraction using principally carbon dioxide is in use in many laboratories across this country, Europe, and Japan. Bench top units are capable of handling up to about 400 g of feedstock, and separation of components is through an increasing pressure profile. There are a few pilot units that truly simulate commercial scale in that large quantities of product can be processed and descending pressure profiling is used.

Commercially, supercritical extraction is in use by the food industry for coffee decaffination, removal of bitter flavor from hops, isolation of essential oils from spices, and purification of omega-3 fatty acids from fish oils. However, more effort has been spent on coffee decaffination than on any other food product to date.

Fundamentals

A supercritical fluid is any fluid at a temperature above its critical value. A phase diagram for a pure component such as carbon dioxide is shown in Figure 1. The solid-liquid and gas-liquid temperature/pressure lines ascend from the triple point to the critical point. At the critical point the properties of both liquid and gas are the same. As a supercritical fluid it has enhanced and unique solvent powers with high density, low viscosity, and no surface tension to provide for easy penetration and diffusion into the food material to be extracted.

Another unique feature of supercritical fluid is that it can show a wide spectrum of solvent characteristics so that it can be fine-tuned to a specified solubility behavior as shown by the curves in Figure 2. The solubility of naphthalene in supercritical ethylene (2, 4) increases dramatically as pressure is raised from 53 kg/cm^2. At 53 kg/cm^2 there is very little solvation, but at pressures greater than about 91 kg/cm^2, the solubility of naphthalene reaches a limiting value of about 1.5 mol/100 mol. Consider the effect when the temperature of the reaction is changed to 35 C and the solvation power is enhanced as might be expected reaching a limiting solubility value of about 5 mol/100 mol. Thus, as expected, there is a pressure-dependent and temperature-dependent solubility characteristic. To expand on this aspect, consider the solubility of soybean triglycerides in supercritical CO_2 (Figure 3). Again, both pressure and temperature alterations would be considered in any supercritical extraction regimen to alter the extraction of triglycerides (4).

TABLE 1. Critical conditions for various supercritical solvents (4).

Solvents	Critical temp.	Critical pressure
	(°C)	(atm)
Carbon dioxide	31.1	72.8
Ethane	32.3	48.2
Ethylene	9.3	49.7
Propane	96.7	41.9
Propylene	91.9	45.6
Cyclohexane	280.3	40.2
Isopropanol	235.2	47.0
Benzene	289.0	48.3
Toluene	318.6	40.6
p-Xylene	343.1	34.7
Chlorotrifluoromethane	28.9	38.7
Trichlorofluoromethane	198.1	43.5
Ammonia	132.5	111.3
Water	374.2	217.6

TABLE 2. Selectivities and distribution coefficients for supercritical extraction trials involving butter.

Trial no. and fraction	Selectivity	Distribution coefficient ($\times 10^3$)
WM14		
1	.66	. . .
2	5.84	3.60
3	5.31	3.90
4	2.81	6.40
5	1.32	10.0
6	.13	. . .
WM21		
1	. . .	. . .
2	.06	. . .
3	6.27	1.50
4	2.92	4.40
5	.72	15.10
6	.16	0
Fish oil		
C_{18}:C_{20}	1.82	
C_{20}:C_{22}	1.65	

The diffusivity behavior of CO_2 is unique over a wide range of pressures and temperatures (Figure 4). For comparison, the typical ranges of diffusivities for solutes in normal liquid solvents is about 10^{-5} cm^2/s. The self-diffusion coefficient for CO_2 is one to two orders of magnitude higher than the diffusivity of solutes in liquids (4).

There are a number of gases and liquids that can be used as supercritical fluids, even water can be a supercritical fluid (4). These are shown in Table 1 along with their critical temperatures and pressures. Clearly, only CO_2 is uniquely inexpensive, nearly as abundant as water, nontoxic in any concentration, nonflammable, noncorrosive, and with its low critical temperature, an attractive solvent.

Processing

Various milk fat products have been evaluated to find the ideal fat source: whole milk, 40% cream, 80% plastic cream, butter, and butter oil made from these creams. The results of supercritical extraction indicate that plastic cream and butter are nearly ideal sources. Another consideration is that the pH in the extractor is about 3.0; this, casein will coagulate.

Two pieces of information are necessary to develop a process flow pattern and preliminary equipment sizing and configuration; the information needed is the distribution coefficient of cholesterol in supercritical CO_2 and the selectivity of CO_2 for cholesterol relative to the triglycerides in butter. These can be calculated given enough data. The distribution coefficient influences the recycle ratio of fluid, the solvent-to-feed ratio, and the diameter of the extraction column; while the selectivity influences the

TABLE 3. Response of cholesterol in butter to treatment by supercritical carbon dioxide.

Trial no. and temperature	Fraction	Pressure	Standard liters CO_2/fraction	Fraction weight	Cholesteroi in milk fat
		(psig)		(g)	(mg/g)
WM-14, 80°C	1	2300	500.00	.86	0
	2	2500	500.00	.13	11.66
	3	2800	300.00	.35	10.62
	4	3050	300.00	.51	5.65
	5	3350	300.00	1.01	2.68
	6	3750	300.00	.99	1.43
	7	6000	300.00	2.05	.25
	Control			6.10	2.02
WM-21, 80°C	1	2300	500.00	.65	.16
	2	2500	1500.00	.51	12.42
	3	2800	1000.00	.85	4.93
	4	3050	300.00	.68	1.44
	5	3400	300.00	.54	.31
	6	6000	300.00	2.65	0
	Control			6.01	2.14

TABLE 4. Fatty acid content in butter oil fractions obtained by CO_2 extraction at 60°C.

Fraction	Fatty acids (mg/g fat)												
	$C_{4:0}$	$C_{6:0}$	$C_{8:0}$	$C_{10:0}$	$C_{12:0}$	$C_{14:1}$	$C_{14:0}$	$C_{15:0}$	$C_{16:1}$	$C_{16:0}$	$C_{17:0}$	$C_{18:1,2,3}$	$C_{18:0}$
WM-38													
1	64.6	33.4	23.4	46.7	50.7	7.7	104.7	11.9	16.2	276.3	3.6	184.5	99.2
2	60.9	29.1	17.9	37.0	42.4	7.6	97.7	11.9	16.8	286.9	3.5	204.4	111.2
3	65.6	30.1	17.0	34.9	40.0	7.7	96.4	12.0	17.4	297.0	3.7	218.1	119.0
4	61.5	28.8	15.2	31.2	36.6	7.5	91.3	11.6	17.3	295.6	4.2	224.9	125.4
5	47.5	25.2	13.4	27.7	31.1	6.5	76.4	9.6	14.3	248.8	3.1	198.0	130.8
6	56.1	26.1	13.3	27.8	31.4	6.9	79.4	10.3	15.2	268.3	4.0	219.2	122.5
7	62.3	26.9	12.9	26.7	31.3	7.1	80.7	10.4	15.8	268.6	3.9	212.9	130.2
8	44.2	23.7	12.0	24.9	27.3	6.7	73.5	9.9	16.0	272.7	3.9	238.0	134.2
9	38.4	22.0	11.8	24.9	26.1	6.7	69.0	9.4	15.5	264.9	3.9	248.1	143.9
10	25.8	18.9	11.7	25.2	25.8	6.9	65.8	9.0	15.2	255.5	4.1	257.6	151.3
11	20.6	16.3	10.3	24.6	26.1	7.5	65.7	8.9	15.6	250.8	4.1	278.7	164.5
12	10.5	10.9	8.4	22.8	26.3	8.1	66.6	9.0	16.3	247.0	4.6	296.6	174.1
13	4.1	6.4	6.0	19.4	25.5	8.5	68.5	9.2	17.6	249.8	5.0	322.4	181.3
14	0	2.4	3.1	12.6	20.4	7.8	63.4	9.0	17.9	243.7	5.1	344.5	183.9
15	0	0.1	.4	3.2	7.8	4.1	36.6	6.4	13.8	205.1	4.7	381.6	231.8
Control	29.2	16.0	9.2	21.9	26.5	6.5	72.8	9.5	16.0	270.3	4.6	293.0	173.5

number of transfer stages or theoretical plates and the height of the column. Furthermore, there are many similarities between the butter separation and the fractionation of fish oil ethyl esters. The information obtained in the fish oils study is directly applicable to the separation of cholesterol from milk fat. The separations of fish oil ethyl esters is practical and can be accomplished commercially. The calculated selectivities are shown for comparison (Table 2). The larger the selectivity value above 1.5, the easier the process will proceed and the smaller the extraction column.

Data used to calculate the distribution coefficients and the selectivities were from results shown in Table 3. Cholesterol values are from analysis of extracts produced by ascending profile separation. Pressures used as well as volume of CO_2 used are shown. The method used for cholesterol assay is the digitonin procedure for β-3-OH sterols as shown in AOAC (5).

Further, it is interesting to observe the fatty acid distribution in fractions collected from the supercritical extraction of butter oil. In this example (Table 4), 15 different fractions were collected in an ascending profile separation. Each fraction was about .5 g. Aliquants of each fraction were saponified and converted to methyl esters using the boron trifluoride technique. Compared with the control, fraction one contains about twice the fatty acid concentrations up to C_{14}. The longer chained fatty acids are soluble at higher pressures; thus, greater concentrations than in the control sample of

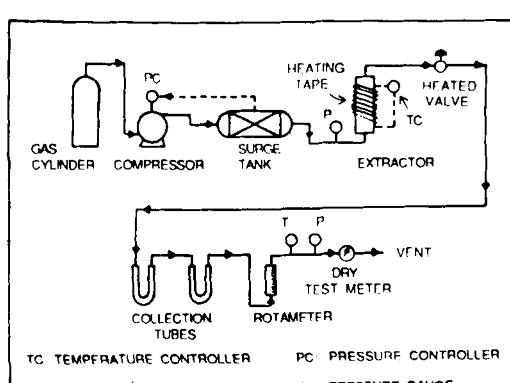

Figure 5. Bench-top scale supercritical fluid extract equipment.

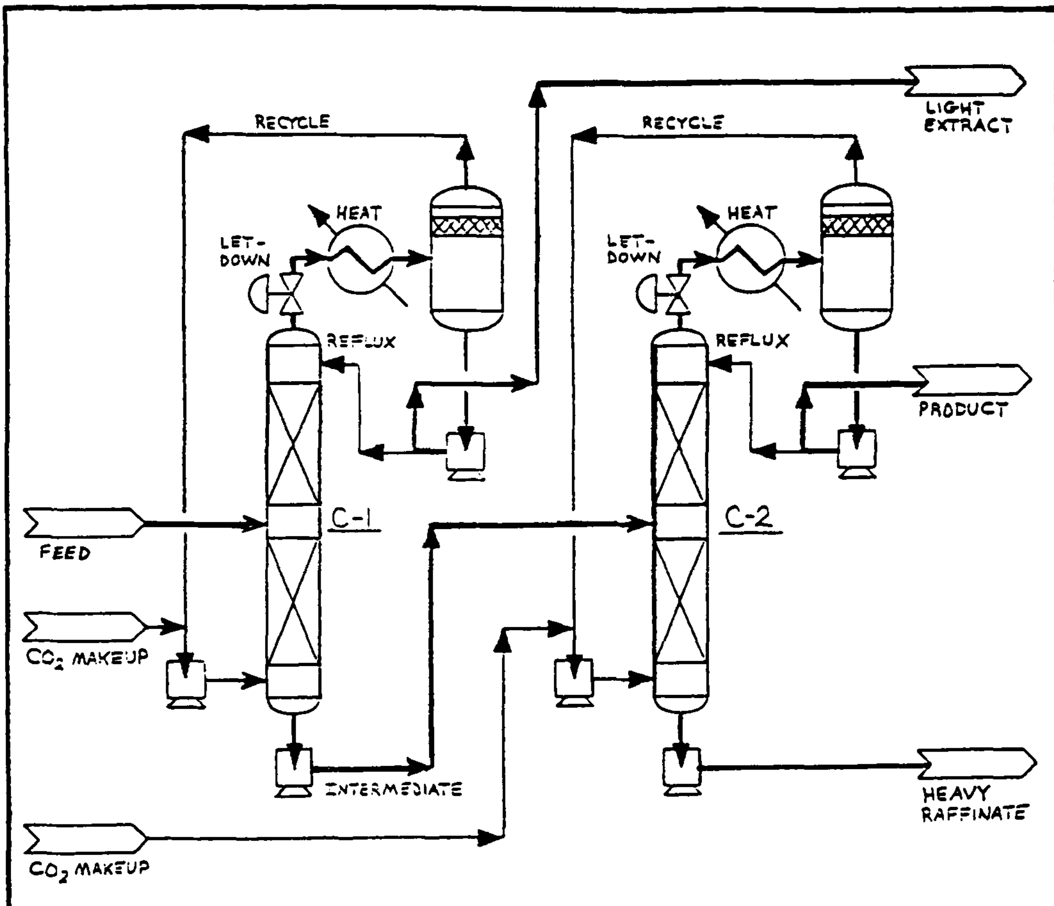

Figure 6. Pilot scale supercritical fluid extraction equipment.

butter oil. Triglyceride analyses are in the final stages of completion.

Equipment

Bench top equipment is designed with flexibility to allow a wide variety of sample fractionation (Figure 5). The sample is held in the extractor at a preset temperature while the supercritical fluid adjusted to the correct pressure with a diaphragm pump is allowed to pass through. Fractions of the product in the extraction vessel are collected in the U-tubes, which are at atmospheric pressure and temperature. The rotameter monitors gaseous flow per unit of time while the test meter monitors total flow. This process can be likened to a chemical fractional distillation where temperature is varied with pressure constant. With supercritical, obviously the temperature is constant and pressure is varied.

Pilot-scale equipment to fractionate cholesterol from milk fat may be designed in more than one manner; an example is in Figure 6. This equipment requires two-stage processing, i.e., the lower pressure first stage is to separate flavors and low melting triglycerides. The remainder with cholesterol is conveyed to the second stage higher pressure unit where cholesterol is separated from the high melting triglycerides. Note that provisions for recycling are a part of the equipment. Cholesterol and a small fraction of milk fat will be removed through the collection port marked "product". By subsequently blending appropriate amounts of light extract and heavy raffinate, the manufacturer has the potential of producing a fat with a melting point range equivalent to butter or to produce a soft-spread butter. Both products with low cholesterol content.

It is difficult before scale-up to set a dollar value for this processing to extract cholesterol. However, knowing the cost of similar processing, one could establish a processing cost of $.15 to .20/lb of milk fat. For butter to be sold as a low cholesterol butter, it would be a direct add-on with limited provision for a value added upcharge. However, for frozen desserts and cheese, the limited amount of fat per pound of finished product introduces a possibility for a value added charge. For example, at $.15/lb of milk fat for a processing cost to reduce the cholesterol to 90% of its original content, the cost per pound of Cheddar cheese would be $.05. This difference would probably by lost in marketing. A similar scenario can be constructed for frozen desserts.

Supercritical extraction costs to produce a low cholesterol butter, cheese, or ice cream can be offset by the value of the by-product. Cholesterol has considerable value currently as an ingredient in the manufacture of steroids and emollients. The current demand for cholesterol suggests a price of $.04/g. If an operator of a commercial-sized supercritical extraction unit could produce a 10% concentrate of cholesterol in milk fat, the value of that product would be $1.77/lb of milk fat. Indeed, a 10% concentrate has more value than the parent compound.

Summary

Research evidence to date has shown that cholesterol can be removed efficiently from milk fat to the 90% level using bench-scale technology. Scale-up using evidence from fish oil processing indicates continued feasibility for a broad range of dairy foods. Furthermore, the final processing cost can be offset by the value of the by-product, cholesterol.

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