Crystallization of Butter Oil and Separation by Filter-Centrifugation

C.J. Breeding and R.T. Marshall*

Food Science and Human Nutrition, University of Missouri, Columbia, Missouri 65211

ABSTRACT: Fractionation by crystallization of melted butter oil can produce fractions that are physically and chemically different. However, many variables affect the formation, growth and separation of the crystalline fat from the entrained oil. The objective of this study was to produce crystalline fractions that contained a minimal volume of entrained oil through optimization of the crystallization and separation conditions. Crystallization was initiated at 33.5°C upon slow cooling from 60°C with stirring (10 rpm). Oil was cooled to 18.5°C and separated by centrifugation, followed by filter-centrifugation. Fractions were analyzed for melting point, solid fat by differential scanning calorimetry, and fatty acid profiles. A split-plot design was used for statistical analyses of data, and the experiment was performed three times. Fractionation caused significant changes in melting profiles of the fractions when compared with the unfractionated butter oil. Melting points of the unfractionated butter oil, liquid and crystalline fractions were 41.6, 25, and 48°C, respectively. The oil content of the crystalline fraction at 18.5°C ranged from 28 to 35%. A 17% increase in C8:0–C10:0, an 11% decrease in C12:0–C16:0, a 32% decrease in C18:0, and a 41% increase in C18:1, C18:2, and C18:3 acids were observed in the liquid fraction when compared with the crystalline fraction. *JAOCS 72,* 449–453 (1995).

KEY WORDS: Butter oil, crystallization, filter-centrifugation, fraction, fractionation.

Milk fat can be fractionated by crystallization from melted fat (1–3) or by partial crystallization with the fat dissolved in an organic solvent (4,5). Crystallization from a polar solvent produces crystals almost instantaneously and yields discrete crystalline and liquid fractions of the fat. However, fractions with different chemical compositions and physical properties can be formed without solvents by manipulation of temperature (2). Melt fractionation is preferred in the dairy industry because the process conforms with the "natural" product image; it requires no solvent or solvent removal (3,5).

Melt crystallization is the process of cooling the completely melted milk fat to a temperature at which the formation of crystal seeds occurs (6). Factors affecting crystal formation, growth, and separation are the initial temperature of the oil, final temperature of fractionation, rate of cooling, rate of agitation, and method of separation. These variables affect the size and shape of the crystals, filtration rate, yield, solid fat content, melting point and fatty acid profile of the liquid and crystalline butter oil fractions.

Grönlund *et al.* (7) reported that, as temperature was reduced, nucleation commenced at about 28°C. Larger crystals were formed when the initial temperature was 33.5°C instead of 29.5°C (3). Voss *et al.* (8) suggested that if the initial temperature was too low, many nuclei were produced so that growth of individual crystals was repressed. Mulder and Walstra (9) reported that for every 5°C lowering of the temperature below 25°C, the rate of crystallization roughly doubled.

Precooling to 33.5°C, followed by linear cooling with agitation for 8 h, gave a faster filtration rate than did exponential cooling for 20 h with agitation (3). The faster filtration rate may have been because the majority of the crystals were of equal size. The slower the rate (0.01–1°C/min) of cooling, the better the separation, yield, and quality of the fractions (10). As the rate of cooling increased above 1°C/min, crystal size increased and reduced the yield of the liquid fraction (10). Slow cooling and no stirring produced spherulite crystals rather than the desired rod-shaped crystals (3,7). Spherulites are unsuitable for vacuum filtration because these crystals cluster randomly and trap oil within the matrix. This reduces the efficiency of separation by increasing the viscosity of the slurry and clogging the filter medium (3,6).

The oil should be stirred to prevent localized increases in temperature (latent heat of crystallization), which can be as much as 5°C during crystallization (9). Slow stirring at a rate of 10 rpm during crystallization produced small, rod-shaped crystals, whereas a fast rate of agitation produced small crystals and a high rate of crystallization (3,5). Grall and Hartel (11) reported that as the impeller velocity increased, the number of crystals increased, and the concentration of large crystals decreased. High shear promotes secondary nucleation; the impeller blades cause tiny crystals to form from disruption of existing crystals. This secondary nucleation results in decreased filtration efficiency and increases the amount of entrained liquid phase during filtration (11). In contrast to previous work, Fouad *et al.* (12) reported that crystals formed without agitation were larger and easier to filter than those formed with agitation, but solid yields were lower.

*To whom correspondence should be addressed at 122 Eckles Hall, University of Missouri, Columbia, MO 65211.

A major problem with melt crystallization is that quantitative separation of the oil from the solid fraction is difficult. Because much oil is entrained, only small differences in chemical composition and physical properties are exhibited between the liquid and solid fractions. A solid fraction produced by step-wise cooling of milk fat to 5°C contained 70% liquid phase after separation with a vacuum filter (13). Furthermore, double fractionation of the solid fraction failed to reduce the percentage of entrained oil. Walstra and Jenness (14) reported that, although milk fat at 5°C appeared to be solid, it contained more than 50% oil (15). Other researchers reported that the solid-fat content of the solid fraction separated at temperatures from 17 to 26°C ranged from 37 to 60% (1,16).

Various methods have been tried to remove the oil from the crystallized fat. Black (6) used a pilot-scale casein dewatering press, which was modified to reduce contamination of one fraction with the other. Separation was accomplished by draining the liquid through a perforated bottom roller, assisted by pressure on the crystals from the top roller; the crystals were scraped from the top roller (6). The Tirtiaux process (10), which has features common to Black's (6) casein dewatering press, is a patented method of dry fractionation. In this method, controlled crystallization is followed by separation of crystals on a continuous filter mounted on a perforated stainless-steel belt. The filters are self-cleaning, and the filtration area is enclosed and air-conditioned, thus maintaining the slurry at the temperature of fractionation (10). At 20°C, the Tirtiaux process yielded a crystalline fraction that was 41% solid fat and exhibited a melting point of 41°C. Larsen and Samuelsson (5) first described filter-centrifugation of fractionated milk fat in combination with a modified Alnarp treatment of the butterfat. Although much research has been performed on milk-fat fractionation, entrained oil in the separated crystalline phase is still a problem. Therefore, the objective of this study was to combine the best conditions for crystallization reported previously and to separate the fractions by filter-centrifugation, thus reducing the amount of oil entrained in the crystalline fraction.

MATERIALS AND METHODS

Summer milk (June–September) was collected at the university dairy farm and separated before cooling. The cream (55 L) was cooled overnight, then churned into butter by vigorous agitation in a 60-L processing vat with a sweep-style agitator and an emulsifying agitator. The butter was melted, and the oil was decanted and stored (24–48 h) at 4°C until time of fractionation.

Crystallization. Butter oil (25 L) was heated to 60°C in a 60-L, jacketed, stainless-steel processing vat and held for 30 min quiescently. While stirring (10 rpm) with a sweep-type agitator, the temperature was lowered at 1°C/min to 33.5°C, then at 4°C/h to 18.5°C. When the temperature of the oil reached 18.5°C, the crystalline slurry was drained from the vat.

Separation. Centrifuge bottles (750 mL) were filled with the crystalline slurry and centrifuged at 1600 × *g* for 20 min at 18.5°C. Gross separation was achieved, producing two layers. The liquid phase, about 30% of the volume, was decanted, and about 100 g of the crystalline layer was transferred to a filter-centrifuge bottle (750 mL) as designed and developed for this study (Fig. 1). This material was separated under the conditions described above, with crystals collected on a single basket-style coffee filter (3¼″ base), while the liquid was deposited in the bottom of the bottle. The entire separation process required about 12 h, and during this time the uncentrifuged slurry and crystalline precipitate from the initial separation were held at room temperature (21°C).

Fatty acid analysis. Fatty acid methyl esters (FAME) were prepared by using 2 mL of a 2% (vol/vol) solution of sulfuric acid in anhydrous methanol per sample (17). Butter oil (20–40 mg) was weighed into a 13 × 100 mm screw-cap culture tube. Sodium sulfate-dried diethyl ether (1 mL), containing 4.5 mg/mL of the internal standard, margaric acid (C17:0), was added to dissolve the fat. Sulfuric acid/methanol was added, and the tubes were tightly sealed with Teflon-lined caps and placed in a heating block at 90°C for 30 min. After vortexing for 20 s and cooling (21°C), distilled water (1 mL) was added, and the tubes were again vortexed (20 s). On standing (1 min), the top layer containing hexane and FAME was removed. Separation of FAME was performed with the injection of the hexane layer (2.4 μL) on a 3 m × 2 mm i.d. glass column packed with 10% Silar-10C® (Applied Science Laboratories, State College, PA). The column was installed on a Model 3700 Varian (Sunnyvale, CA) gas chromatograph. Column temperature was held at 180°C for 1 min, then increased 1°C/min to 210°C. Injection temperature was 210°C, and the flame-ionization detector temperature was maintained at 250°C. Flow rate of the carrier gas, nitrogen, was held constant at 42 mL/min. Integration of FAME peaks was performed with a Spectra-Physics (San Jose, CA) Model SP 4270 recording laboratory integrator. Concentrations of

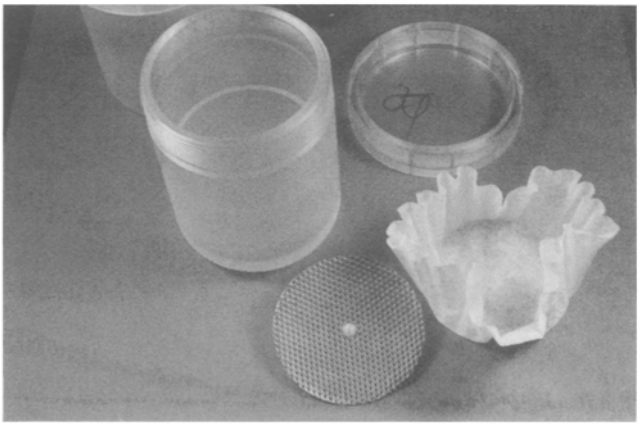

FIG. 1. Centrifuge bottle with stainless-steel perforated filter support and coffee-style filter for filter-centrifugation of crystallized butter oil.

FAME (C8:0–C18:3) were determined by analyses of peak area after correcting for 90% recovery.

Thermal heating curves. Thermal heating curves were produced in triplicate with the original unfractionated butter oil and the liquid and crystalline fractions by differential scanning calorimetry in a DSC-7 (Delta Series; Perkin Elmer Co., Norwalk, CT). Samples (5–10 mg) were quickly cooled to −40°C, held for 3 min, and scanned from −40 to 70°C at the rate of 5°C/min. Thermograms were normalized by rescaling temperature, heat flow, and sample size. Thermal curves were plotted from −20 to 60°C. A straight baseline was drawn between the upper and lower melting limits, and the total area under the curve was determined by using planimetry. The total area was divided into partial areas, encompassing 5°C temperature intervals, and the area of each interval was measured. The percent solid fat at temperature, T, is given by the ratio of the partial area to the left of temperature, T, to the total area.

Statistical analysis. A split-plot design was utilized, and the experiment was performed three times. Significance was preestablished at alpha ≤0.05. Analysis of variance was performed with Proc glm, and significant means were separated by least significant difference and least significant means (18).

RESULTS AND DISCUSSION

Fraction yields. Proportional yields of liquid and crystalline fractions prepared at 18.5°C were 55:45, 53:47, and 62:37 in replications 1, 2, and 3, respectively. Sherbon *et al.* (16) reported that milk fat fractionated at 25.5 and 28°C yielded ratios of 64:32 and 53:47 liquid to crystalline fractions, respectively.

Thermal curves. Fractionation caused marked changes in the melting profiles of the fractions compared with the unfractionated butter oil (Fig. 2). Upper melting limits were about 25°C for the liquid fraction and 48°C for the crystalline fraction, whereas that of the unfractionated milk fat was 41.6°C. Melting thermograms of the three samples exhibited sharp upper temperature limits, although the lower limits were not as easily determined. Thermograms of unfractionated and crystalline samples exhibited melting gaps from 20 to 25°C. The thermogram of the liquid sample exhibited complete melting by 20°C, indicating that the higher melting fraction was removed to an insignificant concentration. The melting thermogram for the unfractionated milk fat showed prominent temperature peak maxima (TPM) at −.4 and 15°C. In addition, there was a fully resolved plateau at 32°C, which was not present in the liquid fraction. The melting thermogram for the liquid fraction showed two partially resolved peaks with TPM at 2° and 16°C. The melting thermogram for the crystalline fraction had two TPM. The TPM at 16°C corresponded to the TPM at 15°C of the unfractionated milk fat and to the TPM at 16°C of the liquid fraction. There were large amounts of triglycerides that melted at 16°C in the unfractionated sample. The crystalline fraction still contained some of the fairly low-melting triglycerides, indicating that some liquid contamination remained. However, the TPM of the unfractionated sample at 0°C was not exhibited by the crystalline fraction. Also, the peak at 42°C on the thermogram of the crystalline fraction probably corresponded to the TPM at 32°C produced by the unfractionated sample. However, this peak was more resolved and skewed, and shifted toward a higher melting limit in the thermogram of the crystalline fraction.

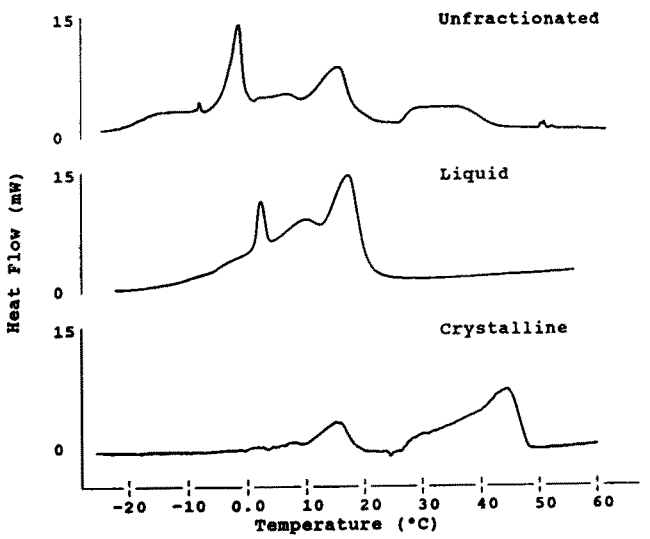

FIG. 2. Melting thermograms of unfractionated butter oil, and its liquid and crystalline fractions.

Sequential crystallization was performed by Grall and Hartel (11) at 30, 20, and 15°C with the liquid fraction from a previously separated lot as the feed. Crystallized milk fat, fractionated at 20°C, produced melting curves similar to those of the unfractionated milk fat from the present research. The crystalline fraction, produced by fractionation at 30°C, exhibited peaks at 8 and 41°C and had an upper limit of 48°C. The liquid phase, fractionated at 15°C, gradually melted, beginning at −20°C, had a TPM at 12.5°C and a melting limit of 19°C (11). Crystallization in a sequential manner did not cause the TPM of the high-melting triglycerides to shift to lower temperatures. This would reflect a change in the physical properties of the triglycerides. Rather, most likely, the higher-melting triglycerides were removed with each sequential drop in temperature, with the resulting liquids possessing greater and greater amounts of the low-melting triglycerides. The thermograms (11) most similar to the thermograms produced by fractionating in the present experiment at 18.5°C were those of the solid and liquid fractions produced at 30 and at 15°C, respectively.

Solid-fat content. Differences in solid-fat content at each temperature interval for the unfractionated butter oil, liquid, and crystalline fractions were easily discernible (Fig. 3). As expected, the integral curves demonstrated that at any temperature the solid-fat content increased in the following order: liquid fraction, unfractionated butter oil, and crystalline fraction. At 10°C, the crystalline fraction contained only 8% en-

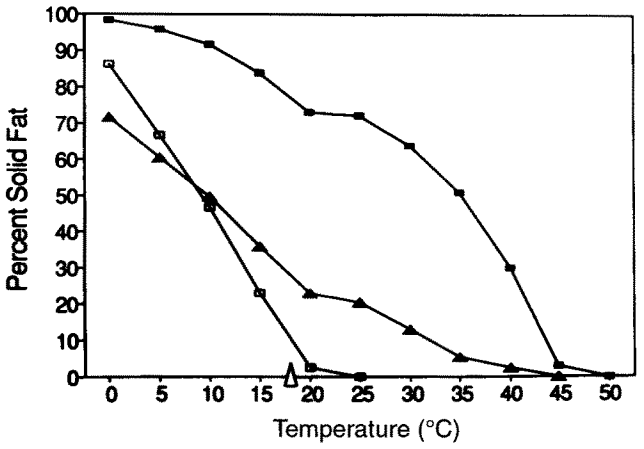

FIG. 3. Integrated thermal heating curves of butter oil fractionated at 18.5°C, indicating the solid-fat content of unfractionated butter oil, ▲; liquid fraction, □; and crystalline fraction, ■. Standard deviations at 50% solid fat of the unfractionated butter oil, liquid, and crystalline fractions are 0.82, 0.70, and 0.34, respectively.

trained oil, whereas the unfractionated sample contained 51%. At 20°C, the crystalline fraction contained 27% liquid phase, while the unfractionated butterfat contained 77% entrained oil. The oil content of the crystalline fraction at the fractionation temperature, 18.5°C, ranged among replications from 28 to 35%. These data indicated a significant improvement, i.e., lower carryover of entrained oil in the crystallization phase, over previous process results in which the contaminating oil phase as 65 to 72% (13,14,16,19,20). Black (6) determined that fractionating milk fat at 18.5°C produced a mean of 58% entrained liquid phase, with a range from 48 to 68% in the crystalline fraction. The higher amount of entrained oil reported by Black (6) may be attributable to the method of separation after crystallization. Black (6) used a modified casein dewatering press, whereas the present work took advantage of the combination of filtration and centrifugation. The oil was forced from the crystalline phase through the filter by centrifugation and deposited in the bottom of the container. Thus, much of the oil was physically separated from the crystalline fraction.

Thermal heating curves of solid-fat content (Fig. 3), representing the unfractionated milk fat and the liquid fraction, were steep at temperatures between 0 and 20°C. From 30 to 45°C, the curves representing the crystalline fraction had slopes similar to those of the liquid fraction at temperatures of 0 to 20°C. Curves representing the solid-fat curves of the present work were similar, within the range of 0 to 25°C, to solid-fat content of olein (10). Likewise, curves, representing the solid-fat curves of the present work, were similar within the range of 25 to 50°C to solid fat of stearin (10). These similarities indicate that thermal heating curves reliably reflect the nature of the fractions.

Upon examination of the solid fractions of anhydrous milk fat, fractionated at 25.5 and 28°C, Sherbon *et al.* (16) reported that melting points and thermograms exhibited melting gaps at 20 to 25°C. In the present research, the thermogram of the unfractionated milk fat displayed little melting at 25°C, whereas the thermogram for the liquid fraction indicated that melting was almost complete at 25°C. Also, the thermogram for the crystalline fraction indicated that no melting occurred between 20 and 25°C, with melting resuming above 25°C. This indicates that the cow produces milk fat in which the triglycerides are distributed bimodally, not randomly. Therefore, the melting gap may be a natural division between the low- and high-melting triglycerides. It may be possible to take advantage of this melting gap to crystallize and separate at 20°C with little melting occurring, even though the temperature may increase to 25°C. This would produce two fractions that would be significantly different in physical properties and chemical composition. Even though crystallization and separation occurred at 18.5°C in the present research, some melting may have ensued because the unseparated slurry and the crystalline fraction from the initial separation were held at room temperature. Because the final temperature of crystallization (18.5°C) was below the melting gap, the amount of solid fat melted to the liquid form was limited by the resistance of the milk fat to melting from 20 to 25°C. The maximal amount of solid fat melted with this change of temperature (18.5–21°C) would have been about 4%, based on the melting curve in Figure 3.

Fatty acid analysis. The two fractions, produced at 18.5°C, had significantly different concentrations of C8:0, C14:0, C16:0, C18:0, and C18:3 fatty acids (Table 1). Percentage differences in amounts of fatty acids in the liquid and crystalline fractions were calculated by using the lower concentration as the divisor. The liquid fraction contained 29% more C8:0 and 77% more C18:3 acids than the crystalline fraction. The crystalline fraction had higher concentrations of C14:0, C16:0, and C18:0 of 7, 14, and 32%, respectively. When changes in the fatty acid concentration were examined by category, a 17% increase in C8:0–C10:0, an 11% decrease in C12:00–C16:0, a 32% decrease in C18:0, and a 41% increase in the long-chain unsaturated fatty acids (C18:1, C18:2, and C18:3) were observed in the liquid fraction compared with the crystalline fraction (Table 2). The fatty acid profiles of the crystalline fraction were similar to those reported by Parodi (21), in which a high-melting glyceride milk-fat fraction was

TABLE 1
Fatty Acid Composition[a] of Milk Fat, Resulting from Melt Crystallization and Fractionation at 18.5°C

FAMEs	Unfractionated	Liquid	Crystalline	$P < 0.05$
C8:0	0.79	0.90	0.70	.002
C10:0	2.92	3.09	2.74	.641
C12:0	3.97	3.98	3.95	.416
C14:0	12.86	12.46	13.50	.001
C16:0	37.46	35.05	40.12	.016
C18:0	12.78	11.44	15.24	.0008
C18:1	25.18	28.39	20.67	.720
C18:2	3.15	3.58	2.44	.078
C18:3	0.88	1.12	0.64	.004

[a]Molar percent. FAMEs, fatty acid methyl esters.

TABLE 2
Fatty Acid Composition[a] Expressed by Groups of Fatty Acids in Unfractionated and Fractionated Milk Fat

	Unfractionated	Liquid	Crystalline
Fatty acid group	Mean SD[b]	Mean SD	Mean SD
Short-chain saturated (C8:0–C10:0)	3.71 ± .11	3.99 ± .27	3.44 ± .43
Medium- and long-chain (C12:0–C16:0)	54.29 ± .44	51.49 ± .71	57.57 ± .73
C18:0	12.78 ± .41	11.44 ± .31	15.24 ± .77
Unsaturated	29.21 ± .31	33.09 ± .51	23.75 ± .69

[a]Molar percent.
[b]Mean value from three replications and standard deviations.

analyzed. The profiles were also similar to those produced by a commercial melt fractionation process (19).

A melting gap has been observed repeatedly in the thermograms of solid fractions obtained from milk fat by melt crystallization (1,16,22). Tempering the fat did not affect the melting gap, indicating that polymorphism is not involved (16). It is also suggested that the appearance of the melting gap may be related to the low proportion of short- and intermediate-chain fatty acids in the crystalline fraction (1,22). Short-chain saturated fatty acids (C4:0–C10:0) were concentrated in the liquid fraction (below the melting gap), and the long-chain saturated fatty acids (C16:0–C20:0) were concentrated in the solid fractions (above the melting gap) (2). The *cis* unsaturated fatty acids (C10:1–C18:3) were also concentrated in the liquid phase. Grall and Hartel (11) reported that the general trend is for the short-chain and unsaturated fatty acids to migrate to the liquid fraction, whereas the long-chain fatty acids remain with the crystalline fraction.

The present study has shown that butter oil can be successfully fractionated by controlled cooling of butter oil, to yield products that are physically and chemically different. Thermal heating curves, solid-fat content, and fatty acid profiles indicated that conditions employed significantly decreased the amount of oil entrained in the crystalline fraction. Suggested uses for fractionated milk fat include: (i) the addition of the liquid fraction to milk powder to improve its reconstituted properties (23); (ii) to improve spreadability of butter (13), (iii) in ice cream and other dairy products to increase the content of short-chain and unsaturated fatty acids; (iv) for the crystalline fraction in puff pastry (5); and (v) as an ingredient in coatings and bakery products (10).

ACKNOWLEDGMENT

This work was supported by the National Dairy Promotion and Research Board.

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[Received May 9, 1994; accepted December 12, 1994]