Thermal Behavior of Butterfat Fractions and Mixtures of Tripalmitin and Butterfat

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The melting and crystallization behavior of blends of tripalmitin and butterfat were compared with that of butterfat fractions, which were prepared by dry fractionation and by solvent extraction. There were marked similarities in the behavior of the blends, the dry fractions and some solvent fractions. This similarity was not shared with the behavior of the hardest solvent fractions. The functionality of hard butterfat fractions seemed to derive from an enrichment in long-chain saturated triglycerides. Improved functionality could therefore be achieved equally well by blending or by fractionation. Blends of tripalmitin and butterfat could be used as model butterfat fractions, or as an alternative to butterfat fractions in some applications.

KEY WORDS: Butter oil, differential scanning calorimetry, dry fractionation, milk fat, solvent extraction, solvent fractionation.

The decline in the consumption of butterfat has been attributed to its high price, poor health image and limited functionality (1–3). Manipulation of the cow's diet can produce a softer butterfat that is high in polyunsaturated fatty acids, but which has limited functional properties and a tendency toward oxidative rancidity (4–6). Butterfat can be separated into harder fractions for use in cold-spreadable butter (7,8), chocolate (2), cookies and pastries (2,9) and coatings (10,11).

Dry (thermal) fractionation is the method of choice for flavor retention (1). Solvent fractionation has problems, such as solvent residues and recovery, extraction with supercritical carbon dioxide can lead to loss of flavor, and short-path distillation is prohibitively expensive (1). The value of fractionation has been questioned (12). The composition of butterfat varies greatly, according to the composition of the feed, seasonal variation, stage of lactation, genetic variation and other factors (13). The resolving power of all fractionation techniques is relatively poor because of the heterogeneity of butterfat. Thus, the natural variation in butterfat composition nearly matches the variation that can be achieved by dry fractionation (12,14) and extraction with supercritical carbon dioxide (15).

Protected feeding and fractionation are costly and of limited effectiveness. Their success has been limited because our understanding of the molecular basis of the functionality of butterfat is also limited. The triglycerides that have a controlling influence on functional properties must be identified. Only then will it be possible to devise techniques to improve functionality that are cheaper and more effective.

Hard dry fractions can be modeled by blends of tripalmitin and butterfat (11). This implies that the functional properties of dry fractions are essentially controlled by the content of long-chain saturated triglyceride. Solvent fractionation has greater resolving power than dry fractionation and can produce fractions that are much less heterogeneous. The aim of this study was to compare the thermal behavior of the model system with that of solvent fractions to test the model system further.

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MATERIALS AND METHODS

Model system. Butterfat blends were prepared from anhydrous butterfat (henceforth referred to as MA butterfat; a gift from Mid-America Dairymen, Inc., Springfield, MO). Tripalmitin (stated purity 95%) was obtained from Fisher Scientific (Pittsburgh, PA), and was used without any further purification. Gas-chromatographic (GC) analysis indicated a purity of 94%, and the principal impurity was identified as trilaurin.

Dry fractions. Hard dry butterfat fractions were a gift from a New Zealand source. Dry fraction A is recommended for use as a shortening, and dry fraction B is recommended for use in chocolate.

Solvent fractions. Butterfat fractions were prepared from anhydrous butterfat (henceforth referred to as CC butterfat; which was a gift from California Co-Operative Creamery, Hughson, CA). Sep-Pak solvent extraction columns (60-mL capacity, octadecane bonded phase) were a gift from Varian Analytichem (Walnut Creek, CA). Methyl *tert*-butyl ether (MTBE), $CHCl_3$, ethanol, acetonitrile and hexane (C_6) were high-performance liquid chromatography-grade solvents from Sigma Chemical Company (St. Louis, MO). GC column DB-HT17 was a gift from J&W (Folsom, CA). Standard triglycerides for GC analysis were obtained from Nu-Chek-Prep Inc. (Elysian, MN).

Preparation of blends of tripalmitin and butterfat. Tripalmitin was blended with butterfat to produce a mock hard fraction rich in long-chain fatty acids. Tripalmitin was chosen because palmitic acid is the most abundant fatty acid in butterfat at 23–48 wt% of the total (16). Previous experiments (11) showed that only a small amount of tripalmitin need be added to butterfat to simulate fractionation. Blends of butterfat were prepared that contained 10, 20 and 30% added tripalmitin by weight. The blends were heated to 90°C for at least half an hour before use to ensure complete mixing and to destroy any crystal nuclei. In the text, the blends are referred to by the ratio of tripalmitin to butterfat. Thus "20:80 blend" indicates butterfat containing 20% added tripalmitin by weight.

Preparation of butterfat fractions by solvent extraction. Butterfat (20 g) was dissolved in chloroform (10 mL); 4 mL of this solution was applied to the column, which had been prewashed with hexane and then with acetonitrile. A low vacuum (10–20 mm Hg) was applied to the column to establish flow. Solvent washes (50 mL) were poured onto the column according to the protocol in Table 1, and the eluents were collected in round-bottomed flasks. The resulting fractions were dried completely under a low vacuum in a rotary evaporator heated to 60°C.

Characterization of solvent fractions by GC analysis. Fractions were recovered from the round-bottomed flasks by washing with chloroform (three washes of about 1 mL). The washes were combined in a 4-mL test tube, dried under nitrogen and then stored under vacuum for 4 h. The samples were analyzed in a Varian (Palo Alto, CA) 3400 gas chromatograph with flame-ionization detection at 355°C and a signal-to-noise ratio of 50. Samples were

TABLE 1

The Elution Protocol Used to Wash the Various Butterfat Fractions off the Solvent Extraction Column[a]

Solvent system[b]	Elution protocol		
	Step 1 (5 washes)	Step 2 (5 washes)	Step 3 (10 washes)
EtOH/C_6/ACN	100:0:0	60:30:10	40:40:20
$CHCl_3$/ACN	100:0	85:15	70:30
MTBE/ACN	100:0	85:15	70:30

[a]The volume of each wash was 50 mL, and the proportion of each solvent in the wash is indicated by the ratio listed below the step number.
[b]EtOH, ethanol; ACN, acetonitrile; C_6, hexane; MTBE, methyl *tert*-butyl ether.

applied to the column with an auto sampler (Varian 8100, injection volume 0.4 μL, solvent plug volume 0.5 μL). The initial injection temperature was 130°C, and the temperature was programmed to rise to 355°C at a rate of 115°C min^{-1}. The column temperature was programmed to rise from 130 to 355°C at a rate of 20°C min^{-1}. The column was held at 355°C for 18.75 min, for a total time of 30 min. The elution time of standard triglycerides was used to identify some of the peaks on the chromatograms.

Characterization of fractions and blends by differential scanning calorimetry (DSC). Melting and crystallization behavior of selected fractions and blends of tripalmitin and butterfat were analyzed in a Perkin-Elmer (Norwalk, CT) DSC IIC. All samples were kept molten at not less than 80°C before preparation for the DSC. About 10 mg of each sample was sealed in an aluminum pan and held at 70°C for at least 10 min to destroy any crystal nuclei. The samples were cooled at a rate of 20°C min^{-1} to −30°C, held at that temperature for approximately 2 min and then reheated to 70°C at a rate of 20°C min^{-1}.

RESULTS AND DISCUSSION

Composition of the solvent fractions. The solvent extraction produced fractions that were liquid, semi-solid and solid at room temperature. Only the results for selected solid fractions will be considered here. The change in composition of these fractions, as determined by GC analysis, is shown in Table 2. The fractions have been ordered according to their crystallization temperatures, and they have been labeled for ease of reference. The identity of each fraction can be determined by reference to Table 3.

The solvent extraction separated triglycerides by fatty acid chainlength without regard to unsaturation. Triglycerides that were rich in short-chain fatty acids had a lower affinity with the C18 solid phase and were washed off the column first. All the solvent fractions considered in this study were greatly depleted in short-chain saturated fatty acid and cholesterol.

Each fraction had a unique pattern of enrichment in some long-chain fatty acids and depletion in others. All the hard fractions were preferentially enriched with myristic, palmitic, oleic and stearic (referred to in compounds as M, P, O and S, respectively) acid. The harder the fraction, the greater was the enrichment with stearic acid and the lesser the enrichment with myristic and palmitic acid. Harder fractions (such as MTBE fraction B, chloroformic fraction B and ethanolic fraction B) were also enriched in species containing oleic acid and at least one stearic acid residue, such as PSO, SSO and SOO (Table 2). Species containing oleic acid and other shorter chain saturated fatty acids, such as MOP, MOS and POO, were abundant in the softer fractions, but greatly depleted in the hardest fractions.

Thermal behavior of butterfat blends and selected fractions. The precise coordinates of the maxima and minima indicated on the DSC curves in Figures 1-8 (shown later) are listed in Table 4.

DSC curves for pure butterfat and 95% pure tripalmitin. The crystallization curves of MA and CC butterfat were

TABLE 2

Percentage of Change (relative to the parent butterfat) in the Content of Selected Triglyceride Species in Selected Butterfat Fractions

Triglyceride species[a]	Butterfat fractions					
	Ethanolic A	MTBE[b] A	Chloroformic A	MTBE[b] B	Chloroformic B	Ethanolic B
MMM	122.7	−63.1	−63.1	−66.7	−100.0	−100.0
MMP	152.3	−6.2	65.4	9.1	−35.4	−89.7
MPP	99.6	129.3	222.2	149.0	172.8	−38.5
MOP	123.7	191.3	111.3	11.8	−64.5	−73.0
MOS	231.7	251.4	40.4	−25.1	−26.2	−37.7
PPP	46.2	86.6	216.0	229.8	564.3	182.8
PPS	0.9	11.3	113.1	154.0	740.8	722.1
PPO	64.9	230.1	258.7	234.7	60.9	27.7
PSS	−38.1	−31.0	19.5	40.7	471.7	1328.3
PSO	10.9	177.6	259.9	439.5	367.0	380.2
POO	77.0	252.9	195.3	147.4	−25.3	0.0
SSS	−23.3	124.7	−100.0	441.1	27.4	445.2
SSO	38.5	241.0	86.3	473.5	468.4	704.3
SOO	129.7	310.8	451.4	10.8	401.4	567.6

[a]M, myristic acid; P, palmitic acid; O, oleic acid; S, stearic acid.
[b]MTBE, methyl *tert*-butyl ether.

TABLE 3

Identities of Selected Solvent Fractions[a]

Fraction	Identity
Ethanolic fraction A	Second wash from second step of EtOH/C_6/ACN extraction
Ethanolic fraction B	Second wash from third step of EtOH/C_6/ACN extraction
MTBE fraction A	Second wash from third step of MTBE/ACN extraction
MTBE fraction B	Fourth wash from third step of MTBE/ACN extraction
Chloroformic fraction A	Second wash from third step of $CHCl_3$/ACN extraction
Chloroformic fraction B	Fifth wash from third step of $CHCl_3$/ACN extraction

[a]Abbreviations as in Table 1.

slightly different (Fig. 1), which was possibly due to differences in composition. The absence of the usual doublet in the peak for MA butterfat was probably a result of the high scan rate. The crystallization temperature of the tripalmitin indicated the expected α form (Fig. 1). The small peak at about 13°C was due to the trilaurin impurity.

The melting curves of both the MA and the CC butterfat (Fig. 2) were typical, except that the characteristic shoulder (which marks the complete melting of butterfat at about 37°C) was not seen (2,17–20). This was mainly a consequence of the high cooling rate used in the previous step (21). The curve for tripalmitin was typical of rapid heating of the α form (Fig. 2). Again, the trilaurin impurity was evident from the small peak at about 22°C. The β melting point of the tripalmitin used here was determined separately as approximately 62.3°C (11).

DSC curves for dry fraction A, ethanolic fraction A, MTBE fraction A and the 10:90 blend. The crystallization curves of ethanolic fraction A and MTBE fraction A suggested that they were harder than dry fraction A, and that all three fractions were softer than the 10:90 blend (Fig. 3). The higher content of pure high-melting triglyceride in the 10:90 blend led to a crystallization peak that occurred at a higher temperature, and which was more distinct from the peak associated with the low-melting component of the blend.

There was more similarity in the melting curves of the fractions and the 10:90 blend than there was in the crystallization curves (Fig. 4). The curves for dry fraction A and the 10:90 blend were most similar. The high-melting peaks on all four curves were similar in form and occurred at similar temperatures (Table 4). There was more difference in the low-melting peaks, with dry fraction A and the 10:90 blend betraying their higher contents of short-chain and unsaturated fatty acids. Nevertheless, the homology between the curves for the 10:90 blend and the MTBE fraction A was notable. The high-melting portions of the melting curves were more alike than those of the crystallization curves because of the interactions between

TABLE 4

Maxima and Minima on the DSC Curves Shown in Figures 1–8[a]

Figure	Minima	Maxima
1a	10.8°C, −0.31; 29.4°C, −4.04	
1b	2.7°C, −0.96	
1c	2.0°C, −0.95; 11.6°C, −0.51	
2a	24.3°C, 0.12	15.8°C, 0.80; 36.6°C, 0.56
2b	26.7°C, 0.25	19.7°C, 0.98; 34.4°C, 0.55
2c		32.1°C, 0.41; 49.2°C, 4.02
3a	4.8°C, −1.00; 18.4°C, −0.87	11.7°C, −0.23
3b	3.1°C, −1.08; 11.9°C, −1.28	8.2°C, −0.76
3c	4.2°C, −1.02; 10.8°C, −0.9	8.2°C, −0.8
3d	0.9°C, −1.04; 10.5°C, −0.6	8.9°C, −0.52
4a	28.3°C, 0.01	13.9°C, 1.04; 38.2°C, 0.95
4b	23.1°C, −0.17	11.4°C, 0.63; 37.5°C, 1.36
4c	14.4°C, 0.66	10.2°C, 0.99; 35.5°C, 1.50
4d	23.9°C, 0.15	14.0°C, 1.00; 35.6°C, 0.87
5a	3.7°C, −0.83; 21.9°C, −1.09	11.2°C, −0.25
5b	8.3°C, −1.29; 20.1°C, −1.67	15.5°C, −0.50
5c	3.6°C, −1.09; 18.0°C, −1.23	11.8°C, −0.40
5d	6.6°C, −1.09; 15.4°C, −1.57	10.8°C, −0.59
6a	26.7°C, 0.13	14.7°C, 0.85; 43.8°C, 1.10
6b	32.2°C, −0.25	18.0°C, 1.10; 42.6°C, 1.70
6c	24.7°C, −0.09	13.8°C, 0.87; 41.3°C, 1.50
6d	27.5°C, −0.19	14.2°C, 0.83; 40.6°C, 1.54
7a	11.4°C, −0.9; 31.4°C, −2.28	17.0°C, −0.25
7b	11.3°C, −0.93; 28.3°C, −2.64	17.1°C, −0.40
7c	4.1°C, −0.74; 24.1°C, −1.46	11.8°C, −0.25
8a	46.4°C, 0.11	22.2°C, 0.88; 43.2°C, 0.96; 54.7°C, 2.08
8b	43.2°C, −0.21	23.5°C, 0.93; 39.5°C, 0.69; 53.3°C, 2.59
8c		14.4°C, 0.91; 46.2°C, 1.35; 53.4°C, 0.34

[a]The y values are given in mcal s^{-1} g^{-1}. DSC, differential scanning calorimetry.

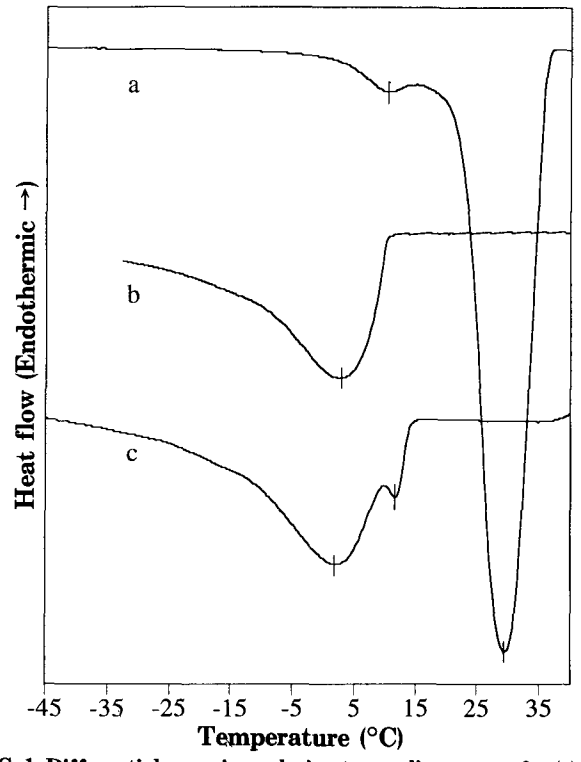

FIG. 1. Differential scanning calorimetry cooling curves for (a) 95% pure tripalmitin, (b) anhydrous butterfat from Mid-America Dairymen, Inc. (Springfield, MO) and (c) anhydrous butterfat from California Co-Operative Creamery (Hughson, CA).

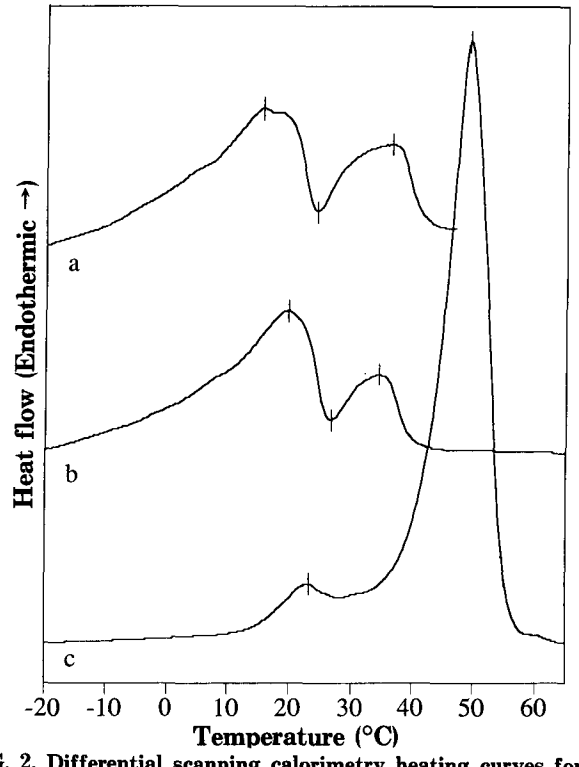

FIG. 2. Differential scanning calorimetry heating curves for (a) anhydrous butterfat from California Co-Operative Creamery, (b) anhydrous butterfat from Mid-America Dairymen, Inc., and (c) 95% pure tripalmitin. See Figure 1 for company sources' locations.

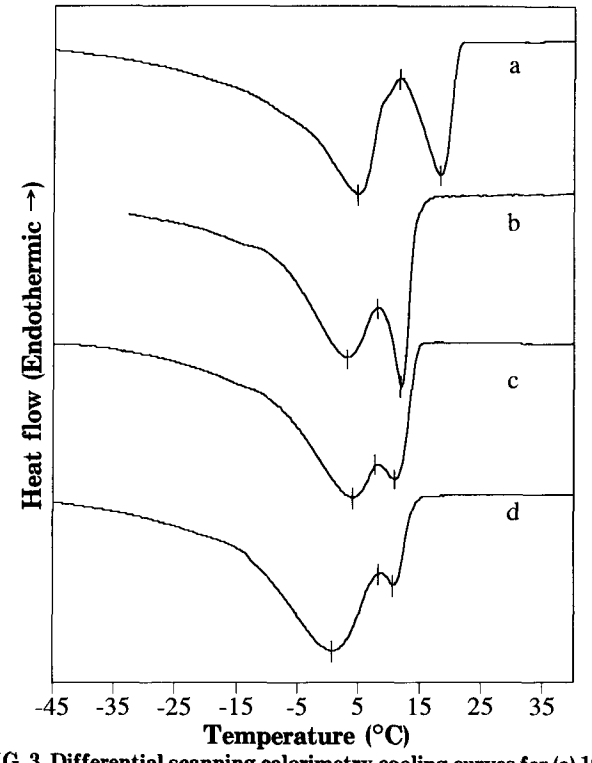

FIG. 3. Differential scanning calorimetry cooling curves for (a) 10:90 blend of tripalmitin and butterfat, (b) methyl *tert*-butyl ether fraction A, (c) ethanolic fraction A and (d) dry fraction A.

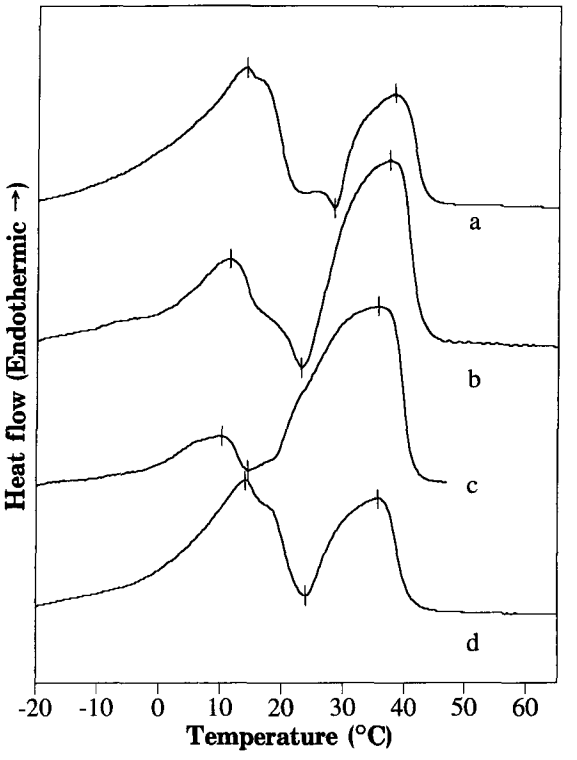

FIG. 4. Differential scanning calorimetry heating curves for (a) 10:90 blend of tripalmitin and butterfat, (b) methyl *tert*-butyl ether fraction A, (c) ethanolic fraction A and (d) dry fraction A.

the low-melting and high-melting components of the fractions and of the blend.

When a molten blend of tripalmitin and butterfat is cooled, the tripalmitin crystallizes initially into the α form and then rapidly transforms to the β form (11). The melting point of the tripalmitin in the mixture is depressed, partly because the low-melting fraction of the butterfat acts as a solvent for the tripalmitin, and partly because the high-melting component of the butterfat forms mixed crystals with the tripalmitin (11). Similar interactions between the low-melting and high-melting components of pure butterfat (and butterfat fractions) mean that the high-melting component tends to transform easily to the β' or β form, depending on the tempering protocol (10,19). Thus, in both the fractions and the blend, the high-melting component is a mixture of triglycerides (rich in long-chain saturated fatty acids), which tends to form mixed crystals in the β form.

DSC curves of chloroformic fraction A, MTBE fraction B, dry fraction B and the 20:80 blend. The crystallization behavior of chloroformic fraction A and MTBE fraction B was quite similar to the behavior of the 20:80 blend (Fig. 5). These fractions, although harder than those considered in the previous section, contained appreciable amounts of oleic acid (see Table 2) and, hence, produced sizable low-melting peaks. However, the separation between the peaks corresponding to the high-melting and low-melting components was more pronounced than in Figure 3, probably because of the reduced heterogeneity of the fractions. There was a strong similarity between the curves for dry fraction B and the 20:80 blend. The crystallization temperature of the tripalmitin component of the 20:80 blend was about 5°C higher than in the 10:80 blend (Table 4) because of the higher mole fraction of tripalmitin in the mixture.

Overall, there was strong homology between the melting curves for all three fractions and for the 20:80 blend (Fig. 6). However, the separation of the peaks corresponding to low- and high-melting components of the 20:80 blend was more noticeable than those for the butterfat fractions. The minima in the curves for MTBE fraction B, chloroformic fraction A and dry fraction B suggested that a little heat was released from the sample during melting. This heat was probably associated with a polymorphic transition of some small portion of the fat.

DSC curves of ethanolic fraction B, chloroformic fraction B and the 30:70 blend. Ethanolic fraction B and chloroformic fraction B crystallized at rather higher temperatures than did the 30:70 blend (Fig. 7). This was evident in the peaks for both the high- and low-melting components (Table 4). The shift occurred because these fractions contained much less short-chain fatty acid than did the 30:70 blend, and much more stearic acid. Thus, blends of tripalmitin and butterfat could not model the hardest solvent fractions. These fractions might be more successfully modeled by adding tripalmitin and tristearin to butterfat.

There were great differences between the curves for the two solvent fractions and the curve for the 30:70 blend on melting (Fig. 8). The enhanced content of stearic acid in the high-melting component of the fractions was evident from the major maxima of the curves in Figure 8 (see

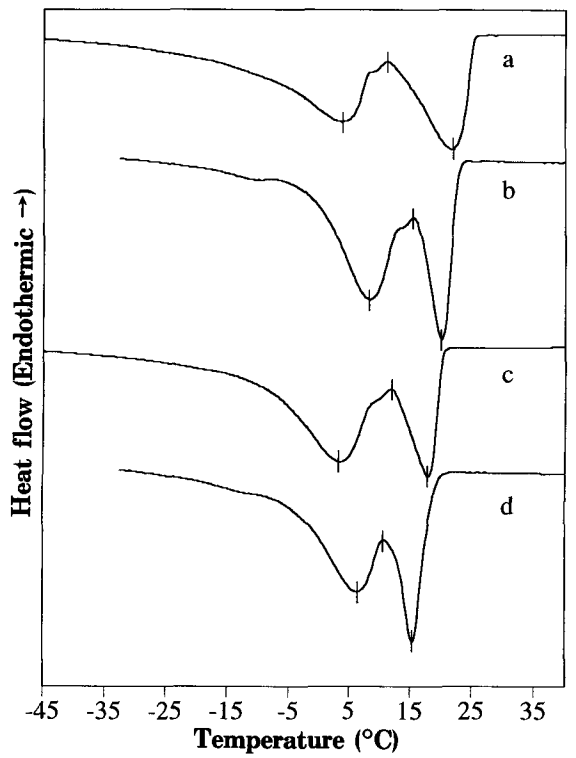

FIG. 5. Differential scanning calorimetry cooling curves for (a) 20:80 blend of tripalmitin and butterfat, (b) methyl *tert*-butyl ether fraction B, (c) dry fraction B and (d) chloroformic fraction A.

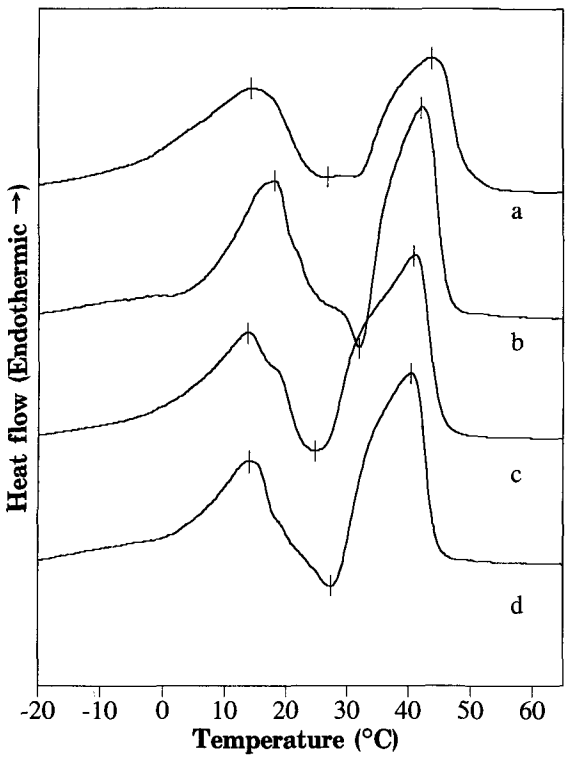

FIG. 6. Differential scanning calorimetry heating curves for (a) 20:80 blend of tripalmitin and butterfat, (b) methyl *tert*-butyl ether fraction B, (c) dry fraction B and (d) chloroformic fraction A.

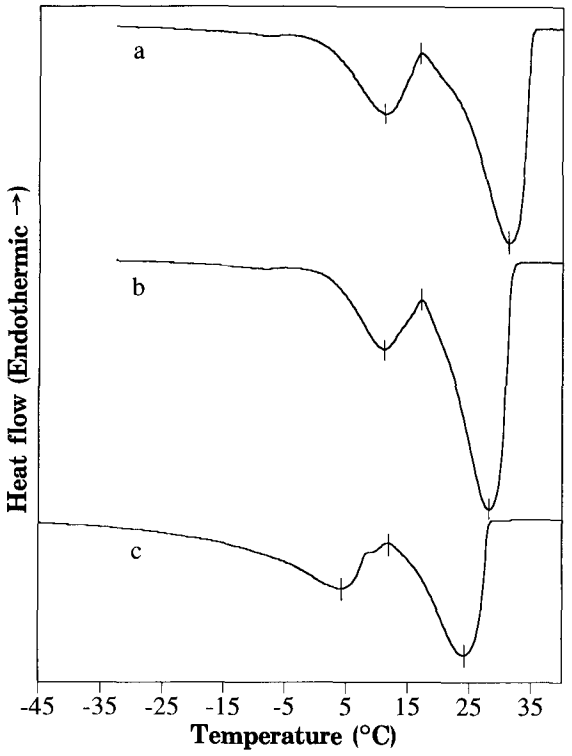

FIG. 7. Differential scanning calorimetry cooling curves for (a) ethanolic fraction B, (b) chloroformic fraction B and (c) 30:70 blend of tripalmitin and butterfat.

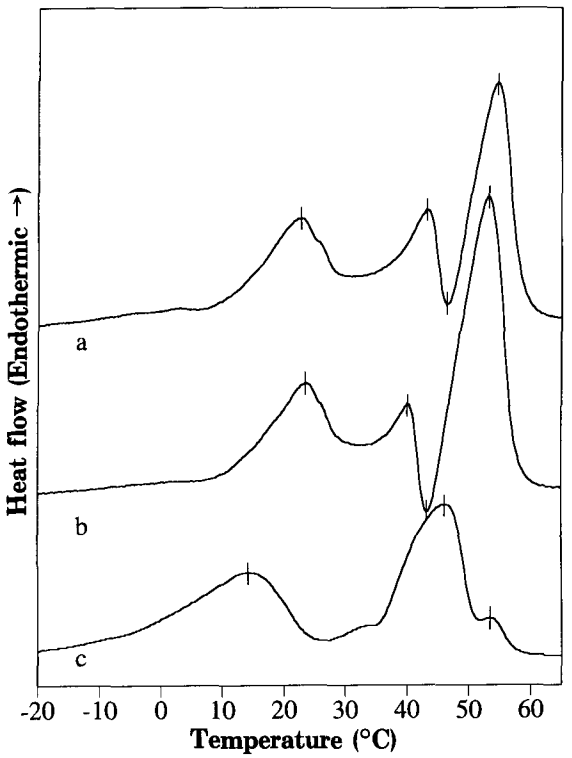

FIG. 8. Differential scanning calorimetry heating curves for (a) ethanolic fraction B, (b) chloroformic fraction B and (c) 30:70 blend of tripalmitin and butterfat.

Table 4 for precise values). There appeared to be a $\beta' \rightarrow \beta$ polymorphic transition in both solvent fractions. In these mixtures, some of the high-melting component either crystallized directly into the β' form or transformed into it shortly after solidification. This polymorphic behavior was not observed in the 30:70 blend because it is not possible to obtain the β' form of tripalmitin from solution (22).

Usefulness of the model system. The similarities in the melting and crystallization behavior of the blends, dry fractions and solvent fractions suggested that the blends generally modeled the fractionation process well. The analogy was particularly good between the blends and the thermal fractions. The blends were a poor model of the hardest solvent fractions, but these are of academic interest only. It would be much more practical to employ vegetable oils as the starting material in any application where a mixture of such hard fats would be required. Production from butterfat would be impractical because of the cost, very low yield, loss of flavor and difficulties in removing solvent residues.

It is well known that some high-melting triglycerides, such as beef tallow and lard, are compatible with butterfat at concentrations of 10% (21). Thus, it should be possible to make simulated hard fractions with a variety of cheap, high-melting triglycerides rather than with pure tripalmitin. In many applications where the functionality and flavor of hard butterfat fractions are desired, the same functionality (and possibly better flavor) could be derived more cheaply from blends of high-melting triglyceride and butterfat.

The hardness of the solvent fractions clearly depended on the content of long-chain saturated triglycerides. Therefore, techniques to improve the functionality of butterfat should concentrate primarily on the content of long-chain saturated triglycerides. We have shown that a relatively slight increase in the content of these acids can make butterfat significantly harder, which implies that a corresponding decrease could make butterfat softer without compromising oxidative stability. This decrease could be achieved through a combination of selective breeding and controlled feeding programs. Although fractionation can produce soft and hard fractions with useful properties, the high cost suggests that alternative strategies to improve functionality should be investigated further. These strategies should be based on an understanding of the molecular basis of functionality.

ACKNOWLEDGMENT

This work was financed by the California Dairy Foods Research Center.

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[Received December 13, 1993; accepted May 12, 1994]