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# Contribution of Triglycerides from Cocoa Butter to the Physical Properties of Milkfat Fractions[1]

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**ABSTRACT:** Milkfat was separated into major chainlength fractions by solid-phase extraction. The effect on thermal behavior and texture of replacing both saturated and monounsaturated long-chain triglycerides from milkfat by long-chain monounsaturated triglycerides with an unsaturated fatty acid in the *sn*-2 position is reported. Increasing proportions of cocoa butter were added to fractions of short- to medium-chain triglycerides ($C_{22}$–$C_{44}$) and medium- to long-chain triglycerides ($C_{36}$–$C_{48}$) isolated from milkfat. Thermal behavior and texture of the mixtures were measured. Results indicated that long-chain monounsaturated triglycerides from cocoa butter enhanced co-crystallization and co-operative melting and did not induce polymorphic transitions upon crystallization and melting of the fractions. At 4°C, they acted as texture builder if present in proportions of more than 30%, whereas below this level, they acted as texture softeners. The effect of the long-chain monounsaturated triglycerides on the texture of fractions that melt at low temperature could not be predicted from the proportion of solid fat at that temperature.

*JAOCS 73,* 955–961 (1996).

**KEY WORDS:** Butterfat, butterfat fractions, cocoa butter, differential scanning calorimetry, fat fractionation, phase transitions, penetrometry, solid-phase extraction, texture.

Milkfat has historically been a valuable component in food formulations because it provides physical properties as texture and structure to foodstuffs (1,2). The plasticity of milkfat (i.e., solid texture) is associated with its composition—the solid structure and plasticity of milkfat is provided by triglycerides that are crystalline within the otherwise liquid milkfat triglycerides at ambient temperature (3). This is associated primarily with long-chain, fully-saturated triglycerides that crystallize within the liquid matrix even at relatively low abundance (4). This unusual property of stabilizing and providing structure to a largely liquid oil mass is relatively specific to animal fats, and particularly to milkfat. Milkfat also suffers from a negative nutritional recognition because of its tendency to elevate human serum lipoproteins, and particularly low-density lipoproteins in predisposed adults (5–9). The predominance of long-chain saturated fatty acids in the *sn*-2 position in milkfat may be one factor contributing to dietary milkfat-induced hyperlipidemia. With the exception of fats in a few tropical plants (10,11), most plant fats do not contain saturated fatty acids in the *sn*-2 position and do not contribute to hyperlipidemia as do animal fats. *In vivo*, modification of the composition of milkfat has been pursued for nutritional improvement and has been realized, for example, by protected fat feeding. This was not accomplished, however, without a dramatic detriment in the physical properties of the resulting milkfat product. Therefore, targets for modification of milkfat need to include retention of the highly desirable yet unusual physical properties.

The contribution of long-chain saturated triglycerides isolated from milkfat to the thermal properties of milkfat has been described (12). Long-chain saturated triglycerides $C \geq 42$ provided the firmness to milkfat as measured by resistance to cone penetration. The phase behavior of milkfat (13), cocoa butter (14), and mixtures of cocoa butter and milkfat and milkfat fractions (15) has been studied, but with most research directed toward understanding the effect of milkfat on the polymorphism of cocoa butter and the appearance of fat bloom. Less is known about the contribution of monounsaturated triglycerides to the texture and thermal behavior of milkfat and milkfat fractions. For example, cocoa butter contains primarily unsaturated triglycerides. The triglycerides of cocoa butter cocrystallize upon cooling and melt cooperatively upon heating, leading to thermal transitions over a narrower temperature range than that exhibited by milkfat. Cocoa butter triglycerides, as a result of their thermal behavior, form a hard solid at room temperature and exhibit a sharp melting just below body temperature (1).

The objective of this work was to investigate the effect of adding cocoa butter, as a model for long-chain monounsaturated triglycerides with the unsaturated species in the *sn*-2 position, to milkfat fractions depleted in long-chain triglycerides on the thermal behavior and textural properties of the fractions. Mixtures of increasing proportions of cocoa butter were added to fractions isolated from milkfat of short- to medium-chain ($C_{22}$–$C_{44}$) and medium- to long-chain triglycerides ($C_{36}$–$C_{48}$) depleted in fully-saturated triglycerides.

## EXPERIMENTAL PROCEDURES

*Fractionation method.* The major chainlength fractions from anhydrous milkfat (obtained from University of Wisconsin,

[1]Presented at the 1995 AOCS Annual Meeting & Expo, San Antonio, Texas, May 1995.

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Madison, WI) were generated by solid-phase extraction using 60-mL capacity $C_{18}$ solid phase columns (Varian Analytichem International, Inc., Harbor City, CA) as described previously (12).

*Analysis of the triglycerides.* Triglycerides were separated and quantified as intact species by high-temperature gas chromatography on a Varian 3400 gas chromatograph (Sunnyvale, CA). A DB-HT17 column (donation of J&W Scientific, Folsom, CA) was used to separate intact triglycerides on the basis of chainlength and unsaturation. The conditions were those described previously (12).

*Combinations of butterfat fractions and cocoa butter.* Cocoa butter (donation of Cocao De Zaan BV, Koog aan de Zaan, The Netherlands) was added in increasing proportions in 10% increments to the fractions. All combinations of the triglyceride species from milkfat and cocoa butter were thoroughly mixed in the molten state at 60°C by vortexing.

*Thermal behavior of the mixtures.* Crystallization and melting behavior of the butterfat fraction and cocoa butter mixtures were monitored by differential scanning calorimetry (DSC) using a Perkin Elmer (Norwalk, CT) DSC-2C. For both heating and cooling modes, the temperature scanning program was set from 340–310 to 240–210 K at 20 K/min depending on the mixture analyzed. Crystal memory was destroyed by maintaining the temperature of the 10-μL samples at 80°C for 5 min. The analyses were performed to induce crystallization in the α form where possible (4).

*Textural properties of the mixtures.* Penetrometry was used to measure texture because this methodology is recognized as the closest indicator of textural spreadability (16,17). Penetration tests were performed using a model 1122 Instron universal testing machine (Canton, MA) (4). Mixtures of Fraction 1 or Fraction 2 and cocoa butter in increasing proportions (20% increments) were tested in triplicate at 4 and at 25°C. The force of penetration at a depth of 2 mm was recorded.

## RESULTS AND DISCUSSION

*Molecular composition and thermal behavior of milkfat, cocoa butter, and milkfat fractions.* Milkfat contained triglyceride species ranging from $C_{24}$ to $C_{54}$ (Fig. 1). About 35% of the total triglycerides were long-chain ($C \geq 42$). Within these long-chain triglycerides, 60% were unsaturated species.

The top curve (a) of each DSC curve represents milkfat as a reference (Figs. 2–5). The crystallization curve displayed two thermal events or exotherms (Figs. 2 and 4), referred to as high temperature or high portion ($T_m$ = 11.5°C) and lower temperature or lower portion ($T_m$ = 2°C) of the curve. How-

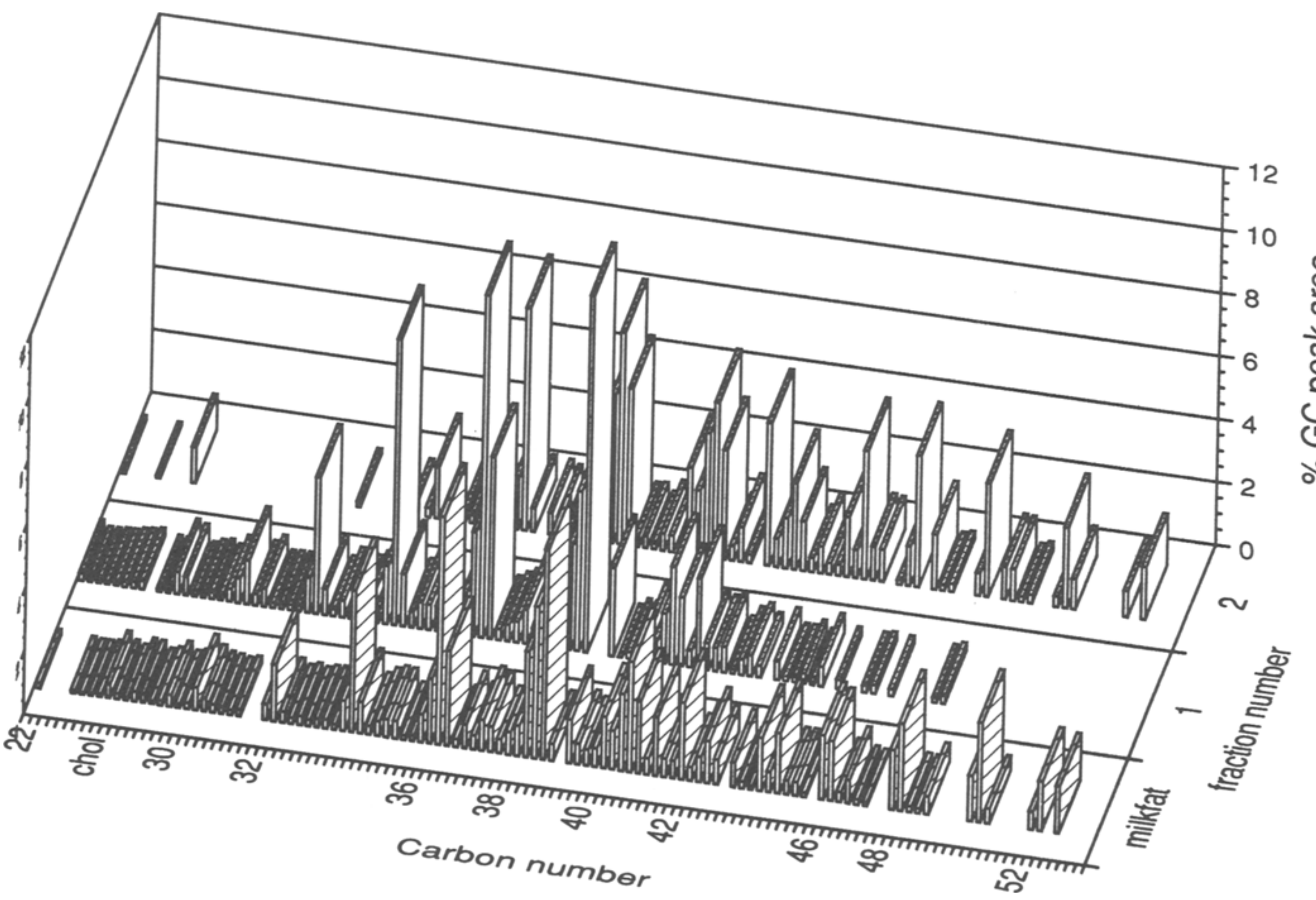


**FIG. 1.** High-temperature chromatography profiles of triglycerides in milkfat vs. Fraction 1 and Fraction 2 separated by solid-phase extraction. GC, gas chromatography; chol, cholesterol.

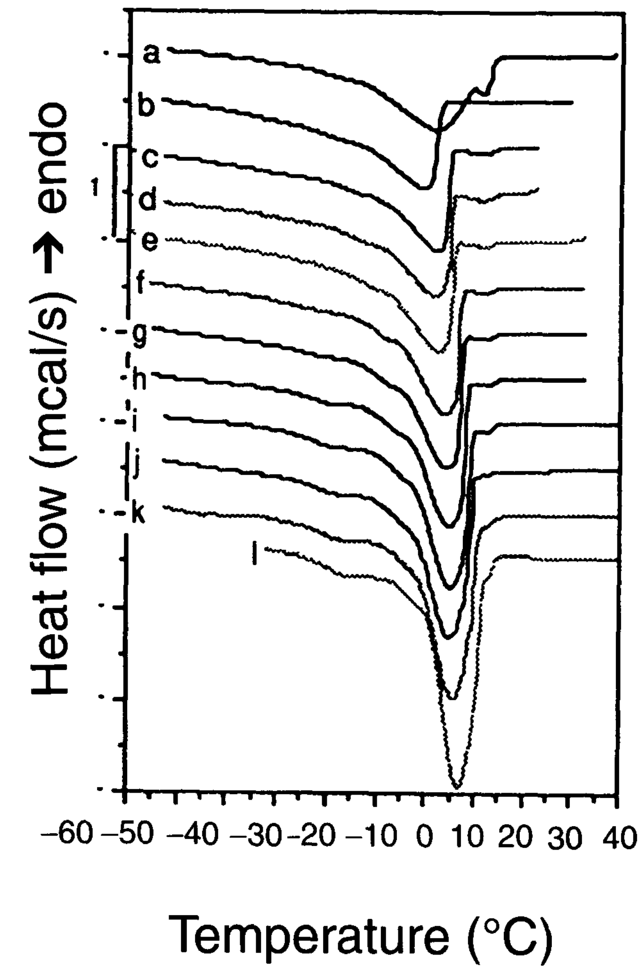


**FIG. 2.** Crystallization curves of Fraction 1 containing increasing amounts of cocoa butter. The proportions of c–k are expressed as the ratio of Fraction 1/cocoa butter; (a) milkfat, (b) Fraction 1, (c) 90:10, (d) 80:20, (e) 70:30, (f) 60:40, (g) 50:50, (h) 40:60, (i) 30:70), (j) 20:80, (k) 10:90, and (l) cocoa butter.

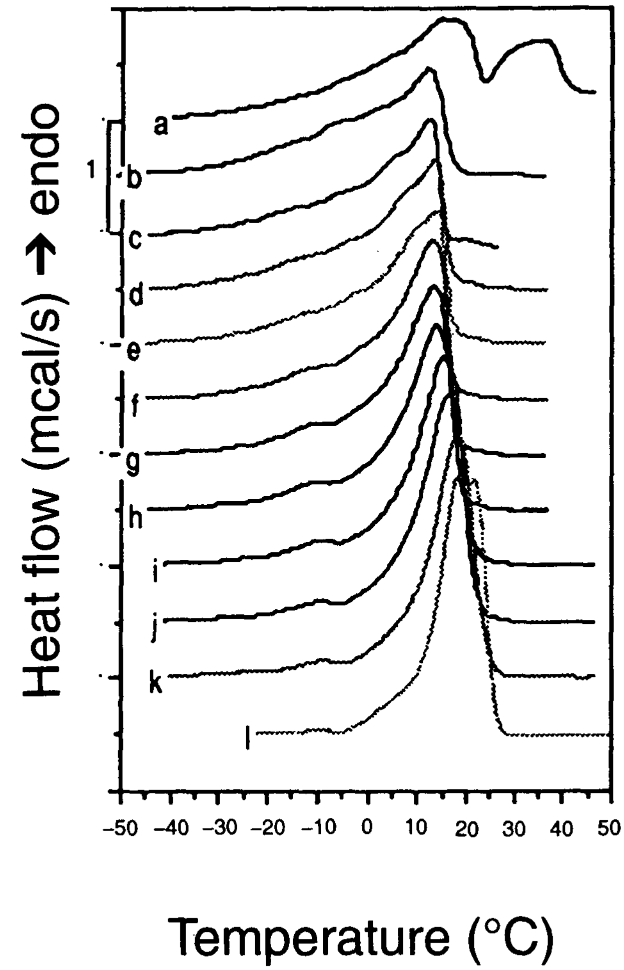


**FIG. 3.** Melting curves of Fraction 1 containing increasing amounts of cocoa butter. The proportions of c–k are expressed as the ratio of Fraction 1/cocoa butter; (a) milkfat, (b) Fraction 1, (c) 90:10, (d) 80:20, (e) 70:30, (f) 60:40, (g) 50:50, (h) 40:60, (i) 30:70, (j) 20:80, (k) 10:90, and (l) cocoa butter.

ever, these thermal events were not distinctly separated. The presence of triglycerides varying in molecular weights, structures, and crystallization temperature leads to their incomplete mutual solubility. Upon crystallization of milkfat, two exotherms were observed on the DSC curves, reflecting the fact that all the triglycerides do not crystallize cooperatively or cocrystallize.

The melting curve of milkfat displayed a low temperature endotherm at $T_m$ = 15 °C and a broad higher temperature endotherm at $T_m$ = 36°C (curve a in Figs. 3 and 5). The polymorphism of milkfat has been reviewed by deMan (13). As discussed by deMan (13), the different crystal forms observed are α, β′ and β, with a preponderance of the β′ form and small amount of β form. The phase behavior of milkfat can be explained in terms of solid solutions (15,18). A solid solution refers to the equilibrium of a liquid mixture of triglycerides with a solid mixture of triglycerides. The concept of solid solution is used to obtain information on the extent of solubility in the solid phase of mixtures of triglycerides. The wide range of triglycerides in milkfat results in incomplete miscibility in the solid phase (19). The phase behavior of milkfat has been explained in terms of three fractions (high-melting, middle-melting, and low-melting) of largely independently-melting solid solutions and is directly related to the molecular composition of milkfat. The fraction melting at low temperature is liquid at ambient temperature and acts primarily as a diluent for the two other fractions, whereas the stable polymorphs for the middle-melting and fractions that melt at high temperature are β′-2 (+ some β′-3) and β-2, respectively (19).

*Cocoa butter.* Ninety-eight percent of cocoa butter is composed of five triglyceride species: palmitic/oleic/palmitic (POP), 19%; palmitic/oleic/stearic (POS), palmitic/oleic/oleic, 46%; stearic/oleic/stearic (SOS), and stearic/oleic/oleic, 33%. The diunsaturated species constitute 20% of the total triglycerides (15).

Cocoa butter crystallizes in six possible crystal modifications (14). The predominant triglycerides of cocoa butter—POP, POS, and SOS—are completely miscible in the solid state in the proportions present in cocoa butter (19), whereas the diunsaturated triglycerides are not completely miscible

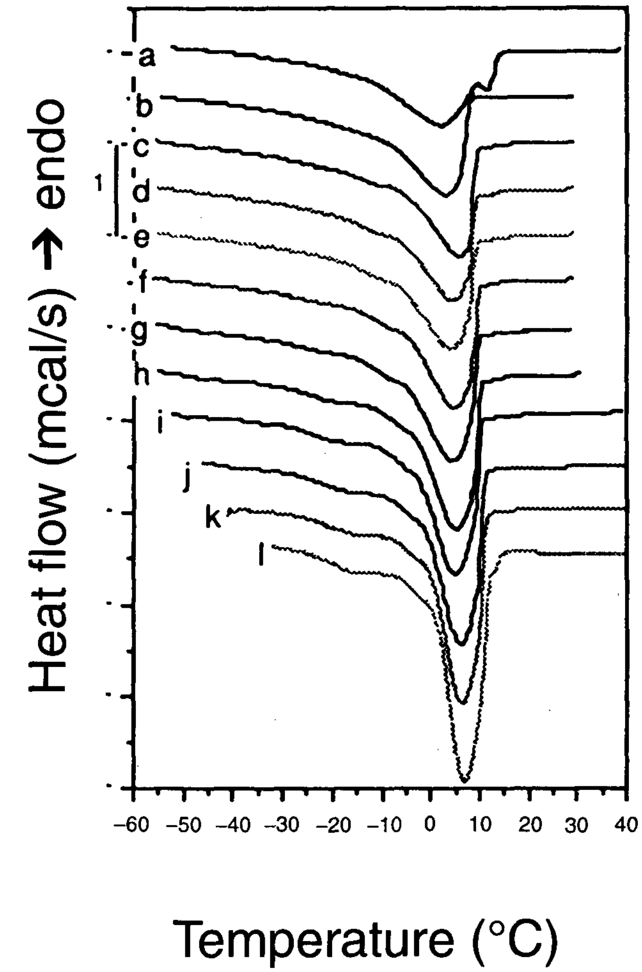


**FIG. 4.** Crystallization curves of Fraction 2 containing increasing amounts of cocoa butter. The proportions in c–k are expressed as the ratio of Fraction 2/cocoa butter; (a) milkfat, (b) Fraction 2, (c) 90:10, (d) 80:20, (e) 70:30, (f) 60:40, (g) 50:50, (h) 40:60, (i) 30:70, (j) 20:80, (k) 10:90, and (l) cocoa butter.

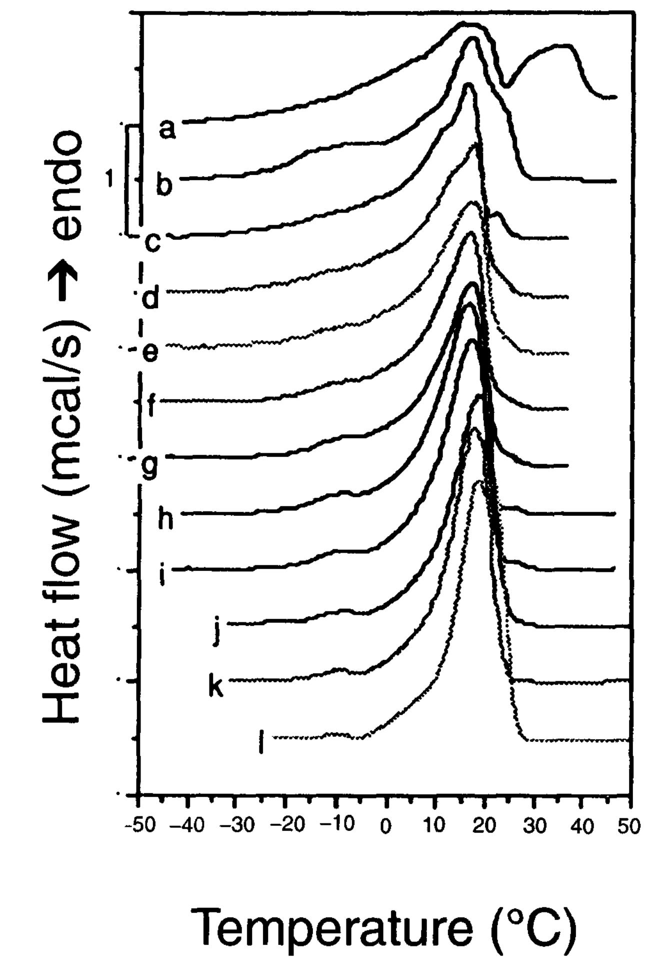


**FIG. 5.** Melting curves of Fraction 2 containing increasing amounts of cocoa butter. The proportions of c–k are expressed as the ratio of Fraction 2/cocoa butter; (a) milkfat, (b) Fraction 2, (c) 90:10, (d) 80:20, (e) 70:30, (f) 60:40, (g) 50:50, (h) 40:60, (i) 30:70, (j) 20:80, (k) 10:90, and (l) cocoa butter.

and thus consist of a liquid phase in equilibrium with the POP, POS, and SOS solid phase at ambient temperatures. The diunsaturated triglycerides are responsible for a small low temperature peak in DSC melting curves (19).

The miscibility of the triglycerides of cocoa butter was exhibited on the crystallization curve by the presence of one exotherm reflecting the cocrystallization of the species upon cooling (Figs. 2 and 4).

At a heating rate of 20°C/min, the melting values of pure cocoa butter at 17.3 and 23.3°C corresponded to the values reported for the $\beta'_3$ (or sub-$\alpha$) and $\alpha$-2 forms, respectively (14).

*Fraction 1.* The first fraction (Fraction 1) consisted of 36% (w/w) of milkfat. The fraction was made up of short- and medium-chain triglycerides composed almost exclusively (95%) of species with a chainlength of less than $C_{42}$ (Fig. 1).

The phase changes of Fraction 1 upon cooling or heating resulted in a single thermal peak. The high temperature thermal events typical of milkfat were absent from this fraction. The crystallization curve of Fraction 1 (curve b, Fig. 2) revealed one exotherm with a sharp onset at $T_i = 5.5$°C. The occurrence of one exotherm upon cooling indicated the cocrystallization of the triglycerides of this fraction upon cooling.

The melting pattern of Fraction 1 (curve b, Fig. 3) exhibited one endotherm at $T_m = 14$°C, which indicated that the different triglycerides of this fraction melted cooperatively.

*Fraction 2.* The second fraction made up 36% (w/w) of milkfat. It contained cholesterol and triglyceride species ranging from medium- to long-chain ($C_{34}$–$C_{52}$) (Fig. 1). Long-chain triglycerides ($C \geq 42$) were 40% of the total triglycerides. These long-chain triglycerides were largely (70%) unsaturated. The fraction did not contain triglycerides of chainlength below $C_{36}$.

The crystallization pattern of Fraction 2 (curve b, Fig. 4) exhibited one exotherm of similar onset and maximum ($T$ = 13°C) as the lower temperature portion of milkfat. The occurrence of one exotherm upon cooling indicated the cocrystallization of the triglycerides of this fraction upon cooling.

The melting pattern of Fraction 2 alone (curve b, Fig. 5) displayed one endotherm at $T_m = 17$°C, which indicated that the different triglycerides of this fraction melted somewhat cooperatively. However, the melting pattern reflected different steps in the heat absorbed upon heating, possibly indicating the melting of different triglyceride species or the melting of different crystal forms of the mixtures of triglycerides.

*Thermal behavior of Fraction 1 and cocoa butter mixtures.* The phase behavior of mixtures of milkfat and milkfat fractions with cocoa butter have been reported (15). The softening effect of milkfat was due to the effect of liquid triglycerides in milkfat. The addition of increasing proportions of cocoa butter to Fraction 1 led to crystallization (i.e., bulk of the heat released) over a narrower temperature range as the percentage of cocoa butter was increased (Fig. 2). The temperature onset of crystallization and the temperature at maximum crystallization rate increased as the percentage of cocoa butter was increased.

The increasing addition of the monounsaturated triglycerides POP, POS, and SOS from cocoa butter to short- and medium-chain triglycerides from milkfat did not lead to the appearance of a higher temperature exotherm, as it did in milkfat. This indicates that all proportions of mixtures of short- and medium-chain triglycerides and cocoa butter, although consisting of triglycerides with different shapes and molecular weights, have a relatively high mutual solubility and form a relatively homogeneous crystalline network. This further suggests that these milkfat fractions are an excellent solvent for cocoa butter triglycerides and may represent the basis for the significant softening effect of milkfat on cocoa butter confections.

The high-temperature events in milkfat are due to fully-saturated triglycerides ($C > 50$) (4). A mixture of Fraction 1 and 5% saturated triglycerides ($C > 50$) also exhibited two exotherms upon cooling. In contrast, crystallization curves of a 90:10 mixture of cocoa butter/Fraction 1 (curve k, Fig. 2) exhibited cocrystallization, which further suggests that only fully-saturated triglycerides have the ability to induce discrete high-temperature crystallization events as in whole milkfat.

The melting behavior of mixtures of Fraction 1 and cocoa butter in increasing proportions revealed a cooperative melting for all the proportions tested. The solubility of the triglycerides of the mixtures in all proportions was displayed by the presence of one large endotherm, and no polymorphic change was discernible. The addition of the monounsaturated triglycerides POP, POS, and SOS from cocoa butter to short- and medium-chain triglycerides from milkfat did not lead to the appearance of a higher temperature endotherm. This suggests that monounsaturated triglycerides from cocoa butter contribute to a cooperative melting and the absence of polymorphic transitions for the crystallization and melting rates tested experimentally.

The bulk of the melting endotherm occurred over a narrower temperature range as the percentage of cocoa butter was increased, which indicated that cooperative melting was increased as the percentage of cocoa butter was increased.

The temperature at maximum melting rate slowly increased as the percentage of cocoa butter was increased above 60%. Below 60% cocoa butter, the increase in temperature at maximum melting rate among the different ratios of Fraction 1 and cocoa butter was negligible.

*Thermal behavior of Fraction 2 and cocoa butter mixtures.* Addition of increasing proportions of cocoa butter to Fraction 2 showed that crystallization of these mixtures of triglycerides proceeded interactively, which was reflected by the presence of one exotherm (Fig. 4). The increasing addition of the monounsaturated triglycerides POP, POS, and SOS from cocoa butter to medium- to long-chain ($C_{36}$–$C_{48}$) triglycerides from milkfat again did not lead to the appearance of a higher temperature exotherm, as was apparent in milkfat. These data suggest that only fully-saturated triglycerides provide the high-temperature crystallization events of whole milkfat. The bulk of the crystallization exotherm also occurred over a narrower temperature range as the percentage of cocoa butter was increased, which indicated that the percentage of cocoa butter increased the extent of cooperative crystallization, and therefore the mutual solubility of the triglycerides present in the mixtures.

The temperature onset of crystallization and the temperature at maximum crystallization rate increased with the addition of 10% cocoa butter and did not significantly change with the further addition of cocoa butter up to 60%. Both then slowly increased as the percentage of cocoa butter was further increased.

Increasing proportions of cocoa butter led to the occurrence of the melting endotherm over a narrower temperature range than for Fraction 2 (Fig. 5). For example, addition of 10% cocoa butter to Fraction 2 led to a more rapid decrease in melting rate (i.e., over a narrower temperature range) after reaching the maximum rate than for Fraction 2 alone.

The melting over a narrower temperature range in one homogeneous endotherm indicated that cocoa butter enhanced the cooperative melting of the triglycerides present in the combination. When cocoa butter was added at 10%, it interacted with the triglycerides of Fraction 2; i.e., it enhanced the solubility of the triglycerides in the solid state, possibly *via* a crystallization into a predominant crystal form. The addition of 10% of monounsaturated long-chain triglycerides also led to a small endotherm at 23°C, which could represent the occurrence of an $\alpha$-2 crystal form of the triglycerides of cocoa butter.

The temperature at maximum melting rate did not change until 30% cocoa butter was present in the mixtures, and then slowly increased as the percentage of cocoa butter was further increased. In mixtures of medium- to long-triglycerides (unsaturated) from milkfat, the increasing addition of the monounsaturated triglycerides POP, POS, and SOS from cocoa butter did not lead to the appearance of a higher temperature endotherm, as was apparent in milkfat. These results indicated that triglycerides from cocoa butter are soluble in the triglycerides present in Fraction 2.

Overall, the thermal behavior at relatively high heating and cooling rates for the combinations tested showed that mixtures of cocoa butter and Fraction 1, as well as Fraction 2, were miscible. More extensive cocrystallization and more cooperative melting of the triglycerides present were observed when cocoa butter was added to Fraction 1 or Fraction 2. The high-temperature crystallization events characteristic of milkfat and milkfat fractions were absent. These events are due to an incomplete solubility of the saturated triglycerides ($C \geq 50$) above 3% (4). These data further indicated that extensive cocrystallization occurred as long as the fractions from milkfat did not contain long-chain saturated triglycerides ($C \geq 50$). Although Fraction 2 was depleted of shorter-chain milkfat triglycerides ($C \leq 36$), it contained 0.3% long-chain saturated triglycerides as opposed to 3% in milkfat, and 6% long-chain unsaturated triglycerides ($C \geq 50$) as in milkfat.

*Textural behavior of mixtures of Fraction 1 or 2 and cocoa butter.* The textural behavior was assessed to relate the phase changes and the presence or absence of cocrystallization with textural properties. Because cocoa butter is solid at room temperature, one question pursued was whether the increase in cocoa butter percentage in the mixtures translated into increased firmness as texture.

The mixtures of Fraction 1 or Fraction 2 and cocoa butter were tested for texture by penetrometry. Samples tested for texture at 4 and 25°C were mixtures of Fraction 1 or Fraction 2 and cocoa butter in 20% increments. The results of penetrometry testing at 4°C for all mixtures (Fig. 6) indicated that combinations of cocoa butter with Fraction 1 and with Fraction 2 could lead to similar firmness (as seen by the force resisting cone penetration) as milkfat. In particular, mixtures of Fraction 2 and cocoa butter in 80:20 and 60:40 proportions displayed similar resistance to penetration as milkfat. The 40:60 mixture of Fraction 1 and cocoa butter also displayed a resistance in the same range as milkfat. Mixtures of Fraction 1 and 40% or less cocoa butter displayed a softer texture than that of milkfat. Mixtures of Fraction 1 and 80% or more cocoa butter, and of Fraction 2 and 40% or more cocoa butter, all displayed a texture significantly firmer than milkfat.

For both Fraction 1 and Fraction 2, the addition of 20% cocoa butter led to a softer texture than that of the fraction alone. For Fraction 2, the amount of cocoa butter that could be added while still leading to a softer texture than the fraction alone was 40%. These data were not expected from the thermal behavior, which indicated higher crystallization temperatures as cocoa butter was added. The thermal behavior indicated that the proportion of solid fat at 4°C would be higher for mixtures with a higher content of cocoa butter, which usually translates into firmer texture (20). In the mixtures tested in the present study, the relationship between the physical state (i.e., % solid vs. % liquid fat) did not simply translate into a firmer texture as seen by the resistance to cone penetration at 2 mm. These results suggested that an interaction between cocoa butter and the triglycerides of the milkfat fractions influenced the textural behavior of the mixtures. In particular, the structure and homogeneity of the crystalline lattice might be of importance, as well as the crystal forms present in the textural experiments. Further research should be directed toward understanding the basis of the textural differences observed.

Results of penetrometry at 25°C (Fig. 7) showed that some mixtures displaying a finite resistance to penetration close to

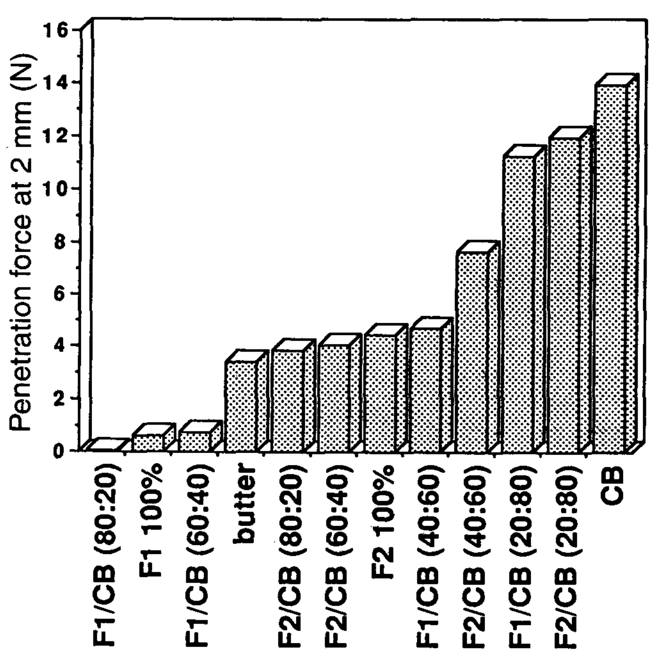


**FIG. 6.** Penetration testing at 4°C. The force at 2-mm penetration was measured using an Instron universal testing machine (Canton, MA). Fractions were brought from the molten state to 4°C for 14 h, and experiments were conducted at 4°C. CB, cocoa butter; F1, Fraction 1; F2, Fraction 2. Ratios are shown in parentheses.

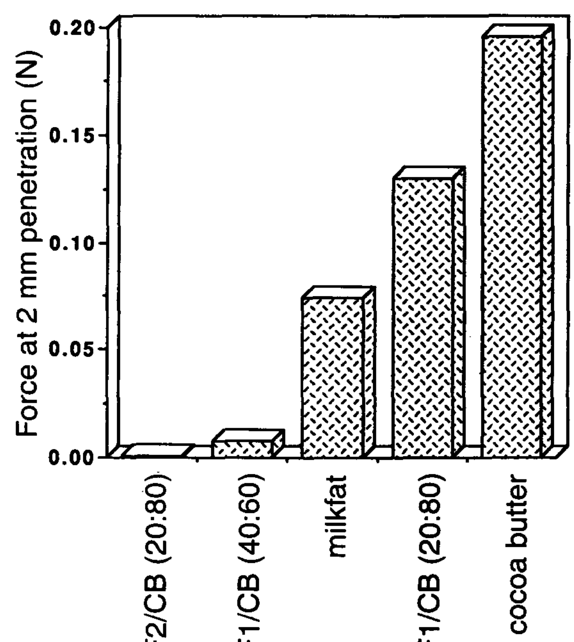


**FIG 7.** Penetration testing at 25°C. The force at 2-mm penetration was measured using an Instron universal testing machine. Fractions were brought from the molten state to 4°C for 14 h and then equilibrated at 25°C for 2 h. Ratios are shown in parentheses. Abbreviations and company source as in Figure 6.

that of milkfat at 4°C did not retain plastic (i.e., solid) properties at 25°C. Mixtures of Fraction 1 and up to 40% cocoa butter, as well as mixtures of Fraction 2 and up to 60% cocoa butter, did not exhibit a measurable resistance to penetration at 25°C, although some fractions were solid and did not exhibit flow. The 20:80 mixture of Fraction 1 and cocoa butter, although presenting a firmness in the range of milkfat at 25°C, was much firmer than milkfat at 4°C.

It should be noted that the 60:40 mixture of Fraction 1 and cocoa butter was a solid at 25°C. This revealed unique relaxation properties—the visible puncture from cone penetration disappeared within less than 30 s, and this mixture was the only one to exhibit such a relaxing capability.

The new and significant findings of this study were: (i) The thermal behavior of mixtures of cocoa butter and fractions of short- to medium-chain and medium- to long-chain unsaturated triglycerides from milkfat generally indicated a slight increase in the temperature of crystallization and melting as cocoa butter was added in increasing percentages to Fraction 1 or Fraction 2. (ii) The addition of cocoa butter at 20–40% to Fraction 1 or Fraction 2 tended to decrease the resistance to cone penetration at 4°C, which could not be inferred from the thermal behavior or proportion of solid fat at 4°C. The data suggested that an interaction between the triglyceride molecules affects the texture. (iii) Overall, long-chain monounsaturated triglycerides from cocoa butter enhanced cocrystallization and cooperative melting of milkfat fractions that melt at low temperature, and acted as a texture builder if present in proportions of more than 30%, whereas below this level they acted as texture softeners.

Particular emphasis needs to be placed on the crystal morphology and crystalline network structure of different combinations of long-chain monounsaturated triglycerides from cocoa butter and milkfat fractions that melt at low temperature.

## ACKNOWLEDGMENTS

The authors gratefully acknowledge the support of the California Dairy Foods Research Center, the Wisconsin Milk Marketing Board, and a University of California Fellowship to C. Simoneau.

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[Received June 14, 1995; accepted October 25, 1995]