

Ternary Phase Behavior of Milk Fat Fractions

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Milk fat was sequentially crystallized from ethyl acetate into three major components—a high melting fraction (HMF), a middle melting fraction (MMF), and a low melting fraction (LMF)—in 12%, 34%, and 54% (w/w) yields, respectively. The phase behavior of mixtures of HMF, MMF, and LMF was studied using solid fat content vs temperature data. Binary and ternary phase diagrams of mixtures of the three components were constructed. Binary HMF–MMF mixtures displayed monotectic solution behavior, forming a solid solution, whereas both LMF–HMF and LMF–MMF binaries formed partial solid solutions. The LMF fraction interacted more strongly with MMF than with HMF. Ternary phase diagrams showed the complex interaction between the three components simultaneously. On average, monotectic solution behavior was observed in mixtures of the three fractions at temperatures between 0 and 15 °C and above 30 °C. A eutectic was observed between a MMF–HMF solid solution and LMF at 0 °C. At temperatures corresponding to the melting range of MMF (15 °C < T < 30 °C), complex solution behavior was observed, with strong interactions between MMF and LMF.

Keywords: Milk fat; fractions; phase diagrams; solution behavior; mixed crystals

INTRODUCTION

Milk fat is a complex mixture of several hundred different triacylglycerols (TAGs) with an extremely heterogeneous fatty acid composition (Gresti et al., 1993). Undoubtedly it is one of the most complex fats found in nature. The physical properties of milk fat, including melting behavior, solid fat content, and polymorphism, are dependent not only on the physical and chemical properties of the constituent TAGs but also on the interaction between these constituent TAGs. For these reasons, several studies have been performed in the past to understand how TAG structure influences phase behavior and polymorphism of milk fat (Mulder, 1953; deMan, 1963; Timms, 1980, 1984).

A typical melting curve of untempered native milk fat determined using differential scanning calorimetry (DSC) shows three endothermic peaks, corresponding to high (>50 °C), medium (35–40 °C), and low (>15 °C) melting fractions (Timms, 1980). According to DSC determinations (ratios of enthalpies), milk fat contains 11% high melting fraction (HMF), 23% middle melting fraction (MMF), and 66% low melting fraction (LMF) (Timms, 1980). These fractions are chemically distinct, with HMF containing principally long-chain saturated fatty acids, MMF containing two long-chain saturated fatty acids and one short or *cis*-unsaturated fatty acid, and LMF containing one long-chain saturated fatty acid and two short-chain or *cis*-unsaturated fatty acids (Timms, 1980).

Knowledge of the chemical composition, phase behavior, and polymorphism of these fractions and their mixtures and how their properties influence each other would help us better understand, predict, and control the physical properties of milk fat. To obtain this understanding, milk fat must first be efficiently separated into three fractions and the phase behavior and

polymorphism of the individual fractions determined. Timms (1980) fractionated milk fat into these three fractions using acetone as a solvent and proved that HMF, MMF, and LMF were, in fact, distinct fractions (at the time, MMF was believed to be a solid solution of HMF and LMF). He also studied the polymorphism of these fractions and the effects of LMF addition to HMF (50% LMF) and MMF (75% LMF). Unfortunately, Timms (1980) never constructed actual phase diagrams for these fraction mixtures. However, to truly understand the complex and subtle interactions between these fractions and their effects on the physical properties of native milk fat, it is necessary to construct such phase diagrams.

Isosolid diagrams are useful tools in the study of the phase behavior of mixtures of natural fats (Timms, 1984). These isosolid diagrams have been used in the study of the phase behavior of mixtures of confectionery fats with milk fat and milk fat fractions (Kaylegian et al., 1993; Ali and Dimick, 1994; Hartel, 1996). The type of solution behavior can usually be discerned with the aid of these diagrams. Their main use has been in the identification of eutectics in mixtures of cocoa butter and cocoa butter substitutes. The softening of chocolate upon incorporation of milk fat is attributed to the formation of a eutectic between cocoa butter and milk fat. This procedure constitutes a useful way of qualitatively judging the compatibility of fats.

Recently, the phase behavior of binary and ternary mixtures of confectionery fats with milk fat and milk fat fractions obtained by dry melt crystallization has been studied (Ali and Dimick, 1994; Hartel, 1996). A better understanding of the complex interactions between milk fat TAGs, cocoa butter TAGs, and palm kernel stearin TAGs and the resulting macroscopic properties of the blends (melting behavior, bloom formation, softening) has been obtained from these studies. Even though milk fat fractions can be produced in high purity by dry melt crystallization for laboratory studies

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(Kaylegian et al., 1993), no effort has been directed toward furthering our understanding of the phase behavior of milk fat fractions and its relationship to the physical properties of native milk fat.

In this study we have constructed binary and ternary phase diagrams for mixtures of HMF, MMF, and LMF obtained by solvent fractionation in order to better understand the complex interactions between these three fractions.

MATERIALS AND METHODS

Multiple-Step Solvent Fractionation. Anhydrous milk fat (200 g) was melted above 80 °C, cooled to 40 °C, and dissolved (1:4 w/w) in room temperature ethyl acetate (Fisher Scientific, St. Louis, MO). The mixture was then transferred to a glass bottle and placed in a thermostated water bath at 5 °C for 1 h. The mixture was mixed by inversion every 5 min and vacuum-filtered at 5 °C using a Buchner funnel. A fast filtering Whatman No. 1 filter paper was used for this purpose. The collected crystal mass was immediately washed with 200 mL of 5 °C ethyl acetate. The crystal mass was completely white and devoid of entrained material after this process. This fraction will be referred to as HMF. The filtrate plus washes were transferred to a glass bottle, which was then placed in a stainless steel bucket filled with ethylene glycol (for improved heat transfer) in a freezer at -28 °C for 1 h. The mixture was mixed by inversion every 10 min and vacuum-filtered at -28 °C using a Buchner funnel. A fast filtering Whatman No. 1 filter paper was used for this purpose. The collected crystal mass was immediately washed with 400 mL of -28 °C ethyl acetate. The crystal mass was completely white and devoid of entrained material after this wash. This fraction will be referred to as MMF. The wet crystal masses were spread as a thin film on stainless steel trays, and the excess solvent was allowed to evaporate overnight at room temperature in a fume hood. The excess solvent present in the -28 °C filtrate and washes was removed by vacuum distillation in a rotary evaporator at 40 °C. This yellow liquid was then spread as a thin film on a stainless steel tray and the solvent allowed to evaporate overnight at room temperature in a fume hood. This fraction will be referred to as LMF.

Chemical and Physical Characterization of the Milk Fat Fractions. Fatty acid analysis of the fractions was performed by gas-liquid chromatography according to the method of Bannon et al. (1985). Triglyceride analysis was performed using high-temperature gas-liquid chromatography as previously described (Rousseau et al., 1996a). Solid fat content was determined by pulsed nuclear magnetic resonance using a Bruker PC20 Series NMR analyzer (Bruker, Milton, ON, Canada) according to the AOCS official method Cd16-81. Dropping points were determined as described in Rousseau et al. (1996b).

Phase Equilibrium Studies. Binary Phase Diagrams. Mixtures (w/w) of HMF in LMF, MMF in LMF, and HMF in MMF were prepared in 10% increments from 0% to 100%. A 5% mixture was prepared as well. The solid fat content (SFC) of the mixtures was determined from 0 °C to 55 °C in 5 °C increments. A cubic spline curve was fitted to the data for interpolation purposes using the software package GraphPad Prism 2.0 (GraphPad Software, San Diego, CA). Isosolid SFC temperatures as a function of blend composition were derived from the data and used in the construction of the binary phase diagrams.

Ternary Phase Diagrams. All combinations of the HMF, MMF, and LMF fractions in 10% (w/w) increments were prepared, and the SFC of the mixtures was measured from 0 °C to 55 °C in 5 °C increments. Ternary phase diagrams of isosolid lines (same SFC at a particular temperature) were constructed in 5 °C increments from 0 to 45 °C using the software package Grapher (Golden Software, Golden, CO).

RESULTS AND DISCUSSION

General Characteristic of the Milk Fat Fractions. The average yields (standard deviation of seven

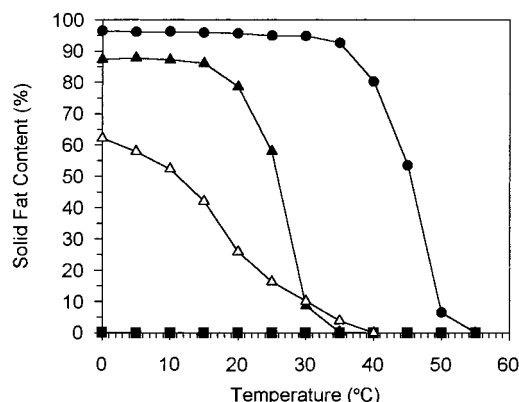


Figure 1. Solid fat content vs temperature profiles for anhydrous milk fat (△-△), high melting fraction (●-●), medium melting fraction (▲-▲), and low melting fraction (■-■).

Table 1. Fatty Acid Composition (% w/w) of Ethyl Acetate Fractionated Anhydrous Milk fat Fractions

fatty acid	HMF	MMF	LMF	AMF
4:0		4.78	5.24	4.51
6:0	0.19	3.50	3.80	3.12
8:0	0.34	1.45	2.03	1.64
10:0	1.74	3.18	4.43	3.86
10:1	0.09	0.47	0.67	0.80
12:0	3.60	3.06	4.78	4.07
14:0	15.40	11.82	11.14	10.99
14:1	0.84	1.25	2.07	1.88
15:0	1.77	1.51	0.88	1.46
16:0	42.50	39.46	19.84	28.73
16:1	1.69	1.70	3.59	3.12
17:0	0.92	0.91	0.83	0.40
18:0	20.55	13.63	6.00	10.45
18:1	9.30	12.31	29.61	20.92
18:2	0.54	0.44	2.17	1.86
18:3	0.39	0.23	1.38	1.65
20:0	0.20	0.50	1.57	0.60

replicates) of the HMF, MMF, and LMF were, respectively, 12% (0.74%), 34.0% (2.5%), and 54% (2.4%) (w/w). These yields are comparable to the in situ, calorimetrically determined (ratios of enthalpies) values of 11%, 23%, and 66% (Timms, 1980). Timms (1980) obtained in his study yields of 6%, 24%, and 70% (w/w) for the acetone fractionation of milk fat into HMF, MMF, and LMF, respectively. The SFC vs temperature profiles of the three fractions plus native milk fat and cocoa butter are shown in Figure 1. Both the HMF and MMF have sharp melting ranges, and the LMF is completely liquid above 0 °C. The dropping points of the HMF and MMF were, respectively, 51.7 and 30.4 °C, while that of native anhydrous milk fat (AMF) was 34.3 °C.

The fatty acid and triglyceride profiles of these fractions and native milk fat are shown in Tables 1 and 2. Long-chain saturated fatty acids such as palmitic and stearic acids are concentrated in the HMF, while short-chain fatty acids and unsaturated fatty acids, predominantly oleic acid, are concentrated in the LMF. The general compositions of our fractions agree with those reported by Timms (1980). Differences in the triglyceride composition of our fractions are quite dramatic as well. The TAG profile of AMF is characterized by two distinct families—from 32 to 42 carbons and from 44 to 54 carbons (excluding glycerol). The HMF contains predominantly the high molecular weight TAGs in the range 42–54 carbons. From the fatty acid composition of this fraction, we can deduce that these

Table 2. Triglyceride Composition (% w/w) of Ethyl Acetate Fractionated Anhydrous Milk fat Fractions

TAG ^a	HMF	MMF	LMF	AMF
22		0.06		0.24
24		0.72	1.02	0.84
26		0.24	0.6	0.37
28		0.17	1.26	0.60
30		0.18	2.37	1.04
32	0.20	0.62	4.74	2.64
34	0.64	4.94	8.16	6.40
36	1.05	18.18	13.37	14.04
38	0.73	17.59	18.72	14.73
40	0.96	11.14	14.73	10.78
42	3.23	8.90	7.66	7.69
44	9.23	6.98	5.66	6.91
46	19.13	6.10	4.80	7.42
48	25.67	7.84	4.34	8.63
50	24.00	9.79	5.14	9.60
52	12.25	6.04	6.28	6.42
54	1.03	0.57	1.18	1.68

^a Number of carbons excluding glycerol.

TAGs contain predominantly saturated fatty acids. The MMF contains TAGs in the range 34–54 carbons, with a peak at 38 carbons. The LMF contains TAGs in the range 28–54 carbons, with a peak at 38 carbons. From the fatty acid composition of the LMF, we can deduce that these TAGs are enriched in oleic and short-chain fatty acids, hence the lower molecular weight. The fatty acid species present in the MMF seem to be a combination of those present in the HMF and LMF.

The important conclusion that can be derived from this characterization is that the HMF, MMF, and LMF have very different chemical compositions, melting points, and molecular volumes.

Phase Behavior—Binary Phase Diagrams. We constructed binary phase diagrams using isosolid lines for mixtures of MMF and HMF, LMF and MMF, and LMF and HMF (Figure 2). Our SFC values were obtained after exhaustive tempering as suggested in the AOCS official method Cd 16-81. The tempering procedure standardizes the effects of environmental conditions on crystallization behavior. Our SFC values are, therefore, approaching their equilibrium SFC value, and the different fractions should be in their equilibrium polymorphic form.

No eutectics were detected in any of the three mixtures. Figure 2A suggests monotectic mixing behavior between HMF and MMF, with a solid TAG solution forming in the solid phase. This type of behavior is observed when the constituent TAGs have similar melting points, molecular volumes, and polymorphic states (Timms, 1984). However, these two fractions have significantly different melting points and molecular volumes but the same polymorphic form (Timms, 1980). This suggests that a high degree of structural complementarity must exist between the HMF and MMF TAGs, making them fully miscible in the solid state. The existence of mixed crystals in milk fat has been suggested by Mulder (1953) and Walstra and Beresteyn (1975). Our results suggest that, indeed, mixed crystals of HMF and MMF form in the solid phase. Timms (1980) reported that both the MMF and HMF, in the absence of LMF, form stable β -2 crystals. The polymorphic form of these two fractions is similar; hence, formation of a mixed crystal lattice is plausible. However, from melting point, molecular volume, and chemical composition arguments, it is perhaps surprising that the HMF and MMF form solid solutions. Our

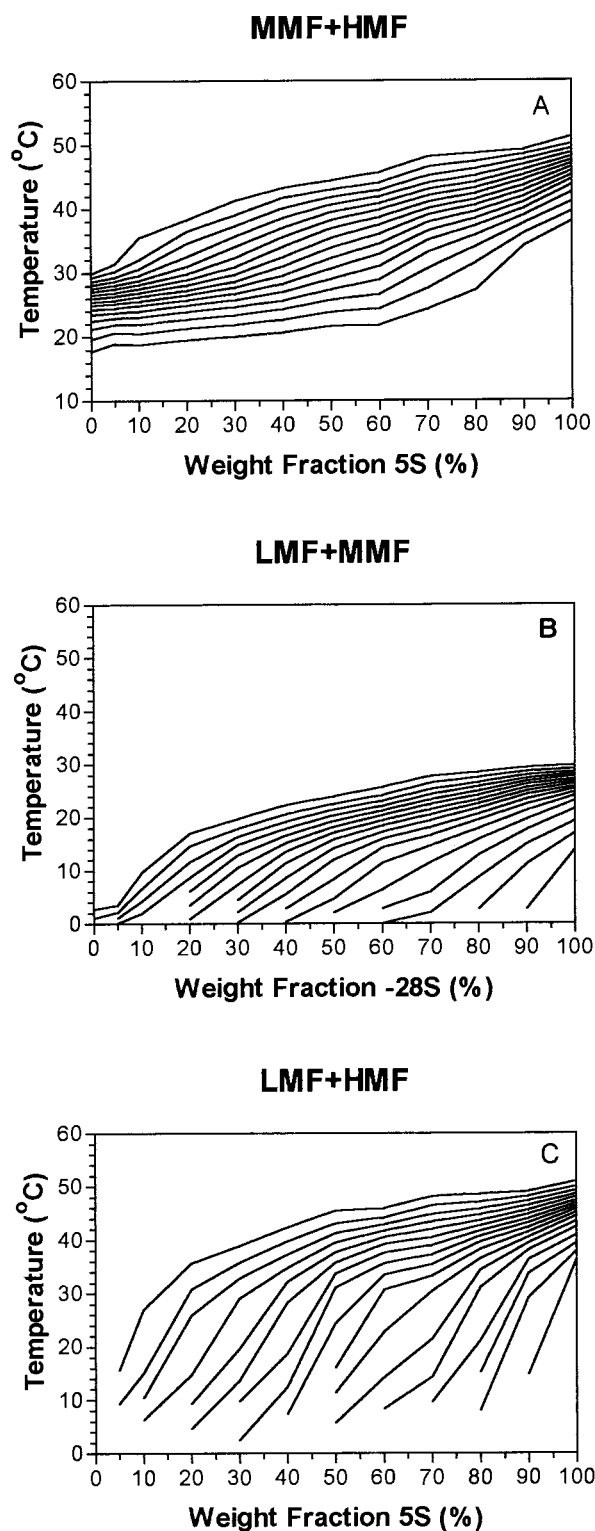


Figure 2. Binary isosolid phase diagrams (temperature for a particular solid fat content) for mixtures of (A) the medium and high melting fractions, (B) the low and medium melting fractions, and (C) the low and high melting fractions. Isosolid lines are spaced in 5% solid fat content intervals from 5% SFC (top curve) to 80% SFC (bottom curve).

results, combined with Walstra's group's results and Timms' study, constitute good evidence for the formation of mixed crystals in milk fat.

The phase behavior of LMF–HMF and LMF–MMF binaries is shown in Figure 2B,C. These phase diagrams suggest monotectic, partial solid solution forma-

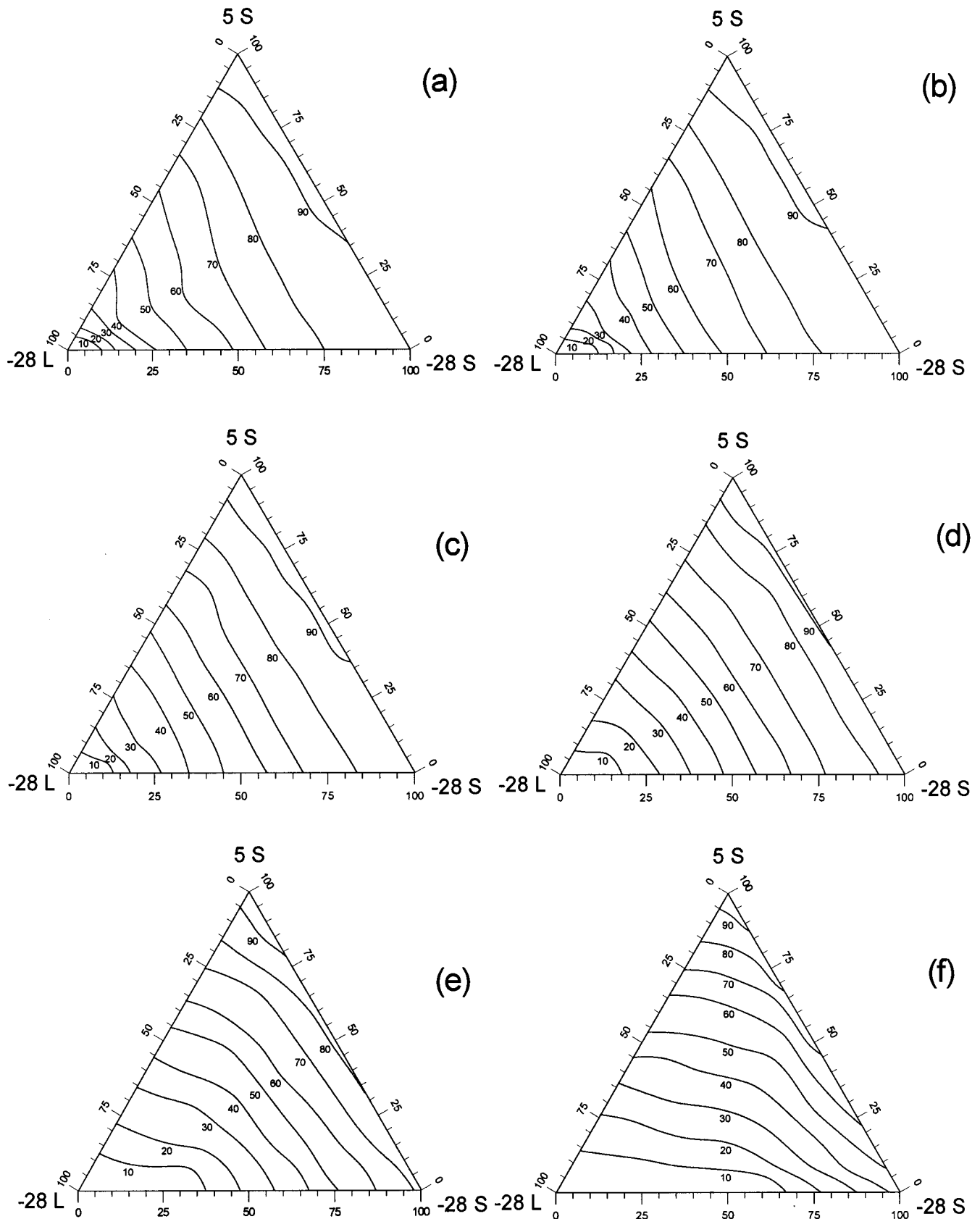


Figure 3. Ternary isosolid phase diagrams (composition at which the solid fat content of a mixture is equivalent) at different temperatures for mixtures of HMF, MMF and LMF: (a) 0, (b) 5, (c) 10, (d) 15, (e) 20, (f) 25, (g) 30, (h) 35, (i) 40, and (j) 45 °C.

tion between LMF and HMF or MMF. This type of behavior is characteristic of eutectic systems which shift to monotectic systems when differences in the melting points of the two components increase (e.g., 20 °C and above), and the *high melting* component dissolves a

substantial amount of the *low melting* component (20–30%) (Timms, 1984). These results suggest that a partial solid solution between LMF and HMF or MMF is forming. LMF is not acting plainly as a diluent of the HMF or MMF but will also enter into the HMF and

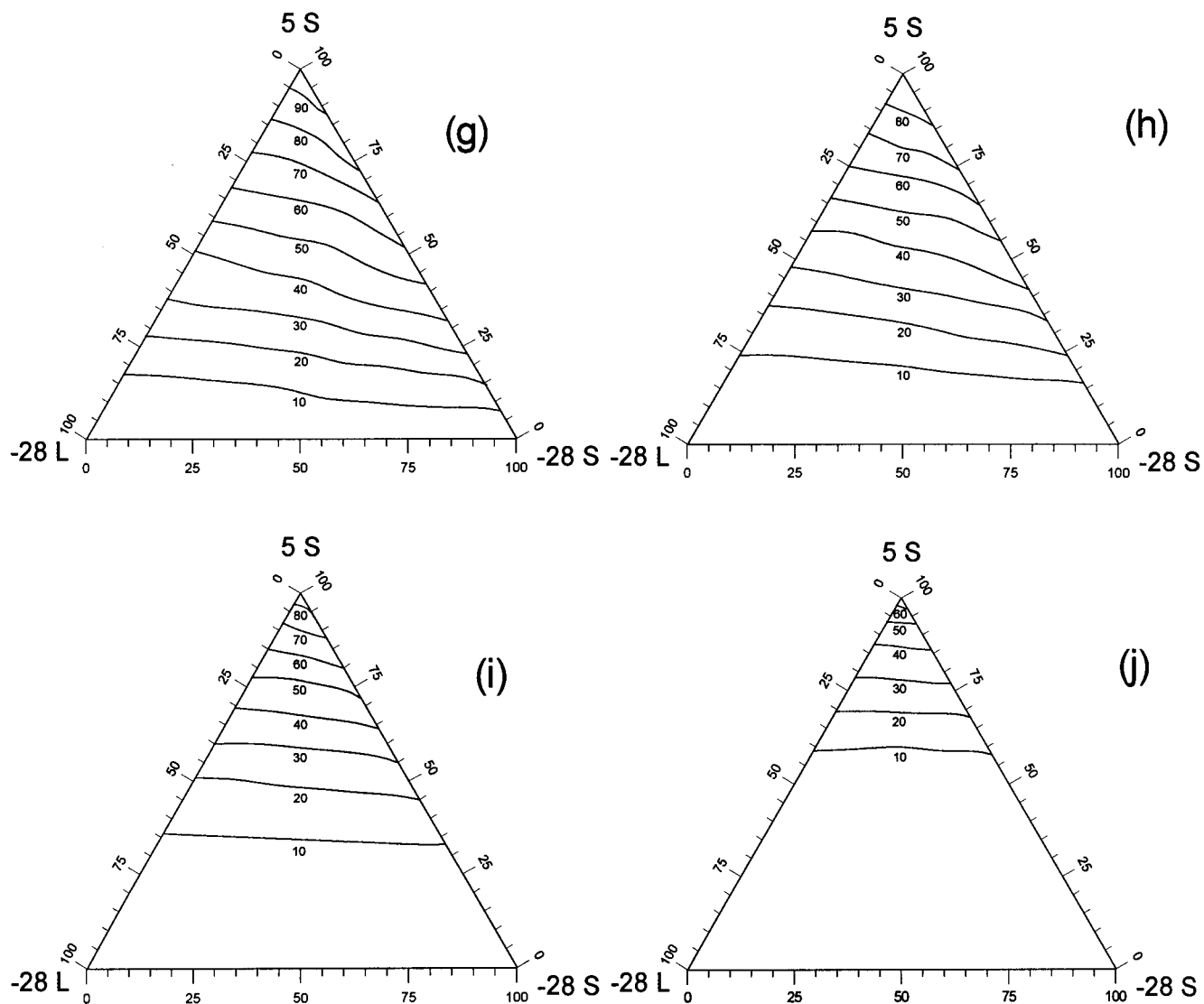


Figure 3 (Continued)

MMF solid crystals. Once again, the strong interaction between these fractions suggests that a high degree of structural complementarity also exists between the LMF and HMF and between LMF and MMF. The reader must remember that this is not a perfect solid solution but rather a partial solid solution whose precise nature has yet to be defined.

In summary, our results suggest that mixed crystals between MMF and HMF form in the solid phase. A solid solution between these two fractions was identified. A partial solid solution between HMF and LMF and MMF and LMF is formed, suggesting that large amounts of LMF are incorporated into HMF and MMF. A high degree of structural complementarity exists between the fractions' constituent triglycerides for these interactions to occur.

Phase Behavior—Ternary Phase Diagrams. Ternary phase diagrams for mixtures of HMF, MMF, and LMF at different temperatures are shown in Figure 3. The lines represent the composition at which the same solid fat content is measured (isosolid lines). In combination with the binary phase diagrams, these ternary phase diagrams allow us to understand the complex solution behavior of ternary mixtures of the three fractions.

During melting, a general shift in isolines from the pattern observed in Figure 3a ($T = 0\text{ }^{\circ}\text{C}$) to that observed in Figure 3j ($T = 45\text{ }^{\circ}\text{C}$) occurs. For ideal mixing behavior, a straight line parallel to the bottom or right axis would be observed for each isoline at each temperature. However, this was not observed for many ternary combinations at a variety of temperatures.

At all temperatures, addition of LMF to MMF leads to a relatively greater reduction in SFC than that when LMF is added to HMF. This suggests that the MMF and LMF TAGs interact strongly. This effect is also evident in the binary phase diagrams (Figure 2B,C), where the temperatures for particular SFC isolines in MMF–LMF mixtures are much lower than those in HMF–LMF mixtures. This effect is not surprising considering the greater similarity in molecular structure (TAG and fatty acid composition) between MMF and LMF as compared to that between HMF and LMF. As well, the isolines are wavy, suggesting the possible formation of solution intermediates between the three components.

As the temperature is increased from 15 to 45 $^{\circ}\text{C}$ (Figure 3c–j), one can notice that the isoline region at higher HMF/MMF ratios (adjacent to the LMF axis) shifts more readily than the isoline region at lower

HMF/LMF ratios (adjacent to the MMF axis). Once again, this effect is due to the strong interaction between LMF and MMF TAGs. As the MMF liquefies, the isolines begin straightening out above 30 °C (Figure 3g). At temperatures in the melting range of MMF, e.g., 15 and 30 °C (Figure 3d–g), one notices wavy isolines, suggesting the formation of solution intermediates between the three components.

Some of these observed changes in SFC behavior could be linked to polymorphic transitions. HMF and MMF crystallize in the β' -2 form, and addition of 50% LMF to the HMF or 75% LMF to the MMF leads to small changes in polymorphism (Timms, 1980). The polymorphic form of MMF is not affected by LMF addition, while some of the HMF crystallizes in a β -2 form upon addition of LMF (Timms, 1980). Differences in the behavior of MMF and HMF upon addition of LMF observed in the binary and tertiary phase diagrams could be due to the partial polymorphic transformation of HMF from the β' -2 to the β -2 form.

In the ranges 0 °C < T < 15 °C and >30 °C, we observe monotectic phase behavior between the three fractions. At 0 °C a eutectic began to form upon dilution of a 50/50 mixture of HMF and LMF with MMF (Figure 3A). At this temperature, HMF and MMF form a solid solution (Figure 2A). LMF solids begin crystallizing out at this temperature and form a eutectic with the MMF–HMF solution solids. Therefore, the eutectic forms because the LMF TAGs and the HMF–LMF TAGs are incompatible in the solid state at this temperature.

Some interesting conclusions can be drawn from this study. First, it was surprising to observe compatibility between the HMF and MMF, since these fractions differ markedly in melting points, molecular volume, and chemical structure. The formation of a solid solution between HMF and MMF is probably due to a structural complementarity between the TAGs from these two fractions. The positional distribution of fatty acids in these fractions is not random but rather must be highly complimentary to the MMF, regardless of the drastic differences in overall fatty acid composition. In classical thermodynamic analysis of phase equilibria for simple molecules, average properties such as molecular volume (assuming sphericity) and average intermolecular forces are typically used to predict observed phenomena. Our results indicate that this approach would be inappropriate for characterizing the phase behavior of milk fat TAGs. The spatial distribution of the three fatty acid chains is a key factor in the formation of the crystal lattice. This “lock and key” view of the interactions between these TAGs has important practical implications as well. For example, dry crystallization processes would not be very efficient in separating HMF from MMF due to their high structural complementarity and

the resulting solid solution formation between these fractions.

Proper separation of the LMF from the HMF or MMF during crystallization and filtration would also pose problems due to formation of a partial solid solution between these components.

Ternary phase equilibrium studies on the solution behavior of the three milk fat fractions are necessary to understand the functional properties of milk fat. These ternary phase diagrams provide a mechanism for the analysis of the complex solution behavior of these three TAG fractions and its effect on the physical properties of milk fat.

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