Solvent Effects on the Crystallization Behavior of Milk Fat Fractions

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The high- and medium-melting fractions of milk fat (HMF and MMF, respectively) were crystallized in the presence of various solvents, including the low-melting fraction of milk fat (LMF), canola oil (CO), hexane, and ethyl acetate. Choice of solvent was shown to have a strong influence on phase behavior and crystallization kinetics. Dilution and solubilization effects were observed for all the blends. More solids were formed in the HMF and MMF blends with LMF than with CO, and complexes were formed between the milk fat fractions possibly because of molecular complementarity. Solids were slightly higher for the more polar ethyl acetate than for hexane. Crystallization proceeded more rapidly in the presence of LMF and ethyl acetate than in the presence of CO and hexane, respectively. According to the Hildebrand equation, HMF and MMF were ideally soluble in LMF and CO. X-ray diffraction spectroscopy (XRD) revealed the existence of liquid-state structure in mixtures of HMF/CO, HMF/LMF, MMF/CO, and MMF/LMF. The observed liquid-state structure was reminiscent of liquid crystals. No differences were observed in the structure of the liquid phase between LMF- and CO-containing mixtures.

Keywords: *Milk fat; fractions; solvent; crystallization; fractionation; phase behavior; solubility; dilution; complex formation*

INTRODUCTION

Milk fat is composed of over 400 different fatty acids and contains literally thousands of triacylglycerol (TAG) species (Jensen and Newburg, 1995). Even though thousands of TAG species are present, differential scanning calorimetric and solvent fractionation studies have clearly demonstrated the existence of three major and chemically distinct families, or fractions, of TAGs in milk fat (Timms, 1980; Marangoni and Lencki, 1998). The high-melting fraction (HMF) TAGs contain mostly long-chain saturated fatty acids, the middle-melting fraction (MMF) TAG contains, on average, two long-chain saturated fatty acids and one short-chain or *cis*-unsaturated fatty acid, and the low-melting fraction (LMF) TAGs contain, on average, one long-chain saturated acid and two short-chain or *cis*-unsaturated acids (Timms, 1980; Marangoni and Lencki, 1998). HMF, MMF, and LMF represent approximately 10%, 35%, and 55% (w/w) of the total milk fat mass (Marangoni and Lencki, 1998).

The physical properties of milk fat are affected by the chemical and physical properties of its constituent TAGs and their interactions (Kaylegian, 1995). In a previous study by our group (Marangoni and Lencki, 1998), binary and ternary phase diagrams of mixtures of HMF, MMF, and LMF were constructed in order to better understand their complex solution behavior. In that study, monotectic solution behavior was detected in mixtures of HMF and MMF, while the formation of partial solid solutions was detected in binary mixtures of HMF−LMF and MMF−LMF. Interactions between MMF and LMF were stronger than between HMF and LMF, particularly at temperatures within the melting range of MMF (15 °C < T < 30 °C). Between 0 and 15 °C, and above 30 °C, ternary mixtures of the three fractions displayed monotectic solution behavior.

The complex solution behavior of milk fat fractions is not fully explainable by classical thermodynamic theory (Marangoni and Lencki, 1998). Although HMF and MMF have very different melting points, fatty acid compositions, and molecular volumes, they exhibit monotectic solution behavior, forming mixed crystals in the solid state. A high degree of structural complementarity between the TAGs in these two fractions was used as an argument to explain mixed crystal formation in HMF−MMF mixtures. Timms (1978) reported that liquid oil addition to milk fat generally causes a reduction in the amount of solid fat present, beyond that expected strictly from dilution, by acting as a solvent for the solid TAGs. However, the fact that partial solid solutions are formed between LMF and HMF or MMF suggests not only that LMF acts as a diluent but that it also has the ability to somehow enhance solid structure (Marangoni and Lencki, 1998). LMF addition caused larger reductions in solid fat content in MMF than in HMF, probably because of the greater molecular similarity between MMF and LMF TAGs than between HMF and LMF TAGs (Marangoni and Lencki, 1998).

The properties of a solvent, including chemical nature, polarity, solubility, viscosity, and structural organization of molecules, can influence the crystallization behavior of TAGs (Wellner et al., 1981; Yang et al., 1992; Hartel, 1992). Solvent polarity has been shown to affect fractionation yield of squid viscera stearin and the solid fat content (SFC) of the resulting fractions (Yang et al., 1992). These authors reported that fractionation yield increased with increasing solvent polarity, while fraction SFC increased with decreasing solvent polarity.

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Solubility of the crystallizing fat in the solvent and solvent viscosity will also influence crystallization behavior (Liu et al., 1995).

Ideal solubility behavior of solid TAGs in oils can be predicted by the Hildebrand equation (Timms, 1978):

$$\log_{10} X = \frac{\Delta H_f}{R}\left(\frac{1}{T_m} - \frac{1}{T_b}\right) \quad (1)$$

where X = mole fraction of the high-melting lipid, ΔH_f = the enthalpy of melting for the high-melting lipid (J/mol), R = universal gas constant (8.314 J/(mol K)), T_m = melting temperature (K) of the high-melting lipid, and T_b = melting temperature (K) of the blend.

The solubility behavior of most edible oil TAGs, consisting mainly of C_{16} and C_{18} fatty acids, obeys eq 1, and the solubility (melting point) of blends of solid TAGs is independent of the nature of the liquid oil used (Timms, 1994). Deviations from this behavior occur at high solvent−solute ratios, because differences in molecular volume between the solute and solvent increase the entropy of mixing upon melting (Timms, 1994). Deviations from ideal behavior also occur when solid solutions or imperfect crystals form, since these have a greater solubility than perfect crystals (Knoester, 1972; Timms, 1978).

Structural organization of molecules in the liquid state can also influence crystallization behavior (Hartel, 1992). Larsson (1972) proposed that TAGs in the melt are arranged in lamellar structures that are not unlike liquid crystals. The size and shape of these structures depend on the rate of diffusion of the molecules, which is determined by the temperature (Larsson, 1972; Hernqvist, 1984). Accordingly, the lamella grow as the temperature is decreased during cooling and finally crystallize into a nucleus. Preexisting structure in the liquid phase of LMF could impact on milk fat crystallization by influencing the alignment of HMF or MMF TAG molecules into lamellae.

In this study, we seek to further our understanding of the phase behavior of milk fat fractions by characterizing the effect of solvent type on their crystallization behavior.

MATERIALS AND METHODS

Materials and Sample Preparation. High-, middle-, and low-melting milk fat fractions (HMF, MMF, and LMF, respectively) were obtained from anhydrous milk fat (AMF) as previously described (Marangoni and Lencki, 1998). Briefly, AMF was crystallized in ethyl acetate and filtered at 5 °C to obtain HMF (solid at 5 °C). The remaining liquor was crystallized at −28 °C to obtain MMF (solid at −28 °C) and LMF (liquid at −28 °C). Blends of HMF in LMF and canola oil (CO), MMF in LMF and CO, HMF in ethyl acetate and hexane, and MMF in ethyl acetate and hexane were prepared in 0%, 10%, 25%, 50%, 75%, and 100% molal ratios (mol/(kg solvent)). Only the highest grade solvents available were used to ensure their purity. HMF, MMF, LMF, and CO were heated at 80 °C for 30 min prior to blend preparation in order to destroy any crystal history. For the mixtures containing ethyl acetate and hexane, HMF and MMF were heated at 80 °C for 30 min and cooled to 35 °C prior to solvent addition. The fatty acid and triacylglycerol compositions of the AMF, HMF, MMF, LMF, and the canola oil used are shown in Tables 1 and 2, respectively. Fatty acid analysis was performed by gas−liquid chromatography (GLC) according to the method of Bannon et al. (1985), and triacylglycerol compositions of the fats and oils were determined by GLC as previously described (Rousseau et al., 1996b).

Table 1. Fatty Acid Composition (% w/w) of AMF, HMF, MMF, LMF, and Canola Oil

fatty acid	AMF	HMF	MMF	LMF	canola oil
4:0	4.5		4.8	5.2	
6:0	3.1	0.2	3.5	3.8	
8:0	1.6	0.3	1.5	2.0	
10:0	3.9	1.7	3.2	4.4	
10:1	0.8	0.1	0.5	0.7	
12:0	4.1	3.6	3.1	4.8	
14:0	11.0	15.4	11.8	11.1	
14:1	1.9	0.8	1.3	20.1	
15:0	1.5	1.8	1.5	0.9	
16:0	28.7	42.5	39.5	19.8	5.8
16:1	3.1	1.7	1.7	3.6	
17:0	0.4	0.9	0.9	0.8	
18:0	10.5	20.6	13.6	6.0	2.8
18:1	20.9	9.3	12.3	29.6	57.9
18:2	1.9	0.5	0.4	2.2	21.7
18:3	1.7	0.4	0.2	1.4	11.8
20:0	0.6	0.2	0.5	1.6	

Table 2. Triacylglycerol Composition (% w/w) of AMF, HMF, MMF, LMF, and Canola Oil

TAG[a]	AMF	HMF	MMF	LMF	canola oil
22	0.2		0.1		
24	0.8		0.7	1.0	
26	0.4		0.2	0.6	
28	0.6		0.2	1.3	
30	1.0		0.2	2.4	
32	2.6	0.2	0.6	4.7	
34	6.4	0.6	4.9	8.2	0.0
36	14.0	1.1	18.2	13.4	0.1
38	14.7	0.7	17.6	18.7	0.3
40	10.8	1.0	11.1	14.7	3.0
42	7.7	3.2	8.9	7.7	0.4
44	6.9	9.2	7.0	5.7	1.2
46	7.4	19.1	6.1	4.8	2.3
48	8.6	25.7	7.8	4.3	
50	9.6	24.0	9.8	5.1	2.9
52	6.4	12.3	6.0	6.3	9.6
54	1.7	1.0	0.6	1.2	73.2
56					5.2

[a] Number of carbons excluding glycerol.

Solid Fat Content Determination. Solid fat contents (SFCs) were measured serially by pulsed nuclear magnetic resonance (pNMR) with a Bruker PC20 Series NMR analyzer (Bruker, Milton, ON, Canada). After preparation, samples were immediately cooled to and held at 0 °C for 5 days, at which time SFC was determined. The temperature of the mixtures was then increased by 5 °C and held for 5 days before the next reading. Subsequent SFC measurements were taken at 5 °C intervals until no solid fat remained in the samples, or up to 45 °C in the case of the ethyl acetate and hexane mixtures.

Solid fat content determined by pNMR, using recommended standardized analytical methods, is a mass fraction quantity due to the use of mass calibration standards. For studies on solution behavior, molar fractions, rather than mass fractions, of solute in solvent are required. When all the molecules in a blend are of similar molecular weight and are protonated to the same degree, the standard mass-SFC determination provides a good approximation to the molar-SFC. However, when the molecular weights and hydrogen proton densities of solvent and solute differ, mass-SFC can differ greatly from molar-SFC, as demonstrated by Marangoni et al. (2000). Adjusting for differences in molecular weight by converting mass-SFC measurements into molar-SFCs, using eq 2, alleviates this problem (Marangoni et al., 2000). Blend ratios, total sample weights, and known molecular weights of the fat and solvent (HMF molecular weight 788 g/mol, MMF molecular weight 756 g/mol, LMF molecular weight 775 g/mol, canola oil molecular weight 879 g/mol, ethyl acetate molecular weight 88 g/mol, and hexane molecular weight 86 g/mol) were required for this purpose. A corrected molar-SFC (SFC_{corr}) quantity can

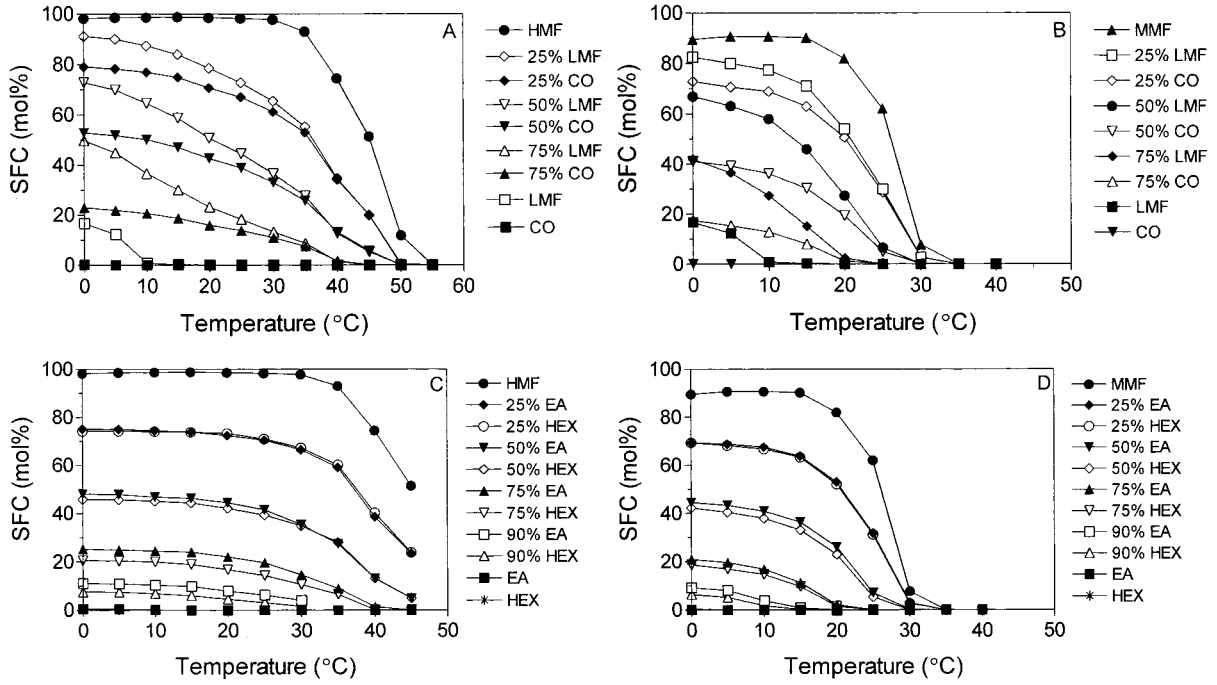

Figure 1. Solid fat content (mol %) as a function of temperature of blends of HMF and MMF with LMF, canola oil (CO), ethyl acetate (EA), and hexane (HEX). (A) HMF−LMF and HMF−CO, (B) MMF−LMF and MMF−CO, (C) HMF−EA and HMF−HEX, (D) MMF−EA and MMF−HEX.

be calculated from the measured SFC as explained by Marangoni et al. (2000):

$$\mathrm{SFC}_{\mathrm{corr}} = \frac{\mathrm{SFC}(M_{\mathrm{A}}^{\mathrm{T}} + M_{\mathrm{B}}^{\mathrm{T}})}{M_{\mathrm{A}}^{\mathrm{T}} + M_{\mathrm{B}}^{\mathrm{T}}\frac{\mathrm{MW_A}}{\mathrm{MW_B}}} \qquad (2)$$

M^{T} corresponds to the total mass of either component A or B; MW corresponds to the molecular weight of either component A or B. SFC is the solid fat content as determined by recommended standard analytical procedures.

For example, a 2.0 g, 20% SFC blend contains 0.4 g of solids and 1.6 g of liquid. Knowledge of the mass of noncrystallizing solvent allows for the calculation of the molar-SFC parameter. The ratio of the calculated molar-SFC to the original mass-SFC provides a conversion factor that can be applied to all other mass-SFC readings for that particular blend. HMF, MMF, LMF, and CO have similar molecular weights and proton densities; hence molar-SFC is nearly equivalent to mass-SFC. However, for blends of compounds of dissimilar molecular weights caution should be exercised. The roughly 10-fold difference in molecular weights between the milk fat fractions (HMF and MMF) and the solvents (hexane and ethyl acetate) resulted in significant conversion factors, particularly at low solute concentrations, where the value of molar-SFC was less than half of the mass-SFC value.

Crystallization Behavior. The crystallization behavior of 50/50 (mol %) mixtures of HMF and MMF in LMF, CO, hexane, and ethyl acetate was studied. Samples were prepared as described above and immediately placed in a thermostated water bath at 10 or 20 °C. SFC readings were taken at appropriate time intervals.

Dropping Points Determination. Dropping points were determined using the Mettler dropping point apparatus as previously described (Rousseau et al., 1996c). Mixtures of HMF and MMF with LMF and CO in 100/0, 80/20, 60/40, 40/60, 20/80, and 0/100% (w/w) ratios were prepared.

Liquid Structure. Mixtures (50/50 mol %) of HMF−LMF, HMF−CO, MMF−LMF, and MMF−CO were prepared as previously described. Samples containing HMF were held at 80 °C for 24 h, while those containing MMF were held at 35 °C for 24 h. Images were taken using an Enraf-Nonius KappaCCD diffractometer with a FR590 X-ray generator. *d* spacings were calculated by comparing the spacing of the rings in these images to those of a standard ($CaSO_4 \cdot 2H_2O$).

Positional Distribution of Fatty Acids. Stereospecific analysis was performed as described in Willis and Marangoni (1999).

RESULTS AND DISCUSSION

Solvent Effects on Solid Fat Content. Changes in molar-SFC as a function of temperature for the binary mixtures (HMF−LMF, HMF−CO, HMF−ethyl acetate, HMF−hexane, MMF−LMF, MMF−CO, MMF−ethyl acetate, and MMF−hexane) are shown in Figure 1. The molar-SFC for the HMF and MMF blends with LMF were higher than for equivalent mixtures with CO (Figure 1A,B). For example, in the 50 mol % HMF blends, the molar-SFC of HMF−LMF at 30 °C was 45%, while the SFC of HMF−CO was 40%. Similarly, when HMF and MMF were blended with organic solvents (Figure 1C,D), the molar-SFCs were slightly higher with ethyl acetate than with hexane. These differences were more evident at lower temperatures and at higher solvent concentrations.

Plots of the molar fraction of solids in the blend as a function of the molal fraction of solute in solvent can reveal three different behaviors: dilution, solubilization, and complex formation. A linear decrease in the molar fraction of solids as a function of an increasing molal fraction of solvent in the mixture represents dilution. Dilution is a colligative effect, depending only on the number of molecules present in a system. At increasing solvent concentrations, there are fewer TAG molecules available to crystallize; hence, less solids are formed. Solubilization of solids in the liquid solvent is demonstrated in these diagrams by a nonlinear curve, where the molar fraction of solids is lower than expected solely on the basis of dilution. Interactions with the solvent cause the solubilization of would-be-solid TAGs, resulting in less solids being formed. Last, the molar fraction

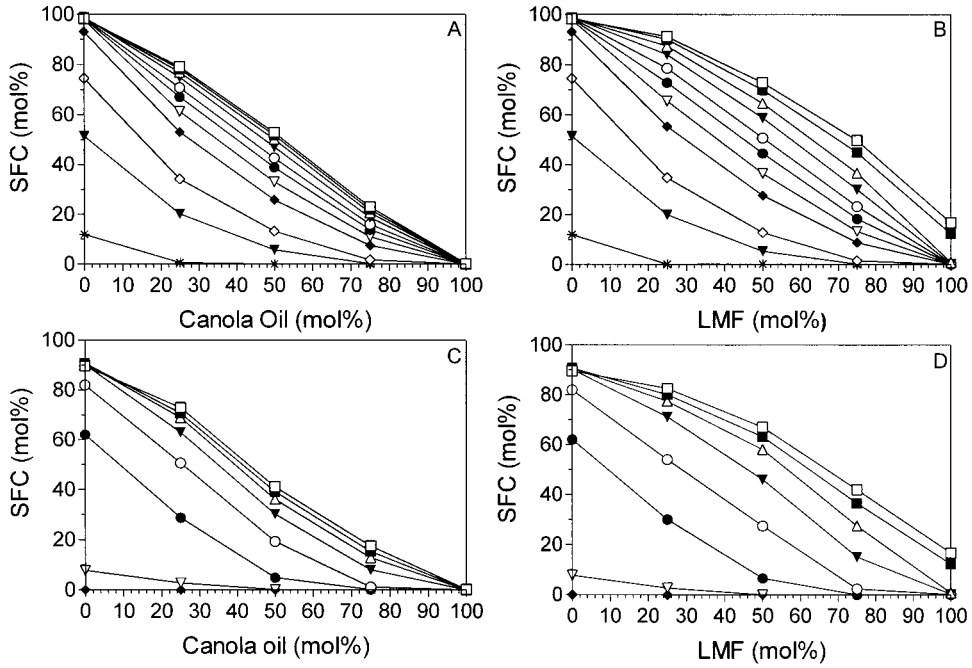

Figure 2. Solid fat content (mol %) of blends of HMF and MMF with LMF and canola oil (CO) at different solvent molal fractions. (A) HMF−CO, (B) HMF−LMF, (C) MMF−CO, (D) MMF−LMF for various temperatures (-□- 0 °C, -■- 5 °C, -△- 10 °C, -▼- 15 °C, -○- 20 °C, -●- 25 °C, -▽- 30 °C, -◆- 35 °C, -◇- 40 °C, -▼- 45 °C, and -∗- 50 °C).

of solids can be higher than expected from dilution effects when complexes form between TAGs of the solute and solvent. The crystallizing solute TAGs pull some of the would-be-liquid solvent TAGs into the solid phase.

Figure 2 shows the effects of increasing solvent content in binary mixtures with HMF and MMF. For the CO blends (Figure 2A,C), dilution effects were observed at lower temperatures, with solubilization becoming more predominant above 15 °C. A very different behavior was observed when LMF was used as the solvent (Figure 2B,D). With LMF at low temperatures, a higher molar fraction of solids than expected due to a dilution effect was observed, possibly due to complex formation. At higher temperatures, however, solubilization of HMF and MMF in LMF was observed.

At 0 and 5 °C, LMF contains some solid fat, which may be contributing to the higher solids found in its blends at these temperatures. However, over most of the temperature range studied (10−55 °C), both LMF and CO are completely liquid; however, the LMF-containing blends still have higher solid contents than expected from dilution effects. Somehow LMF contributes to an increase in solids in the blends. This occurs possibly because of molecular complementarity between LMF and HMF and MMF, which favors interactions in the solid state, or because LMF helps align HMF and MMF molecules in the liquid phase in such a way as to enhance solids formation. To investigate this molecular complementarity, the positional distributions of fatty acids in the milk fat fractions and CO were determined and are shown in Table 3.

The average fatty acid chain length of the three milk fat fractions is much shorter than the average chain length of CO. Also, there is more overall similarity in the type and distribution of fatty acids between LMF and the milk fat fractions than between CO and the milk fat fractions. The formation of mixed crystals becomes increasingly more difficult with greater dissimilarities in molecular structure (Knoester, 1972). Crystallizing TAGs have the tendency to segregate

Table 3. Positional Distribution of Fatty Acids (mol %) of HMF, MMF, LMF, and Canola Oil[a]

fatty	HMF		MMF		LMF		canola oil	
acid	sn-1,3	sn-2	sn-1,3	sn-2	sn-1,3	sn-2	sn-1,3	sn-2
4:0		0.0	20.0		16.1			
6:0	0.5	1.3	0.5	1.3	4.6			
8:0	1.7	8.5	1.1	2.6	4.6			
10:0	0.8	5.3	1.7	3.0	2.4	2.4		
12:0	3.4	7.5	3.0	5.6	4.9	5.2		
14:0	11.6	15.9	9.1	16.4	17.7	17.0		
16:0	38.0	30.0	30.0	45.8	18.4	32.6	7.1	0.2
18:0	28.2	15.3	15.3	17.3	6.2	12.0	1.8	0.1
18:1	15.9	16.2	20.2	8.2	26.3	31.0	67.6	52.7
18:2							13.1	30.6
18:3							6.4	15.7
20:1							2.3	0.3
22:1							0.4	
22:2							0.9	0.1

[a] Ackman et al. (1983).

according to length or degree of saturation of their carbon chains. MMF and LMF have quite similar fatty acid compositions and positional distributions. Because of these strong resemblances, some of the LMF TAGs may be incorporated into the MMF crystal lattice. As with MMF, LMF displays some likeness to HMF, which may allow it to interact with HMF and enter the crystal lattice.

The presence of mixed crystals in milk fat was first proposed by Mulder (1953). Compound crystals and solid solutions are especially common in natural fats, including milk fat, because of partial miscibility of solid phases (Lawler and Dimick, 1998). It is not unlikely that LMF TAGs would form a complex with HMF and MMF TAGs, resulting in a higher solids content than for the CO-containing mixtures. CO TAGs have a very different fatty acid composition and distribution than HMF and MMF TAGs. Interactions between CO TAGs and HMF and MMF TAGs are therefore unlikely. Complex formation between the milk fat fractions supports earlier evidence of partial solid solutions formation between

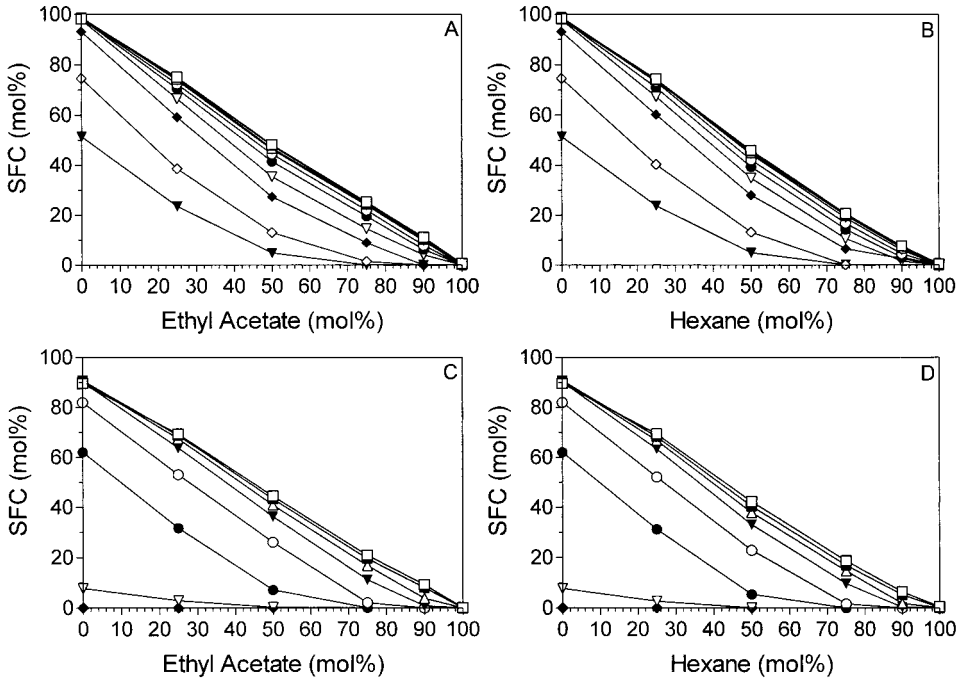

Figure 3. Solid fat content (mol %) of blends of HMF and MMF with ethyl acetate (EA) and hexane (HEX) at different solvent molal fractions. (A) HMF−EA, (B) HMF−HEX, (C) MMF−EA, (D) MMF−HEX for various temperatures (-□- 0 °C, -■- 5 °C, -△- 10 °C, -▼- 15 °C, -○- 20 °C, -●- 25 °C, -▽- 30 °C, -◆- 35 °C, -◇- 40 °C, -▼- 45 °C, and -∗- 50 °C).

MMF and HMF with LMF (Marangoni and Lencki, 1998). Previously, LMF was found to interact more strongly with MMF than HMF, and addition of LMF to MMF led to a relatively greater reduction in SFC than when LMF was added to HMF (Marangoni and Lencki, 1998). Similarly, Figure 2 shows that LMF solubilizes MMF more readily than HMF.

Figure 3 shows the effects of increasing organic solvent molar ratio on the molar-SFC of HMF and MMF binary mixtures with ethyl acetate and hexane. At lower temperatures, decreases in the molar-SFC of mixtures as a function of increasing solvent molal fraction in the mixtures were attributed strictly to dilution effects. HMF and MMF had slightly lower molar-SFCs in blends containing hexane than in mixtures containing ethyl acetate (Figure 1C,D), likely because of differences in the polarity of the solvents. Hexane is a nonpolar solvent, while ethyl acetate is a moderately polar solvent. Hexane is a better solvent for fats and, as a result, solubilizes more solute TAGs; hence fewer crystallize out of the solvent phase. Former work has shown that fractionation yields are higher with more polar solvents (Yang et al., 1992). Differences in polarity between LMF and CO may partially account for differences in the molar-SFCs of blends with these liquid oils. Over 90% of CO fatty acids are 18-carbon species; therefore CO may be less polar than LMF (Rousseau et al., 1996a). LMF contains short chain fatty acids, which would make it more polar than CO and therefore a weaker solvent.

Solvent Effects on Crystallization Behavior. Crystallization dynamics for the different blends were monitored at temperatures where pronounced differences in final molar-SFC had been observed, to determine whether differences existed in the mechanisms and rates of crystallization (Figure 4). Crystallization runs were performed at 10 °C for the 50/50 (mol %) blends of HMF and MMF with LMF, CO, ethyl acetate, and hexane. At 10 °C the blends of MMF and HMF with the organic solvents crystallized too rapidly to follow; therefore, only the crystallization at 20 °C is presented. The degrees of supercooling, defined as the difference in temperature between the 10% molar-SFC temperature and the crystallization temperature, were very similar.

For the MMF−LMF and MMF−CO mixtures, two-stage crystallization patterns were evident. The former had a higher initial rate of crystallization and a more pronounced point of inflection. MMF−LMF briefly levels off at the same final solids content as MMF−canola oil, before increasing again to a higher plateau at about 20% higher molar-SFC. This might suggest that the initial increase in solids corresponds to MMF crystallization, with additional LMF solids formed after the point of inflection, and is supported by the fact that there is roughly a 20% difference in molar-SFC between MMF−CO and the MMF−LMF (50/50 mol %) blends at 10 °C (Figure 2C,D). Similar patterns were observed for the HMF−LMF and HMF−CO blends, although the rates of crystallization were more rapid. The rates of crystallization in blends containing LMF are slightly higher than those containing CO, suggesting that LMF TAGs may favor crystal growth.

When mixtures of MMF−ethyl acetate and MMF−hexane were crystallized at 20 °C, marked differences were evident. Both curves were sigmoidal, but MMF crystallization was more rapid in the presence of ethyl acetate than hexane. Hexane, being less polar, is a better solvent for the fats and therefore tends to dissolve crystallizing TAGs. As a result, it takes longer for stable, solid nuclei and crystals to form. The induction time for ethyl acetate was shorter (105 min) than the induction time for hexane (160 min) (Figure 4C). Induction time was calculated as the time of onset of increase in solids. Eventually, both the MMF−ethyl acetate and MMF−hexane curves plateau, with the MMF−ethyl acetate at a slightly higher solids content than the hexane blend, in agreement with trends previously discussed. At 20 °C, HMF crystallization in both solvents was very rapid.

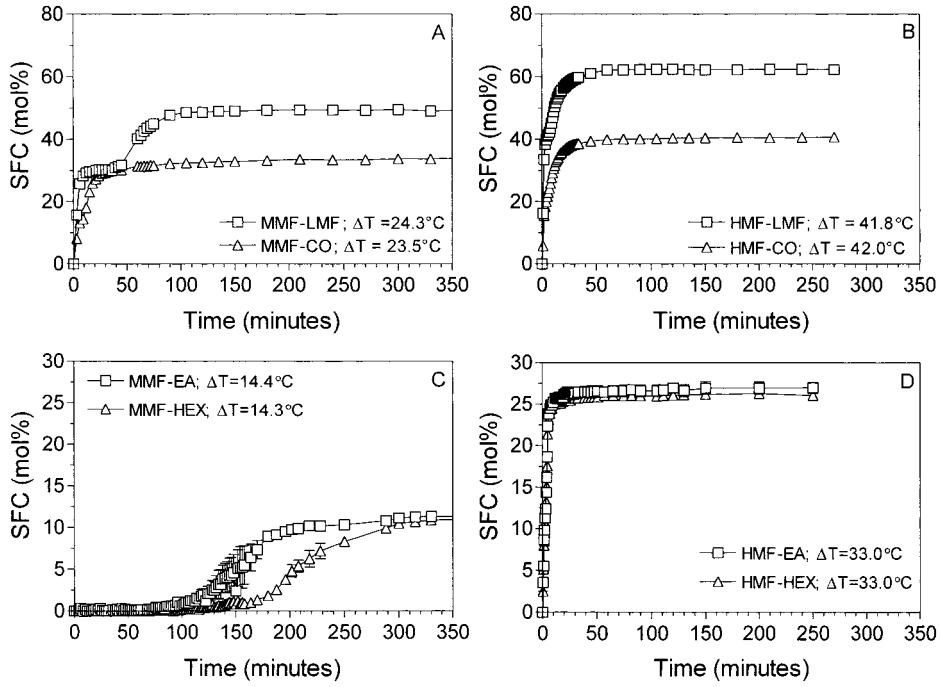

Figure 4. Solid fat content (mol %) vs crystallization time for 50/50 (mol/mol %) blends of (A) HMF−LMF and HMF−CO at 10 °C, (B) MMF−LMF and MMF−CO at 10 °C, (C) HMF−EA and HMF−HEX at 20 °C, (D) MMF−EA and MMF−HEX at 20 °C.

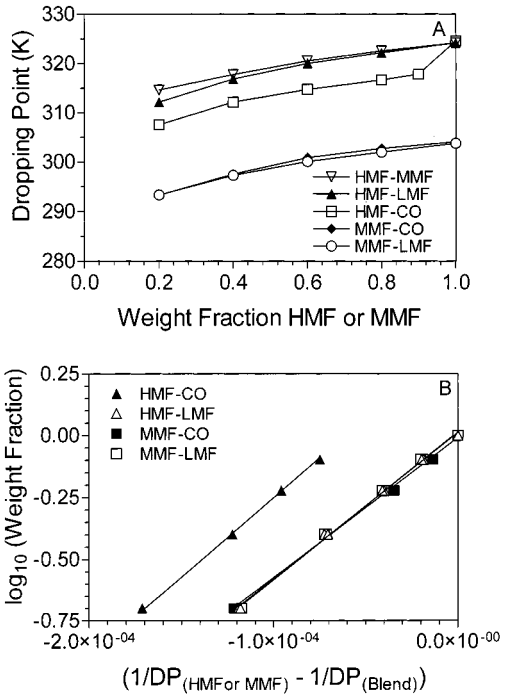

Figure 5. Dropping points of HMF−MMF, HMF−LMF, HMF−CO, MMF−LMF, and MMF−CO blends at different solute molal fractions (A) and corresponding Hildebrand plot (B).

The degree of supercooling is still such that differences are not observed between the kinetics of crystallization for ethyl acetate and hexane.

Solvent Effects on Dropping Points. Mettler dropping points for HMF and MMF blends are shown in Figure 5A. A monotonic decrease in dropping point, as a function of increasing molal fraction of solvent in the mixtures, was observed for the HMF and MMF blends with both LMF and CO. This is an effect generally attributed to increases in melting entropy as a function of increasing dilution. This relationship between melting temperature and melting entropy is defined by the Gibbs−Helmholtz equation (eq 4)

$$\Delta G_m = \Delta H_m - T_m \Delta S_m \quad (4)$$

where ΔG_m is the free energy of melting, ΔH_m is the enthalpy of melting, T_m is the melting temperature, and ΔS_m is the melting entropy. At equilibrium,

$$\Delta G_m = 0$$

and

$$\frac{\Delta H_m}{T_m} = \Delta S_m$$

In order for ΔH_m to remain constant, a decrease in the T_m would have to correspond to an increase in ΔS_m.

Dropping points are not true thermodynamic melting points of the material, since between 5 and 10% solid fat remains in a sample at the temperature corresponding to the dropping point. Clear points provide the best measure of melting temperatures for the Hildebrand analysis, because they represent the temperature at which 100% of the sample is liquid (Sherbon et al., 1972). Although dropping points are not accurate measurements of melting points, they are proportional to them; hence their use in a Hildebrand analysis of solubility behavior is warranted (Timms, 1978).

Interestingly, no differences were observed between the dropping points of the MMF−LMF and MMF−CO blends; however, HMF−CO dropping points were 5 °C lower than HMF−LMF dropping points. The dropping points for pure HMF and MMF were found to correspond to the temperature at which roughly 10% solid fat remains in a sample. The differences between the HMF and MMF systems are probably related to differences in the microstructure of their fat crystal networks. Our results suggest that, at the dropping point temperature, the remaining fat crystal network may strongly

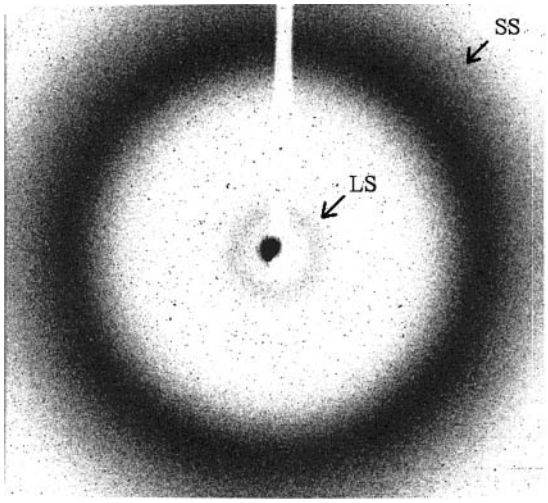

Figure 6. Powder XRD pattern showing existence of low- and high-angle reflections (long and short spacings) in the liquid phase of HMF−CO (50/50 mol/mol %).

Table 4. Liquid-Phase Structure for Mixtures of HMF−LMF, HMF−Canola Oil, MMF−LMF, and MMF−Canola Oil Obtained by Powder XRD

mixture	short spacing (Å)	long spacing (Å)
HMF−LMF	4.48	23.65
HMF−canola oil	4.48	23.65
MMF−LMF	4.48	22.02
MMF−canola oil	4.46	22.81

influence the exact temperature at which the first oil drop falls from the sample. Differences in the microstructure between HMF and MMF crystal networks would allow oil to drop out more, or less, readily from the solid matrix. This could explain the differences between the HMF and MMF blend behaviors.

The linearity in the Hildebrand plot shown in Figure 5b suggests that HMF and MMF exhibit ideal solubility behavior in both LMF and CO. In addition, the dropping points of HMF and MMF were independent of the nature of the solvent used. This ideal solubility was observed within the temperature ranges where HMF and MMF solubilization, by both LMF and CO, was significant (Figure 2), in the vicinity of 50 °C for HMF and 30 °C for MMF. Heats of fusion were calculated from the slopes of the Hildebrand plots. The enthalpies of fusion were 0.146 J/kg and 0.175 J/kg for HMF and MMF, respectively, and were similar to values reported by Timms (1980).

Solvent Effects on Liquid Structure. X-ray diffraction studies were carried out to verify the existence of structure in the liquid phase and to investigate the possibility that differences in this structure may account for the differences observed in the crystallization behavior. Both short and long spacings were detected in the liquid phase at temperatures above the melting point of either HMF or MMF. However, the bands were broad and diffuse. Figure 6 shows a typical powder X-ray diffraction pattern of the liquid phase in the HMF−LMF blend. This image is characteristic of the patterns obtained for all the blends. Short and long spacings of the liquid-phase structure in the different mixtures studied are shown in Table 4. The long and short spacings of HMF−LMF and HMF−CO, as well as MMF−LMF and MMF−CO liquid phases, were similar. While structure was detected in the liquid state, no differences were observed between the liquid phases of different mixtures.

These results provided further evidence for the existence of significant amounts of structure in the liquid state of triacylglycerols (Larsson, 1972; Hernqvist, 1984). The broad short spacings observed around 4.5 Å may indicate the existence of substantial lateral packing in the liquid state. These spacings are also typical of those found in liquid crystals and resemble the spacings at 4.5 Å in trilaurin's melt observed by Cebula et al. (1992). Cebula's group proposed that liquid trilaurin resembles the nematic phase, in which molecules possess some lateral organization, but are not arranged in discrete layers. Using neutron diffraction, they identified peaks in the melt of trilaurin between those that would correspond to (001) and (002) reflections, indicating that structure in the liquid phase is unique from that of the solid phase. The reported broad long spacing peak region was only 15−25 Å, while the typical unit cell of trimyristin is about 33 Å. These relatively short long spacings may be caused by the interdigitation of fatty acid chains from one layer into the adjacent layer (Cebula et al., 1992). The uncharacteristically low long spacings observed in our study correspond to roughly half of the value of a typical unit cell of milk fat triacylglycerols, about 40 Å (Rousseau and Marangoni, 1998). This may also be explained by an arrangement in which triacylglycerols are arranged in a disordered fashion such that their fatty acid chains penetrate into adjacent layers.

We have shown that the choice of liquid oil is an important parameter in the engineering of fats and fat-containing products. The use of a liquid oil with a TAG composition with high degree of molecular complementarity to hardstock TAGs can result in a plastic fat with a relatively higher SFC than expected from straight dilution effects. This would allow for a further reduction in the amount of hardstock used in the formulation. Dilution, solubilization, and complex formation effects should be investigated for different hardstock-oil systems, since this type of information would provide a criterion for choosing the liquid oil in the production of a plastic fat, particulary for a high liquid oil volume fraction spread. Differences in the crystallization behavior of milk fat fractions in ethyl acetate and hexane could have implications for milk fat solvent fractionation processes because of the differences in both the final solids obtained and the kinetics of crystallization. Furthermore, on the basis of the higher SFCs of HMF−LMF and MMF−LMF over the canola oil mixtures, we have provided further evidence of mixed crystal formation between milk fat's fractions. The presence of broad short spacings around 4.5 Å and relatively short long spacings in the liquid phase of the blends also lends support to the argument that triacylglycerols in the melt exist as liquid crystals.

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