Polymorphism and Growth Behavior of Low-*trans* Fat Blends Formulated With and Without Emulsifiers

Marina Cerdeira[a], Silvana Martini[a], Roberto J. Candal[b], and María L. Herrera[a,*]

[a]Departamento de Industrias and [b]Instituto de Química Física de Materiales, Ambiente y Energía, University of Buenos Aires, 1428 Buenos Aires, Argentina

ABSTRACT: Polymorphism and growth behavior of blends of a high-melting fraction of milk fat (HMF) and sunflower oil (SFO) formulated with and without the addition of sucrose esters (SE) P-170, P-1670, and S-170 were studied by pulsed ^{1}H NMR spectroscopy, X-ray diffraction, and polarized light microscopy. The effect of SE on the solid content maximum (S_{max}) or crystallization rate was observed only at low supercooling (values of ΔT below 15°C). The Avrami k_n decreased as n values increased, indicating that SE inhibited growth and impeded nucleation. Addition of SFO modified the polymorphic behavior of milk fat, most likely owing to the increase in the C_{54} fraction (mostly 18:1 *cis*) in crystals composition. P-170 and S-170 modified the polymorphic behavior of HMF when it crystallized in the α-form or in blends with up to 40% SFO at all crystallization temperatures (T_c) selected. Addition of P-170 and S-170 favored crystallization in the β′-form, and the appearance of the β-form was delayed. P-1670 had no effect on polymorphism. When HMF and the blends were crystallized under dynamic conditions, addition of P-170 and S-170 markedly decreased crystal sizes. P-1670, however, showed no effect on microstructure.

Paper no. J11247 in *JAOCS 83*, 489–496 (June 2006).

KEY WORDS: Avrami model, high-melting fraction of milk fat, isothermal growth, polymorphism, sucrose esters, sunflower oil, *trans* fat blends.

Consumer concerns associated with the atherogenic effect of *trans*-FA limit the future of the hydrogenation process as a way to modify the solid-to-liquid ratio in vegetable oils/fats. As an alternative to hydrogenated vegetable oils, modification of high-m.p. stearins by blending with vegetable oils is becoming important, since shortenings with appropriate physical chemical properties and good nutritional characteristics that are free of *trans*-FA and rich in PUFA can be obtained (1–3). The crystallization kinetics and polymorphic behavior of fats (particularly the rate of crystallization and the rate of change from one polymorphic form to another) are very important since they affect the properties of the fat during processing (4).

Emulsifiers are useful functional additives without which many food products could not be made. Emulsifiers typically act in multiphase systems in two ways. The first is as an emulsifying agent to enable two distinct phases to be combined in a stable quasi-homogeneous state for an indefinite length of time. The second function of an emulsifier is often to modify the behavior of the continuous phase of a food product so as to bring about a specific effect or benefit. Sucrose esters (SE) possess both lipophilic and hydrophilic properties. The nature of this property is often expressed as the hydrophilic/lipophilic balance (HLB) on a scale of 0 to 20, with low numbers indicative of the oil-like tendency (5). A few reports have described the effect of SE on induction time of crystallization and development of polymorphic forms of fats in bulk systems (2,6–8). Most of the work was done on hydrogenated oils. Thus, it is of great interest to further study the effects of SE on isothermal crystallization kinetics in bulk low-*trans* fat systems since these blends are a good alternative to the hydrogenation process. Moreover, SE are used in fat products as texturizers and film-formers in addition to their function as emulsifiers.

The Avrami model is the most frequently used approach for the description of isothermal crystallization kinetics of fats. Therefore, in this work it was selected to describe the effects of blending, processing parameters, and emulsifier additions on isothermal crystallization of HMF. The aim of the present study was to investigate the polymorphism and growth behavior of low-*trans* FA blends of a high-melting fraction of milk fat (HMF) and sunflower oil (SFO) formulated with and without emulsifiers.

MATERIALS AND METHODS

Materials. HMF, obtained from Grassland Dairy (Greenwood, WI), had a solid fat content (SFC) of 82% at 5°C and 67% at 20°C, which corresponds to a very high melting fraction, and a *trans* 18:1 content of 2.1 ± 0.4% (Table 1). Three blends were prepared by mixing 20, 40, and 60% of a commercial SFO with HMF. The acyl carbon profile of samples was determined as previously reported (2). The chemical composition and Mettler dropping points (MDP) of all samples are reported in Table 1. Palmitic SE (P-170) with hydrophilic/lipophilic balance (HLB) of 1, Stearic SE (S-170) with HLB of 1, and Palmitic SE (P-1670) with HLB of 16 were commercial esters supplied by Mitsubishi-Kasei Food Corporation (Tokyo, Japan). The SE had MDP of 58.0, 59.5, and 44.0°C, respectively. The monoester content of S-170 and P-170 was 1 wt%, with di-, tri-, and polyesters constituting 99 wt%. P-1670 had 80% monoester and 20% di-, tri-, and polyester. Blends without SE were used as control samples. SE were added at 0.1 or 0.5 wt% concentrations to HMF and the three blends with SFO. The selected concentrations were within the range commonly used in food.

*To whom correspondence should be addressed at University of Buenos Aires, Departamento de Industrias, Intendente Güiraldes S/N, 1428 Buenos Aires, Argentina. E-mail: Lidia@di.fcen.uba.ar

TABLE 1
Chemical Composition and Mettler Dropping Points (MDP) of High- Melting Milk Fat Fraction (HMF), Sunflower Oil (SFO), and Their Blends

Acyl carbon number	Chemical composition of starting materials[a] (wt%)				
	SFO	HMF	20% SFO	40% SFO	60% SFO
C26	0.1	0.2	0.1	0.2	0.2
C28	0	0.3	0.2	0.2	1.0
C30	0	0.7	0.4	0.4	0.2
C32	0	1.5	1.1	0.7	0.5
C34	0	2.9	2.4	1.7	1.1
C36	3.2	3.8	4.5	3.4	2.1
C38	1.8	6.0	7.5	6.0	4.1
C40	0	4.7	4.5	2.6	2.1
C42	0	4.6	4.0	3.0	1.9
C44	0	4.8	4.7	3.9	2.5
C46	0	6.8	7.9	5.7	3.9
C48	0	16.9	11.8	8.3	6.1
C50	1.3	23.4	14.6	12.1	9.0
C52	15.1	15.9	14.1	13.8	13.8
C54 (total)	78.5	7.5	22.2	38.0	51.5
C54 (18:0)	1.1	1.5	0.9	0.5	0.0
C54 (18:1*cis*)	76.0	1.4	16.8	32.5	50.2
C54 (18:1 *trans*)	0	2.1	1.7	1.3	0.8
C54 (other)	1.4	2.5	2.8	3.6	0.5
MDP (°C)	—	48.4	46.2	44.4	41.5

[a]SD for values varied from 0.0 to 1.0%.

SFC determination. SFC during crystallization of the samples were measured by pulsed NMR (p-NMR) spectroscopy in a Minispec PC/120 series NMR analyzer (Bruker). SFC was measured as a function of time for 90 min after the sample temperature had reached crystallization temperature (T_c, at time = 0). Samples were melted at 80°C for 30 min, after which they were placed in NMR tubes, held at 80°C for 10 min, then placed in a water bath at T_c of 10, 15, 20, 25, 30, 35, 37.5, or 40°C. Results are the average of three runs.

X-ray diffraction (XRD). The polymorphic form of crystals formed during isothermal crystallization was analyzed by using a Philips PW 1710 X-ray spectrometer fitted with a system for temperature control. Liquid nitrogen was used as coolant. With this cooling system, T_c was reached in about 15 s. $K_{\alpha 1 \alpha 2}$ radiation from copper was used at 40 kV, 20 mA, and scanning velocity 1° arc/min from 15 to 30°. Samples were melted at 80°C, held at 80°C for 30 min, then cooled within the chamber to T_c, following the same procedure as for NMR experiments. Spectra were taken after 2 and 30 min at T_c. Experiments were performed in duplicate.

Polarized light microscopy (PLM). A Leitz microscope model Ortholux II with a controlled-temperature platform was used to photograph the initial crystals. The samples, *ca.* 80 g, were contained in a jacketed glass cell, the temperature of which was controlled by means of water that was circulated from a programmable bath. Samples were melted and held at 80°C for 30 min and then placed in the glass cell set at a T_c of 35, 37.5, or 40°C. They were crystallized with a cooling rate of 5.5 ± 0.5°C/min (a fast rate as in NMR studies) and an agitation rate of 100 rpm. (With this intermediate agitation rate, the effect of SFO and SE on crystal size was very noticeable. In addition, at this agitation rate there were no significant differences from the results obtained crystallizing the blends on a microscope slide without agitation, as done by NMR and X-ray.) PLM studies were performed with agitation since dynamic crystallization allowed analyzing samples for polymorphism. At the point that the sample temperature reached T_c, samples were taken for PLM observation, with sampling continuing until crystallization was completed (90 min). At each sample point, a drop of slurry was placed between a slide equilibrated at T_c and a cover slide. Images were taken in duplicate at every sample time using a magnification of 250×. Another set of experiments was performed in the same way, but after 30 min samples were filtered and crystals analyzed by XRD.

Avrami model. p-NMR data were fitted to the Avrami equation for isothermal crystallization *(9)*:

$$-\ln(1-f) = k_n t^n \quad [1]$$

where t is time, k_n is the rate constant, f is the fractional extent of crystallization at any time, and n represents the index of the reaction. To obtain the parameters that gave the best fit to Equation 1, the nonlinear section of the Systat software (Point Richmond, CA) was used. Statistical significance of differences in parameters k_n and n with supercooling and between samples was checked using ANOVA. A randomized block design and an additive model that assumes that an observation, γ_{ti}, can be represented as the sum of a general mean, η, a block effect β_I (amount of SFO), a treatment effect τ_t (crystallization temperature), and an error ε_{ti} were selected (10*)*. A multiple comparisons test was used to determine the confidence interval for a particular difference in means.

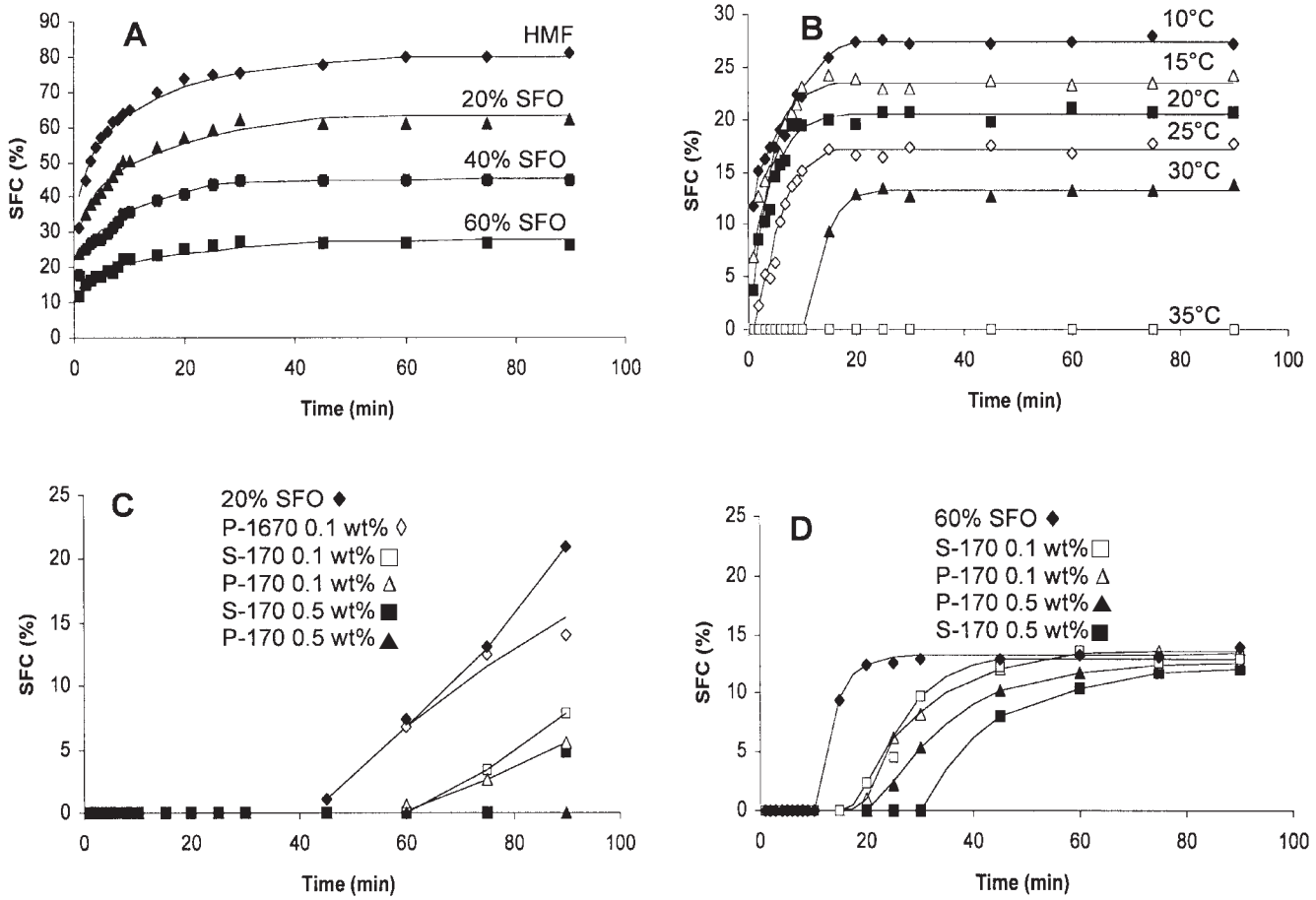

FIG. 1. Solid fat content (SFC) with time for (A) high-melting fat (HMF) and with addition of 20, 40, and 60% sunflower oil (SFO) without sucrose esters (SE) crystallized at 10°C; (B) the 60% SFO blend crystallized at different temperatures; (C) the 20% SFO blend with and without addition of P-1670 [palmitic SE with hydrophilic/lipophilic balance (HLB) of 16] at 0.1 wt%, and P-170 (palmitic SE with HLB of 1) and S-170 (stearic SE with HLB of 1) at 0.1 and 0.5 wt%, crystallized at 37.5°C; (D) the 60% SFO with and without addition of P-170 and S-170 crystallized at 30°C. The lines correspond to best fits of the Avrami equation (except with addition of 0.5 wt% in panel C). Errors were less than 1%.

RESULTS AND DISCUSSION

Isothermal crystallization. p-NMR was used to measure the SFC of the samples. The kinetics of crystallization is indicative of the mechanism of growth and nucleation. As a representative example, Figure 1 shows the increase in SFC with time to monitor the effect of addition of SE or SFO to HMF, and the effect of temperature on crystallization behavior. The temperatures selected in Figure 1C (37.5°C) and Figure 1D (30°C) corresponded to low supercooling (8.7 and 11.5°C for C and D, respectively). Curves obtained are reported here for P-1670 only at 0.1 wt%, because this SE was not soluble in the fat systems used in this study at 0.5 wt%. Zero time for these graphs was the moment at which the samples reached crystallization temperature (T_c).

Figure 1A shows the effect of addition of SFO to HMF. At a T_c of 10°C, no induction period was necessary to start crystallization for all samples, and curves showed a hyperbolic shape. The MDP (T_m) of the HMF was 48.4°C. Addition of 20, 40, and 60% SFO decreased T_m by 2.2, 4.0, and 6.9°C, respectively. Despite the small changes in T_m due to addition of 20 and 40% SFO (Student's *t*-test, $P < 0.05$), the final SFC (after 90 min) decreased considerably as SFO addition increased. Thus, the SFO caused a substantial dilution of the crystalline content of HMF. There was also a small effect on the final melting temperature of the highest-melting TAG at the addition of up to 40% evidenced by changes in MDP, which indicates that there were interactions between the TAG of HMF and SFO. For the 60% SFO the effect on T_m was important ($P < 0.01$). In this case the decrease in SFC was also higher than expected.

When the 60% SFO blend was crystallized at different temperatures (Fig. 1B), there was no induction time of crystallization at crystallization temperatures below 25°C, and curves showed a hyperbolic shape. For low supercoolings (T_c above 25°C), at the beginning, there was an induction time when no fat crystallized, which was followed by a period of rapid crystallization. HMF and the 20 and 40% SFO blends showed the same behavior, but these samples had induction times for T_c above 30°C.

TABLE 2
Best-Fit Parameters of the Avrami Model (Eq. 1) for Mixtures of SFO in HMF With and Without Addition of Sucrose Esters at Different Temperatures[a]

Sample	T_c (°C)	k_n (min^{-n})	n	r^2
HMF	10	0.516 ± 0.032	0.296 ± 0.01	0.997
20% SFO	10	0.368 ± 0.029	0.262 ± 0.01	0.996
40% SFO	10	0.255 ± 0.019	0.221 ± 0.01	0.993
60% SFO	10	0.152 ± 0.008	0.195 ± 0.01	0.993
60% SFO	10	0.152 ± 0.008	0.195 ± 0.01	0.993
	15	0.138 ± 0.007	0.181 ± 0.01	0.984
	20	0.109 ± 0.006	0.203 ± 0.02	0.974
	25	0.058 ± 0.002	0.311 ± 0.02	0.947
	30	0.050 ± 0.009	0.290 ± 0.02	0.995
20% SFO	37.5	0.004 ± 0.0001	1.081 ± 0.09	0.997
20% SFO, 0.1 wt% P 1670	37.5	0.001 ± 0.0001	1.231 ± 0.09	1.000
20% SFO, 0.1 wt% P 170	37.5	$4.5x10^{-5} \pm 2.1x10^{-6}$	1.873 ± 0.09	1.000
20% SFO, 0.1 wt% S 170	37.5	0.002 ± 0.0001	1.068 ± 0.06	0.993
60% SFO	30	0.050 ± 0.002	0.290 ± 0.02	0.995
60% SFO, 0.1 wt% S 170	30	0.046 ± 0.002	0.290 ± 0.02	0.994
60% SFO, 0.5 wt% S 170	30	0.030 ± 0.001	0.378 ± 0.03	0.851
60% SFO, 0.1 wt% P 170	30	0.038 ± 0.002	0.317 ± 0.02	0.995
60% SFO, 0.5 wt% P 170	30	0.033 ± 0.002	0.339 ± 0.02	0.851

[a]Equation 1: $-\ln(1 - f) = k_n t^n$. T_c, crystallization temperature; k_n, rate constant; n, index of the reaction; r^2, correlation coefficient. Values that differ by more than 10% are significantly different at $P < 0.05$.

Figures 1C and 1D show the effect of addition of SE to the 20 and 60% SFO blends. Although addition of 0.1% of P-1670 to the 20% SFO blend delayed crystallization kinetics slightly at 37.5°C ($P < 0.05$), it had no effect at temperatures below 35°C (data not shown). The same effect on crystallization kinetics was found for HMF and the 40% SFO blend. P-1670 showed no effect on the crystallization kinetics of the 60% SFO blend, thus, it was not included in Figure 1D.

At 37.5°C, compared with the control (the 20% SFO blend, Fig. 1C), 0.1 or 0.5% of P-170 and S-170 slowed the crystallization rate and diminished the SFC after 90 min at T_c ($P < 0.05$). At temperatures above 30°C, P-170 and S-170 slowed the crystallization rate of the 60% SFO blend at both addition levels (Fig. 1D).

Avrami analysis. Table 2 shows k_n values, n values, and the correlation coefficients (r^2) for the curves in Figure 1 analyzed by the Avrami model. According to Avrami, n is the reaction order and it describes the type of crystallization mechanism. The Avrami exponent is a function of the number of dimensions in which growth takes place and reflects the details of nucleation and growth mechanisms (11). Christian (12) has tabulated some values of n expected for various transformation conditions. Generally, as the rate of crystallization decreases, the growth mechanism changes from a one- to a multi-dimensional event, as reflected by an increase in the Avrami exponent. An increase in the Avrami exponent can also be due to a change in the type of nucleation, from more instantaneous at higher degrees of supercooling, to more sporadic at lower degrees of supercooling. The Avrami model was able to describe the effects of temperature and of SFO and SE addition on crystallization kinetics of the low-*trans* blends selected for this study. As shown in Table 2, all correlation coefficients but two were above 0.94. Addition of sunflower oil (NMR curves in Fig. 1A) decreased k_n values ($P < 0.001$) at 10°C, indicating that SFO substantially decreased the driving force for crystallization. The effect observed was even greater at higher T_c ($P < 0.001$, data not shown). Not surprisingly, k_n values were higher at lower temperatures ($P << 0.001$), indicating that crystallization proceeded more rapidly at higher driving forces (curves in Fig. 1B). Below 35°C, k_n and n showed no significant differences for addition of sucrose esters to the 20% SFO blend when a multiple comparisons test was used to determine the confidence interval for a particular difference in means ($P < 0.05$). At 37.5°C, k_n decreased with addition of P-170, P-1670, and S-170 at 0.1 wt%. This indicates that, in addition to lengthening the induction times for crystallization, SE inhibited growth. In agreement with the decrease in k_n, n values increased slightly with addition of P-1670 and significantly with addition of P-170 ($P < 0.05$). Crystallization curves with addition levels of 0.5 wt% were not evaluated because crystallization kinetics was too slow to give a good fit (curves in Fig. 1C). k_n and n behaved in a similar way for HMF and the 40% SFO blend as for the 20% SFO blend. For the 60% SFO blend crystallized at 30°C (Fig. 1D), k_n decreased with addition of P-170 and S-170 at both addition levels. n values increased slightly with addition of both P-170 and S-170 at 0.5 wt%. P-1670 showed no effect on crystallization behavior (data not shown).

The SE selected for this study are formed by interesterification of sucrose and the FA palmitic (P) and stearic (S), which are present in high proportions in milk fat. P-170 and S-170

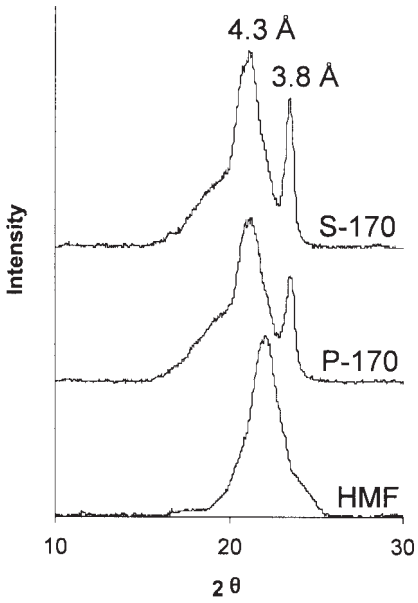

FIG. 2. X-ray patterns corresponding to a high-melting fraction of milk fat (HMF) with and without addition of 0.5% of P-170 and S-170 crystallized at 10°C for 30 min. For other abbreviations see Figure 1.

have an HLB of 1 and therefore they have a high affinity for hydrophobic compounds. On the contrary, P-1670 has an HLB of 16 and low affinity for hydrophobic compounds. The difference in HLB values between P-170 and P-1670 is related to the percentage of mono-, di-, and triesters that constitute these emulsifiers (degree of esterification). Owing to its low hydrophobicity, one might have expected that P-1670 would have a negligible effect on nucleation and growth rates of HMF and the blends. SE with low HLB values (P-170 and S-170) slowed crystallization kinetics at low supercoolings for all samples, whereas SE with high HLB values (P-1670) had no effect when the content of palmitic TAG (C_{48}) was less than 8.3% (the 60% SFO blend), indicating that the more hydrophilic emulsifiers had an effect only when there was more similarity in chain length. In SFO, 93.5% of the TAG had at least one C_{18} FA, and the addition of SFO to HMF decreased the C_{16} TAG content.

As evidenced by the decrease in k_n and the increase in parameter n, P-170 was more efficient than S-170 in delaying crystallization for the 20% SFO blend at 0.1% addition level ($P < 0.05$). For the 60% SFO blend at 0.5% addition level, S-170 was more efficient than P-170 (lower k_n and higher n, $P < 0.05$). The 20% SFO blend had 47.3% of TAG with carbon numbers from C_{34} to C_{48} (C_{16} TAG) and 22.2% of C_{54} (C_{18} TAG), whereas the 60% SFO had 23.8% of TAG with carbon number from C_{34} to C_{48} and 51.5% of C_{54} (Table 1). These results also showed the importance of similarity in chain length. It was reported that both chain length and degree of esterification of the FA were related to the effect on crystallization in oil-in-water emulsions (13). These results are in agreement with those previous studies.

The effect of the SE selected for this study on S_{max} or crystallization rate was observed only at low supercoolings (values of ΔT below 15°C). For high supercoolings, that is, high thermodynamic driving force, addition of the sucrose esters P-1670, P-170, and S-170 had no effect on crystallization kinetics at the selected concentrations.

In all cases, n values reported in Table 2 were less than 2 indicating that the nucleation rate was high, as was expected since the HMF used in this study is a very high melting fraction. However, the Avrami parameter n increased slightly with decreasing driving force (ΔT) and in many cases increased with increased levels of SE (at the same ΔT), indicating that nucleation is impeded.

Effect of addition of SFO and SE on HMF polymorphism. When milk fat is crystallized, it is usually found that, at lower degrees of supercooling or low cooling rates (>0 to 1°C/min) metastable β′ crystals are predominant with trace amounts of α nuclei detectable. High cooling rates (>1°C/min) or high levels of supercooling (>15°C) lead to the rapid formation of α nuclei (14). At 10°C, the α polymorph may be expected as was the case with HMF, which showed the polymorphic behavior previously described for milk fat, that is, a single signal at 4.1 Å (Fig. 2). When SE P-170 and S-170 were added, both at 0.1 and 0.5% levels, the pattern obtained was the one characteristic of the β′-form with two strong signals at 3.8 and 4.3 Å (Fig. 2). P-1670 showed no effect on polymorphism (data not shown).

Figure 3 shows X-ray patterns of the 20% (panels A, C, and E) and 60% (panels B, D, and F) SFO blends at 10°C, panels A and B without SE, panels C and D with the addition of P-170 at 0.5 wt%, and panels E and F with S-170 at 0.5 wt%, respectively. X-ray spectra were obtained after samples had been held 2 and 30 min at crystallization temperature to follow polymorphism with time. As evidenced by the two strong signals at 3.8 and 4.3 Å, the 20% SFO blend crystallized in the β′-form after 2 min at T_c with and without addition of SE (Fig. 3A,C,E). It was expected that the 20% SFO blend would crystallize in the α-form, but no characteristic single signal at 4.1 Å corresponding to the α-form was obtained after 2 min at 10°C. After 30 min, a weak signal at 4.6 Å appeared in the blend with no emulsifiers added, indicating the presence of trace amounts of β crystals. β-Crystals were most likely the result of a polymorphic transition. With addition of the SE P-170 and S-170, the appearance of the β-form was delayed as evidenced by the absence of the 4.6 Å signal after 30 min. The β′-form was present for longer times. The same results were obtained for the 40% SFO blend.

After 2 min at 10°C, the 60% SFO blend had a crystalline phase volume too low to allow well-defined X-ray spectra to be obtained. Patterns were similar to the ones usually shown by amorphous materials. Trace amounts of the β′-polymorph might, however, be present as evidenced by the weak signals at 4.3 and 3.8 Å. After 30 min at 10°C, a 4.6 Å signal was found with and without addition of emulsifiers, showing the predominance of the β-form. The 60% SFO blend had the lowest m.p., and, although supercooling is lower in this sample than for the 20% SFO blend, the thermodynamic driving force is high enough to lead one to expect the α-form. The β-form is uncommon in milk fat because it requires very tight packing of simi-

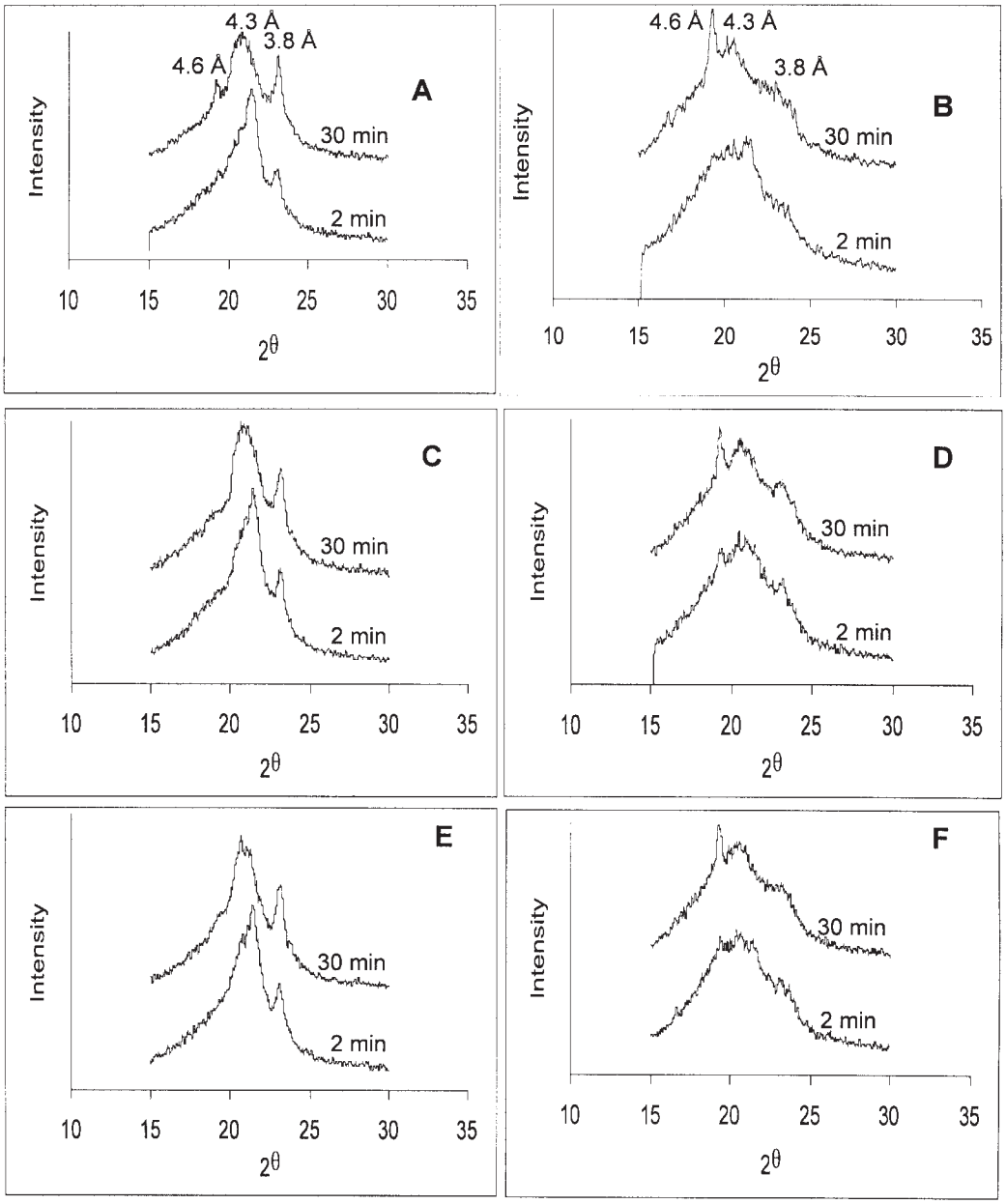

FIG. 3. X-ray patterns of the 20 (panels A, C, E) and 60% (panels B, D, F) SFO blends taken after 2 and 30 min at 10°C: (A) and (B) without SE; (C) and (D) with P-170 at 0.5 wt %; (E) and (F) with S-170 at 0.5 wt%, respectively. Errors in line positions were less than 0.01°.

lar TAG. Ten Grotenhuis *et al*. (14) reported that when milk fat was crystallized at cooling rates varying from 0.5 to 20°C/min, the β-form, which had occasionally been observed in previous studies, was not found. Owing to the wide variety of individual TAG found in low concentrations in milk fat, the conditions required to form ideal β structures are achieved over a long period of crystallization at low degrees of supercooling (14). SFO has a strong tendency to form β crystals, but it is a liquid at 10°C and so it is surprising that the addition of SFO had an effect on HMF polymorphic behavior (Fig. 3). At a 60% addition level the β-form was the most important, most likely owing to an increase in the C_{54} fraction (18:1 *cis*) from 1.4% for HMF to 50.2% for 60% SFO. These results showed that SFO not only was a solvent but also interacted with milk fat. Mixed crystals could have been formed owing to the intersolubility of TAG. A TAG with a lower m.p. could develop a solid solution with another TAG, which could have a higher m.p. Thus, crystals formed at 10°C could contain TAG with a m.p. lower than 10°C as is the case with 18:1 *cis*. When crystals were analyzed for their chemical composition, higher levels of SFO generally led to initial crystals with less (2.5–4.0%) TAG with acyl carbon numbers between 36 and 50 and more (3.6%)

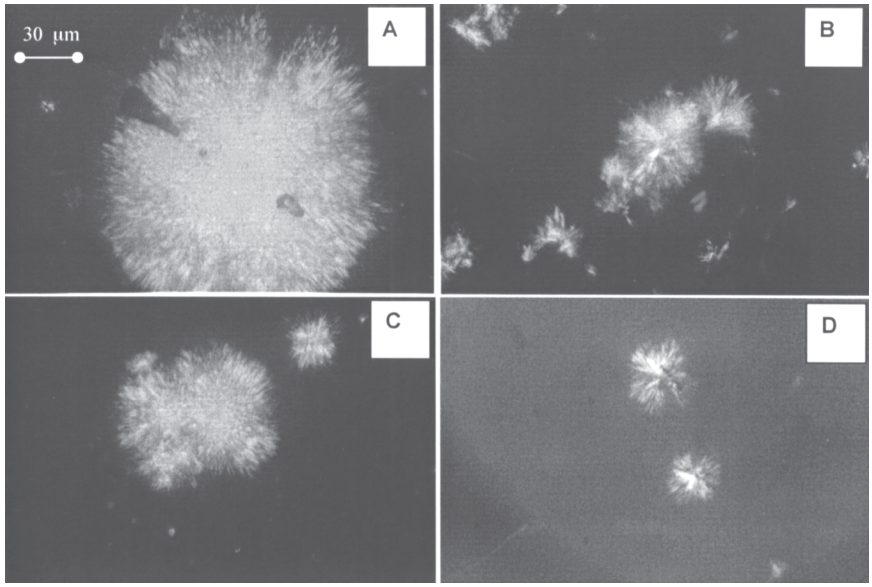

FIG. 4. Polarized light microscopy images of samples taken after 30 min at 37.5°C with an agitation rate of 100 rpm and a cooling rate of 5.5 ± 0.5°C/min: (A) HMF; (B) HMF with S-170 at 0.1 wt%; (C) 60% SFO blend; (D) 60% SFO blend with S-170 at 0.1 wt%. For abbreviations see Figures 1 and 2.

TAG with an acyl carbon number of 52. The C_{54} fraction increased from 3.2% (HMF) to 28.3% (60% SFO), 18:1 *cis* being 0.6 and 23.2%, respectively. TAG composition played a key role in polymorphic behavior of these blends. Addition of P-170 (Fig. 3D) and S-170 (Fig. 3F) did not have an effect on polymorphic behavior of the 60% SFO blend.

Although SE are crystalline solids, diffraction lines corresponded to those characteristics of fats at all temperatures. No new signals were found, which indicates that SE were incorporated into fat crystals.

Two mechanisms have been reported in the literature to interpret the effects of emulsifiers on fat crystallization in bulk systems (15). First, the emulsifier can act as a source of heteronuclei, accelerating nucleation through the catalytic actions of such impurities. During crystal growth, the emulsifiers are adsorbed at steps or kinks on the surface of growing fat crystals and thereby inhibit crystal growth and modify crystal morphology. Second, fats and emulsifiers are able to cocrystallize because of their somewhat similar chemical structure. However, the structural dissimilarities between TAG and emulsifiers can delay nucleation and inhibit growth. In a previous study we demonstrated that, among the mechanisms described in the literature, the cocrystallization hypothesis was the one that better described the effects of SE on crystallization behavior when nucleation was studied by laser polarized light turbidimetry (2). The NMR studies reported here confirmed these findings. SE delayed the overall crystallization rate and diminished S_{max} at low supercoolings. A mechanism involving catalytic actions of impurities would have produced acceleration of nucleation in the bulk medium (16), which did not take place in our systems (2). With regard to the decreases in the extent of fat crystallization, two possibilities were reported (16): retardation of crystal growth, and the formation of impure crystals or aggregates, both of which decrease crystallization rate. XRD results indicated that most likely SE were incorporated into fat crystals in both HMF and the blends.

Morphology and polymorphism in dynamic conditions. Figure 4 shows representative PLM images taken after 30 min at 37.5°C of HMF (panel A), HMF with addition of 0.1% of S-170 (panel B), the 60% SFO blend (panel C), and the 60% SFO blend with addition of 0.1% of S-170 (panel D). The fat crystals observed in all samples were present as well-organized spherulites with needle-shaped crystals oriented radially from the center. Addition of the SE P-170 and S-170 always decreased the size of the spherulites (consistent with the NMR results reported below). However, for HMF and the 60% SFO, they did not alter their polymorphic form at high temperature. X-ray spectra of samples in Figures 3A and 3B show characteristic β′ patterns with two strong signals at d-spacings of 3.9 and 4.3 Å, whereas spectra of samples in Figures 3C and 3D show a β pattern with three strong signals at 4.6, 3.9, and 3.8 Å. For the 20 and 40% SFO blends, P-170 and S-170 favored the β′- form and delayed the formation of the β-form as expected from Figure 3. However, there were no changes in morphology. In agreement with the experiments without agitation, addition of SFO to HMF modified polymorphic behavior. Although P-1670 slightly modified Avrami k_n and n parameters, it affected neither crystal size or morphology nor polymorphism.

ACKNOWLEDGMENTS

María L. Herrera and Roberto J. Candal are associate researchers of the National Research Council (CONICET). This work was supported by the University of Buenos Aires through project X 226 and by CONICET through Project PIP 5380.

REFERENCES

1. Reddy, S.Y., and T. Jeyarani, *Trans*-Free Bakery Shortenings from Mango Kernel and Mahua Fats by Fractionation and Blending, *J. Am. Oil Chem. Soc. 78*:635–640 (2001).
2. Cerdeira, M., S. Martini, R.W. Hartel, and M.L. Herrera, Effect of Sucrose Ester Addition on Nucleation and Growth Behavior of Milk Fat-Sunflower Oil Blends, *J. Agric. Food Chem. 51*:6550–6557 (2003).
3. Campbell, S.D., H.D. Goff, and D. Rousseau, Modeling the Nucleation and Crystallization Kinetics of a Palm Stearin/Canola Oil Blend and Lard in Bulk and Emulsified Form, *J. Am. Oil Chem. Soc. 81*:213–219 (2004).
4. Sato, K., S. Ueno, and J. Yano, Molecular Interactions and Kinetic Properties of Fats, *Prog. Lipid Res. 38*:91–116 (1999).
5. Weyland, M., Emulsifiers in Confectionery, in *Food Emulsifiers and Their Applications*, edited by G.L. Hasenhuettl and R.W. Hartel, Chapman & Hall, New York, 1997, pp. 235–254.
6. Yuki, A., K. Matsuda, and A. Nishimura, Effect of Sucrose Polyesters on Crystallization Behavior of Vegetable Shortening and Margarine Fat, *J. Jpn. Oil Chem. Soc. 39*:236–244 (1990).
7. Herrera, M.L., and F.J. Marquez Rocha, Effects of Sucrose Ester on the Kinetics of Polymorphic Transition in Hydrogenated Sunflower Oil, *J. Am. Oil Chem. Soc. 73*:321–326 (1996).
8. Nasir, M.I., Effect of Sucrose Polyesters and Sucrose Polyester-Lecithins on Crystallization Rate of Vegetable Ghee, in *Crystallization and Solidification Properties of Lipids*, edited by N. Widlak, R.W. Hartel, and S. Narine, AOCS Press, Champaign, 2001, pp. 87–95.
9. Avrami, M., Kinetics of Phase Change. II. Transformation–Time Relations for Random Distribution of Nuclei, *J. Chem. Phys. 8*:212–224 (1940).
10. Box, G.E.P., W.G. Hunter, and J.S Hunter, Randomized Blocks and Two-Way Factorial Designs, in *Statistics for Experimenters,* John Wiley & Sons, New York, 1978, pp. 208–244.
11. Graydon, J.W., S.J. Thorpe, and D.W. Kirk, Determination of the Avrami Exponent for Solid State Transformations from Non-Isothermal Differential Scanning Calorimetry, *J. Non-Cryst. Solids 175*:31–43 (1994).
12. Christian, J.W., *The Theory of Transformations in Metals and Alloys*, Pergamon Press, London, 1975, pp. 26–52.
13. Sakamoto, M., A. Ohba, J. Kuriyama, K. Maruo, S. Ueno, and K. Sato, Influences of Fatty Acid Moiety and Esterification of Polyglycerol Fatty Acid Esters on the Crystallization of Palm Mid-Fraction in Oil-in-Water Emulsion, *Colloids Surf. B: Biointerfaces 37*:27–33 (2004).
14. ten Groetenhuis, E., G.A. van Aken, K.F. van Malssen, and H. Schenk, Polymorphism of Milk Fat Studied by Differential Scanning Calorimetry and Real-Time X-Ray Powder Diffraction, *J. Am. Oil Chem. Soc. 76*:1031–1039 (1999).
15. Garti, N., Effects of Surfactants on Crystallization and Polymorphic Transformation of Fats and Fatty Acids, in *Crystallization and Polymorphism of Fats and Fatty Acids*, edited by N. Garti and K. Sato, Marcel Dekker, New York, 1988, pp. 267–303.
16. Katsuragi, T., N. Kaneko, and K. Sato, Effects of Addition of Hydrophobic Sucrose Fatty Acid Oligoesters on Crystallization Rates of *n*-Hexadecane in Oil-in-Water Emulsions, *Colloids Surf. B: Biointerfaces 20*:229–237 (2001).

[Received October 3, 2005; accepted March 30, 2006]