Effect of Sucrose Esters and Sunflower Oil Addition on Crystalline Microstructure of a High-melting Milk Fat Fraction

S. MARTINI, M.C. PUPPO, R.W. HARTEL, AND M.L. HERRERA

ABSTRACT: The effects of sunflower oil (SFO) and the sucrose esters (SE) P-1670, P-170, and S-170 on crystalline microstructure of a high-melting milk fat fraction (HMF) was studied by polarized-light microscopy (PLM) and confocal laser scanning microsopy (CLSM). The addition of SFO markedly diminished crystal size and delayed crystallization kinetics , as observed in PLM images. Addition of P-1670 modified crystallization behavior only slightly. Addition of P-170 and S-170, however, markedly diminished crystal size and led to more transparent crystals, with lower amount of solids in each crystal. These results show that the microstructural properties of HMF were modifed by addition of SFO and by addition of SPE with low HLB.

Keywords: blending, sucrose esters, microstructure, high melting fraction of milk fat, sunflower oil

Introduction

MILK FAT FRACTIONS HAVE FOUND application in a variety of food products. The high melting point stearins are useful in puffy pastry, whereas the mid-fractions are useful in Danish cookies. Stearins are also used in the reduction in blooming properties of chocolate (Kellens 1997). The modification by blending of milk fat stearins is an interesting and important approach for utilization of milk fat fractions in a number of edible fat products. Milk fat stearin fractions, on blending with liquid oils like sunflower or soybean oil in different proportions, offer nutritionally important fat products with enriched content of essential fatty acids like $C_{18:2}$ and $C_{18:3}$ and almost zero trans fatty acid content (Pal and others 2001).

Sucrose esters (SE) can be used in foods as emulsifiers because they are nontoxic, tasteless, odorless, and are digested to sucrose and fatty acids in the stomach. They can also be used in pharmaceuticals, cosmetics, foods, and in other products where a nonionic, nontoxic, biodegradable emulsifier is required (Gupta and others 1983). In addition to their major function of producing and stabilizing emulsions, SE contribute to numerous other functional roles as texturizer and film former. Typical applications are baked goods, fruit coatings, and confectionery (Hasenhuettl 1997). Their role as fat crystallization modifiers has yet to be explored.

PLM proved to be a very good method for the study of early events in the crystallization of fats (Wright and others 2000). It is also an increasingly used technique for studying the microstructure and composition of food systems in relation to their physical properties and processing behavior (Marangoni and Rousseau 1996). In many applications of edible fats, however, the morphology and number of glyceride crystals determine the suitability of the fat for a given purpose. Controlling size distribution and morphology of fat crystals play a key role in the stabilization of emulsions. For example, small size with narrow distribution and spherical and smooth morphology are necessary to obtain stable emulsions (Garti and others 1998). Early microscopic studies of the polymorphic forms of single-acid TAG have shown that the crystal polymorphs exhibit a wide range of microscopic appearances. More recent studies have confirmed these earlier reports (ten Grotenhuis and others 1999). Complex fat systems were also described by PLM. For example, the isothermal crystallization behavior of hydrogenated sunflower oil was described for different temperatures using PLM (Herrera 1994), and the morphology and crystal size distribution of blends of milk fat fractions was studied by PLM for crystallization under different processing conditions (Grall and Hartel 1992, Herrera and Hartel 2000).

Confocal laser scanning microscopy (CLSM) is a relatively new optical tool that is increasingly being applied in the food area. The primary value of the CLSM to research is its ability to produce optical sections through a three-dimensional (3-D) specimen, for example, a piece of tissue or other thick objects, without procedures that apply substantial shear and compressive forces or without sectioning and chemical processing steps. This property of the CLSM is fundamental for solving 3-D problems where information from regions distant from the plane of focus can blur the image of such objects (Dürrenberger and others 2001).

It is often the case in food products, as well as in confectionery, that a combination of 2 emulsifiers in a recipe or formula containing 2 distinct phases will result in a longer lasting and more uniform product. In these cases, combinations of low and high HLB emulsifiers give the best results (Weyland 1997). This research will supply guidance to food manufacturers interested in controlling crystallization in lipid-based products such as chocolate, butter, margarine, or shortenings in which a certain crystal size distribution gives the desired attributes. The aim of the present work is to observe the effect of sunflower oil and sucrose esters with low and high HLB values on the crystalline microstructure of high-melting fraction of milk fat. The crystal size distributions before storage and the crystalline microstructure of a semisolid product were studied by PLM and CLSM, respectively.

Materials and Methods

Starting materials

High-melting milk fat fraction (HMF) was obtained from Grassland Dairy (Green-

Table 1—Chemical composition and mettler dropping points of high-melting milk fat fractions (HMF), sunflower oil (SFO), and their mixtures

Acyl	Chemical Composition in Weight of Starting Materials (%)				
Carbon nr	SFO	HMF	10% SFO	20% SFO	40% SFO
C26	0.1	0.2	0.2	0.2	0.1
C28	0	0.3	0.3	0.2	0.1
C30	0	0.7	0.5	0.4	0.4
C32	0	1.5	1.2	1.1	0.7
C34	0	2.9	2.4	2.4	1.7
C36	3.2	3.8	5.0	4.5	3.4
C38	1.8	6.0	8.3	7.5	6.0
C40	0	4.7	5.6	4.5	2.6
C42	0	4.6	4.5	4.0	3.0
C44	0	4.8	5.8	4.7	3.9
C46	0	6.9	8.5	7.9	5.7
C48	0	16.9	12.3	11.8	8.3
C50	1.3	23.4	17.5	14.6	12.1
C52	15.1	15.9	13.3	14.1	13.8
C54 (18:0)	1.1	1.5	1.2	0.9	1.0
C54 (18:1cis)	76.0	1.4	8.8	16.8	32.1
C54 (18:1 other)	0	4.6	4.5	4.5	4.9
MDP (°C)	-	48.35	46.95	46.15	44.35

wood, Wis., U.S.A.) and sunflower oil (SFO) from Molinos Rio de La Plata S.A. (Avellaneda, Buenos Aires, Argentina). The HMF of milk fat used in this study had a solid fat content at 5 °C of 82% and at 20 °C of 67%, which correspond to a very high melting fraction. Experimental milkfat fractions are produced from anhydrous milkfat (AMF) using the Tirtiaux fractionation process. The AMF is obtained from a commercial supplier and is made from sweet cream. The fractionation process is a simple physical process that employs no additives. The milkfat is heated until fully melted, cooled under controlled conditions to 45 °C, and pressure filtered to separate the fraction. The fraction is not further processed after fractionation.

Three blends were prepared by mixing 10, 20, and 40% of SFO with HMF. Dropping points (the temperature at which a solid fat just begins to flow under controlled conditions) of the samples were determined with the Mettler FP 80 Dropping Point Apparatus (Mettler Instruments A.G., Greifensee-Zurich, Switzerland), using a heating rate of 1 °C/min. Chemical composition and Mettler Dropping points of all samples are reported in Table 1. Palmitic SE (P-170) with hydrophilic/lipophilic balance (HLB) = 1, Stearic SE (S-170) with HLB = 1 and Palmitic SE (P-1670) with HLB = 16 were supplied by Mitsubishi-Kasei Food Corp. (Tokyo, Japan). The SE had Mettler dropping points of 58.0, 59.5, and 44.0, respectively. Monoester content of S-170 and P-170 was 1 wt%, with di-, tri-, and polyesters comprising 99 wt%. P-1670 had 80% of monoester and 20% of di-, tri-, and polyester. SE were added at concentrations of 0.1 wt% to HMF and the 3 blends with SFO.

GC analysis

Acyl carbon profile of samples was determined with a Hewlett-Packard 5890 Series II (Hewlett- Packard, San Fernando, Calif., U.S.A.) gas chromatography (GC) unit equipped with a flame ionization detector (FID) and on-column injector. The column used was a Heliflex Phase AT-1 with a length of 30 m and an internal dia of 0.25 mm (Alltech and Associates, Deerfield, Ill., U.S.A.). Helium was the carrier gas at a flow rate of 2 mL/min, with hydrogen gas and air also being supplied to the FID. Samples were prepared by using a modified method of Lund (1988). A sample of 10 mg was weighed in GC vials and dissolved in 1.8 ml of iso-octane with 100 ml of internal standard (C27 in iso-octane: 2.02 mg/ml). Samples were stored in a refrigerator prior to analysis. To separate the different TAG according to acyl carbon number, the following temperature profile was used in the GC:initialy hold at 280 °C for 1 min, and then heat at a rate of 3.0 °C/min until a temperature of 355 °C was reached. The detector was held constant at 370 °C. Composition (area %) was based on the area integrated by using ChemStation Chromatography software by Hewlett Packard. Samples were run in duplicate.

X-ray Diffraction (XRD)

Samples were analyzed for their polymorphic form by using a Philips 1730 X-ray spectrometer fitted with a system for temperature control (Philips Argentina S.A., Capital Federal, Argentina). The temperature of the sample holder placed within the refraction chamber was controlled through a programmable Lauda UK 30 cryostat (Werklauda, Königshofen, Germay). Ethylene glycol in water (3:1, vol/vol) was used as coolant. $K_{\alpha 1 \alpha 2}$ radiation from copper was used at 40 kV, 20 mA, and scanning velocity 1 °/min from 5 to 30 °.

Crystallization procedure

500 g of each sample were melted in a water bath at 80 °C and kept at this temperature for 40 min. A combination of 100 mg of Nile blue and 0.5 mg of Nile red were added and the sample was stored in an oven at 60 °C overnight to dissolve the dyes. The dye system used did not modify nucleation kinetics when induction times were measured by turbidimetry (Herrera and Hartel 2000). Nile red is very fluorescent and allows the use of the laser source at 10% or less power, which prevents the dyes from bleaching or samples from melting. Moreover, crystal structure is more defined and air bubbles are easy to distinguish from crystals. However, Nile blue is also necessary to better distinguish the background from the crystals. Nile blue stain was used to negatively stain milk fat crystals. This lipophilic stain diffuses into the oil phase of a sample and generates a deep yellow florescence (l = 488 nm), whereas the solid fat does not fluoresce (Heertje and others 1990).

The melted fat samples were placed in a 1.0L stainless steel, jacketed vessel with a 23 cm height and an 8.4 cm inner dia. A mixer was attached to a Master Servodyne (Servodyne Controller, Chicago, Ill., U.S.A.) drive unit, which maintained constant motor speed. The mixer consisted of a 0.8-cm dia and 39.2-cm length polypropylene shaft with a U-shaped blade paddle assembly for mixing highly viscous liquids. Maximum paddle dia was 6.6 cm. A copper- constantan thermocouple, attached to an aluminum brace and positioned 1 cm from the center of the tank and 2 cm from the top of the sample, was used to determine sample temperature. Bath and sample temperatures were recorded. Characteristic temperature profiles for water bath and sample for slow and rapid cooling were reported elsewhere (Herrera and Hartel 2000). The following processing conditions were used: cooling rates of 0.1 and 5.5 °C/min, agitation rates of 100 rpm, and at a crystallization temperature of 40 °C. Samples were taken periodically for observation by PLM begining from the moment sample reached crystallization temperature. After 90 min at 40 °C, a sample of the slurry in the crystallizer was placed between a slide and a cover slip. The slides were cooled and stored for 24 h at 10 °C before observation in the microscope. During cooling and storage, additional fat crystallized (under quiessent conditions) to reach the final solid fat content

(SFC) at that temperature. All the experiments were run in triplicate.

Light microscopy

At the point when the sample temperature reached crystallization temperature, samples were taken for PLM observation, with sampling continuing for 90 min. At each sample point, a drop of slurry was placed between a warmed slide and a cover slide. Crystals were observed with a polarized-light microscope (Nikon Optiphot, Garden City, N.Y., U.S.A.) equipped with a video camera connected to a computer. Optimas 6.1 (Optimas, Bothell, Wash., U.S.A.) software was used to collect the images. Images were taken in duplicate at every sample time with a 10× objective and a TV relay ocular lens. At that time, at least 20 images were taken. Objectives of 10× and 4× were used. The individual areas of crystals were measured using the Optimas software and the results were exported to Microsoft Excel 7.0 (Microsoft, Redmond, Wash., U.S.A.) software for graphic presentation of data. From 200 to 300 crystals were measured for each sample. The diameters of circles having equivalent areas to the measured areas were reported.

Confocal microscopy

The Bio-Rad MRC-1024 (Bio-Rad Laboratories, Hempsted, England) series confocal laser scanning microscope with a krypton/argon mixed gas laser operated by 24-bit LaserSharp software was used to collect the images. A 10× ocular was used together with a 10× objective for a visual magnification of 100×. The laser intensity was 10%, and the confocal aperture was at setting 2. Images were taken at increasing depths from the surface with increments of 3 μm. Images were recorded by using confocal assistant 4.02 software (Todd Clark, Brelje, Minn., U.S.A.) provided with the MRC- 1024 CLSM.

Results and Discussion

Polymorphic behavior

When polymorphism was studied by XRD, only the β´- form was found for HMF and the blends with SFO with and without SE addition and for both cooling rates. Neither α nor β polymorphs were found. The spectra were characteristic of the β´- form with 2 strong peaks at 3.9 and 4.3 Å. Studies of crystallization of palm mid fraction (PMF) in an emulsion system reported that with the addition of S-170, PMF preferentially crystallized in the β´ form. The α form was found to crystallize below its melting point, 13 °C, when a SE was added (Awad and Sato 2001).

Effect of blending

The melting points (T_m), measured as Mettler dropping points, and TAG composition of the HMF, SFO, and the 3 blends are reported in Table 1. T_m of the HMF was 48.3 °C. Addition of 10% SFO decreased T_m by 1.3 °C, addition of 20% by 2.2 °C, and addition of 40% decreased T_m by only 4 °C. Addition of SFO substantially increased the content of C18:1 and significantly decreased the content of C 48 and C 50 in the blends. It may be expected that different interactions between TAG take place when SFO is added to HMF and as these interactions play a key role in crystallization behavior, different crystal size distribution (CSD) could be expected for these systems.

Figure 1 shows representative PLM images obtained when SFO was added to HMF and crystallized at 40 °C with cooling rate of 5.5 °C/min. Images were taken after 90 min at crystallization temperature. Despite the small changes in T_m (Table 1) due to addition of SFO, the crystallization kinetics of the HMF was substantially slower, as evidenced by the longer induction times for crystallization found when images were taken with time beginning at the moment samples reached crystallization temperature (data not shown; induction time for crystallization is the time interval between crystallization temperature was reached and the start of crystallization). This elongation of induction times for crystallization (as SFO content increased) led to smaller size of crystals formed. In nucleation, molecules need sufficient time to organize and align with their neighbors to form stable nuclei. When induction times are elongated, as is the case of addition of SFO to HMF, that organization takes place for more time at crystallization temperature. As a result, smaller sizes of crystals with a narrow distribution are obtained, with nucleation rate larger relative to the growth rate of the crystals. This effect was observed for any factor that delays nucleation (Herrera and Hartel 2000).

The addition of 10, 20, and 40% SFO (Figure 1 b, c, d) markedly decreased agglomerate size compared to the pure HMF. At an addition level of 40%, SFO markedly slower crystallization rate which was also evidenced by the lower solid content shown in Figure 1d. In all cases, morphology of ag-

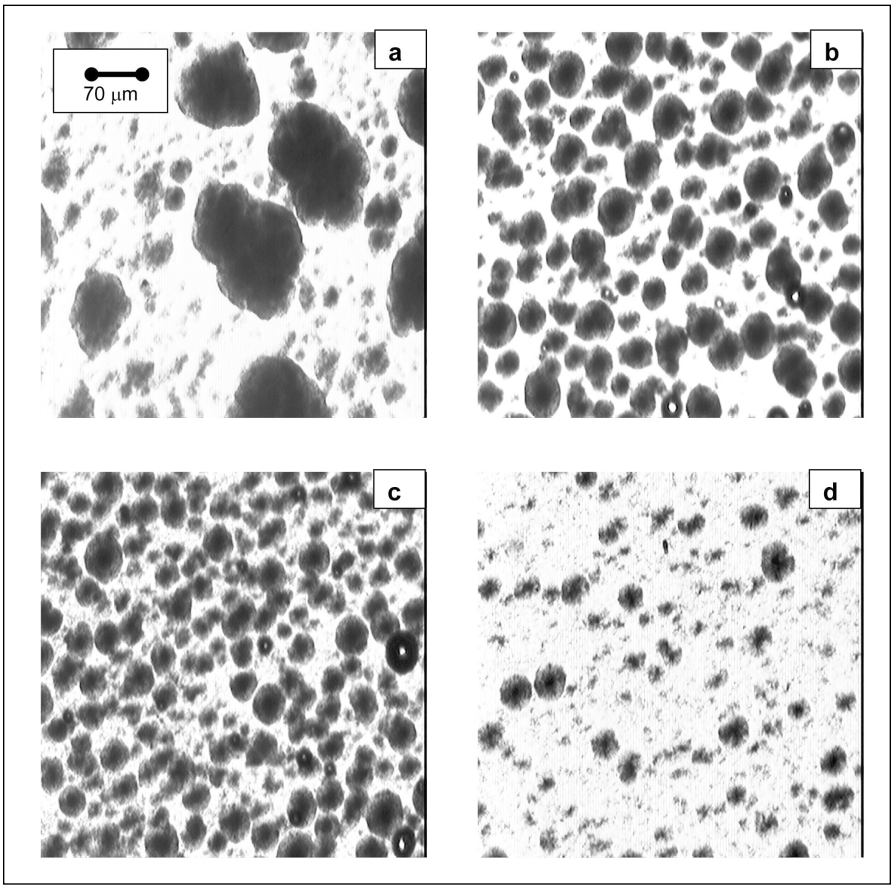

Figure 1—PLM images of (a) high-melting milk fat fraction and addition of (b) 10%, (c) 20%, and (d) 40% SFO crystallized at 40 °C and 100 rpm with a cooling rate of 5.5 °C/min

glomerates was similar. Spherical agglomerates grew by accumulation of small needle crystals. At the beginning of crystallization, only a few crystals were formed. These initial nuclei grew and, at the same time, new crystals were formed. Nucleation and growth continued together and, as can be observed in Figure 1, agglomerates with different size and density (related to opacity of the crystals) were obtained. The fact that agglomerates showed the same morphology indicates that SFO delayed nucleation but did not modified growth mechanism.

Effect of cooling rate

Figure 2 shows representative PLM and CLSM images, as well as crystal size distribution (CSD) of the 10% SFO blend crystallized at 40 °C with either slow or fast cooling and an agitation rate of 100 rpm. PLM images were taken 90 min after the sample reached crystallization temperature. Morphology observed by PLM (Figure 2 a,b) showed no differences with cooling rate. Dense, spherical crystals comprised of small needles were found for the aggregates formed at both cooling rates. The clusters formed at 40 °C with agitation correspond to crystallization at the 1st selected temperature (primary crystallization). After 90 min at 40 °C, the samples were cooled quiescently to 10 °C, and held for 24 h, where secondary crystallization occurred. The crystallization process, in a sense, simulated an industrial process in that samples were crystallized with agitation at a selected temperature, after which the product was packaged and cooled for storage at another temperature. CLSM images for the samples with 10% SFO are shown in Figure 2c and d. In these images, the dyes are only soluble in the liquid phase; therefore, the white zone is the liquid and the different gray zones are the solid phase, which does not fluoresce. Primary crystals, formed under agitation at 40 °C, were generally more dense and distinct from the secondary crystallline structure formed during subsequent cooling of the blend to 10 °C. Most probably, the small and more diffuse crystals that appear in the background were formed during cooling and storage. Some of the primary crystals did not have a well-defined border, perhaps because they grew during cooling to 10 °C. Surprisingly, no observable differences were found between crystalline microstructures (Figure 2c, d) or CSD (Figure 2e, f) that were formed when the samples were cooled at different rates to 40 °C. The thermodynamic driving force (which can be estimated as $\Delta T = T_m - T_c$, where T_m is melting temperature and T_c is crystallization temperature) determines the occurrence of crystallization. However, heat and mass transfer rates during processing can have a significant effect on the rate of crystallization. The manner by which the thermodynamic driving force for crystallization is achieved and the rate of development of this driving force can significantly affect the rates of formation and growth of crystals (Hartel 2001). Previous work had shown that different cooling rates had substantial effect on crystalline microstructure of blends of milk fat fractions (Herrera and Hartel 2000), with faster cooling rates giving smaller and more uniformaly-sized crystals. However, in this study on mixtures of HMF and SFO, no effect of cooling rate on crystalline microstructure was observed, in contrast to our previous study on mixtures of HMF with a low melting fraction of milk fat (LMF). Typically, the effects of processing conditions are minimized when crystallization driving force is high. This may explain the different effects of cooling rate on crystallization of HMF in LMF (lower ΔT) and SFO (higher ΔT).

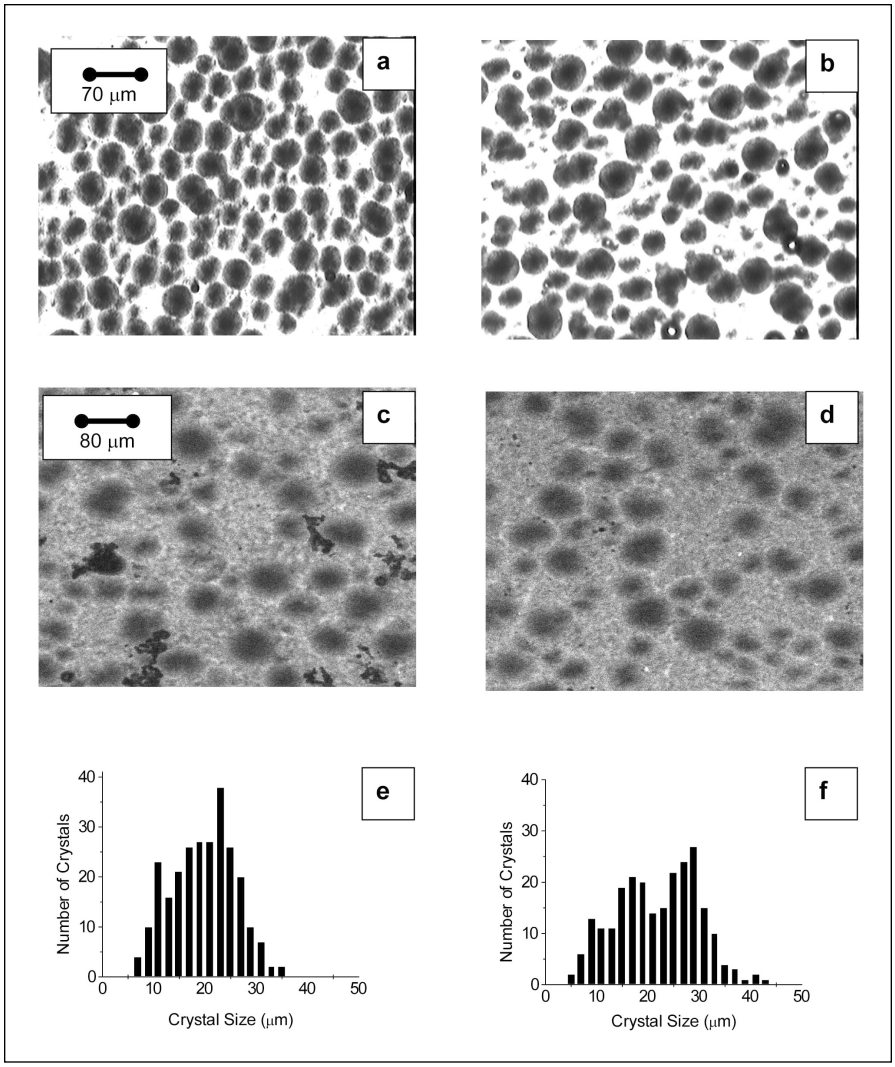

Figure 2—Effect of cooling rate on crystal size for high-melting milk fat fraction with 10% sunflower oil by cooling to 40 °C. Cooling rates are either fast (5.5 °C/min) or slow (0.1 °C/min). (a) and (b): Polarized light microscope images for slow and fast cooling, respectively, after 90 min at 40 °C. (c) and (d): Confocal microscope images for slow and fast cooling, respectively, after 90 min at 40 °C and 24 h at 10 °C. (e) and (f): Crystal size distributions for slow and fast cooling, respectively, after 90 min at 40 °C.

Effect of addition of SE

SE are used in ice cream and other food products in addition levels up to 0.5%. When 0.5% of the emulsifiers used in this study were added to HMF and the blends with SFO, no crystallization occurred after several h at 35 or 40 °C. For this reason, a level of 0.1% was selected for this study. Figure 3 shows representative PLM images of the 10% SFO blend crystallized at 40 °C with a cooling rate of 5.5 °C/min and an agitation

rate of 100 rpm. Figure 3a shows the crystalline microstructure after 15 min at 40 °C without SE, and Figure 3b, c, d shows crystalline microstructure with addition of 0.1 % of P-1670, P-170 and S-170, respectively. Figure 4 shows the CSD after 90 min at 40 °C for the same conditions as in Figure 3. Addition of P-1670 modified crystallization behavior only slightly. No change in morphology was found when compared to the same fat mixture with no added emulsifiers. Spherical crystals with a denser crystal structure covering a range of sizes were present (Figure 3b).

Average crystal size was not significantly different for this 10% SFO blend with HMF upon addition of 0.1% P-1670. However, CSD was slightly narrowed for the sample with added P-1670. Crystals smaller than 10 µm or larger than 40 µm were not found in a population of 300 crystals (Figure 4b). In contrast to P-1670, addition of P-170 and S-170 markedly diminished crystal size. In addition, crystals appeared more transparent, in the microscope images, which suggest a lower amount of solids within each crystal (Figure 3c, d). Samples made with P-170 and S-170 had a narrower CSD than the 10% SFO blend without SPE and a smaller average crystal size ($p < 0.05$) (Figure 4c, d). For all samples, the following trends were observed: addition of the SE used in this study with low HLB values decreased average crystal size and led to more transparent crystals. SE with high HLB values, such as P-1670, had only a slight effect on crystallization behavior of these lipid mixtures. Two mechanisms have been reported in the literature to interpret the effects of emulsifiers on fat crystallization in bulk systems. First, the emulsifier can act as 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 triacylglycerols and emulsifiers can delay nucleation and inhibit growth (Garti 1988). In emulsion systems, another accelerating mechanism has also been observed. S-170 and P-170 are absorbed at the oil-water interface and may accelerate the heterogeneous nucleation of the oil phase at the surface of the droplets when the O/W emulsion was cooled (Katsuragi and others 2001). However, this type of acceleration with added hydrophobic emulsifiers on nucleation has not been observed in bulk fat systems (Garti and Yano

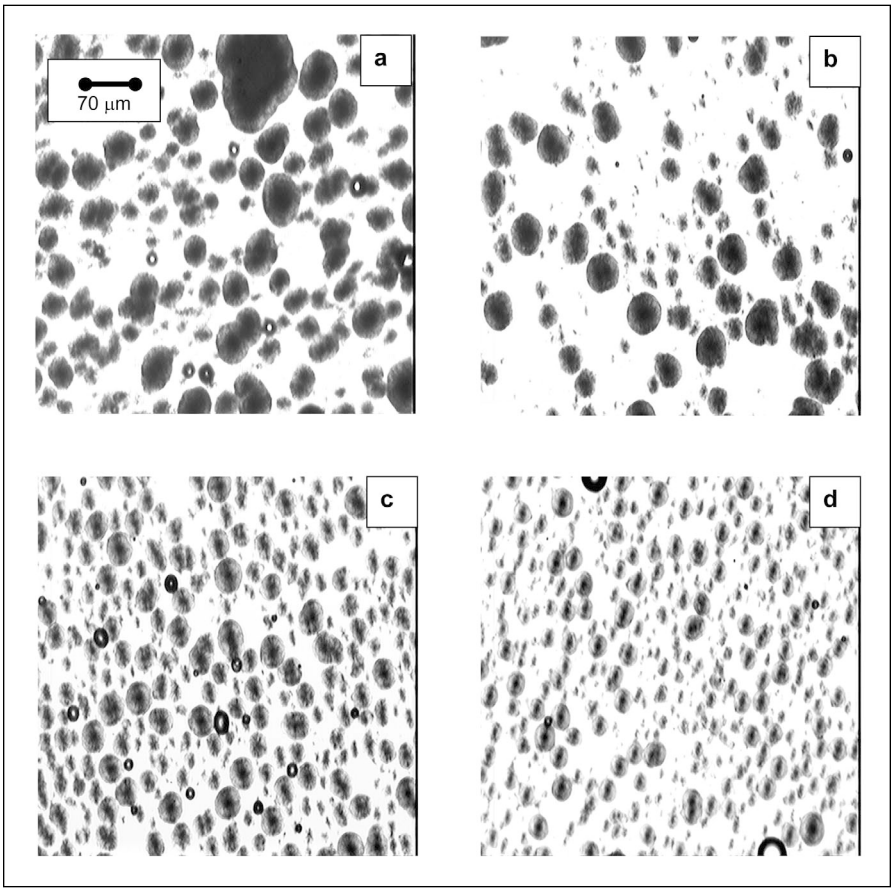

Figure 3—Effect of 0.1% sucrose polyesters (SPE) addition on crystalline microstructure (polarized light microscopy) of a mixture of 10% sunflower oil in high-melting milk fat fraction. Crystallization conditions: rapid (5.5 °C/min) cooling to 40 °C, 100 rpm, images taken after 15 min at 40 °C. (a) no SPE, (b) P-1670, (c) P-170, (d) S-170.

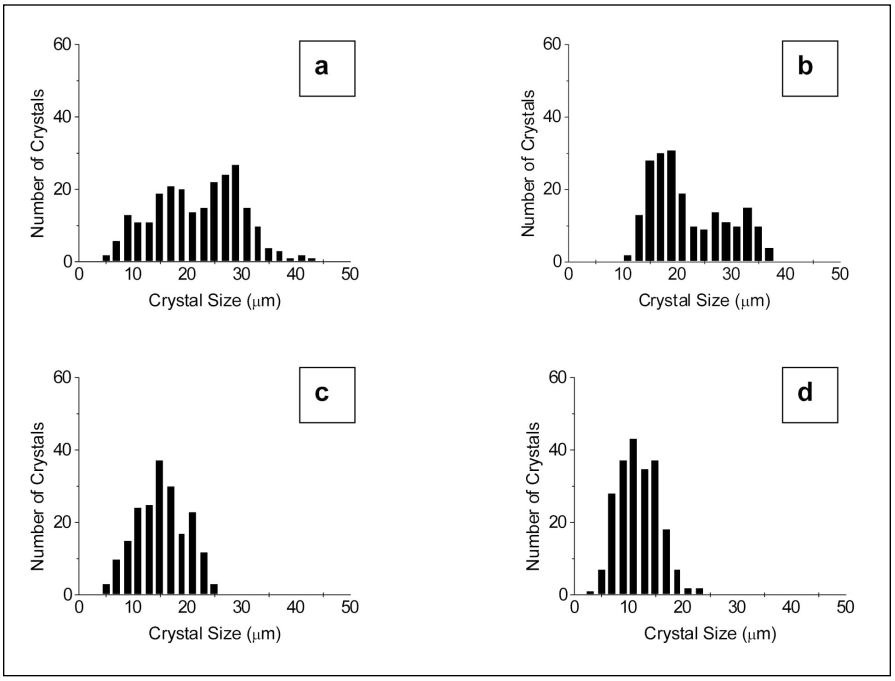

Figure 4—Effects of 0.1% sucrose polyester (SPE) addition on crystal size distribution of a mixture of 10% sunflower oil in high-melting milk fat fraction. Crystallization conditions: rapid (5.5 °C/min) cooling to 40 °C; 100 rpm; samples taken after 90 min at 40 °C. (a) without SPE, (b) P-1670, (c) P-170, (d) S-170.

Table 2—Average crystal size (μm) and standard deviation of the size distribution (μm) for crystallization of high-melting milk fat fraction with added sunflower oil (SFO) and 0.1% sucrose polyester (P-1670, S-170 and P-170). Crystallization conditions: cooling at different rates (0.1 or 5.5 °C/min) to 40 °C with agitation at 100 rpm; samples taken after 90 min at 40 °C.

	Without SPE		P-1670		S-170		P-170	
SFO (%)	0.1 °C/min	5.5 °C/min	0.1 °C/min	5.5 °C/min	0.1 °C/min	5.5 °C/min	0.1 °C/min	5.5 °C/min
0	56.2 ± 6.7	58.1 ± 7.2	56.8 ± 7.4	57.0 ± 8.4	18.4 ± 4.8	19.6 ± 3.5	16.7 ± 4.1	16.8 ± 3.6
10	22.8 ± 6.1	21.9 ± 6.9	20.1 ± 4.5	22.2 ± 4.9	12.5 ± 3.5	11.9 ± 3.7	13.0 ± 3.2	14.3 ± 3.5
20	12.6 ± 5.0	12.8 ± 5.5	12.5 ± 3.0	12.7 ± 3.7	8.5 ± 2.4	8.2 ± 3.4	7.1 ± 2.5	6.9 ± 2.9
40	10.1 ± 3.8	9.7 ± 3.6	10.5 ± 2.4	10.6 ± 3.5	8.6 ± 3.2	7.9 ± 3.1	8.0 ± 2.7	7.7 ± 2.6

Standard deviation for means calculated from different experimental replicates were less than 10%.

2001). The results obtained in this study support a cocrystallization mechanism, since at the concentration selected, the main effect was a delay on nucleation.

Crystal Size Distribution (CSD)

Table 2 reports the effects of SFO and 0.1% SE addition to HMF on the mean crystal size and width of the distribution after 90 min of crystallization at 40 °C and an agitation rate of 100 rpm. Standard deviation (SD) between means obtained from experimental replicates were less than 10% of the mean values. No differences were observed in mean size or SD of the CSD for either cooling rate ($p < 0.05$). Addition of SFO resulted in a marked decrease in mean crystal size (means of the distributions were compared using a Student's t-test at $p < 0.05$), which was observed at both cooling rates. The effect of SFO was to dilute the crystalline solids of HMF (Puppo and others 2002) and it is probably this decrease in SFC that led to the smaller crystal size upon addition of SFO. Addition of P-1670 did not cause a significant change in mean crystal size, as expected. The HLB number for P-1670 is very high (16), which means that it has affinity for hydrophilic compounds and no strong interactions would be expected between this emulsifier and HMF or SFO. P-170 and S-170, in contrast, caused a significant decrease in mean crystal size ($p < 0.05$), as expected, since its HLB number (1) indicates a high affinity for hydrophobic compounds and a high possibility of co-crystallization with the fat. PLM images taken with time showed that addition of SFO and the SE P-170 and S-170 were very effective in delaying nucleation. The sucrose esters used in this study, were reported to delay nucleation in hydrogenated sunflower oil affecting the formation of critical nuclei and prolonging induction times (Herrera and Marquez Rocha 1996). As a result of the elongation of induction times, crystal size was diminished, since the formation of nuclei occurred after more time at crystallization temperature and therefore a significant decrease in mean crystal size was observed experimentally.

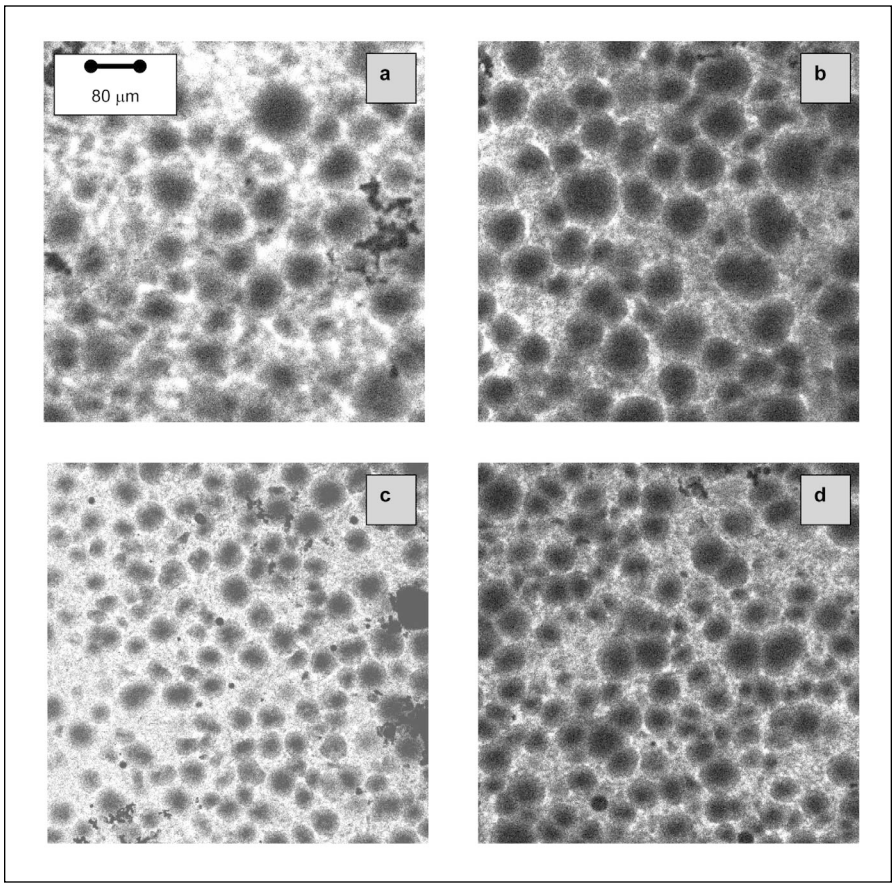

Figure 5—Effects of 0.1% sucrose polyesters addition on crystalline microstructure (confocal microstructure) of a mixture of 10% sunflower oil with HMF. Crystallization conditions: slow (0.1 °C/min) cooling to 40 °C at 100 rpm 90 min at 40 °C; cool to 10 °C; store for 24 h before sampling. (a) without SPE, (b) with P-1670, (c) P-170, (d) with S-170.

Effect of SE on crystalline microstructures of stored samples

Figure 5 shows representative CLSM images of the samples with 20% SFO cooled slowly (0.1 °C/min) to 40 °C (100 rpm) both (a) without and with addition of 0.1 % of (b) P-1670, (c) P-170, and (d) S-170. After 90 min at 40 °C, samples were cooled on microscope slides to 10 °C and held for 24 h to complete crystallization. The large, dense crystals (primary crystallization) were formed during crystallization at 40 °C with the smaller and more diffuse crystals (secondary) in the background formed during cooling and storage at 40 °C. The primary and secondary crystals were dispersed in liquid fat, which remained uncrystallized at 10 °C. The nature of this crystalline structure was dependent on the chemical composition (Table 1) and SE addition. Figure 5 shows that, even after 24 h at 10 °C, the characteristics of the primary crystals formed at 40 °C remained the same. Samples showed the same tendencies found analyzing their CSD on PLM images

before storing at 10 °C. P-1670 had a similar structure than the 20% SFO blend. Addition of S-170 and P-170 diminished crystal size of primary crystals. In the case of P-170, crystal size was markedly smaller in agreement with the most efficient effect in delaying crystallization. Crystal morphology, however, was not modified by the addition of SE, indicating that the growth mechanism was not modified. When an emulsifier acts as a heteronuclei in crystal growth, the emulsifier is adsorbed at steps or kinks on the surface of growing fat crystals and thereby inhibits crystal growth and modifies crystal morphology. The fact that the growth mechanism was not modified in the results presented here supports the cocrystallization mechanism.

Conclusions

PREVIOUS WORK HAD SHOWN THAT different cooling rates had substantial effect on crystalline microstructure. However, in this study on mixtures of HMF and SFO, no effect of cooling rate on crystalline microstructure was observed, in contrast to our previous study on mixtures of HMF with a low melting fraction of milk fat (LMF). The effects of processing conditions are minimized when crystallization driving force is high. Addition of SFO and SE with low HLB value to HMF modified average crystal size, crystal size distribution, and the primary crystalline microstructure, and even after 24 h at 10 °C, the characteristics of the primary crystals formed at 40 °C remained the same.

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