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ORIGINAL ARTICLE

Effects of Addition of a Palmitic Sucrose Ester on Low-*Trans*-Fat Blends Crystallization in Bulk and in Oil-in-Water Emulsions

Cristián Huck-Iriart · Roberto J. Candal · Maria L. Herrera

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Abstract The effect of addition of a highly hydrophobic emulsifier, the palmitic sucrose ester P-170, on isothermal crystallization behavior of a high-melting fraction of milk fat (HMF) and its mixtures with 20% or 40% sunflower oil (SFO), both in bulk and in emulsion systems, were studied by nuclear magnetic resonance (NMR) and by time-resolved in situ small-angle synchrotron X-ray scattering (SAXS). NMR studies showed that the effect of P-170 on the overall isothermal crystallization kinetics in bulk phase could be either acceleration or delay. The effect was strongly dependent on supercooling. For HMF, P-170 retarded crystallization at temperatures above 29.0±0.2 °C, while below that temperature, it accelerated the process. For the blends of HMF with 20% or 40% SFO, the temperature at which the behavior changed was 27.0±0.2 or 26.0±0.2 °C, respectively. In emulsion systems, however, the effect was always acceleration for all temperatures selected. With the aid of SAXS, it was possible to study early stage of crystallization during real time and therefore to improve our understanding of the mechanism of P-170 action. The different effects caused by P-170, as described by SFC curves, were strongly related to the effects of P-170 on fat polymorphism, specially to the value of the time interval of coexistence of the α and β′ forms. These events took place at the very beginning of crystallization and thus could not be described by the traditional X-ray techniques used in previous studies of similar systems.

Keywords High-melting milk fat fraction · Sunflower oil · Nuclear magnetic resonance · Small angle synchrotron X-ray scattering · Palmitic sucrose ester

Abbreviations

HMF	high-melting milk fat fraction
MDP	Mettler dropping point
NaCas	sodium caseinate
NMR	nuclear magnetic resonance
PUFA	polyunsaturated fatty acids
SAXS	small angle X-ray scattering
SE	sucrose ester
SFC	solid fat content
SFO	sunflower oil

Introduction

Consumer concerns associated with the atherogenic effect of *trans* fatty acids limit the future of traditional hydrogenation process as a way to modify the solid-to-liquid ratio in vegetable oils/fats. As one of the alternatives to hydrogenated vegetable oils, modification of high-melting-point 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* fatty acids and rich in PUFA can be obtained[1,2].

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Sucrose esters (SE) can be used in foods as emulsifiers because they are nontoxic, tasteless, and odorless and are digested to sucrose and FA in the stomach. They can also be used in pharmaceuticals and cosmetics and in other products where a nonionic, biodegradable emulsifier is required[3]. In addition to their major function of producing and stabilizing emulsions, SE have numerous other functional roles as texturizers, film formers, and modifiers of crystallization. Several reports have dealt with the effect of SE on the crystallization behavior of fats, both in bulk and in emulsion systems; however, some of these results may be considered contradictory[4–14]. For a selected fat in bulk, none of these authors has reported both effects: acceleration or delay with supercooling. Moreover, differences in behavior between fat in bulk and in emulsion were not explained by the mechanisms proposed in those articles. The use of techniques that does not allow studying in detail the early stage of crystallization can lead to misleading interpretations of the phenomenon. This early stage of crystallization is very important since it determines the later evolution of the system[15]. A synchrotron source allows diffraction patterns to be acquired during real-time crystallization, and therefore, further and less speculative information about mechanisms of action can be obtained.

The purpose of this work was to evaluate the different effects, acceleration or delay, of a palmitic SE addition in the early stage of crystallization of a low *trans* fat cooled isothermally in bulk and in emulsion systems. Its mechanism of action was also deeply analyzed.

Materials and Methods

Materials

The fat phase was either a commercial high-melting fraction of milk fat (HMF) or its blends with 20% or 40% commercial sunflower oil (SFO). Dropping point (the temperature at which a solid fat just begins to flow under controlled conditions) of HMF and the blends with SFO was determined with the Mettler FP 80 dropping point apparatus (Mettler Instruments A.G., Greifensee-Zurich, Switzerland), using a heating rate of 1 °C/min. Mettler dropping point (MDP) of HMF and the 20 and 40 wt.% SFO-in-HMF blends were 40.1±0.6, 38.6±0.7, and 37.1± 0.6 °C. Chemical composition of HMF and the two blends was reported elsewhere[16]. Palmitic SE (P-170) with hydrophilic/lipophilic balance (HLB=1) was supplied by Mitsubishi-Kasei Food Corp. (Tokyo, Japan). The SE had MDP of 58.0 °C and monoester content of 1 wt.% with di-, tri-, and polyesters comprising 99 wt.%. Our previous studies showed that this ester had a notorious effect on nucleation behavior in similar systems[13,14]. It was added to bulk fats at 1 wt.% level. Sodium caseinate (NaCas) was obtained from ICN (ICN Biomedical, Inc., Aurora, OH, USA) and used without any further purification.

Preparation of Emulsions

Six emulsions were prepared. For three of them, 20 g of fat phase was emulsified with 80 g of aqueous phase. In all cases, aqueous phase was a 20 wt.% solution of sucrose with 4 wt.% NaCas. Addition of sucrose to the aqueous phase enhanced stability of emulsions. Three different fat phases were used: HMF and the blends with 20 or 40 wt.% SFO-in-HMF. For the other three emulsions, 20 g of one of the mentioned fat phases, with 1 g of P-170 dissolved in it, was emulsified with 79 g of the described aqueous phase. Fat and aqueous phases were mixed using an Ultra-Turrax T25 high speed blender (GmbH & Co., Staufen, Germany), operated at 20,000 rpm for 1 min. The procedure was repeated twice with 2-min interval. Droplet size distribution was measured by light scattering using a Mastersizer 2000 with a Hydro 2000MU as dispersion unit (Malvern Instruments Ltd, UK). The pump speed was settled at 1,800 rpm. Refraction index was 1.4694. In all cases, emulsions had a monomodal distribution with a median particle size varying from 5 to 7 μm. According to particle size measurements, emulsions were stable for at least 24 h since during this period no changes were found in droplet size distribution.

Measurement of SFC by NMR

The SFC was measured by pNMR with a Bruker mq 20 minispec analyzer (Bruker, Rheinstetten, Germany) using a cell with temperature control. The crystallization process of all samples was studied by measuring SFC as a function of time. Bulk fat sample (4 mL) was placed in NMR tubes and heated at 80 °C for 30 min to destroy any crystal memory. Then, they were kept at 60 °C for 30 min. Emulsions were kept at 60 °C during preparation. After that, bulk fats and emulsions were immediately plunged into a water bath (temperature control 0.1 °C) set at crystallization temperature. As a first approach to select the optimal temperatures to perform crystallization studies, bulk fat were crystallized in a wide range of temperatures from 10 to 33 °C with intervals of 0.5 °C. Temperatures range for emulsions varied from 10 to 24 °C when the fat phase was HMF and from 10 to 22 °C for the blends. During these preliminary scans, it was evident that, depending on supercooling, P-170 has two different effects on crystallization in bulk systems: acceleration or delay.

According to these results, two temperatures close to the one at which the behavior changed were selected to show P-170 effects: one above and the other below it. For bulk HMF, selected crystallization temperatures were 30 and 28 °C, while for emulsions, they were 24 and 22 °C. For the two blends of SFO-in-HMF, selected temperatures were 28 and 26 °C for bulk fat and 22 and 20 °C for emulsions. SFC was measured at time intervals until the crystallization curves reached a plateau. Samples were run in triplicate and the values were averaged. The Student *t* test was used to determine the significance for a particular difference in means.

Study of Polymorphism

The synchrotron X-ray scattering (SAXS) measurements were made at the DO2A-SAXS2 beamline of the Synchrotron National Laboratory (LNLS, Campinas, Brazil) with a 1.488 Å wavelength. The scattering intensity distributions as a function of scattering angle (2θ) were obtained in the 2θ range between 0.1° and 4°. SAXS patterns were recorded as a function of time for at least 30 min with frames of 30 s. One-dimensional curves were obtained by integration of the 2D data using the program FIT-2D. For SAXS experiments in bulk, 15 mg of fat samples was placed in a hermetical aluminum pan with a transparent circle of 4 mm of diameter, in both base and lid. Then, the pan was placed in a cell with temperature control. A MARCCD 2D detector was used with 784.3-mm sample detector distance. For emulsions systems, the liquid sample at 60 °C was mounted on the X-ray diffraction glass plate, placed in a horizontal position, and cooled by using a temperature-controlled sample holder. Sample detector distance was 1186.5 mm. Samples were submitted to the same temperature-time steps as for NMR studies, that is, were kept at 60 °C for 30 min and then were placed in the X- ray cell which temperature was set at crystallization temperature. Zero time was the moment at which sample reached crystallization temperature. Assignment of the subcell packing (α, β′, or β polymorphs) was done on the basis of information from the literature[15,17,18]. In addition to short spacing signals, each polymorphic form showed characteristic long spacing signals. SAXS signals were more intense than wide angle X-ray diffraction (WAXD) peaks. As SFC in the early steps of crystallization was very low, we selected SAXS experiments to describe the effects of P-170 since in those experiments sensitivity was very high. However, in the cases that WAXD experiments were sensitive enough, they confirmed SAXS results. The area under the SAXS peak was integrated using commercial software. The diffraction profiles were fitted to a Gaussian equation and the normalized integrated intensity was plotted as a function of time.

Results and Discussion

Isothermal Crystallization by NMR in Bulk

According to the SFC with time experiments performed at different temperatures, addition of P-170 had different effects on isothermal crystallization depending on crystallization temperature. For the three systems, two temperatures, one above and the other below the temperature at which the effect changed, were selected as an example of each behavior. Figure 1 reports the increase in SFC with time for the samples crystallized in bulk, both with and without addition of P-170. According to the NMR measurements at different

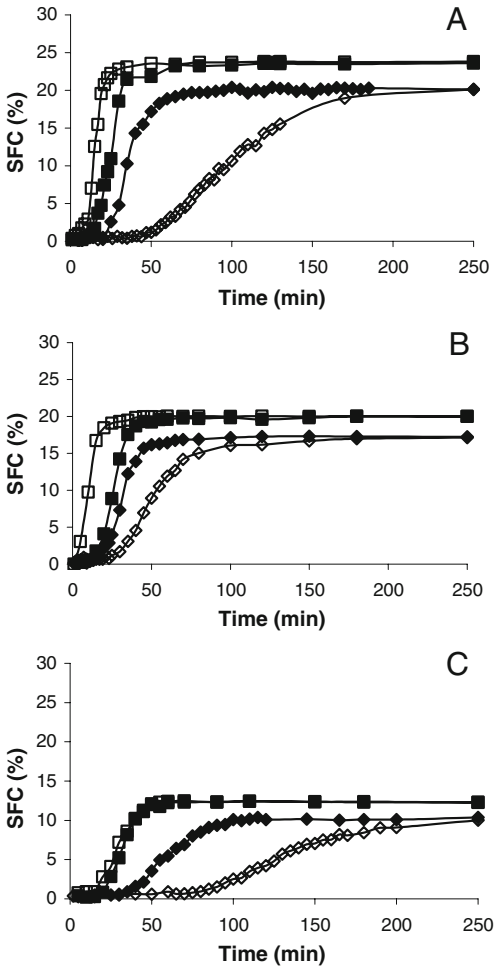

Fig. 1 SFC (%) vs time for bulk systems: **a** HMF isothermally crystallized at 30 °C, without (*filled diamonds*) and with addition of P-170 (*empty diamonds*), and at 28 °C, without (*filled squares*) and with addition of P-170 (*empty squares*). **b** A 80% HMF/20% SFO mixture isothermally crystallized at 28 °C, without (*filled diamonds*) and with addition of P-170 (*empty diamonds*), and at 26 °C, without (*filled squares*) and with addition of P-170 (*empty squares*). **c** A 60% HMF/40% SFO mixture isothermally crystallized at 28 °C, without (*filled diamonds*) and with addition of P-170 (*empty diamonds*), and at 26 °C, without (*filled squares*) and with addition of P-170 (*empty squares*). Error bars were within the symbols' size

temperatures, for HMF, P-170 retarded crystallization at temperatures above 29.0±0.2 °C, while below that temperature, it accelerated crystallization. The same behavior as for HMF was found for the blend of HMF with 20% SFO but in this case the temperature at which the behavior changed was 27.0±0.2 °C. For the 40% SFO-in-HMF blend, there was a slightly significant acceleration or no significant effect on crystallization below 26.0±0.2 °C. Above this temperature, P-170 significantly delayed crystallization ($p<0.05$). The resulting effect, that is, inhibition or acceleration, strongly depended on supercooling (defined as the difference between melting temperature base on MDP and crystallization temperature, $\Delta T = T_m - T_c$). For the three samples, a supercooling lower than 11.0 °C led to inhibition of crystallization, that is, crystallization started later. The temperatures selected in Figure 1 were close to the temperatures at which the effect changed. The delay caused by P-170 increased notoriously with temperature. For HMF, for example, the start of crystallization was delayed 10, 30, 50, and 80 min for 29.5, 30, 30.5, and 31 °C, respectively. At a temperature 4 °C higher than 29 °C, crystallization was delayed for several hours. We have previously reported this behavior in similar systems[13,14].

Isothermal Crystallization by NMR in Emulsion

Figure 2 reports the increase in SFC with time for the emulsions with and without P-170 dissolved in the fat phase. A higher supercooling was always necessary for crystallizing the fat phase in emulsions than in bulk systems[19,20]. For HMF, no solids were detected at temperatures of 28 °C or higher. There was no temperature at which the effect changed in emulsion systems. For all samples and at all temperatures selected, addition of P-170 always accelerated crystallization even when the system had a very low SFC.

In previous studies, we have dealt with similar fat systems in bulk[13,14]. The temperatures selected were very close to the melting point since the aims of those works were to describe nucleation effects at temperatures at which there were measurable induction times. Like many other authors, we did not described both effects for the same fat system in bulk since all temperatures selected were above the one at which the effect changed. Moreover, as we did not study these low *trans* fat in emulsion systems, we were unable to notice acceleration effects. In those studies, we only found the β′ polymorphic form, but they were not performed in real time. With the traditional X-ray technique, it was necessary at least 20 min to obtain a spectrum. Thus, the early stage of crystallization was missed.

It was reported that addition of sucrose esters accelerated crystallization in emulsions formulated with palm kernel oil or palm mid fraction[9–11]. However, this behavior was not found in those fats crystallized in bulk. When highly hydrophobic sucrose esters were added to palm kernel oil or palm, mid fraction crystallization was always retarded. Although the mechanism provided for acceleration of crystallization explained the effect of sucrose esters in emulsions, it did not explain the different behavior found for bulk and emulsion systems. With the aim of understanding the nature of the different effects, the isothermal crystallization kinetics both in bulk and in emulsions systems was studied by time-resolved in situ SAXS.

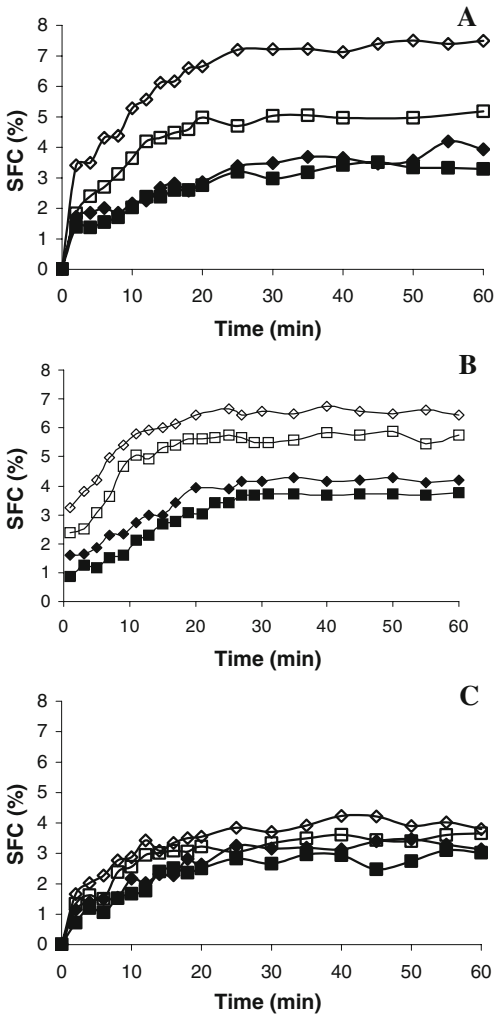

Fig. 2 Solid fat content (%) vs time for emulsions systems: **a** high-melting fraction of milk fat (HMF) isothermally crystallized at 22 °C, without (*filled diamonds*) and with addition of P-170 (*empty diamonds*), and at 24 °C, without (*filled squares*) and with addition of P-170 (*empty squares*). **b** A 80%HMF/20% SFO (sunflower oil) mixture isothermally crystallized at 20 °C, without (*filled diamonds*) and with addition of P-170 (*empty diamonds*), and at 22 °C, without (*filled squares*) and with addition of P-170 (*empty squares*). **c** A 60% HMF/40% SFO (sunflower oil) mixture isothermally crystallized at 20 °C, without (*filled diamonds*) and with addition of P-170 (*empty diamonds*), and at 22 °C, without (*filled squares*) and with addition of P-170 (*empty squares*)

Early Crystallization Studies by SAXS in Bulk

Figure 3 reports SAXS patterns of HMF crystallized isothermally in bulk with and without addition of P-170 at 28 and 30 °C. After 1 min at 30 °C (a), the SAXS spectrum of HMF displayed a single peak in the 0.1° to 3.5° region (2θ) with a *d* of 47.14 Å. According to the literature[15,17,18], these X-ray diffraction patterns are consistent with a bilayer ($2L_{001}$) lamellar packing arrangement and a hexagonal subcell or α polymorph. The intensity of the diffraction pattern of HMF increased until 4 min of isothermal crystallization. After this time, a second SAXS peak began to grow, while the initial diffraction peak began to decrease in intensity. The second diffraction peak with a *d* of 40.60 Å corresponded to the $2L_{001}$ packing of the orthorhombic subcell (β′ polymorph) [15,17,18]. After 5 min at 30 °C, the α peak disappeared. The α and β′ peaks coexisted for 1 min. When P-170 was added to HMF at 30 °C (b), the α and β′ forms coexisted for only 30 s. According to NMR measurements, P-170 delayed crystallization at this temperature. When HMF was crystallized isothermally at 28 °C (c), the α form appeared after 30 s at crystallization temperature. β′ form peak was noticeable after 3.5 min. Both forms, α and β′, coexisted for 30 s. Addition of P-170 (d) expanded the time of coexistence of the α and β′ forms for 1.5 min. NMR measurements showed that P-170 accelerated crystallization at temperatures below 29 °C. Surprisingly, the intensities of diffraction patterns of HMF with addition of P-170, both for delay or acceleration effect, were lower than the ones for the corresponding HMF patterns during the whole run. SAXS intensities showed the crystallinity of solid formed. This indicated that P-170 interacted with HMF disturbing the long-term order.

Figure 4 shows SAXS patterns of 40% SFO-in-HMF crystallized isothermally in bulk with and without addition of P-170 at 26 and 28 °C. Addition of SFO modified the crystallization behavior of HMF. At 28 °C (a), the α form was not detectable. The β′ signal appeared after 1 min of isothermal crystallization at 28 °C and was the only polymorphic form found even until 45 min. When P-170 was added to the 40% SFO-in-HMF blend (b), the α form appeared after 30 s at crystallization temperature. P-170 favored the crystallization in the α form and delayed the formation of the β′ polymorph. A second signal corresponding to the β′ form was found after 2.5 min. Both forms, α and β′, coexisted for 30 s. According to NMR measurements, P-170 delayed crystallization at this temperature. At 26 °C (c), the α form appeared after 30 s. A second signal corresponding to the β′ form was noticeable 2.5 min at crystallization temperature. After 3 min at 26 °C, the signal of the α form was not noticeable, indicating that polymorphic transformation from α to β′ form was complete. Both forms, α and β′, coexisted for 30 s. When P-170 was added to the 40% SFO-in-HMF blend (d), the α form appeared after 30 s at crystallization temperature. A

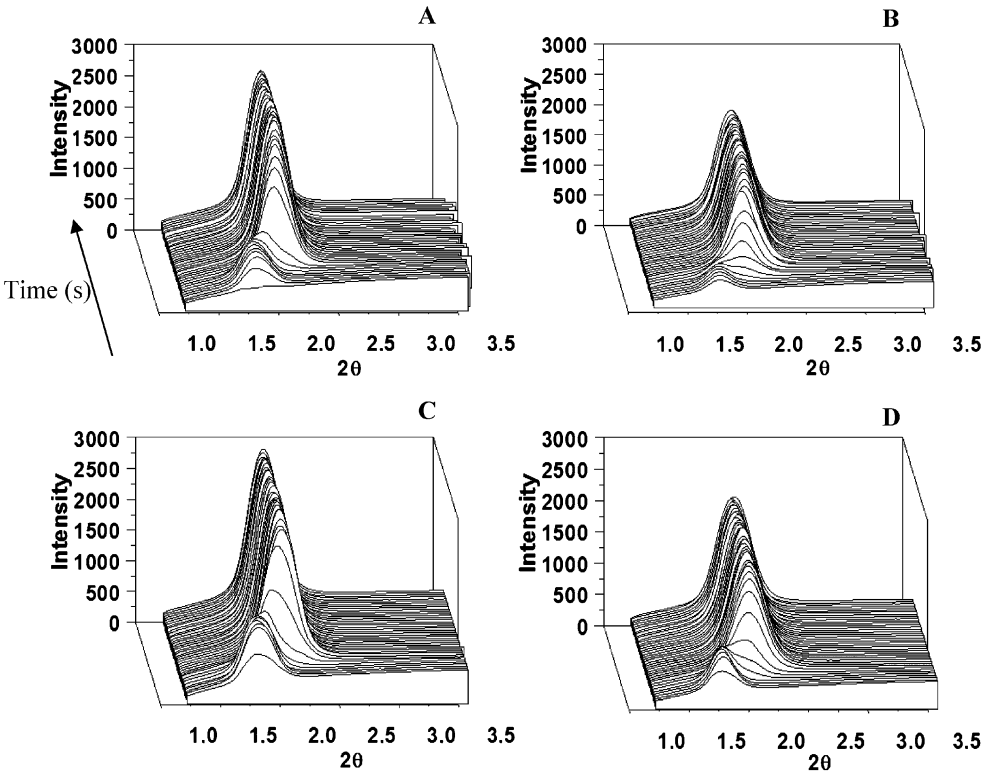

Fig. 3 Three-dimensional plots of SAXS vs the diffraction angle (2θ) recorded as a function of time for bulk high-melting fraction of milk fat (HMF) isothermally crystallized at 30 °C, **a** without and **b** with addition of P-170, and at 28 °C, **c** without and **d** with addition of P-170

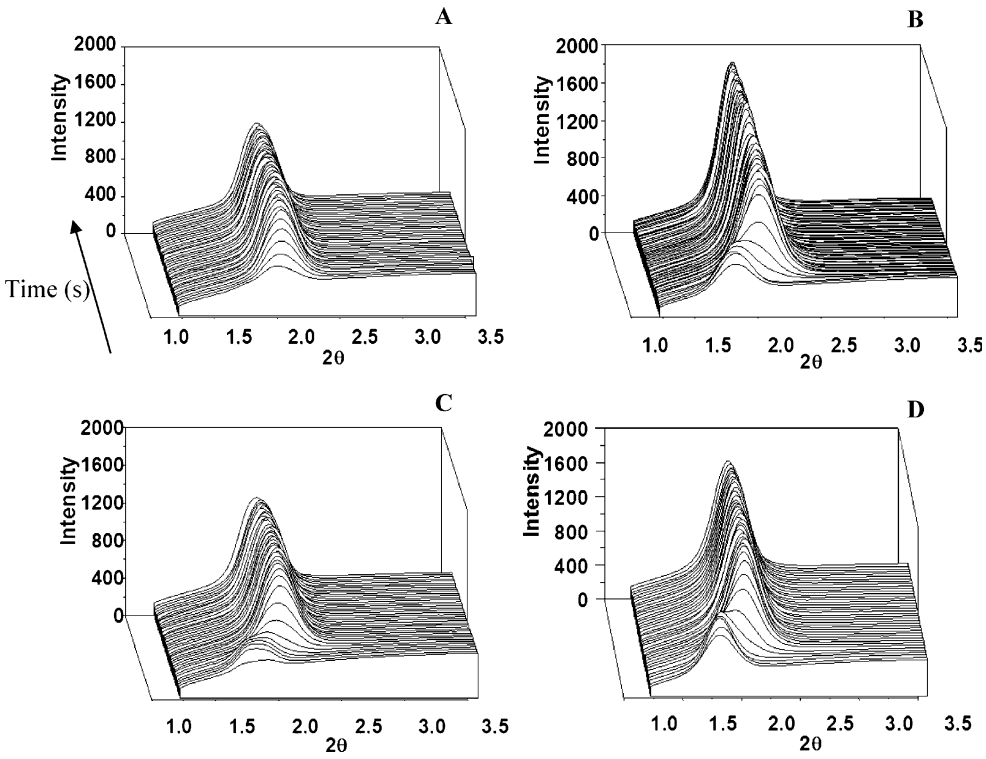

Fig. 4 Three-dimensional plots of SAXS vs the diffraction angle (2θ) recorded as a function of time for bulk 60%HMF/40% SFO mixture isothermally crystallized at 28 °C, **a** without and **b** with addition of P-170, and at 26 °C, **c** without and **d** with addition of P-170

second signal corresponding to the β′ form occurred after 2 min. P-170 accelerated the appearance of the β′ form. Both forms, α and β′, coexisted for 1 min. After 3 min only, the β′ signal was present in the X-ray diffraction pattern. NMR measurements showed that P-170 accelerated crystallization at temperatures below 26 °C if it is likely to have this effect.

The 20% and 40% SFO-in-HMF blends showed similar behaviors at both crystallization temperatures (the 20% SFO-in-HMF spectra are not shown). For HMF/SFO blends, the intensities of the diffraction patterns of the samples with addition of P-170, both for delay or acceleration effect, were notoriously higher than the ones found for the corresponding HMF/SFO patterns during the whole run. The blends had a different chemical composition than HMF, and therefore, the interactions among triglycerides and P-170 were different. In this case, P-170 enhanced crystallinity.

For the three samples, when the effect was acceleration of crystallization, P-170 expanded the time interval during which the α and β′ forms coexisted. In the case of HMF/SFO blends crystallized at 28 °C (effect of retardation of crystallization), the control samples (without emulsifier) showed one signal corresponding to the β′ signal. Thus, the α form was only noticeable with addition of P-170. In addition, α and β′ forms coexisted for shorter times than at lower temperatures.

It would be expected that P-170 always accelerate crystallization of bulk systems because it has higher melting point than HMF or the blends with SFO, which means that, upon cooling, the emulsifiers would crystallize before the oil. These emulsifiers would then have the ability to perform the same roles as catalytic impurities. However, this effect was not found in several fats such as palm mid fraction or palm kernel oil[9–11]. The effect of P-170 proved to be more specific than an impurity effect. The results shown in Figures 3 and 4 clearly indicate that the polymorphic behavior of HMF or its blends with SFO was modified by the addition of P-170. When dissolved in SFO, P-170 crystallizes in the α form in the conditions selected in this study. No other polymorphic forms were present after 1 h at crystallization temperature. However, when added to these samples, the signal of the α form, if present, disappeared after a short time of isothermal crystallization. This seems to indicate that P-170 is incorporated in HMF or blends crystals. It was reported that sucrose esters tend to form molecular aggregates such as inverse micelles even at low concentration[21,22], which may act as template accelerating crystallization of fats[9]. This mechanism occurred with high supercooling when P-170 elongated the time of coexistence of the α and β′ forms of the fat. Under high supercooling, the fat crystallizes in the α form[15], as is the case of P-170. This phenomenon explains the acceleratory effect of P-170, which can act as seed for fat crystallization in the α form. For temperatures close to the melting point, the effect of P-170 on crystallization was the one reported for palm mid fraction and palm kernel oil, a delay

of crystallization. Fats and sucrose esters are able to cocrystallize because of their somewhat similar chemical structure. As at high temperature the α form was either not present or the intensity of the α-form diffraction line was weak, P-170 was not able to accelerate crystallization and was incorporated in β′ fat crystals. However, the structural dissimilarities between triacylglycerols and emulsifiers caused delayed crystallization.

Time-resolved in situ synchrotron X-ray scattering is a more sensitive technique than NMR to monitor isothermal crystallization. Zero time in Figures 1 and 2 is the moment at which the samples in the NMR tubes reached crystallization temperature. The first pattern in X-ray figures corresponded to 30 s at crystallization temperature. The NMR registers crystal appearance when SFC starts increasing. Typical detectable levels of SFC are 0.5% to 1.0%. An SFC of 0.1%, as found in several samples in the first 5 min of crystallization, is below the detection threshold of a pNMR machine. However, X-ray patterns clearly showed early crystallization. Since the effects of P-170 were determined for the events that took place in those early steps of crystallization, they could only be understood by synchrotron X-ray scattering. Conventional X-ray techniques do not allow these studies since they are not performed in real time.

Early Crystallization Studies by SAXS in Emulsion

Figure 5 shows SAXS patterns of emulsions formulated with HMF or the 40% SFO-in-HMF blend with and without P-170. When HMF was crystallized at 24 °C (a), the α form appeared after 30 s at crystallization temperatures. The signal of β′ form was not noticeable until 5.5 min at 24 °C. After 10 min, the intensity of the α form started to diminish until 12.5 min of isothermal crystallization when the polymorphic transformation was complete. Addition of P-170 (b) accelerated 1 min the appearance of the β′ signal and extended the coexistence of α and β′ forms until 20 min at 24 °C. The emulsion formulated with the 40% SFO-in-HMF blend showed a similar behavior. The α and β′ forms coexisted for longer time than in bulk crystallization. The α signal was noticeable after 1 min at 22 °C (c). A second signal corresponding to the β′ form was present at 7.5 min. Then, the α form suffered a polymorphic transformation and disappeared after 23 min at crystallization temperature. When P-170 was dissolved in the fat phase (d), the signal corresponding to the α form appeared after 30 s at crystallization temperature. A second diffraction peak with a *d* of 40.60 Å was present at 4.5 min. Then, after 24 min at 22 °C, the signal of the α form disappeared. In all emulsions, addition of P-170 increases the time of coexistence of the α and β′ forms. In addition, the intensity of the β′ form increases as well. This behavior is opposite to the one found for bulk systems. P-170 effect on crystallinity was complex, and the fact that it is different in bulk than in emulsion suggested that there are further reasons in addition to molecular interactions for this behavior. The compartmentalization might play also an important role.

In an emulsion system, the dispersed phase is divided into a number of droplets that vary in size (polydisperse). Catalytic impurities are therefore distributed unequally through some of the droplets. This leads to isolation of the impurities that catalyze heterogeneous nucleation in the bulk system, and in this manner, the value of supercooling needed for crystallization increased[12]. As may be noticed from Figure 2, temperatures for crystallization in emulsions are lower than the ones selected for bulk systems since for emulsions no crystallization was detected at 28 or 30 °C. For nucleation to be initiated, nuclei must reach a critical radius (size). As was reported, this radius is a function of temperature. The lower the temperature, the smaller the critical radius[23]. Therefore, crystallization is favored by lower temperatures, that is, higher supercoolings. As emulsions crystallized with high supercooling, α and β′ forms coexisted for longer times than in bulk crystallization (Figure 5). As what happened in bulk systems, in this situation, the effect of P-170 was acceleration.

Integrated Intensity for Bulk and Emulsion Systems

Figure 6 shows the area under the SAXS peak, called the integrated intensity, vs time for bulk and emulsion systems. The effect of P-170 in bulk depended on the chemical composition of the system. For HMF, the integrated intensity vs time for both, α and β′ forms, decreased with addition of P-170 (a), while for the blends (b and c), it significantly increased. For both blends crystallized at 28 °C, P-170 promoted the formation of the α form, which was not noticeable for the control samples. In emulsion systems (d), the integrated intensity vs time always increased for addition of P-170. It is clear that the time of coexistence of α and β′ forms is longer in this case than for crystallization in bulk.

Conclusion

By analyzing polymorphism in real time, with the aid of synchrotron X-ray scattering, it was possible to establish that the different effects caused by P-170 during isothermal crystallization of HMF or its blends with SFO, as described by SFC curves, were strongly related to the effects of P-170 on fat polymorphism, specially to the value of the time interval of coexistence of the α and β′ forms.

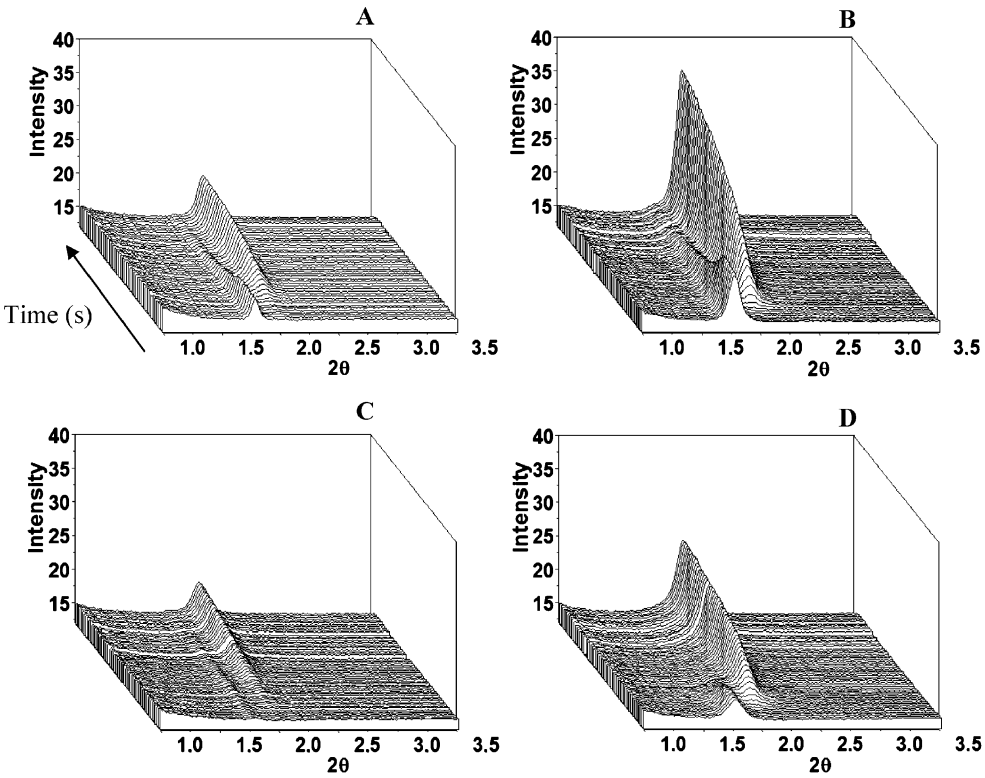

Fig. 5 Three-dimensional plots of SAXS vs the diffraction angle (2θ) recorded as a function of time for emulsion systems. High-melting fraction of milk fat (HMF) isothermally crystallized at 24 °C, **a** without and **b** with addition of P-170. 60% HMF/40% SFO (sunflower oil) mixture isothermally crystallized at 22 °C, **c** without and **d** with addition of P-170

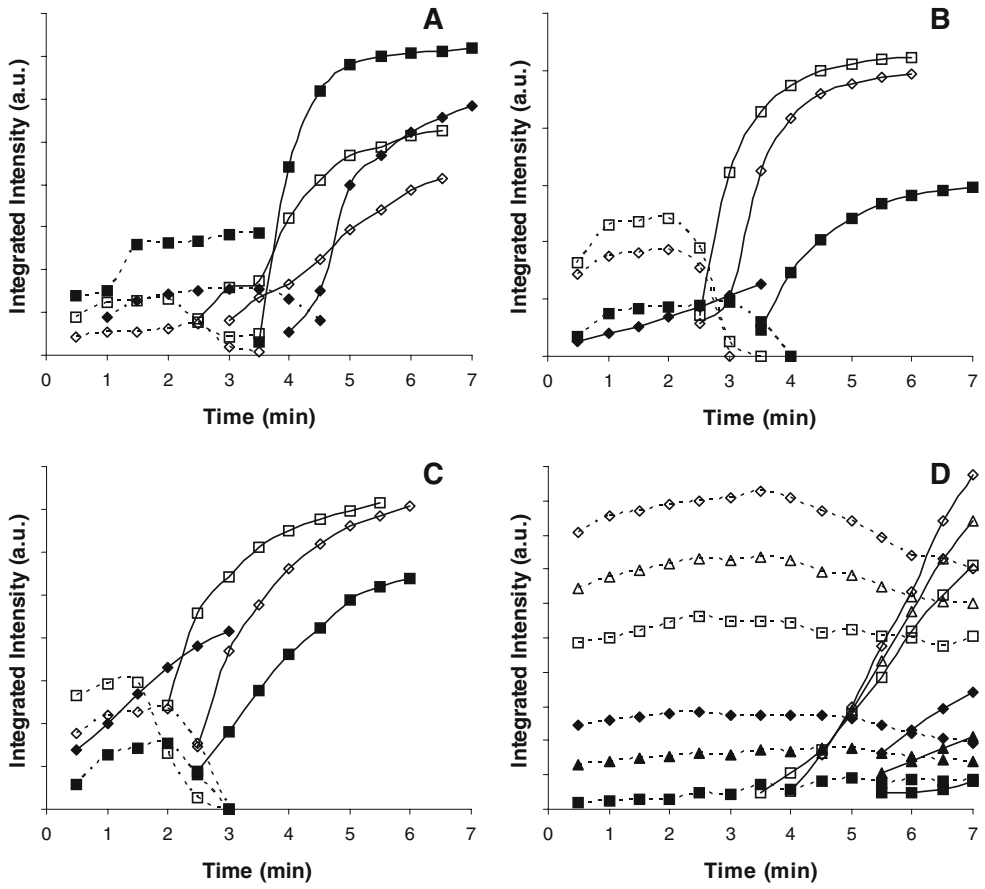

Fig. 6 Normalized integrated intensity (in arbitrary units) of the intensity peaks of the α (*dashed lines*) and β′ (*solid lines*) polymorphs for the controls (*solid symbols*) and with addition of P-170 (*open symbols*). Bulk systems: **a** HMF isothermally crystallized at 30 °C (*rhombuses*) and 28 °C (*squares*); **b** a 80%HMF/20% SFO mixture isothermally crystallized at 28 °C (*rhombuses*) and 26 °C (*squares*); **c** a 60% HMF/40%SFO mixture isothermally crystallized at 28 °C (*rhombuses*) and 26 °C (*squares*). Emulsion systems: **d** HMF (*rhombuses*), 80%HMF/20% SFO mixture (*triangles*), 60%HMF/40%SFO mixture (*squares*)

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