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In situ synchrotron radiation X-ray scattering study on the effect of a stearic sucrose ester on polymorphic behavior of a new sunflower oil variety

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ABSTRACT

The effect of the stearic sucrose ester (SE) S-170 on crystallization behavior and polymorphism of two stearins obtained from a new variety of high stearic high oleic sunflower oil was studied by pulsed nuclear magnetic resonance (p-NMR), small (SAXS) and wide (WAXS) angle X-ray scattering using synchrotron light and differential scanning calorimetry (DSC). p-NMR studies showed that there is always a crystallization temperature below which SE S-170 accelerated crystallization and above which SE S-170 delayed nucleation and growth. The effect of SE S-170 strongly depended on supercooling. It was efficient as a seed for high supercooling (low crystallization temperatures) but this efficiency diminished at low supercooling (temperatures close to the melting point) when few crystals were formed. SAXS and WAXS results demonstrated that depending on crystallization temperature SE S-170 promoted crystallization of α and β forms with more polymorphic similarity and inhibited occurrence of β′ forms especially the β'_2 polymorph. However, in some conditions SE S-170 favored crystallization of β'_1 polymorph. DSC experiments showed that SE S-170 significantly diminished total melting enthalpies when the effect was a delay in crystallization. For other conditions no significant differences were found in melting temperatures or total melting enthalpies. When stearins were stored at 25 °C, crystallization in the β_2 form was promoted. Depending on crystallization temperature, polymorphic forms β'_1 and β_2 may be obtained as the main polymorphic forms. This is very relevant from the technological point of view. Depending on the application, SE S-170 may help obtain the required polymorphic form: β'_1 form for spreads and β_2 polymorph for chocolate production.

1. Introduction

Composition, tempering regime, the presence of other lipids or additives, and mechanical treatment (e.g., shear, agitation) influence how a lipid solidifies from the melt. In the food industry, these parameters are all used to direct polymorphic behavior and morphological development in fats. Polymorphism of fats is a fundamental property that determines food products appearance. For example, in margarine production, maintaining β′-crystallinity is imperative to preserve smooth texture and acceptable spreadability. In chocolate manufacturing, careful tempering is used to promote the crystallization of the metastable β-V form of cocoa butter, responsible for much of chocolate's organoleptic and shelf life properties (Braipson-Danthine & Deroanne, 2004; Loisel, Lecq, Keller, & Ollivon, 1998; Narine & Marangoni, 1999). Small angle X-ray scattering (SAXS) is a powerful tool to study polymorphism. A synchrotron source allows in situ characterization of phase formation in a sample holder and the competition between the different polymorphic species to be followed quantitatively (Loisel, Keller, Lecq, Bourgaux, & Ollivon, 1998; Lopez, Lavigne, Lesieur, Bourgaux, & Ollivon, 2001; Lopez, Lesieur, Bourgaux, Keller, & Ollivon, 2001; Lopez, Lesieur, Bourgaux, & Ollivon, 2005; Lopez et al., 2002; Mazzanti, Guthrie, Sirota, Marangoni, & Idziak, 2003; Peschar et al., 2004). In addition, as a pattern is taken in 10 s, the structural dynamics of sunflower stearins in the early stage of crystallization can be described. This early stage of crystallization is very important since it determines the later evolution of the system (Cisneros, Mazzanti, Campos, & Marangoni, 2006).

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FDA rule about reporting *trans* fats in food labels that was effective on January 1st, 2006 and *trans* fat rules issued in many other countries encouraged food manufacturers to reformulate their products. The modification of fatty acid composition through plant breeding was one of the strategies developed to replace *trans* fats (Kodali, 2005). A new high stearic high oleic (HSHO) sunflower oil variety was developed recently and although it does not contain enough solids at ambient temperature it may be fractionated resulting in hard fractions called stearins. HSHO sunflower oil-stearins' polymorphic behavior during isothermal crystallization was recently described (Rincón-Cardona, Martini, Candal, & Herrera, 2013). Five different polymorphic forms were characterized and the polymorphic transformations that occurred at different temperatures were quantified using small angle X-ray scattering with synchrotron radiation (Rincón-Cardona et al., 2013).

The growing interest on sugar ester surfactants is due to their enhanced performance and environmental compatibility in comparison with petrochemically derived products. Sugar ester surfactants may be obtained from renewable raw materials such as fatty acids and sucrose; they are readily biodegraded in an aqueous environment, and have the potential to be non-toxic and non-allergenic. Hence, they are used in the fields of cosmetics and food additives for a variety of functions, including emulsifying and foaming, in various products such as bread, ice cream, margarine, and fat substitutes (Garti, Aserin, & Fanum, 2000). The effect of some food emulsifiers on the crystal structure of fats crystallized from the melt has been known for many years. Several authors have used in situ X-ray techniques to follow the effects of sucrose esters (SE) and other emulsifiers on fats crystallization in bulk and in emulsion systems (Arima, Ueji, Ueno, Ogawa, & Sato, 2007; Arima, Ueno, Ogawa, & Sato, 2009; Awad & Sato, 2002; Huck-Iriart, Candal, & Herrera, 2009; Kalnin, Schafer, Amenitsch, & Ollivon, 2004; Sakamoto et al., 2004; Sonoda, Takata, Ueno, & Sato, 2006), to name a few. A detailed review of the submicron effects that these minor components have on nucleation, crystal growth, morphology, heat capacity and polymorphic stability have been reported by Smith, Bhaggan, Talbot, and van Malssen (2011). According to these authors, the majority of research on those effects is of an empirical and descriptive nature. In a limited number of studies, underlying mechanisms are proposed, but they are not always properly supported by the experimental results. The aim of the present work is to describe the effect of a stearic sucrose ester, S-170, on polymorphic and crystallization behaviors of HSHO sunflower oil stearins. Isothermal crystallization behavior changes were followed in real time by using SAXS and wide angle X-ray scattering (WAXS), employing a synchrotron source. Nuclear magnetic resonance (NMR) and differential scanning calorimetry (DSC) were also used to document S-170 effects on crystallization.

2. Materials and methods

2.1. Starting materials

Two commercial HSHO sunflower oil stearins from Mar del Plata, Buenos Aires, Argentina were used in this study. Stearins were obtained as previously described (Rincón-Cardona et al., 2013). Briefly, a soft stearin (SS) was obtained through dry fractionation of the sunflower oil while a hard stearin (HS) was obtained using a solvent fractionation of the SS. The fatty acid and triacylglycerol compositions of both stearins were previously reported (Rincón-Cardona et al., 2013). The melting point of SS and HS were determined using AOCS Method Cc 1-25 (1993). SS and HS samples had melting points of 30.6 ± 0.1 and 35.7 ± 0.1 °C, respectively. Stearic sucrose ester, SE S-170, with HLB of 1 was a commercial blend of esters supplied by Mitsubishi-Kasei Food Corporation (Tokyo, Japan). It had a MDP of 59.5 °C. The monoester content of S-170 was 1 wt.%, with di-, tri-, and polyesters constituting 99 wt.%. S-170 was added at a concentration of 1 wt.%. The selected concentration was within the range commonly used in foods.

2.2. Crystallization procedure

Samples were melted at 60 °C in an oven and kept at this temperature for at least 30 min. Then, they were transferred to appropriate sample holders (NMR tubes for solid fat content [SFC] determination). For DSC, SAXS and WAXS studies, samples were melted at 60 °C in the sample holder and kept at that temperature for 2 min and then cooled to crystallization temperature (T_c) at 10 °C/min. Both stearins were crystallized isothermally at T_c. For HS selected temperatures were 10, 21, 22, 23, 24, and 25 °C. SAXS patterns were recorded as a function of time for 50, 70, 70, 80, 80 and 100 min, respectively. SS was crystallized to 5, 15, 16, 17, 18.5, and 19 °C for 50, 40, 40, 70, 120, and 115 min, respectively. Polymorphic transitions and solid fat content (SFC) of the samples were measured as a function of crystallization time, where zero time corresponds to the moment when the samples reached the crystallization temperature. By using this procedure it can be assumed that crystallization occurred mostly isothermally, since for samples without emulsifier (control samples) no signal indicating the presence of solid material appeared in the first X-ray pattern ($t = 0$) in all cases. Selection of temperatures was based on the melting points reported for the crystalline phases of pure TAG present in these samples. In all cases, X-ray analyses were performed long enough to crystallize at least 70% of the equilibrium solid fat material (SFC) at a defined temperature as measured by nuclear magnetic resonance (NMR). It was evident from patterns that intensities did not change significantly at those times. For NMR analyses crystallization was followed for 120 min. HS and SS were also stored for 48 h at T_c. The polymorphism of stearins was analyzed at this time.

2.3. SFC by NMR

The actual SFC of SS and HS was measured by using a Bruker mq20 minispec analyzer (Bruker, Rheinstetten, Germany) equipped with a cell with temperature control. SFC with time was determined at the same temperatures used for X-ray studies. Duplicate runs were performed on consecutive days for each set of samples and three tubes were measured in each run. SFC values were expressed as an average of six data and standard deviations were reported as well.

2.4. SAXS and WAXS studies

The synchrotron X-ray scattering measurements (small and wide angle, SAXS and WAXS, respectively) were made at the SAXS1 beamline of the Synchrotron National Laboratory (LNLS, Campinas, Brazil) with a 1.55 Å wavelength. The scattering intensity distributions as a function of scattering angle (2θ) were obtained in the 2θ range between 0.88° and 27.68°. One pattern per min was acquired in all experiments. One-dimensional curves were obtained by integration of the 2D data using the program FIT-2D. For SAXS experiments, 15 mg of fat samples was placed in a hermetical aluminum DSC pan with a transparent circle of 4 mm of diameter, in both base and lid. Then, the pan was placed in a DCS cell and the sample was crystallized as indicated in Section 2.2. Sample to detector distance was 235.28 mm. Assignment of the subcell packing (α, β′, or β polymorphs) was done on the basis of information from the literature (Cisneros et al., 2006; Lopez, Lavigne, et al., 2001; Lopez, Lesieur, et al., 2001; Mazzanti et al., 2003; Rincón-Cardona et al., 2013). In addition to short spacing signals, each polymorphic form showed characteristic long spacing signals. The area under the SAXS peak (usually called normalized integrated intensity) was integrated using commercial software (OriginPro 8 SR0 v 8.0724, Origin Lab Corporation, Northampton, USA). The diffraction profiles were fitted to a Gaussian equation and the normalized integrated intensity was plotted as a function of time. Induction times for crystallization were also measured from SAXS and WAXS patterns as the interval between the moment crystallization temperature (T_c) was reached and

the start of crystallization (first pattern in which signals were detectable).

2.5. Thermal behavior by DSC

The thermal behavior of the samples was studied with a differential scanning calorimeter, DDSC Mettler Toledo model 822[e] (Mettler Toledo, Schwerzenbach, Switzerland) with a thermal analysis software Mettler Star[e]. Samples were analyzed following the same temperature/time program as used for X-ray experiments. Samples were weighed (15–18 mg) into sealed aluminum pans and after the thermal treatments described in Section 2.2 they were melted from crystallization temperature to 80 °C at 5 °C/min. The calorimeter was calibrated with indium and mercury prior to analysis. Melting peak and onset temperatures were calculated from the DSC profiles. Enthalpies were calculated as the area under the melting curve from the beginning to the end of the melting process. Values reported were the average of two replicates.

3. Results and discussion

3.1. SFC with time

Fig. 1 shows the effect of S-170 on SFC with time for different temperatures. For both, SS (a) and HS (b), SE S-170 accelerated or delayed crystallization depending on crystallization temperature. At lower temperatures, the effect was acceleration of nucleation while at higher temperatures it was delay of crystallization. For soft stearin (a), SE S-170 accelerated crystallization below 17 °C and retarded crystal formation above this temperature. In the case of acceleration, the SFC reached a plateau value that showed no significant differences for SS with or without the addition of SE S-170, indicating that the total amount of solids in a sample was determined by crystallization temperature and supercooling (defined as the difference between melting temperature based on capillary tube method and crystallization temperature, $\Delta T = T_m - T_c$) and was not modified by the presence of the SE S-170. For hard stearin (b), the acceleration effect occurred below 23 °C and the delay of crystallization took place above this temperature. The delay was very noticeable for 25 °C. At this temperature, addition of S-170 inhibited crystallization for 100 min and even after 120 min the amount of solids was very low compared to the SFC measured for HS without the additive. A similar behavior was observed for SS crystallized at 18.5 °C.

Most of the studies in literature about the effect of sucrose esters on nucleation behavior reported a delay in the crystallization of bulk lipids and acceleration in crystallization in emulsions (Smith et al., 2011). The temperatures selected for these studies were within a range in which there was a measurable induction time for crystallization. In those conditions of low supercooling, sucrose esters inhibited nucleation in a degree related to their melting point and molecular structure. However, Huck-Iriart et al. (2009) found that the addition of a palmitic SE (P-170) to blends of high melting fraction of milk fat and sunflower oil isothermally crystallized in bulk phase resulted in an acceleration or delay of crystallization and that the effect was strongly dependent on supercooling. In agreement with those findings, the effect of S-170 on sunflower oil stearins nucleation was affected by supercooling showing either acceleration or delay in crystallization. For SS a supercooling greater than 12.7 °C is needed for SE S-170 to accelerate crystallization, while in the case of HS, acceleration occurred with supercoolings greater than 13.6 °C (SFC studies were performed each 0.1 °C between 16.5 and 17.5 °C for SS and 22.5 and 23.5 °C for HS).

3.2. Polymorphism of SE S-170

SE S-170 was analyzed under the same temperature/time conditions as SS and HS. Fig. 2 shows the SAXS (a) and WAXS (b) patterns obtained at 25 °C. SE S-170 pattern showed a signal at 1.18 nm^{-1} in the SAXS region and a signal at 15.20 nm^{-1} in the WAXS region. These signals correspond to distances (d) of 5.32 and 0.41 nm, respectively, indicating

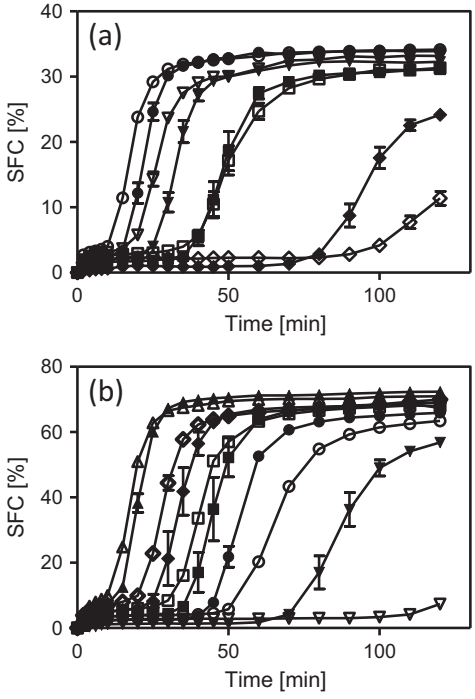

Fig. 1. SFC (%) vs time for sunflower oil stearins: (a) SS isothermally crystallized at different temperatures, without (filled symbols) and with addition of S-170 (empty symbols). Circle: 15 °C, triangle: 16 °C, square: 17 °C, and diamond: 18.5 °C. (b) HS isothermally crystallized at different temperatures, without (filled symbols) and with addition of S-170 (empty symbols). Up pointing triangle: 21 °C, diamond: 22 °C, square: 23 °C, circle: 24 °C, and down pointing triangle: 25 °C. Standard deviations are indicated with bars.

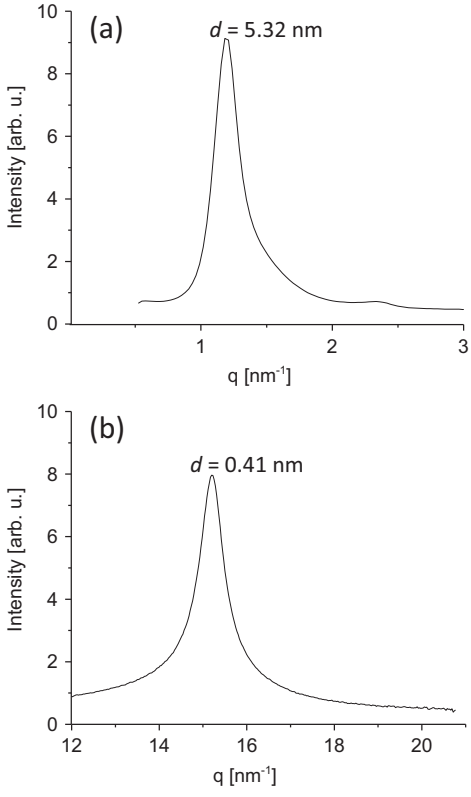

Fig. 2. X-ray patterns of SE S-170 isothermally crystallized to 25 °C for 100 min. (a) SAXS region. (b) WAXS region.

a double chain length structure and a hexagonal lateral packing. The same pattern was obtained for all conditions used in this study indicating that SE S-170 had only one crystalline form. In a previous study the α form of SS presented a pattern with two signals at 1.17 and 14.94 nm^{-1} in the SAXS and WAXS regions, respectively, with d values of 5.37 and 0.42 nm while the α form of HS presented two signals at 1.22 and 14.97 nm^{-1}, with d values of 5.15 and 0.42 nm (Rincón-Cardona et al., 2013). As may be noticed from Fig. 2, SE S-170 showed polymorphic similarity with the α form previously described for both sunflower oil stearins. From X-ray patterns, it may be expected that S-170 was a good modifier of crystallization in these systems.

3.3. Soft stearin (SS)

3.3.1. Polymorphic behavior with time

Fig. 3 shows the effect of the SE S-170 on polymorphic behavior with time for SS isothermally crystallized at 17 °C. When SS, without additives (control sample), was analyzed by SAXS (Fig. 3a) the first pattern obtained corresponded to an amorphous system. Then, after 3 min at T_c, a signal at 1.17 nm^{-1} (d = 5.37 nm) corresponding to the α-form appeared (Rincón-Cardona et al., 2013). After 33 min at T_c, a second polymorphic form, the β'_1 (q = 1.36 nm^{-1}, d = 4.61 nm) appeared, and it was the main form after 36 min.

In the WAXS region (Fig. 3b), the first evidence of crystallization occurred after 5 min. A signal at 15.11 nm^{-1} which corresponded to the α form (d = 0.42 nm) was noticeable in the patterns. After 36 min at T_c, the β'_1 polymorph appeared (q = 14.51 nm^{-1}, d = 0.43 nm). After 50 min at T_c, a weak signal corresponding to the β_2 form (q = 13.78 nm^{-1}, d = 0.46 nm) was present in the patterns. The SAXS signal corresponding to the β_2 polymorphic form was not noticeable in Fig. 3a indicating that β_2 form was present in trace amounts in these conditions.

When S-170 was added (Fig. 3c), the first SAXS pattern showed that crystallization had already started by the time SS reached T_c. A very weak signal indicative of crystallization of S-170 and the α form appeared at zero time. After 21 min at T_c, a signal corresponding to the β'_1 form appeared. The formation of the β'_1 form needed 12 more minutes for SS without S-170 than for SS with addition of S-170. A third polymorphic form that did not appear in the SAXS region without the additive (control sample, Fig. 3a) crystallized, however, after 50 min when S-170 was added to SS (Fig. 3c). The 50 min-pattern presented two very weak signals with q values of 0.85 and 1.93 nm^{-1}, respectively, corresponding to d values of 7.39 and 3.25 nm, characteristics of the β_2 form (Rincón-Cardona et al., 2013). As shown in Fig. 3c, crystallization of the β_2 form was promoted when S-170 was added to SS.

The corresponding WAXS patterns (Fig. 3d) showed that crystallization started after 2 min. The α form (q = 1.17 nm^{-1}, d = 5.37 nm) was the first polymorph that crystallized. The polymorphic transition to the β'_1 (q = 1.36 nm^{-1}, d = 4.61 nm) occurred after 28 min at T_c. Then, a strong signal at 0.46 nm indicative of the β_2 form was noticeable after 55 min. Crystallization of β_2 form was accelerated by the addition of S-170. This result is very relevant from the practical point of view since β_2 form is the required polymorph for chocolate and confections.

It was reported that the occurrence of a small-angle spectrum without wide-angle spectrum was expected when a liquid crystalline phase was formed in a fat system (Sato, 2001). Takeuchi, Ueno, and Sato (2002) studying SOS (1,3-distearoyl-2-oleoyl-*sn*-glycerol) and SSO (1,2-distearoyl-3-oleoyl-*rac*-glycerol) compounds observed that SAXS signals might appear before WAXS signals were noticeable in a variety of triacylglycerols blends. In agreement with these findings, in our

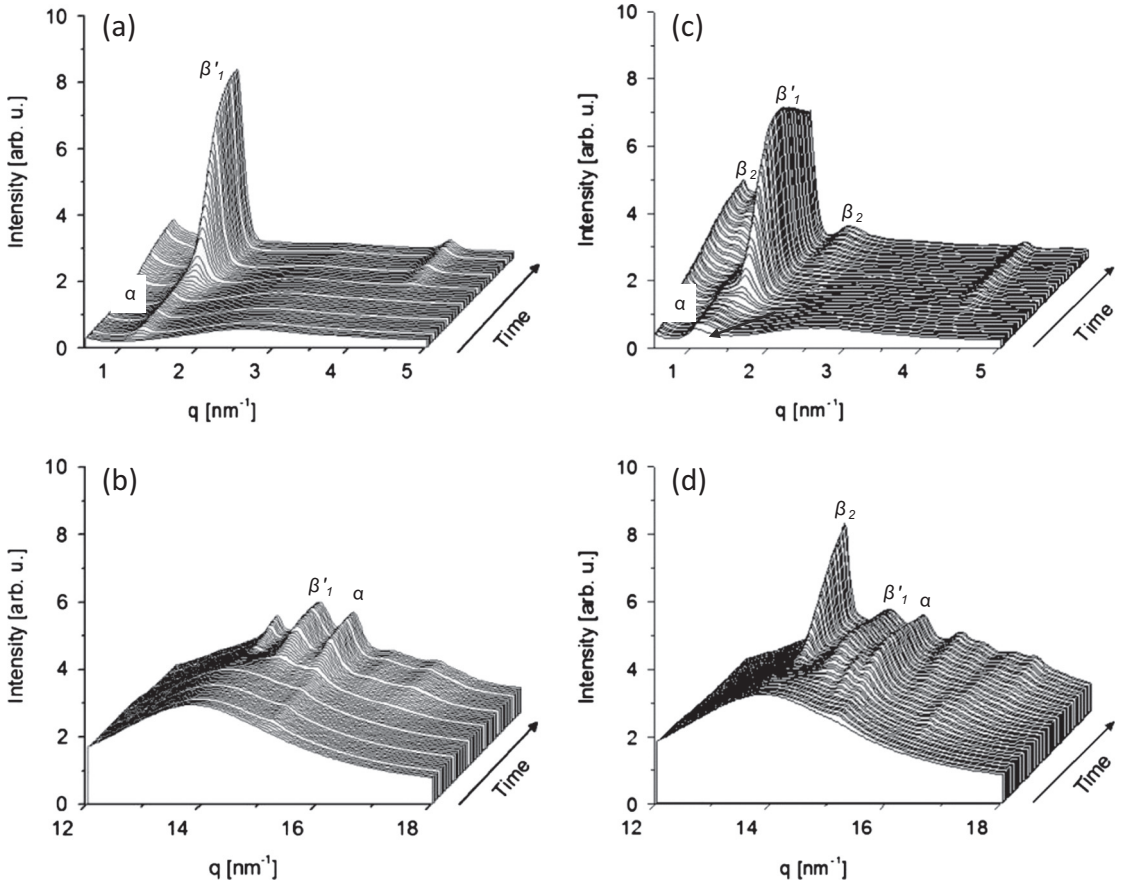

Fig. 3. 3D plots of X-ray scattering measurements of soft stearin (SS) crystallized at 17 °C for 40 min, without SE S-170: (a) small angle region (SAXS), (b) wide angle region (WAXS), and with SE S-170: (c) SAXS, (d) WAXS.

systems, SAXS signals appeared before WAXS signals indicating that lamellar stacking started to organize before lateral chain packing arrangement occurred.

3.3.2. Quantification of polymorphic forms

Fig. 4 shows the results of integrating areas under the SAXS peaks reported in Fig. 3a and c vs. time. The α form was the first form that crystallized in both SS without (Fig. 4a) or with SE S-170 (Fig. 4b). However, when SE S-170 was added the amount of α form was greater and its life longer as evidenced by the fact that α form still remained after 60 min at T_c. Without the additive, the α form had a life of 51 min. According to SAXS results, β_2 form did not crystallize in this temperature/time conditions without additives. However, when SE S-170 was added to SS, crystallization of β_2 form occurred (Fig. 4b). The amount of β'_1 form diminished while the amount of β_2 polymorph increased. SE S-170 promoted those polymorphic forms of sunflower oil stearins that had polymorphic similarity with its crystals. Hachiya, Koyano, and Sato (1989) studied seeding effects on solidification behavior of cocoa butter and dark chocolate. According to these authors the most influential factors in the seeding effects were the physical properties of the seed materials and above all the polymorphic relationship between the seed material and cocoa butter. A similar correlation was found between polymorphic forms of sunflower stearins and the SE S-170.

3.3.3. Induction times for crystallization by SAXS

Table 1 summarizes the effect of S-170 on induction times for crystallization of SS cooled at different temperatures. When the selected temperature was 5 °C, the first polymorph that appeared, both with and without S-170, was the α form. SS without additives showed a second polymorphic form that was the β'_2 form. However, when S-170 was added to SS, crystallization of β'_2 form was inhibited. Only one β′ form was present and was the β'_1 form. At 15, 16 and 17 °C, addition of S-170 accelerated crystallization of α and β'_1 forms. When SS crystallized at 17 °C, a trace of a third polymorph, which was not present without additives, the β_2 form, also appeared after 55 min of isothermal crystallization. This form was promoted by the presence of S-170. At 18.5 and 19 °C, crystallization of the α form was accelerated. However, crystallization of β'_1 form was inhibited and no other crystalline form was noticeable after 120 min at T_c. The global effect on crystallization, as measured by NMR, was delay of nucleation of β'_1 form.

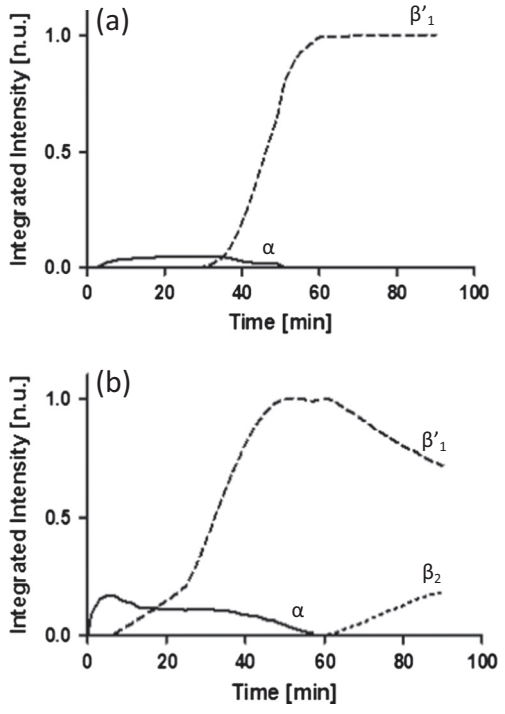

Fig. 4. Normalized integrated intensity (in arbitrary units) of the intensity peaks of the α β'_1, and β_2 polymorphs for soft stearin (SS) isothermally crystallized at 17 °C. (a) Without additives, (b) With addition of SE S-170. Plots in panels (a) and (b) are in the same scale.

It was reported for a variety of systems that induction times of crystallization usually increased with T_c (Cerdeira, Candal, & Herrera, 2004). It was also reported that induction times increased in the order of α, β′ and β polymorphs (Sato, 2001). There is a general tendency that the nucleation rates of less stable forms are much higher than those of the more stable forms, as empirically called by Ostwald rule of steps (Boistelle, 1988). Thus, as expected in many TAG systems more stable polymorphs have longer induction times than less stable forms. SE S-170 had only one crystalline form in all conditions selected for this study. The absence of other polymorphs than α form explains why SE S-170 acted as a good seed to crystalline forms with unit cells similar to the α form. Results indicated that SE S-170 had more polymorphic similarity with the β_2 form than with the β'_1 form since at 19 °C the β'_1 form did not crystallize. A high temperature such as 19 °C increased the probability of crystallization of β_2 form. For this reason, as occurred, it might be expected that no other form than α form was noticeable for the selected time since the β form has longer induction times than the other polymorphic forms. The delay of crystallization measured at high temperatures might be explained in two ways: as the variation of induction times of a polymorphic form with temperature or as consequence of efficiency of seeding effect of SE S-170 at high temperature. Related to the former, as induction time for β_2 form is expected to be longer than for β'_1 form and also longer for nucleation at 19 °C than at 17 °C, and as the β_2 form was favored by the presence of SE S-170, no crystallization occurred for the selected times since β_2 form needed more time to nucleate in those conditions. Regarding the latter, seeding effect of SE S-170 might not be efficient to accelerate crystallization of the β_2 form at higher temperatures since fewer nuclei are formed with low supercooling. Although α form was accelerated as indicated by shorter induction times (Table 1), the number of nuclei were not enough to accelerate nucleation of a form with less polymorphic similarity such as the β_2 form.

SE S-170 promoted the formation of the β'_1 form at temperatures at which β'_2 form was the polymorph expected to crystallize, i.e. 5 °C. This result is also important from the technological point of view. β'_1 is the most stable of β′ forms and addition of S-170 increased its life and stability. If the β′ form is required for a product, addition of S-170 might increase product life. S-170 also promotes crystallization of β_2 form under selected conditions. This is the polymorphic form required for chocolate and confections. Addition of S-170 may be useful in chocolate production.

3.3.4. Thermal behavior

Table 2 summarizes onset and peak temperatures together with total melting enthalpies values of SS isothermally crystallized at different temperatures for the same times as for X-ray studies. Addition of SE S-170 had a slightly significant effect on peak temperatures and total melting enthalpies ($\alpha = 0.05$) at 5, 15, 16, and 17 °C. One of the possible explanations is that DSC thermograms described the overall melting behavior. In a system like SS polymorphic forms can co-exist at different temperatures. For example, it is clear from Fig. 3d that α, β'_1, and β_2 forms co-exist at 17 °C and as is well known these polymorphic forms have different melting points. It was previously reported that this stearin fractionated when crystallized isothermally (Martini et al., 2013; Rincón-Cardona et al., 2013). These different solid solutions may have different TAG compositions and melting points. As a result, global thermal properties did not show significant changes. At 18.5 and 19 °C, SE S-170 delayed crystallization as measured by NMR and in agreement with these data and the fact that only the α form

Table 1
Induction times for crystallization of polymorphic forms of soft stearin (SS) crystallized at different temperatures with and without stearic sucrose ester S-170 (SE S-170) as measured by small angle (SAXS) and wide angle (WAXS) X-ray experiments.

Temperature (°C)	Polymorphic form	Induction times for crystallization of SS (min)		Induction times for crystallization of SS + S-170 (min)	
		SAXS	WAXS	SAXS	WAXS
5	α	0	0	0	0
	β'_2	13	16	ND	ND
	β'_1	ND	ND	13	16
15	α	3	6	1	2
	β'_1	14	16	13	15
16	α	3	6	1	2
	β'_1	24	26	11	12
17	α	3	5	0	2
	β'_1	33	36	21	28
	β_2	ND	55	50	55
18.5	α	4	6	1	2
	β'_1	85	87	ND	ND
19	α	4	7	2	5
	β'_1	90	93	ND	ND

ND: non-detectable.
Standard deviations were less than 1 min.
WAXS times were significantly longer than SAXS times ($\alpha = 0.05$).

crystallized in these conditions, total melting enthalpies decreased significantly ($\alpha = 0.05$).

3.4. Hard stearin (HS)

3.4.1. Polymorphic behavior with time

Fig. 5 shows SAXS patterns corresponding to HS without (Fig. 5a) and with addition of SE S-170 (Fig. 5b) crystallized at 23 °C. At the moment HS reached crystallization temperature ($t = 0$), no presence of crystalline material was detected indicating that crystallization process took place in isothermal conditions (Fig. 5a). After 1 min at 23 °C, SAXS spectra showed the start of crystallization. The first form that appeared was phase α ($q = 1.22\ nm^{-1}$, $d = 5.15$ nm). After 26 min at 23 °C, other intense signal with a $q = 1.82\ nm^{-1}$ ($d = 3.45$ nm), corresponding to a second polymorphic form, the β'_2 form, appeared. A third polymorphic form, β'_1 form with a q value of $1.39\ nm^{-1}$ ($d = 4.52$ nm), crystallized after 36 min. Both β′ forms co-existed indicating that as in the case of SS, HS fractionated. The different solid solutions obtained most likely had crystals with different compositions that crystallized independently since as shown in Fig. 5a, β'_2 form did not transform into β'_1 form. This behavior clearly differs from that reported for pure TAG and for cocoa butter in literature. In those systems, unstable polymorphic forms always suffer a polymorphic transformation to more stable polymorph (Walstra, 2003).

As shown in Fig. 5b, addition of S-170 to HS inhibited crystallization of β'_2 form. The β'_2 form was the crystalline form of HS with less polymorphic similarity than SE S-170 crystals as indicated by long spacing signals (1.82 and $1.18\ nm^{-1}$ for β'_2 and SE S-170 crystals, respectively). When HS with S-170 reached 23 °C, α form crystallized first and after 25 min at T_c transformed into β'_1 form which was the main form after 37 min.

3.4.2. Quantification of polymorphic forms

Fig. 6 shows the results of integrating areas under the SAXS peaks reported in Fig. 5. When HS was cooled to T_c, the first polymorphic form that appeared was the α form (Fig. 6a). Their amount grew until 40 min and then, started to decrease. After 60 min at T_c, a small amount of α form was still present. β'_2 form crystallized after 24 min at T_c. The amount of this form increased until it reached a plateau value. The β'_1 polymorph appeared after 32 min at T_c. It rapidly crystallized reaching a maximum value after 65 min.

When SE S-170 was added to HS (Fig. 6b), only two polymorphic forms crystallized: α and β'_1. The amount of α form always increased and after 25 min crystallization rate was even faster than in the 10–24 min period. β'_2 form did not crystallize in the selected time.

Results indicated that in HS without addition of SE S-170, the α form underwent a polymorphic transition to β'_2 (Fig. 6a). The amount of β'_1 polymorph seemed not to be related to the one of α form. As at 23 °C SE S-170 inhibited the crystallization of β'_2, both α and β'_1 polymorphs, increased with time (Fig. 6b).

3.4.3. Induction times for crystallization by SAXS

Table 3 reports induction times for crystallization of polymorphic forms of HS crystallized at different temperatures with and without SE S-170. SE S-170 had slight or no effect on induction times of crystallization as measured by SAXS for all temperatures. However, it had a noticeable effect on the polymorphic form that crystallized. Depending on T_c it promoted the formation of some polymorphic forms and inhibited others. When HS crystallized at 10 °C, two polymorphic forms were present: α and β'_2. When SE S-170 was added to HS, the β'_1 form also crystallized. This behavior did not exactly match that of SS. In the case of SS, SE S-170 inhibited crystallization of the β'_2 form at all temperatures. SE S-170 did not inhibit the formation of β'_2 form at 10 °C. However, it promoted crystallization of β'_1 form. This β′ form does not crystallize at 10 °C when HS without additives is cooled at 10 °C/min to T_c (Rincón-Cardona et al., 2013). At 21, 22, and 23 °C, β'_2 form did not crystallize when S-170 was added to HS. Most likely, these different behaviors were related to supercooling, the driving force for crystallization. When supercooling was high, β'_2 form of HS was able to crystallize even if SE S-170 was added to HS. For lower supercoolings, SE S-170 was able to inhibit crystallization of β'_2 form. As was previously reported, the presence of β'_2 form was related to crystallization temperature (Rincón-Cardona et al., 2013) and therefore to supercooling. In addition,

Table 2
Onset and peak temperatures together with melting enthalpies of soft stearin (SS) isothermally crystallized at different temperatures for the same times as for X-ray studies.

Crystallization temperature (°C)	Onset temperature (°C)	Peak temperature (°C)	Total melting enthalpies (Jg^{-1})	Onset temperature (°C)	Peak temperature (°C)	Total melting enthalpies (Jg^{-1})
	Without SE S-170			With SE S-170		
5	17.1 ± 1.3^{aA}	24.5 ± 0.6^{aA}	41.13 ± 7.1^{aA}	6.8 ± 0.3^{aB}	27.1 ± 0.5^{aB}	46.4 ± 3.2^{aB}
15	15.4 ± 1.1^{aA}	24.3 ± 0.6^{aA}	45.3 ± 8.4^{aA}	16.4 ± 1.4^{bA}	27.6 ± 0.6^{aB}	47.6 ± 8.6^{bA}
16	17.1 ± 0.8^{aA}	25.1 ± 0.3^{aA}	35.7 ± 5.6^{aA}	17.9 ± 1.0^{bA}	27.1 ± 0.3^{aB}	27.7 ± 5.4^{aA}
17	17.3 ± 0.9^{aA}	30.0 ± 0.9^{bA}	57.7 ± 8.2^{aA}	18.0 ± 0.7^{bA}	30.1 ± 0.2^{bA}	54.1 ± 7.1^{bA}
18.5	21.5 ± 0.7^{bA}	29.5 ± 0.2^{bA}	46.8 ± 6.7^{aA}	19.1 ± 0.5^{bB}	29.5 ± 0.7^{bA}	2.1 ± 1.4^{cB}
19	21.1 ± 0.9^{bA}	30.9 ± 0.4^{bA}	15.1 ± 1.4^{bA}	20.5 ± 0.2^{cA}	29.7 ± 0.6^{bA}	4.1 ± 2.1^{cB}

Data reported are mean values and standard deviations for two replicates.
Significant differences between means were determined by the Student's *t* test. An α level of 0.05 was used for significance. Data in the same column with the same superscript are not significantly different ($\alpha = 0.05$).
For the same parameter (onset temperature, peak temperature and total melting enthalpy), values with the same capital letter are not significantly different ($\alpha = 0.05$).

SS and HS different behaviors may be related to the different TAG compositions of stearins. SS and HS differed in their content of StOSt (stearic–oleic–stearic) TAG. Their values were 23.7 ± 0.6 and 54.6 ± 0.9, respectively. As expected, β'_1 form did not crystallize at 21 and 22 °C, even with addition of SE S-170. However, this form was found at 10 °C. β'_1 form crystallization also depended on crystallization temperature and supercooling. A combination of high supercooling (10 °C) and addition of SE S-170 was effective to promote β'_1 crystallization. At 24 °C, SE S-170 did not affect induction times for crystallization for α nor β'_1 forms. When selected T_c was 25 °C, two forms crystallized during the selected time: α and β'_1. However, when SE S-170 was added only the α form was present. Most likely, SE S-170 promoted formation of β_2 form, but as this polymorph has a very long induction time, it did not crystallized even after 100 min.

In a recent work Shimamura, Ueno, Miyamoto, and Sato (2013) analyzed the effect of polyglycerine fatty acid esters (PGFEs) on palm stearin crystallization and found that crystallization was promoted when the concentration of PGFEs was increased and the cooling rate was increased. On the other hand, they found that all PGFE additives retarded crystallization of palm stearin when the concentration of PGFEs was low and the cooling rate was low. In preliminary studies performed by NMR, we analyzed the effect of SE S-170 concentration on induction times. In our systems, we were unable to find a correlation between emulsifier concentration and acceleration or delay of SS or HS nucleation. The effect on crystallization was only dependent on supercooling. Differences in PGFEs and SEs behaviors could be associated to the fact that SEs contain a sucrose molecule in their formula and therefore there is a greater chemical dissimilarity between SEs and TAG than between PGFEs and TAG. According to Shimamura et al. (2013), nucleation was promoted by the template effects of PGFEs and was retarded by the disturbance of nucleation and subsequent crystal growth processes caused by the PGFE molecules at high temperatures. In agreement with these findings we found that SE S-170 had an efficient seeding effect at high supercooling (lower T_c) and that it promoted polymorphs with unit cell distances similar to the ones of SE S-170. At high temperatures (low supercooling), it inhibited nucleation of β′ forms and as β forms had longer induction times than α and β'_1, the global effect as measured by NMR was delayed. The mechanism could be the one proposed by Shimamura et al. (2013). SAXS and WAXS data showed that TAGS and SE S-170 were able to co-crystallize because of their somewhat similar chemical structure (stearic fatty acid molecules). However, the structural dissimilarities between TAGs and SE S-170 (sucrose molecule) may cause delay in nucleation and crystal growth. The elongation of induction times at high temperatures may be explained as a disturbance of nucleation caused by the presence of molecules with large polar groups. For crystal growth, the retardation mechanisms may be understood in terms of the similarity/dissimilarity concept, with the incorporation of crystallizing materials at the kink sites on the growth steps at the crystal–liquid interface being hindered by the additives. This mechanism was also proposed for the effect of SE P-170 on milk fat blends (Cerdeira et al., 2005).

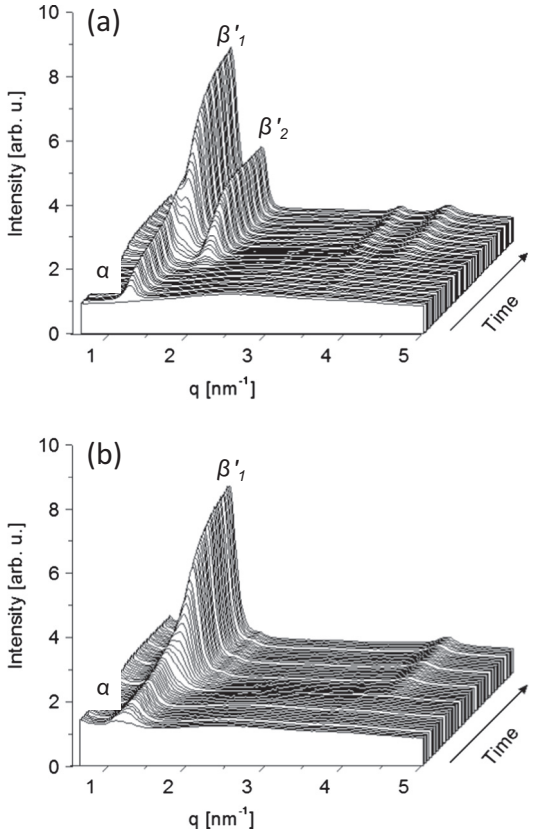

Fig. 5. 3D plots of small angle X-ray scattering (SAXS) measurements of hard stearin (HS) crystallized at 23 °C for 80 min. (a) Without additives, (b) With SE S-170.

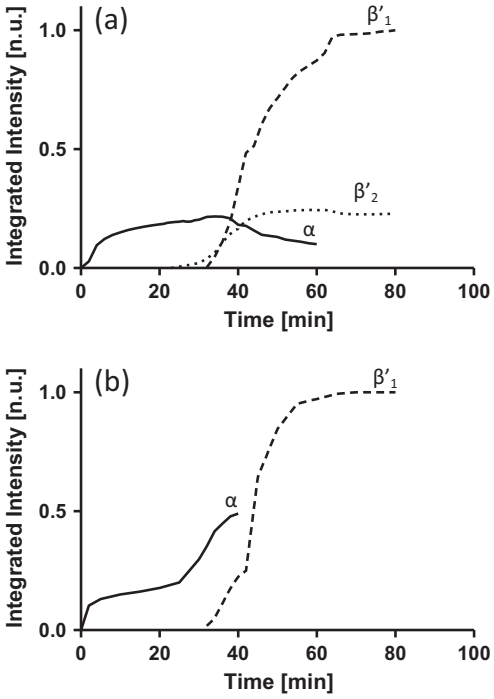

Fig. 6. Normalized integrated intensity (in arbitrary units) of the intensity peaks of the α, β'_2, and β'_1 polymorphs for hard stearin (HS) isothermally crystallized at 23 °C. (a) Without additives, (b) With addition of SE S-170. Plots in panels (a) and (b) are in the same scale.

3.4.4. Thermal behavior

Table 4 summarizes onset and peak temperatures together with total melting enthalpies of HS isothermally crystallized at different temperatures for the same times as for X-ray studies. Addition of SE S-170 did not modify peak temperatures or total melting enthalpies at 10, 21, 22, 23 and 24 °C. DSC thermograms were the result of melting of different polymorphic forms and of crystals with different TAG composition. Therefore, the overall behavior was similar. It was reported that two algal oils with very different composition (one of them rich in saturated fat and the other rich in palmitic and oleic TAG) had identical melting and mechanical properties because the groups of TAG that crystallized had similar melting points and were assembled in similar crystals (Co et al., 2014). In agreement with the delay effect described by NMR, total melting enthalpy significantly diminished by addition of

Table 3
Induction times for crystallization of polymorphic forms of hard stearin (HS) crystallized at different temperatures with and without stearic sucrose ester S-170 (SE S-170) as measured by small angle (SAXS) and wide angle (WAXS) X-ray experiments.

Temperature (°C)	Polymorphic form	Induction times for crystallization of SS (min)		Induction times for crystallization of SS + S-170 (min)	
		SAXS	WAXS	SAXS	WAXS
10	α	0	0	0	0
	β'_2	5	7	4	5
	β'_1	ND	ND	19	21
21	α	2	3	1	2
	β'_2	19	22	ND	ND
22	α	3	4	1	1
	β'_2	17	24	ND	ND
23	α	1	1	2	2
	β'_2	25	34	ND	ND
	β'_1	33	36	27	30
24	α	2	4	3	5
	β'_1	42	47	41	49
25	α	2	3	2	3
	β'_1	72	80	ND	ND

ND: non-detectable.
Standard deviations were less than 1 min.
When different, WAXS times were significantly longer than SAXS times ($\alpha = 0.05$).

SE S-170 at 25 °C. Besides, at this T_c, only the α form was present during the time selected for crystallization studies.

3.5. Storage study

Fig. 7 shows SAXS patterns for HS crystallized at 25 °C and stored for 48 h. As may be noticed from Fig. 7a, four polymorphic forms were present together: α, β'_2, β'_1, and β_2. They were present in similar ratios. It was previously reported that β forms of SS and HS did not crystallize when these stearins were cooled at 10 °C/min to T_c and kept at T_c for 2 h (Martini et al., 2013; Rincón-Cardona et al., 2013). In agreement with previous studies Table 3 shows that in those conditions only α and β'_1 forms were detected. The β forms appeared later during storage. As shown in Fig. 7a, β_2 form co-existed with more unstable forms which was unexpected from the behavior described for pure TAGs in literature (Walstra, 2003). Stearins fractionated and the different fractions had different polymorphic forms. It is clear that a complex system such as high stearic high oleic sunflower oil stearins did not behave as a pure TAG. Unstable forms are not always transformed into more stable forms. When SE S-170 was added to HS (Fig. 7b) only two polymorphic forms were found after 48 h at 25 °C: α and β_2. The β_2 form was the predominant polymorph in those conditions. SE S-170 promoted crystallization of β_2 form and inhibited crystallization of β'_2 and β'_1 forms.

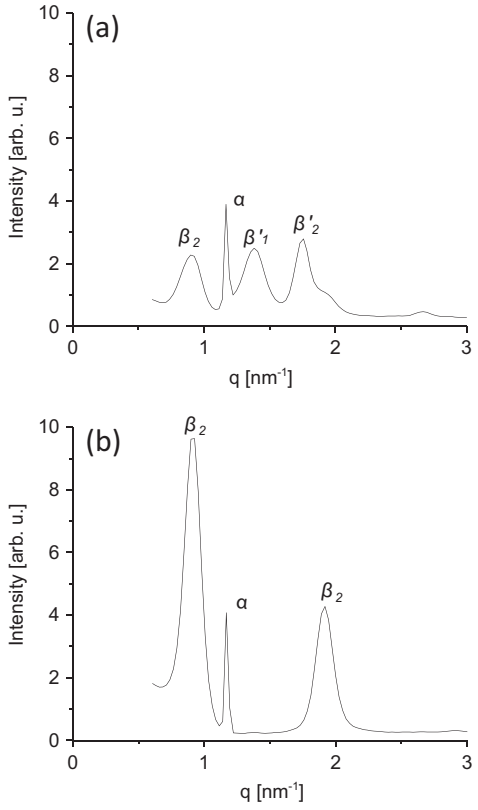

Fig. 7. Small angle X-ray scattering plot (SAXS) of hard stearin (HS) crystallized at 25 °C and kept at that temperature for 48 h. (a) Without additives, (b) With SE S-170.

4. Conclusions

SE S-170 was effective to modify crystallization and polymorphic behaviors of two stearins coming from a new variety of sunflower oil. The effect on crystallization kinetics depended on supercooling. At high supercooling (low crystallization temperatures) the effect was acceleration while at low supercooling (crystallization temperatures close to the melting point or at temperatures at which there is a measurable induction time for nucleation) the effect was delay of nucleation and crystal growth. As SE S-170 is a solid and has a high melting point, it crystallized before stearins at low temperatures and acted as a seed. The seeding effect was more effective when phases formed had polymorphic similarity with SE S-170 crystals. At high temperature, the mechanism of delay could have been promotion of stable polymorphic forms or disruption of nucleation with inhibition of growth due to the large polar group (sucrose molecule) present in SE S-170 molecules. The effect of SE S-170 also was important from the technological point

Table 4
Onset and peak temperatures together with melting enthalpies of hard stearin (HS) isothermally crystallized at different temperatures for the same times as for X-ray studies.

Crystallization temperature (°C)	Onset temperature (°C)	Peak temperature (°C)	Total melting enthalpies (Jg^{-1})	Onset temperature (°C)	Peak temperature (°C)	Total melting enthalpies (Jg^{-1})
	Without SE S-170			With SE S-170		
10	21.6 ± 1.3^{a}	29.5 ± 0.5^{aA}	99.4 ± 7.3^{aA}	22.9 ± 1.8^{a}	30.5 ± 0.3^{aA}	101.6 ± 8.7^{aA}
21	24.2 ± 2.0^{a}	30.1 ± 0.8^{aA}	101.8 ± 9.2^{aA}	23.4 ± 1.2^{a}	30.7 ± 0.2^{aA}	100.8 ± 9.1^{aA}
22	25.0 ± 1.5^{a}	30.4 ± 0.7^{aA}	97.0 ± 5.6^{aA}	24.6 ± 1.3^{a}	31.1 ± 0.5^{aA}	91.9 ± 9.0^{aA}
23	23.9 ± 1.0^{a}	31.7 ± 0.5^{bA}	86.6 ± 9.3^{bA}	24.2 ± 1.7^{a}	33.4 ± 0.4^{bA}	89.8 ± 7.3^{bA}
24	24.9 ± 1.6^{a}	32.2 ± 0.7^{bA}	86.1 ± 8.1^{bA}	24.8 ± 1.5^{a}	32.4 ± 0.3^{bA}	85.5 ± 8.6^{bA}
25	25.9 ± 1.7^{a}	32.1 ± 0.4^{bA}	64.7 ± 2.4^{cA}	25.9 ± 0.9^{a}	33.0 ± 0.4^{bA}	28.2 ± 3.7^{cB}

Data reported are mean values and standard deviations for two replicates.
Significant differences between means were determined by the Student's *t* test. An α level of 0.05 was used for significance. Data in the same column with the same superscript are not significantly different ($\alpha = 0.05$).
For the same parameter (peak temperature and total melting enthalpy), values with the same capital letter are not significantly different ($\alpha = 0.05$).

of view. Depending on crystallization temperature and storage time, the β'_1 or the β_2 form could be the main form obtained. These forms are required forms for bakery and confectionary applications. Therefore, the use of SE S-170 could improve stearins functionality which will be helpful in product manufacturing.

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