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Polymorphic behavior during isothermal crystallization of high stearic high oleic sunflower oil stearins

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ABSTRACT

The polymorphic phases of two high stearic high oleic (HSHO) sunflower oil stearins obtained by dry and solvent fractionation of the oil with the aim of use them as *trans* fat replacers or cocoa butter equivalent were studied by performing in situ isothermal crystallization studies following the appearance of polymorphic forms by X-ray using synchrotron radiation. Thermal behavior, equilibrium and actual isothermal solid fat content and morphology of phases were also analyzed. Three polymorphic forms were observed when samples were crystallized at 10 °C/min to different crystallization temperatures (T_c): α, β'_2, and β'_1. The α form was the first polymorph obtained at all temperatures used and in the opposite way expected, at most crystallization temperatures it did not disappear when β'_2 or β'_1 forms appeared. β'_2 form crystallized below 16 or 23 °C for soft and hard stearins, respectively. Above those temperatures, the obtained polymorph was the β'_1 form. The β polymorphs were not obtained during the times selected for isothermal crystallization. However, β_2 form appeared at least after 6 h at T_c while after 48 h of storage at 25 °C the β_1 polymorph was the main form. The β_2 polymorphic form, which is required for chocolate manufacture, has a very short life and was isolated from β_1 by applying cooling/reheating cycles. The β_1 form was the most frequently observed. Therefore, processing conditions must be carefully controlled to obtain the desired polymorphic form during product manufacturing. This study provides full characterization and quantification of polymorphic phases of HSHO stearins in real time. Results from this study will help optimize processing conditions for the use of HSHO stearins in industrial applications as trans fat replacers and cocoa butter equivalents.

1. Introduction

Polymorphism — the existence of two or more distinct crystalline forms with different types of crystal packing and thermodynamic stabilities of the same substance — is a function of processing conditions, time and temperature of storage (Afoakwa, Paterson, Fowler, & Vieira, 2009). Polymorphism results from the different possibilities of lateral packing of the fatty acid chains and of the longitudinal stacking of molecules in lamellae. These two levels of organization are easily identifiable from the short- and long-spacings observed by X-ray scattering at wide (WAXS) and small (SAXS) angles, respectively (Walstra, 2003).

It has long ago been realized that triacylglycerols (TAG) can crystallize in different monotropic modifications characterized by short-spacings, the three main of which are called α, β' and β, in the order of their increasing stability. The α subcell has hexagonal geometry with each chain surrounded by six others at equal distances. The chains have some freedom to move and therefore there is a partial disorder. The β' polymorph shows orthorhombic subcell with a denser and more perfect packing. The β crystals has triclinic subcell with the densest packing of the three subcells. A fourth crystalline structure, often called sub-α, although it contains a β′ subcell and would be less stable than the α-form, is also reported in the literature. In addition, other polymorphs, γ and δ forms, were also described for some pure TAG such as POP (Ikoda, Ueno, Miyamoto, & Sato, 2010; Minato et al., 1997).

Different polymorphic forms can also be characterized by their long-spacing signals. The lipid long-spacings correspond to the repeated distance in the direction perpendicular to the lamellae. For TAG, long-spacings are commonly double or triple chainlengths (2 L or 3 L) (Aquilano & Sgualdino, 2001). For any polymorphic system, phase transitions toward the most stable phase are unavoidable, due to thermodynamic drive to energy minimization. The high energy of the synchrotron source allows *in situ* characterization of phase formation in a sample holder and the competition between the different

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polymorphic species to be followed quantitatively (Loisel, Keller, Lecq, Bourgaux, & Ollivon, 1998). 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). On-site analysis of fat crystallization using synchrotron radiation was previously performed in several systems such as milk fat (Lopez, Lavigne, Lesieur, Bourgaux, & Ollivon, 2001; Lopez, Lesieur, Bourgaux, Keller, & Ollivon, 2001; Lopez, Lesieur, Bourgaux, & Ollivon, 2005; Lopez et al., 2002) and palm oil (Chong et al., 2007), to name a few.

The physical properties of fat products are closely related to polymorphism of solid fat. For example, only form V of cocoa butter is used by the confectionery industry as the optimal polymorph in chocolate. This is because form V provides chocolate products with snap (ability to break apart easily), good demolding properties (contraction), and a good quality finish in terms of color and gloss. Moreover, form V exhibits resistance to fat bloom, which is a physical defect that appears during storage as undesirable white spots or a streaky grey-white finish on the chocolate surface (Mackley & Sonwai, 2006).

FDA rule about *trans* fat that was effective from January 1st 2006 and *trans* fat rules issued in many other countries encouraged food product manufacturers to eliminate or reduce the *trans* fat from their products. In some applications like baked goods and confectionery a certain amount of solids is crucial to obtain the desired product texture or appearance. The modification of fatty acid (FA) composition through plant breeding was one of the strategies developed to replace *trans* fat (Kodali, 2005). A new high stearic high oleic (HSHO) sunflower oil variety was developed recently and will be commercialized soon. The HSHO sunflower oil does not contain enough solids at high temperature; however, it contains disaturated fatty acids that could be concentrated by oil fractionation resulting in hard fractions called stearins. Although polymorphism is very relevant regarding industrial applications, the isothermal polymorphic behavior in real time of these systems has not been described yet. The present work reports the characterization and quantification of polymorphic phases of HSHO sunflower oil isothermally crystallized at different temperatures following crystallization in-situ with a synchrotron radiation X-ray equipment.

2. Materials and methods

2.1. Sunflower oil stearins

Two commercial HSHO sunflower oil stearins were used in this study. One of them was obtained by dry fractionation (soft stearin) crystallizing the oil in controlled temperature and agitation conditions (18 °C and 30 rpm). The other stearin was obtained by solvent fractionation dissolving the oil in hexane and storing the micelles without stirring at 5 °C (hard stearin). The stearin was collected by Vacuum filtration of the precipitates. Capillary melting points of soft and hard stearins were 30.1 ± 0.5 and 35.0 ± 0.6 °C, respectively.

2.2. Fatty acid composition

The fatty acid composition was determined using a Konik 4000 A HREC model gas chromatography (GC) unit equipped with a flame ionization detector (FID) and on-column injector. The column used was a Chrompack WCOT fused silica, stationary phase CP-sil 88, with a length of 50 m and an internal diameter of 0.25 mm. Helium was the carrier gas at a flow rate of 1.5 mL/min with hydrogen gas and air also being supplied to the FID. The 1-μL injections were from a solution of methyl esters at approximately 200 mg/mL hexane. FAs were identified by comparison of retention times with the ones of known FA methyl ester standards from SIGMA. Composition (area percent) was based on the area integrated by Workstation Borwin 4 chromatography software. The detector and injector temperatures were 200 °C, whereas the oven temperature was kept at 170 °C. Data reported are the average of two duplicates. Standard deviations were lower than 1%.

2.3. TAG analysis

The TAG fractions were determined using a Konik 4000 A HREC model gas chromatography (GC) unit equipped with a flame ionization detector (FID) and on-column injector. The column was the same used for FA methyl esters analysis. Each sample (10 mg) was weighed into a GC vial and dissolved in 1.8 mL of iso-octane, with 100 μL of internal standard (C_{27} in isooctane:2.02 mg/mL) added to the vial. TAGs were identified by comparison of retention times with the ones of known TAG standards from SIGMA. The injector and detector temperatures were 360 and 370 °C, respectively, and the oven temperature was 335 °C. Composition (area percent) was based on the area integrated by Workstation Borwin 4 chromatography software. Data reported are the average of two duplicates. Standard deviations were lower than 1%.

2.4. Study of polymorphism

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°. For isothermal experiments, samples were melted to 60 °C at 10 °C/min, then they were kept isothermally at 60 °C for 2 min to erase any crystal memory, and finally they were cooled to crystallization temperature at 10 °C/min. Zero time was the moment at which sample reached crystallization temperature. By using this procedure it may be assumed that crystallization occurred mostly isothermally since no signal indicating the presence of solid material appeared in the first X-ray pattern ($t = 0$) in all cases. For hard stearin selected temperatures were 10, 23, 24 y 25 °C. SAXS patterns were recorded as a function of time for 50, 80, 80 and 100 min, respectively. Soft stearin was crystallized to 5, 16, 18.5, and 19 °C for 50, 40, 120, and 115 min, respectively. Temperatures were selected taken into account the melting points reported for the crystalline phases of pure TAG present in these samples. Selected crystallization times were long enough to crystallize at least 70% of the equilibrium solid fat material (SFC) at a defined temperature as measured by pulse-nuclear magnetic resonance (p-NMR). It was evident from patterns that intensities did not change significantly at those times. 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 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. 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 et al., 2001a,b; Mazzanti, Guthrie, Sirota, Marangoni, & Idziak, 2004). In addition to short spacing signals, each polymorphic form showed characteristic long spacing signals. 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.

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. Of each sample, 15–18 mg was weighed into sealed aluminum pans and heated from storage temperature (5 °C) to 80 °C at 5 °C/min. Then, samples were cooled from 80 °C to crystallization temperature at 10 °C/min. Samples were kept at crystallization temperature for the same times selected in X-ray studies. Finally, they were melted from crystallization temperature to 80 °C at 5 °C/min. The calorimeter was calibrated with indium and mercury prior to analysis. Peak 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.

2.6. Measurements of SFC by NMR

The solid fat content (SFC) was measured by pulse nuclear magnetic resonance (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 and crystallized to the same temperatures selected for studies of polymorphism during the same times used for X-ray experiments. Equilibrium SFC was measured in all samples applying the pretreatment (tempering) described in the AOCS official method Cd 16b-93 (AOCS, 1989). The tempering procedure was as follows: melt and store for 15 min at 100 °C followed by 5 min at 60 °C; then sample was placed for, 90 ± 5 min at 0 °C, 40 ± 0.5 h at 26 °C, and again 90 ± 5 min at 0 °C and finally, 60 min at each crystallization temperature. SFC was determined at the same temperatures as the ones selected for X-ray studies. Duplicate runs were performed on consecutive days for each set of samples and three tubes were measured in each run. Standard deviations were less than 0.5%.

2.7. Morphology by Polarized Light Microscopy (PLM)

Crystals were observed with a polarized-light microscope model Axio Scope A1 (Carl Zeiss, Jena, Germany) equipped with a controlled temperature platform and a camera. At the point when crystallization temperature was reached, samples were photographed at time intervals for the same temperatures/times as selected for X-ray studies. Images were taken in duplicate for every sample with a 50× (hard stearin) or 20× (soft stearin) objective and a 10× ocular lens.

3. Results and discussion

3.1. Chemical composition

FA and TAG compositions are shown in Tables 1 and 2, respectively. The results shown in Table 1 indicated that the monounsaturated FA contents for soft and hard stearins were 58.8% and 39.9%, respectively; and Table 2 reported that saturated-oleic-saturated (SOS-type) TAGs (POP, POSt, StOSt, StOA, and StOB, with P palmitic acid, O oleic acid, St stearic acid, A arachidic acid, and B behenic acid) were 38.8 and 81.4%, for soft and hard stearins, respectively. For soft stearin, these values do not exactly agree with EU standards to qualify as a cocoa butter equivalent (CBE) material. On the contrary, values for hard stearin reach the requirements, especially to have < 45% unsaturated fatty acids (UFAs) content and > 65% SOS-type TAGs. Dry fractionation produced fractions enriched in saturated FA, that is, soft stearins which could be used for manufacturing structured fats or spreads, or they may be an intermediate step in the production of hard stearins appropriate for confectionary fat formulation.

Table 1
Fatty acid (FA) composition of hard and soft high stearic high oleic (HSHO) sunflower oil stearins.

FA[a]	Soft stearin (wt.%)	Hard stearin (wt.%)
C16:0 (palmitic)	5.3 ± 0.3	4.8 ± 0.3
C18:0 (stearic)	28.1 ± 0.9	46.9 ± 0.9
C18:1 (oleic)	58.8 ± 0.8	39.9 ± 0.5
C18:2 (linoleic)	3.0 ± 0.2	0.9 ± 0.1
C20:0 (arachidic)	2.0 ± 0.1	3.1 ± 0.1
C22:0 (behenic)	2.8 ± 0.1	4.4 ± 0.2
S	38.2	59.2
MU	58.8	39.9
PU	3.0	0.9

[a] Abbreviations: S = saturated, MU = monounsaturated, PU = polyunsaturated. Values of S, MU, and PU are the sum of average values.

3.2. Polymorphic changes during isothermal crystallization. Hard stearin

Fig. 1 reports the wide angle X-ray scattering (WAXS) spectra of hard stearin crystallized at 23 °C for 80 min. Fig. 2 shows the small angle X-ray scattering (SAXS) corresponding to patterns in Fig. 1. At the moment the sample reached crystallization temperature ($t = 0$), no presence of crystalline material was detected indicating that crystallization process took place in isothermal conditions. After 1 min at 23 °C, SAXS and WAXS spectra showed the start of crystallization. During the first 24 min of crystallization, the WAXS patterns shown in panel (a) of Fig. 1 displayed a clear single peak at $q = 14.97\ nm^{-1}$ ($d = 0.42$ nm) which is the characteristic signature of phase α. After 1 min at 23 °C (Fig. 2a) the SAXS patterns displayed a single peak with a q of $1.22\ nm^{-1}$ ($d = 5.15$ nm). According to the literature, these X-ray diffraction patterns are consistent with a bilayer (2 L) lamellar packing arrangement and a hexagonal subcell or α polymorph (Cisneros et al., 2006; Lopez et al., 2001a,b; Mazzanti et al., 2004). After 6 min at 23 °C, a second signal at $q = 3.67\ nm^{-1}$ ($d = 1.71$ nm), less intense, corresponding to a third order diffraction line of the α form was noticeable in SAXS patterns (Fig. 2a). After 26 min at 23 °C, other signals, corresponding to a second polymorphic form, appeared (WAXS Fig. 1b; SAXS, Fig. 2b): two WAXS signals at 30 min with q values of 14.59 and $16.34\ nm^{-1}$ ($d = 0.43$ and $d = 0.38$ nm, respectively) and one intense SAXS signal with a $q = 1.82\ nm^{-1}$ ($d = 3.45$ nm). According to the literature, these X-ray diffraction patterns are consistent with a bilayer (2 L) lamellar packing arrangement and an orthorhombic subcell or β' polymorph (Cisneros, Mazzanti, Campos & Marangoni; Lopez et al., 2001a,b; Mazzanti et al., 2004). After 36 min

Table 2
Triacylglycerol (TAG) composition of hard and soft high stearic high oleic (HSHO) sunflower oil stearins.

TAG[a]	Soft stearin (wt.%)	Hard stearin (wt.%)
POP	0.6 ± 0.1	0.4 ± 0.2
POSt	7.4 ± 0.3	9.3 ± 0.3
POO	5.4 ± 0.7	1.6 ± 0.3
POL	0.5 ± 0.2	0.2 ± 0.1
StOSt	23.7 ± 0.6	54.6 ± 0.9
StOO	22.3 ± 0.5	7.5 ± 0.5
OOO	22.8 ± 0.5	6.1 ± 0.3
StOL	2.1 ± 0.1	0.8 ± 0.2
OOL	3.3 ± 0.2	0.9 ± 0.2
OLL	0.7 ± 0.3	0.3 ± 0.1
StOA	3.6 ± 0.4	8.0 ± 0.2
OOA	1.6 ± 0.2	0.5 ± 0.1
StOB	3.5 ± 0.1	9.2 ± 0.4
OOB	2.3 ± 0.3	0.7 ± 0.2
SUS	38.8	81.4
SUU	34.3	11.2
UUU	26.9	7.3

[a] Abbreviations: A: arachidic acid (C20:0); B: behenic acid (C22:0); L: linoleic acid (C18:2); O: oleic acid (C18:1); P: palmitic acid (C16:0); St: stearic acid (C18:0); S = saturated and U = unsaturated.

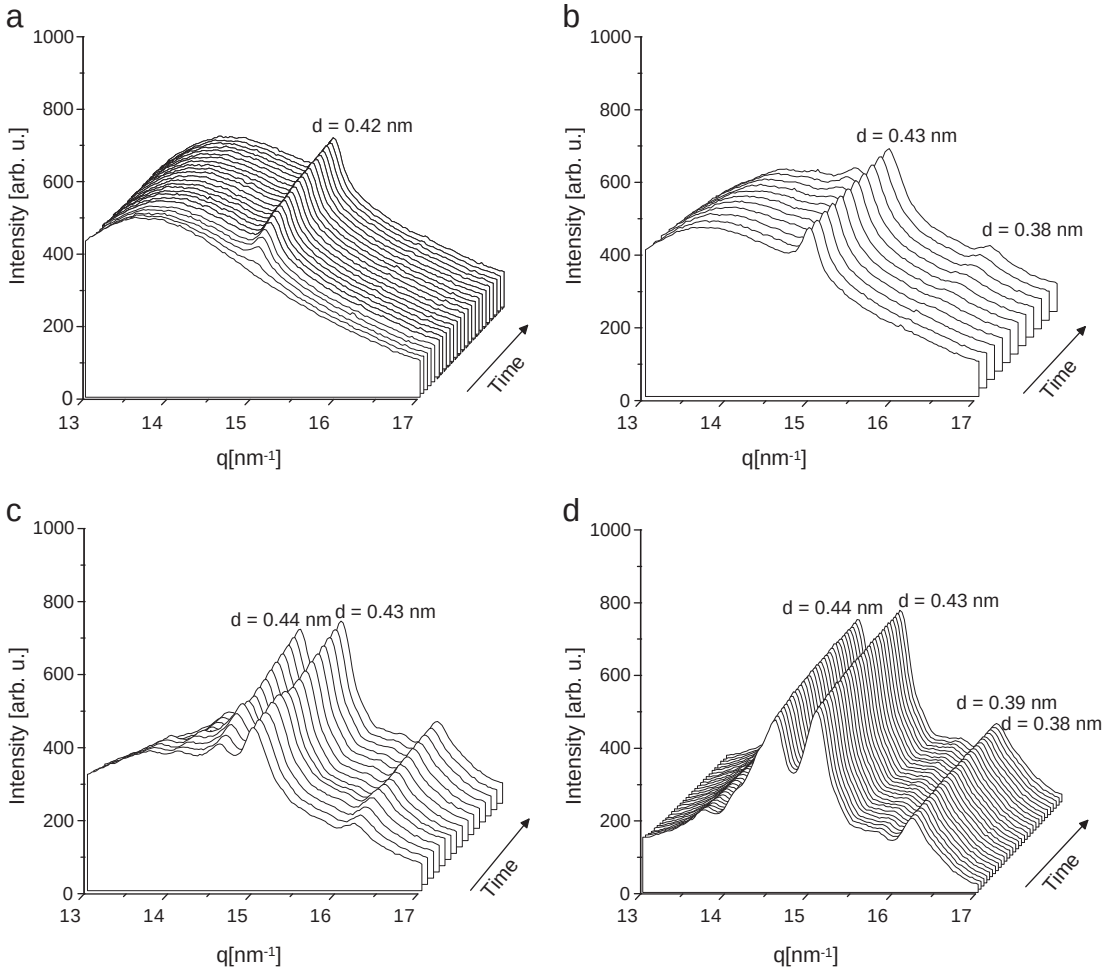

Fig. 1. 3D Plots of wide angle X-ray scattering measurements (WAXS) of hard stearin crystallized at 23 °C for 80 min. (a) 0 to 24 min, (b) 25 to 35 min, (c) 36 to 50 min, (d) 51 to 80 min.

at 23 °C, the appearance of a new polymorphic form can be noticed in WAXS pattern (Fig. 1c). Two new signals at $q = 14.13$ and 16.20 nm^{-1} ($d = 0.44$ and $d = 0.39$ nm) were observed. SAXS patterns (Fig. 2c) showed an intense signal with a q value of 1.39 nm^{-1} ($d = 4.52$ nm) and a weak signal at $q = 4.15$ nm^{-1} ($d = 1.51$ nm). This polymorphic form also corresponded to a β' phase. As the β' form with d values of 0.43 and 0.38 nm was the one obtained first during isothermal crystallization or at lower temperatures, it was called β'_2. The form with d values of 0.44 and 0.39 nm was obtained at longer times or higher temperatures. Therefore it was called β'_1. After 51 min at 23 °C (Figs. 1d and 2d), the intensity of the diffraction patterns of hard stearin remained essentially constant during the length of the study (80 min), indicating that no crystalline growth or polymorphic transformation took place. Only β'_2 and β'_1 forms were present. β_2 or β_1 forms did not appear in the selected conditions.

Similar WAXS and SAXS patterns as the one reported in Figs. 1 and 2 were obtained for the hard and soft stearins crystallized at different T_c. WAXS and SAXS patterns differed on the number and position of the peaks depending on the sample tested (hard or soft stearins) and the T_c used. Details on the different signals and polymorphic forms obtained are described below.

3.3. Polymorphic behavior with time

Fig. 3 reports the results of integrating the normalized SAXS curves vs. time for hard stearin. When hard stearin was isothermally crystallized, the first form that appeared at all temperatures selected was the α form (Fig. 3). At 10 °C WAXS and SAXS signals corresponding to the α form were present after 1 min of crystallization. The β'_2 form appeared at 12 min and was almost completely crystallized at 18 min. At this time point mix of both forms, α and β'_2, co-existed. However, after 30 min at 10 °C the predominant phase was the β'_2 form. At this temperature (10 °C), the amount of α polymorph was greater than at the other selected temperatures (Fig. 3a). It has a maximum before crystallization of β'_2 form. Then, the amount of α form diminished with time. Once the β'_2 form crystallized its amount increased very fast and reached an asymptotic value. After 50 min at 10 °C, the β'_2 was the predominant form. At 23 °C, β'_2 and β'_1 crystallized after the α form. The α form coexisted with these new forms from 36 to 44 min, and then the intensity of signal became weaker but it did not disappeared. β'_2 crystallized 2 min before β'_1 but its amount was considerably lower. Although β'_1 polymorph was the main polymorphic form after 80 min at crystallization temperature (Fig. 3b), α and β'_2 were also present. It is evident from Fig. 3b that β'_2 did not transformed into β'_1 as would have been expected if these stearins had behaved as pure TAG; instead, both polymorphic forms are crystallized from the melt under the experimental conditions tested in this research After 3 min at 24 °C a signal at $q = 1.22$ nm^{-1} ($d = 5.15$ nm) was noticeable in SAXS pattern. A second signal at $q = 3.67$ nm^{-1} ($d = 1.71$ nm) together with a WAXS signal at $q = 14.97$ nm^{-1} ($d = 0.42$ nm) appeared after 5 min at 24 °C. These signals corresponded to a bilayer (2 L) lamellar packing of the α polymorph. The α form was the only one present until 45 min at 24 °C (Fig. 3c). After 46 min at that temperature, a WAXS signal at

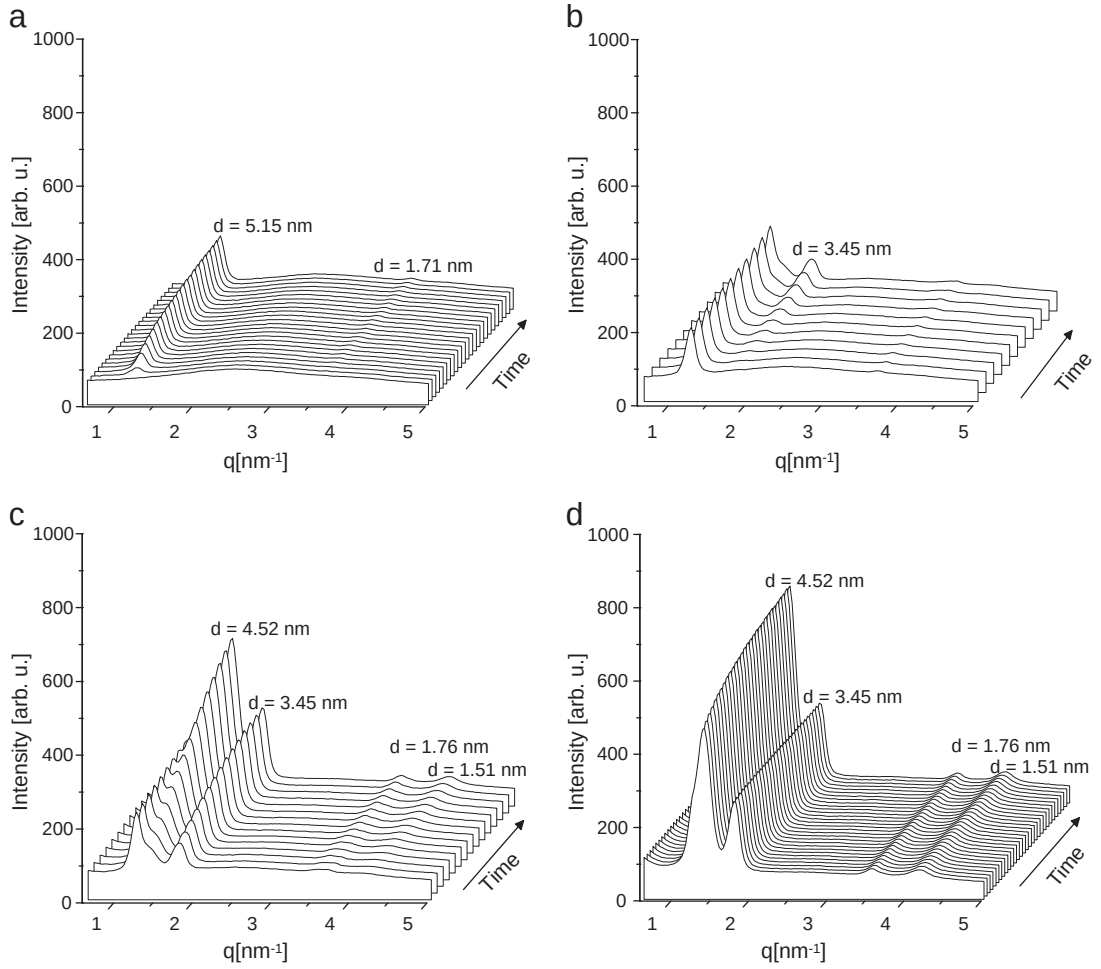

Fig. 2. 3D Plots of small angle X-ray scattering measurements (SAXS) of hard stearin crystallized at 23 °C for 80 min. (a) 0 to 24 min, (b) 25 to 35 min, (c) 35 to 50 min, (d) 51 to 80 min.

$q = 14.13\ nm^{-1}$ ($d = 0.44$ nm) and SAXS signals at q 1.39 nm^{-1} ($d =$ 4.52 nm) and 4.15 nm^{-1} ($d = 1.51$ nm) were present indicating the crystallization of the β'_1 form. This form had also a weak signal at $q = 16.20\ nm^{-1}$ ($d = 0.39$ nm) which was not noticeable before 54 min. After 66 min at 24 °C, both, α and β'_1 forms co-existed. When hard stearin was crystallized at 25 °C, the β'_2 form did not appear during isothermal crystallization for the selected time. After 3 min at 25 °C the α form was present and was the only form until 83 min. Then, the signals corresponding to the β'_1 form were noticeable. After 94 min at 25 °C, the main polymorphic form was the β'_1. In all cases the β_2 and β_1 forms were not noticeable for the selected times. In addition, no γ, sub α or δ forms appeared at these temperatures (10, 23, 24 or 25 °C). It is interesting to note that at 24 °C (Fig. 3c) and 25 °C (Fig. 3d) the β'_1 polymorph was the only β' form present. Once crystallization started, the amount of this form increased very rapidly being the most important form at the end of isothermal crystallization. For a pure TAG, crystallized under a fixed set of conditions (temperature, pressure, composition), only one solid phase will be consistent with a minimum free energy of the system. As previously mentioned, for any polymorphic system, phase transitions toward the most stable phase are unavoidable, due to thermodynamic drive to energy minimization. Multi-component fats tend to form compound crystals, and this significantly affects polymorphic forms and transitions. In some cases, a group of closely related TAG can almost behave like a single triglyceride, especially when crystallizing in the α and β' forms. One example of this is cocoa butter (Walstra, 2003). In cocoa butter only three TAGs, StOSt, POSt and POP, account for more than 85 to 95% of the fat. This specificity gives cocoa butter a thermal and structural behavior similar to that of a pure compound. POP, POSt, and StOSt are very similar molecules that form compound crystals even in the β-form (Rousset, Rappaz, & Minner, 1998). Sunflower oil stearins, however, also contain StOA and StOB. St and B acids have carbons chains than differ in 4 units. Data in Fig. 3b showed that hard stearin did not behave as a pure TAG. On the contrary, it fractionated in more than one solid solution, each of which crystallized in a different polymorphic form. When considering industrial processes, in general, only one phase is required for its technological properties, and hence the crystallization conditions must be controlled to obtain the expected structure only. Regarding hard stearin polymorphic behavior it is very relevant to describe its polymorphic forms and to set the conditions under which a specific polymorphic form is obtained. For baking applications, for example, the β'_1 is the preferred form. This polymorph was the main form obtained when hard stearin was crystallized at 24 and 25 °C and it was stable for at least 2 h.

Fig. 4 shows the same calculations as in Fig. 3 but for soft stearin. When soft stearin crystallized, the first polymorphic form that appeared after 1 min at 5 °C was the α form (Fig. 4a). It was evident from the 0.42 nm WAXS signal. In addition, another signal at $q =$ 16.20 nm^{-1} ($d = 0.39$ nm) that may be indicative of traces amount of γ form was also present from 1 to 15 min. It was reported that StOSt had a γ polymorph more stable than α but still metastable, with two strong WAXS peaks (short spacing) around 0.39 and 0.47 nm and triple chain length structure (Sato et al., 1989). Soft stearin showed a very weak signal at 0.47 nm which might indicate a γ

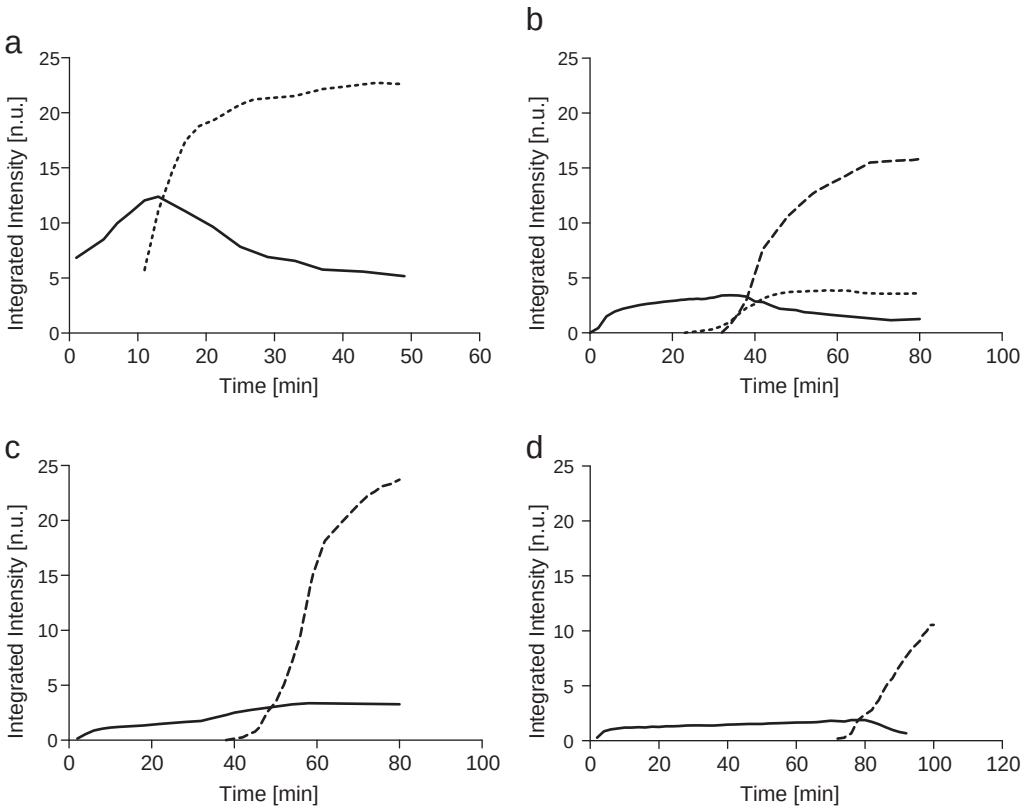

Fig. 3. Normalized integrated intensity (in arbitrary units) of the intensity peaks of the α (solid lines), β'_2 (dotted lines), and β'_1 (dashed lines) polymorphs for hard stearin isothermally crystallized at (a) 10 °C, (b) 23 °C, (c) 24 °C, and 25 °C. Plots in panels (a), (b), (c), and (d) are in the same scale.

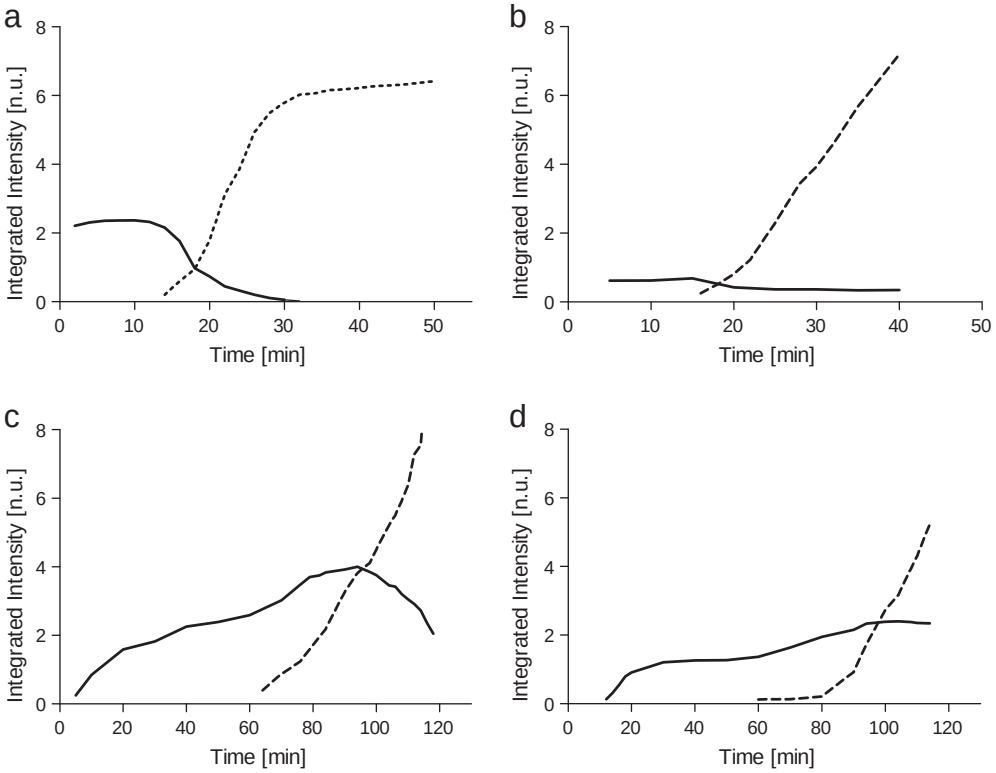

Fig. 4. Normalized integrated intensity (in arbitrary units) of the intensity peaks of the α (solid lines), β'_2 (dotted lines), and β'_1 (dashed lines) polymorphs for soft stearin isothermally crystallized at (a) 5 °C, (b) 16 °C, (c) 18.5 °C, and 19 °C. Plots in panels (a), (b), (c), and (d) are in the same scale.

form. Differing from StOSt polymorphic behavior, SAXS patterns showed a very weak signal at 3.57 nm which suggested double chain length. The traces of γ form disappeared after 16 min at 5 °C, a time at which the β'_2 form was notorious. A strong signal at 3.40 nm corresponding to β'_2 polymorph appeared. From that point both, α and β'_2 forms, were present in similar amounts. The α form lasted until 30 min. Then, the predominant form was β'_2. When the selected crystallization temperature was 16 °C no crystallization was detected for the first 4 min. Then, very weak WAXS and SAXS signals characteristics of the α form appeared. At 16 °C and above this temperature, the β'_2 form did not crystallize (Fig. 4b). In addition, for the soft stearin, there was no temperature at which β'_2 and β'_1 polymorphs were present simultaneously as was the case of hard stearin. After 30 min at 16 °C the characteristics signals of the β'_1 form were present and this form was the predominant polymorphic form after 40 min. At 18.5 °C traces of the α form crystallized after 8 min at crystallization temperature. Then, after 70 min the β'_1 polymorphic form crystallized and was the predominant form after 80 min at 18.5 °C. A similar behavior was found for 19 °C. In all cases the α form was the first form that crystallized from melted fat (Fig. 4). The amount of this form was always lower than the content of β' forms. When the α form was the only polymorphic form present, SFC as measured by NMR had values lower than 3% for all selected temperatures. After 14, 17, 60 and 62 min at 5, 16, 18.5 and 19 °C, respectively, a second polymorphic form appeared: β'_2 at 5 °C (Fig. 4a) and β'_1 at the other temperatures (Fig. 4b, c, and d). Once the second form was present, crystallization was very fast and this second form became the predominant polymorph of soft stearin. This was in agreement with the fact that at those times SFC increased sharply. The α form did not completely disappeared after the crystallization of β'_1 polymorph at 19 °C indicating that some fractionation took also place in soft stearin. There was no temperature at which both β' forms were together as was the case of hard stearin. The different behavior found for both stearins may be related with the fact that soft stearin had lower contents of StOA and StOB than hard stearin. The γ form, if present in soft stearin crystallized at 5 °C, had a signal so weak in SAXS patterns that areas under the normalized peak could not be calculated without making big errors. Therefore, it was not included in Fig. 4. β_2 or β_1 polymorphs were not obtained during isothermal crystallization for the temperatures and times selected in this study.

3.4. Soft stearin

3.4.1. d spacings

Table 3 summarizes short and long spacings for the α, β'_2 and β'_1 forms of hard and soft HSHO sunflower oil stearins. Under isothermal conditions only three main polymorphic forms were obtained after 2 h at crystallization temperature. Since γ form appeared at only one temperature and in trace amounts, the long and short spacings of this polymorphic form were not included in Table 3. When samples were stored at 25 °C, the β_2 form appeared after 6 h. This form had a short life in those conditions and after 48 h the main polymorphic form observed was the β_1 form. Samples stored for two months still remained in the β_1 polymorph. The characteristics WAXS and SAXS signals of β_2 and β_1 form were also included in Table 3.

It is a usual approach to interpret the crystallization properties of a fat system following the behavior of its main TAGs. StOSt was the main TAG in hard stearin and was present in high percentage in soft stearin (Table 2). It might be expected that sunflower stearins had a similar polymorphic behavior than StOSt, especially hard stearin. However, both the type of subcell in selected processing conditions and lamellae organization were different than the ones observed for StOSt. Regarding short spacings, Sato et al. (1989) reported five polymorphic forms for StOSt. They were α, γ, pseudo-β', β_2 and β_1. These forms were also present in other three TAG: POP, AOA and BOB

Table 3
Short and long spacings of main polymorphic forms of hard and soft high stearic high oleic (HSHO) sunflower oil stearins isothermally crystallized at different temperatures.

Sample	Short spacing (nm)	Long spacing (nm)
Soft stearin		
α	0.42 (s)	5.35 (s) 2.72 (m)
β'_2	0.43 (m) 0.38 (m)	3.40 (s) 1.71 (w)
β'_1	0.44 (s) 0.39 (w)	4.61 (s) 1.55 (w)
β_2	0.46 (vs)	6.01 (s) 3.04 (m)
β_1	0.46 (vs)	6.48 (s) 3.69 (m)
Hard stearin		
α	0.42 (s)	5.15 (s) 1.71 (w)
β'_2	0.43 (s) 0.38 (m)	3.45 (s) 1.76 (w)
β'_1	0.44 (s) 0.39 (w)	4.52 (s) 1.51 (w)
β_2	0.46 (vs)	6.06 (s) 3.02 (m)
β_1	0.46 (vs)	6.49 (s) 3.71 (m)

s: strong; m: medium; w: weak; vs: very strong. Values differing in more than 0.009 nm are significantly different.

(Wang, Sato, Sagi, Izumi, & Mori, 1987). Additionally, another intermediate form, β', which reveals short spacings similar to β'_1 of tristearin was detected in POP, AOA, and BOB (Simpson & Hagemann, 1982). Moreover, POP also presented a δ form (Sato et al., 1989). The number of independent polymorphs in POSt was four: α, δ, pseudo-β' and β_1. Interestingly, POSt did not possess β_2-form, which is the second stable form in POP and StOSt (Arishima, Sagi, Mori, & Sato, 1991). Hard and soft stearins showed α, β'_2, β'_1, β_2, and β_1 polymorphs. Soft stearin also presented trace amounts of γ form at low temperature (5 °C). Even for the mixed saturated-unsaturated TAGs analyzed as pure compounds contradictory data were reported in literature. According to Sato et al. (1989) this discrepancy must be attributed to the samples used with different purity and also to experimental techniques. Also changes in polymorphic behavior of pure TAG for bulk or solvent crystallization were reported. In this context it is reasonable to think that other TAGs present in these stearins such as POSt, StOA, StOB, which also crystallized in these conditions, may modify StOSt polymorphic behavior. One of the consequences of TAG interactions is the difficulty of β_1 and β_2 forms to crystallize in the selected conditions, especially β_2 form. Since β_2 form is the polymorphic form required for chocolate manufacture, the polymorphic behavior of these complex systems will involve technological challenges.

The *d* values of long spacings of StOSt polymorphs were not in agreement with the ones of HSHO sunflower oil stearins. Following Sato's approach, the chain length structure was considered by taken into account the length of glycerol bone, and stearic and palmitic lamellae (Sato et al., 1989). α, β'_2, and β'_1, polymorphic forms of soft and hard stearins had arrangements in double chain. On the contrary, β_2 and β_1 form had triple chain structure. In StOSt, however, the α form showed double chain structure while the other forms (more stable) showed triple chain lamellae. In POP, the double chain structure is revealed in α, pseudo-β'_2 and pseudo-β'_1. The other forms, γ, δ, β_2 and β_1, are triple chain arrangements. POSt showed double chain arrangement for the α form and triple chain for δ, pseudo-β' and β form (Arishima et al., 1991). BOB presented triple chain structure in the pseudo-β' and β_2 forms (Hachiya, Koyano, & Sato, 1989). From the data reported in literature, in a pure TAG, the different polymorphic forms had different interlamellar distances which do not correlate with the type of molecule symmetry or the chain length of fatty acids in molecule. Hard and soft stearins presented TAG with A and B acids which are saturated fatty acids with longer carbon number than St acid (chains of 20 and 22 carbons, respectively). StOA and StOB TAGs have not been described yet. Natural fats such as cocoa butter showed a behavior that does not exactly match the one of StOSt or POSt which (depending on the origin) are present in percentages around 27–31% and 41–45%, respectively, and are the

major components. As in the case of sunflower oil stearins, cocoa butter polymorphic forms α, β'_2, and β'_1 showed double chain structure while β_2 and β_1 triple chain arrangement. The differences in polymorphic behavior between stearins and pure TAGs indicated that interactions among TAGs in the complex systems were very relevant and showed that crystallization was also affected by TAGs present in lower percentages such as StOP, StOA and StOB (Table 2).

3.5. Crystal morphology

Fig. 5 shows representative images for microstructure of α, β'_2, and β'_1 polymorphs of hard stearin and Fig. 6 reports microstructure for the same polymorphic forms of soft stearin. In agreement with WAXS and SAXS patterns Figs. 5a and 6a showed no crystallization at the moment the samples reached crystallization temperature indicating that crystallization took place isothermally at the selected temperatures. After 2 min at 23 °C (Fig. 5b), α crystals of hard stearin were noticeable. They grew and showed a needle shape (Fig. 5c). Crystals were isolated and randomly distributed in the microscope field. After 10 min at 23 °C (Fig. 5d), β'_2 crystals appeared. They were the predominant crystals after 35 min at that temperature (Fig. 5e). β'_2 crystals showed needle agglomeration forming ill-defined structures. On the contrary, the β'_1 crystals formed well-organized geometric big clusters of homogeneous crystals (Fig. 5f). For the same supercooling, soft stearin crystallized more slowly than hard stearin resulting in fewer and bigger crystals. Therefore, the 20× objective of the polarized-light microscope was selected to follow crystallization. The α form crystals were also needle shaped (Fig. 6b). Microstructure of the β'_2 form was

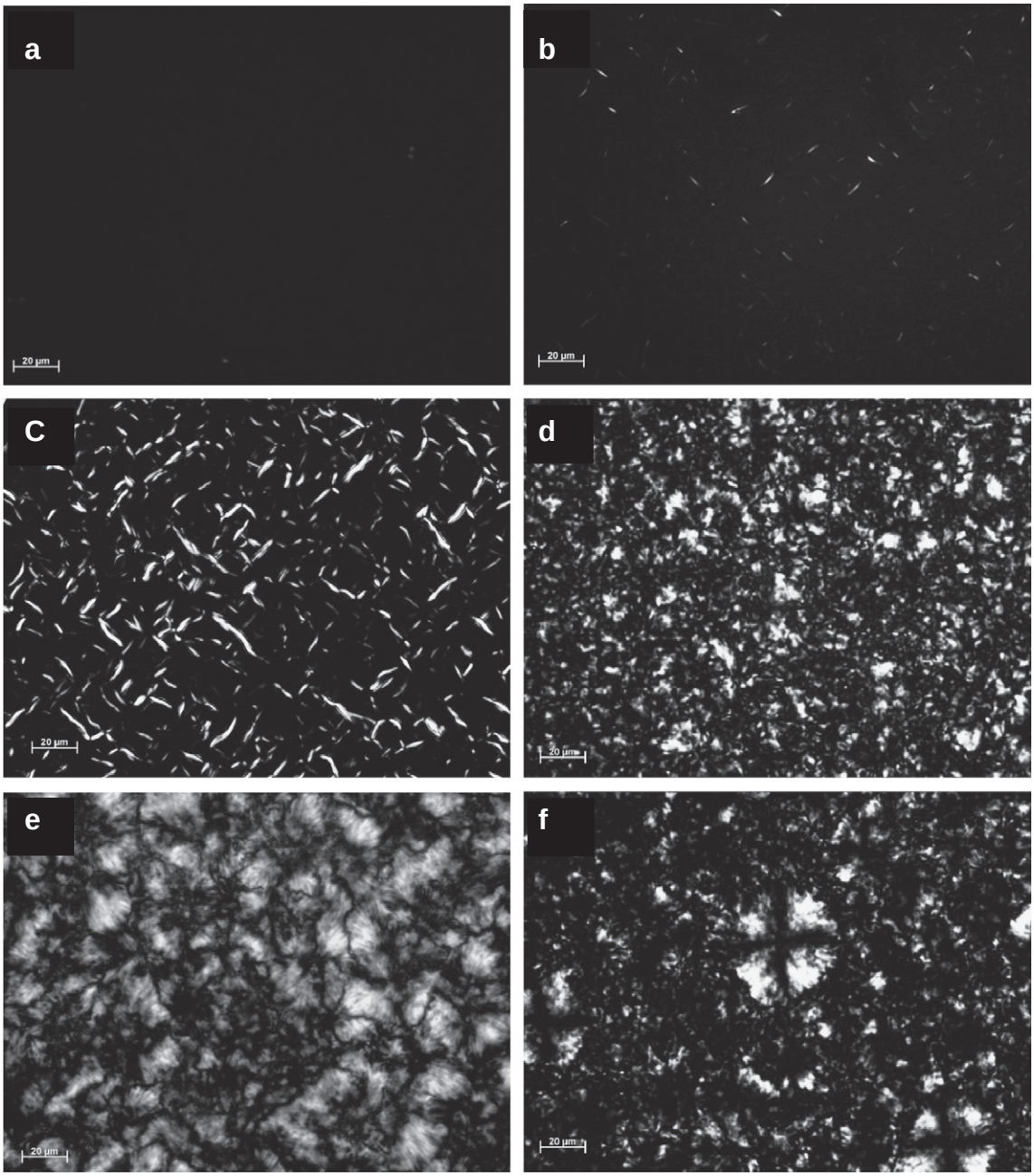

Fig. 5. Representative polarized-light microscopy (PLM) images of polymorphic forms of hard stearin crystallized at 23 °C for 80 min. (a) 0 min, (b) 2 min, (c) 6 min, (d) 10 min, (e) 35 min, and (f) 90 min.

completely different from the one of hard stearin (Fig. 6c and d). Needles agglomerated forming well-defined spherulites. The soft stearin β'_1 form (Fig. 6e and f) had the same morphology as the hard stearin β'_1 form showing well-organized clusters.

Fig. 7 shows a PLM image of hard stearin crystallized at 10 °C/min and stored for 10 h at 25 °C. Image shows 4 different type of crystals: the needles on the background corresponding to the α form, a very well organized β'_1 crystal (on the left), a spherulite corresponding to the β_2 form (right), and β_1 crystals (center). In agreement with X-ray results, this image strongly suggests that polymorphic forms of hard stearin co- existed in the selected conditions which could cause difficulties during processing or storage of final product. It also suggests that these systems fractionated and did not behave as a pure compound in the selected conditions.

3.6. DSC data

3.6.1. Hard stearin

Fig. 8a shows the melting thermograms of hard stearin crystallized under the same temperature/time conditions as X-ray experiments. Table 4 summarizes peak melting-temperature and melting enthalpies of samples shown in Fig. 8a. Fig. 3a shows that the main polymorphic form of hard stearin after 50 min at 10 °C was the β'_2 form. However, trace amounts of the α form were also present. Fig. 8a shows only one melting peak for hard stearin crystallized in those conditions. Its temperature was 29.8 ± 0.1 °C. For the other temperatures (23, 24, and 25 °C) the main polymorphic form was the β'_1. DSC diagrams had a peak with a temperature of 31.9 ± 0.2 °C. It may be assumed that 29.8 ± 0.1 °C and 31.9 ± 0.2 °C were the peak

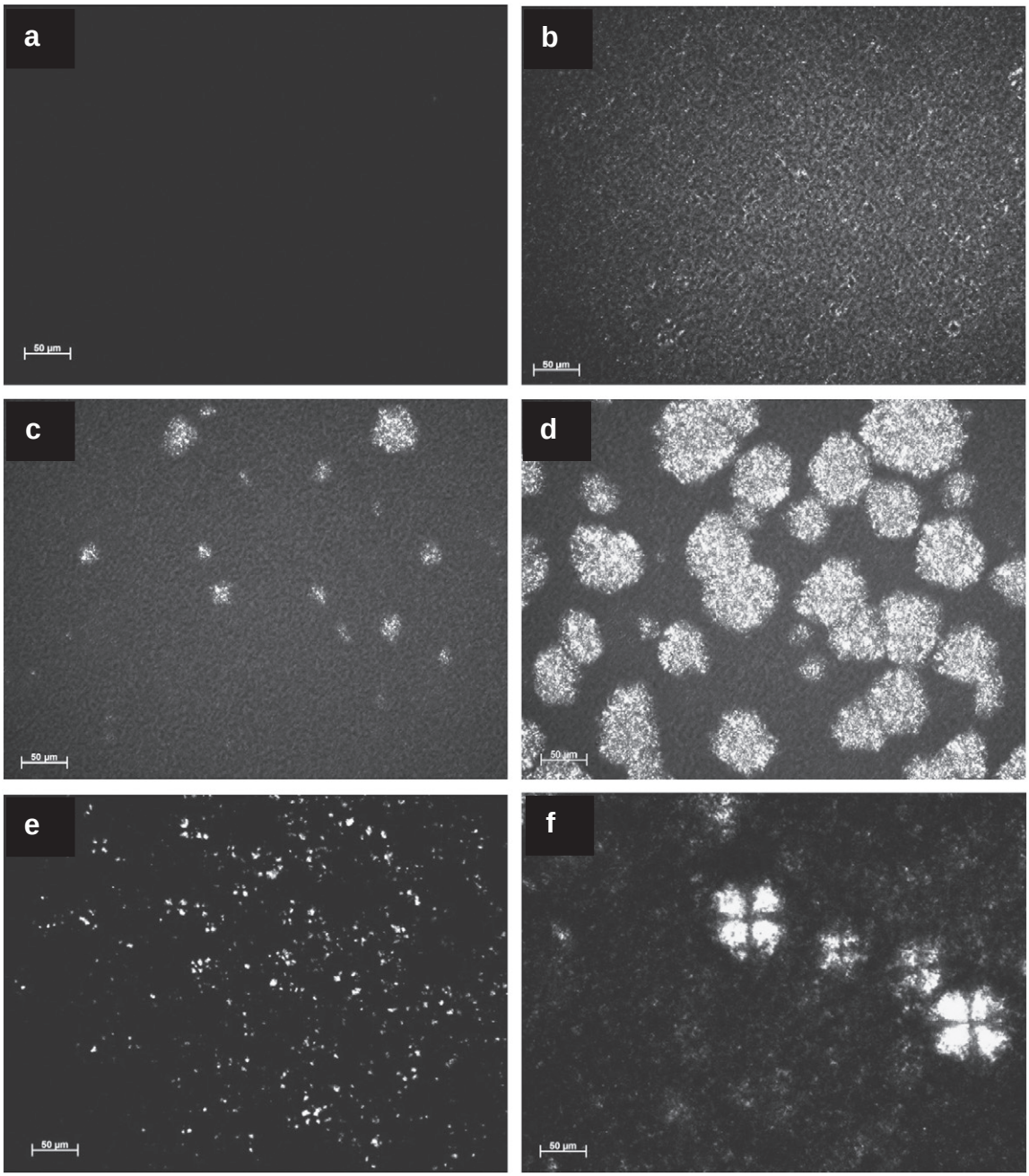

Fig. 6. Representative polarized-light microscopy (PLM) images of polymorphic forms of soft stearin crystallized at 15 °C for 80 min: (a) 0 min, (b) 3 min, (c) 12 min, (d) 75 min, and at 16 °C for 80 min: (e) 15 min, and (f) 72 min.

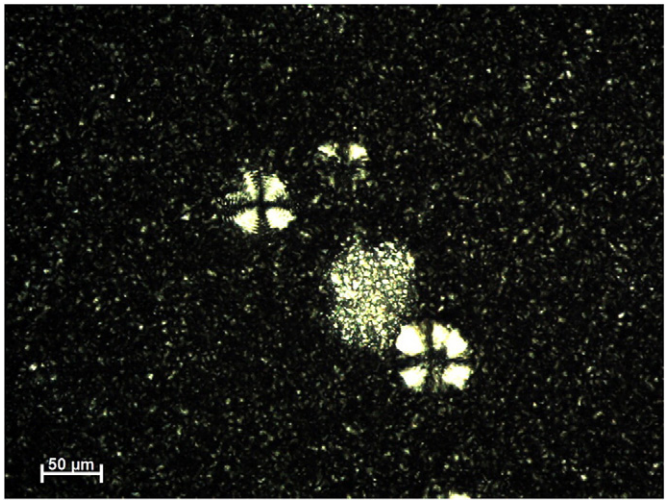

Fig. 7. Representative polarized-light microscopy (PLM) images of polymorphic forms of hard stearin crystallized at 10 °C/min after 10 h of storage at 25 °C.

melting-temperatures of β'_2 and β'_1 forms, respectively. When hard stearin was stored at 10 °C for 48 h, peak melting-temperature was 37.5 ± 0.5 °C and the total enthalpy was 124.1 ± 2.4 Jg^{-1}. After 48 h of storage at 10 °C, peak melting-temperature and total melting enthalpy were significantly higher than the values obtained for hard stearin isothermally crystallized at 10 °C for 50 min. As SFC values for stored and isothermally crystallized hard stearin were within the experimental error, higher peak temperature and enthalpy may indicate the formation of one of the β polymorphs during storage. WAXS and SAXS patterns obtained after 48 h to 2 months of crystallization at 10 °C showed a strong signal corresponding to *d* of 6.49 and 0.46 nm, respectively, which characterized the β_1 polymorph. X-ray studies performed at times from 6 to 24 h showed that β_2 and β_1 forms co-existed in hard stearin. β_2 form was characterized by a strong long spacing at 6.06 nm and a strong short spacing at 0.46 nm. To obtain only the β_2 form, hard stearin was crystallized using the following cooling/reheating process: sample was melted at 60 °C, kept at 60 °C for 15 min, then cooled at 1 °C/min to 26 °C, kept at 26 °C for 90 min, heated from 26 to 32 °C at 1 °C/min, kept at 32 °C for 30 min, cooled from 32 to 26 °C at 1 °C/min, kept at 26 °C for 30 min, and finally heated from 26 to 80 °C at 1 °C/min. DSC melting thermogram obtained after the same temperature/time cycles had an endotherm with a peak temperature of 34.2 ± 0.5 °C that corresponded to the β_2 melting temperature. According to the literature, the normal state of cocoa butter in chocolate is form V (β_2). There is a general agreement that bloom has not been observed for pure form V and does not exists in the absence of form VI (β_1). Therefore, the processing conditions selected for obtaining β_2 and β_1 forms are very relevant for product manufacture since β_2 form is required in chocolate applications and β_1 polymorphs is the form needed to avoid.

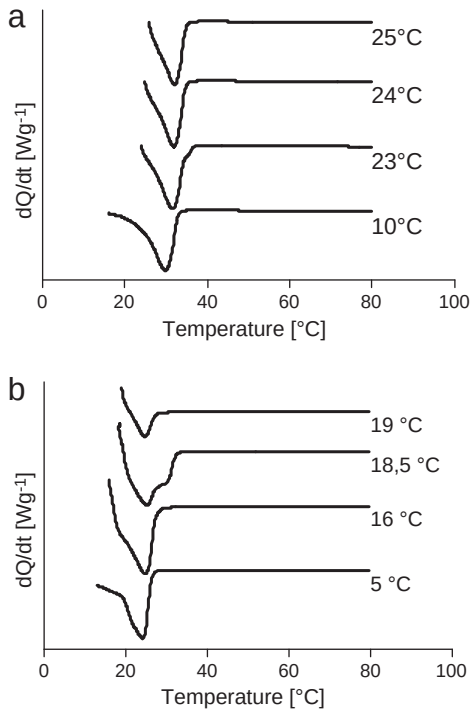

Fig. 8. DSC melting thermograms of a) hard stearin and b) soft stearin, after isothermal crystallization for the same times as in X-ray studies.

3.6.2. Soft stearin

Fig. 8b reports melting behavior for soft stearin. Table 4 also summarizes peak melting-temperature and melting enthalpies of samples in Fig. 8b. According to Fig. 4a, the β'_2 form was the predominant form for soft stearin after 50 min at 5 °C. Peak temperature in melting curve was 24.2 ± 0.2 °C (Fig. 8b). At 16, 18.5, and 19 °C the main polymorphic form was β'_1. Although some fractionation may be observed in DSC diagrams, it may be assumed that the main peak temperature corresponded to β'_1 melting temperature. The average main peak temperature of the three runs (16, 18.5, and 19 °C) was 25.0 ± 0.3 °C. Therefore, 24.2 ± 0.2 and 25.0 ± 0.3 °C may be considered β'_2 and β'_1 HSHO sunflower oil soft stearin melting temperatures, respectively. When soft stearin was stored at 5 °C for 48 h, the melting temperature of the main peak was 32.5 ± 0.5 °C and the total melting enthalpy was 53.4 ± 3.0 Jg^{-1}. Peak temperature was significantly higher than the ones obtained for isothermal crystallization indicating a possible formation of a β polymorph. As in the case of hard stearin, the melting enthalpy of soft stearin crystallized isothermally at 5 °C for the same time at crystallization temperature as in X-ray studies (50 min) was significantly lower than the one obtained after 48 °C of storage at 5 °C. However, the SFC as measured by NMR was within the experimental error. This also suggested the formation of a β polymorph. WAXS and SAXS patterns of stored sample had a very strong spacing peak corresponding to a *d* of 0.46 and 6.48 nm, respectively, indicative of the β_1 form, confirming that this form crystallized during storage. It was reported that the direct crystallization of the phase V of cocoa butter was associated with very long induction times rendering it unsuitable for manufacturing environments using continuous processing (MacMillan et al., 2002). Thus, in production environments the fat is tempered (by analogy with Austenitic steel making) through quenching to the temperature region associated with one of the lower polymorphic phases (forms II/III) and then reheated to phase interconvert the crystals back to the required phase V. HSHO sunflower oil stearins showed a similar behavior. The β_2 form was not obtained after at least 6 h at crystallization temperature for all selected temperatures when a 10 mg sample was stored in an aluminum pan. After a simple cooling, β_2 and β_1 forms were always obtained together. To isolate the β_2 form from β_1 form it was necessary to applied a cooling reheating process to the sample. For soft stearin it was as follows: sample was melted at 60 °C, kept at 60 °C for 15 min, then cooled at 1 °C/min to 20 °C, kept at 20 °C for 90 min, heated from 20 to 26 °C at 1 °C/min, kept at 26 °C for 30 min, cooled from 26 to 20 °C at 1 °C/min, kept at 20 °C for 30 min, and finally heated from 20 to 80 °C at 1 °C/min. DSC melting thermogram obtained after the mentioned program showed an endotherm with a peak temperature of 28.9 ± 0.4 °C. As β_2 was the only form present in those processing conditions, it may be assumed that this is the melting temperature of form β_2.

Table 4
Peak Temperatures and Melting Enthalpies of Soft and Hard High Stearic High Oleic (HSHO) Sunflower Oil Stearins Isothermally Crystallized at Different Temperatures for the Same Times as for X-Ray Studies.

Soft stearin			Hard stearin		
Crystallization temperature (°C)	Peak temperature (°C)	Total melting enthalpies (Jg^{-1})	Crystallization temperature (°C)	Peak temperature (°C)	Total melting enthalpies (Jg^{-1})
5.0	24.2 ± 0.2	45.2 ± 2.5	10.0	29.8 ± 0.1	119.2 ± 6.7
16.0	24.7 ± 0.1	46.9 ± 3.1	23.0	31.6 ± 0.1	106.2 ± 5.4
18.5	25.3 ± 0.2	35.1 ± 2.7	24.0	32.0 ± 0.2	95.4 ± 5.1
19.0	24.9 ± 0.2	8.0 ± 1.3	25.0	32.0 ± 0.2	88.0 ± 4.2

As shown in Table 4 crystallization enthalpy decreased more than expected from 18.5 to 19 °C. There was only a temperature difference of 0.5 °C but the reduction in enthalpy was 77%. The equilibrium SFC at 19 °C as measured by p-NMR was 28.05 ± 0.48%. Under isothermal conditions, SFC was 11.30 ± 0.31% after 4 h at that temperature, significantly lower than equilibrium SFC. Most likely at this temperature, more stable polymorphic forms, β_2 or β_1 forms, should have crystallized but as it has a very long induction time it did not appear even after 4 h of isothermal crystallization and therefore the SFC was still very low at that time. Isothermal behavior of soft stearin was similar to the one reported for cocoa butter indicating that in order to use this fat in a continuous production line it should be tempered, crystallized with addition of emulsifiers or crystallized applying ultrasound treatment to promote crystallization.

The β_2 form is unstable and it is difficult to obtain it isolated from other polymorphic forms. It was reported that cupuassu fat, which three major components were StOSt, POSt, and StOO but also had amounts of StOA around 10%, had two stable thermal states labeled β_2 and β_1 in order of increasing temperature (Silva et al., 2009). These cupuassu β phases were obtained keeping the sample at 25 °C for 1 h and 40 min and 5 days, respectively. Their melting temperatures were similar to those of β_2 and β_1 phases of cocoa butter. However, the structural parameters of the cupuassu fat for the states labeled β_2 and β_1 were very similar. Thus, there was no completely proof of a $\beta_2 \rightarrow \beta_1$ transition in cupuassu fat. The β_1 phase seems to be more stable than cocoa butter form VI, suggesting that the phenomenon of "blooming" could occur more easily in products manufactured with total or partial substitution of cocoa butter by cupuassu fat, since this effect is related to crystallization of the β_1 phase. A similar behavior may be expected for HSHO sunflower oil stearins indicating that processing conditions must be controlled very carefully during chocolate production.

4. Conclusions

This work represents a valuable contribution to food science and technology since there is a great potential for the use of HSHO sunflower oil stearins as *trans* fat replacers in the bakery industry or as cocoa butter equivalent (CBE) in confectionary. However, due to the complex polymorphic behavior of these systems processing conditions must be controlled very carefully. Three polymorphic forms were observed for these stearins under isothermal crystallization: α, β'_2, and β'_1. If intended as trans fat replacer in spreadable products, stearins should be crystallized at fast cooling rate (10 °C/min) to obtain β'_2, and/or β'_1 forms. The β forms did not crystallize under the isothermal conditions (temperature/time) selected for this study. They were obtained after storage for longer times at the selected temperatures. The β_2 form, which is the required polymorph for chocolate manufacture, was only obtained isolated from β_1 form by applying cooling/reheating cycles. The behavior of these complex systems did not exactly match the one of their major components: different polymorphic forms with different long term arrangement were found. However, their behavior was more similar to the one of cocoa butter. Although all the polymorphs of SOS, with the exception of the α form, crystallize in triple chain structure, the β' forms of these stearins showed double chain length structure indicating that TAGs interactions were very relevant to polymorphic behavior.

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