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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,