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Analysis of co-crystallized free phytosterols with triacylglycerols as a functional food ingredient

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ABSTRACT

This research focuses on the analysis of mixtures of free phytosterols (FPSs) with fully hydrogenated soybean oil (FHSO):soybean oil (SO) mixtures as a potential zero-*trans* substitute for various types of shortenings. Oil binding capacity as well as the thermal, rheological and structural properties of FHSO:SO blends containing 0, 20 and 25 wt.% β-sitosterol or stigmasterol were investigated in this study. Differential interference contrast (DIC) microscopy and wide angle X-ray diffraction (WAXRD) revealed that co-crystallization of FPSs with FHSO:SO blends occurred. Polymorphic forms were characterized as a mixture of β′ and β for all samples. The addition of FPSs decreased oil loss (OL) of FHSO:SO samples. Melting profiles of the prepared FPS–TAG (triacylglycerol) blends were extended to higher temperatures compared to a commercial shortening. Rheological properties were comparable to those of commercial puff pastry shortening suggesting that FPS–TAG blends may be acceptable for bakery applications. FPSs co-crystallized with FHSO and SO may be a suitable *trans*-fat free substitute for a number of types of shortening, including puff pastry shortening. The manufacturing of co-crystallized /FPS-TAG matrices will possibly bring large economic benefits as their functionalization can potentially be achieved by using existing simple shear processing.

1. Introduction

Phytosterols are found in all plant foods, with the highest concentrations occurring in vegetable oils (Ostlund, 2002). The general term 'phytosterols' describes plant-derived sterols and stanols with a chemical structure related to cholesterol and having a different side chain configuration (Cantrill, 2008; Spitzer & Maggini, 2013). It has been recognized that high consumption of plant sterols can reduce serum total and LDL cholesterol concentrations in humans (Ostlund, 2002; Piironen, Lindsay, Miettinen, Toivo, & Lampi, 2000); therefore reducing the risk of cardiovascular disease. Phytosterols displace cholesterol from mixed micelles decreasing cholesterol absorption in the intestine (Nissinen, Gylling, Vuoristo, & Miettinen, 2002). Recent research has suggested that plant sterols may have additional biological activities different than cholesterol lowering, such as anti-inflammatory and anti-cancer effects, and changes in cell membrane properties, among others (Awad, Roy, & Fink, 2003; Halling & Slotte, 2004; Navarro, De las Heras, & Villar, 2001; Ratnayake et al., 2000; von Holtz, Fink, & Awad, 1998). In previous studies, an intake of 1–3 g/day of plant sterols has been shown to produce a 10–15% reduction in LDL cholesterol (Kritchevsky & Chen, 2005; Kuhlmann et al., 2005; Ling & Jones, 1995). Nevertheless, the typical Western diet today contains only 150–400 mg/d (Ostlund, 2002). It is evident that a greater intake of phytosterols is necessary to compete for absorption against cholesterol and thus, supplemental doses may be required. Several studies have suggested that the LDL-cholesterol-lowering effect of plant sterols reaches a maximum at doses of 2–3 g/day (Katan et al., 2003; Law, 2000; Musa-Veloso, Poon, Elliot, & Chung, 2011; Ras, Geleijnse, & Trautwein, 2014). For that reason, many health authorities have included 2 g/day PS as an optimal dose in their diet and lifestyle guidelines to manage hypercholesterolemia (American Heart Association Nutrition Committee et al., 2006; International Atherosclerosis Society (IAS) Executive Board, 2013; Gylling et al., 2014).

The enrichment of foods with PSs is challenging from a technological and food quality standpoint, since PSs are insoluble in water and poorly soluble in dietary fats (Salo & Wester, 2005). Therefore, for commercial use, phytosterol and stanol powders are esterified with fatty acids in vegetable oils; a process that allows manipulation of the physical properties of these high melting powders. The characteristics of the esters are similar to edible fats and oils and can be classified as liquid or semi-liquid (Cantrill, 2008). The phytosterol fatty acid esters are incorporated into processed foods such as spreads, juices, oils, and other foods. Therefore, until recently, the majority of studies involving phytosterols or phytostanols have been strictly on the use of esterified phytosterols or phytostanols. There have been few studies addressing the

Abbreviations: FHSO, fully hydrogenated soybean oil; SO, soybean oil; FPS, free phytosterol; β-Sit, β-sitosterol; Stig, stigmasterol; DSC, differential scanning calorimetry; NMR, nuclear magnetic resonance; WAXRD, wide angle X-ray diffraction; DIC, differential interference contrast; OL, oil loss.

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use of free (non-esterified) phytosterols and phytostanols in commercial foods due to their limited solubility and disputes on bioavailability as compared to their esterified counterparts (Hayes, Pronczuk, & Perlman, 2004). However, researchers have reported that when free phytosterols are effectively heated and then recrystallized in fat upon cooling, they become bioavailable and therefore effective in reducing cholesterol absorption (Hayes et al., 2004; Perlman, Hayes, & Pronczuk, 2006). The utilization of non-esterified phytosterols in fat, by recrystallization, would lower the cost and increase convenience of processing to make previously insoluble non-esterified phytosterols bioavailable and soluble in dietary fats (Hayes et al., 2004). Previous strategies of phytosterol utilization include ultrafine powders, chemically modified esterified phytosterols, emulsified phytosterols, and phytosterols in water–oil microparticulate suspensions which are very susceptible to oxidation (Perlman et al., 2006).

Today, there is still a need to develop a stable solid or semisolid food product incorporating free plan sterols. Hence, the justification of this research relies, in part, on the health benefits of phytosterol supplementation as a solid or semisolid ingredient, specifically chemically unmodified free phytosterols to reduce production cost in industry, as well as increase convenience in processing. On the other hand, due to current recommendations to eliminate *trans* fats, there has been a growing demand in the last few years to find appropriate semi-solid fat substitutes that do not increase the risk for CVD (Hunter, Zhang, & Kris-Etherton, 2010). Fully hydrogenated fats have been the focus of studies as a probable replacement of *trans* fatty acids containing fats (Acevedo, Block, & Marangoni, 2012; Acevedo & Marangoni, 2014; Guedes et al., 2014; Ribeiro, Grimaldi, Gioielli, dos Santos, Cardoso & Gonçalves, 2009; Ribeiro, Grimaldi, Gioielli & Gonçalvesa, 2009). Therefore, the purpose of this study was to prepare and evaluate physical blends of fully hydrogenated soybean oil (FHSO) and liquid soybean oil (SO) enriched with free phytosterols, with a view to assessing prospective applications in food products. Our novel approach encompasses the manufacture of free phytosterols (FPSs) and TAG based on their co-crystallization. We hypothesize that this strategy will effectively entrap and protect free-phytosterols in fat processed foods. FPS–TAG blends with 20 wt.% and 25 wt.% β-sitosterol or stigmasterol content were prepared and analyzed in order to meet the recommended daily intake to decrease blood cholesterol with one serving of the blend produced. For example the consumption of one bakery product would be sufficient to achieve the desired effect. In the present study a commercial puff pastry shortening, which contains large amount of *trans* fats was specifically selected as an example of the physico-chemical properties required for use in food manufacturing.

2. Materials and methods

2.1. Materials

Fully hydrogenated soybean oil (FHSO) and liquid soybean oil (SO) were generously donated from ADM oils (Decatur, IL). β-Sitosterol powder (purity ≥70%) was obtained from Sigma-Aldrich (St. Louis, MO). Stigmasterol powder (purity >90%) was obtained from TCI America (Portland, OR). Super Bowl® puff pastry shortening consisting of partially hydrogenated soybean oil and cottonseed oils with artificial flavor and artificial colors was generously provided by Stratas Foods (Memphis, TN).

2.2. Blend preparation

All samples consisted of 48 wt.% solids and 52 wt.% liquid oil. For free phytosterol-triacylglycerol (FPS–TAG) blends, the solid material consisted of either 20 wt.% or 25 wt.% FPS (β-sitosterol or stigmasterol) and 28 wt.% or 23 wt.% FHSO (totaling 48 wt.%); the 52 wt.% liquid portion consisted of soybean oil. The control sample contained 0 wt.% FPS, 48 wt.% FHSO, and 52 wt.% soybean oil. Blends were prepared by heating the mixture up to ~180 °C and agitating with a Caframo Real Torque Digital overhead stirrer (Ontario, Canada) at 200 rpm. Once the mixtures were clear, they were held at 180 °C for additional 20 min to ensure that the crystal memory was erased. Subsequently, all samples were cooled at room temperature until ~20 °C was reached and once crystallization was complete they were stored at 4 °C until use in analysis.

2.3. Wide angle X-ray diffraction (WAXRD)

Wide angle X-ray diffraction (WAXRD) patterns of crystallized FPS, FPS–TAG blends, and control fat blends were measured using a Rigaku Ultima (Rigaku, Japan) IV X-ray diffractometer. The operating conditions during experiments were 44 mA and 40 kV. The angular range using a 10 mm slit was from 1 to 30° (2θ) with steps of 1°, and the measuring time was 1 min/step. XRD patterns were analyzed with MDI Jade 9.0 software (Rigaku, Japan). In this study, three replicates of each sample were performed.

2.4. Differential scanning calorimetry (DSC)

The thermal properties of the samples were measured by differential scanning calorimetry (DSC) using a Perkin Elmer Diamond DSC (Shelton, CT, USA). Heat flow calibration was made by reference to the known melting enthalpy of indium (purity >90%). Temperature calibration was made with zinc (purity >90%). Approximately 10 mg of sample was placed in aluminum pans and sealed hermetically, an empty pan served as a reference. All measurements were performed at a heating rate of 10 °C/min. Thermograms were analyzed using Pyris Series Diamond DSC 9.0 software (Shelton, CT, USA). The peak melting temperature (T_m) and the enthalpy of melting (ΔH_m) were determined. The reported data corresponds to the average and standard deviation (STD) of three replicates for each sample.

2.5. Proton-nuclear magnetic resonance (^{1}H-NMR)

Solid fat content (SFC) was analyzed by proton nuclear magnetic resonance (^{1}H-NMR) by using a Bruker MiniSpec Bench Top NMR (Billerica, MA, USA). Crystallized samples stored at 4 °C were introduced into NMR glass tubes, then incubated for 30 min at 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 °C to allow for a homogenous distribution of temperature at the time of measurement. The reported data correspond to the average and standard deviation (STD) of three replicates for each sample.

2.6. Oil loss determination (OL)

Once crystallized, oil loss (OL) experiments were performed according to previously described techniques (Acevedo et al., 2012; Dibildox-Alvarado, Rodrigues, Gioielli, Toro-Vazquez, & Marangoni, 2004). Blends were molded into PVC molds of 35 mm diameter and 3.2 mm thickness to form disks that were then transferred to filter papers (Whatman #1, 125 mm diameter). The amount of oil lost over time was determined by the difference in weight of the filter papers before and after placing the disk on the paper for the designated time at 20 °C. A "blank" filter paper was included in all experiments to account for differences in possible humidity of the storage environment. Filter papers were large enough to avoid paper saturation with oil during the experiment. The reported data correspond to the average and standard deviation of at least five replicates, each separate disk on an individual filter paper. Oil loss (%) was calculated according to previously described technique as follows:

$$\mathrm{OL}(\%) = \frac{\mathrm{wt.paper(X\ h)} - \mathrm{wt.paper\ (0\ h)}}{\mathrm{wt.paper\ (0\ h)}} \times 100.$$