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Optimization of Solid Fat Content and Crystal Properties of a *trans*-Free Structured Lipid by Blending with Palm Midfraction

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The optimization of solid fat content (SFC) and crystal properties of *trans*-free structured lipids (SL) synthesized by incorporating stearic acid into canola oil was investigated. The SLs were blended with varying amounts of palm midfraction (PMF). The SFC and crystal polymorphism were improved. The addition of sucrose stearate (S-170), sorbitan tristearate (STS), and distilled monoglycerides (DMG) to one of the blends, SL40:PMF (70:30, w/w), did not improve crystal polymorphism but had significant effects on crystal morphology. The emulsifiers significantly delayed crystal growth, resulting in smaller crystal sizes as compared to the control. They were unable to inhibit the formation of granular crystals (30−140 μm), which are undesirable in margarine, after 4 weeks of storage at 0 °C. Blends treated with S-170 and STS showed many small evenly distributed crystals interspersed with large crystal aggregates (after 4 weeks of storage), whereas the blend treated with DMG and the control showed irregularly shaped globular crystals, also interspersed with large crystal aggregates. However, these crystal aggregates were not observed upon visual and physical examination and may therefore not impart the sensory properties of the finished products negatively.

KEYWORDS: Distilled monoglycerides; palm midfraction; trans-free structured lipid; sorbitan tristearate; sucrose stearate

INTRODUCTION

Physical properties such as consistency, solid fat content (SFC), melting profile, crystal polymorphism, and morphology are related to sensory properties, and subsequently, consumer acceptance of margarines. These physical properties depend on the triacylglycerol (TAG) composition, the level of liquid oil present, and temperature fluctuations during storage (*1*). In designing *trans*-free structured lipids (SLs) as alternatives to conventional fats used for margarine formulation, it is imperative that these *trans*-free alternatives possess desirable physical and sensory properties. Previous studies in our laboratory (*2*) have shown that *trans*-free SLs made by incorporating stearic acid into canola oil did not have desirable SFCs and crystal types for tub margarine formulations.

Palm oil and its fractions, including palm midfraction (PMF), have become a major source of fat for the margarine industry because of the number of desirable properties that they impart to the finished product, including high oxidative stability and plasticity at room temperature. PMF is the two-stage fractionation product of palm oil. It is characterized by a high disaturated TAG content (>60%) and a low monosaturated TAG content (<30%), and has a short melting range, which makes it suitable for use in cocoa butter equivalents and margarines (*3*). However, the use of palm oil fractions in blends for the manufacture of margarine has some problems due to poor crystallization properties such as low rate of nucleation and the formation of granular crystals during storage (*4*). These granular crystals are responsible for the sandy mouthfeel of margarine products and are formed by the segregation of 1,3-dipalmitoyl-2-oleoyl glycerol (POP) from other TAG crystals and their eventual polymorphic transition from β' to β forms (*1*). This phenomenon is also dependent on a number of factors such as the level of liquid oil present, the presence of specific TAGs, such as 1,3-dipalmitoyl-2-stearoyl glycerol (PSP) and 1,3-dipalmitoyl-2-elaidoyl glycerol (PEP), and temperature fluctuations during storage. Maintenance of the β' polymorphic form is therefore important in preserving the smooth texture and easy spreadability of margarine. This is accomplished by the inclusion of β'-tending fats in blends and/or by using emulsifiers such as distilled monoglycerides (DMGs), sucrose stearate (S-170), and sorbitan tristearate (STS) that hinder or retard polymorphic transition (*5*). The aim of this study, therefore, was to optimize the SFC, crystal form, and morphology of the *trans*-free SLs synthesized in our previous study (*2*) by blending with PMF and by using emulsifiers. The inclusion of the three emulsifiers in the SL:PMF blends was expected to hinder the growth of undesirable crystal aggregates.

MATERIALS AND METHODS

Stearic acid was purchased from Sigma Chemical Co. (St. Louis, MO). Canola oil (peroxide value, 0.0 mequiv/kg; acid value, 0.28%)

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Table 1. Composition of SL:PMF Blends

blend	composition (wt %)
A	SL40:PMF (80:20)[a]
B	SL40:PMF (70:30)
C	SL40:PMF (60:40)
D	SL40:PMF (50:50)
E	SL50:PMF (80:20)
F	SL50:PMF (70:30)
G	SL50:PMF (60:40)
H	SL50:PMF (50:50)

[a] Abbreviations: SL40, SL made by reacting 40% stearic acid with canola oil; and SL50, SL made by reacting 50% stearic acid with canola oil.

was bought from a local grocery store. PMF (peroxide value, 1.0 mequiv/kg; acid value, 0.1%; iodine value, 43−48) was donated by Fuji Vegetable Oil Inc. (Savannah, Georgia). Immobilized Lipozyme RM IM was purchased from Novo Nordisk A/S (Bagsværd, Denmark). Organic solvents and chemicals were purchased from J. T. Baker Chemical Co. (Phillipsburg, NJ) or Fisher Scientific (Norcross, GA). All other chemicals used were of analytical or high-performance liquid chromatography (HPLC) grade. STS with a hydrophile/lipophile balance (HLB) of 2.1 and DMGs with HLBs of 3.8 were donated by Danisco A/S (Copenhagen, Denmark), and S-170 with a HLB of 1 was supplied by Mitsubishi-Kasei Food Corp. (Tokyo, Japan).

SL synthesis was performed in a stir-batch reactor at 65 °C for 12 h. The reaction mixture typically contained 300 g of canola oil, 40−50% stearic acid (by weight of canola oil), and 10% Lipozyme RM IM (by total weight of reactants). The SLs synthesized with 40 and 50% stearic acid were designated SL40 and SL50, respectively. The product was separated from the enzyme by suction filtration, and free fatty acids were removed by short-path distillation with a KDL-4 (UIC Inc., Joliet, IL) unit under the following conditions: heating oil temperature, 185 °C; cooling water temperature, 55 °C; pump vacuum, < mm Hg; and feed rate, maintained at 100 mL/h. The reaction product was passed through the system twice to reduce the free fatty acid percentage to an acceptable level. The free fatty acid content (0.12−1.05% oleic acid) was determined according to AOCS Official Method, Ca 5a-40 (*6*). A series of blends of the two SLs (SL40 and SL50) containing from 20 to 50% PMF were prepared and analyzed to determine their suitability for margarine formulation in terms of SFC. The composition of the blends is given in **Table 1**.

SFCs of samples were determined according to AOCS Official Method Cd 16-81 (*7*) on a MARAN-20 pulsed NMR spectrometer (Resonance Instruments Ltd., Oxon, United Kingdom). Samples were tempered at 100 °C for 15 min and then kept at 60 °C for 10 min, followed by 0 °C for 60 min and finally at 30 min at each temperature of measurement. The SFC was measured at intervals of 5 °C, from 5 to 45 °C.

Powder X-ray diffraction (XRD) was used to study TAG crystal polymorphism using an ARL Scintag XDS 2000 automated diffractometer (Ecublens, Switzerland). The diffractometer had 2θ configuration, and the generation power was set at 30 KV and 25 mA. The scan range was from 21 to 29°, and the scan rate was 4°/min. The samples were kept at 80 °C for 30 min and then poured into plastic molds and kept at 0 °C for 12 h, after which they were analyzed.

The crystal morphology of the samples was investigated with a Leica DMLB compound microscope (Wetzlar, Germany) equipped with a light polarizer. The microscope was equipped with a digital camera and controlled by a SPOT Basic software. The objective lens was 20×, and the ocular lens was 1×. The temperature of the stage was maintained at 0−5 °C by means of dry ice pellets. Prior to analyses, the samples (5 g) were treated with 2% of S-170, STS, and DMG, kept at 80 °C for 1 h, followed by −20 °C for another 1 h, and finally stored at 0 °C for analysis at 24 h and 4 weeks. The control was a blend of SL40 and PMF (70:30, w/w).

RESULTS AND DISCUSSION

The SFC curve of a fat is a good measure of the spreadability of the finished product. A desirable margarine is one that has at least 7.6% SFC at 10 °C needed to maintain good crystal structure (*8*), easily spreadable once taken out of the refrigerator, and melts completely in the mouth. Complete melting in the mouth ensures the release of flavor and also imparts smooth mouthfeel to the margarine. Fats with moderate SFCs (7.6−13%) at 10 °C and a steep SFC curve at nonrefrigeration temperatures are easily spreadable. The SFC curves of blends containing SLs (SL40 and SL50) and PMF and two commercial margarine fats (MG-A and MG-B) are shown in **Figure 1**. All but one (blend A) of the samples had the minimum SFC (7.6%) at 10 °C needed to maintain good crystal structure, and most of these samples, with the exception of MG-B, were totally melted at 35 °C. Of all of the blends, sample B, a blend of SL40 and PMF (70:30, w/w), had the most desirable SFC curve for tub margarine formulation. It showed moderate SFC (12.4%) at 10 °C and a steep SFC curve at nonrefrigeration temperatures and was completely melted at 30 °C. This blend is expected to impart good spreadability and mouthfeel to the margarine product. Samples D (SL40:PMF, 50:50, w/w) and H (SL50:PMF, 50:50, w/w) had the highest SFC values at 10 °C, showed steep SFC curves at nonrefrigeration temperatures, and were completely melted at 30 and 35 °C, respectively. These two would be suitable for stick margarine formulations. Margarines formulated with samples C (SL40:PMF, 60:40, w/w) and G (SL50:PMF, 60:40, w/w), by the nature of their SFC curves, would have intermediate consistencies relative to margarines formulated with samples B, D, and H.

Equally important, besides SFC, is the crystal habit of the TAG. Fats containing predominantly β' TAG crystals impart

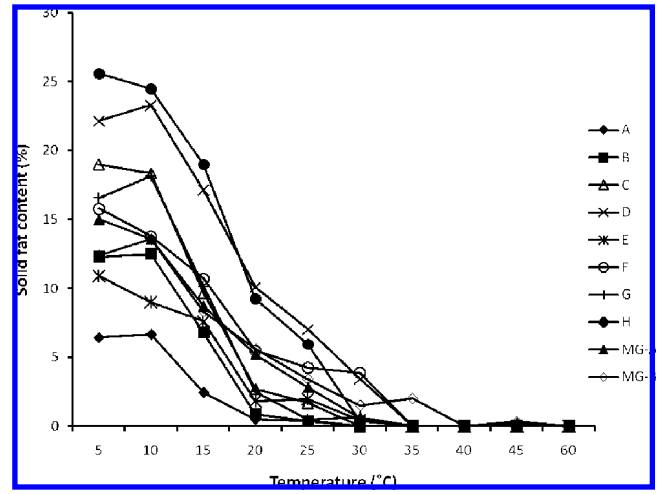

Figure 1. Curves showing SFC of SL and PMF blends (see **Table 1** for compositions of blends) and fat from two commercial margarine products (MG-A and MG-B).

Table 2. Relative Proportion of β and β' Crystals in Each Blend

sample	ratio of intensity at 4.3−4.6 Å	designation
SL50[a]	1.00	β
SL50:PMF (50:50, w/w)	0.51	$\beta' \gg \beta$
SL50:PMF (60:40, w/w)	0.52	$\beta' \gg \beta$
SL40	1.00	β
SL40:PMF (70:30, w/w)[b]	0.56	$\beta' \gg \beta$
2% S-170 in SL40:PMF (70:30, w/w)	0.70	$\beta' > \beta$
2% STS in SL40:PMF (70:30, w/w)	0.66	$\beta' > \beta$
2% DMG in SL40:PMF (70:30, w/w)	0.58	$\beta' \gg \beta$

[a] Abbreviations: SL40, SL made by reacting 40% stearic acid with canola oil; and SL50, SL made by reacting 50% stearic acid with canola oil. [b] SL40:PMF (70:30, w/w) is the control for the emulsifier treatments.

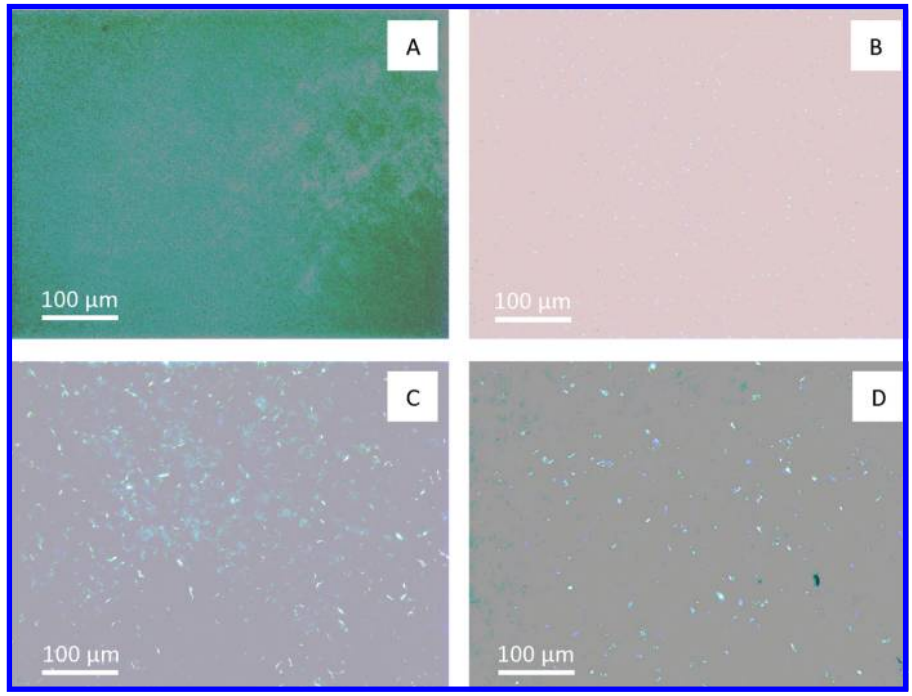

Figure 2. PLM images of SL40:PMF (70:30, w/w) blend treated with (**A**) 2% S-170, (**B**) 2% STS, (**C**) 2% DMG, and (**D**) control, SL40:PMF (70:30, w/w), after 24 h at 0 °C.

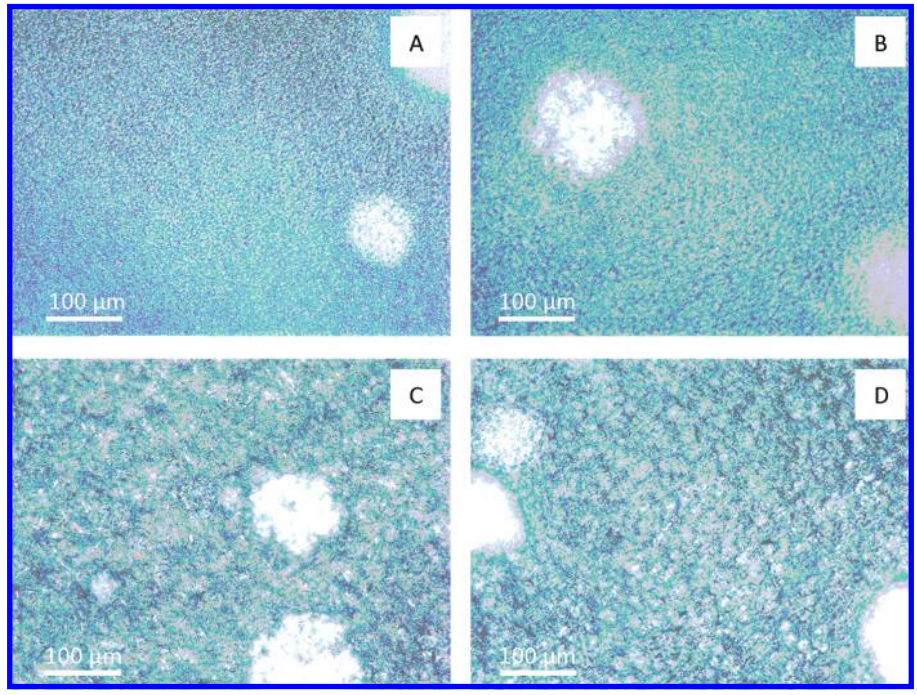

Figure 3. PLM images of SL40:PMF (70:30, w/w) blend treated with (**A**) 2% S-170, (**B**) 2% STS, (**C**) 2% DMG, and (**D**) control, SL40:PMF (70:30, w/w), after 4 weeks at 0 °C.

smooth texture to margarine, whereas those with predominantly β TAG crystals impart grainy texture. Crystal polymorphs are identified by their characteristic *d*-spacings. β' Polymorphs show two strong signals at *d*-spacings of 3.9 and 4.3 Å, whereas β polymorphs show three strong signals at 4.6, 3.9, and 3.8 Å. The relative proportions of β and β' crystals in the blends was calculated by dividing the peak intensity at 4.6 Å (β) by the peak intensity at 4.3 Å (β'). The crystal habits of the samples are given in **Table 2**. The TAG crystals present in SL40 and SL50 were mainly the β polymorphs but became predominantly β' upon blending with PMF. The addition of S-170, DMGs, and STS to the SL40:PMF (70:30, w/w) blend did not improve crystal polymorphism further after 24 h of crystallization. The relative proportions of β to β' crystals were slightly higher in blends treated with S-170 and STS than in the blend treated with DMG and the control (**Table 2**). The β to β' ratio for the blend treated with DMG was very close to that of SL40:PMF (70:30, w/w), the control. These findings suggest that emulsifiers may not always have significant effects on crystal polymorphism. A study by Cerdeira et al. (*9*) showed that TAG composition played a big role in the polymorphic behavior of fats treated with emulsifiers. In that study, sucrose palmitate (P-170) and S-170 had significant effects on the polymorphic behavior of a high melting fraction of milk fat (HMF) but

showed no significant effects when HMF was blended with 60% (w/w) sunflower oil (SFO). Chemical analysis of the TAGs revealed that TAGs with acyl carbon numbers between 36 and 50 decreased upon blending HMF with SFO, while TAGs with acyl carbon number of 54 increased from 3.2% in HMF to 28.3% in the 60% SFO blend.

Figures 2 and **3** show polarized light microscopy (PLM) images of crystal morphologies of blends treated with or without emulsifiers after 24 h and 4 weeks of storage at 0 °C, respectively. The addition of the emulsifiers to the fat blends had significant influence on crystal morphology. The blend treated with S-170 did not show any crystals (**Figure 2A**), whereas small homogeneous crystals were observed in the blend treated with STS (**Figure 2B**). The blend treated with DMG (**Figure 2C**) showed small globular crystals evenly interspersed with needlelike crystal structures. Larger and irregularly shaped crystals were observed in the control (**Figure 2D**), which was a blend of SL40 and PMF (70:30, w/w). The absence of crystals (at the magnification used) in the blend treated with S-170 was due to the tendency of S-170 to lengthen nucleation time and inhibit crystal growth (*9, 10*). S-170 is believed to do this by cocrystallizing with TAG crystals because of their similar acyl chains, but structural dissimilarities between TAGs and the emulsifier result in the delay of nucleation and inhibition of crystal growth. Other emulsifiers or crystal regulators behave in the same manner but to different extents. It can be seen from **Figure 2** that the effect of STS on TAG crystal growth was less pronounced as compared to that of S-170. Two possible theories have been proposed by Garbolino et al. (*10*) to explain this. First, steric hindrance may be a factor during interactions between acyl chains of TAGs and emulsifiers. A bulkier emulsifier (S-170) will most likely disrupt TAG crystallization more than a less bulky one such as STS. Second, the higher solubility of STS in the oil phase should result in smaller disruptive forces that would delay nucleation and hinder crystal growth. The first theory would explain why the blend treated with DMG showed larger crystal growth, since DMG is less bulky than S-170 and STS.

Figure 3 shows the crystal morphologies of the blends after 4 weeks of storage at 0 °C. All blends showed small or globular crystals interspersed with crystal aggregates with sizes ranging between 30 and 140 μm, larger than the range (20−50 μm) reported to be responsible for sandy texture in margarine (*4*). These crystal aggregates were slightly more pronounced in the control (**Figure 3D**). Another study (*11*) reported that crystal aggregates become visible to the naked eye and can be perceived in the mouth when they reach sizes between 100 and 300 μm. Visual and physical (by rubbing in between the fingers) examination of the treated and nontreated fat blends did not show the presence of these bothersome crystal aggregates, even after 5 months of storage. **Figure 3A** (blend treated with S-170) and **3B** (blend treated with STS) show very small evenly distributed crystals interspersed with large crystal aggregates, whereas **Figure 3C** (blend treated with DMG) and **3D** (control) show irregular shapes and sizes of crystals interspersed with very large crystal aggregates. These similarities are accounted for by structural similarities and properties within these two groups. S-170 and STS are nonglycerol-based, bulky with at least three acyl chains, and therefore have more disruptive forces that will delay nucleation and hinder crystal growth as compared to DMG, which is a monoacylglycerol (MAG). The structural similarity between DMG and TAG and the less bulkiness of DMG were responsible for the moderate delay of nucleation and inhibition of crystal growth, as compared to the two nonglycerol-based emulsifiers.

Crystal morphology is of great importance in the manufacture of margarine because it affects product consistency and acceptability (*12*). Smaller crystals lead to firmer fats, while larger crystals produce softer fats. Gabolino et al. (*10*) reported that blends that showed many small crystals upon treatment with sucrose palmitate (P-170) and stearate (S-170) had higher hardness values than blends that showed the presence of large and irregularly shaped crystals. It is thought that samples that have more homogeneously distributed crystals in their fat networks provide higher resistance to penetration by a cone than samples with regions of high and low crystal densities (*13*). In our study, S-170 and STS promoted the formation of homogenously distributed small crystals, while DMG promoted the formation of larger and irregularly shaped crystals. It can therefore be expected that margarine products formulated with blends treated with 2% S-170 and STS will be firmer and less spreadable than margarine formulated with the blend treated with DMG and the control.

This study has shown that blending of PMF with SL40 improved SFC as well as crystal polymorphism. The addition of S-170, STS, and DMG to the SL40:PMF blend did little to improve crystal polymorphism but had significant effects on morphology. S-170, STS, and DMG restricted the growth of TAG crystals relative to the control and may have significantly reduced the extent to which large crystal aggregates were formed but were unable to totally prevent their growth after 4 weeks of storage at 0 °C. However, these crystal aggregates were not detected upon visual and physical (by rubbing in between the fingers) examination and may therefore not impact the sensory properties of the finished products negatively. The choice of emulsifier for use in the formulation of tub margarine will depend on the desired consistency of the margarine product. Use of emulsifiers such as S-170 and STS that promote the formation of very small evenly distributed crystals will result in firmer fats, whereas use of emulsifiers such as DMG that promote the formation of irregularly shaped crystals will result in softer fats.

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