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Characterization of Stearidonic Acid Soybean Oil Enriched with Palmitic Acid Produced by Solvent-free Enzymatic Interesterification

Sarah A. Teichert and Casimir C. Akoh*

Department of Food Science and Technology, The University of Georgia, Athens, Georgia 30602-2610, United States

ABSTRACT: Stearidonic acid soybean oil (SDASO) is a plant source of n-3 polyunsaturated fatty acids (n-3 PUFAs). Solvent-free enzymatic interesterification was used to produce structured lipids (SLs) in a 1 L stir-batch reactor with a 1:2 substrate mole ratio of SDASO to tripalmitin, at 65 °C for 18 h. Two SLs were synthesized using immobilized lipases, Novozym 435 and Lipozyme TL IM. Free fatty acids (FFAs) were removed by short-path distillation. SLs were characterized by analyzing FFA and FA (total and positional) contents, iodine and saponification values, melting and crystallization profiles, tocopherols, and oxidative stability. The SLs contained 8.15 and 8.38% total stearidonic acid and 60.84 and 60.63% palmitic acid at the *sn*-2 position for Novozym 435 SL and Lipozyme TL IM SL, respectively. The SLs were less oxidatively stable than SDASO due to a decrease in tocopherol content after purification of the SLs. The saponification values of the SLs were slightly higher than that of the SDASO. The melting profiles of the SLs were similar, but crystallization profiles differed. The triacylglycerol (TAG) molecular species of the SLs were similar to each other, with tripalmitin being the major TAG. SDASO's major TAG species comprised stearidonic and oleic acids or stearidonic, α-linolenic, and γ-linolenic acids.

KEYWORDS: lipase, stearidonic acid soybean oil, structured lipid, tripalmitin

INTRODUCTION

Stearidonic acid (SDA) soybean oil (SDASO) is a soybean oil that is enriched with SDA (C18:4n-3) consisting of approximately 20% stearidonic, 24% linoleic, and 12% palmitic acids.[1] SDA is an n-3 polyunsaturated fatty acid (n-3 PUFA). PUFAs are often found in plants, fungi, microalgae, and fish. Fish is the main source of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) but contains only small amounts of SDA.[2] The richest sources of SDA are algae and plants. EPA is a very long chain n-3 PUFA that inhibits platelet aggregation and lowers inflammation. James et al.[3] suggested that the daily ingestion of fish or fish oil to obtain the health benefits of n-3 PUFAs is not a sustainable long-term approach. However, increasing ingestion of plant-based sources of n-3 PUFAs is required to increase the tissue concentration of EPA and DHA.[3] SDA is the first metabolite of α-linolenic acid (ALA) in the metabolic pathway leading to EPA by desaturases and elongases.[4] However, this conversion from ALA to EPA is often poor because the initial Δ6 desaturase enzyme is rate limiting in humans.[5] The consumption of SDA, instead of ALA, would skip the rate-limiting step, allowing for better conversion to EPA. Therefore, development of plant-based sources of n-3 PUFAs could be a solution for the supply of these fatty acids in the future. Miles et al.[6] observed that neither SDA nor 20:4n-3 appeared in the peripheral blood mononuclear cell when dietary SDA was ingested at a level of 1.0 g/day, indicating that SDA was readily metabolized to EPA in the body. Dietary SDA was found to increase EPA concentrations by 3−4-fold more effectively than similar levels of ALA.[3,7] SDA was approximately one-third as effective as dietary EPA,[8] and the effectiveness of these FAs in increasing EPA concentrations in tissues is as follows: EPA > SDA > ALA.[3] James et al.[3] conducted a double-blind, parallel group study to examine the effect of dietary SDA on increasing tissue concentrations of EPA in humans and compared SDA's ability with that of ALA and EPA. They concluded that SDA vegetable oils were more effective in increasing EPA tissue concentrations than the currently used ALA vegetable oils. SDA has been noted as a possible potent inhibitor of cancer growth, inhibitor of platelet aggregation, anti-inflammatory pharmaceutical, and provider of cardiovascular benefits.[2] EPA has been linked to reductions in inflammation[9] and neurological disorders.[10] A previous study reported the tocopherol content of SDA soybean as 9.6 mg/100 g of α-tocopherol, 79.3 mg/100 g of γ-tocopherol, and 28.8 mg/100 g of δ-tocopherol.[1] Tocopherols are unsaponifiable materials that are well-known for their cardiovascular benefits and antioxidant capacity.

The ratio of n-6/n-3 PUFA is important in the diet. However, the Western diet is high in n-6 FAs, often resulting in a ratio of 15:1−16.7:1.[11] The high ratio of n-6 FAs can result in increased risk of cardiovascular, inflammatory, and autoimmune diseases and cancer. Conversely, a lower n-6/n-3 PUFA ratio would suppress these negative effects. For example, a lower ratio of 2:1−3:1 suppressed inflammation in patients with rheumatoid arthritis, and a ratio of 5:1 had a beneficial effect on patients with asthma. However, a ratio of 10:1 n-6/n-3 PUFA had adverse consequences.[11] Premature infants often are limited in their ability to make EPA and DHA from ALA.[12]

Structured lipids (SLs) are triacylglycerols (TAGs) that have been modified to change the FA composition and/or their position in the glycerol backbone by chemically and/or enzymatically catalyzed reactions.[13] SLs can be used in a wide variety of food applications such as margarines, shortenings, cookies, and salad dressings. Another possible application of SLs could be their use in infant formula as a human milk fat (HMF) analogue.

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Infants require proper nutrition for growth and development, and infant formulas try to mimic the fat found in breast milk. Palmitic acid (PA) is the second major FA found in breast milk at approximately 18.3−25.9%.[14−16] SDA soybean oil only contains 12% PA, which is lower than that of breast milk. A large portion of PA in breast milk is esterified at the *sn*-2 position of the TAGs. The amount of PA esterified at the *sn*-2 position is >60% (by weight) of HMF, whereas mainly unsaturated FAs are found at the *sn*-1,3 positions.[17−19] As TAGs are metabolized in the body, the FAs esterified at the *sn*-1,3 positions are released by pancreatic lipase and the FAs esterified at the *sn*-2 position remain unhydrolyzed or conserved. The unhydrolyzed *sn*-2 FAs are absorbed by the intestinal mucosa as *sn*-2 monoacylglycerols.[20] The large amount of *sn*-2 esterified PA helps improve the absorption of fat and calcium in infants.[18,19,21,22] Also, PA reduces the formation and disposal of "calcium soaps" that are generated by long-chain saturated FA interactions with calcium.[17,22] However, free PA may be lost as calcium soaps in the feces of the infant. Most infant formulas consist of fats from vegetable oils that contain mostly unsaturated FAs at the *sn*-2 position.[23] Lipids are essential to the growth of an infant. In breast milk, lipids provide essential FAs that are required as structural cell components of membrane tissues, and they provide an essential source of energy that is approximately 50% of the total energy used by infants.[24] Betapol (Loders Croklaan, Glen Ellyn, IL) was the first to commercially produce a HMF analogue by using a 1,3-specific lipase to synthesize SLs by reacting tripalmitin with unsaturated FAs.[23] We are not aware of any other studies, except our previous research,[25] on the enzymatic modification of SDA soybean oil to produce SLs. There are no current studies published on the physical and chemical characterization of SDA soybean oil SLs.

One of our objectives was to scale-up the production of two SLs at 65 °C for 18 h with a substrate mole ratio of 1:2 of SDASO to tripalmitin, catalyzed by immobilized Novozym 435 and Lipozyme TL IM lipases. These conditions were selected on the basis of our previous research using response surface methodology (RSM) to predict the optimal conditions.[25] However, a temperature above 60 °C was used to conduct a solvent-free production of food grade SLs instead of the optimal 50 °C from our previous research.[25] Our next objective was to characterize the physical and chemical properties of Novozym 435 SL (NSL) and Lipozyme TL IM SL (LSL). The reusability of the enzymes was also studied. The overall aim of our research was to characterize SDASO enriched with PA (SLs) produced by solvent-free enzymatic interesterification.

MATERIALS AND METHODS

Materials. SDASO was kindly provided by Monsanto Co. (St. Louis, MO). Tripalmitin was purchased from TCI America (Portland, OR). Immobilized lipases, Novozym 435 (nonspecific *Candida antarctica* lipase) and Lipozyme TL IM (*sn*-1,3 specific *Thermomyces lanuginosus* lipase), were obtained from Novozymes North America Inc. (Franklinton, NC). The specific activity of Novozym 435 was 10000 PLU/g and that of Lipozyme TL IM was 250 IUN/g (PLU is propyl laurate units, and IUN is interesterification units Novo). The lipid standards Supelco 37 Component FAME mix, triolein, 2-oleoylglycerol, tripalmitin, tristearin, 1,2-dioleoyl-3-palmitoyl-*rac*-glycerol, and 1-palmitoyl-2-oleoyl-3-linoleoyl were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO), and C19:0-nonadecanoic acid was purchased from TCI America (Portland, OR). The TAG standard mix (GLC reference standard) was purchased from Nu-chek Prep, Inc. (Elysian, MN). Other solvents and chemicals were purchased from Fisher Scientific (Norcross, GA) and Sigma-Aldrich Chemical Co. (St. Louis, MO).

Scaled-up Interesterification Reaction. The solvent-free interesterification reaction occurred in a 1 L stir-batch reactor at 65 °C for 18 h with a substrate mole ratio of 1:2 (SDASO to tripalmitin) and 10% (w/w) of Lipozyme TL IM or Novozym 435 as the biocatalyst with constant stirring at 200 rpm. The reactor was wrapped with foil to reduce exposure to light. At the end of the reaction, the resulting SLs produced with Lipozyme TL IM (LSL) and Novozym 435 (NSL) were vacuum filtered through a Whatman no. 1 containing sodium sulfate and then through a 0.45 μm membrane filter to dry and separate the SL from the enzymes. If the SLs needed to be stored before short-path distillation, they were stored in an airtight amber container in the dark at 4 °C after being flushed with nitrogen.

Short-Path Distillation. Short-path distillation was used to remove liberated FFAs from the SL substrates. Short-path distillation was performed once using a KDL-4 (UIC Inc.) unit under the following conditions: holding temperature, 65 °C; feeding rate, approximately 100 mL/h; heating oil temperature, 185 °C; coolant temperature, 25−30 °C; and vacuum, <13.33 Pa. After short-path distillation, the FFA content was determined according to AOCS Official Method Ac 5-41.[26] The percent yield was calculated by using the starting weight of the substrates and the final weight of the SL after short-path distillation.

Determination of Fatty Acid Profiles. SDASO and SL samples were converted to FAME following AOAC Official Method 996.01, Section E,[27] with minor modifications.[28] For analysis of the SDASO, 150 mg of the oil was weighed into a Teflon-lined test tube and 100 μL of the internal standard, C19:0 in hexane (20 mg/mL), was added to the sample and dried under nitrogen to remove solvent. For analysis of the SL, 100 μL of the internal standard was added to the SLs after short-path distillation. Two milliliters of 0.5 N NaOH in methanol was added and incubated at 100 °C for 5 min for saponification. Then the samples were cooled under tap water, and 2 mL of 14% BF_3 in methanol was added. The samples were vortexed for 1 min. Again, the sample was incubated at 100 °C for 5 min for methylation and cooled under tap water. To stop the reaction and extract the FAMEs, 2 mL of hexane and 2 mL of saturated NaCl solution were added. The sample was vortexed for 2 min and then centrifuged at 1000 rpm or 104.72 rad/s for 5 min to separate the organic layer from the aqueous layer. The upper organic layer was removed and recovered in an amber GC vial for analysis. Supelco 37 component FAME mix was used as the external standard and was run parallel with the samples.

GC Analysis. The FAMEs (from the SDASO, SLs, and corresponding positional analyses) were analyzed using an Agilent Technology 6890N gas chromatograph (Agilent Technologies, Santa Clara, CA) with a flame ionization detector. A Supelco SP-2560 column, 100 m × 250 μm, 0.20 μm film, was used to attain separation. Injection of 1 μL of sample was made at a split ratio of 20:1. Helium was the carrier gas at a flow rate of 1.1 mL/min and at a constant pressure (45.0 mL/min). The injector temperature and the FID set point were 300 °C. The oven was held at 140 °C for 5 min, then increased to 240 °C at 4 °C/min, and held at 240 °C for 15 min. The relative FAME content was calculated using the online computer. The average and standard deviation of triplicate analyses were reported.

Positional Analysis. A modified version of the Luddy et al.[29] method was used to perform the pancreatic lipase-catalyzed *sn*-2 positional analysis. One hundred milligrams of SDASO or the SLs was placed into Teflon-lined test tubes. Two milliliters of 1.0 M Tris-HCl buffer (pH 8), 0.5 mL of 0.05% sodium cholate solution, and 2.2% calcium chloride solution were added and vortexed for 2 min to emulsify the oil. Then 40 mg of pancreatic lipase was added, vortexed for 1 min, and incubated in a water bath at 40 °C for 3 min with shaking at 200 rpm or 20.94 rad/s. The samples were vortexed again for 2 min. To stop the reaction and extract the hydrolyzed TAG products, 1 mL of 6 N HCl and 4 mL of

diethyl ether were added. The samples were vortexed for 2 min and centrifuged at 1000 rpm or 104.72 rad/s for 3 min. The upper layer, containing the lipid components, was filtered twice through an anhydrous sodium sulfate column. The samples were concentrated under nitrogen until approximately one-third of the original volume was left. A mixture of hexane, diethyl ether, and formic acid (60:40:1.6, v/v/v) was used as the mobile phase after equilibration in the TLC tanks for approximately 30 min. The concentrated sample (~50 μL) was spotted onto the activated silica gel G dried TLC plates and placed into the tank. 2-Oleylglycerol was spotted as the standard and run parallel with the samples for identification of the 2-monoacylglycerol (2-MAG) band. The plates were sprayed with 0.2% 2,7-dichlorofluorescein in methanol and visualized under UV light. The 2-MAG band was scraped off and converted to FAME (as described above). One hundred microliters of the internal standard (20 mg/mL C19:0 in hexane) was used for the SDASO and 50 μL of the internal standard for the SL. The FA content at the *sn*-2 position was quantified by GC, and the FA content at the *sn*-1,3 positions was calculated.

Chemical Properties. The FFA contents, iodine values, and saponification values were determined according to AOCS Official Methods Ac 5-41, Cd 1c-85, and Cd 3a-94, respectively.[26]

Tocopherol Analysis. Tocopherols were identified and quantified in SDASO, NSL, and LSL using a normal phase high-performance liquid chromatography (HPLC) system. An isocratic mobile phase of 0.85% (v/v) isopropanol in hexane, vacuum filtered and degassed by stirring under vacuum, was used at a flow rate of 1.0 mL/min. The column was a LiChrosorb Si 60 column (4 mm, 250 mm, 5 μm particle size, Hiber Fertigsäule RT, Merck, Darmstadt, Germany) with a Shimadzu LC-6A pump equipped with an RF-10AXL fluorescence detector (Shimadzu Corp., Columbia, MD), a Spectra Series As100 autosampler (Thermo Separation Products, Inc., San Jose, CA), and Agilent Chemstation software. The samples were prepared by weighing 0.1 ± 0.001 g into a test tube and adding 5 mL of HPLC-grade hexane. The samples were then vortexed for 30 s, capped, and centrifuged at 1000 rpm (104.72 rad/s) for 10 min at room temperature. A portion of the hexane layer was transferred into an HPLC vial for analysis. The tocopherol standards preparation was performed according to the method of Lee et al.[30] with ~50 mg of α-, β-, and δ-tocopherols and 100 mg of γ-tocopherol dissolved in 25 mL of hexane. The standard purities were measured by pipetting 1 mL of each standard into a 25 mL volumetric flask and evaporating completely under nitrogen. The residue was diluted with 95% (v/v) ethanol, and absorbance was measured with a Shimadzu model UV-1601 UV−vis spectrophotometer and a quartz cuvette. Extinction coefficients ($E^{1\%}_{1\ cm}$), and maximum wavelengths (λ_{max}) were 71.0 and 294, respectively, for α-tocopherol, 86.4 and 297, respectively, for β-tocopherol, 92.8 and 298, respectively, for γ-tocopherol, and 91.2 and 298, respectively, for δ-tocopherol.[31] The following equation was used to determine percent purity:

$$\%\ \text{purity} = (A \times 10/C)/E^{1\%}_{1cm}$$

A is the absorbance, *C* is the concentration (mg/mL), and $E^{1\%}_{1\ cm}$ is the extinction coefficient at λ_{max} of each tocopherol. The purities of α-, β-, γ-, and δ-tocopherols were found to be 96.0, 90.0, 96.0, and 90.0%, respectively, with stock concentrations of 1.88, 1.80, 3.84, and 1.80 mg/mL, respectively. For the daily working standard, the stock concentrations were diluted with the mobile phase containing 0.01% butylated hydroxytoluene (BHT). Standards and samples were injected into the HPLC at a volume of 20 μL.

Oxidative Stability Analysis. Twelve grams of SDASO, NSL, and LSL were weighed into Teflon-lined test tubes and oxidized for 72 h at 65 °C in the dark in a shaking water bath. The samples were removed from the water bath and analyzed at 0, 24, 48, and 72 h. The peroxide value (PV) and *p*-anisidine value (*p*AV) were determined according to AOCS Official Methods Cd 8b-90 and Cd 18-90, respectively.[26] The total oxidation (TOTOX) value was calculated as 2(PV) + (*p*AV).[32] The oxidative stability index (OSI) was determined according to AOCS Method Cd 12b-92[26] using an Oil Stability Instrument (Omnion, Rockland, MA) at 110 °C.

Melting and Crystallization Profile. The melting and crystallization profiles were determined for SDASO, tripalmitin, NSL, and LSL using a differential scanning calorimeter (DSC model DSC7, Perkin-Elmer Co., Norwalk, CT). The method was performed according to AOCS Official Method Cj 1-94[26] with minor modifications using indium and *n*-decane as standards. Sample weight ranged from 8 to 12 mg in aluminum pans. The sample was heated from 25 to 80 °C at 50 °C/min, held for 10 min (to destroy any previous crystalline structure), cooled from 80 to −55 °C at 10 °C/min (for crystallization profiles), held for 30 min, and then heated from −55 to 80 °C at 5 °C/min (for melting profiles). Dry ice and acetone were used as the coolant. The thermograms were analyzed with the DSC (Pyris software, Perkin-Elmer, Shelton, CT).

TAG Molecular Species. A reversed phase HPLC (Agilent Technologies 1260 Infinity, Santa Clara, CA) with a Sedex 85 evaporative light scanning detector (ELSD) was used to analyze the TAG molecular species of SDASO, NSL, and LSL on an Ultrasphere C18 250 mm, 4.6 mm, 5 μm particle size column (Beckman Coulter, Fullerton, CA). The column temperature was kept at 30 °C. The ELSD conditions were 70 °C, 3.0 bar or 300,000 Pa, and gain of 8. Sample concentrations were 5 mg/mL in chloroform. The sample injection volume was 20 μL. The eluent was a gradient of acetonitrile (A) and acetone (B) at a solvent flow rate of 1 mL/min with a gradient of 0 min, 65% B; 45 min, 95% B; and 50 min, 65% B. The equivalent carbon number (ECN) method was used to predict the elution order of TAG. Standards [tripalmitin, tristearin, triolein, TAG Mix, 1,2-dioleoyl-3-palmitoyl-*rac*-glycerol (OOP), and 1-palmitoyl-2-oleoyl-3-linoleoyl (POL)] were also chromatographed to help determine the TAG species.

Reusability of Enzymes. Solvent-free interesterification was performed in a 1 L stir-batch reactor at 65 °C for 18 h with a substrate mole ratio of 1:2 (SDASO/tripalmitin) with both Novozym 435 and Lipozyme TL IM for a total of five times, each reusing the same enzymes. After each use, the enzymes were washed three to four times with hexane, dried under the hood, and placed in a desiccator. Enzymes were stored at 4 °C. The FA composition and positional analyses were performed on the SLs after each use as described above.

Statistical Analysis. All analyses, except melting and crystallization profiles, were performed in triplicate. Melting and crystallization profiles were performed in duplicate. Statistical analysis was performed with the SAS software package (SAS Institute, Cary, NC). Duncan's multiple-range test was performed to determine the significance of difference at $P \leq 0.05$.

RESULTS AND DISCUSSION

SDASO was characterized in this study because it was the starting substrate. The FA profile for SDASO was determined in our previous research[25] and showed that the total and positional FA composition of the oil is in agreement with previous studies.[1] The major FAs in SDASO were linoleic acid (25.78 ± 0.07%), SDA (22.16 ± 0.23%), oleic acid (14.31 ± 0.17%), α-linolenic acid (12.30 ± 0.06), and PA (11.54 ± 0.04%). Linoleic acid was the major FA found at the *sn*-2 position (34.11 ± 4.56%), whereas SDA was the major FA found at the *sn*-1,3 positions of the TAG (25.26 ± 1.40%). SDASO contains a small amount of PA at the *sn*-2 position. Enzymatic interesterification is often used to produce SLs that have improved functionality by incorporating new FAs into the oil or by rearranging the existing FAs.[13] The aim of this research was to produce SLs with immobilized lipases, Novozym 435 and Lipozyme TL IM, by modifying the SDASO's

Table 1. FA Composition (Mole Percent) of SLs[a]

	total		*sn*-2		*sn*-1,3[b]	
FA	NSL[c]	LSL[d]	NSL	LSL	NSL	LSL
C14:0	1.21 ± 0.02 A	1.18 ± 0.01 A	ND[e]	ND	1.82 ± 0.03 A	1.78 ± 0.01 A
C15:0	0.09 ± 0.00 A	0.09 ± 0.00 A	ND	ND	0.14 ± 0.00 A	0.14 ± 0.00 A
C16:0	62.55 ± 0.33 A	61.22 ± 1.45 A	60.84 ± 2.26 A	60.63 ± 1.54 A	63.41 ± 1.44 A	61.51 ± 2.19 A
C17:0	0.16 ± 0.00 A	0.15 ± 0.00 B	ND A	0.35 ± 0.61 A	0.24 ± 0.00 A	0.15 ± 0.31 A
C18:0	2.93 ± 0.04 A	2.88 ± 0.08 A	4.34 ± 1.68 A	3.10 ± 0.07 A	2.22 ± 0.90 A	2.78 ± 0.12 A
C18:1n-9	6.11 ± 0.10 B	6.53 ± 0.17 A	8.32 ± 0.47 A	7.07 ± 0.07 B	5.00 ± 0.38 B	6.25 ± 0.28 A
C18:2n-6	10.34 ± 0.13 A	10.83 ± 0.46 A	12.39 ± 0.55 A	11.49 ± 0.26 A	9.31 ± 0.32 A	10.50 ± 0.72 A
C18:3n-6	2.89 ± 0.03 A	3.01 ± 0.15 A	3.28 ± 0.20 B	4.03 ± 0.18 A	2.69 ± 0.15 A	2.50 ± 0.30 A
C20:1n-9	4.73 ± 0.04 A	4.90 ± 0.21 A	4.24 ± 0.19 A	4.50 ± 0.39 A	4.97 ± 0.15 A	5.10 ± 0.75 A
C18:4n-3	8.15 ± 0.13 A	8.38 ± 0.46 A	6.59 ± 0.80 A	8.82 ± 1.33 A	8.93 ± 0.48 A	8.16 ± 0.75 A
C21:0	0.12 ± 0.01 A	0.13 ± 0.01 A	ND	ND	0.18 ± 0.01 A	0.19 ± 0.01 A
n-6/n-3	1.64 ± 0.02 A	1.67 ± 0.02 A				

[a] Mean ± SD, $n = 3$. Means with the same letter in the same row and same category (i.e.,*sn*-2 column) are not significantly different ($P \leq 0.05$). [b] *sn*-1,3 (mol %) = [3 × total (mol %) − *sn*-2 (mol %)]/2. [c] Novozym 435 SL. [d] Lipozyme TLIM SL. [e] Not detected.

TAG to increase the PA esterified at the *sn*-2 position and to physically and chemically characterize the resulting SLs.

The FA profiles of the resulting SLs, NSL and LSL, are shown in Table 1. The major FA found in the SLs was PA, followed by linoleic acid, SDA, and oleic acid. Enzymatic interesterification decreased linoleic acid from 25.78 ± 0.07% in SDASO to 10.34 ± 0.13 and 10.83 ± 0.46% in the SLs, NSL and LSL, respectively. There was little to no α-linolenic acid in the SLs, indicat ing that palmitic and other fatty acids displaced most of the C18:3n-3 during the interesterification reaction. The SDA content decreased in the SLs, from 22.16 ± 0.23% in SDASO to 8.15 ± 0.13 and 8.38 ± 0.46 in NSL and LSL, respectively. The decrease in SDA content in the SLs is a result of the incorporation of PA in the TAG. For example, the total PA content increased from 11.54 ± 0.04 to 62.55 ± 0.33 and 61.22 ± 1.45% in NSL and LSL, respectively, making PA the major FA in the SLs instead of linoleic acid, which is the main FA found in SDASO. However, SDA remained in the SLs and may provide cardiovascular benefits.[2] The n-6/n-3 PUFA ratio for both SLs was approximately 1.6. This represents an improved ratio of n-6/n-3 PUFAs because the typical Western diet has a high ratio of 15:1−16.7:1.[11] A lower ratio can result in decreased risk of cardiovascular, inflammatory, and autoimmune diseases and cancer. Statistically, the two SLs do differ slightly. It appears that the SLs differ in the position of SDA, but with a $P \leq 0.676$, they are not significantly different. The statistical differences in total FA composition were found only in C17:0 and C18:1n-9. In fact, C18:1n-9 was significantly different in the SLs at the total, *sn*-2, and *sn*-1,3 positions ($P \leq 0.05$). Another significant difference was found for C18:3n-6 at the *sn*-2 position of the SLs. The specificity of Lipozyme TL IM can be influenced by reaction media, substrates, and acyl migration.[33,34] The PA content at the *sn*-2 position increased from approximately 4.77% in SDASO to >60% in both NSL and LSL, resulting in SLs that were enriched with PA. The percent yields of the SLs were 97.05% for NSL and 97.73% for LSL. This high yield is due to the substrates being both in TAG form and not as FFAs for the interesterification reaction. An acidolysis reaction will result in more FFA formation that must be removed by short-path distillation. Enzymatic interesterification of two TAG substrates resulted in little unesterified FFAs, which were removed after short-path distillation.

The physicochemical characteristics were determined for SDASO, NSL, and LSL (Table 2). The iodine value (IV) was calculated, and both SLs had a lower IV than SDASO. The IV measures the degree of unsaturation or number of carbon−carbon double bonds in relation to the amount of fat in the oil. The higher the degree of unsaturation, the more iodine that can be absorbed and the higher the IV.[35] The SLs contained more saturated FAs and resulted in a lower IV. The IVs of SDASO, NSL, and LSL were significantly different ($P \leq 0.05$). The oxidative stability index (OSI) decreased in the SLs compared to the original, more stable, SDASO (6.13 ± 0.04 h). NSL had the lowest OSI value (1.85 ± 0.07 h), whereas LSL's OSI value (3.90 ± 0.28 h) was higher than that of NSL with a significant difference at $P \leq 0.05$. A lower IV or higher amount of saturated FAs usually indicates increased oxidation stability. However, the lower OSI of the SLs may be due to the loss of some tocopherols from the short-path distillation. Tocopherols are antioxidants known to increase the oxidative stability of oils[36] and must be added back to stabilize the oil for food use. The FFA percentage was found to be higher in the SLs than in the original SDASO. The SLs differed significantly ($P \leq 0.05$) from SDASO in FFA content because SLs were not deodorized but did not differ significantly from each other. During enzymatic interesterification, FAs are cleaved off the glycerol backbone, and not all of these FAs reattach to the TAG, resulting in FFAs. Short-path distillation removed most of the FFAs, but did not lower the FFA content to the same percentage as found in the original SDASO. However, repetitive short-path distillation and/or deodorization can remove more FFAs and restore near fresh oil status to a FFA content of below 0.1%.[37] The saponification value (SV) is the measure of the average molecular weight or chain length of all the fatty acids in the oil. The smaller the SV, the longer the average FA chain length is.[35] The SVs of SDASO (59.99 mg/g), NSL (63.19 mg/g), and LSL (63.11 mg/g) were similar, with the SLs having a slightly higher SV. SDASO has a larger molecular weight (902.18 g/mol) than NSL (875.16 g/mol) and LSL (875.16 g/mol). The SV of SDASO shows that it contains a longer average FA chain length than the SLs. However, the SVs of SDASO, NSL, and LSL were not significantly different ($P \leq 0.05$).

Tocopherol contents were also measured and are shown in Table 2. The vitamin E family is composed of α-, β-, γ-, and

Table 2. Physiochemical Characteristics of SDASO and SLs[a]

					tocopherols (ppm)					
substrate	IV	OSI (h)	FFA (%)	SV (mg/g)	α-T	α-T3	β-T	γ-T	γ-T3	δ-T
LSL	36.80 ± 0.92 A	3.90 ± 0.28 B	0.34 ± 0.07 A	63.11 ± 3.07 A	35.73 ± 0.31 B	6.93 ± 0.24 A	2.65 ± 0.02 A	12.55 ± 0.19 B	247.19 ± 4.40 B	85.11 ± 1.46 B
NSL	35.17 ± 0.74 B	1.85 ± 0.07 C	0.35 ± 0.08 A	63.19 ± 0.85 A	31.62 ± 2.07 B	6.26 ± 0.61 A	ND[b] B	12.80 ± 0.48 B	247.29 ± 0.07 B	79.87 ± 0.06 B
SDASO	111.91 ± 0.94 C	6.13 ± 0.04 A	0.05 ± 0.01 B	59.99 ± 0.72 A	107.37 ± 1.53 A	6.94 ± 1.25 A	2.72 ± 1.50 A	28.44 ± 3.22 A	945.10 ± 2.98 A	349.49 ± 0.97 A

[a] Mean ± SD, $n = 3$. Means with the same letter in the same column are not significantly different ($P \leq 0.05$). IV, iodine value; OSI, oxidative stability index; FFA, free fatty acid percentage; SV, saponification value; α-T, α-tocopherol; α-T3, α- tocotrienol; β-T, β-tocopherol; γ-T, γ-tocopherol; δ-T, δ-tocopherol; LSL, Lipozyme TL IM SL; NSL, Novozym 435 SL. [b] Not detected.

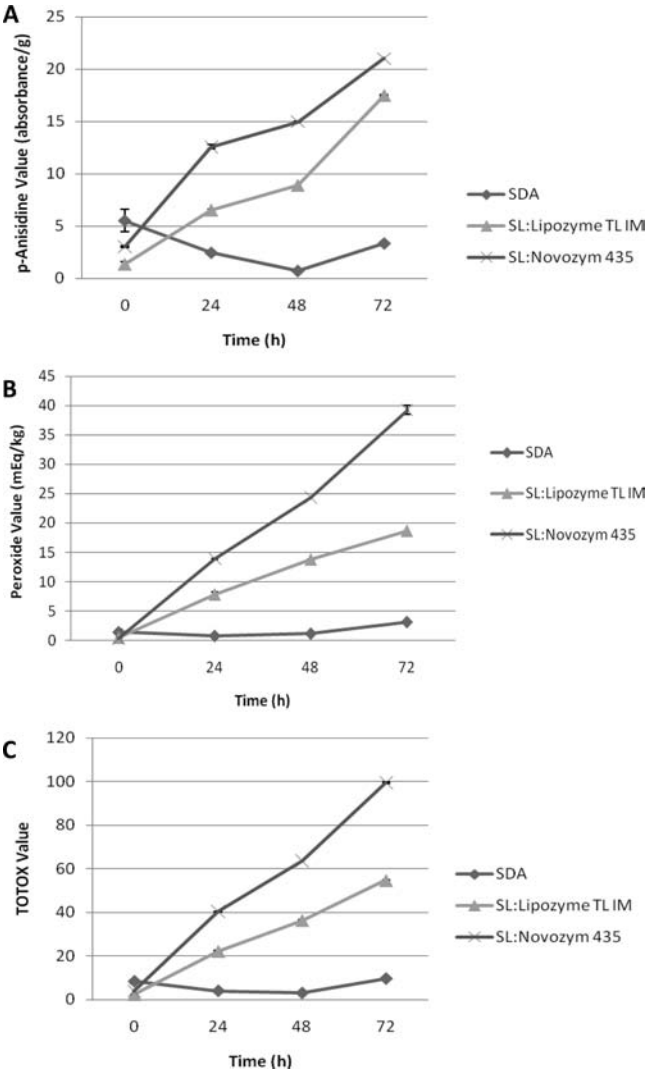

Figure 1. (A) *p*-Anisidine values, (B) peroxide values, and (C) total oxidation values (TOTOX) of SDA soybean oil (SDASO) (◆), Novozym 435 SL (NSL) (×), and Lipozyme TL IM SL (LSL) (▲) over a period of 72 h.

δ-tocopherols that are characterized by a saturated side chain of three isoprenoid units and unsaturated tocotrienols.[35] Tocopherols provide stability and important antioxidant properties to the oil. Compared to SDASO, α-tocopherols, γ-tocopherols, δ-tocopherols, and γ-tocotrienols decreased the most ($P \leq 0.05$) after interesterification and short-path distillation. There were significant differences ($P \leq 0.05$) in the total tocopherol content between SDASO (~1440.06 ppm) and the SLs, NSL (~377.84 ppm) and LSL (~390.16 ppm). The tocopherol losses were approximately 73 and 74% for LSL and NSL, respectively, when compared to SDASO. Tocopherol content was not significantly different between the SLs ($P \leq 0.05$) except β-tocopherol. The difference in oxidative stability between the SLs may be due to the loss of other antioxidants that were not tocopherols and not analyzed. This decrease may also be due to exposure to light, oxygen, and heat during interesterification and/or due to short-path distillation. However, some studies did report an increase in tocopherols after interesterification.[38,39] Other studies suggest that short-path distillation not only affects the FFA content but

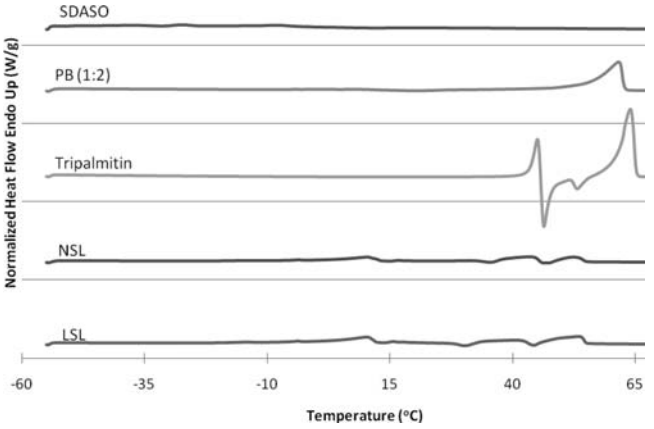

Figure 2. Melting curve using DSC for SDASO, tripalmitin, NSL, LSL, and a physical blend of SDASO to tripalmitin (1:2). PB (1:2), physical blend with a 1:2 substrate mole ratio of SDASO/tripalmitin.

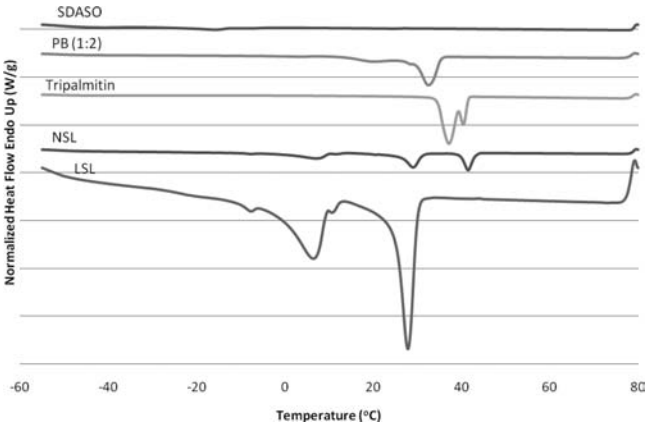

Figure 3. Crystallization curve using DSC for SDASO, tripalmitin, NSL, LSL, and a physical blend of SDASO to tripalmitin (1:2). PB (1:2), physical blend with a 1:2 substrate mole ratio of SDASO/tripalmitin.

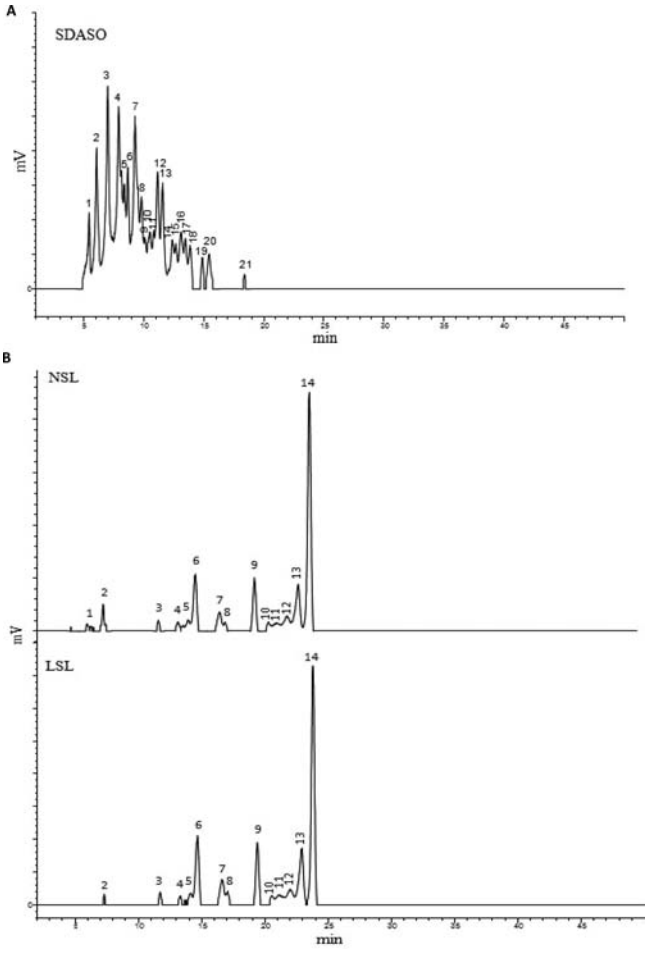

Figure 4. (A) HPLC chromatogram of TAG molecular species of SDASO with relative FA%. Peaks: 1, StLnSt (7.21%); 2, StLnLn (12.38%); 3, StOSt/LnGSt (17.42%); 4, LnLnLn (11.37%); 5, LnGL- (0.97%); 6, StLL (3.84%); 7, LnLnL (12.64%); 8, LnStO (2.10%); 9, LnStP (0.62%); 10, LLLn (1.58%); 11, LnLnO (0.53%); 12, StOO (5.38%); 13, OLLn (5.63%); 14, LLL (1.72%); 15, GLO (0.64%); 16, LnLP (2.06%); 17, LnLnS (1.42%); 18, LnGS (3.44%); 19, OLL (2.97%); 20, PLL (4.88%); 21, PLO/SLL (1.20%). (B) HPLC chromatograms of TAG and relative FA% of NSL and LSL, respectively. Peaks: 1, StLnLn (1.10/ND%); 2, StOSt/LnGSt (4.25/0.75%); 3, OLLn (1.56/1.59%); 4, LnLP (1.63/1.57%); 5, LnGS (0.70/1.43%); 6, OLL (11.04/12.53%); 7, LnOP (3.87/4.34%); 8, OOL (0.90/1.17%); 9, PLP (10.19/11.74%); 10, PPM (1.44/0.88%); 11, OOO (0.84/0.63%); 12, POO (1.78/1.72%); 13, POP (10.48/12.77%); 14, PPP (50.23/48.89%). Abbreviations: M, myristic acid; G, γ-linolenic acid; L, linoleic acid; Ln, α-linolenic acid; O, oleic acid; P, palmitic acid; S, stearic acid; St, stearidonic acid; ND, not detected.

also contributes to the loss of non-TAG components such as tocopherols.[40,41] The loss of tocopherols by short-path distillation may be corrected by supplementation of SLs with antioxidants before food application or storage to stabilize them.

To further analyze the stability of the oils, PV, *p*-AV, and TOTOX were determined and are shown in Figure 1. PV measures the amount of iodine formed by the reaction of peroxides with iodide ion and is the initial product of oxidation. PV measures hydroperoxides that increase and then decrease with time.[35] *p*-AV measures aldehydes or the decay products of the hydroperoxides that continually increase with time. TOTOX continually increases during lipid oxidation.[35] The TOTOX for SDASO remained constant during the 72 h oxidation period. However, the SLs experienced different levels of oxidation. NSL experienced the greatest amount of oxidation and was the least stable during this experiment. LSL oxidation values remained between the TOTOX value of SDASO and that of NSL. This may be explained in part by their tocopherol contents. SDASO had a large amount of tocopherols, followed by LSL and NSL. Tocopherols are important antioxidants that help slow or prevent lipid oxidation of oils.

The melting and crystallization behaviors for SDASO, tripalmitin, NSL, LSL, and a physical blend (1:2 substrate mole ratio, 18 h, 65 °C) without enzymatic catalysis were evaluated with DSC. The melting thermograms and crystallization thermograms are shown in Figures 2 and 3, respectively. SDASO is liquid at both room and refrigeration temperatures (4 °C). SDASO's melting thermogram showed melting from −30.36 to −24.38 °C and from −10.917 to −5.317 °C. Comparison of the SLs, NSL and LSL, to tripalmitin, reveals that the SLs' thermograms shifted to the left and were broader, indicating a better plastic range (Figure 2). NSL experienced melting from 4.11 to 54.90 °C with melting peaks at 10.48, 43.48, and 52.22 °C, with LSL experiencing similar melting properties. The DSC showed an onset of crystallization at −12.86 °C ending at −21.13 °C for SDASO. However,

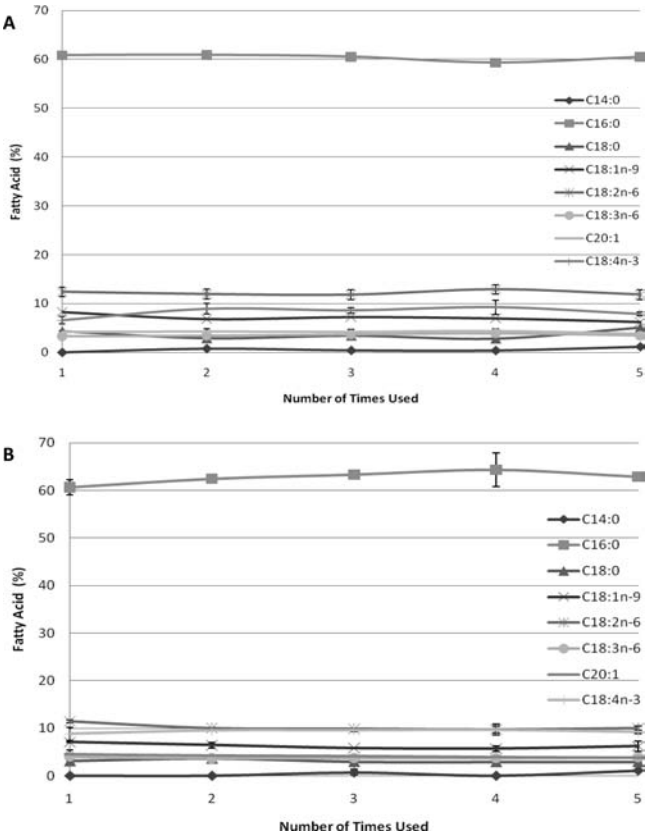

Figure 5. *sn*-2 positional analysis of (A) NSL and (B) LSL with C14:0 (◆), C16:0 (■), C18:0 (▲), C18:1n-9 (×), C18:2n-6 (∗), C18:3n-6 (●), C20:1 (−), and C18:4n-3 (+) after enzyme reuse.

the crystallization properties of the two SLs were very different (Figure 3). NSL showed crystallization from 43.16 to 0.92 °C, whereas LSL experienced crystallization from 29.87 to −9.407 °C. The difference in crystallization behaviors of LSL and NSL may be due to the molecular species of their TAGs. Figure 4 shows the differences in major TAG species among the SLs. LSL has more POP (12.77%) than NSL (10.48%). The SLs will experience softening at room temperature but will not completely melt unless heated to a temperature around 54 °C. According to Tan and Che Man,[42] the presence of multiple peaks in a DSC thermogram of a vegetable oil can be attributed to the complex attributes of the TAG distribution. Saturated TAGs (such as tripalmitin) tend to melt at higher temperatures than the highly unsaturated TAGs.[42] The physical blend showed similar thermograms to those of tripalmitin with a shift to a slightly lower crystallization temperature (Figure 3). This similarity may be due to the fact that the physical blend contained more saturated fat (tripalmitin) than SDASO.

The TAG species were determined using normal-phase HPLC. SDASO TAG species with relative FA percentages are shown in Figure 4A (caption). The SLs' TAG species with relative FA percentages are shown in Figure 4B (caption) for NSL and LSL. On the basis of the relative FA percents from the chromatograms, the main TAG species found in SDASO were StOSt/LnGSt, LnLnL, StLnLn, LnLnLn, StLnSt, OLLn, and StOO. This compares well with the FA profile[23] because the major FAs were L, linoleic (26%); St, SDA (22%); O, oleic (14%); and Ln, ALA (12%). The SLs had different major TAG species because they contained large amounts of PA. NSL's major TAG species were PPP, OLL, POP, PLP, and StOSt, whereas LSL's major TAG species were PPP, POP, OLL, PLP, and LnOP. LSL did contain StOSt/LnGSt, but it was negligible when compared to the major TAG species. This correlates with the FA profiles of the SLs (Table 1) with mainly P, PA (>60%) occupying the TAG molecules followed by L, linoleic (∼10%); St, SDA (8%); and O, oleic (∼6%). Tripalmitin, or PPP, dominated both SLs at approximately 50% of the TAG species. The second major FA in NSL, OLL, is approximately one-fifth of PPP and the second major FA in LSL, POP, is approximately one-fourth of PPP. This is due to the high amount of PA in the TAG molecule and the use of tripalmitin as a substrate for interesterification.

Both Novozym 435 and Lipozyme TL IM lipases were tested for reusability. The enzymes were reused five times under solvent-free conditions and then washed and dried after each reuse. The *sn*-2 FA profiles remained constant over these five uses (Figure 5). The total FA profiles also remained constant over the five reuses (not shown). However, the enzymes did absorb some of the oil during interesterification. After the first use, Novozym 435 increased from the initial 67.67 to 87.65 g, whereas Lipozyme TL IM increased from the initial 67.67 to 83.05 g. After the fifth reuse, Novozym 435 weighed 102.64 g and Lipozyme TL IM weighed 85.62 g. Each enzyme experienced some loss during filtration and transfer. As noted by the weight after the first use, both enzymes absorbed oil, and Novozym 435 continued to absorb more oil through each use. This may be due to the material the lipases were immobilized onto. Novozym 435 was immobilized to macroporous acrylic resin beads, whereas Lipozyme TL IM was immobilized to silica gel. The silica gel appears to be able to release the absorbed oil better when rinsed with hexane than the macroporous acrylic resin beads. Due to the silica gel's polarity, hexane easily extracted the oil. Macroporous resins contain a network of pathways throughout the bead, making it a sponge-like material[43] that appears to absorb more of the oil.

Two SLs (NSL and LSL) were developed from SDASO enriched with PA at the *sn*-2 position with physical and chemical characteristics that could be used for food application purposes, albeit with the addition of antioxidants for stability. A possible food application may be their use as a human milk fat analogue in infant formula. The PA content at the *sn*-2 position of the SLs is >60%, which improves fat and calcium absorption[17−19] and decreases formation of calcium soaps in infants.[17,22] The use of these SLs in actual food products will soon be conducted. We are also exploring the possible addition of beneficial FAs for the infant, such as DHA, into the SL. This may result in a more suitable SL for use as human milk fat analogues than our current SLs.

AUTHOR INFORMATION

Corresponding Author

*Phone: (706) 542-1067. Fax: (706) 542-1050. E-mail: cakoh@uga.edu.

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REFERENCES

(1) Hammond, B. G.; Lemen, J. K.; Ahmed, G.; Miller, K. D.; Kirkpatrick, J.; Fleeman, T. Safety assessment of SDA soybean oil: results of a 28-day study and a 90-day/one generation reproduction feeding study in rats. *Regul. Toxicol. Pharmacol.* **2008**, *52*, 311–323.

(2) Guil-Guerrero, J. L. Stearidonic acid (18:4n-3): Metabolism, nutritional importance, medical uses and natural sources. *Eur. J. Lipid Sci. Technol.* **2007**, *109*, 1226–1236.

(3) James, M. J.; Ursin, V. M.; Cleland, L. G. Metabolism of stearidonic acid in human subjects: comparison with the metabolism of other n-3 fatty acids. *Am. J. Clin. Nutr.* **2003**, *77*, 1140–1145.

(4) Gerster, H. Can adults adequately convert α-linolenic acid (18:3n-3) to eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3)? *Int. J. Vitam. Nutr. Res.* **1998**, *68*, 159–173.

(5) Haung, Y. S.; Smith, R. S.; Redden, P. R.; Cantrill, R. C.; Horrobin, D. F. Modification of liver fatty acid metabolism in mice by omega-3 and omega-6 Δ6-desaturase substrates and products. *Biochim. Biophys. Acta* **1991**, *1082*, 319–327.

(6) Miles, E. A.; Banerjee, T.; Dooper, M. M. B. W.; M'Rabet, L.; Graus, Y. M. F.; Calder, P. C. The influence of different combinations of γ-linolenic acid, stearidonic acid and EPA on immune function in healthy young male subjects. *Br. J. Nutr.* **2004**, *91*, 893–903.

(7) Surette, M. E.; Edens, M.; Chilton, F. H.; Tramposch, K. M. Dietary echium oil increases plasma and neutrophil long-chain (*n*-3) fatty acids and lowers serum triacylglycerols in hypertriglyceridemic humans. *J. Nutr.* **2004**, *134*, 1406–1411.

(8) Ursin, V. M. Modification of plant lipids for human health: development of functional land-based omega-3 fatty acids. *J. Nutr.* **2003**, *133*, 4271–4274.

(9) Calder, P. C. *n*-3 Polyunsaturated fatty acids, inflammation, and inflammatory diseases. *Am. J. Clin. Nutr.* **2006**, *83*, 1505S–1519S.

(10) Hibbeln, J. R.; Nieminen, L. R.; Blasbalg, T. L.; Riggs, J. A.; Lands, W. E. Healthy intakes of *n*-3 and *n*-6 fatty acids: estimations considering worldwide diversity. *Am. J. Clin. Nutr.* **2006**, *83*, 1483S–1493S.

(11) Simopoulos, A. P. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Exp. Biol. Med.* **2008**, *233*, 674–688.

(12) Carlson, S. E.; Rhodes, P. G.; Ferguson, M. G. Docosahexaenoic acid status of preterm infants at birth and following feeding with human milk or formula. *Am. J. Clin. Nutr.* **1986**, *44*, 798–804.

(13) Hoy, C. E.; Xu, X. Structured triacylglycerols. In *Structured and Modified Lipids*; Gunstone, F. D., Ed.; Dekker: New York, 2001; pp 209−239.

(14) Finley, D. A.; Lönnerdal, B.; Dewey, K. G.; Grivetti, L. E. Breast milk composition: fat content and fatty acid composition in vegetarians and non-vegetarians. *Am. J. Clin. Nutr.* **1985**, *41*, 787–800.

(15) Bitman, J.; Wood, D. L.; Hamosh, P.; Mehta, N. R. Comparison of the lipid composition of breast milk from mothers of term and preterm infants. *Am. J. Clin. Nutr.* **1983**, *38*, 300–312.

(16) Fidler, N. T.; Sauerwals, A. P.; Demmelmair, H.; Koletzko, B. Docosahexaenoic acid transfer into human milk after dietary supplementation: a randomized clinical trial. *J. Lipid Res.* **2000**, *41*, 1376–1383.

(17) Tomarelli, R. M.; Meyer, B. J.; Weaber, J. R.; Bernhart, F. W. Effect of positional distribution on the absorption of the fatty acids of human milk and infant formulas. *J. Nutr.* **1968**, *95*, 583–590.

(18) López-López, A.; Castellote-Bergalló, A. I.; Campoy-Folgoso, C.; Rivero-Urgel, M.; Tormo-Carnicé, R.; Infante-Pina, D.; López-Sabater, M. C. The influence of dietary palmitic acid triacylglyceride position on the fatty acid, calcium and magnesium contents of at term newborn feces. *Early Hum. Dev.* **2001**, *65*, 83–94.

(19) Martin, J. C.; Bougnoux, P; Antoine, J. M.; Lanson, M.; Couet, C. Triacylglycerol structure of human colostrums and mature milk. *Lipids* **1993**, *28*, 637–643.

(20) Iwasaki, Y.; Yamane, T. Enzymatic synthesis of structured lipids. *J. Mol. Catal. B: Enzym.* **2000**, *10*, 129–140.

(21) Jandacek, R. Commercial applications of fats in foods. In *Fatty Acids in Foods and Their Health Implications*, 3rd ed.; Chow, C. K., Ed.; CRC: Boca Raton, FL, 2008; pp 473−492.

(22) Kennedy, K.; Fewtrell, M. S.; Morley, R.; Abbott, R.; Quinlan, P. T.; Wells, J. C.; Bindels, J. G.; Lucas, A. Double-blind, randomized trial of a synthetic triacylglycerol in formula-fed term infants: effects on stool biochemistry, stool characteristics, and bone mineralization. *Am. J. Clin. Nutr.* **1999**, *7*, 920–927.

(23) Akoh, C. C.; Xu, X. Enzymatic production of Betapol and other specialty fats. In *Lipid Biotechnology*; Kuo, C. C., Gardner, H. W., Eds.; Dekker: New York, 2002; pp 461−478.

(24) Wells, J. C. K. Nutritional considerations in infant formula design. *Semin. Neonatal.* **1996**, *1*, 19–26.

(25) Teichert, S. A.; Akoh, C. C. Stearidonic acid soybean oil enriched with palmitic acid at the *sn*-2 position by enzymatic interesterification for use as human milk fat analogues. *J. Agric. Food Chem.* **2011**, *59*, 5692–5701.

(26) *Official Methods and Recommended Practices of the American Oil Chemists' Society*; Firestone, D., Ed.; American Oil Chemists' Society: Champaign, IL, 2009; Official Methods Ac 5-41, Cd 1c-85, Cd 3a-94, Cj 1-94, Cd 8b-90, Cd 18-90, and Cd 12b-92.

(27) *Official Methods of Analysis of AOAC International*, 17th ed.; AOAC International: Gaithersburg, MD, 1998; Official Method 996.01.

(28) Pina-Rodriguez, A. M.; Akoh, C. C. Enrichment of amaranth oil with ethyl palmitate at the sn-2 position by chemical and enzymatic synthesis. *J. Agric. Food Chem.* **2009**, *57*, 4657–4662.

(29) Luddy, F. E.; Bardford, R. A.; Herb, S. F.; Magidman, P.; Riemenschneider, R. W. Pancreatic lipase hydrolysis of triacylglycerides as a semi-micro technique. *J. Am. Oil Chem. Soc.* **1964**, *41*, 639–696.

(30) Lee, J.; Landen, W. O., Jr.; Phillips, R. D.; Eitenmiller, R. R. Application of direct solvent extraction to the LC quantification of vitamin E in peanuts, peanut butter, and selected nuts. *Peanut Sci.* **1998**, *25*, 123–128.

(31) Scott, M. L. Vitamin E. In *Handbook of Lipid Research*; DeLuca, H. F., Ed.; Plenum Press: New York, 1978; Vol. 2, pp 133−210.

(32) Shahidi, F.; Wanasundra, U. N. Methods for measuring oxidative rancidity in fats and oils. In *Food Lipids*, 3rd ed.; Akoh, C. C., Min, D. B., Eds.; CRC Press: Boca Raton, FL, 2008; pp 387−408.

(33) Zhang, H.; Xu, X.; Nilsson, J.; Mu, H.; Adler-Nissen, J.; Høy, C.-E. Production of margarine fats by enzymatic interesterification with silica-granulated *Thermomyces lanuginosus* lipase in a large-scale study. *J. Am. Oil Chem. Soc.* **2001**, *78*, 57–64.

(34) Ronne, T.; Pedersen, L.; Xu, X. Triglyceride selectivity of immobilized *Thermomyces lanuginosa* lipase in interesterification. *J. Am. Oil Chem. Soc.* **2005**, *82*, 737–743.

(35) O'Keefe, S. F.; Pike, O. A. Fat characterization. In *Food Analysis*, 4th Ed.; Nielsen, S. S., Ed.; Springer: New York, 2010; pp 239−260.

(36) Turan, S.; Karabulut, I.; Vural, H. Influence of sn-1,3-lipase-catalysed interesterification on the oxidative stability of soybean oil-based structured lipids. *J. Sci. Food Agric.* **2007**, *87*, 90–97.

(37) Jennings, B. H.; Akoh, C. C. Effectiveness of natural versus synthetic antioxidants in a rice bran oil-based structured lipid. *Food Chem.* **2009**, *114*, 1456–1461.

(38) Azadmard-Dmirchi, S.; Dutta, P. C. Stability of minor lipid components with emphasis on phytosterols during chemical interesterification of a blend of refined olive oil and palm stearin. *J. Am. Oil Chem. Soc.* **2008**, *85*, 13–21.

(39) Wang, T.; Jiang, Y.; Hammond, E. G. Effect of randomization on the oxidative stability of corn oil. *J. Am. Oil Chem. Soc.* **2005**, *82*, 111–117.

(40) Akoh, C. C.; Moussata, C. O. Characterization and oxidative stability of enzymatically produced fish and canola oil-based structured lipids. *J. Am. Oil Chem. Soc.* **2001**, *78*, 25–30.

(41) Akoh, C. C. Oxidative stability of fat substitutes and vegetable oils by the oxidative stability index. *J. Am. Oil Chem. Soc.* **1994**, *71*, 211–215.

(42) Tan, C. P.; Che Man, Y. B. Differential scanning calorimetric analysis of palm oil, palm oil based products and coconut oil:effects of scanning rate variation. *Food Chem.* **2002**, *76*, 89–102.

(43) Roberge, P. R. Environments. In *Handbook of Corrosion Engineering*; Roberge, P. R., Ed.; McGraw-Hill: New York, 2000; pp 85−129.