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ORIGINAL PAPER

# Enzymatic Interesterification of Coconut and High Oleic Sunflower Oils for Edible Film Application

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**Abstract** Blends [60:40, 70:30, and 80:20 (*w*/*w*)] of coconut oil (CO) and high oleic sunflower oil (HOSO) were interesterified using immobilized enzyme, Lipozyme® TL IM (Novozymes North America Inc., Franklinton, NC, USA). The structured lipids (SLs), referred to as interesterified products (IPs) IP60:40, IP70:30, and IP80:20, were compared to CO and HOSO for application in edible films. IPs were compared based on fatty acid profile, TAG molecular species, melting profile, moisture vapor permeability, mechanical properties, film transparency, density, and thickness. Interesterification increased oleic acid content at the *sn*-2 position of IPs. CO had 5.50 ± 1.67 mol% oleic acid at the *sn*-2 position, and when interesterified with HOSO (92.81 ± 1.10 mol% oleic acid) the amount of oleic acid significantly increased ($p < 0.05$) at the *sn*-2 position for IP60:40, IP70:30, and IP80:20 (33.86 ± 1.55, 27.34 ± 1.20, 20.61 ± 1.50 mol%), respectively. There was no significant difference between SLs, HOSO, and CO for water vapor permeability and density when applied to emulsion edible films. The HOSO film was significantly different (1.43 ± 0.27 $AUmm^{-1}$) from the rest of the SLs and CO for film transparency. IP60:40 (2.20 ± 0.22 $AUmm^{-1}$) decreased the opacity and was significantly different from HOSO and IP80:20 (2.88 ± 0.08 $AUmm^{-1}$). Tensile strength of IP60:40 was 0.39 ± 0.17 MPa which was significantly different from IP70:30, IP80:20, and HOSO. The elongation at break was significantly different for HOSO and IP60:40. IP60:40 could be used to further investigate the use of SL in edible film for sports nutrition products.

**Keywords** Enzymatic interesterification · Composite edible film · Coconut oil · High oleic sunflower oil

## Introduction

The use of edible films and coatings in food products has seen an increase in the industry. There are multiple types of edible coatings such as mono-layer, bi-layer, and composite (emulsion) films. Composite or emulsion films can contain a carbohydrate or protein component and a lipid component. The lipids incorporated into these films can range from animal and plant waxes, to vegetable oils, and to fatty acids [1]. Lipid incorporation into films improves water vapor barrier properties, which lacks in both carbohydrate and protein films. Unsaturated fatty acids contribute a lower melting temperature and improved moisture barrier to the composite film. Fernandez *et al.* [2] reported that unsaturated fatty acids were attributed to a reduced surface tension compared to saturated fatty acids. In addition, their study discovered that unsaturated fatty acids were more mobile than saturated fatty acids although they did not affect the flexibility of the whey protein isolate films but reduced slightly the tensile strength [2].

Polysaccharides are advantageous in edible film development because they provide structural stability and ability to slow down oxygen transmission. Specifically, maltodextrins have been used in film formulation at water soluble concentrations up to 70% (*w*/*v*). Maltodextrins can slightly reduce the brittleness due to relatively low molecular weight and slightly hygroscopic properties. Typically, maltodextrins are added at 10–20% (*w*/*v*) and help to improve adhesion of the film [3].

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Structured lipids (SLs) are lipids that have been chemically or enzymatically modified from their natural biosynthetic form [4]. SLs, which contain medium-chain fatty acids (MCFAs), may provide a faster hydrolysis and absorption due to the lower number of carbon atoms as well as a greater water solubility in comparison to long chain fatty acids (LCFAs) [5]. LCFAs are more easily absorbed at the *sn*-2 position and typically MCFAs are often targeted at the *sn*-1,3 positions of a triacylglycerol [6].

The objective of this work was to interesterify coconut oil with high oleic sunflower oil using Lipozyme® TL IM lipase derived from *Thermomyces lanuginosus* for potential use in an edible film. Coconut oil was used as the main substrate because it contains a high content of MCFAs, whereas high oleic sunflower oil was selected because it contains a large percentage of oleic acid at the *sn*-2 position. The medium-long-medium chain (MLM) structure was selected for sports nutritional purposes. There has been little previous work utilizing SLs for the development of edible films [7]. In order to determine the best SL formulation, three ratios of coconut:high oleic sunflower oils were tested. The test methods to differentiate the three blends include fatty acid profile, melting profiles, mechanical properties, water vapor permeability, light transmission, film translucency, and film thickness.

## Materials and Methods

### Materials

Frymax sun supreme deep frying oil was donated by Stratas Foods (Memphis, TN, USA). RBD 76 °F(24.44 °C) melting coconut oil was donated by ADM (Chicago, IL, USA). Immobilized lipase, Lipozyme® TL IM (*sn*-1,3 specific *Thermomyces lanuginosus* lipase with a specific activity of 442.0 IUN/g as specified by manufacturer) was obtained from Novozymes North America (Franklinton, NC, USA). Supelco 37 component FAME mix and porcine pancreatic lipase were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA), while heptadecanoic acid (C17:0) was purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). TAG standard mixes (GLC reference standard 437 and 570) were purchased from Nu-Chek Prep, Inc. (Elysian, MN, USA). Other chemicals and solvents were purchased from Fisher Scientific (Norcross, GA, USA), Sigma-Aldrich Chemical Co., and J. T. Baker Chemicals (Center Valley, PA, USA). Film formula ingredients such as maltodextrin STAR-DRI® 1 was donated by Tate & Lyle (Decatur, IL, USA), Dixie sugar was purchased from Publix (Athens, GA, USA), carrageenan and locus bean gums were both donated by Ingredient Solutions Inc. (Waldo, ME, USA), and glycerol was purchased from Hoefer Inc. (San Francisco, CA, USA).

### Determination of Enzyme Load

The time course of interesterification of one blend 70:30 (*w*/*w*) coconut:high oleic sunflower oil was determined by weighing 50 g of sample and placing into an Erlenmeyer flask. One milliliter (in duplicate) of sample was removed to represent the starting oil (substrate) composition and stored in an amber vial in a freezer at −20 °C until RP-HPLC analysis. Then, lipase (Lipozyme® TL IM) was added at three different concentrations (10, 5, and 2.5%, *w*/*w*) of substrates. The flask was closed with a rubber stopper to prevent water from entering the flask. The reaction took place in a shaking water bath at 60 °C for 8 h with shaking at 200 rpm. One milliliter (in duplicate) of oil was sampled every 30 min, and the samples were immediately filtered through anhydrous sodium sulfate column to remove any trace of water and the biocatalyst. The samples were then placed in amber capped vials and stored in a freezer at −20 °C until RP-HPLC analysis to monitor the decrease in triolein as a means of determining the reaction time.

### Gram-Scale Interesterification

The time course of interesterification of coconut and high oleic sunflower oils of three different blends [60:40, 70:30, and 80:20 (*w*/*w*)] were determined by weighing 50 g of sample and placing into an Erlenmeyer flask. Duplicate sampling was done as described above. The interesterified products (IP 60:40, 70:30, 80:20) were then placed in amber capped vials and stored in a freezer at −20 °C until RP-HPLC analysis to monitor the decrease in triolein and to determine the reaction time.

### Large-Scale Interesterification

Two hundred grams of each blend [60:40, 70:30, 80:20 (*w*/*w*)] were weighed out. The interesterified products of the blends are designated as IP 60:40, IP 70:30, and IP 80:20, respectively. All of the interesterification reactions were solvent-free, and they occurred in a 1-L (10 cm inner diameter and 18 cm long) stirred-batch reactor under vacuum with a circulating water bath at 60 °C and mixing at 200 rpm for 3, 2, 2 h, respectively. During the reaction, the reactor was covered with aluminum foil to reduce exposure to light. Each reaction used Lipozyme® TL IM at 10% (*w*/*w*) weight of substrates. At the end of the reaction, the enzyme was removed by vacuum filtration with a Buchner funnel. The enzyme was washed with hexane to recover any remaining product, and the hexane solution was pooled with the structured lipid in the filtrate. After filtration, the

SLs were placed in amber Nalgene bottles and were flushed with nitrogen before being stored at −80 °C in a freezer. Approximately 5 g of each SL was kept for analysis of free fatty acid (FFA) percentage before short-path distillation.

### Short-Path Distillation

Short-path distillation was used to remove excess FFAs from large-scale synthesis of SLs (i.e., IPs). Short-path distillation was performed using a KDL-4 (UIC Inc., Joliet, IL, USA) unit under the following conditions: holding temperature of 50 °C, feeding rate of approximately 100 mL/h, heating oil temperature of 185 °C, coolant temperature of 30–35 °C, and vacuum of <100 mTorr or <13.33 Pa. The IP 60:40, IP 70:30, and IP 80:20 were passed through the short-path distillation once. After short-path distillation, the FFA content as lauric acid equivalents was determined according to AOCS Official Method Ac 5-40 [8]. The percent yield was calculated with Eq. (1) with the initial and final values representing the weight and FFA amount before and after short-path distillation. The SLs were analyzed for their fatty acid profile and positional analysis as described below.

$$\text{Percent yield}\,(\%) = \frac{(\text{Final weight}\,(\text{g}))(1 - \text{Final FFA}\%)}{(\text{Initial weight}\,(\text{g}))(1 - \text{Initial FFA}\%)} \times 100. \quad (1)$$

### Determination of Fatty Acid Profiles

Total fatty acid (FA) percentages were determined by weighing 0.1 g of sample into separate Teflon-lined screw-capped test tubes and 1 mL of 20 mg/mL C17:0 in hexane as internal standard. The lipid samples were then converted into fatty acid methyl esters (FAME) following the AOAC Official Method 996.01, Section E [9] with minor modifications as previously described [10]. An external standard, Supelco 37 component FAME mix, was used for identification of fatty acids (FAs) after GC separation. Samples were analyzed in triplicate and the average and standard deviation were reported.

### Fatty Acids Positional Analysis

Positional analysis (*sn*-2 and *sn*-1,3) of high oleic sunflower and coconut oil was conducted along with each of the physical blends (PBs) and interesterified products (IPs) for the ratios 60:40, 70:30, 80:20 (*w*/*w*) coconut:high oleic sunflower oils. The analysis was performed according to a modified version of the method described by Ifeduba and Akoh [11], which used pancreatic lipase. One modification made was extracting with 2 mL of diethyl ether instead of 4 mL. Samples were analyzed in triplicate and the average and standard deviation were reported.

### GC Analysis

Total and positional FAs were analyzed as FAME on an Agilent Technology 6890 N gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with a Supelco SP-2560 capillary GC column (100 m × 0.25 mm, 0.20 μm film, Sigma-Aldrich Co., St. Louis, MO, USA). Injection of 1 μL of the sample was made at split ratio of 50:1 for total FAs analysis and 5:1 for positional FAs analysis. Helium was the carrier gas at a flow rate of 1.1 mL/min. The detector temperature was set at 250 °C. The oven was held at 140 °C for 5 min, then increased to 240 °C at 4 °C/min and held isothermally for 17 min. The relative FA content was calculated as mol%. Samples were analyzed in triplicate. The average and standard deviations were reported. The *sn*-1,3 position was calculated using the following formula

$$\frac{(3 \times (\text{Average of total fatty acid composition}) - (\text{Average of } sn{-}2 \text{ position}))}{2}. \quad (2)$$

### TAG Molecular Species

Analysis of TAG molecular species of CO and HOSO, in addition to IPs and PBs of ratios 60:40, 70:30, and 80:20 (*w*/*w*) were performed by reversed-phase HPLC on a Agilent 1100 HPLC system (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with a Sedex Model 55 evaporative light scattering detector (ELSD) (Richard Scientific Novato, CA, USA) and a 4 mm × 250 mm, 5 μm particle size, Ultrasphere C18 analytical column (Beckman Coulter Inc., Pasadena, CA, USA). Samples were diluted in chloroform to a concentration of ~5 mg/mL for the IPs, PBs, and CO. The HOSO was diluted in chloroform to a concentration of ~1 mg/mL. Sample injection volume was 20 μL. The column temperature was set at 30 °C. The mobile phase was acetonitrile (A) and acetone (B). The flow rate was set at 1 mL/min. Gradient elution began with 65% B at 0 min to 95% B at

55 min, and was followed by 5 min post-run at 65% B. The drift tube temperature for ELSD was set at 86 °C, and the nebulizer gas pressure was 3.0 bar. The retention times of TAG species depended on polarity and equivalent carbon number (ECN). ECN is defined as $C_N$-$2n$, where $C_N$ is the number of carbon atoms in the TAG excluding the three carbon atoms of glycerol, and n is the number of double bonds. For peak identification, the retention times of sample TAG species were compared with TAG standards of known ECN, namely, tricaprylin (24), tricaprin (30), trilaurin (36), trimyristin (42), tripalmitin (48), triolein (48), tristearin (54), and triarachidin (60). Samples were analyzed in triplicate and average values reported.

### Melting Profiles

The melting profiles of each of the IPs, CO, and HOSO were determined using a 204 F1 Phoenix DSC (NETZSCH Instruments North America, Burlington, MA, USA) following AOCS Official Method Cj 1-94 [12]. Nitrogen was used as the protective gas (purge).

### Emulsion Film Formulation

The emulsion film was dispersed by gelatinizing 10% (*w*/*v*) maltodextrin-1 DE in water at 70 °C at 600 rpm for 30 min. The oil (HOSO, IP 60:40, IP 70:30, IP 80:20 or CO) at 15%, carrageen (5%), and locust bean gum (3.5%) were homogenized using a Polytron® homogenizer PT 10/35 fitted with a PTA7 generator (Brinkmann Instruments Inc., Westbury, NY, USA) at max speed 8 m/s and then heated at 70 °C for 5 min. The sugar (10%) and glycerol (15%) were then added to the oil mixture and mixed at 600 rpm using a VWR (Henry Troemner LLC, Thorofare, NJ, USA). Finally, the (60%) maltodextrin solution was added and mixed at 600 rpm and then homogenized using the Polytron® at max speed 8 m/s for 5 min. The emulsion was then diluted to 50:50 in distilled water. The films were cast onto glass plates (7.5 cm × 11.5 cm) and left to dry at 25 °C for 24 h.

### Mechanical Properties of Edible Films

Mechanical properties such as tensile strength and elongation break were measured using a Ta-XT2i Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, USA). The settings of the texture analyzer were determined based on a method provided by Tang *et al.* [13].

### Film Thickness and Density

The film thickness (FT) was measured using a handheld caliper/micrometer Bel-Art-SP Scienceware (Wayne, NJ, USA), to the nearest 0.001 mm. The mean thickness was calculated from measurements taken randomly at ten points at various locations on each film sample. Density was calculated directly from the film weight and dimensions according to [13]

$$\rho = m/(A \times \mathrm{FT}). \tag{3}$$

where $\rho$ is density which is equal to the m which is the mass (g), $A$ is the film area (1 $cm^2$) and FT is the film thickness (cm). The film density was expressed as the average of three independent determinations.

### Water Vapor Permeability

The water vapor permeability (WVP) was gravimetrically measured according to protocol B of ASTM 96-95 [14] with adaptations proposed specifically for edible films [15]. Circular aluminum cups, with a diameter of 5 cm and a depth of 1.7 cm, were used. Distilled water (15 mL) was placed in each test cup, to expose the lower film face to a high relative humidity (RH). The film surface exposed was 3 cm in diameter. The film samples were mounted and the upper film face was exposed to a RH (50 ± 1%) at a temperature of 27.5 °C. The weight loss of the total cup was monitored over a 72 h period, with weights recorded at 24 h intervals. The WVP (g mm $m^{-2}$ $day^{-1}$ $kPa^{-1}$) of the film was calculated as follows

$$\mathrm{WV}P = (\Delta W \times \mathrm{FT})/(S \times \Delta p). \tag{4}$$

where $\Delta W$ is the weight loss of the cup per day (g $day^{-1}$) (i.e. slope of the linear behavior), FT (mm), $S$ is the area of the exposed film ($m^2$), and $\Delta p$ is the vapor pressure differential across the test film (kPa). Samples were analyzed in triplicate and the standard deviation was calculated.

### Light Transmission and Film Transparency

A modified method from [15] was conducted to determine the ultraviolet (UV) and visible light barrier properties of the dried films at selected wavelengths (in the 300–800 nm range), using a UV-1601 visible Spectrophotometer (Shimadzu Corp., Columbia, MD, USA). The films were cut

into rectangles of 35 mm × 8 mm (length, width) and placed into cuvettes. Film's transmittance was determined at wavelengths of 300–800 nm in order to determine the barrier effect against UV and visible light. The transparency was measured at 600 nm and was calculated

$$\text{Transparency} = A_{600}/\text{FT}. \quad (5)$$

where $A_{600}$ is the absorbance at 600 nm and FT is the film thickness (mm). Three sample strips of each of the five total variables (HOSO, IP 60:40, IP 70:30, IP 80:20, and CO) were averaged and the standard deviation reported.

### Statistical Analysis

Analyses were completed in triplicate or duplicate. All statistical analyses were conducted with the SAS software package (SAS Institute Inc., Cary, NC, USA). Duncan's multiple range test was performed to determine the significance of difference at $p \leq 0.05$.

## Results and Discussion

### Total and Positional Fatty Acid Profiles and Determination of Reaction Parameters

Coconut and high oleic sunflower oils were characterized because they were the starting substrates in this study. The fatty acid profiles are shown in Table 1. The total and positional analyses of the fatty acid composition of high oleic sunflower oil were in agreement with previous studies [16]. The fatty acid profile of coconut oil was in compliance with the manufacture's certificate of analysis. The major fatty acids in coconut oil were caprylic (8.18 ± 0.04 mol%), capric (6.19 ± 0.02 mol%), lauric (46.91 ± 0.27 mol%), myristic (17.66 ± 0.05 mol%), palmitic (8.89 ± 0.05 mol%), and oleic (6.91 ± 0.29 mol%) acids. The *sn*-2 position of coconut oil had high amounts of lauric (80.53 ± 4.92 mol%), myrisitc (6.36 ± 1.90 mol%), and oleic (5.50 ± 1.67 mol%) acids. The amount of oleic acid at the *sn*-2 position of coconut oil was low; therefore, high oleic sunflower oil was added in the interesterification reaction to increase the amount of oleic acid at the *sn*-2 position. The major fatty acid in high oleic sunflower oil was oleic acid (86.38 ± 0.52 mol%). The major fatty acid at the *sn*-2 position is oleic acid (92.81 ± 1.10 mol%). The *sn*-1,3 positions of coconut oil had high amounts of caprylic (11.25 ± 0.32 mol%), capric (8.05 ± 0.57 mol%), lauric (30.11 ± 2.58 mol%), myristic (23.31 ± 0.99 mol%), palmitic (12.47 ± 0.37 mol%) acids. The *sn*-1,3 positions of high oleic sunflower oil had high amount of oleic acid (83.15 ± 1.22 mol%).

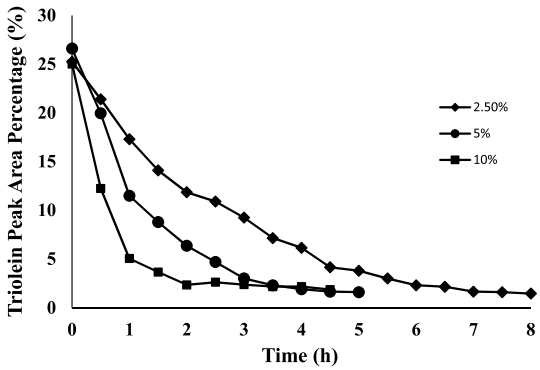


**Fig. 1** Time course showing reduction in triolein (TO) during enzymatic interesterification of coconut and high oleic sunflower oil blend 70:30 (*w*/*w*)

**Table 1** Total and positional fatty acid composition (mol%) of coconut and high oleic sunflower oils

| Fatty acid | Coconut oil[a] | | | High oleic sunflower oil[b] | | |
|---|---|---|---|---|---|---|
| | Total | *sn*-2 | *sn*-1,3 | Total | *sn*-2 | *sn*-1,3 |
| C8:0 | 8.18 ± 0.04 | 1.98 ± 0.30 | 11.25 ± 0.32 | ND | ND | ND |
| C10:0 | 6.19 ± 0.02 | 2.48 ± 1.05 | 8.05 ± 0.57 | ND | ND | ND |
| C12:0 | 46.91 ± 0.27 | 80.53 ± 4.92 | 30.11 ± 2.58 | ND | ND | ND |
| C14: 0 | 17.66 ± 0.05 | 6.36 ± 1.90 | 23.31 ± 0.99 | ND | ND | ND |
| C16:0 | 8.89 ± 0.05 | 1.77 ± 0.31 | 12.47 ± 0.37 | 3.57 ± 0.03 | 1.47 ± 0.36 | 4.62 ± 0.32 |
| C18:0 | 2.58 ± 0.15 | ND | 3.88 ± 0.58 | 2.56 ± 0.23 | 0.39 ± 0.10 | 3.64 ± 0.72 |
| C18:1n9 | 6.91 ± 0.29 | 5.50 ± 1.67 | 7.62 ± 1.16 | 86.38 ± 0.52 | 92.81 ± 1.10 | 83.15 ± 1.22 |
| C18:2n6 | 2.08 ± 0.32 | 1.37 ± 0.29 | 2.43 ± 0.86 | 4.59 ± 0.12 | 4.64 ± 0.08 | 4.56 ± 0.53 |
| C22:1 | ND[c] | ND | ND | 1.27 ± 0.06 | ND | 1.90 ± 0.36 |

Values are mean ± SD ($n = 3$)

[a] Trace amounts of C6:0, C18:3n6, and C18:3n3

[b] Trace amounts C14:0, C16:1n7, C18:3n6, C20:1, C18:3n3, C24:1

[c] *ND* not detected

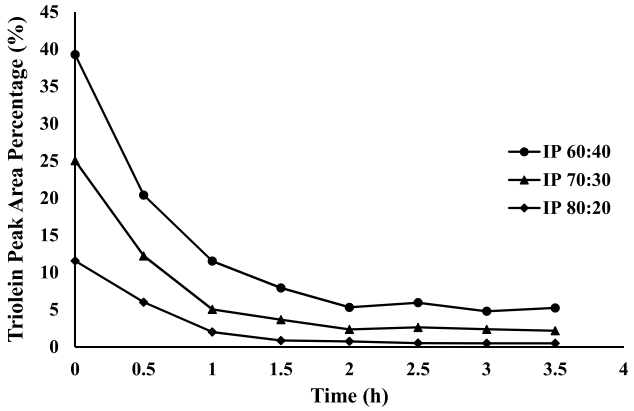


**Fig. 2** Time course showing reduction in triolein (TO) during enzymatic interesterification of coconut and high oleic sunflower oils for three interesterified products (IP)

A preliminary determination of the percentage of enzyme to be used in the interesterification reaction was carried out using a time course. The time course interesterification for triolein disappearance when CO was reacted with HOSO was performed to determine the reaction equilibrium. Figure 1 describes the reaction equilibrium which was 7 h for 2.5%, 4.5 h for 5%, and 2 h for 10% enzyme. We chose 10% enzyme for the small-scale reaction due to the shortest amount of time and cost. In addition, a time course consumption of triolein was constructed to compare the three different ratios 80:20, 70:30, and 60:40 using 10% (*w*/*w*) enzyme. Figure 2 describes the reaction equilibrium which was 2 h for 80:20 and 70:30 and 3 h for the 60:40. The three ratios were then tested on a larger scale to determine which would be the best blend for an edible coating based on chemical and physical characteristics. Such include the total and positional fatty acid profiles, mechanical properties, density, thickness, and light properties.

Comparisons of the total, *sn*-2 and *sn*-1,3 composition of the IPs and the PBs were conducted and data is shown in Table 2. The results for the *sn*-2 oleic acid between physical and interesterified blends differed significantly for each of the three blends. The PB 80:20 was (13.90 ± 2.71 mol%), whilst the IP 80:20 was (20.61 ± 1.50 mol%). The PB 70:30 was (18.52 ± 0.50 mol%) compared to the IP 70:30 (27.34 ± 1.20 mol%). Finally, the PB 60:40 was (24.29 ± 3.94 mol%) and IP 60:40 (33.86 ± 1.55 mol%). Therefore, interesterification resulted in increased oleic acid amount at the *sn*-2 position of the interesterified products. The results for the *sn*-1,3 lauric acid between physical and interesterified blends also differed significantly for each of the three blends. The PB 80:20 was (22.79 ± 1.08 mol%) and the IP was (29.83 ± 0.76 mol%). The PB 70:30 was (16.52 ± 0.65 mol%) and the IP was (24.78 ± 1.31 mol%). The PB 60:40 was (9.75 ± 1.19 mol%) and the IP was (21.66 ± 0.71 mol%).

**Table 2** Total and positional fatty acid composition (mol%) of three physical blends and interesterfied products

| Fatty acid | Total | | | *sn*-2 | | | *sn*-1,3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | 60:40[A] | 70:30[A] | 80:20[A] | 60:40 | 70:30 | 80:20 | 60:40 | 70:30 | 80:20 |
| C8:0 PB | 4.46 ± 0.08$^{g}$ | 5.46 ± 0.04$^{e}$ | 6.12 ± 0.05$^{i}$ | 0.73 ± 0.17$^{kl}$ | 1.02 ± 0.20$^{kl}$ | ND | 6.59 ± 0.44 | 7.65 ± 0.29 | 9.18 ± 0.32 |
| C8:0 IP | 4.53 ± 0.03$^{g}$ | 5.32 ± 0.20$^{e}$ | 5.33 ± 0.13$^{j}$ | 2.53 ± 0.35$^{hijk}$ | 2.57 ± 0.16$^{ij}$ | 2.43 ± 0.47$^{gh}$ | 5.57 ± 0.31 | 6.66 ± 0.67 | 6.71 ± 0.60 |
| C10:0 PB | 3.51 ± 0.04$^{h}$ | 4.23 ± 0.03$^{f}$ | 4.71 ± 0.03$^{k}$ | 2.76 ± 0.46$^{ghij}$ | 3.09 ± 0.35$^{hi}$ | 2.95 ± 0.28$^{gh}$ | 3.88 ± 0.34 | 4.80 ± 0.28 | 5.58 ± 0.29 |
| C10:0 IP | 3.56 ± 0.02$^{h}$ | 4.19 ± 0.11$^{f}$ | 4.51 ± 0.05$^{k}$ | 3.54 ± 0.95$^{gh}$ | 2.64 ± 0.92$^{ij}$ | 3.50 ± 0.08$^{gh}$ | 3.58 ± 0.51 | 4.97 ± 0.68 | 5.02 ± 0.32 |
| C12:0 PB | 27.00 ± 0.19$^{d}$ | 32.52 ± 0.13$^{a}$ | 35.91 ± 0.23$^{b}$ | 61.50 ± 2.82$^{a}$ | 64.53 ± 0.99$^{a}$ | 62.13 ± 1.60$^{a}$ | 9.75 ± 1.19 | 16.52 ± 0.65 | 22.79 ± 1.08 |
| C12:0 IP | 27.99 ± 0.11$^{c}$ | 32.60 ± 0.44$^{a}$ | 36.77 ± 0.11$^{a}$ | 40.67 ± 1.01$^{b}$ | 48.26 ± 1.70$^{b}$ | 50.66 ± 0.91$^{b}$ | 21.66 ± 0.71 | 24.78 ± 1.31 | 29.83 ± 0.76 |
| C14:0 PB | 10.26 ± 0.06$^{e}$ | 12.37 ± 0.02$^{c}$ | 13.58 ± 0.11$^{f}$ | 6.52 ± 0.26$^{f}$ | 7.35 ± 0.35$^{f}$ | 11.55 ± 4.83$^{de}$ | 12.14 ± 0.39 | 14.88 ± 0.25 | 14.61 ± 2.46 |
| C14:0 IP | 10.53 ± 0.03$^{e}$ | 12.25 ± 0.01$^{c}$ | 14.29 ± 0.04$^{e}$ | 8.87 ± 0.14$^{e}$ | 9.70 ± 0.10$^{e}$ | 10.80 ± 0.19$^{e}$ | 11.37 ± 0.27 | 13.52 ± 0.15 | 16.04 ± 0.31 |
| C16:0 PB | 6.72 ± 0.01$^{f}$ | 7.31 ± 0.01$^{d}$ | 7.65 ± 0.05$^{h}$ | 1.12 ± 0.11$^{jkl}$ | 1.76 ± 0.11$^{jk}$ | 3.90 ± 0.73$^{fg}$ | 9.51 ± 0.15 | 10.12 ± 0.13 | 9.55 ± 0.48 |
| C16:0 IP | 6.66 ± 0.11$^{f}$ | 7.12 ± 0.01$^{d}$ | 7.96 ± 0.01$^{g}$ | 4.23 ± 0.09$^{gh}$ | 4.24 ± 0.27$^{gh}$ | 5.02 ± 0.18$^{fg}$ | 7.85 ± 0.51 | 8.60 ± 0.51 | 9.46 ± 0.17 |
| C18:0 PB | 2.72 ± 0.12$^{j}$ | 2.68 ± 0.10$^{g}$ | 2.82 ± 0.31$^{l}$ | ND | ND | ND | 4.07 ± 0.52 | 4.02 ± 0.46 | 4.23 ± 0.84 |
| C18:0 IP | 3.13 ± 0.15$^{i}$ | 2.98 ± 0.09$^{gh}$ | 3.06 ± 0.25$^{l}$ | 1.47 ± 0.44$^{ijkl}$ | 0.15 ± 0.25 $^{l}$ | ND | 3.96 ± 0.62 | 4.39 ± 0.47 | 4.59 ± 0.75 |
| C18:1n9 PB | 39.65 ± 0.58$^{a}$ | 30.73 ± 0.13$^{b}$ | 24.82 ± 0.46$^{c}$ | 24.29 ± 3.94$^{d}$ | 18.52 ± 0.50$^{d}$ | 13.90 ± 2.71$^{d}$ | 47.38 ± 1.80 | 36.84 ± 0.57 | 30.28 ± 2.69 |
| C18:1n9 IP | 38.51 ± 0.13$^{b}$ | 30.98 ± 0.53$^{b}$ | 23.96 ± 0.53$^{d}$ | 33.86 ± 1.55$^{c}$ | 27.34 ± 1.20$^{c}$ | 20.61 ± 1.50$^{c}$ | 40.83 ± 0.93 | 32.80 ± 1.25 | 25.66 ± 0.90 |
| C18:2n6 PB | 3.60 ± 0.05$^{h}$ | 3.29 ± 0.06$^{g}$ | 2.98 ± 0.10$^{l}$ | 3.09 ± 0.12$^{ghi}$ | 3.73 ± 0.80$^{hi}$ | 5.57 ± 3.51 $^{fg}$ | 3.85 ± 0.33 | 3.07 ± 0.47 | 1.68 ± 1.82 |
| C18:2n6 IP | 3.31 ± 0.07$^{hi}$ | 3.14 ± 0.02$^{g}$ | 2.97 ± 0.04$^{l}$ | 4.58 ± 0.07$^{h}$ | 5.11 ± 0.67$^{g}$ | 6.99 ± 0.68$^{fg}$ | 2.67 ± 0.39 | 2.16 ± 0.40 | 0.96 ± 0.45 |

Values are mean ± SD ($n = 3$). Different letters between physical blend (PB) and interesterified product (IP) of the same blend indicate significant difference

*ND* not detected

[A] Trace amounts found of C6:0, C18:3n6, C20:1n9, C18;3n3, C22:1, and C24:1

**Table 3** Relative percentage (%) of peak areas of triacylglycerol (TAG) molecular species based on equivalent carbon number (ECN) of high oleic sunflower oil (HOSO), coconut oil (CO), physical blend (PB), and interesterified products (IP)

| TAG Species[a] | ECN (DB)[b] | CO | PB 60:40 | IP 60:40 | PB 70:30 | IP 70:30 | PB 80:20 | IP 80:20 | HOSO |
|---|---|---|---|---|---|---|---|---|---|
| CyCyLa | 28 | 0.24 ± 0.16 | ND | ND | ND | ND | ND | ND | ND |
| CCyC | 28 | ND | ND | 0.06 ± 0.02 | ND | ND | ND | ND | ND |
| LaCCy | 30 | 2.09 ± 1.09 | 0.74 ± 0.05 | ND | 0.81 ± 0.07 | ND | 0.98 ± 0.13 | 1.10 ± 0.19 | ND |
| CyOCy | 32 (1) | ND | ND | 0.24 ± 0.05 | ND | 0.26 ± 0.08 | ND | ND | ND |
| CyLaLa | 32 | ND | ND | 2.08 ± 0.21 | ND | 1.47 ± 0.10 | ND | ND | ND |
| CLaC | 32 | 15.44 ± 0.46 | 9.58 ± 0.61 | ND | 11.42 ± 0.09 | ND | 13.80 ± 1.33 | 4.71 ± 0.05 | ND |
| LaCLa | 34 | 21.56 ± 2.10 | 15.02 ± 0.29 | ND | 17.90 ± 0.54 | ND | 20.27 ± 0.98 | 8.07 ± 0.64 | ND |
| CyOLa | 36(1) | ND | ND | 3.01 ± 0.38 | ND | 4.05 ± 0.27 | ND | ND | ND |
| CLaM | 36 | ND | ND | 5.25 ± 0.19 | ND | 6.17 ± 0.27 | ND | 23.55 ± 2.44 | ND |
| LaLaLa | 36 | 26.19 ± 3.13 | 18.36 ± 0.51 | ND | 21.40 ± 0.38 | ND | 24.77 ± 0.26 | ND | ND |
| LaOC | 38(1) | ND | ND | 14.67 ± 1.26 | ND | 17.32 ± 0.10 | ND | ND | ND |
| LaLaM | 38 | 18.49 ± 0.88 | 10.63 ± 1.61 | 12.02 ± 1.60 | 13.34 ± 0.08 | 14.91 ± 0.30 | 15.06 ± 0.54 | 18.97 ± 0.27 | ND |
| LaOLa | 40(1) | ND | ND | 27.01 ± 1.48 | ND | 26.80 ± 0.32 | ND | 26.66 ± 1.47 | ND |
| LaMM | 40 | 10.56 ± 1.42 | 3.96 ± 0.24 | ND | 6.06 ± 0.63 | ND | 6.82 ± 0.33 | ND | ND |
| LaOM | 42(1) | ND | ND | ND | ND | 13.12 ± 0.10 | ND | 6.60 ± 0.61 | ND |
| LaMP | 42 | 3.81 ± 1.78 | 1.1 ± 0.08 | 12.14 ± 0.44 | 1.22 ± 0.29 | ND | 1.44 ± 0.13 | 3.29 ± 0.79 | ND |
| LaOP | 44 (1) | ND | ND | ND | ND | 1.14 ± 0.16 | ND | 3.35 ± 0.49 | ND |
| LaOO | 44 (2) | 0.51 ± 0.33 | ND | 18.26 ± 0.30 | ND | 12.64 ± 0.36 | ND | ND | ND |
| LaPP | 44 | 0.77 ± 0.55 | ND | ND | ND | ND | ND | 2.75 ± 0.31 | ND |
| OLO + LOP | 46(4), 46(3) | ND | ND | 2.78 ± 0.25 | ND | 0.94 ± 0.07 | ND | 0.94 ± 0.15 | 2.04 ± 1.56 |
| LaOS | 46(1) | ND | ND | 1.78 ± 0.37 | ND | 0.89 ± 0.01 | ND | ND | ND |
| OOO | 48(3) | ND | 40.62 ± 2.65 | 0.70 ± 0.22 | 27.84 ± 0.83 | 0.38 ± 0.16 | 16.80 ± 2.60 | ND | 96.34 ± 1.80 |
| POS + PPS | 50 | ND | ND | ND | ND | ND | ND | ND | 1.62 ± 0.65 |

Values are mean ± SD ($n = 3$)

*Cy* caprylic acid (C8:0), *C* capric acid (C10:0), *La* lauric acid (C12:0), *M* myristic acid (C14:0), *P* palmitic acid (C16:0), *S* stearic acid (C18:0), *O* oleic acid (C18:1), *L* linoleic acid (C18:2), *ND* not detected

[a] TAG species do not reflect stereochemical configuration

[b] Equivalent carbon number (ECN) = TC − (2 × DB); TC is total carbon number of acyl groups and DB is total number of double bonds in parenthesis

The product reaction yields for the 80:20, 70:30 and 60:40 were 88.50, 93.73, and 94.57%, respectively.

### TAG Molecular Species and Melting Profile

The relative TAG molecular species in CO, HOSO, PBs, and IPs of 80:20, 70:30, and 60:40 were determined in order to follow the change in TAG species due to enzymatic reactions (Table 3). The TAGs shown are not representative of stereochemical configuration. The peak determinations were based on elution time of TAG standards, equivalent carbon number (ECN), and published works on coconut and high oleic sunflower oils [16, 17]. The major TAG species found in HOSO was OOO at (96.34 ± 1.80%). In CO, the major TAG species were LaMM (10.56 ± 1.42%), LaLaM (18.49 ± 0.88%), LaLaLa (26.19 ± 3.13%), LaCLa (21.56 ± 2.10%), and CLaC (15.44 ± 0.46%). In the PB 60:40, the predominant TAG species were CLaC (9.48 ± 0.61%), LaCLa (15.02 ± 0.29%), LaLaLa (18.36 ± 0.51), LaLaM (10.63 ± 1.61%), and OOO (40.62 ± 2.65%), whereas for the IP 60:40, the predominant TAG species were LaOC (14.67 ± 1.26%), LaLaM (12.02 ± 1.60%), LaOLa (27.01 ± 1.48%), LaMP (12.14 ± 0.44%), and LaOO (18.26 ± 0.30%). The predominant TAG species for PB 70:30 were CLaC (11.42 ± 0.09%), LaCLa (17.90 ± 0.54%), LaLaLa (21.40 ± 0.38%), LaLaM (13.34 ± 0.08%), and OOO (27.84 ± 0.83%), whereas the predominant TAG species for the IP 70:30 were LaOC (17.32 ± 0.10%), LaLaM (14.92 ± 0.32%), LaOLa (26.80 ± 0.32%), LaMP (13.12 ± 0.08%), and LaOM (12.64 ± 0.36%). In the PB 80:20, CLaC (13.80 ± 1.33%), LaCLa (20.27 ± 0.98%), LaLaLa (24.77 ± 0.26%), LaLaM (15.06 ± 0.54%), and OOO (16.80 ± 2.60%) were the dominant TAG species whereas for the IP 80:20 LaCLa (8.07 ± 0.64%), CLaM (23.55 ± 2.44%), LaLaM (18.97 ± 0.27%), and LaOLa (26.66 ± 1.47%) were the predominant species.

**Table 4** Film thickness, density, and opacity

| Sample | Thickness (mm)[A] | Density (g/cm$^3$)[B] | Opacity (A600/mm)[B] |
|---|---|---|---|
| HOSO | $0.47 \pm 0.04^{a}$ | $1.69 \pm 0.10^{a}$ | $1.43 \pm 0.27^{c}$ |
| IP 60:40 | $0.46 \pm 0.02^{a}$ | $1.75 \pm 0.16^{a}$ | $2.20 \pm 0.22^{b}$ |
| IP 70:30 | $0.47 \pm 0.03^{a}$ | $1.64 \pm 0.20^{a}$ | $2.64 \pm 0.53^{ab}$ |
| IP 80:20 | $0.45 \pm 0.01^{a}$ | $1.61 \pm 0.13^{a}$ | $2.88 \pm 0.08^{a}$ |
| CO | $0.43 \pm 0.03^{a}$ | $1.53 \pm 0.14^{a}$ | $2.54 \pm 0.10^{ab}$ |

*HOSO* high oleic sunflower oil, *IP* interesterified product 60:40 CO:HOSO SL, IP 70:30 CO:HOSO SL, IP 80:20 CO:HOSO SL, *CO* coconut oil, different letters in each column represent significant difference ($p \leq 0.05$)

[A] Mean ± SD ($n = 10$)

[B] Mean ± SD ($n = 3$)

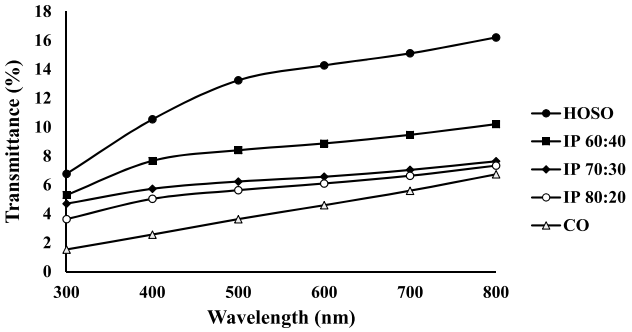


**Fig. 3** Light transmittance changes at different wavelengths ranging from 300 to 800 nm, high oleic sunflower oil (HOSO), interesterified products (IP), coconut oil (CO)

The melting profile of HOSO, IP 60:40, IP 70:30, IP 80:20, and CO was determined using DSC. The melting onset temperature for HOSO was −8.4 °C, IP 60:40 11.5 °C, IP 70:30 16.6 °C, and IP 80:20 18.5 °C, and CO was 24.6 °C. The melting profile was as expected with the IP products having melting temperatures between the two starting oils. Clearly, enzymatic interesterification of CO and HOSO resulted in new TAG molecular species and melting behavior which may affect the physical properties of the products and their application in edible film.

## Film Thickness and Density

Film thickness and density for each of the interesterified products and initial oils can be found in Table 4. The thickness for the films varied from 0.43 ± 0.03 mm to 0.47 ± 0.04 mm. The thickness of the film was greater than that of previously reported films. According to Binisi *et al.* [18], the emulsion film comprised of chitosan and virgin coconut oil had a thickness of 0.075 mm thickness which is thinner than the film thickness we achieved. The film thickness could be reduced by adding less film initially onto each plate which would help improve all parameters as the film thickness affects multiple parameters. The density has no significant difference ($p \leq 0.05$) among lipid types. The density ranged slightly higher from 1.53 to 1.75 (g cm$^{-3}$) than that found by Ramos *et al.* [15] which had density values ranging from (1.16 to 1.29 g cm$^{-3}$).

## Film Light Transmission and Opacity

The results of opacity experiments with the three structured lipids (IP 60:40, IP 70:30, and IP 80:20) and starting substrates are presented in Table 4. The HOSO film was significantly less opaque than the other films (1.43 ± 0.27 AU/mm). However, the IP 80:20 (2.88 ± 0.08 AU/mm) film was significantly different from the HOSO film and the IP 60:40 film, but not IP 70:30 and CO. The structured lipid with the least opacity was IP 60:40 (2.20 ± 0.22 Au/mm), which was significantly different $p \leq 0.05$ from IP 80:20 (2.88 ± 0.08 AU/mm) and HOSO (1.43 ± 0.27 AU/mm). The concentration of oil in the emulsion film was greater than that in previous research conducted by Bisni *et al.* [18] who reported 0.58 and 2.83 opacity index. The opacity differences may be due to various amounts of saturated fatty acids present in CO, IP 60:40, IP 70:30, and IP 80:20 compared to HOSO.

Film light transmission can be found in Fig. 3. Light transmission indicates how much light can penetrate the film barrier. The HOSO film had the highest light transmission and the coconut oil had the lowest light transmission. The UV/Light barrier would be advantageous in protecting the product and has been reported as beneficial in preventing color loss and lipid oxidation [19]. Therefore, the structured lipids containing saturated fatty acids compared to HOSO would be expected to provide barrier to light transmission and reduce lipid oxidation and color loss when used in edible film.

## Water Vapor Permeability

The water vapor permeability (WVP) of the films with the starting substrates and the structured lipids were determined. The WVP was not significantly different from one film to the other (Table 5). The WVP for a film with HOSO was 3.76 ± 0.23 (g mm d$^{-1}$ m$^{-2}$ kPa$^{-1}$), while the range for all structured lipids was 3.48–3.73 g mm day$^{-1}$ m$^{-2}$ kPa$^{-1}$. Monedero *et al.* [20] found similar results of no significant difference ($p \leq 0.05$)

**Table 5** Tensile strength (TS), elongation break (EB), and water vapor permeability (WVP) of test films

| | Tensile strength (Mpa) | Elongation break (%) | Water vapor permeability (g mm $d^{-1}$ $m^{-2}$ $kPa^{-1}$) |
|---|---|---|---|
| HOSO | $0.26 \pm 0.02^{b}$ | $29.60 \pm 2.67^{a}$ | $3.76 \pm 0.23^{a}$ |
| IP 60:40 | $0.39 \pm 0.17^{a}$ | $19.46 \pm 1.22^{c}$ | $3.50 \pm 0.13^{a}$ |
| IP 70:30 | $0.19 \pm 0.02^{b}$ | $23.73 \pm 2.47^{bc}$ | $3.48 \pm 0.36^{a}$ |
| IP 80:20 | $0.16 \pm 0.01^{b}$ | $26.02 \pm 0.35^{ab}$ | $3.73 \pm 0.17^{a}$ |
| CO | $0.26 \pm 0.09^{ab}$ | $22.83 \pm 2.96^{bc}$ | $3.70 \pm 0.60^{a}$ |

*HOSO* high oleic sunflower oil, *IP* interesterified product 60:40 CO:HOSO SL, IP 70:30 CO:HOSO SL, IP 80:20 CO:HOSO SL, *CO* coconut oil, different letters in a column represents significant difference ($p \leq 0.05$)

Mean ± SD ($n = 3$)

between oleic acid and beeswax addition to an edible film, whereas, Binisi *et al*. [18] found WVP to be between 0.41 ± 0.02 for the control to 0.08 ± 0.004 g mm/$m^2$ d kPa after adding virgin coconut oil at 1.5 mL/g chitosan. The WVP recorded by Ramos *et al.* [14] was higher, ranging from 10.1 ± 0.20 to 13.4 ± 0.41 g mm/$m^2$ d kPa than that found by other researchers that included a lipid component. The interaction between the maltodextrin and lipid component impacts the WVP in addition to the thickness of the film.

### Mechanical Film Characteristics

Tensile strength (TS) and elongation at break (Table 5) were determined for all structured lipids and the starting substrates. TS (MPa) for IP 60:40 was significantly higher at $p \leq 0.05$ (0.39 ± 0.17 MPa) than HOSO, IP 70:30, and IP 80:20, but was not significantly different from CO. According to Binsi *et al*. [18], the film containing 1.5 mL/g chitosan of virgin coconut oil had a TS of 57.1 ± 1.53 MPa, whereas the control had a TS of 81.7 ± 1.10 MPa. The TS is affected by the amount of plasticizers present in the film, as well as the type of oil or fatty acids present.

The elongation at break (EB) for HOSO was significantly different ($p \leq 0.05$) with a value of 29.60 ± 2.67% from IP 60:40, IP 70:30, and CO. IP 60:40 was significantly different at a value of 19.46 ± 1.22% from IP 80:20 and HOSO. EB in films with virgin coconut oil and chitosan had EB values between 10.21 and 39.72%, which increased as more oil was added [18]. Conversely, the EB of each of the emulsion films tested was within the ranges described by other researchers. Ramos *et al.* [15] used whey protein isolate and glycerol in an edible film that resulted in a EB between 10 and 20%. Monedero *et al.* [20] incorporated an oleic acid:beeswax mixture into soy protein isolate films. Oleic acid had a greater plasticizing effect than beeswax; therefore, increasing the EB. Furthermore, studies showed the addition of a lipid component to the film helped to increase the EB value. As the percentage of oleic acid increased, the films EB increased.

## Conclusion

Structured lipids were designed for use in an edible film application and their properties compared. The enzyme was successful in increasing oleic acid at the *sn*-2 position of the TAGs. The structured lipids were helpful in reducing the opacity of CO in a film use and this is advantageous. The IP 60:40 provided the strongest TS although it had the weakest EB. The IP 60:40 contained the most oleic acid (33.86 ± 1.55%) at the *sn*-2 position and also resulted in a fairly translucent product. The IP 60:40 will be used to continue further research on the use of SLs in edible films to prepare sports nutrition products in our laboratory.

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