

Continuous Enzymatic Transesterification of Sesame Oil and a Fully Hydrogenated Fat: Effects of Reaction Conditions on Product Characteristics

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Abstract: An immobilized lipase from *Thermomyces lanuginosus* (TL IM) was employed to mediate the continuous transesterification of sesame oil and fully hydrogenated soybean oil (FHSBO) in a packed bed reactor operating at 70°C. Reactions between sesame oil (rich in LLL (15.97%), LOL (31.56%), and OLO (21.15%) [L: linoleic; O: oleic]) and the fully hydrogenated fat ((73.7% SSS, 26.3% SPS) [S: stearic; P: palmitic]) produced semi solid fats. These products are complex mixtures of triacylglycerol (TAG) species whose compositions depend on reaction conditions. The dependence of the steady state product TAG profile on space time was determined for four initial weight ratios of sesame oil to hydrogenated fat (90:10, 80:20, 70:30, and 60:40). Except for the trial involving a weight ratio of sesame oil to FHSBO of 60:40, near equilibrium conditions were achieved at space times of 30 min–1 h. The chemical, physical, and functional properties of the product semi solid fats were characterized. The predominant TAG species in the quasi equilibrium products obtained from the mixture initially containing 90% (w/w) sesame oil and 10% FHSBO were LOL (26.22%) and OLO (21.92%). For transesterification of 80% sesame oil and 20% FHSBO, the major product species were OOP (21.27%), LOL (17.46%), and OLO (13.93%). OOP (24.38%) was the major product for reaction of 70% sesame oil with 30% FHSBO. Appropriate choices of reaction conditions and initial ratios of sesame oil to FHSBO lead to TAG with melting profiles and solid fat contents (SFC) similar to those of a variety of commercial products. © 2006 Wiley Periodicals, Inc.

Keywords: sesame oil; immobilized lipase; transesterification; saturated fat; zero trans fats; HPLC mass spectroscopy

INTRODUCTION

Because potential health problems have recently been associated with consumption of semi solid fats from partially hydrogenated oils, the food industry needs to replace these products by others with similar functional properties. To address this need enzymatic transesterification reactions between a solid fat and a liquid oil can be employed for production of semi solid fats that contain no *trans* fatty acid residues (Kowalski et al., 2004; List et al., 1997). Enzymatic processes based on the use of either *sn* 1,3 specific or non-specific lipases are advantageous because of their selectivity (generation of fewer undesired byproducts), mild reaction conditions, and ease of product recovery. They also facilitate process control and minimize problems of waste disposal (Yang et al., 2003).

Palm stearins have been used as solid substrates to produce semi solid fats; some of the oils used for exchange of acyl groups with palm stearin are coconut oil, sunflower oil, and palm kernel oil (Lai et al., 1998; Ming et al., 1999; Zhang et al., 2001). Tristearin and tripalmitin have also been utilized with similar intent by List et al. (1995) and Seriburi and Akoh (1998). A fully hydrogenated soybean oil (FHSBO) has also been employed for production of fats with low to zero *trans* fatty acid content and properties similar to those of partially hydrogenated fats (Petrauskaite et al., 1998; Zeitoun et al., 1992), and for production of structured lipids with low caloric content (Lopez Hernandez et al., 2005). Enzymatic transesterification of fully hydrogenated sunflower oil with palm oil (or sunflower oil) has been investigated by KMT (the third largest producer of sunflower oil).

Physiological effects of sesame oil include lowering serum cholesterol levels, suppressing certain types of cancer, enhancing liver function, and reducing the effects of aging (Namiki, 1995). Toro Vazquez et al. (2000) have suggested the use of sesame oil to produce value added products (e.g.,

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margarines) by physical mixing/blending with palm stearin. However, very few reports on the lipase catalyzed modification of sesame oil have been published, Shibasaki and Yamane (2000) reported the reduction of the freezing point of sesame oil by self interesterification mediated by an immobilized *Rhizopus sp* lipase in a solvent free batch reaction; Jennings and Akoh (2000) used a lipase from *Rhizomucor miehei* to incorporate capric acid residues into this oil by acidolysis reactions in either the presence or absence of a solvent.

Transesterification between a fat and oil typically involves exchange of fatty acid residues between the precursor acylglycerols and is accompanied by a concomitant change in the properties of the original material. The properties of the product are in large measure governed by a convenient choice of the precursor reagents, their respective proportions in the starting mixture, and the space time (residence time) of the reactants in a packed bed reactor containing an immobilized enzyme. Other factors influencing the properties of the products are the temperature and the water content.

The goal of this research was to conduct a detailed study of the influence of reaction conditions on the compositions and functional characteristics of the products. Hence, we utilized an oil with a high content of polyunsaturated fatty acid residues (sesame oil) and a solid fat (FHSBO) containing none of the undesirable *trans* fatty acid residues produced by partial hydrogenation reactions. Four different semi solid fats were prepared by enzymatic transesterification using four different weight ratios of sesame oil to FHSBO (90:10, 80:20, 70:30, and 60:40). Chemical, physical, and functional properties of the resulting products were characterized to identify potential applications of these materials.

A continuous process employing a packed bed reactor is the most appropriate technology for transesterification of fats and oils for industrial applications. Batch reactors often produce significant acyl migration during lipase catalyzed reactions (Xu, 2000). In the present work, a laboratory scale packed bed reactor containing an immobilized lipase from *Thermomyces lanuginosus* (TL IM) was used to carry out the continuous transesterification reaction. Changes in the triacylglycerol (TAG) profile were monitored by reversed phase HPLC (RP HPLC) analyses. The identities of the newly formed TAG species were confirmed using HPLC mass spectroscopy. Melting profiles and solid fat contents (SFC) of the resulting semi solid product mixtures were determined by differential scanning calorimetry (DSC) and pulsed field NMR spectroscopy, respectively.

MATERIALS AND METHODS

Materials

The sesame oil was a gift from VSP Bolier Group (Nieuwe Tonge, Holland) while the FHSBO ($MP = 70^{\circ}\text{C}$) was donated by AC Humko Oil Products (Cordova, TN). The immobilized lipase preparation (TL IM) was kindly provided by Novozymes

North America (Franklinton, NC). All solvents were HPLC grade (Fisher Scientific, Chicago, IL). Standards of the following triglycerides were used in the HPLC and gas chromatography (GC) analyses: tristearin (SSS), 99% pure, from ICN Biomedicals (Irvine, CA); trilaurin (LLL), tripalmitin (PPP), and triolein (OOO) (approximate purities 99%) were obtained from Sigma Chemical (St. Louis, MO). PUFA No. 3 and FAMEMIX No. 37 standard mixtures of fatty acids and fatty acid methyl esters (no. 4 7085) were purchased from Supelco (Bellefonte, PA). The hexadecane used as an internal standard for the GC analyses was obtained from Aldrich.

Methods

Reaction Protocol

The small packed bed reactor used to perform the reactions consisted of a 5 cm long stainless steel tube (0.5 cm I.D.) packed manually with approximately 0.6 g of the immobilized lipase (TL IM). Losses of the catalyst particles from the bed were prevented by tightly capping both ends of the reactor tube with stainless steel fittings containing welded screens (100 mesh, 140 μm openings). A syringe pump (model 220; KD Scientific, New Hope, PA) was used to continuously supply the different substrate mixtures. Weight ratios (sesame oil to FHSBO) of 90:10, 80:20, 70:30, and 60:40 were investigated. To keep the reaction mixtures in a liquid phase, heating tapes were used to maintain a 50 mL glass syringe and the inlet and outlet tubing at 70°C . Each trial was initiated by flushing the reactor with a total volume of the mixture of substrates equal to at least twice the void volume of the reactor. After quasi steady state operating conditions were achieved at 70°C , several samples of the effluent stream were manually collected over a period corresponding to at least 2.5 space times. (The space time is the ratio of the void volume of the reactor to the total volumetric flow rate of the mixture of liquid fats.)

Compositional Analysis of the Acylglycerols

Gas chromatographic analyses. GC was employed to determine both the overall distribution of fatty acid residues and the manner in which they are distributed among the various positions on the glycerol backbone. Samples (ca. 20 mg/mL) of the precursor reactants (the feed mixtures of sesame oil and FHSBO) and the products of all reaction trials (semi solid fats) were prepared in chloroform solution. Hexadecane was employed as an internal standard.

Complete derivatization of the TAG Samples (400 μL) were methylated as described by Torres et al. (2004) and subjected to gas chromatographic analyses.

Regioselective hydrolysis of TAG Modified versions of the methods of Luddy et al. (1964) and Williams et al. (1995) were employed to release the fatty acid residues from the *sn* 1,3 positions of acylglycerols. Our modification involved the use of longer reaction times and addition of borax to

minimize the possibility of migration of residues from the *sn* 2 position to the *sn* 1,3 positions of the glycerol backbone.

A known weight (ca. 50 mg) of oil and an appropriate weight (ca. 20–50 mg) of porcine pancreatic lipase were added to a 60 mL stoppered flask. Hexadecane was added as an internal standard. Next, 0.65 mL of Tris buffer (1 M, sodium salt, pH 8.0), 0.35 mL of sodium borate (0.19 M), 0.1 mL of CaCl₂ (22%, w/w), and 0.25 mL of bile salts (0.1%, w/w) were added. The resulting mixture (pH = 7.91) was shaken for 8 min at 250 rpm and 40°C. The reaction was stopped by addition of 1 mL of acetic acid (0.1 M). The mixture was then extracted three times with 1 mL of chloroform. The pooled organic phases were then passed through a 0.45 µm syringe filter.

Partial derivatization of the TAG Fatty acid residues at the *sn* 2 position of the TAG subjected to regioselective hydrolysis were methylated using the method of Torres et al. (2002)(2004).

Analyses of fatty acid methyl esters by gas chromatography One microliter of sample was injected into an Agilent (Palo Alto, CA) gas chromatograph (model 6850) fitted with an Omegawax column (30 m × 0.25 mm × 0.25 µm film thickness) purchased from Supelco. Injector and detector temperatures were 250 and 230°C, respectively. The temperature program was as follows: starting at 50°C for 2 min and then heating to 220°C at 30°C/min, followed by heating from 220 to 260°C at 5°C/min. Finally, the temperature was raised to 265°C and held for 5 min. Identification of the various free fatty acids was based on a PUFA No. 3 standard (no. 4 7085) and FAMEMIX No. 37 obtained from Supelco.

HPLC analyses

Normal phase HPLC analyses Samples of the reactor effluent corresponding to different space times were dissolved in chloroform (2.5 mg/mL). Ten microliter aliquots of these solutions were injected to an HPLC system consisting of a Waters 600 (Milford, PA) quaternary pump and an Alltech 500 Evaporative Light Scattering Detector (ELSD). The acylglycerol species were separated using an Econosil Silica 5U column (250 mm × 4.6 mm from Alltech (Deerfield, IL)). The mobile phases were a hexane/2-propanol/ethyl acetate/formic acid mixture (80:10:10:0.1, by volume) and a hexane/formic acid solution (100:0.02, by volume). The solvent gradient was similar to that previously described by Liu et al. (1993).

TAG families in the reactor effluent RP HPLC was used to separate and quantify the TAG families present in samples of the feed stocks (sesame oil and FHSBO) and the effluent from the reactor. The column was an Allsphere ODS 2 5 U (250 mm × 4.6 mm) column from Alltech maintained at 25°C. The ELSD drift tube temperature was 71°C, and the nitrogen flow rate was 1.8 SLPM. The flow rate of the eluent was 1 mL/min for a dichloromethane/acetonitrile solvent system employed according to the gradient in Table I.

Table I. Solvent gradient used to separate TAG families by RP-HPLC.

Time (min)	Dichloromethane (% v/v)	Acetonitrile (% v/v)
0	35	65
30	35	65
35	50	50
45	50	50
50	35	65
55	35	65

Chemical characterization of TAG species by HPLC MS The TAG present in the feed stocks (sesame oil and FHSBO) for the transesterification reaction, as well as the semi solid fats produced by the reaction, were analyzed using RP HPLC coupled to a mass spectrometer through an atmospheric pressure chemical ionization source (RP HPLC/APCI MS). Compositional analyses of the TAG by HPLC/MS were performed using a quadrupole mass analyzer (Hewlett Packard 1100 MSD) with an APCI interface coupled to an HPLC apparatus (HP Series 1100 from Agilent Technologies). The chromatographic separation was carried out in an HPLC apparatus containing a Kromasil C18 column 250 mm × 4.6 mm with a 5 µm particle size from Analisis Vinicos (Ciudad Real, Spain). Several homogeneous (mono acid) TAG containing fatty acid residues with zero two olefinic bonds (trilaurin, tristearin, and trilinolein) were analyzed to evaluate the quality of the mass spectra obtained using the APCI interface and validate the methodology. For the first 20 min of the elution protocol, the mobile phase was a 60:40 (v/v) mixture of acetonitrile and dichloromethane. From 20 to 40 min the volumetric ratio was 55:45. For the period from 40 to 55 min the ratio was 50:50. Subsequently, the column was re-equilibrated for 10 min using a 60:40 ratio of these solvents. Samples (20 µL containing 2.5–4.2 mg/mL of solute) were injected with an autosampler. The eluent flow rate was 1 mL/min. Detection was accomplished by the mass analyzer system. Mass spectrometer parameters were adjusted so as to obtain appropriate conditions. The values selected for the needle potential, gas temperature, drying gas, and nebulizer pressure were 3,500 V, 40°C, 5 L/min, and 35 psi, respectively. The vaporizer temperature and the corona current were 250°C and 4 µA, respectively. The voltage of the fragmentor (100 V) was selected on the basis of the results of previous analyses of reference compounds (trilaurin, tristearin, and trilinolein). The quadrupole was scanned from 125 to 1,500 *uma* to detect an extensive number of compounds. The spectra were recorded in the positive ion mode.

Differential Scanning Calorimetry Analyses of the Reactor Effluent

A Pyris 1 Perkin Elmer (Shelton, CT) differential scanning calorimeter (DSC) was used to determine the thermal profiles of representative samples of the process effluent stream. The DSC was calibrated using indium (*MP* = 156.6°C) and mercury (*MP* = 38.87°C). Samples containing 5–10 mg

of TAG were placed in aluminum pans and sealed. These samples were rapidly heated from 25 to 80°C at 20°C/min, then held at 80°C for 5 min. The samples were then immediately cooled from 80°C to 20°C and heated again to 80°C at a constant rate of 5°C/min. The final temperature of 80°C was again maintained for 5 min.

Solid Fat Content of the Reactor Effluent

The SFC of representative effluent samples were evaluated using a Bruker Minispec Solid Fat Analyzer (Bruker, Canada). NMR tubes (10 mm in diameter) were filled with 1 mL of fat mixture and capped. Tempering pretreatment of all samples was carried out using IUPAC Method 2.150 (Paquot and Hautfenne, 1987). SFC values were determined at 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60°C. Duplicate analyses were conducted for each sample.

RESULTS AND DISCUSSION

Transesterification as a Function of Space Time

For each different mixture of substrates the extent of the lipase catalyzed transesterification reaction was monitored until quasi equilibrium conditions were approached. However, for the trials involving a weight ratio of sesame oil to FHSBO of 60:40, it was not possible to approach equilibrium because at space times longer than 30 min, accumulation of product crystals led to blockage of the reactor exit. Only two samples were collected at space times of 15 and 30 min.

For the experimental trials at weight ratios (sesame oil/FHSBO) of 90:10, 80:20, and 70:30, rapid and nearly complete depletion of the two major components (SSS and SPS, S = stearic; P = palmitic) of the original FHSBO was observed. The residual contents of SSS were always lower than 1% at the time quasi equilibrium conditions were achieved (Fig. 1).

Similar depletion trends were observed for four of the five principal species of TAG present in the original sesame oil, namely LLL, LOL, OLO, and OOO (L = linoleic; O = oleic) (Fig. 2). Marked decreases were noted in the concentrations of these species. For the trials involving 90% (w/w) sesame oil, self transesterification of the sesame oil was very probably occurring. The small changes observed in the peak areas for the main components of the original sesame oil are consistent with those reported by Shibasaki and Yamane (2000) for the self transesterification of refined sesame oil. For other trials a combination of self transesterification of the sesame oil and the higher availability of the residues originating in the TAG of the FHSBO resulted in higher degrees of consumption of LLL, LOL, OLO, and OOO.

For the three trials in which near equilibrium conditions were approached, the transesterification reaction led to increases in the concentrations of eight different TAG species (LOP, OOP, OSO, PPP, SSL, OSP, SOP, and SOS), and near

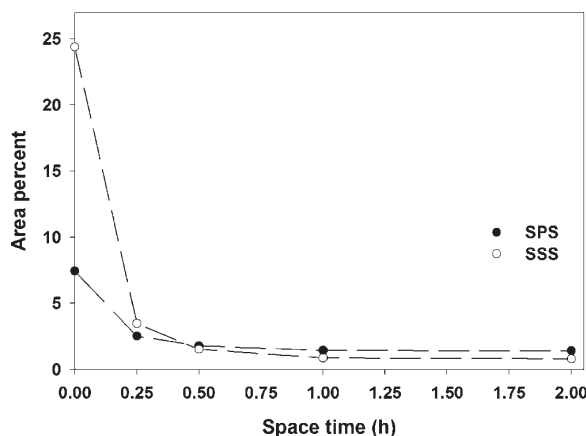


Figure 1. Depletion of the principal TAG species present in the original FHSBO as a function of space time. Conditions: sesame oil:FHSBO = 70:30 (w/w), 70°C.

equilibrium conditions were achieved at relatively short space times (30 min 1 h, Fig. 3). As anticipated, the availability of residues of stearic and palmitic acids governed the type and relative amounts of the newly formed TAG species. Lower initial levels of FHSBO favored formation of TAG species containing only a single saturated fatty acid residue from the original FHSBO (namely OOP or LOP). The formation of other TAG species, for example, SSL and SOS, increased significantly when the initial level of FHSBO in the original mixture of reactants was increased. Similar results were observed in a previous study involving the transesterification of FHSBO and TAG containing medium chain length fatty acid residues (Lopez Hernandez et al., 2005). OOP and LOP are the major TAG products that have been enriched in saturated fatty acid residues (Fig. 3). These species are formed at the expense of the OOO and LOL present in the original sesame oil. It is noteworthy that for the

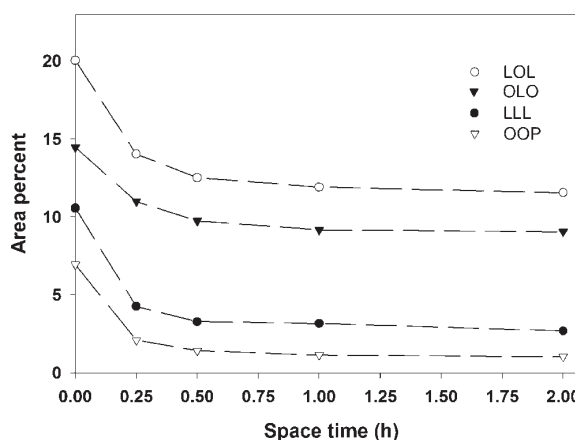


Figure 2. Depletion of the TAG species present in the original sesame oil as a function of space time. Conditions: sesame oil:FHSBO = 70:30 (w/w), 70°C.

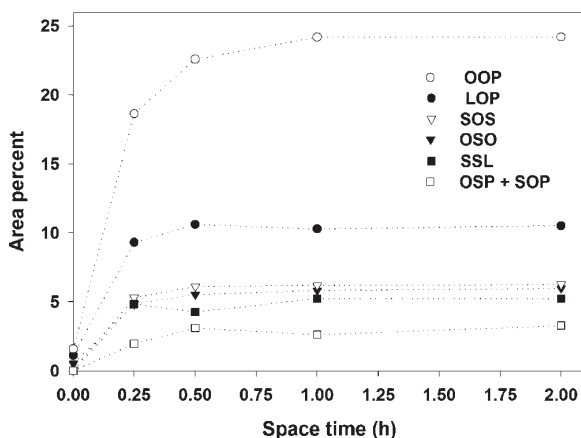


Figure 3. Growth in the levels of representative newly formed product TAG species as a function of space time. Conditions: sesame oil:FHSBO = 70:30 (w/w), 70°C.

trial involving the highest proportion of FHSBO (40% w/w), SOS is produced in even greater amounts than LOP and OOP (data not shown).

Chromatographic Analyses

Distribution of Acylglycerols in the Reaction Products

The HPLC analyses did not reveal the presence of appreciable levels of hydrolysis products in the effluent for any of the weight ratios of reactants or the space times tested. No more than 3% of the effluent consisted of lower acylglycerols in any of the samples tested (data not shown).

Compositional Analyses of the Triacylglycerols (HPLC-MS)

The molecular structures of the TAG species present in both the original fats and the interesterified fats at near equilibrium conditions were determined using reverse phase HPLC/APCI MS. Analysis of the associated mass spectra confirmed that minimal fragmentation occurred when this approach was used. Identification of the various TAG species was based on the corresponding pseudo molecular ion and diacylglycerol fragments (Jakab et al., 2003; Parcerisa et al., 2000). The molecular weights corresponding to each TAG species were determined in each case.

Figure 4A is the HPLC chromatogram of the two precursor reagents (sesame oil and FHSO), while Figure 4B is the HPLC chromatogram of the semi solid fat produced by enzymatic transesterification of the original mixture of 70% (w/w) sesame oil and 30% FHSBO at 70°C for a space time of 2 h. The peaks are labeled with the structure of the corresponding TAG determined using the APCI technique. It should be noted that some of the HPLC MS analyses of the same sample were conducted using two sample sizes differing by a factor of about 4. The chromatogram of the

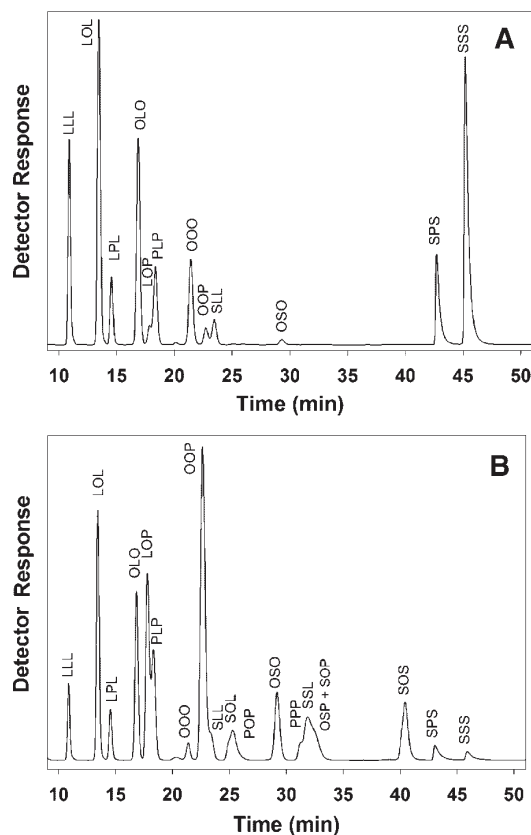


Figure 4. HPLC–ELSD chromatogram including labels for TAG assigned by interpretation of HPLC/APCI-MS data. These chromatograms were obtained under the same conditions as the HPLC/APCI-MS but using the ELSD and a larger sample size to facilitate determination of the residence times associated with the TAG species present in very low concentrations. Panel A: Physical mixture of starting materials 70% (w/w) sesame oil and 30% FHSBO. Panel B: Products of the reaction of the starting materials in Panel A at 70°C for a space time of 2 h.

larger sample was helpful in obtaining peak locations for the TAG species present in very low proportions because the response signal was larger relative to the noise. With the smaller sample, better resolution of the peaks was obtained, thereby facilitating the mass spectrum component of the analysis.

The positional isomers of the various TAG species were identified on the basis of the relative intensities of the $[M - RCO_2]^+$ fragments. Values of the retention time, the molecular weight, the structure of the acylglycerol species, and the area percentage (by HPLC) determined for all of the TAG species present in sesame oil, FHSBO, and three semi solid fats produced using different weight ratios of starting materials (10 30% by weight of FHSBO) are summarized in Table II. The values of the molecular weights listed in Table II are in good agreement with the values calculated from the corresponding molecular formulae using the weight of the most abundant isotope of each atom. Moreover, the sequence of retention times observed for the

Table II. Chemical structures of all TAG species present in sesame oil, FHSBO, and three different semi-solid fats produced by enzymatic transesterification.

RT (min)	MW (amu)	TAG	Sesame oil (area %)	SO:FHSBO 90:10 w/w (area %)	SO:FHSBO 80:20 w/w (area %)	SO:FHSBO 70:30 w/w (area %)	FHSBO (area %)
10.9	878.75 ± 0.05	LLL	15.97	6.87	4.84	3.11	
13.6	880.80 ± 0.10	LOL	31.56	26.22	17.46	12.10	
14.7	854.75 ± 0.05	LLP, LPL ^a	(LLP) 5.71	(LLP) 4.02	(LLP) 3.25	(LPL) 2.55	
17.0	882.85 ± 0.05	OLO	21.15	21.92	13.93	9.20	
17.8	856.80 ± 0.10	LOP	1.64	5.60	9.91	11.10	
18.4	830.80 ± 0.10	PLP	8.58	10.89	9.00	7.61	
21.5	884.85 ± 0.05	OOO	9.37	3.30	1.84	1.18	
22.8	858.80 ± 0.10	OOP	1.86	12.20	21.27	24.38	
23.7	882.85 ± 0.10	SLL	3.15	3.02	2.12	2.02	
25.1	884.85 ± 0.10	SOL	0.08	0.23	0.78	1.22	
25.5	832.75 ± 0.05	POP	0.04	1.02	1.95	2.61	
29.5	886.80 ± 0.10	OSO, OOS ^b	0.77	2.69	4.95	5.69	
31.4	806.80 ± 0.10	PPP		0.10	0.54	1.28	
32.0	886.80 ± 0.15	SSL			3.03	6.04	
32.5	860.85 ± 0.15	OSP + SOP ^c		1.16	1.57	2.05	
40.5	888.90 ± 0.10	SOS		0.48	2.77	5.74	
42.8	862.80 ± 0.10	SPS			0.43	1.14	26.3
45.4	890.90 ± 0.10	SSS			0.07	0.68	73.7

Values are listed for retention times (RT), molecular weights (MW), and area percentages (by HPLC).

Values of the space time of the process fluid correspond to quasi-equilibrium conditions (30 min, except in the case of 70/30 SO/FHSBO sample, for which 2 h was required to approach equilibrium).

^aLLP was present in the effluent from the trials with ratios of 90:10 and 80:20, whereas for a ratio of 70:30, LPL was the species present.

^bThese isomers could not be distinguished on the basis of the mass spectra.

^cFor these three samples, these two isomers were present in similar proportions.

various TAG in our HPLC MS analyses is precisely the same as those reported by Byrdwell et al. (1996) and Neff et al. (2001).

Stolyhwo et al. (1985) have demonstrated that quantitative analysis of TAG mixtures by HPLC with an ELSD detector, carried out without equilibration, give results that are in excellent agreement with data derived from GC. However, Carelli and Cert (1993) have warned that HPLC ELSD analyses yield inaccurate results for those TAG present only in low concentrations. The area percentages of the various TAG species present in our samples (based on the HPLC ELSD chromatogram) are shown in Table II. Examination of this table indicates that the predominant TAG species present in the sesame oil employed in our study was LOL (31.56%) but that four other TAG species are present in significant proportions (OLO: 21.15%, LLL: 15.97%, OOO: 9.37%, and PLP: 8.58%). The composition of this sesame oil differs in minor respects from other compositional analyses described by Lee et al. (2001) for sesame oils obtained from different seeds in Korea, China, and Japan. These investigators reported smaller percentages (7.7–14%) of the predominant TAG, as well as much lower percentages (5.8–10.7% and 3.8–6.6%) of the minor components OLO and LLL, respectively. Our value for OOO is consistent with the ranges reported by Lee et al. (2001) (5.4–10.2%). By contrast, OOS was significantly more abundant (1.3–2.5%) in the sesame oils analyzed by Lee et al. (2001). The sample employed in our study contained only a trace amount of this species (0.77%). Some of the indicated differences in fatty acid residue composition are a consequence of the natural

variations expected as a result of the different origins and growing conditions of the sesame seeds, while others may result from the presence of additives or adulterants possibly present in commercial samples of sesame oil. One can often differentiate between adulterated and authentic oils on the basis of the HPLC chromatograms.

The enzyme catalyzed transesterification reaction between sesame oil rich in LLL (15.97%), LOL (31.56%), and OLO (21.15%) and the fully hydrogenated fat (73.7% SSS, 26.3% SPS) produced semi solid fats, which are complex mixtures of TAG species. Some of the TAG were species that were not present in the precursor starting materials. For the semi solid fats obtained with the reaction mixture containing 90% (w/w) sesame oil and 10% FHSBO, the predominant TAG species were LOL (26.22%) and OLO (21.92%). For the transesterification reaction involving 80% sesame oil and 20% FHSBO, the major product species were OOP (21.27%), LOL (17.46%), and OLO (13.93%). By contrast, for the interesterification of 70% sesame oil with 30% FHSBO, OOP (24.38%) was the major product. Importantly, the reaction mixture originally containing 30% FHSBO yields a semi solid product in which there are significant levels of TAG species that contain only residues of saturated fatty acids (e.g., 1.14% SPS and 0.68% SSS). Fully saturated species, such as SSS and SPS are present only in the semi solid fats obtained in the trials involving 20% or more FHSBO in the starting material. However, the transesterification reactions involving feed mixtures containing 10 and 20% FHSBO give products that are rich in unsaturated fatty acid residues (e.g., 17–26% LOL and 4–7% LLL).

It should be noted that for the minor peak corresponding to OOS, the positional isomers of this TAG could not be distinguished, so the abbreviations given in Table II and Figure 4 (panels A and B) for this TAG identify this molecular species without regard to the positional arrangement of the fatty acid residues. This situation is relatively common in determinations of those TAG species present only in minor proportions (Byrdwell et al., 1996).

Compositional Analyses of the Fatty Acid Residues Present in the Starting Materials and the Process Effluent

Table III contains a summary of the distributions of fatty acid residues at *sn* 2, *sn* 1,3, and in *sn* 1,2,3 sites for both the starting materials and the process effluent for near equilibrium conditions. The entries in Table III are expressed as molar percentages of the four fatty acid residues present. The fatty acid compositions of both starting materials, sesame oil and FHSBO, are in very good agreement with data supplied by their respective manufacturers. Sesame oil contains C18:2 (linoleic acid, L) and C18:1 (oleic acid, O) as its primary fatty acid residues and lesser percentages of C18:0 (stearic acid, S) and C16:0 (palmitic acid, P) residues. The FHSBO contains only saturated fatty acid residues (S and P).

The different semi solid fats produced with four different weight ratios of sesame oil to FHSBO contain variable percentages of linoleic and oleic acid residues. The semi

solid fat prepared with 30% (w/w) FHSBO contains 27.6% more saturated fatty acid residues than sesame oil (43.2% vs. 15.6%). This semi solid fat also contains 18.2% more saturated fatty acid residues than that produced in the trial involving 10% FHSBO (43.2% vs. 25.0%). However, the product of the trial involving 30% (w/w) FHSBO is 27.6% lower in total unsaturated fatty acid residues (C18:1 and C18:2) than the original sesame oil (56.8 vs. 84.4) and 18.2% lower than the semi solid fat obtained in the trial with 10% FHSBO (56.8% vs. 75.0%).

These analyses indicate that the levels of the TAG species containing cholesterolemic saturated fatty acid residues at the *sn* 2 positions of the acylglycerols increased as a direct consequence of increasing the percentage of the FHSBO in the original reaction mixture (Table III). Moreover, only for those samples produced in the trials involving lower amounts of FHSBO (10 and 20%, w/w) was the molar ratio of saturated to unsaturated fatty acid residues approximately the same for the *sn* 2 site as for the *sn* 1,3 positions. When higher percentages of FHSBO were used (e.g., in the trial with the 70:30 weight ratio), the resulting semi solid fats were characterized by a higher ratio of saturated to unsaturated fatty acid residues at the *sn* 2 site than at the *sn* 1,3 sites. This difference is a factor that should be considered in assessing this technology because of the direct implications of dietary ingestion of saturated fatty acid residues for cardiovascular disease, especially those residues located at the *sn* 2 position. In general, vegetable oils contain an elevated proportion of

Table III. Molar percentages of the different fatty acid residues as determined by gas chromatography.

	Molar composition of fatty acid residues (in percent)				Molar ratio
	C16:0	C18:0	C18:1	C18:2	Sat/unsat ^a
Overall composition of TAG:					
Sesame oil (SO)	10.2	5.4	40.1	44.3	0.18
90:10 w/w SO/FHSBO	10.6	14.4	35.6	39.4	0.33
80:20 w/w SO/FHSBO	11.0	23.5	31.2	34.4	0.53
70:30 w/w SO/FHSBO	11.4	31.8	26.9	29.9	0.76
60:40 w/w SO/FHSBO ^b	11.8	37.6	24.1	26.6	0.97
FHSBO	14.2	85.9	—	—	—
Composition at the <i>sn</i> -2 position:					
Sesame oil	9.8	5.5	39.7	44.9	0.18
90:10 w/w SO/FHSBO	10.3	15.0	35.1	39.6	0.34
80:20 w/w SO/FHSBO	10.5	23.9	30.8	34.8	0.52
70:30 w/w SO/FHSBO	11.5	32.5	26.6	29.4	0.79
60:40 w/w SO/FHSBO ^b	6.0	27.8	28.9	37.3	0.51
FHSBO	14.3	85.7	—	—	n.d.
Composition at the <i>sn</i> -1(3) positions:					
Sesame oil	10.4	5.4	40.3	44.0	0.19
90:10 w/w SO/FHSBO	10.7	14.2	35.7	39.4	0.33
80:20 w/w SO/FHSBO	11.1	23.3	31.3	34.2	0.53
70:30 w/w SO/FHSBO	11.4	31.4	27.1	30.1	0.75
60:40 w/w SO/FHSBO ^b	14.6	37.7	23.5	24.1	1.10
FHSBO	14.1	85.9	—	—	n.d.

Data expressed as total composition of the corresponding fat/oil, molar composition of fatty acid residues at the *sn*-2 position, and molar composition of fatty acid residues at the *sn*-1,3 positions of the acylglycerol.

The values of the space time of the process fluid were all 30 min, except for the trial with the 70:30 ratio of sesame oil to FHSBO, for which the space time was 2 h.

^aMolar ratio of saturated to unsaturated fatty acid residues.

^bOnly for the trial with 40% FHSBO were we unable to approach quasi-equilibrium conditions.

unsaturated fatty acid residues at the *sn* 2 position, while for animal fats, saturated fatty acid residues are usually preferentially located at the *sn* 2 position. Oleic acid is the dominant species at the *sn* 2 position in chicken fat while for pork fat, this position is rich in palmitic acid residues (Mottram et al., 2001).

The data in Table III were plotted in the form of the percentage of a particular fatty acid residue at the *sn* 2 position versus the corresponding percentage in the precursor sesame oil (not shown). Highly linear plots were obtained for the C16:0, C18:0, C18:1, and C18:2 residues. The high degrees of linearity strongly suggest that very little, if any, reaction occurs at the *sn* 2 position of the TAG and that during transesterification the distribution of residues at this position remains substantially unchanged. Moreover, these results also suggest that very little acyl migration occurs as the substrates move through the packed bed reactor. Acyl migration is usually associated with such factors as the water content of the lipase and the reaction medium, the chemical nature of the support material, reaction times and temperatures, and the type of reactor employed (Xu et al., 1998). The lipase used in this study (TL IM) is reportedly *sn* 1,3 selective, although Kim et al. (2002) found that reaction conditions can induce acyl migration when this biocatalyst is used. The relatively short space times necessary to reach quasi equilibrium conditions, together with the positional specificity of the TL IM lipase, lead to conditions under which one expects to observe minimal migration of acyl groups, as we found in those trials in which equilibrium was approached. In addition, it should be noted that in none of the trials did the effluent contain more than 3% lower acylglycerols. Hence, hydrolysis was not appreciable.

For those trials involving 40% FHSBO in the starting mixture, the results of our analyses for the residues esterified at the *sn* 2 position showed an unexpectedly low concentration of saturated fatty acid residues (palmitic and stearic) in that position. We attribute these results to problems with the regiospecific analytical protocol for the trial involving a high percentage of solid fat in the reaction mixture. Because of poor digestion of the high melting fraction of the reaction products by the pancreatic lipase, the solid fat fraction present in the reaction products (enriched in palmitic or stearic acid residues) might not have been effectively emulsified by the bile salts. Poor emulsification, in turn, could have constrained the ability of this lipase to catalyze the hydrolysis reaction of the ester bonds in the substrate TAG.

DSC Analyses

The thermal behavior of edible fats is commonly characterized by two physical events, namely melting and crystallization. Generally, melting curves of oil samples contain complex features that are not readily interpretable, such as shoulders not separable from peaks (Tan and Che Man, 2000). Melting thermograms of samples corresponding to near equilibrium reaction conditions for the trials involving weight ratios of sesame oil to FHSBO of 90:10, 80:20, and

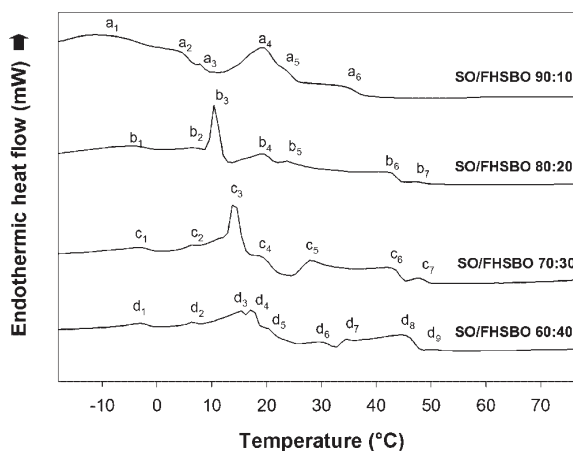


Figure 5. DSC melting thermograms of the transesterified products of sesame oil and FHSBO for four different weight ratios of substrates. Samples correspond to space times of 30 min, except for the sample containing 70 weight percent sesame oil, for which the space time was 2 h.

70:30 are shown in Figure 5. A melting profile for a sample corresponding to a space time of 30 min for the trial with a 60:40 weight ratio was also studied by DSC. The peaks for the endothermic melting transitions labeled in Figure 5 were assigned for each curve, based on the temperature at which a maximum value of heat flow occurred. Simple inspection of the thermograms revealed that the number of endothermic peaks increased with the initial amount of solid fat in the mixture of reactants. In addition, more peaks associated with high melting point TAG species were observed as the concentration of saturated fatty acid residues in the reaction mixture was increased.

The thermal properties of various oil samples from DSC melting and crystallization curves can be characterized by various transition temperatures. For melting curves the offset temperature (T_{offset}), represents a point where the extrapolated leading edge of the last endotherm intersects with the baseline, whereas the onset temperature (T_{onset}) is related to the start of crystallization transition, and is graphically obtained from the intersection of the extrapolated slope of the transition state with an extrapolated baseline (Tan and Che Man, 2002). Our results showed a gradual decrease in the crystallization T_{onset} corresponding to the presence of lower percentages of saturated high melting TAG. T_{onset} values of 29.5, 25.5, 24.5, and 20.2°C were obtained from the crystallization thermograms (data not shown) of the interesterified mixtures corresponding to initial substrate weight ratios of 60:40, 70:30, 80:20, and 90:10, respectively. The melting T_{offset} values increased with the amount of hydro generated fat in the initial reaction mixture, the values observed for the trials with 10, 20, 30, and 40% initial FHSBO were 52.9, 49.8, 49.2, and 38.5°C, respectively. A third DSC parameter, the temperature range ($T_{\text{offset}} - T_{\text{onset}}$) was calculated for each interesterified product. These ranges decreased as the amount of saturated fatty acid residues in the starting material increased. Temperature ranges of 32.7, 25.3,

23.7, and 9°C were observed for the samples corresponding to initial substrate weight ratios of 90:10, 80:20, 70:30, and 60:40, respectively.

The interesterified products formed from the mixtures initially containing 10% FHSBO exhibited a very broad melting profile, ranging from 10 to 35°C, although the more representative peaks are those between 10 and 25°C. These peaks can be largely attributed to the substitution of a few saturated fatty acid residues for some of the primary unsaturated residues present in the native sesame oil to produce new TAG species, namely LOP, PLP, OOP, and OOS, as determined by HPLC MS. The samples corresponding to initial substrate weight ratios of 80:20, 70:30, and 60:40 exhibited melting profiles slightly broader than that for the ratio of 90:10. The former samples were completely melted at temperatures between 45 and 50°C. In these samples, an important fusion region can be clearly observed between 25 and 45°C. The presence of TAG species containing two saturated residues (SSL, SOS) and residual amounts of SSS, SSP, and PPP in these samples are responsible for the shift in the melting profile towards the melting temperatures of tripalmitin (65–68°C) and tristearin (71–73°C).

Solid Fat Contents of the Products of Transesterification

SFC of the four samples subjected to DSC analyses were determined at different temperatures (Fig. 6). The SFC profile is responsible for many important characteristics of fats, for example, the physical appearance, organoleptic properties, and spreadability. This profile also influences the melting properties of a specific fat and the plasticity or physical consistency of an edible product. The variation of SFC with temperature and the sharpness of melting range, together with other factors such as the crystal morphology, determine the range within which a fat can be considered plastic (Rao et al., 2001; Seriburi and Akoh,

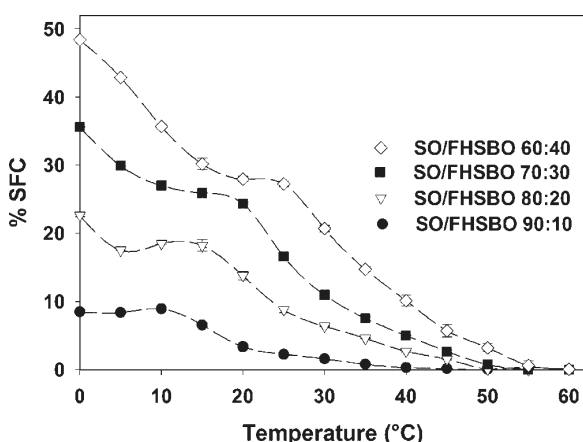


Figure 6. SFCs of reaction products at different temperatures. Samples correspond to space times of 30 min, except for the sample containing 70 weight percent sesame oil, for which the space time was 2 h.

1998). Desirable spreadability occurs within a range of roughly 15–35% solids, the so called plastic range of fats. An SFC value at 10°C of less than 32% is essential for good spreadability while the SFC values between 4 and 10°C determine the spreadability of the product at refrigeration temperatures. In addition, SFC values between 33 and 38°C influence “mouth feel” or waxy sensations (Brekke, 1980; DeMan, 1992).

Inspection of Figure 6 indicates that the SFC values were higher for those samples produced from starting mixtures containing larger proportions of saturated fatty acid residues (from the FHSBO). The observed plastic ranges for the trials corresponding to weight ratios of 60:40, 70:30, and 80:20 covered 25, 28, and 20°C, respectively. The high solid content at refrigeration temperatures is attributed to the enhanced presence of high melting fractions in the 60:40 interesterified mixtures. However, this mixture exhibited SFC values within the plastic range at temperatures between 10 and 35°C. For the mixtures with initial weight ratios of 70:30 and 60:40, the SFC values lie within the plastic range corresponding to good spreadability. Except for a small portion of the SFC curve corresponding to the 60:40 trials, none of the transesterified products exhibited the high SFC values that can result in an appreciable waxy taste in the mouth. For temperatures between 0 and 60°C, only the curve corresponding to the 90:10 trial exhibited SFC values below the plastic range.

For the 60:40 mixtures, the high SFC values observed are attributed to the presence of high melting TAG (SSL, SOS, and residual amounts of SSS, SPS, and PPP from the FHSBO) in the interesterified samples. The low concentrations of saturated residues in the TAG present in the products obtained for the trials involving an initial weight ratio of 90:10 are reflected in the low SFC values and the observed melting range. For these trials, the SFC of the product was always less than 10% and this mixture was completely melted at 40°C. The differences in the SFC values for the samples from the 80:20 and 70:30 trials can be attributed to differences in the concentrations of TAG species containing two saturated fatty acid residues, namely SOS and SSL, and to a minor extent to the unreacted TAG residues present in the original FHSBO.

Comparison of the results presented in Figure 6 with those obtained by Petrauskaite et al. (1998) indicates that the SFC profile of the product from the 90:10 trial is similar to those of tub margarines, while the profile for the sample from the 80:20 trial is similar to those typical of confectionary fats and shortenings. For the samples from the trials carried out with weight ratios of 70:30 and 60:40, the corresponding SFC curves for the interesterified mixture closely match those for fats used in formulation of stick margarines.

CONCLUSIONS

Semi solid fats have been prepared via enzymatic transesterification of sesame oil and FHSO. The effects of the most

important reaction conditions on the chemical and functional characteristics of the products have been elucidated. HPLC mass spectroscopy analyses were used to identify the various TAG product species, including positional isomers of the fatty acid residues. DSC and SFC analyses of the quasi equilibrium products indicated that by appropriate choice of the initial ratio of sesame oil to FHSBO, one can produce fats with melting profiles and SFCs similar to those of traditional products ranging from tub margarines through confectionary fats and shortenings to stick margarines.

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