Synthesis, Purification, and Characterization of Structured Lipids Produced from Chicken Fat[1]

Ki-Teak Lee and Thomas A. Foglia*

USDA, ARS, ERRC, Wyndmoor, Pennsylvania 19038

ABSTRACT: A structured lipid (SL) was synthesized enzymatically from chicken fat by incorporating a medium-chain length fatty acid (caprylic acid) into chicken fat triacylglycerols. *Carica papaya* latex was used as the biocatalyst. The optimal substrate mole ratio found was 1:2 (chicken fat fatty acids/caprylic acid). At this ratio of reactants, the incorporation of caprylic acid ($C_{8:0}$) at 65ºC was 23.4 mol%, whereas at 55°C the incorporation of caprylic acid was 17.6 mol%. A packed-bed column bioreactor was designed for the synthesis of SL from chicken fat. In using ground crude *C. papaya* latex ($a_w < 0.1$), 7.1 mol% of caprylic acid was incorporated into the chicken fat triacylglycerols after 117 min of reactor residence time. After purification of the SL, the acyl positional distribution of fatty acids on the glycerol backbone was determined by ^{13}C nuclear magnetic resonance (NMR) spectroscopy. From the NMR spectrum of the SL, it was determined that saturated fatty acyl residues at the 1,3-positions of the SL triacylglycerols increased to 62% over that of the starting chicken fat triacylglycerols, suggesting that caprylic acid was preferentially incorporated at the 1,3-positions. In addition, differential scanning calorimetry thermograms were obtained to compare the crystallization characteristics of the starting chicken fat with the SL prepared from it.

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KEY WORDS: *Carica papaya* latex, chicken fat, medium-chain length fatty acid, packed-bed column bioreactor, structured lipid.

In 1998 approximately 27,900 million pounds (lb) of chicken was produced in the United States, which exceeded beef production (1). Despite the high production of chicken, poultry fats are usually not utilized separately in other food or nonfood uses. Chicken fat is composed of about 60% unsaturated fatty acids and has a lower titer and higher degree of unsaturation than tallow (2). Among unsaturated fatty acids, monounsaturated fatty acids (MUFA), such as oleic acid, are considered as desirable in regard to ameliorating the risk of coronary artery disease (3).

Usually animal fats contain a higher proportion of saturated fatty acids (SFA) compared to vegetable oils, which is of concern to health conscious consumers. Because SFA in chicken fat triacylglycerols (TAG) are located primarily at the *sn*-1,3 positions of the glycerol backbone, they can be selectively replaced with a more nutritionally desirable fatty acid by lipase-catalyzed acidolysis using a 1,3-regioselective lipase.

The synthesis of tailored fats and oils, which are commonly referred to as structured lipids (SL), has become an important topic in the oil processing industry. Such materials are made to tailor the physical/chemical characteristics of a fat/oil so as to provide specific functional properties. The definition, synthesis, and applications of SL have been described previously (4,5). To attain the maximal benefits of SL, the structural/compositional modification of TAG often is carried out with a selective lipase, since chemical modification does not selectively replace fatty acyl residues because of its random nature. After modification, the nutritional, physical, and chemical characteristics, such as digestibility, titer, or solid fat content, of the modified TAG are improved or changed from the starting TAG. Thus, SL with defined characteristics can be used to provide specific metabolic effects for nutritive or pharmaceutical purposes, and hence hold promise for wider usage in nutritional, medical, or food applications (4–6).

The latex from *Carica papaya* is widely used in the food and beverage industry because of its proteolytic activity. The latex also has lipolytic activity and in hydrolysis of TAG shows maximal activity for short-chain fatty acids and *sn*-3 stereospecificity (7,8). In esterification reactions, *C. papaya* latex (CPL) exhibited a medium-chain fatty acid (MCFA) and *sn*-1,3 selectivity, but acyl exchange was more pronounced at the *sn*-3 position (9). Other studies suggested that CPL favors *cis*-9 unsaturated fatty acids, such as oleic and α-linolenic acid, in esterification reactions (10).

In this study, chicken fat was modified by incorporating caprylic acid (MCFA) into the chicken fat TAG to produce a functional SL composed of MUFA and MCFA. CPL in a packed-bed column reactor was used as the biocatalyst. After the transesterification reaction, the SL was purified by stepwise column chromatography, and its fatty acid distribution and thermal properties characterized.

MATERIALS AND METHODS

Materials. CPL (crude papain), caprylic acid (*n*-octanoic acid, 99%), olive oil, and 14% boron trifluoride (BF_3)/

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*To whom correspondence should be addressed at USDA, ARS, ERRC, 600 E. Mermaid Lane, Wyndmoor, PA 19038. E-mail: tfoglia@arserrc.gov

methanol complex were purchased from Sigma Chemical Company (St. Louis, MO). Chicken fat was obtained from Tyson Foods (Springdale, AR).

Preparation of buffer-washed papain. Crude ground papain (20 g) was stirred with 100 mL of 0.1 M phosphate buffer (pH 7.3) containing 0.1% Triton X-100 for 10 min and centrifuged at 9000 rpm for 15 min. After discarding the supernatant, the precipitate was washed with two 100-mL portions of 0.1 M phosphate buffer (pH 7.3) and vacuum-dried for 48 h. Water activity (a_w) of each papain preparation was determined with an Aqualab CX-2 (Decagon Devices Inc., Pullman, WA) after vacuum-drying at room temperature.

Measurement of lipase activity. Lipase activity was measured by titration of the free fatty acids released from olive oil with 0.05 N NaOH using a VIT 90 Video Titrator (Radiometer, Copenhagen, Denmark). The olive oil emulsion was prepared by mixing and sonicating 20 mL of deionized water, 9 mL of olive oil, and 21 mL of 10% gum arabic solution. Lipolytic activity was measured by vigorously mixing 40 mg of papain (crude or buffer-washed) and 5 mL of emulsion for 30 min at 30°C. An online computer monitored titration results. One unit of lipase activity is defined as μmol of fatty acids released per min per mg of papain (11).

*Batch acidolysis reaction*s. To study the effects of temperature on CPL-catalyzed acidolysis, a 1:1 mole ratio of chicken fat (500 mg) to caprylic acid (264 mg) as substrates was mixed with crude ground papain (380 mg, $a_w < 0.1$) in 1 mL hexane at 35, 45, 55, and 65°C. Experiments were performed as batch reactions in screw-top vials that were placed in a water-jacketed beaker. Circulating water through the jacket from a constant temperature water bath regulated reaction temperatures. The effect of substrate mole ratio on extent of C_8 incorporation was ascertained by conducting a series of reactions where the mole ratio of chicken fat fatty acids to caprylic acid was 1:1, 1:2, 1:3, and 1:4. All experiments were run using 50 wt% (total weight of substrates) of ground crude papain ($a_w < 0.1$) in 1 mL of hexane at 65°C. Each acidolysis reaction was carried out for 18 h with magnetic stirring at 200 rpm.

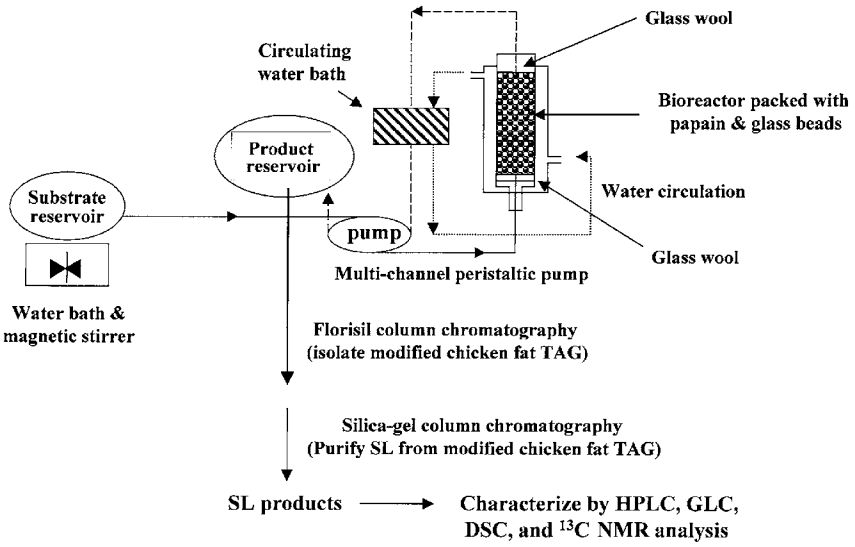
Analytical procedures. After the reaction, 25-μL aliquots of product were analyzed by thin-layer chromatography (TLC) on silica gel G plates developed with hexane/diethyl ether/acetic acid (50:50:1, vol/vol/vol) to separate the TAG. The TAG band contained the synthesized SL and the unreacted chicken fat TAG. The bands were visualized under ultraviolet light after spraying with 0.2% 2,7-dichlorofluorescein in methanol. Bands corresponding to TAG were scraped from the plate and the TAG isolated by extraction with isooctane. Solvent was removed from the TAG by evaporation under nitrogen.

Gas chromatography (GC) and GC–mass spectrometry (GC–MS). TAG were converted to fatty acid methyl esters (FAME) by adding 2 mL of 14% BF_3 in methanol and heating at 100°C for 15 min. After cooling, 1 mL of saturated sodium chloride solution and 2 mL of isooctane were added and the mixture was agitated in a vortex mixer. The isooctane layer was isolated, passed through anhydrous sodium sulfate, and FAME were analyzed by GC. Analyses were done on a Hewlett-Packard (HP) Model 5890 Series II GC equipped with an automatic split injector, a flame-ionization detector, and a cross-linked polyethylene glycol column, 30 m × 0.25 mm i.d., 0.25 micron film thickness (HP-INNOWAX, Hewlett-Packard, Avondale, PA). The carrier gas was helium at a flow of 5.5 mL/min. The column was held at 120°C for 2 min and programmed to 230ºC at a rate of 5ºC/min. The final column temperature was held for 22 min. The injector and detector temperatures were 260ºC.

GC–MS was used for identification of individual FAME on an HP Model 5890 Series II GC equipped with an HP Model 5972 Mass Selective Detector (MSD). The MSD was set to scan from *m/z* 10 to 600 at 1.2 scans per second. The capillary column used was 30 m × 0.25 mm, coated with 0.25 μm of 5% cross-linked phenyl methyl silicone (HP-5MS). The column was held at 80°C for 2 min and programmed to 230ºC at a rate of 10ºC/min and held at this temperature for 10 min. The carrier gas was helium at a flow of 1 mL/min and a split ratio of 50:1. The temperatures of the injector and detector were 230 and 280ºC, respectively.

Acidolysis reactions in a packed-bed column reactor. The bioreactor system constructed for this study is illustrated in Scheme 1. Crude ground CPL (14 g) was mixed with glass beads (8–12 mesh, 20 g) and packed into a water-jacketed glass column (1 cm i.d. × 25 cm). The glass beads increased the column volume and reduced the column backpressure. Total volume and void volume of the reactor were 14.6 and 7.5 mL, respectively. The reaction temperature and molar ratio of reactants used were selected from the results of the batch acidolysis studies. Water through the column jacket was maintained at 65ºC by a constant temperature water bath. The substrate mixture (chicken fat/caprylic acid, 1:2 mole ratio) was fed into the bottom of the reactor and pumped out the top with a peristaltic tubing pump (ISCO Inc., Lincoln, NE). Residence time was calculated from the void volume of the reactor divided by the flow rates of 0.064, 0.032, and 0.022 mL/min, providing 117, 234, and 340 min as residence times, respectively.

Column chromatography/high-performance liquid chromatography (HPLC). Products from the reaction were applied to a Florisil (100–200 mesh, Floridin Company, Hancock, WV) chromatography column to separate the TAG consisting of both the synthesized SL and unreacted chicken fat from free fatty acids and esters. The SL was further separated from unreacted chicken fat TAG present in the initially separated TAG (modified chicken fat) by silica-gel column chromatography. Scheme 1 illustrates the schematic diagram for the purification scheme. Forty grams of Florisil were packed into a column (30 cm × 10 mm i.d.), and the crude product (3.5 g) in 70 mL of hexane was applied and the flow rate adjusted to 2.3 mL/min. After sample loading, 70-mL portions of elution solvent, composed of hexane with increasing amounts of diethyl ether (1–35%, vol/vol), were sequentially applied to the column to separate TAG from fatty acids and esters in the reaction product. Each fraction was analyzed by HPLC to iden-



SCHEME 1

tify fractions containing TAG species. A Hewlett-Packard Model 1050 HPLC equipped with a Varex (Burtonville, MD) ELSD II mass detector was used along with a Phenomenex (Torrance, CA) cyanopropyl (1B-Sil, 5 micron, 250 × 4.6 mm i.d.) column with accompanying guard column (30 × 4.6 mm i.d.) for TAG analysis. An elution gradient using methyl-*t*-butyl ether and hexane as solvent described by Foglia and Jones was used (12). Fractions containing TAG were combined, and after the solvent was removed with nitrogen, approximately 1.0 g of SL-TAG was recovered. Forty grams of silica gel (230–400 mesh, 60Å, Aldrich Chemical Company, Milwaukee, WI) were packed into a column (30 cm × 10 mm i.d.) and the initially separated SL product (1.0 g) was further purified using the same solvent system described above except that the flow rate was 1.5 mL/min. After separation, two fractions (SL-I and SL-II, 0.6 and 0.2 g, respectively) containing the newly synthesized SL were obtained.

The chicken fat, separated TAG (modified chicken fat) composed of unmodified chicken fat and synthesized SL, and purified SL (SL-I and SL-II) were analyzed by nonaqueous reverse-phase HPLC on a Beckman/Altex Ultrasphere ODS 5-µm (4.6 mm × 25 cm) column. Partition numbers (PN) of TAG peaks were assigned by comparison with a standard TAG mix (G-1) obtained from Nu-Chek-Prep (Elysian, MN). The solvent separation gradient of acetone/acetonitrile and other HPLC conditions used were as described in Foglia *et al.* (13).

Differential scanning calorimetry (DSC). A PerkinElmer Model Pyris 1 (PerkinElmer Corp., Norwalk, CT) was used to determine the melting profiles of the purified SL and starting chicken fat. Thermograms were obtained with the following program temperature profile. The instrument temperature was increased to 80°C and after 10 min at this temperature, the cooling curve was obtained by cooling at 10°C/min to −60°C. After holding for 10 min at −60°C, the temperature was returned to 80°C at 5°C /min.

^{13}C Nuclear magnetic resonance (NMR) for fatty acyl positional analysis of TAG. High-resolution NMR spectra of TAG were obtained on Varian Unity Plus 400, operating at 400 MHz with proton decoupling. Spectra were recorded at concentrations of 8% wt/vol (80 mg of sample in 1 mL chloroform-*d*) in 5-mm NMR tubes. Typical spectrometer conditions were 54K data points; 3.5 KHz spectra width; 4.0 microseconds pulse width, and 8.1-second recycle times. Chemical shifts were assigned relative to chloroform (77.0 ppm).

RESULTS AND DISCUSSION

Among the TAG species present in chicken fat, TAG composed of three unsaturated (U) fatty acyl residues (UUU, about 28%) and TAG composed of one saturated (S) and two unsaturated fatty acyl residues (SUU, about 38%) are the most abundant forms. In contrast, major tallow TAG species are composed of three saturated fatty acyl residues (SSS, about 29%) and two saturated and one unsaturated fatty acyl residues (SUS and SSU, about 33%) (14). According to Brockerhoff *et al.* (15), the *sn*-2 position of chicken fat TAG is composed of approximately 80% unsaturated and 20% saturated fatty acyl residues, respectively. Generally, fatty acyl groups located at the *sn*-2 position of TAG are regarded as being more nutritionally and physiologically available than similar fatty acyl groups located at the *sn*-1,3 positions of TAG (4,5). In this work an *sn*-1,3 selective lipase (CPL) was used to incorporate caprylic acid, an MCFA, into the chicken fat TAG. This was done because chicken fat provides an SL-TAG that contains about 80% unsaturated fatty acyl residues at the 2-position that is not replaced by caprylic acid due to the selectivity of the lipase. It was anticipated that the MCFA would selectively replace the saturated long-chain fatty acyl groups, which are predominately located at the 1,3-positions of the chicken fat TAG.

TABLE 1
Water Activity and Lipolytic Activity of Crude and Buffer-Washed *Carica papaya* Latex (CPL)

	Neat papain	Buffer-washed papain
Recovered weight (%)	—	20
Water activity after drying	0.06	0.07
Lipolytic activity (unit)[a]	17.1	27.3

[a]A unit of lipolytic activity is defined as 1 μmol equivalent of fatty acids liberated from olive oil per min per mg of CPL. Reaction conditions are described in the Material and Methods section.

It appears that the lipase present in CPL is associated with the latex particle and is not water-soluble. According to Giordani *et al*. (7), the lipolytic activity of the latex was found only in the water-insoluble sediment, not in the supernatant, after washing and centrifugation, suggesting that solubilization of other constituents from the latex occurred. In the present study, the lipolytic activities measured were 17.1 units for crude CPL and 27.3 units for buffer-washed CPL, respectively (Table 1). Thus, a 60% increase in lipolytic activity for the sediment was obtained after washing. However, we used crude CPL in our experiments because the weight recovery of the latex pellet was low (only 20% weight of crude CPL was recovered after washing and drying). Moreover, the recovered latex pellet had a gummy texture after buffer washing even after drying the latex to $a_w < 0.1$.

In the transesterification of chicken fat with caprylic acid, the crude CPL lipase favored higher reaction temperatures; incorporation of caprylic acid increased by 105% when the reaction temperature was raised from 35 to 65ºC (Table 2). Giordani *et al*. (7) also reported increased hydrolytic activity for CPL at higher reaction temperatures.

In this study, for batch acidolysis reactions the best substrate mole ratio (chicken fat fatty acids/caprylic acid) found was 1:2. When the mole ratio was increased from 1:1 to 1:2, incorporation of caprylic acid into chicken fat TAG increased to 28% (Table 2). However, higher mole ratios of acid to substrate, such as 1:3 and 1:4, resulted in decreased incorporation (Table 2). A similar result was found previously when caprylic acid and peanut oil were reacted in a stirred batch reactor (16). One possible explanation for this may be substrate inhibition in which the excess amount of acid in the reaction medium causes deactivation of the lipase.

Villeneuve *et al*. (17) obtained a higher esterification activity for CPL lipase with $a_w < 0.1$ than in reactions with $a_w = 0.56$. Because the reverse reaction, hydrolysis, is expected above a critical water content in the system, we maintained the CPL lipase with $a_w \leq 0.1$ for further studies.

After acidolysis of chicken fat in the packed-bed column reactor for 117 min, 7.1% of caprylic acid was incorporated into the chicken fat TAG. Increasing the residence time of the substrate mixture in the reactor beyond 117 min, resulted in increased caprylic acid incorporation, as expected. For example, incorporation of caprylic acid into chicken fat increased up to 24.1% when the residence time was increased to 340 min (Table 2). Thus, increased residence time allows for

TABLE 2
Effect of Reaction Conditions on the CPL Lipase-Catalyzed Incorporation of Caprylic Acid into Chicken Fat

Parameter	Caprylic acid incorporated (%)
Temperature (°C)[a]	
35	11.4
45	13.9
55	17.6
65	23.4
Substrate mole ratio[b] (chicken fat fatty acids to caprylic acid)	
1:1	23.4
1:2	30.0
1:3	25.5
1:4	19.3
Reactor residence time (min)[c]	
117	7.1
234	16.1
340	24.1

[a]Mole ratio of substrates was 1:1 (chicken fat fatty acids/caprylic acid) in the batch reactor.
[b]Reaction temperature of 65ºC in the batch reactor.
[c]Mole ratio of chicken fat fatty acids to caprylic acid of 1:2 and reaction temperature of 65ºC in the continuous packed-bed column reactor. For abbreviation see Table 1.

longer contact time between substrates and lipase for reaction to occur. However, increased formation of partial glycerides (mono- and diacylglycerols) and acyl migration were observed when the residence (reaction) time was increased (18). Therefore, to minimize partial glyceride formation and to produce SL with limited acyl migration, the acidolysis reactions were limited to 117 min of reactor residence time.

To purify the SL product, TAG (modified chicken fat) from the reaction mixture were initially separated from the acid and ester by-products by Florisil column chromatography. It was found by HPLC analysis that crude CPL itself contains esters in its latex. Even though Florisil column chromatography provided a rapid separation, it did not allow a complete separation of SL products from unreacted chicken fat TAG due to the similar elution characteristics of their TAG species. Therefore, a second purification step using silica gel column chromatography was subsequently used to further separate the SL product from unreacted chicken fat (Scheme 1). After purification, two fractions, SL-I and SL-II, were obtained that were composed predominantly of newly synthesized SL-TAG.

The fatty acid profiles for chicken fat, modified chicken fat after removing fatty acids and esters, and purified SL (SL-I and SL-II) are shown in Table 3. For SL-I, caprylic acid constituted 19.6% of the fatty acids and palmitic acid 19.7%, which is a 22% decrease in palmitic acid content compared to chicken fat. For SL-II, caprylic acid constituted 27.3% of the constitutive fatty acids and palmitic acid 16.4%, which is a 35% decrease from chicken fat.

Analysis of fractions SL-I and SL-II by reversed-phase HPLC indicated that they were composed of distinct TAG species. Reversed-phase HPLC showed that TAG species with PN over the range of 46 to 48 carbons represented 81.5% of the chicken fat TAG. Among them, LOO (one linoleic and

TABLE 3
Fatty Acid Profile of Chicken Fat, Modified Chicken Fat, and Structured Lipid (SL) Products

Fatty acid	Chicken fat	Modified chicken fat[a]	SL-I[b]	SL-II[b]
8:0	—	7.1	19.6	27.3
14:0	0.7	1.6	0.7	0.5
14:1	0.3	ND[c]	0.2	0.2
16:0	25.2	20.3	19.7	16.4
16:1	7.8	5.3	5.2	5.0
18:0	5.9	8.2	5.9	4.3
18:1	40.5	38.7	34.2	29.7
18:2	18.4	16.0	13.7	15.8
18:3	0.7	1.8	0.4	0.5
20:1	0.5	1.0	0.5	0.4

[a]SL-TAG (triacylglycerol) product obtained from the continuous packed-bed column reactor after 117 min residence time and purified by Florisil column chromatography.
[b]SL-I and SL-II purified by silica-gel chromatography. Unreacted chicken fat removed from modified chicken fat. Abbreviations SL-I and SL-II are described in the Results and Discussion section.
[c]Not detected.

two oleic acyl residues, 23.5%) and POO (one palmitic and two oleic acyl residues, 18.8%) were found as the major TAG species (Fig. 1A). This analysis was made from a comparison of chicken fat TAG retention times with those previously reported for tallow and standard TAG mixtures (13).

After the TAG fraction (modified chicken fat) was separated by Florisil column chromatography, about 28% of the TAG species present were seen to be newly synthesized SL, and 72% were unreacted chicken fat (Fig. 1B). Fatty acid composition showed that the incorporated caprylic acid was 7% in the modified chicken fat (Table 3). As shown in Figure 1B, the composition of TAG species in unreacted chicken fat was similar to that in the starting chicken fat.

For SL-I, TAG species were separated into three distinct TAG groups with PN in the range of 28–34, 36–44, and 46–50 carbon atoms (Fig. 1C). Since caprylic acid ($C_{8:0}$) was incorporated into the SL, we assigned the first TAG group with PN between 28–34 as SL-TAG composed of two medium-chain caprylic acyl residues and one long-chain fatty acid (C_{16}-C_{18}) residue from the chicken fat TAG, which are referred to as MML-TAG. SL-1 with TAG peaks with PN 32 were assigned as SL having two caprylic and one palmitic acyl or oleic acyl residues, which are the most abundant fatty acids in chicken fat based on their theoretical PN of 32. The first SL-TAG group species (PN 28–34) in SL-I accounted for 40% of this product. The second TAG group in SL-I with PN between 36–44 are TAG containing one medium-chain capryloyl and two long-chain fatty acyl residues from chicken fat, which are referred to as MLL-TAG and accounted for 37.6% of the TAG species in SL-I. The third TAG group with PN ≥ 46 are long-chain TAG from chicken fat (LLL-TAG), which were not separated by silica-column chromatography. The fatty acid composition of fraction SL-I is presented in Table 3.

The majority of TAG present in the second fraction (SL-II) were MML-TAG, which accounted for 90.4% of the TAG species present in this fraction (Fig. 1D). From the foregoing, it was concluded that newly formed SL-TAG species represented 77.6% (SL-I) and 90.4% (SL-II) of the TAG species present, respectively (Fig. 1C and 1D). Of the two SL products,

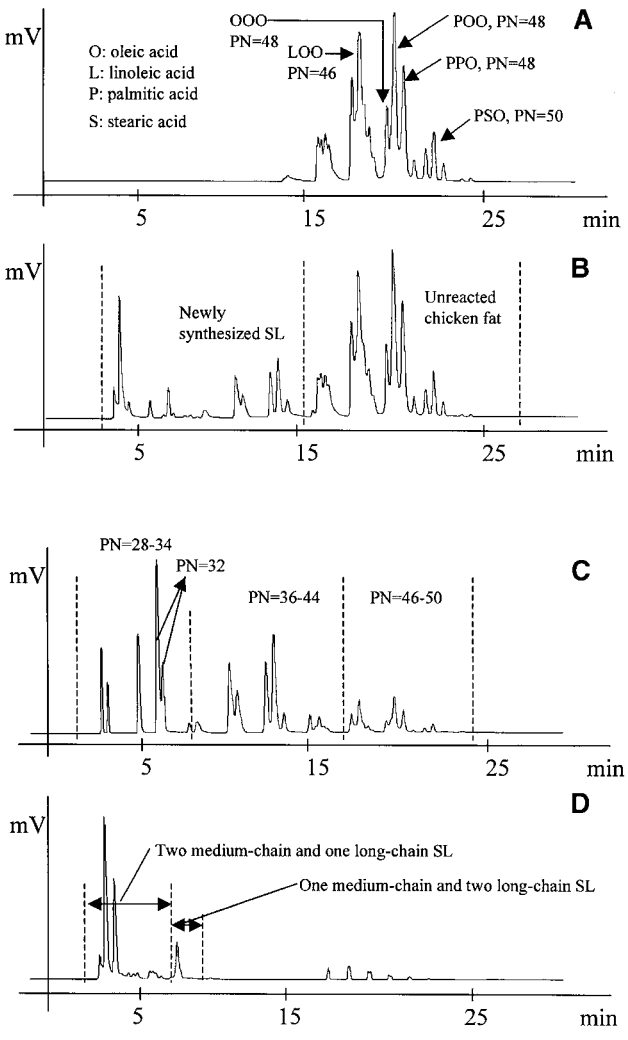


FIG. 1. Chromatograms from the reversed-phase high-performance liquid chromatographic separation of: (A) chicken fat triacylglycerols (TAG), (B) modified chicken fat TAG after Florisil column chromatography, (C) structured lipid (SL)-I TAG, and (d) structured lipid SL-II TAG. Analysis conditions are given in the Materials and Methods sections.

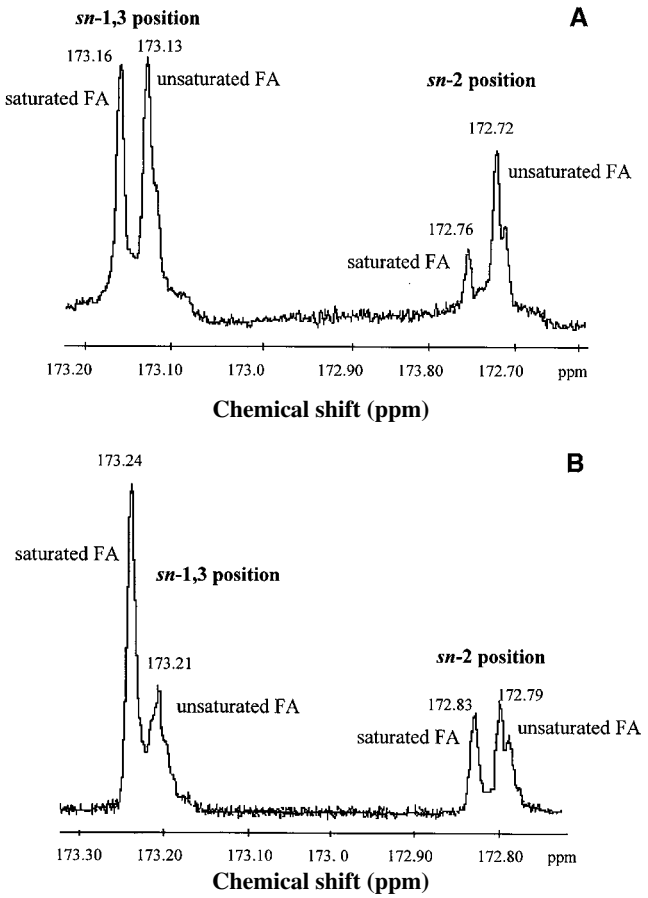


FIG. 2. High field (400 MHz) proton noise decoupled ^{13}C nuclear magnetic resonance spectrum of the carbonyl carbon regions of (A) chicken fat TAG and (B) structured lipid product SL-I TAG. FA, fatty acid; for other abbreviations see Figure 1.

SL-I was selected for further analysis by ^{13}C NMR and DSC because SL-I contained both MML- and MLL-TAG species.

NMR is a powerful method for studying the fatty acyl positional distribution in TAG molecules since it is a simple nondestructive method requiring short analysis time. Several studies have examined the ^{13}C NMR spectra of vegetable oil TAG and showed that the carbonyl region can be used to differentiate the position of the saturated and unsaturated fatty acyl residues on the glycerol backbone in the TAG (19–21). The chemical shifts (ppm) for the carbonyl groups in chicken fat are found at 173.13 and 173.16 for the unsaturated and saturated fatty acyl groups at the *sn*-1,3 positions and 172.72 and 172.76 for the unsaturated and saturated fatty acyl residues at the *sn*-2 position, respectively. The carbonyl signals in SL-I had similar chemical shift assignments (Fig. 2). However, ^{13}C NMR does not differentiate among the unsaturated (e.g., palmitoleoyl and oleoyl) carbonyl signals or among the saturated (e.g., palmitoyl and stearoyl) carbonyl signals in TAG because of the similar ^{13}C carbonyl chemical shifts of the fatty acyl classes (Fig. 2) (22).

Our NMR results showed a similar fatty acyl distribution for chicken fat to that reported by Brockerhoff *et al.* (15). For chicken fat, 61 and 76% of the unsaturated fatty acyl groups are located at the *sn*-1,3 and *sn*-2 positions, respectively (Table 4). From its NMR spectrum, the saturated fatty acyl content at the *sn*-1,3 positions for structured lipid SL-1 was 62% compared to 39% for chicken fat TAG (Table 4), indicating that caprylic acid was preferentially incorporated at the *sn*-1,3 position of the chicken fat TAG. Because the CPL lipase is regarded as more stereoselective to *sn*-3 than *sn*-1 position (8), most of the caprylic acid was thought to be at the *sn*-3 position. However, the saturated fatty acyl content at the *sn*-2 position also increased from 23.8 to 34.5% after transesterification, suggesting that acyl migration of saturated fatty acids to the *sn*-2 position occurred during the reaction.

The synthesized SL had different physical properties, such as melting point, as well as other chemical properties from the chicken fat used as substrate (23). Chicken fat and purified SL-I showed distinct DSC thermograms. Cooling curves are presented in Figure 3A and 3B. In Figure 3A, the exothermic peaks that represent the crystallization (solidification) of chicken fat showed broad peaks with minima at 9.8 and –0.6°C. These results are similar to those reported by Coni *et al.* (24). However, the exothermic thermogram for SL-I had shifted crystallization minima at 3.4 and –46°C (Fig. 3B). The shifting of exothermic peaks is explained by the incorporation of caprylic acid, which has a lower melting point than long-chain (C_{16} or C_{18}) fatty acids, into chicken fat to give a more complex TAG composition, which contributes to a lower crystallization temperature of the SL-I product.

TABLE 4
Distribution of Saturated and Unsaturated Fatty Acyl Residues in Chicken Fat and Structured Lipid TAG by ^{13}C-NMR Spectroscopy

		Chicken fat		SL-I[a]	
	Fatty acid type	Within TAG molecule	Within each position	Within TAG molecule	Within each position
sn-1,3	Saturated	27	39	44	62
	Unsaturated	43	61	27	38
	SUM	70	100	71	100
sn-2	Saturated	7	24	10	35
	Unsaturated	23	76	19	65
	SUM	30	100	29	100

[a]Unreacted chicken fat was removed from modified chicken fat by silica-gel chromatography. NMR, nuclear magnetic resonance; for other abbreviation see Table 3.

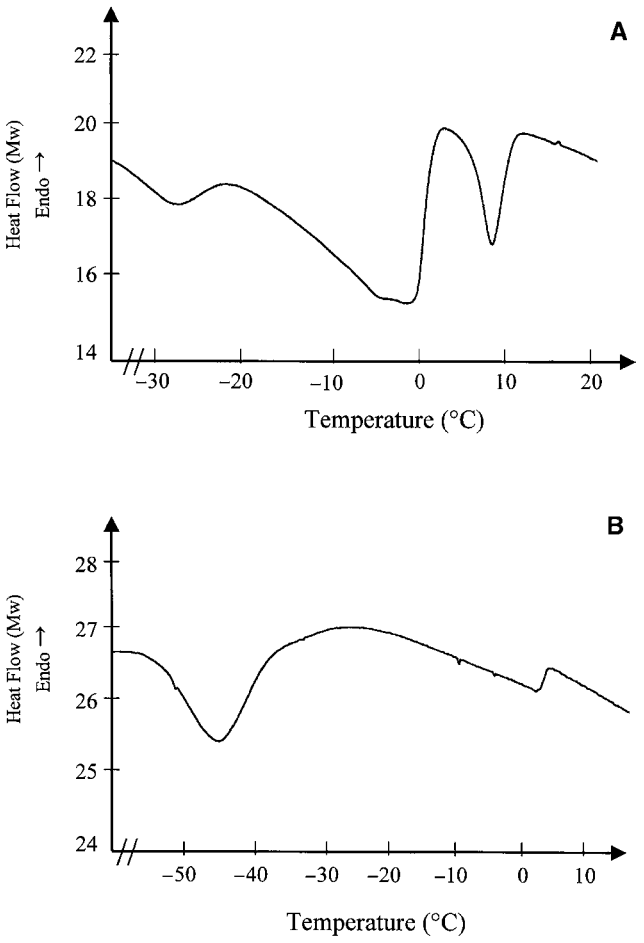


FIG. 3. Dynamic differential scanning calorimetry cooling curves for (A) chicken fat and (B) structured lipid product SL-I. For abbreviation see Figure 2.

When SL are synthesized from natural fats or oils, purification of the SL products from unreacted substrate (fat/oil) is not readily accomplished. Generally, natural fats and oils are a complex mixture of individual TAG species. After acidolysis with other acyl moieties, an even more complex mixture of TAG species is expected. Often the SL synthesized has physical and chemical characteristics similar to the fat or oil used as substrate so that purification of the SL by distillation or chromatography is difficult at best, especially when larger quantities are required. In most instances therefore, TAG mixtures that are prepared from natural lipids and separated from starting TAG can be considered as either modified lipids, tailored lipids, or SL products. Thus, conversion of substrate to product should be increased to eliminate the purification steps.

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REFERENCES

1. U.S. Department of Agriculture, *Livestock Dairy and Poultry Situation and Outlook*, LDP-M-55, Economic Research Service, Washington DC, 1999.
2. Bockisch, M., *Fats and Oils Handbook*, AOCS Press, Champaign, 1998, pp. 121–173.
3. Nicolosi, R.J., A.F. Stucchi, and J. Loscalzo, Effect of Dietary Fat Saturation on Low Density Lipoprotein Metabolism, in *Health Effects of Dietary Fatty Acids*, edited by G.J. Nelson, American Oil Chemists' Society, Champaign, 1991, pp. 77–82.
4. Lee, K.-T., and C.C. Akoh, Structured Lipid: Synthesis and Applications, *Food Rev. Int. 14*:17–34 (1998).
5. Willis, W.M., R.W. Lencki, and A.G. Marangoni, Lipid Modification Strategies in the Production of Nutritionally Functional Fats and Oils, *Crit. Rev. Food Sci. Nutr. 38*:1–36 (1998).
6. Lee, K.-T., C.C. Akoh, and D.L. Dawe, Effects of Structured Lipid Containing Omega-3 and Medium Chain Fatty Acids on Serum Lipids and Immunological Variables in Mice, *J. Food Biochem. 23*:197–208 (1999).
7. Giordani, R., A. Moulin, and R. Verger, Tributyroylglycerol Hydrolase Activity in *Carica papaya* and Other Latices, *Phytochemistry 30*:1069–1072 (1991).
8. Villeneuve, P., M. Pina, D. Montet, and J. Graille, *Carica papaya* Latex Lipase: *sn*-3 Stereoselectivity or Short-Chain Selectivity? Model Chiral Triglycerides Are Removing the Ambiguity, *J. Am. Oil Chem. Soc. 72*:753–755 (1995).
9. Villeneuve, P., M. Pina, A. Skarbek, J. Graille, and T.A. Foglia, Specificity of *Carica papaya* Latex in Lipase-Catalyzed Interesterification Reactions, *Biotechnol. Tech. 11*:91–94 (1997).
10. Mukherjee, K.D., and I. Kiewitt, Specificity of *Carica papaya* Latex as Biocatalyst in the Esterification of Fatty Acids with 1-Butanol, *J. Agric. Food Chem. 44*:1948–1952 (1996).
11. Klein, R.R., G. King, R.A. Moreau, G.P. McNeill, P. Villeneuve, and M.J. Hass, Additive Effects of Acyl-Binding Site Mutations on the Fatty Acid Selectivity of *Rhizopus delemar* Lipase, *J. Am. Oil Chem. Soc. 74*:1401–1407 (1997).
12. Foglia, T.A., and K.C. Jones, Quantitation of Neutral Lipid Mixtures Using High-Performance Liquid Chromatography with Light Scattering Detection, *J. Liq. Chromatogr. Rel. Technol. 20*:1829–1838 (1997).
13. Foglia, T.A., K. Petruso, and S.H. Feairheller, Enzymatic Interesterification of Tallow-Sunflower Oil Mixtures, *J. Am. Oil Chem. Soc. 70*:281–285 (1993).
14. Belitz, H.D., and W. Grosch, *Food Chemistry*, Springer-Verlag Inc., New York, 1984, pp. 128–200.
15. Brockerhoff, H., R.J. Hoyle, and N. Wolmark, Positional Distribution of Fatty Acids in Triglycerides of Animal Depot Fats, *Biochim. Biophys. Acta 116*:67–72 (1966).
16. Lee, K.-T., and C.C. Akoh, Solvent-Free Enzymatic Synthesis of Structured Lipids from Peanut Oil and Caprylic Acid in a Stirred Tank Batch Reactor, *J. Am. Oil Chem. Soc. 75*:1533–1537 (1998).
17. Villeneuve, P., A. Skarbek, M. Pina, J. Graille, and T.A. Foglia, Catalytic Behavior of *Carica papaya* Latex in Transesterification Reactions, *Biotechnol. Tech. 11*:637–639 (1997).
18. Xu, X., S. Balchen, C.-E. Høy, and J. Adler-Nissen, Pilot Batch Production of Specific-Structured Lipids by Lipase-Catalyzed Interesterification: Preliminary Study on Incorporation and Acyl Migration, *J. Am. Oil Chem. Soc. 75*:301–308 (1998).
19. Sacchi, R., F. Addeo, I. Giudicianni, and L. Paolillo, Analysis of the Positional Distribution of Fatty Acids in Olive Oil Triacylglycerols by High Resolution ^{13}C NMR of the Carbonyl Region, *Ital. J. Food Sci. 2*:117–123 (1992).
20. Ng, S., Analysis of Positional Distribution of Fatty Acids in Palm Oil by ^{13}C NMR Spectroscopy, *Lipids 20*:778–782 (1985).
21. Wollenberg, K.F., Quantitative High Resolution ^{13}C-Nuclear

Magnetic Resonance of the Olefinic and Carbonyl Carbons of Edible Vegetable Oils, *J. Am. Oil Chem. Soc. 67*:487–494 (1990).
22. Pouchert, C.J., and J. Behnke, *Aldrich Library of ^{13}C and ^{1}H NMR Spectra*, Aldrich Chemical Company, Milwaukee, 1993, pp. 751–847.
23. Lee, K.-T., and C.C. Akoh, Characterization of Enzymatically Synthesized Structured Lipids Containing Eicosapentaenoic, Docosahexaenoic, and Caprylic Acids, *J. Am. Oil Chem. Soc. 75*:495–499 (1998).
24. Coni, E., D. Pasquale, P. Coppolelli, and A. Bocca, Detection of Animal Fats in Butter By Differential Scanning Calorimetry: A Pilot Study, *Ibid. 71*:807–810 (1994).

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