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Lipase-catalyzed Transesterification of Medium-chain Triacylglycerols and a Fully Hydrogenated Soybean Oil

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ABSTRACT: A commercial preparation of medium-chain TAG (MCT) was enzymatically transesterified with a fully hydrogenated soybean oil (FHSBO) at different weight ratios (40:60, 50:50, 60:40, and 70:30). All reactions were carried out at 70 °C in flasks placed in an orbital shaker (300 rpm). Three different lipase preparations were tested: TL IM (*Thermomyces lanuginosus*), lipase PS (*Burkholderia cepacia*), and Chirazyme L2 (*Candida antarctica*). The progress of the reaction was monitored by following the changes in the triacylglycerol (TAG) profile of the reaction mixture with reversed-phase high-performance liquid chromatography (HPLC). The rates of disappearance of the TAG originally present in both the MCT and the FHSBO were fastest when lipase TL IM was used; the slowest rates were observed for lipase PS. Although the relative compositions of the newly formed TAG at equilibrium depended on the particular lipase used and the initial weight ratio of the substrates, the TAG families containing 2 stearic residues and a residue of either capric acid or caprylic acid were the most abundant product species. All samples were analyzed by differential scanning calorimetry (DSC) to determine the effect of this reaction on the melting profile of the resulting products.

Keywords: lipase, medium chain TAG, structured lipids, transesterification

Introduction

Structured lipids (SL) are, by definition, triacylglycerols (TAG) modified by using either chemical or enzymatic methods to change the composition of the fatty acid residues and/or their positional distribution on the glycerol backbone. The main purpose of such modifications is to improve the physical and/or chemical characteristics of natural triacylglycerols such as melting point, solid fat content, iodine value, or saponification number, to obtain fats suitable for specific applications (Lee and Akoh 1998a). The enzymatic synthesis of SL is a relatively new concept in lipid modification, and lipases are the biocatalysts of choice for this application. Although lipases are named for their ability to catalyze lipolysis (hydrolysis) reactions, the use of these enzymes in systems with very low water contents leads to catalysis of a variety of reactions, including ester synthesis, alcoholysis, acidolysis, and interesterification (Akoh and others 2002). Enzyme-catalyzed transesterification reactions have been studied by several research groups because they provide a viable technology for producing SL, including acylglycerols containing medium-chain-length fatty acid residues. The present investigation is focused on the production of structured TAG lipids that are rich in medium-chain fatty acid residues.

Medium-chain TAG (MCT) contain primarily saturated fatty acid residues with chain lengths from 6 to 12 carbon atoms. They are very stable to oxidation and are liquids at room temperature, and they have low viscosities and melting points (Lee and Akoh 1998b). Their small molecular size and relatively high solubility in water have important implications for the metabolism of these species. The MCT are readily absorbed in the digestive tract and are metabolized more like carbohydrates than as conventional oils and fats. Hence, MCT can be used as a source of quick energy because of their rapid absorption, especially for infants and individuals with lipid absorption disorders (Yankah and Akoh 2000).

Several authors have recently reported the incorporation of medium-chain-length fatty acids in acylglycerols to produce SL via lipase-catalyzed acidolysis reactions. For example, Fomuso and Akoh (2002) carried out the lipase-catalyzed acidolysis of olive oil with caprylic acid using a bench-scale packed bed bioreactor containing a lipase from *Rhizomucor miehei* as the biocatalyst. Kawashima and others (2002) incorporated caprylic acid into an oil that was rich in gamma linoleic acid residues using a fixed-bed bioreactor packed with an immobilized lipase from *Rhizopus oryzae*. Ledochowska and others (2001) prepared SL via acidolysis of rapeseed oil with capric acid in the presence of Lipozyme.

Interesterification reactions involving only TAG as reactants have also been studied as a method of producing SL. An interesting advantage of interesterification is that if the water content of the reaction medium is properly controlled, the production of byproducts such as free fatty acids and lower acylglycerols can be minimized (Xu 2003). This situation constitutes an important economic advantage of this reaction because the need for further purification procedures is reduced or eliminated. Mangos and others (1999) have described a batch reaction procedure for the synthesis of low-calorie SL from hydrogenated soybean oil and triacetine. This synthesis takes advantage of the specificity of a lipase from *Carica papaya* for short-chain acyl groups. Mogi and others (2000) used this approach to produce novel TAG based on the reaction of MCT with a mixture of TAG containing long-chain saturated fatty acid residues ranging from palmitic (C16:0) to lignoceric (C24:0), as well as some monounsaturated fatty acid residues. These researchers used a surfactant-modified lipase as the biocatalyst in an organic reaction medium.

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Here, we describe the use of enzymatic technology to produce MCT-enriched SL by interesterification of a commercially available MCT oil and a totally hydrogenated soybean oil in a solvent-free system. Because of the composition of the substrates (they contain only saturated fatty acid residues) and the mild reaction conditions, these reactions are not likely to generate *trans* fats. The reactions were performed using 3 different commercial enzymes and 4 weight ratios of substrates. The effects of the biocatalyst and the substrate mixture on the final TAG compositions and the corresponding melting profiles of the products are also reported and discussed.

Materials and Methods

Materials

A commercial nonflavored mixture of MCT (Original Thin Oil®) was purchased from Sound Nutrition (Dover, Idado, U.S.A.). The fully hydrogenated soybean oil (FHSBO) was a gift from AC Humko Oil Products (Cordova, Tenn., U.S.A.). An immobilized lipase preparation from *Thermomyces lanuginosus* (TL IM) was obtained from Novozymes North America (Franklinton, N.C., U.S.A.); a lipase from *Candida antarctica* fraction B (Chirazyme L2 c.f. 2) was purchased from Boehringer (Indianapolis, Ind., U.S.A.); and a free lipase preparation from *Burkholderia cepacia* was obtained from Amano (Nagoya, Japan). Molecular sieves from Sigma-Aldrich (St. Louis, Mo., U.S.A.) were used to control the water activity in the reaction medium. All solvents used were high-performance liquid chromatography (HPLC) grade (Fisher Scientific, Chicago, Ill., U.S.A.). Standards of capric, caprylic, palmitic and stearic acids, as well as the TAG standards for tricaprin, tricaprylin, tripalmitin, and tristearin, were purchased from Sigma Chemical.

Transesterification reaction

All reactions were carried out in duplicate at 70 °C in sealed plastic Erlenmeyer flasks placed in a water bath shaker/incubator operated at 300 rpm. Ten grams of a fat mixture were placed in each flask. These mixtures consisted of MCT and FHSBO at weight ratios of 40:60, 50:50, 60:40, and 70:30. The enzyme loading was 5% of the total weight of reactants. As recommended by Torres and others (2002), molecular sieves were also added (1 g) to minimize the extent of an undesired side reaction (hydrolysis).

Gas chromatographic analyses

The fatty acid compositions of the MCT and the FHSBO were determined using a Hewlett Packard (Avondale, Pa., U.S.A.) Model 5890 gas chromatograph equipped with a 30 m × 0.32 mm × 0.25 μm Supelcowax-10 capillary column (Supelco, Bellefonte, Pa., U.S.A.) and a flame ionization detector. Samples were derivatized according to the procedure described by Williams and others (1995) to form the corresponding methyl esters of the fatty acid residues present by treatment with 0.1 *N* methanolic NaOH for 30 min at 60 °C. The oven temperature was programmed for the gas chromatographic analysis as follows: start at 150 °C, heat to 225 °C at 20 °C/min, and then hold at 225 °C for 16.25 min. The injector temperature was 220 °C, and the detector temperature was 250 °C. One microliter of sample was injected and a split ratio of 20:1 was used. Individual fatty acids were identified by comparison with the retention times of the methyl esters of capric, caprylic, palmitic, and stearic acids.

Analyses of reaction products

Samples of the reaction mixture (200 μL) were periodically withdrawn from the flask during the course of the reaction. These samples were diluted immediately with chloroform (1.8 mL) to ensure complete dissolution of tristearin. The enzyme present in the samples was removed by filtration of the chloroform solution using 0.45-μm Whatman (Clifton, N.J., U.S.A.) nylon syringe filters.

Reversed-phase HPLC (RP-HPLC) was used to separate and quantify the TAG families present in the original fats and those being formed and consumed during the course of the reaction. For this purpose, we used a 250 mm × 3.2 mm Econosil C18 column from Alltech (Deerfield, Ill., U.S.A.) maintained at 45 °C. The chromatographic system consisted of a Waters 600 (Milford, Pa., U.S.A.) quaternary pump and an Alltech 500 Evaporative Light Scattering Detector (ELSD). An acetone/acetonitrile solvent system was developed to perform the separation at a constant flow rate of 1 mL/min according to the gradient shown in Table 1. In this analysis, 100-μL aliquots from the original dilution were mixed with 1.9 mL chloroform. Then 10 μL of the final dilution were injected into the HPLC column. Calibration curves for tricaprin, tricaprylin, tripalmitin, and tristearin were also prepared.

HPLC analysis of acylglycerols

The types and concentrations of the various acylglycerols present in the samples taken during the transesterification reaction were determined using normal-phase HPLC analysis. The main purpose of these analyses was to determine the extent of net hydrolysis via quantification of the total free fatty acids present in the reaction mixture as the reaction proceeded. The analyses were performed using the chromatographic system described earlier but using an Econosil-Silica 5U column (250 × 4.6 mm from Alltech) for this analysis. The mobile phases used were hexane/2-propanol/ethyl acetate/formic acid (80:10:10:0.1, by volume) and hexane/formic acid (100:0.02, by volume). The solvent gradient was identical to that previously described by Liu and others (1993). Aliquots of 50 μL from the original dilution were mixed with 1.95 mL chloroform and then analyzed (10 μL injection volume) using the conditions noted previously.

Differential scanning calorimetry (DSC) of the final products

A Pyris 1 Perkin-Elmer DSC (Shelton, Conn., U.S.A.) was used to determine the thermal profiles of the final TAG mixtures. The DSC was calibrated using indium (m.p. = 156.6°C) and mercury (m.p. = –38.87 °C). Samples containing 5 to 10 mg of TAG were placed in aluminum pans and sealed. All samples were heated to 80 °C and held at this temperature for 1 min and then cooled from 80 °C to –20 °C. This temperature was held for 5 min before the samples were heated again to 80 °C at a constant rate of 5 °C/min. All samples had previously been dissolved in chloroform, filtered using a 0.45-μm Whatman nylon syringe filter to remove the lipase and then dried under a nitrogen stream to recover the fat.

Results and Discussion

Substrate composition

Fatty acid profiles were determined by gas chromatographic analysis for each of the substrates. The MCT oil contained only caprylic (octanoic) and capric (decanoic) acid residues at levels of approximately 70% and 30% (w/w), respectively. The FHSBO contained only residues of stearic (87.12%) and palmitic (12.88%) acids. Gas chromatographic analyses of the reaction products revealed the presence of only the 4 fatty acids present in the original fats. Identities of the peaks were confirmed by comparison with the retention times of the individual standards: capric (3.1 min), caprylic (4.3 min), palmitic (10.1 min), and stearic acid (12.4 min).

Product acylglycerol profiles

The HPLC analyses on the silica column did not reveal the pres-

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Table 1—Solvent gradient used to separate triacylglycerol (TAG) families

Time (min)	% Acetone (v/v)	% Acetonitrile (v/v)
0	0	100
8	0	100
15	75	25
35	75	25
40	0	100
45	0	100

ence of appreciable levels of hydrolysis during any stage of the reaction. Normally a simple chromatographic peak that encompasses all of the TAG in a mixture is observed in the HPLC analysis. However, for MCT, 2 peaks were observed in every analytical run, but neither peak corresponded to a characteristic retention time for a free fatty acid. Moreover, a separate analysis of the pure MCT oil revealed that, under standard conditions, the silica column accomplished the separation of this oil into its constituent TAG species. To confirm the absence of free fatty acids in the intermediate and final product samples, we injected standards of the different free fatty acids corresponding to the residues present in both substrates. None of the observed retention times corresponded to the peaks observed in the MCT sample. Moreover, no peaks corresponding to monoacylglycerols or diacylglycerols were observed. Consequently, we concluded that at no time was the level of hydrolysis appreciable for any of the reactions we carried out.

Transesterification reactions

Changes in the TAG composition were used to monitor the progress of the reaction. Standards of several pure TAG containing only saturated fatty acid residues were subjected to triplicate HPLC analyses. A regression analysis was then performed to fit an empirical model that relates the elution time (RT) for a specific TAG with the total number of carbon atoms (CN) present in the fatty acid residues attached to the glycerol backbone. The model used here was a simple linear relation of the following form:

$$RT = m^*CN + b \quad (1)$$

This model provides a very good fit of the data (not shown). Identification of the peaks observed in the RP-HPLC analyses (Figure 1) was based on a comparison of the observed retention times with those predicted by the empirical model of Eq. 1 (Figure 1; Table 2).

The HPLC analyses of the 2 original fats revealed the presence of 3 primary families of TAG in each sample. For the MCT oil, these families were those with CN of 24, 26, and 28, whereas for the FHSBO, the carbon numbers were 48, 52, and 54. In the MCT oil, the principal TAG was that with a CN equal to 24, corresponding to tricaprylin (CpCpCp). The other 2 peaks were those with CN of 26 and 28, corresponding to combinations of 2 caprylic acid and 1 capric acid residues and 1 capric acid and 2 caprylic acid residues, respectively. The average content, in weight percent, observed for such TAG species was as follows: 47.91% CN 24, 42.54% CN 26, and 9.55% CN 28. The FHSBO was composed primarily of tristearin (63%), with small amounts of tripalmitin (9%) and a TAG family containing 2 stearic acid and 1 palmitic acid residues (28%).

In general, depletion of tristearin (SSS) was fastest for those reactions in which lipase TL IM was used as the biocatalyst. This TAG disappeared almost completely within 4 h when the weight ratio of substrates (MCT:FHSBO) was 70:30. However, tristearin remained in the reaction mixture after 24 h (at levels ranging from about 2% to 10% [w/w] of the total TAG content) for the other weight ratios studied. Higher residual contents of SSS were observed when the amount of FHSBO in the initial mixture was increased. When comparing our results with those previously reported by Torres and others (2002) for the interesterification of SSS and corn oil, we observed that in our case, approximate equilibrium was always reached within the 1st 4 to 6 h of reaction and that SSS depletion levels were higher. Several factors can be cited to explain the observed differences. For example, the reaction temperatures used in the 2 studies were very different (45 °C for the work of Torres and others versus 70 °C for the present work). The effect of this change is clearly evident when one compares the rates of reaction; SSS was, in general, highly depleted in our trials, even when the reaction mixtures contained a large proportion of this TAG.

For the trials in which the other 2 lipases (L2 and PS) were used, very similar trends for depletion of tristearin (Figure 2) were observed, although SSS disappeared at a slower rate relative tothat observed when the TL IM lipase was the biocatalyst. Final SSS contents were usually between 5% and 13%. The rate at which this TAG was consumed in the presence of the lipase from *C. antarctica* was slightly less than was the case for the reactions catalyzed by lipase PS. The final concentrations of SSS observed when the lipase from *C. antarctica* was used as biocatalyst are comparable to those reported by Seriburi and Akoh (1998) for the interesterification of SSS and triolein in hexane solution after 24 h of reaction at a lower temperature (55 °C).

For most of the conditions investigated, the other 2 components of the FHSBO disappeared to similar extents (data not shown). In the presence of lipase TL IM, the TAG corresponding to a CN of 48 was completely consumed at a very fast rate when the weight ratio of substrates was 70:30 (MCT:FHSBO). In all other cases, this component disappeared only after 4 h of reaction. Similar behavior was

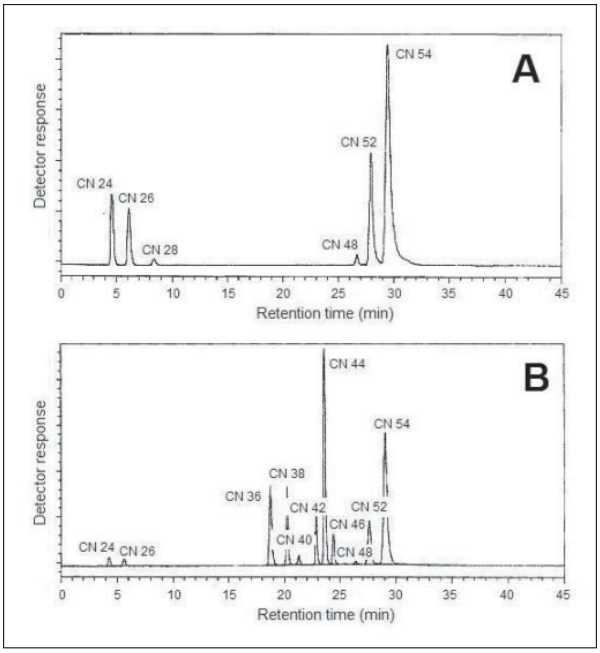

Figure 1—Reversed-phase high-performance liquid chromatography (HPLC) separation of the triacylglycerols (TAG) present in the starting reaction mixture (A) and the TAG formed by the transesterification reaction between medium-chain TAG (MCT) oil and fully hydrogenated soybean oil (FHSBO) (B) after 8 h of reaction at 70 °C in the presence of the lipase from *Candida antarctica* (Chirazyme L2)

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Table 2—Relation of carbon number (CN) to triacylglycerol (TAG)[a]

CN	TAG
24	CpCpCp
26	CaCaCp
28	CaCpCp
36	CpCpP/CaCpS
38	CpCpS
40	CaPP
42	CpPP/CaPS
44	CaSS
46	CpSS
52	SSP
54	SSS

[a]Ca = capric; Cp = caprylic; P = palmitic; S = stearic.

observed for the TAG with CN 52 when small amounts of FHSBO were present in the initial mixture. For the trials involving weight ratios of 50:50 and 60:40, small residual amounts of this TAG were present. In both trials, the concentrations were about 5%. For the trials in which the lipases from *C. antarctica* and *B. cepacia* were used as the catalyst, the TAG with CN 48 were completely consumed within 4 h for the 70:30 trials and within 6 h for the trials at 60:40 and 50:50 weight ratios of substrates; only small residual contents (about 3%) were observed for the 40:60 trials in which lipase PS was used as the biocatalyst. In all cases, the residual concentration of the TAG with CN 52 was less than 6%.

For each weight ratio of substrates, the TAG present in the original MCT substrate (CN 24, 26, and 28) disappeared in a very similar fashion for each lipase. Figure 3 contains a typical disappearance profile of the MCT TAG when lipase TL IM was used as the catalyst. The amounts of these TAG remaining at long reaction times (approaching equilibrium) were greater for those trials in which higher initial amounts of the MCT oil were used. TAG with CN of 24 and 26 were consumed at approximately the same rate, and the concentrations of these species in the final mixture were approximately 20% for each of the reactions involving a weight ratio of 70:30, but only 12% to 15%, 5% to 9%, and 5% for the trials at weight ratios of 60:40, 50:50, and 40:60, respectively. Akoh and Yee (1997) have suggested that the medium-chain-length substrates probably have an inhibitory effect at high mole ratios of this substrate for the lipase-catalyzed reactions between either tricaprin or tricaprylin with tristearin in hexane solution. The 3rd component of the original MCT oil, CN 28, was completely depleted in those trials involving weight ratios of 50:50 and 40:60 MCT:FHSBO. In the trials with the other 2 ratios (70:30 and 60:40), the final levels of this TAG were about 5% of the total TAG, except for the trials at a 60:40 initial weight ratio using lipase L2 where a total depletion was observed after 24 h .

Six different types of newly formed TAG were observed in all 12 experimental trials. These species were all characterized by carbon numbers between 36 and 46 (Figure 1B). For those reactions in which lipase TL IM was used as the biocatalyst, the TAG fractions corresponding to CN 36 and 38 (TAG containing 1 residue of palmitic or stearic acid) were part of the main compounds formed in the trials at weight ratios of 70:30, 60:40, and 50:50. These TAG reached maximum levels of 16% and 18% at long reaction times for the 3 weight ratios. A slight decrement in the concentrations of these TAG families (CN 36 and 38) in the final product mixture was observed for the experiments with the lowest initial content of MCT. The TAG with CN 42 and 46 were present at similar concentrations (about 5%) for the trials at initial weight ratios of 70:30 and 60:40 but were present at a level of about 10% for the trials at weight ratios of 50:50 and 40:60. Except for the trials at a weight ratio of 40:60, the TAG fraction corresponding to CN 40 reached a maximum level of about 5% for the

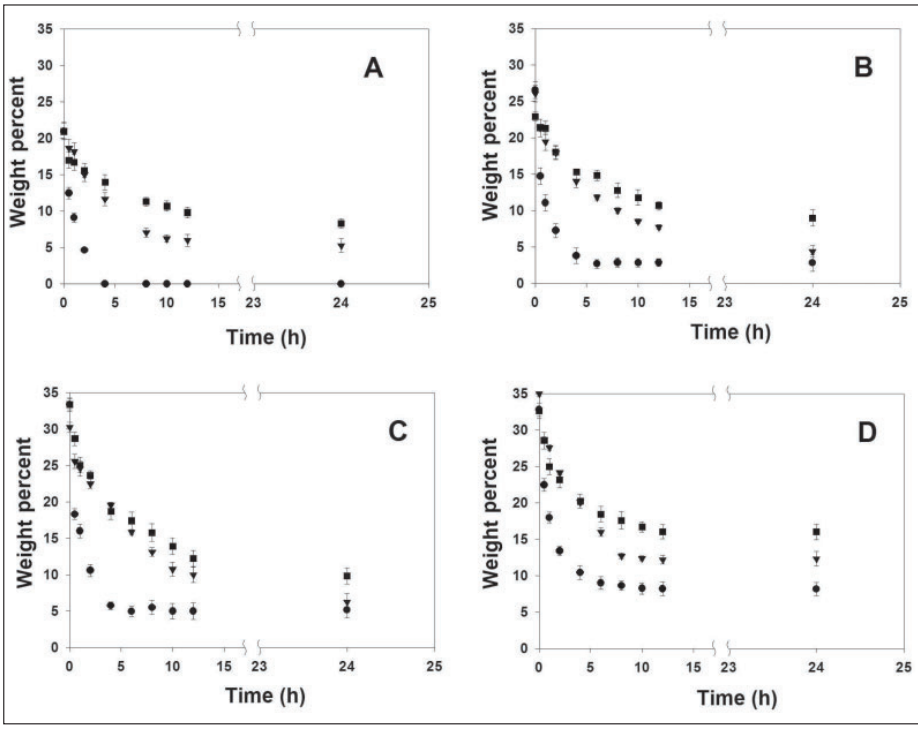

Figure 2—Evolution of the tristearin content for reactions at 70 °C using different initial weight ratios of medium-chain TAG (MCT) to fully hydrogenated soybean oil (FHSBO) (A) 70:30; (B) 60:40; (C) 50:50; (D) 40:60) (● = lipase TL IM; ▼ = lipase L2; ■ = lipase PS).

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conditions studied. For the species with CN 44 (TAG containing 2 stearic acid residues), there was a concomitant increase in the concentration of this species at long times when the initial level of the FHSBO was increased, although no appreciable variations were observed at the highest contents of initial hydrogenated fat. For the 2 lowest initial levels of the MCT oil, this TAG was the major constituent of the newly formed TAG, whereas at the 2 high initial levels of the MCT, the species with CN 36 was the most representative TAG (Figure 4). The results observed for reactions catalyzed by lipase L2 were similar to those obtained with lipase TL IM, although nearly equilibrium compositions were reached after longer reaction periods (4 h for lipase TL IM, and 12 h for lipase L2). The main differences observed were that in all cases, the TAG with CN 44 was usually the major constituent of the final mixture. The level of this TAG increased as the percentage of the MCT oil in the original mixture decreased. In addition, the TAG with CN 36 was always present at a level of 12% to 15% for all of the conditions tested. For those reactions carried out using lipase PS as the biocatalyst, the CN 44 species was not only the major product formed, but also the product with the fastest rate of appearance. In all trials, near equilibrium conditions were reached after about 12 h of reaction. The TAG species corresponding to CN of 36 and 38 were observed at levels near 15% and 10% in the experiments in which the L2 and PS lipases were used, respectively. For these lipases, the levels of the TAG corresponding to CN 42 and 46 varied from 5% in the trials with the highest initial levels of the MCT oil (70:30) to 10% at a weight ratio of 40:60. For all the reactions catalyzed by the *C. antarctica* and *B. cepacia* lipases, the highest level of the CN 40 TAG was approximately 2%. Interestingly, for the reactions mediated by lipase PS, the relative composition of the new TAG was substantially constant for the 4 initial weight ratios tested. The only effect observed was a corresponding overall increase in the levels of the TAG with CN ranging from CN 42 to CN 46 at long times when the initial amount of the FHSBO was increased. The differences observed between the 3 lipases tested for their ability to mediate the interesterification reactions can be explained by taking into account their differences in thermal stability and water content. The lipase from *B. cepacia* is very stable at high temperatures, but has optimum activity at 45 °C. However, lipase technical information provided by the supplier indicates that this enzyme loses its activity rapidly when the reaction temperature is increased. The lipases from *C. antarctica* and *T. lanuginosus* are both very heat-tolerant enzyme preparations, although our results showed marked differences in their abilities to exchange fatty acids between the MCT oil and the FHSBO. These differences can be explained if one considers the importance of the water content on the rate of reaction. The lipase from *C. antarctica* has a lower water content than TL IM (about 4.2% for the *C. antarctica* lipase and 6.7% for the TL IM, as determined by oven-drying before carrying out the reactions). In light of the restricted water content in the system, this factor can be critical in maintaining the lipase in a conformation in which there is ready access to the active site, a situation that directly affects the catalytic properties of these lipases.

In general, when the hydrogenated fat was present at the higher levels, the major components of the final product mixtures were families containing 2 stearic acid residues combined with 1 capric acid residue. For these feed mixtures, the higher concentration of stearic acid residues increased the level of species with CN 44 in the final reaction products. For the trials at low ratios of stearic acid to MCT, the main components of the newly formed TAG products were TAG containing a stearic acid residue and 2 medium-chain-length fatty acid residues. The levels of the TAG with CN 36 and CN 38 in the product were relatively constant for substrate weight ratios of 40:60 and 50:50 for each of the lipases tested. These species were the primary components of the products when fewer stearic or palmitic acid residues were available for exchange.

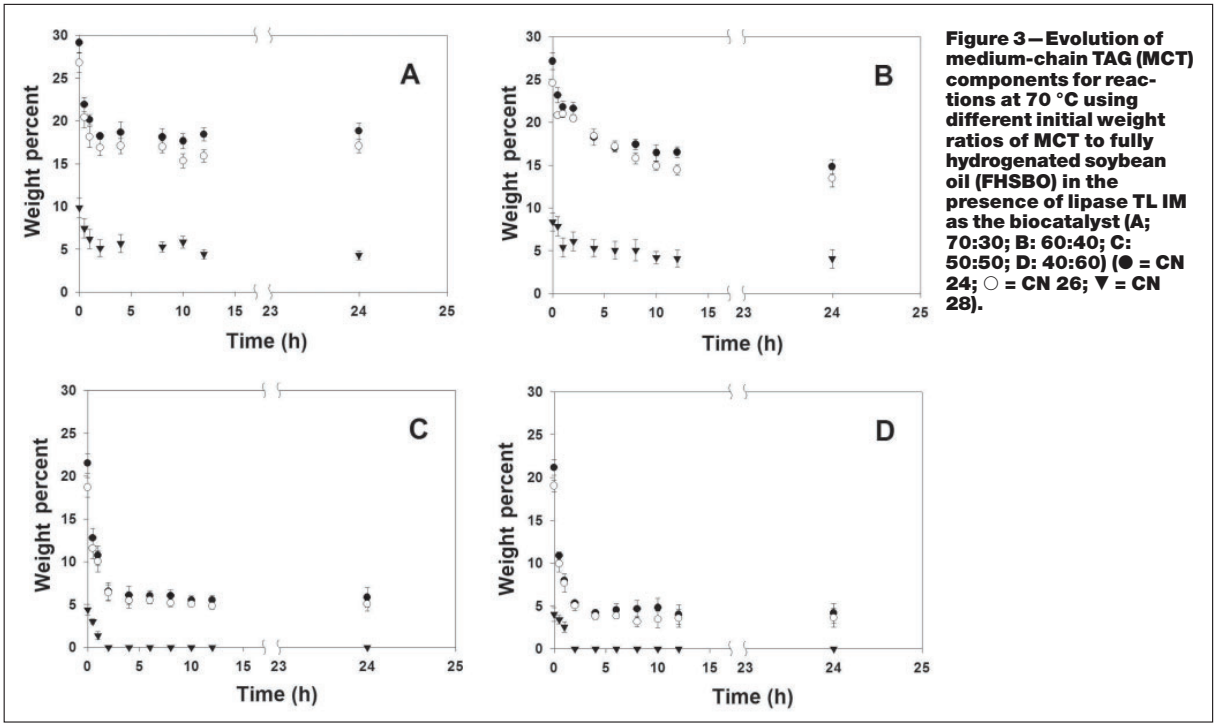

Figure 3—Evolution of medium-chain TAG (MCT) components for reactions at 70 °C using different initial weight ratios of MCT to fully hydrogenated soybean oil (FHSBO) in the presence of lipase TL IM as the biocatalyst (A; 70:30; B: 60:40; C: 50:50; D: 40:60) (● = CN 24; ○ = CN 26; ▼ = CN 28).

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Table 3—Average triacylglycerol (TAG) compositions (in weight percent) after 24 h of reaction at 70 °C for the several feed compositions used

	CN 24	CN 26	CN 28	CN 36	CN 38	CN 40	CN 42	CN 44	CN 46	CN 48	CN 52	CN 54
Lipase TL IM												
70:30	18.81	17.07	4 29	18.56	16.16	4.61	4.78	11.79	3.93	0.00	0.00	0.00
60:40	13.81	13.45	3.64	18.84	15.82	4.91	6.49	16.96	6.73	0.00	0.00	1.82
50:50	5.83	5.06	0.00	18.86	16 25	6.08	9.35	21.86	8.67	0.00	5.37	5.16
40:60	3.15	3.62	0.00	14.94	15.04	12.73	5.55	22.21	9.91	0.00	5.58	8.17
Lipase L2												
70:30	22.80	20.64	3.68	12.13	9 96	3.78	4.43	10.24	4.36	0.00	3.17	5.26
60:40	12.83	11.75	0.00	16.96	14.75	5.06	6.95	16.62	6.46	0.00	3.82	4.40
50:50	4.88	4.49	0.00	17.62	15.17	6.40	9.34	22.18	8.58	0.00	3.94	6.26
40:60	4.13	3 58	0.00	14.39	12 57	5.04	9.97	25.72	9.28	0.00	5.58	9.73
Lipase PS												
70:30	22.88	19.79	5.71	9.71	7 98	3.11	4.35	12.89	4.91	0.00	3.12	8.29
60:40	16.99	15.24	3.89	12.04	9 94	3.94	5.64	16.73	5.90	0.00	3.48	8.99
50:50	8.74	7.64	0.00	13.44	12.16	6.60	8.37	21.19	8.51	0.00	5.34	9.83
40:60	6.00	5.10	0.00	12.62	10.44	3.88	8.15	25.18	8.04	2.63	5.56	12.41

Table 3 contains a summary of the quasi equilibrium compositions for each of the 12 experimental conditions screened. The tabular entries correspond to the averages of duplicate runs. Variations between trials were less than 5% for all TAG.

DSC analyses

Figure 5 contains the melting profiles of the 2 starting materials (MCT oil and FHSBO) as determined by DSC. The melting curve obtained for the fully hydrogenated fat exhibited 2 melting components (at 53 °C and 63 °C), probably 2 polymorphic forms similar to those reported by Seriburi and Akoh (1998) for the α to β and β to liquid transitions of tristearin, the main component of the hydrogenated fat. DSC analysis of the MCT oil revealed only a single peak at approximately –8 °C. All of the modified lipids melted at temperatures between those observed for the melting thermograms of the original fats, starting at –5 °C and ending around 53 °C. Their peaks were much smaller and broader than those of the original pure substrates. The appearance of high-melting fractions in the transesterified products is related to the presence of the FHSBO. In those samples obtained in trials in which the lipase PS was used as the biocatalyst and the ultimate levels of SSS were relatively high, a peak was always present at temperatures above 50 °C. This peak is also present in the samples from the reactions catalyzed by lipases TL IM and L2. This peak is more pronounced when the initial level of FHSBO was high, as was the content of tristearin in the final product. In general, more peaks (up to 6) were observed in the thermograms as

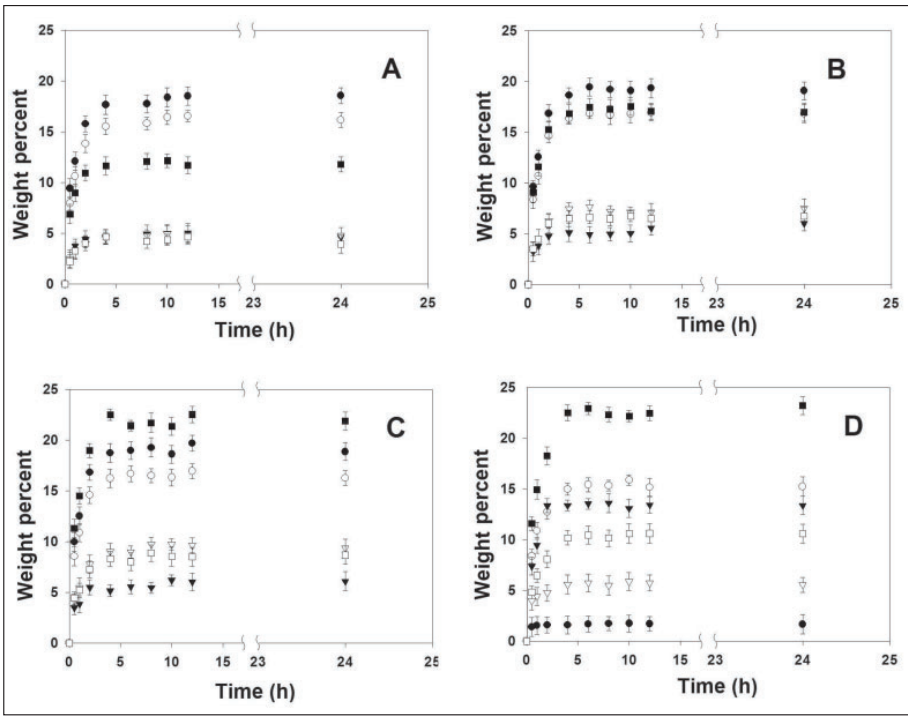

Figure 4—New triacylglycerols (TAG) formed by transesterification reactions at 70 °C catalyzed by lipase TL IM for several initial weight ratios of medium-chain TAG (MCT) to fully hydrogenated soybean oil (FHSBO). (A) 70:30; (B) 60:40; (C) 50:50; (D) 40:60) (● = CN 36; ○ = CN 38; ▼ = CN 40; ▽ = CN 42; ■ = CN 44; □ = CN 46).

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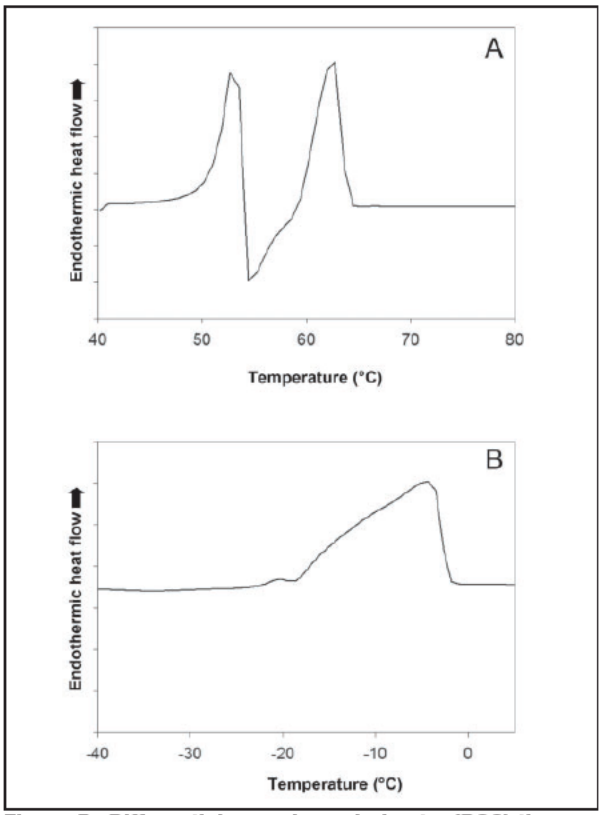

Figure 5—Differential scanning calorimetry (DSC) thermograms of the original substrates (A) fully hydrogenated soybean oil (FHSBO); (B) medium-chain TAG (MCT) oil

the initial level of FHSBO was increased. This condition leads to the formation of greater amounts of the new TAG (Figure 6). Further studies are necessary to determine the functional properties and possible applications of the different types of products obtained using these transesterification reactions.

Conclusions

Structured lipids containing different levels of MCT were produced by lipase-catalyzed interesterification reactions. Studies involving 3 different commercial lipase preparations led to the conclusion that the rate of disappearance of SSS was highest when lipase TL IM was used as the biocatalyst. Six different families of new TAG were formed under all of the conditions tested, although the rates of formation, and relative concentrations of these families, depended on the initial weight ratios of the substrates and the particular biocatalyst used. The changes in the melting profiles of the final products corresponded directly to the observed depletion of high-melting-point TAG present in the FHSBO. The SL generated in these experiments contain only saturated fatty acid residues (as confirmed by GC). These SL can be considered as non-*trans* fats that provide a low-calorie alternative for several applications (baking, frying, and coating). The principal long-chain fatty acid component (stearic acid) is known to be less well absorbed in the human body. Moreover, the presence of medium-chain length fatty acid residues also confers to them a reduced caloric value relative to conventional fats and oils containing only long-chain fatty acids. The absence of unsaturated fatty acids residues implies that the products of the synthesis reaction have enhanced stability with regard to oxidation processes.

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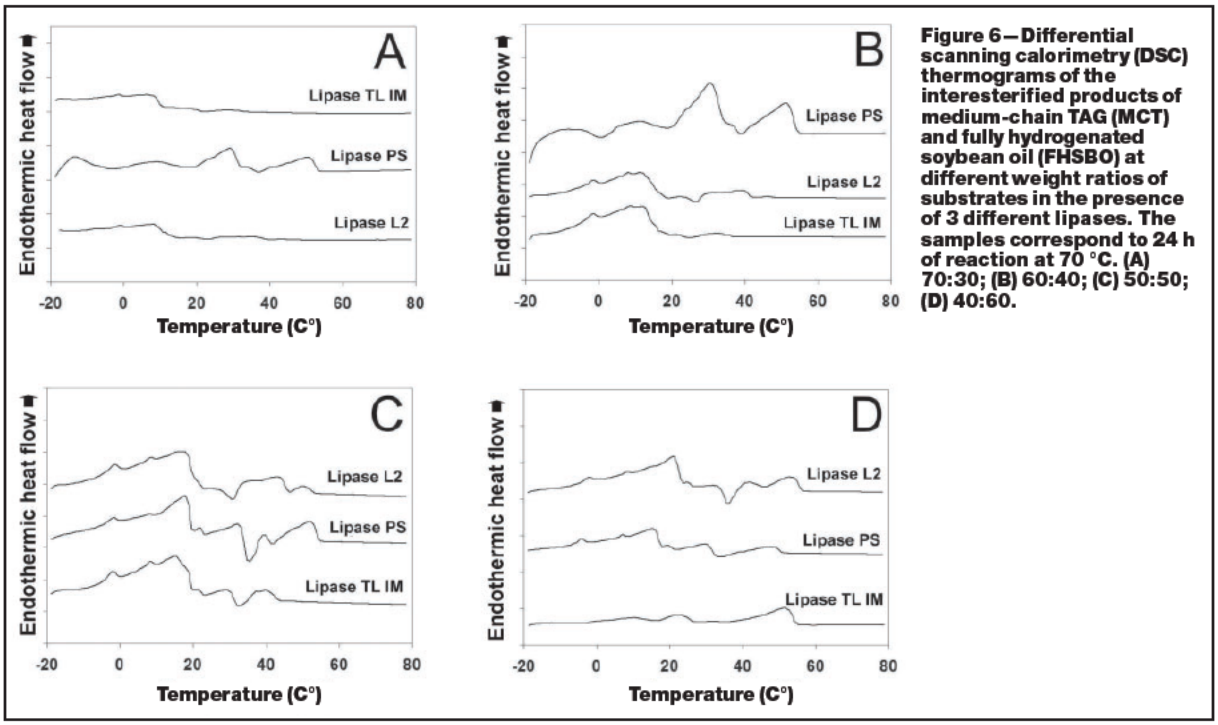

Figure 6—Differential scanning calorimetry (DSC) thermograms of the interesterified products of medium-chain TAG (MCT) and fully hydrogenated soybean oil (FHSBO) at different weight ratios of substrates in the presence of 3 different lipases. The samples correspond to 24 h of reaction at 70 °C. (A) 70:30; (B) 60:40; (C) 50:50; (D) 40:60.

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