Catalytic Transesterification of Corn Oil and Tristearin Using Immobilized Lipases from *Thermomyces lanuginosa*

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ABSTRACT: *Thermomyces lanuginosa* lipase was employed to catalyze the interesterification reaction between corn oil and tristearin at 45°C in a solvent-free system. HPLC and GC analyses were used to monitor both the distribution of TAG and the concentrations of MAG, DAG, and TAG as the reaction progressed. The positional distribution of the FA residues in the interesterified product was also determined for comparison to that of the original corn oil. Two different weight ratios of corn oil to tristearin were studied. Addition of molecular sieves to the reaction medium reduced the percentage of hydrolysis from 15 to 7. In order to improve the results obtained with Lipozyme TL IM, an immobilization of *T. lanuginosa* lipase was carried out. At a solids loading of 10% (w/w), the new immobilized lipase reduced the concentration of tristearin from 6 to 0.5% in only 30 min.

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KEY WORDS: Interesterification, lipase, lipozyme TL IM, margarine, structured lipids.

Interest in the development of zero-*trans* solid fats with properties suitable for use in dairy spreads (margarines) has increased in the past decade because of reports of undesirable effects of dietary *trans* FA (1). The adverse effects of *trans* FA are one factor responsible for the increased interest of the edible oils industry in producing structured lipids (2). For many years interesterification reactions have been employed by this industry to modify the spreadability and baking properties of several fats and oils (3). Lipase-catalyzed interesterification reactions offer possibilities for other transformations of fats and oils beyond those that can be achieved using chemical interesterification reactions (4).

In the present work, immobilized *Thermomyces lanuginosa* lipases were used to catalyze the interesterification reactions between corn oil and tristearin to produce modified lipids. The time dependence of the composition of the interesterification products was monitored by HPLC analyses to determine both the TAG profiles and the distribution of MAG, DAG, and TAG. The positions of FA residues in both the original corn oil and the interesterified products were also determined. The objective of the study was to characterize the products of the lipase-mediated reactions as a function of time for each enzyme of interest.

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MATERIALS AND METHODS

Materials. Corn oil was obtained from Archer Daniels Midland (Decatur, IL). The mole percentages of the various FA residues in the corn oil were: 14% palmitic (P), 2% stearic (S), 21% oleic (O), 58% linoleic (L), with the remaining 5% consisting of small amounts of various other FA. The lipases were from *T. lanuginosa*, expressed in *Aspergillus oryzae*. The commercial preparation was Lipozyme TL IM, kindly donated by Novo Nordisk (Franklinton, NC), whereas the soluble precursor of the enzyme immobilized in our laboratory was a crude extract (Lipopan) from the same microbial source donated by Novo Nordisk (Bagsvaerd, Denmark). Vendor specifications indicate that these lipases are 1,3-specific. Silica was a generous gift from PQ Corporation (Conshohocken, PA). Tristearin (90%) was from ICN Biomedicals (Aurora, OH) The tristearin employed in the present study contained *ca.* 10% (w/w) of distearoyl-palmitoyl-glycerol. Type II porcine pancreatic lipase (L-3126), *p*-nitrophenyl propionate (pNPP), molecular sieves (pore diameter 4 Å), and tributyrin were from Sigma Chemical (St. Louis, MO). All solvents used were HPLC grade from Fisher (Chicago, IL).

Methods. (i) Reaction protocol. The reaction mixture consisted of a 10-g sample of a corn oil–tristearin mixture together with 10% (w/w) immobilized lipase in a 50-mL sealed flask. The specific amounts of corn oil and tristearin employed in each experiment appear in the figure legends. Where indicated, molecular sieves (1 g) were added to sequester water present in the system. (Experience in our laboratory indicates that this loading of molecular sieves provides an appropriate amount of water, which is necessary to accomplish the first step in interesterification, namely, hydrolysis of the reacting TAG, while minimizing the amounts of lower acylglycerols present in the final product.) The flask was placed in an orbital shaker (300 rpm) maintained at 45°C in a water bath. The reaction mixture was held at these conditions for 48 h unless otherwise indicated.

(ii) HPLC. Analysis of reaction products. Samples (200 μL) of the reaction mixture were periodically withdrawn from

the flask during the course of the reaction. These samples were diluted with 1.8 mL of chloroform or 1.8 mL of 2:1 (vol/vol) chloroform/methanol. The first solvent was used for samples containing high concentrations of tristearin (samples drawn at short reaction times) in order to ensure complete dissolution of the tristearin. The enzyme was rapidly removed from the diluted sample using a 0.45 μm Whatman (Clifton, NJ) nylon syringe filter. Aliquots (500 and 50 μL) of the final transparent solution were analyzed by HPLC.

HPLC analysis of the reaction mixture was conducted using a Nova-Pak C_{18} column (150 × 3.9 mm) from Waters (Milford, MA). The instrument was equipped with a light-scattering detector (Alltech, Deerfield, IL). The compositions of the TAG were calculated using the method of Perona and Ruiz-Gutierrez (5) based on equivalent carbon numbers (ECN).

The protocol employed for the mobile phase involved linear elution gradients of 20% (vol/vol) acetone in acetonitrile increasing to 45% (vol/vol) acetone in 12 min, followed by an increase to 74% (vol/vol) acetone at 60 min. Next, the system was restored to its initial conditions by again passing the 20:80 (vol/vol) acetone/acetonitrile mixture through the column for 7 min. To conduct the analysis, 10 μL of sample [25 mg/mL in chloroform or 2:1 (vol/vol) chloroform/methanol] was injected. The flow rate was 1.0 mL/min.

A second HPLC analysis (for detection of MAG, DAG, and TAG) employed an Econosil-Silica 5U column (250 × 4.6 mm from Alltech). The mobile phases (A and B) were hexane/2-propanol/ethyl acetate/formic acid (80:10:10:0.1, by vol) and hexane/formic acid (100:0.02, vol/vol). An eluant flow rate of 2 mL/min was used, together with a sample concentration of 2.5 mg/mL in 2:1 (vol/vol) chloroform/methanol. The sample was first eluted for 6 min with a 15:85 (vol/vol) mixture of phase A and phase B and then with a 98:2 mixture for an additional 7 min. Next, the column was restored to its initial conditions by passing a 15:85 mixture of phases A and B through the column for 6 min. The retention times for the TAG, FA, 1,3-DAG, 1,2-DAG, 1(3)- and 2-MAG were 1.7, 3, 4.7, 7.9, 13, and 14 min, respectively. No significant amounts of MAG were detected in any experiment.

(iii) GC analysis of reaction products. The following analytical procedure was based on that of Williams *et al.* (6) to effect methylation of the esterified FA residues present in the samples. The samples (200 μL) were mixed with 1800 μL of a mixture of chloroform and methanol (volume ratio = 2:1) and immediately filtered with a 0.45 μm nylon syringe filter. Aliquots of the final transparent solution (400 μL) were methylated by addition of 1 mL of 0.1 M methanolic NaOH. This mixture was allowed to stand for 30 min at 60°C. Then, 200 μL water was added. The resulting mixture was extracted with two 1-mL portions of *n*-hexane. Samples were dried with sodium sulfate and centrifuged for 2 min at 5035 × *g*.

A 1-μL sample was injected into a gas chromatograph fitted with a 60 m HP Supelcowax 10 column (Bellefonte, PA) (0.32 mm i.d.). Injector and detector temperatures were set at 220 and 230°C, respectively. The temperature program was as follows: starting at 100°C and then heating to 180°C at 20°C/min; followed by heating from 180 to 220°C at 15°C/min. The final temperature (220°C) was held for 30 min. Identification of the various FFA was based on a menhaden oil standard (#4-7085) obtained from Supelco (Bellefonte, PA).

(iv) Purification of the TAG in the reaction mixture. Separation of the TAG was performed by solid-phase extraction on Accu-Bond (1000 mg) prepacked columns (J&W Scientific, Folsom, CA). Silica columns were conditioned before use by washing with 4 mL hexane. Care was taken to prevent the columns from becoming completely dry. The sample (10 mL containing a nominal concentration of 10 mg/mL) was applied to the column and was eluted under vacuum (5 mm Hg) using a mixture of solvents of increasing polarity. The mobile phases used for the TAG separation were 8 mL hexane/diethyl ether (200:3, vol/vol) and 40 mL hexane/diethyl ether (96:4, vol/vol). The eluate from the second elution (containing the TAG) was collected and evaporated for subsequent analysis of the distribution of FA residues along the glycerol backbone.

(v) Positional distributions of FA in TAG. A modified version of the methods of Williams *et al.* (6) and Luddy *et al*. (7) was employed to release FA from the *sn*-1,3 positions of acylglycerols. This modification involves the use of borax to minimize the possibility of migration of residues from position 2 to positions 1 and 3 of the glycerol backbone. A known weight of TAG and an appropriate (*ca.* 20–50 mg) weight of porcine pancreatic lipase were added to a 60-mL stoppered flask. Next, 0.65 mL Tris-HCl buffer (1 M, sodium salt, pH 8.0), 0.35 mL sodium borate (0.19 M), 0.1 mL $CaCl_2$ (22%, w/w), and 0.25 mL bile salts (0.1%, w/w) were added. The resulting mixture (pH = 7.91) was maintained at 40°C for 1 min without shaking, then shaken at 300 rpm at 40°C for 7 min. The reaction was stopped by addition of 1 mL acetic acid (0.1 M). The mixture was extracted three times with 1 mL of chloroform. The pooled organic phases were passed through a 0.45-μm syringe filter and then methylated with 0.1 M methanolic NaOH as described above in the section on GC analysis. This protocol provides information concerning the distribution of FA residues at the *sn-2* position. The distribution of FA residues at the *sn*-1,3 positions was then calculated by subtracting the amount of a FA residue at the *sn*-2 position from the total quantity of this FA present in the corresponding unhydrolyzed TAG as determined by GC.

(vi) Lipase immobilization: Lipase (20 g) from *T. lanuginosa* solid extract (Lipopan) were added to 100 mL of 25 mM sodium phosphate (pH 7.0) and stirred vigorously for 1 h. Then the suspension was centrifuged at 13,173 × *g* for 30 min, and the supernatant decanted. Immobilization was performed by adding 40 mL of this enzyme solution to 1 g of octyl-silica. The suspension was maintained under mild stirring at room temperature during 75 min. Immobilization yields were determined by assaying for the esterase activities of the suspension and supernatant. When the activity of the supernatant was negligible, the suspension was filtered under vacuum with a sintered glass funnel. The volume of supernatant

was measured and an equivalent amount of phosphate buffer (25 mM, pH 7.0) was added to the solid catalyst. This new suspension was gently stirred for 15 min, and the activities of suspension and supernatant were again measured in order to check that no desorption had occurred. Then, the suspension was washed five times with phosphate buffer (50 mM, pH 7.0), and vacuum filtered. Analyses indicated that the activity of the suspension then remained constant, and the activity of supernatant was negligibly small. Finally, the catalyst was suspended in dry acetone (approximately the same volumes of solid catalyst and acetone were employed) and then vacuum filtered for 30 min.

(vii) Enzymatic assays. Dry catalyst (5 mg) was suspended in 1 mL phosphate buffer (50 mM, pH 7.0). The suspension was cooled in an ice bath, and the silica beads were then broken up using vigorous magnetic stirring (in order to minimize potential intraparticle diffusional limitations on reaction rates). Aliquots (50 µL) of this suspension were withdrawn at different times and assayed for esterase activity as described below until the activity reached an asymptotic limit. In a separate assay, 5 mg of a commercial preparation (Lipozyme from Novo Nordisk) was suspended in 1 mL phosphate buffer (50 mM) and subjected to the same assay procedure.

(viii) Esterase activity. Although an assay for ester hydrolysis activity does not provide a specific test for lipase activity, this assay was selected for use as a routine assay because it is easy to conduct *via* spectrophotometric measurements and it provides a rapid assessment of relevant enzymatic activity. Hydrolysis of pNPP was followed at 348 nm in a spectrophotometer equipped with stirring devices and constant temperature capability. The cuvette contained 1.9 mL of substrate solution at 25°C (0.4 mM pNPP in 50 mM sodium phosphate buffer, pH 7.0). Aliquots (50 µL) of the suspension were diluted with 0.95 mL 50 mM phosphate buffer (pH 7.0) prior to being added to the cuvette to facilitate the analysis. Aliquots from the supernatant were not diluted: 50 µL were added directly to 1.9 mL substrate solution in the cuvette. One unit of esterase activity corresponds to consumption of 1 micromole of pNPP per minute ($\varepsilon_{\text{pNPP pH 7.0, 25°C}} = 5150\ M^{-1}\ cm^{-1}$).

(ix) Tributyrin activity. Hydrolysis of tributyrin was monitored titrimetically using 100 mM sodium hydroxide. One unit of lipase activity corresponds to consumption of 1 micromole of NaOH per minute. Potassium phosphate buffer (48.5 mL 10 mM, pH 7.0) was incubated in a thermostatted vessel at 25°C. Then 1.47 mL tributyrin was added and the pH-stat was started to keep the pH at 7.0. When the pH stabilized, 5 mg catalyst was added and the consumption of NaOH was determined.

RESULTS AND DISCUSSION

Inspection of Figure 1 indicates that for a weight ratio of corn oil to tristearin of 90:10, the course of the reaction in the absence of molecular sieves is characterized by very significant decreases in the concentrations of some TAG species while increases are observed in the concentrations of other TAG. In terms of ECN, species with ECN values of 42 (LLL), 44.5 (PLL), and 54 (SSS) decreased from 23 to 14, from 14 to 9, and from 6 to 0.5% (w/w), respectively. By contrast, species with ECN values of 46 (LLS), 47(LOS), and 48 (LPS) increased from 11 to 16, from 3 to 8, and from 1 to 3% (w/w).

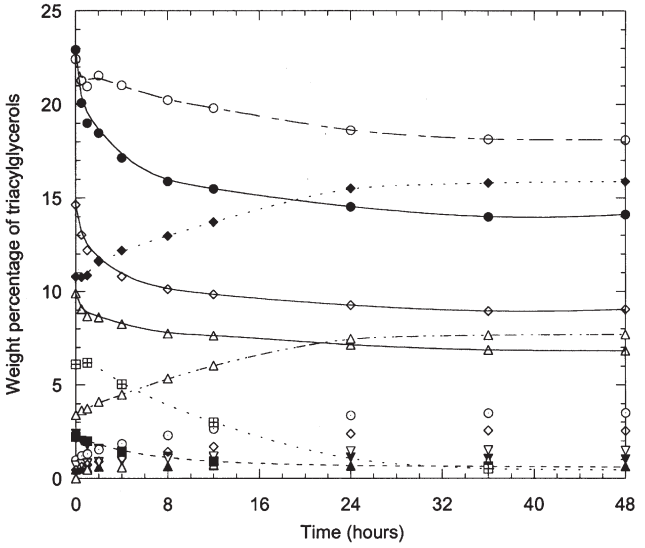

FIG. 1. Time course of the interesterification reaction between corn oil and tristearin. Conditions: 9 g corn oil, 1 g tristearin, 1 g Lipozyme TL IM, 45°C, 300 rpm. TAG residues: –●– LLL, –◇– LLP, –△– LOO, --◆-- LLS, --△-- LOS, --□-- SSS, –-○–- LLO, ⊙ LPS, ▽ OOS/OPS, △ OSS, --■-- PSS, ▼ OPP, ▲ OOP, ◇ PPS/SSL, where L represents linoleic, O is oleic, P is palmitic, and S is stearic acid.

In the presence of the desiccant (molecular sieves), similar profiles of these TAG are observed (*cf.* Figs. 1 and 2). However, there are differences in the extents to which the speci-

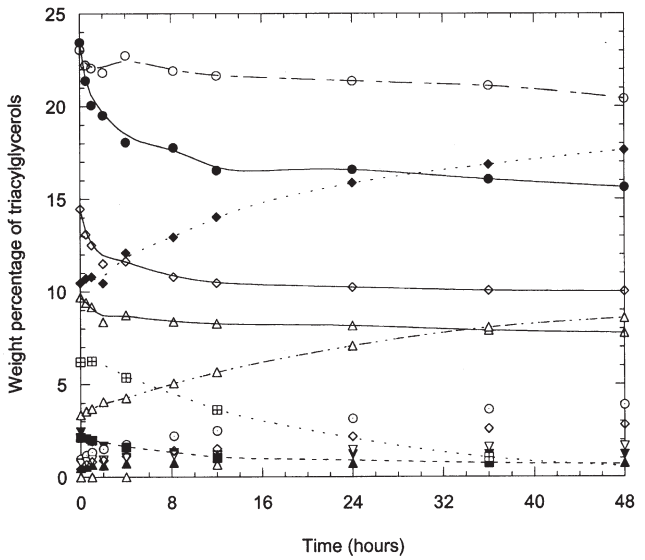

FIG. 2. Time course of the interesterification reaction between corn oil and tristearin. Conditions: 9 g corn oil, 1 g tristearin, 1 g Lipozyme TL IM, 1 g molecular sieves, 45°C, 300 rpm. The symbols and residues are the same as for Figure 1.

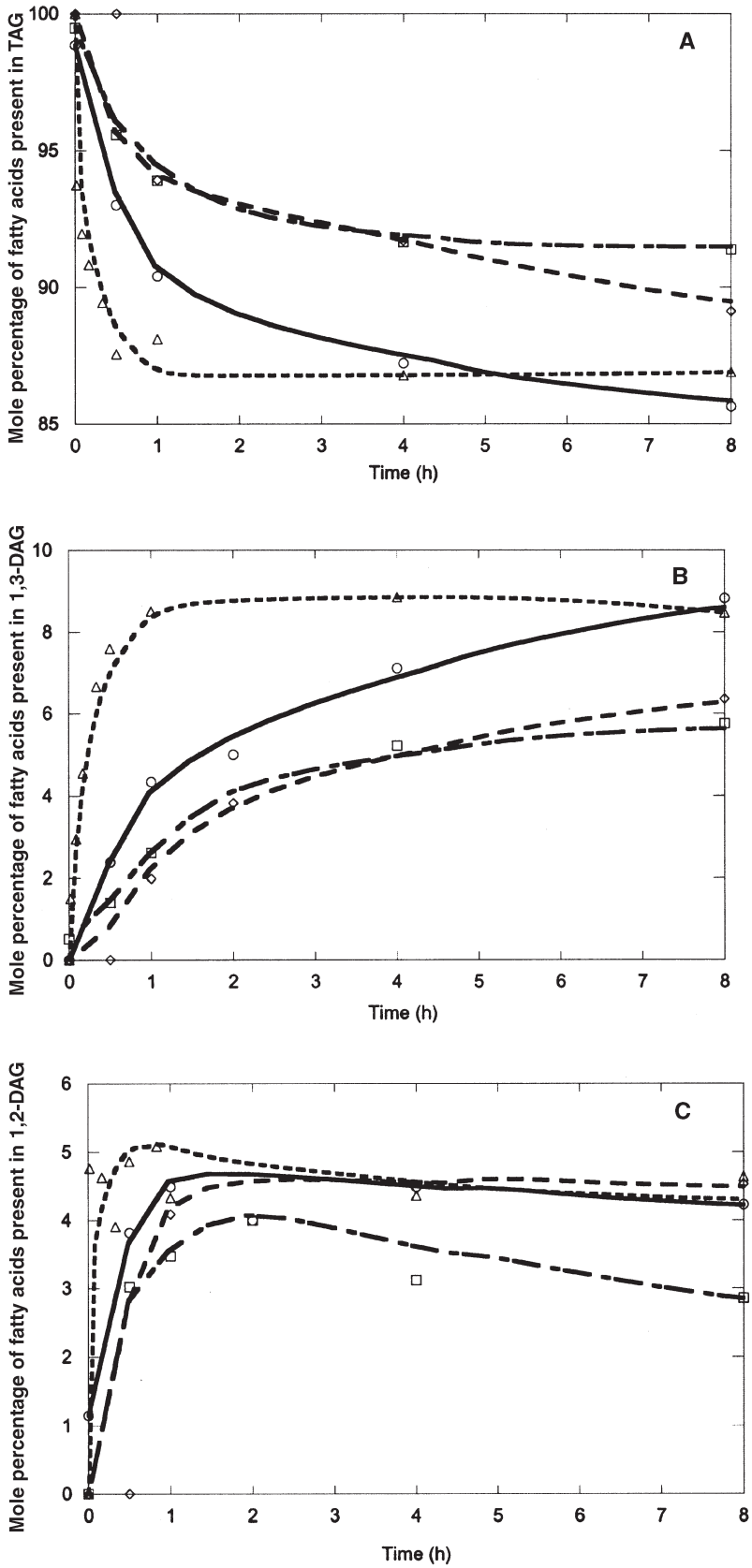

FIG. 3. Distribution of acylglycerols in the interesterification reaction between corn oil and tristearin (45°C, 300 rpm). Conditions: (–○–) 9 g corn oil, 1 g tristearin, 1 g Lipozyme TL IM; (–□–) 9 g corn oil, 1 g tristearin, 1 g Lipozyme TL IM, 1 g molecular sieves; (–◇–) 8 g corn oil, 2 g tristearin, 1 g Lipozyme TL IM, 1 g molecular sieves; (--△--) 9 g corn oil, 1 g tristearin, 1 g immobilized *Thermomyces lanuginosa* lipase.

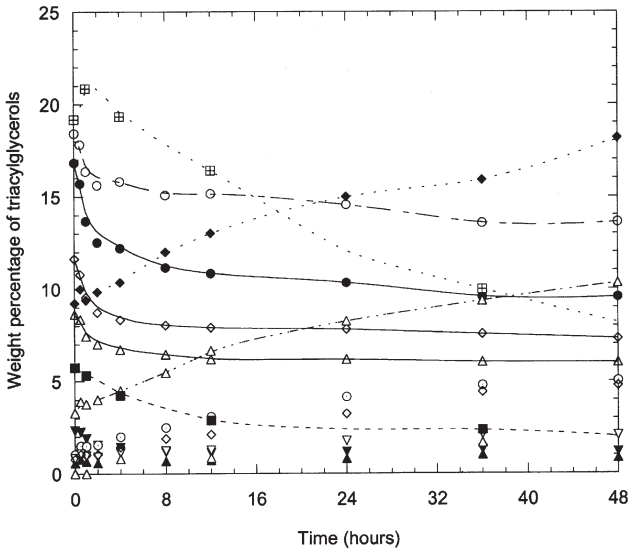

FIG. 4. Time course of the interesterification reaction between corn oil and tristearin. Conditions: 8 g corn oil, 2 g tristearin, 1 g Lipozyme TL IM, 1 g molecular sieves, 45°C, 300 rpm. The symbols and residues are the same as for Figure 1.

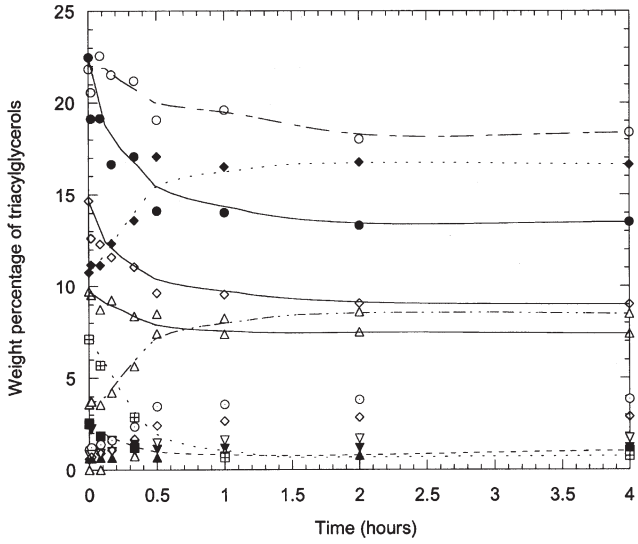

FIG. 5. Time course of the interesterification reaction between corn oil and tristearin. Conditions: 9 g corn oil, 1 g tristearin, 1 g immobilized *Thermomyces lanuginosa* lipase, 45°C, 300 rpm. The symbols and residues are the same as for Figure 1.

fied TAG decrease or increase. In the presence of molecular sieves, LLL and LLO underwent decreases that were smaller than those obtained in the absence of molecular sieves. Other TAG were formed in marginally higher concentrations when molecular sieves were utilized. Hence after 48 h, LLS increased from 16% in the absence of molecular sieves to 18% in the presence of this desiccant, whereas the corresponding increase for LPS was from 3.5 to 4.0%. However, the primary advantage of adding the molecular sieves is not to produce small changes in the relative proportions of the various TAG, but rather to markedly reduce the extent of hydrolysis reactions relative to transesterification reactions (*cf.* Fig. 3), thereby giving a higher overall yield of TAG.

An experiment involving a weight ratio of corn oil to tristearin of 80 to 20 was also carried out in the presence of molecular sieves (Fig. 4). Under these conditions, the reaction does not appear to reach equilibrium within 48 h. At 45°C, the presence of 20% by weight of tristearin produces a much more viscous reaction medium, which may in part be responsible for the longer reaction times required to approach equilibrium.

In an effort to improve the results obtained with Lipozyme TL IM, a soluble extract from *T. lanuginosa* was immobilized on silica. The objective of the immobilization experiment was to obtain an immobilized lipase with a higher specific activity that could be employed to increase the rate of the transesterification reaction, thereby minimizing the detrimental effect of acyl migration. Tributyrin and esterase activities of the immobilized derivative, as well as for Lipozyme TL IM, were determined (Table 1). Inspection of Figure 5 indicates that for this preparation the final TAG composition of the product mixture was similar to that obtained with lipozyme TL IM. However, comparison of the plots in Figures 1 and 5 indicates that at the same loading of solid catalyst, the reaction rate is much faster for our preparation. After 30 min of reaction, consumption of the original tristearin was *ca.* 93% in the presence of our immobilized *T. lanuginosa* lipase whereas for the Lipozyme TL IM the corresponding value is less than 5%.

For the same conditions as employed in Figure 1, the distributions of the FA residues among the different acylglycerol species (tri-, di-, and mono-) are presented in Figure 3. For times between 8 and 48 h, no major changes in the concentrations of the various acylglycerols were observed. In no case were MAG detected in the reaction products. However, for the weight ratio of corn oil to tristearin of 90:10 in the absence of molecular sieves, the sum of the mole percentages of the 1,2- and 1,3-DAG was *ca.* 15%. A much lower hydrolysis level was observed for this weight ratio when molecular sieves were present. In the latter case, the sum of 1,2- and 1,3-DAG never exceeded 7 mol%. For the experiment involving a weight ratio of corn oil to tristearin of 80:20, the level of hydrolysis obtained in 48 h was *ca.* 9%. This value is slightly greater than that obtained in the corresponding experiment for the weight ratio of corn oil to tristearin of 90:10. It is widely recognized that interesterification reactions occur in two consecutive steps (partial hydrolysis of the precursor TAG and subsequent addition of an acyl group to the intermediate

TABLE 1
Esterase and Tributyrin Activities of Lipozyme TL IM and the Immobilized Derivative of the Lipase of *Thermomyces lanuginosa*

	pNPP hydrolysis[a] (units/g)	Tributyrin hydrolysis (units/g)
Immobilized *T. lanuginosa*	1,000	16,000
Lipozyme TL IM	100	10,000

[a]pNPP, *p*-nitrophenyl propionate.

TABLE 2
Positional Distribution of FA Residues in Native Corn Oil and the Initial Corn Oil/Tristearin Mixture [90:10 (w/w)]

	Original corn oil			Corn oil/tristearin mixture [90:10 (2/2)]		
FA	% of total	% of *sn*-1,3	% of *sn*-2	% of total	% of *sn*-1,3	% of *sn*-2
Linoleic	56.6	51.3	67.3	51.0	46.2	60.6
Oleic	25.2	25.6	24.6	22.7	23.0	22.1
Stearic	2.0	2.5	1.1	10.8	11.3	10.0
Palmitic	11.0	15.6	1.9	9.9	14.0	1.7

DAG). The medium containing 20% (w/w) tristearin is more viscous, and the higher viscosity may impose mass transfer/diffusional limitations on the esterification step that follows the hydrolysis reaction. With respect to the reaction mediated by our immobilized *T. lanuginosa* lipase preparation, the final level of hydrolysis obtained was *ca.* 13%, a value similar to that obtained with Lipozyme TL IM in the absence of molecular sieves.

The positional distributions of the various FA in the original corn oil, the initial mixture of corn oil and tristearin, and the interesterified products are presented in Tables 2 and 3. The entries labeled "% of total" in columns labeled 1 to 4 in Table 3 correspond to the compositions of the purified TAG produced by the reaction, i.e., what is left after the DAG, FFA, and the like that are produced by concurrent hydrolysis reactions are removed. If one neglects the residues present in the lower acylglycerols, stoichiometric considerations require that the total column in the broader column labeled 4 in Table 3 be generally consistent with the total column for the corn-oil/tristearin mixture in Table 2. Comparison of these entries indicates that this is indeed the case. Now, if the *T. lanuginosa* lipase is completely *sn*-1,3 specific, the entries in the *sn*-2 column for the unreacted corn oil/tristearin mixture should be generally consistent with those for the interesterified products of the reaction mediated by the *T. lanuginosa* enzyme. The entries in the *sn*-2 column within the broader column 4 of Table 3 are slightly different from those in the right-most column in Table 2 (57.2 vs. 60.6, 22.7 vs. 22.1, 10.9 vs. 10.0, and 4.5 vs. 1.7). These differences can be attributed to either imperfect specificity or to acyl migration, but are generally consistent with *sn*-1,3 selectivity for the *T. lanuginosa* lipase. If the indicated selectivity is characteristic of this enzyme, one would also expect the distribution of residues at the *sn*-1,3 sites to be comparable in the products and starting materials. This is indeed the case if one compares the entries in the *sn*-1,3 column of the broader column in Table 3 labeled 4 with those of the starting materials shown in Table 2 (47.4 vs. 46.2, 23.2 vs. 23.0, 11.0 vs. 11.3, and 13.4 vs. 14.0 for L, O, S, and P, respectively). Discrepancies can be attributed to the removal of lower acylglycerols in the purification process. Although we do not regard this analysis as definitive, it is consistent and supportive of the working hypothesis that the *T. lanuginosa* enzyme exhibits *sn*-1,3 selectivity. The fact that there is a significant difference in the percentages of the L residues at the different sites does not support the hypothesis that this lipase is not selective.

The differences in the percentages of P and L residues located at the *sn*-1,3 positions of the glycerol backbone, relative to the percentages at the *sn*-2 position in the native and the interesterified forms of the corn oil product, can be used as indicators of ester exchange *via* acyl migration. Because the level of DAG is appreciable (Fig. 3), migration of acyl groups may occur. The extent of acyl migration has previously been correlated with the amount of DAG present (8). Inspection of the entries in Table 3 indicates that the percentages of P at the *sn*-2 position and L at the *sn*-1,3 positions have increased in three of the four interesterified products studied. However, in the case of the new immobilized *T. lanuginosa*, the extent of migration of P from the *sn*-1,3 positions to the *sn*-2 position was relatively small. Because the level of DAG resulting from these reaction conditions was similar to that obtained in the presence of Lipozyme TL IM,

TABLE 3
Positional Distribution of FA Residues in the Interesterified Products of the Reaction Between Corn Oil and Tristearin[a]

	1			2			3			4		
FA	% of total	% of *sn*-1,3	% of *sn*-2	% of total	% of *sn*-1,3	% of *sn*-2	% of total	% of *sn*-1,3	% of *sn*-2	% of total	% of *sn*-1,3	% of *sn*-2
Linoleic	49.6	49.1	50.6	47.8	47.7	47.9	43	45.4	38.1	50.1	47.4	57.2
Oleic	23	23.8	21.4	24	24.8	22.9	19.9	21.5	16.7	23.1	23.2	22.7
Stearic	11.5	10.3	13.9	11.7	10.4	13.6	21.2	16.4	31.4	10.9	11.0	10.9
Palmitic	10.9	11.8	9.1	11.5	12.1	10.6	10.8	11.8	8.8	10.9	13.4	4.5

[a]Products were obtained as follows: (1) Corn oil/tristearin mixture [90:10 (w/w)] interesterified in the absence of molecular sieves. This interesterified corn oil/tristearin mixture was obtained with Lipozyme TL IM. The positional distribution corresponds to the purified TAG obtained after 24 h of reaction. (2) Corn oil/tristearin mixture [90:10 (w/w)] interesterified in the presence of molecular sieves. This interesterified corn oil/tristearin mixture was obtained with Lipozyme TL IM. The positional distribution corresponds to the purified TAG obtained after 24 h of reaction. (3) Corn oil/tristearin mixture [80:20 (w/w)] interesterified in the presence of molecular sieves. This interesterified corn oil/tristearin mixture was obtained with Lipozyme TL IM. The positional distribution corresponds to the purified TAG obtained after 24 h of reaction. (4) Corn oil/tristearin mixture [90:10 (w/w)] interesterified in the absence of molecular sieves. This interesterified corn oil/tristearin mixture was obtained with the immobilized *Thermomyces lanuginosa* lipase. The positional distribution corresponds to the purified TAG obtained after 0.5 h of reaction.

one can conclude that the extent of acyl migration depends not only on the presence of DAG but also on the time available for reaction. This result is in agreement with that of Xu (8), who reported that acyl migration can be attributed primarily to the longer residence times characteristic of stirred tank reactors relative to those in a tubular reactor. The shorter reaction time in the presence of the new immobilized lipase allows one to discriminate between the effect of acyl migration and the positional selectivity of this lipase. The results presented in Table 3 show that in the absence of acyl migration (case 4) the lipase preferentially interesterifies those residues located at *sn*-1,3 positions. For the interesterified corn oil/tri-stearin mixture [80:20 (w/w)], the reaction had not reached equilibrium in 24 h. The large amount of S residues located at the *sn*-2 position is attributed to the presence of unreacted tristearin in the purified TAG (more than 12% by weight). Both the melting point of tristearin (74°C) and the results obtained with the corn oil/tristearin weight ratio of 80:20 indicate that at higher weight ratios of tristearin to corn oil it would be desirable to increase the rate of the interesterification reaction, perhaps by increasing the temperature, by using a higher loading of catalyst, or by using a more active immobilized lipase. Increasing the temperature may be problematic because of the potential for deactivation of the lipase and because the activation energies for acyl migration and the enzyme-mediated reaction are not known. Nonetheless, the goal is to reduce the requisite reaction time to produce a concomitant decrease in the extent of migration of acyl groups during the course of the reaction.

The present study illustrates the technical feasibility of using Lipozyme TL for interesterification of corn oil with tristearin. The presence of a desiccant (molecular sieves) in the reaction mixture markedly reduces the hydrolysis level, although in the presence of the desiccant the extent of acyl migration to the *sn*-2 position was comparable to that observed in the absence thereof. The results of the present study also indicate that ratios of corn oil to tristearin of 80:20 (w/w) or below produce more viscous media in which a temperature of 45°C is not sufficient to eliminate mass transfer/diffusional limitations on the reaction kinetics.

It is well known that the positions of specific FA residues in the TAG constitute an important factor governing the atherogenic character (9), physical properties (10,11), and oxidative stability (12) of a particular fat or oil. Consequently, one should try to avoid a random distribution of FA residues in the glycerol backbone in order to improve the nutritional and therapeutic properties of these fats and oils. Our results indicate that, to reduce randomization of the FA residues in transesterified acylglycerols, one should employ short reaction times. This process variable is more important than the level of DAG in the final product in determining the extent of acyl migration that occurs.

This work has involved development of analytical methodologies for monitoring the kinetics of the enzymatic synthesis of tailored fats and oils. These methodologies permit one to follow changes in the relative amounts of the individual TAG species present in the mixture as time evolves. The technology described above permits one to circumvent some of the disadvantages associated with partial hydrogenation of vegetable oils (production of *trans* FA) and also avoids the necessity for using a strong base to catalyze interesterification *via* a purely chemical route.

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REFERENCES

1 Mensink, R.P., and M.B. Katan, Dietary Saturated Fatty Acids and Low-Density or High-Density Lipoprotein Cholesterol—Reply, *N. Engl. J. Med. 322*:404 (1990).
2. Gunstone F.D., Movements Towards Tailor-Made Fats, *Prog. Lipid Res. 37*:277–305 (1998).
3. Zeltoun, M.A.M., W.E. Neff, G.R. List, and T.L. Mounts, Physical Properties of Interesterified Fat Blends, *J. Am. Oil Chem. Soc. 70*:467–471 (1993).
4. Macrae, A.R., Tailored Triacylglycerols and Esters, *Biochem. Soc. Trans. 17*:1146–1148 (1989).
5. Perona, J.S., and V. Ruiz-Gutierrez, Characterization of the Triacylglycerol Molecular Species of Fish Oil by Reversed-Phase High Performance Liquid Chromatography, *J. Liq. Chromatogr. Rel. Technol. 22*:1699–1714 (1999).
6. Williams, J.P., M.U. Khan, and D. Wong, A Simple Technique for the Analysis of Positional Distribution of Fatty Acids on Di- and Triacylglycerols Using Lipase and Phospholipase A_2, *J. Lipid Res. 36*:1407–1412 (1995).
7. Luddy, F.E., R.A. Barford, S.F. Herb, P. Magidman, and R.W. Riemenschneider, Pancreatic Lipase Hydrolysis of Triglycerides by a Semimicro Technique, *J. Am. Oil Chem. Soc. 41*:693–696 (1964).
8. Xu, X., Enzymatic Production of Structured Lipids: Process Reactions and Acyl Migration, *inform 11*:1121–1131 (2000).
9. Kritchevsky, D., Effect of Triglyceride Structure on Lipid Metabolism, *Nutr. Rev. 46*:177–181 (1988).
10. Formo, M.W., E. Jungermaann, F.A. Norris, and N.O. Sonntag, *Bailey's Industrial Oil and Fat Products*, Wiley-Interscience Publications, New York, 1979, Vol. 1, pp. 177–232.
11. 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).
12. Wada, S., and C. Koizumi, Influence of the Position of Unsaturated Fatty Acid Esterified Glycerol on the Oxidation Rate of Triglyceride, *Ibid. 60*:1105–1109 (1983).

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