Assessment of Lipase- and Chemically Catalyzed Lipid Modification Strategies for the Production of Structured Lipids

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ABSTRACT: The purpose of the present study was to devise a two-step reaction to produce partial glycerides, which would subsequently be used as substrates in both lipase-catalyzed and chemically catalyzed esterification reactions with caprylic acid. The yields and kinetics of these two-step reactions were compared to established lipase-catalyzed acidolysis and transesterification as well as to chemical transesterification reactions. Acyl migration did not occur during the hydrolysis or short-path distillation steps in the preparation of free fatty acid–free partial glycerides for esterification reactions. No significant differences in final yields (59.9% to 82.8% w/w of total triacylglycerols) of new structured lipids were detected among lipase-catalyzed (24 h) and chemically catalyzed (5 h) reactions; however, the yield of new structured triacylglycerols (TAG) after 2 h was lower for acidolysis than for the other lipase-catalyzed reactions ($P \leq 0.05$). Since no differences in final yields were detected among the reactions, chemical esterification using hydrolyzed oil could represent the best synthetic option, since it offers the advantage of positional distribution control associated with lipase-catalyzed reactions as well as rapid reaction times associated with chemically catalyzed reactions. Attempts to evaluate the positional distribution of caprylic acid using allyl magnesium bromide (Grignard reagent) were hampered by production of unknown species, which prevented accurate determination of the concentration of some key fatty acids.

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KEY WORDS: Acidolysis, canola oil, caprylic acid, esterification, lipase, structured lipids, transesterification.

An increased understanding of the nutritional properties of lipids, in particular the metabolic effects associated with consumption of triglycerides with specific fatty acid compositions and positional distributions, has led to the development of novel lipid modification technologies for the improvement of the nutritional properties of fats and oils (1,2). During the past two decades, much attention has been focused on the negative health effects associated with excessive intakes of certain fats and oils, particularly in adults. Recently, however, we have begun to realize that consumption of certain fats and oils has positive health effects, because they contain compounds that are essential for growth, health maintenance, and disease prevention in infants and adults (1,2).

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Lipid modification strategies for the production of physiologically functional fats and oils include genetic engineering of oilseed crops, production of high-polyunsaturated fatty acid-(PUFA) oil concentrates using fungi and algae, and lipase-catalyzed or chemically catalyzed interesterification reactions (1,2). In terms of postproduction modification of vegetable oils, lipase-catalyzed and chemically catalyzed interesterification reactions are the methods of choice. Chemical interesterification is inexpensive and easy to scale up; however, the reaction lacks specificity and offers little or no control over the positional distribution of fatty acids in the final product (3,4). On the other hand, lipase-catalyzed interesterification reactions, including transesterification and acidolysis, offer the advantage of greater control over the positional distribution of fatty acids in the final product, due to lipases' fatty acid selectivity and regiospecificity (3,5). However, difficulties associated with process scale-up and control, as well as the high cost of lipases, have prevented their widespread industrial use as catalysts for the modification of food lipids. Lipase-catalyzed transesterification and acidolysis reactions are a combination of hydrolysis and esterification reactions. The degree to which hydrolysis occurs relative to esterification is governed by the concentration of water present. In the presence of excess water, hydrolysis predominates, resulting in the accumulation of free fatty acids (FFA), monoglycerides (monoacylglycerols, MAG), and diglycerides (diacylglycerols, DAG), whereas under water-limiting conditions esterification predominates (3,5). To "optimize" one-step lipase-catalyzed transesterification and acidolysis reactions, it is necessary to strike a balance between hydrolysis and esterification. This is not always easy, because excess water favors hydrolysis, whereas water-limiting conditions favor esterification. By splitting lipase-catalyzed transesterification and acidolysis reactions into their component hydrolytic and esterification phases, it is possible to optimize reaction conditions for each individual phase of the reaction, possibly resulting in higher yields of modified TAG. Such enhanced control would improve productivity and probably make lipase-catalyzed reactions economically viable. In the present work, the performance of two-step hydrolysis and esterification reactions was compared to standard one-step lipase-catalyzed and chemically catalyzed reactions, using canola oil and caprylic acid as a model system (Table 1). The yields of

TABLE 1
Lipase-Catalyzed and Chemically Catalyzed Reactions Assessed in This Study

Reaction	Substrates
Lipase-catalyzed	
Acidolysis	Free fatty acids + triacylglycerols
Transesterification	Triacylglycerols + triacylglycerols
Fatty acid esterification	Free fatty acids + mono- and diacylglycerols
Triacylglycerol esterification	Triacylglycerols + mono- and diacylglycerols
Chemically catalyzed	
Transesterification	Triacylglycerols + triacylglycerols
Fatty acid esterification	Free fatty acids + mono- and diacylglycerols

TABLE 2
Potential New Structured Triacylglycerol Species Produced from Both Chemically Catalyzed and Lipase-Catalyzed Reactions

Carbon number[a]	Fatty acids present
32	16:0/16:1, 8:0, 8:0
34	18:0/18:1/18:2, 8:0, 8:0
42	16:0/16:1, 18:0/18:1/18:2, 8:0
44	18:0/18:1/18:2, 18:0/18:1/18:2, 8:0
46	20:1, 18:0/18:1/18:2, 8:0

[a]Carbon number does not include glycerol.

new modified TAG (Table 2), degree of hydrolysis, and fatty acid positional distribution in the final product were determined for the different reactions.

EXPERIMENTAL PROCEDURES

Materials. Food grade canola oil was obtained from Daminco Inc. (West Seneca, NY). Caprylic acid was purchased from ICN Biomedical Inc. (Aurora, Ohio), and tricaprylin was obtained from Sigma Chemical Co. (Toronto, Ontario, Canada). Lipozyme IM (from *Mucor miehei*), containing 9.9% (w/w) water, was a generous gift from Novo Nordisk Inc. (Franklinton, NC). Unless otherwise stated, all chemicals, standards, and reagents were obtained from Sigma Chemical Company (Toronto, Ontario, Canada), while all solvents were obtained from Fisher Scientific (Unionville, Ontario, Canada).

Degree of hydrolysis and TAG composition. Lipid samples were analyzed according to the method of Goh and Timms (6) to assess the degree of hydrolysis through the production of FFA, MAG, and DAG. A sample weighing between 10 and 30 mg was placed in a 2-mL screw-cap vial with a Teflon/silicone septum. A volume of 400 μL of pyridine was added and mixed until the sample was dissolved. The sample was derivatized using 100 μL of trimethylsilylimidazole and mixed for 30 s. Derivatized samples were used within 4 h of preparation.

The derivatized samples were analyzed using a Shimadzu 8A (Kyoto, Japan) gas–liquid chromatograph (GLC) and a Shimadzu C-R3A Chromatopac integrator. A 0.5-m × 0.3-mm glass column packed with 3% OV-1 on 80/100 mesh Supelcoport (Supelco Chromatography Products, Mississauga, Ontario, Canada) was used with a temperature gradient of 155 to 360°C at 8°C/min. The flow rate of the carrier gas, nitrogen, was 75 mL/min, while hydrogen and compressed air for the detector were set at 50 and 500 mL/min, respectively. Since the composition of the samples was such that the retention times of the FFA, MAG, DAG, and structured TAG peaks did not overlap, all could be analyzed simultaneously. The concentration of caprylic acid was determined by difference, since it eluted in the void volume.

Standards of oleic acid, monoolein, and diolein were run to identify the FFA and partial acylglycerols, and theoretical response factors for the individual components were used after the accuracy of the experimental response factors had been verified.

Fatty acid composition. The fatty acid composition of the individual partial acylglycerols extracted from the silica from the thin-layer chromatography (TLC) plates was determined using GLC according to the method of Bannon and coworkers (7). The lipid sample was dissolved in 2 mL of isooctane and converted to fatty acid methyl esters using 2 N potassium hydroxide in methanol. This solution was vortexed for 1 min and held at room temperature for 5 min, after which 2 drops of methyl orange were added and the sample was neutralized with 2 N HCl. A volume of 1–3 μL of the organic layer was injected into the gas chromatograph. The column used was packed with 10% DEGS-PS on 80/100 mesh Supelcoport Silar 9CP (Chromatographic Specialties, Brockville, Ontario, Canada). The temperature gradient was 60 to 220°C at 8°C/min. The accuracy of the results was verified using a fatty acid methyl ester standard containing a complete range of fatty acids, from butyric to linolenic acid (Nu-Chek-Prep. Inc., Elysian, MN).

Chemical esterification. Chemical esterification between the hydrolyzed, distilled oil and caprylic acid was performed according to a standard organic chemistry protocol (8). The substrates were dried using molecular sieves, and 2.6 g of caprylic acid and 17.4 g of hydrolyzed canola oil were weighed into a 100-mL round-bottom flask. A volume of 167 μL of sulfuric acid was added, and the mixture was heated under reflux in an oil bath at 150°C for 5 h. After completion of the reaction, the sample was cooled and transferred to a separatory funnel using 10 mL of chloroform, then washed with 2 vol of 25 mL of water and 3 vol of 25 mL of 5% (w/w) sodium bicarbonate, or until the aqueous phase attained a

basic pH. Any emulsion formed was broken with 20 mL of saturated sodium chloride solution. The organic layer was dried using molecular sieves (Aldrich Chemical Co., Milwaukee, WI), and the chloroform was evaporated under a stream of nitrogen. The composition of the sample was analyzed by GLC, as described previously.

Chemical interesterification. Chemical interesterification was performed in a flask attached to a rotary evaporator under a vacuum of 0.1 torr at 60°C. A mass of 3.90 g of tricaprylin was mixed with 16.10 g of canola oil in the presence of 0.2% (w/w) sodium methoxide. The oils were dried using molecular sieves prior to running the reaction. After addition of the catalyst, the reaction was run for 5 h, with samples being taken after 1, 3, and 5 h. After 5 h, the reaction was stopped with the addition of 100 mL of dilute citric acid, then washed with 3 vol of 100 mL of water. The oil phase was dried using centrifugation and molecular sieves. The composition of the sample was analyzed by GLC.

Hydrolysis. Previous experiments in our laboratory had determined that optimal conditions for hydrolysis of canola oil using Lipozyme IM were 10% (w/w) water, 2.5% (w/w) Lipozyme IM, at 50°C. These parameters were used to hydrolyze canola oil in order to produce a high concentration of MAG and DAG, which could then be used in lipase-catalyzed and chemically catalyzed esterification reactions. The oil and water were mixed together to form an emulsion using an Osterizer Blender on the "whip" setting for 1 min, then poured into the reaction vessel, a 50-mL glass screw-cap test tube. The lipase was added to the emulsion and mixed gently by hand. The sample was placed in a temperature-controlled water-jacketed container and stirred using a magnetic stir plate. The reaction was performed with samples being taken every hour, and it was determined that under these conditions, 6 h were required to obtain high concentrations of MAG and DAG. For all lipase-catalyzed and chemically catalyzed reactions, the concentrations of individual substrates used were calculated based on the combined number of available sites on the MAG and DAG produced during the hydrolysis of canola oil. A molar excess of 1.7:1 (ratio of mol of caprylic acid to mol of available sites) was used in all lipase-catalyzed and chemically catalyzed experiments. The mass of substrate was adjusted depending on whether caprylic acid or tricaprylin was used, in order to keep the molar ratios of caprylic acid to available sites the same for all reactions.

Short-path distillation. A Pope short-path, wiped-film distillation unit (Pope Scientific, Menomonee Falls, WI) was used to remove fatty acids from the hydrolyzed canola oil. The fatty acids were removed under a vacuum of 0.1 torr, with the evaporator set at 155°C and the condenser set at 45°C. The flow rate of the oil was 4 mL/min. Two passes through the unit were required to reduce the FFA concentration from around 40% (w/w) to less than 1% (w/w). The resulting hydrolyzed, distilled oil was used in lipase-catalyzed esterification and chemical esterification reactions.

Lipase-catalyzed transesterification, acidolysis, and esterification. The lipase used in all reactions was Lipozyme IM 60 lipase from *M. miehei* immobilized on an anion exchange resin. The moisture content of Lipozyme IM was determined to be 9.9% (w/w), which was sufficient for all lipase-catalyzed reactions (interesterification and esterification) to be performed in the absence of any additional water. Preliminary experiments were performed to evaluate the required water level, using 0.1 and 0.2% added water, and in all reactions the samples with no added water experienced the lowest degrees of hydrolysis. Lipase-catalyzed transesterification was performed using 0.975 g tricaprylin and 4.025 g fresh canola oil, while lipase-catalyzed acidolysis was performed using 0.647 g caprylic acid and 4.353 g fresh canola oil. Lipase-catalyzed esterification was performed using 0.975 g caprylic acid, which was added to 4.025 g of hydrolyzed canola oil. In all experiments, 3% (w/w) lipase was added to the reagent and mixed gently. The reaction was run, with stirring, in a 50-mL glass test tube in a jacketed water bath placed over a stir plate, at 50°C. Volumes of 100 µL were taken at 30, 60, 90, 120, 360, 540, 720, and 1440 min, and any residual lipase in the samples was inactivated by heating the samples at 100°C for 10 min. All samples were analyzed for both the degree of hydrolysis and TAG composition using gas–liquid chromatography.

Positional distribution using Grignard reagent. While the positional distribution of fats and oils has typically been determined using hydrolysis by pancreatic lipase followed by TLC and analysis of the composition of the 2-MAG band, this method could not be applied to these samples. Since pancreatic lipase possesses slightly more specificity toward short- and medium-chain fatty acids, a lipid containing both medium- and long-chain fatty acids cannot be analyzed using this method. A chemical method by Becker and coworkers (9) was used, which involved hydrolysis by a Grignard reagent to produce a random mixture of 1/3-MAG, 2-MAG, 1,2/2,3-DAG, 1,3-DAG, TAG, and tertiary alcohols.

A mass of 100 mg of oil was dissolved in 5 mL of ethyl ether, then reacted under nitrogen with 600 µL of Grignard reagent (allyl-magnesium bromide) for 30 s, after which an additional 5 mL of ethyl ether was added. The reaction was inactivated using 25 mL of 0.4 M boric acid containing 3% (vol/vol) hydrochloric acid, followed by two volumes of 25 mL of 0.4 M boric acid. The organic layer was removed, and the diethyl ether was evaporated under a stream of nitrogen until the sample contained a lipid concentration of 0.8 mg fat/µL diethyl ether. This was applied to TLC plates for separation of the different partial acylglycerols.

TLC. Lipid samples were dissolved in ethyl ether at a concentration of approximately 0.08 mg/µL and applied to TLC plates (Whatman K5 Silica gel 150A, Fisher Scientific). In order to prevent acyl migration and to separate 1/3-MAG from 2-MAG and 1,2/2,3-DAG from 1,3-DAG, the plates were sprayed with 4% (wt/vol) boric acid until saturated then dried for 1 h at 100°C. The solvent system used was chloroform/acetone in a ratio of 96:4 (w/w). The plates were developed in an iodine tank, and the bands were identified using MAG and DAG standards (Sigma Chemical Co., St. Louis,

MO). Retention factors were not measured because, in order to separate the DAG adequately from each other and from FFA, the solvent was allowed to run off the top of the plate. The bands were identified as running in the following order, from lowest to highest degree of acyl migration: 1/3-MAG, 2-MAG, 1,2/2,3-DAG, FFA, 1,3-DAG, tertiary alcohols, TAG. This order of migration was in agreement with the results of Becker and coworkers (9).

RESULTS AND DISCUSSION

Preparation of hydrolyzed, distilled canola oil. Hydrolysis of canola oil and subsequent short-path distillation to produce hydrolyzed oil with low concentrations of FFA and high concentrations of MAG and DAG was a priority in this study. The hydrolysis reaction was run for 6 h, after which the lipase was inactivated and the composition of the products determined. The FFA concentration was reduced in one pass through the short-path distillation unit to 3.5% (w/w) and required a second pass to obtain concentrations of less than 1.0% (w/w) (Table 3). Some MAG were lost due to the similarity in molecular weight between MAG and FFA. The expected concentration of DAG was around 26% (w/w), based on the reduction in FFA and MAG. The experimentally determined concentration of 30.2% (w/w) was therefore slightly higher than expected.

Lipase-catalyzed and chemically catalyzed lipid modification reactions. Lipase-catalyzed fatty acid esterification and TAG esterification reactions using hydrolyzed canola oil as the substrate were compared to lipase-catalyzed acidolysis and transesterification reactions using fresh canola oil as the substrate. All reactions were performed in duplicate, and increases in the concentration of new structured TAG (% w/w of all new TAG species combined within the TAG fraction) as a function of time are shown in Figure 1. Changes in specific TAG species as a function of time for the different reactions are shown in Figures 2 and 3. An unidentified 28-carbon species was produced over the course of the reaction using both nonhydrolyzed and hydrolyzed oil. This species was probably an oxidized reaction product, but since its concentration increased over the course of the reaction, and caprylic acid was used up in its formation, its concentration was included in the calculation of total yield of structured TAG.

TABLE 3
Composition of Hydrolyzed Canola Oil After 6 h Hydrolysis and Short-Path Falling-Film Distillation

		Distilled oil (% w/w)	
Component[a]	Hydrolyzed oil (% w/w)	Pass 1	Pass 2
FFA	20.6	3.5	0.7
MAG	3.7	3.7	2.1
DAG	20.6	28.8	30.2
TAG	55.1	64.0	67.0

[a]FFA, free fatty acids; MAG, monoacylglycerols; DAG, diacylglycerols; TAG, triacylglycerols.

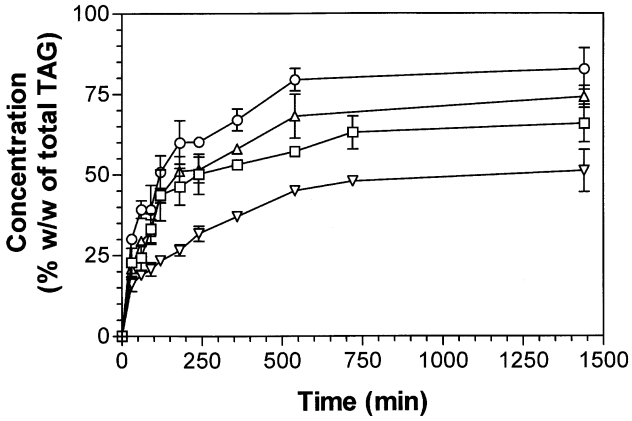

FIG. 1. Progress curves for the enzymatic production of structured triacylglycerols (TAG) over 24 h (fatty acid esterification, □; transesterification, △; acidolysis, ▽; triacylglycerol esterification, ○). Values represent means and corresponding standard errors of three replicates.

Results from these experiments were examined in two different ways. First, the lipase-catalyzed and chemically catalyzed reactions were allowed to reach completion in order to determine the maximum yields of structured TAG. Second, an appropriate shorter reaction time was chosen where the production of structured TAG was still relatively high. Minimizing the length of the lipase-catalyzed reactions would reduce production costs and decrease the extent of acyl migration (10).

The only significant difference in the final yields of structured TAG among all reactions studied was observed between the TAG esterification reaction and acidolysis, with TAG esterification producing significantly ($P \leq 0.05$) more structured TAG than acidolysis (Table 4). The yields for chemical esterification and transesterification reactions were not significantly higher than for the lipase-catalyzed reactions; however, it must be kept in mind that reaction times were substantially shorter (5 h). These reaction times could probably be further reduced to 2–3 h on further optimization of chemically catalyzed reactions. In our experiments, 5 h of chemical esterification were required to achieve high yields of new structured TAG. However, the concentration of structured TAG did not differ significantly between samples taken at 1, 3, and 5 h of chemical interesterification (not shown). Acidolysis and fatty acid esterification were expected to produce lower yields than their corresponding transesterification and TAG esterification reactions, since FFA, particularly medium-chain fatty acids, may inhibit lipase activity (11,12); however, such an effect was not observed in this study.

The yield of structured TAG was also compared among all lipase-catalyzed reactions after a 2-h reaction time (Table 5). The concentration of new TAG produced *via* acidolysis was significantly lower than for all other reactions ($P \leq 0.05$), including its two-step fatty acid esterification counterpart. Lower yields were probably the result of decreased lipase activity due to a reduction in the pH of the aqueous phase surrounding the lipase, or to accumulation of FFA at the

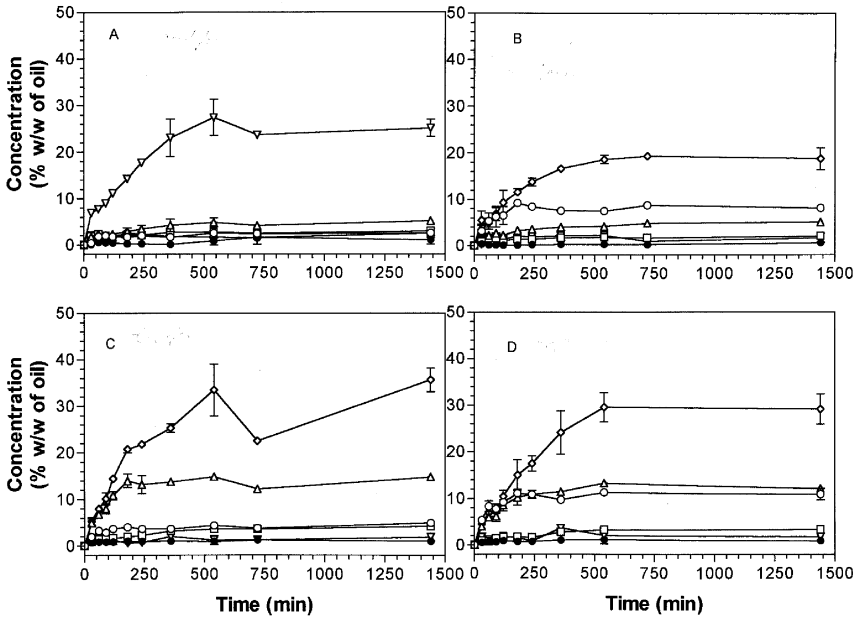

FIG. 2. Composition of new structured lipids produced from lipase-catalyzed acidolysis (A), fatty acid esterification (B), transesterification (C), and TAG esterification (D). (C28, ○; C32, ●; C34, △; C42, □; C44, ◇; C46, ▽). Values represent means and corresponding standard errors of three replicates. For abbreviation see Figure 1.

oil–water interface preventing access of the substrate to the lipase (11,12). Therefore, at short reaction times, fatty acid esterification was an improvement over acidolysis, and no significant differences ($P > 0.05$) in yields between transesterification, fatty acid esterification, and TAG esterification reactions were detected. Yields of structured TAG derived from chemical transesterification and esterification (5 h reaction time) were also similar to those obtained by lipase-catalyzed reactions.

Degree of hydrolysis; FFA and partial acylglycerol accumulation. The partial acylglycerols and long-chain free fatty acids (LCFFA) remaining in the oil after 2 h and 24 h for lipase-catalyzed reactions and 5 h for chemically catalyzed reactions are presented in Table 6. Different criteria had to be used to evaluate the degree of hydrolysis, due to differences in initial substrates and final products of each reaction. It was difficult to evaluate the degree of hydrolysis based on LCFFA

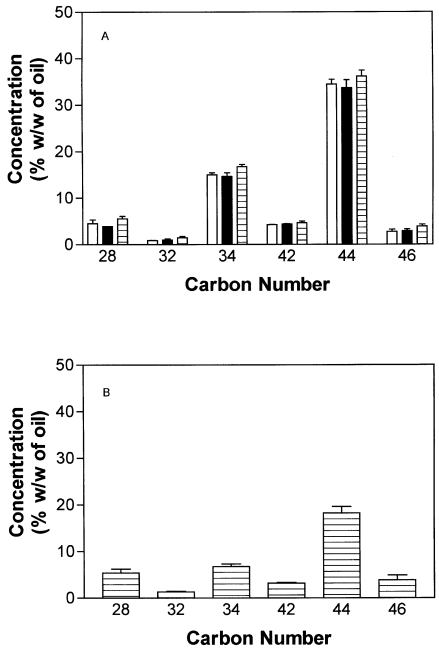

FIG. 3. Production of new structured TAG by chemical transesterification (A) and chemical esterification (B). (1 h, open bar; 3 h, solid; 5 h, striped). Values represent means and corresponding standard errors of two replicates for chemical esterification and three replicates for chemical transesterification. For abbreviation see Figure 1.

TABLE 4
Average Concentration of New TAG[a]

Reaction	New TAG (% w/w of total TAG[b]) (*n*)	Standard error (% w/w)
Lipase-catalyzed		
Acidolysis	59.9[a] (3)	4.7
Transesterification	74.1[a,b] (3)	3.4
Fatty acid esterification	65.8[a,b] (3)	5.8
TAG esterification	82.8[b] (3)	6.5
Chemically catalyzed		
Transesterification	77.2[a] (3)	2.5
Fatty acid esterification	63.9[a] (2)	0.1

[a]After 24 h for the lipase-catalyzed reactions and after 5 h for the chemically catalyzed reactions.
[b]Values with different superscript letters are significantly different ($P \leq 0.05$).
n, number of replicates; for other abbreviations see Table 3.

TABLE 5
Average Concentration of New TAG After 2 h for Lipase-Catalyzed Reactions, Using Nonhydrolyzed and Hydrolyzed Canola Oil

Reaction	New TAG[a] (% w/w of total TAG)	Standard error
Acidolysis	23.4 (3)[b]	1.2
Transesterification	43.5 (3)[a]	2.2
Fatty acid esterification	43.7 (2)[a]	7.9
TAG esterification	50.9 (3)[a]	5.0

[a]Values with different superscript letters are significantly different (≤0.05)

content, since long-chain fatty acids (LCFA) were released during the course of acidolysis, fatty acid esterification, and chemical esterification reactions. Increases in DAG and MAG concentrations are not necessarily observed even if some hydrolysis occurs during the reactions. This is because the consumption of one tricaprylin substrate molecule can lead to the conversion of three DAG substrate molecules into new structured TAG. The 1,3-specific lipase can hydrolyze tricaprylin at positions *sn*-1 and *sn*-3. Further hydrolysis, after acyl migration, would release the remaining fatty acid at position *sn*-2. Of course, this one-to-three conversion does not occur quantitatively; hence, decreases in partial glyceride contents smaller than 2/3 are observed. Moreover, if hydrolysis is occurring in the absence of acyl exchange, smaller decreases in MAG and DAG concentrations would also be observed.

Acidolysis produced a low degree of hydrolysis as shown by the minimal change in MAG and DAG concentrations after both 2 and 24 h compared to the nonhydrolyzed canola oil. There was a slight increase in hydrolysis products for lipase-catalyzed transesterification, with 2.4% (w/w) LCFFA after 2 h, 3.3% (w/w) LCFFA after 24 h, and a corresponding slightly increase in DAG concentrations for both times. Some hydrolysis was evident for TAG esterification, with an increase in the LCFFA concentration from 0.7 to 3.4 and 3.5% (w/w) after 2 and 24 h, respectively. Fatty acid esterification had the highest degree of hydrolysis, with 12.4% (w/w) LCFFA being produced after 2 h, which increased to 27.8% (w/w) after 24 h. However, some acidolysis could have occurred during the reaction as well, releasing more canola oil-derived LCFA, as those fatty acids in the TAG were replaced with caprylic acid. As expected, minimal hydrolysis was observed during chemical transesterification, since the sample had to be very dry in order for the reaction to proceed. The chemical esterification reaction produced high concentrations of LCFFA, which was attributed to hydrolysis by sulfuric acid. In spite of the problems with some hydrolysis products, the main consideration was not the source of partial acylglycerols and FFA, but rather the total concentration in products from each reaction, since their removal would add to the cost of the reaction. For this reason, lipase-catalyzed acidolysis, TAG esterification, and fatty acid esterification as well as chemical esterification would all require a higher degree of refining to remove the high concentrations of FFA and partial acylglycerols remaining. In this respect, chemical transesterification and lipase-catalyzed transesterification were superior. Further optimization of the esterification reactions would improve yields and reduce refining costs by using more of the partial acylglycerols. However, there would still be some partial glycerides, caprylic acid, or tricaprylin remaining, which would have to be removed.

Positional distribution. While the method of Becker and

TABLE 6
Partial Glyceride Concentrations (% w/w) in Original, Hydrolyzed, and Distilled Canola Oil, or as Produced in Lipase-Catalyzed (2,24 h) and Chemically Catalyzed (5 h) Reactions[a]

Sample	LCFA	MAG	DAG
Canola oil			
Fresh	0.0	1.0	7.8
Hydrolyzed	20.6	3.7	20.6
Distilled	0.7	2.1	30.2
Lipase-catalyzed			
2 h			
Acidolysis	9.9 (4.5)	0.5 (0.3)	7.8 (0.6)
Transesterification	2.4 (0.2)	0.7 (0.4)	10.3 (1.4)
Fatty acid esterification	12.4 (3.7)	2.1 (0.2)	21.8 (3.8)
TAG esterification	3.4 (0.9)	2.1 (0.2)	18.7 (1.3)
24 h			
Acidolysis	23.9 (1.1)	0.4 (0.2)	6.5 (1.2)
Transesterification	3.3 (0.9)	0.4 (0.1)	9.6 (0.2)
Fatty acid esterification	27.8 (0.1)	1.7 (0.2)	16.9 (1.6)
TAG esterification	3.5 (0.4)	1.5 (0.3)	18.5 (0.3)
Chemically catalyzed			
Transesterification	0.9 (0.5)	0.2 (0.1)	8.3 (2.1)
Fatty acid esterification	16.6 (1.8)	0.6 (0.4)	13.3 (0.5)

[a]Values for lipase-catalyzed and chemically catalyzed reactions represent means and standard error (in parentheses) of three replicates (except fatty acid esterification and chemical esterification, where n = 2). LCFA, long-chain fatty acids. For other abbreviations see Table 3.

TABLE 7
Composition of 1,3 Positions (mol%) as Determined from the 1,3-DAG Fraction for Canola Oil, Before and After Hydrolysis and Distillation[a]

Fatty acid	Fresh oil (se)	Hydrolyzed (se)	Distilled (se)
16:0	8.30 (0.2)	9.4 (1.3)	7.6 (0.2)
18:0	4.42 (0.8)	4.9 (0.8)	4.2 (0.2)
18:1	61.5 (3.8)	69.4 (3.2)	68.6 (5.1)
18:2	15.1 (1.4)	11.7 (2.8)	13.4 (3.3)
18:3	10.1 (0.9)	4.2 (0.5)	6.2 (2.0)

[a]se, standard error; for other abbreviations see Table 3.

coworkers (9) worked very well when tested on standard mixtures of tricaprylin and canola oil, as well as with the hydrolyzed, distilled canola oil, difficulties were encountered with the actual samples. Resolution of individual 1/3-MAG, 2-MAG, 1,2/2,3-DAG, and 1,3-DAG bands by TLC was good, but the difficulty arose with the gas–liquid chromatographic analysis. In most cases, there was not a sufficiently high concentration of MAG present for analysis, so only the 1,3-DAG were analyzed.

The positional distributions of fatty acids in the hydrolyzed oil and distilled oil samples were compared to each other and to the original canola oil to assess the degree of acyl migration caused by the reactions (Table 7). There was no significant change in the positional distribution of any of the fatty acids, except a decrease in the concentration of linolenic acid between the fresh canola oil and hydrolyzed canola oil ($P \leq 0.05$). Therefore, the degree of acyl migration caused by 6 h of hydrolysis and two passes through the short-path distillation unit was minimal.

The positional distribution of caprylic acid in positions *sn*-1 and *sn*-3 in all the lipase-catalyzed and chemically catalyzed reaction products was lower than expected (data not shown). We believe that caprylic acid could be forming tertiary alcohols and fatty acid methyl esters, which could be eluting at the same time as palmitic acid and stearic acid. This problem made it very difficult to assess the positional distribution of fatty acids accurately in reaction products, especially caprylic acid. A lower than expected concentration of caprylic acid and inflated palmitic and stearic acid values were also identified in the chemically reacted samples (not shown). Positional distributional analysis of lipids containing caprylic acid did not work well using the Grignard reagent and made it impossible to evaluate the concentration of caprylic acid in the *sn*-1,3 positions, as well as the degree of acyl migration in final reaction products. However, since minimal acyl migration occurred after 6 h of lipase-catalyzed hydrolysis and two passes through a short-path distillation column, we believe that 2 h lipase-catalyzed transesterification, acidolysis, or esterification reactions would not significantly increase the degree of acyl migration in the final reaction products.

No significant differences in final yields of new structured lipids were detected between lipase-catalyzed (24 h) and chemically catalyzed (5 h) reaction, however, the yield of new structured TAG after 2 h was lower for acidolysis relative to the other lipase-catalyzed reactions ($P \leq 0.05$). Lipase-catalyzed fatty acid esterification and TAG esterification reaction yields were similar to lipase-catalyzed transesterification and acidolysis reactions. Since no differences in final yields were detected among the reactions, chemical esterification using hydrolyzed oil could potentially constitute the best option, because it offers the control over final product fatty acid positional distribution associated with lipase-catalyzed reactions, as well as the rapid reaction time associated with chemically catalyzed reactions. The major drawback associated with this reaction is product charring by the use of sulfuric acid at elevated temperatures. Optimization of chemical esterification conditions to reduce charring, degree of hydrolysis, and decomposition is required. Further work on the use of the Grignard reagent to determine the positional distribution of fatty acids in structured lipids is also required.

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REFERENCES

1. 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. Nutri. 38*:1–36 (1998).
2. Willis, W.M., and A.G. Marangoni, Biotechnological Strategies for the Modification of Food Lipids, *Biotechnol. Gen. Eng. Rev*. (1999, in press).
3. Marangoni, A.G., and D. Rousseau, Engineering Triacylglycerols: The Role of Interesterification, *Trends Food Sci. Technol. 6*:329–335 (1995).
4. Rousseau, D., and A.G. Marangoni, Chemical Interesterification, *Food Lipids*, edited by C.C. Akoh and D.B. Min, Marcel Dekker Inc., N.Y., 1998, pp. 251–281.
5. Willis, W.M., and A.G. Marangoni, Enzymatic Interesterification, *Ibid.*, pp. 665–698.
6. Goh, E.M., and R.E. Timms, Determination of Mono- and Diglycerides in Palm Oil, Olein and Stearin, *J. Am. Oil Chem. Soc. 62*:730–734 (1985).
7. Bannon, C.D., J.D. Craske, and A.E. Hilliker, Analysis of Fatty Acid Methyl Esters with High Accuracy and Reliability. IV. Fats with Fatty Acids Containing Four or More Carbon Atoms, *Ibid. 62*:1501–1507 (1985).
8. Pavia, D.L., G.M. Lampman and G.S. Kriz, Jr., *Introduction to*

Organic Laboratory Techniques: A Contemporary Approach, 2nd edn., Saunders College Publishing, Washington, 1982, pp. 86–88.
9. Becker, C.C., A. Rosenquist, and G. Holmer, Regioselective Analysis of Triacylglycerols Using Allyl Magnesium Bromide, *Lipids 28*:147–149 (1993).
10. Sil Roy, S., and D.K. Bhattacharrya, Distinction Between Enzymically and Chemically Catalyzed Interesterification, *J. Am. Oil Chem. Soc. 66*:343–347 (1987).
11. Trevan, M.D., *Immobilized Enzymes*, John Wiley & Sons, New York, pp. 16–26.
12. Kuo, S.-J., and K.L. Parkin, Substrate Preference for Lipase-Mediated Acyl-Exchange Reactions with Butteroil Are Concentration-Dependent, *J. Am. Oil Chem. Soc. 70*:393–399 (1993).

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