Enzymatic Interesterification of Triolein with Tripalmitin in Canola Lecithin-Hexane Reverse Micelles

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Lipase-catalyzed interesterification of tripalmitin with triolein in canola lecithin-hexane reverse micelles allowed for the successful modification of triolein and tripalmitin to yield a fat of intermediate properties between the two initial substrates. Acetone-insoluble canola lecithin (AIL) reverse micelles containing *Rhizopus arrhizus* lipase in buffer, or plain 0.1M sodium phosphate buffer of pH 7.0, formed readily in hexane. Both had an average Stokes' radius of approximately 40Å, as determined by quasi-elastic light-scattering determinations. The reverse micelle system was stable and did not form higher-order micelle oligomers or aggregates. Biotransformation of the triglycerides was performed at 47°C in a 50-mM AIL-hexane reverse micelle system containing 50% (w/w) oil at a water-to-surfactant ratio (w_o) of 5.5. Dynamic (oscillatory) mechanical analysis indicated that the crystallization temperature of the fat dropped from 47.7 to 37.5°C as judged by the storage (G′) and loss (G″) modulus *vs.* temperature profiles after 48 h of reaction. Differential scanning calorimetric studies showed that the melting point of the fat dropped from 61 to 57°C after 48 h of reaction. Triglyceride analysis of the fat mixture by gas–liquid chromatography (GLC) indicated that, after 48 h of reaction, the tripalmitin content dropped from 34.5 to 29% (w/w), the triolein content dropped from 64.5 to 52.1% (w/w) and the 1-oleyl-2,3-dipalmitin content reached 7.5% (w/w) while the 1-palmitoyl-2,3-diolein content reached 7.2% (w/w). 1,2-Dipalmitoyldiglyceride and 1,2-dioleyldiglyceride contents reached 1.6 and 2.4% (w/w), respectively, after 48 h. Free fatty acid analysis of the fat mixture by GLC revealed that the free palmitic acid content increased from 0.28 to 2.4% (w/w) while the free oleic acid content increased from 1.4 to 5.4% (w/w) in the initial 24 h, after which the levels remained constant. The relatively high initial free fatty acid content of the mixture was due to free fatty acids present in the canola lecithin and not in the oils. This enzymatic interesterification protocol utilizes, for the first time, an organic solvent commonly used in food processing operations and a food-grade and inexpensive surfactant that readily forms reverse micelles and yields a modified fat with improved rheological properties for use as an edible plastic fat.

KEY WORDS: Canola lecithin, enzymatic interesterification, enzymes, lipases, organic solvents, reverse micelles, structured lipids.

Interesterification of fats and oils for the manufacture of structured lipids is the subject of intense research around the world today. The ability to synthesize fats and oils for specific applications from the vast pool of fat and oil sources available has great appeal, both from a textural and a nutritional point of view (1).

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Structured lipids are important from a textural point of view in the manufacture of plastic fats, such as margarines, modified butters and shortenings. A 1,3-specific lipase-catalyzed interesterification of 1,3-dipalmitoyl-2-monolein (POP), from palm oil midfraction, with stearic acid or tristearin gives products enriched in 1-palmitoyl-3-stearoyl-2-monolein (POS) and 1,3-distearoyl-2-monolein (SOS), which are important components of cocoa butter (2). This forms the basis for a lipase-catalyzed process for the production of a valuable cocoa butter substitute. Yet another structured lipid of interest is caprenin. It appears to be a structured lipid of medium-chain fatty acids and behenic acid. This fat has similar properties as cocoa butter with half the calories (1). A sodium methylate or sodium metal-catalyzed chemical interesterification product of palm oil with coconut oil can be blended with 40% of sunflower oil, thereby producing a margarine not only lacking *trans* fatty acids but high in medium-chain triglycerides (3). Advantages of manufacturing plastic fats by enzymatic/nonenzymatic interesterification rather than hydrogenation include the minimization of *trans* isomerization of fatty acids in the finished product. There is a concern for the metabolic effects of *trans* fatty acids on serum high-density and low-density lipoprotein cholesterol levels (4).

Structured lipids are also important from a nutritional and pharmacological point of view. The ability to synthesize triglycerides and phospholipids with a defined structure to target a specific disorder or to improve health has tremendous potential. Triglycerides containing specific balances of medium-chain, ω-3, ω-6, ω-9 and saturated long-chain fatty acids could be synthesized to help lower serum low-density lipoprotein cholesterol levels, reduce serum triglyceride levels, help prevent thrombosis, improve immune function, lessen the incidence of cancer or improve nitrogen balance (1,5,6).

There are several advantages in the use of enzymes rather than inorganic catalysts, such as sodium metal or sodium alkoxides, to catalyze interesterification reactions. The most important one is the specificity of most microbial lipases toward the *sn*-1 and *sn*-3 positions on the triacylglycerol molecules (7,8). This allows for the synthesis of specific lipid structures. In the case of cocoa butter substitutes, in particular, this becomes extremely important, because the relative contents of POP, POS and SOS give cocoa butter its desirable textural characteristics (7–9). Synthesis of these lipids would not be possible with nonspecific chemical catalysts or with nonspecific lipases.

The reason for the *sn*-1,*sn*-3 specificity of lipases seems to be related to steric factors in the alcohol moiety, which prevent access of the *sn*-2 carbon ester to the active site of the lipase. Steric factors seem to predominate over electronic factors in this reaction (10). Also, there does not seem to be any stereospecificity in the reaction because the *sn*-1 and *sn*-3 esters on enantiomeric forms of phosphatidylcholine (PC) are hydrolyzed at similar rates (11).

The reaction displays strictly positional specificity (7). Performing interesterifications with enzyme catalysts rather than inorganic catalysts eliminates the need to remove and purify the products and by-products of the reaction from the catalysts' residues (12). Concern from consumers over "chemical" residues in foods is an important consideration as well.

There exist several ways of performing enzymatic esterification reactions in organic solvents. These include simple biphasic systems (13), free enzymes in microaqueous environments (12,13), reverse micellar systems (13–17), immobilized enzymes in microaqueous environments (2,18) and immobilized substrate (glycerol) at oil-water interfaces in microaqueous systems (19,20). Most of these are esterification reactions between glycerol and a fatty acid, a fatty acid and an alcohol or a free fatty acid and a triglyceride. Of interest to the fats and oils industry is the development of commercial interesterification reactions between two or more triacylglycerol molecules, in which a fatty acid from one triacylglycerol molecule is exchanged with a fatty acid from another triacylglycerol molecule. Ideally, after removal or inactivation of the enzyme, the fat can then be used without the need for further processing. Also, the process engineering behind many of these processes has not been developed, leaving the whole area of applicability of these methods in doubt. Some efforts have been made in membrane bioreactors with two-phase systems (21), large-scale microaqueous systems (22) and in batch or packed-bed reactors with immobilized lipases (2).

In this paper, we report on the development of a biocompatible, food-grade reverse micelle system for the lipase-catalyzed interesterification of triolein, akin to a vegetable oil, with tripalmitin, akin to palm tripalmitin, a common hard stock.

MATERIALS AND METHODS

Chemicals. Hexane (technical-grade) was purchased from Fisher Scientific (St. Louis, MO). *Rhizopus arrhizus* lipase was purchased from Boehringer Mannheim (Hamburg, Germany). Trimethylphenylammonium iodide was purchased from Sigma Chemicals (St. Louis, MO). Silver oxide, petroleum ether, chloroform, methyl acetate and anhydrous sodium carbonate were purchased from Fisher Scientific. Triolein, tripalmitin and heptadecanoic acid were purchased from Sigma Chemicals and were of the highest available purity.

Lipase preparation. Rhizopus arrhizus lipase in 4M ammonium sulfate was dialyzed overnight at 4°C against excess 0.1M sodium phosphate buffer, pH 7.0. The dialyzed enzyme preparation was then concentrated at 4°C in a Centricon 10 (Amicon, Danvers, MA) centrifugal concentrator with a molecular weight cutoff of 10,000. The final lipase stock solution concentration was 3 mg/mL as determined by the direct spectrophotometric method of Whitaker and Granum (23). This commercially available enzyme preparation was of the highest purity and contained a single molecular species (24).

Surfactant preparation. Canola lecithin was isolated from raw canola oil by a standard water degumming procedure. The precipitated wet gum was then purified by washing the lecithin twice with excess cold acetone, which solubilizes triglycerides but not phospholipids. The acetone-washed pellet was then resolubilized in petroleum ether to release any entrapped oil, followed by two additional acetone precipitation steps (AOCS Official Method Ja 4-46 Acetone Insoluble Matter). The residual acetone in this lecithin preparation was allowed to evaporate in a fume hood at room temperature until the lecithin acquired a sandy texture and was light brown in color. This lecithin preparation will be referred to as "acetone-insoluble lecithin" or AIL. At this point, the material was transferred to a glass container and stored at −18°C until needed. It was found that, if all the acetone was removed from the purified lecithin before frozen storage, the lecithin became extremely hard and unmanageable and turned dark brown in color.

Reverse micelle formation. Stock solutions of AIL in hexane (200 mM concentrations were attainable) were prepared assuming an average molcular weight for the lecithin phospholipids of 880. An amount of AIL was removed from the freezer, placed on a weighing dish and allowed to come to a constant weight upon evaporation of the residual acetone in a fume hood at room temperature. The AIL-hexane stock solution was briefly centrifuged in a benchtop centrifuge, and the supernatant was decanted and filtered through a 0.45-μm nylon 66 membrane (Micron Separations, Inc., Honeyoye Falls, NY) by using a glass syringe.

Different volumes of aqueous phase, either 0.1M sodium phosphate buffer pH 7.0 or lipase in 0.1M sodium phosphate buffer pH 7.0, were added to the AIL-hexane stock solutions and vigorously vortexed and mixed with a micropipette until a clear solution was obtained. This clear solution was then centrifuged briefly, decanted carefully so as to leave any precipitate at the bottom of the tube and refiltered through a 0.45-μm nylon 66 membrane with a glass syringe. This AIL-hexane reverse micelle constituted the basic catalytic component of our interesterification system.

Interesterification reaction. Triolein and tripalmitin were added in a 2:1 (w/w) proportion to a 10-mL glass scintillation vial fitted with a Teflon-lined cap (Fisher). This mixture was heated until all tripalmitin was melted, vortexed and cooled down to 50°C. Lipase-containing 100 mM AIL-hexane reverse micelles were added to the oil mixture in proportions of 1:1 (w/w), and the vial was quickly sealed, vortexed and mixed with a micropipette until a clear solution was obtained. The lipase-catalyzed reactions were then allowed to take place at 47°C for 48 h. Samples for analysis were withdrawn at 0, 6, 24 and 48 h and heated to 85°C for 10 min to inactivate the enzyme and evaporate the hexane. Samples were immediately frozen to −18°C until required for analysis.

Quasi-elastic light scattering (QELS). QELS was performed with an appartus and set-up described previously (25). Data analysis was performed as previously described (25,26). Samples for photon correlation spectroscopy were prepared by adding 3 μL of either 0.1M sodium phosphate buffer pH 7.0, or 3 μL of a 3 mg/mL *R. arrhizus* lipase solution in 0.1M sodium phosphate buffer pH 7.0, to 1 mL of 25 mM AIL-hexane and vortexing until a clear solution was obtained. These samples were then briefly centrifuged in a benchtop clinical centrifuge, and the supernatant was decanted and filtered through 0.45-μm nylon membranes. The reported size distribution corresponds to the average of thirty-two 30-min accumulated runs.

Dynamic (oscillatory) mechanical analysis (DMA). DMA was performed with a Carri-med CSL 100 controlled stress rheometer (Dorking, Surrey, England). The fat samples were melted and loaded between parallel plates (diameter 4 cm, gap 300 μm) at 65°C, the temperature was adjusted to 60°C and held for 10 min. The temperature was subsequently reduced at a rate of 0.5°C/min down to 30°C. The storage modulus (G′) and the loss modulus (G″) were monitored throughout the cooling process at a frequency of 1 Hz and a constant stress of 50N/m^2. This value was selected from within the linear viscoelastic region of the melted sample.

Differential scanning calorimetry (DSC). DSC was performed with a DuPont 2000 series Thermal Analysis system equipped with a DSC cell (Boston, MA). A 10.5-mg sample of the fat was hermetically sealed into DSC pans. The fat samples were incubated at 5°C for 30 min and heated to 70°C at a rate of 5°C/min. Fat melting midpoint temperatures and enthalpies were determined from the DSC runs with software provided by the manufacturer.

Free fatty acid content. The free fatty acid content of the fat samples as a function of reaction time was determined by the method of Williams and MacGee (27) with trimethylphenylammonium hydroxide. Heptadecanoic acid was used as the internal standard. Gas–liquid chromatography (GLC) was performed in a Hewlett-Packard 5830 gas chromatograph equipped with a flame-ionization detector (FID) (Palo Alto, CA). A 2-m glass column packed with 10% Silar 10C on Gas-ChromeQ 100-120 mesh (Chromatographic Specialties, Brockville, Ontario, Canada) was used in this analysis. Nitrogen was used as the carrier gas at a flow rate of 30 mL/min. The column was run isothermally at 180°C. The injector temperature was set at 195°C while the detector temperature was set at 230°C. The free fatty acid content is expressed as the weight of fatty acid present per unit weight of fat. Values reported are the average of five determinations with their corresponding standard deviations.

Triglyceride analysis. The conversion of tripalmitin and triolein into 1-palmitoyl-2,3-diolein (POO) and 1-oleyl-2,3-dipalmitin (OPP) and diglycerides was monitored by GLC of the fat as a function of reaction time (AOCS Official Method Ce 5-86 Triglycerides by Gas Chromatography). In our determination we utilized 10% OV-1 (methyl polysiloxane) on Gas-ChromQ (Applied Science, State College, PA) as the stationary phase. In the quantitation of triglycerides, triolein was also included as an external standard. GLC was performed in a Hewlett-Packard 5830 gas chromatograph equipped with an FID. The lipid content is expressed as the relative proportions of species present (diglycerides plus triglycerides only) on a weight/weight basis. Values represent averages of four determinations and their corresponding standard errors.

RESULTS AND DISCUSSION

AIL and sodium phosphate buffer of pH 7.0 formed reverse micelles in hexane. Figure 1 shows the number distribution of the reverse micelle population in the presence (A) and absence (B) of *R. arrhizus* lipase, as determined by QELS. Canola lecithin dissolved readily in hexane and formed reverse micelles upon addition of the appropriate aqueous phase. The average radius of the empty reverse micelles was 39.5Å, while for reverse micelles containing lipase it was 41.2Å. The number distribution of the empty reverse micelles is more discrete than the distribution of the lipase-containing reverse micelles. Upon incorporation of the lipase into certain reverse micelles, a structural rearrangement of the system could result in the more continuous number distribution observed. In both cases, however, the population of reverse micelles was relatively monodisperse, as judged by a single number distribution in each case. The reverse micelle system was stable during the 16-h duration of the QELS experiment as judged from the thirty-two individual 30-min determinations. No higher-order aggregates or changes in size distribution were evident in this period.

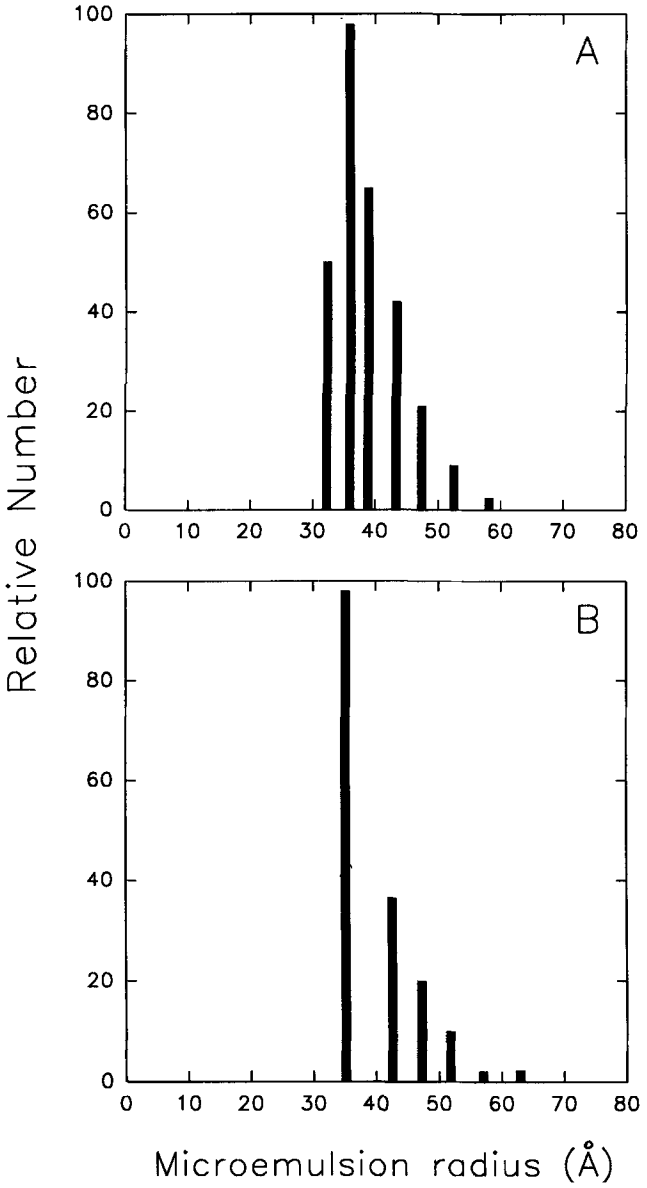

FIG. 1. Quasi-elastic light-scattering determination of population number distribution for 25 mM acetone-insoluble canola lecithin-hexane reverse micelles (w_o = 3.3 at 20°C). (A) Reverse micelles containing 3 μL of 3 mg/mL *Rhizopus arrhizus* lipase in 0.1M sodium phosphate buffer pH 7.0; (B) reverse micelles containing 3 μL of 0.1M sodium phosphate buffer pH 7.0.

The ease with which canola lecithin formed reverse micelles was surprising because phospholipid-based reverse

micelles do not form readily. Lecithin is slightly too lipophilic in water-oil systems to spontaneously form the zero mean curvature amphiphile layers needed for microemulsions. Cosurfactants are generally required for the formation of lecithin-based reverse micelles (28). A possible alternative to cosurfactant addition is to adjust the polarity of the aqueous phase. The hydrophilic-lipophilic balance balance can be shifted by making the polar solvent less hydrophilic, by making the nonpolar solvent more lipophilic or by making the lecithin more hydrophilic (28).

Hydrated phospholipid molecules form superstructures that adopt specific phases, depending on several factors. One of the key factors that influence the phase behavior of phospholipids is the shape factor of the molecule. The shape factor is defined as v/al, where v is the volume of the phospholipid, a is the area per headgroup and l is the approximate thickness of the lipid hydrocarbon layer. Table 1 summarizes the preferences of phospholipid molecules for particular phases (29). It is imperative, however, that the reader keep in mind that many factors influence the phase behavior of phospholipid molecules (temperature, pH, ionic strength, metal ions) and that the same molecule may adopt different phases, depending on environmental conditions (30).

In reverse micelles, phospholipids adopt inverted hexagonal (H_{II}) phases; hence, those phospholipids that adopt this configuration under the chosen conditions will favor reverse micelle formation. It is not surprising then that PC reverse micelles do not form unless some cosurfactant, generally free fatty acid, is added (see Table 1 and Ref. 31). To make reverse micelles from phospholipids, it is necessary to destabilize the lamellar phase, which many phospholipids adopt, and favor the adoption of the inverted hexagonal phase (28). Decreasing hydrocarbon unsaturation and decreasing temperature favor the H_{II} phase, while increasing headgroup ionization, increasing water content and increasing the headgroup size will favor the lamellar (L_α) phase (29). Considering the structure of Aerosol-OT, the most common surfactant used in reverse micelle research, it is not surprising that this surfactant forms reverse micelle structures so well.

The phospholipid compositions of canola, soybean and sunflower lecithins have been determined previously (32). The predominant phospholipid species are PC and phosphatidylethanolamine (PE). The balance is made up of phosphatidylinositol (PI), phosphatidic acid (PA), phosphatidylglycerol (PG) and lysophosphatidylcholine (LPC). For a water-degummed AIL, the ratio of PE, which forms H_{II} phases, to PC + PI + PA + PG, which form lamellar phases, is 0.28 for canola lecithin, 0.53 for soybean and 0.50 for sunflower lecithin. This indicates that lecithins from soybean and sunflower, and probably other sources, should work equally well or better than canola lecithin due to their high content of H_{II}-forming phospholipids.

From our free fatty acid analysis at time 0, we observe that 0.28% (w/w lipid) palmitic acid, 1.44% (w/w lipid) oleic acid, 0.12% (w/w lipid) linoleic acid and trace amounts of stearic acid were present in the mixture, derived exclusively from the lecithin. Table 1 shows that free unsaturated fatty acids promote the formation of H_{II} phases, hence their presence probably aids in the formation of reverse micellar structures.

These findings suggest that a more highly purified lecithin, stripped of fatty acids or probably hot ethanol-fractionated (which enhances the PC content of the lecithin), may not be adequate for this application. Most probably, any process that would enhance the PE content of the lecithin would increase its value as a biosurfactant for reverse micelle enzymology. Some free fatty acids may always be required for proper reverse micelle formation.

TABLE 1

Polymorphic Phase Preferences of Unsaturated Lipids[a]

Lipid	Physiological conditions[b]	Other conditions
Phosphatidylcholine	L	H_{II}—low hydration and high temperature
Sphingomyelin	L	
Phosphatidylethanolamine	H_{II}	L—pH > 8.5 —low temperatures
Phosphatidylserine	L	H_{II}—pH < 3.5
Phosphatidylglycerol	L	H_{II}—high temperatures and high salt concentrations
Phosphatidylinositol	L	
Cardiolipin	L	H_{II}—divalent cations —pH < 3 —high salt
Phosphatidic acid	L	
Monoglucosyldiglyceride	H_{II}	
Diglucosyldiglyceride	L	
Monogalactosyldiglyceride	H_{II}	
Digalactosyldiglyceride	L	
Cerebroside	L	
Cerebroside sulfate	L	
Ganglioside	M	
Lysophosphatidylcholine	M	
Cholesterol		Induces H_{II} phase in mixed lipid systems
Unsaturated fatty acids		Induce H_{II} phase

[a]Reproduced with permission from Ref. 29.
[b]L, lamellar; H_{II}, hexagonal II; M, micellar.

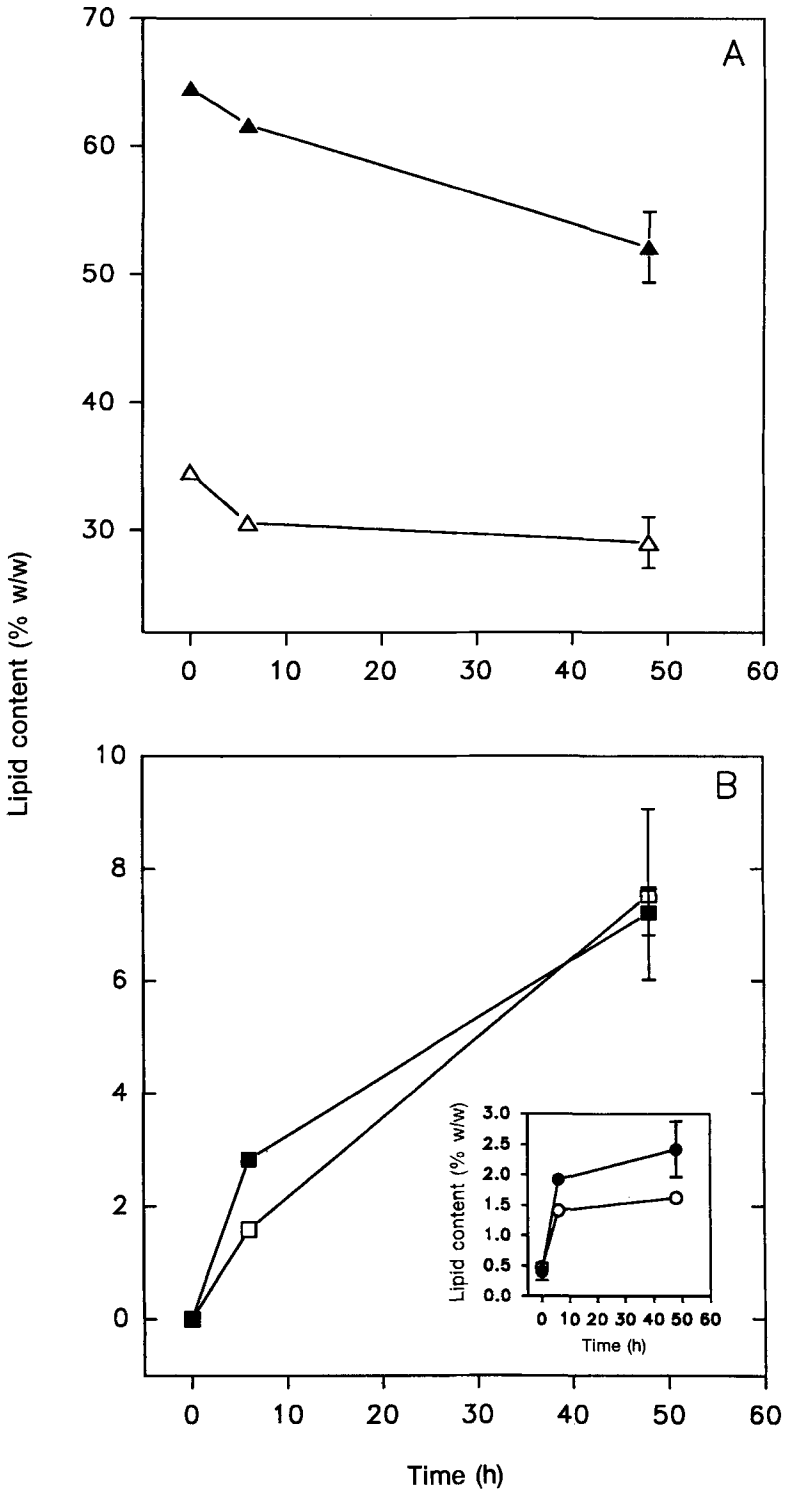

FIG. 2. Triglyceride analysis of the triolein-tripalmitin-canola lecithin fat mixture as a function of enzymatic interesterification reaction time. (A) Decreases in triolein (▲—▲) and tripalmitin (△—△) contents as a function of reaction time; (B) increases in the interesterified reaction products 1-oleyl-2,3-dipalmitin (□—□) and 1-palmitoyl-2,3-diolein (■—■) as a function of reaction time. The inset depicts the increases in dipalmitoyldiglyceride (○—○) and dioleyldiglyceride (●—●) as a function of reaction time. Values represent the average of four determinations and their corresponding standard errors.

We also found that pH (6-9) or the addition of salt (50 mM NaCl) did not affect the AIL/hexane reverse micelle system as judged by visual inspection. Attempts at making AIL-vegetable oil reverse micelles failed because AIL could not be dispersed into oil. Attempts included solvent replacement, where the lecithin was dissolved in petroleum ether, dispersed throughout the oil and evaporated off with stirring, as well as sonication, peristaltic pumping, extrusion under high pressures and low pressures and microhomogenization. The AIL would coagulate and precipitate upon removal of the petroleum ether. A 50% (w/w) oil/hexane reverse micelle system was stable, however; and other oil/hexane proportions may prove useful.

The progress on the interesterification reaction in our system was monitored by GLC of the lipids present. The content of tripalmitin and triolein decreased as a function of reaction time (Fig. 2A) from 34.5 to 29.0% (w/w) and from 64.5 to 52.1% (w/w), respectively. The decrease in tripalmitin content seemed more biphasic than the decrease in triolein content. This was accompanied by a simultaneous increase in the interesterified products, POO and OPP from 0 to 7.2% (w/w) and from 0 to 7.5% (w/w), respectively (Fig. 2B). Also evident was a slight increase in 1,2-dipalmitoyldiglyceride (PPG) and 1,2-dioleyldiglyceride (OOG) contents as a function of reaction time (Fig. 2B, inset). PPG content increased from 0.47 to 1.62% (w/w) while OOG content increased from 0.40 to 2.42% (w/w). Free fatty acid content also increased during the course of the reaction in a rectangular hyperbolic fashion (Fig. 3). Hydrolysis of triolein was more predominant than that of tripalmitin, probably suggesting a greater affinity of the enzyme for this substrate. Molecular packing defects induced by the *cis* double bond on the oleic acid moiety could aid in the binding of the enzyme to the substrate, while the more ordered structure of tripalmitin could sterically hinder binding and, hence, catalysis. The entropy of activation for the reaction is probably greater for the enzyme-triolein complex than for the enzyme-tripalmitin complex because the enzyme-triolein complex should be able to adopt a greater number of configurations in the transition state. This would manifest itself as a greater rate of triolein breakdown. The greater free oleic acid content and the higher dioleyldiglyceride content of the oil support the argument that triolein was hydrolyzed more readily than tripalmitin.

A 14.7% (PPO plus OOP) content of interesterified products in the modified fat was sufficient to cause dramatic changes in the physical properties of the fat. DMA of the interesterified fat indicated that the crystallization midpoint temperature dropped from 47.7 to 37.5°C in the course of the 48-h reaction as judged by the storage (G′) and loss (G″) moduli *vs.* temperature profiles (Figs. 4A and 4B). The storage modulus reflects the elastic component of a viscoelastic material, while the loss modulus (G″) reflects the viscous component. From these cooling curves, we can observe that at 60°C, when the system is in the liquid state, G″ is greater than G′. As the temperature drops, G′ and G″ show little change until the lipid components begin to crystallize. At this point, G′ increases sharply as the system becomes more elastic; G″ also increases, although by less than its elastic counterpart. After crystallization has occurred, the system exhibits properties similar to those of solid materials, where G′ is greater than G″. Fluctuations in dynamic moduli after crystallization are attributed to less well resolved wave forms (due to the small strains observed). To our knowledge, this is the first time that oscillatory DMA has been used with fat systems. The technique proved to be sensitive to changes in lipid structure and rheological properties. We foresee that this technique will become useful in fats and oils research in the future.

DSC of the fat was also used to determine whether any changes in the physical structure of the fat had resulted from the enzymatic interesterification process. Melting endotherm transition midpoint temperatures dropped from 60.8 to 57.1°C in the course of the reaction while transition enthalpy dropped from 67.3 to 41.4 J/g (Table 2). The lack of agreement between crystallization midpoint temperatures and DSC transition midpoint temperatures is not surprising due to the nonequilibrium nature of the

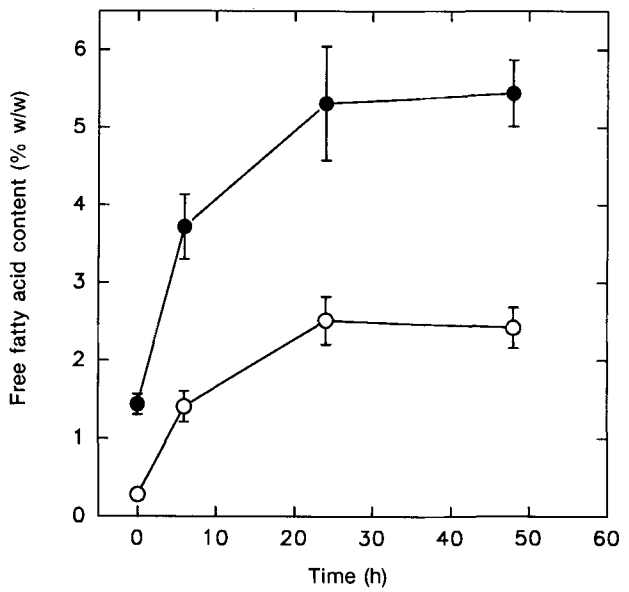

FIG. 3. Free fatty acid analysis of the triolein-tripalmitin-canola lecithin fat mixture as a function of enzymatic interesterification reaction time. Oleic acid (● — ●) and palmitic acid (○—○) contents increased as a function of reaction time. Values represent the average of five determinations and their corresponding standard deviations.

TABLE 2

Transition Midpoint Temperatures and Transition Enthalpies for Fat Samples Containing Triolein-Tripalmitin (2:1, w/w) Enzymatic Interesterification Reaction Products Catalyzed by *Rhizopus arrhizus* Lipase[a]

Time (h)	Transition midpoint[b] (°C)	Transition enthalpy (H)[b] (J/g)
0	60.8 (0.26)	67.3 (0.84)
6	58.7 (0.06)	48.8 (4.00)
24	57.4 (0.08)	44.8 (0.94)
48	57.1 (0.20)	41.4 (0.40)

[a]The reaction was carried out in acetone-insoluble lecithin-hexane reverse micelles at 47°C.

[b]Values represent means (standard deviations) of two (n = 2) separate determinations.

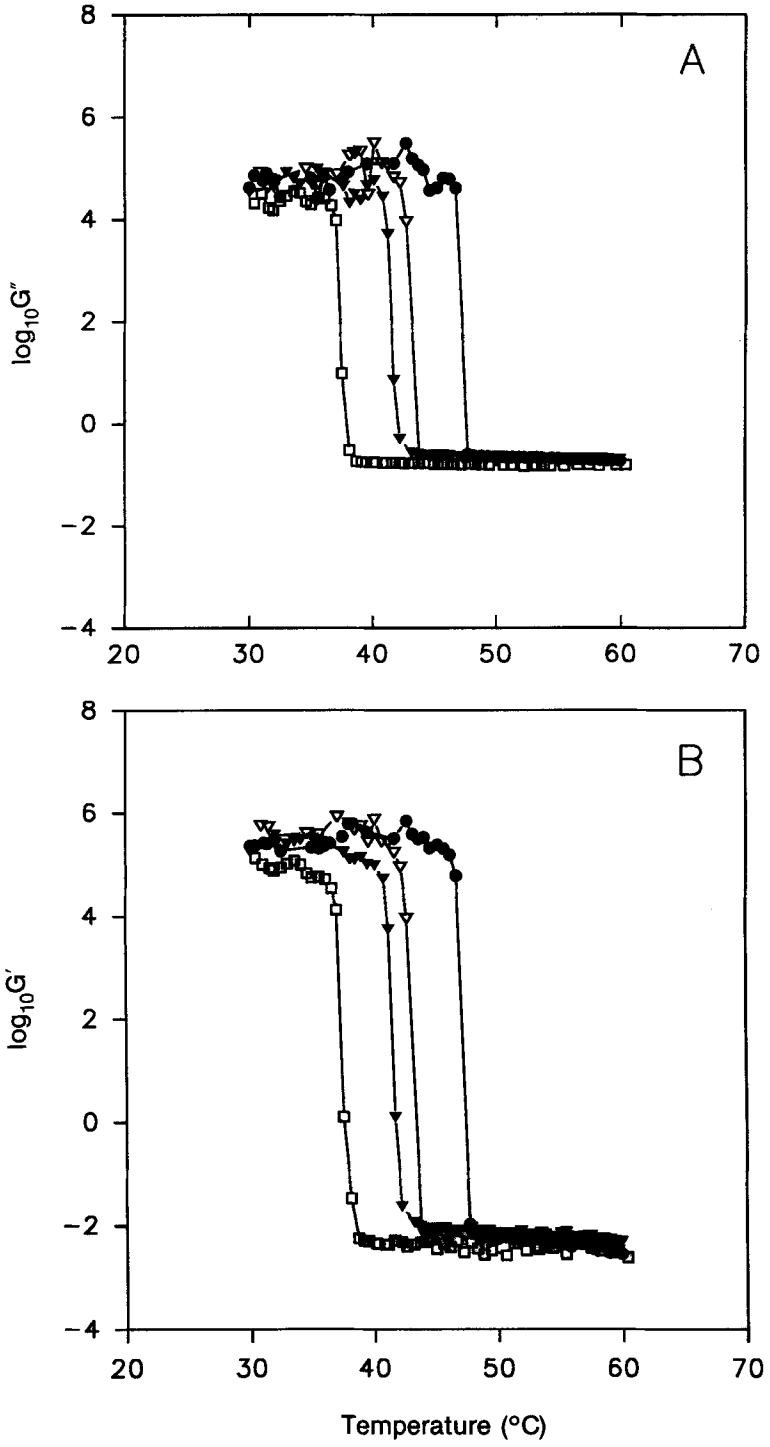

FIG. 4. Dynamic oscillatory mechanical analysis of the triolein-tripalmitin-canola lecithin fat mixture as a function of enzymatic interesterification time. (A) Effect of reaction time on the loss modulus (G″) *vs.* temperature profile of the fat. Curves correspond to reaction times of 0 h (●—●), 6 h (▽—▽), 24 h (▼—▼), 48 h (□—□). (B) Effect of reaction time on the storage modulus (G′) *vs.* temperature profile of the fat. Curves correspond to reaction times of 0 h (●—●), 6 h (▽—▽), 24 h (▼—▼), 48 h (□—□).

DSC experiment at heating rates of 5°C/min as opposed to cooling rates of 0.5°C/min in the DMA experiment. Also, melting and crystallization, being different physical processes, would be expected to display different physical parameter-temperature behavior. However, the DSC experiments still indicated that the average melting temperature of the fat had dropped considerably.

In conclusion, a food-grade canola lecithin/hexane/oil reverse micelle system proved successful in the lipase-catalyzed interesterification of triolein with tripalmitin. The enzymatic conversion led to the synthesis of a plastic fat with potentially desirable texture. Future work will be directed toward the optimization of reaction conditions and the application of this technology to the modification of vegetable oils and dairy fat because much promise has recently been shown in PC-butteroil reverse micelle systems for the hydrolysis of milkfat (33).

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