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Lipase-Catalyzed Synthesis of Structured Low-Calorie Triacylglycerols

T.J. Mangos, K.C. Jones, and T.A. Foglia*
ARS, USDA, ERRC, Wyndmoor, Pennsylvania 19038

ABSTRACT: Because of their unique fatty acid specificities and regioselectivities, lipases have been found to be effective catalysts for the synthesis of structured lipids that have a predetermined composition and distribution of fatty acyl groups on the glycerol backbone. The prospective plant-derived lipase found in the exudate of *Carica papaya* is known for its short-chain acyl group specificity, 1,3-glycerol regioselectivity, and *sn*-3 stereoselectivity. *Carica papaya* latex (CPL) was therefore examined for its potential ability to synthesize structured low-calorie short- and long-chain triacylglycerols (SLCT). In this paper, we describe the utility of CPL in the lipase-catalyzed interesterification reaction of triacetin and hydrogenated soybean oil. Normal-phase high-performance liquid chromatography, combined with mass spectrometry, was used to distinguish the structured SLCT synthesized using the lipase from the corresponding SLCT produced by chemical synthesis.

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KEY WORDS: *Carica papaya* latex, *C. papaya* lipase, hydrogenated soybean oil, interesterification, low-calorie fats, *Rhizomucor miehei* lipase, triacetin, triacylglycerols.

Interesterification is one of the major reactions used by industry for the modification of natural fats and oils. In its simplest form, interesterification corresponds to an exchange of acyl residues between two triacylglycerols (TAG), resulting in the formation of new TAG that have chemical and physical properties distinct from the starting TAG (1). Presently, the interesterification of TAG is conducted either chemically or enzymatically. Chemical interesterifications are generally catalyzed by metal alkoxides, which are relatively inexpensive, readily available, and easy to use. Chemical interesterification produces TAG that have a predetermined composition, but random distribution of fatty acyl groups on the glycerol backbone and are referred to as tailored lipids (TL). On the other hand, because of their unique specificities, the use of lipases allows for the design of TAG with a predetermined composition and distribution of fatty acyl groups on the glycerol backbone (2). Accordingly, TAG prepared by lipase-catalyzed interesterification are referred to as structured lipids (SL). Examples of lipase-catalyzed interesterification (or transesterification) reactions abound in the literature (3–6). However, the applications of lipase-catalyzed interesterification reactions on an industrial scale are limited by the high cost of lipases. Consequently, the use of plant biocatalysts may have advantages owing to their lower cost and ready availability in comparison to their microbial and animal counterparts (7).

Carica papaya latex (CPL) is well known for containing proteases, such as papain, a thiol protease with many industrial applications, e.g., as a meat tenderizer, contact lens cleaner, digestive aid, or bloodstain remover in detergents. This plant exudate, however, also exhibits a lipase activity (8), which several groups have characterized (9–12). For example, in interesterification reactions, it was shown that CPL lipase has an *sn*-3 stereoselectivity (11) and selectivity for short-chain fatty acyl groups (12). Exploiting these CPL selectivities, we recently reported the synthesis of structured low-calorie TAG by interesterification of hydrogenated soybean oil (HSO) with tributyrin (13).

Presently, low-calorie SL are mainly designed for use in selected nutritional applications (14), and as such are characterized by a combination of short-chain (C_{2-4}) and long-chain (C_{16-22}) acyl residues into a single triacylglycerol structure. Interest in this class of lipids stems from the fact that they contain only 5 cal/g (15) compared with the 9 cal/g of natural fats and oils because of the lower caloric content of short-chain acyl residues (C_{2-4}) compared to their long-chain (C_{16-22}) counterparts. The most familiar class of these low-calorie type fats is the so-called Salatrim™ family, which is obtained by chemical interesterification (15). These TL are a mixture of two types of TAG structures; the first contain two short-chain and one long-chain acyl residues (SSL-TAG), and the second contain two long-chain and one short-chain acyl residues (LLS-TAG). By predetermining the fatty acid composition and ratio of SSL- and LLS-TAG, it is possible to produce a range of products useful in several food applications. For example, low-calorie TAG are intended for use in baking chips, coatings, dips, baked products, or as cocoa butter substitutes (16). Here, we describe the synthesis of SL based on CPL lipase-catalyzed interesterification of HSO with triacetin, a reaction that failed using our previously reported procedure (13). The low-calorie SL obtained was similar in composition to the chemically produced TL as determined by

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

high-performance liquid chromatographic (HPLC)–mass spectrometric (MS) analysis of both product mixtures.

MATERIAL AND METHODS

Materials. Crude CPL and triacetin were obtained from Sigma Chemical Co. (St. Louis, MO). The CPL was ground in a mortar to a powder before use. Lipozyme™ IM was a gift from Novo Nordisk (Franklinton, NC). The CPL was conditioned by placing the ground latex into a desiccator over anhydrous calcium sulfate to constant weight. Silica gel (60 Å, 35–75-micron diameter, 7 wt% water content) was obtained from Alltech (Newark, DE) and was conditioned by drying at 100°C for several days to constant weight. HSO (12% C_{16}, 88% C_{18}, iodine value < 5) was a gift from Nabisco Brands (Indianapolis, IN). Salatrim™ (23HSO), a gift from Pfizer Central Research (Groton, CT), was used as a reference standard for gas–liquid chromatography (GLC) and HPLC analysis.

Methods. Interesterification reactions were conducted as follows: triacetin (0.545 g, 2.5 mmol) and HSO (2.20 g, 2.5 mmol) were weighed into a screw-top vial. The mixture was placed in an 80°C water bath to melt the hydrogenated oil. The vial was then placed into a water-jacketed beaker and 70°C water was circulated through the jacket from a constant-temperature bath. CPL (10% wt/wt of lipid) was added to the vial and the mixture was stirred magnetically at 200 rpm throughout the reaction.

Analysis of the reaction mixtures by GLC. Over the time course of the reactions, approximately 5 mg of lipids were removed periodically and added to 4 mL of hexane. The samples were analyzed by GLC as follows: cold on-column capillary injector; a methyl-silicone capillary column (DB1-HT; J&W Scientific, Folsom, CA), 15 m × 0.32 mm i.d.; film thickness, 0.1 μm. The chromatographic conditions were: on-column injection, flame-ionization detection at 370°C, He carrier gas at 5.5 mL min^{-1}. Separations were obtained with the following oven temperature profile: initial temperature 70°C to a final temperature of 350°C at 20°C min^{-1}; final time 4 min.

Analysis of reaction mixtures by HPLC. Over the time course of the reactions, approximately 25 mg of lipids were removed periodically and added to 1 mL of a hexane/chloroform/acetic acid mixture (190:50:1, vol/vol/vol). The samples were filtered through disposable Fluoropore™ poly(tetrafluoroethylene) membrane filters (Millipore, Bedford, MA). An aliquot of the filtrate (100 μL) was diluted in 0.5 mL hexane/methyl-*t*-butyl ether (MTBE)/acetic acid (95:5:0.4, vol/vol/vol) and analyzed by HPLC using a Hewlett-Packard (Wilmington, DE) 1050 series liquid chromatograph with solvent cabinet, autosampler, and quaternary pump modules. A Varex (Burtonsville, MD) model IIA evaporative light-scattering detector was used for analyte detection. Separations were made on a Phenomenex (Torrance, CA) normal-phase cyanopropyl (NP_{CN}) column (250 × 4.6 mm i.d.) with a guard column (30 × 4.6 mm i.d.) of the same phase. A binary mobile-phase gradient of hexane (solvent A) and methyl-*t*-butyl ether (solvent B), each fortified with acetic acid (0.4%), at a flow rate of 1.0 mL/min was used. The previously reported (17,18) gradient was modified for the purpose of separating TAG and di- and monoacylglycerols (DAG and MAG, respectively) from the newly formed mixed short- and long-chain TAG molecules. Separations were obtained with the following solvent gradient profile: initial condition solvent A (100); increase linearly to 90:10 (A/B) over 6 min; increase linearly to 20:80 (A/B) over 10 min; return to solvent A (100) over 6 min.

Analysis of reaction mixtures by HPLC–MS. HPLC–MS analyses were conducted with a Hewlett-Packard 1050 series liquid chromatograph with solvent cabinet, autosampler, and quarternary pump modules. Analyte separations were obtained on a Hewlett-Packard Hypersil 5-μm, 200 × 4.6-mm (NP_{sil}) column. The mobile phase used was hexane/MTBE/acetic acid (82.5:17.5:0.4, vol/vol/vol) at an isocratic flow rate of 0.5 mL/min. Sample volume was 20 μL. The mass spectrometer hardware and system parameters were an online LC–MS model HP5989A quadrupole mass spectrometer coupled to a model HP1084B liquid chromatograph *via* direct liquid atmospheric pressure chemical ionization interface model HP5998A (Hewlett-Packard, Palo Alto, CA), operated in the positive-ion mode. Parameters: EM voltage, 2906; HED voltage, 10^4; scan range 100–1000; threshold, 50; sampling, 2; Quadrupole temperature, 150°C; CapEx, 100; drying gas, N_2, 330°C; nebulizing gas, N_2, 350°C. Computer method: APCI-POS. Full-mass spectra were taken every 0.7 s over the mass range of 100–1000 over the entire elution profile and stored on hard disk. Subsequently, single-ion profiles were recalled from the stored data for peak identification.

RESULTS AND DISCUSSION

In our previous study, the CPL lipase-catalyzed interesterification of HSO with tributyrin gave satisfactory yields of short- and long-chain TAG (SLCT) product (13). In contrast, the reaction between HSO and triacetin under the same conditions was severely limited with regard to the formation of SLCT in that only minor amounts (<1%) of the desired SLCT were formed after 24 h of reaction. Determination of CPL activity for hydrolysis of TAG of increasing chain length from slightly water-soluble triacetin through the partially water-soluble tripropionin, tributyrin, and tricaproin, and the water-insoluble tricaprylin, trilaurin, and triolein corroborated incomplete data from previous studies (9). Results from that work and our own studies showed that hydrolysis was best with tributyrin (100% initial hydrolysis rate), whereas triacetin and triolein were poorly hydrolyzed (<15% hydrolysis, relative to tributyrin). CPL displayed intermediate activity on tripropionin and the other TAG studied. Similar results were observed in CPL-catalyzed interesterification reactions between tricaprylin and a series of TAG ranging in carbon number from C_4–C_{20} (12), but triacetin and tripropionin were not tested.

Tributyrin is soluble in HSO and thus forms a monophasic medium. In contrast, triacetin is immiscible in HSO and

hence presents itself as a biphasic medium to the enzyme. It was assumed therefore that the immiscibility of these substrates was the main reason for poor results obtained in the aforementioned CPL-catalyzed reactions. Ester interchange is known to be limited in biphasic systems due to the lack of a sufficient surface area for the enzyme to react with the substrates. However, ultrasonication of the mixture prior to addition of the enzyme did not result in a distinct improvement. An exchange of CPL with Lipozyme™, an immobilized 1,3-regioselective lipase from *Rhizomucor miehei*, gave similar results in interesterification reactions between triacetin or tributyrin with HSO. Subsequent experiments conducted to improve reaction rates and product yields therefore used Lipozyme™ because of its uniformity with regard to performance and its well-characterized lipolytic and synthetic activities. However, further attempts at optimizing the dispersion of the two-phase triacetin–HSO reaction failed to improve the yields of interesterified SLCT products.

Lipozyme™ had a measured water content of 10% (wt/wt) which accounted for a detectable hydrolysis of HSO in the absence of triacetin (12% after 24 h). As hydrolysis of HSO did not occur to any appreciable extent (<1%) in the presence of triacetin, it was assumed that the latter compound or one of its hydrolysis products might be inhibiting enzyme activity. Addition of a moderately polar solvent, such as MTBE or chloroform, to partially dissolve both substrates improved reaction yields to 8 and 6%, respectively. This indicated that physical incompatibility was one major factor for the low yields of SLCT in the triacetin–HSO interesterification reactions.

Effect of silica on interesterification reactions. The immobilization of organic solvent-insoluble polar substrates onto macroporous materials, such as silica or diatomaceous earth, has been described recently as a way to improve the synthesis of various mono- and diesters of hydrophilic diols (19,20). In our experiments, it was found that the addition of silica-adsorbed triacetin preparations to melted HSO significantly improved the SLCT yields compared to reactions without silica. The porous silica served to physically support the polar triacetin in the interesterification experiments with HSO and thus served as a "polar substrate reservoir." The results suggested a simple mechanism of action of silica. Without silica, the triacetin visibly accumulated on the enzyme particles, leaving the enzyme particles physically entrapped within the triacetin droplets as the inner phase of the two-phase liquid–liquid system, thus creating a polar barrier between the hydrophobic bulk media and the enzyme. This mass transport problem resulted in a significant decrease in SLCT yields.

When silica was added to a blocked reaction between nonabsorbed triacetin and melted HSO after a short period of preincubation with Lipozyme™, an immediate increase in conversion to SLCT products was observed until a yield comparable to that obtained with a preformed silica–triacetin preparation was obtained. This suggested that triacetin has a higher affinity for silica than for the enzyme support. Accordingly, triacetin was removed from the enzyme support allowing the blocked reaction to proceed. However, when silica was added to an inhibited Lipozyme™ reaction after about 24 h of reaction of nonabsorbed triacetin in HSO, an increase in product yield was not observed. This suggested that triacetin or one of its hydrolysis products inhibited the catalytic activity of the enzyme, possibly by removing essential water or by denaturation of the enzyme.

Effect of triacetin concentration on product formation. Mixtures were tested at various mole ratios of HSO to triacetin, e.g., 1:1, 1:2, and 1:4, using an amount of silica equaling the amount of triacetin by weight. As expected, increasing concentrations of triacetin resulted in increased formation of SLCT products (Fig. 1). The higher decrease in HSO substrate compared to SLCT formed was a result of HSO hydrolysis measured as free fatty acids (FFA) and DAG. With increased amounts of silica, there was a concomitant increase in hydrolysis because of the increased amounts of water introduced with the silica.

The 1:1 reaction mixture of HSO and triacetin/silica did not show a distinct increase in viscosity compared to the HSO/triacetin melt without silica. However, the mixtures of HSO and triacetin/silica became much more viscous at a ratio of 1:2, and almost gel-like at a ratio of 1:4. Yet even with the latter mixture, an almost quantitative interesterification of HSO with triacetin could be achieved. Further increases in the triacetin/silica preparation (1:6, 1:8) not only were limited in view of the manageabilility of the mixtures prepared but also led to a decrease in SLCT yields (data not shown). In the latter instance, the volume of the powdered triacetin–silica preparation surpassed the volume of melted HSO. Apparently, diffusion was a limiting factor, as the lipid melt became

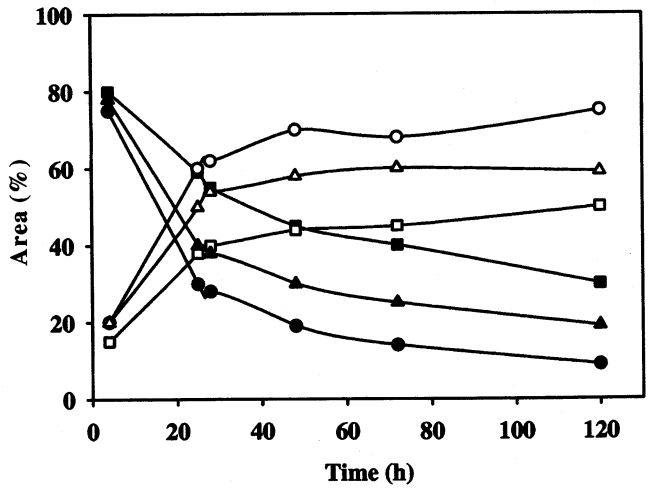

FIG. 1. Effect of molar ratio of reactants on the Lipozyme™-catalyzed interesterification of hydrogenated soybean oil (HSO) with silica-supported triacetin; ■/□ HSO/triacetin, 1:1 (mol/mol); triacetin/silica, 1:1 (wt/wt): ▲/△ HSO/triacetin, 1:2 (mol/mol); triacetin/silica, 2:2 (wt/wt); ●/○ HSO/triacetin, 1:4 (mol/mol); triacetin/silica, 4:4 (wt/wt). Solid markers, substrate (HSO); open markers, products [Σ of short, short, long-chain (SSL) and long, long, short-chain (LLS) triacylglycerols (TAG) formed].

the minor phase while the triacetin–silica powder became the major phase.

Effect of the triacetin/silica ratio on product formation. In other experiments, the mole ratio of triacetin to HSO was increased (experiment number, mol ratio HSO/triacetin: exp. 1, 1:1; exp. 2, 1:2; and exp. 3, 1:4). In each experiment, however, the absolute amount of silica was held constant resulting in weight ratios of silica/triacetin of: exp. 1, 1:0.5; exp. 2, 1:1; and exp. 3, 1:2. Accordingly, experiment 1 contained excess silica and experiment 3 contained less silica than that used in the previous experiments. The data in Figure 2 show a sharp decrease in the formation of newly formed SLCT when going from a HSO/triacetin mole ratio of 1:2 (exp. 2) to 1:4 (exp. 3). This is in contrast to the results shown in Figure 1, where an increase in yield was observed at the higher triacetin concentration (1:4) and constant silica/triacetin weight ratio. This observation suggested that about 1 g of silica was needed to adsorb 1 g of triacetin. This same observation was noted when preparing the silica/triacetin mixtures. At a 1:1 weight ratio, the powder character of the silica did not change, whereas at ratios greater than 1:1, the silica particles were suspended in triacetin, with the nonadsorbed triacetin forming an outer phase resulting in a gel-like structure. The excess triacetin in these mixtures was not a good reaction substrate for lipase-catalyzed interesterification reactions in that lower yields of SLCT were obtained in experiments where insufficient or no silica was used. In contrast, when excess silica was used, the initial conversion to SLCT was comparable to the 1:1 weight ratio reaction. However, the residual amount of water introduced with the excess silica promoted hydrolysis of HSO, even when the silica was oven dried.

Effect of HSO/triacetin mol ratio on SLCT formation. The chemically synthesized SLCT, a product of HSO and acetic and propionic acids, used in this study as reference material, was a mixture of TAG with the SSL-TAG dominating the composition of the product (about 90 wt%). The LLS-TAG were a minor component of the product (about 10 wt%). According to a recent publication (15), this product was made by using a 12-fold excess of short-chain TAG (triacetin and tripropionin) over HSO, followed by the removal of nonreacted substrate at the end of the reaction. The results shown in Figures 1 and 2 were obtained by GLC (13) and therefore show the formation of SLCT as a single product and the decrease of long-chain substrate TAG, respectively. The appearance of newly formed SSL- and LLS-TAG was followed by an HPLC method developed for the separation of neutral lipid and SLCT mixtures (17,18). The chromatographic data for the enzyme-catalyzed product showed that the ratio of LLS- and SSL-TAG was distinctly different from that of the chemically synthesized SLCT (Fig. 3). For example, using a fourfold molar excess of triacetin over HSO resulted in a product mixture containing about 40% SSL-TAG and 25% LLS-TAG after 120 h of reaction. To prepare a SLCT comparable to the composition of the chemically synthesized material, a larger molar excess of triacetin was needed over HSO.

Effect of multiple doses of triacetin on product composition. Instead of Lipozyme™, the following experiments were conducted with CLP to test the influence of multiple doses of triacetin over the time course of the reaction on the yield of SLCT and the ratio of SSL- and LLS-TAG. The initial reaction was started using a twofold molar excess of triacetin over HSO and an equal weight of silica to triacetin. The amount of silica initially added to the medium was not increased with the further addition of triacetin. Additional volumes of triacetin (2 molar equivalents) were added to the reaction after

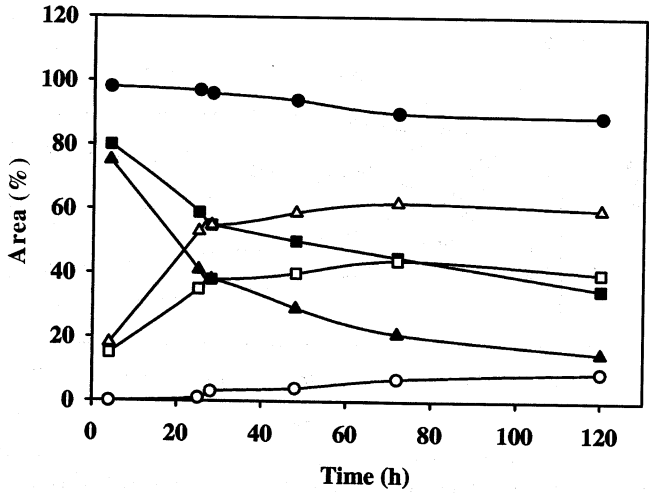

FIG. 2. Effect of triacetin on the interesterification of HSO catalyzed by Lipozyme™. ■/□ HSO/triacetin, 1:1 (mol/mol); silica/triacetin, 1:0.5 (wt/wt); ▲/△ HSO/triacetin, 1:2 (mol/mol); silica/triacetin, 1:1 (wt/wt); ●/○ HSO/triacetin, 1:4 (mol/mol); silica/triacetin, 1:2 (wt/wt). Solid markers, substrate (HSO); open markers, products (Σ of SSL and LLS TAG formed). See Figure 1 for abbreviations.

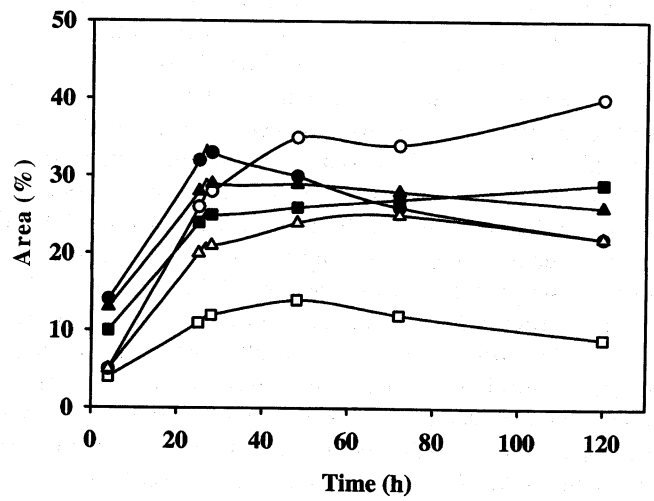

FIG. 3. Effect of triacetin on product composition (SLL- and SSL-TAG) in the interesterification with HSO, catalyzed by Lipozyme™. ■/□ HSO/triacetin, 1:1 (mol/mol); triacetin/silica, 1:1 (wt/wt); ▲/△ HSO/triacetin, 1:2 (mol/mol); triacetin/silica, 2:2 (wt/wt); ●/○ HSO/triacetin, 1:4 (mol/mol); triacetin/silica, 4:4 (wt/wt). Solid markers, LLS_2, open markers, SSL_1 [the subscripts 1 and 2 indicate the elution order of the major short- and long-chain triacylglycerols (SLCT) isomers]. See Figure 1 for abbreviations.

the reaction reached an apparent equilibrium. Initially, it was found that the conversions to SLCT increased with each addition of triacetin (Fig. 4). More importantly, however, it was found that the ratio of SSL- to LLS-TAG increased with each dose of triacetin. A final ratio of SSL- to SLL-TAG of 90:10, comparable to the chemically synthesized product, was obtained after three subsequent additions of triacetin to the initial reaction (final HSO/triacetin mole ratio was 1:8). Weight percentages were calculated from the area percentages for the isomers using calibration curves (17).

Figure 5 (panel A2) shows the NP_{CN}-HPLC chromatogram of the SLCT product obtained from CPL-catalyzed interesterification of HSO with stepwise addition of triacetin. The chromatogram displays the appearance of two sets peaks for each of the two major SLCT products, LLS_1 and LLS_2 (elution time between 5–7 min) and SSL_1 and SSL_2 (elution time between 8–11 min), that are representative for the isomeric sets of SLCT structures. It was this HPLC chromatographic method that was used for optimizing the enzymatic interesterification of triacetin and HSO described above. The chromatogram for the chemically synthesized SLCT reference, which contains acetyl and propionyl residues, is superimposed for comparison (Fig. 5, panel A1).

Separation of the two diacetylated SLCT compounds into four SLCT isomers (elution time between 16 and 21 min) for both lipase-catalyzed (Fig. 5, panel B2) and chemically synthesized SLCT (Fig. 5, A2) products was achieved on a normal-phase nonmodified silica column (NP_{sil}) using a mass spectrometer for detection and characterization. Values for characteristic ions $[M\text{-}RCOO]^+$ were taken from the literature

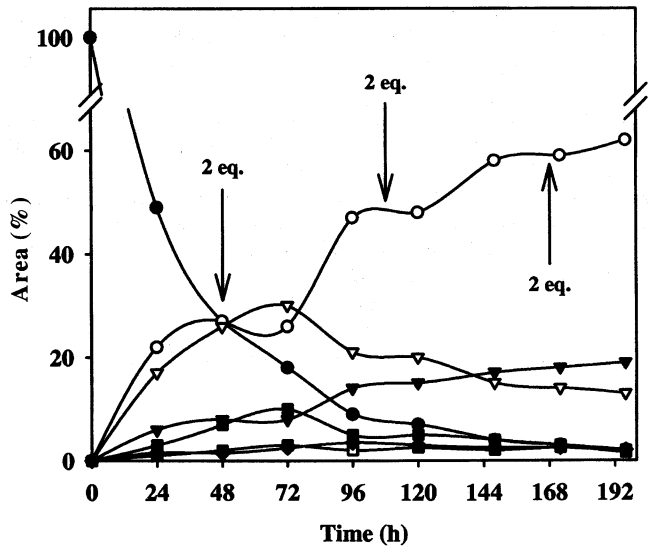

FIG. 4. Effect of multiple doses of triacetin on conversion rates during interesterification of HSO and triacetin (1:2, mol/mol), lipase from *Carica papaya* latex (CPL), and triacetin/silica (2:2, wt/wt). ●, LLL (HSO); ○, SSL_1; ▼, SSL_2; ▽, LLS_1; ■, LLS_2; □, DAG; ◆, free fatty acid (FFA) (the subscripts 1 and 2 indicate the sequence of elution of the respective SLCT isomers: LLS_1, LLS_2, SSL_1, and SSL_2). Arrows: points at which 2 molar equivalents of triacetin were added to the reaction mixture. See Figure 1 for abbreviations.

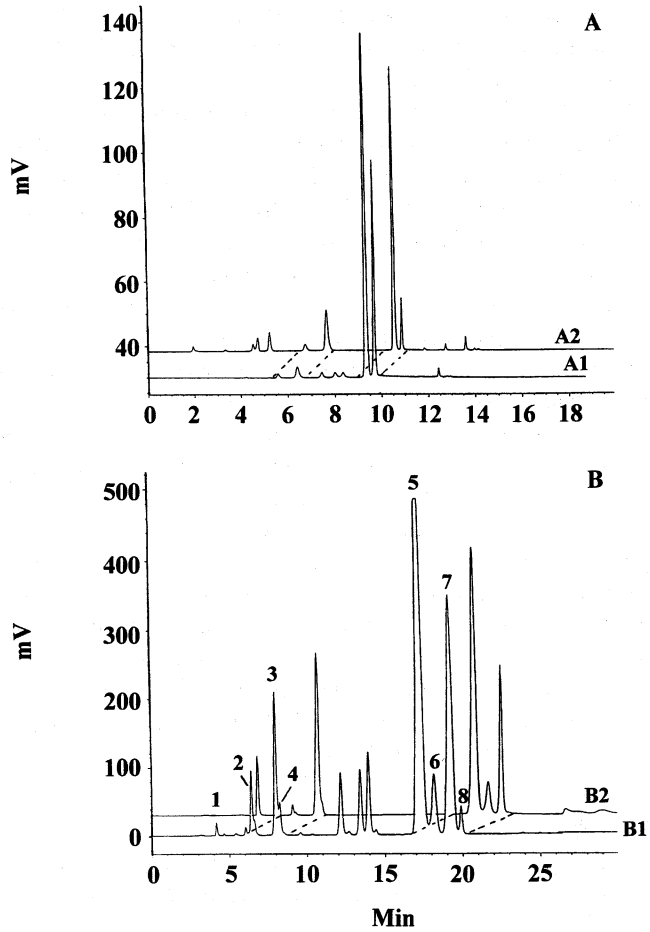

FIG. 5. Panel A, high-performance liquid chromatography with a normal-phase cyanopropyl column (NP_{CN}-HPLC) of the chemically synthesized 23 short- and long-chain triacylglycerols (SLCT) (A1) and the CPL-catalyzed 2SLCT (A2) of HSO and triacetin. Panel B, Hypersil column (NP_{sil})-HPLC of the chemically synthesized 23SLCT (B1) and the CPL-catalyzed 2SLCT (B2) of HSO and triacetin. Abbreviations: 23SLCT, SCLT containing acetyl C_2 and propionyl C_3; 2SLCT, SLCT containing only acetyl groups incorporated into HSO; for other abbreviations see Figures 1 and 4.

(21). Peak numbers (Table 1) correspond to SLCT structures found in Figure 5 (panels B1 and B2). The chromatogram (Fig. 5, B2) displaying the separation of CPL-catalyzed SLCT

TABLE 1
Identification of Peaks in the Final CPL-Catalyzed Product

Peak	R_t (min)	Major ions (*m/z*)	Proposed structure[a]
1	4.85	607, 579	18:0-18:0-16:0
2	7.10	383, 607	18:0-18:0-2:0
3	8.70	383, 607	18:0-2:0-18:0
4	8.90	579, 355	18:0-2:0-16:0
5	18.75	383, 159	18:0-2:0-2:0
6	19.65	355, 159	16:0-2:0-2:0
7	20.48	383	2:0-18:0-2:0
8	20.61	355	2:0-16:0-2:0

[a]Masses for ions $[M\text{-}RCOO]^+$ for the peaks separated by high-performance liquid chromatography with a Hypersil column were calculated for the following diglyceride fragments: *m/z* = 607, DAG 36:0 (18:0-18:0); *m/z* = 579, DAG 34:0 (18:0-16:0); *m/z* = 383, DAG 20:0 (18:0-2:0); *m/z* = 355, DAG 18:0 (16:0-2:0); *m/z* = 159, DAG 4:0 (2:0-2:0); CPL, *Canica papaya* latex; DAG, diacylglycerol.

did not contain the peak at R_t = 20.61 min found in the randomized product of the chemical product 23HSO (Fig. 5, B1), which accounts for the compound with the proposed structure of 2:0-16:0-2:0. For this TAG no fragment ion of the m/z = 159 was found. This result confirmed the nonrandomized and structured nature of the SLCT product of the enzyme-catalyzed interesterification of triacetin and HSO. This conclusion was made because the palmitic acid in soybean oil is not located at the *sn*-2 position and can therefore only be found in the final product either at the *sn*-1 or *sn*-3 position when using a 1,3-regioselective lipase as catalyst.

CPL, known for its proteolytic activity, also should be viewed as a prospective biocatalyst for fat and oil modification. Its interesting lipolytic activity and specificity, as well as its relatively inexpensive price ($25–30/kg) (22) make CPL a potential biocatalyst of choice for various enzymatic processes involving oils and fats. The shortcoming with regard to enzyme inhibition using polar TAG can be overcome by physical adsorption of the polar substrate onto silica. Also, a solvent-free reaction system can be used, which should allow the opportunity to adapt the process to industrial scale. We also found that CPL can be useful in lipase-catalyzed synthesis of low-calorie structured TAG, particularly in the production of nonrandomized SLCT.

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