Solvent-Free Enzymatic Synthesis of Structured Lipids from Peanut Oil and Caprylic Acid in a Stirred Tank Batch Reactor

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ABSTRACT: Structured lipids were synthesized by transesterification of peanut oil and caprylic acid in a stirred-batch reactor. Different substrate molar ratios (1:1 to 1:4, peanut oil/caprylic acid) were used. The reaction was performed for 72 h at 50°C catalyzed by IM60 lipase from *Rhizomucor miehei* (10 g, 2% w/w substrate) in the absence of organic solvent. The highest incorporation of caprylic acid was obtained with a 1:2 molar ratio (peanut oil/caprylic acid) after 72 h reaction. With a 1:2 molar ratio, the incorporation increased by 28% from 1:1. On the other hand, a 1:4 molar ratio gave the lowest incorporation during the reaction. The effect of different mixing speeds (200, 640, or 750 rpm) on reaction was studied with a 1:2 substrate molar ratio for 24 h. A high incorporation of caprylic acid (14.3 mol%) was obtained at 640 rpm, while 200 rpm gave the lowest incorporation (2.2 mol%), suggesting that good mixing is essential in a stirred-batch reactor. After 24 h of reaction at different rpm, IM60 lipase was recovered, washed with hexane, and reacted with substrates to study its stability after reaction at different mixing speeds. The results showed that caprylic acid incorporation was similar (24.9, 24.3, 24.2 mol%) at 200, 640, and 750 rpm, respectively. When 20 g of IM60 lipase (4% w/w substrate) instead of 10 g was used in a 1:1 substrate molar ratio reaction, the incorporation of caprylic acid increased by 26% after 72 h. To study enzyme reuse, 10 g of IM60 lipase was used in a 1:1 substrate molar ratio for 24 h at 640 rpm. The incorporation of caprylic acid gradually decreased with increased number of reuses. During five times of reuse, 15, 13.9, 9.6, 6.7, and 9.7 mol% of caprylic acid were incorporated into peanut oil, respectively.

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KEY WORDS: Caprylic acid, peanut oil, solvent-free, stirred-batch reactor, structured lipids, transesterification.

Georgia is one of the largest producers of peanuts in the United States. Peanut oil, known as groundnut oil, earthnut oil, or arachis oil, has a high content of unsaturated fatty acids such as oleic and linoleic acid. Oleic acid is the major fatty acid in peanut oil, comprising about 14 to 40% depending on the variety (1).

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It is well known that the atherogenicity of edible oil and the degree of saturation in its composite fatty acids are closely related. Unsaturated fatty acids seem to be less cholesterolemic/atherogenic than saturated fatty acids. However, in spite of relatively high unsaturation of composite fatty acids, peanut oil has been known to be unexpectedly atherogenic in some animals (2,3). This atherogenic effect was reduced when randomized peanut oil (same fatty acid composition but different triacylglycerol structures) was fed to male rabbits for 8 wk instead of native peanut oil, suggesting that the atherogenicity of peanut oil may be attributed to the structure of composite triacylglycerols (4). The high amount of very long-chain fatty acids (C20–C24), which are predominantly located at the *sn*-3 position in triacylglycerol, and the high ratio of linoleic-to-oleic acid at the *sn*-2 position also accounts for their atherogenicity (5,6).

Structural modification of triacylglycerol can be achieved enzymatically with several advantages over chemical modification. Through enzymatic transesterification, it is possible to incorporate a desired acyl group onto a specific position of the triacylglcerol, whereas chemical transesterification does not possess this regiospecificity due to the random nature of the reaction (7).

Medium-chain fatty acids (MCFA) have several distinctive characteristics such as high oxidative stability, low viscosity and melting points, and high solubility in water. MCFA are metabolized mainly *via* the portal vein and provide quick energy (8). Thus structural and compositional changes in triacylglcyerols of peanut oil through transesterification with MCFA can alter the physiological effects of peanut oil.

In this study, structured lipids were synthesized by a solvent-free enzymatic transesterification of peanut oil and caprylic acid. The reactions were performed in a stirred tank batch reactor in quantities of 500 g. The effects of different substrate molar ratios, mixing speeds, enzyme loads, and time intervals are presented in this paper, as well as the results of mechanical stability and reusability of IM60 lipase studies.

EXPERIMENTAL PROCEDURES

Materials. Peanut oil (C.F. Sauer Co., Richmond, VA) and caprylic acid (Sigma Chemical Company, St. Louis, MO)

were purchased. Immobilized lipase, IM60 from *Rhizomucor miehei*, was provided by Novo Nordisk Biochem North America Inc. (Franklinton, NC).

Stirred tank batch reactor. The transesterification reaction was carried out in a 1-L capacity stirred tank batch reactor (Fig. 1). A four-blade stirring propeller was used to facilitate the mixing of the substrates and immobilized lipase. The length and width of each blade were 2 cm and 1 cm, respectively. The dimensions of the tank were 10.5 cm diameter and 15 cm length, respectively. The propeller was placed at a distance of 5 cm from the bottom of the reactor and rotated by stirrer motor (model # SL 2400; Fisher Scientific, Pittsburgh, PA). Agitation speed was controlled by a speed controller (Cole Parmer Instrument Co., Niles, IL). The temperature of the reactor (50°C) was maintained by circulating water in a water bath.

Transesterification reaction. To compare the effect of different substrate molar ratios on transesterification reaction, 500 g of mixed substrates of peanut oil and caprylic acid at different molar ratios (1:1 to 1:4, peanut oil/caprylic acid) were prepared. The solvent-free reaction was performed for 72 h at 50°C with IM60 lipase (10 g, 2% w/w substrate). The mixing speed was 640 rpm.

To compare the effects of different amounts of immobilized lipase on the reaction, 10 g (2% w/w substrate) or 20 g (4%) lipase was used for 72 h. Five-hundred grams of prepared substrate (molar ratio, 1:1) was mixed with lipase at 640 rpm and the reaction was performed at 50°C.

To compare the effect of different mixing speeds (200, 640, or 750 rpm) on the reaction, 500 g of prepared substrate (molar ratio, 1:2) and 10 g lipase were incubated for 24 h at 50°C.

After 24 h of reaction at different rpm, IM60 lipase was recovered, washed with hexane, dried in a desiccator at room temperature, and reacted with substrates to study its stability after being used at different mixing speeds. One gram substrate mixture (peanut oil and caprylic acid) of 1:1 molar ratio was mixed in 3 mL hexane with 100 mg (10%, w/w substrate) of recovered IM60 and incubated at 50°C in an orbital shaking water bath for 24 h at 200 rpm.

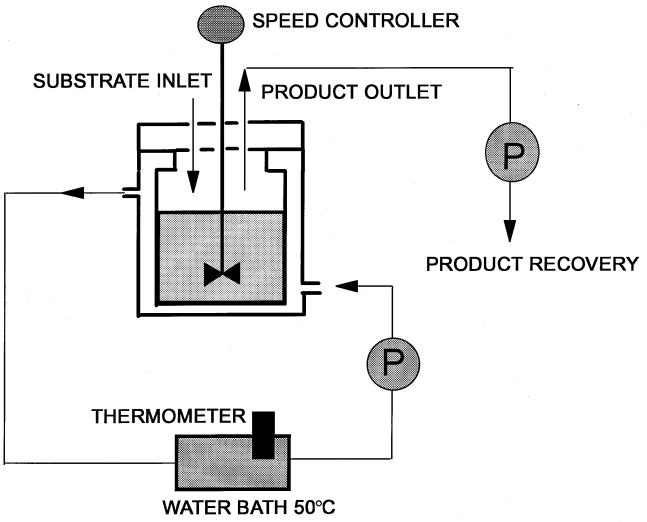

FIG. 1. Schematic diagram of stirred tank batch reactor for transesterification. P, pump.

The mechanical stability of IM60 lipase after repeated reactions (five times) was studied with a 1:1 substrate molar ratio. At 50°C, 10 g of lipase was reacted in a reactor with 500 g of prepared substrate for 24 h at 640 rpm. Fresh substrate was used in every reaction. In all reactions, samples were withdrawn at designated time intervals and analyzed by gas chromatography after thin-layer chromatography (TLC) separation and methylation. The separation and methylation were described in our previous paper (9). Transesterification activities were calculated during 24 h of reaction and expressed in units where 1 unit was defined as 0.01 mol% incorporation of caprylic acid/min. Mol% incorporated caprylic acid was obtained at the end of 120 min of reaction at the initial rates (mol% incorporated C8/min).

Viscosity. The viscosity of peanut oil, caprylic acid, and prepared substrate mixtures with different molar ratios (1:1, 1:2, 1:3, and 1:4) was measured at room temperature with a Brookfield viscometer (Brookfield Engineering Lab, Inc., Stoughton, MA).

GC analysis. For fatty acid composition analysis, a Hewlett-Packard 5890 series II gas chromatograph (GC) equipped with flame-ionization detector (Hewlett-Packard, Avondale, PA) was used. The column and analysis condition were described in our previous paper (9). Peanut oil was analyzed by GC, and its compositions (mol%) were: 5.4, 16:0; 2.3 16:1; 3.3, 18:0; 58.3, 18:1; 22.3, 18:2; 1.3, 18:3; 1.6, 20:0; 2.1, 22:0; and 1.3, 24:0 from which 277 was calculated as an approximate molecular weight.

RESULTS AND DISCUSSION

The viscosity of peanut oil and caprylic acid were 0.0734 and 0.021 kg/m·s, respectively, and the viscosity of each substrate mixture are presented in Table 1. The highest incorporation of caprylic acid was obtained with a 1:2 molar ratio (peanut oil/caprylic acid) after 72 h reaction (Fig. 2). With a 1:2 molar ratio, the incorporation increased by 28% from 1:1. The 1:3 and 1:4 molar ratios led to a decrease in incorporation of caprylic acid. The lowest incorporation was obtained using a 1:4 substrate molar ratio. From the standpoint of reaction efficiency, the ideal substrate molar ratio for the highest incor-

TABLE 1
Content and Viscosity of Substrate Mixtures

Substrate	Content (peanut oil/caprylic acid, g)	Viscosity (kg/m·s)
Peanut oil	500	0.0734
Caprylic acid	500	0.021
1:1 (peanut oil/caprylic acid)	327.8:172.2	0.0513
1:2	244.8:255.2	0.0429
1:3	195:305	0.0373
1:4	162.2:337.8	0.035

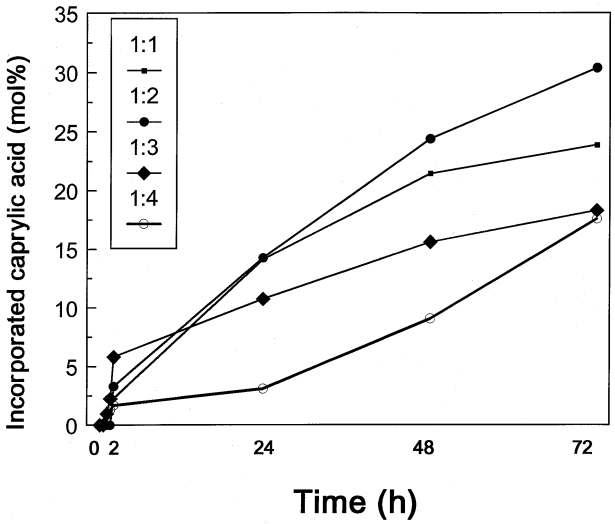

FIG. 2. Progress curve of caprylic acid incorporation at different substrate ratios (peanut oil:caprylic acid) in a batch reactor catalyzed by IM60 lipase.

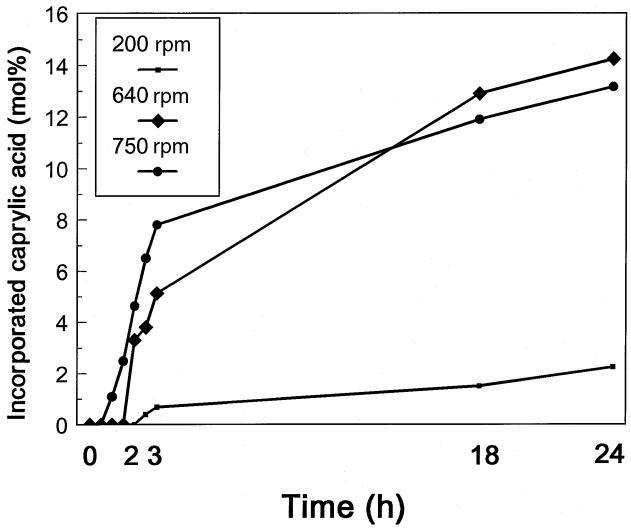

FIG. 4. Effect of stirring speed on caprylic acid incorporation. Molar ratio of substrates (peanut oil:caprylic acid) was 1:2 and the incubation time was 24 h.

poration of caprylic acid into peanut oil was 1:2 for this reaction condition, even though the highest initial rate was obtained with 1:3 molar ratio. Initial rate was calculated as mol% incorporation of caprylic acid/min after 2 h of reaction. Molar ratio 1:4 gave the lowest initial rate (Fig. 3). The results indicated that an increase in concentration above the critical value of caprylic acid in substrate mixture seems to result in a decreased initial rate and incorporation. It may be that the decreased incorporation with a high amount of caprylic acid in the substrate mixture was due to acidic substrate inhibition. High amounts of caprylic acid used as a substrate may inhibit the activities of IM60 lipase during the transesterification reaction.

The reaction mixture, substrates and immobilized lipases, were subjected to continuous mixing for 24 h with different rotational speeds at a fixed viscosity of 0.0429 kg/m·s, in which the substrate molar ratio was 1:2 (peanut oil:caprylic acid). Among the different mixing speeds (200, 640, or 750 rpm), the reaction at 750 rpm gave the highest initial incorporation of caprylic acid. After 24 h of reaction, 14.3 and 13.2 mol% incorporation were obtained at 640 and 750 rpm, respectively (Fig. 4). Incorporation of caprylic acid into peanut oil increased with increasing rotational speed from 200 to 640 or 750 rpm. With 200 rpm, immobilized lipase (IM60) settled to the bottom of the reactor, and was not suspended and mixing well with substrates. We presume that this settlement of immobilized enzyme led to the lowest incorporation of caprylic acid, suggesting that enough rotational speed to keep the enzyme in suspension is essential for a stirred-batch reactor. No further investigation was conducted above 750 rpm because mixing by propeller was too vigorous above that speed.

Although good mixing is essential to catalyzing the reaction, Reese and Ryu (10) suggested that mechanical shear, such as stirring, may denature the catalytic site of enzymes, causing loss of activity. In addition, there is a possibility that lipase can leach out from the support used for the immobi-

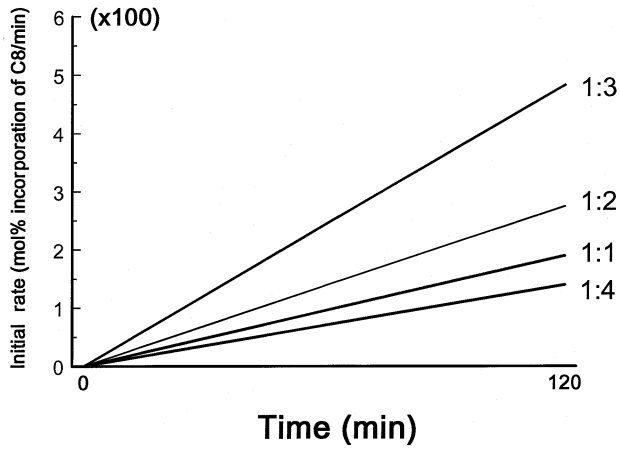

FIG. 3. Initial reaction rate at different substrate ratios (peanut oil:caprylic acid). The amount of IM60 lipase was 2%, w/w substrate.

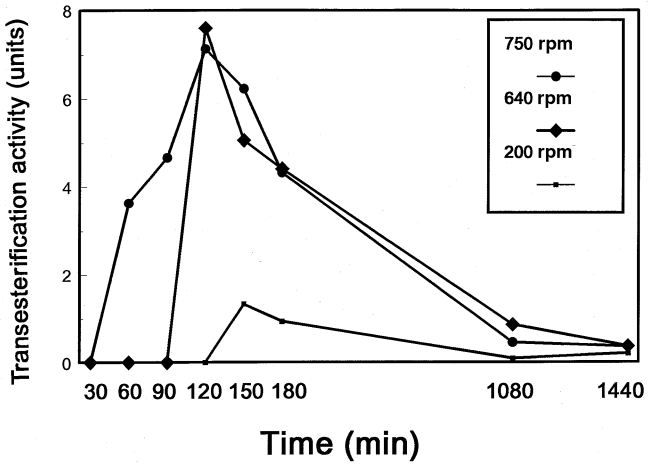

FIG. 5. Change in transesterification activity of IM60 reaction at different time intervals and at different mixing speeds.

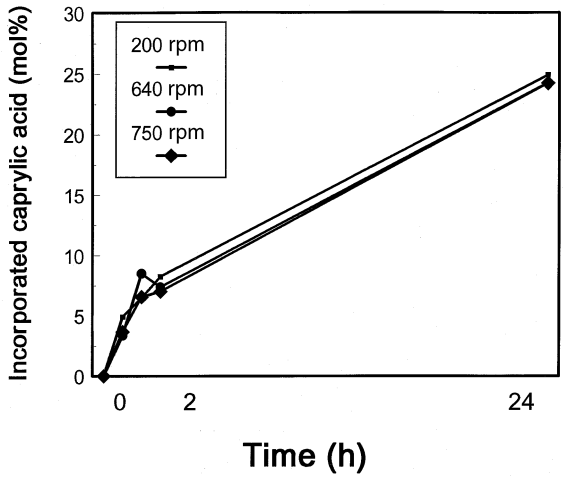

FIG. 6. The effect of rotational speed on IM60 stability after 24 h incubation.

lization. During 24 h of reaction, transesterification activities of IM60 decreased after being sheared for 150 min at a rotational speed of 200 rpm and 120 min at both 640 and 750 rpm. After reacting for 120 min at 640 and 750 rpm, the activities decreased with an increase in reaction time. The lipase activity was not much different at these rotational speeds, suggesting that the loss of IM60 activity was affected more by the shearing time than the rotational speed under this reaction condition (Fig. 5).

Enzyme (IM60) stability was studied after 24 h of reaction at different mixing speeds. After the reaction, IM60 lipase was recovered, washed with hexane, and reacted with fresh substrates again. The results showed that the values of incorporated caprylic acid were similar (24.9, 24.3, 24.2 mol%) after the reaction at 200, 640, and 750 rpm, respectively (Fig. 6). Thus it seems that the increase in stirring speed from 200 to 750 rpm did not have any significant effect on the stability of immobilized enzyme such as enzyme leaching or denaturation under our assay condition.

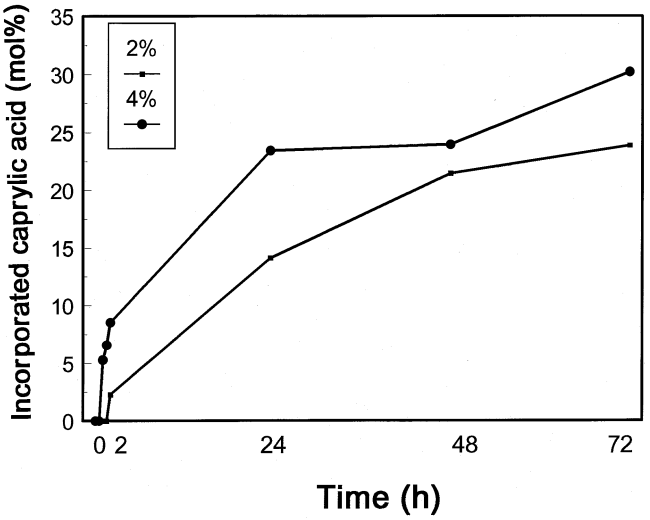

FIG. 7. The effect of enzyme load (2 or 4% , w/w substrate) on caprylic acid incorporation.

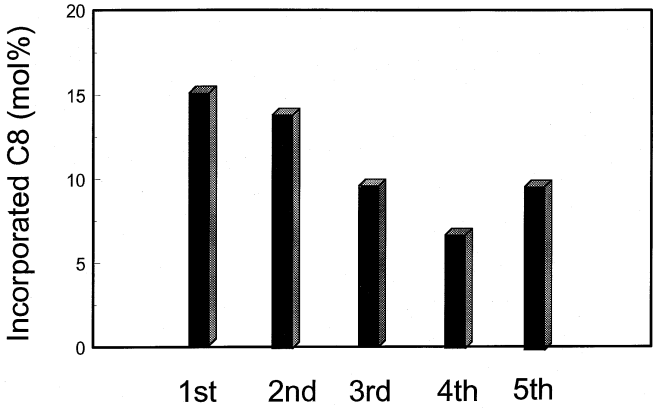

FIG. 8. IM60 stability after reuse. Molar ratio of peanut oil to caprylic acid was 1:1.

As expected, the higher load of IM60 (4% w/w substrate compared to 2%) in the reaction led to higher incorporation of caprylic acid. After 72 h the incorporation had increased by 26%. In the enzyme reusability study, the incorporation of caprylic acid gradually decreased with repeated reuse (Fig. 7). During five times of reuse, 15, 13.9, 9.6, 6.7, and 9.7 mol%, respectively, of caprylic acid were incorporated into peanut oil (Fig. 8). Thus the reaction yield was influenced by the repeated use or prolonged incubation time of immobilized enzyme in the stirred tank batch reactor.

From our results, it appears that IM60 lipase from *R. miehei* immobilized on a macroporous anion exchange resin can be successfully used for solvent-free transesterification reactions under a condition where substrates and the immobilized enzyme are suspended by continuous mixing.

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