Synthesis of Medium-Chain Glycerides Using Lipase from *Candida rugosa*

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ABSTRACT: Enzymatic synthesis of medium-chain glycerides (MCG) from capric acid and glycerol was studied using lipase from *Candida rugosa*. The effects of various reaction parameters such as time, molar ratio of substrates (mmol capric acid/ mmol glycerol), amount of lipase, type of organic solvents, and initial water activity (a_w) were studied. The best conditions tested for MCG synthesis at 37°C were, respectively, time, 24 h; molar ratio of substrates, 2.5; and amount of lipase, 100.0 mg. The use of organic solvents greatly influenced the activity of lipase in the synthesis of MCG. Generally, activity of lipase was high in nonpolar solvents with log *P* values from 3.50 to 4.50, where *P* is the partition coefficient between water and 1-octanol. The enzymatic synthesis of MCG was preferably carried out at an initial a_w of 0.328, which resulted in maximal yield. Analysis of the products of reaction using gas chromatography showed that lipase from *Candida rugosa* seemed to produce more dicaprin and tricaprin than monocaprin.

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KEY WORDS: Esterification, lipase, medium-chain glycerides, solvents, water activity.

Medium-chain glycerides (MCG) are glycerides containing mono-, di-, and triglycerides of caprylic and capric acids (1). Their manufacture involves the hydrolysis of high-grade vegetable oils such as coconut oil, which is rich in medium-chain fatty acids, followed by the fractionation of the resulting fatty acids to concentrate caprylic and capric acids, and reesterification with glycerol to form glycerides. Medium-chain mono-, di-, and triglycerides have unique properties that make them attractive for use in cosmetics, toiletries, the pharmaceutical industry, and human nutrition. They are also used as a solvent or carrier for dyes, flavors, vitamins, and pharmaceuticals and to dissolve gallstones in humans (2).

Industrially, MCG are produced by reacting medium-chain fatty acids and glycerol under elevated temperature and pressure; extensive product purification is required (1). An alternative method is the use of an enzyme as a catalyst whereby the reactions can be carried out at ambient temperature and pressure. The product obtained with an enzyme is usually much purer than that at higher temperatures and pressures.

In this paper, the synthesis of MCG using capric acid and glycerol, an abundant by-product in the oleochemical industry in Malaysia, was carried out using lipase from *Candida rugosa*. The effects of various parameters on esterification reaction were investigated.

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EXPERIMENTAL PROCEDURES

Materials. Lipase from *C. rugosa* (Type VII) and capric acid were purchased from Sigma Chemical Co. (St. Louis, MO). Glycerol was obtained from BDH Supplies (Poole, England) and PORIM (Kuala Lumpur, Malaysia). All other reagents were of analytical grade.

Esterification reaction. Glycerol (1.0 mmol), capric acid (3.0 mmol), hexane (3.0 mL), and lipase from *C. rugosa* (50.0 mg) were mixed in reaction vials. The mixture was incubated at 37°C for 16 h (unless otherwise stated) with continuous shaking at 150 rpm in a horizontal shaker waterbath. The reaction was terminated by dilution with 3.5 mL of ethanol/acetone (1:1, vol/vol). The remaining free fatty acid in the reaction mixture was determined by titration with 0.15 M NaOH in an automatic titrator (ABU 90, Radiometer, Copenhagen, Denmark) to an end point of pH 9.5. The percentage conversion for each investigated time was expressed as number of moles of capric acid consumed as a percentage of moles of initial capric used.

Effect of organic solvent. The effects of various organic solvents in the reaction mixture were investigated. The percentage conversion for the indicated solvent was expressed as moles of capric acid consumed as a percentage of moles of initial capric used.

Effect of molar ratio of substrates. The reaction mixture containing lipase (50.0 mg) was incubated with different molar ratios of substrates (3 mmol capric acid/*n* mmol glycerol). The percentage conversion for each investigated molar ratio was expressed as the number of moles of capric acid consumed as a percentage of the number of moles of initial capric used.

Effect of amount of enzyme. The effect of increasing the amount of enzyme used in the MCG synthesis was studied. The percentage conversion for each investigated amount of enzyme was expressed as the number of moles of capric acid consumed as a percentage of the number of of moles of initial capric used.

Effect of initial water activity (a_w). Lipase, hexane, and substrates were pre-equilibrated with the vapor of saturated salt solutions with different a_w values at 25°C in separate containers. The salts used were LiCl (a_w = 0.113), $MgCl_2 \cdot 6H_2O$

(a_w = 0.328), $Mg(NO_3)_2 \cdot 6H_2O$ (a_w = 0.529), KI (a_w = 0.689), NaCl (a_w = 0.753), and KCl (a_w = 0.843). The reaction was started by mixing the reaction mixture and enzyme followed by incubating at 37°C for 16 h at 150 rpm. The percentage conversion for each investigated a_w was expressed as the number of moles of capric acid consumed as a percentage of the number of moles of initial capric acid used.

Identification of product composition. The esterification reaction was carried out at optimal test conditions. These were time, 24 h; molar ratio of stubstrates, 2.5; and amount of lipase, 100.0 mg.

Products of the synthetic reaction were examined using thin-layer chromatography (TLC) on precoated silica gel plates (Merck, Darmstadt, Germany). The mobile phase of TLC consisted of chloroform/acetone (9.5:0.5, vol/vol).

In the gas chromatographic analysis, the organic phase of the reaction mixture was withdrawn and diluted with isooctane and internal standard (tricaprylin). The mixture was injected into a gas chromatograph (Shimadzu 9A, Kyoto, Japan) equipped with an Rtx-65TG column (0.32 mm × 30 m) from Restek Corporation (Bellefonte, PA). Helium was used as carrier gas with flow rate 50.0 mL/min. The split ratio used was 50:1. The injector and detector temperatures were set at 320°C. The initial column temperature was 150°C, and the final temperature was 280°C. The amounts of product formed were determined using the internal standard method. The mole percentage of the products formed was expressed as a percentage of the number of moles of initial capric acid used.

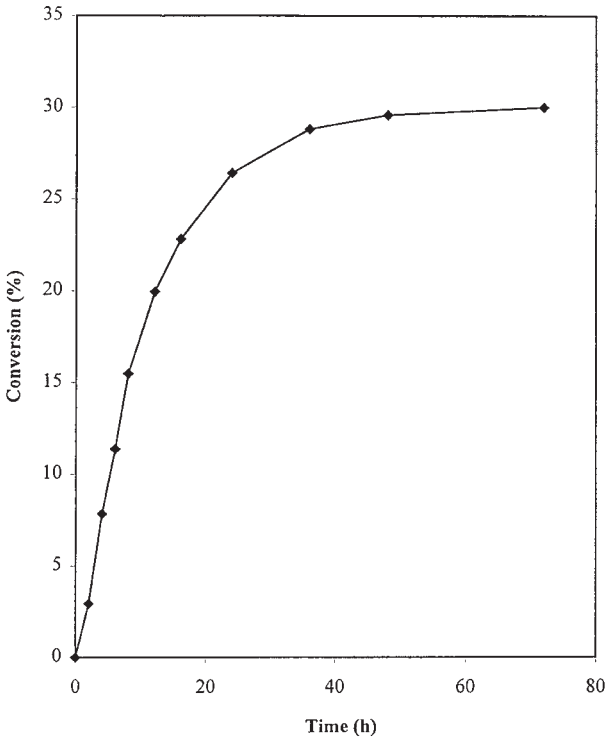

FIG. 1. Time course of the synthesis of medium-chain glycerides. The reaction mixture consisted of glycerol (1.0 mmol), capric acid (3.0 mmol), hexane (3.0 mL), and lipase (50.0 mg). The percentage conversion for each investigated time was expressed as moles of capric acid consumed in the reaction as a percentage of moles of initial capric acid used.

RESULTS AND DICUSSION

Time course of the synthesis of MCG. Figure 1 shows the time course of enzymatic synthesis of MCG in hexane catalyzed by lipase from *C. rugosa*. The percentage of conversion increased with increasing reaction time. The initial rate of esterification continued from 0 until 16 h. Beyond that time, there was a slower rate of increase in the percentage conversion. In the enzymatic esterification, the first synthetic product expected is monocaprin. Thereafter, monocaprin will be converted to dicaprin, which is subsequently converted to tricaprin. It is believed that dicaprin did not easily convert to tricaprin (3). It is not clear why the final reaction step in synthesis is slower than those of former steps. After 24 h, the percentage conversion remained constant, probably because water was produced and acted as a substrate in the hydrolysis of the acyl-enzyme intermediate (4).

Effect of organic solvent. The effect of various organic solvents on MCG synthesis is shown in Table 1. Lipase is more active in the nonpolar organic solvents, such as hexane, isooctane, *n*-octane and *n*-heptane, than the polar ones. Nonpolar organic solvents with log *P* (the partition coefficient of a given solvent between water and 1-octanol) values >2.00 proved to be the best solvents. Similar results were reported by Laane *et al.* (5). No activity was detected in acetone, whereas a relatively lower activity was detected in polar organic solvents. This may be due to the ability of the more polar solvents to strip off the water layer from enzyme molecules (6). This water layer around the enzyme molecule greatly influences the dynamic and catalytic properties of lipase (7). The lower lipase activity in *n*-decane (log *P* = 5.60) may be due to the relatively high viscosity of the solvent, which hinders efficient interaction between the catalyst and the substrates (8). A relatively low activity (1.10%) was detected when no solvent was used in this study. The presence of an organic solvent is necessary to solubilize capric acid and decrease its viscosity in order to achieve the esterification reaction. Organic solvents also increased the mixing of the two-phase system. In this study, hexane and isooctane seemed to be the best solvents for the esterification reaction.

TABLE 1
Effect of Various Solvents on the Esterification Reaction

Solvent	log P^a	Conversion^b (%)
No solvent	—	1.10
Acetone	–0.23	0.00
Ethyl acetate	0.68	2.06
Chloroform	2.00	6.52
Hexane	3.50	22.81
Heptane	4.00	21.02
Isooctane	4.20	22.96
n-Octane	4.50	21.69
n-Decane	5.60	14.42

[a]From Laane *et al.* (5).
[b]Conversion (%) for the indicated solvent is expressed as moles of capric acid consumed in the reaction as a percentage of moles of initial capric acid used.

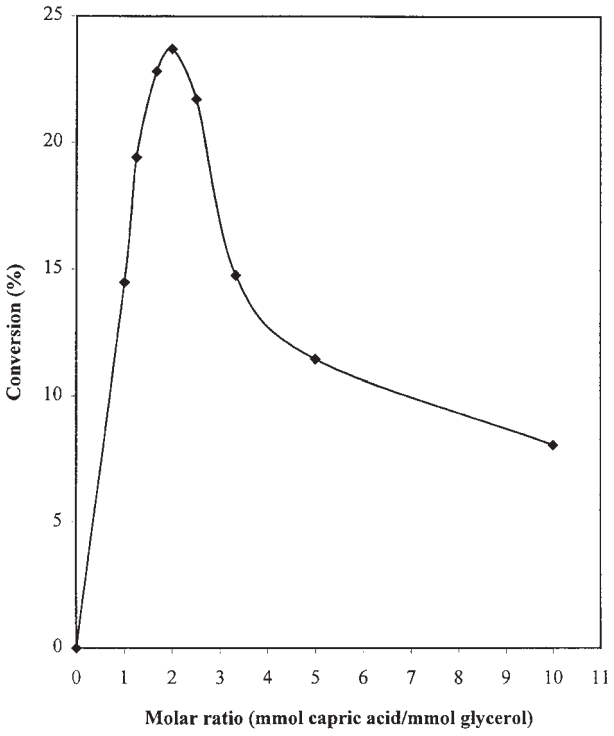

FIG. 2. Effect of molar ratio of substrates (capric acid, 3 mmol/glycerol, *n* mmol) on the esterification reaction. The reaction mixture consisted of substrates, hexane (3.0 mL), and lipase (50.0 mg). The percentage conversion for each investigated molar ratio is expressed as moles of capric acid consumed in the reaction as a percentage of moles of initial capric acid used.

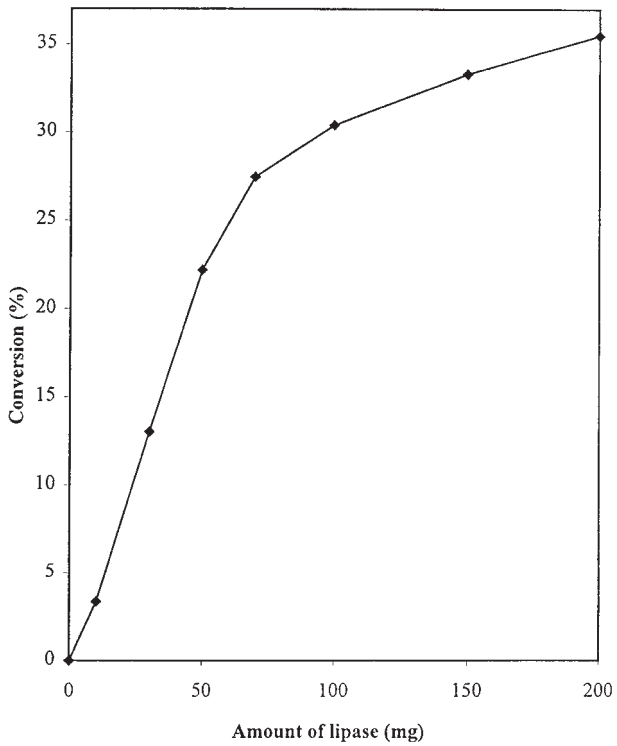

FIG. 3. Effect of amount of enzyme on the esterification reaction. The percentage conversion for each investigated amount of enzyme is expressed as moles of capric acid consumed in the reaction as a percentage of moles of initial capric acid used.

Effect of molar ratio of substrates. The effect of molar ratio of substrates in the reaction mixture on MCG synthesis by lipase from *C. rugosa* was investigated (Fig. 2). The optimal molar ratio of capric acid to glycerol was 2.5, and conversion decreased above the ratio. Excess glycerol in the reaction mixture seemed to inhibit the activity of the enzyme. During this experiment, high volumes of glycerol caused high solubility of enzyme in glycerol, and thus decreased the lipase concentration at the interface, probably decreasing the reaction rate. The inhibition by the alcohol substrate was also reported by Basri *et al.* (9) and Claon *et al.* (10).

Effect of amount of enzyme. Figure 3 shows the effect of the amount of enzyme on MCG synthesis. As expected, MCG synthesis increased when the amount of lipase was increased. No significant increase in MCG yield was observed for lipase loading of more that 100.0 mg.

Effect of initial water activity (a_w). The effect of initial a_w on MCG synthesis is shown in Figure 4. In this study, hexane was preequilibrated separately with reaction mixture (capric acid and glycerol) to ensure that at equilibrium, the a_w value would be the same for all phases present. Lipase activity exhibited a characteristic bell-shaped curve. Wehtje and Adlercreutz (11) reported a similar finding although the substrates used were different. They concluded that the a_w profile (bell-shaped form) of a lipase was not affected by the type of reaction used. However, they also reported that immobilized lipase from *C. rugosa* showed an optimum at a_w = 0.53. In our experiment, native lipase from *C. rugosa* seemed to require a lower optimum a_w (0.328). Immobilization of the lipase can affect the a_w profile, as reported by Bovara *et al.* (12). At a_w higher than 0.529, the activity of lipase was sharply decreased (Fig. 4). This may have resulted from the hygroscopicity of glycerol. At high a_w, glycerol absorbed a lot of water during initial a_w equilibration. High water content causes a decrease in glycerol viscosity. More lipase will be dissolved in the glycerol, and this may decrease the concentration of lipase at the interfacial area, therefore decreasing the rate of reaction. As water is continuously produced during the esterification reaction, it can give rise to an increase in the a_w of the reaction mixture (13) and causes a gradual shift of reaction equilibrium in favor of hydrolysis as the reaction proceeds.

Product composition analysis. A typical gas chromatogram of the product mixture is shown in Figure 5. The reaction was carried at the optimal conditions (incubation period, 24 h; molar ratio of substrates, 2.5; and amount of lipase, 100.0 mg. The organic solvent and a_w used were isooctane and 0.38, respectively), and the products of the reaction were determined by the internal standard method. Peak A is the solvent (isooctane), and peak B represents the capric acid remaining in the mixture. Peak E is the internal standard (tricaprylin). Peaks C, D, and F are the products of the reaction, which are monocaprin, dicaprin, and tricaprin, respectively. The remaining mole percentage of free capric acid was deter-

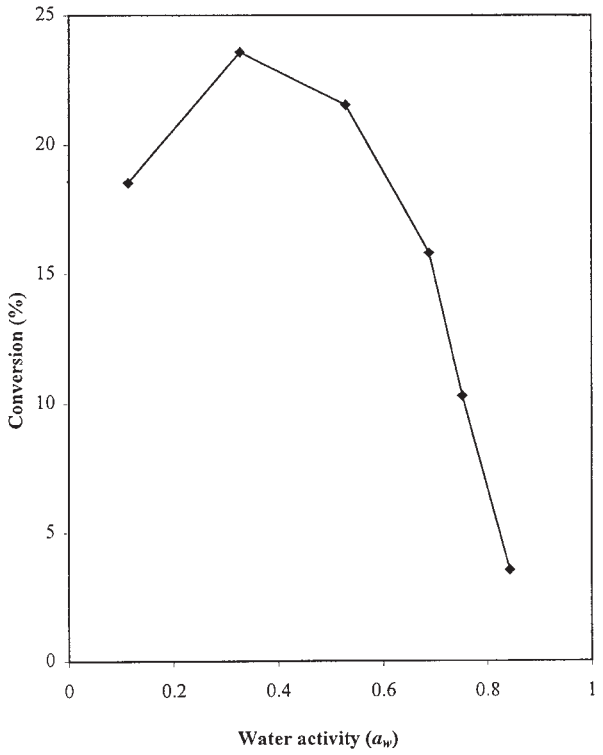

FIG. 4. Effect of initial water activity a_w on the esterification reaction. The percentage conversion for each investigated a_w is expressed as moles of capric acid consumed in the reaction as a percentage of moles of initial capric acid used.

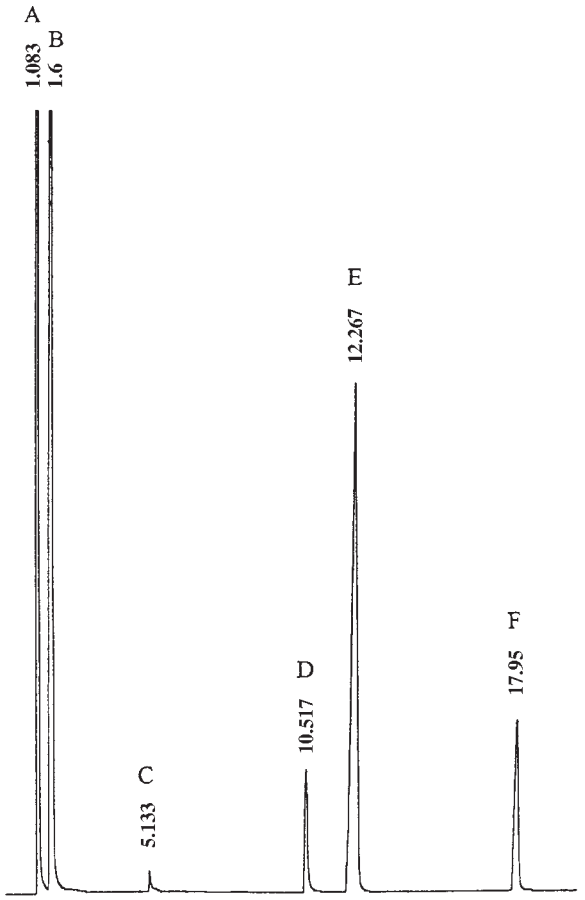

FIG. 5. Gas chromatogram of esterification of capric acid with glycerol. Symbols: isooctane (A), capric acid (B), monocaprin (C), dicaprin (D), tricaprylin (internal standard) (E), and tricaprin (F). For gas chromatographic conditions see the Experimental Procedures section.

mined to be 66.76%, and thus 33.24% of the capric acid was converted to MCG. The percentages of monocaprin, dicaprin, and tricaprin produced were 0.99, 14.97, and 17.28%, respectively. These observations suggest that lipase from *C. rugosa* could be used for the production of dicaprin and tricaprin. Similar results were reported by Kwon *et al.* (3).

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