Lipase Biocatalysis in the Production of Esters

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ABSTRACT: Lipase biocatalysis was investigated as a tool for the production of butyl oleate and rapeseed oil 2-ethyl-1-hexyl ester by esterification and transesterification, respectively. We screened 25 commercially available lipases and found that butyl oleate was produced at high yields from oleic acid and 1-butanol by lipases from *Candida rugosa, Chromobacterium viscosum, Rhizomucor miehei, and Pseudomonas fluorescens.* The initial water content of the system, lipase quantity, and the molar ratio of 1-butanol to oleic acid were important factors in influencing the ester yield. In general, no ester was formed without the addition of water. The exception was *Ch. viscosum* lipase, which yielded 98% of ester in 12 h with 1-butanol excess without additional water. The addition of 3.2% water increased the initial rate of reaction. With an oleic acid excess and only 0.3% lipase, *C. rugosa* and *R. miehei* lipases yielded 94 and 100% esters with initial water contents of 3.2 and 14%, respectively. Lipase-catalyzed alcoholysis of low-erucic acid rapeseed oil and 2-ethyl-1-hexanol without additional organic solvent also was studied in stirred batch reactors. In this case, *C. rugosa* lipase was the best biocatalyst with an optimal 2-ethyl-1-hexanol to rapeseed oil molar ratio of 2.8, a minimum of 1.0% added water, and 37°C. An increase in temperature up to 55°C increased the rate of reaction but did not affect the final ester yield. The enzyme was inactivated at 60°C. Under optimal conditions, the ester yield increased from 88% in 7 h to nearly complete conversion in 1 h when the lipase content was increased from 0.3 to 14.6%. In a 2-kg small pilot scale, up to 90% conversion (97% of theoretical) was obtained in 8 h at 37°C with 3.4% lipase in the presence of Amberlite XAD-7 resin with 3% added water.
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KEY WORDS: Alcoholysis, biocatalysis, butyl oleate, *Candida rugosa,* enzyme, esterification, 2-ethyl-1-hexanol, 2-ethyl-1-hexyl ester, lipase, rapeseed oil, *Rhizomucor miehei,* transesterification.

There is an increasing interest in obtaining biodegradable esters that will be useful as lubricants, biodiesel, surface-active agents, solvents, etc., from vegetable oils. The benefits of employing lipase biocatalysis in ester production are obvious (1–4). Consequently, lipase biocatalysis has been intensively studied during recent years (5,6). Lipases (triacylglycerol acylhydrolase, EC 3.1.1.3) catalyze the hydrolysis and synthesis of glycerol esters. In transesterification, the acyl moiety is exchanged either between ester and acid (acidolysis), ester and alcohol (alcoholysis), or two esters (acyl exchange) (7,8). Complete transesterification between one mole of triacylglycerol and three moles of alcohol yields three moles of ester and one mole of glycerol. Acyl exchange between two molecules is also called interesterification, and between two acyl groups within a molecule it is called intraesterification.

Recently, special attention has been paid to lipases in food-related lipid modifications (9,10), lipase-catalyzed ester synthesis (6,11,12), transesterification (5,6,13–17), biodegradable polyesters (18,19), fatty acids, glycerol, and mono- and diglycerides (6,20), etc. Lipase-catalyzed ester synthesis and alcoholysis in the absence of a solvent are important in industrial applications, especially for food uses. Although it has been claimed that the presence of additional organic solvent may be useful, for example in controlling water activity and microbial contamination (21,22), the absence of solvent allows higher substrate and product concentrations (23), simplifies downstream processing (24), and improves safety (25). Nevertheless, enzymic ester synthesis and alcoholysis of vegetable oils without an additional organic solvent have not been investigated that extensively. Ishii *et al.* (26) studied the esterification of oleic acid with various alcohols in an aqueous system using *Rhizopus oligosporus* lipase. Mittelbach (27) employed *Pseudomonas fluorescens* lipase to study the alcoholysis of sunflower oil, both in petroleum ether as solvent and without an additional solvent for the biosynthesis of methyl and ethyl esters as diesel oil substitutes. Shaw *et al.* (28) used *P. fluorescens* lipase immobilized on Celite as a biocatalyst for the alcoholysis of olive oil. Trani *et al.* (24) used Lipozyme IM-20 (Novo Nordisk, Bagsvaerd, Denmark), a *Rhizomucor miehei* lipase immobilized on a weak anion exchange resin, to produce stearyl oleate by transesterification of triolein and stearyl alcohol, and erucyl erucate, the main component of jojoba oil, by transesterification of rapeseed oil and erucyl alcohol.

The aim of the present work is to develop environmentally friendly processes that are based on lipase biocatalysis for the production of butyl oleate and rapeseed oil fatty acid esters of 2-ethyl-1-hexanol. Butyl oleate is useful as biodiesel additive, polyvinylchloride (PVC) plastisizer, water-resisting agent, and in hydraulic fluids. Rapeseed oil fatty acid esters of 2-ethyl-1-hexanol can be used as a solvent for printing ink and car washing detergent.

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MATERIALS AND METHODS

Materials. 1-Butanol was obtained from E. Merck (Darmstadt, Germany), and oleic acid from Eastman Kodak (Rochester, NY). Refined, low-erucic acid rapeseed oil and synthetic rapeseed oil 2-ethyl-1-hexylester were obtained from Raisio Group (Raisio, Finland). The approximate fatty acid composition of the oil was 57% oleic acid, 22% linoleic acid, 12% linolenic acid, 4% palmitic acid, 2% eicosaenoic acid, 1% stearic acid, <1% erucic acid, and 1% others. 2-Ethyl-1-hexanol (water solubility at 25°C *ca.* 2.5%) was obtained from Fluka Chemie AG (Buchs, Switzerland). Mono-, di-, and triolein standards were from Sigma (St. Louis, MO), and glycerol from May & Baker (Dagemham, United Kingdom).

Enzymes. The following powdered microbial lipases were obtained from Biocatalysts Ltd. (Pontypridd, United Kingdom): *Candida rugosa* (ex. *cylindracea*) (42,500 U/g; water 5.0%), *Chromobacterium viscosum* (13,300 U/g; water 5.9%), *R. miehei* (7,200 U/g; water 7.4%), and *P. fluorescens* (11,900 U/g; water 3.1%).

1-Butyl oleate synthesis. 1-Butyl oleate syntheses were carried out at 37°C in 8 mL screw-capped flasks containing varying quantities of different lipases and the substrates at different molar ratios, with or without various quantities of added water. The contents were stirred with a magnetic stirrer at 200 rpm. At a given time, the reaction was stopped with a mixture (1:1, vol/vol) of diethyl ether and ethanol, and the free oleic acid was titrated with 0.1 M sodium hydroxide. The yield, in %, was calculated from oleic acid consumed on the basis of the limiting substrate.

Transesterification. A preliminary study with the four lipases (10 mg; 3.3%) was carried out with 0.680 mmol (107 µl) 2-ethyl-1-hexanol and 0.227 mmol (*ca.* 0.2 g) rapeseed oil (molar ratio 3) in capped, 13-mL test tubes under magnetic stirring at 200 rpm, 3.0% added water. Transesterification was allowed to continue for 72 h, after which lipase was separated by centrifugation for 5 min at 2000 rpm (Type UJ3; Martin Christ, Osterode, Germany), and the supernatant was pipetted into Eppendorf tubes for storage at –20°C and later analysis. Further transesterification reactions were carried out for up to 72 h with varying substrate molar ratios (ethyl hexanol to rapeseed oil from 10 to 0.5), *C. rugosa* lipase (from 0.3 to 14.6%) added water (from none to 50%), and temperatures (from 37 to 60°C). In the semi-pilot-scale experiments, the reaction mixture contained 25 mL (20 g) to 1.0 L (829 g) 2-ethyl-1-hexanol and 50–2000 g rapeseed oil (substrate molar ratio of 2.8), 3.4% (w/w) lipase, and 1–5% (w/w) added water. The reaction was carried out for up to 5 h at 37°C in a stirred vessel of varying shape, either with free powdered lipase or with enzyme in the presence of various carriers. Glass beads (20 g/100 g rapeseed oil and 5 g lipase), polyurethane foam (0.8 g), and adsorption resins Amberlite XAD-2 (2.5 g) and Amberlite XAD-7 (Rohm and Haas Nordiska AB, Bromma, Sweden) (2.5–15 g) were used as carriers.

Lipase activity. Lipase activity was determined according to the Biocatalysts Ltd. assay method *Lipase Assay* (29), which is based on the hydrolysis of 50% (vol/vol) olive oil emulsion (Product No. 800-1; Sigma Chemical Company) as substrate at pH 7.7, 37°C in one hour. The quantity of free fatty acids formed was titrated with 0.1 M sodium hydroxide. One unit of lipase activity was defined as the quantity of enzyme that catalyzes the release of one µ mole of free fatty acid from olive oil in one minute at these conditions.

Analytical methods. Qualitative analyses were carried out by thin-layer chromatography (TLC). Samples were diluted 1:10 (vol/vol) with ethanol, and 0.01 mL of the diluted samples was used for TLC analysis. Hexane/diethyl ether/acetic acid (80:20:1) was used as solvent on Kieselguhr 60 F_{254} plates (E. Merck, Darmstadt, Germany) with a 1-h running time. Slightly dried plates were sprayed with 0.1% 2′,7′-dichlorofluorescein (Aldrich-Chemie, Steinheim, Germany) in 99.5% ethanol (Alko Ltd., Finland) for detecting spots at 254 and 360 nm.

Rapeseed oil conversion (% rapeseed oil used) and ester yield (% of theoretical) were determined by reversed-phase high-performance liquid chromatography (HPLC) as modified from El-Hamdy and Perkins (30) and Forssell *et al.* (10), employing a Perkin-Elmer (Norwalk, CT) 4-pump module, ISS-100 sampler, and 101 oven, Novapack C18 3.9 × 150 mm column with 4 µm silica particles, HP 1047A refractive index detector, PE 316 integrator, and PE 7500 professional computer. The running solvent was acetone/acetonitrile (1:1) at 1.0 mL/min, 37°C, 30 min. Residual 2-ethyl-1-hexanol could not be determined by the HPLC method because the alcohol overlapped with the solvent peak. Consequently, any excess 2-ethyl-1-hexanol was determined by TLC as described previously. Samples were diluted with acetone to 10 to 20 mg/mL, filtered through a Millex-LCR4 disposable filter of 0.5 µm porosity (Millipore, Bedford, United Kingdom), and 0.02 mL of the filtrate was used for the analysis.

Moisture content of the enzyme preparations was determined by drying about 4-g samples overnight at 105°C.

RESULTS AND DISCUSSION

Effect of type of lipase on esterification and transesterification. Commercial lipases are sold on the basis of their hydrolytic activity, and there is no guarantee regarding their esterification activity. There does not appear to be any correlation between the hydrolytic and synthetic activity and, as has been shown recently, even lipases from different fermentation batches of a similar hydrolytic activity may vary widely in their esterification activities (31). Consequently, it is necessary to screen lipases for their desired synthetic activity under various processing conditions. Preliminary experiments were carried out with 25 commercial lipases for *n*-butyl oleate synthesis (12) to identify the most suitable enzymes for subsequent esterification and transesterification trials. Table 1 summarizes the results. Under the screening conditions, *C. rugosa, Ch. viscosum, R. miehei,* and *P. fluorescens* lipases exhibited the highest ester synthesis activity, resulting in

TABLE 1
Screening for Butyl Oleate Synthesis with Different Lipases[a]

Lipase from	Yield (%) 2 h	20 h
Achromobacter sp.	13.8	49.2
Alcaligenes sp.	33.1	84.4
Aspergillus niger 1	0.9	3.0
A. niger 2	4.5	66.5
A. niger 3	0.4	5.7
***Candida rugosa* 1**	**62.6**	**85.5**
C. rugosa 2	7.6	48.0
C. rugosa 3	24.7	78.7
C. lipolytica	7.0	9.7
Chromobacterium viscosum	**76.5**	**87.3**
Geotrichum candidum	0.0	1.2
Humicola languinosa	7.6	44.9
Mucor javanicus	6.8	62.3
Penicillium cyclopium	10.0	67.3
P. roqueforti	0.0	0.7
Pseudomonas fluorescens	**55.9**	**87.0**
Rhizomucor miehei 1	4.4	40.0
R. miehei 2	11.7	50.8
***R. miehei* 3**	**47.1**	**84.6**
R. arrhizus	2.8	31.8
R. delemar	6.0	61.2
R. japonicus 1	0.0	1.8
R. japonicus 2	0.2	5.7
R. javanicus	7.1	52.8
R. niveus	2.5	5.0

[a]Reaction mixture contained 3.27 mM 1-butanol, 0.7 mM oleic acid, 0.005 g (1.13%) lipase, at 40°C and 200 rpm (lipases selected for further studies are shown in **bold type**).

about 85–87% yield in 20 h with 1.13% enzyme. Consequently, these four lipases were selected for further studies.

A substrate molar ratio of 3, 3.3% of lipase and 3.0% of added water was used in the synthesis of 2-ethyl-1-hexyl esters of rapeseed oil fatty acids. Table 2 shows that the use of *C. rugosa* lipase as biocatalyst resulted in a 98% conversion of rapeseed oil in 24 h with no residual rapeseed oil and few by-products detectable by TLC. Previously, we reported a superior cost/benefit ratio for this lipase in direct biocatalytic

TABLE 2
The Production of 2-Ethyl-1-Hexanol Rapeseed Oil Fatty Acid Ester by Different Lipases[a]

Lipase from	Conversion (%) 24 h	48 h
Candida rugosa	98	98
Chromobacterium viscosum	96	97
Pseudomans fluorescens	96	99
Rhizomucor miehei	45	87

[a]2-Ethyl-1-hexanol to rapeseed oil molar ratio 3.0, 3.3% lipase, and 3% added water.

synthesis of *n*-butyl oleate (13). The use of *P. fluorescens* and *Ch. viscosum* lipases also resulted in relatively high ester production, with 96% conversion in 24 h and 97% conversion or higher in 48 h. As has already been shown for the butyl oleate synthesis, poorest results were again obtained with *R. miehei* lipase. Consequently, *C. rugosa* lipase was chosen for further studies.

Effect of lipase quantity on esterification and transesterification. As could be expected, an increase in lipase quantity markedly increased butyl oleate synthesis and rapeseed oil conversion during the first few hours, but the differences were almost leveled off in about 7 to 12 h. Figure 1 shows that, only with the *R. miehei* lipase, the ester yield increased markedly when the lipase quantity was increased from 0.3 to 1.5%. However, in this experiment, the specific activity of the *R. miehei* lipase was lower than that of the other lipases investigated, and the added water amount of 3.2% was suboptimal for *R. miehei* lipase.

Figure 2 illustrates the rapeseed oil conversion as function of time with 0.3, 3.3, and 14.6% lipase, substrate molar ratio of 2.8, and 3.0% added water. The reaction was nearly complete in 1 h with the highest lipase quantity used, and in 7 h with 3.3% lipase, whereas with 0.3% lipase the conversions in 1 and 7 h were only about 26 and 88%, respectively. Interestingly, Goldberg *et al.* (32) reported that an increase in the quantity of powdered *C. rugosa* lipase resulted in a decrease in the apparent enzyme activity in the production of heptyl

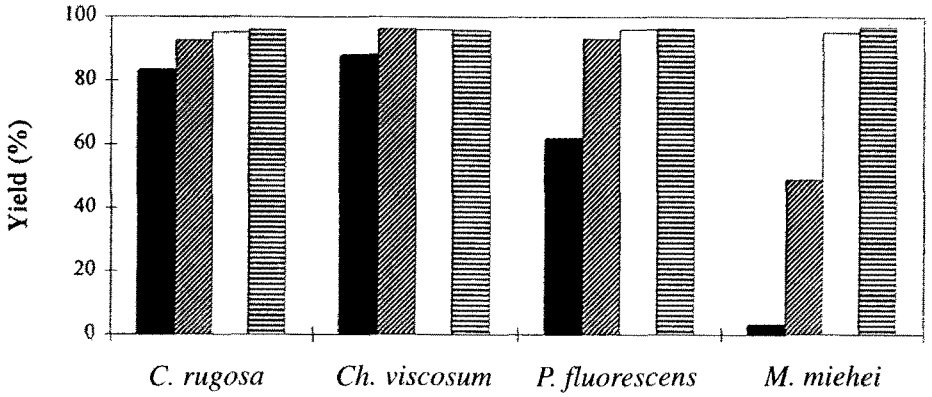

FIG. 1. Effect of lipase quantity (black bar, 0.3%; cross-hatched bar, 0.6%; white bar, 1.5%; and striped bar, 3.2% lipase) on the yield of butyl oleate in 12 h with 1-butanol to oleic acid molar ratio 2 and 3.2% added water.

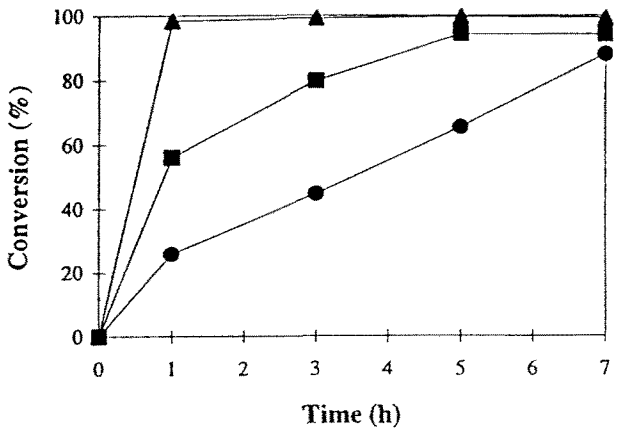

FIG. 2. Time course and the effect of lipase quantity (● 0.3%, ■ 3.3%, and ▲ 14.6% lipase) on the synthesis of 2-ethyl-1-hexyl ester of rapeseed oil fatty acids by transesterification (2-ethyl-1-hexanol to rapeseed oil molar ratio 2.8; and 3% added water).

oleate, owing to an increase in diffusion limitation, a problem that in large-scale experiments may be minimized by optimal biocatalyst and bioreactor design.

Effect of added water on butyl oleate synthesis and transesterification with different lipases. The importance of the control of water content (and of water activity) in lipase-catalyzed esterifications has been emphasized (8,33–35). Although a minimum quantity of water is necessary for enzyme catalysis to take place (36), ester synthesis is favored under restricted water availability (low water activity) (37). Ester synthesis and hydrolysis are reversible processes, and the equilibrium may be shifted toward synthesis either by an excess of one of the substrates or by controlling the water content of the reaction system. By using 1.416 mM 1-butanol, 0.708 mM oleic acid, and 0.3% commercial lipase preparation in butyl oleate synthesis (Fig. 3), about 90% yields were obtained with 3.2% added water by *C. rugosa* and *P. fluorescens* lipases in 30 h; whereas, with the *Ch. viscosum* lipase, a 98% yield was reached in 12 h without any water addition. About 3% of added water also was found optimal for transesterification of rapeseed oil with 2-ethyl-1-hexanol for *C. rugosa* lipase and Amberlite XAD-7 resin as carrier. *Rhizomucor miehei* lipase showed little activity for butyl oleate ester synthesis at 3.2% water, but with 14% added water, a 90% ester yield was obtained in 24 h (Fig. 3). With an oleic acid excess and 3.2% added water, *C. rugosa* lipase showed an excellent ester yield of 87% in 2 h and 94% in 12 h. Again,

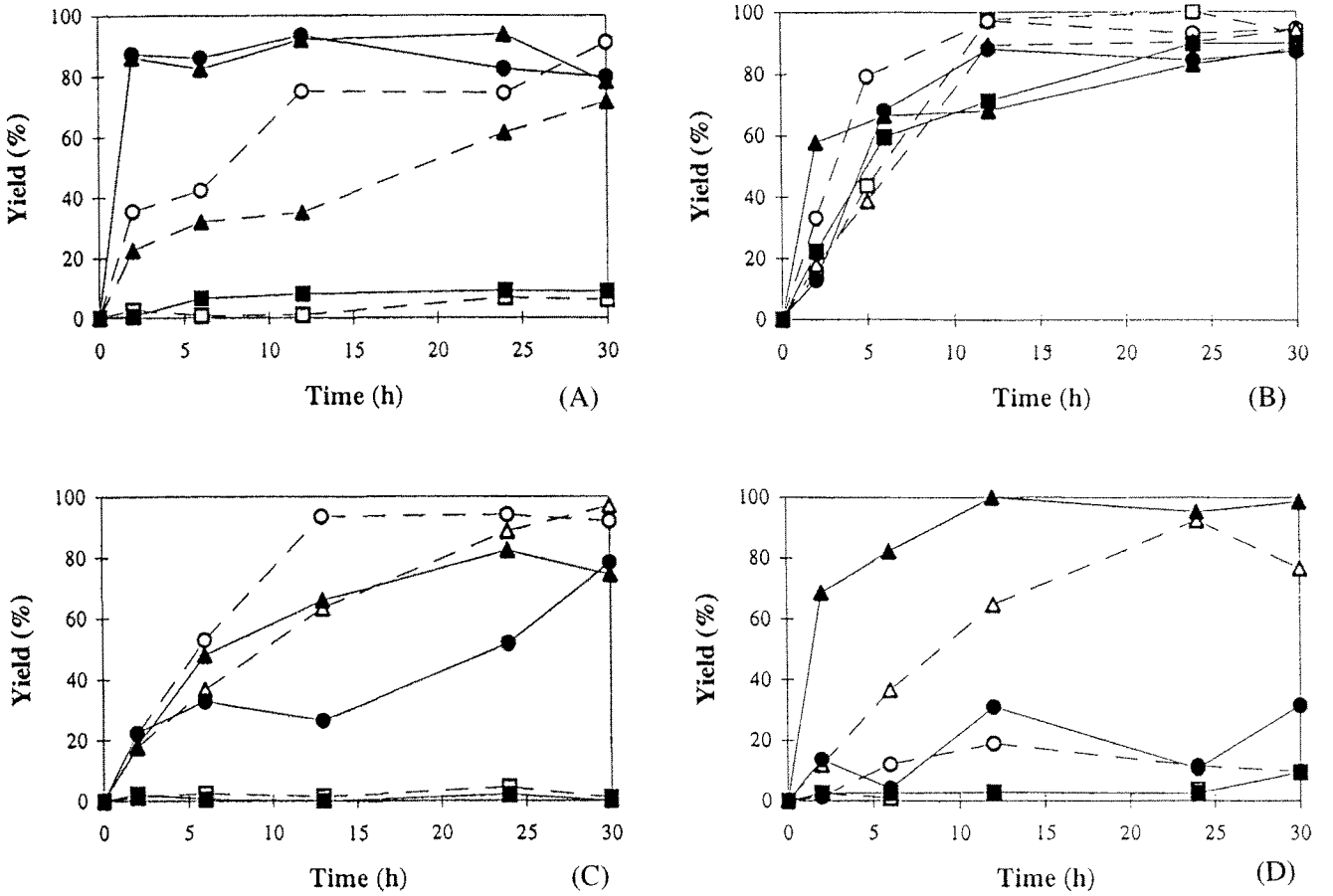

FIG. 3. Effect of added water on the yield of butyl oleate synthesis; (A) *Candida rugosa*, (B) *Chromobacterium viscosum*, (C) *Pseudomonas fluorescens*, and (D) *Rhizomucor miehei* lipase; 1-butanol to oleic acid molar ratio of 0.5 (—) or 2 (---); ■ and □ 0%, ○ and ● 3.2%, and ▲ and △ 14.0% water.

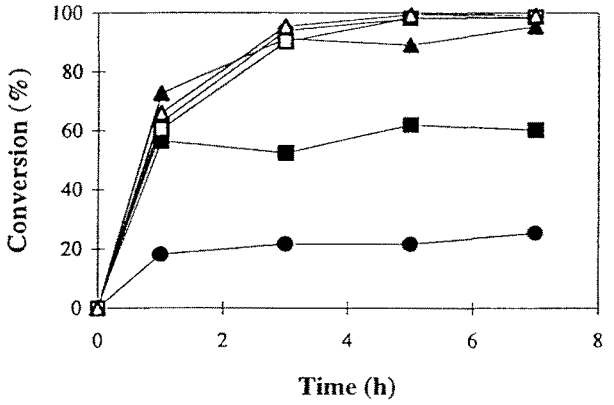

FIG. 4. Effect of water on transesterification by *Candida rugosa* lipase with 2-ethyl-1-hexanol to rapeseed oil molar ratio 2.8 (● 0%, ■ 0.25%, ▲ 1.0%, ○ 3.0%, □ 5.0%, and △ 50% added water).

R. miehei lipase catalyzed little ester synthesis at 3.2% water, but gave a complete conversion in 12 h with 14% added water.

Figure 4 shows the effect of added water on transesterification with 3.3% *C. rugosa* lipase when the substrate molar ratio was 2.8. No phase separation took place with up to 5% added water. At higher water quantities, the organic phase was used for HPLC analyses. The water present in the lipase preparation (*ca.* 5%) was insufficient, and only about 25% conversion was reached in 7 h without added water. With 0.25% added water, about 60% conversion was reached in one hour. Further increase in reaction time did not bring about further increase in rapeseed oil conversion. With a minimum of about 1.0% added water, about 50% conversion was reached in one hour, and a nearly complete conversion in five hours. Little difference in conversion was observed between 1.0 and 3.0% added water. Additional increases in the initial water content neither hurt nor improved the reaction. Although lipase-catalyzed ester synthesis may not be directly compared with transesterification, it is of interest to note that 90% or higher butyl oleate yields have been reported in the presence of excess water (13,38).

Effect of substrate molar ratio on ester synthesis with different lipases. Butyl oleate yield in excess of 80% was obtained with the *C. rugosa* lipase, both with a 1-butanol to oleic acid molar ratio of 0.5 and of 2.0 (Fig. 5). Both the *Ch. viscosum* and *P. fluorescens* lipases gave an ester yield in excess of 90% with a 2 × molar excess of 1-butanol. *Rhizomucor miehei* lipase gave only 40% ester yield, even with a 3 × molar excess of 1-butanol. In general, *Ch. viscosum* lipase is less affected by the substrate molar ratio.

One of our aims in rapeseed oil fatty acid ester transesterification was to obtain a maximum rapeseed oil conversion with little or no residual 2-ethyl-1-hexanol. Unlike the butyl oleate synthesis, rapeseed oil conversion was always low when an alcohol excess was used. The product mixture contained large quantities of residual alcohol and, in some cases, residual oil as well. The relative ester yield decreased with an increase in the alcohol molar excess. Consequently, in this transesterification, the use of alcohol excess was not further investigated. When the substrate molar ratio was between 2.8 to 3.0, lipase quantity 3.3%, and added water 3.0%, about 50% rapeseed oil conversion was reached in 1 h, and a nearly complete conversion was reached in 10 h (Fig. 6). It is concluded that the highest ester yield with the least residual alcohol is obtained at a substrate molar ratio of 2.8. Our results agree well with the previous reports that an excess amount of alcohol is beneficial in alcoholysis by suppressing the hydrolytic side reaction (36,37). Consequently, the molar ratio of 2.8 was used in most of the subsequent trials.

Effect of temperature on transesterification. Figure 7 illustrates the effect of temperature on the time course of rapeseed oil conversion by *C. rugosa* lipase. There was little difference in conversion with the temperature ranging from 37 to 55°C. They all reached about 90% conversion in 2–3 h and nearly complete conversion in 7 h. Lipase was clearly inactivated at

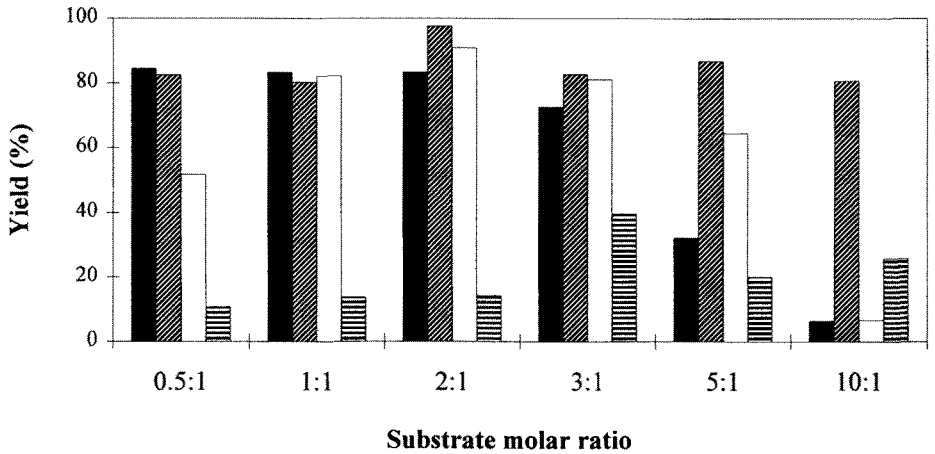

FIG. 5. Effect of 1-butanol to oleic acid substrate molar ratio on the yield of butyl oleate in 24 h at 3.2% added water and 0.3% lipase (black bar, *Candida rugosa;* cross-hatched bar, *Chromobacterium viscosum;* white bar, *Pseudomonas fluorescens;* and striped bar, *Rhizomucor miehei* lipase).

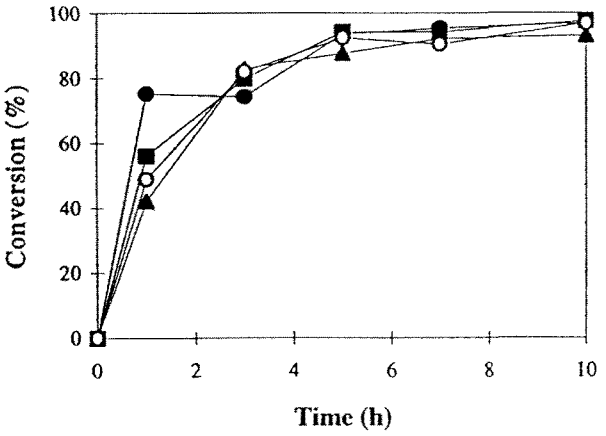

FIG. 6. Effect of 2-ethyl-1-hexanol to rapeseed oil substrate molar ratio (● 2.5, ■ 2.8, ▲ 2.9, and ○ 3.0) on rapeseed oil conversion in transesterification with 3.3% *Candida rugosa* lipase and 3% added water.

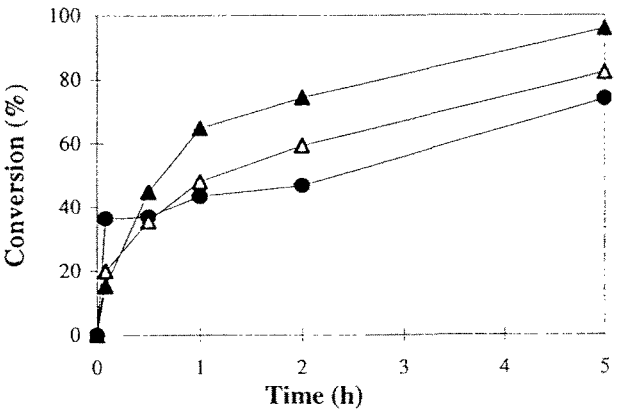

FIG. 8. Effect of the quantity of Amberlite XAD-7 resin (Rohm and Haas Nordiska AB, Bromma, Sweden) on conversion of 50 g of rapeseed oil in transesterification by *Candida rugosa* lipase (● 3.75 g, ▲ 5.0 g, and △ 7.5 g).

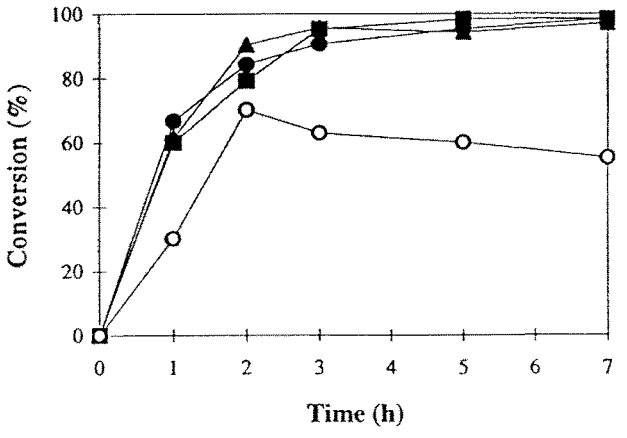

FIG. 7. Effect of temperature on transesterification with 3.3% *Candida rugosa* lipase, 3.0% added water, and 2-ethyl-1-hexanol to rapeseed oil molar ratio 2.8 (● 37°C, ■ 45°C, ▲ 55°C, and ○ 60°C).

60°C under the experimental conditions. The results agreed well with those of Mittelbach (27), in which the optimal temperature for *Candida* sp. lipase-catalyzed sunflower oil alcoholysis was between 45 and 50°C. Hirata *et al.* (35) also reported 50°C as the optimal temperature for the transesterification of tributyrin and 1-octanol with *C. rugosa* lipase.

Effect of mixing on rapeseed oil transesterification. The effect of different types of mixing on rapeseed oil conversion was investigated in a series of experiments with substrate molar ratio of 2.8, 1.0% (w/w) added water, and 3.4% (w/w) (25 g) lipase. With 50 mL (41 g) 2-ethyl-1-hexanol and 100 g rapeseed oil in a 500-mL flat-bottomed flask under 300 rpm magnetic stirring, the conversions were 65, 80, and 89% (94% of theoretical maximum) in a few minutes, 1.5 and 5 h, respectively. The solid lipase was "immobilized" on the walls within 30 min of the reaction. Ester production was 21.7 g per g lipase and 108.4 g per 100 g rapeseed oil. In another experiment with 500 g oil and 700-rpm mechanical stirring for the first 5 h, followed by 300 rpm for a further 5 h, the final conversion was only 61% (66% of theoretical). During the first 5 h, lipase remained evenly suspended, but the conversion was only 46% (50% of theoretical). The decrease of mixing rate resulted in the aggregation of lipase at the bottom, with an increase in conversion. This agrees well with the observation of Goldberg *et al.* (32), during studies on *C. rugosa* lipase-catalyzed heptyl octanoate synthesis, that, when the mixing rate was increased sufficiently for complete lipase suspension, the enzyme was inactivated. Lee and Choo (39) also reported that *C. rugosa* lipase is sensitive to shear forces in a 0.1% solution with the activity decreasing with an increase both in mixing rate and time. The degree of lipase inactivation could be markedly reduced by the addition of propylene glycol.

When 200 g rapeseed oil was used under similar conditions with mechanical stirring at 300 rpm, the conversion increased to about 73% (78% of theoretical) in 30 min, with no further increase thereafter (12). As much as 89% of the product was recovered by simple decanting. Similar results were obtained when the batch quantity of rapeseed oil was increased to 550 g. Only a slight increase in conversion was observed when the flask was filled to minimize the air/liquid boundary, as suggested by Lee and Choo (39).

Kilogram-scale production of rapeseed oil 2-ethyl-1-hexanol ester. Most previous papers on lipase-catalyzed esterification and transesterification involved immobilized lipase (9–11,14,24,28). Previously, we investigated a number of carriers for the immobilization of *C. rugosa* lipase. The carrier was also thought to help in keeping the enzyme more evenly dispersed. No improvement was found with glass beads, polyurethane foam, or Amberlite XAD-2 resin addition (16). However, when we studied the effect of relative quantity of the Amberlite XAD-7 resin (carrier) on conversion, we found that about 95% (100% of theoretical) rapeseed oil conversion (50 g oil) was achieved in 5 h with 7.5 g resin per 2.5 g lipase (Fig. 8). The optimal conditions for a 2-kg batch operation were: 1 L (829 g) 2-ethyl-1-hexanol, 3% added water, 100 g lipase with 300 g XAD-7 resin, 170 rpm, and 37°C. The time course of the transesterification is shown in Figure 9. About 90% conversion (97% of theoretical) was obtained in 8 h,

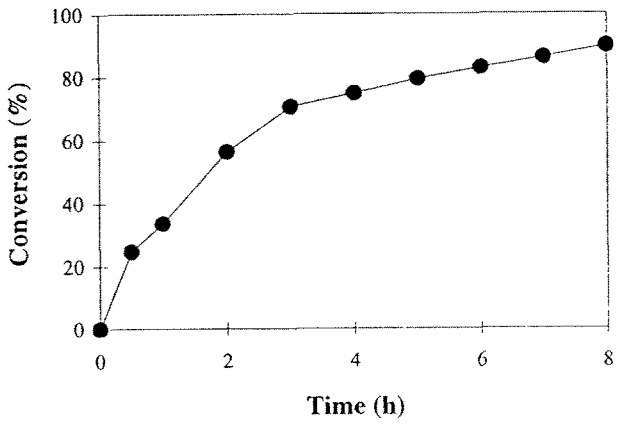

FIG. 9. Time course of transesterification of 2 kg rapeseed oil and 829 g 2-ethyl-1-hexanol (molar ratio 2.8) with 100 g (3%) *Candida rugosa* lipase, 300 g Amberlite XAD-7 resin as carrier, and 3% added water at 170 rpm and 37°C. See Figure 8 for company source.

equal to 22.4 g ester per g lipase or 112 g ester per 100 g rapeseed oil. Although the total reaction time increased somewhat in the presence of the Amberlite XAD-7 carrier, the final conversion was increased at least by about 20% to nearly theoretical.

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