Production of Specific-Structured Lipids by Enzymatic Interesterification in a Pilot Continuous Enzyme Bed Reactor

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ABSTRACT: Production of specific-structured lipids (interesterified lipids with a specific structure) by enzymatic interesterification was carried out in a continuous enzyme bed pilot scale reactor. Commercial immobilized lipase (Lipozyme IM) was used and investigations of acyl migration, pressure drop, water dependence, production efficiency, and other basic features of the process were performed. The extent of acyl migration (defined as a side reaction) occurring in the present enzyme bed reactor was compared to that in a pilot batch reactor. The continuous enzyme bed reactor was better than the batch reactor in minimizing acyl migration. Generally the former produced about one-fourth the acyl migration produced by the latter at a similar extent of incorporation. Pressure drop and production efficiency were evaluated in order to obtain a suitable yield in one reaction step. High incorporation was favored by high substrate ratios between acyl donors and oils, requiring long reaction times on the enzyme bed. Under these conditions, the pressure drop of the reactor was modeled statistically and theoretically. Residence time, water content, and effects of mass transfers were also investigated. Incorporation of medium-chain fatty acids increased with increased residence time. Approximately 40% of lipase activity was lost after a 4-wk run. External mass transfer was not a major problem in the linear flow range, but internal mass transfer did impose some transfer limitations. *JAOCS 75,* 1573–1579 (1998).

KEY WORDS: Acyl migration, enzyme bed reactor, incorporation, lipase-catalyzed interesterification, Lipozyme IM, pressure drop, *Rhizomucor miehei,* specific-structured lipids.

Modification of lipids by lipase-catalyzed reactions has been reported in a large number of publications in the past 15 years. Industrial applications are, however, still limited; the main reasons for this limitation are (i) few industrially promising applications and few high-value-added products; (ii) high cost of commercial lipases; and (iii) low efficiency of available processes. At the same time, new products have been studied based on new nutritional or functional properties (1,2). In particular, structured lipids (or specific-structured lipids) are currently attracting worldwide attention in both technology and nutrition (3–8). Furthermore, new developments in the production of high-quality lipases by genetic engineering, the improvement of lipase stability by protein engineering, and theoretical insights and practical improvements of microaqueous media have occurred recently (9–12). High-stability lipases at relatively low prices will soon be available for commercial applications. Thus the development of efficient processes and reactors will become the prerequisite for the commercial or economic potential of the products. Applications of lipases or lipase-catalyzed interesterification have been reviewed recently (13–15).

Fixed bed reactors have been investigated and applied in a wide range of enzymatic applications both in the laboratory and in industry, especially for immobilized enzymes (16). Lipase-catalyzed lipid modifications in fixed bed reactors have also been proposed, and a few studies have been published on the applications of fixed bed reactors (17–20). Macrae (21–23) commented on the advantages of fixed bed reactors for immobilized lipase-catalyzed reactions under different reaction conditions including the use of solvents. Hansen and Eigtved (20) and Posorske *et al*. (19) used the continuous fixed enzyme bed reactor in their laboratories using Lipozyme and solvent-free media. They evaluated the problems for industrial applications and considered potential processes of continuous fat modification. Bauer and co-workers (18,24,25) examined the kinetics, engineering parameters, and modeling of lipase-catalyzed interesterification in a fixed bed reactor. They used Lipozyme IM as the biocatalyst and a solvent-free system for a reaction between olive oil and trimyristin. Forssell *et al*. (17,26) also investigated some of the features of enzymatic transesterification of rapeseed oil and lauric acid in a continuous reactor. Parallel with the pilot plant work in the present study, basic process parameters have been tested in our laboratory in a small-scale fixed bed reactor for the production of specific-structured lipids by lipase-catalyzed interesterification (27). Fixed bed reactors are very promising for future developments of lipase-catalyzed lipid modifications.

In the present study, we scaled up the lipase-catalyzed reaction between oils and free fatty acids. We examined some

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new aspects of the pilot plant production of specific-structured lipids in a solvent-free medium, including acyl migration, water dependence, pressure drop, bed properties, and the effect of the combination of substrates on the efficiency of the process. Pressure drop in the enzyme bed was modeled, and parameters such as residence time and stability were also examined and discussed.

MATERIALS AND METHODS

Materials. Refined rapeseed oil (LEAR) was a donation from Aarhus Oliefabrik A/S (Aarhus, Denmark). Safflower oil was purchased from RO-CO (Birkeroed, Denmark). Capric acid was purchased from Henkel Kimianika Sdn. Bhd. (Selangor, Malaysia) (purity: 99.6 mol%). Caprylic acid and oleic acid were purchased from Riedel-de Haen (Seelze, Germany) (purity: caprylic acid 99.5 and oleic acid 72.8 mol%). Medium-chain triacylglycerol (MCT), containing 60.0 mol% caprylic acid and 40.0 mol% capric acid by our analysis, was purchased from Grünau GmbH (Illertissen, Germany). The characteristics of the rapeseed oil and safflower oil used are further clarified in Table 1. Lipozyme IM, a commercial *sn*-1,3-specific lipase from *Rhizomucor miehei*, immobilized on a macroporous ion resin, was donated by Novo Nordisk A/S (Bagsvaerd, Denmark). All solvents and reagents for analyses were analytical grade.

Apparatus. A diagram of the apparatus is shown in Figure 1. A jacketed glass column, from Pharmacia Biotech Europe GmbH (Freiburg, Germany), was used to form the bed (i.d. 50 mm × length 100 cm). The column temperature was maintained by a water bath. Before pumping through the enzyme bed, the substrates were preheated to a set temperature in a feed container by circulating heated water from a water bath. Homogeneous distribution of water in the substrate mixture was achieved by stirring at the set temperature. In a few cases, substrates were saturated with water. The product container was cooled by a jacketing system. Both the substrate and product containers were nitrogen-protected. Both the enzyme bed column and feed pipelines were insulated to reduce heat loss.

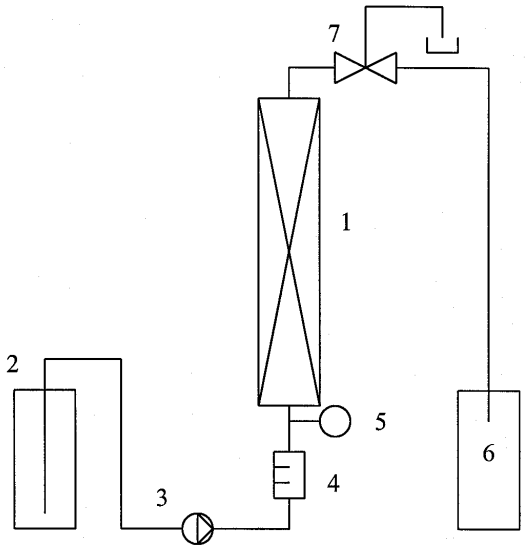

FIG. 1. Process diagram of lipase-catalyzed interesterification for the production of specific-structured lipids in an enzyme bed reactor. 1, Enzyme bed reactor, i.d. 5 cm × length 100 cm, jacketed; 2 and 6, substrate and product containers, volume 35 L, coiled heater or cooler; 3, gear pump, flow range 2 to 30 mL/min; 4, flow meter; 5, manometer; 6, sampling valve.

Packing of the column. Prior to column packing, the required amount of water was added to the commercial Lipozyme IM and the mixture allowed to equilibrate for 12 h. The pretreated enzyme was then loaded onto the column. The upper end of the column was attached after gentle shaking for a short time. Nitrogen was passed through the packed column to remove air, and the substrates were pumped in from the bottom. Bubbles in the column were removed by running for a couple of hours. Another packing method, similar to that used to pack liquid chromatographic columns, was also utilized. The pretreated enzyme was gently mixed with the substrates and the mixture poured into the column. The enzyme gradually settled to the bottom after gentle shaking. Substrates were eluted from the bottom of the column until the liquid level reached the top of the bed. The upper end of the column was attached and the column connected to the process line.

Determination of the bed void fraction (ε). Substrates were fed slowly into the newly packed column at room temperature. The column was gently shaken to remove bubbles in the liquid until the enzyme bed was completely filled with the substrates. The volume of the substrates (*Vs*) was measured and the volume of the enzyme bed (*V*) was calculated from the diameter of the column and length of the bed. The void fraction was then calculated as $\varepsilon = Vs/V$. The density of Lipozyme IM was nearly unaffected by changes in temperature. Thus the calculated ε at room temperature could be used for other temperatures without introducing large errors. The ε was also calculated by measuring the weight of the column

TABLE 1
Characteristics of Rapeseed Oil and Safflower Oil[a]

	Rapeseed oil		Safflower oil	
FFA (wt%)	0.12		0.10	
PV (meq/kg)	0.3		4.7	
Water (wt%)	0.05		0.04	
Fatty acid composition (mol%)	Total	2-Position	Total	2-Position
$C_{16:0}$	6.0	3.6	8.1	0.5
$C_{16:1}$	0.2	0.3	—	—
$C_{18:0}$	1.6	—	2.5	0.2
$C_{18:1n-9}$	58.8	45.0	13.8	13.6
$C_{18:2n-6}$	21.9	34.6	74.5	84.8
$C_{18:3n-3}$	10.0	16.3	0.6	0.5
Σ	98.5	99.8	99.5	99.6

[a]FFA, free fatty acid content; PV, peroxide value.

before and after filling the bed with substrates. *V* was calculated using the substrate weight and density. Another method to calculate ε was to change the substrates and measure the time until new substrates appeared from the column, the *Vs* being calculated using the measured flow rate. This method required a very stable flow. The three methods gave similar values for ε.

Experimental methods. Most of the lipase-catalyzed interesterifications (acidolysis) were performed using rapeseed oil and capric acid as substrates. Some additional runs on the same scale were carried out using safflower oil and caprylic acid. With each new experiment with specific set parameters, preheated and conditioned substrates were pumped into the enzyme bed. The first 1.5–2.0 L from the enzyme bed, equivalent to 3–4 enzyme bed void volumes (*Vs*), were discarded. When a new enzyme bed was used, short-time equilibration with new substrates was performed to stabilize the bed, especially for parameter optimization. When an enzyme bed was reused, the original substrates were removed and equilibration was performed with the new substrates.

Experimental design for pressure drop modeling. In the examination of pressure drop and water retention by the bed, the more hydrophilic substrates, MCT and oleic acid, were used. A factorial design with star points was used, and 27 experimental settings were generated with four factors. These factors were water content (W_c), reaction temperature (T_e), flow rate (V_f), and substrate ratio (S_r, molar capric acid/triacylglycerols). The levels of each variable were chosen on the basis of the above single-factor experiments. The settings were 0.08–0.12 wt%, 40–60°C, 2–6 mL/min, and 2–6 mol/mol, respectively. Second-order or first-order coefficients were generated by regression analysis. The fit of the model was evaluated by the coefficients of determination (R^2) and the analysis of variance.

Fatty acid composition analysis. The fatty acid composition (FAC) of triacylglycerols from the samples containing free fatty acids was determined by isolation using thin-layer chromatography (TLC), preparation of fatty acid methyl esters of the extracted triacylglycerols by methylation, extraction of the fatty acid methyl esters with hexane, and analysis by gas–liquid chromatography (28).

Grignard analysis. The structure of the oils and product samples was determined by Grignard degradation with allyl magnesium bromide followed by isolation, methylation, and fatty acid composition analysis of the *sn*-2 monoacylglycerol fraction (29).

Other analyses. The water content of oils, substrates, products, and enzymes was determined by the Karl Fischer method with a 720 KFS Titrino (Herisau, Switzerland), and using Hydranal titrant and solvents. The standard deviations for the water content of the oils, fatty acids, and enzymes were 0.0007, 0.0012, and 0.0164%, respectively. Triplicate measurements were made and the average was taken for the results. The free FAC and peroxide value of the oils were determined with the alkali titration method and the thiosulfate titration method, respectively (30).

RESULTS AND DISCUSSION

Enzyme bed properties and operation. When using a continuous enzyme bed reactor, some process phenomena that are not important in the operation of a batch reactor need attention. The enzyme bed has a residence time and a void fraction. The residence time was calculated as $V \times \varepsilon/V_f$, where *V* is the enzyme bed volume, ε is the void fraction, and V_f is the flow rate. The residence times of the substrates in the bed are Gauss-distributed, but the distribution is relatively wide owing to mass transfer limitations. This phenomenon was checked by changing the substrates during steady flow conditions from the mixture of safflower oil and capric acid to MCT at a linear flow rate of 4.6×10^{-5} m/s (Fig. 2). When the retention time (defined in Fig. 2) was similar to the residence time, the outlet purity of the second substrate was 99.2%, reflecting some contamination by the previous substrate mixture used. Hence, a longer retention time was needed if higher outlet purity was to be obtained. For practical operation, 3–4-fold void volumes were run before collecting samples or products. The product purity from the outlet was more than 99.99% with this operational practice. In general, the bed behavior was not dependent on the method of bed packing. However, the void fraction was somewhat dependent on the packing method, probably because particle size was affected by the packing method, and Lipozyme IM may be swollen by substrates. The average void fractions using the first packing method were 0.44 ± 0.01 and the second 0.47 ± 0.02.

Residence time. In previous work, incorporation of acyl donors was affected more by residence time than by other parameters in our small-scale experiments (27). In the present pilot experiments, residence time was also a very important factor. The reaction reached equilibrium in about 2 h (Fig. 3) with the incorporation curve consistent with that in the pilot batch reactions (28). But the time to reach half-maximum incorporation (51 min) was much less than in the batch reactor [19 h (28)]. Acyl migration increased almost linearly with time in a similar way to that in batch systems (28,31).

Acyl migration in enzyme bed reactors. In our previous work using a batch stirred tank reactor (STR) (28,31), acyl

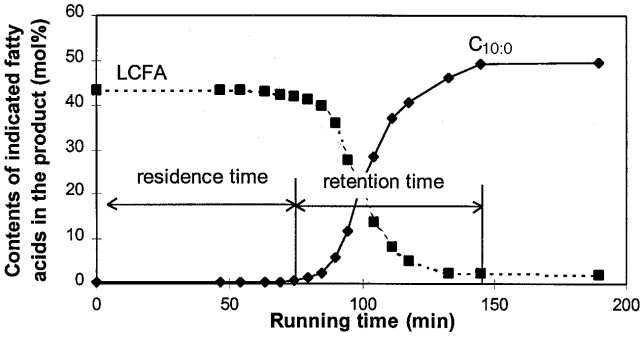

FIG. 2. Determination of operational properties (residence time and retention time) of the enzyme bed reactor by changing the feeding substrates from safflower oil/caprylic acid to medium-chain triacylglycerols at time zero. Linear flow rate 4.6×10^{-5} m/s, temperature 60°C, and water content 0.05%. LCFA, long-chain fatty acids; $C_{10:0}$, capric acid.

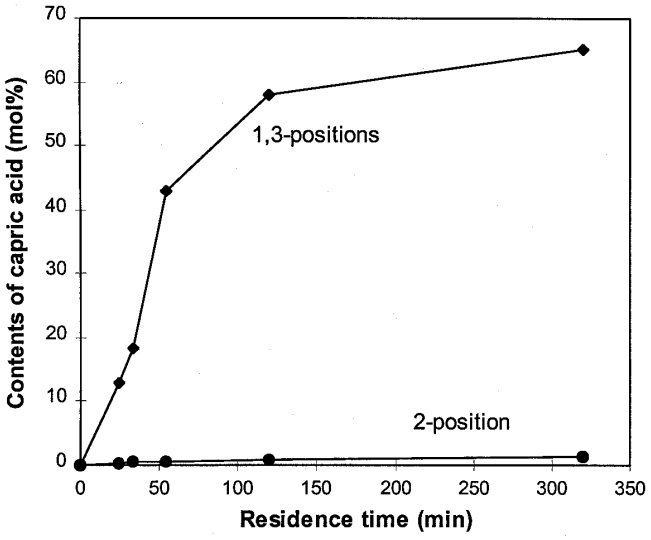

FIG. 3. Residence time course of the lipase-catalyzed interesterification between rapeseed oil and capric acid in the enzyme bed reactor. Water content in the substrates 0.03 wt%, substrate molar ratio 4, temperature 60°C.

migration occurred in the lipase-catalyzed interesterification, probably due to the existence of diacylglycerols produced as reaction intermediates. In the present enzyme bed reactor, acyl migration also occurred, but on a much smaller scale (Fig. 3). A relevant and precise comparison of the two types of reactors is difficult since the systems operate under different optimal conditions. However, a general comparison can be made based on three ratios: (i) acyl migration/1,3-incorporation ($M_f/I_{f1,3}$); (ii) acyl migration/enzyme load (M_f/E_1); and (iii) acyl migration/reaction time (M_f/T_r). $M_f/I_{f1,3}$ was plotted as the function of reaction time for both reactors (Fig. 4), the curve of the batch reactor performance being recalculated from our previous work (28). The plot shows that $M_f/I_{f1,3}$ in the batch reaction system was much higher than in the enzyme bed system. For both reactors $M_f/I_{f1,3}$ displayed a minimal value. M_f/E_1 for the batch reaction system (160 mol%/wt% recalculated from Ref. 28) was much higher than for the enzyme bed reaction system (1 mol%/wt% calculated from Fig. 3, enzyme load calculated as the substrate enzyme ratio in the enzyme bed) at 60% incorporation. This suggests that the enzyme itself does not catalyze acyl migration. The degree of acyl migration for the two reactors was compared based on M_f/T_r (the linear slope of the M_f-T_r plots). This was valid because in both reactors acyl migration increased linearly in the normal range. For similar products, the linear slope for the batch reaction system (0.0037 mol%/min recalculated from Ref. 28) was similar to that for the enzyme bed reaction system (0.0043 mol%/min calculated from Fig. 3 by regression). However, the batch reaction system required a much longer reaction time (at least 10-fold higher) than that required with the enzyme bed reaction system, and consequently resulted in more acyl migration for the former than for the latter. Generally, acyl migration is not a key issue for the enzyme bed reactors when the process or production is optimized, compared to that in the batch reactors.

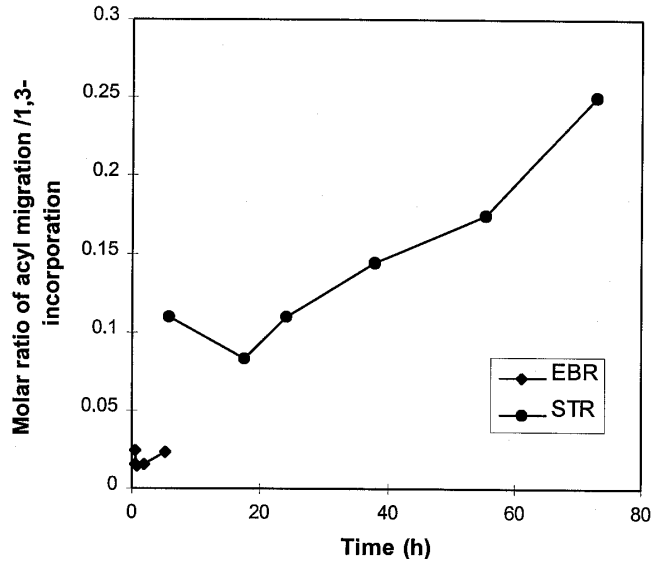

FIG. 4. The ratio between acyl migration and 1,3-incorporation as the function of reaction time (STR, stirred tank reactor, recalculated from Ref. 28) or residence time (EBR, enzyme bed reactor, recalculated from Fig. 3) in the Lipozyme IM-catalyzed interesterification between rapeseed oil and capric acid.

Mass transfer. Mass transfer is much more important for enzyme bed reactors than for batch reactors, the difference being due to the high enzyme-to-substrate ratio with the former. To achieve optimal yield of the interesterification, the process should be performed under kinetically controlled conditions without mass transfer limitations. Bauer and co-workers (18,24,25) demonstrated that for Lipozyme IM external mass transfer could be neglected, but that internal mass transfer reduced the reaction rate. Forssel *et al*. (17,26) found that external mass transfer limitations could be neglected by using a linear flow rate higher than 3×10^{-5} m/s for Lipozyme IM. We did not observe the external mass transfer limitations with the linear flow rates (minimum 3×10^{-5} m/s) used. To test the effect of internal mass transfer on the process, we performed a simple experiment by using a crushed Lipozyme IM under the same batch conditions as in previous work (28). The incorporation and acyl migration are compared in Figure 5, showing that crushed Lipozyme IM gave slightly faster incorporation but also higher acyl migration. The faster incorporation was caused by less internal mass transfer limitation, but the higher acyl migration was probably caused by the enzyme support. A similar effect of the enzyme support was observed in the batch reactor (31).

Water content. Lipase-catalyzed interesterification involves a hydrolysis and an esterification. In the first step, water is a reactant. In the second step it is a product. Thus a suitable water content is necessary to maximize the reaction rates of both steps. In addition, water is crucial to maintain enzyme structure and stability (32,33). However, an excess of water has negative effects on enzyme activity and stability. It is therefore necessary to monitor and control the water content of the components in the enzyme bed. We found, in one

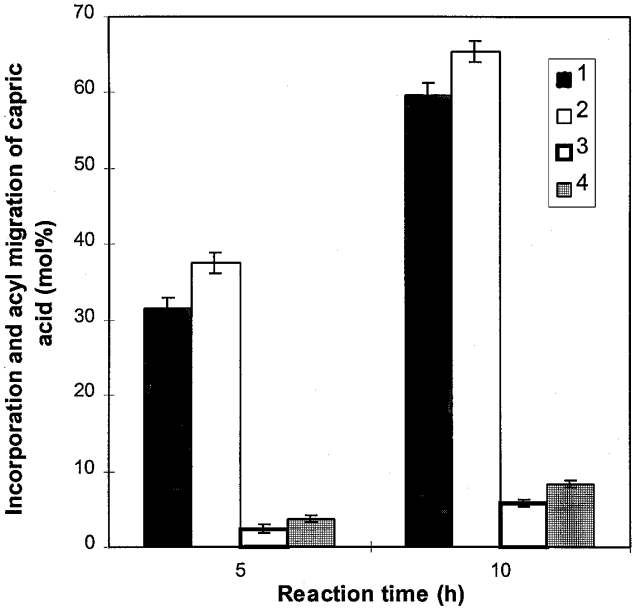

FIG. 5. Internal mass transfer detection using normal Lipozyme IM and crushed Lipozyme IM in the batch reaction system (same as in Ref. 28) between rapeseed oil and capric acid. Substrate molar ratio, 6; water content, 6%; temperature, 60°C; and enzyme load, 10 wt%. (1) 1,3-Incorporation for original Lipozyme IM, (2) 1,3-incorporation for crushed Lipozyme IM, (3) acyl migration for original Lipozyme IM, and (4) acyl migration for crushed Lipozyme IM.

case, that an increase in water content from 0.03 to 0.09% in the substrates regained its initial activity of the enzyme (Fig. 6). The inhibition of enzyme activity by water in this case was reversible after a run of 1 or 2 d. In another case, however, the activity could not be recovered by increasing the water content (Fig. 7) after a run of 25 d. The association of water with components in the bed was also investigated by changing the water content in the substrates. Such changes showed that water content in the products was unrelated to that in the

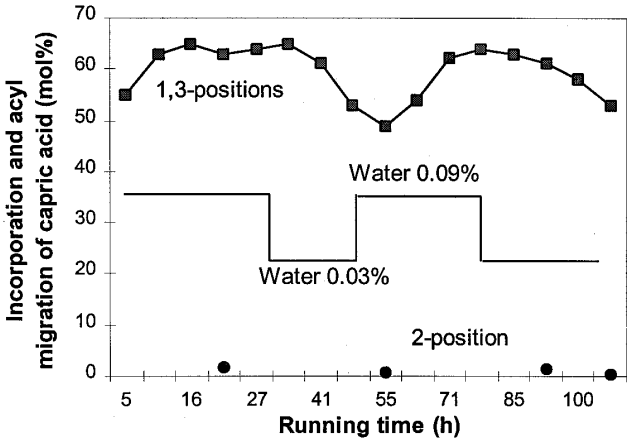

FIG. 6. Effects of water content on the 1,3-incorporation and acyl migration in a long-term running of the enzyme bed reactor for the production of specific-structured lipids by Lipozyme IM-catalyzed interesterification between rapeseed oil and capric acid. Residence time, 2 h; temperature, 60°C; and substrate molar ratio, 6.

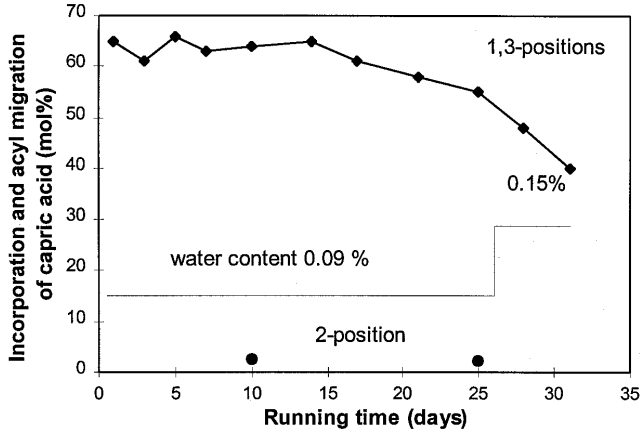

FIG. 7. Stability of the pilot enzyme bed reactor and Lipozyme IM for continuous production of specific-structured lipids by enzymatic interesterification. Residence time, 90 min; temperature, 60°C; rapeseed oil and capric acid as substrates; and substrate molar ratio, 5.

substrates (Fig. 8). We also found that there was no correlation between the water content in the products and the number of experiments performed with the same bed. This phenomenon is very much related to the water content of the enzyme, which currently cannot be accurately measured. The amount of water remaining in the bed was largely related to the parameters used, such as temperature and the identity of the substrates. Thus, maintenance of a constant thermodynamic water activity is probably the best way to control the distribution of water among the components in the enzyme bed, although it is presently not possible (34).

Pressure drop. Pressure drops in the enzyme bed are very important for the operational stability of the beds. A high pressure drop not only complicates operation of the bed but also changes the particle size of the immobilized enzyme, decreasing bed stability. A longer enzyme bed has to be used to increase the residence time when a higher substrate ratio is used, and in this case smaller enzyme particles are preferred to reduce internal mass transfer limitations. Both situations will increase pressure drop. This depends upon the length of the enzyme bed, the size of particles, the viscosity of substrates, and the linear flow rates of the liquids, according to the Kozeny-Carman equation (16):

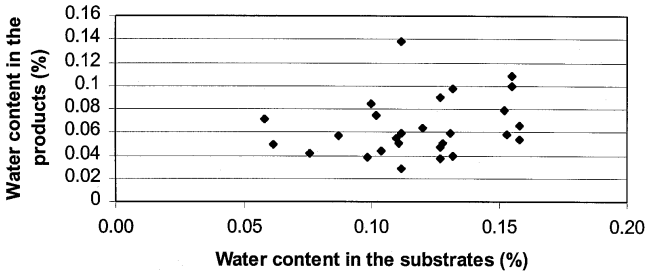

FIG. 8. The relationships between the water content in the products and the water content in the substrates in the enzyme bed reactor. Lipozyme IM was used as biocatalyst and substrates were medium-chain triacylglycerols and oleic acid.

$$\Delta P = k \times L \times S^2 \times \upsilon \times \mu \times (1 - \varepsilon)^2/\varepsilon^3 \quad [1]$$

where ΔP is the pressure drop (bar), k is a constant, L is the length of the bed, S is the specific area of the immobilized enzyme (m^2/m^3), υ is the linear flow rate (m/s), μ is the viscosity of the liquid (Pa·s), and ε is the void fraction. We set up a practical statistical model for a specific production and enzyme bed in which pressure drop was related only to the flow rate of the substrates and the temperature. The equation was a linear model as follows:

$$\Delta P = \beta_0 + \beta_1 V_f + \beta_2 T_e \quad [2]$$

where ΔP is pressure drop (bar), V_f is the flow rate (mL/min), T_e is the temperature (°C), and β_0, β_1, and β_2 are coefficients which change only with different enzyme beds and substrates. For the enzyme bed and substrates (MCT and oleic acid) used here, β_0, β_1, and β_2 were 1.141, 0.545, and −0.246, which were calculated by multiple regression. The errors associated with the estimated values of all the coefficients were very low ($P < 5 \times 10^{-9}$) and the correlation between ΔP and the two parameters (V_f, T_e) was very high ($R^2 = 0.95$). Equation 2 is valid within the range applied for the two parameters in these experiments. The Kozeny-Carman equation can be modified into Equation 3 in such a specific situation because all other variables are constant and V_f also correlates linearly with υ:

$$\Delta P = K \times V_f \times \mu \quad [3]$$

where K is a constant. ΔP has a linear relationship with V_f in both Equations 2 and 3 when a constant temperature is used, because μ is constant in the latter case. However, Equation 2 contains an intercept that does not exist in Equation 3, probably owing to the control of the different modeling systems.

We attempted to reduce the pressure drop by setting up a flexible layer on the top of the enzyme bed. We used glass wool and cotton to form the layer, and its use resulted in a 20–30% reduction in pressure drop. The reason is probably an increase in the void fraction (ε) as described by the Kozeny-Carman equation; this observation may be useful in the future design of commercial enzyme bed reactors.

Production efficiency. Theoretical maximum incorporation depends only on substrate ratios, since lipase-catalyzed interesterification is reversible. When equilibrium is reached, the incorporation cannot be increased by additional process optimization (28). Higher substrate ratios between acyl donors (free fatty acids) and triacylglycerols (oils) result in higher incorporation but also increase the need for purification of the products. We calculated the time needed to reach 50% maximum conversion at different substrate ratios and found that a higher substrate ratio needed longer time to reach equilibrium (Fig. 9). This results in a longer enzyme bed and a longer residence time. This conclusion is kinetically reasonable because the concentration of the triacylglycerols in the substrates determines the reaction rate in the hydrolysis step, which is kinetically a limiting step. Substrate or product inhibition is probably another reason for longer reaction times with higher substrate ratios. Thus, a higher incorporation can be achieved by using either a single reaction stage with a higher substrate ratio or two reaction stages in the process with a lower substrate ratio and a purification step between the two stages, but the production efficiency may be reduced.

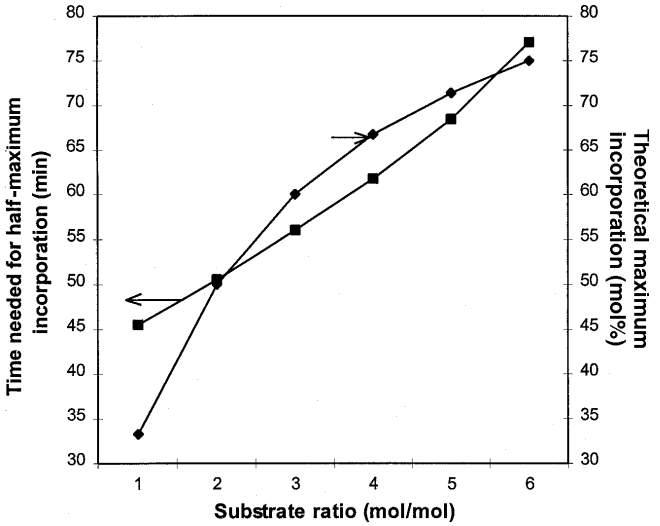

FIG. 9. Theoretical maximum 1,3-incorporation and the time needed for half maximum 1,3-incorporation as the function of substrate ratios in the lipase-catalyzed interesterification between rapeseed oil and capric acid. The time was calculated by interpolation according to basic incorporation developments. Water content in the substrates, 0.09%; rapeseed oil and capric acid as substrates; and temperature, 60°C.

Production of structured lipids. A few structured lipid products were produced by the pilot enzyme bed reactor for nutritional applications. The results are summarized in Table 2. The general qualities of the products were greatly improved compared to products from the batch reactor (28). In general, acyl migration was relatively small even though very long residence times (up to 5 h) were used. Acyl migration was of little importance when choosing parameters to optimize the enzyme bed. A high incorporation can be achieved by increasing the residence time. The amount of diacylglycerols in the products was measured by HPLC and was found to be generally smaller than those in batch reactors (35).

TABLE 2
Parameters and Product Quality of the Specific-Structured Lipids Produced in the Pilot Enzyme Bed Reactor[a]

Substrates	S_r	T_e (°C)	τ_R (min)	W_c (wt%)	$I_{f1,3}$ (mol%)	M_f (mol%)	DAG (wt%)
Safflower oil and caprylic acid	6	60	278	0.09	71.5	2.8	7.9
Safflower oil and caprylic acid	6	50	134	0.09	63.6	1.2	5.5
Rapeseed oil and capric acid	6	60	151	Sat.	66.2	4.1	—
Safflower oil and caprylic acid	7	55	220	0.09	75.8	2.1	7.8
MCT and oleic acid	6	50	175	0.05	57.6	3.2	5.8

[a]The enzyme beds in each production were not newly packed. Some of them were used beds without special regulation of the usage. Diacylglycerols were measured and calculated based on the glycerides in the products. Abbreviations: τ_R, residence time; $I_{f1,3}$, 1,3-incorporation; M_f, acyl migration; DAG, content of diacylglycerols; Sat., substrates saturated with water; W_c, water content in substrates (wt%); T_e, reaction temperature (°C); V_f, flow rate (mL/min); S_r, substrate ratio (mol/mol); MCT, medium-chain triacylglycerols.

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