

A Kinetic Study on Lipase-Catalyzed Interesterification of Soybean Oil with Oleic Acid in a Continuous Packed-Bed Reactor

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Abstract

To provide a mathematical basis for the design and operation of a continuous, packed-bed reactor for the interesterification of soybean oil, soybean oil that contains 22.7% oleoyl and 54.3% linoleoyl moieties as molar acyl moiety composition was interesterified in hexane with oleic acid, using an immobilized *sn*-1,3-specific lipase (Lipozyme IM) from *Mucor miehei*. The reaction was carried out in a U-shaped Pyrex glass-made packed-bed reactor at 37°C in the following system: concentration of soybean oil in the feed stream = 12.5 wt%, molar ratio of fatty acid to soybean oil = 3.0, and water content in the feed stream = 1340–2340 ppm. At these water contents, Lipozyme IM gave practically the same catalytic activity, and the content of triacylglycerols in the product oil was 91–94 wt%. Rate equations for the change in oleoyl and linoleoyl moiety compositions in soybean oil were derived and their validity was confirmed experimentally. On the other hand, the catalytic activity of Lipozyme IM decayed in the first-order fashion. Based on these deactivation kinetics, the flow rate of the feed stream is simulated for the operation of a continuous, packed-bed reactor at 37°C that produces an oil of a fixed composition of oleoyl moiety.

Index Entries: Interesterification; acidolysis; soybean oil; oleic acid; linoleic acid; lipase; Lipozyme IM; packed-bed reactor; kinetics; deactivation.

Introduction

The nutritional properties of fats and oils depend on the composition of acyl moieties on the glycerol backbone. Recent studies have indicated

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Table 1
Compositions of Acyl Moieties
in Soybean Oil and Reagent Grade Oleic Acid Used

	Composition of acyl moieties (mol %)							Average M.W. ^b
	14:0 ^a	16:0	16:1	18:0	18:1 ω 9	18:2 ω 6	18:3 ω 3	
Soybean oil	0	11.5	0	2.2	22.7	54.3	9.3	873.4 ^c
Oleic acid ^c	3.9	3.5	6.9	0.6	73.2	10.8	1.1	277.7
Rapeseed oil ^d	0	3.9	0	1.8	57.9	21.8	11.3	—

^a14:0:myristoyl; 16:0:palmitoyl; 16:1:palmitoleoyl; 18:0:stearoyl; 18:1 ω 9:oleoyl; 18:2 ω 6:linoleoyl; 18:3 ω 3:α-linolenoyl.

^bAverage molecular weight.

^cReagent grade.

^dLow ercic type (9).

^eAs a mixture of triacylglycerols.

that excess intake of dietary linoleic acid promotes incidence and growth of mammary tumors in rats and mice (1–3). By contrast, dietary α-linolenic acid has been reported to have an inhibiting effect on the growth and metastasis of mammary tumors (4–7). It has also been reported that dietary oleic acid has neither a tumor-promoting effect nor a tumor-inhibiting effect (1). Nowadays, soybean oil is produced much more than any other edible vegetable oil. Judging from the typical composition of acyl moieties in soybean oil (Table 1), the major acyl moiety in soybean oil is linoleoyl (18:2 ω 6). Hence, soybean oil may not be a nutritionally safe vegetable oil.

To reduce the percentage of 18:2 ω 6 moiety in soybean oil to the levels found in rapeseed oil (21.8%, see Table 1), we previously investigated the interesterification of soybean oil with oleic acid, using an immobilized *sn*-1,3-specific lipase (Lipozyme IM) (8). The reaction was carried out in a batch reactor at 37°C. At the molar ratio of fatty acid to soybean oil of 3.0 and the reaction time of 6 h, the product oil that contains 50.8% oleoyl (18:1 ω 9), 38.8% 18:2 ω 6, and 5.4% α-linolenoyl (18:3 ω 3) moieties was obtained in the reaction with biochemical reagent grade oleic acid. The product oil that contains 40.3% 18:1 ω 9, 42.1% 18:2 ω 6, and 6.5% 18:3 ω 3 moieties was obtained with reagent grade oleic acid. Approximately 86–88% of the interesterification of 18:2 ω 6 moiety took place within 1 h (8).

The present work aims to provide a mathematical basis for the design and operation of a continuous, packed-bed reactor for the interesterification of soybean oil with oleic acid. The proper content of water in the feed stream is first determined, and rate equations for the interesterification reaction and deactivation kinetics for Lipozyme IM during the time in operation are investigated. Based on these kinetics, the flow rates of the feed stream per unit mass of enzyme after different times in operation are simulated for the production of an oil at 37°C in which the composition of 18:1 ω 9 moiety is fixed.

Materials and Methods

Materials

Refined soybean oil (1.06 wt% H₂O content), dehydrated hexane (H₂O content < 0.003%), and guaranteed reagent grade hexane (H₂O content < 0.05%) were purchased from Kanto Chemicals (Tokyo, Japan). Diethyl ether, acetic acid, acetyl chloride, and iodine, all of which are of guaranteed reagent grade, and reagent grade oleic acid (H₂O content = 0%) and dehydrated methanol (H₂O content < 0.005%) were from Wako Chemical Industries, Osaka, Japan. Table 1 gives the compositions of acyl moieties in soybean oil and reagent grade oleic acid used in the present work. Activated aluminum oxide (90, basic, activity I, 70–80 mesh) for column chromatography (CC) and silica gel plate (Silica gel 60, 5 × 20 cm) for thin-layer chromatography (TLC) were from Merck (Darmstadt, Germany). Prior to use, 50 g of this aluminum oxide was well mixed with 1 g of water in a rubber-stoppered flask and allowed to stand for at least 1 d to equilibrate.

Immobilized lipase of the fungus *Mucor miehei* supported on a macroporous anion-exchange resin (Lipozyme IM) was supplied from Novo Nordisk (Chiba, Japan). It was composed of particles of 0.2–0.6 mm diameter and contained 5.0 wt% water. This lipase (triacylglycerol acylhydrolase, EC 3.1.1.3) specifically hydrolyzes the acyl moieties from the *sn*-1- and *sn*-3-positions of triacylglycerol. The activity of Lipozyme IM was 108 batch interesterification units (BIUs)/g of dry particles; one BIU corresponds to 1 μmol of palmitic acid incorporated into trioleoylglycerol in 1 min from an equimolar mixture at 37°C.

Interesterification Reaction

The reaction system consisted of a U-shaped Pyrex glass-made packed-bed reactor (4.6 mm id, 150 mm height), reservoirs to contain the feedstock mixture and the effluent or the product stream, and a pump to maintain a constant flow through the system. The reactor was heated in a continuously stirred water bath of which temperature was controlled at 37°C. A Shimadzu LC-10AD-type pump (Shimadzu, Kyoto, Japan) was used. The amount of Lipozyme IM packed into the reactor was 0.1–0.5 g; the length of packed-bed ranged from 1.7 to 7.7 cm. The top and bottom of the catalyst bed were tightly fixed with silica wool.

The feedstock consisted of a mixture of soybean oil, reagent grade oleic acid, and a small amount of water dissolved in dehydrated hexane. The concentrations of reactants were 12.5 wt% soybean oil (0.0987 mol/L), 12.0 wt% reagent grade oleic acid (0.296 mol fatty acid/L), and 1340–2340 ppm water. The molar ratio of fatty acid to soybean oil was 3.0. The feedstock mixture was degassed at room temperature, and air in the reservoir was replaced with atmospheric nitrogen gas. Two portions of the product stream (100 μL each) were sampled several times during the time in operation and were analyzed for acyl moiety composition and lipid content in the product oil.

Fatty Acid Analysis

The composition of acyl moieties in the product oil was analyzed by means of CC and gas chromatography (GC), as described in our previous work (8). That is, lipids were separated from the product sample (100 μ L) by CC on the activated aluminum oxide (2.0 g) with diethyl ether (4 mL) as the eluting solvent. Lipids thus obtained were transmethylated with 6 wt% methanolic HCl, and fatty acid methyl esters (FAMES) formed were extracted with hexane and then analyzed by GC (8).

Lipid Content Analysis

The contents of monoacylglycerols (MGs), diacylglycerols (DGs), and triacylglycerols (TGs) in the product oil were analyzed by means of TLC and GC. Another product sample (100 μ L) was separated into MGs, DGs, TGs, and the other components by TLC on silica gel plate, using a hexane-diethyl ether-acetic acid mixture (80:30:1, v/v/v) as the developing solvent (10). The silica gel layers, on which MGs, DGs, or TGs were adsorbed, were scraped off and then transmethylated with 6 wt% methanolic HCl. FAMES formed were extracted with hexane and analyzed by GC. From the amounts of FAMES formed, the amount of MGs, DGs, or TGs was determined.

Results and Discussion

Effect of Water Content in the Feed Stream on Catalytic Activity

On a molecular level, interesterification reaction involves mechanistically hydrolysis of acylglycerols, followed by resynthesis. As a result, there must be at least a catalytic amount of water present in the reactant mixture (11,12). Posorske et al. (13) reported that when an immobilized lipase bed was operated continuously, oil in the feed stream would absorb water to eventually dry out the bed, which stopped the reaction. To maintain continuous column operation over a long period, it is thus necessary to supply water with the reactant stream. When the content of water in the feed stream is high, however, the hydrolysis of oil is rather promoted (14). Our feedstock mixture contained 1340 ppm of water even when no water was added. Therefore, we compared the catalytic activity of Lipozyme IM at the water contents of 1340, 1840, and 2340 ppm in the feed stream. Figure 1 shows the compositions of acyl moieties in the product oils formed after different times in operation. The content of water in the feed stream was 1840 ppm. The percentage of 18:2 ω 6 moiety in the product oil decreased quickly to 44.3% within 1 h, and then leveled off at about 44.0% during the operation time of 2–6 h. The percentages of palmitoyl (16:0), stearoyl (18:0), and 18:3 ω 3 moieties similarly decreased within 1 h and then leveled off at about 7.0, 1.4, and 7.9%, respectively, during the same interval of operation time. By contrast, the percentage of 18:1 ω 9 moiety increased quickly to 36.5% within 1 h, and then leveled off at about 36.7% during the operation time of 2–6 h (Fig. 1). The percentages of myristoyl (14:0) and palmitoleoyl (16:1)

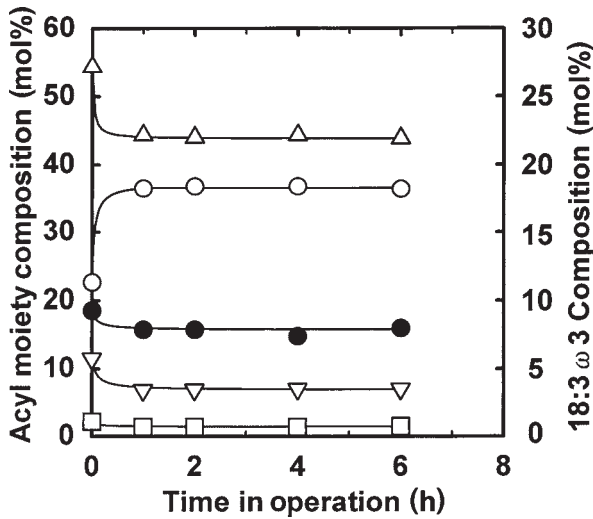


Fig. 1. Compositions of acyl moieties in the product oils formed after different times in operation. Reaction conditions: Lipozyme IM, 0.475 g (dry); flow rate of the feed stream, 0.287 mL/min; water content in the feed stream, 1840 ppm. ∇ , 16:0; \square , 18:0; \circ , 18:1 ω 9; \triangle , 18:2 ω 6; \bullet , 18:3 ω 3.

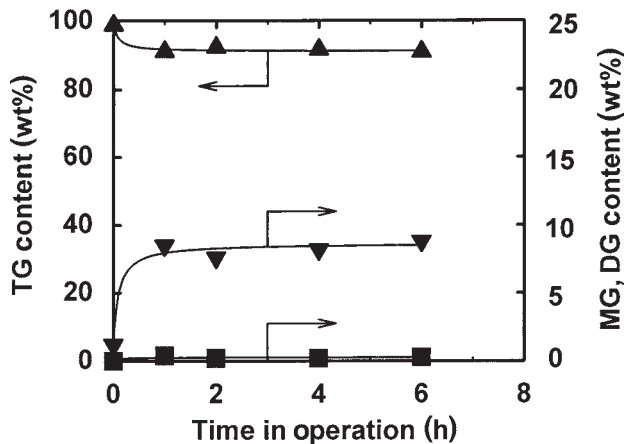


Fig. 2. Lipid contents in the product oils formed after different times in operation. Reaction conditions are the same as those in Fig. 1. \blacktriangle , TG; \blacktriangledown , DG; \blacksquare , MG.

moieties were 1.0–1.1 and 2.1–2.2%, respectively, during the same interval of operation time (data not shown). The contents of MGs, DGs, and TGs in the product oils were also nearly constant—0.2–0.3, 7.5–8.8, and 91.0–92.3 wt%, respectively—during the operation time of 2–6 h (Fig. 2). These results indicate that at the water content of 1840 ppm, in the feed stream, the catalytic activity of Lipozyme IM remained constant during the operation time of 2–6 h.

Figure 3 shows the effect of water content in the feed stream on the acyl moiety compositions in the product oil formed after the operation time of

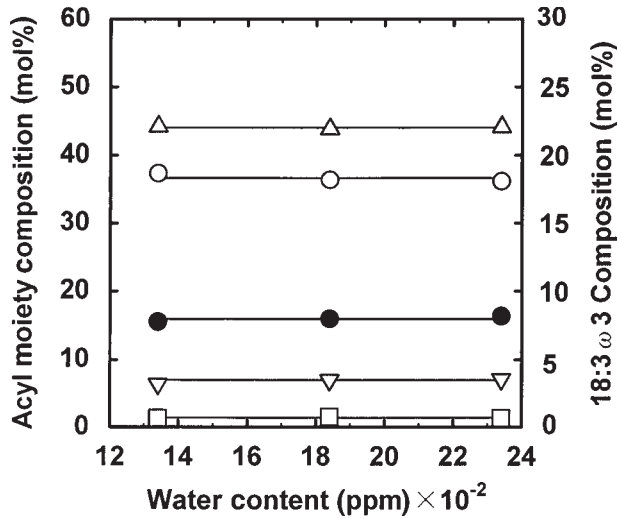


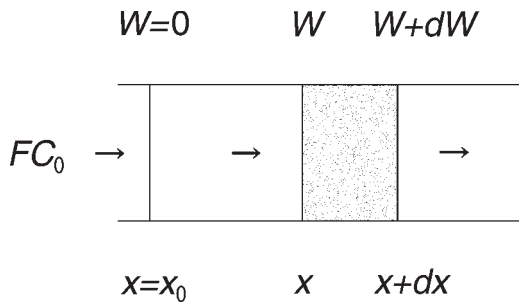
Fig. 3. Compositions of acyl moieties in the product oils formed at different contents of water in the feed stream. Reaction conditions and symbols are the same as those in Fig. 1 except for the water content in the feed stream. The product stream was sampled for analysis after the operation time of 6 h.

6 h. Although the water content in the feed stream changed from 1340 to 2340 ppm, the percentages of 16:0, 18:0, 18:1 ω 9, 18:2 ω 6, and 18:3 ω 3 moieties in the product oil remained nearly constant—about 7.0, 1.4, 36.7, 44.0, and 7.9%, respectively (Fig. 3). The percentages of 14:0 and 16:1 moieties were 1.0–1.1 and 2.0–2.2%, respectively (data not shown). Also, the lipid contents in the product oil were practically the same at these water contents: 0.1–0.2 wt% MGs, 6.8–8.8 wt% DGs, and 91.0–93.1 wt% TGs (data not shown). At the water content of 1340–2340 ppm in the feed stream, Lipozyme IM therefore gave practically the same catalytic activity for the interesterification of soybean oil at 37°C. Hence, the fixed water content of 1840 ppm in the feed stream was used throughout this work.

Rate Equations

Rate equations for the interesterification of soybean oil in a continuous, packed-bed reactor are derived. We assume a plug flow model as shown in Scheme 1, where F = flow rate of the feed stream (mL/min); C_0 = concentration of soybean oil in the feed stream (mol TGs/mL); x = molar composition of an acyl moiety in oil (-); x_e = equilibrium molar composition of an acyl moiety in oil (-); x_0 = molar composition of an acyl moiety in soybean oil (-); x_f = molar composition of a fatty acid in reagent grade oleic acid (-); W = dry Lipozyme IM (g of enzyme); and r = rate for interesterification of an acyl moiety in oil (mol/[g of enzyme · min]).

The compositions of an acyl moiety in oil are x and $x + dx$, respectively, when the feed stream contacts with W and $W + dW$ g of Lipozyme IM. When neither MG nor DG is contained in soybean oil and the oil formed, the



Scheme 1. A plug flow model.

following equation can be derived for the reaction over dW g of Lipozyme IM on the basis of the mass balance of an acyl moiety in oil:

$$3FC_0 dx = rdW \tag{1}$$

We pay attention to two important reactions: the reaction by which the composition of 18:1 ω 9 moiety in soybean oil increases, and the reaction by which the composition of 18:2 ω 6 moiety in soybean oil decreases. Here, we again assume that surface reactions over Lipozyme IM are rate controlling for these two reactions. For the former reaction, we propose second-order reaction kinetics, in which the rate of reaction increases in proportion to the product of “the difference in 18:1 ω 9 moiety composition in oil from the equilibrium value” and “the concentration of oleic acid in the reactant mixture.” Since the molar ratio of reactant fatty acid to soybean oil is 3.0, the following rate equation is obtained:

$$r = k_{OA} [3C_0 (x_e - x)] [3C_0 (x_0 + x_f - x)] = k_{OA} (3C_0)^2 (x_e - x) (x_0 + x_f - x) \tag{2}$$

Here, k_{OA} is the rate constant for the former reaction. From Eqs. 1 and 2,

$$F dx = 3k_{OA} C_0 (x_e - x) (x_0 + x_f - x) dW \tag{3}$$

By integrating Eq. 3 between $x = x_0$ at $W = 0$ and $x = x$ at $W = W$, we obtain Eq. 4:

$$\frac{1}{x_0 + x_f - x_e} \ln \left[\left(\frac{x_0 + x_f - x}{x_e - x} \right) \left(\frac{x_e - x_0}{x_f} \right) \right] = 3k_{OA} C_0 \left(\frac{W}{F} \right) \tag{4}$$

For the latter reaction, we propose similar second-order reaction kinetics, in which the rate of reaction increases in proportion to the product of “the difference in 18:2 ω 6 moiety composition in oil from the equilibrium value” and “the total concentration of fatty acids other than linoleic acid in the reactant mixture.” As in the case of the former reaction, the following equation is obtained:

$$\frac{1}{1 + x_e - x_0 - x_f} \ln \left[\left(\frac{1 + x - x_0 - x_f}{x - x_e} \right) \left(\frac{x_0 - x_e}{1 - x_f} \right) \right] = 3k_{LA} C_0 \left(\frac{W}{F} \right) \tag{5}$$

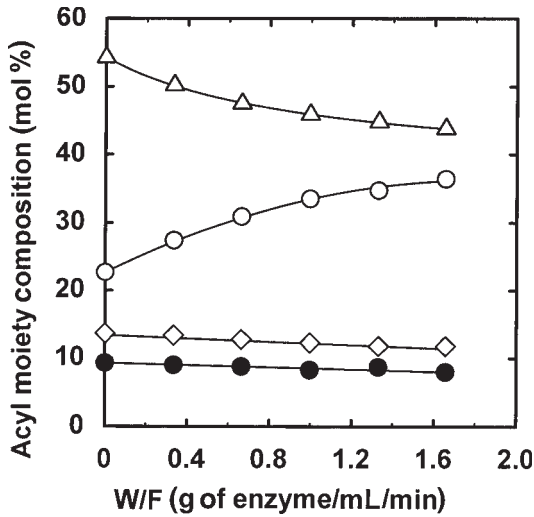


Fig. 4. Compositions of acyl moieties in the product oils formed at different contact times of the reaction. Reaction conditions and symbols are the same as those in Fig. 1 except for the amount of Lipozyme IM used. The product stream was sampled for analysis after the operation time of 6 h. \diamond , 14:0 + 16:0 + 16:1 + 18:0.

Here, k_{LA} is the rate constant for the latter reaction. A previous experiment revealed that doubling of both the amount of Lipozyme IM used (0.097 \rightarrow 0.190 g) and the flow rate of the feed stream (0.153 \rightarrow 0.300 mL/min) at the fixed contact time of the reaction ($W/F = 0.633$ g of enzyme/mL/min) caused only a negligible change in the composition of acyl moieties in the product oil (8.3–8.4% for 16:0; 1.3–1.4% for 16:1; 1.6% for 18:0; 30.7–31.0% for 18:1 ω 9; 48.1–48.6% for 18:2 ω 6; and 8.5–8.7% for 18:3 ω 3). In Lipozyme IM, lipase is immobilized on a 0.2- to 0.6-mm macroporous anion-exchange resin. Hence, we believe that the external and intrapore diffusions of the reactants do not affect the rate of interesterification of soybean oil with oleic acid.

To check the validity of Eqs. 4 and 5, the effect of contact time (W/F) on the composition of acyl moieties in the product oil was investigated (Fig. 4). The percentage of 18:2 ω 6 moiety decreased at higher contact times of the reaction; it was 43.8% at the contact time of 1.66 g of enzyme/mL/min). The percentage of 18:3 ω 3 moiety similarly decreased; it was 8.0% at the contact time of 1.66 g of enzyme/mL/min). By contrast, the percentage of 18:1 ω 9 moiety increased at higher contact times of the reaction; it was 36.4% at the contact time of 1.66 g of enzyme/mL/min) (Fig. 4). The contents of MGs, DGs, and TGs in the product oil did not change much at the contact times of the reaction employed in the present work; they were 0.3–1.0, 5.1–8.8, and 90.9–93.9 wt%, respectively (data not shown).

Based on these results of the reaction (Fig. 4), the validity of Eqs. 4 and 5 was checked by means of a linear plotting method. In the calculations, the values of x_o , x_p , and x_e employed were 0.227, 0.732, and 0.403, respectively,

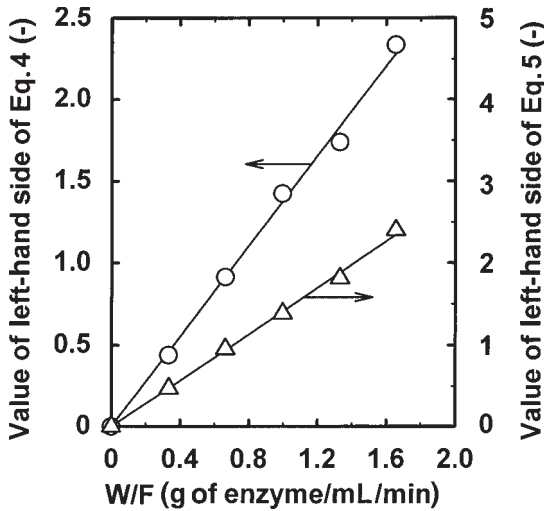


Fig. 5. Linear plots of Eqs. 4 and 5. The compositions of acyl moieties in the product oils used are those in Fig. 4. ○, Eq. 4; △, Eq. 5.

for Eq. 4, whereas they were 0.543, 0.108, and 0.421, respectively, for Eq. 5. Here, the percentages of 18:1 ω 9 and 18:2 ω 6 moieties (0.403 and 0.421, respectively) in the product oil, which was formed after the reaction time of 6 h in a batch reaction over 10 BIU of Lipozyme IM (8), were used as the values of x_e , respectively. The value of C_0 is 9.87×10^{-5} mol TGs/mL. Figure 5 shows that good linearity exists between the values of the left-hand side of Eqs. 4 and 5 and the contact times of reaction (W/F), respectively. This indicates that surface reactions are rate controlling and that Eqs. 4 and 5 are valid in the Lipozyme IM-catalyzed interesterification of soybean oil with oleic acid.

Deactivation Kinetics

The deactivation behavior of Lipozyme IM during the time in operation was investigated next. In this experiment, the product stream was first sampled after the operation time of 4 h, and then it was sampled daily for analysis. By using Eqs. 4 and 5, the rate constants, k_{OA} and k_{LA} , were calculated and were then plotted against the time in operation by means of semilog plots (Fig. 6). The logarithmic values of k_{OA} and k_{LA} decreased linearly with the time in operation, and the values of k_{OA} and k_{LA} were about 26% of the initial values after the operation time of 7 d (Fig. 6). The contents of MGs, DGs, and TGs in the product oil were 0.1–0.2, 5.5–6.1, and 93.8–94.3 wt%, respectively, during the operation time of 1–7 d (data not shown). These high contents of TGs in the product oil are advantageous from the viewpoint of commercialization.

The deactivation behavior of Lipozyme IM shown in Fig. 6 was successfully described by using first-order deactivation kinetics. That is:

$$k = k_0 e^{-\alpha t} \tag{6}$$

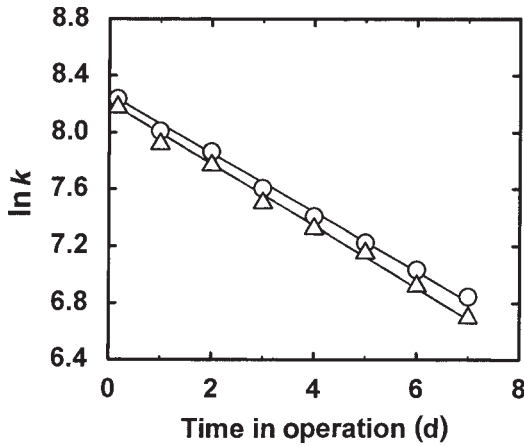


Fig. 6. Variation of rate constants, k_{OA} (○) and k_{LA} (△), with time in operation. Reaction conditions: Lipozyme IM, 0.261 g (dry); flow rate of the feed stream, 0.250 mL/min; water content in the feed stream, 1840 ppm.

Here, k , t , and α are rate constants for the interesterification reaction, time in operation (d), and deactivation constant (d^{-1}), respectively; and k_0 is the rate constant at $t = 0$. We applied Eq. 6 to the deactivation behaviors of k_{OA} and k_{LA} shown in Fig. 6 and obtained the following two equations:

$$k_{\text{OA}} = 3692e^{-0.198t} \quad (7)$$

$$k_{\text{LA}} = 3351e^{-0.191t} \quad (8)$$

The correlation factors for k_{OA} and k_{LA} were 0.9979 and 0.9939, respectively. Nearly the same values of deactivation constants (0.198 and 0.191) for k_{OA} and k_{LA} indicate that the active sites for these two reactions are identical.

Reactor Operation for Fixed 18:1 ω 9 Moiety Composition in the Product Oil

For industrial reaction processes, it is desirable to maintain the constant composition of the product stream over a long period. Then we simulate the flow rate of the feed stream per unit mass of enzyme (F/W) at various times in operation for the production of an oil in which the composition of 18:1 ω 9 moiety remains unchanged. We consider a catalytic reactor operated at the same reaction conditions as those in Fig. 6. In this reactor, reaction temperature was fixed to be 37°C, and the percentages of 18:1 ω 9 and 18:2 ω 6 moieties in the product oil formed after the operation time of 4 h were 32.3 and 46.9%, respectively (Fig. 6). By using Eqs. 4 and 7, we simulated the values of F/W (mL/[min · g of enzyme]) after various times of operation to keep the percentage of 18:1 ω 9 moiety in the product oil at 32.3% (Fig. 7). The percentages of 18:2 ω 6 moiety in the product oils formed after various times of operation were calculated by using Eqs. 5 and 8 and

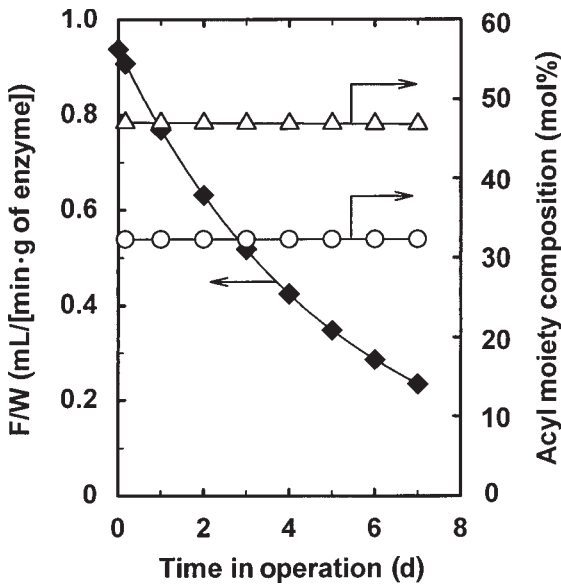


Fig. 7. Simulation of F/W and acyl moiety compositions in the product oils for the Lipozyme IM-catalyzed interesterification of soybean oil with reagent grade oleic acid. Reaction conditions: Lipozyme IM, 0.261 g (dry); water content in the feed stream, 1840 ppm. The percentage of 18:1 ω 9 moiety in the product oil is fixed to be 32.3%. Symbols used are the same as those in Fig. 1.

are shown in Fig. 7. The value of F/W starts from 0.938 mL/(min · g of enzyme), and it decreases after longer times in operation and reaches 0.234 mL/(min · g of enzyme) after the operation time of 7 d. The percentage of 18:2 ω 6 moiety in the product oil remains practically the same: 46.9–47.7% (Fig. 7). The area under the curve of F/W represents the total amount of the feed stream that has been processed within 7 d. Combination of Eqs. 4 and 7 followed by the integration between $t = 0$ and $t = 7$ d gives that this area is 5118 mL/g of enzyme. Hence, the total amount of soybean oil that has been processed within 7 d is $5118 \times 9.87 \times 10^{-5} \times 873.4 = 441$ g/g of enzyme.

Posorske et al. (13) already investigated the Lipozyme-catalyzed interesterification of soybean oil with lauric acid in a packed-bed reactor at 60°C. They reported that Lipozyme gave about 68% of the initial catalytic activity even after the operation time of 10 d (13). However, our Lipozyme deactivated more rapidly than that reported by Posorske et al. (13) the values of k_{OA} and k_{LA} after the operation time of 7 d were as low as about 26% of the initial values (Fig. 6). Usually, the deactivation of catalyst takes place markedly when the flow rate of the feed stream per unit mass of catalyst is high. Posorske et al. (13) used the initial flow rate of 3.5 g of TGs/(h · g of enzyme) whereas it was 4.85 g of TGs/(h · g of enzyme) in our experiment (Fig. 6). At the contact time of the reaction (W/F) of 1.66 g of enzyme/(mL · min), the percentages of 18:1 ω 9 and 18:2 ω 6 moieties in the product oil formed after the operation time of 6 h were 36.4 and 43.8%, respectively

(Fig. 4). If this contact time, which corresponds to the initial flow rate of 3.12 g of TGs/(h · g of enzyme), had been used in the deactivation studies, we could have obtained a slower deactivation kinetics of Lipozyme IM than that found in Fig. 6. The product oil formed must have contained a higher composition of 18:1 ω 9 moiety than that in Fig. 7 (32.3%) as well.

The present work provides a mathematical basis for the design and operation of a continuous, packed-bed reactor in the interesterification of soybean oil with oleic acid. Rate equations and deactivation kinetics found in this work could also be useful in the lipase-catalyzed interesterification of other vegetable oils in continuous, packed-bed reactors.

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