0273-2289/03/104/0105/$20.00

Lipase-Catalyzed Interesterification of Soybean Oil with an ω-3 Polyunsaturated Fatty Acid Concentrate Prepared from Sardine Oil

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Received September 1, 2001; Revised March 1, 2002;
Accepted March 1, 2002

Abstract

To reduce the content of linoleoyl moiety in soybean oil, soybean oil that contains 53.0% linoleoyl moiety as molar acyl moiety composition was interesterified with an ω-3 polyunsaturated fatty acid (PUFA) concentrate (24.0 mol% eicosapentaenoic acid [EPA], 40.4 mol% docosahexaenoic acid [DHA]) prepared from sardine oil, using an immobilized sn-1,3-specific lipase from *Rhizomucor miehei* (Lipozyme IM). The reaction was carried out in a batch reactor at 37°C under the following conditions: 500 μmol of soybean oil, molar ratio of ω-3 PUFA concentrate to soybean oil = 1.0–6.0, 5.0 mL of heptane, and 30 batch interesterification units of enzyme. After the reaction time of 72 h, modified soybean oil, which contains 34.9% linoleoyl, 10.1% eicosapentaenoyl, and 14.2% docosahexaenoyl moieties, was produced at the molar reactant ratio of 6.0. In this oil, the total ω-3 acyl moiety composition reached 34.1%; the molar ratio of ω-3 to ω-6 acyl moieties was enhanced by five times compared with soybean oil. Compared with palmitic acid, DHA was kinetically six times less reactive, although the EPA was by 16% more reactive.

Index Entries: Interesterification; acidolysis; kinetics; soybean oil; eicosapentaenoic acid; docosahexaenoic acid; *Rhizomucor miehei*; lipase; Lipozyme IM; sardine oil.

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Introduction

Recent studies have demonstrated beneficial effects of feeding fish oil in animal mammary tumor models *(1–6)*. Fish oils are rich in dietary ω-3 polyunsaturated fatty acids (PUFAs), particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The remarkable preventing effect of these two dietary ω-3 fatty acids on the growth of a human lung carcinoma in athymic mice has also been reported *(7)*. By contrast, fats and oils rich in ω-6 PUFAs—linoleic acid, in particular—appear to enhance mammary tumorigenesis and tumor cell growth *(4,6,8,9)*. The preventing and curing effects of EPA and DHA on thrombosis and arteriosclerosis have also been reported *(10,11)*. These desirable fatty acids are bound to glycerides, which is the natural form of fatty acids in fish oils *(12)*. Thus, preparation of triacylglycerols (TGs) highly enriched with EPA and DHA via lipase-catalyzed esterification of glycerol *(13–18)* and interesterification of fish oils *(19–24)* has been investigated extensively. The most commonly observed feature of these two reactions was the drastically low reactivity of free EPA and DHA and their acyl moieties in fish oils. Based on this low reactivity of these ω-3 acyl moieties, the lipase-assisted concentration of ω-3 PUFAs in acylglycerols via partial alcoholysis *(25–28)* and partial hydrolysis *(29–35)* of fish oils has also been investigated.

Nowadays, soybean oil is produced much more than any other edible vegetable oil, and approx 70% of the product oil is consumed directly as a cooking oil. The major acyl moiety in soybean oil is linoleoyl (18:2 ω-6) (*see* Table 1). In order to reduce the content of linoleoyl moiety in soybean oil, we previously investigated the lipase-catalyzed interesterification of soybean oil with oleic acid and α-linolenic acid *(36,37)*. The present work aims to reduce the content of 18:2 ω-6 moiety in soybean oil via interesterification with ω-3 PUFAs. For this purpose, an ω-3 PUFA concentrate was prepared from sardine oil, and an immobilized lipase from *Rhizomucor miehei* (Lipozyme IM) was used. The reactivity of acyl moieties in soybean oil and that of free EPA and DHA in the interesterfication with soybean oil are also kinetically studied to evaluate the usefulness of this immobilized lipase in the reaction.

Materials and Methods

Materials

Refined soybean oil and sardine oil were purchased from Kanto Chemicals (Tokyo, Japan) and Yamakei Sangyo Co. Ltd. (Osaka, Japan), respectively. Table 1 gives the composition of acyl moieties in the soybean oil and sardine oil used. Organic solvents, fatty acids, and inorganic compounds used in the present work were purchased from Wako Pure Chemical Industries (Osaka, Japan). They were of the highest purities available. Palmitic acid and oleic acid used were of 100% purity. α-Linolenic acid used was of practical grade and it contained 1.9 mol% oleic acid, 17.3 mol%

Table 1
Composition of Acyl Moieties in Soybean Oil, Sardine Oil, and ω-3 PUFA Concentrate Used

	Composition of acyl moieties (mol%)													Average MW[b]
	14:0[a]	16:0	16:1	18:0	18:1 ω-9	18:2 ω-6	18:3 ω-3	18:4 ω-3	20:4 ω-6	20:5 ω-3	22:5 ω-3	22:6 ω-3	24:1	
Soybean oil	0	11.9	0	3.4	22.9	53.0	8.8	0	0	0	0	0	0	873.4[c]
Sardine oil	4.9	18.8	5.8	3.5	30.9	14.1	3.9	4.3	3.4	3.3	0.6	5.1	1.5	873.1[c]
ω-3 PUFA[d]	0.7	0.2	0.9	0	1.5	4.7	2.0	6.0	4.7	24.0	2.3	40.4	12.8	317.8

[a] 14:0 = 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; 18:4 ω-3 = octadecatetraenoyl; 20:4 ω-6 = arachidonoyl; 20:5 ω-3 = eicosapentaenoyl; 22:5 ω-3 = docosapentaenoyl; 22:6 ω-3 = docosahexaenoyl; 24:1 = nervonoyl.

[b] Average molecular weight.

[c] As a mixture of triacylglycerols.

[d] ω-3 PUFA concentrate.

linoleic acid, and 80.8 mol% α-linolenic acid. Fatty acid methyl esters used as the standard for gas chromatography (GC) were purchased from Sigma Chemical Co. (St. Louis, MO). Activated aluminum oxide (90, basic, activity I, 70–80 mesh) for column chromatography (CC) was 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.

Lipozyme IM was supplied from Novo Nordisk (Chiba, Japan). It contained 4.9 wt% moisture. This lipase (triacylglycerol acylhydrolase, E.C. 3.1.1.3) specifically hydrolyzes the acyl moieties at sn-1- and sn-3-positions of triacylglycerol. Because its enzymatic activity was reported to be maximal at 10 wt% moisture content, commercial Lipozyme IM was hydrated to contain 10 wt% moisture by the method reported *(36)*. The activity of Lipozyme IM thus obtained was 155 batch interesterification units (BIUs) per gram of dry enzyme; 1 BIU corresponds to 1 μmol of palmitic acid incorporated into trioleoylglycerol in 1 min from an equimolar mixture at 37°C.

The ω-3 PUFA concentrate was prepared from sardine oil by saponification, extraction of fatty acids, crystallization of potassium salts from acetone solution, and extraction of fatty acids using the method reported *(38,39)*. The ω-3 PUFA concentrate obtained was kept at –20°C under nitrogen atmosphere. Table 1 also gives the fatty acid composition of ω-3 PUFA concentrate obtained. Its total ω-3 PUFA content was 74.7 mol%.

Interesterification Reaction

The reaction was carried out at 37°C in a silicone-rubber-stoppered 50-mL Erlenmeyer flask under nitrogen atmosphere, as reported previously *(36)*. Soybean oil (500 μmol) and ω-3 PUFA concentrate (500–3000 μmol) were dissolved in 5.0 mL of heptane. Lipozyme IM was added and the mixture was shaken at 0.30 *g*. A sample for analysis (approx 100 μL) was periodically withdrawn from the reaction mixture and modified soybean oil was separated by CC on the activated aluminum oxide (2.0 g) with diethyl ether (3–4 mL) as the eluting solvent.

Fatty Acid Analysis

The composition of acyl moieties in modified soybean oils was analyzed by GC. The ether solution of modified soybean oil obtained in CC was completely evaporated at room temperature under vacuum, and the oily residue obtained was transmethylated with 5 mL of 6 wt% methanolic HCl for 1 h at 90–92°C. Fatty acid methyl esters formed were extracted with hexane and then analyzed by GC using a 5 wt% Advance DS/Chromosorb W glass column (3 mm i.d. [inner diameter] × 2.0 m) at 160°C (methyl esters of C_{14}–C_{18} fatty acids) and 190°C (methyl esters of C_{20}–C_{24} fatty acids), respectively. Methyl heptadecanoate and methyl lignocerate were used as the internal standards for GC at 160°C and 190°C, respectively. A Shimadzu GC-8A-type gas chromatograph, equipped with a flame ionization detec-

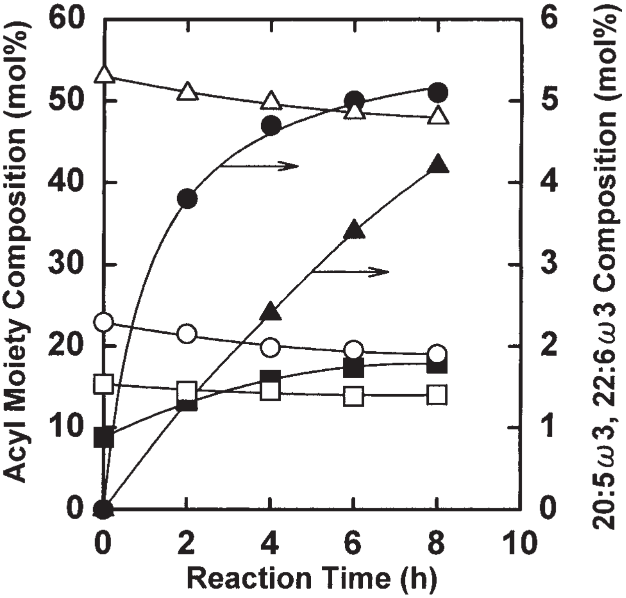

Fig. 1. Acyl moiety composition of modified soybean oils produced at different reaction times. Reaction conditions: 500 μmol soybean oil, 1500 μmol ω-3 PUFA concentrate, 20 BIU Lipozyme IM, 5.0 mL heptane, reaction temperature 37°C. ○: 18:1 ω-9; Δ: 18:2 ω-6; ●: 20:5 ω-3; ▲: 22:6 ω-3; ■: total ω-3 acyl moieties (= 18:3 ω-3 + 18:4 ω-3 + 20:5 ω-3 + 22:5 ω-3 + 22:6 ω-3); □: 14:0 + 16:0 + 16:1 + 18:0 + 20:4 ω-6 + 24:1.

tor and a Shimadzu CR-6A-type integrator (Shimadzu, Kyoto, Japan) were used. The plotting software package Delta Graph Pro. 3.5 (Delta Point, Monterey, CA) was used for curve-fitting calculations.

Results and Discussion

Effect of the Amount of Enzyme

Figure 1 shows the acyl moiety composition of modified soybean oils produced at different reaction times in the interesterification of soybean oil with ω-3 PUFA concentrate. The amount of Lipozyme IM used was 20 BIU and the molar ratio of ω-3 PUFA concentrate to soybean oil was 3.0. The percentage of 18:2 ω-6 moiety in soybean oil decreased slowly and reached 48.0% after the reaction time of 8 h. That of 18:1 ω-9 moiety also decreased to 19.0% after this reaction time. In contrast, the percentage of 22:6 ω-3 moiety increased to 4.2% after the reaction time of 8 h. The percentage of 20:5 ω-3 moiety increased more rapidly than that of 22:6 ω-3 moiety and reached 5.1% after the reaction time of 8 h. However, the percentages of these two ω-3 acyl moieties did not level off within this reaction time (*see* Fig. 1).

Figures 2 and 3 show the acyl moiety composition of modified soybean oils produced at different reaction times in the interesterification over 30 BIU of Lipozyme IM. In this run, the reaction was carried out up to the

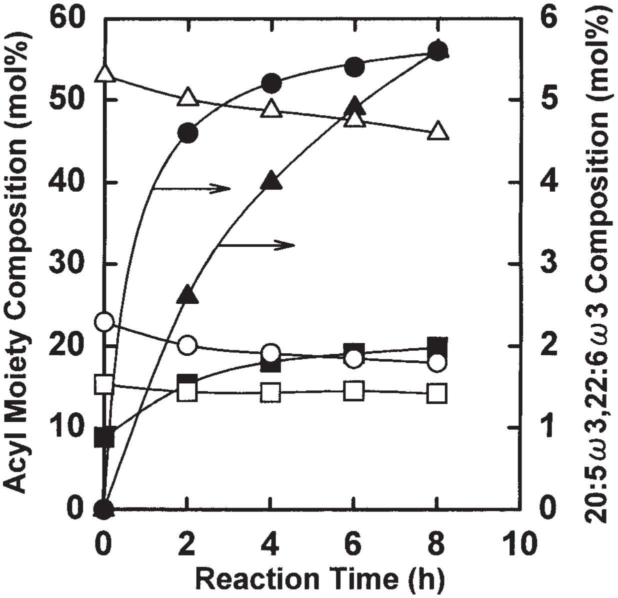

Fig. 2. Acyl moiety composition of modified soybean oils produced at different reaction times (continues to Fig. 3). Reaction conditions and symbols used are the same as those in Fig. 1 except for the use of 30 BIU Lipozyme IM.

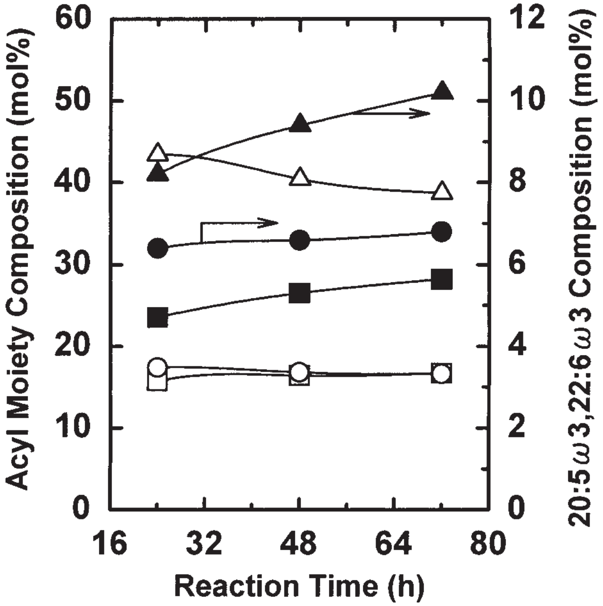

Fig. 3. Acyl moiety composition of modified soybean oils produced at different reaction times (continues from Fig. 2).

reaction time of 72 h. The percentage of 18:2 ω-6 moiety decreased more rapidly than that in the reaction over 20 BIU of Lipozyme IM (e.g., 46.0% at 8 h) and reached 38.7% after the reaction time of 72 h. That of 18:1 ω-9 moiety also decreased to 16.6% after this reaction time (*see* Figs. 2 and 3). The percentages of 20:5 ω-3 and 22:6 ω-3 moieties reached the same value (5.6%) after the reaction time of 8 h (*see* Fig. 2). The percentages of these two ω-3 moieties increased further at longer reaction times and reached 6.8% and 10.2%, respectively, after the reaction time of 72 h (*see* Fig. 3). At this reaction time, the total ω-3 acyl moiety composition in the modified soybean oil was 28.2%. This value is three times larger than that in soybean oil.

The relation of the composition of acyl moieties with reaction time could be satisfactorily expressed by the equation $y = ax/(x + b) + c$, where x and y are the reaction time (h) and the percentage of an acyl moiety, respectively, and a, b, and c are constants. For 20:5 ω-3 moiety in the run shown in Figs. 2 and 3, a, b, and c were 6.674, 1.089, and 0 ($R^2 = 0.9918$, R = regression factor), respectively, whereas for 22:6 ω-3 moiety in the same run, they were 10.948, 7.289, and 0 ($R^2 = 0.9977$), respectively. By the extraporation $x \to \infty$ in these equations, the maximum percentages of 20:5 ω-3 and 22:6 ω-3 moieties, which could be incorporated into soybean oil at the molar reactant ratio of 3.0, were calculated to be 6.7% and 11.0%, respectively. We tentatively call these maximum percentages the equilibrated values. At the reaction time of 72 h with 30 BIU of Lipozyme IM, EPA was incorporated almost equilibrated (6.8% $\longleftrightarrow$ 6.7%), whereas DHA was incorporated up to 93% of the equilibrium value (10.2% $\longleftrightarrow$ 11.0%) (*see* Fig. 3).

Effect of the Reactant Ratio

Because EPA and DHA could be incorporated into soybean oil up to more than 90% values of the equilibrium 20:5 ω-3 and 22:6 ω-3 moiety compositions (*see* Figs. 2 and 3), the effect of reactant feed ratio on the acyl moiety composition of modified soybean oil was investigated under the reaction conditions employed in Figs. 2 and 3. As shown in Fig. 4, the percentage of 18:2 ω-6 moiety decreased to 34.9% at the molar reactant ratio of 6.0, which was the highest reactant ratio used in this work. Thus, 34% of the 18:2 ω-6 moiety originally present in soybean oil could be replaced at this reactant ratio. The percentage of 18:1 ω-9 moiety also decreased to 13.8% at the highest reactant ratio. By contrast, the percentages of 20:5 ω-3 and 22:6 ω-3 moieties were maximum, 10.1% and 14.2%, respectively, at the highest reactant ratio. The total percentage of ω-3 acyl moieties was also maximum, 34.1% (*see* Fig. 4). Although arachidonic acid was incorporated up to 2.3% 20:4 ω-6 moiety composition at the highest reactant ratio, the molar ratio of ω-3 to ω-6 acyl moieties was enhanced to 0.92, which is five times larger than that in soybean oil (0.17, Fig. 4).

Reactivity of Acyl Moieties in Soybean Oil

To evaluate the usefulness of Lipozyme IM in the interesterification of soybean oil, the reactivity of acyl moieties in soybean oil was kinetically investigated at the molar reactant ratio of 3.0. We assume a completely mixed reactor model for our batch reactor, in which mass transfer is not rate determining *(36)*, and pay attention to the reaction by which 18:2 ω-6 moiety

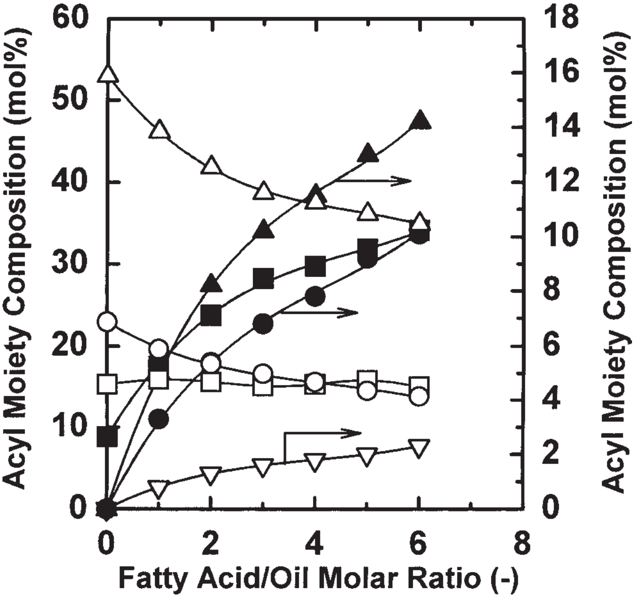

Fig. 4. Acyl moiety composition of modified soybean oils produced at different molar reactant ratios. Reaction conditions: 500 μmol soybean oil; 500–3000 μmol ω-3 PUFA concentrate; 30 BIU Lipozyme IM; 5.0 mL heptane; reaction temperature 37°C; reaction time 72 h. ▽: 20:4 ω-6; □: 14:0 + 16:0 + 16:1 + 18:0 + 24:1. The other symbols used are the same as those in Fig. 1.

composition in soybean oil decreases. When neither monoglyceride nor diglyceride is contained in soybean oil and its modified oils, the following mass balance can be derived for a small decrease in the concentration of 18:2 ω-6 moiety (dC) within a short reaction time (dt):

$$dC = 3C_0\,dx = rW\,dt \tag{1}$$

where C is the 18:2 ω-6 moiety concentration of oil in the reactant mixture (mol/mL), C_0 is the initial concentration of soybean oil in the reactant mixture (mol/mL), x is the molar composition of 18:2 ω-6 moiety in oil, r is the rate for interesterification of 18:2 ω-6 moiety in soybean oil (mol/mL · h · BIU), W is the amount of Lipozyme IM used (BIU), and t is the reaction time (h). Here, we propose the 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 concentration of oil from the equilibrium value" and "the total concentration of fatty acids other than linoleic acid in the reactant mixture." Because the molar ratio of reactant fatty acid to soybean oil is 3.0, the following rate equation is obtained:

$$r = -k_{\mathrm{LA}}\,[3C_0(x - x_e)][3C_0(1 - x_f - x_0 + x)] = -9k_{\mathrm{LA}}\,C_0^2(x - x_e)(1 + x - x_0 - x_f) \tag{2}$$

Here, k_{LA} is the rate constant for the interesterification of 18:2 ω-6 moiety in soybean oil, x_e is the equilibrium molar composition of 18:2 ω-6 moiety in oil, x_0 is the molar composition of 18:2 ω-6 moiety in soybean oil, and x_f is

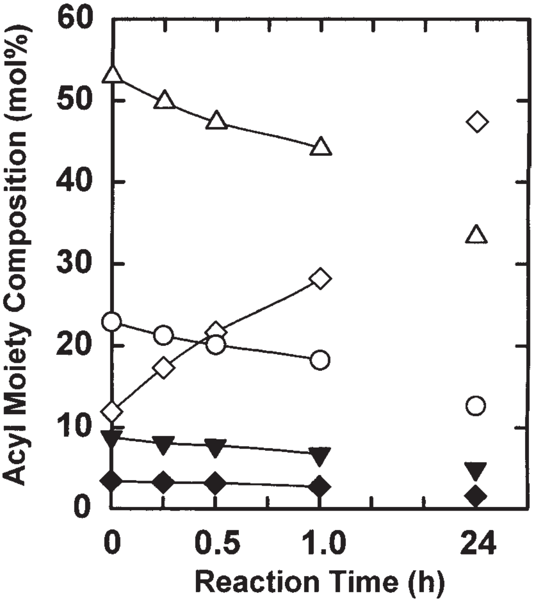

Fig. 5. Acyl moiety composition of modified soybean oils produced at different reaction times. Reaction conditions: 500 μmol soybean oil; 1500 μmol palmitic acid; 10 BIU Lipozyme IM; 5.0 mL heptane; reaction temperature 37°C. ◇: 16:0; ◆: 18:0; ○: 18:1 ω-9; Δ: 18:2 ω-6; ▼: 18:3 ω-3. x_e = 0.495 (16:0), 0.121 (18:1 ω-9), 0.323 (18:2 ω-6), and 0.047 (18:3 ω-3).

the molar composition of linoleic acid in the reactant fatty acid. From Eqs. (1) and (2),

$$dx = -3k_{\mathrm{LA}}WC_0(x - x_e)(1 + x - x_0 - x_f)\,dt \qquad (3)$$

By integrating Eq. (3) between $x = x_0$ at $t = 0$ and $x = x$ at $t = t$, we obtain

$$[1/(1 + x_e - x_0 - x_f)] \ln [(1 + x - x_0 - x_f)(x_0 - x_e)/(x - x_e)(1 - x_f)] = 3k_{\mathrm{LA}}WC_0t \qquad (4)$$

For the decrease in the composition of 16:0 and 18:1 ω-9, and 18:3 ω-3 moieties in soybean oil, equations similar to Eq. (4) are derived.

Figure 5 shows the composition of acyl moieties in modified soybean oils produced at different reaction times in the interesterification with palmitic acid. The percentages of 18:1 ω-9, 18:2 ω-6, and 18:3 ω-3 moieties decreased at longer reaction times and reached 12.7%, 33.4%, and 4.9%, respectively, after the reaction time of 24 h (*see* Fig. 5). By the same curve-fitting procedure used in a previous part of this work, the equilibrium percentages of these acyl moieties were determined to be 12.1% ($R^2 = 0.9997$), 32.3% ($R^2 = 0.99997$), and 4.7% ($R^2 = 0.9928$), respectively. By using these equilibrium acyl moiety compositions, the validity of Eq. (4) was confirmed and rate constants for the interesterification of 18:1 ω-9, 18:2 ω-6, and 18:3 ω-3 moieties in soybean oil were determined (*see* Fig. 6 and Table 2). Similarly, soybean oil was separately interesterified with oleic acid and α-linolenic acid and rate constants for the interesterification of acyl moieties

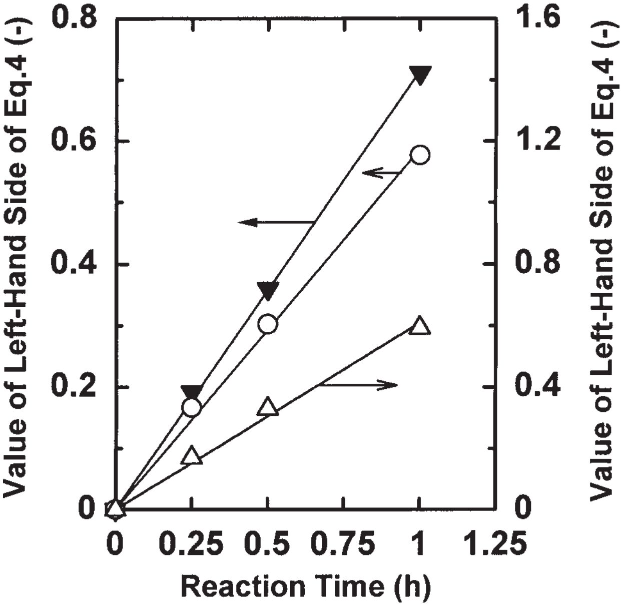

Fig. 6. Linear plots of Eq. (4) in the interesterification with palmitic acid. Acyl moiety compositions shown in Fig. 5 are used for calculations. ○: 18:1 ω-9; Δ: 18:2 ω-6; ▼: 18:3 ω-3.

Table 2
Reactivity of Acyl Moieties and Fatty Acids Presented by the Rate Constants in Lipozyme IM-Catalyzed Interesterification of Soybean Oil at 37°C

	Rate constant *k* (mL/mol · h · BIU)				
	Acyl moiety			Average value	Fatty acid
16:0	—	221	203	212	206
18:1 ω-9	196	—	190	193	202
18:2 ω-6	203	184	231	206	—
18:3 ω-3	238	236	—	237	241
20:5 ω-3	—	—	—	—	239
22:6 ω-3	—	—	—	—	34

in soybean oil were determined (*see* Table 2). The reactivity of acyl moieties in soybean oil followed the order: 18:3 ω-3 > 16:0 ≥ 18:2 ω-6 > 18:1 ω-9 (*see* Table 2). Interesterification of oils and fats with fatty acids takes place via the partial hydrolysis of TGs followed by the re-esterification of partially hydrolyzed species *(40)*. Hence, the order of the reactivity of acyl moieties in soybean oil presented here must be that of the reactivity of acyl moieties at the hydrolysis step. Hara et al. *(41)* reported the following orders of the reactivity of acyl moieties in the hydrolysis of some vegetable oils and TGs with lipase derived from *Candida cylindracea*: 18:3 ω-3 > 16:0 > 18:1 ω-9 > 20:5 ω-3 > 22:6 ω-3, and 18:3 ω-3 > 18:2 ω-6 > 18:1 ω-9 > 18:0. Shimada et al. *(42)*

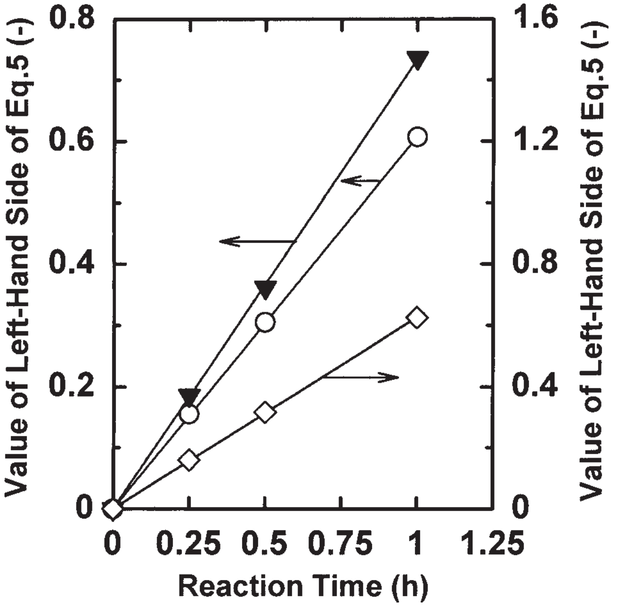

Fig. 7. Linear plots of Eq. (5) in the interesterification with various fatty acids. Reaction conditions: 500 μmol soybean oil; 1500 μmol fatty acid; 10 BIU Lipozyme IM; 5.0 mL heptane; reaction temperature 37°C. ◇: palmitic acid; ○: oleic acid; ▼: α-linolenic acid. x_e = 0.495 (16:0), 0.568 (18:1 ω-9), and 0.416 (18:3 ω-3).

also reported the highest reactivity of 18:3 ω-3 moiety within C_{18} acyl moieties in the hydrolysis of a randomized TG with lipases derived from *Candida cylindracea* and *Rhizopus delemar*.

Reactivity of Fatty Acids

The reactivity of palmitic acid, oleic acid, α-linolenic acid, EPA, and DHA in the reaction with soybean oil was kinetically investigated at 37°C. Here, we pay attention to the reaction by which the composition of an acyl moiety in soybean oil increases. We again propose the second-order reaction kinetics, in which the rate of reaction increases in proportion to the product of "the difference in the concentration of an acyl moiety in oil from the equilibrium value" and "the concentration of fatty acid having the same acyl moiety in the reactant mixture." As in the case of Eq. (4), the following equation is obtained:

$$[1/(x_0 + x_f - x_e)] \ln [(x_0 + x_f - x)(x_e - x_0)/x_f (x_e - x)] = 3kWC_0t \qquad (5)$$

Figures 7 and 8 show the linear plots of Eq. (5) in the interesterification of soybean oil with various fatty acids at 37°C. From the slopes of these straight lines, rate constants for these fatty acids were determined (*see* Table 2). Compared with palmitic acid, oleic acid has nearly the same reactivity, whereas α-linolenic acid and EPA are 16–17% more reactive. In contrast, DHA is six times less reactive than palmitic acid. Thus, the reactivity of

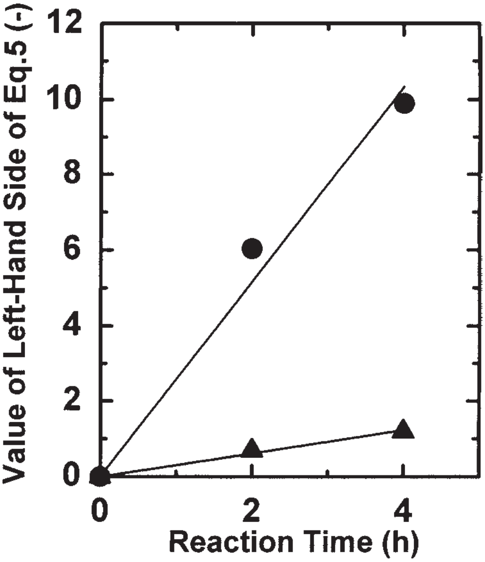

Fig. 8. Linear plots of Eq. (5) in the interesterification with ω-3 PUFA concentrate. Acyl moiety compositions shown in Fig. 2 are used for calculations. ●: EPA; ▲: DHA. $x_e = 0.067$ (20:5 ω-3) and 0.110 (22:6 ω-3).

fatty acids in the interesterification of soybean oil followed the order: α-linolenic acid ≥ EPA > palmitic acid ≥ oleic acid > DHA. This order must be the order of the reactivity of fatty acids at the re-esterification step. Osada and Hatano *(43)* reported the following order of the reactivity of free PUFAs in Lipozyme-catalyzed interesterification of triolein: EPA > linoleic acid > palmitic acid > α-linolenic acid > DHA. Yamashita et al. *(44)* also reported the following orders in Lipozyme-catalyzed esterification of glycerol: oleic acid ≥ linoleic acid ≥ α-linolenic acid > stearic acid and oleic acid > EPA > DHA. These three orders of the reactivity of fatty acids partially agree with each other although they cannot be compared strictly because of the different experimental procedures used. At any rate, the order of the reactivity of fatty acids found in the present work agreed with that of the reactivity of acyl moieties at the hydrolysis step (*see* Table 2), suggesting that the steric and electronic properties of acyl moieties in TGs and free fatty acids affect in a similar manner their reactivity at these two reaction steps. Also, the present work showed the low catalytic activity of Lipozyme IM for the reaction of DHA in the interesterification, as reported by many workers. The authors believe that the drastically low reactivity of DHA in the interesterification is attributed to its bulky molecular structure.

Preparation of soybean oils enriched with 20:5 ω-3 and 22:6 ω-3 moieties via lipase-catalyzed interesterification was already reported. For example, Li and Ward *(45)* prepared modified soybean oil (20:5 ω-3 plus 22:6 ω-3 moiety content = 10.5 wt%) by using ω-3 PUFA concentrate obtained from cod liver oil. Huang and Akoh *(46)* and Khare and Nakajima *(47)* also prepared modified soybean oils (22:6 ω-3 moiety content = 32.9%, 25%) by the reaction with DHA and its ethyl ester. In the present work, modified soybean oil having 6.8% 20:5 ω-3 and 10.2% 22:6 ω-3 moiety compositions was prepared at the molar reactant ratio of 3.0, whereas that having 10.1% 20:5 ω-3 and 14.2% 22:6 ω-3 moiety compositions was at the molar reactant ratio of 6.0. At this reactant ratio, the total ω-3 acyl moiety composition reached 34.1% after the reaction time of 72 h (*see* Fig. 4). The ω-3 acyl moiety compositions obtained in the present work are not always low compared with those already reported for soybean oil *(45–47)*.

It was reported that excess intake of ω-3 PUFAs resulted in an enhancement of aortic atherosclerosis in rabbits because of the significantly high serum peroxide levels *(48)*. Thus, oils extraordinarily highly enriched with 20:5 ω-3 and 22:6 ω-3 moieties are not always desirable. Dietary oleic acid has neither a tumor-promoting effect nor a tumor-inhibiting effect (8). However, 18:1 ω-9 moiety composition in soybean oil decreased in the interesterification (Figs. 1–4). As pointed out previously, soybean oil may not be a nutritionally safe vegetable oil. However, modified soybean oils obtained in the present work do hold promise for specialty nutrition and other therapeutic uses.

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