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Nutritional Improvement of Soybean Oil via Lipase-Catalyzed Interesterification

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ABSTRACT

In order to decrease the content of linoleoyl moiety in soybean oil, soybean oil that contains 22.8% oleoyl, 54.8% linoleoyl, and 7.1% α-linolenoyl moieties as molar acyl moiety composition was interesterified in hexane with oleic acid or α-linolenic acid, using an immobilized sn-1,3-specific lipase (Lipozyme® IM) from *Mucor miehei*. The reactions were carried out in a batch reactor at 37°C in the following system: molar ratio of fatty acid to soybean oil = 1.0 ~ 6.0, 5.0 mL of hexane/500 μmol soybean oil, and 10.0 or 15.0 batch interesterification units of enzyme/500 μmol soybean oil. Under these reaction conditions, the rates of interesterification of acyl moieties in soybean oil were of the order: stearoyl > palmitoyl > linoleoyl > oleoyl > α-linolenoyl, and the reaction with oleic acid occurred without a significant loss of α-linolenoyl moiety. At the molar ratio of 3.0 and the reaction time of 6 h, triacylglycerols (TGs), which contain 50.8% oleoyl, 38.8% linoleoyl, and 5.4% α-linolenoyl moieties, were produced in the reaction with oleic acid; TGs that contain 13.5% oleoyl, 40.8% linoleoyl, and 40.4% α-linolenoyl moieties were obtained with α-linolenic acid. Approximately 86–88% of the interesterification of linoleoyl moiety, which occurred in 10 h, took place within 1 h.

Index Entries: Interesterification; acidolysis; soybean oil; oleic acid; linoleic acid; α-linolenic acid; lipase; Lipozyme.

Table 1
Compositions of Acyl Moieties in Soybean Oil and Reactant Fatty Acids Used in the Present Work

	Composition of acyl moieties (mol%)							Average mol wt[f]
	14:0[a]	16:0	16:1	18:0	18:1 ω 9	18:2 ω 6	18:3 ω 3	
Soybean oil	0	12.6	0	2.7	22.8	54.8	7.1	872.9[g]
Rapeseed oil[b]	0	3.9	0	1.8	57.9	21.8	11.3	–
Oleic acid[c]	0	0	0	0	100	0	0	282.4
Oleic acid[d]	3.9	3.5	6.9	0.6	73.2	10.8	1.1	277.7
α-Linolenic acid[e]	0	0	0	0	1.9	17.3	80.8	279.4

[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.
[b] Low ercic type (ref. *12*).
[c] Biochemical reagent grade.
[d] Reagent grade.
[e] Practical grade.
[f] Average mol wt.
[g] As a mixture of triacylglycerols.

INTRODUCTION

It is desirable to alter the composition of acyl moieties in triacylglycerols (TGs) occurring naturally in fats and oils, in order to modify their nutritional properties. Nowadays, soybean oil is produced much more than any other edible vegetable oils (e.g., 18.2×10^6 tons in 1995), and about 70% of the product oil is consumed directly as a cooking oil. Soybean oil contains exclusively C_{16}- and C_{18}-acyl moities in TG; their typical molar composition is given in Table 1. The major acyl moiety in soybean oil is thus linoleoyl (18:2 ω 6). Recent studies have indicated that excess intake of dietary linoleic acid promotes incidence and growth of mammary tumors in rats and mice *(1–3)*. Hence, soybean oil is not always a nutritionally safe vegetable oil. In contrast dietary α-linolenic acid has been reported to have an inhibiting effect on the growth and metastasis of mammary tumors. *(4–7)* Also, this ω 3-type fatty acid is essential to the development of retina and brain in mammals *(8–11)*. On the other hand, dietary oleic acid has no significant tumor-promoting or -inhibiting effect *(1)*. Included for comparison is the typical acyl moiety composition of rapeseed oil in Table 1. If the percentage of 18:2 ω 6 moiety in soybean oil could be reduced to the levels found in rapeseed oil by the interesterification with oleic acid, soybean oil would be a much more nutritionally safe vegetable oil.

The present work investigates the interesterification of soybean oil with oleic acid in hexane, using an immobilized lipase (Lipozyme IM)

from the fungus *Mucor miehei,* supported on a macroporous anion-exchange resin. This lipase (triacylglycerol acylhydrolase, EC 3.1.1.3) specifically hydrolyzes the acyl moieties from the sn-1- and sn-3-positions of TG. The interesterification with α-linolenic acid was also carried out, and the effects of reaction conditions (amount of enzyme, and hexane/soybean oil and fatty acid/soybean oil ratios) on the composition of acyl moieties in TGs formed were investigated.

MATERIALS AND METHODS

Materials

Refined soybean oil, hexane (dehydrated, H_2O content < 0.003%), methanol (dehydrated, H_2O content < 0.025%), and Celite 545 were purchased from Kanto Chemicals (Tokyo, Japan). Guaranteed reagent grade trioleoylglycerol, biochemical reagent grade oleic acid, reagent grade oleic acid, and practical grade α-linolenic acid were from Wako Pure Chemical Industries, Osaka, Japan. The compositions of acyl moieties in soybean oil, and the reactant fatty acids used in the present work, are given in Table 1. Palmitic acid and methyl esters of fatty acids as the standard for gas chromatography (GC) were purchased from Sigma (St. Louis, MO). Activated aluminium oxide (90, basic, activity I, 70–80 mesh) for column chromatography (CC) was from Merck (Darmstadt, Germany). Prior to use, 50 g of this aluminium 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 *M. miehei* (Lipozyme IM) was supplied from Novo Nordisk (Chiba, Japan). Because its enzymatic activity was reported in the catalog to be maximal at 10 wt% water content, 3.0 g of commercial Lipozyme IM (4.9 wt% water content) was mixed with a piece of wet filtration paper (H_2O content = 170 mg) in a stoppered glass bottle, and allowed to stand at 5°C for 1 wk to equilibrate. The activity of Lipozyme IM thus obtained was 172 batch interesterification units (BIU)/g of dry granules; 1 BIU corresponds to 1 μmol of palmitic acid incorporated into trioleoylglycerol/min from an equimolar mixture at 37°C. Immobilized nonspecific lipase was prepared using Celite 545 and lipase LP from Chromobacterium viscosum (Asahi Chemical Industry, Tokyo, Japan) by the method of Coleman and Macrae *(13).* Its water content was similarly adjusted to 5.2 wt% *(14).* The activity of this immobilized lipase was 23.0 BIU/g of dry powder.

Interesterification Reactions

The reactions were carried out at 37°C in a Teflon-stoppered 50-mL glass flask under nitrogen atmosphere. Soybean oil (500 μmol) and fatty

acid (500–3000 μmol) were dissolved in 2.5–10.0 mL of hexane. Lipozyme IM was added and the mixture was shaken at 0.30*g*. Samples (ca. 100 μL) were withdrawn from the reaction mixture and TGs were separated by CC on the activated aluminium oxide (2.0 g) with diethyl ether (3–4 mL) as the eluting solvent, as described by Osada et al. *(14)*. A thermostatic shaking water bath, "Thomastat T-22S" (Thomas Kagaku Kikai, Tokyo, Japan) was used.

Fatty Acid Analysis

The composition of acyl moieties in TG was analyzed by GC. The ether solution of TG 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 3 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 dia × 2.0 m) at 160°C. Methyl heptadecanoate was used as the internal standard for GC. The methanolic HCl was prepared by adding guaranteed reagent grade acetyl chloride into dehydrated methanol. A Shimadzu GC-8A-type gas chromatograph, equipped with a flame ionization detector and a Shimadzu CR-6A-type integrator (Shimadzu, Kyoto Japan), were used.

RESULTS

Interesterification with Oleic Acid

Figure 1 shows the effects of the amount of enzyme and reaction time on the conversion of oleic acid in the equimolar interesterification. When the amount of enzyme was 2.5 BIU, the conversion of oleic acid increased with reaction time, and was 41.6% after the reaction time of 10 h. The conversion of oleic acid increased more quickly when larger amounts of enzyme were used. At 10.0 BIU, it nearly leveled off at ca. 46% after the reaction time of 6–10 h (Fig. 1).

Figure 2 shows the compositions of acyl moieties in TGs formed at different reaction times in the equimolar reaction. The amount of enzyme used was 10.0 BIU. The percentage of 18:2 ω 6 moiety decreased quickly to 45.4% within 1 h, and then decreased slowly to 43.8% after the reaction time of 10 h. Thus, ca. 86% of the interesterification of 18:2 ω 6 moiety, which occurred in 10 h, took place within 1 h. The percentage of oleoyl (18:1 ω 9) moiety increased quickly to 36.9% within 1 h, and then increased slowly to 39.0% after the reaction time of 10 h. The percentages of palmitoyl (16:0) and stearoyl (18:0) moieties decreased to 8.3 and 1.7%, respectively, within 1 h, and were 7.6 and 1.6%, respectively after the reaction time of 10 h. In

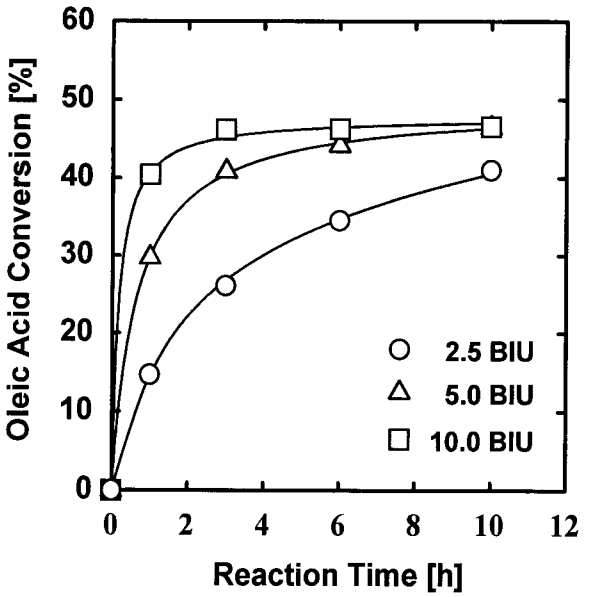

Fig. 1. Effects of the amount of enzyme and reaction time on the conversion of oleic acid. Reaction conditions: 500 μmol soybean oil, 500 μmol oleic acid (biochemical reagent grade), 2.5–10.0 BIU Lipozyme IM, 5.0 mL hexane, reaction temperature 37°C.

contrast, the percentage of α-linolenoyl (18:3 ω 3) moiety remained nearly constant during the course of the reaction (Fig. 2). Thus, the interesterification of 18:2 ω 6 moiety occurred preferentially to 18:3 ω 3 one moiety.

The percent decreases of acyl moieties after the reaction time of 1 h were 34.1% (16:0), 37.0% (18:0), 17.2% (18:2 ω 6), and ca. 0% (18:3 ω 3), respectively (Fig. 2). Thus, the reactivities of acyl moieties in soybean oil in the interesterification with Lipozyme IM followed the order: 18:0 > 16:0 > 18:2 ω 6 > 18:3 ω 3. A similar experiment with palmitic acid gave the order: 18:0 > 18:2 ω 6 > 18:1 ω 9 > 18:3 ω 3. These orders must be those of the reactivities of acyl moieties in the hydrolysis reaction, because the interesterification takes place via partially hydrolyzed species of TG.

On the other hand, percent yield of TG, i.e., TG formed (g)/soybean oil fed (g) × 100, in the equimolar reaction (Fig. 2) was as high as 96–100% after the reaction time of 6–10 h (data not shown). This indicates that TGs almost free from any di- and monoacylglycerols were formed at these reaction times. This agreed with the Lipozyme-catalyzed interesterification of cod liver oil by C_{20} and $C_{22}\omega$ 3 polyunsaturated fatty acids reported *(15)*. Hence, it was decided to use the enzyme amount of 10.0 BIU and the reaction time of 6 h in later experiments. The use of immobilized nonspecific lipase, instead of Lipozyme IM, at experimental conditions otherwise identical to those of Fig. 2, gave nearly the same acyl moiety composition of TG formed.

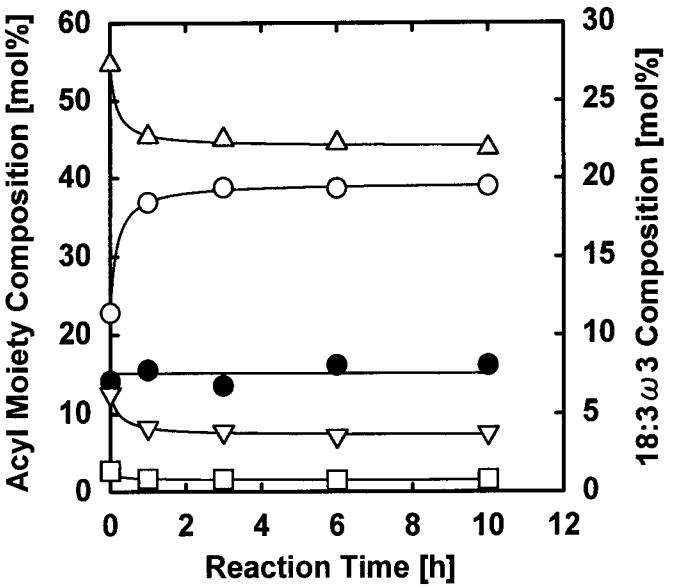

Fig. 2. Compositions of acyl moieties in TGs formed at different reaction times in the reaction with oleic acid. Reaction conditions are the same as those in Fig. 1 (the amount of Lipozyme IM: 10.0 BIU). ▽, 16:0; □, 18:0; ○, 18:1 ω 9; △, 18:2 ω 6; ●, 18:3 ω 3.

Effect of the Amount of Hexane

Doubling or halving the reactant concentrations, at experimental conditions otherwise identical to those of Fig. 2, led to practically the same composition of acyl moieties after the reaction time of 6 h (data not shown). Therefore, the constant amount of hexane to soybean oil (5.0 mL/500 μmol) was used throughout this work.

Effect of Oleic Acid/Soybean Oil Molar Ratio

Figure 3 shows the composition of acyl moieties in TGs formed after the reaction time of 6 h at different molar ratios of oleic acid to soybean oil. The percentages of acyl moieties changed remarkably at the molar ratios of less than 3.0, and then slightly at higher molar ratios. Thus, the use of molar ratios higher than 3.0 was not always beneficial. The percentage of 18:2 ω 6 moiety was 38.8% at the molar ratio of 3.0, and it was 37.1% at the molar ratio of 5.0. Thus, 32.3% of 18:2 ω 6 moiety originally present in soybean oil was substituted at the molar ratio of 5.0. The percentage of 18:3 ω 3 moiety decreased slightly, and was 5.4–5.7% at the molar ratios of 3.0–5.0. In contrast, the percentage of 18:1 ω 9 moiety in TG was 50.8% at the molar ratio of 3.0, and it leveled off at 52.1% at the molar ratios of 4.0–5.0 (Fig. 3). The

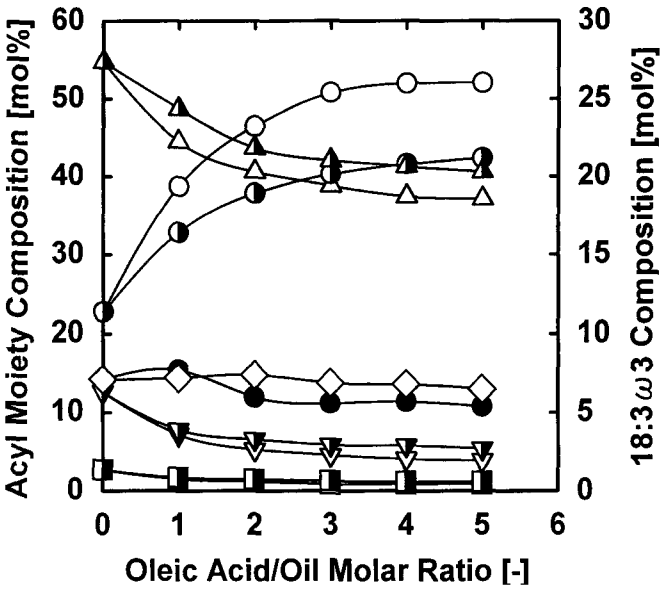

Fig. 3. Compositions of acyl moieties in TGs formed at different oleic acid/soybean oil molar ratios. Reaction conditions: 500 μmol soybean oil, 500–2500 μmol oleic acid (biochemical reagent grade or reagent grade), 10.0 BIU Lipozyme IM, 5.0 mL hexane, reaction temperature 37°C, reaction time 6 h. Symbols used are the same as those in Fig. 2. For the reaction with reagent grade oleic acid, ▽, 16:0; ■, 18:0; ◑, 18:1 ω 9; ▲, 18:2 ω 6; ◇, 18:3 ω 3.

conversion of oleic acid decreased from 46.2% at the molar ratio of 1.0 to 17.6% at the molar ratio of 5.0 (data not shown in Fig. 3).

The reaction at the molar ratio of 3.0 by use of a magnetically stirred (13–30*g*) flask, at experimental conditions otherwise identical to those of Fig. 3, gave almost the same acyl moiety composition of TG. On the other hand, the addition of fresh Lipozyme IM (10.0 BIU) to the reaction mixture obtained after the reaction time of 6 h at the molar ratio of 3.0, which was followed by the further reaction for 17 h, gave rise to slight change in the acyl moiety composition of TG (0.5% for 18:1 ω 9 and 1.8% for 18:2 ω 6).

Figure 3 also shows the results of the interesterification of soybean oil with reagent-grade oleic acid. The reaction conditions used were the same as those in the reaction of biochemical reagent grade oleic acid, except for the use of a fatty acid mixture containing 73.2% oleic acid. The percentages of 18:1 ω 9, 18:2 ω 6, and 18:3 ω 3 moieties in TG were 40.3, 42.1, and 6.9%, respectively, at the molar ratio of 3.0, and they were 42.4, 40.6, and 6.5%, respectively, at the molar ratios of 5.0. Compared with the case of biochemical reagent-grade oleic acid, these percentages of 18:1 ω 9 moiety are

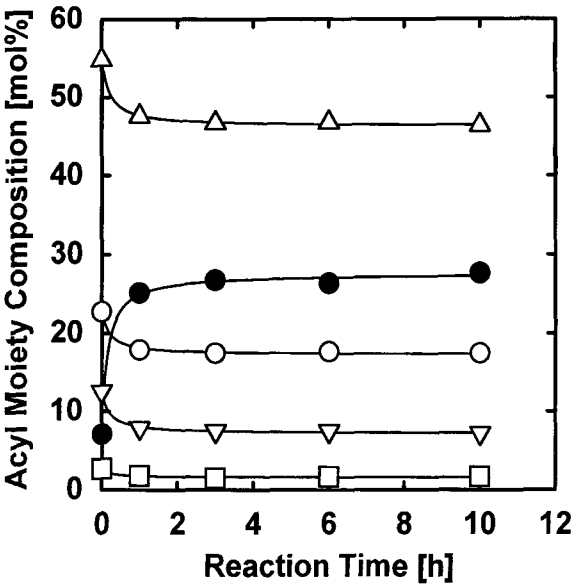

Fig. 4. Compositions of acyl moieties in TGs formed at different reaction times in the reaction with α-linolenic acid. Reaction conditions: 500 μmol soybean oil, 500 μmol α-linolenic acid (practical grade) as a mixture of fatty acids, 15.0 BIU Lipozyme IM, 5.0 mL hexane, reaction temperature 37°C. Symbols used are the same as those in Fig. 2.

clearly lower, whereas those of 18:2 ω 6 and 18:3 ω 3 moieties are higher (Fig. 3). The combined percentages of 16:0 and 18:0 moieties were 7.2 and 6.6%, respectively, at these molar ratios. Myristoyl (14:0) and palmitoleoyl (16:1) moieties were also incorporated into TG, although their combined percentages were low (0.6–2.7%); (data not shown).

Interesterification with α-Linolenic Acid

Figure 4 shows the compositions of acyl moieties in TGs formed at different reaction times. In this equimolar interesterification, 15.0 BIU of Lipozyme IM was used instead of 10.0 BIU, in order to obtain practically the constant acyl moiety composition of TG after the reaction time of 6–10 h. The percentages of 18:1 ω 9, 18:2 ω 6, and 18:3 ω 3 moieties in TG after the reaction time of 6 h were 17.7, 46.8, and 26.4% respectively. As the percentage of 18:2 ω 6 moiety in TG formed after the reaction time of 1 h was 47.6%, ca. 88% of the interesterification of this acyl moiety, which occurred in 10 h, took place within 1 h.

Figure 5 shows the composition of acyl moieties in TGs formed after the reaction time of 6 h at different molar ratios of α-linolenic acid to soybean oil. The percentages of 18:1 ω 9, 18:2 ω 6, and 18:3 ω 3 moieties were 13.5, 40.8, and 40.4%, respectively, at the molar ratio of 3.0. They were 12.1, 38.9, and 44.6%, respectively, at the molar ratio of 6.0. Thus, 29.0% of 18:2

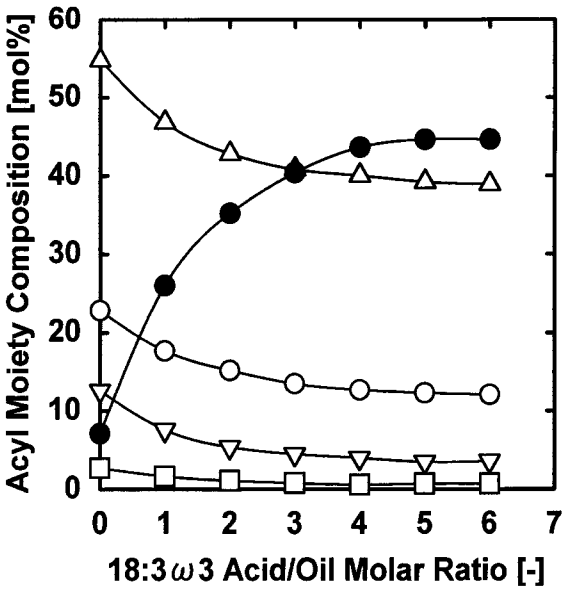

Fig. 5. Compositions of acyl moieties in TGs formed at different α-linolenic acid/soybean oil molar ratios. Reaction conditions: 500 μmol soybean oil, 500–3000 μmol α-linolenic acid (practical grade) as a mixture of fatty acids, 15.0 BIU Lipozyme IM, 5.0 mL hexane, reaction temperature 37°C, reaction time 6 h. Symbols used are the same as those in Fig. 2.

ω 6 moiety originally present in soybean oil was substituted at the highest molar ratio under study.

DISCUSSION

As found above, the maximum percent substitution of 18:2 ω 6 moiety in TG obtained in the present study was 32.3%. Here, the percentages of 18:2 ω 6 and 18:1 ω 9 moieties in TG were 37.1 and 52.1%, respectively. These values were obtained at the oleic acid/soybean oil molar ratio of 5.0 (Fig. 3). This maximum percent substitution of 18:2 ω 6 moiety is not as high as expected. In this reaction, the conversion of oleic acid was only 17.6%. This low conversion of oleic acid suggests that the acyl moiety composition of TG is not always controlled by the equilibrium of the reaction especially at high fatty acid/soybean oil molar ratios. On the other hand, no effects of the reactant concentrations and the method of reaction (shake flask vs magnetically stirred flask) on the acyl moiety composition of TG (*see* Results) indicate that mass transfer is not rate-controlling in the reaction. This is in contrast to the interesterifications of trioleoylglycerol and sardine oil with C_{20} and $C_{22}\omega$ 3 polyunsaturated fatty acids reported *(16)*. In addition, negligible effects of the sn-specificity of lipase (1,3-specific vs

nonspecific), and the addition of fresh enzyme on the acyl moiety composition (*see* Results), indicate that the acyl moiety composition of TG is not controlled kinetically, either. The reason for this enzymatic reaction behavior of soybean oil remains unknown. Kimura et al. *(17)* also reported that the maximum percentage of 16:0 moiety in TGs formed in the sn-1,3-specific lipase-catalyzed interesterification of trioleoylglycerol with palmitic acid (molar ratio = 1:7) was 47.6%.

In the present work, the percentage of 18:2 ω 6 moiety in soybean oil could not be decreased to be as low as in rapeseed oil (21.8%). When the molar ratio of fatty acid to soybean oil was 3.0 (i.e., fatty acid/acyl moiety molar ratio = 1), however, TGs that contain 50.8% 18:1 ω 9, 38.8% 18:2 ω 6, and 5.4% 18.3 ω 3 moieties could be produced in the reaction with biochemical reagent-grade oleic acid. Similarly, TGs that contain 40.3% 18:1 ω 9, 42.1% 18:2 ω 6, and 6.9% 18:3 ω 3 moieties could be obtained by using reagent-grade oleic acid (Fig. 3). The author believes that these TGs are fairly improved nutritionally. For industrial purposes, use of reagent-grade oleic acid is desirable from the viewpoint of economical costs. On the other hand, TGs that contain 26.4–44.6% 18:3 ω 3 moiety could be obtained in the reaction with practical-grade α-linolenic acid (Fig. 5). These TGs cannot undergo usual thermal cooking, because their 18:3 ω 3 moieties are easily oxidized in atmosphere, especially at high temperatures. These TGs should be consumed directly as diets having antitumor and antiallergic activities *(4–7,18)*. Approximately 86–88% of the interesterification of 18:2 ω 6 moiety in soybean oil, which occurred in 10 h, took place within 1 h (Figs. 2 and 4). This is advantageous for the design of industrial reactors.

REFERENCES

1. Tinsley, I. J., Schmitz, J. A., and Pierce, D. A. (1981), *Cancer Res.* **41,** 1460–1465.
2. Chan, P. C., Ferguson, K. A., and Dao, T. L. (1983), *Cancer Res.* **43,** 1079–1083.
3. Braden, L. M., and Carroll, K. K. (1986), *Lipids* **21,** 285–288.
4. Hori, T., Moriuchi, A., Okuyama, H., Sobajima, T., Tamiya-Koizumi, K., and Kojima, K. (1987), *Chem. Pharm. Bull.* **35,** 3925–3927.
5. Yonekura, I., and Sato, A. (1989), *J. Clin. Exp. Med.* (Igaku no Ayumi) **150,** 233–234.
6. Cameron, E., Bland, J., and Marcuson, R. (1989), *Nutr. Res.* **9,** 383–393.
7. Fritsche, K. L., and Johnston, P. V. (1990), *J. Nutr.* **120,** 1601–1609.
8. Lampty, M. S., and Walker, B. L. (1976), *J. Nutr.* **106,** 86–93.
9. Holman, R. T., Johnson, S. B., and Hatch, T. F. (1982), *Am. J. Clin. Nutr.* **35,** 617–623.
10. Neuringer, M., Connor, W. E., Lin, D. S., Barstad, L., and Luck, S. (1986), *Proc. Natl. Acad. Sci.* USA **83,** 4021–4025.
11. Watanabe, I., Kato, M., Aonuma, H., Hashimoto, A., Naito, Y., Moriuchi, A., and Okuyama, H. (1987), *Adv. Biosci.* **62,** 563–570.
12. Japan Inspection Institute of Fats and Oils (1988), *Annual Report for Grades of Edible Vegetable Oils by Japanese Agricultural Standards,* 1978, p. 48.

13. Coleman, M. H., and Macrae, A. R. (1976), UK Patent. 1,577,933.
14. Osada, K., Takahashi, K., and Hatano, M. (1990), *J. Jpn Oil Chem. Soc.* **39,** 467–471.
15. Haraldsson, G. G., Hoskuldsson, P. A., Sigurdsson, S. Th., Thorsteinsson, F., and Gudbjarnason, S. (1989), *Tetrahedron Lett.* **30,** 1671–1674.
16. Osada, K., Nakamura, M., Nonaka, M., and Hatano, M. (1992), *J. Jpn Oil Chem. Soc.* **41,** 39–42.
17. Kimura, M., Hasegawa, K., Takamura, H., and Matoba, T. (1991), *Agri. Biol. Chem.* **55,** 3039–3043.
18. Hashimoto, A., Katagiri, M., Torii, S., and Okuyama, H. (1988), *Jpn. J. Allerg.* **37,** 157–165.