Production of Structured TAG Rich in 1,3-Capryloyl-2-arachidonoyl Glycerol from *Mortierella* Single-Cell Oil

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ABSTRACT: Two oils containing a large amount of 2-arachidonoyl-TAG were selected to produce structured TAG rich in 1,3-capryloyl-2-arachidonoyl glycerol (CAC). An oil (TGA58F oil) was prepared by fermentation of *Mortierella alpina,* in which the 2-arachidonoyl-TAG content was 67 mol%. Another oil (TGA55E oil) was prepared by selective hydrolysis of a commercially available oil (TGA40 oil) with *Candida rugosa* lipase. The 2-arachidonoyl-TAG content in the latter was 68 mol%. Acidolysis of the two oils with caprylic acid (CA) using immobilized *Rhizopus oryzae* lipase showed that TGA55E oil was more suitable than TGA58F oil for the production of structured TAG containing a higher concentration of CAC. Hence, a continuous-flow acidolysis of TGA55E oil was performed using a column (18 × 125 mm) packed with 10 g immobilized *R. oryzae* lipase. When a mixture of TGA55E oil/CA (1:2, w/w) was fed at 35°C into the fixed-bed reactor at a flow rate of 4.0 mL (3.6 g)/h, the degree of acidolysis initially reached 53%, and still achieved 48% even after continuous operation for 90 d. The reaction mixture that flowed from the reactor contained small amounts of partial acylglycerols and tricaprylin in addition to FFA. Molecular distillation was used for purification of the structured TAG, and removed not only FFA but also part of the partial acylglycerols and tricaprylin, resulting in an increase in the CAC content in acylglycerols from 44.0 to 45.8 mol%. These results showed that a process composed of selective hydrolysis, acidolysis, and molecular distillation is effective for the production of CAC-rich structured TAG.

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KEY WORDS: Arachidonic acid, caprylic acid, distillation, fixed-bed bioreactor, immobilized enzyme, *Mortierella alpina, Rhizopus oryzae* lipase, single-cell oil, structured TAG.

Arachidonic acid (AA; 20:4n-6) is a precursor of local hormones (prostaglandins, leukotrienes, and thromboxanes) involved in the AA cascade (1,2). After ingestion or synthesis by linoleic acid, AA is incorporated into the 2-position of membrane phospholipids, which contributes to maintenance of normal membrane structure and fluidity (3,4). In addition, AA is contained in human milk and accelerates the growth of preterm infants, as does DHA (22:6n-3) (5,6).

Highly absorbable lipids, which contain functional FA including AA, are beneficial as nutrients for patients with maldigestion and malabsorption of lipids and as a nutraceutical supplement for the elderly. Structured TAG with medium-chain FA at the 1,3-positions and long-chain FA at the 2-position (MLM-type) were recently reported to be absorbed extensively into intestinal mucosa (7,8). The structured TAG can be produced by exchanging FA in natural oils and fats with medium-chain FA using an immobilized 1,3-position-specific lipase (9–12).

An MLM-type structured TAG containing AA was produced by acidolysis of a single-cell oil from *Mortierella alpina* with caprylic acid (CA; 8:0) using immobilized *Rhizopus oryzae* (13). In the acidolysis, an oil containing 25 wt% AA in which the content of 2-arachidonoyl-TAG was 32.7 mol% was used as a substrate. Hence, the content of 1,3-capryloyl-2-arachidonoyl glycerol (CAC) in the acidolysis product could not exceed 32.7 mol% even when all FA at the 1,3-positions were exchanged with CA. Structured TAG containing a higher concentration of CAC could be expected to show a strong physiological effect even if consumed in only small amounts. We thus attempted to produce structured TAG rich in CAC. This paper shows that the AA-rich oil produced by selective hydrolysis with *Candida rugosa* lipase is suitable as a substrate for the production of structured TAG containing a high content of CAC.

MATERIALS AND METHODS

Oils. TGA40 oil, containing 39.3 wt% (37.7 mol%) AA, was a commercial product of Suntory Ltd. (Osaka, Japan). TGA58F oil, containing 57.9 wt% (57.5 mol%) AA, was prepared by cultivation of *M. alpina* (14), followed by refinement from the mycelia by an oil refining method of Suntory Ltd. In brief, the cultivation was conducted aerobically at 28°C for 10 d in a 2-kL fermentor (Kansai Chemical Engineering Co., Ltd.) containing 1400 L medium composed of 2% glucose, 1% yeast extract, and 0.2% olive oil, pH 6.0. During the cultivation, glucose was added periodically to maintain a 1.5–2.5% concentration (total glucose added: 100 g/L). To increase the AA content in the oil, the harvested mycelia were allowed to stand for an additional 5 d. CA was purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan).

Enzymatic preparation of oil containing a high concentration of 2-arachidonoyl-TAG. An oil containing a high concentration of AA was prepared by selective hydrolysis of TGA40 oil with *C. rugosa* lipase according to the method in a previous paper (15). A mixture of 8 kg TGA40 oil, 8 kg water, and 15,000 U/kg reaction mixture of *C. rugosa* lipase was agitated

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at 30°C for 20 h in a 30-L reactor (Mitsuwa Co. Ltd.) at 200 rpm (degree of hydrolysis: 33.4%).

To remove FFA in the oil layer separated from the reaction mixture, it was first distilled at 170°C and 0.05 mm Hg using an MS-150 molecular distillation apparatus (Nippon Sharyo Ltd.). Because the acid value of the residue was still high (35 mg KOH/g), a second distillation was conducted at 200°C and 0.05 mm Hg. The resulting residue (4.44 kg; acid value, 4.1 mg KOH/g) contained 2.0 wt% FFA, 2.2 wt% MAG, 7.4 wt% DAG, and 88.4 wt% TAG. Selective hydrolysis increased the AA content in the acylglycerol fraction from 37.7 to 54.5 mol%. The oil was referred to as TGA55E oil.

Regiospecific analysis of TGA40, TGA55E, and TGA58F oils. The three oils contained partial acylglycerols. To remove the partial acylglycerols, 10 g of the oil was put on a silica gel 60 column (80 g; 30 × 260 mm; Merck, Darmstadt, Germany), and TAG were eluted with a mixture of *n*-hexane/ethyl acetate (98:2, vol/vol). Regiospecific analysis of their TAG content was conducted by Grignard degradation with allyl magnesium bromide (16), followed by isolation and analysis of 1,3-DAG. The 1,3-DAG were isolated by TLC with a silica gel 60 plate (Merck), which was developed with a mixture of chloroform/acetone/acetic acid (94:4:1, by vol). FA located at the 2-position were calculated from the FA compositions of TAG and 1,3-DAG.

Lipases. *Candida rugosa* lipase (Lipase-OF) and *R. oryzae* lipase (Ta-lipase) were donated by Meito Sangyo Co. (Aichi, Japan) and Tanabe Seiyaku Co. Ltd. (Osaka, Japan), respectively. Lipase activity was measured by titrating FA liberated from olive oil (Wako Pure Chemical Industries Ltd., Osaka, Japan) with 50 mM KOH as described previously (17). One unit (U) of lipase activity was defined as the amount of enzyme that liberated 1 μmol FA per min.

Immobilization of *R. oryzae* lipase was performed according to a procedure reported previously (18). In brief, after 50 g Dowex WBA (Dow Chemical Co., Midland, MI) was suspended in 40 mL of *R. oryzae* lipase solution (125 mg/mL; 6000 U/mL), immobilized lipase was prepared by drying under reduced pressure. Because the preparation did not express the full activity, it was activated by incubating it in a substrate mixture containing a small amount of water (18,19). In a batch reaction, the lipase preparation was shaken at 35°C for 24 h in a substrate mixture containing 2 wt% water. In continuous flow reactions, the substrate mixture, saturated with water (water content, 1.2%), was fed at 35°C for 24 h into a cylindrical fixed-bed bioreactor at the same flow rate as that in the main reaction.

Reactions. Batch acidolyses of TGA40, TGA58F, and TGA55E oils were conducted with two weight parts of CA using 5 wt% activated immobilized *R. oryzae* lipase. The reaction mixtures were incubated at 35°C in 10- or 50-mL screw-capped vessels with shaking at 130 oscillations/min. Repeated acidolyses were performed as follows. Acylglycerols were first extracted from the reaction mixture with *n*-hexane according to the method in a previous paper (20). In brief, they were extracted with 100 mL *n*-hexane after adding 70 mL of 0.5 N KOH (20% ethanol solution) to 4–8 g of reaction mixture. The resulting acylglycerols were then subjected to acidolysis with two weight parts of CA under conditions similar to those in the first reaction.

The continuous flow reaction was performed in a fixed-bed bioreactor (18 × 120 mm) packed with 10 g immobilized *R. oryzae* lipase. The substrate mixture of TGA55E oil/CA (1:2, w/w) was continuously fed into the reactor by a peristaltic pump at 30°C at a flow rate of 4.0 mL (3.6 g)/h.

Analyses. All analyses were conducted three to five times under the same experimental conditions.

FA in acylglycerols were methylated in methanol with Na-methylate as a methylating reagent. The methyl esters were analyzed by GC on a DB-23 capillary column (0.25 mm × 30 m; J&W Scientific, Folsom, CA) as described previously (21). The column temperature was raised from 150 to 210°C at 2°C/min, and the injector and detector temperatures were set at 250°C.

The contents of MAG, DAG, TAG, and FFA were determined with a TLC/FID analyzer (Iatroscan MK-5; Iatron Laboratories Inc.) after developing them with a mixture of *n*-hexane/ethyl acetate/acetic acid (90:10:1, by vol). TAG composition was analyzed by HPLC and GC. The HPLC analysis was performed on an ODS column (4.6 × 250 mm; Cosmosil 5C18-MA; Nacalai Tesque Inc., Kyoto, Japan). The mobile phase of acetone/acetonitrile (1:1, vol/vol) was used at a flow rate of 1.0 mL/min and 40°C, and the peaks of TAG were detected with a refractometer. Because CAC, 1,3-capryloyl-2-dihomo-γ-linolenoyl glycerol, and 1,3-capryloyl-2-γ-linolenoyl glycerol were not separated by HPLC, a GC analysis was performed to determine the contents of these TAG with an Ultra Alloy UA-17-15M-0.1F capillary column (0.25 mm × 15 m; Frontier Laboratories Ltd., Fukushima, Japan). The column temperature was raised from 260 to 290°C at 1°C/min and from 290 to 390°C at 10°C/min, and maintained at 390°C for 5 min. The injector and detector temperatures were set at 310 and 400°C, respectively. The carrier gas was helium at a flow rate of 40 cm/min. The positional isomers of CAC were analyzed on a Chrompack silver ion chromatography column (4.6 × 250 mm; Chrompack, Middelberg, The Netherlands) as described by Irimescu *et al*. (22).

Water contents of the substrate and reaction mixture were determined by Karl Fischer titration (Moisture Meters CA-07; Mitsubishi Chemical Corp.).

Molecular distillation. The reaction mixture obtained by the acidolysis of TGA55E oil was dehydrated at 70°C and 3 mm Hg for 30 min before applying molecular distillation. The water content was reduced to <100 ppm by dehydration. Removal of FFA was performed with a molecular distillation apparatus (Wiprene type 2-03; Shinko Pantec Co. Ltd.), by a stepwise distillation as follows: at 130°C and 0.2 mm Hg; at 180°C and 0.2 mm Hg; and at 200°C and 0.1 mm Hg.

RESULTS AND DISCUSSION

FA compositions at the 2-positions of TGA40, TGA55E, and TGA58F oils. When an AA-containing oil undergoes acidolysis with CA, the CAC content in the product depends on the amount of TAG with AA at the 2-position. Hence, we first conducted a regiospecific analysis of the substrate oils. TGA58F

and TGA55E oils are AA-rich oils produced by fermentation and by selective hydrolysis of TGA40 oil with *C. rugosa* lipase, respectively. Because the three oils (TGA40, TGA55E, and TGA58F) contained partial acylglycerols, TAG were purified from the oils by silica gel column chromatography. Significant differences were not observed between FA compositions before and after the purification, showing that the FA compositions of TAG in the oils were almost the same as those of the partial acylglycerols. FA compositions at the 1,3- and 2-positions of the purified TAG are shown in Table 1. Although TGA40 oil contained 17.8 mol% of 2-oleoyl-TAG and 12.7 mol% of linoleoyl-TAG, their contents of TGA55E oil were reduced to 2.8 and 1.8 mol%, respectively. The decrease in these two TAG species resulted in an increase in the relative content of TAG with AA at the 2-position. The content of 2-arachidonoyl-TAG in TGA58F was almost the same as that in TGA55E, although the contents of 2-oleoyl- and 2-linoleoyl-TAG in TGA58F were higher than those in TGA55E. These results show that TGA58F and TGA55E oils may be suitable as substrates for the production of CAC-rich structured TAG.

TGA55E oil contained 10 wt% partial acylglycerols, whereas TGA40 and TGA58F oils contained only <3 wt% partial acylglycerols (DAG). As clarified previously, when a mixture of partial acylglycerols and TAG underwent acidolysis with CA, part of the partial acylglycerols was converted to tricaprylin and MLM-type structured TAG by simultaneous esterification and acyl migration (23). The following experiments were therefore performed using the three oils containing partial acylglycerols as substrates.

Acidolyses of TGA oils with CA. TGA oils were allowed to react with >2 weight parts of CA (*ca*. 15 molar amounts against the oil), but significant increases in the degree of acidolysis were not observed. Acidolysis was therefore performed at 35°C by shaking a mixture of oil/CA (1:2, w/w) and 5% of immobilized *R. delemar* lipase by weight of the reaction mixture. Figure 1 shows typical time courses of the reactions. In all cases, the CA contents in acylglycerols completely agreed with the amount by which the constituent FA decreased, indicating that the degree of acidolysis can be expressed by the CA content in acylglycerols. In the acidolysis of TGA40 oil, initial velocity was 1.5 times faster than those of TGA58F and TGA55E oils. The degree of acidolysis of TGA40 oil reached >50% after 72 h, but those of TGA58F and TGA55E oils did not. The constituent FA in TGA oils could be divided into roughly two groups from their pattern of decrease. One group consisted of AA, GLA (18:3n-6), and dihomo-GLA (DHGLA; 20:3n-6), whose decrease continued even after 48 h (the contents of GLA and DHGLA were not plotted). The decrease in the other constituent FA reached a constant value after 48 h, as exemplified by linoleic acid (LnA; 18:2n-6) and lignoceric acid (24:0).

Rhizopus oryzae lipase is a 1,3-position-specific lipase and shows weak activities toward AA, GLA, and DHGLA. As shown in Table 1, TGA58F and TGA55E oils contained larger amounts of these poor FA at the 1,3-positions than did TGA40 oil. Therefore, the slow velocity and low degree of acidolysis in the reactions of TGA58F and TGA55E oils (Fig. 1) can be explained by the FA specificity of the lipase.

Repeated acidolysis of TGA oils with CA. If all the FA at the 1,3-positions in TGA oils were exchanged with CA, the CAC content in the acidolysis product could increase to that of the AA content at the 2-position. However, because FA on which *R. oryzae* lipase shows weak activity were esterified at the 1,3-positions, all of the FA cannot be exchanged with CA. The relationship between the degree of acidolysis and the CAC content in the product should offer good information for production of a CAC-rich structured TAG. As the degree of acidolysis did not exceed 46% by a single 48-h reaction (Fig. 1), repeated acidolysis was attempted.

Acidolyses of TGA40, TGA58F, and TGA55E oils were repeated as follows. A mixture of 10 g oil/CA (1:2, w/w) and 0.5 g immobilized *R. oryzae* lipase was shaken at 35°C for 48 h. The acylglycerols were extracted with *n*-hexane from the reaction mixture and allowed to react again with two weight

TABLE 1
FA Compositions of TGA40-, TGA58F-, and TGA55E-TAG[a]

	FA composition (mol%)[b]								
Oil	16:0	18:0	18:1	18:2[c]	18:3[c]	20:3[c]	20:4[c]	22:0	24:0
TGA40-TAG									
Total	13.7	6.0	15.1	7.3	3.6	4.5	37.7	2.0	3.7
1,3-Position	19.9	8.9	13.8	4.6	1.5	2.1	33.1	2.9	5.4
2-Position	1.3	0.3	17.8	12.7	7.9	9.2	46.7	0.2	0.2
TGA58F-TAG									
Total	6.7	3.9	5.4	7.0	2.2	2.8	57.5	3.1	7.4
1,3-Position	9.5	5.7	4.8	6.0	0.3	1.1	52.8	4.5	11.0
2-Position	1.2	0.2	6.5	9.1	5.9	6.3	66.8	0.3	0.2
TGA55E-TAG									
Total	6.8	4.9	7.1	3.7	4.2	6.4	53.6	2.6	4.8
1,3-Position	8.8	7.2	9.3	4.6	2.4	3.9	46.4	3.7	7.0
2-Position	2.8	0.3	2.8	1.8	7.8	11.3	68.2	0.3	0.4

[a]TGA40 oil, a commercial product (Suntory, Osaka, Japan); TGA58F oil, a single-cell oil produced by fermentation; TGA55E oil, an oil prepared by selective hydrolysis of TGA40 oil with *Candida rugosa* lipase (see the Materials and Methods section for details of the preparation).
[b]Relative SD were less than ±8.5% for the average value of <1%, less than ±2.7% for the values of 1–3%, less than ±2.0% for 3–10%, and less than ±1.0% for >10%.
[c]n-6 PUFA.

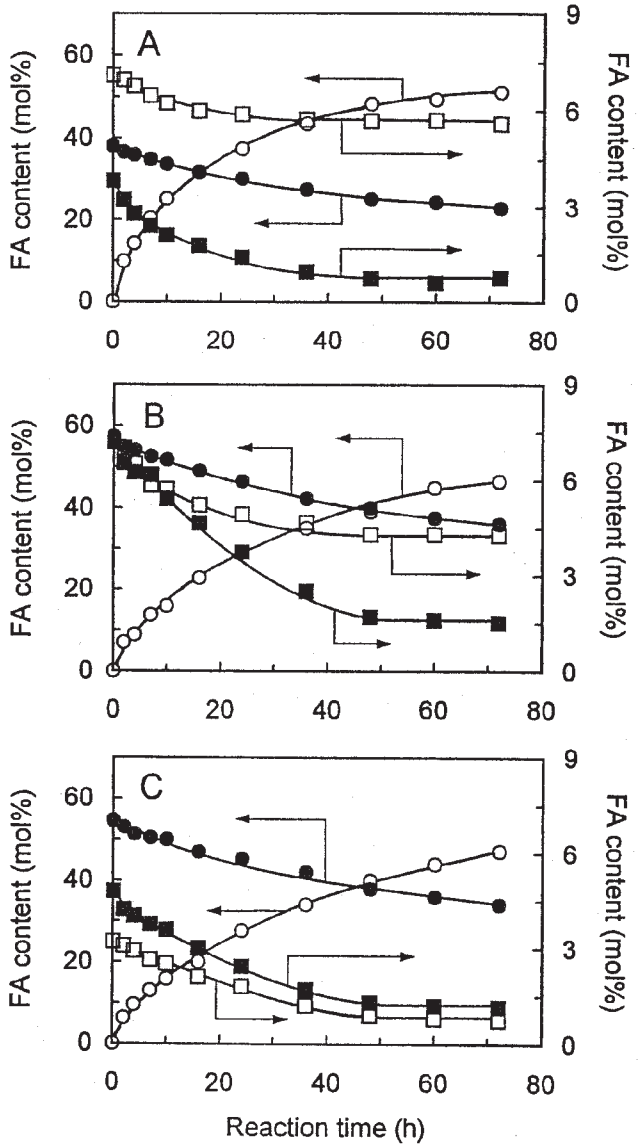

FIG. 1. Acidolyses of TGA40, TGA58F, and TGA55E oils with caprylic acid (CA) using immobilized *Rhizopus oryzae* lipase. A mixture of 12 g oil, 24 g CA, and 1.8 g immobilized lipase was shaken at 35°C. An aliquot of the reaction mixture (*ca.* 1 g) was periodically withdrawn to analyze the FA composition in acylglycerols. (A) TGA40 oil; (B) TGA58F oil; (C) TGA55E oil. ○, CA content in acylglycerols; ●, arachidonic acid; □, linoleic acid; ■, lignoceric acid. See the Materials and Methods section for preparation of TGA58F and TGA55E oils. The direction of arrows indicates the axes of plots.

parts of CA under similar conditions. The acidolysis was repeated three times in total. Table 2 shows the acylglycerol compositions in the acidolysis product obtained by each reaction. TGA40 and TGA58F oils contained 2.9 and 2.6 wt% DAG, respectively. Repeated acidolysis of the two oils slightly increased the DAG content. In contrast, TGA55E oil contained 2.2 wt% MAG and 7.6 wt% DAG, and the contents of these partial acylglycerols were slightly decreased by repeating the acidolysis. Because the water content in the substrate mixture was 300 to 500 ppm and the content after the reaction scarcely changed, these results indicated that hydrolysis and/or esterification occurred slightly along with acidolysis, and that the content of partial acylglycerols at the equilibrium state settled down to several percent. The TAG content in each reaction product was >90 wt%.

Table 3 shows the TAG composition of each product obtained by repeated acidolysis. TAG species are represented by three capital letters, for example, CAO means TAG with CA and OA at the 1,3-positions and with AA at the 2-position, and the 1,3-positions are not distinguished. The FA detected were: CA (C), palmitic acid (P; 16:0), oleic acid (O; 18:1n-9), LnA (L), GLA (G), DHGLA (D), and AA (A). CXX indicates TAG with 1 mol of CA (except for CAA), and XXX shows TAG not containing CA (except for AAA). In the first acidolysis of TGA58F oil (40.6% acidolysis), the CAC content was 24.4 mol%. The three-time repetition approach increased the degree of acidolysis to 64.6%, and the CAC content reached 43.1 mol%. In the first acidolysis of TGA40 oil (45.5% acidolysis), the CAC content was 23.4 mol%, which was increased to only 36.0 mol% by the three-time repetition (62.8% acidolysis). The acidolysis of TGA55E increased the CAC content to 36.7% by the first reaction (42.4% acidolysis) and to 50.7% by the three repetitions (64.8% acidolysis). The high content of CAC achieved by triple acidolysis of TGA55E oil was largely attributed to the elimination of 2-oleoyl- and 2-linoleoyl-TAG by selective hydrolysis of TGA40 oil with *C. rugosa* lipase. These results showed that TGA55E oil is a superior substrate for producing CAC-rich structured TAG.

Continuous-flow acidolysis of TGA55E with CA. TGA55E oil was selected to produce CAC-rich structured lipids. We attempted continuous-flow acidolysis of the oil in a fixed-bed bioreactor with the aim of developing an industrial process. The effect of flow rate on the acidolysis of TGA55E oil with

TABLE 2
Acylglycerol Compositions of Reaction Products Obtained by Acidolysis of TGA40, TGA58F, and TGA55E Oils with Caprylic Acid (CA)[a]

Oil Treatment	Degree of acidolysis (%)	Acylglycerol composition (wt%)[b] MAG	DAG	TAG
TGA40	—	<0.5	2.9	97.1
First	45.5	<0.5	2.4	97.6
Second	56.7	<0.5	3.3	96.7
Third	62.8	<0.5	4.6	95.4
TGA58F	—	<0.5	2.6	97.4
First	40.6	<0.5	2.6	97.4
Second	56.6	<0.5	3.1	96.9
Third	64.6	<0.5	4.1	95.9
TGA55E	—	2.2	7.6	90.2
First	42.4	1.9	7.4	90.7
Second	54.4	1.5	7.0	91.5
Third	64.8	1.2	5.2	93.6

[a]Acidolyses of TGA40, TGA58F, and TGA55E oils were performed at 35°C for 48 h with two weight parts of CA using 5 wt% immobilized *Rhizopus oryzae* lipase. After the reactions, acylglycerols were recovered with *n*-hexane and then allowed to react again under similar conditions. The acidolysis was repeated three times in total.

[b]Relative SD were less than ±35.3% for the average value of <0.5%, less than ±30.1% for the values of 1–2%, less than ±17.7% for 2–4%, less than ±11.8% for 4–8%, and less than ±3.4% for >90%. See Table 1 for other abbreviations.

TABLE 3
TAG Compositions of Reaction Products Obtained by Acidolysis of TGA40, TGA58F, and TGA55E Oils with CA[a]

Oil	TAG composition (mol%)[b]										
Treatment	CCC	CPC	COC	CLC	CGC	CDC	CAC	CAA	CXX	AAA	XXX
TGA40											
First	2.5	1.2	15.4	9.3	6.0	6.1	23.4	11.0	13.1	2.3	9.7
Second	4.9	1.3	18.0	11.3	6.4	7.6	30.1	7.5	5.9	0.8	6.2
Third	6.3	1.4	17.2	12.5	6.9	8.2	36.0	6.4	4.2	<0.5	0.9
TGA58F											
First	2.5	1.1	4.7	6.3	3.7	4.0	24.4	18.5	8.8	10.4	15.6
Second	5.0	1.0	5.9	7.5	4.6	5.2	36.3	18.0	6.3	4.4	5.8
Third	7.2	1.2	6.0	8.6	6.1	6.0	43.1	12.7	5.4	2.9	0.8
TGA55E											
First	3.5	2.7	2.0	1.6	4.3	7.5	36.7	18.2	6.8	5.7	11.0
Second	9.3	2.2	2.3	1.6	6.3	9.7	46.7	12.0	4.0	2.0	3.9
Third	10.9	2.2	2.6	1.7	6.9	10.9	50.7	8.0	2.6	0.9	2.6

[a]The products were the same as those obtained in Table 2.
[b]TAG are expressed by three of the following capital letters: C, CA; P, palmitic acid; O, oleic acid; L, linoleic acid; G, γ-linolenic acid; D, dihomo-γ-linolenic acid; A, arachidonic acid. CXX, TAG with 1 mol of CA except for CAA; XXX, TAG not containing CA except for AAA. Relative SD were less than ±15.8% for the average value of <0.5%, less than ±10.7% for the values of 0.5–1.5%, less than ±5.9% for 1.5–3%, less than ±4.1% for 3–10%, and less than ±2.0% for >10%. See Tables 1 and 2 for other abbreviations.

CA was first investigated by feeding a substrate mixture of TGA55E/CA (1:2, w/w) into a column (18 × 125 mm) packed with 10 g immobilized *R. oryzae* lipase at 35°C at different flow rates. The degree of acidolysis increased with a decrease in the flow rate: CA contents in product acylglycerols were 35, 41, and 53 mol% at flow rates of 8.0, 5.6, and 4.0 mL/h, respectively. Because feeding the substrate at <4 mL/h did not increase the degree of acidolysis, the flow rate was fixed at 4.0 mL (3.6 g)/h. Under these conditions, the reactor was operated continuously for 90 d (Fig. 2). The degree of acidolysis decreased from 53 to 48%, showing that the immobilized lipase preparation was stable under these conditions.

Purification of CAC-rich structured lipids. The reaction mixture that flowed from the above reactor after 10–30 d was collected, and 1400 g was subjected to molecular distillation (Table 4). The mixture was dehydrated at 70°C and 3 mm Hg, and subjected to molecular distillation at 130°C and 0.2 mm Hg. CA was recovered in the distillate 1 (870 g) with a 96.8% recovery. The CA content was 93.1 wt%, showing that the preparation can be used as a substrate for the next reaction. Additional FFA in the residue were removed by distillation at 180°C and 0.2 mm Hg. However, because the residue still contained 16.5 wt% FFA, the distillation was conducted again at 200°C and 0.1 mm Hg. The residue recovered after distillation (341 g) had a FFA content of 3.5 wt% (recovery of TAG: 95.1%).

Table 5 shows the TAG compositions before and after distillation. The CCC content was decreased from 7.6 to 3.9 mol%, showing that a part of CCC was removed by distillation at 200°C and 0.1 mm Hg together with partial acylglycerols (distillate 3 in Table 4), because the M.W. of CCC is only 470. Consequently, the content of CAC increased slightly to 45.8 mol%. To investigate the presence of positional isomers of CAC, the purified preparation was analyzed by HPLC with a silver ion column; these results showed that CAC was composed of 97 mol% 1,3-dicapryloyl-2-arachidonoyl glycerol and 3 mol% 1(3),2-dicapryloyl-3(1)-arachidonoyl glycerol. These facts indicated that removal of partial acylglycerols in AA-rich oil is not necessary for the production of CAC-rich structured TAG.

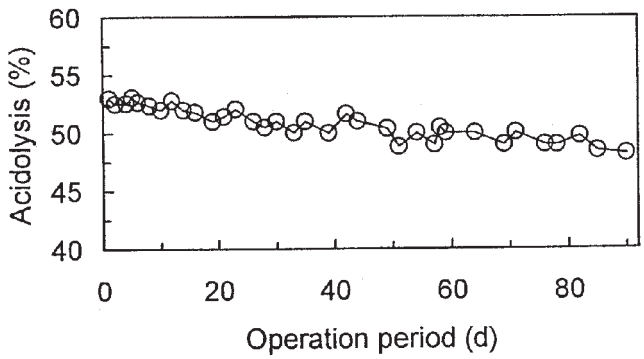

FIG. 2. Stability of immobilized *R. oryzae* lipase in a continuous-flow reaction. A substrate mixture of TGA55E oil/CA (1:2, w/w) was continuously fed into a fixed-bed bioreactor (18 × 120 mm) packed with 10 g immobilized lipase at 35°C and a flow rate of 4.0 mL/h. See Figure 1 for abbreviations and the Materials and Methods section for preparation of TGA55E oil.

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TABLE 4
Purification of Structured TAG by Molecular Distillation

Step	Total weight (g)	Acid value (mg KOH/g)	Acylglycerols (g)				FFA (g)		
			Total	MAG	DAG	TAG	Total	8:0	20:4
Reaction mixture[a]	1400	263	362	7	29	326	1038	837	102
Distillate 1[b]	870	368	ND[c]	ND	ND	ND	870	810	15
Distillate 2[d]	92	215	ND	ND	ND	ND	92	13	48
Distillate 3[e]	65	168	15	3	5	7[f]	50	ND	29
Residue 3[e]	341	7	329	2	18	310	12	ND	6

[a]Degree of acidolysis, 51.6%.
[b]Distillation at 130°C, 0.2 mm Hg.
[c]ND, not detected.
[d]Distillation at 180°C, 0.2 mm Hg.
[e]Distillation at 200°C, 0.1 mm Hg.
[f]Tricaprylin.

TABLE 5
TAG Compositions of Reaction Products Before and After Molecular Distillation

Molecular distillation	TAG composition (mol%)[a]										
	CCC	CPC	COC	CLC	CGC	CDC	CAC	CAA	CXX	AAA	XXX
Before[b]	7.6	2.5	2.1	1.7	6.0	9.0	44.0	14.7	5.3	2.3	4.8
After[c]	3.9	2.4	2.2	1.7	6.3	9.4	45.8	15.1	5.5	2.4	5.3

[a]Relative SD were less than ±5.1% for >3%, less than ±4.0% for 3–10%, and less than ±1.8% for >10%.
[b]The sample is the reaction mixture shown in Table 4 (TAG/acylglycerols = 90.0 wt%).
[c]The sample is residue 3 shown in Table 4 (TAG/acylglycerols = 94.2 wt%). See Table 3 for abbreviations of TAG and Table 1 for other abbreviations.

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