Purification of Steryl Esters from Soybean Oil Deodorizer Distillate

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ABSTRACT: Soybean oil deodorizer distillate (SODD) contains steryl esters in addition to tocopherols and sterols. Tocopherols and sterols have been industrially purified from SODD, but no purification process for steryl esters has been developed. SODD was efficiently separated to low b.p. substances (including tocopherols and sterols) and high b.p. substances (including 11.2 wt% DAG, 32.1 wt% TAG, and 45.4 wt% steryl esters) by molecular distillation. The high b.p. fraction is referred to as soybean oil deodorizer distillate steryl ester concentrate (SODDSEC). We attempted to purify steryl esters after a lipase-catalyzed hydrolysis of acylglycerols in SODDSEC. Screening of industrially available lipases indicated that *Candida rugosa* lipase was most effective. Based on the study of several factors affecting hydrolysis, the reaction conditions were determined as follows: ratio of SODDSEC/water, 1:1 (w/w); lipase amount, 15 U/g reaction mixture; temperature, 30°C. When SODDSEC was agitated for 24 h under these conditions, acylglycerols were almost completely hydrolyzed and the content of steryl esters did not change. However, study with a mixture of steryl oleate/trilinolein (1:1, w/w) indicated that about 20% of constituent FA in steryl esters were exchanged with constituent FA in acylglycerols. Steryl esters in the oil layer obtained by the SODDSEC treatment with lipase were successfully purified by molecular distillation (purity, 97.3%; recovery, 87.7%).

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KEY WORDS: Acylglycerols, *Candida rugosa,* hydrolysis, lipase, molecular distillation, soybean oil deodorizer distillate, steryl ester.

The cholesterol-lowering effect of sterols, which has been studied since the 1950s (1–5), is believed to be caused by an inhibition of cholesterol absorption resulting from the higher solubility of sterols than of cholesterol in bile salt micelles (4,5). Steryl esters show the same physiological effects as free sterols (6–8). This effective physiological activity has led to the development of several functional foods, such as salad oils and dressings with added sterols and margarine blended with steryl esters. In particular, because steryl esters and oils (TAG) completely dissolve each other, a lot of attention is being focused on the addition of steryl esters in oil-related foods.

Tocopherols and sterols are industrially purified from vegetable oil deodorizer distillate (VODD) produced in the deodorization step of oil refining. VODD contains about 10% steryl esters, but no process for recovering them has been developed. A preliminary experiment showed that the high b.p. fraction (M.W. >600) separated from VODD was mainly composed of DAG, TAG, and steryl esters. This paper reports that *Candida rugosa* lipase almost completely hydrolyzes only acylglycerols in the high b.p. fraction, and that molecular distillation is very effective for recovering steryl esters from the hydrolysate.

MATERIALS AND METHODS

Materials. Soybean oil deodorizer distillate (SODD) was obtained from Yashiro Co. Ltd. (Osaka, Japan). High b.p. substances (referred to as soybean oil deodorizer distillate steryl ester concentrate; SODDSEC) containing steryl esters and acylglycerols were the residue obtained by distillation of SODD at 250°C and 0.02 mm Hg with a molecular distillation apparatus (Wiprene type 2-03; Shinko Pantec Co. Ltd., Hyogo, Japan). Soybean oil and linoleic acid (LnA; purity, 98.7%) were obtained from Yashiro. Sterols were purified from SODD by Yashiro, and the preparation contained 2.1 wt% brassicasterol, 26.3 wt% campesterol, 21.3 wt% stigmasterol, and 45.4 wt% β-sitosterol. Oleic acid (OA; 91.6%) was purchased from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan). All other reagents were of analytical grade.

Lipases were gifts from the following companies: *Candida rugosa*, *Pseudomonas stutzeri*, and *Burkholderia cepacia* lipases and *Alcaligenes* sp. lipase (Lipase QLM) were from Meito Sangyo Co. Ltd. (Aichi, Japan); *Pseudomonas* sp. lipases (Lipase PS and Lipase AK) and *Aspergillus niger* lipase were from Amano Enzyme Inc. (Aichi, Japan); *Rhizopus oryzae* lipase was from Tanabe Seiyaku Co. Ltd. (Osaka, Japan); *P. aeruginosa* lipase was from Toyobo Co. Ltd. (Osaka, Japan); and *B. glumae* lipase was from Asahi Chemical Industry Co. Ltd. (Osaka, Japan). The lipase activity was measured by titrating FA liberated from olive oil (Wako Pure Chemical Co. Ltd., Osaka, Japan) with 50 mM KOH, according to our previous paper (9). In brief, the reaction was performed at 30°C for 30 min with stirring at 500 rpm. One unit (U) of lipase was defined as the amount that liberated 1 μmol FA/min.

Preparation of trilinolein (TriLnA) and steryl oleate (steryl OA). TriLnA was synthesized in a 100-mL flask fitted to a rotary evaporator using immobilized *C. antarctica* lipase

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(Novozymes, Bagsvaerd, Denmark) according to Kawashima *et al.* (10). A medium of 30 g LnA/glycerol (3:1, mol/mol) and 1.5 g of the immobilized lipase was mixed at 40°C and 15 mm Hg for 30 h (esterification, 94.2%). Acylglycerols in the reaction mixture were recovered by *n*-hexane extraction (11). In brief, 400 mL *n*-hexane was added to the reaction mixture from which immobilized lipase had been removed by filtration, and the solution was then washed twice with 200 mL of 0.5 N KOH (20% ethanol solution) to remove unesterified LnA. The organic solvent was finally evaporated, and 25.4 g acylglycerols were obtained (TAG/DAG = 92:8, w/w).

Steryl OA was basically synthesized as described previously (12). A mixture of 24 g sterols/OA (1:3, mol/mol), 6 mL water, and 200 U/g *C. rugosa* lipase was stirred at 30°C for 30 h (esterification, 84.3%). Synthesized steryl OA was extracted with *n*-hexane under alkaline conditions as described above. After evaporation of *n*-hexane, the extracts were applied to a silica gel 60 column (120 g; 30 × 390 mm; Merck, Darmstadt, Germany), and steryl OA was eluted with a mixture of *n*-hexane/ethyl acetate (98:2, vol/vol) (yield, 8.9 g).

Reactions. SODDSEC was treated at 30°C in a mixture of 50% water and 15 U/g mixture of *C. rugosa* lipase. The small-scale reaction was conducted in a 50-mL vessel with stirring at 500 rpm, and the large-scale reaction was performed in a 5-L reactor (MDL-500; Marubishi Bioengineering Co. Ltd., Tokyo, Japan) with agitation at 300 rpm.

Large-scale purification of steryl esters. A mixture of 1.0 kg SODDSEC, 1.0 L water, and 30,000 U *C. rugosa* lipase was agitated at 30°C for 24 h. The reaction mixture separated into oil and water layers, and the oil layer was dehydrated at 80°C and 3 mm Hg for 60 min (water content, 95 ppm). Steryl esters in the oil layer were recovered in the residue by a two-step molecular distillation: first, 180°C and 0.2 mm Hg; second, 250°C and 0.02 mm Hg.

Analysis. Contents of steryl ester, sterol, TAG, DAG, MAG, and FFA were analyzed with a Hewlett-Packard 5890 gas chromatograph (Avondale, PA) connected to a DB-1ht capillary column (0.25 mm × 5 m; J&W Scientific, Folsom, CA). The column temperature was raised from 120 to 280°C at 15°C/min and from 280 to 370°C at 10°C/min, and then maintained for 3 min. The injector and detector (FID) temperatures were set at 370 and 390°C, respectively. FA and sterol compositions in steryl esters were analyzed as follows. Steryl esters (50 mg) were heated at 75°C for 30 min in a mixture of 4 mL methanol and 5% Na-methylate. The resulting FAME were extracted with *n*-hexane and analyzed by GC with a DB-5 capillary column (0.25 mm × 10 m; J&W Scientific) (12). The column temperature was raised from 190 to 290°C at the rate of 5°C/min, and then raised to 320°C at 10°C/min. The injector and detector temperatures were set at 245 and 340°C, respectively.

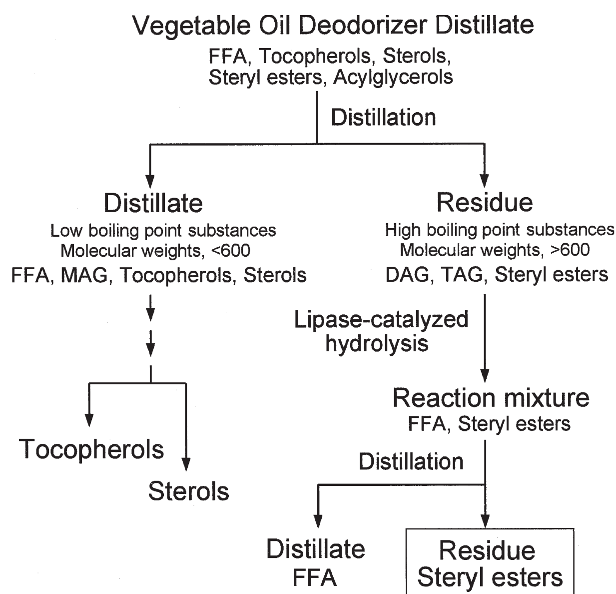
All analyses were conducted three times under the same experimental conditions. The relative SD were less than ±8.2% for the average value of <1%, less than ±2.9% for the value of 1–3%, less than ±1.9% for >3–10%, and less than ±1.0% for >10%.

RESULTS AND DISCUSSION

Strategy for purification of steryl esters in VODD. Scheme 1 shows the strategy for purification of steryl esters. VODD produced in the deodorization step of vegetable oil refining includes FFA, tocopherols, sterols, steryl esters, and acylglycerols. VODD is first fractionated to low b.p. and high b.p. substances by molecular distillation. The low b.p. fraction contains tocopherols and sterols and can be used as a starting material for their purification (13). On the other hand, the high b.p. fraction (M.W. >600) contains mainly DAG, TAG, and steryl esters. Next, only DAG and TAG in the fraction are selectively hydrolyzed by a lipase, resulting in the production of a mixture composed of FFA and steryl esters. Finally, steryl esters are purified from the reaction mixture by molecular distillation.

Fractionation of SODD. SODD (5 kg) was distilled at 250°C and 0.02 mm Hg, and yielded 3.48 kg distillate (low b.p. fraction) and 1.42 kg residue (high b.p. fraction). The composition of the two fractions is shown in Table 1. FFA, MAG, and tocopherols were efficiently distilled, and large portions of the sterols were also recovered in the distillate. On the other hand, all of the steryl esters and TAG and most of the DAG remained in the residue, and a part of the sterols remained. This high b.p. fraction (SODDSEC) was used as a material for purification of steryl esters.

Suitable lipase for hydrolysis of acylglycerols in SODDSEC. SODDSEC was hydrolyzed at 30°C for 20 h in a mixture containing 50% water with 10 U/g of various lipases (Table 2). All lipases hydrolyzed acylglycerols in SODDSEC, and *C. rugosa* lipase was most effective. Surprisingly, *C. rugosa* lipase hydrolyzed acylglycerols in SODDSEC almost completely. The degree of hydrolysis was 99% based on the amount of FA in the acylglycerols before and after the reaction, as the M.W. of MAG, DAG, and TAG were 354, 616, and 878, respectively. In



SCHEME 1

TABLE 1
Fractionation of Soybean Oil Deodorizer Distillate (SODD) by Molecular Distillation[a]

	Content (wt%)		
Component	SODD	Low b.p. fraction	High b.p. fraction[b]
FFA	30.1	41.4	1.4
MAG	4.1	5.6	0.4
DAG	3.5	0.3	11.2
TAG	9.5	ND[c]	32.1
Tocopherols	10.4	14.8	0.7
Sterols	10.3	12.8	4.0
Steryl esters	12.8	ND	45.4
Unknown[d]	19.3	25.4	4.8

[a]SODD (5 kg) was subjected to molecular distillation at 250°C and 0.02 mm Hg, and yielded 3.48 kg distillate (low b.p. fraction) and 1.42 kg residue (high b.p. fraction).
[b]Referred to as soybean oil deodorizer distillate steryl ester concentrate, SODDSEC.
[c]Not detected (<0.1 wt%).
[d]Mainly hydrocarbons.

this reaction, the content of steryl esters did not change. Hence, we selected *C. rugosa* lipase for the following experiments.

To determine the amount of lipase necessary for hydrolysis of acylglycerols, a mixture of SODDSEC, 50% water, and 1–25 U/g of *C. rugosa* lipase was incubated at 30°C for 20 h with stirring. Because complete hydrolysis of acylglycerols was attained with >15 U/g of the lipase, the lipase amount was set at 15 U/g by weight of reaction mixture.

Effect of water content and temperature on hydrolysis of acylglycerols in SODDSEC. The amount of water is generally an important factor in the hydrolysis of acylglycerols by a lipase (14). Hence, SODDSEC was hydrolyzed at 30°C for 20 h with 15 U/g of *C. rugosa* lipase in the presence of 5–90% water (Table 3). The content of acylglycerols decreased with an increasing amount of water. The results showed that >50% water is necessary for complete hydrolysis of acylglycerols in SODDSEC.

TABLE 2
Hydrolysis of Acylglycerols in SODDSEC with Various Lipases[a]

	Composition (wt%)					
Lipase	FFA	MAG	DAG	TAG	Sterol	Steryl ester
None	1.4	0.4	11.2	32.1	4.0	45.4
Rhizopus oryzae	25.4	2.1	8.3	10.7	4.1	45.9
Aspergillus niger	28.2	0.7	7.1	9.4	4.0	46.4
Candida rugosa	45.1	ND[b]	0.2	0.3	4.0	47.2
Pseudomonas aeruginosa	26.6	1.4	7.1	10.5	4.9	45.3
Pseudomonas stutzeri	24.0	1.4	7.1	13.9	3.9	46.4
Pseudomonas sp.[c]	27.9	1.9	7.1	8.4	4.0	46.7
Pseudomonas sp.[d]	26.7	3.6	8.5	6.7	3.7	46.8
Burkholderia cepacia	38.3	0.4	2.5	4.1	4.3	46.0
Burkholderia glumae	34.8	0.2	3.3	6.9	3.9	46.6
Alcaligenes sp.[e]	30.0	2.6	7.0	6.1	4.0	46.1

[a]A mixture of 2.5 g soybean oil deodorizer distillate steryl ester concentrate (SODDSEC), 2.5 mL water, and 50 U lipase was stirred at 30°C for 20 h.
[b]Not detected (<0.1 wt%).
[c]Lipase PS (Amano enzyme).
[d]Lipase AK (Amano enzyme).
[e]Lipase QLM (Meito Sangyo).

TABLE 3
Effect of Water Content and Temperature on Hydrolysis of Acylglycerols in SODDSEC with *C. rugosa* Lipase

Reaction conditions[a]		Composition (wt%)					
Water (%)	Temperature (°C)	FFA	MAG	DAG	TAG	Sterol	Steryl ester
5	30	35.1	1.2	3.4	5.1	4.0	46.3
10	30	40.5	0.3	1.3	2.2	3.9	46.7
30	30	43.1	ND[b]	0.2	1.3	4.0	46.9
50	30	44.5	ND	ND	ND	3.9	47.0
70	30	44.1	ND	ND	ND	3.9	47.6
90	30	44.7	ND	ND	ND	4.0	46.9
50	20	37.2	0.5	2.2	3.1	3.9	46.5
50	40	44.3	ND	ND	ND	3.9	46.8
50	50	40.0	0.3	1.4	2.5	4.0	46.7

[a]A 5-g mixture of SODDSEC and water was stirred at 20–50°C for 20 h with 75 U *C. rugosa* lipase. For abbreviations see Tables 1 and 2.
[b]Not detected (<0.1 wt%).

To study the effect of temperature, a 5-g mixture of SODDSEC/water (1:1, w/w) and 75 U of the lipase was stirred for 20 h at 20 to 50°C (Table 3). Complete hydrolysis was obtained at a range of temperatures between 30 and 40°C. The optimal conditions of 30–40°C in the presence of 50% water were the same as those obtained in selective hydrolyses of tuna oil, borage oil, and arachidonic acid-containing single-cell oil with *C. rugosa* lipase for production of DHA-, γ-linolenic acid-, and arachidonic acid-rich oils (15).

Time course of lipase treatment of SODDSEC. Based on the results stated previously, the reaction conditions were determined as follows: ratio of SODDSEC/water, 1:1 (w/w); lipase amount, 15 U/g reaction mixture; temperature, 30°C. Figure 1 shows a typical time course under these conditions. TAG and DAG contents decreased rapidly, and only a small amount of MAG accumulated at the early stage of the reaction. FFA content increased along with the decrease of acylglycerols. The contents of steryl esters and sterols scarcely changed during the reaction. The reaction reached nearly

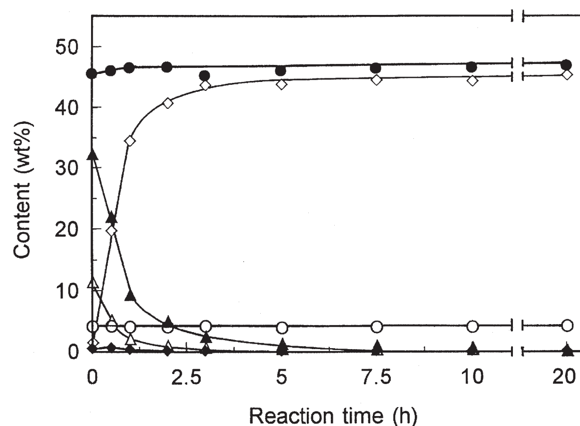


FIG. 1. Hydrolysis of acylglycerols in soybean oil deodorizer distillate steryl ester concentrate (SODDSEC) with *Candida rugosa* lipase. A mixture of 4 g SODDSEC, 4 mL water, and 120 U *C. rugosa* lipase was stirred at 30°C. ○, Sterol; ●, steryl ester; ◇, FFA; ◆, MAG; △, DAG; ▲, TAG.

TABLE 4
Purification of Steryl Ester from SODDSEC

Step	Weight (g)					
	Total	FFA	DAG	TAG	Sterol	Steryl ester
SODDSEC	1000	14	112	321	40	454
Hydrolysis[a]	910	410	ND[b]	ND	36	421
Distillation						
Distillate 1[c]	399	376	ND	ND	ND	ND
Distillate 2[d]	78	24	0.7	ND	33	9
Residue 2[d]	409	1.9	0.5	1.8	2.6	398

[a]A mixture of 1 kg SODDSEC, 1 L water, and 30,000 U *C. rugosa* lipase was agitated at 30°C for 24 h. After the hydrolysis, the resulting oil layer was recovered. For abbreviation see Tables 1 and 2.
[b]Not detected (<0.1 wt%).
[c]Distilled at 180°C and 0.2 mm Hg.
[d]Distilled at 250°C and 0.02 mm Hg.

steady state after 3 h, and acylglycerols were not detected (<0.1 wt%) after 20 h.

Purification of steryl esters from the reaction mixture. Purification of steryl esters from SODDSEC is summarized in Table 4. SODDSEC (1.0 kg) was agitated at 30°C for 24 h with 15 U/g-mixture in the presence of 50% water. The reaction mixture separated into oil and water layers, and the oil layer was dehydrated. The resulting oil layer (910 g) contained 45.1 wt% FFA, 4.0 wt% sterols, and 46.3 wt% steryl esters. To remove FFA, the oil layer was distilled at 180°C and 0.2 mm Hg. The distillation process separated the oil layer into 399 g distillate 1 (FFA content, 94.2 wt%) and 494 g residue 1 that contained 5.2 wt% FFA, 0.2 wt% DAG, 0.3 wt% TAG, 7.3 wt% sterols, 83.6 wt% steryl esters, and a negligible amount (<0.1 wt%) of MAG. To remove the remaining FFA and sterols, residue 1 was then distilled at 250°C and 0.02 mm Hg. The distillation separated residue 1 into 78 g distillate 2 and 409 g residue 2. Residue 2 contained only a small amount of FFA (0.5 wt%) and sterols (0.6 wt%), and steryl esters were purified to 97.3% with a recovery of 87.7% of the initial content. This result showed that a process comprised of lipase treatment and molecular distillation was very effective for the purification of steryl esters from SODD.

Kinetics of SODDSEC treatment with lipase. Candida rugosa lipase catalyzes esterification of sterols with FA (13,16) and hydrolysis of steryl esters (17), indicating that the lipase recognizes FA, sterols, and steryl esters as substrates. The reaction mixture for SODDSEC treatment contained substrates for hydrolysis of steryl esters and for esterification of sterols (water and FA), but the contents of sterols and steryl esters did not change during the SODDSEC treatment with *C. rugosa* lipase (Fig. 1). To solve this apparent inconsistency, purified steryl esters obtained in Table 4 were hydrolyzed at 30°C for 24 h in a mixture containing 50% water, 15 U/g *C. rugosa* lipase, and different amounts of soybean oil (TAG). The result is shown in Table 5.

TAG were almost completely hydrolyzed regardless of the amount of steryl esters, showing that hydrolysis of TAG is not affected by addition of steryl esters. Meanwhile, hydrolysis of steryl esters was significantly affected by addition of TAG. In the reaction mixture without TAG, the ratio of FA to sterols was 1:1, mol/mol. The degree of hydrolysis of steryl esters in this reaction reached 49.8% after 24 h (at the equilibrium state). An increase in the amount of TAG led to an increase in FFA content in the reaction mixture, and resulted in a decrease of the degree of hydrolysis of steryl esters. The reaction mixture of TAG/steryl esters (1:2, w/w) contains 2.1 molar amounts of FA against sterols. When the reaction was performed for 24 h, the degree of hydrolysis of steryl esters reached 11.7%. Even though the amount of TAG was

TABLE 5
Treatment of TAG/Steryl Esters with *C. rugosa* Lipase[a]

Reaction TAG/steryl ester (g/g)		Composition (wt%)						Hydrolysis of steryl esters[b] (%)
		FFA	MAG	DAG	TAG	Sterol	Steryl ester	
3.0/0	Before	ND[c]	ND	ND	99.1	ND	0.9	—
	After	97.2	0.6	0.8	0.6	ND	0.8	—
2.5/0.5	Before	0.1	ND	ND	82.1	0.2	17.4	—
	After	79.5	0.6	0.5	0.5	1.1	17.3	9.4
2.0/1.0	Before	0.2	ND	ND	65.3	0.3	34.2	—
	After	63.3	ND	0.2	0.1	2.3	33.3	10.2
1.5/1.5	Before	0.2	ND	ND	49.2	0.3	49.7	—
	After	48.4	ND	0.1	ND	3.9	47.0	12.0
1.0/2.0	Before	0.3	ND	ND	32.9	0.4	65.9	—
	After	31.2	ND	ND	ND	5.1	63.1	11.7
0.5/2.5	Before	0.4	ND	0.1	16.7	0.5	81.5	—
	After	22.0	ND	ND	ND	15.8	59.1	30.5
0/3.0	Before	0.5	ND	0.1	0.4	0.6	96.8	—
	After	16.9	ND	ND	ND	30.7	50.7	49.8

[a]A mixture of 3 g TAG/steryl esters, 3 mL water, and 90 U *C. rugosa* lipase was stirred at 30°C for 24 h. TAG was soybean oil.
[b]Degree of hydrolysis is expressed as a mole percentage of sterols based on the total amount of sterols and steryl esters. Molar amounts of sterols and steryl esters were calculated from their average M.W.: sterols, 410; steryl esters, 672.
[c]Not detected (<0.1 wt%). For abbreviation see Table 2.

TABLE 6
Treatment of Steryl Oleate (Steryl OA)/Trilinolein (TriLnA) or Steryl OA/Linoleic Acid (LnA) with *C. rugosa* Lipase[a]

	Time	Composition (wt%)						FA composition in steryl esters (wt%)		
	(h)	FFA	MAG	DAG	TAG	Sterol	Steryl ester	18:0	18:1	18:2
Substrate										
Steryl OA	—	—	—	—	—	—	100	2.1	91.0	4.4
TriLnA	—	—	—	8.1	91.9	—	—	0.5[b]	98.8[b]	0.7[b]
LnA	—	100	—	—	—	—	—	0.7[b]	98.7[b]	0.6[b]
Reaction mixture										
Steryl OA/TriLnA	0	ND[c]	ND	4.1	46.6	ND	49.3	2.1	91.0	4.4
	10	49.0	ND	0.1	ND	2.8	48.1	1.8	75.0	20.9
	24	49.4	ND	0.1	ND	2.9	47.6	1.7	69.5	25.3
Steryl OA/LnA	0	48.6	ND	ND	ND	ND	51.4	2.1	91.0	4.4
	10	48.9	ND	ND	ND	2.6	47.7	1.8	74.0	21.1
	24	49.1	ND	ND	ND	2.8	47.3	2.0	68.7	26.6

[a]A mixture of 3 g steryl OA/TriLnA (or steryl OA/LnA) (1:1, wt/wt), 3 mL water, and 15 U/g *C. rugosa* lipase was stirred at 30°C. For other abbreviation see Table 2.
[b]FA compositions of TriLnA and LnA preparations.
[c]Not detected (<0.1 wt%).

increased to 5 weight parts against steryl esters (FA, 12.5 molar amounts against sterols), the degree of hydrolysis of steryl esters decreased slightly (9.4%).

These results showed that TAG is completely hydrolyzed to FFA and glycerol at the equilibrium state in the reaction of TAG/steryl esters/water with *C. rugosa* lipase. Meanwhile, the ratio of sterols/steryl esters at the equilibrium state depended on the amount of FA in the reaction mixture. The amount of FA in SODDSEC was 2.8 times the molar amounts for sterols (Table 1), and the FA amount in a mixture of TAG/steryl esters (1:1, w/w) was 3.2 times the molar amounts for sterols (Table 5). The ratio of sterols/steryl esters in SODDSEC was 13:87 (mol/mol) (Table 1), and the ratio did not change after the lipase treatment (Fig. 1). Meanwhile, the ratio of sterols/steryl esters after the reaction of TAG/steryl esters (1:1, w/w) changed from 1:99 to 12:88 (mol/mol) (Table 5). The ratios of sterols/steryl esters after the two reactions agreed well. Hence, the phenomenon that the contents of sterols and steryl esters did not change during the SODDSEC treatment can be explained by the equilibrium state that depends on the amount of FA for sterols in the reaction mixture.

Exchange degree of FA in steryl esters in the treatment of SODDSEC. Table 5 indicated the possibility that at least a part of FA in steryl esters was exchanged with FA originating from acylglycerols. We thus studied the degree of FA exchange in the treatment of SODDSEC with lipase. In this experiment, a mixture of steryl OA/TriLnA (1:1, w/w) was used as a substrate, because SODDSEC contained almost equal weights of acylglycerols and steryl esters. A mixture of steryl OA/TriLnA, 50% water, and 15 U/g *C. rugosa* lipase was stirred at 30°C. After the reaction, steryl esters were extracted with *n*-hexane under alkaline conditions, and the FA composition was analyzed (Table 6). The 10-h reaction hydrolyzed almost all TriLnA, and the resulting steryl esters contained 75.0 wt% OA and 20.9 wt% LnA. The FA composition of the substrate steryl OA was 91.0 wt% OA and 4.4 wt% LnA, indicating that 16% of the FA in steryl esters was exchanged with FA originating from acylglycerols. Similarly, the 24-h reaction exchanged 21% of the FA in steryl esters with FA in acylglycerols. It was found from these results that the FA exchange reaction continues beyond the completion of hydrolysis of TAG.

Acylglycerols in SODDSEC were rapidly hydrolyzed, and the components in the reaction mixture became steryl esters and FFA (Fig. 1). Hence, we also studied the FA exchange of steryl OA with LnA. The reaction was performed at 30°C in a mixture of steryl OA/LnA (1:1, w/w), 50% water, and 15 U/g *C. rugosa* lipase with stirring. As shown in Table 6, the 10- and 24-h reactions exchanged 17 and 22% of FA in steryl esters with LnA, respectively.

These results indicated that about 20% of the FA in steryl esters was exchanged with FA originating from acylglycerols after the 24-h lipase-treatment of SODDSEC. However, because the FA composition of steryl esters in SODDSEC is the same as that of acylglycerols, the FA composition of steryl esters did not change before and after the lipase treatment.

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