Enzymatic Pretreatment of Deodorizer Distillate for Concentration of Sterols and Tocopherols

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Separation of sterols and tocopherols from fatty acids in deodorizer distillate was facilitated through lipase-catalyzed modification of fatty acids in canola, mixed and soya deodorizer distillates. The fatty acid esterification with methanol catalyzed by SP-382 (an immobilized nonspecific lipase) proceeded rapidly, with conversion of fatty acid to methyl ester in 5 h being 96.5, 83.5 and 89.4%, respectively. A model mixture of pure oleic acid and dl-α-tocopherol was used to study any potential side reactions that may lower the tocopherol content during the esterification reaction. Under the conditions employed, the loss of tocopherol was less than 5%. Simple vacuum distillation (1–2 mm Hg) was employed to remove the volatile fraction (methyl esters of fatty acids, some fatty acids and other volatiles) of the esterified deodorizer distillate, leaving behind sterols, sterol esters and tocopherols. Sterols and tocopherols were almost completely retained in the residue fraction with recoveries in the range of 95%. Overall recoveries of sterols and tocopherols after esterification and distillation were over 90% for all the deodorizer distillate samples.

KEY WORDS: Deodorizer distillate, esterification, fatty acids, lipase, methyl esters of fatty acids, sterols, tocopherols, vitamin E.

Tocopherols are extensively used as antioxidants, with an estimated worldwide use exceeding 150 metric tons annually (1). There is a growing trend of consumer preference for natural antioxidants. Natural tocopherols are recovered from vegetable oil deodorizer distillate, a by-product of the vegetable oil refining process. Deodorizer distillate is composed of fatty acids, sterols, tocopherols, sterol esters, hydrocarbons, breakdown products of fatty acids, aldehydes, ketones and acyl glycerol species. This by-product is sold on the basis of tocopherol content, which is valued both for its vitamin E activity and antioxidant property (2). Sterols are used as starting materials or as intermediates for the manufacture of pharmaceutical drugs and steroids for medicinal purposes. Fatty acids constitute 25–75% of the distillate depending on the raw material being refined, the type of refining process and the conditions employed therein. Deodorizer distillate can have significantly different characteristics, uses and value. When derived from soybean or other unsaturated vegetable oils, it can be a good raw material for production of vitamin E and sterols. From other fats and oils the distillate may be useful only for fatty acid production (3). Fatty acids from deodorizer distillates are limited to nonfood, low-cost applications because they are contaminated. We have shown that fatty acids in deodorizer distillate could be converted to their methyl esters with lipase as the catalyst (4). Some key points from our previous study included: (i) The enzyme efficiently catalyzed the conversion of fatty acids to their methyl esters; (ii) the lipase tolerated the presence of large amounts of impurities in the substrate and the lipase was active at high temperatures; (iii) the product of reaction did not require any washing and the reaction was conducted under mild conditions, therefore not detrimental to heat-labile compounds such as tocopherols; and (iv) the reaction was close to completion in a relatively short period of time.

Few studies on the recovery of tocopherols and sterols from deodorizer distillate have appeared in the literature. Kim and Rhee (5) studied solvent extraction, chemical treatment and molecular distillation for separation of sterols and tocopherols from soy oil scum but they reported low recoveries. Even the use of high vacuum ($3–7 \times 10^{-3}$ torr) could not prevent the co-distillation of sterols and tocopherols with free fatty acids. Sheabar and Neeman (6) have tried solvent extraction as well as chemical treatment for separation of tocopherols and sterols. It required the use of copious amounts of organic solvents at low temperatures. Recently, an attempt has been made to concentrate tocopherols through supercritical fluid extraction of tocopherols from esterified soy sludge (7). They reported increased solubility of the esterified soy sludge in the extracting fluid. However, they have used an acid catalyst for esterification, with excess methanol, which requires alkali neutralization and subsequent treatment to remove the salt that was formed. The effect of alkali treatment on the tocopherol molecule is deleterious, and some loss of tocopherol at this stage of processing can be expected. In this paper we report the lipase-catalyzed esterification of deodorizer distillate from canola oil, soybean oil and also a mixed deodorizer distillate, with varying tocopherol and sterol contents. The esterified deodorizer distillate was subjected to vacuum distillation to separate the methyl esters of fatty acids from tocopherols and sterols. Concentration and recovery of sterols and tocopherols were estimated. A model mixture of oleic acid and dl-α-tocopherol was used to study the effect of processing on tocopherol during esterification.

MATERIALS AND METHODS

Substrate and enzyme. Canola oil deodorizer distillate was supplied by CSP Foods Ltd., Saskatoon, Saskatchewan, Canada. Soya deodorizer distillate was obtained from Central Soya, Bellevue, OH. Mixed deodorizer distillate was obtained from Distillation Products Industries, Rochester, NY. The characteristics of deodorizer distillate samples are listed in Table 1. Oleic acid (>99%) was purchased from Sigma Chemical Co., St. Louis, MO. The immobilized lipase Randozyme SP-382 was a gift of Novo Industri A/S, Copenhagen, Denmark. The moisture content of the enzyme was adjusted to 3% before use in the reaction. Methanol used for the esterification reaction was of Omnisolv grade (BDH Chemicals, Toronto, Canada).

Chemicals. Unless otherwise specified, all chemicals were of reagent grade. Tocopherol standards (α-, β-, γ- and δ-) were the kind gift of Henkel Corporation, La Grange, IL. dl-α-Tocopherol, squalene, stigmasterol, campesterol, cholesterol and β-sitosterol were purchased from Sigma Chemical Co. Fatty acid standards were purchased from Nu-Chek-Prep, Inc., Elysian, MN.

Esterification reaction. The esterification reaction with deodorizer distillate was conducted as described previously (4). Reactions with pure oleic acid as the substrate were

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TABLE 1

Characteristics of Canola, Mixed and Soya Deodorizer Distillate Samples

	Canola	Mixed	Soya
% Moisture	0.02 ± 0.01	0.70 ± .02	0.10 ± .02
Acid value	157.6 ± 0.1	64.6 ± 0.1	44.8 ± 0.1
Saponification value	163.1 ± 1.0	127.4 ± 1.3	72.8 ± 1.6
Iodine value	74.3 ± 0.5	93.3 ± 0.8	120.2 ± 0.7
% Unsaponifiable matter	14.6 ± 0.1	23.5 ± 0.2	58.1 ± 1.5

conducted in 1-mL Reacti-vials™ (Pierce, Rockford, IL) placed in a thermostated Gyrotory Shaker (New Brunswick Scientific Company, New Brunswick, NJ). The reaction was initiated by adding the immobilized lipase to a specific amount of oleic acid, dl-α-tocopherol and methanol. Samples of 1-μL were taken from the reaction vial at specific intervals of time by a 10-μL syringe. Trimethyl silyl derivatives of the unesterified fatty acids and tocopherol were prepared by adding 0.3 mL of a mixture of Sylon BFT (Supelco, Oakville, Ontario, Canada) and pyridine (1:7, vol/vol). The contents were heated at 105°C for 10 min. After cooling down to room temperature, 1 μL was injected on gas chromatography (GC) for simultaneous analysis of fatty acid, fatty acid methyl ester and tocopherol.

Estimation of conversion. Percentage conversion of fatty acids to methyl esters during the enzymatic esterification of deodorizer distillate was estimated as described previously (4).

Fatty acid analysis. Free fatty acids in the deodorizer distillate were separated by thin-layer chromatography (TLC). A solution (60 μL) of deodorizer distillate in chloroform (1:10 wt/vol) was spread as a band on a precoated TLC plate (20 × 20 cm, silica gel 60 F254, aluminum sheets) and developed with hexane/diethyl ether/glacial acetic acid (60:40:0.5, vol/vol/vol). After drying, the plate was sprayed with 2′,7′-dichlorofluorescein (1% wt/vol solution in ethanol) and visualized under ultraviolet (UV) light. The fatty acid band was identified by referring to an oleic acid standard, scraped off the TLC plate and recovered by eluting with ether. After evaporating the solvent, the fatty acids were derivatized with methylating reagent (methanol/toluene/sulfuric acid in the ratio 20:10:1, vol/vol/vol) in a nitrogen-flushed screw-cap centrifuge tube at 100°C for 30 min, extracted with hexane and analyzed on GC.

Total fatty acid compositions of the deodorizer distillates were determined after saponification. The unsaponifiables were extracted with diethyl ether and the remaining soap was acidified with HCl. The fatty layer was extracted by ether, washed and derivatized as described above for fatty acid analysis through GC. Capillary gas chromatography was executed with a 3400 series Varian gas chromatograph equipped with a flame-ionization detector (FID). The fused silica capillary column, BPX-70 (Rose Scientific, Edmonton, Alberta, Canada), 50 m × 0.22 mm i.d. had film thickness of 0.25 μm. Helium with a flow rate of 0.5 mL/min was used as the carrier gas, and the split ratio was 140:1. The column was held initially for 15 min at 170°C, raised to 180°C at the rate of 1°C/min, then to 250°C at the rate of 5°C/min and held there for 5 min. The injector was maintained at 250°C and the FID detector at 280°C. The response factors of the fatty acids were estimated from standards run under identical conditions.

Analysis of tocopherols and sterols. Tocopherols and sterols were analyzed according to AOCS Recommended Practice Ce 7-87 (8) with heptadecanyl stearate as the internal standard and d-α-tocopherol (Eastman Kodak, Rochester, NY) as the primary standard. Response factors of α-, β-, γ- and δ-tocopherol, and all the sterols (except brassicasterol, for which the response factor was assumed to be equal to 1) were estimated by injecting known amounts of standards. The response factors are listed in Table 2. The capillary column BP-5 (Rose Scientific) 23 m × 0.25 mm i.d. had a film thickness of 0.25 μm. The carrier gas was helium at 1.2 mL/min and the split ratio was 65:1.

Sterol esters. Sterol esters were estimated in the deodorizer distillates according to Feeter (9).

Simultaneous analysis of fatty acids, fatty acid methyl esters and tocopherol. The conventional acid value measurement (10) to determine the percent conversion of fatty acids to methyl esters required substantially large amounts of samples (2–5 g). We needed a simple and efficient method to detect accurately fatty acids, fatty acid methyl esters as well as dl-α-tocopherol (in our model system) simultaneously within a short period of time. We tried HT-5 (Rose Scientific), a high-temperature nonpolar column with a siloxane-carborane backbone to effect the separation. Trials with direct injection of the sample or the sample after silylation showed that resolution of fatty acids and the methyl esters of the fatty acids was excellent after silylation by using a 3-m column (0.22 mm i.d., 0.25 μm film thickness). A flow rate of 1.8 mL/min was maintained with helium as the carrier gas, and the split ratio was 35:1. The column was temperature-programmed from 80 to 290°C at the rate of 10°C/min. Figure 1 shows the response of fatty acids and methyl esters of fatty acids, which is linear for varying ratios of fatty acid and the methyl ester. Figure 2 shows a typical chromatogram of a mixture of oleic acid, methyl oleate and dl-α-tocopherol.

Distillation. Esterified deodorizer distillate samples (25 g) were placed in a preweighed 50-mL round-bottom flask. The flask was connected to a vacuum pump through a distillation head, an air condenser and a preweighed 50-mL round-bottom collecting flask. Volatile fractions from 160 to 185°C (1–2 mm Hg) were collected, following which the residue in the distillation flask was cooled to room temperature before releasing the vacuum. The feed, residue and the volatile fraction were weighed and analyzed for acid value, tocopherol and sterol content and their composition according to the methods described above.

RESULTS AND DISCUSSION

Table 1 lists the characteristics of deodorizer distillate samples used in this study. Canola oil deodorizer distillate

TABLE 2

Response Factors of Tocopherols, Sterols and Squalene Obtained by Gas Chromatography (GC) Analysis of Standard Compounds (GC conditions as in Materials and Methods section)

Compound	Response factor
Tocopherol	
α-tocopherol	1.03
β-tocopherol	0.90
γ-tocopherol	0.96
δ-tocopherol	0.87
Sterol	
cholesterol	1.25
campesterol	0.49
stigmasterol	1.22
β-sitosterol	0.82
Squalene	1.21

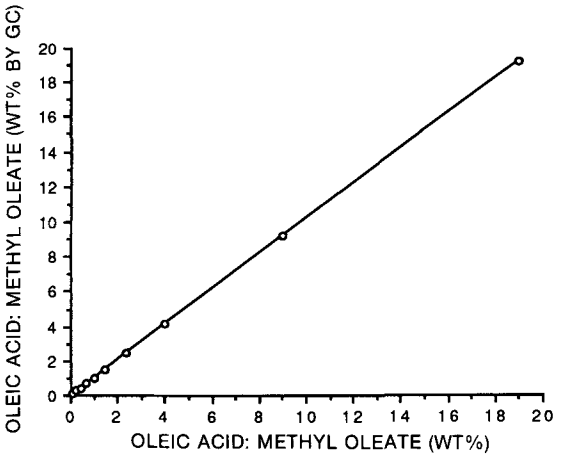

FIG. 1. Linearity of response for oleic acid-methyl oleate. Results obtained by analyzing standard solutions of mixtures containing known amounts of oleic acid and methyl oleate. Purity of oleic acid and methyl oleate were checked on gas chromatography (GC) (GC conditions as in Materials and Methods section).

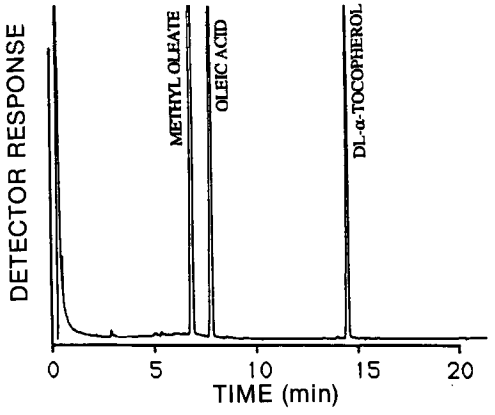

FIG. 2. Typical chromatogram of a silylated sample mixture of oleic acid, methyl oleate and dl-α-tocopherol used in the experiment with model mixture.

(obtained from deodorization of chemically refined canola oil and partially hydrogenated canola oil) is solid at room temperature and is yellowish brown in color. Mixed deodorizer distillate (from a mixture of vegetable oils) and soya deodorizer distillate (from chemically refined soybean oil) are semi-solid at room temperature and are dark brown in color. The deodorizer distillate samples could be classified on the basis of unsaponifiable content. Canola deodorizer distillate had a low content of unsaponifiables and represented one end of the spectrum, while soya deodorizer distillate with high unsaponifiable content represented the other end. Mixed deodorizer distillate fell in between the above two types of deodorizer distillates. The free fatty acid content of the canola deodorizer distillate was high, comprising nearly 79%, while in soya deodorizer distillate only 24% was free fatty acid. Thus, soya deodorizer distillate was an ideal raw material for recovery of sterols and tocopherols. Mixed deodorizer distillate could be upgraded for sterols and tocopherols in times of demand, and canola deodorizer distillate could find use as a cheap fatty acid source.

Table 3 lists the free and total fatty acid composition of the deodorizer distillate samples. The fatty acid composition of canola deodorizer distillate did not match the fatty acid composition of canola oil. The high percentage of stearic acid in the deodorizer distillate was due to the processing of an admixture of canola oil and partially hydrogenated canola oil. The total fatty acid composition of the deodorizer distillate was similar to that of the free fatty acid fraction. Canola deodorizer distillate had only 0.9% steryl esters, and the calculated ester value (saponification value-acid value) was only 5.5. Thus, the amount of fatty acids esterified to other compounds (steryl esters or acyl glycerol species) was low. The mixed deodorizer distillate had about 32% free fatty acids and contained 1.09% steryl esters. The calculated ester value was ~63.0, indicating that a large amount of fatty acids were present in the esterified form, probably in the form of acyl glycerol species because the deodorizer distillate had low amounts of steryl esters. The soya deodorizer distillate with a calculated ester value of 28.0 contained 13.7% steryl esters. Vegetable oils contain steryl esters, mainly sterols esterified to fatty acids. It has been previously shown that the fatty acids esterified to sterols may differ in composition to that of the whole oil (11). In soya deodorizer distillate, the short-chain and long-chain fatty acids might be found as steryl esters and as acyl glycerol species.

Tocopherol and sterol contents of the deodorizer distillate samples and their compositions are reported in Table 4. Soya deodorizer distillate contained the highest amounts of tocopherols and sterols. Typical specifications for deodorizer distillates used as the raw material for recovery of sterols and tocopherols are 2.5% min stigmasterol, 6.0% min tocopherol and 2.0% max. water (2). In soya deodorizer distillate, γ- and δ-tocopherol formed about 56 and 37% of the total tocopherols, respectively. α-Tocopherol was present at less than 10% while β-tocopherol was present in very small quantities. Soybean oil is a poor source of α-tocopherol, unlike cottonseed oil and sunflower oil. However, the presence of three tocopherols (γ-, δ- and α-) in sufficient levels provides the manufacturer the option to produce designer antioxidants. The sterol fraction in soya deodorizer distillate was composed of approximately 47% β-sitosterol, 34% stigmasterol and 19% campesterol. Together, sterols and tocopherols constituted only 2.6% of canola deodorizer distillate. The tocopherol fraction was composed of δ-, α- and γ-tocopherol with no traceable quantity of β-tocopherol. Brassicasterol is a uni-

TABLE 3

Free Fatty Acid and Total Fatty Acid Composition of Deodorizer Distillate Samples[a]

	Canola		Mixed		Soya	
Fatty acid	FFA	FA-total	FFA	FA-total	FFA	FA-total
C12:0	0.1	trace	0.4	0.4	0.0	0.2
C14:0	0.6	0.4	1.0	0.6	0.0	0.2
C16:0	8.8	8.1	31.5	28.2	15.6	15.8
C16:1	0.1	trace	0.0	0.1	0.0	0.0
C18:0	40.1	37.9	22.0	14.7	3.7	3.5
C18:1	42.2	45.7	26.7	30.9	17.8	18.0
C18:2	5.7	5.8	15.6	20.7	55.9	55.9
C18:3	1.5	1.3	0.8	1.0	7.0	5.7
C20:0	0.6	0.6	0.6	0.4	0.0	0.2
C20:1	0.2	0.2	0.0	0.0	0.0	0.0
C22:0	0.0	0.0	0.9	0.6	0.0	0.4
C24:0	0.0	0.0	0.4	0.4	0.0	0.2

[a]Components expressed as wt%; trace (present at <0.1%). Fatty acids represented as C number of carbon atoms/number of double bonds. FFA, free fatty acids, FA, fatty acids.

TABLE 4

Squalene, Sterol and Tocopherol Composition of Deodorizer Distillate Samples[a]

Deodorizer		Tocopoherol				Sterol				Total	Total
distillate	Squalene	δ—	β—	γ—	α—	brassica-	campe-	stigma-	β sito-	tocopherol	sterol
Canola	0.24 ± .02	0.11 ± .01	0.00	0.65 ± .03	0.24 ± .01	0.46 ± .02	0.33 ± .02	0.00	0.78 ± .04	1.00	1.58
Mixed	0.94 ± .10	0.82 ± .00	0.00	2.70 ± .00	1.00 ± .00	0.00	0.81 ± .00	1.49 ± .01	3.69 ± .00	4.51	5.99
Soya	2.62 ± .01	4.73 ± .04	0.18 ± .01	7.16 ± .67	0.68 ± .01	0.00	2.13 ± .02	3.88 ± .01	5.38 ± .06	12.74	11.39

[a]Components expressed as wt%.

que sterol found in the canola oil deodorizer distillate but is absent in both soya and mixed deodorizer distillate. About 30% of the sterol fraction was composed of brassicasterol. Kircher and Rosenstein (12) have isolated brassicasterol from rapeseed oil deodorizer distillate through derivatization and crystallization. The sterol fraction of canola deodorizer distillate also contained campesterol and β-sitosterol. Mixed deodorizer distillate contained 4.51% total tocopherols and 5.99% sterols and fell short of the specification limits set for deodorizer distillate. The tocopherol fraction was composed of about 60% γ-tocopherol, 22% α-tocopherol and 18% δ-tocopherol. Mixed deodorizer distillate contained substantially more α-tocopherol than soya deodorizer distillate and less δ-tocopherol, while the γ-tocopherol content was about the same. β-Sitosterol was the major component of the sterol fraction in the mixed deodorizer distillate at nearly 61.5%, the rest being stigmasterol (25%) and campesterol (13.5%).

Esterification of deodorizer distillate with methanol. The esterification reaction of deodorizer distillate and methanol was carried out under optimum conditions at temperatures close to the melting point of the deodorizer distillates. Figure 3 shows the course of esterification of the deodorizer distillate samples. The equilibrium conversion was attained for all the samples in approximately 24 h. For canola oil deodorizer distillate, up to 96.5% conversion was achieved within 3 h and it remained at that level. In the case of mixed and soya deodorizer distillates, during the same time period of 3 h the conversions were slightly above 80%, after which the esterification reaction proceeded slowly to reach equilibrium in 24 h. This could be the direct result of a low percentage of free fatty acids

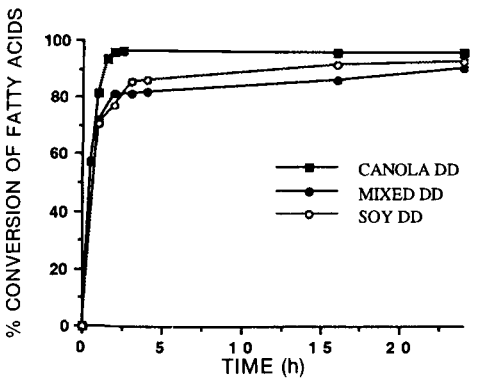

FIG. 3. Course of enzymatic esterification of fatty acids in deodorizer distillate samples with methanol (conditions given in the Materials and Methods section).

in mixed and soya deodorizer distillates to start with. These raw materials represented diluted substrates, and the reaction proceeded slowly to attain equilibrium. The esterification reactions with mixed and soya deodorizer distillate were carried out for 5 h, and these products were taken as the feed for subsequent distillation. The percentage conversions were 96.5, 83.5 and 89.4% for canola, mixed and soya deodorizer distillates, respectively.

Esterification reaction with a model mixture. Since the enzymatic esterification of fatty acids in the deodorizer distillate is suggested as a preliminary treatment for the recovery of sterols and tocopherols, it was necessary to find if there were any losses of these components, especial-

ly tocopherols, because they are labile molecules. During the esterification reaction, loss of tocopherol can occur either due to oxidation or due to the formation of esters with fatty acids. In both cases, alteration in the structure of the molecule (at the hydroxyl group) will render it useless for application as an antioxidant. We used a model system comprised of pure oleic acid (>99%), dl-α-tocopherol and methanol to study any possible loss of tocopherol during the esterification reaction. Esterification reactions were conducted in 1-mL reacti-vials at 25°C with shaker speed set at 220 rpm. In the reaction system consisting of oleic acid, methanol and immobilized lipase, the esterification reaction proceeded rapidly. About 96.5% of oleic acid was converted to methyl oleate in 3 h. The reaction attained 97.5% equilibrium conversion in a 24-h time period. Following initial experiments, we then performed the esterification reaction of oleic acid in the presence of tocopherol (usually, tocopherol was taken as 10% by weight of the total weight of oleic acid and tocopherol). The significant observations were as follows. There was no appreciable loss of tocopherol during the esterification reaction, and the oleic acid was converted to methyl oleate to the same extent (about 96.5%) as in the absence of tocopherol. Thus, presence of tocopherol did not hinder the conversion of oleic acid to methyl oleate, and because no loss of tocopherol was observed, it is fair to assume that both undesired reactions, oxidation of tocopherol and the potential side reaction for the formation of tocopheryl ester of oleic acid did not proceed to any observeable extent. In the absence of immobilized lipase, the esterification reaction did not proceed and there was minimum loss of tocopherol (<1%). In the absence of methanol, to check for the formation of tocopheryl esters of fatty acids, both oleic acid and tocopherol remained unchanged during 24 h. Reactions with oleic acid, methanol, tocopherol and the enzyme were conducted with varying headspace in the reaction vial. No significant change in tocopherol was observed in all cases in a 24-h reaction period. Thus, we concluded that structural integrity of the tocopherol molecule was preserved with a minimum loss (less than 5%) during the esterification of oleic acid, and this conclusion could be extended to lipase-catalyzed methyl esterification of deodorizer distillate samples.

Recovery of sterols and tocopherols. Table 5 gives the material balance of sterols, tocopherols, sterol esters, squalene and fatty acids during esterification and distillation for canola, mixed and soya deodorizer distillate samples. For canola oil deodorizer distillate, the recoveries of sterols and tocopherols during esterification were 95.5 and 93.1%, respectively. The amount of sterol esters increased by about 15% (calculated from amounts in the deodorizer distillate and in the esterified deodorizer distillate) and formed 1% of the esterified deodorizer distillate. Possibly, some of the free sterols esterified with free fatty acids to form sterol esters as reflected in the recovery of free sterols. Nearly 7% of the free tocopherols were lost during the esterification reaction. As suggested earlier, the loss could be the direct result of oxidation of tocopherols or could be due to the formation of esters of tocopherol. To estimate the latter, the procedure adopted for sterol esters could not be applied, as tocopherols are alkali-labile. Analysis of tocopherols in the unsaponifiable matter extracted from the deodorizer distillate and back calculating the content in the deodorizer distillate consistently yielded lower values compared to direct analysis of tocopherol contents in the deodorizer distillate. This clearly showed the sensitive nature of tocopherols in the presence of alkali (hence the use of the model mixture to estimate loss of tocopherol and a method for direct analysis of the components). The possibility of esterifica-

TABLE 5

Material Balance of Sterols, Tocopherols, Sterol Esters and Squalene and Their % Recoveries After Esterification of Deodorizer Distillate Samples and After Distillation of the Esterified Deodorizer Distillate

Component	Deodorizer distillate[a]	Esterified deodorizer distillate	Residue fraction	Volatile fraction	% Recovery		% Overall recovery[d]
					Esterification[b]	Distillation[c]	
Canola deodorizer distillate							
FFA[e]	79.19	2.97	5.60	2.79	—	—	—
Tocopherols	1.02	0.91	6.83	trace	93.07	98.68	91.84
Sterols	1.58	1.45	10.45	trace	95.53	94.77	90.53
Sterol esters	0.91	1.00	7.60	—	114.56	99+	114.56
Squalene	0.24	0.21	1.34	trace	99+	80.00	80.00
Mixed deodorizer distillate							
FFA[e]	32.26	5.25	4.00	5.06	—	—	—
Tocopoherols	4.51	4.30	11.11	0.18	96.43	97.27	93.80
Sterols	5.99	5.64	14.33	0.15	95.16	95.83	91.19
Sterol esters	1.09	1.24	3.32	—	114.70	99+	114.70
Squalene	0.94	0.80	1.93	0.15	86.21	99+	86.21
Soya deodorizer distillate							
FFA[e]	23.62	2.49	1.11	3.50	—	—	—
Tocopherols	12.74	12.75	21.48	0.19	100.00	99.32	99.32
Sterols	11.39	11.11	17.56	0.21	98.60	93.81	92.50
Sterol esters	13.73	13.21	22.44	—	97.14	99+	97.14
Squalene	2.62	2.52	3.69	0.82	96.18	86.21	82.92

[a]Components expressed as wt%.
[b]% Recovery (esterification) = (amount in esterified deodorizer distillate/amount in deodorizer distillate) × 100.
[c]% Recovery (distillation) = (amount of component in residue/amount of component in feed) × 100.
[d]% Overall recovery = (amount of component in residue/amount of component in deodorizer distillate) × 100.
[e]FFA, free fatty acids.

tion of tocopherol can be ruled out due to the results from experiments with the model system. However, there is a strong possibility of tocopherols being oxidized, especially when a large reaction vessel is used for the esterification of deodorizer distillate (only the jacketed portion of the wheaton's flask was used for the reaction, which left about 50% headspace volume). Flushing of the reaction vessel headspace with nitrogen gas after addition of methanol was avoided, so as to prevent loss of methanol. Esterified deodorizer distillate (~25 g) was used as the feed for distillation. The volatile fraction represented the portion collected from 160 to 180°C at 1-2 mm Hg vacuum and accounted for 84.4% of the feed. The residue retained in the distillation flask was 13.2% of the feed. If desired, the volatile fraction could be fractionated to yield material for specific end use. Methyl esters of fatty acids are almost exclusively used as intermediate materials for the synthesis of other chemicals. However, the specifications would demand greater purity and higher quality. The methyl esters recovered from the distillation step could be put to use in formulation of cheaper materials or as fuels. While analyzing for sterols and tocopherols in the distillate and the residue, we found that the volatile fraction did contain only trace amounts of sterols and tocopherols. Significant loss of sterols and tocopherols was reported by Kim and Rhee (5) while attempting to separate sterols and tocopherols from free fatty acids in the deodorizer distillate by direct molecular distillation,

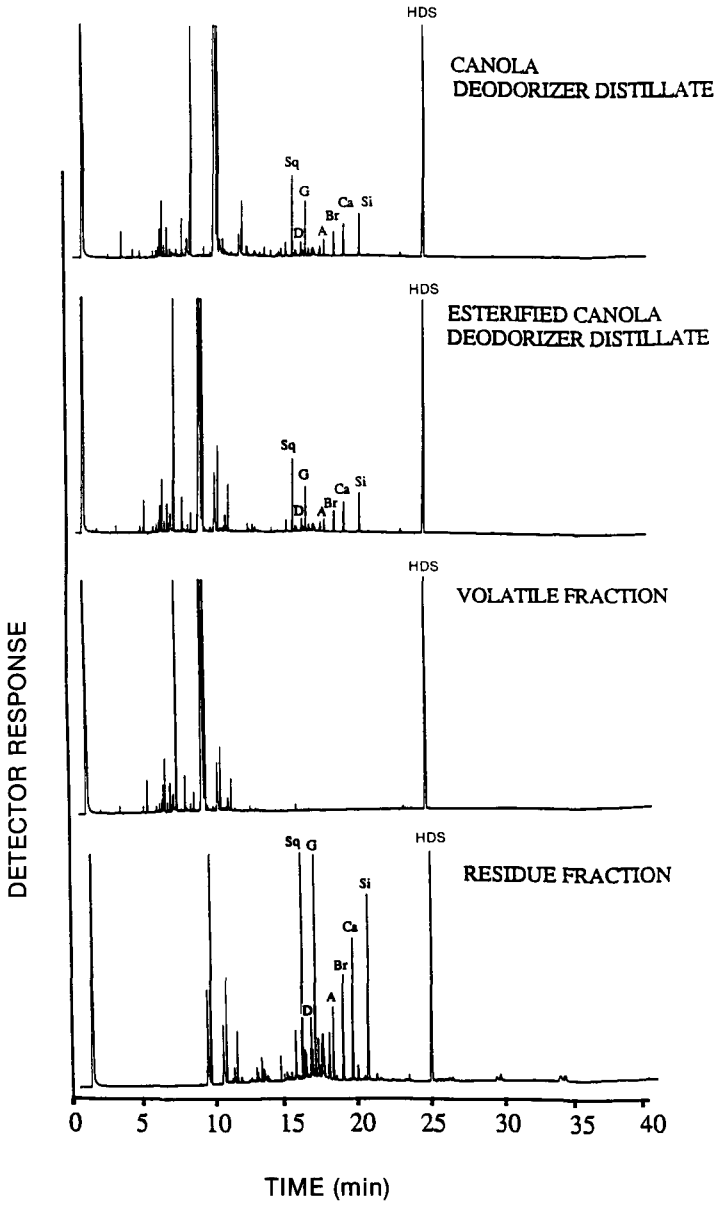

FIG. 4. Chromatograms showing qualitatively the concentration of sterols and tocopherols in canola deodorizer distillate, esterified canola deodorizer distillate and in the two fractions obtained from distillation of esterified canola deodorizer distillate, volatile fraction and residue fraction (gas chromatography conditions as mentioned in Materials and Methods section). Peak Sq, Squalene; D, δ-tocopherol; G, γ-tocopherol; A, α-tocopherol; Br, brassicasterol; Ca, campesterol; Si, β-sitosterol; and HDS, heptadecanyl stearate.

due to the similar boiling points. The boiling point difference has been greatly increased between the fatty acids and the valuable fraction, sterols and tocopherols, by converting the free fatty acids in the deodorizer distillate to their methyl esters. The recovery of sterols and tocopherols during the simple distillation of esterified canola deodorizer distillate in our study was 94.8 and 98.7%, respectively. The overall recoveries (including both the esterification and distillation steps) of sterols and tocopherols were 90.5 and 91.8%, respectively. Thus, starting with a raw material, which had 1% tocopherol and 1.6% sterol, we end up with a concentrate, free of most of the fatty acids, that had 6.8% tocopherol and 10.5% sterols. The concentration has been approximately 6.6-fold with high (>90%) recoveries. Because sterol esters have higher molecular weight than sterols, it was assumed that all the sterol esters present in the feed would be retained in the residue. About 20% of the squalene was lost in the distillation step. The chromatograms of canola deodorizer distillate, esterified deodorizer distillate, fractions obtained from distillation of esterified deodorizer distillate, namely, volatile fraction and residue, are shown in Figure 4.

Table 6 gives the composition of sterols and tocopherols of the deodorizer distillate, esterified deodorizer distillate and in the volatile fraction and residue for all three deodorizer distillate samples. The relative composition of sterols and tocopherols in esterified canola deodorizer distillate and the residue fraction was similar to that of the starting material. While the canola deodorizer distillate did not contain measurable β-tocopherol and cholesterol, after concentration their presence was detected at low levels in the residue. The presence of cholesterol in deodorizer distillates has been reported previously (8,13).

The recoveries of sterols and tocopherols were 95.2 and 96.4%, respectively, during esterification of mixed deodorizer distillate. As with canola deodorizer distillate, the amount of sterol esters in mixed deodorizer distillate increased by about 15%. Residual free fatty acids represented 5.3% of the esterified deodorizer distillate. After the distillation step, most of the sterols and tocopherols were recovered in the residue fraction, as shown in Table 5. The volatile fraction collected was 61.5% of the feed, and the residue retained was 37.8%. The recoveries of sterols and tocopherols during distillation were 95.8 and 97.3%, which resulted in overall recoveries of 91.2 and 93.8%, respectively. Therefore, the sterols and tocopherols had been concentrated 2.4 times. The residue fraction contained 4% fatty acids compared to the deodorizer distillate that contained 32.3% free fatty acids. Figure 5 shows the gas chromatograms of the mixed deodorizer distillate, esterified mixed deodorizer distillate and the two fractions from distillation, the volatile fraction and the residue.

Soya deodorizer distillate is a rich source of sterols, tocopherols and sterol esters. However, it does contain about 23.6% free fatty acids, of which ~80% is unsaturated. As methyl esters of fatty acids are more stable and as they lend themselves easily to processing, it would be convenient to modify the free fatty acids to their methyl esters (14). Soybean oil distillates are the most common deodorizer distillates in the United States (2). Because about 90% of the free fatty acids in the deodorizer distillate was converted to methyl esters, the esterified soya deodorizer distillate contained only 2.5% free fatty acids. During esterification, recoveries of sterols and tocopherols were high, being 98.6% and 100%. Sterol ester formation during the esterification reaction was not observed for soya as it was for mixed and canola deodorizer distillates. After distillation of the esterified

TABLE 6

Squalene, Sterols and Tocopherols Composition of Deodorizer Distillate, Esterified Deodorizer Distillate and the Two Fractions Obtained from the Distillation of Esterified Deodorizer Distillate, Volatile Fraction and Residue Fraction[a]

		Tocopherol				Sterol				Total	Total
	Squalene	δ—	β—	γ—	α—	campe-	stigma-	β sito-	brassica-	tocopherol	sterol
Canola deodorizer distillate											
Deodorizer distillate	0.24 ± .02	0.11 ± .01	0.00	0.65 ± .03	0.24 ± .01	0.33 ± .02	0.00	0.78 ± .04	0.46 ± .02	1.00	1.58
Esterified deodorizer distillate	0.21 ± .02	0.10 ± .01	0.00	0.60 ± .03	0.21 ± .00	0.31 ± .00	0.00	0.71 ± .00	0.43 ± .00	0.91	1.45
Residue fraction[b]	1.34 ± .29	0.75 ± .06	0.09 ± .02	4.22 ± .07	1.77 ± .09	2.16 ± .12	0.00	5.12 ± .02	3.00 ± .06	6.83	10.45
Volatile fraction	traces[c]	traces	0.00	traces	traces	traces	0.00	traces	traces	traces	traces
Mixed deodorizer distillate											
Deodorizer distillate	0.94 ± .10	0.82 ± .00	0.00	2.70 ± .00	1.00 ± .00	0.81 ± .00	1.49 ± .01	3.69 ± .00	0.00	4.51	5.99
Esterified deodorizer distillate	0.80 ± .10	0.78 ± .00	0.00	2.57 ± .01	0.95 ± .00	0.76 ± .01	1.40 ± .01	3.48 ± .00	0.00	4.30	5.64
Residue fraction	1.93 ± .23	1.99 ± .02	0.00	6.66 ± .06	2.46 ± .03	1.92 ± .01	3.59 ± .04	8.82 ± .06	0.00	11.11	14.33
Volatile fraction	0.15 ± .02	0.00	0.00	0.18 ± .01	0.00	0.00	0.00	0.18 ± .01	0.00	0.18	0.18
Soya deodorizer distillate											
Deodorizer distillate	2.62 ± .01	4.73 ± .04	0.18 ± .01	7.16 ± .67	0.68 ± .01	2.13 ± .02	3.88 ± .01	5.38 ± .06	0.00	12.74	11.39
Esterified deodorizer distillate	2.52 ± .00	4.50 ± .01	0.16 ± .01	7.44 ± .02	0.65 ± .01	2.09 ± .02	3.78 ± .01	5.24 ± .05	0.00	12.75	11.11
Residue fraction	3.69 ± .05	7.37 ± .03	0.26 ± .02	12.71 ± .03	1.14 ± .01	3.30 ± .01	5.93 ± .01	8.33 ± .02	0.00	21.48	17.56
Volatile fraction	0.82 ± .00	0.08 ± .01	0.00	0.11 ± .00	0.00	0.06 ± .00	0.00	0.15 ± .01	0.00	0.19	0.21

[a]Components expressed as wt%.
[b]Residue fraction contained 0.17 ± .04% cholesterol.
[c]Traces: present at < .01%.

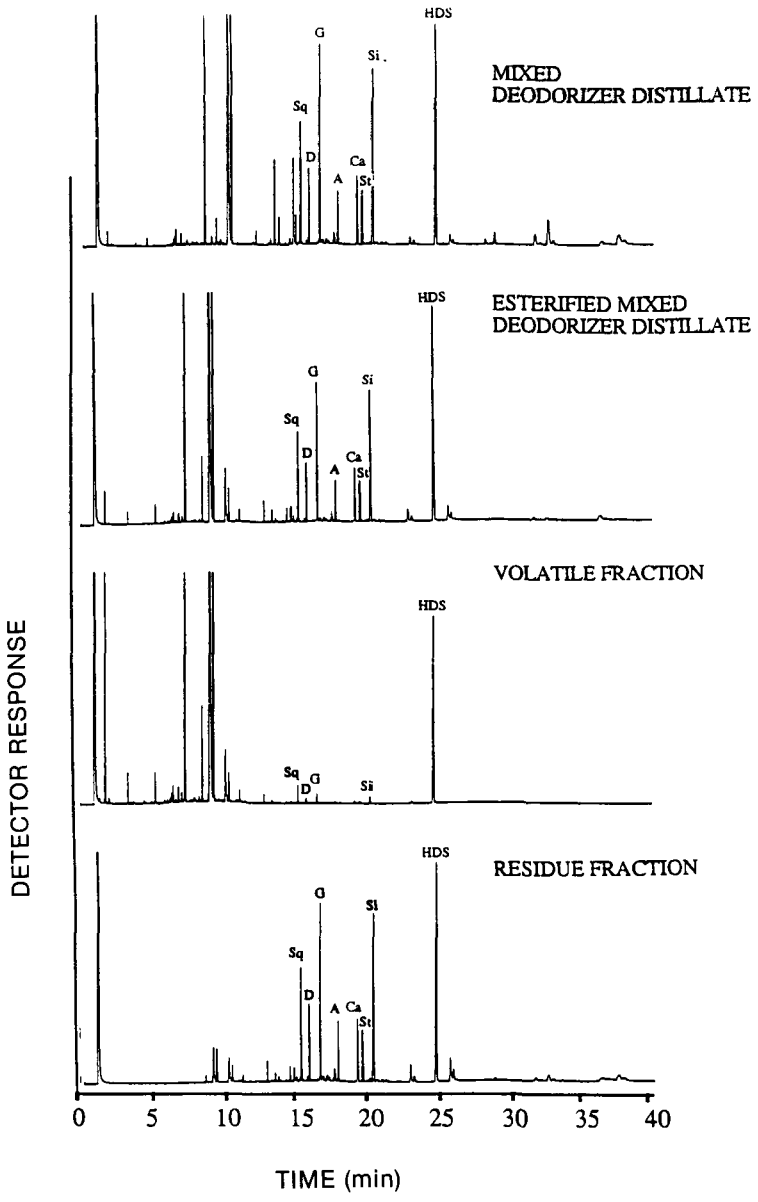

FIG. 5. Chromatograms showing qualitatively the concentration of sterols and tocopherols in mixed deodorizer distillate, esterified mixed deodorizer distillate and in the two fractions obtained from distillation of esterified mixed deodorizer distillate, volatile fraction and residue fraction (gas chromatography conditions as mentioned in Materials and Methods section). Peak Sq, Squalene; D, δ-tocopherol; G, γ-tocopherol; A, α-tocopherol; Ca, campesterol; St, stigmasterol; Si, β-sitosterol; and HDS, heptadecanyl stearate.

soya deodorizer distillate, 41.1% of the feed was collected as the volatile fraction, while the remaining formed the residue (58.9%). About 93.8% of the sterols and 99.3% of the tocopherols were recovered in the distillation step. Sterols were concentrated 1.5 times, while tocopherols were concentrated 1.7 times. The composition of sterols and tocopherols shown in Table 6 indicates no change in relative ratios of the individual tocopherols and sterols in the residue when compared with the starting material. Figure 6 shows the chromatograms of the soya deodorizer distillate during different stages of processing.

In conclusion, we have shown that immobilized lipase is an efficient catalyst for esterification of fatty acids with methanol. Immobilized lipase can be readily applied to modification of deodorizer distillate at mild operating conditions (with no requirement for product clean-up) to get a product rich in sterols and tocopherols after distilling off the methyl esters of fatty acids. However, the residue obtained after distillation will require another step for the separation into sterol-rich and tocopherol-rich fractions.

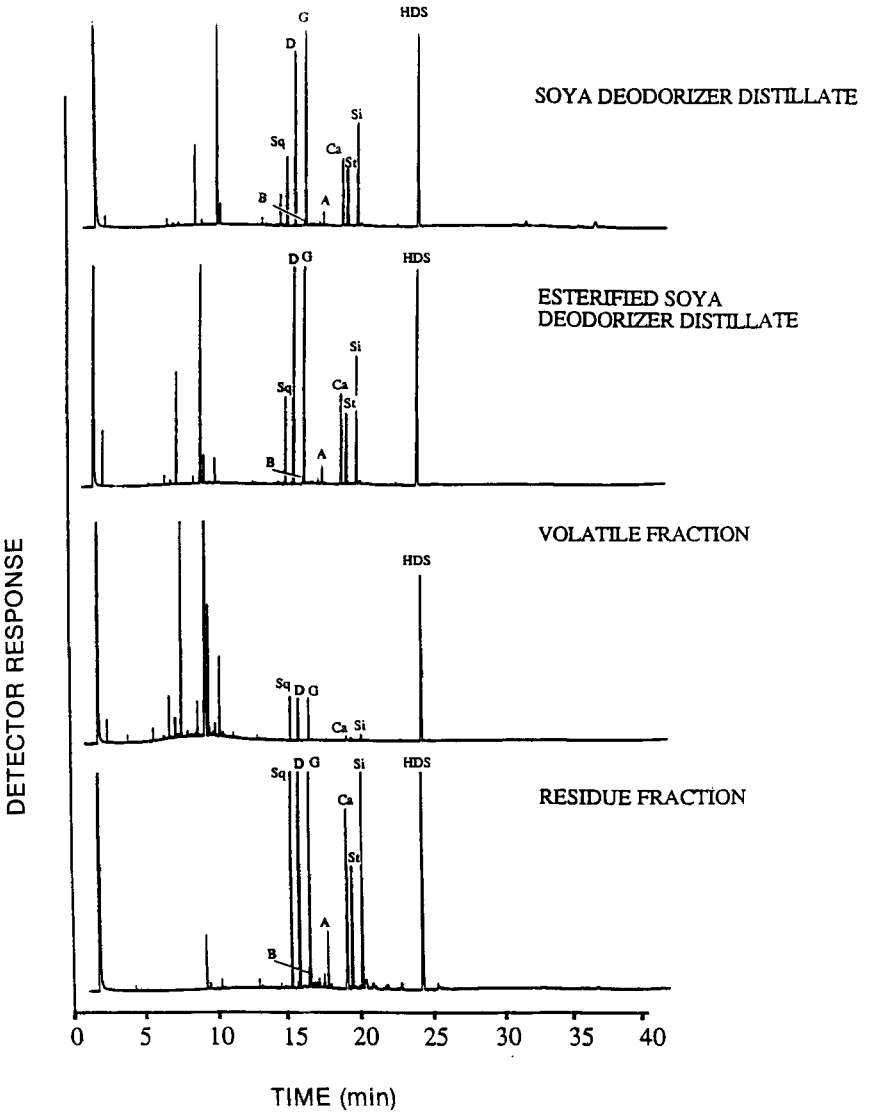

FIG. 6. Chromatograms showing qualitatively the concentration of sterols and tocopherols in soya deodorizer distillate, esterified soya deodorizer distillate and in the two fractions obtained from distillation of esterified soya deodorizer distillate, volatile fraction and residue fraction (gas chromatography conditions as mentioned in Materials and Methods section). Peak Sq, Squalene; D, δ-tocopherol; B, β-tocopherol; G, γ-tocopherol; A, α-tocopherol; Ca, campesterol; St, stigmasterol; Si, β-sitosterol; and HDS, heptadecanyl stearate.

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