Isolation of Tocopherol and Sterol Concentrate from Sunflower Oil Deodorizer Distillate

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ABSTRACT: The isolation of tocopherols and sterols together as a concentrate from sunflower oil deodorizer distillate was investigated. The sunflower oil deodorizer distillate was composed of 24.9% unsaponifiable matter with 4.8% tocopherols and 9.7% sterols, 28.8% free fatty acid (FFA) and 46.3% neutral glycerides. The isolation technology included process steps such as biohydrolysis, bioesterification and fractional distillation. The neutral glycerides of the deodorizer distillates were hydrolyzed by *Candida cylindracea* lipase. The total fatty acids (initial FFA plus FFA from neutral glycerides) were converted into butyl esters with *Mucor miehei* lipase. The esterified product was then fractionally distilled in a Claisen-vigreux flask. The first fraction, which was collected at 180–230°C at 1.00 mm of Hg for 45 min, contained mainly butyl esters, hydrocarbons, oxidized products and some amount of free fatty acids. The fraction collected at 230–260°C at 1.00 mm Hg for 15 min was rich in tocopherols (about 30%) and sterols (about 36%). The overall recovery of tocopherols and sterols after hydrolysis, esterification and distillation were around 70% and 42%, respectively, of the original content in sunflower oil deodorizer distillate.
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KEY WORDS: *Candida cylindracea* lipase, deodorizer distillate, *Mucor miehei* lipase.

Sunflower oil deodorizer distillate is an important refining by-product of the fatty materials. It contains about 9% tocopherols, mainly α-tocopherol, and 18% sterols, and the rest is hydrocarbons, fatty acids, flavor components, and neutral glycerides (1). The recovery of tocopherols, sterols, and other components is important from a commercial point of view for making value-added products.

Tocopherol, which is physiologically active as vitamin E, is a major natural antioxidant and is used for protection of fats and oils from atmospheric oxidation. Sterols are used as starting materials for the synthesis of steroids for pharmaceutical purposes.

Some technical information is available pertaining to the recovery of constituents, particularly tocopherols and sterols. Solvent extraction, chemical treatment, and molecular distillation were employed for recovery of tocopherols and sterols from soybean oil scum by Kim and Rhee (2), but the yield was poor. Due to the similar volatility of sterols, tocopherols, and fatty acids, it is quite difficult to separate sterols and tocopherols from fatty acids during fractional distillation and steam-stripping at high vacuum. Separation of sterols and tocopherols has also been tried by Sheabar and Neeman (3) with a combination of solvent extraction and chemical treatment. Supercritical fluid extraction technology is promising, and attempts have been made to isolate tocopherols from soya sludge (4) by this process. Recently, Ramamurthi and McCurdy (5) have tried to concentrate sterols and tocopherols from canola deodorizer distillate (which contained initially 1.00% w/w tocopherols and 1.58% w/w sterols) and soybean deodorizer distillate (initially containing tocopherols at 12.74% w/w and sterols at 11.39% w/w) by an enzymatic process. They converted the fatty acids into methyl esters by random SP 382 lipase action, whereupon sterols and tocopherols were concentrated in the residue fraction by distilling off the methyl esters and other volatile fractions. The conversion of fatty acids into methyl esters was 96%, while a small amount of fatty acids was left in the residue. From soybean deodorizer distillate, they concentrated sterols 1.5 times (17.56% w/w) and tocopherols 1.7 times (21.48% w/w) over the originally present levels in the deodorizer distillate.

There appears to be a need for development of processes that are more suitable in terms of conversion and separation of various constituents. Microbial lipase-catalyzed reactions, such as hydrolysis, alcoholysis, and esterification with specific or nonspecific lipases or their appropriate combinations, can be utilized in the production of sterols, tocopherols, fatty acids, and fatty acid esters.

The present study aims at the recovery of tocopherols and sterols and also of fatty acids and fatty acid esters from the sunflower oil deodorizer distillate by a combination of lipase-catalyzed hydrolysis and esterification reactions, followed by fractional distillation of the derived ester product.

MATERIALS AND METHODS

Sunflower oil deodorizer distillate (deodorized conditions: temperature *ca.* 225°C; 6 mm Hg pressure; 4 kg/t stripping steam) was provided by ITC (Agro-tech.) Ltd. (Hyderabad, India). The immobilized lipase *Mucor miehei* was a gift of NOVO Industri A/S (Copenhagen, Denmark), and *Candida*

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cylindracea lipase was purchased from Sigma Chemical Co. (St. Louis, MO), while *n*-butanol was purchased from E. Merck India Ltd. (Bombay, India).

Hydrolysis of sunflower oil deodorizer distillate by Candida cylindracea *lipase.* Sunflower oil deodorizer distillate contained 28.8% free fatty acids (FFA), 46.3% neutral glycerides, and 24.9% unsaponifiable matter. To hydrolyze the neutral glycerides, 100 g deodorizer distillate was taken in a 250-mL conical flask, stirred by a magnetic stirrer at 35 ± 2°C with 27.8 mL water (60% on the weight of neutral glycerides present in the deodorizer distillate) and 0.4 g *C. cylindracea* lipase powder. The hydrolyzed fatty acid was collected periodically after breaking the emulsion, which was formed due to stirring, by heating the reaction mixture at 80°C and centrifuging out the water layer, which contained enzyme and glycerol. The FFA content in the hydrolyzed products was determined by a standard method (6). After complete hydrolysis, the oily layer (hydrolyzed deodorizer distillate), consisting predominantly of fatty acids and unsaponifiable matter, was collected for esterification. To prevent oxidation of tocopherols, the reaction was carried out in the dark and under nitrogen.

Esterification reaction of hydrolyzed deodorizer distillate with butanol. The hydrolyzed deodorizer distillate (100 g) was reacted with *n*-butanol (28 g) (1:1.5 mole on the basis of FFA present for 5 h) in a 250-mL round-bottom flask with continuous stirring by a magnetic stirrer bar at 60 ± 2°C in the presence of *M. miehei* lipase (contained 2% w/w water) at 10% level (w/w) on the weight of total fatty acids and butanol. The reaction was carried out in the dark and in nitrogen atmosphere. The reaction course was followed by determining the acid value of the product as a function of time. After completion of the reaction, the product mixture was separated from the enzyme by filtration. Excess butanol was removed by vacuum distillation.

Distillation of the ester fraction. The ester fraction, obtained from the deodorizer distillate sample by the combination of enzymatic hydrolysis and esterification reactions, was fractionally distilled in a Claisen-vigreux flask without any stripping medium. The fraction from 180–230°C at 1.0 mm of Hg was collected for 45 min as the first fraction. The distillation was continued at 1 mm Hg at 230–260°C, and the second fraction was distilled and collected for 15 min by changing the collecting flask. The residue in the distillation flask was cooled to room temperature before releasing the vacuum. The feed, residue, and two fractions were weighed and analyzed for FFA, tocopherol, and sterol content according to the methods described below.

Determination of acid value. Acid value and FFA was determined by standard method (6).

Determination of unsaponifiable matter. Unsaponifiable matter was determined by following standard procedures (7).

Fatty acid analysis. The FFA and total fatty acid (FFA + equivalent fatty acid from neutral glycerides and sterol esters) compositions of deodorizer distillate were determined by following standard procedures (8).

Analysis of tocopherols and sterols. Tocopherols and sterols were analyzed according to AOCS recommended practice (9) with hexadecyl palmitate as the internal standard and d-α-tocopherol as the primary standard. A glass column of size 6′ × 1/8″ i.d., packed with 3% OV-1, was used for analysis. The flow rate of the carrier gas nitrogen was 32 mL/min. The oven was programmed from 250–300°C at a rate of 5°C/min with a holding time at 250°C for 14 min. The injector and detector block temperatures were maintained at 320 and 330°C, respectively.

Tocopherol is expressed as a total tocopherol content (sum of α, β, γ, and δ tocopherols), although sunflower oil is rich in α-tocopherol, and the sterols as a sum of total sterols. The amounts of hydrocarbons and other components were estimated by difference of total sterols and tocopherols from unsaponifiable matter.

RESULTS AND DISCUSSION

Table 1 lists the characteristics of the sunflower oil deodorizer distillate sample. It contains about 28% FFA, 46% neutral glycerides, and 25% unsaponifiable matter. The analysis of unsaponifiable matter shows the occurrence of 4.8% tocopherols and 9.7% sterols as its two important components. The tocopherol and sterol content in the sunflower deodorizer distillate is much less than that of the reported value (1). This may be due to different conditions of deodorization.

The fatty acid compositions of the FFA fraction and of the total fatty acids of the deodorizer distillate were identical (Table 2).

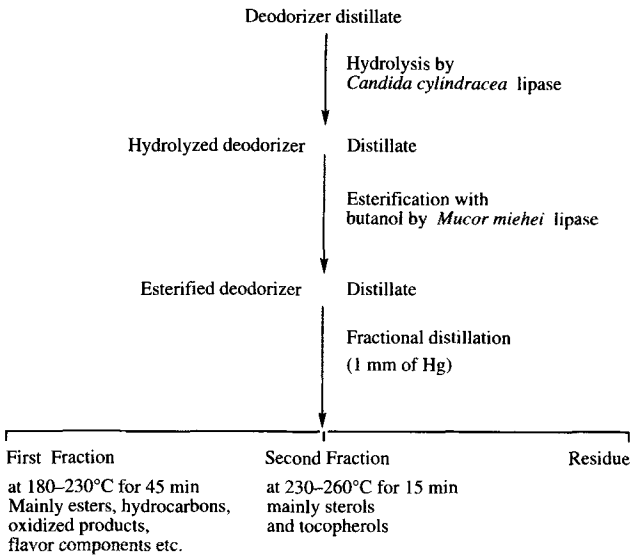
To get the maximum conversion of neutral glycerides to esters, the deodorizer distillate fatty material was first hydrolyzed by *C. cylindracea* lipase (Scheme 1). The hydrolyzed deodorizer distillate was then converted into butyl esters with the help of *M. miehei* lipase. The esterified de-

TABLE 1
Characteristics and Composition of Sunflower Oil Deodorizer Distillate

Characteristics and compositions	Sunflower deodorizer distillate
Moisture (% w/w)	0.15 ± 0.01
Free fatty acid (% w/w) (as oleic)	28.8 ± 0.03
Saponification value	138.1 ± 0.36
Neutral glycerides (% w/w)	46.3 ± 0.25
Unsaponifiable matter (% w/w)	24.9 ± 0.32
Total tocopherol (% w/w)	4.8 ± 0.04
Total sterols (% w/w)	9.72 ± 0.03

TABLE 2
Fatty Acid Composition (% w/w) of the Free Fatty Acid Part and the Total Fatty Acids of Sunflower Oil Deodorizer Distillate

	Composition of fatty acids (% w/w)				
	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Free fatty acids	13.8	3.2	38.5	44.0	0.5
Total fatty acids	14.7	4.3	35.7	44.5	0.8



SCHEME 1

odorizer distillate was then fractionally distilled. The fraction distilled over at 180–230°C, and 1.0 mm of Hg for 45 min was collected as the first fraction, and the second fraction was obtained and collected for 15 min at 230–260°C at the same pressure. The first fraction was mainly butyl esters, along with hydrocarbons, oxidized products, flavor components, etc. The second distillate was rich in tocopherols and sterols.

The rate of hydrolysis with time, as depected in Figure 1, was high. Within 3 h, as much as 92% hydrolysis was achieved. Afterward, the rate slowed down and reached an equilibrium in 24 h, attaining more or less complete hydrolysis.

The esterification reaction with butanol was also fast (Fig. 2) in the presence of immobilized *M. miehei* lipase in a solvent-free system. The esterification reaction with methanol was also tried with *M. miehei* lipase, but the conversion was poor (about 5.6%), supporting published information (10), and there was invariably a much higher amount of fatty acids in the reaction mixture. Ramamurthi and McCurdy (5) esterified the deodorizer distillate with methanol with a nonspecific SP 382 lipase, and about 4–10% fatty acids were retained in the reaction mixture.

The yield of hydrolyzed deodorizer distillate was about 6% less (Table 3), due to the loss of glycerol by hydrolysis of the neutral glycerides. The yield of esterified deodorizer distillate was about 14% higher, obviously due to an increase of molecular weight of the ester formed. Most of the butyl esters, fatty acids, hydrocarbons, and oxidized products were recovered in the first fraction, with a negligible content of sterol and tocopherol. The yield of the fraction was 80% on the weight of feed. The second distillate fraction (11% yield) was composed of mainly sterols (about 36%) and tocopherols (about 30%), along with some fatty acids, alcohol esters, and the hydrocarbons. The residue (10% yield) contained mostly

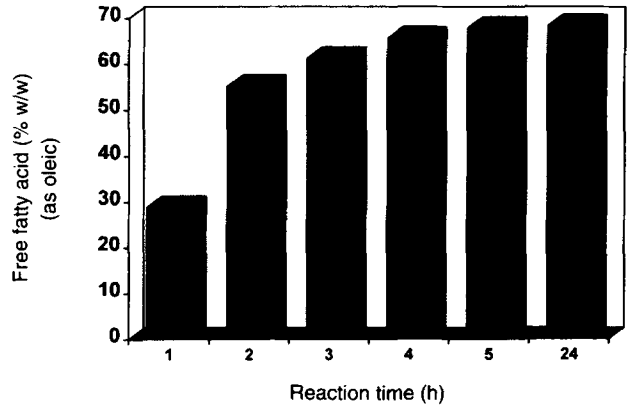


FIG. 1. Free fatty acids vs. time of hydrolysis of sunflower oil deodorizer distillate with *Candida cylindracea* lipase at 35 ± 2°C.

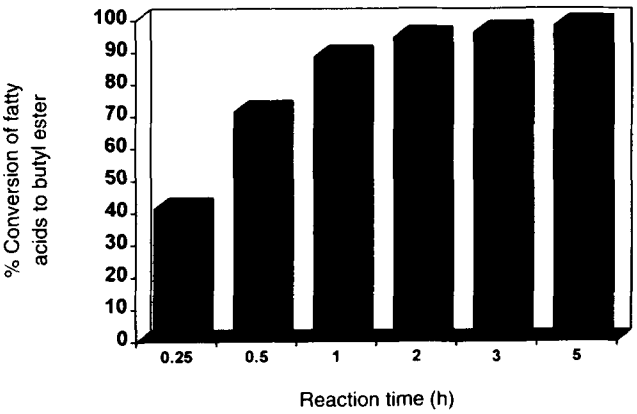


FIG. 2. Percentage conversion vs. time of esterification reaction of hydrolyzed deodorizer distillate with butanol in the presence of *Mucor miehei* lipase (10% w/w on total reactants); temperature, 60°C.

sterols and other unidentified components, along with a small amount of tocopherols.

About 9% tocopherols and 4% sterols were lost in the hydrolysis and esterification steps (Table 4). This loss of tocopherol was due to oxidation with the head-space air of the reaction vessel. Esterified deodorizer distillate was the distillation feed, and during distillation, the loss of tocopherol was about 10%. This loss of tocopherol was also due to oxidation with dissolved oxygen at high temperature. This type of oxidation loss during esterification and distillation was reported by Ramamurthi and McCurdy (5).

About 72% tocopherol and 42% sterols could be concentrated in the second distillate (Table 4). It appears that, in the second distillate, the tocopherols are concentrated nearly 6 times (30.1%) and sterols nearly 3.7 times (36.4%) over those present in original sunflower oil deodorizer distillate (tocopherol 4.8% and sterols 9.72%). The overall recoveries of tocopherols and sterols were 70 and 42%, respectively, on the basis of sunflower oil deodorizer distillate.

TABLE 3
Material Balance at Different Steps of Sunflower Oil Deodorizer Distillate Processing[a]

Components (% w/w)	Deodorizer distillate (original)	Hydrolyzed deodorizer distillate	Esterified deodorizer distillate	Fractions after distillation (% w/w) of: Esterified First fraction	Deodorizer Second fraction	Distillate Residue fraction
Yield	—	94.3 ± .25	114.3 ± .55	79.0 ± .45	11.2 ± .85	9.8 ± .36
Free fatty acids	28.8 ± .03	68.7 ± .56	1.4 ± .02	1.3 ± .04	1.0 ± .15	—
Butyl esters	—	—	76.2 ± 1.2	87.9 ± 1.5	23.8 ± .56	—
Unsaponifiable matter	24.9 ± .32	ND	21.7 ± 1.1	7.2 ± .23	75.2 ± .35	84.6 ± 1.2
Tocopherols	4.8 ± .04	ND	4.1 ± .03	trace	30.1 ± .21	3.0 ± .12
Sterols	9.72 ± .03	ND	8.5 ± .34	trace	36.4 ± .25	41.8 ± .03
Others (unidentified compounds)	10.38 ± .51	ND	9.1 ± 1.0	7.2 ± .23	8.7 ± .07	55.2 ± .10
Neutral glycerides	46.3 ± .35	trace	trace	—	—	—

[a]ND = Not determined. Temperature: 180–230°C (first fraction); 230–260°C (second fraction). Pressure: 1.0 mm of Hg.

TABLE 4
Percentage Recovery of Sterols and Tocopherols at Different Steps of Processing

Components	% Recovery[a] esterified deodorizer distillate	% Recovery[b] in the distillation stages of the esterified product: First fraction	Second fraction	Residue fraction	% Overall recovery[c] in second distillate
Tocopherol	91.45 ± .02	trace	71.9 ± .24	6.2 ± .10	70.2 ± .31
Sterol	94.95 ± .36	trace	42.0 ± .27	42.2 ± .04	41.9 ± .21

[a]% Recovery (esterification) = amount in esterified deodorizer distillate amount in deodorizer distillate × 100.
[b]% Recovery (distillation) = amount of component in fraction amount of component in feed × 100.
[c]% Overall recovery (second fraction) = amount of component in second distillate amount of component in deodorizer distillate × 100.

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