Influence of the Vegetable Oil Refining Process on Free and Esterified Sterols

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ABSTRACT: The influence of the refining process on the distribution of free and esterified phytosterols in corn, palm, and soybean oil was studied. Water degumming did not affect the phytosterol content or its composition. A slight increase in the content of free sterols was observed during acid degumming and bleaching due to acid-catalyzed hydrolysis of steryl esters. A significant reduction in the content of total sterols during neutralization was observed, which was attributed to a reduction in the free sterol fraction. Free sterols probably form micelles with soaps and are transferred into the soapstock. The steryl ester content remained constant during all neutralization experiments, indicating that hydrolysis of steryl esters did not take place during neutralization. During deodorization, free sterols are distilled from the oil, resulting in a gradual reduction in the total sterol content as a function of the deodorization temperature (220–260°C). A considerable increase in the steryl ester fraction was found during physical refining, probably owing to a heat-promoted esterification reaction between free sterols and FA.

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KEY WORDS: Chemical neutralization, deodorization, esterified sterols, free sterols, refining, vegetable oils.

The influence of the vegetable oil refining process on minor components has been studied extensively (1–4). Several minor components (e.g., phospholipids, FFA, and coloring pigments) need to be removed during the vegetable oil refining process. Phytosterols are partly removed during refining, and the magnitude of phytosterol loss largely depends on the refining conditions applied. Several factors might contribute to phytosterol loss, including adsorption, partitioning, oxidation (5), and dehydration (6,7). Nevertheless, the relative proportion of individual phytosterols is not altered during the refining process (7–9).

Generally, complete refining of vegetable oils results in a variable phytosterol loss ranging between 10 and 70% (10). Although studies present data on sterol glucosides and acetylated sterol glucosides, it is generally accepted that water degumming effectively removes sterol glucosides to a nondetectable level (10,11). During neutralization, a large part of the phytosterols (9–21%) are transferred by liquid–liquid partitioning to the soapstock (12–16). The unsaponifiable fraction of the soapstock contains on average 70% phytosterols (12). Although bleaching hardly affects phytosterols (17), some nonpolar dehydration products may be formed (6,7,10) and hydrolysis of steryl esters has been observed (17). During deodorization, a significant reduction in the total sterol content has been reported, mainly due to distillation (12,16,18). Free sterols in particular are distilled during the deodorization process. At the same time, an increase in the steryl ester content has been observed after deodorization (17). The deodorizer distillate is a good source of free phytosterols, as phytosterols are present in a concentration ranging from 2 to 20% (12,17,19).

Data from the literature on the influence of the vegetable oil refining process on esterified and free sterols is both scarce and incomplete. In this study, the influence of chemical and physical refining of corn, palm, and soybean oil on the distribution of free and esterified phytosterols is reported. Finally, the effects of process parameters during neutralization and deodorization on esterified and free sterols were studied in detail.

MATERIALS AND METHODS

Materials. Analytical-grade solvents were purchased from Merck (Darmstadt, Germany). All analytical-grade reference substances, cholesteryl stearate (5-cholesten-3β-yl-octadecanoate), and betulin [lup-20(29)-ene-3β,28-diol] were purchased from Sigma Chemical Company (St. Louis, MO) and were at least 96% pure. All chemicals and reagents were of analytical grade and were used without further purification. Crude vegetable oils (corn, palm, and soybean oils) and neutralized, bleached soybean oil were obtained from Extraction De Smet (Edegem, Belgium). All oil samples were refrigerated at 4°C until analyzed.

Analytical procedures. Total sterols and the contents of free and esterified sterols were analyzed according to our method described previously (20). Tocopherols were analyzed by normal-phase HPLC (21). The FFA content was determined by titration according to AOCS Official Method Ca 5a-40 (22) and was expressed as oleic acid ($C_{18:1}$) for corn and soybean oil and as palmitic acid ($C_{16:0}$) for palm oil. The phosphorus and iron contents were analyzed by inductive coupled plasma according to AOCS Method Ca 20-99 (22).

Refining procedure. Vegetable oils were refined on a lab scale either by chemical refining (including water degumming, chemical neutralization, bleaching, and deodorization) or physical refining (acid degumming, bleaching, and deodorization).

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Water degumming. Crude oil (800 g) was heated to 70°C under gentle agitation (175 rpm). Water (3% w/w) was mixed with the oil at 250 rpm for 30 min. After centrifuging (10 min at 1700 × *g*), the supernatant oil layer was decanted.

Chemical neutralization. Water-degummed oil (600 g) was heated under gentle agitation to 55°C. A calculated amount of NaOH (10%) in relation to the FFA level, as calculated by Hodgson (2), with 10% excess was added under agitation at 250 rpm, followed by continued agitation at 175 rpm for 40 min. The oil was heated to 75°C and allowed to rest for 15 min without mixing to break the emulsion. After centrifuging for 10 min at 1700 × *g*, the oil phase was collected and washed twice with water (10% w/w) under gentle mixing at 175 rpm.

Acid degumming. Crude oil (600 g) was heated to 60°C, and 0.15% (w/w) of a citric acid solution (30% w/w) was added under high-shear mixing (UltraTurrax homogenizer at 10,000 rpm for 1 min; IKA-Werke GmbH, Staufen, Germany). After adding 0.05% (w/w) NaOH solution (20% w/w), and 2% (w/w) water, the oil was mixed for another minute at 10,000 rpm and allowed to mature for 60 min at 40°C, followed by centrifuging for 10 min at 1700 × *g*. Oil and phospholipids were separated by decantation.

Bleaching. The degummed oil was preheated to 95°C under reduced pressure (50 mbar) in a rotary evaporator (Buchi, Flawil, Germany). After addition of bleaching earth (1% w/w, Tonsil 210 Optimum; Südchemie, Germany), the oil was mixed at 85 rpm for 30 min followed by filtering through a Büchner filter.

Deodorization. Lab-scale deodorization was performed with specially designed glassware installed in an oven. Deodorization was carried out on 200 g of vegetable oil weighed into a 1-L flask. A glass piece was mounted vertically onto the flask, which included a steam injection unit and a tube in which to insert a thermocouple. A peristaltic pump connected to a buret filled with boiled water regulated the water flow and consequently the steam flow. The pumped water was converted into steam before injecting it into the oil by preheating in the oven. Vapors left the oven through a horizontal glass piece connected to a condenser and an electronic manometer. The pressure of the deodorizer was regulated by a microvalve. Before reaching the vacuum pump, vapors were rapidly cooled in a countercurrent spiral condenser set at 0°C followed by a cold trap cooled with liquid nitrogen. Physical and conventional deodorization were carried out at a temperature of 240°C and a pressure of 2 mbar, with injection of 1.5 and 1% steam, respectively.

RESULTS

Overview of chemical and physical refining. A general evaluation of the vegetable oil refining process, including data on

TABLE 1
Overview of the Tocopherol, Phosphorus (P), Iron (Fe), and FFA Contents[a] in Corn, Palm, and Soybean Oil During Chemical and Physical Refining

	Tocopherols (ppm)				P	Fe	FFA
	α	γ	δ	Total	(ppm)	(ppm)	(%)
Crude soybean oil	179.3	636.8	330.3	1146.4	327.8	4.9	2.3
Physical							
Degummed	158.1	607.9	323.8	1089.8	5.7	0.9	2.31
Bleached	132.9	585.1	320.0	1038.0	1.7	<0.1	2.26
Deodorized	111.2	429.5	189.5	730.2	0.2	<0.1	0.11
Chemical							
Degummed	157.9	601.8	316.3	1076.0	33.2	3.13	2.3
Neutralized	153.3	602.7	312.5	1068.5	0.8	<0.1	0.18
Bleached	94.2	504.4	293.3	891.9	0.3	<0.1	0.1
Deodorized	58.1	353.1	167.0	578.2	0.3	<0.1	0.08
Crude corn oil	265.7	1008.8	54.1	1328.6	109.7	0.9	1.3
Physical							
Degummed	261.4	968.4	51.6	1281.4	1.24	<0.1	1.26
Bleached	261.5	963.4	50.2	1275.1	0.3	<0.1	1.2
Deodorized	141.5	506.0	23.2	670.7	0.3	<0.1	0.08
Chemical							
Degummed	218.6	842.0	47.3	1107.9	7.5	0.16	0.31
Neutralized	217.3	810.8	45.2	1073.3	0.6	<0.1	0.16
Bleached	198.4	798.4	41.9	1038.7	0.1	<0.1	0.14
Deodorized	82.6	458.2	19.4	560.2	0.1	<0.1	0.09
Crude palm oil	Mv[b]	Mv	Mv		19.1	4.9	3.45
Physical							
Degummed	Mv	Mv	Mv		5.7	<0.1	3.41
Bleached	Mv	Mv	Mv		0.7	<0.1	3.28
Deodorized	Mv	Mv	Mv		0.5	<0.1	0.10

[a] Expressed as oleic acid for corn and soybean oil and palmitic acid for palm oil.
[b] Mv, missing value.

the residual FFA, phosphorus, and tocopherol contents, is given in Table 1. Acid degumming and chemical neutralization removed phospholipids and iron to acceptably low levels. In the early stages of the refining process, tocopherols were only slightly affected. On the other hand, the tocopherol content was drastically reduced during deodorization due to distillation. In chemical refining, the neutralization process effectively removed FFA as sodium soaps, whereas in physical refining, FFA were distilled during deodorization. These observations are in accordance with data published earlier (7,12,14,18,23).

The effects of chemical and physical refining on the total phytosterol content and on the ratio of free and esterified sterols in soybean oil were studied in detail. As illustrated in Table 2, a good relationship was obtained between the free and esterified sterol contents and the total sterol content. Qualitative observations of the distribution of phytosterols in corn and soybean oil are in accordance with Reference 20. In both chemical and physical refining, the total phytosterol content decreased. During refining no variation in the relative distribution of the individual phytosterols was observed, which is in accordance with data published earlier (7,9).

Physical and chemical refining of soybean oil. In physical refining of soybean oil, degumming and bleaching hardly affected the total phytosterol content. After bleaching, a slight reduction in the esterified sterol content (from 59.0 to 46.0 mg/100 g) and a slight increase in the free sterol content were observed. This reduction might be explained by acid-catalyzed hydrolysis of the steryl esters on the acid-activated bleaching earth (16,17). The phytosterol fraction was strongly affected by the deodorization process. In the physically refined oil, an increase in the steryl ester fraction from 46.0 to 96.7 mg/100 g was observed owing to a sterol esterification reaction promoted by the deodorization temperature and a reduction in the free sterol content caused by distillation. These observations are in accordance with data from the literature (17).

In chemical refining of soybean oil, a considerable reduction in the total sterol content from 322 to 257 mg/100 g or 20% was observed during neutralization. The reduced phytosterol content was attributed to reduced free sterol content (from 251 to 184 mg/100 g), as the steryl ester fraction remained constant. This decrease was probably due to a loss of free phytosterol in the soapstock (12,13,16). The effects of different neutralization parameters on the esterified and free sterol fractions are described in detail later in this paper. Unlike in physical refining, deodorization in chemical refining did not result in the formation of steryl esters. The low level of FFA in the oil prior to deodorization made esterification a less probable outcome. For this reason, the reduction in the total sterol content was attributed to the reduced free sterol fraction brought about by distillation (a decrease from 90.1 to 73.9 mg/100 g).

During industrial processing of oils and fats, phytosterols undergo dehydration resulting in the formation of steradienes (6,7). Steradienes are formed during bleaching due to acid-catalyzed dehydration on the surface of the acid-activated bleaching earth or to dehydration promoted by heat during the deodorization process (7).

Refining of corn and palm oil. The influence of the refining process on corn oil phytosterols (Table 3) was very similar to that of soybean oil. Corn oil is rich in phytosterols (976 mg/100 g), with the major part being esterified (55.5%) (Table 3). Acid degumming and bleaching both resulted in reduced esterified phytosterol contents of 9.09 and 4.08%, respectively, probably due to acid-catalyzed hydrolysis. After deodorization (physical refining), an increase in the steryl ester content was observed (from 460 to 491 mg/100 g), whereas the content of free phytosterols was reduced by 42.3%. In chemical refining, neutralization greatly reduced the total sterol content (from 930 to 869 mg/100 g). This reduction was attributed to a loss in the free sterol content (from 485 to 390 mg/100 g) since no hydrolysis of steryl esters was detected. Similar to soybean oil, no significant formation of steryl esters could be detected during deodorization.

Palm oil can contain a high content of FFA and is usually physically refined. Results showing the phytosterol distribution during physical refining are listed in Table 4. Acid degumming and bleaching hardly affected the ratio of esterified/free sterols. As in the physical refining of corn and

TABLE 2
Influence of Physical and Chemical Refining on Esterified and Free Sterols in Soybean Oil

	Esterified/free sterol analysis (mg/100 g)											Total sterol analysis (mg/100 g)				
	Esterified sterols[a]					Free sterols					E + F total[b]	Total sterols				
	Camp	Stigm	Sito	Δ5-Ave	Total	Camp	Stigm	Sito	Δ5-Ave	Total		Camp	Stigm	Sito	Δ5-Ave	Total
Crude soybean oil	5.6	4.7	40.1	8.6	59.0	62.7	54.9	137.1	—	254.7	313.7	71.0	61.4	183.7	10.5	326.6
Physical																
Degummed	5.7	4.7	40.6	8.5	59.5	65.9	57.7	143.4	—	267.0	326.5	77.5	64.2	192.2	12.1	346.0
Bleached	4.3	3.9	31.6	6.2	46.0	63.0	55.1	142.3	—	260.4	306.4	74.6	59.9	183.4	11.5	329.4
Deodorized	12.6	12.3	63.0	8.8	96.7	48.6	43.2	112.5	—	204.3	301.0	60.6	51.1	162.7	10.9	285.3
Chemical																
Degummed	5.9	4.7	42.5	8.8	61.9	62.1	54.0	134.8	—	250.9	312.8	71.1	58.8	180.4	11.4	321.7
Neutralized	6.0	4.7	43.2	9.0	62.9	45.5	39.5	98.8	—	183.8	246.7	54.4	44.7	146.0	12.2	257.3
Bleached	6.4	4.4	42.8	8.3	61.9	35.9	35.4	90.1	—	161.4	223.3	55.0	44.8	147.9	11.5	259.2
Deodorized	5.7	5.2	45.8	8.7	65.4	28.2	28.7	73.9	—	130.8	196.2	41.1	35.5	123.9	15.4	215.9

[a] Camp, campesterol; Stigm, stigmasterol; Sito, sitosterol; Δ5-Ave, Δ5-avenasterol; Total, sum of a–d.
[b] Sum of free and esterified sterol contents.

TABLE 3
Influence of Physical and Chemical Refining on Esterified and Free Sterols in Corn Oil

	Esterified/free sterol analysis (mg/100 g)											Total sterol analysis (mg/100 g)				
	Esterified sterols[a]					Free sterols					E + F total[b]	Total sterols				
	Camp	Stigm	Sito	Δ5-Ave	Total	Camp	Stigm	Sito	Δ5-Ave	Total		Camp	Stigm	Sito	Δ5-Ave	Total
Crude corn oil	103.2	41.9	390.7	12.1	547.9	108.9	29.6	300.3	—	438.8	986.7	224.2	68.6	671.9	10.9	975.6
Physical																
Degummed	87.7	36.1	345.6	10.5	479.9	109.4	30.3	308.6	—	448.3	928.2	212.8	66.2	619.4	11.6	910.0
Bleached	84.6	34.7	329.1	11.9	460.3	110.9	32.0	317.7	—	460.6	920.9	191.9	65.7	629.3	11.1	898.0
Deodorized	104.6	34.5	344.9	7.5	491.4	56.0	17.8	183.3	—	257.1	748.5	149.7	53.8	528.5	10.9	742.9
Chemical																
Degummed	86.0	34.3	327.1	13.2	460.7	118.7	34.6	331.4	—	484.7	945.4	198.4	66.9	653.0	11.4	929.5
Neutralized	87.7	35.3	339.0	10.9	473.0	92.9	27.0	269.9	—	389.8	862.8	182.4	63.6	611.1	12.2	869.3
Bleached	85.2	41.1	309.8	11.1	447.2	90.8	27.3	269.6	—	387.7	834.9	177.0	58.3	598.0	11.7	845.0
Deodorized	77.3	39.5	329.1		455.1	70.8	22.6	242.1	—	335.5	790.6	164.3	54.9	563.1	10.7	793

[a] For abbreviations see Table 2.
[b] Sum of free and esterified sterol contents.

soybean oils, a significant increase in the ratio of esterified/free sterols was observed after deodorization. The steryl ester fraction increased from 17.3 to 27.6 mg/100 g, which represented an increase of 60%.

Influence of chemical neutralization on esterified and free sterols. A significant reduction in the total sterol content has been reported during the chemical neutralization process (12–16), which is attributed to a liquid–liquid partitioning of phytosterols into the soapstock. No data on the influence of neutralization parameters on the phytosterol fraction are available. In this research, the influence of two main neutralization parameters, strength of the NaOH solution added and the degree of excess, were studied in detail. Neutralization temperature and mixing parameters were not included in the study as they are believed to have only a minor influence. The strength of the caustic solution (% NaOH) added ranged between 2.5 and 15%. The excess NaOH, calculated by the level of FFA to be neutralized, varied between 0 and 40%. Although some experimental conditions performed in this study are not realistic for industrial application, they were nevertheless selected in order to elucidate the influence of these neutralization process parameters on the phytosterol fraction in detail.

A significant reduction in the total sterol content of soybean oil was observed during the neutralization process (Table 5). The reduced sterol content was attributed to a reduction in the free sterol fraction (from 288 mg/100 g to 206–286 mg/100 g). It should be stressed that the esterified sterol content was not altered during chemical neutralization (60.7 ± 1.3 mg/100 g).

The influence of the strength of the NaOH solution on free sterol loss upon addition of stoichiometric amounts of NaOH is represented in Figure 1. Similar graphs (not shown here) were obtained for different levels of excess. A gradual reduction in the loss of free sterols as a function of weaker caustic solutions was observed. Neutralization with 2.5% NaOH solution reduced the free phytosterol fraction by about 25%. Neutralization with stronger NaOH solutions (7.5–15%) gradually reduced the loss of the free sterol fraction from 6.4 to 3.2%. As free sterols are very weak bases, the hydroxyl function cannot be alkoxylated. Nevertheless, free sterols have been identified in the soapstock fraction (24). The free sterols probably formed micelles with the soap and were transferred into the soapstock. Those micelles built of soaps/water/free sterols might have formed only upon addition of a weak base (2.5–5% NaOH), whereas they would not have formed upon addition of a stronger base (>7.5% NaOH), consequently leaving all free sterols in the oil.

Addition of excess NaOH had almost no influence on the free and esterified sterol fraction. The steryl ester content remained constant during all neutralization reactions, indicating that no caustic-catalyzed hydrolysis had taken place. Steryl esters were probably not hydrolyzed because of steric

TABLE 4
Influence of Physical Refining on Esterified and Free Sterols in Palm Oil

	Esterified/free sterol analysis (mg/100 g)											Total sterol analysis (mg/100 g)				
	Esterified sterols[a]					Free sterols					E + F total[b]	Total sterols				
	Camp	Stigm	Sito	Δ5-Ave	Total	Camp	Stigm	Sito	Δ5-Ave	Total		Camp	Stigm	Sito	Δ5-Ave	Total
Crude palm oil	3.7	1.8	9.7	1.1	16.3	9.8	6.9	32.5	—	49.2	65.4	14.9	9.1	41.8	1.6	67.4
Physical																
Degummed	4.2	1.8	9.8	1.8	17.6	9.5	7.6	33.8	—	50.9	68.5	16.3	9.3	40	3	68.5
Bleached	4.2	1.9	10	1.2	17.3	9.8	5.9	27.4	—	43.1	60.4	15.2	8.8	39.8	2.6	66.4
Deodorized	6.0	3.2	16.8	1.6	27.6	6.3	4.1	18.5	—	28.9	57.1	13.7	7.1	36.5	2.6	59.9

[a] For abbreviations see Table 2.
[b] Sum of free and esterified sterol contents.

TABLE 5
Influence of Chemical Neutralization on Free and Esterified Sterols in Soybean Oil

		Esterified/free sterol analysis									Total sterol analysis			
		Esterified sterols (mg/100 g)[c]				Free sterols (mg/100 g)					Total sterols (mg/100 g)			
NaOH[a] (%)	Excess[b] (%)	Camp	Stigm	Sito	Total	Camp	Stigm	Sito	Total	E + F total[d]	Camp	Stigm	Sito	Total
Degummed soybean		7.3	5.9	48.2	61.5	67.5	68.2	152.7	288.4	349.9	74.3	73.5	200.1	347.9
2.5	0	6.6	5.4	47.4	59.4	46.0	51.8	118.4	216.2	275.6	57.2	56.1	163.4	276.7
2.5	10	6.6	4.1	46.4	57.1	44.1	51.1	123.3	218.5	275.6	54.7	54.1	155.3	264.1
2.5	20	5.6	8.1	45.8	59.6	44.4	49.4	112.1	205.9	265.5	55.3	53.8	159.3	268.4
5.0	0	7.3	5.7	48.3	61.2	59.3	60.3	135.4	255.0	316.2	63.8	63.4	178.3	305.5
5.0	10	7.0	5.5	48.6	61.1	56.1	54.4	120.8	231.3	292.4	61.2	60.5	172.8	294.5
7.5	0	7.2	6.3	46.3	59.8	59.8	60.5	149.5	269.8	329.6	71.0	70.9	193.2	335.1
7.5	20	6.8	5.6	47.6	59.9	60.4	61.0	139.7	261.1	321.0	67.5	66.4	184.8	318.7
10	0	7.6	4.9	49.1	61.6	63.0	64.7	145.5	273.2	334.8	71.2	70.5	193.9	335.6
10	10	5.8	8.2	45.5	59.5	64.2	65.5	148.5	278.2	337.7	70.7	70.2	193.4	334.3
10	20	7.8	6.8	47.3	62.0	62.2	65.7	149.0	276.9	338.9	71.5	70.4	194.5	336.4
10	40	6.9	5.6	47.9	60.4	62.3	64.1	144.6	271.0	331.4	69.7	69.0	191.1	329.8
12.5	0	7.8	5.6	48.2	61.6	63.3	64.7	149.6	277.6	339.2	73.8	73.1	200.4	347.3
12.5	20	7.6	6.5	48.5	62.6	62.0	66.1	148.9	277.0	339.6	71.8	70.4	193.2	335.4
15	0	7.5	6.4	48.2	62.1	63.4	65.4	150.2	278.9	341.0	73.3	71.9	196.7	341.9
15	20	7.4	6.1	47.8	61.3	65.6	67.6	152.9	286.1	347.4	74.1	73.4	199.8	347.3

[a] Strength (%) of the NaOH solution added.
[b] Excess (%) of NaOH added.
[c] For abbreviations see Table 2.
[d] Sum of free and esterified sterol contents.

hindrance of the sterol ring structure, making the ester function less accessible for saponification.

Influence of deodorization on esterified and free sterols. To study the influence of the deodorization process on the content of free and esterified sterols in detail, soybean oil was deodorized at temperatures ranging between 220 and 260°C. Deodorization experiments were carried out with a chemically neutralized, bleached soybean oil (FFA = 0.074%). Additional deodorization experiments were carried out with the same soybean oil to which oleic acid ($C_{18:1}$) was added to a FFA level of 1.188%. The influence of the deodorization temperature was studied, since it has a major influence on distillation and may also promote the level of sterol esterification taking place during deodorization. Other process parameters that might influence the esterification reaction (concentration of FFA, deodorization pressure, amount of stripping agent, etc.) were not included in this study. Results showing the esterified/free sterol and FFA contents are listed in Table 6.

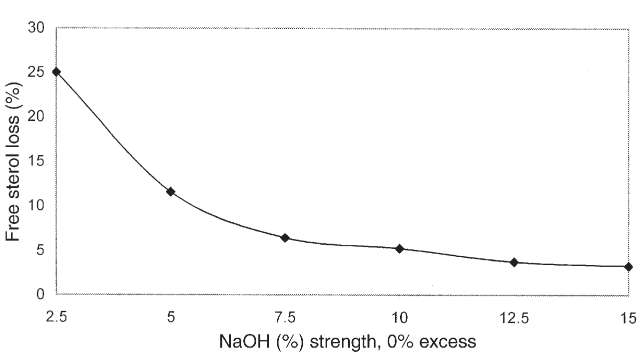

FIG. 1. Influence of NaOH strength on free sterol loss during chemical neutralization.

A gradual reduction in the total sterol content due to distillation was observed as deodorization temperature increased (16,23). Increasing the deodorization temperature from 220 to 260°C resulted in a gradual reduction of total sterol recovery from 90.4 to 67.7% in the physical refining process and from 93.0 to 62.7% in the chemical refining process. Little difference was found between chemical and physical refining in the contents of residual total sterols.

In physically refined soybean oil, an increase in the steryl ester fraction from 67.3 mg/100 g in the bleached oil to 94.4 mg/100 g in the deodorized oil (260°C) was observed, representing a relative increase of 40%. However, in chemically refined oil a slight trend toward an increase in the steryl ester fraction from 62.7 mg/100 g in the bleached oil to 67.2 mg/100 g in the deodorized oil (260°C) was found. The increase in the steryl ester content during the deodorization process might be explained by esterification, promoted by temperature, between a free sterol and a FA. As heat promoted the esterification reaction, a gradual increase in the steryl ester fraction as a function of the deodorization temperature was observed.

In physical refining, FFA are present in considerable amounts prior to deodorization and will react with free sterols to form steryl esters. However, as FFA are gradually stripped during deodorization, their concentration is constantly being reduced, and the chance for esterification with the free sterols reduces as well. FFA are scarcely present in chemically neutralized oil prior to deodorization. This explains the significantly lower formation of steryl esters during deodorization

TABLE 6
Influence of Deodorization on Free and Esterified Sterol and Residual FFA During Physical and Chemical Refining of Soybean Oil[a]

		Esterified/free sterol analysis									Total sterol analysis			
		Esterified sterols (mg/100 g)				Free sterols (mg/100 g)					Total sterols (mg/100 g)			
Temperature (°C)	FFA (%)	Camp	Stigm	Sito	Total	Camp	Stigm	Sito	Total	E + F total[b]	Camp	Stigm	Sito	Total
Physical refining														
Feed[c]	1.188	8.9	7.5	50.9	67.3	65.6	64.3	146.4	276.3	343.6	76.3	67.8	180.7	324.8
220	0.086	12.7	9.1	59.5	81.3	53.0	52.8	120.7	226.5	307.8	69.5	63.9	160.2	293.6
230	0.045	14.2	10.6	63.0	87.8	44.2	44.3	105.8	194.3	282.1	68.3	61.8	159.2	289.3
240	0.030	16.9	11.4	66.5	94.8	48.0	43.8	100.6	192.4	287.2	61.9	54.7	163.6	280.2
250	0.033	15.6	11.9	64.9	92.4	36.4	39.1	93.8	169.3	261.7	55.0	49.0	154.6	258.6
260	0.019	15.5	12.2	66.6	94.3	27.6	29.3	73.5	130.4	224.7	43.6	38.3	138.0	219.9
Chemical refining														
Feed	0.074	6.9	5.0	50.8	62.7	58.0	63.5	146.2	267.7	330.4	66.6	69.6	195.7	331.9
220	0.015	6.8	6.2	53.7	66.7	53.0	57.9	131.9	242.8	309.5	62.1	63.3	183.4	308.8
230	0.014	6.6	5.3	52.2	64.1	50.3	55.5	125.0	230.8	294.9	57.4	59.8	175.9	293.1
240	0.013	7.6	6.1	52.9	66.6	42.9	46.9	111.5	201.3	267.9	52.6	53.9	164.4	270.9
250	0.011	8.5	5.2	52.1	65.8	36.4	40.4	99.1	175.9	241.7	46.3	47.3	151.7	245.3
260	0.008	8.3	6.1	52.8	67.2	26.3	28.7	74.4	129.4	196.6	37.0	38.2	132.8	208.0

[a] Deodorization conditions: time 60 min, steam 1.5% (w/w), pressure 2 mbar.
[b] Sum of free and esterified sterol contents.
[c] Industrially neutralized bleached soybean oil to which oleic has been added.

in the chemical refining process. In addition, some interesterification between free sterols and TAG at high deodorization temperatures should not be excluded (13).

Consequently, the decrease in the total sterol content during deodorization can be totally attributed to the reduced content of free sterols (Table 6). Little difference in free sterol content was observed between chemical and physical deodorization. In both chemical and physical deodorization experiments, free sterols were distilled during the deodorization step, going from ±276 mg/100 g in the bleached oil to 130 and 129 mg/100 g in the physically and chemically deodorized oils (at 260°C), respectively. However, the physically refined oil contained slightly higher amounts of total sterols, attributed to its higher content of steryl esters.

The influence of the amount of steam injected during deodorization on the level of esterified phytosterols was not studied. Although hydrolysis of TAG during steam deodorization has been reported (25), hydrolysis of steryl esters during steam deodorization was not expected because of steric hindrance by the sterol ring structure, which made the ester function less accessible.

Phytosterols are progressively lost during refining, altering the ratio of esterified/free sterols. During chemical neutralization, the free sterol content is significantly reduced, mainly when a diluted NaOH (2.5%) solution is used. Hydrolysis of steryl esters may occur during acid degumming and bleaching. Free sterols are distilled during deodorization at elevated temperatures. At the same time, deodorization conditions induce an esterification reaction between free sterols and FFA, resulting in an increase in the content of steryl esters after deodorization.

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