Analysis of Free and Esterified Sterols in Vegetable Oils

T. Verleyen[a,*], M. Forcades[a], R. Verhe[a], K. Dewettinck[b], A. Huyghebaert[b], and W. De Greyt[c]

Departments of [a]Organic Chemistry and [b]Food Technology and Nutrition, 9000 Gent, Belgium, and [c]De Smet Engineering, Edegem, Belgium

ABSTRACT: In vegetable oils, phytosterols occur as free sterols or as steryl esters. Few analytical methods report the quantification of esterified and free sterols in vegetable oils. In this study, esterified and free sterols were separated by silica gel column chromatography upon elution with *n*-hexane/ethyl acetate (90:10 vol/vol) followed by *n*-hexane/diethyl ether/ethanol (25:25:50 by vol). Both fractions were saponified separately and the phytosterol content was quantified by GC. The analytical method for the analysis of esterified and free sterols had a relative standard deviation of 1.16% and an accuracy of 93.6–94.1%, which was comparable to the reference method for the total sterol analysis. A large variation in the content and distribution of the sterol fraction between different vegetable oils can be observed. Corn and rapeseed oils were very rich in phytosterols, which mainly occurred as steryl esters (56–60%), whereas the majority of the other vegetable oils (soybean, sunflower, palm oil, etc.) contained a much lower esterified sterol content (25–40%). No difference in the relative proportion of the individual sterols among crude and refined vegetable oils was observed.

Paper no. J10067 in *JAOCS 79,* 117–122 (February 2002).

KEY WORDS: Analysis, esterified sterols, free sterols, gas chromatography, vegetable oils.

In vegetable oils, plant sterols mainly occur as free sterols or steryl esters of FA. The biosynthesis, biological function, and nutritional importance of phytosterols has recently been reviewed (1). The conventional method for sterol analysis involves a saponification of the TAG followed by an extraction of the unsaponifiable fraction with an organic solvent in order to determine the total sterol content (1). However, important information on the phytosterol fraction is lost through saponification. The steryl ester fraction is a complex mixture consisting of a variety of phytosterols esterified to various FA. The distribution of FA esterified to sterols differed considerably from the FA in the TAG of the vegetable oil. All steryl esters contained significantly higher levels of unsaturated FA compared to the corresponding TAG composition of the oil (2,3).

Only a few literature reports are available on the combined quantification of free and esterified sterols. Methods reported so far have separated esterified and free sterols by either silica gel column chromatography, preparative TLC, or normal-phase solid-phase extraction followed by saponification and GC quantification of both fractions (2–7). The esterified/free sterol content has been published for some vegetable oils (8).

*To whom correspondence should be addressed at Faculty of Agricultural and Applied Biological Sciences, Department of Organic Chemistry, Coupure Links 653, 9000 Gent, Belgium. E-mail: tom.verleyen@rug.ac.be

Some analytical methods have reported the quantification of steryl esters without saponification of the TAG. Steryl esters, sometimes in combination with free sterols, were isolated from TAG by TLC or normal-phase LC and quantified by GC or HPLC (3,9–13). This direct analysis of the steryl ester fraction always resulted in an incomplete separation of the different steryl esters.

In summary, few analytical reports deal with a separation of the esterified/free sterol fraction, and quantitative data on the free and esterified sterol content in vegetable oils are scarce. This study reports on the optimization and evaluation of an analytical method to determine the level of free and esterified sterols in vegetable oils followed by a quantification of the free and esterified sterol content in a variety of vegetable oils.

MATERIALS AND METHODS

Analytical-grade solvents were purchased from Merck (Darmstadt, Germany). All analytical-grade reference substances, cholesterol (5-cholesten-3β-ol), stigmasterol (3β-hydroxy-24-ethyl-5,22-cholestadiene), cholesteryl stearate (5-cholesten-3β-yl-octadecanoate), 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. Technical-grade triolein and silica gel were purchased from Sigma and contained no detectable amounts of cholesterol or phytosterols.

The analysis of the free and esterifed sterol content in combination with the total sterol content was performed for several vegetable oils. Vegetable oils (crude oils, oil samples taken out of the refining process, or refined samples) were obtained from Extraction De Smet (Edegem, Belgium). All oil samples were stored in the refrigerator at 4°C until analysis.

Synthesis of sitosteryl stearate. As there are no vegetable oils that only contain either free or esterified phytosterol, optimization of the analytical method was carried out by use of a model system consisting of technical grade triolein spiked with two different sterols, cholesterol and sitosteryl stearate, respectively. Sitosterol stearate was prepared according to the following procedure.

Technical sitosterol (2 g) containing 10% stigmasterol was dissolved in dichloromethane (20 mL), and triethylamine (0.3 g) was added. Stearoylchloride (3 g) dissolved in dichloromethane (3 mL) was injected under nitrogen flow, and the solution was stirred under reflux overnight. The reaction mixture was then washed with water (2 × 25 mL), 0.5 M NaOH (3 × 25 mL), and dried over magnesium sulfate. After

evaporation of the solvent, a crystalline product was obtained. Impurities were removed by crystallization in acetone (25 mL) at 0°C. Sitosteryl stearate was obtained after silica gel (20 g) column chromatography (40 × 1 cm internal diameter) upon elution with *n*-hexane/ethylacetate (97:3 vol/vol).

NMR spectrometry was performed on a JEOL JNM-EX270 spectrophotometer (Tokyo, Japan). ^{1}H NMR and ^{13}C NMR spectra were recorded in deuterated solvents with tetramethylsilane as internal standard at 270 and 68 MHz, respectively. Sitosteryl stearate had ^{1}H NMR δ 5.30–5.37 (1H, *m*, >C=CH–), 4.55–4.67 (1H, *m*, >CH–O–CO), 2.23 (2H, *t*, *J* = 7.6 Hz, C2′), 0.67–2.03 (*m*); ^{13}C NMR δ 11.8 (C18), 11.9 (C29), 14.1 (C18′), 18.7 (C21), 19.0 (C27), 19.2 (C19), 19.8 (C26), 21.0 (C11), 22.6–29.7 (14 × C′), 22.7 (C28), 24.3 (C15), 26.0 (C23), 28.2 (C16), 29.0 (C25), 31.7 (C2), 31.8 (C7), 31.9 (C8), 32.0 (C3′), 34.1 (C2′), 34.7 (C22), 36.1 (C20), 36.5 (C10), 37.0 (C1), 39.7 (C12), 42.1 (C4), 42.3 (C13), 45.8 (C24), 50.0 (C9), 56.0 (C17), 56.6 (C14), 73.6 (C3), 122.5 (C6), 139.6 (C5), 173.2 (C1′).

Total sterol analysis. The total sterol content was analyzed according to the DGF method (14). Oil samples (5 g) were saponified with 10 M KOH (5 mL) in ethanol (45 mL), together with 5 mg betulin as internal standard to quantify losses during saponification and extraction. The solution was heated for 30 min at 70°C. After saponification, 100 mL water was added and the unsaponifiables were extracted twice with 100 mL diethyl ether. The combined diethyl ether fractions were washed with 0.5 M KOH (2 × 20 mL), water (4 × 20 mL), and dried over anhydrous sodium sulfate. The residue obtained after evaporation was derivatized by dissolving the sample in 0.5 mL of pyridine and addition of 1 mL *N,O*-bis(trimethylsilyl)trifluoracetamide containing 1% trimethylchlorosilane solution as derivatizating and silylating agent. The test tube was placed in an oven at 70°C for 20 min for completion of the silylation. Afterward, the derivatized sample was transferred into a vial and the sample was ready for injection. The derivatized samples were analyzed within 6 h of preparation. GC conditions are described below.

Separation of free and esterified sterols. The polarity difference between free and esterified sterols was utilized for optimization of their separation. Oil samples (1.5 g) were weighed into a small beaker and transferred by aid of *n*-hexane (2 × 5 mL), rinsing onto a silica gel column (15 g silica, column 2.5 cm i.d.). Elution started with 75 mL *n*-hexane/ethyl acetate (90:10 vol/vol) to collect the steryl ester fraction, followed by elution with 75 mL *n*-hexane/diethyl ether/ethanol (25:25:50 by vol) for collection of the free sterol fraction. After evaporation of the solvent, both fractions were saponified, extracted, and derivatized according to the procedure described for the total sterol analysis, using proportionately lower amounts of materials, reagents, and solvent.

For the analysis of vegetable oil samples, a quality parameter was included into the method in order to verify the perfect separation between esterified and free sterols on the silica gel column. Therefore, 5 mL of a cholesteryl stearate stock solution (50 mg of cholesteryl stearate/50 mL *n*-hexane) was added to the oil before transferring the solution onto the silica gel column.

GC conditions for sterol analysis. GC separations were performed by an HP 6890 series GC (Hewlett-Packard, Avondale, PA) using an Alltech EC5 capillary column (30 m × 0.25 mm, 0.25 μm; Alltech, Deerfield, IL) using helium as the carrier gas at a pressure of 124.8 kPa and a split ratio of 15:1. The GC operating parameters were as follows: Initial oven temperature was 290°C. After an isothermal period of 20 min, the oven temperature was increased at 10°C/min to 300°C and was held at this temperature for 10 min. Detection was done by flame ionization with the detector temperature set at 360°C. Peak identification was carried out by comparison of relative retention times of standards.

Concentration of the different sterols present in the oil was expressed as mg sterol/100 g of oil. Cholesterol was the reference sterol used for determination of the response factor.

RESULTS AND DISCUSSION

Optimization of the method. The analytical procedure described was based on the polarity difference between free and esterified sterols. Free sterols remain longer on a silica gel column upon elution with a nonpolar solvent, as free sterols are more polar than esterified sterols. The most critical point in the optimization of the method was to define a good solvent ratio in order to elute all steryl esters in a first fraction, while keeping all free sterols on the column. In the second step, the silica gel column was eluted with a polar solvent allowing the recovery of free sterols. Both fractions were saponified separately, in order to hydrolyze esterified sterols and TAG, and quantification of the free sterol level in the nonsaponifiable fraction thus obtained was done by GC.

As the ratio of free and esterified sterols in a real vegetable oil was unknown, optimization of the analytical method described was carried out by use of a model system, which consisted of technical-grade triolein spiked with cholesterol and sitosteryl stearate. For financial reasons, technical grade sitosteryl stearate was used, which contained stigmasteryl stearate (10%) as well. Actually, this was not encountered as a problem, as on calculation of the quantitative data both phytosterols were taken into account. Using the described model system, which consisted of cholesterol and sitosteryl stearate, allowed a perfect evaluation of the esterified/free sterol separation. To obtain a good separation, all sitosteryl stearate had to elute with the nonpolar solvent in a first fraction and all cholesterol had to elute in the second fraction.

Experiments were carried out with different mixtures of nonpolar solvents. Polarity of the nonpolar solvent, which consisted of *n*-hexane, was altered by addition of small amounts of ethyl acetate as polar modifier. Free sterols were eluted in a second fraction upon elution with a polar solvent mixture of *n*-hexane/diethyl ether/ethanol 25:25:50 (by vol).

On elution of the silica gel column with *n*-hexane/ethyl acetate ratios of 98:2 and 95:5 (vol/vol), respectively, retention of sitosteryl stearate on the column was observed. Elution of the silica gel column with a ratio of *n*-hexane/ethyl acetate 90:10 (vol/vol) gave a good separation of all esterified sitosterol stearate in the first fraction and free sterol (cholesterol) in the

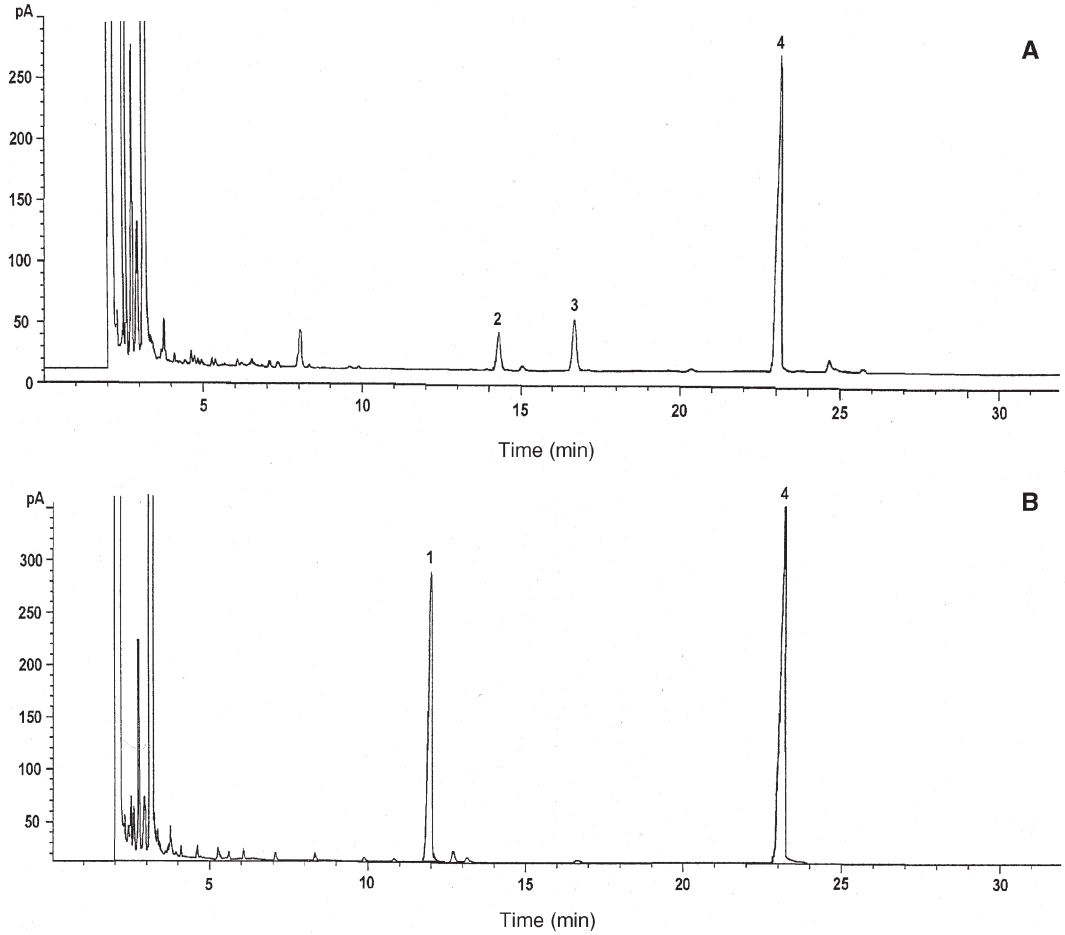

FIG. 1. GC chromatogram of the (A) esterified and (B) free sterol fraction from the model study (triolein spiked with sitosteryl stearate and free cholesterol) obtained after silica gel chromatography with *n*-hexane/ethyl acetate (90:10 vol/vol) and *n*-hexane/diethyl ether/ethanol (25:25:50 by vol), respectively, followed by saponification and derivatization (1, cholesterol; 2, stigmasterol; 3, sitosterol; 4, betulin).

second fraction. A chromatogram of both fractions is presented in Figure 1, indicating the perfect separation between sitosteryl stearate and cholesterol. Upon increasing the solvent ratio *n*-hexane/ethyl acetate to 85:15 (vol/vol), elution of cholesterol in the steryl ester fraction did occur.

These data indicate it is possible to separate esterified and free sterols with a minimal amount of solvent on a small silica gel column with *n*-hexane/ethyl acetate (90:10 vol/vol) and *n*-hexane/diethyl ether/ethanol (25:25:50 by vol), respectively. However, a good polarity of the first solvent is of primary importance.

Evaluation of the method. In this study, repeatability and accuracy of the analytical method were evaluated by comparing analytical data of the esterified and free sterol analysis with the total sterol analysis, which was regarded as the reference sterol analysis. Therefore, cholesterol and sitosteryl stearate were dissolved in technical grade triolein toward a realistic sterol concentration present in vegetable oils. Results obtained for repeatability and accuracy are listed in Table 1.

Results obtained by repeated analysis ($n = 6$) of the model mixture indicate a perfect relationship between both analytical

TABLE 1
Repeatability for the Analysis of Esterified Sitosterol and Cholesterol in Triolein

	Esterifed/free sterol analysis[a]			Total sterol analysis[b]		
	Sitosterol	Cholesterol	Total	Sitosterol	Cholesterol	Total
Mean[c] (mg/100 g)	98.26	240.20	338.46	100.97	239.40	340.37
SD (mg/100 g)	4.59	2.41	3.92	4.49	6.69	9.52
RSD[d] (%)	4.67	1.00	1.16	4.45	2.80	2.80
Accuracy[e] (%)	93.31	94.20	93.94	95.88	93.88	94.47

[a]Free and esterified sterols analyzed by silica gel column separation followed by saponification, extraction, derivatization.
[b]Total sterol content after saponification, extraction, derivatization.
[c]Mean of six replicates.
[d]Relative standard deviation.
[e]Based on the gravimetric amount added.

methods. Upon separation of the esterified and free sterol fraction, mean concentrations of 98.26 mg/100 g sitosterol and 240.2 mg/100 g cholesterol, respectively, were found. The total sterol analysis resulted in a sitosterol (steryl ester) and cholesterol (free sterol) concentration of 100.97 and 239.4 mg/100 g.

A relative standard deviation (RSD) of 2.8% was obtained for the total sterol analysis, which is good in comparison to the RSD found for the total sterol analyses in literature (13,15,16). For the total sterol content obtained after separation of the esterified and free sterols, an even lower RSD of 1.16% was found. The esterified sterol fraction (sitosterol) had a slightly higher RSD of 4.67% in comparison to the RSD of the free sterol fraction (cholesterol) of 1.1%. In addition, the results obtained by both analytical methods resulted in a sterol content that was comparable to the gravimetrically calculated sitosterol and cholesterol content. Acceptable accuracy levels, relative to the gravimetric concentration, ranging between 93.6 and 94.1% were obtained for both analytical methods.

Thus, a reproducible and accurate method for the analysis of esterified and free sterols was established. A good separation on the silica gel column between the esterified and free sterol fraction was of primary importance for obtaining accurate results. Therefore, a quality parameter was included in the method for the analysis of vegetable oils. As cholesterol does not naturally occur in vegetable oils and fats, with exception of a few mg/kg in coconut and palm oil (4,17), cholesteryl stearate was added to the oil before column chromatography. All cholesteryl stearate eluted in the esterified sterol fraction, and recovery levels of 100 ± 2% were always obtained. Detection of cholesterol in the free sterol fraction would indicate an incomplete separation between the free and esterifed sterol fraction on the silica gel column.

Overview of the free and esterified sterol content in vegetable oils. A variety of vegetable oils were analyzed for their esterified/free sterol and total sterol content. For both analytical methods, quantitative data, expressed as mg sterol/100 g of oil, are listed in Table 2. An overview of the mean esterified/free sterol ratio and the absolute esterified/free sterol content for the vegetable oils studied is presented in Figures 2 and 3, respectively.

A large variation in the content and distribution of the phytosterol fraction between different vegetable oils can be observed. The predominant sterol class present in vegetable oils is 4-desmethyl sterol, with sitosterol usually contributing more than 50% of the total phytosterol content (Table 2). Other important phytosterols are campesterol, stigmasterol, and Δ5-avenasterol. Factors causing variation and inconsistency in the results (including genetic species, growing and storage conditions, influence of the refining process, and specificity of the analytical method applied) should be borne in mind when the sterol content and composition of different vegetable oils are evaluated and compared (1).

No difference in the relative proportion of the individual sterols among crude and refined vegetable oils was observed. During oil refining, a reduction in the free sterol content and an absolute increase of the esterified sterol fraction was detected for all vegetable oils analyzed. The influence of the refining process on free and esterified sterols has been considered in detail (Verleyen, T., U. Sosinska, S. Ionadis, K. Dewettinck, A. Huyghebaert, and W. De Greyt, unpublished data).

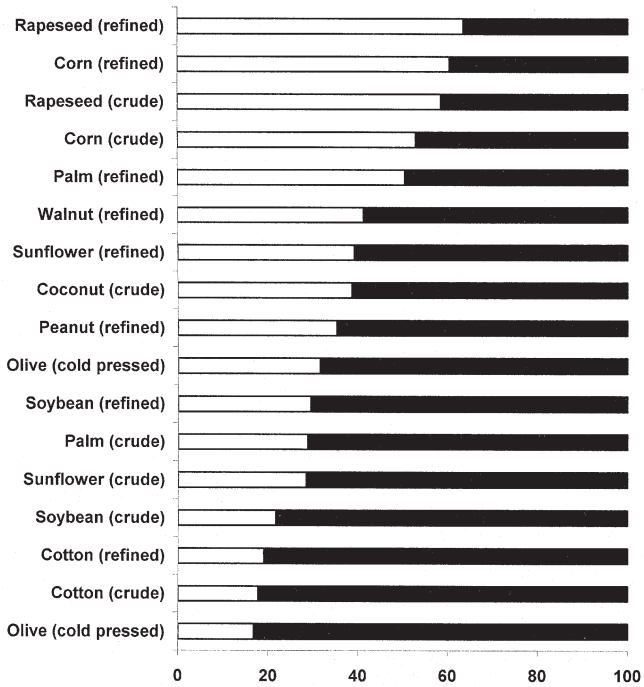

FIG. 2. Schematic overview of the esterified (open bar) and free (solid bar) sterol proportions (%) for several vegetable oils.

The highest phytosterol content was detected in corn and rapeseed oils, with a total phytosterol content ranging between 770 and 920 mg/100 g. The reported phytosterol distribution is in good accord with literature data (2,3,7,17,19,20).

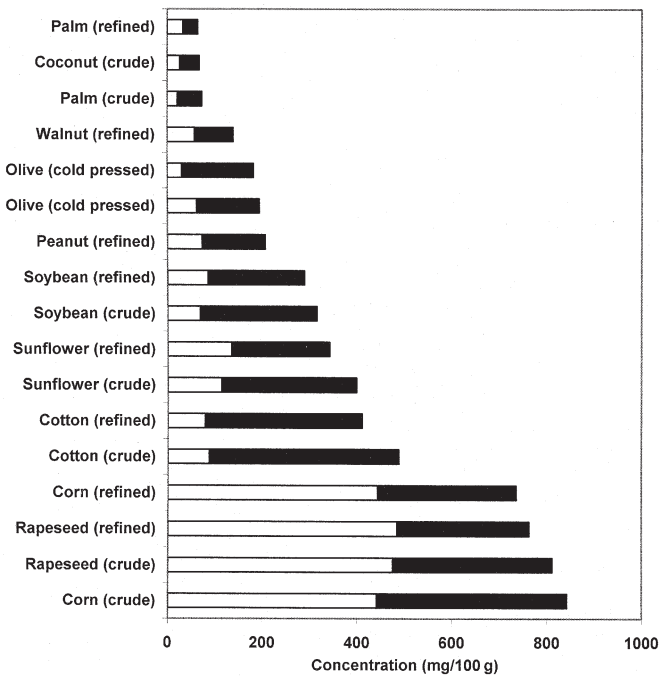

FIG. 3. Schematic overview of the free (solid bar) and esterified (open bar) sterol content (mg/100 g) for several vegetable oils.

TABLE 2
Sterol Content (mg/100 g) Analyzed by the Esterified/Free Sterol Analysis and Total Sterol Analysis in Vegetable Oils[a]

	Esterified/free sterol analysis											Total sterol analysis				
	Esterified sterols (mg/100 g)					Free sterols (mg/100 g)					E + F	Total sterols (mg/100 g)				
Sample	Camp	Stigm	Sito	Δ5-Ave	Total[b]	Camp	Stigm	Sito	Δ5-Ave	Total	total[c]	Camp	Stigm	Sito	Δ5-Ave	Total
Coconut (crude)	2.9	4.5	18.9	—	26.3	4.6	7.7	29.2	—	41.5	67.8	7.8	12.5	48.6	—	68.9
Corn (crude)	76.4	33.4	302.8	10.7	423.3	112.6	32.1	340.8	—	485.5	908.8	200.5	67.7	645.7	10.4	924.3
Corn (crude)	91.1	35.7	323.9	9.3	460.0	70.8	21.1	224.4	—	316.3	776.3	168.9	58.3	541.0	11.4	779.6
Corn (refined)	77.3	29.5	319.1	9.17	435.1	70.8	22.6	242.1	—	335.5	770.6	164.3	54.9	543.1	10.7	773
Corn (refined)	80.6	34.9	323.6	12.9	452.0	53.9	18.0	177.5	—	249.4	701.4	148.8	52.0	474.3	10.4	685.5
Cotton (degummed)	8.3	1.6	68.7	8.5	87.1	26.8	5.9	360.6	8.7	402.0	489.1	33.3	5.0	401.8	19.4	459.5
Cotton (refined)	8.6	0.8	62.0	7.2	78.6	22.8	4.4	301.6	4.1	332.9	411.5	32.1	3.9	357.2	15.0	408.2
Olive (cold pressed)	1.4	—	19.9	9.3	30.6	—	—	116.4	35.0	151.4	182.0	1.7	—	130.3	44.3	176.3
Olive (cold pressed)	—	—	45.2	16.3	61.5	4.4	—	89.8	38.1	132.3	193.8	5.3	—	127.7	60.1	193.1
Palm (crude)	3.7	1.8	9.7	1.1	16.3	9.8	6.9	32.5	—	49.2	65.4	13.9	9.5	42.6	3.3	69.3
Palm (crude)	5.5	2.3	16.9	—	24.7	10.4	4.9	35.8	—	51.1	75.8	19.8	7.3	52.3	—	79.4
Palm (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	40.0	3.0	68.6
Palm (refined)	10.0	6.6	20.7	—	37.3	6.2	2.5	26.1	—	34.8	72.1	17.7	9.5	40.5	—	67.7
Palm (refined)	6.0	3.2	17.1	1.9	28.2	6.3	4.1	18.5	—	28.9	57.1	13.7	7.1	36.5	2.6	59.9
Peanut (refined)	12.4	4.2	56.6	—	73.2	17.3	16.1	100.0	—	133.4	206.6	37.8	21.9	169.0	—	228.7
Rapeseed (bleached)	192.3	—	238.4	37.7[d]	468.4	107.7	—	173.5	49.2[d]	330.4	798.8	309	—	404.6	86.5[d]	800.1
Rapeseed (crude)	193.0	—	256.6	25.8[d]	475.4	97.4	—	170.6	68.2[d]	336.2	811.6	293.0	—	419.8	110.9[d]	823.8
Rapeseed (refined)	191.1	—	254.7	38.9[d]	484.7	93.3	—	158.5	26.5[d]	278.3	763	299.7	—	390.0	77.4[d]	767.1
Palm olein (crude)	4.5	2.4	16.1	—	23.0	13.7	6.8	35.3	—	55.8	78.8	19.5	10.5	51.0	—	81.0
Soybean (bleached)	8.7	5.9	49.3	10.5	74.4	43.5	45.4	114.2	—	203.1	277.5	62.4	57.1	171.8	10.0	301.3
Soybean (crude)	8.9	6.0	52.5	11.3	78.7	49.2	52.3	137.9	—	239.4	318.1	57.1	57.7	173.4	13.5	301.7
Soybean (crude)	5.6	4.7	40.1	8.6	59.0	62.6	54.9	137.1	—	254.6	313.6	71.0	61.4	183.7	10.5	326.6
Soybean (refined)	10.7	3.2	51.3	10.1	75.3	43.9	39.2	108.6	—	191.7	267.0	60.4	47.8	164.2	12.1	284.5
Soybean (refined)	10.8	9.3	57.8	9.9	87.8	39.5	40.0	113.2	—	192.7	280.6	47.5	48.3	159.4	11.9	267.1
Soybean (refined)	12.7	9.1	59.4	12.1	93.3	53.0	52.8	120.7	—	226.6	319.8	69.5	63.9	160.2	13.8	307.4
Sunflower (bleached)	12.4	—	80.9	20.7	114.0	34.9	33.2	213.7	3.7	285.5	399.5	41.0	33.7	265.3	43.2	383.2
Sunflower (refined)	14.9	4.1	97.2	28.6	144.8	24.8	29.9	167.0	13.2	234.9	379.7	41.1	32.0	257.1	45.3	375.5
Sunflower (refined)	12.9	4.2	81.1	26.3	124.5	18.9	22.3	138.3	12.2	191.7	316.2	35.8	26.8	225.4	42.4	330.4
Sunflower (refined)	20.4	4.4	91.3	34.0	150.1	25.7	30.3	161.0	12.7	229.7	379.8	43.8	30.8	237.7	55.5	367.8
Sunflower high-oleic (refined)	7.6	5.9	90.0	17.3	120.8	20.0	22.5	143.3	5.1	190.9	311.7	34.2	26.2	208.5	25.7	294.6
Sunflower (refined)	25.8	3.9	85.0	19.0	133.8	25.8	20.9	140.3	4.6	191.6	325.3	54.5	24.3	229.0	33.3	341.1
Walnut (refined)	2.7	—	54.8	—	57.5	4.9	—	76.5	—	81.4	138.9	7.8	—	136.2	—	144.0

[a]Abbreviations: Camp, campesterol; Stigm, stigmasterol; Sito, sitosterol; Δ5-Ave, Δ5-avenasterol; —, not detected.
[b]Sum of Camp, Stigm, Sito, Δ5-Ave, and brassicasterol.
[c]Sum of esterified and free sterols.
[d]Brassicasterol.

As observed in Figure 2, both corn and rapeseed oils were very rich in steryl esters. In corn oil an equal distribution of the several phytosterols between the esterified and free sterol fraction is observed with exception of Δ5-avenasterol, which was only present in the esterified sterol fraction. In rapeseed oil the free sterol fraction contained more brassicasterol (14.9%) compared to the esterified fraction (7.2%). This observation is in accord with the literature (7).

The total sterol content of the soybean oil samples analyzed ranged between 267.1 and 326.6 mg/100 g of oil. Those results are in accord with the literature (6,17,18,20). The distribution of the different phytosterols differed between the esterified and free sterol fraction. For example, in the free sterol fraction no Δ5-avenasterol could be detected.

The total sterol content of sunflower oil ranged between 294.6 and 383.2 mg/100 g of oil, which agreed with the literature (17,18,20). Sunflower oil contains more free sterols (62.4%) than esterified sterols (37.6%). In crude and bleached sunflower oil, no stigmasterol could be detected in the steryl ester fraction. However, in refined sunflower oil, esterified stigmasterol was present in low concentrations (4–6 mg/100 g) probably due to a sterol esterification promoted by the temperature during the deodorization process (Verleyen, T., U. Sosinska, S. Ionadis, K. Dewettinck, A. Huyghebaert, and W. De Greyt, unpublished data). In contrast to corn and soybean oils, Δ5-avenasterol was present in the free sterol fraction as well (3.7–13.2 mg/100 g, or 1.3–6.4%).

A large variation in the total sterol content of virgin olive oils ranging between 87.6 and 188.3 mg/100 g is reported in literature (1,5,10,17,21,22). Olive oil is very rich in sitosterol (70%) and Δ5-avenasterol (28.1%) and contains traces of campesterol and stigmasterol. In addition, large variations in the esterified (86–27%) and free (14–73%) sterol content are reported (5,6,10,23), which is confirmed in our research as well. This large variation in the composition of different olive oils is probably due to the different varieties of olives

TABLE 3
Linear Correlation Coefficients for the Phytosterol Content Analyzed by the Esterified/Free Sterol Analytical Method and the Total Sterol Analysis

Phytosterol	Linear equation[a]	R^2
Campesterol	$y = 1.0229x + 1.011$	0.9947
Stigmasterol	$y = 1.0067x - 0.2968$	0.9897
Sitosterol	$y = 0.9794x + 0.4667$	0.9968
Δ5-Avenasterol	$y = 1.0455x + 2.2181$	0.9943
Total phytosterol	$y = 0.9913x + 3.3916$	0.9976

[a]x = phytosterol content (mg/100 g) analyzed by the esterified/free sterol analysis method, y = phytosterol content (mg/100 g) analyzed by the total sterol method.

employed and the different process techniques applied in the extraction process (10,21,22,24).

Total phytosterol content for coconut, cottonseed, peanut, and walnut oils was compared with literature data (2,8,17). Coconut, peanut, and walnut oils contained considerable esterified sterol contents of 38.8, 35.4, and 41.4%, respectively, whereas cottonseed oil contained a much lower esterified phytosterol content of 18.5%. A similar phytosterol distribution for these oils was observed in the esterified and free sterol fraction.

In comparison to other vegetable oils, palm oil contained little phytosterol, ranging between 71 and 117 mg/100 g (1,21). Palm oil mainly contains free sterols, which is in accord with observations in this study. A homogeneous distribution of the different phytosterols in the esterified and free sterol fraction was found. During the refining process a considerable increase in the ratio of esterified/free sterol was observed, which has been discussed in detail (Verleyen, T., U. Sosinska, S. Ionadis, K. Dewettinck, A. Huyghebaert, and W. De Greyt, unpublished).

In summary, a good relationship between the analysis of esterified/free sterol content compared to the total sterol content was obtained upon analyzing more than 90 samples in our laboratory. As indicated in Table 3, a good relationship between both analytical methods was obtained as well as for the individual phytosterols and for the total phytosterol content.

REFERENCES

1. Piironen, V., D.G. Lindsay, T.A. Miettinen, J. Toivo, and A.M. Lampi, Plant Sterols: Biosynthesis, Biological Function and Their Importance to Human Nutrition, *J. Sci. Food Agric. 80*: 939–966 (2000).
2. Worthington, R.E., and H.L. Hitchcock, A Method for the Separation of Seed Oil Steryl Esters and Free Sterols: Application to Peanut and Corn Oils, *J. Am. Oil Chem. Soc. 61*:1085–1088 (1984).
3. Ferrari, R.A., W. Esteves, K.D. Mukherjee, and E. Schulte, Alteration of Sterols and Steryl Esters in Vegetable Oils During Industrial Refining, *J. Agric. Food Chem. 45*:4753–4757 (1997).
4. Homberg, E., and B. Bielefeld, Freie und gebundene Sterine in rohen und raffinierten Palmölen, Teil I: Gehalt und Zusammensetzung der freien Sterine, Sterinester, freien und acylierten Steringlucoside, *Fette Seifen Anstrichm. 84*:141–146 (1982).
5. Boskou, D., and I. Vlachopoulou, On the Level of Steryl Esters in Olive Oil, *Lebensm. Wiss. Technol. 19*:156–157 (1986).
6. Phillips, K.M., J.I. Toivo, M.A. Swank, R.S. Whiton, and D.M. Ruggio, Free and Esterified Sterol Content of Food Oils and Fats, Presented at the 90th Annual AOCS Meeting and Expo, Orlando, May 9–12, 1999.
7. Johansson, A., and L.A. Appelqvist, The Sterol Composition of Freshly Harvested Compared to Stored Seeds of Rape, Sunflower, and Poppy, *J. Am. Oil Chem. Soc. 56*:995–997 (1979).
8. Homberg, E., and B. Bielefeld, Freie und gebundene Sterine in Pflanzenfetten, *Fette Seifen Anstrichm. 87*:61–64 (1985).
9. Grob, K., and M. Lanfranchi, Determination of Free and Esterified Sterols and of Wax Esters in Oils and Fats by Coupled Liquid Chromatography–Gas Chromatography, *J. Chromatogr. A 471*:397–405 (1989).
10. Grob, K., M. Lanfranchi, and C. Mariani, Evaluation of Olive Oils Through the Fatty Alcohols, the Sterols and Their Esters by Coupled LC–GC, *J. Am. Oil Chem. Soc. 67*:626–634 (1990).
11. Gordon, M.H., and R.E. Griffith, A Comparison of the Steryl Esters of Coconut and Palm Kernel Oils, *Fat Sci. Technol. 94*: 218–221 (1992).
12. Gordon, M.H., and L.A.D. Miller, Development of Steryl Ester Analysis for the Detection of Admixtures of Vegetable Oils, *J. Am. Oil Chem. Soc. 74*:505–510 (1997).
13. Lechner, M., B. Reiter, and E. Lorbeer, Determination of Tocopherols and Sterols in Vegetable Oils by Solid-Phase Extraction and Subsequent Capillary Gas Chromatographic Analysis, *J. Chromatogr. A 857*:231–238 (1999).
14. DGF Gemeinschaftarbeitung, Sterine isolierung und GC-untersuchung, *Fat Sci. Technol. 92*:189–192 (1990).
15. Zurcher, K., H. Hadorn, and C. Strack, Einfache leistungsfähige Methode zur gaschromatographischen Trennung und Bestimmung der Sterine in Ölen und Fetten, *Dtsch. Lebensm. Rundsch. 72*:345–352 (1976).
16. Frega, N., F. Bocci, and G. Lecker, Direct Gas Chromatographic Analysis of the Unsaponifiable Fraction of Different Oils with a Polar Capillary Column, *J. Am. Oil Chem. Soc. 69*:447–450 (1992).
17. Gunstone, F.D., J.L. Harwood, and F.B. Padley, eds., Occurrence and Characteristics of Oils and Fats, in *The Lipid Handbook*, Chapman and Hall, London, 1986, pp. 130–140.
18. Ferrari, R.A., E. Schulte, W. Esteves, L. Bruhl, and K.D. Mukherjee, Minor Constituents of Vegetable Oils During Industrial Processing, *J. Am. Oil Chem. Soc. 73*:587–592 (1996).
19. Prior, E., V.S. Vadke, and F.W. Sosulski, Effect of Heat Treatment on Canola Press Oils I. Non-triglyceride Components, *Ibid. 68*:401–406 (1991).
20. Homberg, E., and B. Bielefeld, Sterinzusammensetzung und Steringehalt in 41 verschiedenen pflanzlichen und tierischen Fetten, *Fette Seifen Anstrichm. 91*:23–27 (1989).
21. Ranalli, A., M.L. Ferrante, G. De Mattia, and N. Costantini, Analytical Evaluation of Virgin Olive Oil of First and Second Extraction, *J. Agric. Food Chem. 47*:417–424 (1999).
22. Ranalli, A., N. Costantini, G. De Mattia, and M.L. Ferrante, Evaluating Two Kinds of Centrifuged Virgin Oils Arising from Continuous Olive Processing, *J. Sci. Food Agric. 80*:673–683 (2000).
23. Grob, K., A. Artho, and C. Mariani, Gekoppelte LC–GC für die Analyse von Olivenölen, *Fat Sci. Technol. 93*:494–500 (1991).
24. Koutsaftakis, A., F. Kotsifaki, and E. Stefanoudaki, Effect of Extraction System, Stage of Ripeness, and Kneading Temperature on the Sterol Composition of Virgin Olive Oil, *J. Am. Oil Chem. Soc. 76*:1477–1481 (1999).

[Received August 22, 2001; accepted December 6, 2001]