Development of Steryl Ester Analysis for the Detection of Admixtures of Vegetable Oils

Michael H. Gordon* and Luke A.D. Miller

Department of Food Science and Technology, The University of Reading, Whiteknights, Reading RG6 6AP, United Kingdom

ABSTRACT: The steryl ester content and composition of 28 samples from 10 vegetable oil types have been determined by isolation of the steryl esters by high-performance liquid chromatography and analysis by gas chromatography. The oils can be classified into oils with a high content (>4000 mg/kg) of steryl esters (corn and rapeseed); oils with a medium content (1400–2400 mg/kg) of steryl esters (sunflower oil and high-oleic sunflower oil); and oils with a low content (<1200 mg/kg) of steryl esters (safflower, soybean, cottonseed, groundnut, olive, and palm oils). The composition of the steryl ester fraction varies to a greater extent for different oil types than for different varieties of the same oilseed. The developed method is promising for authentication of some oils, and is particularly suitable for detecting admixtures of low levels of corn or rapeseed oils. *JAOCS 74*, 505–510 (1997).

KEY WORDS: Analysis, authentication, steryl esters, vegetable oils.

Many edible oils are similar in physical properties, and consequently, substitution or blending with alternative oils may not affect product properties. However, good analytical procedures that are capable of identifying oils and detecting admixtures are required to monitor trade and to ensure compliance with legislation.

Several procedures can be used in authenticating edible oils. These include analysis of triacylglycerols, fatty acid methyl esters (FAME), lipolysis-FAME, sterols, tocopherols, and the carbon isotope ratio (1). Each of these methods is useful for some blends, but no method is suitable for all blends, and consequently, additional methods are required either for confirmation or for extension of the range of blends that can be identified.

Sterols in oils are present as mixtures of free sterols and sterols esterified with fatty acids. Methods for the analysis of intact steryl esters have been described (2), but the conventional method of sterol analysis involves saponification to allow the total sterol content to be determined. However, potentially useful information may be lost by this procedure because the steryl esters are complex mixtures that differ in composition from that expected by a random esterification of total fatty acids and total sterols in the oil (3). The steryl ester fraction is reduced less by processing than the free sterol content, which is reduced particularly during the bleaching stage (4,5). A previous study has shown that the composition of the steryl ester fraction varies between different oil types (6). The steryl ester fraction from rapeseed oil, isolated from seeds grown in five regions of Sweden (3) and from summer and winter varieties of *Brassica napus* and *B. campestris* (7), showed only minor differences in composition. The present study aims to investigate the range of steryl ester content and composition of selected vegetable oils, and the potential of steryl ester analysis as an additional method for the authentication of vegetable oils.

MATERIALS AND METHODS

Oils and seeds. Authentic seeds were supplied by Leatherhead Food RA (Leatherhead, United Kingdom) (USA corn and 2 × Argentinean corn); Natural Resources Institute (Ashford, UK) (Ghanaian groundnuts); University of Reading Farm (Reading, United Kingdom) (5 × rapeseed); University of Bologna (Italy) (2 × Italian sunflower seeds); Poznan Agricultural University (Poland) (Polish rapeseed); and Setuza a.s. (Usti Nad Labem, Czech Republic) (2 × Czech rapeseed). Authentic samples of cottonseed oil (United States), groundnut oil (West Africa), and palm oil (Indonesia) were supplied by The Ministry of Agriculture, Fisheries and Food (London, United Kingdom). Extra virgin olive oil, described as Arbequina, Hojiblanca, Pajarera, Picual and an unknown variety, was supplied by Instituto de la Grassa (Seville, Spain).

Oil extraction. Oil was obtained from seeds (20 g) by Soxhlet extraction with hexane for 6 h. The extraction was performed in duplicate.

Preparative high-performance gas chromatography (HPLC) isolation of steryl esters. Oil (0.3 g) and cholesteryl acetate (1.0 mg) as internal standard were accurately weighed into a screw-top vial, and hexane (3.0 mL) was added. This solution (50 µL) was then injected into an HPLC system comprised of a Perkin Elmer Binary LC 250 pump (Norwalk, CT), a 10 × 8 mm guard column, a 250 × 8 mm i.d. Hypersil 5sa column (Hichrom Ltd., Reading, Berkshire, United Kingdom), a switching valve and an ultraviolet detector, set at 205

*To whom correspondence should be addressed at Department of Food Science and Technology, The University of Reading, Whiteknights, P.O. Box 226, Reading RG6 6AP, United Kingdom.

nm, coupled to a Hewlett-Packard integrator, model 3396A (Palo Alto, CA). The mobile phase was hexane (99.5%)/isopropanol (0.5%) with a flow rate of 0.5 mL/min. The steryl ester fraction was collected, and the column was back-flushed with solvent for *ca.* 30 min to remove the triacylglycerols. The steryl esters collected from 10 individual runs on each sample were combined to provide sufficient sample for subsequent gas chromatographic (GC) analysis.

GC analysis of intact steryl esters. GC analysis of steryl esters was carried out on a fused-silica wall-coated open tubular capillary column (25 m × 0.25 mm i.d.) coated with TAP CB (0.1 μm film thickness, 50% -phenyl, 25%- methyl, 25% -X-polysiloxane phase) (Chrompack UK Ltd., London, United Kingdom) with the temperature program 50°C (0.5 min), ramp 30°C/min; 250°C (5 min), ramp 5°C/min; 355°C (15 min). A Perkin Elmer 8500 gas chromatograph, fitted with a cooled on-column injector and a flame-ionization detector, was used with hydrogen as carrier gas, flow 85 $cm \cdot s^{-1}$ (2.5 $mL \cdot min^{-1}$). Six replicates were analyzed.

Relative retention times (RRT) and concentrations of GC peaks were calculated relative to the internal standard cholesteryl palmitate, which was added before GC analysis. Response factors were assumed to be 1.0.

Electron impact GC–mass spectrometry (MS) identification of intact steryl esters. GC–MS of the intact steryl esters in crude rapeseed oil was carried out at Bristol University on a Finnigan 4500 quadrupole mass spectrometer (San Jose, CA), which was directly coupled to a Carlo Erba 5160 gas chromatograph (Milano, Italy), fitted with a Restek 30 m × 0.25 mm i.d. Rtx-65TG (35% dimethyl, 65% diphenyl polysiloxane, 0.1 μm film thickness) capillary column (Thames Chromatography, Windsor, Berkshire, United Kingdom) and an on-column injector. The carrier gas was helium with a flow rate of 68 $cm \cdot s^{-1}$ (2.5 $mL \cdot min^{-1}$). The GC temperature program was 50°C, 20°C/min to 270°C, 5°C/min to 355°C (20 min). The MS source temperature was 170°C, electron energy 70 eV, emission current 350 μA.

Hydrogenation of steryl esters. Hydrogenation of rapeseed oil steryl esters (1 mg) in toluene (3 mL) was performed at 60°C with hydrogen (1 bar) in the presence of tris(triphenylphosphine) rhodium chloride (Wilkinson's catalyst, 1 mg).

Statistical analysis. Student's *t*-test was used to assess significance of differences in individual steryl ester contents between oil types, and principal-component analysis was used to identify overall differences in steryl ester patterns.

RESULTS AND DISCUSSION

Analytical method. The main requirements for the method of isolation of the steryl ester fraction were that the method should give good recovery of the steryl esters and complete separation from the triacylglycerols. Isolation by HPLC, with cholesteryl acetate added as an internal standard, allowed accurate quantitation with approximately 95% recovery and complete removal of triacylglycerols, which was confirmed by HPLC. The steryl ester fraction eluted at approximately 18.5 min, with the triacylglycerol peak starting to elute at approximately 21 min. Small peaks on either side of the steryl ester peak were commonly present. We suspect that these peaks were due to methyl- and dimethylsteryl esters, and partial, but not total, separation from these components was achieved. Backflushing the column before the triacylglycerol peak eluted reduced the time between successive injections.

A second internal standard, cholesteryl palmitate, was added before GC analysis. The GC conditions, when optimized, typically gave 19 peaks for the steryl esters isolated from rapeseed oil, with retention times relative to the internal standard of 1.01–1.20 (Fig. 1). Saponification of the steryl ester fraction and analysis of the separated sterols and fatty acids indicated that 4 sterols and 7 fatty acids were present at concentrations >1% of the total (Table 1). Consequently, if these fatty acids and sterols were esterified with each other, 28 steryl esters would have been present in this fraction with further steryl esters present at low concentrations. GC retention data for steryl esters confirm that some steryl esters coelute (6). Although complete separation of steryl esters was not achieved in the GC analysis, separation of most major steryl esters was achieved. The GC analysis was checked for selective on-column losses of polyunsaturated steryl esters by comparing the steryl ester content of rapeseed oil determined before and after hydrogenation of the steryl ester fraction. The ratio of the sums of all steryl ester peaks after and before hy-

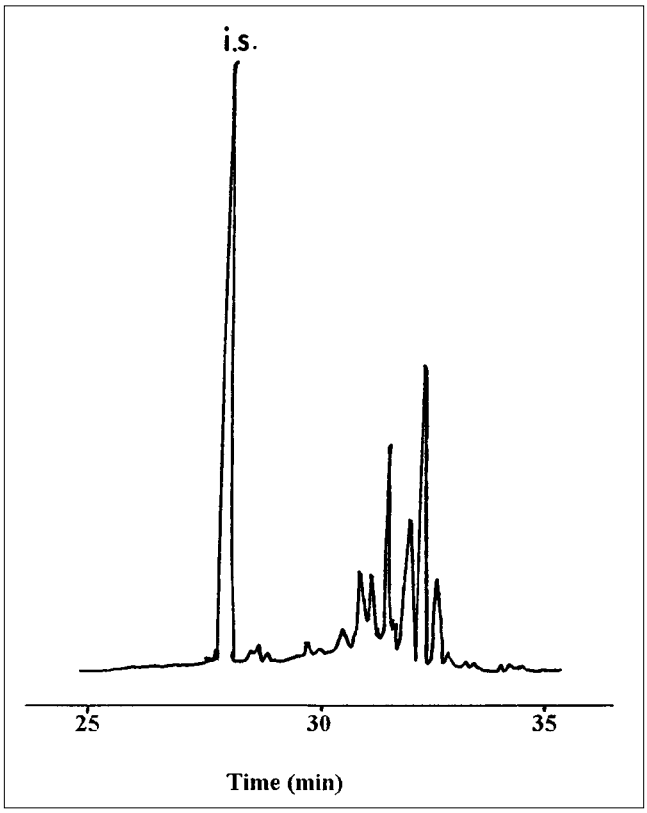

FIG. 1. Gas chromatogram of steryl esters isolated from rapeseed oil (i.s. = cholesteryl palmitate).

TABLE 1
Rapeseed Oil Fatty Acids in (a) the Steryl Esters and (b) the Whole Oil

	Fatty acid							
	14:0	16:0	18:0	18:1	18:2	20:0	18:3	22:1
(a)	3.1	17.5	18.4	30.9	20.5	0.8	7.6	1.2
(b)	0.5	5.6	2.1	58.1	21.6	0.4	11.2	0.4

TABLE 2
Rapeseed Oil Sterols (%) Isolated after Saponification from (a) the Steryl Esters and (b) the Whole Oil

	Cholesterol	Brassicasterol	Campesterol	Stigmasterol	Unknown	β-Sitosterol	Avenasterol
(a)	—	5.1	32.0	0.0	0.0	55.2	7.7
(b)	0.6	12.7	31.1	0.0	0.6	52.2	1.9

drogenation was >0.93:1, indicating minimal losses of polyunsaturated steryl esters on-column.

The method of HPLC and GC analysis was consequently chosen as acceptable for determining the steryl ester content and for achieving a fingerprint characteristic of the steryl ester components in the oil.

Composition of the steryl ester fraction. The concentrations of sterols and fatty acids in the steryl ester fraction of rapeseed oil were significantly different from their concentrations in the total sterols and fatty acids obtained by saponification of the oil (Tables 1,2). In particular, palmitic and stearic acids were present at higher concentrations in the steryl ester fraction, whereas oleic acid was present at higher concentration in the triacylglycerols. Brassicasterol was present at higher concentration in the total sterols, whereas avenasterol was present at higher concentration in the steryl ester fraction. The differences in sterols and oleic acid between the steryl esters and total sterol and fatty acids are qualitatively in agreement with the earlier findings of Johansson and Appelqvist (3). However, the earlier study found a much higher content of linoleic acid in the steryl esters than in the whole oil, whereas the present study does not demonstrate a difference in linoleic acid but instead finds a higher level of myristic, palmitic, and stearic acids in the steryl ester fraction. GC–MS analysis of the steryl ester fraction of rapeseed oil confirmed the presence of brassicasteryl, campesteryl, β-sitosteryl, and avenasteryl esters in rapeseed oil, and stigmasteryl, campesteryl, β-sitosteryl and avenasteryl esters in sunflower oil. MS with electron impact ionization only allowed the sterol moiety to be identified owing to ion fragmentation.

Steryl esters in oils. The steryl esters in 28 samples of oils were isolated and analyzed. All oils from seeds were extracted in the laboratory to ensure authenticity, but samples of palm oil and olive oil were supplied from reliable sources. Between 1 and 5 samples of each oil were analyzed. Mean steryl ester content (and standard deviations when samples from at least 3

TABLE 3
Steryl Ester Content of Vegetable Oils (mg·kg^{-1})

		Corn		GN		Olive		Rapeseed		Soybean				Oleic	Linoleic	
RRT	Corn	SD	Groundnut	SD	Olive	SD	Rapeseed	SD	Soybean	SD	Palm	Safflower	sunflower	sunflower	Cottonseed
1.01	11.40	13.36	1.35	0.26	1.78	0.99	8.85	5.25	1.95	0.88	2.30	9.50	2.10	1.30	1.10
1.02	25.68	19.49	1.33	0.36	6.02	2.01	21.80	2.18	3.23	0.96	0.00	1.10	1.60	1.60	0.00
1.03	110.35	29.57	17.85	5.61	8.06	5.79	38.70	26.64	7.00	4.36	16.3	8.10	6.20	3.70	13.00
1.04	92.30	17.71	6.60	2.87	2.36	3.90	26.95	6.12	2.03	1.41	11.10	9.60	58.30	62.50	26.50
1.05	64.00	11.50	57.78	19.01	19.20	14.55	92.70	5.80	27.33	9.40	58.90	25.60	27.90	26.50	55.60
1.06	308.35	89.55	5.93	4.74	53.14	13.71	0.00	0.00	13.63	7.86	3.10	6.10	15.60	9.50	7.70
1.07	59.10	13.00	16.85	8.79	22.86	34.91	49.70	41.21	3.98	2.59	1.50	20.20	10.70	11.60	16.50
1.08	129.88	34.65	6.13	2.56	54.84	24.59	226.05	32.47	17.15	12.79	6.30	15.90	36.60	35.70	29.30
1.09	182.48	43.04	14.98	6.00	41.62	23.54	326.00	27.21	12.38	6.66	13.50	54.10	99.40	92.00	23.70
1.1	288.90	98.00	43.98	16.71	10.90	6.58	380.85	37.81	23.40	15.07	4.20	59.50	64.30	69.30	69.50
1.11	742.30	88.50	94.40	23.27	32.20	56.03	800.60	32.47	30.60	16.15	6.50	68.80	123.70	144.90	116.50
1.12	326.95	37.01	26.63	8.13	97.36	54.02	113.00	30.55	24.05	11.04	18.70	125.30	279.30	119.88	25.10
1.13	821.40	190.50	126.55	57.25	38.34	6.83	649.55	39.54	121.05	56.35	6.50	109.90	57.10	181.70	143.40
1.14	1819.53	306.87	253.90	68.49	67.92	22.57	1058.00	36.81	20.23	12.20	1.80	61.20	80.60	208.20	402.00
1.15	194.35	99.44	56.88	20.47	147.80	65.72	315.15	51.11	63.10	31.81	23.40	166.00	367.40	256.60	59.90
1.16	115.50	52.90	44.98	16.76	60.72	44.37	71.00	5.31	57.15	41.86	9.60	103.70	75.10	383.10	38.20
1.17	125.15	113.86	28.55	10.68	41.90	18.40	18.05	0.68	34.03	37.94	2.60	49.90	150.30	65.10	16.60
1.18	68.65	12.66	23.43	8.01	27.16	15.78	13.10	4.79	40.55	30.49	4.70	31.30	185.80	184.60	27.20
1.19	141.63	69.54	23.03	4.72	18.36	12.56	9.10	3.29	53.58	37.36	7.60	11.70	17.50	165.50	22.90
1.2	26.93	10.04	6.23	3.77	6.22	5.51	12.35	3.18	20.03	12.67	18.20	13.90	27.70	45.50	10.20
Total	5654.80	771.79	857.30	245.47	758.76	267.26	4231.50	80.06	576.40	331.41	216.80	951.40	1687.20	2068.78	1104.90
number	4	4	4	4	5	5	3	3	4	4	1	2	2	2	1

sources were analyzed) for 10 vegetable oils is quoted in Table 3. It is clear that major differences exist in the content and composition of steryl esters from different vegetable oils. It is useful to classify oils according to their steryl ester content for authenticity studies because it is clear that steryl ester analysis is most useful for detecting oils with a high steryl ester content as admixtures in oils with a low steryl ester content, particularly if the composition of the steryl ester fraction also differs between the oils.

Based on the content of steryl esters, rapeseed and corn oils can be classified as Class I oils because they have a high content of steryl esters (>4000 mg/kg); sunflower oil can be classified as a Class II oil with a steryl ester content of 1400–2400 mg/kg for both high-oleic and high-linoleic varieties. Safflower, soybean, cottonseed, groundnut, olive, and palm oils can be described as Class III oils with a steryl ester content <1200 mg/kg. The values of 4151–4311 mg/kg steryl esters in rapeseed oil from commercial seeds compare with values of 7000–12,000 mg/kg reported for samples of this oil in earlier studies (3,5). The present study was therefore extended to include noncommercial cultivars, and the analysis of Starlight, Mars, Sprinter, Apex, and Bristol cultivars showed levels of 8470, 6540, 7310, 8550, and 7180 mg/kg steryl esters in the oil. It therefore appears that the steryl ester content of rapeseed oil from different cultivars can vary widely, although no level of <4151 mg/kg has been found in the samples analyzed to date.

The levels found in this study of about 4200 $mg \cdot kg^{-1}$ steryl esters in commercial varieties of rapeseed oil correspond to approximately 2446 $mg \cdot kg^{-1}$ sterols compared with a total sterol content of 6900 $mg \cdot kg^{-1}$, which indicates a free sterol content of 4454 $mg \cdot kg^{-1}$. These values compare with levels of approximately 3000 $mg \cdot kg^{-1}$ of sterols in both the free and the esterified forms in the seeds of *B. campestris* (7). The literature (8) reports more sterols in the free form than in the esterified form in cottonseed and safflower oils, and this has been confirmed by this study. Olive oil from Greece has been reported to contain 100–350 $mg \cdot kg^{-1}$ steryl esters compared with the range of 329–1015 $mg \cdot kg^{-1}$ for Spanish oil in the present study (9). The level of steryl esters in sunflower oil found by the current study (1409–2319 $mg \cdot kg^{-1}$) is less than the value (4600 $mg \cdot kg^{-1}$) reported in the literature (10). Levels of steryl esters in corn and soybean oils (4585–6262 and 192–999 $mg \cdot kg^{-1}$, respectively) are also less than those reported for these oils in another study (5), where values of 11,139 and 3295 $mg \cdot kg^{-1}$ were found. However, full experimental details and validation of the method used in this latter study have not been published.

Principal-component analysis (Fig. 2) showed that the corn oil samples and rapeseed oil samples could clearly be distinguished as groups from each other and from the other oils on the basis of the first and second principal components. The four sunflower oil samples were also separated from other oils in this plot; 70.5% of the variance in the data was predicted by the first and second principal components.

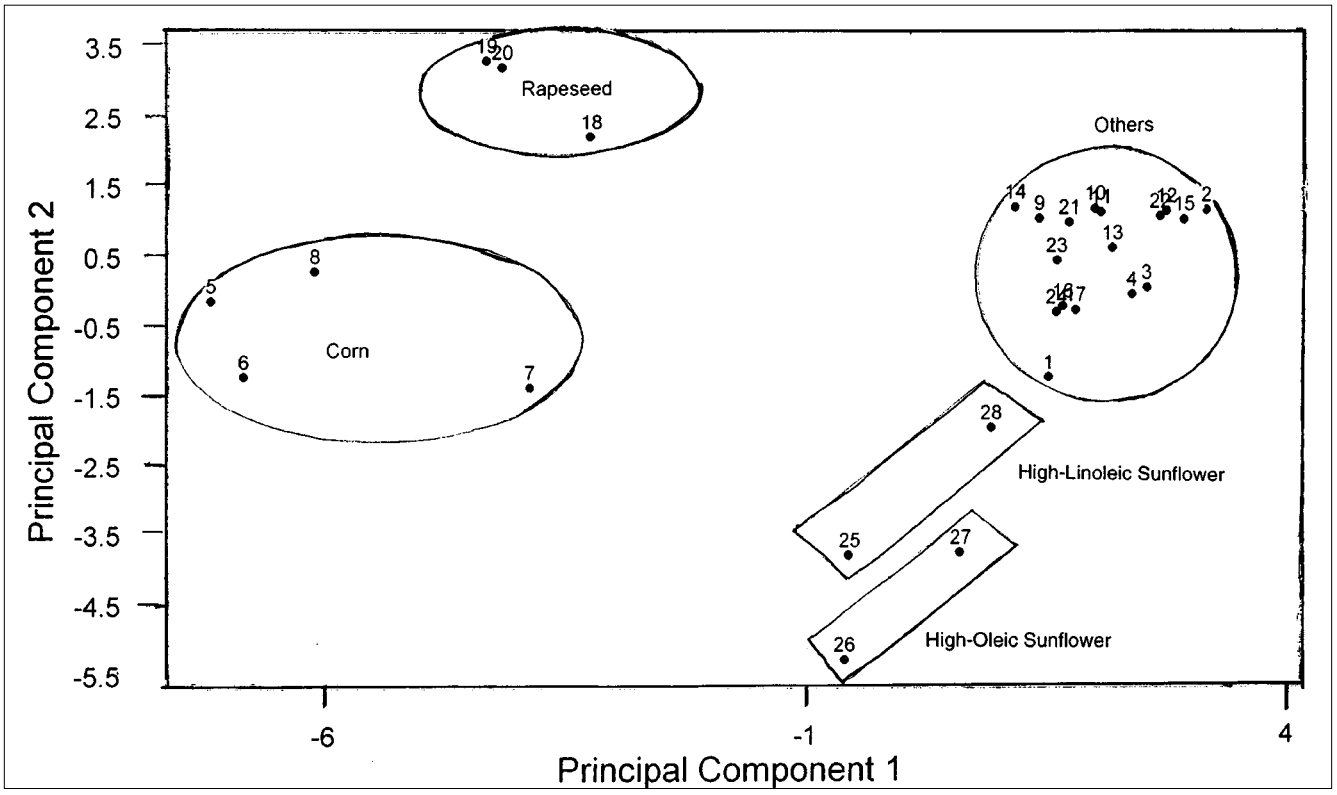

FIG. 2. Principal-components plot for steryl ester gas chromatographic data (1–4 = soybean; 5–8 = corn; 9–12 = groundnut; 13–17 = olive; 18–20 = rapeseed; 21 = cottonseed; 22 = palm; 23,24 = safflower; 25,28 = high-oleic sunflower; 26,27 = high-linoleic sunflower).

TABLE 4
Mean Steryl Ester Composition (%) and Standard Deviation for Five Oils

Relative retention time	Soybean		Rapeseed		Olive		Groundnut		Corn	
	Mean (%)	SD	Mean (%)	SD	Mean (%)	SD	Mean (%)	SD	Mean (%)	SD
1.01	0.55	0.64	0.25	0.12	0.23	0.09	0.17	0.09	0.21	0.24
1.02	0.80	0.72	0.50	0.06	0.80	0.08	0.16	0.02	0.43	0.31
1.03	1.15	0.40	1.08	0.62	1.10	0.60	2.10	0.27	1.97	0.5
1.04	0.55	0.47	0.71	0.14	0.37	0.51	0.77	0.20	1.65	0.35
1.05	5.62	2.42	2.13	0.17	2.61	1.54	6.69	0.44	1.14	0.19
1.06	2.38	0.09	0.00	0.00	7.42	1.66	0.60	0.44	5.46	1.34
1.07	0.68	0.16	1.42	0.96	2.73	3.77	1.85	0.57	1.04	0.13
1.08	2.54	1.25	5.46	0.67	7.85	3.35	0.70	0.20	2.31	0.58
1.09	2.32	0.73	7.49	0.76	6.00	3.41	1.70	0.24	3.19	0.4
1.1	4.34	1.52	9.13	0.74	1.33	0.59	5.08	0.69	4.99	1.18
1.11	5.27	1.95	18.65	1.06	3.82	6.10	11.14	0.73	13.19	1.1
1.12	4.37	0.62	2.41	0.76	12.12	3.44	3.11	0.24	5.83	0.73
1.13	22.31	3.21	15.50	0.67	5.61	2.04	14.37	2.88	14.55	2.69
1.14	3.35	0.63	25.34	0.62	9.32	1.95	29.99	4.29	32.09	2.3
1.15	11.17	1.10	7.06	1.33	19.48	4.70	6.86	2.27	3.34	1.44
1.16	9.19	1.74	1.69	0.10	7.24	3.66	5.25	0.95	2.17	1.38
1.17	5.43	3.09	0.43	0.01	5.35	0.77	3.32	0.51	2.14	1.88
1.18	6.44	1.38	0.28	0.12	3.29	1.41	2.69	0.29	1.23	0.25
1.19	8.36	2.76	0.19	0.08	2.46	1.46	2.78	0.54	2.59	1.44
1.2	3.16	1.29	0.27	0.08	0.88	0.66	0.70	0.26	0.48	0.19

Although corn oil resembles rapeseed oil by virtue of the main peak occurring at RRT 1.14 with major peaks at 1.11 and 1.13, the oils differ in other areas of the chromatogram especially in peaks at 1.06 and 1.17–1.19. The analysis of standard mixtures that contained campesteryl linoleate, β-sitosteryl oleate, and β-sitosteryl linoleate indicated that they occurred at RRT 1.11, 1.13, and 1.14, respectively, and the main peaks in these oils are expected to be mainly due to these components. However, GC–MS analysis of the sterol ester fraction indicated that a β-sitosteryl ester was coeluting at RRT 1.11, and a campesteryl ester was coeluting at RRT 1.13.

Differences occur in the steryl ester composition of most oils examined. The 1.06 peak of corn oil is significantly higher than that in any other oil analyzed ($P = 0.001$). The 1.08 peak of rapeseed oil is significantly higher than that for any other oil analyzed ($P = 0.01$). The main features that characterize corn and rapeseed oils are the high contents of steryl esters with RRT 1.11, 1.13, and 1.14. Groundnut oil is rather similar to corn oil in the relative concentration of the steryl esters (Table 4), but the total steryl ester content is much lower. The relative concentrations of the sterols in groundnut oil are similar to those in corn oil (Table 5), but the differences in the fatty acid composition (Table 6) of the two oils are not reflected in the steryl ester pattern. Olive oil has a low content of steryl esters, with the peak at RRT 1.15 being largest. Soybean oil samples varied in steryl ester content from 192–999 mg/kg, with the peak at RRT 1.13 being most prominent for all samples, followed by the peak at 1.15.

Sunflower oil was characterized by the presence of at least 5 peaks at >100 mg/kg in the range RRT 1.11–1.18. This was true for both high-oleic and high-linoleic acid sunflower oils, although the differences in fatty acid composition are reflected in differences in the relative intensities of peaks in this region.

In general, the differences in composition of the steryl ester fraction between oil types are much greater than differences between different samples of the same oil type. Olive oil appears to show quite a wide variation in steryl ester content.

TABLE 5
Mean Sterol Composition (%) and Content (mg/kg) in Vegetable Oils

Sterol	Corn	Groundnut	Olive	Rapeseed	Soybean	Safflower	Oleic sunflower	Linoleic sunflower	Cottonseed
Brassicasterol	trace	3.6	trace	14.3	trace	5.4	trace	trace	2.9
Campesterol	18.9	12.5	4.8	27.6	25.9	10.6	7.7	6.5	8.6
Stigmasterol	7.6	8.5	1.6	0.0	16.8	6.9	10.4	7.8	2.3
Sitosterol	60.4	58.3	64.9	52.3	44.8	43.3	60.6	56.1	75.8
Δ^5-Avenasterol	7.8	9.6	10.0	2.5	6.5	2.5	10.6	14.8	7.2
Δ^7-Stigmasterol	1.8	3.1	5.2	2.3	1.4	23.7	5.8	7.1	1.6
Δ^7-Avenasterol	2.1	4.5	5.6	0.9	2.8	7.5	3	5.9	1.7
Total (mg/kg)	9700	3000	1900	6900	4600	2000	4600	4100	4600

TABLE 6
Mean Fatty Acid Content of Vegetable Oils

Fatty acid	Corn	Groundnut	Olive	Rapeseed	Soybean	Palm	Safflower	Oleic sunflower	Linoleic sunflower	Cottonseed
14:0	0.0	trace	trace	trace	0.1	1.2	0.4	trace	0.1	0.8
16:0	11.7	10.5	10.9	4.5	10.3	45.3	7.5	3.8	5.9	25.4
16:1	0.1	trace	0.5	0.2	0.1	0.1	0.1	0.1	0.1	0.5
18:0	2.2	3.4	3.7	2.4	3.5	4.2	2.4	3.7	2.3	2.2
18:1	31.6	56.9	76.6	64.5	21.3	38.1	12.7	86.4	37	15.5
18:2	53.0	26.5	6.8	17.7	55.2	10.5	76.6	5.4	54.3	55.1
18:3	0.8	0.0	0.6	8.6	9.4	0.4	0.3	0.3	0.3	0.3
20:0	0.4	1.1	0.4	0.8	0.3	0.0	0.0	0.0	0.0	0.2
20:1	0.1	0.8	0.3	0.6	0.1	0.3	0.0	0.1	0.0	0.0
22:0	0.0	0.7	trace	0.1	0.0	0.0	0.0	0.0	0.0	0.0
22:1	0.0	0	trace	0.5	0.0	0.0	0.0	0.0	0.0	0.0

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