Sterol Fractions in Hazelnut and Virgin Olive Oils and 4,4′-Dimethylsterols as Possible Markers for Detection of Adulteration of Virgin Olive Oil

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ABSTRACT: Reports on the methylsterol fractions of hazelnut oils are scarce. The objectives of this study were to characterize methylsterols in hazelnut and virgin olive oils and to study the possibility of detection of adulteration of virgin olive oils. In hazelnut oils, 4-desmethylsterols were present in higher proportions (86 to 91%) than in virgin olive oils where this fraction was *ca.* 50% of the total sterol. In the 4-monomethylsterol fraction, citrostadienol was the major component in both kinds of oils followed by cycloeucalenol and obtusifoliol in virgin olive oils, and obtusifoliol in hazelnut oils. 24-Methylenecycloartanol was predominant in both kinds of oils in the 4,4′-dimethylsterols. For the first time, δ-amyrin was tentatively identified by comparing published mass spectral data in the analyzed samples of both kinds of oils. An unknown compound X (containing a lupane skeleton) and lupeol were detected only in the 4,4′-dimethylsterols fraction of hazelnut oils at a level of 2–8 and 6–10%, respectively. GC–MS analysis showed that adulteration of virgin olive oil by hazelnut oil could be detected at a level less than 4% by using these two compounds as possible potential markers.

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Olive oils, particularly virgin olive oils, are highly valued because they are traditionally pressed from olives without the use of heat and because they are considered to be better tasting and nutritionally favorable (1,2). Because of the high price of virgin olive oils, there is a great temptation to adulterate them with oils of similar FA and sterol profiles (3). It is estimated that in the European Union a loss of 4 million Euros per year occurs because of this adulteration (4). Hazelnut oil has been used to adulterate olive oils owing to the similarity of its composition of TAG, FA, and major sterols to those of olive oils (5,6).

Free Δ^7-sterols in combination with ΔECN42 (calculated from the difference between the theoretical and experimental equivalent carbon number 42 in TAG) and esterified sterol fraction have been applied to discriminate virgin olive oil and a mixture of virgin olive oil with 10% hazelnut oil (5,7). It has also been reported that hazelnut oil at levels lower than or equal to 5% could not be detected in olive oils by using FA profile, ΔECN42, and parameters based on the differences in TAG and FA composition (6).

Polar components of hazelnut and virgin olive oils have been studied for tracing adulteration (8). But owing to the large variability of the marker component in the polar fraction of pressed hazelnut oils, this method could not be used for the quantitative determination of the level of adulteration. Filbertone (*E*-5-methylhept-2-en-4-one) has been suggested as a marker; however, use of this compound cannot detect crude hazelnut oils at concentrations less than 7% in virgin olive oil (9).

In a recent multinational European Union project, methods to detect adulteration of olive oil with hazelnut oil were evaluated. Among the parameters summarized was the use of an empirical mathematical model whose variables were based on the amounts of the three 4-desmethylsterols found in the oil as sterol esters and free sterols: campesterol, Δ^7-stigmasterol, and Δ^7-avenasterol. This method has drawbacks, as false positives occur that confuse the analysis, particularly when oil from roasted hazelnuts is present or an adulteration of less than 5% occurs (9).

The sterol fraction of vegetable oils can be divided into three main groups: 4-desmethylsterols, 4-monomethylsterols, and 4,4′-dimethylsterols (triterpene alcohols) (10). Structural formulae of sitosterol (a 4-desmethylsterol), citrostadienol (a 4-monomethylsterol), and 24-methylenecycloartanol (a 4,4′-dimethylsterol) are shown in Scheme 1. Details of the composition of different sterols of olive oils are known (10–13). In contrast, published papers have mainly focused on the 4-desmethylsterol fraction of hazelnut oils (14–17). Only a few papers have been published on the 4-monomethyl and 4,4′-dimethylsterols content of hazelnut oils (18–20).

The main objective of this paper was to carry out a qualitative and quantitative characterization of 4-desmethyl-, 4-monomethyl-, and 4,4′-dimethylsterols with emphasis on 4,4′-dimethylsterols of some selected samples of hazelnut and virgin olive oils collected from different countries. This was done to determine whether any characteristic sterol component could be used as a marker of adulteration of virgin olive oils with hazelnut oils. After saponification of oils, total sterols were fractionated by preparative TLC followed by GC and GC–MS analyses.

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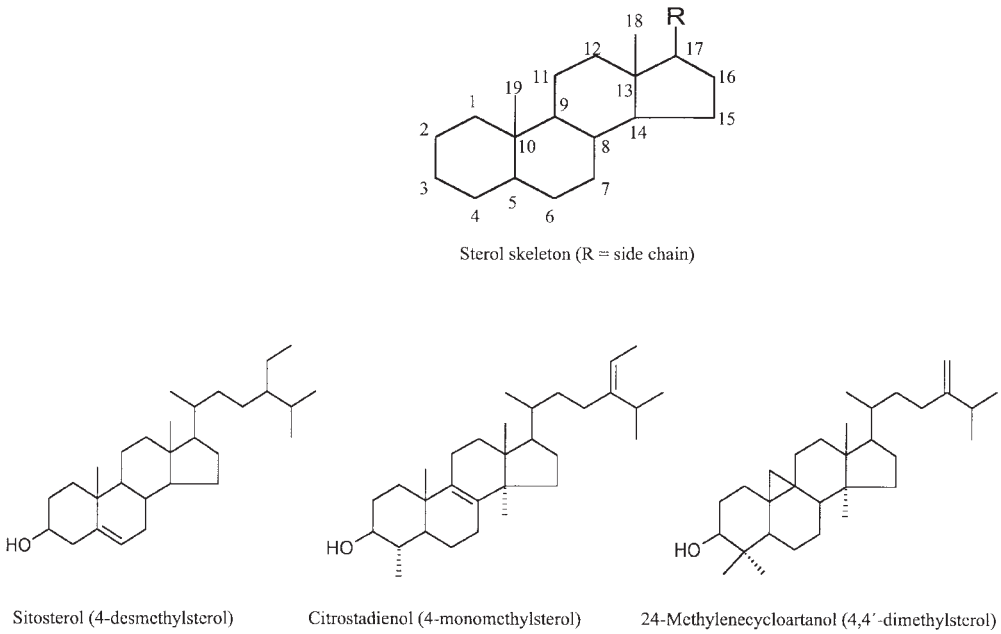

SCHEME 1

MATERIALS AND METHODS

Samples. Hazelnuts were collected from Iran (Rodsar, Iran), and Italian hazelnuts (Besana, Italy) were bought from a local market (Uppsala, Sweden), a refined and winterized hazelnut oil from Turkey (Ordu Soya Industries, Inc., Ordu, Turkey), a cold-pressed hazelnut oil sample (BayOils Co., Blenheim, New Zealand), and a hazelnut oil made from fresh-roasted hazelnuts (Hazelwood Hazelnuts, Amberley, New Zealand). Virgin olive oil samples from Italy (Bertolli, Italy) and Spain (Sierra de Genave, Genave, Jaén- Spain) were obtained from a local market (Uppsala, Sweden). Another virgin olive oil sample was obtained from Norwood Olive Oil (Canterbury, New Zealand).

Oil extraction. Oil samples were extracted from hazelnuts originating from Iran and Italy according to the method described by Savage *et al.* (17). In brief, chopped nuts (*ca.* 10 g) were extracted with 30 mL hexane/isopropanol (3:2, vol/vol) at room temperature with vigorous shaking for 1 h in steel tubes containing four steel balls to facilitate homogenization. After filtration of homogenates through defatted filter papers on a Buchner funnel under vacuum, the residues were washed twice with 20 mL of the same solvent; then 35 mL of 6.7% sodium sulfate was added, and the upper layer was separated and rotary-evaporated under reduced pressure at 40°C. The extracted oils were stored at −20°C for further analysis.

Saponification for sterol analysis. Saponification for sterol analysis was done according to the method described by Savage *et al.* (17). The weighed oil sample (*ca.* 0.1 g) was mixed thoroughly with 10 mL of 2 M KOH in 95% ethanol in ground-glass-stoppered tubes and shaken in a water bath at 60°C for 45 min. After cooling the tubes under running cold water, 10 mL of water was added and the solution was extracted three times with 10 mL diethyl ether. The combined extracts were washed once with 0.5 M KOH in ethanol and again washed with distilled water. The diethyl ether layer was passed through anhydrous sodium sulfate. The solvents were removed in a rotary vacuum evaporator at 30°C. The dry unsaponifiable matter was dissolved in 0.5 mL dichloromethane for further analysis.

Sterol fractionation by preparative TLC. Fractionation of sterols was carried out using a modified method described by Kornfeldt and Croon (12). The unsaponifiable materials were dissolved in dichloromethane and then applied to the TLC plate (Silica gel 60, 20 × 20 cm, 0.25 mm thickness; Merck, Darmstadt, Germany). To correctly identify the sterol bands, a reference sample of purified sterol fractions was applied by alongside of the sample band on the TLC plate. The plate was developed twice in hexane/diethyl ether/acetic acid (70:30:1). After developing, the reference band was exposed to iodine vapor while the sample area was covered with a glass plate. On the basis of the reference spots, three zones (4-desmethyl-, 4-monomethyl-, and 4,4′-dimethylsterols) were identified and marked out. The zones on the TLC were then scraped off and, after adding 20 μg 5-α-cholestane (Sigma Chemical Co., St, Louis, MO) as internal standard, each fraction was extracted three times with 2 mL dichloromethane. The solvent was then evaporated to dryness using N_2.

Preparation of trimethylsilyl (TMS) ether derivatives of sterol fractions. TMS ether derivatives of sterol fractions were prepared according to method described by Savage *et al.* (17). Each fraction was derivatized to TMS ether by adding 100 μL of Tri-Sil reagent (Pierce Chemical Co., Rockford, IL) and mixing in an ultrasonic bath, incubating at 60°C for 45 min, and then resuspending again using the ultrasonic bath. Thereafter, the solvent was evaporated under a stream

of nitrogen, and the TMS ether derivatives were dissolved in 200 µL hexane for further analysis by GC and GC–MS.

Analysis of sterol fractions by GC. For this purpose, a fused-silica capillary column DB-5MS 30 m × 0.25 mm, 0.50 µm (J&W Scientific, Folsom, CA) was used. The column was connected to a Chrompack CP 9001 gas chromatograph (Chrompack, Middelburg, The Netherlands) equipped with an FID. The analysis conditions were: (i) injector 260°C, (ii) oven 60°C for 1 min, rate 40º C/min, final temperature 310°C for 27 min, (iii) helium as a carrier gas and nitrogen as a makeup gas at a flow rate of 30 mL/min, (iv) detector 310°C. The peak areas were integrated by Mastro version 2.4 (Chrompack), and quantification was done relative to the 5-α-cholastane internal standard. All samples were analyzed in triplicate, and mean results are reported.

Analysis of sterol fractions by GC–MS. The GC–MS analyses were performed on a GC8000 Top Series gas chromatograph (Thermo Quest Italia S.P.A., Rodano, Italy) coupled to a Voyager mass spectrometer with MassLab data system version 1.4V (Finnigan, Manchester, United Kingdom). The column and conditions for the analysis were the same as used for GC analysis. The mass spectra were recorded at an electron energy of 70 eV, and the ion source temperature was 200°C. The spectra were scanned in the range of *m/z* 50–600. The sterols were identified by comparing the mass spectra with pure samples of cholesterol, campesterol, stigmasterol, brassicasterol, sitosterol, and cycloartenol. The other sterols were identified by comparing the mass spectra with published values (12,21–23).

Recovery test. The recovery of sterol fractions was evaluated to determine the level of sterol loss during the workup procedure. Authentic samples were supplied as a mixture of reference sterols (campesterol, stigmasterol, and sitosterol) (Research Plus, Inc., Bayonne, NJ). Unsaponifiable materials were fractionated by TLC; the 4-desmethylsterols fraction was analyzed by GC as described above. The same amount of authentic sample was also derivatized to TMS ether and analyzed by GC in triplicate. The recovery level was calculated individually for stigmasterol, campesterol, and sitosterol.

A preliminary study to detect hazelnut oil in virgin olive oil. Mixtures of hazelnut and virgin olive oils were prepared at two levels. A hazelnut oil sample (Hazelwood Hazelnuts) was mixed with a virgin olive oil sample (Norwood olive oil) at 3.5 and 5% (w/w). The 4,4-dimethylsterol fraction was analyzed by GC–MS following the sample workup procedure as described earlier.

RESULTS AND DISCUSSION

Hazelnut oils from Iran, Italy, New Zealand, and Turkey and virgin olive oils from Italy, New Zealand, and Spain were characterized for their detailed sterol composition. Since detailed reports on sterol fractions and their levels in olive oils are available, we have included only a few olive oil samples in this study.

After separation by preparative TLC (Fig. 1), the three sterol fractions were analyzed by GC and GC–MS. The main reason for the separate analyses of the three sterol fractions by GC is that some 4-desmethyl-, 4-monomethyl-, and 4,4′-dimethylsterols could not be separated on the GC column (Tables 1–3). For example, sitosterol and δ-amyrin [relative retention time (RRT) = 1.43]; Δ^5-avenasterol, Δ^7-(4)-monomethylsterol, and taraxerol (RRT = 1.45); and Δ^7- and Δ^8-(4)-monomethylsterols and Δ^7-(4,4′)-dimethylsterols (RRT = 1.58) were overlapped. Moreover, 4-monomethyl- and 4,4′-dimethylsterols in vegetable oils are generally present in much lower amounts compared with 4-desmethylsterols. For this reason, it was necessary to enrich 4-monomethyl- and 4,4′-dimethylsterols from total sterols.

Considerable quantitative differences were observed in the relative proportions of sterol fractions for both types of oils, based on GC analysis (Table 4). In hazelnut oils, 4-desmethylsterols had the highest portion (ranging from 86 to 91%), and 4-monomethyl and 4,4′-dimethylsterols had lower amounts (4–8 and 3–8%, respectively) of total sterols. Olive oils showed the lowest amount of 4-monomethylsterols (9–11%) while 4-desmethyl and 4,4′-dimethylsterols constituted 51–57 and 32–40%, respectively, of total sterol. Total unsaponifiables of hazelnut and virgin olive oil samples were separated by preparative TLC to enrich each sterol fraction (Scheme 1). It was also visually observed on the TLC plate that quantitative differences among sterol fractions in hazelnut and olive oils were considerable (Fig. 1). The 4-desmethylsterol contents of hazelnut oils were qualitatively and quantitatively rather similar to those of virgin olive oils. But 4-monomethyl and 4,4′-dimethylsterols were about 3 and 8 times lower, respectively, in hazelnut oils

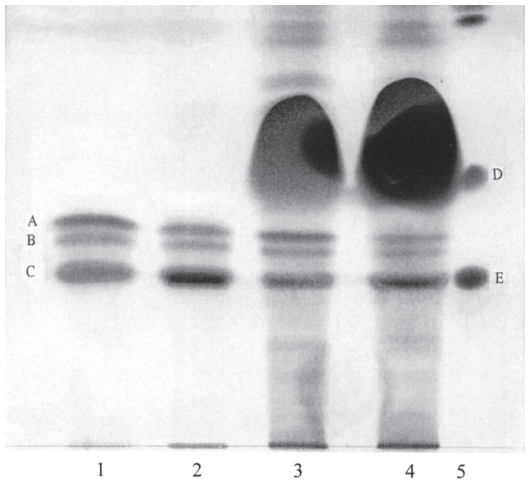

FIG. 1. Separation of sterol fractions by TLC showing quantitative differences in sterol fractions of hazelnut oil and virgin olive oil. For TLC conditions see the Materials and Methods section. Lane 1, purified sterol fractions of virgin olive oil; lane 2, purified sterol fractions of hazelnut oil; lanes 3 and 4, total unsaponifiables extracted from virgin olive and hazelnut oils, respectively; lane 5, mixture of standard lipid samples containing 4-desmethylsterol (cholesterol) and FFA. A, 4,4′-Dimethylsterol fraction; B, 4-monomethylsterol fraction; C, 4-desmethylsterol fraction; D, FFA; E, 4-desmethylsterol (cholesterol).

TABLE 1
Contents of 4-Desmethylsterol Fraction of Hazelnut and Virgin Olive Oil Samples[a]

		Hazelnut oil µg/g oil (%)					Virgin olive oil µg/g oil (%)		
Compound	RRT[b]	NZ (BayOils)[c]	NZ (Hazelwood)[c]	Iran[d]	Italy[d]	Turkey[e]	Italy[f]	New Zealand[f]	Spain[f]
Cholesterol	1.19	4.3	4.1	6.2	6.2	6.9	6.1	3.6	7.6
		(0.8)	(0.6)	(0.8)	(0.6)	(1.0)	(1.2)	(0.4)	(1.3)
Campesterol	1.30	33.8	41.3	39.4	40.3	33.5	18	29.6	20.2
		(5.5)	(4.4)	(5.2)	(3.9)	(4.5)	(3.3)	(3.5)	(3.5)
Campestanol	1.32	2.0	2.6	2.4	2.1	3.5	1.5	2.1	1.9
		(0.3)	(0.3)	(0.3)	(0.2)	(0.5)	(0.2)	(0.2)	(0.2)
Stigmasterol	1.34	4.4	5.2	8.1	12.1	10.3	4.2	4.5	4.3
		(0.8)	(0.7)	(1.1)	(1.3)	(1.3)	(0.6)	(0.6)	(0.5)
Sitosterol	1.43	476.2	649.2	635.1	784.6	554.8	413.8	756.6	507.7
		(83.5)	(85.9)	(83.4)	(78.4)	(78.6)	(81.0)	(83.8)	(87.8)
Δ^5-Avenasterol	1.45	24.8	38.8	39.5	74.5	38.1	50.6	66.5	31.2
		(4.5)	(4.8)	(5.1)	(7.1)	(5.4)	(10.1)	(8.4)	(5.4)
$\Delta^{5,24}$-Stigmastadienol	1.48	3.4	3.2	3.8	11.3	5.0	2.4	2.9	2.5
		(0.5)	(0.4)	(0.5)	(0.9)	(0.7)	(0.5)	(0.2)	(0.3)
Δ^7-Stigmastenol	1.50	1.7	1.4	2.1	5.4	2.2	1.0	0.7	0.7
		(0.3)	(0.2)	(0.3)	(0.5)	(0.4)	(0.2)	(0.1)	(0.1)
Δ^7-Avenasterol	1.52	2.6	2.1	5.1	6.1	7.2	0.8	1.1	0.8
		(0.3)	(0.3)	(0.6)	(0.6)	(2.6)	(0.1)	(0.1)	(0.1)
Unknown		40.7	13.7	15.7	41.4	52.6	7.6	16.8	8.5
		(6.7)	(1.6)	(2.1)	(4.2)	(7.4)	(1.5)	(2.0)	(1.5)
Total 4-desmethylsterols		593.2	761.6	757.0	984.0	715.1	506.0	884.4	585.4

[a]Means of triplicate analyses (CV is generally less than 5%).
[b]Relative retention time compared with 5-α-cholastane.
[c]Two commercial samples of cold-pressed hazelnut oils from New Zealand.
[d]One hazelnut sample from each country, oil extracted in the laboratory (see Materials and Methods section).
[e]A commercial hazelnut oil sample from Turkey (refined and winterized).
[f]A commercial sample of virgin olive oil from each country.

than in olive oils. Similar results were demonstrated previously on the levels of 10 individual 4-monomethyl and 4,4′-dimethylsterols, which were up to nine times lower in hazelnut oils than in olive oil (19).

To check on the loss of sterols during enrichment, a recovery test was also performed under conditions similar to those used to fractionate the total unsaponifiable matter of oils. The recoveries were 61, 61, and 65% for campesterol, stigmasterol,

TABLE 2
Contents of 4-Monomethylsterol Fraction of Hazelnut and Virgin Olive Samples[a]

		Hazelnut oil µg/g oil (%)					Virgin olive oil µg/g oil (%)		
Compound	RRT[b]	NZ (BayOils)[c]	NZ (Hazelwood)[c]	Iran[d]	Italy[d]	Turkey[e]	Italy[f]	New Zealand[f]	Spain[f]
Obtusifoliol	1.42	2.6	4.8	6.0	5.2	10.8	9.4	21.1	14.4
		(10.1)	(9.1)	(19.8)	(9.1)	(16.5)	(10.6)	(13.0)	(13.2)
Δ^7-Sterol	1.45	0.9	0.7	1.1	0.4	1.0	2.7	5.1	2.6
		(2.2)	(1.5)	(3.9)	(0.6)	(1.5)	(2.9)	(3.1)	(2.3)
Gramisterol	1.51	1.6	2.8	0.7	1.9	4.1	4.2	8.3	6.8
		(4.9)	(6.0)	(1.9)	(3.3)	(6.3)	(4.2)	(5.2)	(6.2)
Cycloeucalenol	1.53	Trace[g]	Trace	Trace	Trace	Trace	12.7	10.4	16.4
							(14.0)	(7.9)	(14.4)
Δ^7- and Δ^8-Sterol[h]	1.58	1.6	1.5	1.4	4.2	4.5	1.6	2.0	2.5
		(4.6)	(2.4)	(4.3)	(7.0)	(6.7)	(1.8)	(1.8)	(2.2)
$\Delta^{7,22}$-Sterol	1.62	0.8	1.4	0.7	1.8	1.7	2.8	4.4	2.3
		(2.3)	(3.4)	(2.3)	(3.0)	(0.7)	(3.0)	(2.5)	(2.2)
Citrostadienol	1.69	11.2	19.9	12.1	29.7	30.1	40.2	80.9	43.3
		(39.0)	(49.3)	(40.0)	(51.5)	(45.6)	(44.1)	(50.8)	(38.5)
Unknown		9.8	9.4	8.9	13.4	13.4	17.7	20.0	19.0
		(34.4)	(23.2)	(28.8)	(23.6)	(20.4)	(19.4)	(13.1)	(17.7)
Total 4-monomethylsterols		28.5	40.5	30.9	56.6	65.6	91.3	152.2	107.3

[a]Means of triplicate analyses (CV is generally less than 5%).
[b-f]See Table 1.
[g]Trace amount (<0.5 µg/g).
[h]Δ^7-Sterol and Δ^8-sterol overlapped.

TABLE 3
Contents of 4,4′-Dimethylsterol Fraction of Hazelnut and Virgin Olive Samples[a]

		Hazelnut oil µg/g oil (%)					Virgin olive oil µg/g oil (%)		
Compound	RRT[b]	NZ (BayOils)[c]	NZ (Hazelwood)[c]	Iran[d]	Italy[d]	Turkey[e]	Italy[f]	New Zealand[f]	Spain[f]
X[g]	1.40	4.4	5.5	1.7	1.9	1.1	ND[h]	ND	ND
		(8.5)	(7.5)	(3.8)	(6.1)	(2.3)			
δ-Amyrin	1.43	4.6	7.0	2.5	1.5	2.5	10.5	20.1	12.3
		(8.7)	(10.4)	(5.9)	(5.9)	(5.7)	(2.6)	(3.5)	(3.7)
Taraxerol	1.45	ND	ND	ND	ND	ND	6.7	23.9	7.0
							(1.7)	(3.4)	(2.5)
β-Amyrin	1.47	1.6	2.0	1.1	1.2	1.4	25.7	58.3	30.7
		(3.2)	(2.8)	(2.5)	(3.9)	(3.1)	(6.1)	(8.9)	(9.8)
Cycloartenol	1.54	5.8	4.6	2.3	2	3.6	151.9	182.1	75.0
		(10.7)	(8.6)	(5.2)	(6.5)	(8.1)	(35.2)	(26.0)	(22.0)
Lupeol	1.56	5.7	8.5	8	3.4	8.9	ND	ND	ND
		(11.8)	(13.6)	(18.1)	(13.5)	(18.8)			
Δ^7-Sterol	1.58	1.3	1.8	1.1	1.3	1.3	2.5	2.4	2.3
		(2.6)	(2.6)	(2.5)	(4.5)	(2.9)	(0.6)	(0.4)	(0.8)
24-Methylene-cycloartanol	1.64	11.7	18.1	10.8	6.2	10.1	168.5	302.8	173.4
		(19.2)	(28.3)	(25.1)	(19.2)	(19.5)	(36.3)	(44.8)	(52.8)
Unknown		17.2	19.0	16.5	13.4	17.7	36.8	105.2	28.5
		(32.9)	(28.6)	(37.5)	(43.3)	(37.9)	(9.8)	(15.2)	(8.3)
Total 4,4′-dimethylsterols		52.3	66.5	44.0	30.9	46.6	402.6	694.7	329.2

[a]Means of triplicate analyses (CV is generally less than 5%).
[b–f]See Table 1.
[g]Compound unknown X detected exclusively in hazelnut oil samples.
[h]Not detected.

and sitosterol, respectively. Losses may have occurred during the extraction of unsaponifiable matter from the saponified oil sample and the recovery of sterol fractions in silica gel scraped from TLC plates. Further efforts to optimize the recoveries of the sterol fractions were not carried out.

Sitosterol was the predominant sterol in both kinds of oils. Hazelnut oils contained higher amounts of campesterol, Δ^7-stigmastenol and $\Delta^{5,24}$-stigmastadienol and lower amounts of Δ^5-avenasterol except for Italian hazelnut oil, compared with virgin olive oils (Table 1). These results are generally comparable with published results (9,15,24).

The results from the purified 4-monomethylsterol fraction in hazelnut and olive oils are shown in Table 2. In general, the total and identified 4-monomethylsterols in Turkish hazelnut oil are considerably higher among the hazelnut oil samples. Citrostadienol was the major 4-monomethylsterol in both kinds of oils followed by obtusifoliol. In virgin olive oils cycloeucalenol content was similar to the level of obtusifoliol. In contrast, cycloeucalenol was present at only a trace level in all hazelnut oil samples. In the literature the presence of obtusifoliol is controversial. It has been reported that obtusifoliol was not detected in an unspecified variety of hazelnut oil (15), but that it was present up to 18 and 6 µg/g in European and Turkish hazelnut oils, respectively (19). In this study, we confirmed the presence of obtusifoliol by GC–MS analysis by comparing our data with published MS data. In both types of oils, quite a

TABLE 4
Contents of Sterol Fractions in Hazelnut and Virgin Olive Oil Samples[a]

	Sterol fractions µg/g oil (%)		
Sample	4-Desmethyl	4-Monomethyl	4,4′-Dimethyl
Hazelnut oils			
NZ (BayOils)[b]	593.2	28.5	52.3
	(88.0)	(4.2)	(7.8)
NZ (Hazelwood)[b]	761.6	40.5	66.5
	(87.7)	(4.7)	(7.6)
Iran[c]	757.0	30.9	44.0
	(90.9)	(3.7)	(5.3)
Italy[c]	984.0	56.6	30.9
	(91.8)	(5.3)	(2.9)
Turkey[d]	715.1	65.6	46.6
	(86.4)	(7.9)	(5.6)
Olive oils			
Italy[e]	506.0	91.3	402.6
	(50.6)	(9.1)	(40.3)
New Zealand[e]	884.4	152.2	694.7
	(51.1)	(8.8)	(40.1)
Spain[e]	585.4	107.3	329.2
	(57.3)	(10.5)	(32.2)

[a]Means of triplicate analyses (CV is generally less than 3%).
[b]Two commercial samples of cold-pressed hazelnut oils from New Zealand.
[c]One hazelnut sample from each country; oil was extracted in the laboratory (see the Materials and Methods section).
[d]A commercial hazelnut oil sample from Turkey (refined and winterized).
[e]A commercial sample of virgin olive oil from each country.

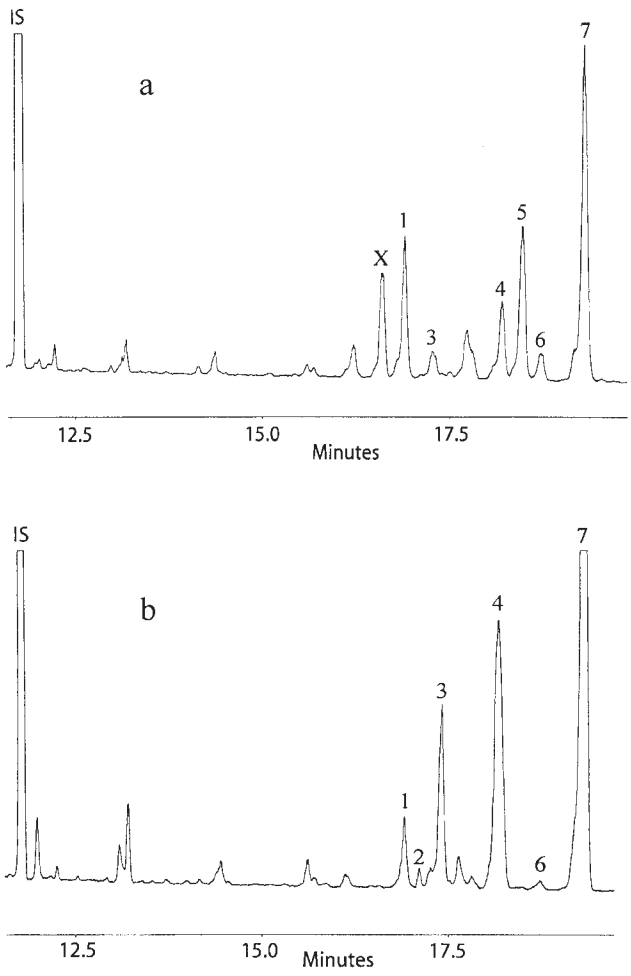

FIG. 2. Gas chromatograms showing separation of trimethylsilyl ether derivatives of the purified 4,4′-dimethylsterols of hazelnut oil (a) and virgin olive oil (b). For GC conditions see the Materials and Methods section. Peaks: IS, internal standard (5-α-cholastane); X, unknown compound (detected only in hazelnut oil samples); 1, δ-amyrin; 2, taraxerol (detected only in virgin olive oil samples); 3, β-amyrin; 4, cycloartenol; 5, lupeol (detected only in hazelnut oil samples); 6, Δ^7-sterol; 7,24-methylencycloartanol.

few unidentified 4-monomethylsterols were observed. Their levels were in the range of 10–30%. All of these unidentified peaks were common in both types of oils, and further attempts to identify them were not made. Only a few reports (18,19) record values for 4-monomethylsterol compositions in hazelnut oils, and our results confirm these values.

The composition and amounts of the 4,4′-dimethylsterols of hazelnut and virgin olive oils are presented in Table 3. The total amount of 4,4′-dimethylsterols was about 90% lower in hazelnut oils than olive oils. 24-Methylenecycloartanol was the major component, 19–28 and 36–53% in hazelnut and virgin olive oils, respectively. Cycloartenol and β-amyrin were the other predominant components in virgin olive oils, 22–25 and 6–10%, respectively. In hazelnut oils, lupeol and cycloartenol were the other two major components, 12–19 and 5–11%, respectively. GC separation of purified 4,4′-dimethylsterols of hazelnut oil and virgin olive oils is shown in Figure 2. All the identified peaks were well separated by the GC capillary column used in this study. From these chromatograms, one can clearly see that an unknown compound, X, and lupeol (peak 5) were exclusively detected in hazelnut oils (Fig. 2, Table 3). These two compounds are present in lower amounts in Italian hazelnut oil compared with other samples. Lupeol has been reported in hazelnut oils (*Corylus avellana* and *C. maxima*) at levels of 17–38% in the 4,4′-dimethylesterols fraction (18–20).

Similar to 4-monomethylsterols, negligible research has been carried out on the level and composition of 4,4′-dimethylsterols in hazelnut oil. It has been reported that 4,4′-dimethylsterol of *Corylus* oils contained β-amyrin (6–8%), cycloartenol (13–14%), lupeol (30–38%), and 24-methylenecycloartanol (35–43%) (18). Ollivier *et al.* (20) reported that β-amyrin (5.5%), butyrospermol (5.8%), α-amyrin (6.7%), lupeol (16.6%), cycloartenol (13.9%), and 24-methylenecycloartanol (46.9%) were present in hazelnut oils. Sanchez *et al.* (19) quantified cyclobranol, β-amyrin, butyrospermol, cycloartenol, and 24-methylenecycloartanol in hazelnut oils. Our results on the amounts of detected and identified compounds generally concur with those studies except with respect to the amount of β-amyrin (12 to 192 μg/g oil), which was considerably lower (1–2 μg/g oil) in this study (Table 3). In addition, we could not detect α-amyrin, cyclobranol, and butyrospermol in the samples of hazelnut oils as were reported (19,20). 24-Methylenecycloartanol was reported as a major compound in addition to cycloartenol, β-amyrin, cyclobranol, butyrospermol, taraxerol, and α-amyrin in olive oils (10–13). Our results generally concur with those published reports except that we could not detect cyclobranol, butyrospermol, and α-amyrin in these virgin olive oil samples.

The mass spectra of the known compounds in hazelnut and virgin olive oils—δ-amyrin, β-amyrin, cycloartenol, Δ^7-sterol, 24-methylenecycloartanol, and taraxerol—are shown in Figure 3a–f, respectively. The identification of these compounds concurs with previously published MS data (12,21–23). We have also used an authentic sample of cycloartenol to check the identity of this compound in GC and in GC-MS analyses (peak no. 4, Figs. 2a, 2b).

From the published mass spectrum (22), we have tentatively identified δ-amyrin in both oils. The levels of δ-amyrin were 2–7 and 11–20 μg/g in hazelnut and virgin olive oils, respectively (peak 1, Figs. 2a, 2b). The mass spectrum of δ-amyrin showed a molecular ion at *m/z* 498 (25%), a base peak at *m/z* 189 (100%), and the typical ions *m/z* 190 (75%), 203 (17%), and 218 (10%) (Fig. 3a), which concurs with published data (22). Further studies are necessary to confirm the identity of δ-amyrin. To our knowledge, δ-amyrin has not been reported previously in hazelnut and olive oils (10–13,25)

An unknown compound X was eluted close to δ-amyrin in GC analysis of the 4,4′-dimethylsterol fraction (see discussion below), which was present in hazelnut oil but could not be detected in any of the olive oil samples analyzed in this study (Fig. 2a). A full-scan mass spectrum of the TMS ether derivative of this compound is shown in Figure 3g. The mass spectrum shows the molecular ion at *m/z* 498 (27%), and other

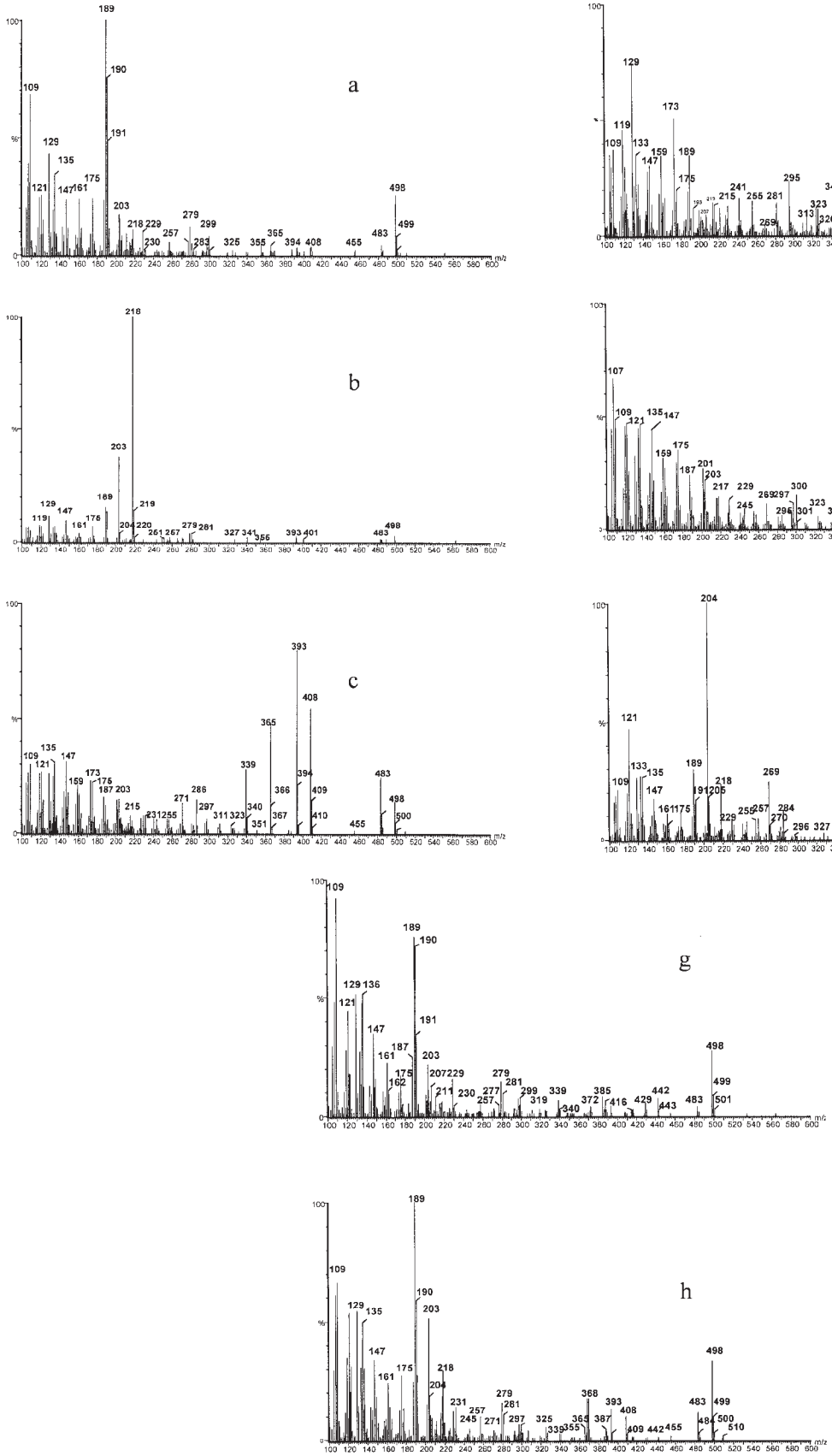

FIG. 3. Full-scan mass spectra of 4,4′-dimethylsterols fraction of hazelnut and olive oils. For GC–MS conditions see the Materials and Methods section. (a) δ-Amyrin; (b) β-amyrin; (c) cycloartenol; (d) Δ^7-sterol; (e) 24-methylencycloartanol; (f) taraxerol (detected only in virgin olive oil samples); (g) unknown compound X (detected only in hazelnut oil samples); (h) lupeol (detected only in hazelnut oil samples).

major ion fragments are at *m/z* 136 (51%), 149 (16%), 189 (76%), 190 (68%), 191 (38%), 203 (22%), 205 (6%), 218 (6%), and 483 (5%). These mass fragments were described for compounds having a lupane skeleton (14-α-methylsterols) (26). Confirmation of the identity of this unknown compound requires further study. This compound was consistently present in all hazelnut oils but absent in all olive oil samples analyzed. This compound could be used as a potential marker to identity the presence of hazelnut oil in olive oil.

In this study lupeol was exclusively detected in hazelnut oil samples at levels that were 12–19% (4-9 μg/g oil) of the 4,4′-dimethylsterols fraction. Use of this compound as a potential marker to detect hazelnut oil in olive oils has been suggested previously (20). We confirmed the identity of lupeol in all hazelnut oil samples by comparison with the published mass spectrum (Fig. 3h) (21). It was reported that α–amyrin can be used to detect 5% hazelnut oil in olive oil (20). However, we could not detect α–amyrin in any of the hazelnut oil samples analyzed. In contrast, we detected the presence at considerable levels of another potential marker (compound X) having a lupane skeleton only in hazelnut oil.

A preliminary study (results not shown) of the 4,4′-dimethylsterol fraction of the admixture of hazelnut and virgin olive oils showed that it was possible under the present analytical conditions to detect 3.5% hazelnut oil mixed with virgin olive oil by using lupeol as a marker in GC–MS. Similar to lupeol, the unknown compound X also was detected by GC–MS analysis at this level of admixture.

Qualitative and quantitative differences were found among three sterol fractions of hazelnut and olive oils in this study. At least two 4,4′-dimethylsterols have been identified in the analyzed samples of hazelnut oils that are absent in olive oils. Many known and unknown compounds have been suggested as potential markers of detection of hazelnut oil in olive oils (7–9,20). Our preliminary results show that lupeol and an unknown compound X can be used as possible markers to detect virgin olive oil adulteration with hazelnut oil at levels less than 4%.

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