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Analysis of olive and hazelnut oil mixtures by high-performance liquid chromatography–atmospheric pressure chemical ionisation mass spectrometry of triacylglycerols and gas–liquid chromatography of non-saponifiable compounds (tocopherols and sterols)

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Abstract

We analysed the triacylglycerol, tocopherol and sterol composition of hazelnut oil, olive oil and their mixtures (90% olive oil with 10% hazelnut oil, 70% olive with 30% hazelnut oil and 50% olive oil with 50% hazelnut oil). The main triacylglycerols were 1,2,3-trioleylglycerol, 2,3-dioleyl-1-palmitoylglycerol, 2,3-dioleyl-1-linoleylglycerol and 2,3-dioleyl-1-stearoylglycerol. Non-saponfiable compounds (tocopherols and sterols) were derivatised as *O*-trimethylsilyl ethers. α-Tocopherol was the main vitamin E isomer in all samples; however, small amounts of β-tocopherol and γ-tocopherol were also found. β-Sitosterol and Δ^5-avenasterol were the principal sterols in all samples; campesterol and stigmasterol were minor sterol compounds in all samples. Obtusifoliol, which was a major sterol in olive oil and oil mixtures, was not found in hazelnut oil. The discriminant analysis showed that hazelnut oil, olive oil and oil mixtures were clearly separated according to their triacylglycerol composition.

Keywords: Oils; Olive oil; Food analysis; Tocopherols; Vitamins; Sterols; Triacylglycerols

1. Introduction

Olive and hazelnut oils are mainly produced and consumed in those countries on the shores of the Mediterranean Sea. The first of these, olive oil, is one of the principal ingredients in the Mediterranean diet and is used in particular for frying, roasting and seasoning. Olive oil is frequently sold as virgin olive oil, which is the purest and most expensive of its type on sale in the supermarkets of Southern European countries. The raw material for the second of these, hazelnuts, are harvested in Turkey, Italy and Spain, the main hazelnut-producing countries in the Mediterranean area [1], during the fall. They are then dried and stored. Hazelnuts are retailed in supermarkets as raw and roasted products and used widely as an ingredient in other manufactured food products: Chocolate, ice cream, cookies, biscuits, etc. Hazelnut oil, which accounts for 60% of hazelnut kernel, is used as an ingredient in certain bakery products [2].

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Although hazelnut oil is considerably less expensive than olive oil it is rarely used as a raw ingredient in Mediterranean cuisine, and almost never for frying. Hazelnut fatty acid composition is very similar to that of olive oil [3–7]: Oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) are the main fatty acids in both oils. Nevertheless, it is difficult to distinguish between their respective fatty acid compositions as certain factors, such as climate and geographical origin influence the fatty acid composition of the hazelnut lipid fraction [7–9].

As olive oil might be adulterated with oils from other sources, including hazelnut oil, there are several methods that can be applied to identify adulterated olive oil such as: Raman spectroscopy [10,11], near-infrared spectroscopy [12,13], nuclear magnetic resonance [14–16] and high–performance liquid chromatography (HPLC) [17,18]. Recently, a particularly useful technique, i.e., combustion–gas chromatography–isotopic ratio mass spectrometry, has been developed for use with adulterated oils [15,19–21].

Ruiz et al. investigated hazelnut and olive oil mixtures based on filbertone content [22]; yet, no research work has been conducted on triacylglycerol and non-saponifiable compounds to the best of our knowledge. In this study we examine hazelnut, olive oil and their mixtures by high-performance liquid chromatography–mass spectrometry of triacylglycerols and gas–liquid chromatography of non-saponifiable compounds.

2. Experimental

2.1. Analysis of triacylglycerols

2.1.1. Samples

Hazelnut and olive oil solutions were prepared as follows: 5 g of hazelnut oil was poured into a 100 ml volumetric flask and was made up with chloroform (5%, w/v), 5 g of olive oil was poured into a 100 ml volumetric flask and also made up with chloroform (5%, w/v). Oil mixtures were prepared as follows: (a) One ml of the hazelnut oil solution (5% w/v) was mixed with 9 ml of the olive oil solution (5% w/v), (b) 3 ml of the hazelnut oil solution (5% w/v) was mixed with 7 ml of the olive oil solution (5% w/v) and (c) 5 ml of the hazelnut oil solution (5% w/v) was mixed with 5 ml of the olive oil solution (5% w/v).

2.1.2. High-performance liquid chromatography–atmospheric pressure chemical ionisation mass spectrometry of triacylglycerols

Sample oil solutions were passed through a 25 mm diameter PTFE (ethylene polytetrafluoride) luer-lock filter (0.45 μm pore) (Lida, Sant Cugat del Vallés, Barcelona, Spain). Five μl of each solution was injected into the HPLC system, which consisted of a Waters 2690 separation module (Waters Chromatography, Mildford, MA, USA). The HPLC separation system was connected to a model Platform II mass spectrometer (Micromas, Manchester, UK) fitted with an atmospheric pressure chemical ionisation (APCI) source as described by Mottram et al. [23]. This was typically operated according to the following conditions: Tip of the source at 3000 V and 400°C, source block at 180°C, counter-electrode at 100 V and 280°C, sampling cone at 50 V and a corona current of 5 μA [23]. High purity nitrogen was used as nebulizer gas at a flow of 2.5 l min^{-1}, courtain nitrogen flow was set at 6.67 l min^{-1}. Spectra were obtained over the range of 200 to 1000 u, scan duration of 2 s, inter scan delay 0.2 s, analyser pressure at less than $5 \cdot 10^{-6}$ kPa.

The stationary phase was a 0.25 m×0.46 cm I.D. Spherisorb ODS2 (octadodecylsilane) column (5 μm pore) (Supelco, Sant Cugat del Vallés, Barcelona, Spain). The mobile phase was a mixture of HPLC-grade acetone–acetonitrile (64:36, v/v) that passed through the column at a flow-rate of 1 ml min^{-1} at 25°C for 50 min as described in previous studies [24,25].

2.1.3. Identification of triacylglycerol compounds

Peaks were identified from their mass spectra produced by the total ion chromatogram based on their molecular ion ($[M+H]^+$) and diacylglycerol fragments [23,26].

2.2. Analysis of non-saponifiable constituents

2.2.1. Samples

Samples consisted of hazelnut oil, olive oil and their mixtures prepared as follows: 1.0 g of hazelnut

oil was mixed with 9.0 g of olive oil, 3.0 g of hazelnut oil was mixed with 7.0 g of olive oil and 5.0 g of hazelnut oil was mixed with 5.0 g of olive oil. Approximately 200 mg of the oil sample was weighed in a 15 cm×1.5 cm screw capped glass-test tube. Fifty μl of 5,7-dimethyltocol (5.0 mg ml^{-1} in isooctane) was mixed as internal standard (I.S.) [27]; then 8 ml of a solution of 3% (w/v) ethanolic pyrogallol was added, followed by 0.5 ml of a saturated solution of potassium hydroxide in water. The mixture was vortexed for 30 s at high speed, then heated in a water bath at 90°C for 10 min. The solution was vortexed again and cooled to room temperature. Then it was transferred into a separatory funnel. Twenty ml of cyclohexane and 12 ml of distilled water were added consecutively. The mixture was shaken gently and then centrifuged at 650 *g* in a Meditronic centrifuge (Selecta, Abrera, Spain) for 10 min at 25°C. The upper layer was suctioned with a Pasteur pipette and dried out with anhydrous sodium sulphate. Then it was filtered and concentrated in a rotary vacuum pump. The concentrated cyclohexane solution was transferred to a 10 ml screw-capped glass test tube.

2.2.2. Derivatisation of non-saponifiable constituents

The remaining cyclohexane was evaporated under a stream of nitrogen. Then 50 μl of pure dry pyridine was added followed by 50 μl of a mixture containing *N*,*O*-bis(trimethylsilyl)acetamide–trimethylsilylchlorosilane–trimethylsilylimidazole (3:2:3, v/v/v), commercially available as Sylon BTZ. This solution was shaken gently for 1 min and left at room temperature for 15 min.

2.2.3. Gas–liquid chromatography–mass spectrometry of O-trimethylsilyl ether derivatives

One μl of the sample was injected into the GC system, a Hewlett-Packard 5890 Series II connected to a HP5989A mass spectrometer. The GC system was equipped with a 30 m×0.25 mm I.D. (0.25 μm film thickness) HP-5 fused-silica capillary column (Anorsa, Barcelona, Spain) coated with a stationary phase of 5% crosslinked phenylmethylsilicone. The oven temperature was as follows: 210–250°C at a rate of 6°C min^{-1}, held at 250°C for 11 min, then 250–310°C at a rate of 3°C min^{-1}. This final temperature was held isothermally for 12 min. The injector and detector temperatures were 290 and 350°C, respectively. Grade 5.0 helium (Air Liquide España, Madrid, Spain) was used as carrier gas at a pressure of 75.0 kPa. *O*-Trimethylsilyl derivatives eluted from the column were passed into the mass spectrometer using electron impact (EI) with an ion source temperature of 350°C.

2.2.4. Gas–liquid chromatography with flame ionisation detection (FID) of O-trimethylsilyl ether derivatives

The sample, 1.5–2.0 μl, was injected into the GC system, a Sigma 2000 Perkin-Elmer (PE) GC system (Norwalk, CT, USA) with a FID system, coupled to a PE 1010 integrator. The GC system was equipped with a 25 m×0.25 mm I.D., (0.13 μm film thickness) wall-coated, open-tubular, fused-silica capillary column (WCOT) coated with a stationary phase of CP-Sil 5CB (Chrompack, Middelburg, Netherlands). The oven temperature was programmed as follows: 230–264°C at a rate of 2°C min^{-1}, held at 264°C for 5 min, then raised to 294°C at a rate of 2°C min^{-1}. This final oven temperature was maintained isothermally for 5 min. The injector and detector temperatures were 290 and 350°C, respectively. Grade 4.7 helium (Air Liquide España) was used as carrier gas at a pressure of 103.4 kPa.

O-Trimethylsilyl ether tocopherol and sterol derivatives were identified from their mass spectra, produced by the total ion chromatogram based on the molecular ion ($[M+H]^+$) and its fragmentation profile, and by comparison of their retention time, obtained by GLC–FID, to those of pure sterol and tocopherol standard *O*-trimethylsilyl ether derivatives. Quantification of *O*-trimethylsilyl ether derivatives of tocopherol and sterol compounds was performed by GLC–FID. In this way, a calibration curve was calculated using several solutions containing increasing amounts of α-tocopherol (15.0, 30.0, 45.0, 60.0 and 75.0 μg) and β-sitosterol (16.6, 41.5, 83.0, 166.0 and 249.0 μg). To each solution, 500 μg of 5,7-dimethyltocol was added as I.S. (100 μl of a solution containing 5 μg μl^{-1} of I.S. in dry isooctane).

2.3. Reagents and standards

Cyclohexane, chloroform, ethanol and isooctane were all reagent grade purchased from Panreac (Montcada i Reixach, Barcelona, Spain). Acetone and acetonitrile were both HPLC grade obtained from SDS (Peypin, France). Dry pyridine was of analytical grade obtained from Merck (Darmstadt, Germany).

Anhydrous sodium sulphate and potassium hydroxide were both analytical grade purchased from Panreac. Pyrogallol was of analytical grade purchased from Merck. Sealed ampoules of Sylon BTZ were purchased from Supelco (Alcobendas, Madrid, Spain).

Standard α-tocopherol, β-tocopherol and γ-tocopherol compounds were purchased from Merck as part of a kit. Campesterol (24α-methyl-5-cholesten-3β-ol), stigmasterol (3β-hydroxy-24-ethyl-5,22-cholestadiene), β-sitosterol (24β-ethylcholesterol), and Δ^5-avenasterol were obtained from Sigma. The I.S., 5,7-dimethyltocol was purchased from Matreya (Pleasant Gap, PA, USA).

2.4. Data processing

Statistical calculations by the one-way analysis of variance (one-way ANOVA) were carried out on data obtained by several determinations of each of the compounds.

All statistical analyses were conducted on a personal computer using the Statistical Package for Social Sciences (SPSS), version 8.0 (Hispanoportuguesa, Madrid, Spain) for Windows 95 (Microsoft Ibérica, Madrid, Spain).

HPLC mass spectral data were collected using the MassLynx software, release 2.1 (Micromass).

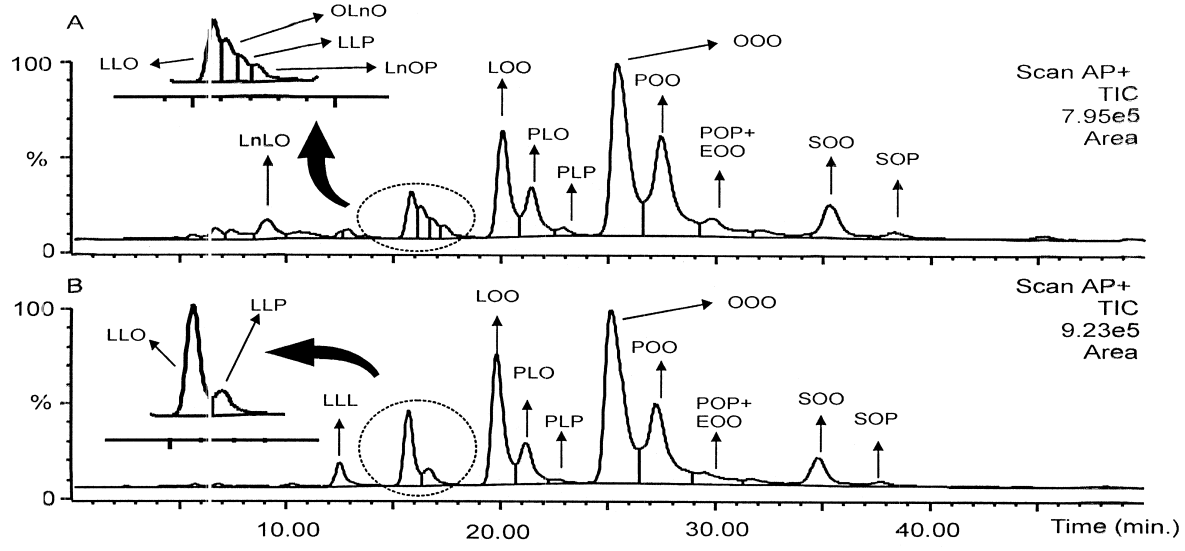

Fig. 1. Triacylglycerol profiles of olive oil (A) and hazelnut oil (B) as obtained by the hyphenated technique HPLC–APCI–MS. Peak identity: LLL, 1,2,3-trilinoleylglycerol; LnLO, 1-linolenyl-2-linoleil-3-oleylglycerol; LLO, 1,2-dilinoleyl-3-oleylglycerol; OLnO, 1,3-dioleyl-2-linoleylglycerol; LLP, 1,2-dilinoleyl-3-palmitoylglycerol; LnOP, 1-linolenyl-2-oleyl-3-palmitoylglycerol; LOO, 2,3-dioleoyl-1-linoleylglycerol; PLO, 2-linoleyl-3-oleyl-1-palmitoylglycerol; PLP, 2-linoleyl-1,3-palmitoylglycerol; OOO, 1,2,3-trioleylglycerol; POO, 2,3-dioleyl-1-palmitoylglycerol; POP, 1,3-dipalmitoyl-2-oleylglycerol; EOO, 2,3-dioleyl-1-eicosenoilglycerol; SOO, 2,3-dioleyl-1-stearoylglycerol; SOP, 2-oleyl-3-palmitoyl-1-stearoylglycerol. HPLC–MS conditions: Spherisorb ODS2 column (0.25 m×0.46 cm I.D., 5 μm pore); mobile phase, acetone–acetonitrile (64:36, v/v); instrument: A waters 2690 separation module (Mildford, MA, USA) fitted with a 100 μl Rheodyne Loop and connected to a Micromas model Platform II atmospheric pressure chemical ionisation source (Manchester, UK). Ordinate, total chemical ionisation current intensity with major peak as 100%; abscissa, time (min).

3. Results and discussion

3.1. Triacylglycerol content

Triacylglycerols were identified by their molecular ions ($[M+H]^+$) and diacylglycerol fragments observed in the HPLC–APCI–MS mass spectra [23,26,28–33]. Fig. 1 shows the total ion HPLC–APCI–MS chromatogram (total ion current, TIC) of pure hazelnut and olive oil in which the main triacylglycerols were assigned. Also, Fig. 1 shows a good separation of mayor peaks. These results are in good agreement with data collected using other detection systems by HPLC such as the refractive index detector and the light-scattering detector [24,25,34].

The area of the peaks in the chromatogram obtained recording the addition of signals in the region 880–884 u and 850–857 u in positive mode, from the TIC recorded data, mostly comprises those triacylglycerols that have linoleyl and palmitoyl fatty acid moieties. The ratio of the peak areas obtained from both chromatograms ([880–884]/[850–857]) over the range 15.00 to 20.00 min (Fig. 2) gave the following results (standard deviation in parenthesis): Olive oil = 2.91 (0.18), olive oil 90%-hazelnut oil

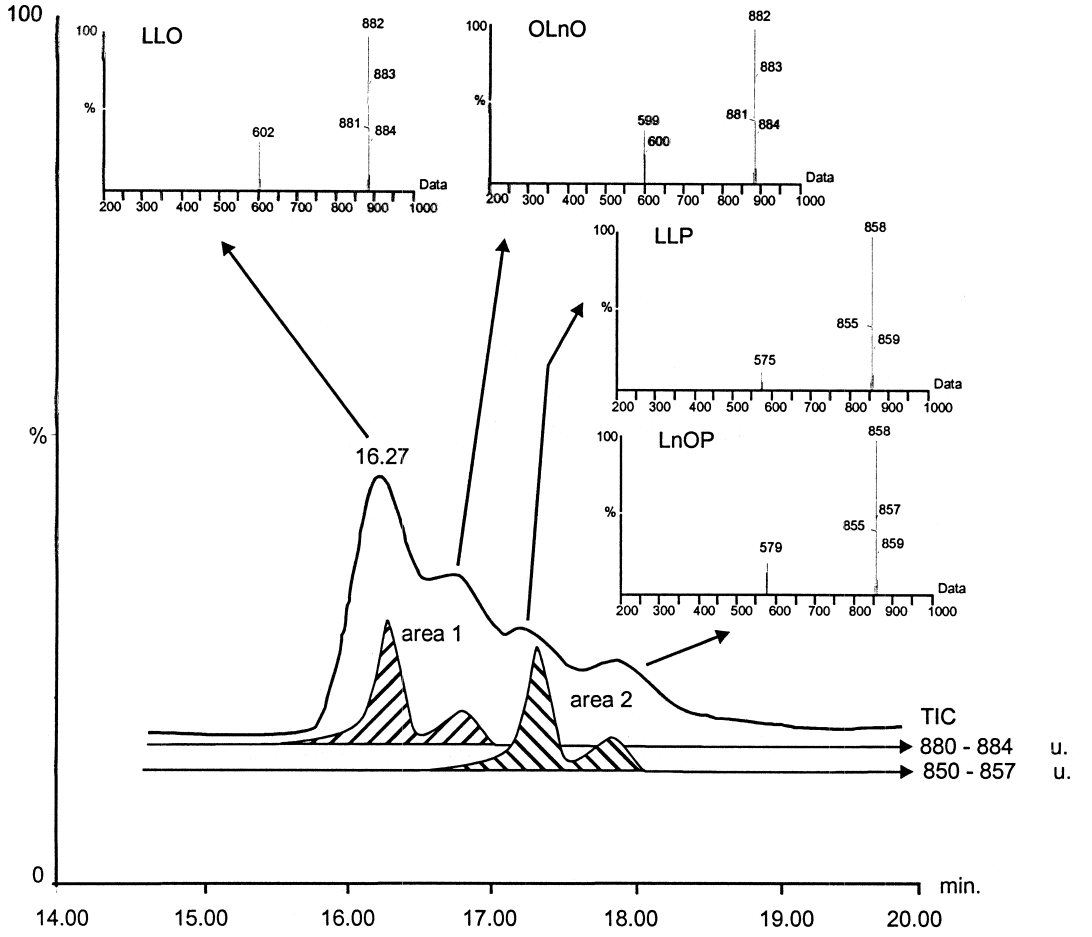

Fig. 2. As an example, in the region 15–20 min of the total ion chromatograms (TIC), differences between 880 and 884 u and 854–857 u were observed. Recording the chromatogram corresponding to both mass regions (post-process of TIC data) shows two chromatograms that were used to study the oil mixtures. The ratio of the total areas obtained from post-processed chromatograms (area 1/area 2) were used in order to recognise pure olive oil and mixtures of olive and hazelnut oil.

Table 1
Triacylglycerol content (%) of hazelnut oil, olive oil and their oil mixtures[a]

Sample	LLL		LnLO		LLO		OLnO		LLP		LnOP		LOO		PLO		PLP		OOO		POO		POP+EOO		SOO		SOP	
	x[f]	SD[g]	*x*	SD	*x*	SD	*x*	SD	*x*	SD	*x*	SD	*x*	SD	*x*	SD	*x*	SD	*x*	SD	*x*	SD	*x*	SD	*x*	SD	*x*	SD
Olive–hazelnut (90:10)	0.48	0.03	0.60	0.11	4.03[b]	0.40	2.54	0.44	1.55[b]	0.07	1.30	0.13	14.84[b]	1.09	7.56[b]	0.39	0.84[b]	0.13	33.13	0.85	22.21[b]	1.14	4.00	0.73	5.75	1.20	1.17	0.76
Olive–hazelnut (70:30)	1.20[b]	0.62	0.53	0.25	5.03	0.59	2.06[b]	0.24	1.74	0.15	1.28	0.17	15.45[b]	0.97	7.14	0.20	0.85[a]	0.05	32.81	1.21	20.20	1.25	5.44	2.42	5.51	1.01	0.78	0.19
Olive–hazelnut (50:50)	1.28[b]	0.13	0.58	0.03	5.83[c]	0.44	1.94[b]	0.31	1.74	0.17	1.13	0.11	16.49	0.56	7.20	0.23	0.73	0.08	34.08	1.35	20.59	1.01	2.57	0.15	5.12	0.35	0.73	0.22
Hazelnut oil	2.82[c]	0.24	n.d.[h]	n.d.	8.40[d]	0.46	n.d.	n.d.	2.02[c]	0.19	n.d.	n.d.	18.40[c]	0.70	6.51[c]	0.10	0.35[c]	0.30	35.26[b]	0.56	18.76[c]	0.87	2.18	0.77	4.45	0.31	0.87	0.50
Olive oil	n.d.	n.d.	0.83	0.03	4.28[e]	0.37	3.03[c]	0.21	1.57[b]	0.10	1.49	0.11	14.94[b]	0.79	7.83[b]	0.21	1.06[b]	0.15	32.05[c]	0.21	22.33[a]	0.60	4.00	1.16	5.69	0.31	0.91	0.24

[a] Data are means of duplicate results. LLL, 1,2,3-trilinoleylglycerol; LnLO, 1-linolenyl-2-linoleil-3-oleylglycerol; LLO, 1,2-dilinoleyl-3-oleylglycerol; OLnO, 1,3-dioleyl-2-linoleylglycerol; LLP, 1,2-dilinoleyl-3-palmitoylglycerol; LnOP, 1-linolenyl-2-oleyl-3-palmitoylglycerol; LOO, 2,3-dioleoyl-1-linoleylglycerol; PLO, 2-linoleyl-3-oleyl-1-palmitoylglycerol; PLP, 2-linoleyl-1,3-palmitoylglycerol; OOO, 1,2,3-trioleylglycerol; POO, 2,3-dioleyl-1-palmitoylglycerol; POP, 1,3-dipalmitoyl-2-oleylglycerol; EOO, 2,3-dioleyl-1-eicosenoilglycerol; SOO, 2,3-dioleyl-1-stearoylglycerol; SOP, 2-oleyl-3-palmitoyl-1-stearoylglycerol.

[b] Denotes statistically significant differences (Bonferroni).

[c] Denotes statistically significant differences (Bonferroni).

[d] Denotes statistically significant differences (Bonferroni).

[e] Denotes statistically significant differences (Bonferroni).

[f] *x*; mean.

[g] SD; standard deviation.

[h] n.d.; not detected.

10%=3.75 (0.16), olive oil 70%-hazelnut oil 30%= 4.29 (0.04), olive oil 50%-hazelnut oil 50%=4.83 (0.18) and hazelnut oil=6.35 (0.13) (data were means of triplicate results). The one-way ANOVA showed significant differences ($p<0.001$) between these values. These results suggests that hazelnut oil increases the value of the peak area ratio [880–884]/ [850–857] over the range 15.00 to 20.00 min (Fig. 2).

Results for triacylglycerol content (calculated according to the area percentage of each triacylglycerol from the TIC as a semiquantitative method) of oil samples are shown in Table 1. The main triacylglycerols were 1,2,3-trioleylglycerol (OOO), 2,3-dioleyl-1-palmitoilglycerol (POO), 2,3-dioleyl-1-linoleylglycerol (LOO) and 2,3-dioleyl-1-stearoylglycerol (SOO). Other minor triacylglycerols were 2,3-dioleyl-1-eicosenoylglycerol (EOO), 2-oleyl-3-palmitoyl-1-stearoylglycerol (SOP), 1,3-dipalmitoyl-2-linoleylglycerol (PLP), 1-linolenoyl-2-oleyl-3-palmitoylglycerol (LnOP), 1,2-dilinoleyl-3-palmitoylglycerol (LLP), 1,3-dioleyl-2-linolenoylglycerol (OLnO), 1-linolenoyl-2-linoleyl-3-oleylglycerol (LnLO) and 1,2,3-trilinoleylglycerol (LLL).

In relation to the main triacylglycerols (OOO, POO and LOO), hazelnut oil showed the highest value for OOO (35.26%) and LOO (18.40%), whereas olive oil and the mixture olive oil 90%-hazelnut oil 10% showed the lowest values for OOO (32.05%) and LOO (14.84%), respectively (Table 1). Olive oil showed the highest value for POO (22.33%), while hazelnut oil had the lowest value (18.76%) (Table 1). Regarding hazelnut and olive oil triacylglycerols, our results are consistent with previously published data [24,25,35–38].

A one-way ANOVA showed significant differences ($p<0.05$) between samples for: LLL, LLO, OLnO, LLP, LOO, PLO, PLP, OOO and POO contents (Table 1). Discriminant analysis using individual triacylglycerol contents as the variables showed that hazelnut oil, olive oil and oil mixture samples were grouped according to their triacylglycerol composition (Fig. 3). These results are consistent with other research in which mixtures of olive oil with other oils from seed sources were investigated on the bases of their triacylglycerol composition [17,18]

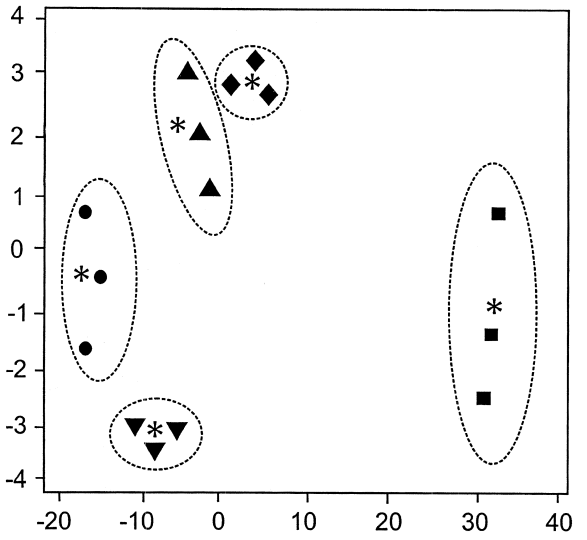

Fig. 3. Plot of the values of the two discriminant scores for each case using individual triacylglycerol contents as variables (discriminant analysis). Cases are identified as follows: Olive oil–hazelnut oil (50:50) (♦), olive oil–hazelnut oil (70:30) (▲), olive oil–hazelnut oil (90:10) (▼), olive oil (●), hazelnut oil (■), group centroids (*).

3.2. Tocopherol and sterol composition

Trimethylsilyl (TMS) ether derivatives were identified by comparing of their retention times to those of pure standard TMS derivatives of tocopherols and sterols by GLC. Moreover TMS derivatives of tocopherols and sterols were also assigned by their mass spectrometric data (GC–MS) [39–43]. Fig. 4 shows a typical chromatogram by GLC of an olive oil–hazelnut oil (50:50) mixture in which TMS derivatives of tocopherols and sterols have been identified.

Table 2 shows the contents (mg kg^{-1}) for tocopherols (α-tocopherol, β-tocopherol and γ-tocopherol) and sterols (campesterol, stigmasterol, β-sitosterol, Δ^5-avenasterol and obtusifoliol) of oil samples. In relation to tocopherol content, α-tocopherol is the main isomer. Hazelnut oil showed the highest value (333.50 mg kg^{-1}) whereas olive oil had the lowest value (111.35 mg kg^{-1}). γ-Tocopherol and β-tocopherol were minor compounds in comparison to α-tocopherol (Table 2). Regarding sterol content, the main compounds were β-sitosterol

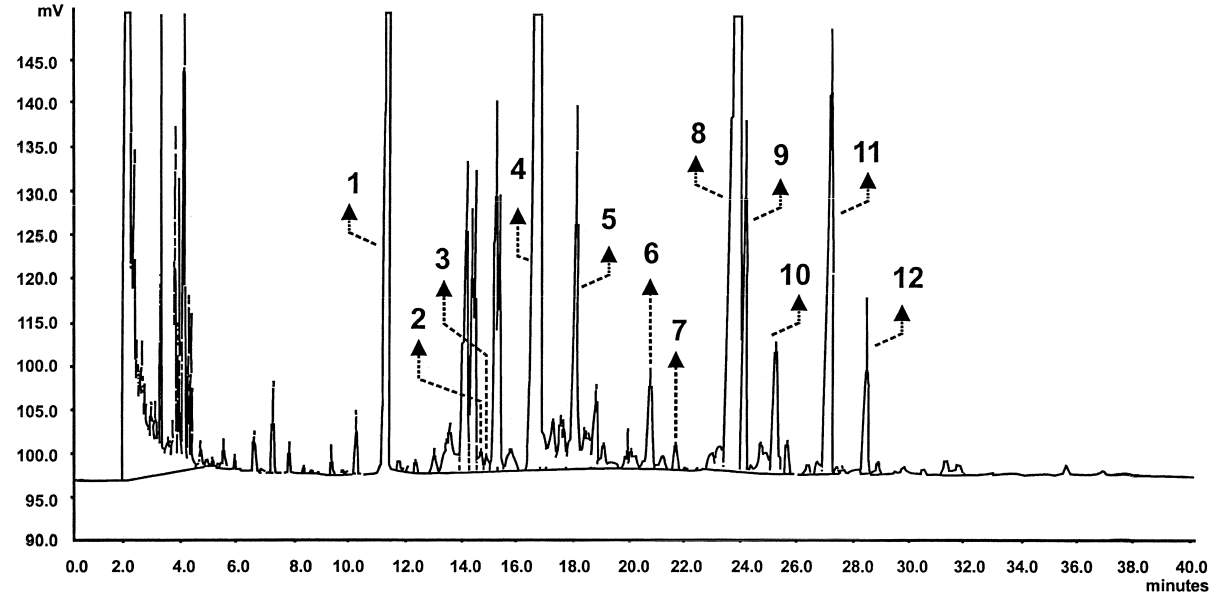

Fig. 4. Typical chromatogram of *O*-trimethylsilyl ether derivatives of the non-saponifiable lipid fraction of an olive oil–hazelnut oil (50:50) mixture. Ordinate, miliVolts; abscissa, time (min).1, not identified; 2, γ-tocopherol; 3, β-tocopherol; 4, internal standard; 5, α-tocopherol; 6, campesterol; 7, stigmasterol; 8, β-sitosterol; 9, Δ^5-avenasterol; 10, not identified; 11, obtusifoliol; 12, not identified.

and Δ^5-avenasterol (Table 2). Hazelnut oil showed the highest in β-sitosterol and Δ^5-avenasterol values (1564.43 mg kg^{-1} and 209.95 mg kg^{-1}, respectively), whereas olive oil recorded the lowest (1163.65 mg kg^{-1} and 152.72 mg kg^{-1}, respectively). Olive oil had the highest obtusifoliol content (793.38 mg kg^{-1}) whereas the sample olive oil–hazelnut oil (50:50) had the lowest content (441.73 mg kg^{-1}). No trace of obtusifoliol was found in hazelnut oil. Campesterol and stigmasterol contents showed the lowest values in all samples (Table 2). These results are consistent with data published elsewhere [4,27,44–53].

A one-way ANOVA showed significant differences ($p<0.05$) between samples for: α-tocopherol, β-sitosterol, Δ^5-avenasterol and obtusifoliol contents (Table 2). The discriminant analysis using individual tocopherol and sterol contents as variables showed that hazelnut oil and olive oil samples were grouped according to their tocopherol and sterol composition. However, the discriminant analysis did not clearly separate oil mixtures (Fig. 5).

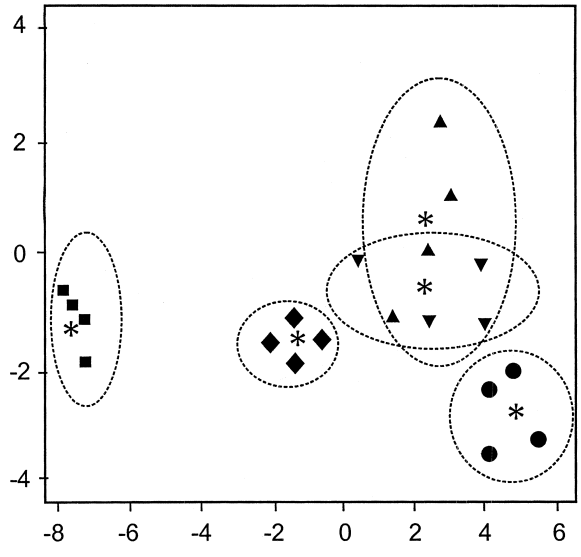

Fig. 5. Plot of the values of the two discriminant scores for each case using individual tocopherol and sterol contents as variables (discriminant analysis). Cases are identified as follows: Olive oil–hazelnut oil (50:50) (♦), olive oil–hazelnut oil (70:30) (▲), olive oil–hazelnut oil (90:10) (▼), olive oil (●), hazelnut oil (■), group centroids (*).

Table 2
Tocopherol and sterol contents of hazelnut oil, olive oil and their mixtures[a]

Sample	Tocopherols (mg kg^{-1})						Sterols (mg kg^{-1})									
	γ-Tocoppherol		β-Tocopherol		α-Tocopherol		Campesterol		Stigmasterol		β-Sitosterol		Δ^5-Avenasterol		Obtusifoliol	
	x[e]	SD[f]	x	SD	x	SD	x	SD	x	SD	x	SD	x	SD	x	SD
Olive–hazelnut (90:10)	22.32	12.32	16.12	0.88	199.92[b]	66.07	56.03	9.90	19.71	7.43	1264.86[b,d]	128.90	158.92[b]	22.79	591.91[b]	145.41
Olive–hazelnut (70:30)	30.53	13.56	22.53	4.23	214.51[b]	18.13	60.86	12.08	16.31	11.72	1249.84[b,d]	80.54	157.93[b]	10.61	550.63[b]	13.14
Olive–hazelnut (50:50)	14.98	5.66	18.41	3.06	258.69[b,c]	56.76	66.61	12.50	19.14	4.49	1492.75[b,c]	260.41	192.12[b]	36.72	441.73[b]	61.09
hazelnut oil	10.19	2.42	15.81	4.02	333.50[c]	12.83	70.65	2.61	20.93	2.95	1564.43[c]	11.25	205.95[c]	4.59	n.d.[g]	n.d.
Olive oil	27.05	6.30	23.36	7.33	111.35[d]	34.19	55.58	18.34	15.10	4.22	1163.65[d]	23.28	152.72[b]	2.82	793.38[c]	23.22

[a] Data are means of triplicate results.
[b] Denotes statistically significant differences (Bonferroni).
[c] Denotes statistically significant differences (Bonferroni).
[d] Denotes statistically significant differences (Bonferroni).
[e] x; mean.
[f] SD; standard deviation.
[g] n.d.; not detected.

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