Some physiochemical properties of olive and olive oil of three jordanian olive varieties

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The physiochemical properties of olives and olive oils of Nabali Baladi (NB), Nabali Muhassan (NM) and Shami (SH) olive varieties were studied during the crop season 2008/2009 in Bani Kananeh district (in the North of Jordan). The olives were taken from 4 different farms in the district. Olive and its oil were analyzed using various parameters. The olive of NM showed higher moisture content than other varieties, but the oil percentage was lower. The fatty acid compositions of NM and NB oils were insignificantly different. The C16:0 content in SH olive oil was significantly higher than that in NM and NB oils, while C18:0 was lower. The C18:1, C18:2 and C18:3 for the three oil samples were insignificantly different. The olive oil of NM had the highest total sterols, squalene and α-tocopherol, while those of NB oil were the lowest. Three new methylsterols components were tentatively identified in these oils.
Keywords: olive, olive oil, fatty acids, sterols.

Proprietà fisico-chimiche delle olive e degli oli d'oliva di tre varietà di olive giordane

Sono state studiate le proprietà fisico-chimiche della varietà delle olive e degli oli d'oliva di Nabali Baladi (NB), Nabali Muhassan (NM) e Shami (SH), varietà di olive raccolte nel corso della stagione 2008/2009 nel distretto di Bani Kananeh (nel nord della Giordania).
Le olive sono state raccolte da quattro diverse aziende agricole distribuite lungo il distretto.
Sono stati analizzati vari parametri per le olive e per l'olio ricavato.
Le olive di NM hanno mostrato un peso del frutto e un tenore di umidità più alto rispetto alle altre varietà, ma la percentuale di olio è risultata inferiore.
La differenza di composizione degli acidi grassi degli oli d'oliva di NM e di NB non era significativa.
Il contenuto di C16:0 nell'olio d'oliva di SH è risultato significativamente superiore a quello dell'olio di oliva di NM e a quello di NB, mentre il C18:0 era inferiore.
Gli acidi grassi C18:1, C18: 2 e C18: 3 non mostravano differenze significative.
L'olio d'oliva di NM aveva il più alto contenuto di steroli totali, squalene e α-tocoferolo, mentre nell'olio di oliva NB erano più bassi.
In questi oli d'oliva sono stati individuati tentativamente tre nuovi metilsteroli.
Parole chiave: olive, olio di oliva, acidi grassi, steroli.

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INTRODUCTION

The olive tree (*Olea europaea L.*) is a major agricultural crop in the Mediterranean Basin. Its cultivation originated in the eastern part of this basin over 6000 years ago. Jordan is one of the cultivated olives homeland were the olive tree is the most important it covers about 72% of the total area is planted with olive trees and 36% of the total cultivated area. Increasing demand for olive oil has motivated a fast increase in the planted area of about 5% annually over the last 15 years. Approximately 127,000 hectares of olive trees are grown in Jordan [1].

Olives are found almost all over the Kingdom going from the highlands to the Jordan Valley and to the desert. There are two main olive producing regions in Jordan; the western mountainous region and the north eastern desert region. The former is non-irrigated and covers a surface area of 96,923 hectares with an average density of 140 trees per hectare, and it produces 70% of the country's total olive production. The olive area of this region is distributed in the governorates of Irbid (26%), Balqa (19%), Jerash and Ajloun. The olive trees in these governorates are mostly non-irrigated. The olive area in Bani Kanana region, which belongs to Irbid governorate, is about 9000 hectares representing about 31.9% of the cultivated olive area in this governorate, 7.3% of the total cultivated olive area in and 9.3% of the total non-irrigated and cultivated olive area in Jordan. The olive oil produced in this region in 2008 was 4744 tons which represents 65.4% and 25.7% of that produced in Irbid governorate (7257.8 tons) and in Jordan (18472 ton), respectively [1]. Many olive varieties are grown in Jordan; however, the predominant indigenous ones are 'Nabali Baladi', 'Nabali Muhassan (is cald also Rasei)' and 'Shami'which is dominant in Irbid governorate. Other cultivars are also grown in Jordan such as Nasouhi Jaba'i [2], Grossa di Spagna, and Barnea (K-18). Nabali olive accounts for more than 70% of the cultivars grown in Jordan because it is suitable for all locations as it resists drought and diseases, is suitable for both pickling and oil production, and provides higher percentage of extracted oil (20-35%) compared to other cultivars [1].

Little information about the physio-chemical properties of the three predominate varieties has been published. The aim of this study is to study the physio-chemical properties of 'Nabali Baladi', 'Nabali Muhassan and 'Shami' olives and their olive oil grown in Bani kanana district which is located in the north-west of Jordan.

MATERIALS AND METHODS

OLIVE SAMPLES

Four olive orchards were selected in Bani kenaneh in the north of Jordan. These farms were in Al-shajarah, Saham, Hatem and Om Qais, which belongs to the Bani Kenanah region. At each farm 4 trees (15-20 years old) of each of 'Nabali Baladi', 'Nabali Muhassan and 'Shami' olive varieties were selected. The olive fruits of the 4 trees of each variety were picked by hand when 75% of the olive fruits became black (during 10-25 November, 2008) and mixed well to give a representative sample.

The fat yield (Soxhlet method) and moisture content (oven drying) were carried out according to the AOAC methods [2]. The average weight of 100 olives and 100 seeds, the seed % and the percentage of the extracted oil on both dried and fresh olives were also determined.

OLIVE OIL SAMPLES

The olive extraction of the collected olives was carried out in Al- Sadoon olive press located in Bani Kenaneh district in which three-phase Alpha Laval centrifugal press was used. All samples were cold pressed (28-32C) within 24 hrs after collection. The extracted oil was collected in olive oil tin cans (16 kg). Sub-samples of 2 litres were taken from these cans and kept in dark glass bottles in a refrigerator (5-7°C) for further chemical analysis.

CHEMICAL ANALYSIS

Fatty acid composition

Fatty acid methyl esters (FAMEs) of the olive oil samples were prepared according to EC Regulation no. 2568/91 method [3]. Briefly: 50 mg of lipid extract was weighed, dissolved in 2 ml hexane (GC grade) and mixed by vortex for 1 min. A 200 µl of 2 M-potassium hydroxide prepared in anhydrous methanol was added and mixed for 30 sec. until the solution became clear, and then 200 µl of acetic acid was added and mixed for 30 sec. The prepared methyl esters were analyzed using capillary GC column (Restek, Rtx-225, USA, cross bond 50%-cyanopropylmethyl 50%-phenylmethyl polysiloxane, 60 m, 0.25 mm/D, 0.25 µm df) immediately after esterification by injecting 1 µl of the hexane layer through the injection port of the GC (model GC-2010, Shimadzu. Inc., Koyoto, Japan). The FAMEs were injected after adjusting the GC conditions; column oven temperature was 165°C for 10 min, increased to 185°C at 1°C/min and kept at 185°C for 1 min, then increased to 220°C 3°C/min and kept at 220°C for 20 min. Injector temperature was 240°C, flame ionization detector temperature was 260°C, helium flow rate 0.8 ml/min Helium and split ratio used was 80.

The fatty acids methyl esters were identified using the chromatogram of the corresponding fatty acid methyl ester standards (Supelco Inc, Bellefonte, USA)

Saponification and Extraction of the olive oils unsaponifiables

Olive oil samples (5 g), with 1 mg α-cholestanol as internal standard were saponified with 50 ml of 2 M KOH in methanol for 1 h under reflux. The unsaponifiable portions were extracted three times with 80, 70 and 60 ml diethyl ether. The pooled extract was then washed several times with 50 ml water until the washing solution became colorless with phenolphthalein. The solvent was removed under low pressure using a vacuum rotary evaporator. The Trimethylsilyl (TMS) derivatives of the whole unsaponifiables were prepared using a solution composed of 5 volume pyridine, 2 volume of hexamethyldisilazan and 1 volume of trimethylchlorosilan at 40°C for 20 min, and then TMS solution was evaporated using extra pure nitrogen gas. The derivatized TMS sterols were redissolved in 1 ml hexane (GC grade), and mixed for 30 second in vortex mixer and then centrifuged for 5 min. The derivatized unsaponifiables were ready for GC-MS (model QP2010, Shimadzu. Inc., Koyoto, Japan) and GC-FID analysis [4].

GC-MS analysis (identification)

The derivatized unsaponiables were identified with Shimadzu GC coupled to mass spectrometer. They were separated on GC polarcolumn (Restek, USA, cross-bond 35%-diphenyl 65%-dimethyl polysiloxane, 30 m, 0.25 mm/id, 0.1 µm df). Oven temperature was programmed from 200 to 300°C and held at this temperature for 10 min; Helium was the carrier gas at flow rate of 0.8 ml/min. The injector and detector temperatures were 310°C [4]. Some of the unsaponifialbe components were identified by the examination of their fragmentation pattern and the mass spectra were also compared to those of the Shimadzu library. GC-FID analysis of the unsaponifiable portion for quantitation of sterols, squalene and α-tocopherol, Shimadzu GC-FID (model 2010) analyses were carried out following the same analytical conditions used for GC-MS identification.

The sterols were expressed as mg/g oil and each desmethylsterol and methylsterol was also expressed as percentage of the total amount of each.

Statistical analysis

Statistical analysis of data was carried out using statistical analysis system package (SAS Inc., 2000) [5].

RESULTS AND DISCUSSION

The samples are identified with letters; NM, NB and SH which refer to the Nabali Muhassan, Nabali Baladi and Shami olive varieties selected in this study. The farm locations were found to have no significant effect on the results of the analyzed parameters. Therefore, the mean value of the analyzed parameters of each olive and olive oil variety obtained from the 4 farms was reported in this study.

FRUITS

The results of the some parameters analyzed in olive fruits are shown in Table I. The data show that there are significant differences in olive fruit weight of the three olive varieties. The greatest weight was for the olive fruits of SH (7.1 g/fruit), while the lowest was for NB (2.9 g/fruit). The fruit length of NB (1.9 cm) and NM (2.0 cm 0 olives was insignificantly different. However, the fruit length of these two olive varsities were significantly lower than that of SH (2.9 cm). A different trend was observed for the seeds percentage. NB olives had the greatest seeds percentage (20.2%), while NM had the lowest one (11.3%). The results also indicate that the seed weight was 0.55, 0.59 and 1.1 g for NM, NB and SH olive fruits, respectively. These results agree with that reported in the annual report (2008) of Ministry of Agriculture of Jordan [1].

MOISTURE CONTENT

Table I shows that there was no significant difference in moisture content between either the whole or the flesh of the olive fruits of NB and SH varieties.

Table I - Average analytical characteristics of NM, NB and SH olives in Banin Kananeh [a]*

	Nabali Muhassan	Nabali Baladi	Shami
Weight of olive fruit (g)	4.9 ± 0.5[b]	2.9 ± 0.2[c]	7.1 ± 0.5[a]
Seed weight (g)	0.55 ±0.04 [b]	0.59 ± 0.03[b]	1.1 ± 0.3[a]
Seed (%)	11.3 ± 0.4[c]	20.2 ± 1.3 [a]	15.8 ± 0.5[b]
Fruit length (cm)	1.9 ± 0.3	2.0 ± 0.2 [b]	2.9 ± 0.2 [a]
Moisture of flesh (%)	66.2 ± 2.2 [a]	48.0 ± 1.5[b]	49.4 ± 1.5[b]
Moisture of whole olive	51.8 ± 2.5 [a]	40.7 ± 0.8[b]	43.1 ± 1.5[b]
Fat (%) in dried flesh	52.0 ± 3.2[c]	60.2 ± 2.7[b]	65.7 ± 3.1[a]
Fat (%) in fresh flesh	25.6 ± 1.3[b]	35.7 ± 1.1 [c]	38.6 ± 1.1[a]
Fat (%) in whole olive	19.2 ± 0.7 [c]	23.5 ± 0.6 [b]	28.2 ± 1.3[a]

[a] Values are given as means (n =3)

*Different superscript letters after mean values within the same raw are significantly different ($P < 0.05$).

Table II - Fatty acid composition of NM, NB and SH olive oils in Bani Kananeh*

Fatty acid	Nabali Muhassan	Nabali Baladi	Shami
C14:0	0.03 ± 0.001[a]	0.02± 0.00[ab]	0.01 ± 0.00[b]
C16:0	12.4 ± 0.05[b]	12.5 ± 0. 2[b]	16.0 ± 0.3[a]
C16:1	1.06 ± 0.02[b]	1.08 ± 0.02[b]	1.2 ± 0.2[a]
C17:0	0.32 ± 0.003[a]	0.29 ± 0.03 [a]	0.12 ± 0.02[b]
C17:1	0.32 ± 0.005[a]	0.31 ± 0.01[a]	0.25 ± 0.03[a]
C18:0	3.00 ± 0.2[a]	3.1 ± 0.05[a]	2.17 ± 0.1[b]
C18:1	67.1± 0.3[a]	67.00 ± 0.4[a]	67.10 ± 0.5[a]
C18:2	13.80 ± 0.2 [a]	13.90 ± 0.2 [a]	11.4 ± 0.3[b]
C18:3	0.74 ± 0.03[a]	0.74 ± 0.05a	0.75 ± 0.1[a]
C20:0	0.6 ± 0.005[a]	0.6 ± 0.05[a]	0.4± 0.03b
C20:1	0.33 ± 0.003[a]	0.32± 0.01[a]	0.28± 0.02[a]
C22:0	0.16 ± 0.001[a]	0.16± 0.005[a]	0.11± 0.02b
C24:0	0.07 ± 0.004[b]	0.08± 0.01 [ab]	0.09± 0.00[a]
Unsat/sat	5.03 [a]	4.97[a]	4.29[a]

*Different superscript letters after mean values within the same raw are significantly different ($P < 0.05$).

However, the moisture contents of the olive fruits of these two varieties were significantly lower than that of NM olive fruits. The moisture content of the olive fruits of NM was 1.3 and 1.2 time greater than those of olive fruits of NB and SH, respectively. These results partially agree with results of Al-Maaitah et al. [6] who reported that the moisture content of the olive fruits of NM was higher than that of NB olives throughout the entire olive harvesting time (from Oct, 15 to Dec, 15, 2006).

OIL CONTENT

The olive oil content both in the dried flesh and whole fresh olive fruits was significantly influenced by the olive variety. The highest olive oil content was found in SH olives, while the lowest in NM olives (Table I). The olive oil content in the fresh olive fruits of SH variety was 1.2 and 1.5 times greater than NB and NM olives, respectively.

The results of this study agree with that of Al-Maaitah et al. [6] who reported that the olive oil content of NM and NB olives grown in Al-Mshaqer in 2006/2007 season and harvested during the same period was about 23% and 20%, respectively. The results of the olive oil content of NB olives are in agreement with Freihat et al. [7] who reported that the oil content in 2001 season of NB olive grown in Soum and Om Al-dananer, which have similar climatic conditions, was 53% and 49% on dry matter bases.

FATTY ACIDS COMPOSITION

The fatty acid composition of the oil samples of the three olive varieties studied in this work is shown in Table II. The data show that no significant differences were found in the fatty acids content of NM olive oil and the corresponding fatty acids of NB olive oil. Freihat et al. [7] reported that the average C16:0 and C18:0 contents in NB oil during the 2001 season in two different locations with different altitudes (400 and 700m above sea) were about 9.4 and 1.8%, respectively. The content of these two acids were lower than their content found in NB oil of this study (Table II), while the content of C16:1 (1.5%), C18:1 (69.5%) and C18:3 (1.15%) were higher (Table II). Furthermore, the C18:3 content of their study was higher than the maximum level (0.8%) set by the Jordanian standards for extra virgin olive oil [8].

The fatty acid content of C16:0, C16:1, C18:0, C18:1, C18: 2 and C18:3 of the NM olive oil obtained from irrigated olive trees in an arid area in Jordan (Al-Hashimeah) was 13.8, 1, 2.6, 66.8, 14.1 and 0.72%, respectively [9]. These results agree with the corresponding fatty acids found for NM olive oil of this study. These findings may indicate that the level of these fatty acids was not affected by the level of irrigation water and the climate conditions.

On the other hand, the data in Table II show that C18:0, C18:2, C20:0, C22:1 contents in SH olive oil were significantly lower than the corresponding fatty acids observed in NM and NB olive oils, while that of C16:0 was higher. The oleic acid content and unsaturated/saturated ratio were insignificantly different among the three different varieties.

UNSAPONIFIABLES

The GC-MS analysis of the unsaponifiable portion, as shown in Figure 1 confirmed the results of Frega et al. (1992) [4] who reported that the GC analysis of the unsaponifiables using polar column was adequate for fractionation of desmethylsterols, methylsterols and alcohols. The results obtained confirmed the identity of the peaks 1 (Squalene), 3 (α- Tocopherol), 4 (Campesterol), 5 (Stigmasterol), 6

Table III - The content of some of the unsaponifiable components in NM, NB and SH olives in Bani Kananeh*

	Nabali Baladi	Nabali Muhassan	*Shami
Squalene	613.6 ± 10^{b}	880 ± 8^{a}	555.6 ± 11^{c}
α-Tocopherol	10.8 ± 0.6^{c}	27.1± 2^{a}	16.1± 0.9^{b}
Campesterol	3.5 ± 0.2^{c} (2.8)**	7.5 ± 0.4^{a} (2.9)	4.9 ± 0.3^{b} (2.6)
Stigmasterol	1.0 ± 0.7^{c} (0.8)	4.2 ± 0.3^{a} (1.6)	1.8 ± 0.2^{b} (0.94)
β-Sitosterol	114.7 ± 3^{c} (90.5)	224.3 ± 5^{a} (86.5)	174.6 ± 5^{b} (91.2)
Δ^{5}-Avenasterol	7.6 ± 0.5^{c} (6.0)	23.3 ± 1^{a} (9.0)	10.2 ± 0.4^{b} (5.3)
β-amyrine (tentative)	2.2 ± 0.06^{c} (4.5)	6.4 ± 0.3^{a} (2.7)	3.4 ± 0.2^{b} (4.6)
Lanosterol isomer 1	5.8 ± 0.1^{c} (11.9)	17.5 ± 0.8^{a} (7.3)	7.5 ± 0.3^{b} (10.1)
Cycloartenol	7.4 ± 0.3^{c} (15.2)	47.4 ± 2^{a} (19.8)	11.1 ± 0.7^{b} (14.9)
Lanosterol isomer 2	6.2 ± 0.2^{c} (12.7)	26.5 ± 0.7^{a} (11.1)	12.5 ± 0.5^{b} (16.8)
24-methylecycloartanol	14.3 ± 0.8^{c} (29.4)	111.0 ± 3^{a} (46.3)	21.2 ± 1^{b} (28.4)
Citrostadienol	12.8 ± 1^{c} (26.3)	31.1^{a} (13.0)	18.8 ± 0.8^{b} (25.2)
Total desmethylsterols	127.9^{c}	239.2^{a}	191.5^{b}
Total methylsterols	48.7^{c}	259.3^{a}	74.6^{b}
Total des and methyl sterol	175.5^{c}	499.2^{a}	266.1^{b}

*Different superscript letters after mean values within the same raw are significantly different ($P < 0.05$).
** The values in parenthesis are the percentage of desmethylsterol and methylsterols of their total amount.

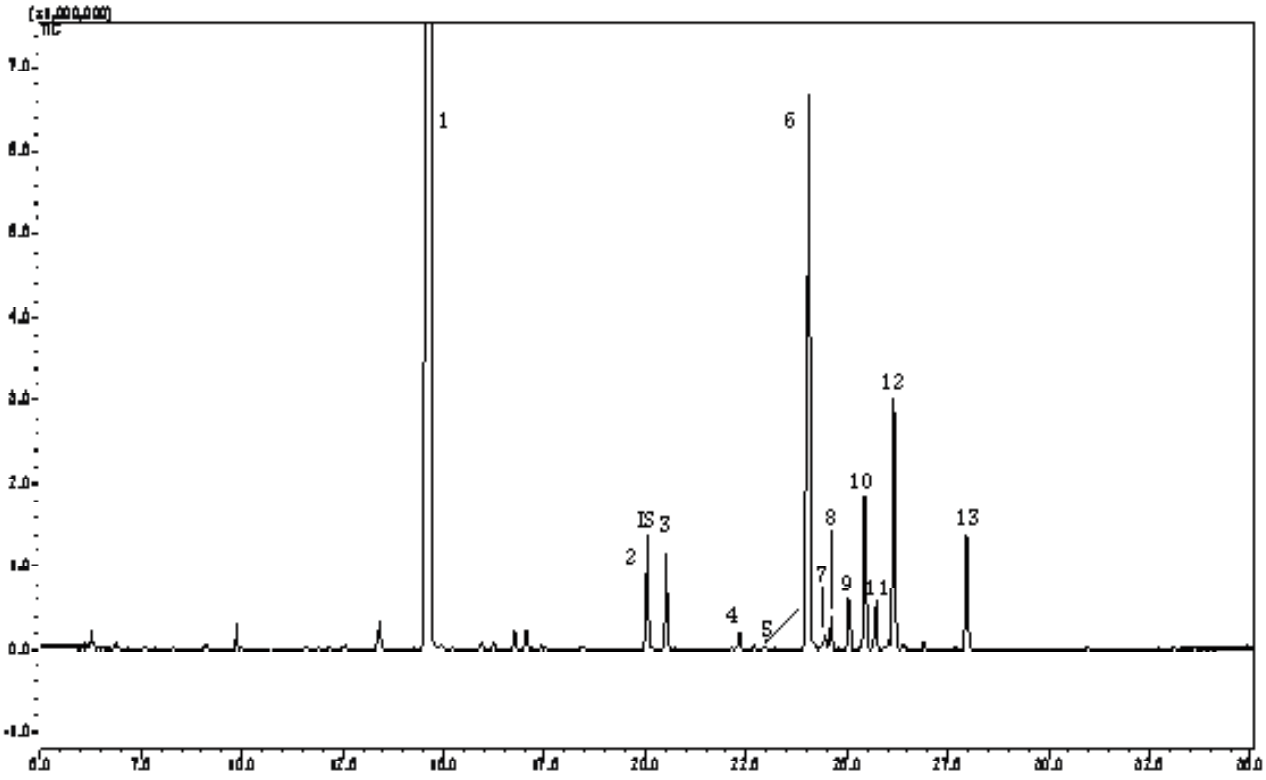

Figure 1 - GC-MS trace of the unsaponifiables of olive oil: 1) squalene; 2) α-cholestanol (Internal standard); 3) α-tocopherol; 4) campesterol; 5) stigmasterol; 6) β-sitosterol; 7) β-amyrin (tentative); 8) and 11) lanosterol isomers (tentative); 9) Δ^{5}-Avenasterol; 10) Cycloartenol; 12) 24-methylecycloartanol; 13) Citrostadienol

(β-Sitosterol), 9 (Δ^{5}-Avenasterol), 10 (Cycloartenol), 12 (24-methylecycloartanol), and 13 (Citrostadienol). However, the components 7, 8 and 11 were not reported in their study. The results of the library research provided with the GC-MS indicated that peak 7 could be β-amyrin with a probability of 86% (Figure

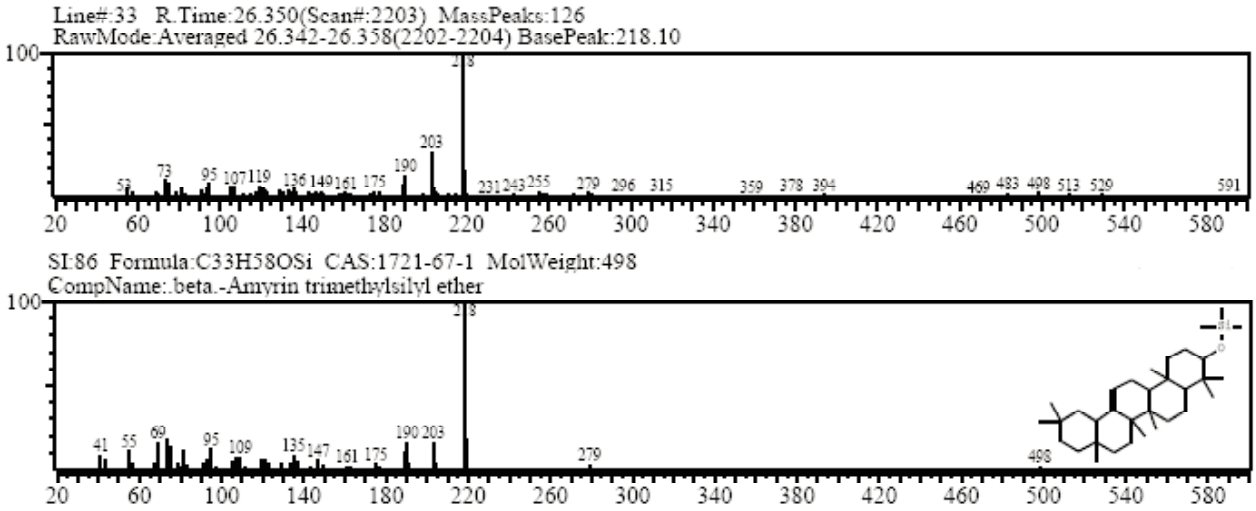

Figure 2 - Mass spectra of peak n. 7 reported in Figure 1 and that of the β-amyrin

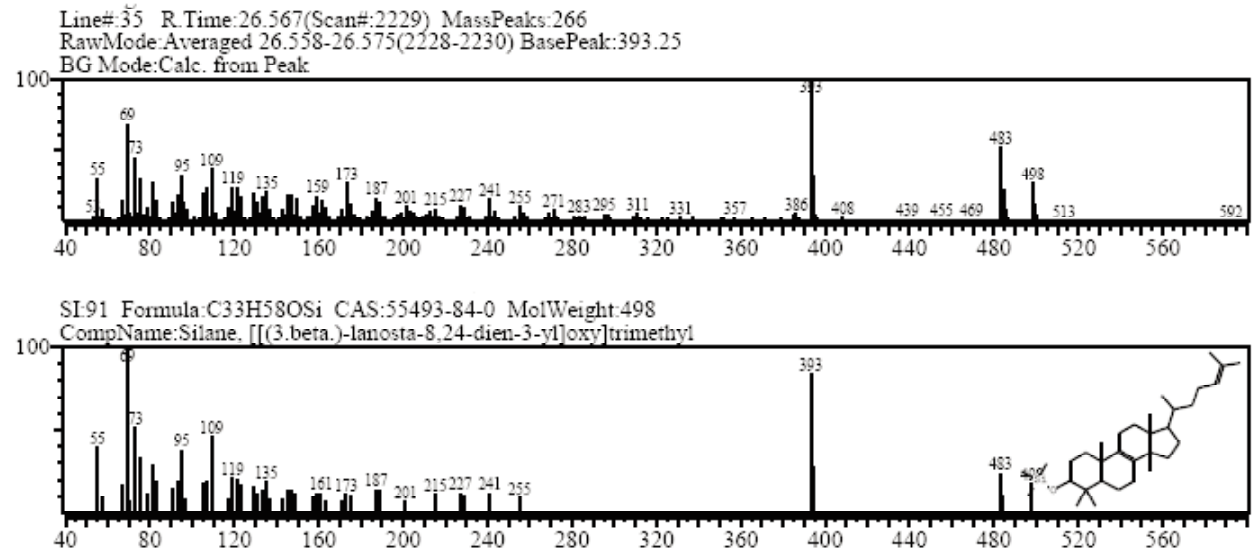

Figure 3 - Mass spectra of peak n. 8 reported in Figure 1 and that of lanosterol

2), while the peaks 8 and 11 have the same MS spectra and they could be isomers of lanosterols with a probability of 91% (Figures 3 and 4).

Virgin olive oil is the major source of phytosqualene, with a content ranging from 80 to 1200 mg/100g depending on the cultivar, harvesting date and purity [10]. Furthermore, epidemiological studies of breast and pancreatic cancer in several Mediterranean populations have demonstrated that increased dietary intake of olive oil is associated with a small decreased risk or no increased risk of cancer, despite the higher proportion of overall lipid intake [11]. The squalene content in the oils of the three olive varieties ranged from 555 to 880 mg/100 g oil, which lies within the aforementioned range reported for olive oil. The squalene content in NM olive oil was about 1.4 and 1.6 times greater than that observed for NB and SH olive oils, respectively.

α-Tocopherol content of the oils of the three olive varieties varied significantly. The content of α-Tocopherol in NM oil was the highest, while that in NB oil was the lowest. The content of α-Tocopherol in the three studied oils was comparable to that reported by others. Garcia et al. [12] found that the α-Tocopherol content of oils of 5 different olive varieties was between 11-39 mg/100 g oil.

Phytosterols are of a great importance because of their antioxidant activity and their impact on health, since they may decrease blood cholesterol levels [13,14]. Sterols have been considered as the major fraction of the unsaponifiables in many oils [15].

The desmethylsterols and methylsterols contents for NB, NM and SH olive oils are presented in Table III. The mean sterol content of both desmethyl- and methylsterols in these three oils was significantly different and it was in the order: NM > SH > NB. The desmethylsterols content in NM oil was 2.1 and 1.4 times greater than that in NB and SH olive oils,

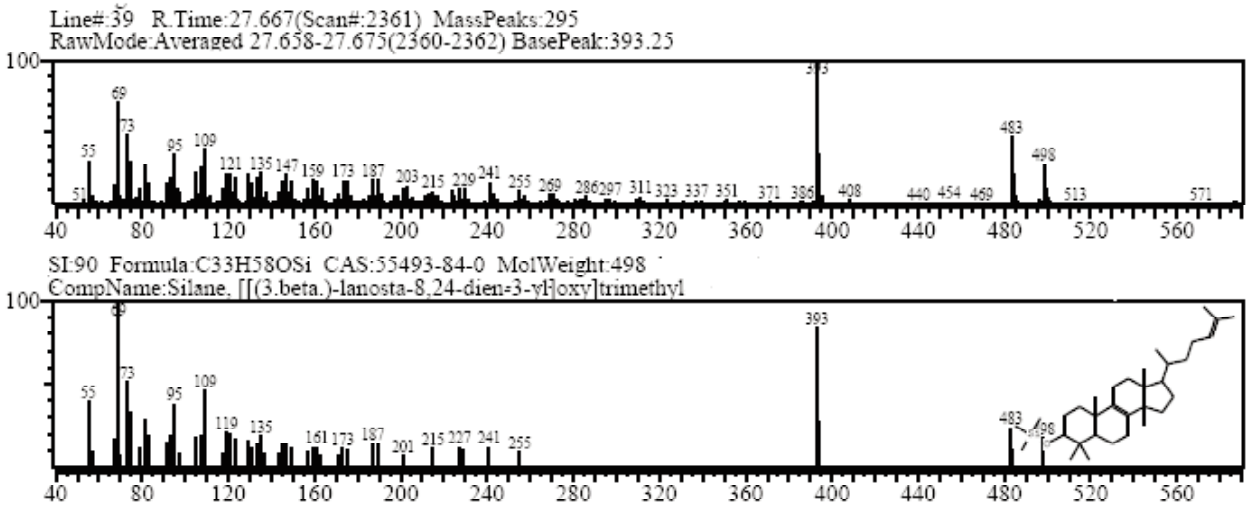

Figure 4 - Mass spectra of peak n. 11 reported in Figure 1 and that of lanosterol

whereas the total sterols (the sum of des- and methylsterols) content in NM oil was 3.5 and 2.3 times higher than that in NB and SH olive oils, respectively. The total content and the content of each desmethylsterol in the three studied oils lie within the established regulatory limits [3].

Campesterol occurs at higher levels in NM oil than in the oils of the other two varieties. The differences in campesterol level in these oils might be mainly due to the variety of the olive from which they were obtained, since this sterol is insensitive to variations such as geographical location, water shortage and storage conditions [16, 17].

It has been reported that the high content of stigmasterol is correlated with the high acidity and low organoleptic quality [12,18]. The stigmasterol content in the olive oils of the three olive varieties was low, and lower than those of campesterol. This indicates that the oil of these samples was obtained from healthy fruits and the condition of oil extraction was excellent [18] (Sanchez et al., 2004).

β-Sitosterol is the predominate component of the desmethylsterols of most of the vegetable oils. The β-Sitosterol content (expressed as mg/100g oil) was the greatest for NM olive oil and was the lowest for NB olive oil with significant differences among them. The β-Sitosterol content for NM olive oil was 2 and 1.3 times greater than that in NB and SH olive oils, respectively. However, when the content of desmethylsterol components are expressed as percentage of their total amount, β-Sitosterol of NM olive oil (86.5%) became lower than that of the NB (90.5%) and SH (91.5%) olive oils.

Δ^5-Avenasterol represents the second major sterol among the desmethylsterols. The percentages of this sterol in NM, NB and SH olive oils were 9%, 6% and 5.3%, respectively. These results agree with the results of other workers who reported that the level of β-Sitosterol and Δ^5-Avenasterol was negatively correlated [16,17].

The total methylsterols level of the NM olive oil was significantly higher than that of NB and SH olive oils. This is due to the elevated content of 24-methylcycloartanol and to some extent to cycloaretenol level. The content of 24-methylcycloartanol in NM olive oil was about 8 and 5 times higher, respectively than that of NB and SH olive oils. However, the content of 24-methylcycloartanol expressed as percentage of the total methylsterols in NM, NB, SH oils was about 46%, 29% and 28%, respectively. This means, that the 24-methylcycloartanol percent of NM oil was about 1.6 greater than those of the other two oil samples. The content of 24-methylcycloartanol in NM olive oil is in agreement with that reported by Freqa et al. [4], while that of the other two studied oils was significantly lower.

The level of citrostadienol in NB olive oil was 2.4 and 1.7 times greater than that in NB and SO olive oils, respectively. However, this result was different when the level of citrostadienol was expressed as percentage, since its level in NM olive oil was about two times lower than that in NB and SH oils (Table III). Similar conclusion can be observed for β-amyrine and lanosterol isomers.

The results indicated that the conclusion that might be obtained about the sterol levels in oils will depend on the way of expressing the sterol content; i.e mg/100g or percentage.

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