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MALDI-TOF/MS Fingerprinting of Triacylglycerols (TAGs) in Olive Oils Produced in the Israeli Negev Desert

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Triacylglycerols (TAGs), composed of three esterified fatty acids with an attached glycerol backbone, are the main component of vegetable oil (~95%) and an important source of energy and nutrition for humans, so their compositional analysis merits extensive interest. Intact TAG composition of oil in native form is highly important, rather than the fatty acid profile itself. This paper reports the analysis of the TAG profile of olive oils produced from the six common olive cultivars grown in the Negev desert of Israel (Barnea, Souri, Arbequina, Picual, Leccino, and Koroneiki) together with the content of some additional common oil quality parameters closely associated with TAG composition and integrity. Matrix-assisted laser ionization−desorption time-of-flight/mass spectrometry (MALDI-TOF/MS) fingerprintings were employed for TAG profiling. With 2,5-dihydroxybenzoic acid (DHB), MALDI-TOF/MS was able to fingerprint the intact TAG profiles in olive oils in a fast and easy manner without any derivatization. Triolein (31.53%) was found to be the main intact TAG followed by dioleoyl−palmitoyl (23.06%) and dioleoyl−linoleoyl (14.31%). MALDI-TOF/MS also enabled calculation of the main fatty acids and their compositions in a simple manner from the TAG profiles; the results are found to be very similar to conventional methods determined by GC and HPLC. Average free fatty acids and peroxide value were found to be less than 0.8% and 10 mequiv of O_2/kg of oil, respectively, in all of the tested oils. Relatively high levels of tocopherols (av = 325 μg/kg) and phytosterols (av = 2375 mg/kg) were found. This study demonstrates MALDI-TOF/MS technology as an easy and fast methodology for TAG and fatty acid profile analysis in olive oils. Additionally, this study also shows the high levels of tocopherols and phytosterols in the olive oils produced from the common cultivars grown in the Israeli Negev desert.

KEYWORDS: Olive oil; Negev desert; triacylglycerols; fatty acids; free fatty acids; peroxide value; phytosterols; tocopherols; MALDI-TOF/MS

INTRODUCTION

Olive oil produced from the *Olea europaea* fruit is one of the most important vegetable oils currently used. In terms of volume, olive oil accounts for only 2% of the world trade in vegetable oils, but in terms of value it accounts for 30% of trade (*1*). About 98% of the total area of olive cultivation in the world is concentrated in countries of the Mediterranean basin, but recently there has been a trend in olive production in other areas, and this trend is likely to continue in the foreseeable future (*2*). Olive cultivation in these newly expanded regions with some saline irrigation has been showing considerable success; however, little study about the effect of saline irrigation on oil quality has so far been conducted (*3*).

This study is the continuation of our efforts to evaluate olive oil from olive tree cultivation in the Israeli Negev desert. Our first study focused on the evaluation of different saline water irrigation levels for Barnea olive (*4*), and the second study focused on the vegetative and reproductive response of olives under these desert environments (*5*). The present study focuses on the triacylglycerol (TAG) characterization in olive oils produced in the Israeli Negev desert. Considering the great importance of TAG structure and integrity, rather than fatty acid profiles by themselves, special emphasis has been given to TAG fingerprinting. TAG was chosen as the main parameter for this study because there is also a fear that the hot conditions present in the Israeli Negev desert may affect and reduce oil quality due to faster cleavage and oxidation of the TAG molecules.

Triacylglycerols are composed of three esterified fatty acids with a glycerol backbone attached and are the main saponifiable component of olive oil (~95−98%). Other minor nonsaponifiable components of the olive oils, squalene, precursor of phytosterols (*6*), polyphenols, tocopherols, and carotenoids, mainly protect the TAG molecules that dominate the properties

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of the oil (*7*) by preserving their integrity (*8*). Due to their specific, unique, and typical composition, TAGs in different kinds of fats and oils are used to confirm authenticity in the food industry (*9*).

It is suggested that the TAG structure, and not only its fatty acid profile, is of special importance with regard to its physiological effect (*10*). The molecular composition of the TAG mixture is typically very complex due to a combination of the variety of fatty acids, differing in their chain length, degree of unsaturation, and distribution between the sn-1, sn-2, and sn-3 positions of the glycerol backbone. Therefore, an easy to run, fast, and reliable analytical method enabling determination of both the fatty acid profile and TAG composition is greatly needed (*11*).

Different methods have been applied for the analysis of intact TAGs. Recently, soft ionization mass spectrometry (MS) techniques, mainly matrix-assisted laser desorption ionization−time-of-flight/mass spectrometry (MALDI-TOF/MS), have been found to be powerful and important tools for a fast and accurate method of analysis, even for lipids, not requiring any prior derivatization stage and supplying results within minutes (*12*−*14*).

Because fatty acids do not stand alone in oils, a method enabling determination of the intact TAGs, rather than from the derivatization, has great value. The aim of the present study was to analyze the TAG profiles of olive oils using MALDI-TOF/MS fingerprinting, as well as analyzing some additional quality parameters closely associated with TAG composition and integrity. These parameters include free fatty acids (FFA), peroxide value (PV), and two lipophilic antioxidant compounds (tocopherols and phytosterols) in the olive oil produced from the six common olive cultivars grown in the Israeli Negev desert.

MATERIALS AND METHODS

Oil Samples. Samples for this study were obtained from olive oils produced from the olive trees grown in the Ramat Negev Desert Agro Station located in the semiarid region of the southern Israeli Negev desert. The details of the geo-climatic situation of the area can be found elsewhere (*5*). Six of the most common olive cultivars grown in the region−Barnea and Souri (Middle Eastern cultivars), Arbequina and Picual (Spanish cultivars), and Leccino and Koroneiki (Italian and Greek cultivars, respectively)−were chosen for this study. The olive plants are grown in an intensive manner with medium saline irrigation (EC = 4.2 dS/m) with specific leaching management to reduce any damage from the saline water to the olive tree, twice a year during March and November as described earlier by Weissbein (*15*) and Weissbein et al. (*5*). Oil was extracted in a commercial olive mill located in the Ramat Negev Desert Agro Experimental Station, following the protocol for extra virgin olive oil extraction recommended by the International Olive Council, immediately after fruit harvesting using a dual-phase extracting system. The bottled oil was kept in the laboratory under conditions of low light and temperature until analysis, within 1 month of oil extraction. Although this study was conducted for five years, the oil analysis data from two consecutive crop years as on and off years (2005 and 2006) that fit well with analysis of the previous years (2002−2004) were used in this study.

TAG Analysis by MALDI-TOF/MS. Oil samples were dissolved in hexane to 1 mg/mL. 2,5-Dihydroxybenzoic acid (DHB) was used as matrix. Matrix solution was prepared by dissolving DHB in 90% methanol to about 10 mg/mL. For MALDI analysis, small volumes of samples and matrix were mixed in a ratio of 1:4, and 1 μL was then applied directly to a stainless steel MALDI target. Samples were analyzed on a Reflex IV (Bruker Daltonik GMBH, Bremen, Germany) MALDI-TOF mass spectrometer using 337 nm radiations from a nitrogen laser. An accelerating voltage of 20 kV was used. The spectra were recorded in reflectron mode within a mass range *m*/*z* of 450−2400 as described by Kaufman and Wiesman (*14*). Before the sample was used, standard TAGs, triolein (MW 885) and tripalmitin (MW 807), were also tested (**Figure 1**).

Data Processing. Calculations were made to determine the theoretical molecular mass of the different optional TAGs as described by Lay et al. (*16*). The four major fatty acids of olive oils−palmitic acid (P), stearic acid (S), oleic acid (O), and linoleic acid (L), as previously described (*8, 15*)− were taken into consideration; linolenic acid (Ln), palmitoleic acid (Po), and other fatty acids that are present to a smaller extent (usually <1%) were therefore not included in the calculations. The molecular mass of these fatty acids was calculated according to their chemical formulas as described earlier by Kaufman and Wiesman (*14*).

The results of the possible TAG combinations and their expected molecular masses are shown in **Table 1**. The carbon number (CN) in each TAG composition with the number of double bonds (DB) in the molecule and equivalent chain numbers (ECN) are also shown in this table. After subtraction of the background, correction for isotope contributions as described earlier by Kaufman and Wiesman (*14*), and comparison with the calculated TAGs, the TAG of each oil sample produced from the MALDI-TOF/MS was identified.

Fatty Acid Profile Determination by GC-MS. Fatty acid methyl esters (FAME) of the oil were prepared as described by AOCS official methods (*17*) and slightly modified by Kaufman and Wiesman (*14*) using a 6890N model gas chromatograph (Agilent Technologies) equipped with a 30 m × 0.25 mm i.d., 0.25 μm, column. Fatty acids were identified both by their retention time and by mass spectra obtained with a 5973 Network Mass Selective Detector (Agilent) operating at 70 eV according to the GC-MS library.

Free Fatty Acid (FFA) Determination. The FFA, also referred to as the acidity of the oil, was measured as described by the IOC regulation using acid/base titrations with phenolphthalein as indicator (*18*). Oil samples were dissolved in an organic solvent and titrated with sodium hydroxide solution; results were noted as percent FFA as oleic acid.

Peroxide Value (PV) Determination. The peroxide value, which is indicative of the quantity of hydroperoxide in the oil, was determined using the standard method for measuring peroxide, by titrating a mixture of the oil, chloroform, acetic acid, and saturated potassium iodide solution with sodium thiosulfate in darkness (*18*) and expressed in milliequivalents (mequiv) of active oxygen per kilogram.

Determination of Tocopherols. Standards for α-, β-, γ-, and δ-tocopherols were purchased from Sigma Chemical Co. (St. Louis, MO), and the concentration of these tocopherol isomers in various olive oil samples was determined as described in the procedure suggested by the IOC (*18*) and IUPAC (*19*) and modified by Maranz and Wiesman (*20*) using HPLC (Agilent 1100 series, Palo Alto, CA, with a G1314A detector and a 250 mm × 4 mm, 5 μm, Spherisorb SS NH_2 column). Results were adjusted for the average specific gravity of olive oil and expressed in micrograms per gram.

Determination of Phytosterols. The composition of the phytosterols was determined as described earlier by Damirchi et al. (*21*) with some minor modification using GC-MS (*14*). The details of the preparation of the unsaponifiables, separation of the sterol fraction, preparation of trimethylsilyl ethers, peak identification, and methods of result expression are described elsewhere (*14*).

Statistical Analysis. Data of the oil sample analyses of two years were used in this study. Each year at least 10 samples were analyzed for each cultivar, and the average from the pool data of two years was used. Statistical analysis of the data was performed with JMP (version 4, SAS Institute, Inc., Cary, NC) using the Tukey−Kramer HSD test for determining the significant difference among treatments at the $P = 0.05$ level of significance.

RESULTS AND DISCUSSION

TAG Composition Determination by MALDI-TOF/MS. Using DHB as a matrix, MALDI-TOF/MS easily produced the mass profile of the components of oil in a very clear and systematic manner (**Figure 2A**). After subtraction of the background and correction for isotope contributions, the spectral

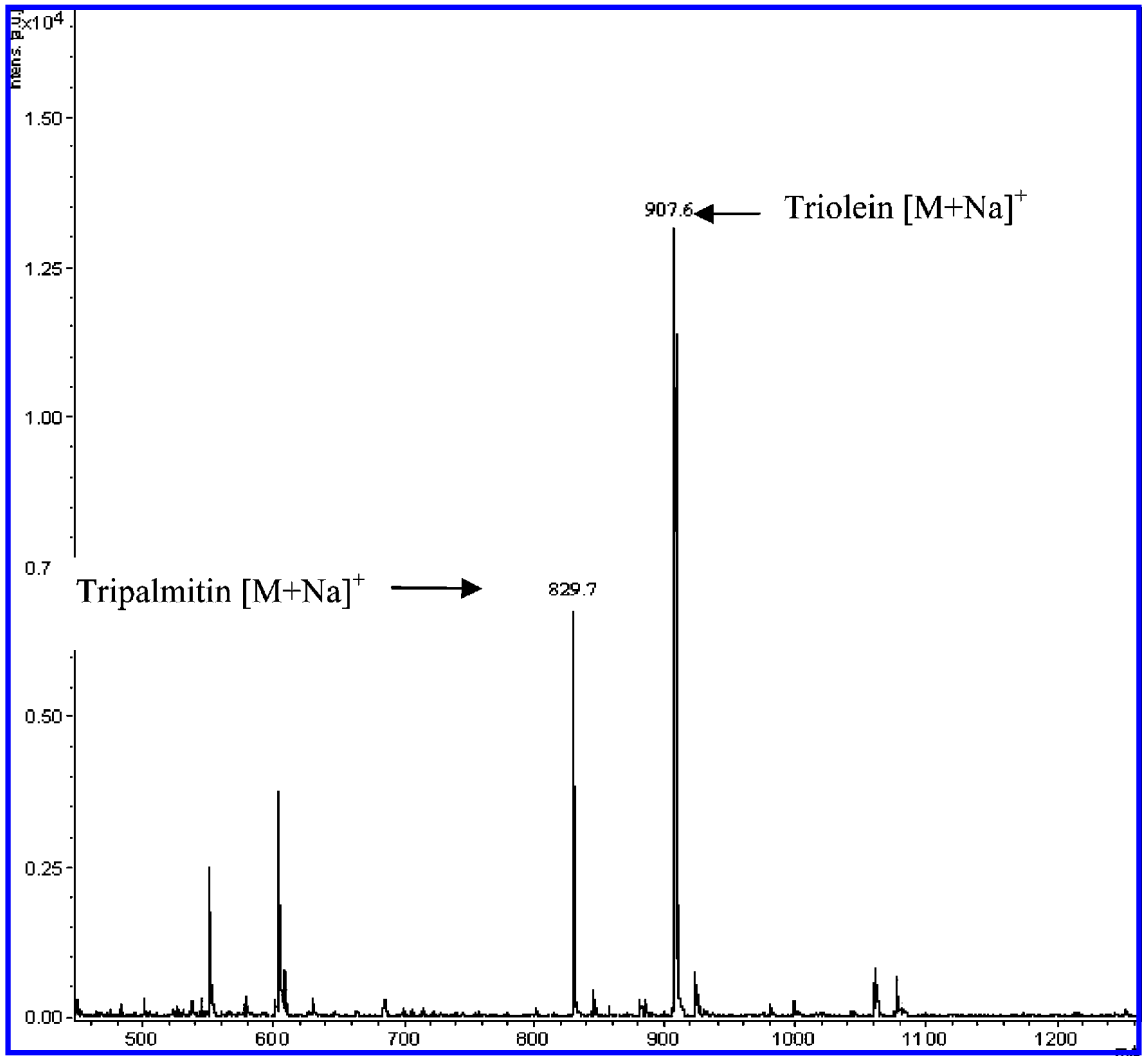

Figure 1. MALDI-TOF/MS spectra of standard TAGs: triolein and tripalmitin in positive ion mode.

Table 1. Calculated Molecular Mass of Possible TAG Compositions of Olive Oil

mass[a]	TAG[b]	CN:DB[c]	ECN[d]
853.2	PLP	53:2	46
855.3	POP	53:1	48
877.3	LPL	55:5	52
879.3	POL	55:3	48
881.4	OOP	55:2	48
885.4	SPP	53:2	50
901.3	LLL	57:6	46
903.3	LOL	57:5	46
905.4	OOL	57:4	42
907.4	OOO	57:3	44
909.5	OOS	57:2	42
911.5	SOS	57:1	46

[a] Mass is for sodium adduct ions. [b] L, linoleic acid, 18:2; O, oleic acid, 18:1; P, palmitic acid, 16:0; S, stearic acid, 18:0. [c] CN:DB, carbon number:number of double bonds. [d] ECN, equivalent chain number equal to CN − (2 × DB).

data produced from the MALDI-TOF/MS TAGs and their profile of the oil samples were identified on the basis of the calculation of molecular mass as shown in **Table 1**. As described in the literature, all of the TAGs yielded the corresponding sodium adduct exclusively; no proton adducts were detected (*22*, *23*), which can also be seen in the standard triolein and tripalmitin (**Figure 1**). The MALDI spectra of olive oils of six cultivars commonly grown in the Israeli Negev desert are presented in **Figure 2B**.

Nine major TAG species were identified in all studied oil samples. These identified TAGs were PPL, PPO, LLP, POL, OOP, LLO, OOL, OOO, and OOS (**Table 2**; **Figure 2B**). As expected, in all samples of the six cultivars, the OOO (triolein) composition was the most abundant, followed by the OOP (diolein−palmitoyl), OOL (dioleyol−linoleoyl), and POL (palmitoyl−linolenoyl). The obtained pattern of TAGs demonstrated variations among the olive cultivars. Koroneiki and Barnea had especially high OOO fractions (42.4 and 36.4%, respectively). Arbequina and Souri showed lower OOO profiles (25.89 and 24.59, respectively); TAGs were quite dominant among the compositions with both OOO and OOP fractions, followed by the OOL and POL compositions. However, the lowest TAG composition, OOS, was highest in Barnea (3.8%), and Arbequina possessed the lowest level (0.52%). Among the cultivars tested, Koroneiki demonstrated the highest percentage of OOO (42.4%) and Souri the lowest (24.59%). Picual, Barnea, and Leccino were in the middle. On average, of the total, 31.53% (OOO), 23.06% (OOP), 14.31% (OOL), 12.16% (POL), 5.28% (LOL), 4.85% (POP), 3.57% (LPL), 3.19% (PLP), and 2.10% (OOS) TAGs were found (**Table 2**).

Comparison of the TAG profiles obtained from MALDI-TOF/MS in the present study with literature data obtained from various conventional methods (GC and HPLC) as reported by Fedeli (*24*), Kiritsakis et al. (*25*), and Rezanka and Rezankova (*26*) showed similar patterns (**Table 3**). The MALDI-TOF/MS

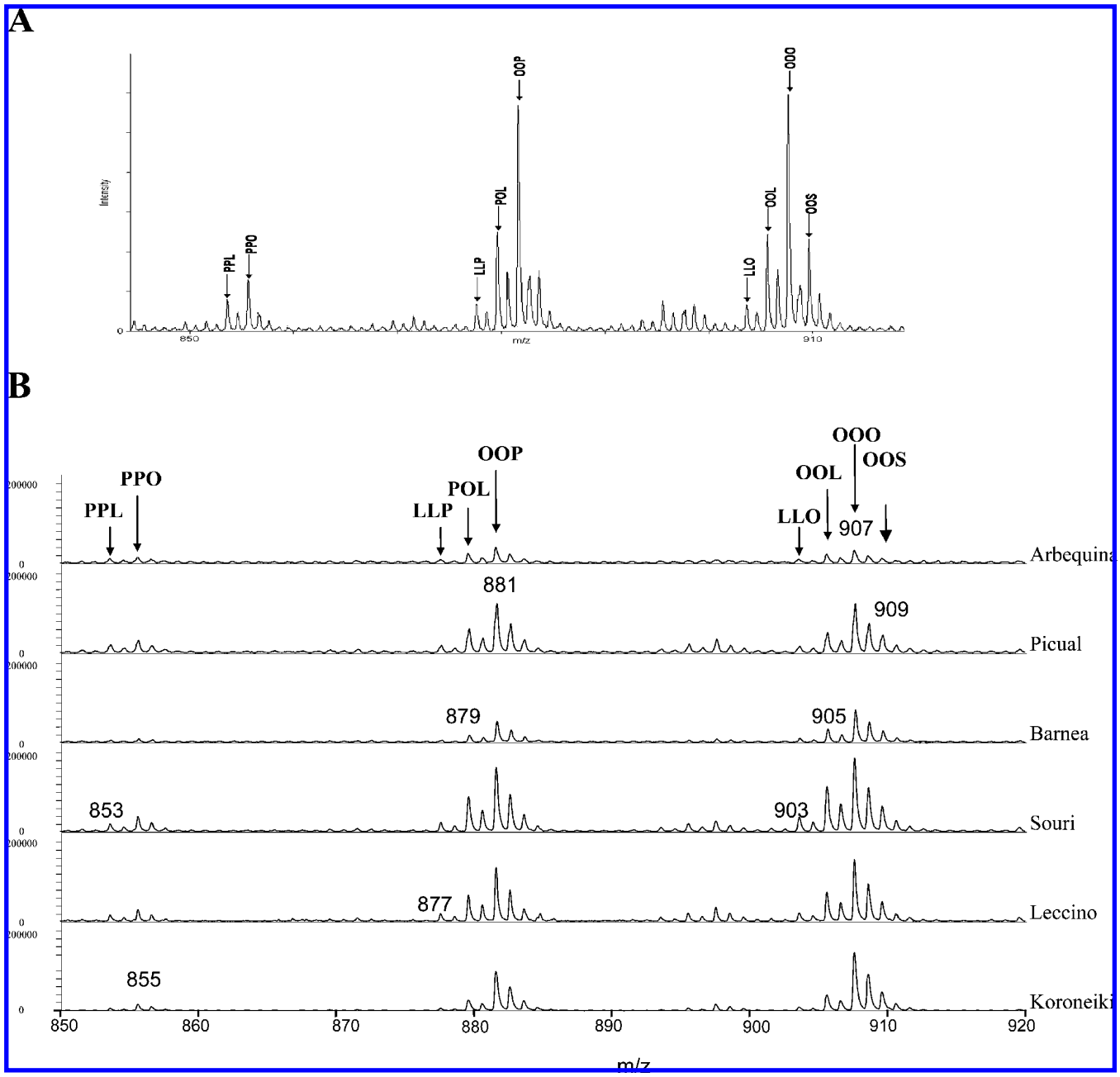

Figure 2. TAG spectra obtained from MALDI-TOF/MS of olive oils in positive ion mode: (**A**) model of a single oil sample; (**B**) comparison of six different cultivars.

Table 2. Relative TAG Composition of Olive Oils from Six Common Cultivars Grown in the Israeli Negev Desert Determined by MALDI-TOF/MS

	TAG composition (%)								
cultivar	PLP	POP	LPL	POL	OOP	LOL	OOL	OOO	OOS
Arbequina	2.96	5.78	4.98	14.41	21.35	7.88	16.23	25.89	0.52
Picual	3.67	4.56	4.84	13.57	24.37	4.53	11.22	30.74	2.50
Barnea	2.96	4.11	1.20	9.46	22.46	4.85	14.94	36.41	3.80
Souri	3.71	5.09	4.18	14.07	20.95	6.63	17.62	24.59	3.16
Leccino	3.87	4.83	4.42	13.17	23.22	4.97	14.68	29.13	1.70
Koroneiki	1.97	4.72	1.78	8.25	25.98	2.83	11.16	42.40	0.90
av[a]	3.19	4.85	3.57	12.16	23.06	5.28	14.31	31.53	2.10

[a] Average value of all cultivars.

results fit very closely with HPLC-RI results reported by Kiritsakis et al. (*25*), which is the official method of determining the TAG profile. This clearly points out that MALDI-TOF/MS could easily fingerprint the TAG profile in a fast and easy way without performing long derivatization procedures. The next aspect of TAG profile obtained from the MALDI-TOF/MS fingerprinting regarding determination of the fatty acid profiles will be discussed later.

Table 3. Comparison of the Major TAGs of Olive Oil Obtained by Various Methods

	methods			
major TAG	HPLC-UV[a]	HPLC-RI[b]	GC[c]	MALDI-TOF/MS
OOL	6.8	15.7	2.4	11.16–17.23
POL	5.9	8.8	9.5	8.25–14.41
OOO	43.5	31.7	36.7	25.89–42.40
OOP	18.4	19.7	23.4	20.95–25.98
POP		3.7	4.9	4.11–5.78
OOS	5.1	3.7	5.7	0.52–3.8

[a] Fedeli (*24*). [b] Kiritsakis et al. (*25*). [c] Rezanka and Rezankova (*26*).

Fatty Acid Profile by GC-MS. The fatty acids and their compositional profile (on a percent basis) in the olive oils from the six cultivars grown in the Negev desert as determined by the GC-MS are presented in **Table 4**. All oil samples demonstrated typical fatty acid profiles as reported in the literature (*8*). As expected, oleic acid (18:1) was the most dominant fatty acid by far, followed by palmitic (16:0) and linoleic (18:2) acids, each constituting a similar fraction of the total fatty acids (~15%); stearic (18:0) and palmitoleic (16:1) acids ranged from 1.3 to 3.4% and from 0.6 to 2.2%, respectively. Other fatty acids did not exceed 1%. However, significant variations were observed among the cultivars in all FAs. The highest level of oleic acid was found in Picual (69.89%) and the lowest in Arbequina (59.28%), and vice versa for palmitic acid. The average (of all cultivars) oleic acid content was 64.97% and the averager linolenic acid content, 0.65%. These results show that the compositional distribution of fatty acids of these oil samples are within the normal range expected for olive oil as defined by EEC regulations (*27*). Variations were also found in the ratios of unsaturated versus saturated fatty acids among the cultivars; however, the ratio in all cultivars was >4 (calculation not shown). This ratio also indicates relatively high quality oil (*28*). Although the composition range of oleic and linoleic acids was within the range of EEC regulations, the level of linoleic was higher and oleic was lower in all olive cultivars tested in this study, compared to the same cultivars cultivated in the typical olive-growing areas (*29, 30*). This phenomenon might be the effect of the saline water; a similar phenomenon was also found in previous studies carried out on Tunisian olive oils (*31*).

Fatty Acid Profiles Determined from MALDI-TOF/MS. From the TAG profiles of the MALDI-TOF/MS, profiles of each fatty acid can easily be calculated according to its relative part in each TAG and the abundance of the particular TAG; these profiles can then be compared with the fatty acid profile determined by the GC. The fatty acid profile of the olive oils from different olive cultivars obtained from MALDI-TOT/MS fingerprint and GC-MS (mentioned previously) are presented in **Table 5**. The results obtained from GC-MS can be easily interpreted as the relative area of the fatty acid peaks; however, the results obtained from MALDI-TOF/MS need to be calculated. For example, the relative part of palmitic acid (P) in the TAG PPL is 2/3, and this TAG's abundance in the Arbequina variety is 2.96% (**Table 2**). Hence, the contribution of this TAG to the P fraction in Arbequina will be (2/3) × 2.96%, which is 1.97%. A total of the contributions of the different TAGs results in the fraction of the specific fatty acid for a specific cultivar (**Table 5**).

Some differences were observed in the fatty acid profiles obtained from GC-MS and MALDI-TOF/MS (**Table 5**). There might be a number of reasons for this, but the first is connected with the methodology used to calculate the possible TAG compositions using MALDI-TOF/MS fingerprinting. During the calculation only four major fatty acids of the olive oils were considered (see data processing above). This is because TAGs containing other, less abundant, fatty acids were either not identified or difficult to recognize, so there were no fatty acids defined as "others", as in the GC-MS results (**Table 5**). This kind of problem has already been reported by Hlongwane et al. (*32*). Second, some MALDI peaks with a particular mass can be attributed to a few different TAG compositions (*33*). In these cases, choosing the specific composition and identifying the peak were done according to the most probable combination of fatty acids (the probability was calculated according to the abundance of each fatty acid). The composition chosen was in all cases more probable than the others by an order of at least 10^2. As a result of this process, some TAG compositions that might exist (it is difficult to identify whether a few peaks with the same mass have combined) at a very small level cannot be identified, and thus the fatty acids composing them will also be disregarded (*16*).

Free Fatty Acids and Peroxide Value. The data presented in **Table 6** show the free fatty acid (FFA, acidity) and peroxide value (PV) of the olive oil of the six common olive cultivars grown in the Israeli Negev desert. The results show on average a low level of FFA (av = 0.52%) and PV (av = 6.43 mequiv of O_2/kg) with significant variation among the cultivars tested. The highest FFA was observed in Arbequina (0.72%) and the lowest in Leccino and Picual (<0.4%). Koroneiki demonstrated the highest PV (8.53 mequiv of O_2/kg) and both Arbequina and Leccino demonstrated the lowest PV (~5 mequiv of O_2/kg) among the tested cultivars. This demonstrated that all oil samples produced from Negev desert grown cultivars meet the standard for extra virgin olive oil regarding FFA and PV.

The presence of FFA and PV in vegetable oils, including olive oil, is directly related to the intact TAG composition of oil and its integrity and quality (*34, 35*). The low FFA and PV results obtained in all oil samples showed a high intact TAG and low oxidation. These results help avoid the fear of obtaining low-quality olive oils from cultivars grown in the Israeli Negev desert due to cleavage of TAG molecules because of the relatively high temperature in this region. These results were also found to be very close to those of the study carried out by Haddada et al. (*31*) on Tunisian olive cultivars produced in a comparatively dry region. Furthermore, these results agree with the earlier findings: olive oil quality—mainly the free fatty acid and peroxide values—are practically independent of salinity and other mainly environmental factors (*3*); rather, these qualities are directly connected with the oil extraction and handling techniques (*36*).

Tocopherol Compositions. Two-year average data of the α-, β-, γ-, and δ-tocopherol composition of the olive oils from different cultivars grown in the Israeli Negev desert are shown in **Table 7**. These data show that Negev desert grown olives contain a far higher level of total tocopherols (ranging from 197 to 413 μg/g) compared to the previous literature data of traditional olive-growing areas (*37, 38*). As reported by these authors, the α-tocopherol fraction was found at the highest level in all tested oil samples in this study. A possible reason for the higher tocopherol level, especially the α-isomer, in our study compared to the traditional olive-growing areas might be the high temperature (hot growing environment). A high level of tocopherols in hot weather has already been reported in the literature, not only in olive oil (*39*) but in shea butter (*20*) and soybean as well (*40*). These data also pointed out a low percent of α-tocopherol content in oil from Negev desert grown olive

Table 4. Fatty Acid Profile (Percent of Total) of Olive Oils Determined by GC-MS from Six Common Cultivars Grown in the Israeli Negev Desert[a]

	olive cultivar						
fatty acid	Barnea	Souri	Arbequina	Picual	Leccino	Koroneiki	av[b]
myristic (C14:0)	0.05 a	0.05 a	0.05 a	0.02 c	0.03 b	0.05 a	0.04
palmitic (C16:0)	13.26 c	15.84 b	18.16 a	11.81 d	15.8 b	14.99 bc	14.98
palmitoleic (C16:1)	1.9 ab	1.4 b	1.0 c	0.6 d	1.8 ab	2.2 a	1.48
heptadecanoic (C17:0)	0.1 b	0.2 a	0.2 a	0.2 a	0.1 a	0.2 a	0.18
stearic (C18:0)	2.57 a	2.06 b	1.45 c	1.55 c	2.8 a	2.2 b	2.11
oleic (C18:1)	66.17 b	62.93 c	59.28 d	69.89 a	64.48 bc	67.08 b	64.97
linoleic (C18:2)	14.44 c	16.24 b	17.92 a	14.61 c	13.37 c	11.73 d	14.72
linolenic (C18:3)	0.8 a	0.7 ab	0.8 a	0.6 b	0.5 c	0.5 c	0.65
arachidic (C20:0)	0.3 c	0.2 c	0.5 a	0.2 c	0.4 b	0.3 c	0.32
gadoleic or eicosenoic (C20:1)	0.2 b	0.1 c	0.3 a	0.1 c	0.2 b	0.2 b	0.18
behenic (C22:0)	0.05 b	0.07 b	0.1 a	0.1 a	0.1 a	0.1 a	0.09
lignoceric (C24:0)	0.1 b	0.1 b	0.1 b	0.2 a	0.2 a	0.1 b	0.13

[a] Values followed by the same letter in each row are not significant at a 5% level, determined by Tukey−Kramer HSD. [b] Average value of all cultivars.

Table 5. Comparison of Fatty Acid Profiles of Olive Oil from Six Common Cultivars Grown in the Israeli Negev Desert Determined by GC-MS and MALDI-TOF/MS Methodologies

		fatty acid profile (%)				
cultivar	method	palmitic	stearic	oleic	linoleic	others
Arbequina	GC-MS	18.16	1.45	59.28	17.92	3.29
	MALDI-TOF/MS	19.41	0.17	60.64	19.78	
Picual	GC-MS	11.81	1.55	69.89	14.61	2.24
	MALDI-TOF/MS	19.75	0.83	63.68	15.74	
Barnea	GC-MS	13.26	2.57	66.17	14.44	3.76
	MALDI-TOF/MS	15.76	1.27	70.02	13.16	
Souri	GC-MS	15.84	2.06	62.93	16.24	2.33
	MALDI-TOF/MS	18.93	1.05	61.01	19.01	
Leccino	GC-MS	15.8	2.8	64.48	13.37	2.27
	MALDI-TOF/MS	19.41	0.57	63.19	16.84	
Koroneiki	GC-MS	14.99	2.2	67.08	11.73	3.91
	MALDI-TOF/MS	16.46	0.30	73.03	10.20	

Table 6. Free Fatty Acid and Peroxide Values of Olive Oil from Six Common Cultivars Grown in the Israeli Negev Desert[a]

cultivar	FFA (% oleic acid)	PV (mequiv of O_2/kg)
Barnea	0.49 bc	6.48 bc
Souri	0.60 ab	5.99 bc
Arbequina	0.72 a	5.19 c
Picual	0.39 c	7.23 ab
Leccino	0.34 c	5.22 c
Koroneiki	0.59 ab	8.53 a
av[a]	0.52 ± 0.03	6.43 ± 0.31

[a] Values followed by the same letter are not significant at a 5% level of significance determined by Tukey−Kramer HSD. [b] Average value of all cultivars.

Table 7. Tocopherol Compositions of Olive Oil from Six Common Cultivars Grown in the Israeli Negev Desert

	tocopherol (μg/g of oil)				
cultivar	α-	β-	γ-	δ-	total[a]
Barnea	368 (89.1)[b]	2.2 (0.5)	42 (10.2)	0.7 (0.2)	413 a
Souri	273 (82.7)	4.2 (1.3)	51 (15.4)	2 (0.6)	330 b
Arbequina	168(85.5)	3.4 (1.7)	24 (12.2)	1.2 (0.6)	197 d
Picual	284 (87.1)	4.1 (1.3)	36 (11.0)	2 (0.6)	326 c
Leccino	311(85.6)	2.4 (0.7)	47 (12.9)	3 (0.8)	363 b
Koroneiki	285 (88.2)	2.8 (0.9)	33 (10.2)	2.5 (0.8)	323 c
av[c]	281.5 (86.5)	3.2(1.0)	38.8 (11.9)	1.9 (0.6)	325

[a] Values followed by the same letter in total column are not significant at 0.05% level, determined by Tukey−Kramer HSD. [b] Values in parentheses represent percent of the total for the cultivar. [c] Average value of all cultivars.

cultivars (86%) compared to the other traditional growing areas, where the percent of α-tocopherol was reported to be >90% of the total (*8*). Similarly, the γ-isomer is found to reach ∼12% of the total. The reason for the higher γ-isomer is not clearly known, but possibly may be because of the higher temperature. Earlier studies have shown that with rising temperature the percent of the γ-isomer increases, compared to the α-isomer (*20*). Because the difference between α- and γ-isomers is only one methyl group (the α-isomer contains three and the γ-isomer contains two), this is quite possible. There is also a significant variation in the different isomers of the tocopherols, as well as in the total among the studied cultivars. The variation in tocopherol level among the olive cultivars was also mentioned in earlier papers (*37*−*39*). Among the tested cultivars, Barnea demonstrated the highest total tocopherol (413 μg/g) and Arbequina the lowest (197 μg/g) level.

Tocopherols are known as effective antioxidants, and the optimum concentration and combination improve the oxidative stability of edible oils (*41*). Hence, the findings of the comparatively greater integrity of TAG in olive oil from the Israeli Negev cultivated olive cultivars may have a direct correlation with the relatively higher level of tocopherol concentration. Although a minor proportion (∼2% of the total), the availability of the δ-tocopherol in these studied olive oils may have played a crucial role (*42*).

Table 8. Phytosterol Composition (Milligrams per Kilogram) of Olive Oil from Six Common Cultivars Grown in the Israeli Negev Desert

	olive cultivar					
phytosterol	Barnea	Souri	Arbequina	Picual	Leccino	Koroneiki
cholesterol	0.60 (0.03)[a]	0.41 (0.02)	1.66 (0.08)	1.37 (0.05)	0.99 (0.04)	0.7 (0.02)
brassicasterol	0.22 (0.01)	0.23 (0.01)	0.07 (<0.01)	0.15 (0.01)	0.56 (0.03)	0.32 (0.01)
24-methylenecholesterol	0.74 (0.03)	1.41 (0.08)	1.35 (0.06)	1.04 (0.04)	3.01(0.14)	2.89 (1.41)
campesterol	35.85 (1.69)	12.65 (0.74)	17.89 (0.82)	23.77 (0.81)	26.99 (1.21)	12.27 (0.39)
campestanol	3.48 (0.16)	2.10 (0.12)	0.70 (0.03)	3.05 (0.10)	0.61(0.03)	3.17 (0.10)
stigmasterol	6.32 (0.30)	5.61 (0.33)	8.58 (0.40)	5.96 (0.20)	6.34 (0.28)	6.79 (0.22)
Δ-7-campesterol	2.58 (0.12)	2.31 (0.14)	0.40 (0.02)	0.76 (0.03)	1.0 (0.04)	0.96 (0.03)
Δ-5,23-stigmastadienol	2.58 (0.12)	0.45 (0.03)	0.72 (0.03)	1.48 (0.05)	0.17 (0.01)	1.55 (0.05)
clerosterol	15.93 (0.75)	5.62 (0.33)	28.65 (1.32)	12.35 (0.42)	9.02 (0.40)	8.87 (0.29)
β-sitosterol	1999.66 (94.50)	1569.01 (92.23)	1965.9 (90.63)	2814.32 (96.11)	2057.15 (92.27)	3031.9 (97.54)
sitostanol	2.83 (0.13)	4.60 (2.83)	4.29 (0.20)	1.66 (0.06)	3.51 (0.16)	5.13 (00.17)
Δ-5-avenasterol	35.20 (1.66)	86.30 (5.07)	128.6 (5.93)	53.02 (1.81)	110.10 (4.94)	22.69 (0.73)
Δ-5,25-sigmastadienol	2.46 (0.12)	4.60 (0.27)	5.12 (0.24)	4.18 (0.14)	4.99 (0.22)	5.56 (0.18)
Δ-7-stigmastenol	4.20 (0.20)	2.10 (0.12)	2.20 (0.10)	2.53 (0.09)	2.0 (0.09)	2.15 (0.07)
Δ-7-avenasterol	3.48 (0.16)	3.80 (0.22)	3.05 (0.14)	2.57 (0.09)	3.08 (0.14)	3.29 (0.11)
total[b]	2116.13 d	1701.17 e	2169.12 d	2928.22 b	2229.53 c	3108.27a

[a] Values in parentheses in each column are the percentage of the total phytosterols in each cultivar. [b] Values followed by the same letter in the total row are not significant at a 5% level, determined by Tukey−Kramer HSD.

Phytosterol Composition. Phytosterol comprises a major portion of the unsaponifiable matter in most vegetable oils and is considered a characteristic of the purity of oils. Phytosterols are considered to be a very useful parameter for detecting adulteration of the oils (*23*). The profile of the phytosterol composition in the oils of the six common olive cultivars grown in the Israeli Negev desert as detected by GC-MS is presented in **Table 8**. In each cultivar 15 different phytosterols were found. Among the cultivars, Souri contained the lowest concentration of total phytosterols (1701 mg/kg); all other varieties had >2000 mg/kg of total phytosterols, and among them Koroneiki had the highest level (3108 mg/kg). Among the phytosterols, β-sitosterol was found in the highest concentration (92−97% of the total in all cultivars). Other main phytosterols found were Δ-5-avenasterol, clerosterol, and campesterol. These results clearly point out the higher total phytosterol results of olive oil in desert-cultivated oil than the common results of olive oil from trees grown in traditional areas (*43*).

According to regulations, extra virgin olive oil should contain ≥1000 mg/kg total phytosterols, ≤0.5% cholesterol, ≤4.0% campesterol, ≤0.5% Δ-7-stigmastenol, and ≥93% sitosterol (β-sitosterol + sitostanol + Δ-5,25-sigmastadienol) (*27*). Considering these limits, the phytosterol composition found in our study shows that all of the oils tested are within extra virgin categories. Moreover, the percentage of stigmasterol in all tested samples was less than that of campesterol, which indicates that the oil samples came from healthy fruits (*34*).

Studies have shown that phytosterols could undergo faster oxidation only when the temperature exceeds 100 °C; however, below this temperature they are rather stable (*44*). In natural conditions, production, extraction, and storage of olive oil occur below 100 °C. Olive oils produced in the Israeli Negev desert show a fairly high concentration of phytosterols in all cultivars tested, which may help to stabilize the oil, thus helping to integrate TAGs. Furthermore, phytostanols (which are saturated compounds and found at a significant concentration in olive oil) are less prone to oxidation even in the higher temperature condition (*45*).

Olive oil is considered to be among the healthiest oils on the basis of its balanced fatty acid composition and high levels of antioxidant components. This study clearly points out that olive oils from Israeli Negev desert cultivated olive cultivars effectively maintain this ratio and even have relatively high antioxidant compositions including tocopherols and phytosterols. The results of the low levels of FFA and PV found in the oil samples of this study, as in many past studies conducted in main olive production areas, show that there should not be any fear about the cleavage of intact TAG composition in the olive oil produced in the hot environment of the Israeli Negev desert. However, clear influences of genetic factors (significantly different among cultivars) were observed in most of the quality parameters tested.

Although it needs further work, at this level the MALDI-TOF/MS technology shows a tentative method for determining TAG and fatty acid profile in virgin olive oils; however, there is no doubt that this study demonstrates the high potential of MALDI-TOF/MS technology as an easy, reliable, and fast method for effective determination of intact TAG profiles and to profile the fatty acids without any derivatization.

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