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Quality Properties, Fatty Acid and Sterol Compositions of East Mediterranean Region Olive Oils

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Abstract: In this study, important physicochemical properties, fatty acid and sterol compositions of olive oils from the olives which harvested from Mersin (Buyuk Topak Ulak, Gemlik, Sari Ulak), Adana (Gemlik), Osmaniye (Gemlik) and Hatay (Gemlik, Kargaburun, Hasebi, Halhali) in the Eastern Mediterranean region of Turkey have been investigated. Ripening index and oil yield analysis of the olives and free fatty acids, peroxide value, UV absorbance (K_{232}, K_{270}), fatty acid composition, sterol composition, erythrodiol+uvaol content, and total sterol analysis of the olive oil samples were carried out. The levels of free acidity in the olive oil samples ranged from 0.39% (Hatay Gemlik: HG3) to 2.23% (Mersin Gemlik: MG). Peroxide value ranged from 8.87 to 18.87 meq O_2/kg. As K_{232} values in the oils fluctuated between 1.4370 and 2.3970, K_{270} values varied between 0.1270 and 0.1990. The results showed that all ΔK values were lower than the maximum legal limit of 0.01. The main fatty acid in all oil samples was oleic acid, ranging from 58.72% (Hatay Hasebi: HHs) to 74.54% (Hatay Gemlik: HG2). Palmitic acid values were within the percentage of 12.83% (Hatay Kargaburun: HK) to 18.50% (HHs). Total sterol content varied from 720.41 mg/kg (Hatay Kargaburun: HK) to 4519.17 mg/kg (Buyuk Topak Ulak: BTU). The β-sitosterol percentage of olive oils ranged from 76.12% (Adana Gemlik: AG) to 94.23% (Buyuk Topak Ulak: BTU). The results of this study indicated that variety significantly affect the quality indices, fatty acid and sterol compositions of olive oils significantly varied among varieties.

Key words: olive oil, quality indices, fatty acids, sterols

1 Introduction

Virgin olive oil (VOO) is natural vegetable oil obtained from fruit of the olive tree without chemical treatments[1]. VOO is mainly consisted of the saponifiable (98-99%) and unsaponifiable matters (1-2%) which are called minor compounds[2, 3]. The saponifiable matters are triacyglycerols, phosphatides, and waxes whereas the unsaponifiable matters are sterols, tocopherols, hydrocarbons (squalen), pigments and polyphenols[4, 5]. Free fatty acid (FFA) is an important criterion which refers to the quality and classification of olive oil[6]. Generally FFA level is high at the ending of physiological maturity of fruits[7]. K_{232}, K_{270} and peroxide values are considered as the indicators of the degree of oxidation[6].

The main fatty acids like oleic (C18:1), linoleic (C18:2), palmitic (C16:0) and stearic (C18:0) acids are found in olive oil[8]. The high percentage of oleic acid is an important component of the nutritional profile of olive oils[9]. Moreover, oleic acid is less susceptible to oxidation, having an important role in terms of contributing to the high stability[10−12]. Mailer *et al.*[13] determined the quality indices and the chemical composition of 10 olive cultivars viz. Arbequina, Barnea, Coratina, Corregiolo, Frantoio, Koroneiki, Leccino, Manzanillo, Pendolino and Picual which are grown in Australia. They indicated that the palmitic, palmitoleic and linoleic acid contents in oils are higher, but the oleic acid content is lower when plants grown under warmer climatic conditions.

Phytosterols are the main constituents of the unsaponifiable fraction of olive oil[14, 15]. Sterol composition is contributed to the quality of the oil and it is broadly used for checking oil genuineness and detecting adulteration, since sterols are known as the real fingerprint of oils[4, 16−18]. For example brassicasterol is present in rapeseed/canola oil;

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therefore, it is possible to use brassicasterol to detect olive oils's adulteration with canola oil[19]. The sterol fraction of vegetable oils can be divided into three main groups: 4-desmethylsterols, 4-monomethylsterols, and 4,4′-dimethylsterols (triterpene alcohols)[20]. Beta-sitosterol (29 carbons), campesterol (28 carbons), and stigmasterol (29 carbons) are the three most common sterols[21]. Olive oil contains 75-90% β-sitosterol, 5-20% Δ-5-avenasterol and 2-4% campesterol of total sterol composition. Furthermore, the triterpene alcohols such as erythrodiol and uvaol are found in VOOs[17, 22]. However, various factors such as olive cultivar, ripening degree, extraction method and storage conditions influence the sterol content and profile of VOO[23]. Clinical studies have shown that the dietary intake of phytosterols can reduce blood cholesterol levels inhibiting its absorption from the small intestine[2, 24, 25]. In addition, it has anti-inflammatory, antibacterial, antifungal, antioxidant and antitumoral activities[2, 24]. The chemical composition of olive oil varies according to variety[26], growing regions and climate conditions[27, 28], degree of ripening[29, 30], oil extraction methods and storage conditions[31, 32].

Turkey is one of the most important olive and olive oil production country in the Mediterranean regions alike Spain, Italy, Greece[33], and has adequate soil and climatic conditions for olive cultivation. The main aim of this study is to determine quality indices (FFA, peroxide value, K_{232}, K_{270} and ΔK values), fatty acid and sterol compositions of olive oils from Kargaburun, Hasebi, Halhalı, Sarı Ulak, Büyük Topak Ulak and Gemlik olive varieties harvested from Mersin, Adana, Osmaniye and Hatay provinces located in the East Mediterranean region of Turkey.

2 Experimental

2.1 Chemicals

All reagents used in the experiments were of analytical grade. These were Tri-methyl chlorosilane, hexamethyl chlorosilane, pyridine, 2,7 dichlorofluorescein, 5 α-cholestan-3beta ol, sodium carbonate, beta sitosterol, campesterol, stigmasterol, cholesterol, methanol, *n*-hexane, cyclohexane, ethyl ether, ethanol, diethyl ether, acetone, toluene, formic acid, chloroform, acetic acid, sodium sulfate and potassium iodine were purchased from Merck (Germany) and Sigma-Aldrich (Germany). The fatty acid methyl ester (FAME) mix was obtained from Supelco (Bellefonte, USA).

2.2 Olive sampling

This study was carried out during the crop season of 2015 - 2016. Olive samples of common Turkish olive varieties; Gemlik, Halhalı, Hasebi, Kargaburnu, Büyük Topak Ulak, Sarı Ulak were picked by hand from their growing area in the East Mediterranean region of Turkey. The olive samples were harvested from four trees (as replicates) of the different orchard for each cultivar from Hatay, Adana, Mersin and Osmaniye provinces. A representative 3 kg sample of olives was collected to represent the whole tree and coded with respective growing regions, varieties and harvesting dates of olive samples are illustrated in Table 1. Moreover, geographical characteristics about the locations from which olives were collected and given are listed in Table 2.

2.3 Ripening index (RI)

The RI was determined by using 100 olive fruits randomly selected from each olive fruit batch. It is reported earlier that the RI values ranged from 0 (100% intensely green skin) to 7 (100% purple flesh and black skin)[14].

Table 1 Codes, varieties, growing regions and harvesting dates of olive samples.

Code	Variety	Growing Region	Harvest date
HH	Halhalı	Hatay/Altınözü	10.11.2015
HK	Kargaburun	Hatay/Antakya	08.11.2015
HHs	Haşebi	Hatay/Altınözü	10.11.2015
HG3	Gemlik	Hatay/Reyhanlı	17.11.2015
HG2	Gemlik	Hatay/Altınözü	10.11.2015
HG1	Gemlik	Hatay/Antakya	08.11.2015
AG	Gemlik	Adana	14.11.2015
BTU	Buyuk Topak Ulak	Mersin	14.11.2015
MG	Gemlik	Mersin	13.11.2015
MS	Sarı Ulak	Mersin	13.11.2015
OG	Gemlik	Osmaniye	12.11.2015

Table 2 Geographical characteristics about the locations from which olives were obtained.

Code	Altitude (m)	Latitude	Longitude
HH	380	36° 00' 38.0" N	36° 14' 02.9" E
HK	120	36° 17' 00.9" N	36° 05' 50.9" E
HHs	380	36° 00' 38.0" N	36° 14' 02.9" E
HG3	155	36° 17' 54.5" N	36° 35' 48.1" E
HG2	380	36° 00' 38.0" N	36° 14' 02.9" E
HG1	120	36° 17' 00.9" N	36° 05' 50.9" E
AG	310	37° 15' 23.9" N	35° 04' 04.1" E
BTU	53	36° 54' 39.9" N	34° 51' 07.7" E
MG	33	36° 38' 01.8" N	34° 20' 48.2" E
MS	33	36° 38' 01.8" N	34° 20' 48.2" E
OG	79	37° 22' 20.4" N	36° 00' 59.5" E

2.4 Olive oil extraction

Three kg of olive samples selected from each variety were eliminated from damaged and decay fruits. Olive oil extraction was performed using a laboratory scale mechanical extractor (Hakkı Usta, Turkey) with a crusher, a vertical malaxer and a two-phase centrifuge. Malaxation and centrifuge processes was carried out at 25℃ for 30 min and at 3000 rpm, respectively. The oil was separated by decanting and put into dark glass bottles. Oil samples were kept at 4℃ until chemical analysis which were duplicated.

2.5 Oil content (%)

Oil content was performed according to the method described in American Oil Chemists' Society (AOCS) Official Methods Am 2-93[34] by Soxhlet extraction method using *n*-hexane at 80℃ for 6 h.

2.6 Quality indices

Free fatty acids (% oleic acid), peroxide value (meq O_2/kg) and spectrophotometric indices (K_{232}, K_{270}, ΔK) were performed according to the AOCS Official Methods Ca 5a-40, Cd 8-53, Ch 5-91[34], respectively.

2.7 Fatty acid compositions

The fatty acid composition of the oils was performed according to the method proposed by the International Olive Oil Council, COI/T.20/Doc.No.24[35]. Fatty acid methyl esters (FAME) were prepared by vigorous shaking of a solution of oil in *n*-heptane (0.1 g in 2 mL) with 0.2 mL of 2 N methanolic potassium hydroxide. The analysis of FAME was performed by Thermo Focus gas chromatography system (ThermoFisher Scientific, Milan, Italy,) using a hydrogen flame ionization detector (FID) and a capillary column SGE BPX 90 (100 m length, 0.25 mm i.d. and 0.25 μm film thickness). The temperatures of detector and injector were set at 290 and 250℃, respectively. Helium was employed as carrier gas with a flow rate of 1 mL/min and the split ratio was 1:10. The injection volume was 1 μL. The results were expressed as a relative area percentage of total. Fatty acids were determined by comparing their retention times with those of reference compounds.

2.8 Sterol and triterpene diols (erythrodiol+uvaol) composition

Sterol compositions were determined depending on the official method IOC/T.20/No10[36]. Identification and quantification of sterols and diols as trimethylsilyl ethers was performed by gas chromatography (GC 2010, Shimadzu, Japan) equipped with a SGE BPX5 capillary column (25 m, 0.32 mm i.d. and 0.25 μm film thickness) and a flame ionization detector (FID). Injector, column and detector temperatures were 280, 265 and 290℃, respectively. Helium was used as the carrier gas with a flow rate of 1 mL/min and the split ratio of 10:1. Individual sterols and two triterpendiols (erythrodiol and uvaol) in oils were identified based on their relative retention times with respect to the internal standard, cholestanol, according to the standardized reference method.

2.9 Statistical analysis

Statistical analysis was carried out using the SPSS 10.0 statistical software (SPSS Inc., Chicago, USA). Data were analyzed by a one-way analysis of variance (ANOVA). Significant differences between samples were determined by Duncan's multiple range test at the 5% confidence level[37].

3 Results and Discussion

3.1 Quality indices

Ripening index, oil content of olives and quality indices (FFA, peroxide value and K_{232}, K_{270}, ΔK values) of olive oils are shown in Table 3. The RI of olives ranged from 1.40 (BTU)– 6.30 (OG, HG1). Oil content of olives varied between 13.92% (BTU) and 32.37% (HHs). Several factors like variety, ripeness, climatic conditions and temperature during oil synthesis are among the factors that can affect significantly on the amount of oil content as reported by Yorulmaz and Bozdogan Konuskan[3], and Rallo *et al.*[8]; Fernandez-Cuesta *et al.*[23]; Noorali *et al.*[38]. All the studied olive oils exhibited the values of some quality indices (peroxide value ≤ 20 meq O_2/kg; K_{232} ≤ 2.5; K_{270} ≤ 0.22 and ΔK ≤ 0.01) below the limits established by EU regulation for extra virgin olive oil. However, free fatty acidity values of the samples were over the limit of 0.8% fixed for extra virgin olive oil category except for HG1 and HG3. Our results were higher than those reported by Bouarroudj *et al.*[39] whereas in they were in agreement with those by Yorulmaz and Bozdogan Konuskan[3]. Free fatty acid which is a quality criteria for olive oils can show variations according to variety, location, olive fly and unsuitable storage conditions[8, 40, 41].

3.2 Fatty acid compositions

The fatty acid compositions of olive oils samples are presented in Table 4. The major fatty acids were palmitic (C16:0), oleic (C18:1) and linoleic (C18:2) acids while palmitoleic (C16:1), linolenic (C18:3), stearic (C18:0), arachidic (C20:0), behenic (C22:0) acids occurred in minor amounts. According to the results, the fatty acid content of olive oil samples was within the legal limits proposed by EU regulation for extra virgin olive oil. The fatty acid compositions were significantly influenced by variety in the oil samples ($p < 0.05$). The major fatty acid in all oil samples was oleic acid, varying between 58.72% (Hatay Hasebi) and 74.54% (Hatay Gemlik2). Xiang *et al.*[15] reported that the oleic acid content of four olive oil varieties from China was in the range of 60.94-74.03%. Our results showed similarity with those of the results obtained by Xiang *et al.*[15]. In another study by Manai-Djebali *et al.*[2] oleic acid content of five Tunisian olive oil varieties varied between 67.2 (Aloui) and 78.9% (Hor Kesra), indicating that they obtained higher values of oleic acid content when compared with our results. Palmitic acid, the main saturated fatty acid of olive oils, ranged from 12.83 (HK) to 18.50% (HHs). Lopez-Cortes *et al.*[42] stated that palmitic acid content from eight Spanish varieties ranged from 9.84 to 18.44% as in accordance with our findings. However, Arbequina olive oil during 2011 crop season contained higher palmitic acid than our varieties according to the work by Abenoza *et al.*[41]. Linoleic acid content varied between 4.88% (HG2) and 17.18% (BTU). These results were in agreement with those obtained by Bouarroudj *et al.*[39] Laincer *et al.*[43] and Xiang *et al.*[15], although the linoleic acid content of Brazilian olive oil samples in the study carried out by Ballus *et al.*[44] was lower than our values. Stearic, palmitoleic, linolenic, arachidic, and behenic acids were present in the range between 2.49 and 3.77, 0.46 and 1.58, 0.46 and 0.87, 0.39 and 0.58 and 0.10 and 0.16%, respectively. The fatty acid composition of olive oil is significantly influenced by the cultivar, ripeness stage of the fruit, climatic conditions, latitude, irrigation management and zone of production[38, 45, 46].

3.3 Sterol and triterpene diols (erythrodiol + uvaol) composition

The sterol and triterpene diols (erythrodiol + uvaol) compositions of olive oil samples are given in Table 5. The sterols found with the highest amounts were β-sitosterol, Δ-5-avenasterol and campesterol was found in the sterols which made up over 90% of total sterol content, while cholesterol, stigmasterol, campesterol, Δ-5,24-stigmastadienol, Δ-5,23-stigmastadienol, Δ-7-stigmastenol, Δ-7-avenas-

Table 3 Ripening index, water and oil content of olive varieties and quality criterion of olive oils.

Cultivars and Locations	Ripening Index	Oil content %	Free fatty acids (oleic %)	Peroxide value (meq O_2/kg)	K_{232}	K_{270}	ΔK
HH	$3.90^{e} \pm 0.05$	$25.47^{b,c} \pm 0.55$	$1.82^{c} \pm 0.10$	$11.99^{g} \pm 0.10$	$1.8330^{i} \pm 0.002$	$0.1800^{c} \pm 0.002$	$0.0033^{b} \pm 0.0003$
HK	$4.60^{c} \pm 0.05$	$22.06^{e} \pm 0.18$	$1.23^{g} \pm 0.00$	$18.87^{a} \pm 0.08$	$2.2040^{c} \pm 0.002$	$0.1320^{h} \pm 0.002$	$0.0005^{f} \pm 0.0000$
HHs	$3.80^{f} \pm 0.05$	$32.37^{a} \pm 0.36$	$1.92^{b} \pm 0.20$	$14.19^{e} \pm 0.08$	$1.9000^{g} \pm 0.002$	$0.1720^{d} \pm 0.002$	$0.0013^{e} \pm 0.0003$
BTU	$1.40^{h} \pm 0.05$	$13.92^{g} \pm 0.73$	$0.87^{i} \pm 0.10$	$9.95^{h} \pm 0.19$	$1.8410^{h} \pm 0.002$	$0.1440^{g} \pm 0.002$	$0.0005^{f} \pm 0.0000$
AG	$4.50^{d} \pm 0.05$	$25.01^{c} \pm 0.64$	$0.90^{h} \pm 0.06$	$12.63^{f} \pm 0.12$	$1.8290^{j} \pm 0.002$	$0.1270^{i} \pm 0.002$	$0.0020^{d} \pm 0.0000$
MG	$4.70^{b} \pm 0.05$	$25.57^{b,c} \pm 0.57$	$2.23^{a} \pm 0.06$	$15.44^{c} \pm 0.13$	$2.3140^{b} \pm 0.002$	$0.1510^{f} \pm 0.002$	$0.0030^{c} \pm 0.0000$
MS	$2.80^{g} \pm 0.05$	$18.81^{f} \pm 0.27$	$1.54^{e} \pm 0.01$	$14.68^{d} \pm 0.12$	$2.1900^{d} \pm 0.002$	$0.1990^{a} \pm 0.002$	$0.0003^{f} \pm 0.0003$
OG	$6.30^{a} \pm 0.05$	$23.97^{d} \pm 0.44$	$1.34^{f} \pm 0.02$	$11.83^{g} \pm 0.07$	$2.3970^{a} \pm 0.002$	$0.1830^{b,c} \pm 0.002$	$0.0050^{a} \pm 0.0000$
HG1	$6.30^{a} \pm 0.05$	$23.60^{d} \pm 0.52$	$0.69^{j} \pm 0.06$	$16.55^{b} \pm 0.15$	$2.1140^{e} \pm 0.002$	$0.1840^{b} \pm 0.002$	$0.0013^{e} \pm 0.0003$
HG2	$3.80^{f} \pm 0.05$	$25.43^{b,c} \pm 0.23$	$1.59^{d} \pm 0.06$	$8.87^{i} \pm 0.13$	$1.4370^{k} \pm 0.002$	$0.1710^{d} \pm 0.002$	$0.0005^{f} \pm 0.0000$
HG3	$4.50^{d} \pm 0.05$	$26.13^{b} \pm 0.23$	$0.39^{k} \pm 0.01$	$10.00^{h} \pm 0.17$	$1.9240^{f} \pm 0.002$	$0.1570^{e} \pm 0.002$	$0.0013^{e} \pm 0.0003$

Results are signified as mean ± SD of three sample replicates. Different small letters express significant statistical differences (Duncan's Test $p < 0.05$) among varieties.

Table 4 Fatty acid composition of olive oils (%).

Cultivars	Palmitic %	Palmitoleic %	Heptadecanoic %	Heptadecenoic %	Stearic %	Oleic %	Linoleic %	Linolenic %	Arachidic %	Eicosenoic %	Eicosatetra-enoic %	Behenic %	*Trans* oleic (C18:1T) %	*Trans* Linoleic + Linolenic (C18:2T + C18:3T)%
HH	13.55^{g} ± 0.024	1.20^{d} ± 0.006	0.11^{e,f} ± 0.0060	0.20^{c,d} ± 0.0075	3.02^{g} ± 0.0135	74.50^{a} ± 0.0785	4.97^{j} ± 0.0005	0.47^{h} ± 0.0055	0.44^{h} ± 0.0000	0.23^{d} ± 0.0005	1.12^{d} ± 0.0275	0.11^{g} ± 0.0000	0.024^{b,c} ± 0.0005	0.018^{g} ± 0.0000
HK	12.83^{i} ± 0.031	0.46^{j} ± 0.001	0.13^{c,d} ± 0.0070	0.16^{f} ± 0.0040	3.69^{c} ± 0.0040	70.78^{c} ± 0.0395	9.37^{g} ± 0.0130	0.46^{h} ± 0.0075	0.49^{e} ± 0.0030	0.28^{b} ± 0.0200	1.07^{e} ± 0.0115	0.12^{f} ± 0.0005	0.016^{d} ± 0.0065	0.025^{c} ± 0.0000
HHs	18.50^{a} ± 0.075	1.58^{a} ± 0.007	0.11^{f} ± 0.0070	0.13^{g} ± 0.0015	3.75^{b} ± 0.0035	58.72^{j} ± 0.1085	14.70^{b} ± 0.0295	0.59^{e} ± 0.0040	0.58^{a} ± 0.0020	0.21^{e,f} ± 0.0005	0.91^{g} ± 0.0035	0.15^{b} ± 0.0015	0.027^{b} ± 0.0005	0.024^{c} ± 0.0015
BTU	14.54^{d} ± 0.043	0.71^{i} ± 0.003	0.05^{g} ± 0.0005	0.06^{h} ± 0.0010	2.49^{j} ± 0.0145	62.27^{i} ± 0.0725	17.18^{a} ± 0.0125	0.87^{a} ± 0.0010	0.49^{d} ± 0.0010	0.33^{a} ± 0.0000	0.75^{i} ± 0.0035	0.15^{b} ± 0.0010	0.024^{b,c} ± 0.0025	0.034^{a} ± 0.0040
AG	14.22^{e} ± 0.069	1.27^{c} ± 0.004	0.10^{f} ± 0.0005	0.20^{e} ± 0.0020	2.93^{h} ± 0.0025	68.74^{e} ± 0.0840	10.37^{e} ± 0.0160	0.58^{e} ± 0.0035	0.39^{j} ± 0.0005	0.22^{e} ± 0.0045	0.81^{h} ± 0.0005	0.10^{i} ± 0.0005	0.020^{c,d} ± 0.0015	0.020^{e,f} ± 0.0025
MG	13.41^{h} ± 0.036	0.88^{g} ± 0.005	0.12^{d,e} ± 0.0160	0.20^{d,e} ± 0.0065	3.51^{d} ± 0.0045	67.56^{f} ± 0.0070	10.95^{d} ± 0.0235	0.47^{h} ± 0.0115	0.53^{b} ± 0.0025	0.25^{c} ± 0.0030	1.91^{a} ± 0.0255	0.14^{d} ± 0.0020	0.035^{a} ± 0.0015	0.031^{a,b} ± 0.0030
MS	15.19^{c} ± 0.050	0.84^{h} ± 0.006	0.20^{a} ± 0.0005	0.25^{a} ± 0.0000	3.34^{e} ± 0.0055	64.78^{h} ± 0.0320	12.49^{c} ± 0.0195	0.82^{b} ± 0.0035	0.57^{a} ± 0.0005	0.26^{c} ± 0.0005	0.97^{f} ± 0.0060	0.16^{a} ± 0.0005	0.025^{b,c} ± 0.0005	0.033^{a,b} ± 0.0020
OG	15.82^{b} ± 0.020	1.49^{b} ± 0.004	0.13^{d} ± 0.0025	0.23^{b} ± 0.0010	2.87^{i} ± 0.0025	66.79^{g} ± 0.0060	10.04^{f} ± 0.0195	0.72^{c} ± 0.0030	0.43^{i} ± 0.0010	0.21^{f} ± 0.0000	1.07^{e} ± 0.0005	0.11^{h} ± 0.0010	0.024^{b,c} ± 0.0035	0.030^{b} ± 0.0015
HG1	13.93^{f} ± 0.008	0.87^{g} ± 0.011	0.16^{b} ± 0.0550	0.23^{b} ± 0.0005	3.77^{a} ± 0.0105	70.11^{d} ± 0.0560	8.45^{h} ± 0.0285	0.68^{d} ± 0.0045	0.52^{c} ± 0.0020	0.23^{d} ± 0.0030	0.84^{h} ± 0.0115	0.13^{e} ± 0.0010	0.028^{b} ± 0.0045	0.023^{c,d} ± 0.0005
HG2	13.34^{h} ± 0.003	1.06^{e} ± 0.005	0.12^{d,e} ± 0.0025	0.20^{d,e} ± 0.0040	3.14^{f} ± 0.0075	74.54^{a} ± 0.0005	4.88^{k} ± 0.0060	0.49^{g} ± 0.0015	0.48^{f} ± 0.0025	0.25^{c} ± 0.0000	1.27^{b} ± 0.0090	0.14^{c} ± 0.0005	0.027^{b} ± 0.0005	0.019^{g} ± 0.0015
HG3	13.60^{g} ± 0.005	0.93^{f} ± 0.001	0.15^{c} ± 0.0005	0.21^{c} ± 0.0005	3.51^{d} ± 0.0005	72.48^{b} ± 0.0070	6.46^{i} ± 0.0045	0.53^{f} ± 0.0015	0.48^{g} ± 0.0010	0.23^{d} ± 0.0015	1.23^{c} ± 0.0080	0.12^{e} ± 0.0020	0.033^{a} ± 0.0000	0.019^{e,f} ± 0.0010

Results are signified as mean ± SD of three sample replicates. Different small letters express significant statistical differences (Duncan's Test $p < 0.05$) among varieties.

Table 5 Sterol composition of olive oils (%).

Cultivars and Locations	Cholesterol %	Campesterol %	Stigmasterol %	Δ-5,23 Stigmastadienol %	β-Sitosterol %	Δ-5 Avenasterol %	Δ-5,24 Stigmastadienol %	Δ-7 Stigmastenol %	Δ-7 Avenasterol %	Apparent β-Sitosterol %	Total Sterol (mg/kg)	Erythrodiol + Uvaol %
HH	0.13^{d} ± 0.0020	1.32^{d,e} ± 0.0335	1.03^{b} ± 0.0290	0.51^{d} ± 0.0005	86.07^{g} ± 0.2230	10.29^{c} ± 0.2840	0.30^{f} ± 0.0005	0.08^{e} ± 0.0015	0.26^{h} ± 0.0035	97.18^{a} ± 0.0600	788.80^{j} ± 0.0950	1.32^{e} ± 0.0100
HK	tedb^{e} ± 0.0000	1.52^{c,d} ± 0.0080	1.35^{a} ± 0.0450	0.59^{c} ± 0.0210	88.07^{e} ± 0.1680	6.83^{g} ± 0.1455	0.69^{b} ± 0.0085	0.43^{a} ± 0.0170	0.54^{d} ± 0.0090	96.17^{d} ± 0.0100	720.41^{k} ± 4.6300	3.25^{b} ± 0.0100
HHs	tedb^{e} ± 0.0000	2.90^{a} ± 0.0405	0.80^{d} ± 0.0020	0.58^{c} ± 0.0055	90.17^{c} ± 0.3100	4.86^{h} ± 0.2500	0.17^{g} ± 0.0140	tedb^{f} ± 0.0000	0.53^{d} ± 0.0090	95.78^{e} ± 0.0515	1804.38^{e} ± 4.6325	2.49^{c} ± 0.1510
BTU	tedb^{e} ± 0.0000	2.58^{b} ± 0.0435	0.61^{f} ± 0.0150	0.52^{d} ± 0.0130	94.23^{a} ± 0.0240	1.82^{j} ± 0.0440	0.08^{h} ± 0.0060	0.02^{f} ± 0.0015	0.14^{j} ± 0.0010	96.65^{c} ± 0.0270	4519.17^{a} ± 25.0800	tedb^{g} ± 0.000
AG	tedb^{e} ± 0.0000	0.96^{e,f} ± 0.0275	0.87^{c} ± 0.0465	0.62^{b,c} ± 0.0080	76.12^{i} ± 0.1250	18.91^{a} ± 0.0710	0.90^{a} ± 0.0585	0.35^{b} ± 0.0430	1.27^{a} ± 0.0040	96.55^{c} ± 0.1205	1924.95^{d} ± 107.1405	4.27^{a} ± 0.0045
MG	0.30^{a} ± 0.0000	1.14^{d,e} ± 0.0330	0.58^{f} ± 0.0255	0.71^{a} ± 0.0075	87.13^{f} ± 0.1195	8.21^{e} ± 0.0015	0.50^{c} ± 0.0045	0.37^{b} ± 0.0425	0.99^{b} ± 0.0140	96.55^{c} ± 0.1240	2057.38^{c} ± 3.1575	tedb^{g} ± 0.000
MS	0.27^{b} ± 0.0025	2.62^{b} ± 0.0000	0.71^{e} ± 0.0370	0.66^{b} ± 0.0155	92.84^{b} ± 0.0195	2.25^{i} ± 0.0185	0.32^{e,f} ± 0.0055	0.10^{e} ± 0.0100	0.20^{i} ± 0.0100	96.07^{d} ± 0.0590	3094.66^{b} ± 3.6955	0.07^{g} ± 0.0040
OG	0.28^{b} ± 0.0270	0.75^{f} ± 0.4910	0.89^{c} ± 0.0170	0.64^{b} ± 0.0195	86.88^{f} ± 0.0640	8.81^{d} ± 0.0915	0.53^{c} ± 0.0460	0.20^{c} ± 0.0130	0.49^{e} ± 0.0070	96.87^{b} ± 0.0010	1586.72^{g} ± 5.7935	0.21^{f} ± 0.0025
HG1	0.26^{b} ± 0.0190	1.33^{d,e} ± 0.0315	0.64^{f} ± 0.0150	0.72^{a} ± 0.0025	88.41^{d} ± 0.0040	7.73^{f} ± 0.0190	0.36^{e} ± 0.0425	0.14^{d} ± 0.0165	0.41^{f} ± 0.0115	97.22^{a} ± 0.0600	1648.25^{f} ± 12.0235	tedb^{g} ± 0.000
HG2	0.18^{c} ± 0.0050	1.63^{c} ± 0.0395	0.90^{c} ± 0.0370	0.74^{a} ± 0.0880	87.07^{f} ± 0.2195	8.68^{d} ± 0.3045	0.34^{e,f} ± 0.0155	0.09^{e} ± 0.0055	0.30^{g} ± 0.0250	96.84^{b} ± 0.0145	880.18^{i} ± 3.1560	1.81^{d} ± 0.0625
HG3	0.28^{b} ± 0.0210	1.23^{d,e} ± 0.0035	0.62^{f} ± 0.0625	0.76^{a} ± 0.0150	81.18^{h} ± 0.1175	14.56^{b} ± 0.0470	0.44^{d} ± 0.0095	0.10^{e} ± 0.0230	0.84^{c} ± 0.0085	96.94^{b} ± 0.0950	980.65^{h} ± 2.9885	tedb^{g} ± 0.000

Results are signified as mean ± SD of three sample replicates. Different small letters express significant statistical differences (Duncan's Test $p < 0.05$) among varieties.

terol and two triterpene dialcohols (erythrodiol and uvaol) were identified in small amounts. There was a significant differentaition between cultivars in relation to the sterol composition of olive oil samples ($p < 0.05$). The total sterol content of the studied olive oils which ranged from 720.41 (HK) to 4519.17 (BTU) mg/kg was found to be above the allowable limits (1000 mg/kg) for extra virgin olive oils specified by EU regulations except for 720.41 mg/kg with HK, 788.80 mg/kg with HH, 880.18 mg/kg with HG2 and 980.65 mg/kg with HG3. Additionally, Gemlik oil samples from different locations of Hatay showed significant changes in terms of total sterol content. β-sitosterol was the predominant phytosterol in all oil samples, varying between 76.12 (AG) and 94.23% (BTU). The studied Turkish oils exhibited high contents of β-sitosterol when compared with those from other olive oils, namely the Tunisian ones worked by Baccouri *et al.*[17], the Iranian ones studied by Noorali *et al.*[38] and the Spanish ones studied by Fernandez-Cuesta *et al.*[23]. Apparent β-sitosterol content of the all samples, calculated as the sum of the contents of β-sitosterol and other sterols (sitostanol, Δ-5,24-stigmastadienol, clerosterol, and Δ-5-avenasterol), were higher than the legal minimum of 93% established by EU regulations. These results were in good harmony with those of Baccouri *et al.*[17] who found the values of apparent β-sitosterol content of the oils from different Tunisian varieties ranging from 92.63 to 95.33%.

Δ-5-avenasterol, which is the second most plentiful sterol in olive oils, fluctuated between 1.82 (BTU) and 18.91% (AG). These results were in consistent with those of Lukic *et al.*[14] Hannachi *et al.*[47] and Damirchi *et al.*[20] even though Fernandez-Cuesta *et al.*[23] obtained higher values of the Δ-5-avenasterol content of Spanish olive oils. Campesterol content of the oil samples was below the maximum limit of 4% required by EU regulations, varying between 0.75 (OG) and 2.90% (HHs). Manai-Djebali *et al.*[2] obtained higher campesterol content of Tunisian olive oils while our values were in agreement with those of the results found by Temime *et al.*[27]. The content of campesterol in olive oils can show variations according to olive ripening and variety[3]. Stigmasterol is the main sterol associated to the low quality of virgin olive oil[38]. The contents of stigmasterol of oils were lower than those of campesterol required by EU regulations, ranging from 0.58 (MG) to 1.35 (HK). Moreover, Δ-7 stigmastenol and cholesterol contents of all oils were within the legal maximum of 0.5%. The triterpenic dialcohols (erythrodiol and uvaol), which are the part of unsaponifiable fraction of olive oil, are analyzed together with the sterol fraction[2]. The percentage of triterpenic dialcohols (erythrodiol and uvaol) was below the maximum limit of 4.5% required by EU regulations, varying between 0.07 (MS) and 4.27% (AG). Gorassini *et al.*[48] determined the erythrodiol and uvaol contents of natural olive oils between 1.6-3.8% using different methods. These findings are similar to our study.

4 Conclusion

This study showed that olive oils from different varieties (Halhalı, Gemlik, Kargaburun, Büyük Topak Ulak, Hasebi, Sarı Ulak) in different growing regions of East Mediterranean have significant variations based on chemical properties, fatty acid and sterol compositions, and these results were in accordance with the internationally accepted limits. Gemlik oils obtained from five different locations had significant variations especially in terms of oleic acid, β-sitosterol content, total sterol content. The varieties growing in East Mediterranean should be intensely cultivated and certified with Protected Designation of Origin due to their higher quality properties.

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Conflict to Interest

The authors certify that there is no conflict of interest.

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