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ORIGINAL PAPER

Effects of Variety, Maturation and Growing Region on Chemical Properties, Fatty Acid and Sterol Compositions of Virgin Olive Oils

Dilsat Bozdogan Konuskan[1] · **Burcu Mungan**[1]

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Abstract Chemical properties, fatty acid and sterol compositions of olive oils extracted from Gemlik and Halhalı varieties grown in Hatay and Mardin provinces in Turkey were investigated during four maturation stages. The olive oil samples were analyzed for their chemical properties such as free acidity, peroxide value, total carotenoid, total chlorophyll, total phenolic contents, antioxidant activity, fatty acid and sterol compositions. Chemical properties, fatty acids and sterol profiles of olive oil samples generally showed statistically significant differences depending on the varieties, maturation and growing areas ($p < 0.05$). As free fatty acid contents and total phenolic contents increased, total carotenoid and chlorophyll contents decreased throughout the maturity stages. Total carotenoid and chlorophyll contents of oil samples from Mardin were higher than those of Hatay. The total phenolic compounds of olive oil samples ranged from 20.62 in Gemlik to 525.22 mg GAE/kg oil in Halhalı from Hatay. In general, the phenolic contents and antioxidant activities of olive oil samples were positively associated. Oleic acid content was the highest 71.53 % in H1 samples in Hatay. Total sterol contents were 1194.33 mg/kg in Halhalı and 2008.66 mg/kg in Gemlik from Hatay. Stigmasterol contents of oils obtained from Hatay were lower than those of Mardin. Oleic acid, palmitic acid, β-sitosterol, Δ-5-avenasterol and campesterol contents fluctuated with maturation for each of variety from both growing regions. These results showed that the variety, growing area and maturation influence the chemical properties, fatty acid and sterol compositions.

✉ Dilsat Bozdogan Konuskan
dilsat@mku.edu.tr

[1] Food Engineering Department, Faculty of Agriculture, Mustafa Kemal University, Hatay, Antakya, Turkey

Keywords Olive cultivar · Maturity · Growing region · Fatty acid · Sterol

Introduction

Virgin olive oil (VOO) has nutritional, sensorial and functional characteristics that make it unique among other vegetable oils and a basic component of the Mediterranean diet [1, 2]. VOO is a juice obtained by solely mechanical or other physical means from the fruit of the olive tree, without the use of chemicals [3]. Thus generally lipophilic components of the drupe are transferred to the oil, which in turn retains the organoleptic properties of olives [4]. The composition of VOO is mainly constituted of triacylglycerols and minor compounds (0.5–2 %) which is called unsaponifiable or nonglycerol fraction [5]. Olive oil has high content of monounsaturated fatty acids (%56–84) mainly oleic acid [6, 7]. The high percentage of oleic acid is an important component of the nutritional profile of olive oils, particularly in relation to its effect on cardiovascular system health [8]. Olive oil naturally contains main minor compounds such as hydrocarbons, sterols, aliphatic alcohols, polyphenols, tocopherols, pigments and flavor components [9, 10]. Phytosterols are main constituents of the unsaponifiable fraction of olive oil [11]. Sterol composition is related to the quality of the oil and are broadly used for checking its genuineness and detecting adulteration, since sterols can be considered as its real fingerprint [9, 11, 12]. Clinical studies have demonstrated that the dietary intake of phytosterols may reduce blood cholesterol levels inhibiting its absorption from the small intestine. Also it has anti-inflammatory, antibacterial, antifungal, antioxidant and antitumoral activities [13–15]. The main olive oil sterols are β-sitosterol (%75–90), Δ-5-avenasterol (%5–20)

and campesterol (%1–4). Total sterol content of olive oil is affected by the cultivar, stage of maturity of the olives, extraction method, environmental conditions and storage time prior to oil extraction [16, 17]. Phenolic compounds which are important minor compounds and main antioxidants in VOO are used in the characterization and authentication with respect to geographical origin and cultivars [15, 18, 19]. They have potent antioxidant activity and contribute significantly to the remarkable stability of VOO against oxidation [10, 20]. The benefits of phenolic on human health are correlated with their antioxidant activity [7, 21]. They are also responsible for the nutritional and organoleptic properties such as bitter and pungent taste of VOO [4, 22]. Olive oil color is correlated with its pigment composition, which is considered quality criterion. Chlorophyll and carotenoid pigments are mainly responsible for the color of VOO, ranging from yellow–green to greenish gold [23, 24]. Chlorophyll pigments act as photosensitizers, promoting oxidation, while antioxidant activity was reported in the dark [25, 26]. Carotenoids, as singlet oxygen quenchers, protect oils from photo-oxidation [23, 27]. Therefore, these minor compounds show great importance since their antioxidant activity, nutritional and organoleptic properties effects on VOO. Antioxidant content of the oil is not constant; it depends on the cultivar, maturation stage, agroclimatic conditions and olive growing techniques [23, 28]. Turkey is one of the most important Mediterranean countries, such as Spain, Italy, Greece because of olive and olive oil production [29]. Turkey has adequate climate and soil conditions for olive production [30]. Despite the great production and economic importance of olive oil, relatively little information is available in the literature about the characteristics particularly sterol profiles of Turkish olive oil. The aim of this work was to determine the effect of the cultivar, maturation and growing area on the chemical compositions (free fatty acids, peroxide value, total chlorophyll, total carotenoid, total phenolic contents, antioxidant activity, fatty acids) and sterol profiles of olive oils.

Materials and Methods

Reagents and Standards

All reagents used in the experiments were of analytical grade. Folin-Ciocalteu's reagent, gallic acid, 2,2′-diphenyl-1picryhydrazyl (DPPH), 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).

Olive Sampling

This study was performed during the growing seasons between 2014 and 2015. Olive samples of two common Turkish olive varieties; Gemlik and Halhalı, were picked by hand in their growing area in Hatay (Mediterranean region of Turkey) and Mardin (south eastern Anatolia region of Turkey) provinces. The olive samples were collected from three trees (as replicates) of the same orchard for each individual cultivar and at four different harvesting times, 20 day intervals, beginning from the 5th of September to the 5th of November and coded accordingly from 1 to 4 as H1-H4 for Halhalı and G1–G4 for Gemlik cultivars. Only healthy olive drupes, without any infection or physical damage were selected.

Olive Oil Extraction

Oil extraction was carried out within 24 h from the harvested olives. A representative 3 kg olive sample was extracted to obtain the oil by a laboratory scale mechanical mill (Hakkı Usta, Turkey) with a crusher, a vertical malaxer and a two-phase centrifuge. Malaxation and centrifuge processes was performed at 25 °C for 30 min and at 5000 rpm, respectively. The oil was separated by decanting and put into dark glass bottles. Oil samples were kept at 4 °C until chemical analysis which were duplicated.

Chemical Properties

Free acidity (% oleic acid) and peroxide value (mequiv O_2/kg) were performed according to the AOCS official methods Ca 5a-40 and Cd 8–53, respectively [31].Chlorophyll and carotenoid compounds were determined at 670 and 470 nm, respectively, in cyclohexane using the specific extinction values, by the method of Minguez-Mosquera *et al*. [32]. The chlorophyll and carotenoid contents are expressed as mg of pheophytin "a" and lutein per kg of oil, respectively.

Extraction of Phenolics

The phenolic extraction procedure was performed according to the method described by Pirisi *et al*. [33]. Firstly, a 2-ml oil sample was weighed into a centrifuge tube and 1 ml hexane and 2 ml methanol:water (60:40, v/v) were added. This mixture was stirred for 2 min in a vortex and the tube was then centrifuged at 3000 rpm for 5 min. The methanol:water phase was separated and the extraction was

repeated twice. Finally, phenolic extracts were recovered with a syringe and then filtered through a 0.45 μm membrane filter (Millipore, USA) before analysis.

Total Phenolic Content

THe total phenolic content was colorimetrically determined according to the Folin-Ciocalteu procedure as reported by Montedoro *et al*. [34], with some modifications. Firstly, 0.2 ml phenolic extract was mixed with 0.5 ml Folin-Ciocalteu reagent. Then, 1 ml saturated sodium carbonate solution was added and the mixture shaken for 0.5 min. Finally, the solution was brought up to a volume of 10 ml with distilled water. After 45 min of reaction at ambient temperature in the dark, the absorbance was measured at 765 nm in a UV–Vis spectrophotometer (Hitachi U1900, Japan). The quantitative results were calculated using an analytical curve of gallic acid equivalents per kg of VOO (mg GAE/kg).

Antioxidant Activity

The antioxidant activities (free radical scavenging capacity) of phenolic extracts, was measured using 2-2′-diphenyl-1-picrylhydrazyl radical (DPPH) according to the procedure of Brand-Williams [35] with some modifications. Briefly, 1 ml of extracts were diluted with 1.9 ml DPPH methanolic solution. Samples were incubated at room temperature in the dark for 60 min and then the absorbance was measured at 515 nm against a blank (MeOH). The percentage of inhibition was calculated from the following equation:

$$\text{DPPH inhibition (\%)}: \frac{\text{Ac} - \text{As}}{\text{Ac}} \times 100.$$

Ac and As refer to the absorbances at 515 nm of DPPH in the control and sample solutions, respectively. All measurements were carried out in triplicate.

Fatty Acid Composition

Determination of the fatty acid composition was made according to the International Olive Oil Council, COI/T.20/Doc.No.24 [36]. Methyl-esters 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 and anajyzed by Agilent gas chromatography system (Agilent 6850, USA) equipped with a hydrogen flame ionization detector (FID) and a capillary column DB-23 of 60 m length × 0.25 mm i.d. and 0.25 μm of film thickness. The carrier gas was helium at 1.0 ml/min ratio. Injector, oven and detector temperatures were 250, 230 and 280 °C, respectively. The results were expressed as the relative area percentage of the total. The injection volume was 1 μL. Fatty acids were identified by comparing their retention times with those of reference compounds.

Sterol and Triterpene Dialcohols

Sterol composition were carried out according to International Olive Council method COI/T.20/Doc.No30/Rev. 1 [37]. Identification and quantification of sterols and diols as trimethylsilyl ethers was performed by gas chromatography (GC 2010, Shimadzu, Japan) equipped with a Supelco (SPBTM-5 24034, Bellefonte, USA) capillary column (30 m × 0.25 mm i.d × 0.25 mm film thickness) and flame ionization detector (FID). Injector, column and detector temperatures were 280, 260 and 290 °C, respectively. Helium was used as the carrier gas with a flow rate of 1 ml/min and the split ratio of 50:1. 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.

Statistical Analysis

Statistical analysis was performed using SPSS 15 statistical software (SPSS Inc., Chicago, USA).Data were analyzed according to Analysis of Variance (one way ANOVA). Significant differences between samples were determined by Duncan's multiple range test at the 5 % confidence level. Interaction analysis was performed by using the Student *t* test [38].

Results and Discussion

Chemical Properties

The chemical properties of olive oil such as free fatty acidity, peroxide value, total carotenoid, total chlorophyll, total phenol contents and antioxidant activities are shown in Table 1. As can be seen in Table 1, free fatty acids of olive oils ranged between %0.34 (H2 from Mardin)– %2.49 (G4 from Hatay). Free fatty acids of oils were within the limits (≤2) established by European regulations for virgin olive oil except for G1 from Hatay. The free fatty acid contents of oils from Mardin was lower than those of from Hatay and it was observed that this content increased during ripening slightly. The free acidity of oils showed statistically significant differences depending on the varieties, ripening and growing area ($p < 0.05$). Peroxide values varied between 7.10 (H1 from Hatay) and 18.36 (G1 from Mardin) mequiv O_2/kg oil and peroxide values of all samples were below the legal limit (<20) for virgin olive oil. There

Table 1 Chemical properties of olive oil samples

Chemical properties	Gemlik					Halhalı			
	Grow. region	G1	G2	G3	G4	H1	H2	H3	H4
Free fatty acids (%C18:1)	Hatay	$1.56 \pm 0.05^{C;X;E}$	$1.65 \pm 0.07^{C;X;E}$	$1.84 \pm 0.07^{B;X;E}$	$2.49 \pm 0.17^{A;X;E}$	$0.63 \pm 0.09^{B;F}$	$0.56 \pm 0.06^{B;X;F}$	$0.74 \pm 0.09^{B;X;F}$	$1.60 \pm 0.17^{A;F}$
	Mardin	$0.56 \pm 0.1^{B;Y}$	$0.47 \pm 0.1^{B;Y}$	$0.94 \pm 0.0^{A;Y;E}$	$0.93 \pm 0.1^{A;Y;F}$	0.54 ± 0.09^{B}	$0.34 \pm 0.07^{C;Y}$	$0.70 \pm 0.05^{B;Y;F}$	$1.65 \pm 0.13^{A;E}$
Peroxide value (mequiv O_2/kgoil)	Hatay	$8.05 \pm 0.67^{C;Y}$	$12.17 \pm 0.65^{B;Y;E}$	$12.70 \pm 0.42^{B;X}$	18.12 ± 2.74^{A}	$7.1 \pm 0.87^{C;Y}$	$7.72 \pm 0.71^{C;Y;F}$	$13.06 \pm 0.38^{B;X}$	$18.6 \pm 0.16^{A;X}$
	Mardin	$18.36 \pm 0.52^{A;X;E}$	$14.31 \pm 0.21^{C;X}$	$10.64 \pm 0.71^{D;Y}$	$17.45 \pm 0.90^{B;E}$	$11.29 \pm 0.44^{A;X;F}$	$13.68 \pm 0.67^{A;X}$	$8.56 \pm 2.25^{B;Y}$	$13.97 \pm 1.34^{A;Y;F}$
Carotenoid (mg/kg)	Hatay	$3.18 \pm 0.26^{A;Y;F}$	$2.08 \pm 0.25^{B;Y;F}$	$1.68 \pm 0.15^{C;Y;F}$	$1.56 \pm 0.06^{C;Y;F}$	$5.66 \pm 0.15^{A;Y;E}$	$4.88 \pm 0.10^{B;Y;E}$	$4.24 \pm 0.15^{B;Y;E}$	$3.08 \pm 0.78^{C;Y;E}$
	Mardin	$5.04 \pm 05^{A;X;F}$	$4.42 \pm 0.10^{B;X;F}$	$3.48 \pm 0.31^{C;X;F}$	$2.32 \pm 0.20^{D;X;F}$	$6.40 \pm 0.26^{A;X;F}$	$5.26 \pm 0.12^{B;X;E}$	$5.28 \pm 0.24^{B;X;E}$	$4.74 \pm 0.15^{C;X;E}$
Chlorophyll (mg/kg)	Hatay	$8.03 \pm 0.65^{A;F}$	$7.00 \pm 0.16^{B;F}$	$4.5 \pm 0.48^{C;F}$	$3.57 \pm 0.66^{C;F}$	$12.29 \pm 0.34^{A;E}$	$10.36 \pm 0.51^{B;Y;E}$	$7.69 \pm 0.33^{C;Y;E}$	$5.46 \pm 0.22^{D;Y;E}$
	Mardin	9.25 ± 0.51^{F}	$7.36 \pm 0.50^{B;F}$	$5.27 \pm 0.80^{C;F}$	$3.69 \pm 0.30^{D;F}$	$13.59 \pm 1.00^{A;E}$	$12.15 \pm 0.51^{B;X;E}$	$11.57 \pm 0.34^{B;X;E}$	$11.04 \pm 0.50^{B;X;E}$
Total phenolic content (mg/kg)	Hatay	$74.57 \pm 13.09^{A;Y;F}$	$20.62 \pm 16.85^{C;Y;F}$	$34.23 \pm 8.31^{BC;Y;F}$	$43.31 \pm 9.44^{B;Y;F}$	$525.22 \pm 65.59^{A;X;E}$	$322.13 \pm 38.14^{C;Y;E}$	$415.38 \pm 53.80^{B;X;E}$	$120.77 \pm 13.90^{D;E}$
	Mardin	$355.28 \pm 41.4^{B;X;E}$	$421.23 \pm 19.4^{AB;X;E}$	$465.20 \pm 71.9^{A;X;E}$	$188.48 \pm 31.7^{C;X;E}$	$225.81 \pm 27.91^{A;Y;F}$	$232.09 \pm 24.62^{A;X;F}$	$188.82 \pm 37.81^{B;Y;F}$	$92.16 \pm 12.49^{B;F}$
%Inhibition	Hatay	$51.19 \pm 5.21^{A;X;F}$	$23.73 \pm 4.50^{C;Y;F}$	$34.73 \pm 6.21^{BC;Y;F}$	$31.73 \pm 4.30^{C;Y;F}$	$59.39 \pm 8.42^{A;X;E}$	$45.48 \pm 5.20^{C;E}$	$48.42 \pm 7.56^{B;X;E}$	$34.82 \pm 8.60^{D;Y;E}$
	Mardin	$37.63 \pm 6.21^{B;Y;F}$	$46.15 \pm 8.14^{A;X}$	$45.93 \pm 10.52^{A;X;E}$	$37.29 \pm 9.23^{B;X}$	$53.73 \pm 6.27^{A;Y;E}$	$45.81 \pm 9.40^{B;}$	$42.53 \pm 7.65^{C;Y;F}$	$36.20 \pm 8.62^{D;X}$

[A–C] For each parameter different letters for the same cultivar and region indicate statistically significant differences ($p < 0.05$) between maturation

[X–Y] For each parameter different letters for the same cultivar and maturity indicate statistically significant differences ($p < 0.05$) between region

[E–F] For each parameter different letters for the same maturity and region indicate statistically significant differences ($p < 0.05$) between cultivar

were statistically significant differences between the peroxide values of the cultivars in growing area and ripening. The carotenoid and chlorophyll contents decrease significantly throughout ripening in both olive oils from two regions. These results were in accordance with those of Abenoza *et al*. [28]. Carotenoid and chlorophyll contents ranged from 1.56 to 6.40 mg/kg and from 3.57 to 13.59 mg/kg, respectively. Carotenoid and chlorophyl contents were found to be the highest in Halhalı from Mardin while their contents were the lowest in Gemlik from Hatay.

Total Phenolic Contents

Total phenolic contents (Table 1) decreased generally during ripening. The highest (525 mg/kg) and the lowest (20.62 mg/kg) total phenol contents were determined in Halhalı and Gemlik from Hatay, respectively. There were significant differences among the cultivars, ripening and location the in total phenolic contents of the olive oil samples ($p < 0.05$). Kesen *et al*. [19] reported that the total phenolic contents of two olive oil varieties from different regions in Turkey ranged from 74.71 to 96.97 mg of GA/kg of oil. Also, Del Monaco *et al*. [7] studied 12 olive oils from different regions and cultivars in Italy to characterization of olive oils with respect to varieties, geographical region and harvest date. They reported that the phenolic content of olive oils showed large variation based on varieties, ripening and geographical region, ranging from 290 to 2180 mg/kg.

Antioxidant Activity

Radical scavenger capacity of oil samples was performed by using a spectrophotometric method based on the color change of the radical DPPH. Results were expressed as %Inhibition. The highest antioxidant activity was determined as 59.39 % (H1 from Hatay) while the lowest 23.73 % (G2 from Hatay). Antioxidant activities were in accordance with total phenolic content in all oil samples with minor exceptions. The differences in antioxidant activities may depend on the composition and profile of phenolic compounds rather than total phenol contents as in the previous works [39].

Fatty Acid Compositions

The fatty acid compositions of olive oils samples are shown in Table 2. The major fatty acids were palmitic (C16:0), oleic (C18:1) and linoleic (C18:2) acids. Palmitoleic (C16:1), linolenic (C18:3), stearic (C18:0), arachidic (C20:0), behenic (C22:0) acids were detected in minor amounts. The fatty acid compositions were significantly affected by variety, maturation and location in oil samples with minor exceptions ($p < 0.05$). The main fatty acid in all oil samples was oleic acid, ranging from 62.34 % (G2 from Hatay) to 71.53 % (H1 from Hatay). Oleic acid contents of Halhalı olive oils were higher than Gemlik and these content showed fluctuation for olive oils from both locations. Similarly, stearic acid contents also showed apparent variation in olive oils. Palmitic acid, the main saturated fatty acid of olive oils, ranging from 13.87 % (G1 from Mardin) to 16.70 % (H1 from Mardin). Palmitic acid content increased slightly during ripening in Gemlik oils while this content decreased in Halhalı oils from both of two locations. Linoleic acid content ranged between 5.02 % (H1 from Hatay) and 11.93 % (G2 from Hatay). Linoleic acid content of Gemlik olive oils was higher than Halhalı olive oils. Linoleic acid content increased during maturation, confirming the results reported by Yorulmaz *et al*. [40] which is probably because of the results of oleate desaturase enzyme activity transforming oleic into linoleic acid. The fatty acid compositions of oils from both olive varieties were within the legal limits established by the EU regulations, with a minor exception that could have been due to genetic factors and climatic conditions [41]. Palmitoleic, linolenic, arachidic and behenic acids were between 0.75–1.67, 0.56–1.06, 0.50–0.83, 0.08–0.18 %, 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 [42, 43].

Sterol and Triterpene Dialcohol Compositions

The sterol and triterpene diols (erythrodiols + uvaol) compositions of olive oil were presented in Table 3. While β-sitosterol, Δ-5-avenasterol and campesterol were determined as the main sterols in the oil samples, cholesterol, brassicasterol, 24-methylenecholesterol, campestanol, stigmasterol, Δ-7-campesterol, clerosterol, sitostanol, Δ-5,24-stigmastadienol, Δ-7-stigmastenol, Δ-7-avenasterol and two triterpene dialcohols (erythrodiol and uvaol) were found as minor sterols. These results were in good agreement with data reported elsewhere [1, 16, 44, 45]. As shown in Table 3., campesterol, stigmasterol, Δ-5-avenasterol, β-sitosterol, Δ-5,24-stigmastadional, erythrodiol + uvaol and total sterols contents were significantly affected by the variety, maturity and growing region ($p < 0.05$) with minor exceptions. The total sterol compositions of olive oil samples were higher than the minimum limits (1000 mg/kg) established by EU regulation, ranging from 1194 (H2 from Hatay) to 2008 mg/kg (G1 from Hatay) and the apparent β-sitosterol contents signified 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 threshold

Table 2 Fatty acid compositions of olive oil samples(%)

Fatty acids	Gemlik					Halhalı			
	Grow. region	G1	G2	G3	G4	H1	H2	H3	H4
C16:0	Hatay	$15.33 \pm 0.22^{D;X;E}$	$16.23 \pm 0.96^{A;X;E}$	$15.62 \pm 0.54^{B;E}$	$15.13 \pm 0.41^{C;X;E}$	$14.47 \pm 0.34^{A;Y;F}$	$14.46 \pm 0.14^{A;Y;F}$	$14.37 \pm 0.18^{B;Y;F}$	$14.30 \pm 0.28^{C;Y;F}$
	Mardin	$13.87 \pm 0.30^{C;Y;F}$	$13.96 \pm 0.20^{C;Y;F}$	14.57 ± 0.31^{B}	$14.74 \pm 0.15^{A;Y;F}$	$16.70 \pm 1.19^{A;X;E}$	$16.40 \pm 0.48^{B;X;E}$	$15.92 \pm 0.42^{C;X}$	$15.75 \pm 0.44^{D;X;E}$
C16:1	Hatay	$0.75 \pm 0.29^{C;Y;F}$	$1.63 \pm 0.28^{A;X;E}$	$1.67 \pm 0.20^{A;X;E}$	$1.56 \pm 0.20^{B;X;E}$	$1.11 \pm 0.12^{B;E}$	$0.83 \pm 0.32^{C;Y;F}$	$0.82 \pm 0.15^{C;Y;F}$	$1.25 \pm 0.22^{A;F}$
	Mardin	$0.85 \pm 0.20^{C;X;F}$	$1.05 \pm 0.20^{B;Y;F}$	$1.35 \pm 0.10^{A;Y;E}$	$1.34 \pm 0.11^{A;Y;E}$	1.26 ± 0.14^{E}	$1.22 \pm 0.23^{;X;E}$	$1.23 \pm 0.14^{X;F}$	1.22 ± 0.10^{F}
C18:0	Hatay	$3.61 \pm 0.32^{A;X}$	$2.58 \pm 0.20^{D;Y;F}$	$3.07 \pm 0.06^{C;Y;F}$	$3.57 \pm 0.26^{B;X;E}$	$3.60 \pm 0.15^{A;X}$	$3.53 \pm 0.21^{B;Y;E}$	$3.50 \pm 0.17^{B;Y;E}$	$2.99 \pm 0.27^{C;Y;F}$
	Mardin	$2.85 \pm 0.31^{D;Y;F}$	$3.11 \pm 0.30^{C;X;F}$	$3.44 \pm 0.32^{A;X;F}$	$3.27 \pm 0.20^{B;Y;F}$	$2.96 \pm 0.21^{D;Y;E}$	$3.44 \pm 0.23^{C;X;E}$	$3.67 \pm 0.39^{A;X;E}$	$3.51 \pm 0.21^{B;X;E}$
C18:1	Hatay	$68.29 \pm 0.19^{A;X;F}$	$62.34 \pm 0.23^{D;Y;F}$	$65.50 \pm 0.27^{C;Y;F}$	$67.07 \pm 0.32^{B;F}$	$71.53 \pm 0.32^{A;X;E}$	$67.20 \pm 0.22^{D;Y;E}$	$70.21 \pm 0.14^{B;X;E}$	$68.28 \pm 0.30^{C;E}$
	Mardin	$64.14 \pm 0.22^{D;Y;F}$	$67.90 \pm 0.42^{A;X;E}$	$65.98 \pm 0.30^{C;X;F}$	$66.33 \pm 0.22^{D;F}$	$66.32 \pm 0.18^{D;Y;E}$	$67.45 \pm 0.13^{C;X;F}$	$68.75 \pm 0.27^{B;Y;E}$	$69.08 \pm 0.38^{A;E}$
C18:2	Hatay	$7.68 \pm 0.26^{D;Y;E}$	$11.93 \pm 0.14^{A;X;E}$	$10.25 \pm 0.13^{B;Y;E}$	$8.86 \pm 0.33^{C;Y;E}$	$5.02 \pm 0.32^{D;Y;F}$	$5.23 \pm 0.12^{C;Y;F}$	$7.48 \pm 0.23^{B;X;F}$	$7.88 \pm 0.31^{A;X;F}$
	Mardin	$7.54 \pm 0.20^{D;X;E}$	$8.29 \pm 0.42^{C;Y;E}$	$10.55 \pm 0.30^{B;X;E}$	$11.13 \pm 0.12^{A;X;E}$	$6.64 \pm 0.21^{B;X;F}$	$6.33 \pm 0.24^{C;X;F}$	$6.82 \pm 0.35^{A;Y;F}$	$6.84 \pm 0.16^{A;Y;F}$
C18:3	Hatay	$0.56 \pm 0.27^{C;Y;F}$	$0.88 \pm 0.31^{A;Y;E}$	$0.86 \pm 0.22^{A;E}$	$0.76 \pm 0.34^{B;X}$	$0.87 \pm 0.14^{A;Y;E}$	$0.74 \pm 0.20^{B;Y;F}$	$0.68 \pm 0.26^{C;Y;F}$	$0.77 \pm 0.26^{B;Y}$
	Mardin	$1.01 \pm 0.02^{A;X;E}$	$0.95 \pm 0.21^{B;X;E}$	0.87 ± 0.42^{C}	$0.67 \pm 0.20^{D;Y;F}$	$0.97 \pm 0.18^{A;X;F}$	$0.84 \pm 0.29^{BC;X;F}$	$0.87 \pm 0.22^{B;X}$	$0.84 \pm 0.11^{C;X;E}$
C20:0	Hatay	$0.58 \pm 0.19^{B;Y;E}$	$0.69 \pm 0.22^{A;F}$	$0.50 \pm 0.29^{C;Y;F}$	$0.54 \pm 0.29^{BC;X}$	$0.51 \pm 0.21^{A;Y;F}$	$0.59 \pm 0.32^{A;X;E}$	$0.60 \pm 0.29^{B;Y;E}$	$0.54 \pm 0.14^{C;Y}$
	Mardin	$0.65 \pm 0.02^{B;X;E}$	$0.63 \pm 0.30^{A;E}$	$0.58 \pm 0.11^{C;X;F}$	$0.44 \pm 0.20^{D;Y;F}$	$0.56 \pm 0.19^{B;X;F}$	$0.57 \pm 0.30^{B;Y;F}$	$0.62 \pm 0.28^{A;X;E}$	$0.65 \pm 0.34^{A;X;E}$
C20:1	Hatay	$0.32 \pm 0.02^{C,;X;F}$	$0.38 \pm 0.02^{A;X,E}$	$0.35 \pm 0.01^{B;X;E}$	$0.34 \pm 0.02^{C;Y}$	$0.36 \pm 0.01^{B;X;F}$	$0.37 \pm 0.00^{B;X;E}$	$0.32 \pm 0.01^{A;X;F}$	0.34 ± 0.01^{A}
	Mardin	$0.30 \pm 0.02^{C,;Y;F}$	$0.32 \pm 0.02^{A;Y,E}$	$0.34 \pm 0.01^{B;Y;E}$	$0.36 \pm 0.02^{A;X;E}$	$0.33 \pm 0.01^{B;Y;E}$	$0.31 \pm 0.00^{C;Y;F}$	$0.31 \pm 0.01^{C;Y;F}$	$0.34 \pm 0.01^{A;F}$

$^{A–C}$ For each parameter, different letters for the same cultivar and region indicate statistically significant differences ($p < 0.05$) between maturation

$^{X–Y}$ For each parameter different letters for the same cultivar and maturity indicate statistically significant differences ($p < 0.05$) between region

$^{E–F}$ For each parameter different letters for the same maturity and region indicate statistically significant differences ($p < 0.05$) between cultivar

Table 3 Sterol compositions of olive oil samples (%)

Sterol	Gemlik					Halhalı			
	Grow region	G1	G2	G3	G4	H1	H2	H3	H4
Cholesterol	Hatay	$0.21 \pm 0.01^{A;F}$	$0.17 \pm 0.02^{B;F}$	$0.18 \pm 0.01^{B;F}$	$0.17 \pm 0.01^{B;Y;E}$	$0.31 \pm 0.01^{B;X;E}$	$0.39 \pm 0.01^{A;X;E}$	$0.23 \pm 0.01^{C;E}$	$0.24 \pm 0.01^{C;F}$
	Mardin	0.20 ± 0.00^{F}	0.22 ± 0.00^{F}	0.19 ± 0.00^{F}	0.22 ± 0.00^{X}	$0.27 \pm 0.02^{B;Y;E}$	$0.31 \pm 0.01^{A;Y;E}$	$0.23 \pm 0.01^{C;E}$	0.21 ± 0.01^{C}
Brassicasterol	Hatay	$0.23 \pm 0.05^{A;X;E}$	0.02 ± 0.00^{B}	$0.06 \pm 0.02^{B;X;E}$	$0.02 \pm 0.00^{B;F}$	$0.02 \pm 0.01^{B;F}$	0.02 ± 0.01^{B}	$0.01 \pm 0.00^{B;Y;F}$	$0.05 \pm 0.01^{A;E}$
	Mardin	$0.02 \pm 0.00^{A;Y}$	$0.01 \pm 0.00^{B;F}$	$0.01 \pm 0.00^{B;Y}$	$0.01 \pm 0.00^{B;F}$	0.01 ± 0.00	0.03 ± 0.01^{E}	0.03 ± 0.55^{X}	0.05 ± 0.01^{E}
24-Methylene-cholesterol	Hatay	0.08 ± 0.01^{Y}	0.06 ± 0.02^{Y}	$0.06 \pm 0.01^{Y;F}$	0.08 ± 0.01^{Y}	$0.06 \pm 0.01^{C;Y}$	0.06 ± 0.01^{C}	$0.09 \pm 0.02^{B;E}$	$0.14 \pm 0.01^{A;X}$
	Mardin	$0.17 \pm 0.00^{X;E}$	$0.16 \pm 0.00^{X;E}$	$0.16 \pm 0.00^{X;E}$	$0.15 \pm 0.00^{X;E}$	$0.09 \pm 0.01^{X;F}$	0.08 ± 0.01^{F}	0.09 ± 0.02^{F}	$0.08 \pm 0.01^{Y;F}$
Campesterol	Hatay	$2.98 \pm 0.05^{A;X;F}$	$2.96 \pm 0.02^{A;X;F}$	$2.27 \pm 0.02^{B;X;F}$	$1.87 \pm 0.01^{C;X}$	$3.11 \pm 0.01^{B;Y;E}$	$3.49 \pm 0.01^{A;Y;E}$	$2.91 \pm 0.01^{C;Y;E}$	$2.27 \pm 0.01^{D;Y}$
	Mardin	$1.93 \pm 0.00^{B;Y;F}$	$2.01 \pm 0.00^{A;Y;F}$	$1.53 \pm 0.01^{C;Y;F}$	$1.52 \pm 0.20^{C;Y;F}$	$3.68 \pm 0.01^{A;X;E}$	$3.60 \pm 0.01^{B;X;E}$	$3.66 \pm 0.02^{A;X;E}$	$3.62 \pm 0.03^{B;X;E}$
Campestanol	Hatay	$0.06 \pm 0.01^{Y;F}$	$0.05 \pm 0.00^{Y;E}$	0.06 ± 0.01^{E}	0.07 ± 0.02	$0.13 \pm 0.01^{B;X;E}$	$0.17 \pm 0.01^{A;X;F}$	$0.10 \pm 0.02^{B;C;F}$	0.09 ± 0.02^{C}
	Mardin	0.12 ± 0.00^{X}	0.08 ± 0.00^{X}	0.07 ± 0.00	0.07 ± 0.00	0.09 ± 0.01^{Y}	0.08 ± 0.02^{Y}	0.09 ± 0.02	0.08 ± 0.01
Stigmasterol	Hatay	$0.90 \pm 0.01^{C;Y;E}$	$0.92 \pm 0.02^{C;Y;E}$	$1.69 \pm 0.05^{B;X;E}$	$1.84 \pm 0.02^{A;X;F}$	$0.66 \pm 0.02^{D;Y;F}$	$0.78 \pm 0.01^{C;Y;F}$	$0.93 \pm 0.02^{B;Y;F}$	$1.96 \pm 0.02^{A;Y;E}$
	Mardin	$1.16 \pm 0.00^{B;X;F}$	$1.27 \pm 0.00^{A;X;E}$	$0.96 \pm 0.01^{C;Y;F}$	$1.14 \pm 0.01^{B;Y;F}$	$1.27 \pm 0.02^{C;X;E}$	$1.15 \pm 0.01^{D;X;F}$	$1.35 \pm 0.01^{B;X;E}$	$2.72 \pm 0.01^{A;X;E}$
Δ-7-Campesterol	Hatay	0.28 ± 0.06	0.06 ± 0.01^{X}	0.03 ± 0.01	0.02 ± 0.01	$0.11 \pm 0.01^{A;X}$	0.05 ± 0.01^{B}	$0.05 \pm 0.01^{B;X}$	0.02 ± 0.00^{C}
	Mardin	0.03 ± 0.01^{B}	$0.01 \pm 0.00^{B;Y;F}$	0.04 ± 0.01^{B}	0.09 ± 0.01^{A}	0.04 ± 0.01^{Y}	0.03 ± 0.01^{E}	0.02 ± 0.01^{Y}	0.02 ± 0.01
Clerosterol	Hatay	$0.83 \pm 0.01^{C;Y;F}$	$0.86 \pm 0.01^{C;Y;F}$	0.98 ± 0.03^{B}	$1.07 \pm 0.02^{A;X}$	$0.99 \pm 0.02^{B;Y;E}$	$0.99 \pm 0.01^{B;E}$	$1.02 \pm 0.02^{BA;X}$	$1.06 \pm 0.01^{A;X}$
	Mardin	$1.10 \pm 0.0^{B;X;E}$	$1.16 \pm 0.0^{A;X;E}$	1.02 ± 0.0^{C}	$0.97 \pm 0.0^{D;Y}$	$1.06 \pm 0.01^{A;X;F}$	$1.03 \pm 0.02^{A;F}$	$0.98 \pm 0.02^{B;Y}$	$0.96 \pm 0.02^{B;Y}$
β-Sitosterol	Hatay	$88.03 \pm 0.07^{A;X;E}$	$87.96 \pm 0.16^{A;X;F}$	$87.24 \pm 0.09^{B;X;E}$	$84.73 \pm 0.02^{C;X;F}$	$83.21 \pm 0.02^{D;Y;F}$	$85.87 \pm 0.03^{A;X;E}$	$83.70 \pm 0.02^{C;Y;F}$	$84.45 \pm 0.03^{B;Y;E}$
	Mardin	$76.60 \pm 0.1^{B;Y;F}$	$77.13 \pm 0.1^{A;Y;F}$	$71.21 \pm 0.1^{D;Y;F}$	$72.10 \pm 0.1^{C;Y;F}$	$88.21 \pm 0.03^{B;X;E}$	$88.19 \pm 0.02^{B;Y;E}$	$88.69 \pm 0.03^{A;X;E}$	$86.95 \pm 0.02^{C;X;E}$
Sitostanol	Hatay	$0.23 \pm 0.02^{D;Y;F}$	$0.33 \pm 0.08^{C;Y;F}$	0.69 ± 0.01^{B}	$1.03 \pm 0.01^{A;X;E}$	$1.06 \pm 0.02^{AB;X;E}$	$1.39 \pm 0.02^{A;X;E}$	$0.52 \pm 0.39^{C;X}$	$0.72 \pm 0.01^{BC;X;F}$
	Mardin	$0.75 \pm 0.0^{A;X;E}$	$0.70 \pm 0.0^{AB;X;E}$	$0.65 \pm 0.0^{B;E}$	$0.59 \pm 0.0^{C;Y}$	$0.53 \pm 0.01^{Y;F}$	$0.50 \pm 0.02^{Y;F}$	$0.46 \pm 0.02^{Y;F}$	0.31 ± 0.02^{Y}
Δ-5-Avenasterol	Hatay	$4.07 \pm 0.08^{C;Y;F}$	$4.16 \pm 0.05^{C;Y;E}$	$4.83 \pm 0.06^{B;X;F}$	$6.88 \pm 0.02^{A;Y;F}$	$7.41 \pm 0.01^{B;X;E}$	$5.37 \pm 0.02^{D;Y;F}$	$7.87 \pm 0.02^{A;X;E}$	$7.06 \pm 0.02^{C;X;E}$
	Mardin	$15.53 \pm 0.0^{C;X;E}$	$15.05 \pm 0.0^{D;X;E}$	$21.30 \pm 0.1^{A;Y;E}$	$20.93 \pm 0.1^{B;X;E}$	$3.42 \pm 0.02^{B;Y;F}$	$3.73 \pm 0.01^{A;X;F}$	$3.22 \pm 0.02^{C;Y;F}$	$2.74 \pm 0.01^{D;Y;F}$
Δ-5,24-Stigmastadional	Hatay	$1.51 \pm 0.02^{A;X;E}$	$1.43 \pm 0.09^{A;X;E}$	$0.90 \pm 0.02^{B;E}$	$0.87 \pm 0.02^{B;X;E}$	$0.75 \pm 0.02^{A;X;F}$	$0.47 \pm 0.02^{B;F}$	$0.75 \pm 0.02^{A;X;F}$	$0.75 \pm 0.03^{A;X;F}$
	Mardin	$0.85 \pm 0.0^{A;Y;E}$	$0.91 \pm 0.0^{A;Y;E}$	$0.85 \pm 0.0^{A;E}$	$0.76 \pm 0.0^{B;Y;E}$	$0.50 \pm 0.02^{A;Y;F}$	$0.46 \pm 0.02^{AB;F}$	$0.49 \pm 0.02^{AB;Y;F}$	$0.44 \pm 0.03^{B;Y;F}$
Δ-7-Stigmastenol	Hatay	$0.43 \pm 0.03^{X;F}$	$0.44 \pm 0.01^{X;F}$	$0.43 \pm 0.02^{X;F}$	$0.45 \pm 0.01^{X;E}$	$0.55 \pm 0.02^{A;X;E}$	$0.47 \pm 0.01^{B;X;E}$	$0.49 \pm 0.02^{B;X;E}$	$0.40 \pm 0.01^{C;X;F}$
	Mardin	$0.22 \pm 0.0^{C;Y;F}$	$0.23 \pm 0.0^{BC;Y}$	$0.38 \pm 0.0^{A;Y;E}$	$0.28 \pm 0.0^{B;Y}$	$0.30 \pm 0.01^{AB;Y;E}$	$0.27 \pm 0.01^{AB;Y}$	$0.31 \pm 0.03^{A;Y;F}$	$0.26 \pm 0.02^{B;Y}$
Δ-7-Avenasterol	Hatay	$0.77 \pm 0.02^{B;Y;F}$	$0.68 \pm 0.02^{C;Y;E}$	$0.62 \pm 0.02^{D;Y;F}$	$0.92 \pm 0.01^{A;Y;E}$	$1.02 \pm 0.01^{A;X;E}$	$0.43 \pm 0.01^{D;Y;F}$	$0.97 \pm 0.01^{B;X;E}$	$0.66 \pm 0.03^{C;X;F}$
	Mardin	$1.28 \pm 0.0^{B;X;E}$	$1.17 \pm 0.0^{C;X;E}$	$1.69 \pm 0.0^{A;X;E}$	$1.08 \pm 0.0^{D;X;E}$	$0.49 \pm 0.02^{A;Y;F}$	$0.47 \pm 0.01^{A;X;F}$	$0.31 \pm 0.01^{B;Y;F}$	$0.31 \pm 0.01^{B;Y;F}$

Table 3 continued

Sterol	Gemlik					Halhalı			
	Grow region	G1	G2	G3	G4	H1	H2	H3	H4
Apparent β-sitosterol (%)	Hatay	$94.53 \pm 0.03^{B;X;E}$	$94.59 \pm 0.08^{AB;X;E}$	$94.64 \pm 0.01^{A;Y;E}$	$94.63 \pm 0.03^{A;Y;E}$	$93.43 \pm 0.03^{C;Y;F}$	$94.09 \pm 0.02^{B;Y;F}$	$94.13 \pm 0.02^{AB;X;F}$	$94.46 \pm 0.36^{A;X;F}$
	Mardin	$94.86 \pm 0.10^{B;X;E}$	$94.88 \pm 0.01^{B;X;E}$	$94.95 \pm 0.10^{B;X;E}$	$95.46 \pm 0.10^{A;X;E}$	$93.74 \pm 0.03^{A;X;F}$	$93.91 \pm 0.02^{A;Y;F}$	$91.55 \pm 0.02^{B;Y;F}$	$91.55 \pm 0.02^{B;Y;F}$
Total sterol (mg/kg)	Hatay	$2008.66 \pm 1.52^{A;X;E}$	$1988.00 \pm 1.73^{A;X;E}$	$1770.66 \pm 20.20^{B;X;E}$	$1761.33 \pm 18.50^{B;X;E}$	$1195.33 \pm 13.31^{C;Y;F}$	$1194.33 \pm 12.42^{C;Y;F}$	$1279.16 \pm 6.42^{B;F}$	$1477.33 \pm 8.50^{A;X;F}$
	Mardin	$1397.67 \pm 7.6^{B;Y;F}$	$1325.33 \pm 4.2^{D;Y;F}$	$1354.67 \pm 6.8^{C;Y;E}$	$1472.67 \pm 3.1^{A;Y;E}$	$1503.00 \pm 6.24^{B;X;E}$	$1568.66 \pm 7.76^{A;X;E}$	$1277.66 \pm 6.65^{D;F}$	$1455.00 \pm 4.35^{C;Y;F}$
Erythrodiol + uvaol	Hatay	$1.16 \pm 0.01^{D;Y;F}$	$1.34 \pm 0.00^{C;Y;F}$	$1.80 \pm 0.01^{A;Y;F}$	$1.71 \pm 0.02^{B;F}$	$3.50 \pm 0.03^{A;X;E}$	$3.13 \pm 0.01^{B;X;E}$	$2.74 \pm 0.01^{C;E}$	$2.33 \pm 0.15^{D;Y;E}$
	Mardin	$2.08 \pm 0.1^{A;X;E}$	$2.11 \pm 0.2^{A;X}$	$1.56 \pm 0.1^{C;X;F}$	$1.86 \pm 0.1^{B;X;F}$	$1.93 \pm 0.02^{C;F}$	$2.30 \pm 0.02^{B;Y}$	$1.83 \pm 0.01^{D;E}$	$4.09 \pm 0.03^{A;X;E}$

A–C For each parameter different letters for the same cultivar and region indicate statistically significant differences ($p < 0.05$) between maturation

X–Y For each parameter different letters for the same cultivar and maturity indicate statistically significant differences ($p < 0.05$) between region

E–F For each parameter different letters for the same maturity and region indicate statistically significant differences ($p < 0.05$) between cultivar

value (93 %) except for Halhalı from Mardin (91.55 %). β-sitosterol was found as major sterol in all oil samples, varying between 71.21 (G3) and 88.69 % (H3) in oils from Mardin. β-sitosterol contents of Gemlik oils from Hatay were higher than those of from Mardin. The second most abundant sterol was Δ-5-avenasterol, fluctuating between 2.74 (H4) and 21.30 % (G3) in oils obtained from Mardin. Gutierrez *et al*. [46] and Lukic *et al*. [11] reported that β-sitosterol contents generally decrease during maturation, while Δ-5-avenasterol increase. Campesterol contents in oil samples ranged from 1.52 % (G4 from Mardin) to 3.68 % (H1 from Mardin). Cholesterol and campesterol percentages were below the limit (0.5 and 4.0 %, respectively) established by EU regulation. Stigmasterol is the main sterol related to the quality of virgin olive oil and its high level contents are correlated with high acidity and low organoleptic quality [1, 47]. The mean content in the oil samples ranged from 0.66 to 1.96 % in Halhalı oils from Hatay. Stigmasterol percentages were lower than those of campesterol in all samples. These results are in consistent with other research which showed the existence of differences according to variety and growing region [17, 48]. Many researchers reported that geographical region had significant effect on sterol amounts this may be related to the different climate conditions at each growing area, such as rainfall, temperature and humidity [1, 41, 49].

The triterpene dialcohols (erythrodiol and uvaol) were anajyzed together with the sterol fraction of the oil samples. As can be seen in Table 3, the mean content of erythrodiol + uvaol was below the accepted limit of 4.5 % from the EU regulation, ranging from 1.16 (G1 from Hatay) to 4.09 % (H4 from Mardin). Moreover, the sum of erythrodiol and uvaol contents was influenced by variety, maturity and geographical region and it was determined the amounts of those in Halhalı olive oils were higher than Gemlik from both locations. These results were consistent with those of Mailer *et al*. [48] who pointed out erythrodiol and uvaol contents of olive oils of ten olive cultivars from different maturation stages and regions of Australia ranged from 0.4 to 4 %.

Conclusions

Analysis of olive oils obtained from two cultivars (Halhalı and Gemlik) at four maturation stages in two different growing regions (Hatay and Mardin) indicated that oil samples have largely significant variations in terms of chemical properties, fatty acid and sterol compositions, and this findings were in accordance with the internationally accepted ranges. Total phenolic contents, antioxidant activity, oleic acid, β-sitosterol, erythrodiol and uvaol contents of the Halhalı olive oils were higher than Gemlik oils. Halhalı

variety generally had better than Gemlik in terms of oil quality properties and sterol composition. β-sitosterol and total sterol contents of Gemlik oils from Hatay were higher than those of from Mardin. Therefore, the Halhalı variety should be intensely cultivated and certified with Protected Designation of Origin especially in Hatay. The results of this study showed that cultivar, maturation and growing region conditions had major roles in determining the oil quality properties. To conclude, the investigation indicated that sterol compositions can be used as reliable indicators for determining authenticity of the Halhalı and Gemlik varieties according to growing region.

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Compliance with Ethical Standards

Conflict to interest The authors certify that there is no conflict of interest.

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