

# Characterization of Turkish Olive Oils by Triacylglycerol Structures and Sterol Profiles

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**Abstract** A characterization study of Turkish monovarietal olive oils using chemical variables such as fatty acid, *sn*-2 fatty acid, triacylglycerol, and sterol composition is presented. A total of 101 samples of *Olea europaea* L. fruits from 18 cultivars were collected for two crop years from west, south, and southeast regions of Turkey. Olives were processed to oil and olive oil samples were evaluated for their triacylglycerol structures and sterol composition. Oleic acid content ranged from 60.15 to 80.46 % of total fatty acids and represented 70.90–89.02 % of *sn*-2 position triacylglycerols. Major triglycerides of oil samples were triolein, palmitodiolein, dioleolinolein, palmitooleolinolein, dipalmitoolein, and stearodiolein. Triolein values were between 24.72 and 48.64 % and compatible with the fatty acid composition. Total sterol content varied from 1,145.32 to 2,211.77 mg/kg and Edremit yağlık stood out because of its high sterol content. A one-way analysis of variance revealed significant differences for variables among cultivars. Principle component analysis enabled the classification of common varieties on the basis of analytical data. Sterol composition achieved more relevant discrimination than fatty acid and triglyceride composition. Classification according to geographical origin was performed by discriminant analysis.

**Keywords** Characterization · Fatty acids · Olive oil · Triacylglycerols · Sterols

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## Introduction

Virgin olive oil (VOO) is obtained from healthy olive drupes using only mechanical processing and it is therefore edible without further refining. Its consumption has increased throughout the world owing to its sensory properties and nutritional value deriving from its high mono-unsaturated/polyunsaturated fatty acid ratio [1] together with natural antioxidant content [2]. There are many types of olive oils available on the market with typical chemical composition and sensorial features depending on the variety and geographical district where the olives are grown. The European Union established a system known as Protected Designation of Origin (PDO) and Protected Geographical Indications (PGI) for certification of foods according to their designation of origin to ensure and improve quality of products. Olive oils with PDO certificates have higher market prices, which favors adulterations with lower grade oils or oils from different areas of origin. Hence, particular attention has been paid to accumulate knowledge regarding the chemical composition of oils obtained from various cultivars and geographical area in order to check authenticity and determine the identity of oils. Qualitative and quantitative analysis of triacylglycerols, fatty acids, sterols, tocopherols, hydrocarbons, phenols, and volatiles followed by chemometric treatments have been widely used for characterizing and confirming authenticity of virgin olive oils on the basis of their variety, growing area, and harvest year.

Triacylglycerols (TAG) are major components of oils and are derived from the esterification of glycerol with three fatty acids. Physical, nutritional, and chemical properties of oils are highly associated with the composition and location of fatty acids in TAG molecules [3]. The *sn*-1 and *sn*-3 positions of triglycerides are usually bonded to

saturated fatty acids, whereas the *sn*-2 position includes unsaturated ones. The fatty acid composition of VOO is significantly influenced by the type of cultivar, ripeness stage of the fruit, climatic conditions, latitude, irrigation management, and zone of production [4]. There are plenty of works revealing the TAG and fatty acid profiles of olive oils for Italian [5, 6], Spanish [7, 8], Greek [9], Iranian [10], French [11, 12], Tunisian [13, 14], and Moroccan [15] cultivars. Although a moderate number of papers have been published on the fatty acid composition of Turkish olive oils [16–21], few are available on the triglyceride distribution of varieties including only prevalent ones [22–25].

Phytosterols are the main components of the unsaponifiable fraction of lipids. Sterol composition is important for assessing authenticity, detecting adulteration, and characterization purposes because it is specific for each oil. Major sterols of olive oil are  $\beta$ -sitosterol,  $\Delta$ -5-avenasterol, and campesterol. The sterol profile of olive oil is affected by the cultivar, degree of ripening of the fruits, agronomic and climatic conditions, oil extraction technique, and storage conditions [26, 27]. Spanish [28, 29], Italian [30, 31], Portuguese [32, 33], Tunisian [13, 34], and Turkish [21, 22, 35, 36] olive varieties have been evaluated for their sterol composition.

Chemometric methods such as principal component analysis (PCA), hierarchical cluster analysis (HCA), and discriminant analysis (DA) have gained increasing importance in recent years owing to promising results from characterization studies. Analytical data obtained by chromatographic methods and quantitative information determined by spectroscopic procedures utilizing near-infrared (NIR) spectroscopy [37], Fourier transform infrared (FTIR) spectroscopy [38], and fluorescence spectroscopy [39] were evaluated by multivariate statistical techniques for classification issues in previous works. Turkish olive oils have been characterized according to mainly fatty acid composition [19, 20, 40, 41], TAG [23], fatty acid and TAG profile [25], aroma [42, 43], fatty acid and mid-infrared spectra [16], and phenolic compounds [44]. The first aim of this study was to determine fatty acid, *sn*-2 fatty acid, triacylglycerol, and sterol composition of olive oils obtained from 18 Turkish olive cultivars including both common and domestic ones for two successive crop years in order to establish a database for authentication and identification purposes. The second aim was to characterize and classify oils according to variety and growing region by using the acquired data. This study is the second part of a wider project and intends to complete the characterization of Turkish olive oils which began by evaluating Turkish olive cultivars and their corresponding oils in terms of their phenolic profiles [45].

## Materials and Methods

### Reagents and Standards

Isopropyl alcohol, isooctane, lipase, silica gel, and  $5\alpha$ -cholestan- $3\beta$ -ol were obtained from Sigma (St-Louis, USA); sodium cholate, acetonitrile, hydrochloric acid, methanol, tris(hydroxymethyl)aminomethane, hexane, formic acid,  $\beta$ -sitosterol, pyridine, and chloroform were purchased from Merck (Darmstadt, Germany), *N,O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) with trimethylchlorosilane (TMSC), and a 37 fatty acid methyl ester mix were from Supelco (Bellefonte, USA). The reagents used in HPLC were of HPLC grade.

### Olive Sampling and Olive Oil Extraction

The study was performed in 2006–2007 and 2007–2008 harvest years with 18 olive cultivars collected from 13 provinces placed at four different olive growing areas in Turkey (Table 1). The North Aegean region included Canakkale, Bursa, Balıkesir, Izmir, and Manisa; the South Aegean region contained Aydın and Muğla; the Mediterranean region involved Antalya, Adana, Mersin, and Hatay provinces; and the Southeast Anatolia region was composed of Gaziantep and Kilis. Memecik, Edremit yağlık (Ayvalık), and Gemlik are predominant olive varieties of Turkey and grown in more than one region; hence, more samples were available for these varieties. Halhalı was obtained from two different regions, although it has a low production potential. Kilis yağlık and Nizip yağlık are economically important varieties of Southeast Anatolia and grown in large areas. Uslu and Erkence are generally used for table olive production and are specific to the North Aegean. The remaining varieties were relatively domestic. Olive samples were mainly harvested from their respective traditional growing areas where they are cultivated as the unique and well-known variety. Olives were also tested for their physical characteristics such as fruit weight, endocarp properties, and flesh/pit ratio for clear identification of cultivars. Samples of younger trees, especially the Gemlik variety, were collected from certified orchards to ensure the correct determination of varieties.

*Olea europaea* L. fruits were collected from two trees in each case during the harvest period starting from the November to the end of December. After being transported to the laboratory, olives were deleafed and washed. A representative 5-kg sample of fruits was processed to oil by a laboratory scale mill (Spremoliva, Italy). The mill features a cylinder where crushing, malaxing, olive oil extraction, and waste discharge can be executed. Malaxation was performed at 27 °C for 25 min. Olive oil samples were kept at 4 °C under a nitrogen atmosphere until analyses.

**Table 1** Variety, geographical origin, harvest year, and sample size of olives

Variety	Growing area	Harvest year (sample size)
Memecik	North Aegean–South Aegean	06/07 ( <i>n</i> = 9)–07/08 ( <i>n</i> = 15)
Uslu	North Aegean	06/07 ( <i>n</i> = 3)–07/08 ( <i>n</i> = 4)
Gemlik	North Aegean–Mediterranean–Southeast Anatolia	06/07 ( <i>n</i> = 5)–07/08 ( <i>n</i> = 7)
Edremit yağlık	North Aegean–Mediterranean–South Aegean	06/07 ( <i>n</i> = 12)–07/08 ( <i>n</i> = 16)
Gulumbe	South Aegean	06/07 ( <i>n</i> = 1)–07/08 ( <i>n</i> = 1)
Antalya yağlık	Mediterranean	06/07 ( <i>n</i> = 1)–07/08 ( <i>n</i> = 1)
Mersin yağlık	Mediterranean	06/07 ( <i>n</i> = 1)–07/08 ( <i>n</i> = 1)
Saurani	Mediterranean	06/07 ( <i>n</i> = 1)–07/08 ( <i>n</i> = 2)
Nizip yağlık	Southeast Anatolia	06/07 ( <i>n</i> = 2)–07/08 ( <i>n</i> = 2)
Kilis yağlık	Southeast Anatolia	06/07 ( <i>n</i> = 2)–07/08 ( <i>n</i> = 2)
Erkence	North Aegean	06/07 ( <i>n</i> = 2)–07/08 ( <i>n</i> = 2)
Celebi	South Aegean	07/08 ( <i>n</i> = 1)
Girit	Mediterranean	07/08 ( <i>n</i> = 1)
Sarı ulak	Mediterranean	07/08 ( <i>n</i> = 1)
Halhalı	Mediterranean–Southeast Anatolia	07/08 ( <i>n</i> = 3)
Sarı hasebi	Mediterranean	07/08 ( <i>n</i> = 1)
Kalamata	Mediterranean	07/08 ( <i>n</i> = 1)
Domat	North Aegean	07/08 ( <i>n</i> = 1)

## Analyses

### Fatty Acid Composition

Fatty acid methyl esters were prepared as described by the International Union of Pure and Applied Chemistry [46] and analyzed using a GC-2010 gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a split/splitless injector ( $T = 290\text{ }^{\circ}\text{C}$ ) and flame ionization detector ( $T = 240\text{ }^{\circ}\text{C}$ ). A DB-23 fused silica capillary column (60 m  $\times$  0.25 mm i.d. and 0.25  $\mu\text{m}$  film thickness; J&W Scientific) was employed to separate individual fatty acids. The oven temperature was 195  $^{\circ}\text{C}$ . The carrier gas was helium with a flow rate of 1 ml/min and the split ratio was 80:1. The results were expressed as percentage of each fatty acid.

### Fatty Acids at the 2 Position of Triglycerides

Fatty acids at the 2 position of triglycerides were determined according to AOCS Official Method Ch 3-91 [47]. The gas chromatographic conditions were the same as those used for individual fatty acids.

### Iodine Value and Saponification Number

The iodine value and saponification number were calculated from the fatty acid composition of the oils according to AOCS Cd 1c-85 and Cd 3a-94 methods, respectively [47].

### Triglyceride Composition

Oil samples (3 %) were dissolved in acetonitrile/2-propanol/hexane (2:2:1) prior to analysis. Chromatographic separation was achieved according to the method described by Herslöf [48]. An HPLC apparatus (Shimadzu, Kyoto, Japan) equipped with a differential refractometer detector and Nucleosil 100 C18 column (25  $\times$  0.46, 5  $\mu\text{m}$  particle size; Teknochroma, Barcelona, Spain) was employed. The mobile phase was acetonitrile/2-propanol/hexane (500:118:100) with a flow rate of 1.0 ml/min. The column temperature was held at 25  $^{\circ}\text{C}$  and the injection volume was 50  $\mu\text{l}$ . Triacylglycerols were separated according to equivalent carbon number (ECN) and the peaks were identified by comparing with literature data [12].

### Sterol Composition

Sterol compositions were determined according to AOCS Official Method Ch 6-91 [47]. Sterol derivatives (silyl ethers) were analyzed with a gas chromatograph (GC-2010, Shimadzu, Japan) equipped with a flame ionization detector ( $T = 290\text{ }^{\circ}\text{C}$ ) and split/splitless injector ( $T = 280\text{ }^{\circ}\text{C}$ ). An HP-5 fused silica capillary column (30 m, 0.25 mm i.d., and 0.25 mm film thickness; Chrom Tech., Apple Valley, MN, USA) was employed to separate individual sterols. The oven temperature was 260  $^{\circ}\text{C}$ . The carrier gas was helium with a flow rate of 0.8 ml/min and the split ratio was 80:1. 5 $\alpha$ -Cholestan-3 $\beta$ -ol was used as the internal standard for quantification.

**Table 2** Fatty acid composition of Turkish olive oils (%)

Variety	Fatty acids (%)												
	14:0	16:0	16:1	17:0	17:1	18:0	18:1	18:2	18:3	20:0	20:1	22:0	24:0
Uslu ( <i>n</i> = 4)	0.01 <sup>a</sup>	12.65 <sup>bcd</sup>	1.34 <sup>ab</sup>	0.09 <sup>bc</sup>	0.22 <sup>def</sup>	1.70 <sup>abc</sup>	72.34 <sup>def</sup>	10.18 <sup>b</sup>	0.81 <sup>cde</sup>	0.29 <sup>ab</sup>	0.24 <sup>ab</sup>	0.05 <sup>ab</sup>	0.01 <sup>a</sup>
Memecik ( <i>n</i> = 15)	0.01 <sup>a</sup>	12.90 <sup>bcd</sup>	1.02 <sup>bcd</sup>	0.03 <sup>a</sup>	0.06 <sup>a</sup>	2.08 <sup>abcd</sup>	73.31 <sup>ef</sup>	9.08 <sup>ab</sup>	0.75 <sup>abcde</sup>	0.33 <sup>abcd</sup>	0.25 <sup>abc</sup>	0.09 <sup>bcd</sup>	0.02 <sup>a</sup>
Gemlik ( <i>n</i> = 7)	0.01 <sup>a</sup>	13.17 <sup>bcd</sup>	1.22 <sup>bcd</sup>	0.12 <sup>c</sup>	0.21 <sup>cdef</sup>	2.56 <sup>de</sup>	72.61 <sup>def</sup>	8.55 <sup>ab</sup>	0.74 <sup>abcde</sup>	0.38 <sup>bcdef</sup>	0.24 <sup>ab</sup>	0.10 <sup>bcd</sup>	0.04 <sup>a</sup>
Edremit yağlık ( <i>n</i> = 15)	0.01 <sup>a</sup>	12.98 <sup>bcd</sup>	1.02 <sup>abcd</sup>	0.12 <sup>c</sup>	0.21 <sup>cdef</sup>	2.25 <sup>bcd</sup>	71.57 <sup>def</sup>	10.37 <sup>b</sup>	0.65 <sup>abc</sup>	0.36 <sup>bcdef</sup>	0.27 <sup>bc</sup>	0.10 <sup>bcd</sup>	0.03 <sup>a</sup>
Erkençe ( <i>n</i> = 2)	0.01 <sup>a</sup>	12.20 <sup>abcd</sup>	0.82 <sup>ab</sup>	0.13 <sup>c</sup>	0.21 <sup>cde</sup>	2.31 <sup>bcd</sup>	67.93 <sup>cde</sup>	14.82 <sup>c</sup>	0.72 <sup>abcd</sup>	0.35 <sup>abcde</sup>	0.23 <sup>ab</sup>	0.08 <sup>abc</sup>	0.12 <sup>b</sup>
Mersin yağlık ( <i>n</i> = 1)	0.01 <sup>a</sup>	13.88 <sup>bcd</sup>	0.85 <sup>abc</sup>	0.12 <sup>c</sup>	0.20 <sup>cde</sup>	2.26 <sup>bcd</sup>	65.75 <sup>bc</sup>	15.37 <sup>c</sup>	0.79 <sup>bcde</sup>	0.37 <sup>bcdef</sup>	0.25 <sup>abc</sup>	0.07 <sup>abcd</sup>	0.04 <sup>a</sup>
Saurani ( <i>n</i> = 2)	0.01 <sup>a</sup>	12.75 <sup>bcd</sup>	0.83 <sup>ab</sup>	0.18 <sup>d</sup>	0.23 <sup>ef</sup>	3.70 <sup>f</sup>	70.06 <sup>cde</sup>	8.49 <sup>ab</sup>	0.65 <sup>abc</sup>	0.47 <sup>efg</sup>	0.22 <sup>ab</sup>	0.16 <sup>f</sup>	0.05 <sup>a</sup>
Antalya yağlık ( <i>n</i> = 1)	0.01 <sup>a</sup>	14.22 <sup>cde</sup>	1.02 <sup>abcd</sup>	0.11 <sup>bc</sup>	0.20 <sup>cde</sup>	2.04 <sup>abcd</sup>	62.69 <sup>ab</sup>	18.29 <sup>c</sup>	0.70 <sup>abcd</sup>	0.33 <sup>abcd</sup>	0.22 <sup>ab</sup>	0.08 <sup>abcd</sup>	0.02 <sup>a</sup>
Nizip yağlık ( <i>n</i> = 2)	0.01 <sup>a</sup>	15.91 <sup>ef</sup>	1.20 <sup>bcd</sup>	0.13 <sup>c</sup>	0.18 <sup>bcd</sup>	3.16 <sup>ef</sup>	68.97 <sup>cde</sup>	8.92 <sup>ab</sup>	0.65 <sup>abc</sup>	0.46 <sup>defg</sup>	0.20 <sup>a</sup>	0.10 <sup>bcd</sup>	0.04 <sup>a</sup>
Kilis yağlık ( <i>n</i> = 2)	0.02 <sup>ab</sup>	14.96 <sup>def</sup>	1.02 <sup>abcd</sup>	0.12 <sup>c</sup>	0.17 <sup>bcd</sup>	3.17 <sup>ef</sup>	70.26 <sup>cde</sup>	8.69 <sup>ab</sup>	0.64 <sup>abc</sup>	0.49 <sup>fg</sup>	0.24 <sup>ab</sup>	0.12 <sup>def</sup>	0.06 <sup>ab</sup>
Domat ( <i>n</i> = 1)	–	17.40 <sup>f</sup>	1.34 <sup>de</sup>	0.12 <sup>c</sup>	0.19 <sup>bcd</sup>	2.44 <sup>cd</sup>	60.15 <sup>a</sup>	16.29 <sup>c</sup>	1.04 <sup>f</sup>	0.44 <sup>cdefg</sup>	0.21 <sup>ab</sup>	0.12 <sup>def</sup>	0.19 <sup>c</sup>
Celebi ( <i>n</i> = 1)	–	12.15 <sup>abcd</sup>	1.13 <sup>bcd</sup>	0.11 <sup>bc</sup>	0.15 <sup>bc</sup>	2.63 <sup>de</sup>	71.59 <sup>def</sup>	10.59 <sup>b</sup>	0.93 <sup>ef</sup>	0.40 <sup>bcdef</sup>	0.22 <sup>ab</sup>	0.10 <sup>bcd</sup>	0.02 <sup>a</sup>
Kalamata ( <i>n</i> = 1)	0.01 <sup>a</sup>	11.80 <sup>abc</sup>	1.72 <sup>e</sup>	0.07 <sup>b</sup>	0.19 <sup>bcd</sup>	1.63 <sup>ab</sup>	73.48 <sup>ef</sup>	9.86 <sup>b</sup>	0.72 <sup>abcd</sup>	0.21 <sup>a</sup>	0.21 <sup>ab</sup>	0.04 <sup>a</sup>	0.01 <sup>a</sup>
Sarı ulak ( <i>n</i> = 1)	–	13.89 <sup>bcd</sup>	0.69 <sup>a</sup>	0.18 <sup>d</sup>	0.21 <sup>def</sup>	3.69 <sup>f</sup>	68.51 <sup>cde</sup>	11.03 <sup>b</sup>	0.78 <sup>bcde</sup>	0.54 <sup>g</sup>	0.22 <sup>ab</sup>	0.14 <sup>ef</sup>	0.05 <sup>ab</sup>
Halhalı ( <i>n</i> = 3)	0.03 <sup>b</sup>	14.05 <sup>cde</sup>	0.92 <sup>abcd</sup>	0.10 <sup>bc</sup>	0.13 <sup>b</sup>	3.41 <sup>f</sup>	69.81 <sup>cde</sup>	9.99 <sup>b</sup>	0.56 <sup>a</sup>	0.57 <sup>g</sup>	0.21 <sup>ab</sup>	0.10 <sup>cde</sup>	0.05 <sup>a</sup>
Sarı hasebi ( <i>n</i> = 1)	–	10.96 <sup>ab</sup>	0.92 <sup>abcd</sup>	0.02 <sup>a</sup>	0.04 <sup>a</sup>	1.88 <sup>abcd</sup>	67.59 <sup>bcd</sup>	17.31 <sup>c</sup>	0.61 <sup>abc</sup>	0.27 <sup>ab</sup>	0.25 <sup>abc</sup>	0.06 <sup>abc</sup>	0.01 <sup>a</sup>
Girit ( <i>n</i> = 1)	0.01 <sup>a</sup>	9.77 <sup>a</sup>	1.28 <sup>cd</sup>	0.03 <sup>a</sup>	0.07 <sup>a</sup>	1.46 <sup>a</sup>	80.46 <sup>g</sup>	5.34 <sup>a</sup>	0.89 <sup>def</sup>	0.26 <sup>ab</sup>	0.27 <sup>bc</sup>	0.08 <sup>abc</sup>	0.02 <sup>a</sup>
Gulumbe ( <i>n</i> = 1)	0.01 <sup>a</sup>	10.97 <sup>ab</sup>	0.94 <sup>abcd</sup>	0.12 <sup>c</sup>	0.26 <sup>f</sup>	1.79 <sup>abc</sup>	76.25 <sup>fg</sup>	8.32 <sup>ab</sup>	0.60 <sup>ab</sup>	0.30 <sup>abc</sup>	0.31 <sup>c</sup>	0.06 <sup>abc</sup>	0.01 <sup>a</sup>

<sup>a</sup> Different superscript letters in the same column indicate significant difference between values at the  $p < 0.05$  level

## Statistical Analysis

Statistical analysis was performed using SPSS 15 statistical software (SPSS Inc., Chicago, USA). Data were evaluated by one-way ANOVA procedure using the Duncan's multiple range test to determine if there were any significant differences between cultivars. A  $p$  value of less than 0.05 was considered significant. Correlation analysis was performed using Pearson's test. Data were also processed by PCA and DA using XLSTAT 2014 version (Addinsoft, New York, NY).

## Results

### Fatty Acid Composition

Table 2 shows the average fatty acid composition of Turkish olive oils. The main fatty acids were palmitic (C16:0), oleic (C18:1), and linoleic (C18:2) acids. Palmitoleic (C16:1), linolenic (C18:3), arachidic (C20:0), myristic (C14:0), heptadecanoic (C17:0), heptadecenoic (C17:1), stearic (C18:0), gadoleic (C20:1), behenic (C22:0), and lignoceric (C24:0) acids were determined in low amounts. The predominant fatty acid in all samples was oleic acid ranging from 60.15 % (Domat) to 80.46 % (Girit). Linoleic acid was the second most important fatty acid varying

between 5.34 % (Girit) and 18.29 % (Antalya yağlık). The oleic/linoleic acid ratio is important for oxidative stability of the oils. The Girit variety was characterized by its high oleic/linoleic acid ratio (15.06), whereas this ratio is considerably lower for Antalya yağlık (3.43), Domat (3.69), and Sarı hasebi (3.90) cultivars. Palmitic acid was the main saturated fatty acid of oil samples and present at between 9.77 (Girit) and 17.40 % (Domat). Samples having higher oleic acid content had lower palmitic or linoleic acid contents as in the Girit variety. Oils of Nizip yağlık, Kilis yağlık, Domat, Sarı Ulak, and Halhalı varieties have higher palmitic acid content, whereas oils of Antalya yağlık and Sarı hasebi varieties have higher linoleic acid content than the other cultivars.

The fatty acid compositions of the Turkish olive cultivars were within the legal limits proposed by the Turkish Food Codex [49] which is consistent with EU regulations, except for the linolenic acid content of the Domat cultivar (1.04 %), which is a local variety cultivated in a restricted area. The minimum and maximum percentages of remaining fatty acids were as follows: C14:0, 0–0.03 % (Halhalı); C16:1, 0.69 (Sarı ulak)–1.72 % (Kalamata); C17:0, 0.02 (Sarı hasebi)–0.18 % (Saurani, Sarı ulak); C17:1, 0.04 (Sarı hasebi)–0.26 % (Gulumbe); C18:0, 1.46 (Girit)–3.70 % (Saurani); C18:3, 0.56 (Halhalı)–1.04 % (Domat); C20:0, 0.21 (Kalamata)–0.57 % (Halhalı); C20:1, 0.20 (Nizip yağlık)–0.31 % (Gulumbe); C22:0,

**Table 3** *sn*-2 Fatty acid composition of Turkish olive oils (%)

Variety	<i>sn</i> -2 Fatty acids (%)						
	16:0	16:1	17:1	18:0	18:1	18:2	18:3
Uslu ( <i>n</i> = 4)	0.90 <sup>abc</sup>	1.08 <sup>f</sup>	0.23 <sup>de</sup>	0.11 <sup>abc</sup>	82.37 <sup>cd</sup>	14.26 <sup>bc</sup>	1.02 <sup>bcd</sup>
Memecik ( <i>n</i> = 15)	0.86 <sup>ab</sup>	0.74 <sup>abcdef</sup>	0.06 <sup>a</sup>	0.15 <sup>abc</sup>	84.04 <sup>cde</sup>	12.82 <sup>bc</sup>	1.30 <sup>de</sup>
Gemlik ( <i>n</i> = 7)	1.23 <sup>cd</sup>	1.09 <sup>f</sup>	0.22 <sup>de</sup>	0.14 <sup>abc</sup>	84.71 <sup>cde</sup>	11.72 <sup>bc</sup>	0.86 <sup>abc</sup>
Edremit yağlık ( <i>n</i> = 15)	0.90 <sup>abc</sup>	0.78 <sup>abcdef</sup>	0.22 <sup>de</sup>	0.13 <sup>abc</sup>	81.96 <sup>cd</sup>	14.99 <sup>bc</sup>	0.99 <sup>bcd</sup>
Erkence ( <i>n</i> = 2)	0.85 <sup>ab</sup>	0.51 <sup>a</sup>	0.22 <sup>de</sup>	0.08 <sup>ab</sup>	74.64 <sup>ab</sup>	22.58 <sup>d</sup>	1.08 <sup>bcd</sup>
Mersin yağlık ( <i>n</i> = 1)	1.09 <sup>bcd</sup>	0.55 <sup>ab</sup>	0.18 <sup>cde</sup>	0.18 <sup>abc</sup>	74.16 <sup>ab</sup>	22.75 <sup>d</sup>	1.06 <sup>bcd</sup>
Saurani ( <i>n</i> = 2)	1.25 <sup>cde</sup>	0.62 <sup>abcd</sup>	0.25 <sup>de</sup>	0.23 <sup>c</sup>	84.14 <sup>cde</sup>	12.71 <sup>bc</sup>	0.77 <sup>ab</sup>
Antalya yağlık ( <i>n</i> = 1)	1.25 <sup>cde</sup>	0.78 <sup>abcdef</sup>	0.25 <sup>e</sup>	0.13 <sup>abc</sup>	70.90 <sup>a</sup>	25.50 <sup>d</sup>	1.16 <sup>cde</sup>
Nizip yağlık ( <i>n</i> = 2)	1.33 <sup>de</sup>	0.99 <sup>def</sup>	0.17 <sup>cd</sup>	0.19 <sup>abc</sup>	81.32 <sup>cd</sup>	15.18 <sup>bc</sup>	0.79 <sup>ab</sup>
Kilis yağlık ( <i>n</i> = 2)	1.18 <sup>bcd</sup>	0.74 <sup>abcdef</sup>	0.17 <sup>cd</sup>	0.19 <sup>abc</sup>	84.61 <sup>cde</sup>	12.17 <sup>bc</sup>	0.91 <sup>abc</sup>
Domat ( <i>n</i> = 1)	1.03 <sup>bcd</sup>	1.06 <sup>ef</sup>	0.23 <sup>de</sup>	0.10 <sup>abc</sup>	72.97 <sup>a</sup>	23.24 <sup>b</sup>	1.35 <sup>e</sup>
Celebi ( <i>n</i> = 1)	0.65 <sup>a</sup>	0.92 <sup>cdef</sup>	0.17 <sup>cd</sup>	0.07 <sup>a</sup>	79.24 <sup>bc</sup>	17.23 <sup>c</sup>	1.70 <sup>f</sup>
Kalamata ( <i>n</i> = 1)	1.59 <sup>e</sup>	0.87 <sup>bcdef</sup>	0.15 <sup>bc</sup>	0.13 <sup>abc</sup>	84.24 <sup>cde</sup>	12.42 <sup>bc</sup>	0.60 <sup>a</sup>
Sarı ulak ( <i>n</i> = 1)	1.28 <sup>de</sup>	0.59 <sup>abc</sup>	0.23 <sup>de</sup>	0.16 <sup>abc</sup>	80.77 <sup>c</sup>	15.79 <sup>bc</sup>	1.16 <sup>cde</sup>
Halhali ( <i>n</i> = 3)	1.11 <sup>bcd</sup>	0.71 <sup>abcde</sup>	0.15 <sup>bc</sup>	0.15 <sup>abc</sup>	80.84 <sup>c</sup>	16.24 <sup>c</sup>	0.80 <sup>ab</sup>
Sarı hasebi ( <i>n</i> = 1)	1.10 <sup>bcd</sup>	0.67 <sup>abcd</sup>	0.05 <sup>a</sup>	0.10 <sup>abc</sup>	73.27 <sup>a</sup>	24.01 <sup>d</sup>	0.78 <sup>ab</sup>
Girit ( <i>n</i> = 1)	1.99 <sup>f</sup>	0.97 <sup>def</sup>	0.09 <sup>ab</sup>	0.38 <sup>e</sup>	89.02 <sup>e</sup>	6.30 <sup>a</sup>	1.24 <sup>de</sup>
Gulumbe ( <i>n</i> = 1)	0.85 <sup>ab</sup>	0.48 <sup>a</sup>	0.32 <sup>e</sup>	0.21 <sup>bc</sup>	86.69 <sup>de</sup>	10.33 <sup>ab</sup>	1.07 <sup>bcd</sup>

<sup>a</sup> Different superscript letters in the same column indicate significant difference between values at the  $p < 0.05$  level

0.04 (Kalamata)–0.16 % (Saurani); C24:0, 0.01 (Uslu, Kalamata, Sarı hasebi, Gulumbe)–0.19 % (Domat). The fatty acid profiles of the Turkish olive oil samples were also in good agreement with the values obtained for the same cultivars in some other harvest seasons [16–18, 40].

Elaidic acid (*tr* C18:1) was not observed in any of the samples. The total contents of *trans* forms of linoleic and linolenic acids (*tr* C18:2 + *tr* C18:3) were between 0 and 0.03 % (data not shown in the table), within the limits of international and Turkish regulations. A correlation study was carried out to elucidate the relationships between variables. There were high and positive correlations between C17:0 and C17:1 ( $r = 0.819$ ) and C18:0 and C20:0 ( $r = 0.749$ ), whereas there was a negative and strong correlation between C18:1 and C18:2 ( $r = -0.807$ ).

The saponification numbers were between 192 and 193 mg KOH/g for olive oil samples. The iodine values of the samples were between 75.63 and 89.88 (data not shown), similar to those found in previous work [23, 40].

#### Fatty Acids at the 2 Position of Triglycerides

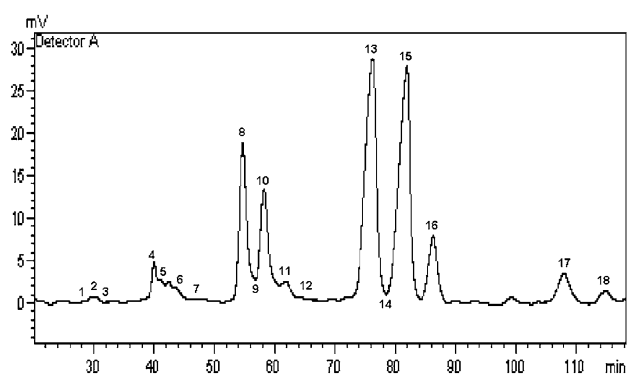
Table 3 shows the fatty acid composition of the *sn*-2 position of triglycerides. C16:0, C16:1, C17:1, C18:0, C18:1, C18:2, and C18:3 were identified in the *sn*-2 position of triglycerides. The *sn*-2 fatty acid compositions have been used for characterization of VOO and also for checking the presence of synthetic triglycerides. Unsaturated fatty acids generally occupy the 2 position of triacylglycerols. Oleic

acid ranged from 70.90 % (Antalya yağlık) to 89.02 % (Girit), whereas linoleic acid ranged from 6.30 % (Girit) to 25.50 % (Antalya yağlık). Oleic acid contents were quite lower whereas linoleic acid percentages were rather higher than the ratios determined for the Cornicabra variety [7]. Linolenic acid content was between 0.60 % (Kalamata) and 1.70 % (Celebi) which is higher than the ratios reported by Aranda et al. [7] and Tekin [50]. The main saturated fatty acids, the combination of palmitic and stearic acids, were considerably higher in oil from Nizip yağlık (1.52 %), Kalamata (1.72 %), and Girit (2.37 %) varieties, which are generally cultivated in the south region of Anatolia peninsula. Positive and high correlations were determined between *sn*-2 C16:1 and C16:1 ( $r = 0.772$ ), *sn*-2 C17:1 and C17:0 ( $r = 0.735$ ), *sn*-2 C17:1 and C17:1 ( $r = 0.715$ ), *sn*-2 C18:1 and C18:1 ( $r = 0.755$ ), and *sn*-2 C18:2 and C18:2, whereas strong and negative ones were observed between *sn*-2 C18:1 and C18:2 ( $r = -0.911$ ), *sn*-2 C18:2 and C18:1 ( $r = -0.741$ ), and *sn*-2 C18:1 and *sn*-2 C18:2 ( $r = -0.994$ ). To our knowledge, this is the first report evaluating the fatty acids in the *sn*-2 position of triglycerides of Turkish monovarietal olive oils.

#### Triglyceride Composition

The triacylglycerol composition of the Turkish olive oils is given in Table 4 and an HPLC chromatogram of an oil sample is shown in Fig. 1. The main triglycerides determined for the Turkish olive oils were triolein (OOO), palmitodiolein

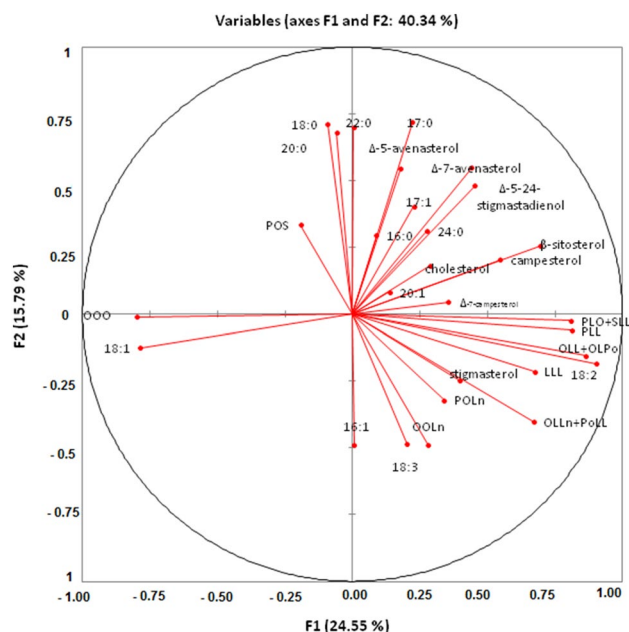




**Fig. 1** HPLC triglyceride chromatogram of olive oil. 1 LLL, 2 OLLn + PoLL, 3 PLLn, 4 OLL + OLPo, 5 OOLn, 6 PLL, 7 POLn, 8 OOL + PPLn, 9 OOPo, 10 PLO + SLL, 11 PoPO, 12 PPL, 13 OOO, 14 SOL, 15 OOP, 16 POP, 17 SOO, 18 POS

(OOP), dioleolinolein (OOL), palmitooleolinolein (PLO), dipalmitoolein (POP), and stearodiolein (SOO). Trilinolein (LLL), oleolinoleolinolenin (OLLn), palmitolinoleolinolenin (PLLn), oleodilinolein (OLL), dioleolinolenin (OOLn), palmitodilinolein (PLL), palmitooleolinolenin (POLn), palmitoleodiolein (OOPo), palmitoleopalmitoolein (PoOP), dipalmitolinolein (PPL), stearooleolinolein (SOL), and palmitostearoolein (POS) were also observed in all analyzed samples. The major triglyceride, triolein (OOO), was present at between 24.72 % (Domat) and 48.64 % (Gulumbe) in agreement with the fatty acid results. Aranda et al. [7] reported 51.7 % for the Cornicabra variety, Graciani [51] determined 48.4 % for Picual, Ollivier et al. [11] reported 27.32–58.76 % for French cultivars, whereas Baccouri et al. [52] described 24.6–49.2 % and 45.6–63.2 % for Tunisian and Sicilian varieties, respectively. OOP, the second most important triglyceride, had values between 21.09 % (Erkence) and 28.86 % (Nizip yağlık), higher than the results obtained for some Tunisian [13, 34] and French [12] cultivars but in accordance with the results obtained for Italian [53] and Iranian [10] varieties. OOL (accomplished with PPLn) was between 8.19 % (Girit) and 15.80 % (Domat) consistent with the values reported by Piravi-Vanak et al. [10], Ollivier et al. [12], and Chiavaro et al. [53], but higher than those described by Ben Temime et al. [34] and Baccouri et al. [52]. PLO (accomplished with SLL) was between 4.82 % (Girit) and 13.87 % (Domat) comparable to the previous work [13]. Similar triglyceride profiles were reported for Memecik, Edremit yağlık [22]; Gemlik [24]; and Ayvalık (Edremit yağlık), Uslu, Nizip yağlık, Memecik varieties [25].

Olive oil has mainly triglycerides with equivalent chain numbers (ECNs) of 44, 46, 48, and 50. ECN 42 triglycerides are indicators of more unsaturated oils. Trilinolein and ECN 42 levels of the Turkish olive oils ranged from 0.04 % (Celebi) to 0.51 % (Sarı hasebi) and 0.19 % (Gulumbe) to 1.00 % (Antalya yağlık), respectively. ECN



**Fig. 2** Loading plot of Turkish olive oils obtained from PCA of data using relevant fatty acids, triacylglycerols, and sterols on the plane identified by two principal components

44, ECN 46, ECN 48, and ECN 50 values of samples were 2.35 (Gulumbe)–8.77 % (Antalya yağlık), 15.49 (Girit)–36.27 % (Antalya yağlık), 50.01 (Antalya yağlık)–75.15 % (Girit), and 3.60 (Mersin yağlık)–8.52 % (Saurani) in the same order.

Strong positive correlations were determined between OLL and C18:2 ( $r = 0.886$ ), PLL and C18:2 ( $r = 0.735$ ), PLO and C18:2 ( $r = 0.792$ ), OOO and C18:1 ( $r = 0.776$ ), OLL and *sn*-2 C18:2 ( $r = 0.910$ ), OLL and LLL ( $r = 0.757$ ), OLL and PLL ( $r = 0.782$ ), PoOP and POLn ( $r = 0.702$ ), PLO and OLL ( $r = 0.739$ ), PLO and PLL ( $r = 0.730$ ), PLL and *sn*-2 C18:2 ( $r = 0.746$ ), PLO and *sn*-2 C18:2 ( $r = 0.794$ ), and OOO and *sn*-2 C18:1 ( $r = 0.764$ ). Conversely, there were high negative correlations between OLL and C18:1 ( $r = -0.728$ ), PLO and C18:1 ( $r = -0.733$ ), OOO and C18:2 ( $r = -0.747$ ), OLL and *sn*-2 C18:1 ( $r = -0.911$ ), PLL and *sn*-2 C18:1 ( $r = -0.756$ ), PLO and *sn*-2 C18:1 ( $r = -0.799$ ), and OOO and *sn*-2 C18:2 ( $r = -0.744$ ).

#### Sterol Content and Composition of Turkish Olive Oils

Table 5 shows the individual sterol content and triterpene dialcohol ratio of the Turkish olive oils. The main sterols found in olive oil were  $\beta$ -sitosterol,  $\Delta$ -5-avenasterol, and campesterol. Cholesterol, brassicasterol, 24-methylene-cholesterol, campestanol, stigmasterol,  $\Delta$ -7-campesterol, clerosterol, sitostanol,  $\Delta$ -5,24-stigmastadienol,  $\Delta$ -7-stigmastanol,  $\Delta$ -7-avenasterol, and two triterpene

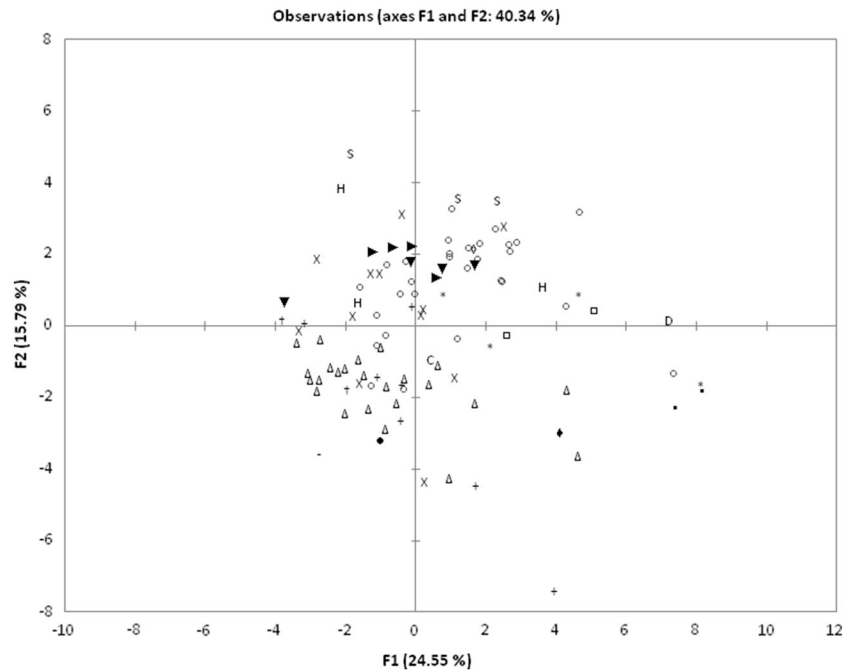
**Table 5** Sterol profile of Turkish olive oils

Variety	Sterol (mg/kg) and erythrodiol- $\alpha$ -vaol (%) content														
	Cholesterol	Brassica-sterol	24-Methylene-cholesterol	Campesterol	Campestanol	Stigma-sterol	$\Delta$ -7-Campesterol	Clero-sterol	$\beta$ -Sitosterol	Sito-sterol	$\Delta$ -5-Avenasterol	$\Delta$ -5,24-Stigmastadienol	$\Delta$ -7-Stigmasteno	$\Delta$ -7-Avenasterol	Total sterols + Erythrodiol + $\alpha$ -vaol
Uslu	5.32 <sup>a</sup>	0.34 <sup>ab</sup>	1.75 <sup>a</sup>	40.36 <sup>ab</sup>	1.21 <sup>ab</sup>	9.76 <sup>ab</sup>	1.33 <sup>a</sup>	13.59 <sup>a</sup>	1,301.51 <sup>abc</sup>	8.96 <sup>ab</sup>	128.99 <sup>abcd</sup>	11.87 <sup>a</sup>	4.83 <sup>a</sup>	11.33 <sup>ab</sup>	1,541.20 <sup>ab</sup>
Memecik	5.75 <sup>a</sup>	0.45 <sup>ab</sup>	1.50 <sup>a</sup>	46.42 <sup>ab</sup>	1.37 <sup>ab</sup>	15.49 <sup>abc</sup>	1.84 <sup>ab</sup>	14.73 <sup>a</sup>	1,356.67 <sup>abc</sup>	5.94 <sup>a</sup>	56.67 <sup>ab</sup>	6.14 <sup>a</sup>	4.67 <sup>a</sup>	5.32 <sup>ab</sup>	1,523.03 <sup>ab</sup>
Gemlik	6.02 <sup>b</sup>	0.63 <sup>ab</sup>	1.78 <sup>a</sup>	42.48 <sup>ab</sup>	1.27 <sup>ab</sup>	15.71 <sup>abc</sup>	1.37 <sup>a</sup>	14.93 <sup>a</sup>	1,406.13 <sup>abc</sup>	12.21 <sup>ab</sup>	147.00 <sup>bcd</sup>	14.15 <sup>ab</sup>	6.74 <sup>a</sup>	13.74 <sup>abc</sup>	1,683.16 <sup>ab</sup>
Edremit yağılık	6.43 <sup>b</sup>	0.53 <sup>ab</sup>	2.17 <sup>a</sup>	67.50 <sup>b</sup>	1.11 <sup>ab</sup>	12.46 <sup>ab</sup>	2.32 <sup>ab</sup>	17.12 <sup>a</sup>	1,836.26 <sup>bc</sup>	11.00 <sup>ab</sup>	197.77 <sup>d</sup>	26.90 <sup>b</sup>	11.83 <sup>a</sup>	18.43 <sup>bc</sup>	2,211.77 <sup>b</sup>
Erkence	5.51 <sup>b</sup>	1.69 <sup>b</sup>	1.95 <sup>a</sup>	52.52 <sup>ab</sup>	1.47 <sup>ab</sup>	13.27 <sup>abc</sup>	2.76 <sup>ab</sup>	15.97 <sup>a</sup>	1,819.59 <sup>bc</sup>	9.88 <sup>ab</sup>	101.40 <sup>abc</sup>	14.73 <sup>ab</sup>	12.99 <sup>a</sup>	18.16 <sup>bc</sup>	2,071.94 <sup>b</sup>
Mersin yağılık	7.03 <sup>b</sup>	0.35 <sup>ab</sup>	1.63 <sup>a</sup>	52.23 <sup>ab</sup>	0.81 <sup>a</sup>	13.64 <sup>abc</sup>	1.04 <sup>a</sup>	17.97 <sup>a</sup>	1,703.72 <sup>abc</sup>	10.41 <sup>ab</sup>	86.07 <sup>abc</sup>	17.62 <sup>ab</sup>	18.62 <sup>a</sup>	25.98 <sup>cd</sup>	1,957.17 <sup>ab</sup>
Saurani	7.37 <sup>b</sup>	0.30 <sup>ab</sup>	0.93 <sup>a</sup>	31.09 <sup>a</sup>	1.82 <sup>ab</sup>	9.90 <sup>ab</sup>	0.67 <sup>a</sup>	20.24 <sup>a</sup>	1,592.93 <sup>abc</sup>	20.81 <sup>bc</sup>	180.70 <sup>bc</sup>	15.35 <sup>ab</sup>	17.53 <sup>a</sup>	33.49 <sup>d</sup>	1,933.18 <sup>ab</sup>
Antalya yağılık	6.85 <sup>a</sup>	0.69 <sup>ab</sup>	1.40 <sup>a</sup>	52.04 <sup>ab</sup>	0.80 <sup>a</sup>	40.67 <sup>c</sup>	2.33 <sup>ab</sup>	17.16 <sup>a</sup>	1,932.13 <sup>c</sup>	8.67 <sup>ab</sup>	71.16 <sup>ab</sup>	14.92 <sup>ab</sup>	10.72 <sup>a</sup>	14.01 <sup>abc</sup>	2,173.59 <sup>b</sup>
Nizip yağılık	6.38 <sup>b</sup>	0.72 <sup>ab</sup>	1.07 <sup>a</sup>	37.60 <sup>ab</sup>	1.21 <sup>ab</sup>	15.72 <sup>abc</sup>	2.45 <sup>ab</sup>	13.65 <sup>a</sup>	1,176.08 <sup>abc</sup>	8.01 <sup>ab</sup>	102.35 <sup>abc</sup>	7.74 <sup>a</sup>	5.59 <sup>a</sup>	9.91 <sup>ab</sup>	1,388.54 <sup>ab</sup>
Kilis yağılık	7.25 <sup>b</sup>	1.02 <sup>ab</sup>	5.01 <sup>a</sup>	39.02 <sup>ab</sup>	2.20 <sup>ab</sup>	10.17 <sup>ab</sup>	2.67 <sup>ab</sup>	135.89 <sup>a</sup>	1,094.24 <sup>ab</sup>	15.01 <sup>abc</sup>	85.05 <sup>abc</sup>	7.71 <sup>a</sup>	7.84 <sup>a</sup>	14.60 <sup>abc</sup>	1,427.73 <sup>ab</sup>
Domat	8.73 <sup>b</sup>	0.25 <sup>a</sup>	0.75 <sup>a</sup>	67.50 <sup>b</sup>	0.82 <sup>a</sup>	26.76 <sup>c</sup>	4.87 <sup>b</sup>	16.80 <sup>a</sup>	1,765.60 <sup>bc</sup>	6.62 <sup>a</sup>	66.34 <sup>ab</sup>	7.90 <sup>ab</sup>	2.97 <sup>a</sup>	4.61 <sup>a</sup>	1,980.52 <sup>ab</sup>
Celebi	6.33 <sup>b</sup>	0.62 <sup>ab</sup>	1.95 <sup>a</sup>	67.19 <sup>b</sup>	3.18 <sup>b</sup>	15.54 <sup>abc</sup>	0.49 <sup>a</sup>	16.98 <sup>a</sup>	1,561.71 <sup>abc</sup>	20.84 <sup>bc</sup>	90.92 <sup>abc</sup>	8.22 <sup>a</sup>	4.11 <sup>a</sup>	5.96 <sup>ab</sup>	1,804.04 <sup>ab</sup>
Kalamata	3.90 <sup>b</sup>	0.39 <sup>ab</sup>	0.71 <sup>a</sup>	22.82 <sup>a</sup>	1.20 <sup>ab</sup>	12.32 <sup>ab</sup>	0.49 <sup>a</sup>	11.36 <sup>a</sup>	976.66 <sup>a</sup>	8.92 <sup>ab</sup>	80.12 <sup>ab</sup>	9.46 <sup>ab</sup>	4.74 <sup>a</sup>	12.20 <sup>ab</sup>	1,145.32 <sup>a</sup>
Sarı ulak	5.53 <sup>b</sup>	0.66 <sup>ab</sup>	0.54 <sup>a</sup>	66.05 <sup>b</sup>	2.66 <sup>ab</sup>	21.77 <sup>bc</sup>	3.91 <sup>ab</sup>	18.28 <sup>a</sup>	1,605.79 <sup>abc</sup>	27.00 <sup>c</sup>	38.54 <sup>a</sup>	5.66 <sup>ab</sup>	4.31 <sup>a</sup>	5.30 <sup>ab</sup>	1,806.03 <sup>ab</sup>
Halhali	5.91 <sup>b</sup>	0.48 <sup>ab</sup>	0.84 <sup>a</sup>	37.36 <sup>ab</sup>	1.11 <sup>ab</sup>	13.60 <sup>abc</sup>	1.05 <sup>a</sup>	10.85 <sup>a</sup>	1,189.25 <sup>abc</sup>	6.92 <sup>a</sup>	81.28 <sup>ab</sup>	7.11 <sup>a</sup>	5.60 <sup>a</sup>	6.77 <sup>ab</sup>	1,368.18 <sup>ab</sup>
Sarı hasebi	4.76 <sup>b</sup>	0.18 <sup>a</sup>	0.56 <sup>a</sup>	42.29 <sup>ab</sup>	1.06 <sup>ab</sup>	5.50 <sup>a</sup>	1.19 <sup>a</sup>	13.88 <sup>a</sup>	1,279.00 <sup>abc</sup>	7.16 <sup>a</sup>	87.57 <sup>abc</sup>	14.32 <sup>ab</sup>	10.60 <sup>a</sup>	14.30 <sup>abc</sup>	1,482.40 <sup>ab</sup>
Girit	7.52 <sup>a</sup>	0.37 <sup>ab</sup>	2.09 <sup>a</sup>	55.50 <sup>ab</sup>	1.99 <sup>ab</sup>	8.84 <sup>ab</sup>	0.86 <sup>b</sup>	12.75 <sup>a</sup>	1,192.29 <sup>abc</sup>	6.37 <sup>a</sup>	52.99 <sup>ab</sup>	6.48 <sup>a</sup>	5.27 <sup>a</sup>	3.43 <sup>a</sup>	1,356.75 <sup>ab</sup>
Gulumbé	5.50 <sup>b</sup>	0.35 <sup>ab</sup>	1.95 <sup>a</sup>	51.37 <sup>ab</sup>	0.98 <sup>ab</sup>	16.42 <sup>abc</sup>	0.87 <sup>a</sup>	13.56 <sup>a</sup>	1,302.19 <sup>abc</sup>	6.37 <sup>a</sup>	51.52 <sup>ab</sup>	6.61 <sup>a</sup>	4.04 <sup>a</sup>	3.47 <sup>a</sup>	1,465.26 <sup>ab</sup>

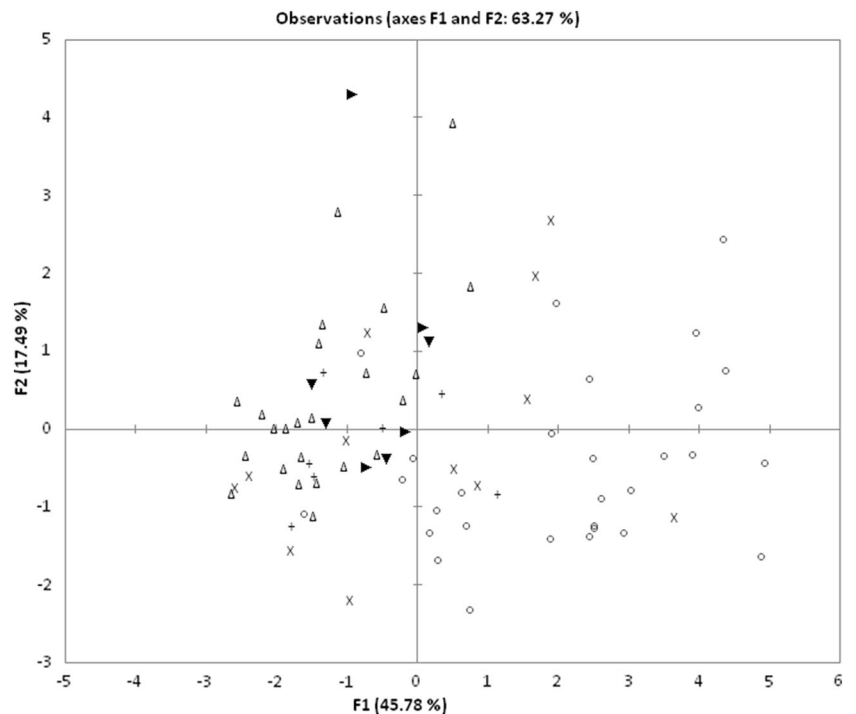
<sup>a</sup> Different superscript letters in the same column indicate significant difference between values at the  $p < 0.05$  level



**Fig. 3** Factor score plot of Turkish olive oils obtained from PCA of data using relevant fatty acids, triacylglycerols, and sterols on the plane identified by two principal components (Uslu: *plus*, Gemlik: *multiplication*, Memecik: *open triangle*, Edremit: *open circle*, Nizip yağlık: *filled inverted triangle*, Kilis yağlık: *right filled triangle*, Erkençe: *asterik*, Gulumbe: *dagger*, Mersin yağlık: *open square*, Saurani: S, Antalya yağlık: *filled square*, Domat: D, Celebi: C, Sarı ulak: *open diamond*, Halhalı: H, Sarı hasebi: *filled diamond*, Girit: *minus*, Kalamata: *filled circle*)



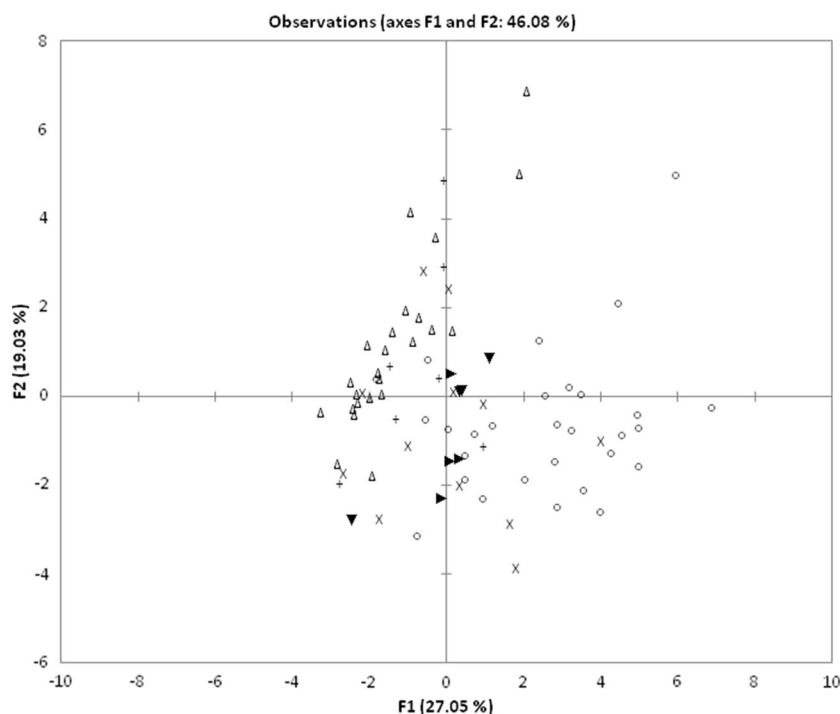
**Fig. 4** Factor score plot of Turkish olive oils of common varieties obtained from PCA of data using sterols on the plane identified by two principal components (Uslu: *plus*, Gemlik: *multiplication*, Memecik: *open triangle*, Edremit: *open circle*, Nizip yağlık: *filled inverted triangle*, Kilis yağlık: *right filled triangle*)



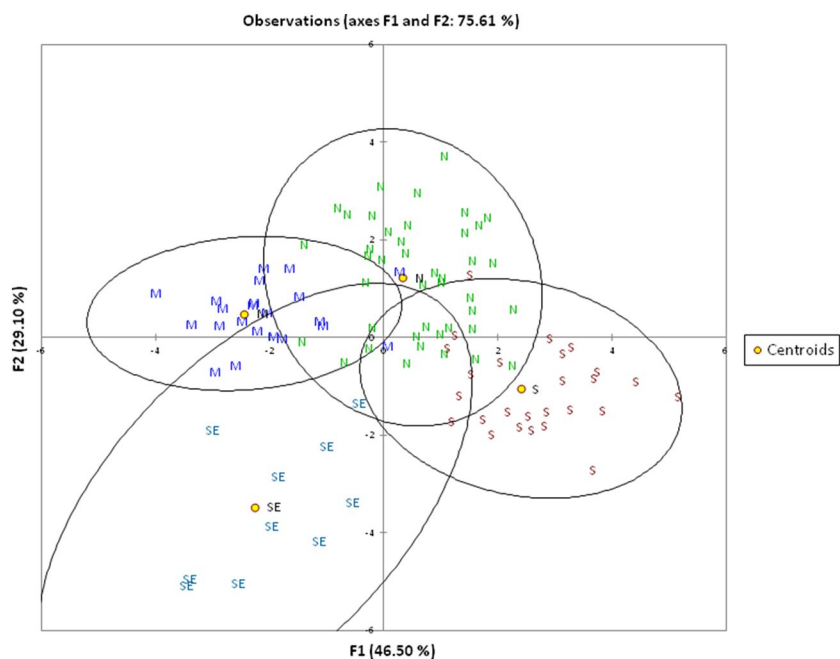
dialcohols (erythrodiol and uvaol) were also determined in smaller amounts. The total sterol contents of all samples were above the legal threshold value (1,000 mg/kg) determined for extra virgin olive oil by EU regulations, ranging from 1,145.32 (Kalamata) to 2,211.77 mg/kg (Edremit yağlık). Edremit yağlık has also been well discriminated with its high sterol content in previous works [22, 36].

$\beta$ -Sitosterol, the predominant sterol of olive oil, varied between 976.66 (Kalamata) and 1,836.26 mg/kg (Edremit yağlık).  $\beta$ -Sitosterol was the most densely present in the Memecik variety (89.25 %) and least concentrated in Saurani (82.04 %). The second most abundant sterol was  $\Delta$ -5-avenasterol fluctuating between 38.54 (Sarı ulak) and 197.77 mg/kg (Edremit yağlık). Matthäus and Özcan [21]

**Fig. 5** Factor score plot of Turkish olive oils of common varieties obtained from PCA of data using fatty acids, triacylglycerols and sterols on the plane identified by two principal components (Uslu: *plus*, Gemlik: *multiplication*, Memecik: *open triangle*, Edremit: *open circle*, Nizip yağlık: *filled inverted triangle*, Kilis yağlık: *right filled triangle*)



**Fig. 6** Discriminant plot of Turkish olive oils according to four geographical origins based on fatty acid, triacylglycerol, and sterol composition (*S* South Aegean, *N* North Aegean, *M* Mediterranean, *SE* South East Anatolia)



reported similar  $\Delta$ -5-avenasterol values for Edremit and Domat, but lower values for Gemlik and Sarı ulak varieties.  $\beta$ -Sitosterol and  $\Delta$ -5-avenasterol contents have a strong negative correlation ( $r = -0.982$ ), possibly because of desaturase enzyme activity transforming  $\beta$ -sitosterol to  $\Delta$ -5-avenasterol [54]. Regarding campesterol, Edremit yağlık had the highest (67.50 mg/kg) and Kalamata had the lowest (22.82 mg/kg) values. The amounts of the other sterols, such as 24-methylene-cholesterol, campestanol, and  $\Delta$ -7-campesterol, were less than 6 ppm. Sarı ulak, Edremit

yağlık, and Saurani varieties contained the highest levels of sitosterol,  $\Delta$ -5,24-stigmastadienol, and  $\Delta$ -7-avenasterol correspondingly.

Sterol percentages of the Turkish olive oils were in agreement with both national and international directives with some exceptions. The Girit variety exceeded both cholesterol and campesterol upper limits with 0.54 and 4.09 % ratios. Likewise,  $\Delta$ -7-stigmastenol contents of Erkençe, Mersin yağlık, Saurani, Kilis yağlık, and Sarı hasebi oils were over the regulatory maximum limit of 0.5 %,

**Table 6** Statistically significant chemical parameters of olive oils with respect to olive cultivar at each growing region

	<b>Gemlik</b>		
	<b>North Aegean</b>	<b>Mediterranean</b>	<b>Southeast Anatolia</b>
C 16:1	13.32 <sup>ab</sup>	11.24 <sup>a</sup>	14.73 <sup>b</sup>
LLL	0.08 <sup>a</sup>	0.15 <sup>b</sup>	0.07 <sup>a</sup>
OLLn	0.11 <sup>a</sup>	0.20 <sup>a</sup>	0.30 <sup>b</sup>
PLLn	0.03 <sup>a</sup>	0.02 <sup>a</sup>	0.08 <sup>b</sup>
<b>Edremit</b>			
	<b>North Aegean</b>	<b>Mediterranean</b>	<b>South Aegean</b>
C17:1	0.20 <sup>a</sup>	0.25 <sup>b</sup>	0.20 <sup>a</sup>
C18:2	10.89 <sup>a</sup>	7.96 <sup>b</sup>	9.99 <sup>ab</sup>
C22:0	0.11 <sup>b</sup>	0.07 <sup>a</sup>	0.10 <sup>b</sup>
POLn	0.06 <sup>ab</sup>	0.08 <sup>b</sup>	0.03 <sup>a</sup>
OOPo	0.15 <sup>a</sup>	0.93 <sup>b</sup>	0.34 <sup>a</sup>
Campesterol	70.51 <sup>ab</sup>	48.76 <sup>a</sup>	75.49 <sup>b</sup>
$\beta$ -sitosterol	1938.81 <sup>b</sup>	1399.31 <sup>a</sup>	1934.15 <sup>b</sup>
$\Delta$ -5-avenasterol	229.15 <sup>b</sup>	190.57 <sup>ab</sup>	139.75 <sup>a</sup>
$\Delta$ -5,24-stigmastadienol	32.08 <sup>ab</sup>	17.09 <sup>a</sup>	23.52 <sup>ab</sup>
$\Delta$ -7-Stigmastenol	7.95 <sup>ab</sup>	4.38 <sup>a</sup>	12.22 <sup>b</sup>
<b>Memecik</b>			
	<b>North Aegean</b>	<b>South Aegean</b>	
C18:0	2.36 <sup>a</sup>	2.03 <sup>b</sup>	
<i>sn</i> -2 C16:0	1.18 <sup>a</sup>	0.85 <sup>b</sup>	
<i>sn</i> -2 C 18:0	0.25 <sup>b</sup>	0.13 <sup>a</sup>	
<b>Halhah</b>			
	<b>Mediterranean</b>	<b>Southeast Anatolia</b>	
C17:1	0.17 <sup>b</sup>	0.05 <sup>a</sup>	
C18:1	73.05 <sup>b</sup>	63.35 <sup>a</sup>	
C18:2	7.33 <sup>a</sup>	15.32 <sup>b</sup>	
C18:3	0.55 <sup>a</sup>	0.59 <sup>b</sup>	
C20:0	0.65 <sup>b</sup>	0.43 <sup>a</sup>	
<i>sn</i> - C17:1	0.19 <sup>b</sup>	0.05 <sup>a</sup>	
<i>sn</i> -2 C18:1	86.02 <sup>b</sup>	72.39 <sup>a</sup>	
<i>sn</i> -2 C 18:2	10.84 <sup>a</sup>	24.98 <sup>b</sup>	
LLL	0.07 <sup>a</sup>	0.21 <sup>b</sup>	
PLLn	0.03 <sup>a</sup>	–	
OLL	1.04 <sup>a</sup>	4.07 <sup>b</sup>	
PLL	0.33 <sup>b</sup>	1.41 <sup>b</sup>	
OOO	38.30 <sup>b</sup>	25.66 <sup>a</sup>	
PLO	7.63 <sup>a</sup>	11.74 <sup>b</sup>	
PoOP	0.58 <sup>a</sup>	1.45 <sup>b</sup>	
Cholesterol	5.91 <sup>b</sup>	4.19 <sup>a</sup>	
Brassicasterol	0.48 <sup>a</sup>	6.76 <sup>b</sup>	
24-methylene-cholesterol	0.86 <sup>a</sup>	0.00 <sup>b</sup>	
Campesterol	37.36 <sup>a</sup>	80.19 <sup>b</sup>	
Campestanol	1.11 <sup>a</sup>	1.77 <sup>b</sup>	
Clerosterol	10.85 <sup>a</sup>	–	
$\beta$ -sitosterol	1189.25 <sup>a</sup>	1704.86 <sup>b</sup>	
Sitostanol	6.93 <sup>a</sup>	22.40 <sup>b</sup>	
$\Delta$ -5-avenasterol	81.28 <sup>a</sup>	170.63 <sup>b</sup>	
$\Delta$ -5,24-stigmastadienol	7.11 <sup>a</sup>	14.38 <sup>b</sup>	
$\Delta$ -7-avenasterol	6.77 <sup>a</sup>	13.66 <sup>b</sup>	

specifically 0.57, 0.90, 0.80, 0.53, and 0.70 %, respectively. Although Mersin yağlık, Saurani, Sarı hasebi, and Girit are relatively domestic varieties, Kilis yağlık and Erkence have higher economic value because they comprise 2.8 % and 3.5 % of the total olive trees in Turkey [55]. All olive varieties having incompatible ratios with legal regulations in terms of individual sterols account for about 8 % of the total olive trees.

The sum of triterpene dialcohols (erythrodiol and uvaol) must be lower than 4.5 % of total sterols. The totals of those triterpene dialcohols were within the limits varying between 0.69 (Sarı ulak) and 4.42 % (Antalya yağlık).

### Multivariate Analysis

PCA is a multivariate analysis method frequently used for classification purposes. The method reduces the variables to a relatively small number of principal components but maximizes the variability. In this study, analytical data were arranged in a matrix of 101 samples  $\times$  52 variables. PLLn, OOPo, PoOP, PPL, 24-methylene-cholesterol, clerosterol, and  $\Delta$ -7-stigmastanol were discarded because they did not generate significant differences among cultivars. Variables which had a Kaiser–Meyer–Olkin measure of sampling adequacy index lower than 0.5 were also removed. The remaining adequate variables were C16:0, C16:1, C17:0, C17:1, C18:0, C18:1, C18:2, C18:3, C20:0, C20:1, C22:0, C24:0, LLL, OLLn + PoLL, OLL + OLPo, OOLn, PLL, POLn, PLO + SLL, OOO, POS, cholesterol, campesterol, stigmastanol,  $\Delta$ -7-campesterol,  $\beta$ -sitosterol,  $\Delta$ -5-avenasterol,  $\Delta$ -5,24-stigmastadienol, and  $\Delta$ -7-avenasterol, forming a matrix of 101 samples  $\times$  29 variables. Loading plot and factor score plots are given in Figs. 2 and 3, respectively. The first two principle components explained 40.34 % of the total variance [factor 1 (F1), 24.55 %; factor 2 (F2), 15.79 %]. F1 shows high and positive correlations with C18:2, OLL + OLPo, PLL, and PLO + SLL, whereas high and negative correlations with OOO, C18:1, C18:0, and C17:0 positively affect F2. The score plot revealing the relations among varieties shows that Antalya yağlık, Mersin yağlık, Erkence, Celebi, Sarı ulak, Sarı hasebi, and Domat varieties have positive scores, whereas Gulumbe, Girit, and Kalamata varieties have negative scores on F1. Memecik oils were all negatively correlated with F2.

In this study, some common cultivars were represented with higher numbers of samples than some local cultivars. To avoid the scattering of values in PCA, less common cultivars (Gulumbe, Antalya yağlık, Mersin yağlık, Erkence, Celebi, Girit, Domat, Kalamata, Sarı hasebi, Sarı ulak, Halhalı, Uslu, Saurani) represented with fewer samples were eliminated to obtain clearer separations among widespread varieties (Memecik, Gemlik, Edremit, Uslu, Nizip yağlık, Kilis yağlık). Nizip and Kilis yağlık were

chosen to be economically important cultivars for their growing areas. Additional PCAs were performed using fatty acid, triacylglycerol, and sterol composition of common olive oils separately. Results revealed that sterol composition was more evident than fatty acid and triacylglycerol profiles for discrimination of varieties. PCA based on sterol profile allowed one to group Memecik and Edremit yağlık cultivars as shown in Fig. 4. The first two principle components explained 63.27 % of the total variance (F1, 45.78 %; F2, 17.49 %). Memecik oils had negative scores (except one sample) and Edremit yağlık oils had positive scores on F1. The first principle component was highly correlated with  $\Delta$ -5,24-stigmastadienol and  $\beta$ -sitosterol. A global PCA including all relevant analytical data was also employed to distinguish common cultivars resulting in a total variance of 46.08 % (F1, 27.05 %; F2, 19.03 %) (Fig. 5). Memecik and Edremit yağlık oils were grouped on negative and positive sides of F1 respectively. But a clear cluster was not observed for other varieties. F1 was highly correlated with  $\Delta$ -5,24-stigmastadienol,  $\beta$ -sitosterol, and campesterol.

The classification of olive oils according to geographical origin was achieved by discriminant analysis. Oils obtained from North Aegean, South Aegean, Mediterranean, and Southeast Anatolia were grouped according to their origin as shown in Fig. 6. The first two discriminant functions explained 75.61 % of the total variance. Olive oils from various geographical areas were not separated clearly and partially overlapped.  $\beta$ -Sitosterol,  $\Delta$ -5,24-stigmastadienol, C18:1, C18:2, OLL, OOL, PLO, OOO, and OOP had strong standardized discriminant coefficients. The percentage of correct classification of the samples was 93.14 %.

In the context of the current work, variability between two crop years and the contribution of harvest time to the classification of oils were also investigated. However an apparent discrimination according to harvest year could not be accomplished. Also, the relations between geographic area and cultivar were examined using one-way ANOVA procedure. Four of the 18 varieties were collected from more than one location; Gemlik and Edremit yağlık were harvested from three different growing areas, whereas Memecik and Halhalı were obtained from two locations. There were in total 53 variables (fatty acids, *sn*-2 fatty acids, triacylglycerols, sterols). Among them, four variables generated statistically significant differences for Gemlik, three variables for Memecik, 10 variables for Edremit, and 26 variables for Halhalı (Table 6). Halhalı was clearly affected by the growing region because half of the variables differed because of the geographic area and those variables were composed of major lipid components such as C18:1, *sn*-2 C18:1, OOO, and  $\beta$ -sitosterol. A limited number of variables of Gemlik and Memecik oils were affected by location and they were mainly minor ones except the

palmitic acid content of Gemlik. Edremit oils' individual sterols were influenced by the growing area.

## Conclusion

This work reports the varietal and geographical differentiation of Turkish olive oils based on fatty acid, *sn*-2 fatty acid, triglyceride, and sterol composition. The results expose that some chemical compounds exceed the legal limits as a result of varietal characteristics which should be taken into consideration in commercial dealings and regulatory studies. In Turkey, there are four regions with PDO certificates: Edremit Gulf Olive Oils, Ayvalık Olive Oils, South Aegean Olive Oils, and Nizip Olive Oils. Olive oils certified with PDOs have higher market values owing to their quality and authenticity. The data reported herein can be used for newer PDO applications.

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

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