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Fatty Acid and Sterol Compositions of Turkish Monovarietal Olive Oils with Regard to Olive Ripening

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Abstract: This study was conducted in crop season of 2018 and the olive fruits from three Turkish varieties Saurani, Karamani and Halhalı under the same pedoclimatic conditions (with no irrigation and no fertilization) were assessed. Oil content, fatty acid, and sterol compositions of three monovarietal 'Halhalı', 'Karamani', and 'Saurani' virgin olive oils were examined at green, spotted and ripe olives. The oil content of olives ranges between 23.77-34.77% and the highest oil yield was observed in the ripe Karamani variety. In terms of fatty acids, the lowest oleic acid values were found in the ripe period of Karamani variety (59.78%), and the highest oleic acid values in the green period of Halhalı variety (69.97%). The oleic and palmitic acid contents decreased, while linoleic and stearic acid contents increased with olive ripening. Total sterol amounts of olive oils varied between 946-1782 mg/kg and showed a significant increase with ripening ($p < 0.05$). The highest β-sitosterol amount was detected in the green period of Saurani variety (91.66%), and the lowest β-sitosterol amount in the spotted period of Halhalı variety (86.16%). The highest Δ^5-avenasterol amounts were detected in the ripe period of Saurani variety (6.54%), the lowest Δ^5-avenasterol amounts were detected in the green period of Halhalı variety (2.36%). Total β-sitosterol, stigmasterol and erythrodiol+uvaol contents of olive oils are changed with ripening. Accordingly, these results showed that fatty acid and sterol compositions can be used as indicators of variety and ripening degree among monovarietal virgin olive oils.

Key words: olive oil, ripening index, Halhalı, Karamani, Saurani

1 Introduction

Virgin olive oil (VOO) is a juice obtained from the olive (*Olea europaea* L.) fruits by completely physically processes[1, 2]. The health benefits of VOO are closely related to its chemical composition, which can strongly affect the oil's quality and oxidative stability[3]. Olive oil is consist of the saponifiable and unsaponifiable fractions representing about 98 and 2% of the total weight, respectively[4]. The saponifiable fraction is mainly composed of the fatty acids, while the unsaponifiable fraction contains chemical compounds, such as hydrocarbons, terpenes, phenolic compounds, tocopherols, phytosterols, pigments and volatile components, among others[5, 6]. The VOO has a high prominence due to its fatty acids composition with high proportion of monounsaturated oleic acid (55-83%) and the biologically active minor components (phenolic compounds, sterols, tocopherols) which result in higher nutritional value and oil quality[7, 8]. Numerous studies carried out on VOO and demonstrated that several factors such as variety, ripening degree, climate and geographic conditions, extraction methods, and preservation conditions significantly influence the chemical composition and quality of VOO[9–16]. Variety and ripening degree of the fruit are the most important factors which contribute to characterize the composition of the oils[2]. Olive fruit can be harvested in different growth stages from immature to mature, or even overripening stages, and from green fruits to black[15]. Fatty acid composition of olive oil are significantly affected by olive fruit ripening[17]. Esti *et al.*[18] found that the content of oleic acid increased at the expense of palmitic acid during ripeness of the cultivars of Leccino and Frantoio, while the content of linoleic acid rose with ripeness in the cultivar Coratina, and decreased in the cultivars Leccino and Frantoio. Several studies have concluded that the relative influence of environmental conditions on fatty acid composition is relatively low, with cultivar being the most relevant factor impacting variability in the fatty acid profile[5, 19]. Sterols and triterpene diols are mainly constituents of olive oil, and constitute the major proportion of its unsaponifiable fraction. Sterols are primary constituents of cell mem-

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branes[20, 21]. 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[22, 23]. The sterol fraction in olive oil is largely dominated by β-sitosterol, which typically accounts for 75 to 90% of the sterols, and to a lesser extent by Δ^5-avenasterol, which usually ranges between 5 and 20%[24]. β-Sitosterol and Δ^5-avenasterol are considered the main carriers of antioxidant activity and other beneficial effects among the sterols from olive oil owing to their abundance and structural specificities[22]. Variety and olive ripening are among the most important factors affecting the sterol and triterpene diol fractions in olive oil. Therefore, it is very important to take the olive ripening into account when considering chemical compounds for oil variety authentication[22, 25]. The aim of this study is to determine the fatty acid and sterol compositions of monovarietal olive oils during olive ripening. For this study, the monovarietal oils from three olive varieties grown in the same growing area and produced at three different ripening stages were studied in this study.

2 Experimental Procedures

2.1 Olive sampling

This study was carried out in the crop season of 2018. Olive fruits from three Turkish varieties Saurani, Karamani and Halhalı under the same pedoclimatic conditions (with no irrigation and no fertilization) were evaluated. The olive cultivars obtained from three single trees of a given variety were collected from same olive growing area in Hatay (36°02'36"N 36°21'26"E). Only intacked fruits considered as healthy were hand-picked from each younger tree for different cultivars. For each olive variety, three harvesting dates (from September to November with 30 days intervals) corresponding to three different ripening stages (green–spotted–ripe) was selected. A representative 3 kg sample of olives was collected to represent the whole tree. At the end of each harvest, the samples were labelled, and immediately transported to the laboratory. They were extracted to olive oil within 24 h.

2.2 Chemicals

Analytical grade campesterol, 5 alpha-cholestan-3beta ol, stigmasterol, cholesterol, beta sitosterol, methanol, *n*-hexane, diethyl ether, cyclohexane, ethyl ether, ethanol, acetone, toluene, formic acid, acetic acid, chloroform, potassium iodine and sodium sulfate were purchased from Merck (Germany) and Sigma-Aldrich (USA). The fatty acid methyl ester (FAME) mixture were obtained from Supelco (Bellefonte, USA).

2.3 Ripening index

The ripening index (RI) was determined from one hundred olive fruits randomly drawn from each olive variety. This index which is based on the assessment of skin and pulp color on a scale ranging from 0 (deep green skin 100%) to 7 (100% purple flesh and black skin), was determined according to International Olive Council[26].

2.4 Oil content (%)

Oil content was determined according to the method by Emmanouilidou *et al.*[6] by soxhlet extraction method using *n*-hexane at 80℃ for 6 h.

2.5 Olive oil extraction

Monovarietal olive 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 28℃ for 45 min and at 3000 rpm, respectively. The oil was separated by decanting and put into dark glass bottles. Oil samples were stored in darkness at 4℃ until chemical analysis which were triplicated.

2.6 Fatty acid compositions

Fatty acid composition of monovarietal virgin olive oil samples was determined according to the analytical method described in COI/T.20/Doc.No.24[27]. 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 analyzed 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℃, respectively. The injection volume was 1 μL. Results are given as weight percentage by comparing retention time of FAMEs of samples with the retention times of standard FAME mixture.

2.7 Sterol and triterpene dialcohols

Sterol and triterpene dialcohols compositions were performed from the unsaponifiable fraction of the olive oil, according to IOC/T.20/No10/Rev. 1[28]. Identification and quantification of sterols and diols as trimethylsilyl ethers was determined 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 245, 300 and 330℃, respectively. Helium was used as the carrier gas with a flow rate of 1.2 mL/min and the split ratio

of 25:1. 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.

2.8 Statistics

Statistical analysis was carried out using the SPSS 17.0 statistical software (SPSS Inc., Chicago, USA). Data were analyzed by a one-way analysis of variance One-way ANOVA. Duncan's multiple range tests was used to determine if there were any statistical differences ($p < 0.05$) between the samples[29].

3 Results and Discussion

3.1 Ripening index

The ripening index (RI) and oil content of olive varieties are shown in Table 1. The RI value was determined in the range of 0.1 (green Saurani) – 3.68 (ripe Karamani). The RI of olive varieties showed significant differences in all harvest dates. It was determined that the RI values of olive varieties increased with the harvest time. The RI value ranged from 1.3 to 3.6[30], 0.65 to 3.87[31], 0 to 2.5[13]. The analysis and the chemical composition of the oil during the fruit ripening process are very important for establishing the variety specificity, determining the harvesting time[32].

3.2 Oil content

Olive oil yield is mainly determined by the oil content of the olive fruit[6]. The maximum oil content is reported to occur between 60th to 75th days after the start of the ripening process. In this study, oil content values were determined in the range of 23.77 (green Saurani)-34.59% (ripe Karamani). Moreover, the values of oil content increased during olive ripening for each olive variety. There was significant differences among the degree of ripening as regards the oil content of olives ($p < 0.05$). In similar studies, this value was found to be 25-41% by Bakshi *et al.*[33] and 24.66% by Koyuncu and Cabaroğlu[34]. Olive biosynthesis occurs rapidly when the olives are at the green stage until they turn fully black, during which oil content levels are high[22].

3.3 Fatty acid compositions

During the ripening process, a series of metabolic routes such as enlargement of olive, increase in oil content increases and darkening of colour occur, which can modify the composition of the oil in the olive, generating changes in the composition of the fatty acids[35]. The chemical characteristics and composition of fatty acids gradually change as the fruit ripens, and it affects the overall quality of the oil[33]. Table 2 reports the fatty acids composition of analysed three monovarietal VOOs. Major fatty acids that were found in olive oils were palmitic (C16:0), oleic (C18:1), linoleic (C18:2) acid. The minor fatty acids were palmitoleic (C16:1), stearic (C18:0), linolenic (C18:3), arachidic

Table 1 Ripening index and oil content of Halhalı, Karamani, and Saurani olive varieties.

	Halhalı			Karamani			Saurani		
	Green	Spotted	Ripe	Green	Spotted	Ripe	Green	Spotted	Ripe
Ripening index	$0.3^{e} \pm 0.04$	$2.00^{c} \pm 0.05$	$2.62^{b} \pm 0.04$	$1.68^{d} \pm 0.04$	$2.00^{c} \pm 0.05$	$3.68^{a} \pm 0.04$	$0.1^{e} \pm 0.02$	$1.92^{c,d} \pm 0.04$	$3.66^{a} \pm 0.05$
Oil content (%)	24.44 ± 0.45	25.23 ± 0.29	33.14 ± 0.61	27.87 ± 0.57	30.82 ± 0.66	34.59 ± 0.53	23.77 ± 0.28	28.78 ± 0.39	34.30 ± 0.47

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

Table 2 Fatty acid composition (%) of Halhalı, Karamani, and Saurani olive oils.

Fatty acids	Halhalı			Karamani			Saurani		
	Green	Spotted	Ripe	Green	Spotted	Ripe	Green	Spotted	Ripe
16:0	$16.23^{a-c} \pm 0.04$	$16.07^{a-c} \pm 0.02$	$15.59^{a-d} \pm 0.03$	$15.38^{b-d} \pm 0.05$	$15.23^{c,d} \pm 0.04$	$14.94^{d} \pm 0.03$	$17.47^{a,b} \pm 0.05$	$16.51^{a} \pm 0.06$	$13.92^{e} \pm 0.07$
16:1	$1.02^{b,c} \pm 0.02$	$0.96^{c,b} \pm 0.04$	$0.87^{d} \pm 0.03$	$1.10^{b} \pm 0.05$	$0.96^{c,d} \pm 0.04$	$1.07^{b,c} \pm 0.06$	$1.42^{a} \pm 0.05$	$1.39^{a} \pm 0.04$	$1.08^{b} \pm 0.03$
18:0	$3.32^{e} \pm 0.04$	$3.47^{d} \pm 0.06$	$3.97^{a} \pm 0.03$	$3.22^{e,f} \pm 0.02$	$3.57^{c,d} \pm 0.07$	$3.67^{c} \pm 0.05$	$3.07^{g} \pm 0.04$	$3.19^{f} \pm 0.02$	$3.79^{b} \pm 0.03$
18:1	$69.97^{a} \pm 0.06$	$68.66^{b} \pm 0.05$	$66.50^{c} \pm 0.07$	$61.74^{e} \pm 0.04$	$60.25^{g} \pm 0.01$	$59.78^{h} \pm 0.02$	$64.78^{d} \pm 0.11$	$61.44^{e,f} \pm 0.06$	$61.08^{f} \pm 0.05$
18:2	$5.65^{i} \pm 0.01$	$7.20^{h} \pm 0.04$	$8.65^{g} \pm 0.06$	$14.73^{d} \pm 0.07$	$16.16^{c} \pm 0.03$	$16.86^{a} \pm 0.05$	$10.05^{f} \pm 0.04$	$13.81^{e} \pm 0.0$	$16.46^{b} \pm 0.05$
18:3	$1.03^{d} \pm 0.04$	$1.02^{d} \pm 0.03$	$1.04^{c} \pm 0.03$	$1.01^{d} \pm 0.06$	$0.98^{e} \pm 0.07$	$0.90^{f} \pm 0.04$	$1.10^{a} \pm 0.06$	$1.04^{b,c} \pm 0.03$	$1.06^{b} \pm 0.04$
20:0	$0.64^{e} \pm 0.05$	$0.69^{d} \pm 0.02$	$0.74^{b} \pm 0.01$	$0.70^{c,b} \pm 0.06$	$0.76^{a,b} \pm 0.04$	$0.78^{a} \pm 0.05$	$0.57^{g} \pm 0.02$	$0.61^{f} \pm 0.05$	$0.70^{c} \pm 0.04$
20:1	$0.16^{c,d} \pm 0.03$	$0.17^{b,c} \pm 0.06$	$0.18^{a,b} \pm 0.06$	$0.15^{d} \pm 0.04$	$0.17^{b,c} \pm 0.05$	$0.19^{a} \pm 0.06$	$0.13^{e} \pm 0.04$	$0.12^{e} \pm 0.02$	$0.13^{e} \pm 0.04$
22:0	1.58 ± 0.04	1.32 ± 0.05	0.96 ± 0.03	1.70 ± 0.06	1.52 ± 0.08	1.34 ± 0.04	1.06 ± 0.06	0.94 ± 0.05	0.71 ± 0.03
24:0	0.11 ± 0.02	0.09 ± 0.01	0.10 ± 0.02	0.11 ± 0.01	0.12 ± 0.01	0.11 ± 0.02	0.09 ± 0.03	0.08 ± 0.02	0.09 ± 0.01

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

(C20:0), gadoleic (C20:1), behenic (C22:0), lignoseric (C24:0) acid. Olive oil characterized by a high level of oleic acid[22]. Among fatty acids, the oleic acid was the most abundant one with percentages between 59.78 (ripe Karamani)-69.97% (green Halhalı), and it was determined that there was a decrease in oleic acid content with fruit ripening. In a study reported by Pardo *et al.*[35], in which the changes due to variety and ripening were examined, the oleic acid values were found in the range of 74.21%-78.46%, and were higher than the values found in the our study. The oleic acid values were determined by Demirağ and Konuşkan[36] ranging from 66.32 to 68.79%, and by Gao *et al.*[37] ranging from 66.33 to 79.61%, and these results are in agreement with the our results. Navajas-Porras *et al.*[38] reported that the oleic acid content of olives decreased with ripening, which was similar to our study. Palmitic acid, which was the second most abundant fatty acid, ranged from 13.92 (ripe Saurani) to 17.42% (green Saurani).

It was determined that the palmitic acid values of the cultivars decreased with the harvest time. In the study conducted by Emmanouilidou *et al.*[6], they found this value in the range of 12.58-17.68%, and it is in agreement with the study. In a similar study, Kafkaletou *et al.*[39] found it in the range of 9.85-11.16%, which is lower than the values in our results. Concerning the linoleic acid content of olive oils, it varied between 5.65 (green Halhalı) and 16.86% (ripe Karamani). Moreover, the linoleic acid composition of monovarietal olive oils was significantly influenced by olive ripening stage and variety. It was determined that there was an increase in linoleic acid values with the harvest time. In the study conducted by Emmanouilidou *et al.*[6], this value was found in the range of 7.71-16.97%, while Sicari *et al.*[40] determined it in the range of 8.12-10.43%, which is in agreement with the study. The percentages of other fatty acids varied as follows: C16:1, 0.87 (Halhalı) and 1.42% (Saurani); C18:0, 3.07 (Saurani) and 3.97% (Halhalı); C18:3, 0.90 (Karamani) and 1.10% (Saurani); C20:0, 0.57 (Saurani) and 0.78% (Karamani); C20:1, 0.12 (Saurani) and 0.19% (Karamani); C22:0, 0.71 (Saurani) and 1.70% (Karamani); C24:0, 0.08 (Saurani) and 0.12% (Karamani). Similar results of fatty acid compositions have been reported in different virgin olive oils[13, 14, 39]. The concentration of fatty acids may differ due to the effect of environmental factors in the variety year and stage of fruit growth or ripening[7].

3.4 Sterol and triterpene dialcohols

Sterol composition is an important nutritional and authenticity parameter[17]. In all the samples analysed, the levels of sterols and triterpenic dialcohols content were found to be within the intervals required by Commission Regulation (EEC) No. 1989/2003[41] for extra virgin olive oils (cholesterol 0.5%; campesterol 4.0%; stigmasterol < campesterol; apparent sitosterol 93.0%; D-7-stigmasterol 0.5%; total sterols 1000 mg/kg; erythrodiol+uvaol 4.5%) with the exception of total sterol content. The sterol and triterpene diols (erythrodiols + uvaol) compositions of olive oil are presented in Table 3. While β-sitosterol, Δ^5-avenasterol and campesterol were determined as the main sterols in the oil samples, cholesterol, stigmasterol, clerosterol, sitostanol, $\Delta^{5,24}$-stigmastadienol, Δ^7-stigmastenol, Δ^7-avenasterol and two triterpene dialcohols (erythrodiol and uvaol) were found as minor sterols. As shown in Table 3, campesterol, stigmasterol, Δ^5-avenasterol, β-sitosterol, $\Delta^{5,24}$-stigmastadienol, erythrodiol + uvaol and total sterols contents were significantly affected by the variety and ripening ($p < 0.05$) with minor exceptions. The total sterol compositions of olive oil samples were higher than the minimum limits (1000 mg/kg) established by EU regulation (except spotted Halhalı), ranging from 949 (spotted Halhalı) to 1782 mg/kg (ripe Halhalı). Changes in total sterol amounts were deter-

Table 3 Sterol compositions of Halhalı, Karamani and Saurani olive oils (mg/kg).

Sterols	Halhalı			Karamani			Saurani		
	Green	Spotted	Ripe	Green	Spotted	Ripe	Green	Spotted	Ripe
Cholesterol	$0.20^{f} \pm 0.01$	$0.07^{e} \pm 0.02$	$0.05^{f} \pm 0.00$	$0.28^{b} \pm 0.01$	$0.20^{d} \pm 0.00$	$0.05^{f} \pm 0.00$	$0.24^{c} \pm 0.01$	$0.31^{a} \pm 0.01$	$0.06^{f} \pm 0.00$
Campesterol	$3.11^{b} \pm 0.01$	$2.41^{d} \pm 0.02$	$2.08^{g} \pm 0.04$	$2.37^{f} \pm 0.03$	$4.3^{a} \pm 0.04$	$2.06^{h} \pm 0.01$	$2.47^{c} \pm 0.01$	$2.39^{e} \pm 0.03$	$1.94^{j} \pm 0.01$
Stigmasterol	$1.02^{b} \pm 0.01$	$0.76^{e} \pm 0.00$	$0.88^{d} \pm 0.02$	$0.94^{c} \pm 0.01$	$1.61^{a} \pm 0.03$	$0.88^{d} \pm 0.02$	$0.97^{b,c} \pm 0.01$	$0.97^{b,c} \pm 0.02$	$0.88^{d} \pm 0.00$
Clerosterol	$1.02^{c} \pm 0.01$	$0.99^{d} \pm 0.00$	$0.94^{e} \pm 0.01$	$1.08^{a} \pm 0.02$	$0.95^{e} \pm 0.02$	$0.93^{e} \pm 0.00$	$1.05^{b} \pm 0.01$	$1.05^{b} \pm 0.02$	$0.94^{e} \pm 0.01$
β-sitosterol	$89.94^{d} \pm 0.12$	$86.16^{g} \pm 0.08$	$87.67^{f} \pm 0.05$	$91.25^{c} \pm 0.07$	$88.95^{e} \pm 0.05$	$87.75^{f} \pm 0.08$	$91.66^{a} \pm 0.22$	$91.61^{b} \pm 0.14$	$87.63^{f} \pm 0.07$
Sitostanol	$0.79^{a} \pm 0.01$	$0.55^{c} \pm 0.03$	$0.20^{g} \pm 0.00$	$0.49^{d} \pm 0.00$	$0.57^{b} \pm 0.02$	$0.20^{g} \pm 0.00$	$0.50^{d} \pm 0.01$	$0.47^{e} \pm 0.00$	$0.23^{f} \pm 0.00$
Δ^5-avenasterol	$2.36^{h} \pm 0.03$	$4.31^{c} \pm 0.04$	$6.35^{b} \pm 0.03$	$2.82^{d} \pm 0.01$	$2.55^{e} \pm 0.03$	$6.34^{b} \pm 0.04$	$2.38^{g} \pm 0.03$	$2.41^{f} \pm 0.01$	$6.54^{a} \pm 0.04$
$\Delta^{5,24}$-stigmastadienol	$0.29^{d} \pm 0.00$	$0.26^{e} \pm 0.01$	$0.41^{a} \pm 0.02$	$0.31^{c} \pm 0.00$	$0.29^{d} \pm 0.01$	$0.37^{b} \pm 0.01$	$0.32^{c} \pm 0.03$	$0.32^{c} \pm 0.00$	$0.36^{b} \pm 0.002$
Δ^7-avenasterol	$0.69^{d} \pm 0.04$	$0.86^{c} \pm 0.02$	$1.00^{b} \pm 0.02$	$0.23^{f} \pm 0.00$	$0.37^{e} \pm 0.01$	$1.00^{b} \pm 0.03$	$0.22^{f} \pm 0.00$	$0.23^{f} \pm 0.00$	$1.02^{a} \pm 0.02$
Δ^7-stigmastenol	$0.58^{a} \pm 0.00$	$0.62^{a} \pm 0.01$	$0.42^{b} \pm 0.00$	$0.22^{c} \pm 0.01$	$0.22^{c} \pm 0.02$	$0.42^{b} \pm 0.01$	$0.19^{c} \pm 0.00$	$0.23^{c} \pm 0.00$	$0.42^{b} \pm 0.01$
Toplam sterol (ppm)	$949^{e} \pm 6.21$	$1782^{a} \pm 8.14$	$1315^{c} \pm 13.57$	$1236^{d} \pm 12.41$	$1334^{b} \pm 14.29$	$1310^{c} \pm 7.28$	$1223^{d} \pm 4.46$	$1223^{d} \pm 10.22$	$1317^{b,c} \pm 15.89$
Eritrodiol+uvaol	$1.15^{b} \pm 0.05$	$0.84^{e} \pm 0.01$	$0.47^{f} \pm 0.02$	$0.91^{d} \pm 0.03$	$1.53^{a} \pm 0.05$	$0.46^{f,g} \pm 0.00$	$0.91^{d} \pm 0.01$	$0.93^{c} \pm 0.04$	$0.45^{g} \pm 0.01$

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

mined with olive ripening. In the study conducted by Gao *et al.*[37], this value was determined as 985.14-1904.77 (mg/kg) and is in harmony with the our findings. β-sitosterol was found as major sterol in all oil samples, varying between 86.16 (ripe Halhalı) and 91.66% (spotted Saurani). As ripening progressed from green to ripe stage, the total sterol content of all olive oils tended to increase significantly ($p<0.05$). It was determined that the amount of β-sitosterol of Saurani and Karamani olive oil varieties decreased with ripening, and changes in the amount of β-sitosterol of Halhalı olive oil variety.

In the study performed by Boulfane *et al.*[21], this value was found to be between 79.36 and 92.48%, and these results show similarities with our results. The second most abundant sterol was Δ^5-avenasterol, changes between 2.36 (spotted Halhalı) and 6.54% (green Saurani) in oils. There was a general increase in the amount of Δ^5-avenasterol of Saurani and Halhalı olive oil varieties with ripening, and changes in the amount of Δ^5-avenasterol of Karamani olive oil varieties. β-Sitosterol and Δ^5-avenasterol were found to strongly positively correlate with the oxidative stability of olive oil[22]. 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[12]. The mean content in the oil samples ranged from 0.76 (ripe Halhalı) to 1.61% (ripe Karamani). Stigmasterol percentages were lower than those of campesterol in all samples. It was determined that the stigmasterol amounts of olive oil varieties fluctuated with ripening. In a study carried out by Sicari *et al.*[40], this value was found to be between 0.97 and 1.50%, which is accordance with the study. The triterpene dialcohols (erythrodiol and uvaol) were analyzed 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 0.45 (green Saurani) to 1.53% (ripe Karamani). With ripening, erythrodiol + uvaol amounts of Saurani and Karamani olive oil varieties fluctuated, while a decrease was determined in Halhalı variety. In the study conducted by Konuşkan[42], this value was determined in the range of 0.00-4.27%, and it is similar to the study conducted. This results indicated that the erythrodiol + uvaol content of the olive oils was significantly affected by variety and ripening, and this compounds fluctuated during olive ripening as similar to work by De Mendoza *et al.*[43].

4 Conclusions

This study showed the variations in oil content, ripening index, fatty acid and sterol compositions of monovarietal olive oils depending on three different varieties and ripening stages. As ripening progressed, a series of changes occurred in olive oil samples and with a strong influence especially on authenticity parameters such as fatty acid composition and sterol composition. Karamani variety was distinguished from other varieties with its higher oil content. Halhalı variety had higher oleic acid content and total sterol. The results of this study indicated that the ripening degree and variety significantly affect the quality of olive oils. The results of this study provided useful information to establishing criterion of variety. Finally, further study is also suggested to comprehensively investigate other objective factors and their behaviors in affecting Turkish monovarietal olive oils.

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Conflict of Interest

Authors declare no conflict of interest.

Authorship

Gulcin Gunduz: Investigation, original draft, formal analysis. Dilsat Bozdogan Konuskan: Conceptualization, supervision, investigation, writing, review and editing.

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