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ORIGINAL ARTICLE

Antioxidant activity, sterol and fatty acid compositions of Turkish olive oils as an indicator of variety and ripening degree

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Abstract This study was carried out to determine the effect of three ripening stages (green, spotted and ripe) on fatty acid, sterol composition and antioxidant activity of olive oils from three olive varieties (Sarı Hasebi, Gemlik and Halhalı) widely grown in the eastern Mediterranean region of Turkey. The variety had a significant effect on the fatty acids, sterols and total phenolic content. Halhalı oil had the lowest oleic acid content (67.28%), while Sarı Hasebi oil had the highest (75.61%). Total phenolic content varied between 163.02 mg GAE/kg oil and 749.28 mg GAE/kg oil. Halhalı oil showed the highest antioxidant activity (IC_{50} = 66 µg/ml) whereas Sarı Hasebi oil showed the lowest one (IC_{50} = 2617 µg/ml). The total content of sterols in olive oils ranged from 358 mg/kg in Sarı Hasebi to 1092.33 mg/kg in Halhalı. The β-sitosterol content of olive oils varied between 80.72 (Sarı Hasebi) and 87.81% (Halhalı). Δ-5-avenasterol content ranged between 3.34 (Halhalı) and 7.30% (Gemlik). Variety and ripening degree significantly affected the β-sitosterol, Δ-5-avenasterol and erythrodiol + uvaol contents of oils. Finally, these results showed that sterol and fatty acid compositions can be used as indicators of variety and ripening degree among virgin olive oils.

Keywords Olive oil · Fatty acids · Antioxidant activity · Sterols · Ripening · Variety

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Introduction

The virgin olive oil (VOO) extracted from the healthy and intact fruits of olive trees (*Olea europea* L.) only by mechanical procedure is regarded as one of the basic ingredients of Mediterranean diet (Gouvinhas et al. 2015). More than 95% of the world olive oil production occurs in these countries such as Spain, Italy, Greece and Turkey (Arslan and Schreiner 2012; Bengana et al. 2013). Virgin olive oil is mainly composed of two different fractions: the saponifiable fraction and the unsaponifiable fraction. The saponifiable fraction represents nearly 98% of the total composition, including the fatty acids and triacylglycerols. The main components in the unsaponifiable fraction are sterols, alcohols, vitamin E, hydrocarbons, carotenoids, volatile compounds and phenolic compounds, representing only 2% of the total (Antonini et al. 2015). Olive oil has a source of monounsaturated fatty acids, especially oleic acid (60–80%), which is less susceptible to oxidation, having an important role in terms of contributing to the high stability and long shelf life of olive oil (Anastasopoulos et al. 2012). The content of phenolic compounds is an important parameter considering the evaluation of the quality of virgin olive oil since phenols are mainly responsible for oil flavour and aroma (Cioffi et al. 2010; Franco et al. 2014). The phenolic compounds in olive oil have raised attention due to their antioxidant properties and positive health effects along the last years (Condelli et al. 2015). Sterols are bioactive compounds present in all vegetable oils and represent the major constituent of the olive oil unsaponifiable fraction (Lukic et al. 2013). β-sitosterol is in the range of 75-90% of the total sterol composition in olive oils and Δ-5-avenasterol and campesterol include 5-20% and 2-4% of the total, respectively. Moreover, the triterpene dialcohols erythrodiol and uvaol

are found in VOOs (Guillaume et al. 2012). The sterol composition is considered as an important parameter for determining the adulteration or authenticity since each variety has a specific sterol profile known as "fingerprint" (Piravi-Vanak et al. 2012). Nevertheless, several factors affect the sterol content and profile of VOOs such as olive cultivar, stage of maturity of olives, extraction method, storage conditions and olive oil category (Fernandez-Cuesta et al. 2013). In fact, previous studies have focused on the quality criteria such as free fatty acids and peroxide value. However, few studies has been focused on the examination of the influence of ripening on antioxidant activity, fatty acid and sterol compositions of VOOs from the traditional Turkish varieties especially grown in Hatay province. Hatay, which is located in the southern part of Turkey and borders of the Mediterranean Sea, has adequate climate and soil conditions for olive production. The most important olive varieties cultivated in this province are Halhalı, Sarı Hasebi and Gemlik. According to our knowledge, there is no comprehensive study on fatty acid composition, antioxidant activity and sterol composition depending on ripening in olive oils from indigenous varieties cultivated in Hatay province, which is accepted as "the motherland of olives".

Materials and methods

Olive sampling

This study was conducted during the crop season of 2013–2014. Olive fruits from three Turkish varieties: "Sari Hasebi", "Gemlik" and "Halhalı" under the same pedo-climatic 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 areas in Hatay. Only undamaged fruits which were considered healthy were hand-picked from each younger tree for different cultivars. For each olive variety, three harvesting dates (from September to October with 20 days intervals) corresponding to three different ripening stages (Green–spotted–ripe) was selected. 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.

Chemicals

All reagents used in the experiments were of analytical grade. Gallic acid, 2,2′-diphenyl-1picryhydrazyl (DPPH), Folin–Ciocalteu's reagent, tri-methyl chlorosilane, hexamethylchlorosilane, pyridine, 2,7 dichlorofluorescein, sodium carbonate, 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, sodium sulfate and potassium iodine were obtained from Merck (Germany) and Sigma-Aldrich (Germany). The fatty acid methyl ester (FAME) mix were obtained from Supelco (Bellefonte, USA).

Experimental equipments

Olive extractor was purchased from Hakkı Ustaogulları, Turkey. The analysis of fatty acid methyl esters (FAME) was performed by Agilent gas chromatography system (Agilent 6850, USA) using a hydrogen flame ionization detector (FID) and a capillary column DB-23 (60 m length × 0.25 mm i.d. and 0.25 μm film thickness). Colorimetric analyses were carried out by using UV–Vis spectrophotometer (Hitachi U-1900, Japan). Separation and quantification of the silylated sterol fraction were performed by capillary gas chromatography (GC-2010, Shimadzu, Japan).

Virgin olive oil processing

Two kg of olive samples selected from each variety were eliminated from unhealthy and decay fruits. Olive oil extraction was performed using a laboratory scale mechanical extractor. It is equipped with a crusher, a malaxer and a decanter. In fact, olives were crushed and then slowly kneaded for 40 min at 28 °C. Next, the obtained paste was centrifuged at 5000 rpm for 1 min with the decanter. The oil was put into dark glass bottles under a nitrogen atmosphere of 150–200 ml without headspace. The oil obtained was kept at 4 °C in the dark area until analyses which were duplicated.

Ripening index

The ripening index (RI) was determined from one hundred olive fruits randomly drawn from each olive variety. This parameter, which is based on evaluating the color of both skin and pulp of olives, was determined according to International Olive Council (IOOC 2001a, b). Ripening degrees, harvest dates and ripening index of olives for each cultivar are shown in Table 1.

Water and oil contents

To determine water content, about 10 g of olive samples were weighed and then dried in an oven at 105 °C for 24 h. Being cooled in a desiccator, the samples were reweighed (Yorulmaz et al. 2013). Oil content was determined

Table 1 Ripening indices and ripening degrees of Sarı Hasebi, Gemlik and Halhalı olive varieties

Variety	Ripening degree	Harvest date	Ripening index
Sarı Hasebi	Green	15/09/2013	2.0
	Spotted	06/10/2013	3.0
	Ripe	26/10/2013	4.4
Gemlik	Green	15/09/2013	2.1
	Spotted	06/10/2013	3.3
	Ripe	26/10/2013	4.8
Halhalı	Green	15/09/2013	1.9
	Spotted	05/10/2013	3.1
	Ripe	26/10/2013	4.5

according to the method described in American Oil Chemists' Society (AOCS) Official Methods Am 2–93 (AOCS 2003) by Soxhlet extraction method using n-hexane at 80 °C for 6 h.

Free fatty acid and peroxide value

Free fatty acid (given as % oleic acid) and peroxide value (meq O_2/kg of oil) analysis of the samples was carried out following the AOCS Official Method Ca 5a-40 and Cd 8-53, respectively (AOCS 2003).

Total carotenoid and chlorophyll contents

Carotenoid and chlorophyll contents of the samples (mg/kg of oil) were found out following the method previously described by Ceballos et al. (2003). The absorbances of the samples were read at 470 and 670 nm, respectively using UV spectrophotometer.

Total phenolic content

The content of total phenolic compounds was determined according to the Folin–Ciocalteu's method as proposed by Montedoro et al. (1992) with some modifications. After the extraction process, 0.2 ml of phenolic extract was mixed with 0.5 ml of Folin–Ciocalteu reagent. One ml of saturated sodium carbonate solution was added to this mixture. After vigorous shaking, the volume of the solution was increased to 10 ml with distilled water. The mixture was left to stand for 45 min in the dark at room temperature. The absorbance was recorded at 765 nm in a UV–Vis spectrophotometer (Hitachi U-1900, Japan). Quantification was carried out using a standard curve built with 50–100–200–400–600–800–1000 mg/l prepared in an aqueous solution of methanol (70%) The results were expressed as milligrams of gallic acid equivalents per kilogram of oil (mg GAE/kg).

Antioxidant activity

The antioxidant activity (free radical scavenging capacity) of the olive oil phenolic extracts was determined by using the method of 2,2-diphenyl-1-picrylhydrazil (DPPH) radical according to the procedure of Brand Williams et al. (1995) with some modifications. Briefly, 1 ml of extracts was diluted with 1.9 ml DPPH methanolic solution. After 60 min of incubation in the dark at room temperature, the absorbance was measured at 515 nm against a blank (MeOH). The percentage of inhibition was calculated from the following equation:

$$\%\text{Inhibition (DPPH)} = \left[\left(\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}}\right)/\text{Abs}_{\text{Control}}\right] \times 100$$

Fatty acid composition

The fatty acid composition of the oils was determined according to the method proposed by the International Olive Oil Council, COI/T.20/Doc.No.24 (2001). Fatty acid methyl esters (FAME) were prepared by shaking a solution of oil samples in n-heptane (0.1 g in 2 ml) potassium hydroxide. The analysis of FAME was performed by Agilent gas chromatography system (Agilent 6850, USA) using a hydrogen flame ionization detector (FID) and a capillary column DB-23 (60 m length × 0.25 mm i.d. and 0.25 µm film thickness). The temperatures of detector and injector were set at 230 and 280 °C, respectively. Helium was employed as carrier gas with a flow rate of 1 ml/min and the split ratio was 1:50. The injection volume was 1 µl. The results were expressed as a relative area percentage of total fatty acid methyl esters. Fatty acids were determined by comparing their retention times with those of reference compounds.

Sterol composition

The content and composition of the sterols were determined according to the official method IOC/T.20/No10/Rev. 1 (IOOC 2006). Two ml of internal standard (0.1% cholesterol in chloroform) was added to the samples which were then saponified using 2 N ethanolic potassium hydroxide solution. The unsaponifiable fraction was removed with diethyl ether. The unsaponifiable sterol fraction was separated using thin layer chromatography. Separation and quantification of the silylated sterol fraction were performed by capillary gas chromatography (GC-2010, Shimadzu, Japan) using a Supelco (SPBTM-5 24034, Bellefonte, USA) capillary column (30 m, 0.25 mm i.d. and 0.25 mm film thickness) and a flame ionization detector (FID). The column temperature was 260 °C. Detector and injector temperatures were 290 and 280 °C,

respectively. Helium was used as a carrier gas with 1 ml/min flow rate and the split ratio was 50:1. Individual sterols and two triterpene diols (erythrodiol and uvaol) in oils were determined depending on their relative retention times according to the internal standard cholestanol.

Statistical analysis

Statistical analysis was carried out using the SPSS 10 statistical software (SPSS Inc., Chicago, USA). Data were analyzed by a one-way analysis of variance (ANOVA). Duncan's multiple range tests was used to determine if there were any statistical differences between the samples ($P < 0.05$). Student t test was used for correlation analysis (Ozdamar 1999).

Results and discussion

Olive properties

The moisture and oil contents of Sarı Hasebi, Gemlik, and Halhalı olives according to different maturity stages are shown in Table 2. According to the results, the moisture content of olives varied between 31.40 (spotted Gemlik) and 55.51% (ripe Halhalı). The moisture content of Sarı Hasebi and Gemlik olives decreased from green to spotted maturation stage and then increased remarkably at ripe maturation stage. However, there were no fluctuations in the moisture content of Halhalı olives between different ripening stages. The results indicated that the moisture content of all olive samples showed significant differences depending on the olive ripening and variety ($P < 0.05$). As similar to our results, a fluctuation in the moisture content of olives was reported during the ripening process. In fact, the water content of Spanish olives (Picudo) obtained throughout nine different maturation stages ranged from 48.87 to 56.42% (Jimenez et al. 2013). Concerning the oil content of olives, the results showed that it varied between 16.19 (green Gemlik) and 33.93% (ripe Sarı Hasebi). Moreover, the values of oil content increased during olive ripening for each olive cultivar. There were significant differences among the varieties and stages of maturity as regards the oil content of olives ($P < 0.05$). Olive biosynthesis occurs rapidly when the olives are at the green stage until they turn fully black, during which oil content levels are high (Essiari et al. 2014).

Chemical properties

The chemical properties (free fatty acids and peroxide value) of olive oils are shown in Table 3. Statistical differences in the free fatty acids of olive oils were observed depending on the maturation and olive variety ($P < 0.05$). In fact, the free fatty percentages of oil samples were between 0.28 (green Gemlik) and 1.13 (ripe Sarı Hasebi) % of oleic acid. Besides, the free fatty acid content of oils was below the limit of 0.8% established by Turkish Food Codex and Regulation EC/1989/2003 (European Union Comission 2003; Turkish Food Codex 2014) for extra virgin olive oils with the exception of ripe Sarı Hasebi and Gemlik oils (1.13 and 0.95%, respectively). Moreover, the free fatty acid percentages of our olive oil samples were higher than those of the values reported by Antonini et al. (2015). Furthermore, there was a remarkable increase in the free fatty acids of all olive oil samples during olive ripening. Similar results were observed by De Mendoza et al. (2013) who had related the highest acidity value of olive oils during olive ripening to the progressive action of the lipolytic activity.

Concerning peroxide values, there were statistically significant differences in peroxide values depending on the

Table 2 Water and oil content of three olive varieties

Variety	Ripening stage	Moisture content (%)	Oil content (%)
Sarı Hasebi	Green	$46.10^{A,b} \pm 0.52$	$19.15^{B,b} \pm 1.92$
	Spotted	$41.84^{B,c} \pm 0.07$	$20.05^{B,b} \pm 1.41$
	Ripe	$48.17^{B,a} \pm 0.86$	$33.93^{A,a} \pm 0.09$
Gemlik	Green	$33.18^{B,b} \pm 1.27$	$16.19^{C,c} \pm 1.25$
	Spotted	$31.40^{C,b} \pm 0.43$	$18.27^{C,b} \pm 0.33$
	Ripe	$42.25^{C,a} \pm 2.33$	$30.03^{C,a} \pm 0.64$
Halhalı	Green	$47.75^{A,c} \pm 0.43$	$24.49^{A,c} \pm 0.83$
	Spotted	$50.36^{A,b} \pm 0.33$	$29.12^{A,b} \pm 0.14$
	Ripe	$55.51^{A,a} \pm 0.36$	$32.67^{B,a} \pm 0.21$
Interactions maturity x variety		*	*

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. Different capital letters express significant statistical differences (Duncan's test $P < 0.05$) among varieties

* Significant interaction

Table 3 Chemical properties and total phenolic content of olive oils

Variety	Ripening stage	Free fatty acids (% oleic)	Peroxide value (meq O_2/kg)	Total chloroyphyll content (mg pheophytin/kg)	Total carotenoid content (mg lutein/kg)	Total phenolic content (mg/kg)
Sarı Hasebi	Green	$0.50^{A,b} \pm 0.01$	$7.91^{B,b} \pm 0.50$	$17.30^{B,a} \pm 0.18$	$8.77^{B,a} \pm 0.06$	$566.08^{B,a} \pm 10.46$
	Spotted	$0.54^{A,b} \pm 0.03$	$8.23^{B,b} \pm 0.74$	$12.87^{A,b} \pm 0.32$	$6.25^{B,c} \pm 0.07$	$304.35^{B,b} \pm 31.41$
	Ripe	$1.13^{A,a} \pm 0.05$	$14.22^{A,a} \pm 1.59$	$10.17^{B,c} \pm 0.56$	$7.26^{B,b} \pm 0.17$	$163.02^{B,c} \pm 15.70$
Gemlik	Green	$0.28^{B,c} \pm 0.01$	$9.85^{A,a} \pm 0.07$	$15.22^{C,a} \pm 0.38$	$8.44^{C,a} \pm 0.04$	$163.03^{C,c} \pm 36.64$
	Spotted	$0.37^{B,b} \pm 0.02$	$10.09^{A,a} \pm 0.23$	$8.46^{B,b} \pm 0.40$	$5.32^{C,c} \pm 0.08$	$272.94^{B,b} \pm 10.46$
	Ripe	$0.95^{B,a} \pm 0.05$	$10.73^{A,a} \pm 0.76$	$8.04^{C,b} \pm 0.41$	$5.54^{C,b} \pm 0.11$	$477.09^{A,a} \pm 68.05$
Halhalı	Green	$0.52^{A,b} \pm 0.03$	$5.62^{C,b} \pm 0.58$	$23.70^{A,a} \pm 0.16$	$12.09^{A,a} \pm 0.14$	$749.28^{A,a} \pm 15.70$
	Spotted	$0.53^{A,b} \pm 0.02$	$6.90^{C,b} \pm 0.02$	$12.28^{A,c} \pm 0.38$	$7.71^{A,c} \pm 0.14$	$592.25^{A,b} \pm 36.64$
	Ripe	$0.79^{C,a} \pm 0.02$	$14.72^{A,a} \pm 2.92$	$14.53^{A,b} \pm 0.05$	$8.02^{A,b} \pm 0.17$	$466.62^{A,c} \pm 26.17$
Interactions maturity x variety		*	*	*	*	*

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. Different capital letters express significant statistical differences (Duncan's test $P < 0.05$) among varieties

* Significant interaction

variety and maturation. In fact, the peroxide values of oils ranged from 5.62 (green Halhalı) up to 14.72 (ripe Halhalı) meq O_2/kg oil. In the results obtained, the peroxide values of all olive oil samples were below 20 meq/O_2 kg which is accepted as the legal limit for extra virgin olive oils by Turkish Food Codex and Regulation EC/1989/2003 (European Union Commission 2003; Turkish Food Codex 2014). Moreover, an increase in the peroxide values of all olive oil samples was found as ripening stage advanced. The fluctuation in peroxide values throughout ripening is the result of enzymatic activity of lipoxygenase (Bengana et al. 2013).

Chlorophyll and carotenoid contents

As can be seen in Table 3, stage of ripening and variety had a significant effect on the content of chlorophylls. In fact, the total chlorophyll content of olive oil samples varied among 8.04 (ripe Gemlik)–23.70 (green Halhalı) mg pheophytin/kg oil. Similar results were obtained by Yorulmaz et al. (2013) and Condelli et al. (2015) who reported that the total chlorophyll content of oils varied between 0.70 and 25.90 and between 8.68 and 20 mg pheophytin/kg oil, respectively. Moreover, a general decline was observed in the chlorophyll content of olive oil samples during olive ripening. The total carotenoid contents varied significantly according to ripening stage and variety. In fact, the total carotenoid content of oil samples was found to be at concentration between 5.32 (spotted Gemlik) and 12.09 (green Halhalı) mg lutein/kg oil. Our results were higher than those obtained by Zegane et al. (2015). Generally, a decrease in the carotenoid content of olive oil samples was determined when ripening progressed. Similar to our work, a decline in the carotenoid content of olive oils during olive ripening was reported by Arslan and Schreiner (2012) who stated that the total carotenoid content of oils was between 5.30 and 12.60 mg lutein/kg oil.

Total phenolic content

The evolution of total phenolic contents in Turkish olive oils during olive ripening is shown in Table 3. Significant differences were observed in the total phenolic content of olive oils according to olive maturation and variety. In fact, the total phenolic content of olive oils was between 163.02 (ripe Sarı Hasebi) and 749.28 (green Halhalı) mg/kg oil. Previous studies demonstrated that the total phenolic content of oils varied between 348 and 960 mg GA/kg oil (Gouvinhas et al. 2015) and between 343 and 353 mg/kg (Cioffi et al. 2010). These differences between values could be related to the olive variety, the geographical area, the climate, the degree of olive ripening and the crop season (Manai-Djebali et al. 2012; Condelli et al. 2015). Moreover, the results showed that the total phenolic content of Sarı Hasebi and Halhalı oils decreased during olive ripening as reported by Bengana et al. (2013) who stated that the loss in phenolic content during olive ripening could effect extra virgin olive oil quality. On the other hand, an increase in the total phenolic content of Gemlik oils was determined throughout ripening. Significant variations in the total phenolic contents were observed pertaining to variety and stage of ripening.

Antioxidant activity

Determining the amount and effect of antioxidant compounds is quite important when considering their beneficial effect on health (Vasilescu et al. 2015). It is accepted that the higher the value of percent inhibition is, the higher the antioxidant activity is. IC_{50} value is another sign of the scavenging effect of DPPH. This value is defined as the amount of antioxidant activity needed to scavenge half of DPPH. A lower IC_{50} value indicates a higher antioxidant activity (Bucak 2011). IC_{50} values of olive oils and the percentage inhibitions of DPPH are shown in Table 4. According to the results, Halhalı oil was the most effective as DPPH radical scavenging agent among varieties with IC_{50} value of 66 μg/ml. Moreover, the antioxidant activity of Halhalı and Sarı Hasebi oils decreased with the maturation of olives. Nevertheless, the antioxidant activity of Gemlik oils increased as ripening advanced. When the correlation between the antioxidant capacity and total phenolic content was evaluated, the higher antioxidant activity Halhalı oil could be explained by the higher content of total phenols according to our results. These results were in accordance to previous studies reporting that the antioxidant activity of plant materials was well correlated with their content of phenolic compounds (Gouvinhas et al. 2014). Concerning IC_{50} values of olive oil samples, they ranged from 66 (green Halhalı) to 2617 (ripe Sarı Hasebi) μg/ml. Franco et al. (2014) reported that Carrasquena, Arbequina, and Corniche were the varieties possessing the most powerful antioxidant activity (IC_{50} = 14.8, 16.9, and 17.0 μg/ml, respectively) while Morisca and verdial de Badajoz possessed the poorest one (IC_{50} = 25.7 and 26.6 μg/ml, respectively). They also determined significant differences in the antioxidant activity of olive oils according to olive variety ($P < 0.05$). Furthermore, according to Condelli et al. (2015), IC_{50} values of oils from five different Italian varieties varied between 31.9 (Coratina variety) and 53.4 μl (Maiatica variety). They also pointed out that this variation in the values of antioxidant activities may arise from the profile of phenolic compounds rather than the total phenolic content.

Fatty acid composition

Fatty acid composition is an important parameter determining the quality and the authenticity of olive oil (Essiari et al. 2014). The evolution of fatty acid content in olive oils during olive ripening is presented in Table 5. 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 (C20:0), eicosatrienoic (C20:3) acid.

Among fatty acids, oleic acid was the most abundant one with percentages between 67.28 (ripe Halhalı) and 75.61% (ripe Sarı Hasebi). No significant differences were found between green and spotted olive ripening stage in terms of oleic acid content of olive oils although oleic acid content changed significantly according to olive ripening stages for each olive variety ($P < 0.05$). Oleic acid contents followed different patterns throughout olive maturation. Contrary to our findings, De Mendoza et al. (2013) observed an increase in the oleic acid content of Spanish oils during three different ripening stages with percentages between 69.4 and 71.6%. Our results showed similarity with those of the results obtained by Manai-Djebali et al. (2012).

Palmitic acid, which was the second most abundant fatty acid, ranged from 15.23 (ripe Gemlik) to 19.30% (green Sarı Hasebi). Moreover, it varied significantly with olive maturation and variety. Generally, a decline in the palmitic acid content of olive oils was observed during olive ripening. Lopez-Cortes et al. (2013) reported that palmitic acid content from eight Spanish varieties varied between 9.84 and 18.44% as in accordance with our values. However, Chemlal cultivar had lower palmitic acid content than our varieties (Bengana et al. 2013).

Concerning the linoleic acid content of olive oils, it varied between 3.19 (green Sarı Hasebi) and 8.49% (ripe

Table 4 IC_{50} values of olive oils and % inhibitions of DPPH

Varieties	Ripening stage	% inhibition of DPPH	IC_{50} values (μg/ml)
Sarı Hasebi	Green	23.6	915
	Spotted	26.1	880
	Ripe	21.8	2617
Gemlik	Green	22.4	1248
	Spotted	25.33	906
	Ripe	25.8	837
Halhalı	Green	46.22	66
	Spotted	37.19	122
	Ripe	32.89	194
BHT		33.63	365

Table 5 Fatty acid composition of olive oils (%)

Fatty acids	Sarı Hasebi			Gemlik			Halhalı			Interactions maturity × variety
	Green	Spotted	Ripe	Green	Spotted	Ripe	Green	Spotted	Ripe	
16:0	$19.30^{A,a} \pm 0.27$	$19.27^{A,a} \pm 0.12$	$15.36^{B,b} \pm 0.13$	$17.18^{B,a} \pm 0.11$	$15.97^{C,b} \pm 0.19$	$15.23^{B,b} \pm 1.00$	$16.09^{C,c} \pm 0.30$	$17.04^{B,b} \pm 0.31$	$18.37^{A,a} \pm 0.34$	*
16:1	$0.31^{C,a} \pm 0.05$	$0.31^{C,a} \pm 0.09$	$0.22^{B,b} \pm 0.10$	$1.79^{A,a} \pm 0.04$	$1.50^{A,a} \pm 0.01$	$0.86^{A,b} \pm 0.40$	$1.05^{B,c} \pm 0.01$	$1.37^{B,a} \pm 0.03$	$1.23^{A,b} \pm 0.04$	*
18:0	$3.62^{A,b} \pm 0.05$	$3.83^{B,b} \pm 0.18$	$4.20^{A,a} \pm 0.01$	$2.94^{B,b} \pm 0.04$	$3.28^{C,b} \pm 0.01$	$3.82^{B,a} \pm 0.43$	$3.62^{A,b} \pm 0.01$	$4.22^{A,a} \pm 0.15$	$3.65^{B,b} \pm 0.06$	*
18:1	$73.11^{A,b} \pm 0.40$	$72.66^{A,b} \pm 0.02$	$75.61^{A,a} \pm 0.48$	$71.84^{A,b} \pm 1.31$	$72.41^{A,b} \pm 1.95$	$74.57^{B,a} \pm 1.85$	$73.20^{A,b} \pm 0.92$	$70.28^{A,b} \pm 0.65$	$67.28^{C,c} \pm 0.11$	*
18:2	$3.19^{C,c} \pm 0.06$	$3.37^{C,b} \pm 0.05$	$4.04^{C,a} \pm 0.62$	$4.97^{B,b} \pm 0.07$	$4.83^{B,b} \pm 0.21$	$5.72^{B,a} \pm 0.08$	$6.04^{A,c} \pm 0.08$	$7.10^{A,b} \pm 0.14$	$8.49^{A,a} \pm 0.36$	*
18:3	$0.16^{B,a} \pm 0.05$	$0.23^{B,a} \pm 0.05$	$0.17^{C,a} \pm 0.05$	$0.23^{B,b} \pm 0.05$	$0.63^{A,a} \pm 0.06$	$0.65^{A,a} \pm 0.01$	$0.43^{A,a} \pm 0.01$	$0.16^{B,c} \pm 0.05$	$0.26^{B,b} \pm 0.05$	*
20:0	$0.21^{C,b} \pm 0.01$	$0.24^{A,a} \pm 0.06$	$0.23^{B,a} \pm 0.05$	$0.36^{B,a} \pm 0.05$	$0.26^{A,b} \pm 0.05$	$0.32^{A,a} \pm 0.04$	$0.48^{A,a} \pm 0.01$	$0.28^{A,b} \pm 0.06$	$0.23^{B,b} \pm 0.05$	*
20:3	$0.22^{B,b} \pm 0.01$	$0.16^{B,b} \pm 0.01$	$0.23^{A,a} \pm 0.05$	$0.60^{A,a} \pm 0.01$	$0.53^{A,b} \pm 0.05$	$0.23^{A,c} \pm 0.05$	$0.14^{C,b} \pm 0.05$	$0.23^{B,a} \pm 0.05$	$0.26^{A,a} \pm 0.06$	*
∑SFA	23.13	23.34	19.79	20.48	19.51	19.37	20.19	21.54	22.25	
∑MUFA	73.32	72.97	75.83	73.63	73.91	75.43	74.25	71.65	68.51	
∑PUFA	3.35	3.60	4.21	5.8	5.46	6.37	6.61	7.49	9.01	
MUFA/PUFA	21.88	20.26	18.01	12.69	13.53	11.84	11.23	9.57	7.60	

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. Different capital letters express significant statistical differences (Duncan's test $P < 0.05$) among varieties

SFA saturated fatty acids, *MUFA* monounsaturated fatty acids *PUFA* polyunsaturated fatty acids

* Significant interaction

Halhalı). Moreover, the linoleic acid content of all olive oils was significantly influenced by olive ripening stage and variety. Generally, an increase in the linoleic acid content of olive oils was observed during olive ripening as previously reported by Yorulmaz et al. (2013) and Dag et al. (2015). Bengana et al. (2013) pointed out that the increase in linoleic acid content occurs due to the fact that additionally to the triglycerides biosynthesis, the enzyme oleate desaturase was active, turning oleic acid into linoleic acid.

The fatty acid composition of the Sarı Hasebi, Gemlik, and Halhalı oils met the standards for EVOO committed by Turkish Food Codex which is in consonant with EU regulations with the exception of the palmitoleic acid content of ripe Sarı Hasebi (0.22%) and linoleic acid content of green Sarı Hasebi (3.19%). The percentages of other fatty acids varied as follows: C16:1, 0.22 (Sarı Hasebi) and 1.79% (Gemlik); C18:0, 2.94 (Gemlik) and 4.22% (Halhalı); C18:3, 0.16 (Sarı Hasebi) and 0.65% (Gemlik); C20:0, 0.21 (Sarı Hasebi) and 0.48% (Halhalı); C20:3 0.14 (Halhalı) and 0.60% (Gemlik).

The percentages of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) and the ratio of MUFA/PUFA were also calculated. Total SFA was in the range of 19.37 (ripe Gemlik)–23.61% (spotted Sarı Hasebi). These results were higher than those pointed out by Anastasopoulos et al. (2012) for Mavrolia and Koroneiki cultivar. The total SFA showed fluctuation with ripening in oil samples. Ripe Sarı Hasebi was rich in terms of MUFA with 75.83% due to the high oleic acid content. Considering PUFA, ripe Halhalı had the highest PUFA (9.01%) while green Sarı Hasebi had the lowest one (3.35%). A general increase in PUFA was observed during ripening. Green Sarı Hasebi had the highest MUFA/PUFA ratio (21.88%), whereas ripe Halhalı had the lowest one (7.60%). Moreover, the results demonstrated that MUFA/PUFA ratio decreased throughout olive ripening.

Sterol composition

The sterol content of olive oil samples during olive ripening is listed in Table 6. The sterols determined with the highest amounts were β-sitosterol, Δ-5-avenasterol and campesterol which made up over 90% of total sterol content, whereas cholesterol, brassicasterol, 24-methylene-cholesterol, 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 within small amounts. The total sterol content of the studied olive oils was above the legal threshold (1000 mg/kg) for extra virgin olive oils required by EU regulations, attaining 1061.33, 1000.66, 1034.66 and 1092.33 in ripe Sarı Hasebi, ripe Gemlik, spotted Halhalı and ripe Halhalı, respectively. However, the total sterol contents of green and spotted Sarı Hasebi (358 and 521.66 mg/kg, respectively), green and spotted Gemlik (728 and 828.66 mg/kg, respectively) and green Halhalı (897.66 mg/kg) were determined to be below this limit. As ripening progressed from green to ripe stage, the total sterol content of all olive oils tended to increase significantly ($P < 0.05$). This result was in agreement with Lukic et al. (2013) who stated that the levels of sterol in oils show at a certain peak point during ripening after which they decrease due to the enzymatic activity of sterol biosynthesis. The total sterol contents of Arbequina and Picual varieties were 989 and 1123 mg/kg, respectively as proposed by Fernandez-Cuesta et al. (2013). Apparent β-sitosterol content of the samples which signified the sum of the contents of β-sitosterol and other sterols (sitostanol, Δ-5,24-stigmastadienol, clerosterol, and Δ-5-avenasterol) was higher than 93% determined by EU regulations as minimum level except for all ripening stages of Sarı Hasebi oils (90.73, 91.79 and 92.55%, respectively), fluctuating between 90.73 (green Sarı Hasebi) and 94.96% (spotted Gemlik). It was determined that the variety and ripening stage influenced significantly the apparent β-sitosterol content of oils. Ayvalık cultivar was characterized by higher apparent β-sitosterol content, varying between 96.18 and 96.59% followed by Memecik variety with the range of 94.01 and 95.08% during different harvest time (Ilyasoglu et al. 2010).

Our results were in good agreement with those of Manai-Djebali et al. (2012) and Noorali et al. (2014). The mean content of β-sitosterol was the most predominant one among all sterols, varying between 80.72 (green Sarı Hasebi) and 87.81% (green Halhalı). The β-sitosterol percentages of Gemlik and Halhalı oils decreased during maturation while the β-sitosterol percentages of Sarı Hasebi oils increased. The second most plentiful sterol was Δ-5-avenasterol, ranging from 3.34 (green Gemlik) to 7.30% (ripe Gemlik). An increase in the Δ-5-avenasterol content of Gemlik and Halhalı oils was observed during ripening. Both the β-sitosterol and Δ-5-avenasterol contents of olive oils showed statistical differences depending on the maturity and variety. A negative correlation was found between β-sitosterol content and Δ-5-avenasterol contents of Gemlik and Halhalı oils during olive ripening as reported by Yorulmaz et al. (2013), Fernandez-Cuesta et al. (2013) and Lukic et al. (2013). Many researchers stated that β-sitosterol is minimum and Δ-5-avenasterol is maximum when olives are obtained at their optimum stage of maturation (Manai-Djebali et al. 2012; De Mendoza et al. 2013; Noorali et al. 2014). Campesterol percentages of the samples were below the maximum limit of 4% required by EU regulations, fluctuating between 1.78 (ripe

Table 6 Sterol composition of olive oils (%)

Sterols	Sarı Hasebi			Gemlik			Halhalı		
	Green	Spotted	Ripe	Green	Spotted	Ripe	Green	Spotted	Ripe
Cholesterol	$1.08^{A,a} \pm 0.01$	$0.82^{A,b} \pm 0.01$	$0.37^{A,c} \pm 0.01$	$0.56^{B,a} \pm 0.01$	$0.37^{B,b} \pm 0.01$	$0.22^{C,c} \pm 0.01$	$0.41^{C,a} \pm 0.01$	$0.34^{C,b} \pm 0.01$	$0.34^{B,b} \pm 0.01$
Brassicasterol	$0.04^{A,b} \pm 0.01$	$0.03^{A,b} \pm 0.01$	$0.23^{A,a} \pm 0.01$	$0.04^{A,b} \pm 0.01$	$0.03^{A,b} \pm 0.01$	$0.16^{B,a} \pm 0.01$	$0.03^{A,b} \pm 0.01$	$0.02^{A,b} \pm 0.01$	$0.06^{C,a} \pm 0.01$
24-methylene cholesterol	$0.23^{A,a} \pm 0.05$	$0.13^{A,b} \pm 0.01$	$0.09^{B,b} \pm 0.01$	$0.09^{B,c} \pm 0.01$	$0.13^{A,b} \pm 0.01$	$0.15^{A,a} \pm 0.01$	$0.05^{B,a} \pm 0.01$	$0.05^{B,a} \pm 0.01$	$0.05^{C,a} \pm 0.01$
Campesterol	$3.11^{B,a} \pm 0.03$	$3.10^{B,a} \pm 0.02$	$3.17^{A,a} \pm 0.05$	$2.50^{C,a} \pm 0.01$	$2.35^{C,b} \pm 0.01$	$1.78^{C,c} \pm 0.01$	$3.60^{A,a} \pm 0.01$	$3.52^{A,b} \pm 0.01$	$3.11^{B,c} \pm 0.01$
Campestanol	$0.36^{A,a} \pm 0.01$	$0.24^{A,b} \pm 0.02$	$0.17^{A,c} \pm 0.01$	$0.18^{B,a} \pm 0.01$	$0.14^{B,b} \pm 0.01$	$0.13^{A,b} \pm 0.01$	$0.15^{C,a} \pm 0.01$	$0.13^{B,a} \pm 0.01$	$0.10^{A,a} \pm 0.05$
Stigmasterol	$2.10^{A,b} \pm 0.02$	$2.20^{A,a} \pm 0.02$	$1.74^{A,c} \pm 0.02$	$1.44^{B,a} \pm 0.01$	$1.16^{B,c} \pm 0.01$	$1.29^{B,b} \pm 0.01$	$1.11^{C,a} \pm 0.01$	$0.94^{C,b} \pm 0.01$	$0.86^{C,c} \pm 0.05$
Δ-7-Campesterol	$0.11^{A,a} \pm 0.02$	$0.08^{A,a} \pm 0.01$	$0.09^{A,a} \pm 0.01$	$0.02^{B,a} \pm 0.01$	$0.03^{B,a} \pm 0.01$	$0.02^{B,a} \pm 0.01$	$0.05^{B,b} \pm 0.01$	$0.07^{A,a} \pm 0.01$	$0.08^{A,a} \pm 0.05$
Clerosterol	$1.00^{A,a} \pm 0.04$	$0.92^{C,b} \pm 0.01$	$1.03^{A,a} \pm 0.02$	$1.06^{A,a} \pm 0.06$	$1.03^{A,a} \pm 0.01$	$1.00^{A,a} \pm 0.01$	$1.00^{A,a} \pm 0.01$	$0.98^{B,b} \pm 0.01$	$1.01^{A,a} \pm 0.01$
β-sitosterol	$80.72^{C,b} \pm 0.01$	$83.53^{C,a} \pm 0.01$	$83.72^{B,a} \pm 1.12$	$86.53^{B,a} \pm 0.01$	$85.72^{B,b} \pm 0.03$	$83.97^{B,b} \pm 0.02$	$87.81^{A,a} \pm 0.01$	$86.57^{A,b} \pm 0.05$	$84.24^{A,c} \pm 0.01$
Sitostanol	$2.82^{A,a} \pm 0.01$	$1.67^{A,b} \pm 0.01$	$0.96^{A,c} \pm 0.02$	$1.32^{B,a} \pm 0.04$	$1.02^{B,b} \pm 0.01$	$0.83^{B,c} \pm 0.01$	$0.89^{C,a} \pm 0.01$	$0.65^{C,b} \pm 0.01$	$0.59^{C,c} \pm 0.01$
Δ-5-avenasterol	$5.29^{A,a} \pm 0.01$	$4.87^{C,c} \pm 0.01$	$5.09^{C,b} \pm 0.07$	$4.78^{B,c} \pm 0.06$	$6.48^{A,b} \pm 0.01$	$7.30^{A,a} \pm 0.01$	$3.34^{C,c} \pm 0.01$	$4.94^{B,b} \pm 0.01$	$6.96^{B,a} \pm 0.01$
Δ-5,24-stigmastadienol	$0.87^{A,a} \pm 0.03$	$0.79^{A,b} \pm 0.01$	$0.56^{C,c} \pm 0.01$	$0.80^{B,b} \pm 0.02$	$0.71^{B,c} \pm 0.01$	$0.94^{A,a} \pm 0.01$	$0.58^{C,b} \pm 0.01$	$0.57^{C,b} \pm 0.01$	$0.69^{B,a} \pm 0.01$
Δ-7-stigmastenol	$0.88^{A,a} \pm 0.01$	$0.64^{A,b} \pm 0.03$	$0.67^{A,b} \pm 0.01$	$0.29^{C,b} \pm 0.01$	$0.27^{C,b} \pm 0.01$	$0.40^{C,a} \pm 0.01$	$0.38^{B,c} \pm 0.01$	$0.41^{B,b} \pm 0.01$	$0.64^{B,a} \pm 0.01$
Δ-7-avenasterol	$1.40^{A,a} \pm 0.03$	$0.97^{A,b} \pm 0.03$	$0.83^{C,c} \pm 0.01$	$0.35^{C,c} \pm 0.01$	$0.52^{C,b} \pm 0.01$	$1.80^{A,a} \pm 0.01$	$0.55^{B,c} \pm 0.01$	$0.81^{B,b} \pm 0.01$	$1.25^{B,a} \pm 0.01$
Apparent β-sitosterol	$90.73^{C,c} \pm 0.01$	$91.79^{C,b} \pm 0.01$	$92.55^{C,a} \pm 0.01$	$94.49^{A,b} \pm 0.01$	$94.96^{A,a} \pm 0.04$	$94.05^{A,c} \pm 0.01$	$93.65^{B,b} \pm 0.02$	$93.72^{B,a} \pm 0.04$	$93.50^{B,c} \pm 0.01$
Total Sterol (mg/kg)	$358^{C,c} \pm 1.00$	$521.66^{C,b} \pm 0.57$	$1061.33^{B,a} \pm 1.15$	$728^{B,c} \pm 1.00$	$828.66^{B,b} \pm 0.57$	$1000.66^{C,a} \pm 0.57$	$897.66^{A,c} \pm 0.57$	$1034.66^{A,b} \pm 0.57$	$1092.33^{A,a} \pm 1.15$
Erythrodiol + uvaol	$4.52^{A,a} \pm 0.09$	$2.35^{B,c} \pm 0.04$	$2.68^{B,b} \pm 0.03$	$2.33^{C,a} \pm 0.01$	$1.78^{C,c} \pm 0.01$	$1.83^{C,b} \pm 0.02$	$3.16^{B,b} \pm 0.01$	$2.74^{A,c} \pm 0.24$	$3.97^{A,a} \pm 0.03$

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. Different capital letters express significant statistical differences (Duncan's test $P < 0.05$) among varieties

Gemlik) and 3.60% (green Halhalı). The content of campesterol in olive oils was significantly influenced by olive maturity and variety. Guillaume et al. (2012) obtained higher campesterol content in Australian olive oils as our values were in harmony with those of the results found by Dag et al. (2015).

Stigmasterol is associated to different parameters of the quality of virgin olive oil and its high contents are linked with low sensory quality and high acidity (Noorali et al. 2014). In our study, the percentages of stigmasterol were low and their mean percentages were lower than those of campesterol required by EU regulations, which revealed that all olive samples were obtained from healthy fruit (Manai-Djebali et al. 2012). Green and spotted Sarı Hasebi and green Gemlik were above the legal limit for cholesterol (%0.5) with 1.08, 0.82 and 0.56%, respectively. Similarly, the Δ-7-stigmastenol content of all three ripening stages of Sarı Hasebi (0.88, 0.64 and 0.67%, respectively) and ripe Halhalı (0.64%) were higher than the maximum limit of 0.5%. This result was in accordance with the study carried out by Yorulmaz (2008) who stated that the most of the olive oils produced in the south regions of Turkey had higher Δ-7-stigmastenol content. Triterpene dialcohols (erythrodiol and uvaol) form a part of the unsaponifiable fraction of olive oil and they are analysed together with the sterol fraction (Noorali et al. 2014). The sum of erythrodiol and uvaol content ranged from 1.78 (spotted Gemlik) to 4.52% (green Sarı Hasebi). The results indicated that the erythrodiol + uvaol content of the samples was significantly affected by variety and ripening and this content fluctuated during olive ripening as similar to work by De Mendoza et al. (2013). Erythrodiol + uvaol content in all the oil samples studied was below the limit of 4.5% with the exception of green Sarı Hasebi with 4.52%.

Conclusion

The present study showed the variations in various chemical properties, sterol compositions and antioxidant activity of 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 some parameters such as total phenolic content, total chlorophyll and carotenoid content, fatty acid composition and sterol composition. Halhalı variety was distinguished from other varieties with its higher oil content, total phenolic content, β-sitosterol content, total sterol content and its powerful antioxidant activity. Therefore, Halhalı variety should be intensely cultivated and certified with Protected Designation of Origin especially in Hatay. Gemlik variety had higher monounsaturated fatty acid content, Δ-5-avenasterol content and lower free fatty acids. The results of this study indicated that the ripening degree and variety significantly affect the quality of olive oils. To conclude, the investigation indicated that sterol compositions can be used as reliable indicators for determining the authenticity of Halhalı, Sarı Hasebi and Gemlik varieties according to ripening degree.

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