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Oxidation Stability of Virgin Olive Oils from Some Important Cultivars in East Mediterranean Area in Turkey

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Abstract Some important olive cultivars (Kilis yağlık, Halhalı, Karamani, Haşebi, Nizip yağlık) in East Mediterranean Area were studied. Olive fruits were processed on a low-scale mill equipped with a basket centrifuge. Basic quality parameters, the content of total polyphenols, *o*-diphenols, oxidation stability (Rancimat) and antiradical activity [1,1-diphenyl-2-picrylhydrazyl (DPPH)] were determined in oil samples. The highest induction period (IP) was 36.42 h, found in the Halhalı cultivar (from Gaziantep province), which also had strong radical scavenging activity (RSA) (96.72% in methanol:water extract and 94.91% in total oil) in all samples. The total phenol and *o*-diphenol content for this cultivar were 495.42 and 76.89 mg caffeic acid/kg oil, respectively. The oxidation stability and antiradical activity of the Kilis yağlık cultivar (from Kilis province) was very poor when compared to other cultivar (IP; 10.40 h, RSA in methanol: water extract; 30.94%, RSA in total oil; 52.31%). In addition total phenol and *o*-diphenol content for this cultivar were 38.31 and 5.03 mg caffeic acid/kg oil, respectively.

Keywords Kilis yağlık · Halhalı · Karamani · Haşebi · Nizip yağlık · Rancimat · DPPH · Polyphenols

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Introduction

The olive tree (*Olea europaea* L.) originated in upper Mesopotamia and South Front Asia and this includes a part of the southeastern Anatolia region of Turkey and Syria. The first cultivation and improvement were done by the Semitic people in this region as early as 3000 BC. From this region, olive cultivation spread northward into the Anatolia, then into the Aegean Sea islands, Greece, Italy and Spain, southward into Egypt, then to Tunisia, and Morocco [1].

There are many well known local olive cultivars in Turkey, many of which are region specific. Ayvalık, Çakır, Çekişte, Çilli, Domat, Edincik su, Erkence, İzmir Sofralık, Kiraz, Memecik, Memeli, and Uslu cultivars are grown in the Aegean region; Çelebi, Gemlik, Karamürsel Su, and Samanlı cultivars are grown in the Marmara region; Büyük Topak Ulak, Sarı Haşebi, Sarı Ulak, and Tavşan Yüreği cultivars are grown in the Mediterranean region; Saurani, Halhalı, Sayfi, Karamani, Elmacık, and Haşebi cultivars are grown in the Eastern Mediterranean, especially in Hatay province; Eğri Burun, Halhalı, Kalembezi, Kan Çelebi, Kilis Yağlık, Nizip Yağlık, and Yağ Çelebi cultivars are grown in the Southeast Anatolia region; Samsun Tuzlamalık, Trabzon Yağlık, Hastos, Butko, and Otur cultivars are grown in the Black Sea region [2].

Virgin olive oil has stronger oxidation stability because of high monounsaturated and low unsaturated fatty acid composition and minor components such as phenolic compounds, tocopherols and sterols. This stability is affected by a number of factors such as cultivar, location, harvesting, processing, and storage. Oils obtained from different cultivars have different characteristics because of their chemical composition. Environmental conditions also influenced oil properties such as phenolic compounds, tocopherols, and sterols in the same cultivar [3–7].

The purpose of this work was to study oxidation stability and antiradical activity of important olive oils from important cultivars (Kilis yağlık, Halhalı, Karamani, Haşebi, Nizip yağlık) growing in the eastern Mediterranean area in Turkey. These five cultivars were collected from their main growing areas in the Kilis, Gaziantep and Hatay provinces. Since Halhalı is so important for Hatay and Gaziantep (Nizip), two different samples were collected for it.

Experimental Procedures

Material

Six olive oil samples from five cultivars were analyzed. Olive cultivars were collected by hand from different provinces in East Mediterranean Area (Table 1). Olives were processed immediately using a low scale mill (Hakki Usta Ogulları, Aydin-Turkey) equipped with a hammer crusher, a vertical malaxator, and basket centrifuge. Malaxation temperature was 30 °C and duration of malaxation was 60 min.

Methods

The maturation index (MI) was determined according to International Olive Oil Council (IOOC) [8] and varied between 0 and 7. One hundred olive fruit were randomly taken from 1 kg of newly harvested olives from MI determination. MI was calculated by the following formula [1]:

$$\mathrm{MI} = [(0 \times n_0) + (1 \times n_1) + (2 \times n_2) + (3 \times n_3) + (4 \times n_4) + (5 \times n_5) + (6 \times n_6) + (7 \times n_7)]/100 \quad (1)$$

Free fatty acid, UV absorption characteristics and peroxide value (PV) were determined by the Ca 5a-40, Ch 5-91 and Cd 8-53 methods of in the American Oil Chemist's Society, respectively [9–11].

Table 1 Areas and cultivars of olive samples

Sample	Cultivar	Location	Altitude (meter)	Harvest date
A1	Halhalı	Hatay	115	10.12.2007
A2	Halhalı	Nizip-Gaziantep	584	26.12.2007
B	Kilis yağlık	Kilis	523	24.11.2007
C	Karamani	Hatay	112	15.12.2007
D	Hasebi	Hatay	126	15.12.2007
E	Nizip yağlık	Nizip-Gaziantep	567	26.12.2007

Fatty acids were converted to their methyl esters [12] and injected into a Shimadzu (Kyoto, Japan) gas chromatograph, fitted with a FID detector. Separations were performed using a DB-23 capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness) (Agilent J&W, USA). Helium was used as a carrier gas at a flow rate of 1.00 mL/min. Injector and detector temperatures were 230 and 240 °C, respectively. Column temperature was kept at 190 °C for 40 min. Samples of 1 μL were injected by an autoinjector in a split mode (1:80). FAMEs were identified by comparison of their retention times with those of the reference standards. The content (percentage by weight) of fatty acids was calculated from their corresponding integration data.

Phenols were extracted following the method proposed by [13]. Five grams of olive oil were dissolved in 10 mL *n*-hexane to remove oil, extracted with 10 mL of a methanol:water mixture (60:40, v/v) and then shaken vigorously by means of a vortex and centrifuged at 3,500 rpm for 10 min.

The total phenols content (TPC) of the extracts from the method [13] used above was determined using Folin–Ciocalteau reaction at 725 nm. Two hundred microliters of phenol extract were diluted with water to a total volume of 5 mL, followed by the addition of 0.5 mL Folin–Ciocalteau reagent. After 3 min, 1.0 mL of sodium carbonate solution (35%, w/v) was added, mixed, and diluted with water to 10 mL. The mixture allowed to stand for 1 h. The absorbance of the solution was measured after 1 h against a blank sample by UV–vis spectrophotometer at 725 nm. The results were expressed in mg caffeic acid/kg oil [13].

o-diphenol concentrations were determined from a 4 mL sample of each phenolic extract from method [13] used above. These samples were added to 1 mL of a 5% solution of sodium molybdate dihydrate in ethanol/water (1:1, v/v) and shaken vigorously. After 15 min, the absorbance at 370 nm was measured using caffeic acid for the calibration curve. The results were expressed in mg caffeic acid/kg oil [14].

Oxidation stability test was performed using the Rancimat equipment at 120 °C (Metrohm Ltd, Herisau, Swiss) with a continuous air flow of 20 l/h passing through the samples. The inflection point of the curve was assigned as the induction time and given in hour [15, 16].

The antiradical activity was measured by DPPH method in total olive oil samples and in methanol:water extracts from oil samples.

Determination of antiradical activity of methanol/water extract used the following method. The extract solution (0.5 mL) was added to 3 mL of a 0.1 mM methanolic solution of DPPH and vortex-mixed. After 30 min, the absorbance was measured at 515 nm [13] and antiradical action toward DPPH radical was estimated from the difference in absorbance with or without sample (control) and

the percent of inhibition was calculated from the following equation:

$$\%\text{Inhibition} = [(\text{Absorbance of control} - \text{absorbance of test sample})/\text{absorbance of control}] \times 100 \quad (2)$$

Antiradical activities in total olive oil samples were determined according to a reported procedure [13]. One milliliter of oil solution (10% w/v) was added to 4 mL of a freshly prepared DPPH solution (0.1 mM) in a 20 mL test tube, which was immediately closed and vigorously mixed for 10 s in a vortex apparatus. Ethyl acetate was used as a solvent. Absorbance of the mixture was measured after 30 min at 515 nm and the percent of inhibition was calculated using [2] formula.

Statistical Analysis

Statistical analysis was carried out using SPSS 11.5 (2002) software. One-way ANOVA followed by "Duncan's test" was applied to evaluate differences (at 95% level) between mean values of olive oil samples according to some basic quality parameters. The Pearson correlation test was used to determine correlations between total phenol, *o*-diphenol, RSA and, oxidation stability. Six olive oil samples from five cultivars were analyzed with two replications.

Results and Discussion

Oil quality characteristics are presented in Table 2. The MI of samples ranged between 2.95 and 6.02. Free fatty acid content ranged between 0.41 and 0.64% and did not exceed the limit of 0.8%, which is an accepted standard for virgin olive oil. Peroxide values (4.30–8.81 meq/kg) of the samples were below 20 meq/kg limit. K_{232}, K_{270}, and ΔK values were 1.78–2.71, 0.12–0.20 and −0.0010–(−0.0090), respectively. K_{232} value of one sample (sample C) exceeded the limit value ($K_{232} < 2.50$) established by the IOOC's trade standard for olive oil and olive–pomace oil [17]. ΔK values of all samples were below the limit value.

The fatty acid composition varied among samples and but the level of all fatty acids were within the standart limits (Table 3). Oleic and palmitic acid were the major fatty acids and ranged between 64.56–73.31 and 10.90–16.72%, respectively. Oleic acid/linoleic acid ratio (O/L) changed between 4.76 and 8.98. Margaric, margaroleic, behenic, gadoleic, and lignoceric acid were present in small amounts in all the samples. The ratio of oleic to linoleic is reported to be indicative of the oxidation stability of olive oil. However our results showed that the oleic to linoleic acid ratio showed no correlation to oxidation stability, antiradical activity, total phenol or *o*-diphenol. Cerratani et al. [14] observed that the oleic to linoleic acid ratio of oil samples were similar but the other parameters, namely TPC, *o*-diphenol and oxidation stability index were different, and that their values were well correlated. Our results were similar with the data of this author [14].

Total phenol and *o*-diphenol content of samples varied significantly ($P < 0.05$) depending on cultivar (Table 4). Sample A2 had the highest total phenol content (495.42 mg caffeic acid/kg oil). While sample B had a poor phenol content (38.31 mg caffeic acid/kg oil). Differences in *o*-diphenol content among samples were similar to total phenol data. *o*-diphenol content (mg caffeic acid/kg oil) varied between 5.03 (Sample B) and 76.89 (Sample A2).

Oxidation stability and antiradical activity data are shown in Table 5. RSA was measured both in methanol:water extracts and in total oil. Both RSA activity of methanol:water extracts and RSA activity in total oil varied with respect to olive cultivar. Sample A2 was more effective as a DPPH radical scavenger than other samples. However, Sample B reduced DPPH radical slowly, thus the RSA of this sample was very poor compared to all samples. A good correlation ($r^2 = 0.895$) was observed between RSA of methanol:water extract and RSA in total oil ($P < 0.01$). Oxidation stability ranged from a minimum of 10.40 h for sample B to 36.42 h for sample A2.

Pearson's correlation test was used to determine correlations between total phenol, *o*-diphenol, RSA, and oxidation stability (Table 6). A strong correlation between total phenol and *o*-diphenol content was observed

Table 2 Some quality parameters of olive oil samples

Samples	MI	FFA (%, oleic acid)	PV (meq/kg)	K_{232}	K_{270}	ΔK
A1	3.21 ± 0.07	0.63 ± 0.01	5.50 ± 0.77	2.13 ± 0.08	0.14 ± 0.02	–0.0045 ± 0.00
A2	6.02 ± 0.03	0.56 ± 0.00	4.85 ± 0.01	1.94 ± 0.24	0.20 ± 0.01	–0.0015 ± 0.01
B	2.95 ± 0.07	0.41 ± 0.01	6.24 ± 1.05	1.78 ± 0.30	0.14 ± 0.00	–0.0010 ± 0.00
C	5.99 ± 0.01	0.64 ± 0.01	8.81 ± 1.42	2.71 ± 0.31	0.15 ± 0.02	–0.0035 ± 0.00
D	5.01 ± 0.04	0.38 ± 0.04	4.76 ± 0.76	2.08 ± 0.11	0.12 ± 0.01	–0.0055 ± 0.01
E	5.01 ± 0.01	0.41 ± 0.00	4.30 ± 0.70	2.13 ± 0.08	0.18 ± 0.01	–0.0090 ± 0.01

Values are mean ± SD of two measurements

Table 3 Fatty acid (%) composition of samples

Fatty acids	A1	A2	B	C	D	E
Myristic acid	0.01	0.02	0.01	0.01	0.01	0.01
Palmitic acid	15.66	16.72	15.13	12.27	10.90	14.70
Palmitoleic acid	1.02	1.52	0.91	0.73	0.57	0.95
Margaric acid	0.14	0.31	0.12	0.15	0.24	0.14
Margaroleic acid	0.17	0.45	0.15	0.22	0.28	0.16
Stearic acid	3.83	3.16	3.68	2.67	4.20	4.60
Oleic acid	69.85	64.56	67.96	67.74	73.31	67.63
Linoleic acid	7.78	11.71	10.44	14.23	8.66	10.25
Linolenic acid	0.61	0.80	0.69	1.11	0.84	0.60
Arachidic acid	0.56	0.44	0.54	0.41	0.52	0.59
Gadoleic acid	0.21	0.19	0.21	0.28	0.30	0.20
Behenic acid	0.11	0.08	0.10	0.12	0.10	0.11
Lignoceric acid	0.06	0.03	0.06	0.05	0.04	0.06
Oleic acid/linoleic acid ratio	8.98	5.51	6.51	4.76	8.46	6.60

Values are means of two measurements

Table 4 Total phenol and *o*-diphenol content of samples

Samples	Total phenol (mg caffeic acid/kg oil)	*o*-Diphenol (mg caffeic acid/kg oil)
A1	138.03 ± 0.72 b	12.32 ± 0.02 b
A2	495.42 ± 2.00 f	76.89 ± 1.34 e
B	38.31 ± 0.29 a	5.03 ± 0.02 a
C	161.38 ± 0.72 c	12.50 ± 0.06 b
D	114.08 ± 0.72 d	6.60 ± 0.31 c
E	241.55 ± 0.72 e	15.76 ± 0.17 d

Values are mean ± SD of two measurements

Table 5 Antiradical activity and oxidation stability of oil samples

Samples	RSA[a] (%)	RSA[b] (%)	Oxidation stability (h)
A1	86.65 ± 1.17 b	69.95 ± 3.11 b	24.89 ± 0.21 b
A2	96.72 ± 0.18 c	94.91 ± 0.07 e	36.42 ± 0.51 f
B	30.94 ± 0.45 a	52.31 ± 0.44 a	10.40 ± 0.25 a
C	95.17 ± 0.85 c	85.38 ± 0.98 c	14.66 ± 0.27 c
D	49.17 ± 0.06 d	68.05 ± 4.06 b	20.61 ± 0.35 d
E	96.29 ± 0.06 c	92.69 ± 1.37 d	23.85 ± 0.01 e

Means within a column with different letters are significantly different ($P < 0.05$) by one-way ANOVA analysis. RSA expressed as % reduction in concentration of DPPH after a 30 min reaction

[a] Antiradical activity of methanol:water extracts

[b] Antiradical activity of oils

($r^2 = 0.957$, $P < 0.01$). Oxidation stability was positively correlated to total phenol ($r^2 = 0.889$, $P < 0.01$) and *o*-diphenol ($r^2 = 0.837$, $P < 0.01$). A good correlation ($r^2 = 0.895$, $P < 0.01$) observed between RSA of methanol:water extracts and RSA in total oil. Average correlations ($r^2 = 0.608$, 0.669, respectively, $P < 0.05$) were established between oxidation stability with RSA of methanol:water extracts and RSA in total oil.

Rancimat values are influenced by factors such as fatty acids (position and unsaturation degree of fatty acids), tocopherols, carotenoids, and sterols [18]. RSA is a measure of antiradical capacity of extracts from oil samples and the antiradical substances react with compounds dissolved within the solvent. This assay may not show good correlations to rancimat. Ramadan and Moersel [19] found that antiradical differences in vegetable oils was based on the content and composition of polar bioactive and non-saponifiable substances, phenolic antioxidants in the oil, and that there is a synergism of polar compounds with other components present in each oil and differences in kinetic behaviors of potential antioxidants.

Tura et al. [5] found there are differences in oxidative stabilities among olive oil cultivars. Varied correlations between oxidation stability and TPC were found depending on olive cultivar. Cerratani et al. [14] observed a high correlation between oxidation stability and *o*-diphenol while there was a weak correlation was found between TPC and oxidation stability. A high correlation between oxidation stability with total phenol and *o*-diphenol was determined by previously reported [20, 21]. Our results were in agreement with previous results [5, 14, 20, 21]. Correlations of oxidation stability and total phenol varied according to olive cultivar. These differences may be attributed to different cultivars and locations.

Poerio et al. [22] reported multiple correlations between total phenol, *o*-diphenol, DPPH, and oxidation stability. According to their results, the DPPH test showed good correlations with oxidation stability, total phenol and *o*-diphenol ($r^2 = 0.93$, 0.97, and 0.87, $P < 0.05$). With those exception of *o*-diphenol, our results were in agreement with those reported by Poerio et al. [22].

The sample A2 from Halhalı cultivar (Nizip-Gaziantep) had better stability than other samples. Its strong oxidative stability was expected because of the high phenol and *o*-diphenol content, which contributed to oxidation stability. However, sample B from Kilis yağlık cultivar (Kilis) had poor stability because of low total phenol and *o*-diphenol content. Another Halhalı cultivar from Hatay (sample A1) showed stronger oxidation stability after sample A2. These results are in agreement with a previous report, on oil quality from five different geographical sites of Umbria and Tuscany Regions: phenols, tocopherols, and fatty acids were influenced by area with diverse climates and soil characteristics [23]. Moreover, Salvador et al. [24] observed that the cultivar area affected the concentration of natural antioxidants, phenols, and tocopherols, and therefore the oxidative stability of the Cornicabra virgin olive oil.

Table 6 Correlations ($P < 0.01$) among the main parameters related to oxidative stability of samples

	Total phenol	*o*-Diphenol	Oxidation stability	RSA[a]	RSA[b]
Total phenol	1.000	0.957**	0.889**	0.664*	0.812**
o-diphenol	0.957**	1.000	0.837**	0.486	0.626*
Oxidation stability	0.889**	0.837**	1.000	0.608*	0.669*
RSA[a]	0.664*	0.486	0.608*	1.000	0.895**
RSA[b]	0.812**	0.626*	0.669*	0.895**	1.000

RSA expressed as % reduction in concentration of DPPH after a 30 min reaction

** Correlation is significant at the 0.01 level (two-tailed)

* Correlation is significant at the 0.05 level (two-tailed)

[a] Antiradical activity of methanol:water extracts

[b] Antiradical activity of oils

Olive production in the Kilis region is mainly done under drought conditions. Kilis is drier than other regions. Hatay is more humid than the Gaziantep–Nizip region. The reason of variation in oxidation stability within cultivars could be related to the characteristics of cultivars and also to ecological factors. Higher oxidation stabilities were observed in the samples of cultivars collected from its origin region. This could be the reason of variation in the amount of some components, which have antioxidant characteristics related with the effects of ecological factors on oil stabilities. Laroussi et al. [25] studied olive oil of Arbequnia olive cultivars from Spain which had been grown in different locations of Tunisia and found oxidation stabilities varying from 3.5 to 7.4 h. Differences in oxidation stability were due to the amount of antioxidant substances (phenolic compounds) related with location.

Mousa et al. [26] suggest that the altitude at which the olive trees are grown also affect the quality characteristics of olive oil and mainly its composition. Olive oil from high elevation has higher oxidative stability compared with the oil from lower elevations [27]. Within the same growing area, as the temperature decreases (higher elevation) the percentage of unsaturated fatty acids increases [28]. The altitude (100 and 800 m) of olive oil samples were different (Table 1). This differences contribute to oxidation stability of oil samples. Especially, the altitude of samples (A1 and A2) collected area for Halhali in Hatay (115 m) and Nizip-Gaziantep (584 m), the differences among oxidation stability of these samples can be explained.

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