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Effect of Olive Leaves Addition before Extraction of Turkish Olive Cultivars on Olive Oil Minor Components and Antioxidant Activity

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Abstract

This research aimed to study the effect of olive leaves addition (0, 1, 3%) on minor components of Ayvalık and Memecik olive oils, during 18 months storage, in Turkey. The olives were harvested from Olive Research Station orchard, 2008/09 and 2009/10 crop seasons. In each year, Ayvalık and Memecik olive fruits harvest date was decided according to maturation index. The leaves were collected from the trees at the same times with the olive fruits and added during crushing of olive fruits. Total chlorophyll, α-tocopherol, total phenolic contents and the antioxidant activity (DPPH• and $ABTS^{\bullet+}$ radical scavenging) were analyzed. Total chlorophyll, α-tocopherol, total phenolic contents and the antioxidant activity of Memecik olive oils were determined higher than Ayvalık olive oils ($P<0.001$). Olive leaf addition induced a significant increase in total chlorophyll, α-tocopherol and total phenolic content, and antioxidant activities, in both years ($P<0.001$). During the storage period antioxidant content and antioxidant activities were significantly decreased, in both years ($P<0.001$). After storage, total chlorophyll content ($P<0.001$) and DPPH• radical scavenging activity ($P<0.01$ and $P \leq 0.001$) of control samples were lower than 3% leaf added samples. The results obtained from research suggest that with addition of olive leaf it is possible to obtain more nutritional olive oils with higher antioxidant content.

Keywords: α-tocopherol; DPPH; ABTS; Total phenolic content; Chlorophyll; Shelf life

Introduction

Olive tree is a great economic fruit trees in Mediterranean area. It is one of the most important agricultural activities in the Mediterranean countries, where there are about 8 million ha of cultivated olive trees [1]. Turkey is the world's sixth largest producer of olive oil. The Aegean coast, Turkey's leading olive growing region, accounts for 75-80 % of the total production, Ayvalık and Memecik are the most widespread, dominant and economically important olive cultivars [2].

The importance of virgin olive oil (VOO) is concerned to its high levels of monounsaturated fatty acids and to the presence of minor components such as chlorophyll, α-tocopherol, phenolics, volatile compounds, aliphatic and triterpenic alchols, sterols and several antioxidants. The phenolic compounds important among them because of nutritional and sensorial benefit [3]. Phenolic compounds and tocopherols play a protective role against oxidative stress [4]. And also phenolic compounds are of primary importance to the shelf life of virgin olive oils due to their antioxidative properties [5]. Olive oil color is correlated with its pigment composition, especially chlorophyll. Chlorophyllic pigments show antioxidant activity in the dark but prooxidant in the light for that reason presence of chlorophyll compound in the oils are very important [6,3]. The olive oils are being compared to other vegetable oils, it is more susceptible to oxidation because of higher content of unsaturated fatty acids and natural antioxidant compounds. Synthetic antioxidants, such as Butylated Hydroxy Anisole (BHA), Butylated Hydroxyl Toluene (BHT), Ter-Butyl Hydroquinone (TBHQ) have been used as food additives to overcome the stability problems. However, new data has shown that synthetic antioxidants can include many health risks [7]. Therefore, many recent researches have been targeted at the identification of alternative new antioxidants from natural sources which have similar characteristics. Olive leaf extract is one of them [8]. Olive leaves are by-product of olive culture and can easily be found either from pruning or in olive oil industry [1]. Recent studies have highlighted olive leaves high-added value because of great antioxidant properties, especially phenolic compounds, and exhibiting a strong preventive effect against oxidation [7,9-11].

The aim of the present research was to determine the changes in total chlorophyll, α-tocopherol, total phenolic contents and DPPH• and $ABTS^{\bullet+}$ radical scavenging activities of Ayvalık and Memecik olive oils during storage and compared the effect of olive leaf addition. For this purpose, olive fruits Ayvalık and Memecik were harvested during 2008/09 and 2009/10 crop years and mixed with the leaves of the same variety at three different percentages (0, 1 and 3%: w:w) prior to oil extraction.

Materials and Methods

Olive leaves and fruit sampling

The research was conducted during the harvest seasons of 2008/09-2009/10 in the Olive Research Station of Izmir/Turkey. In both years, olive fruits of Ayvalık and Memecik cultivar harvested by hand at 3.9 maturity index (MI), which is proposed by the International Olive Oil Council [12]. Olive leaves were also collected from the same trees during the harvest. The following percentages of olive leaves by weight were added (w/w) to fruits prior to crushing: 0% (control), 1% and 3%.

Oil extraction and storage

The washed fruits were crushed immediately to obtain oil by using an Abencor System (MC2 Ingenierias y Sistemas Sevilla, Spain) which

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has fruit crushing, malaxation and centrifuge parts. All oil samples were stored at room temperature in darkness using amber glass bottles (100 ml), which were completely filled. Oil samples were analyzed after the extraction prior to storage, and after 6, 12 and 18 months of storage.

Total chlorophyll content

Total chlorophyll was determined according to the official method of American Oil Chemists Society (AOCS) [13].

Total phenolic content

Total phenolic content in olive oil was determined according to the methods of Hrncirik and Fritsche [14]. Oil (2.5 g) was dissolved in 5 mL hexane and the phenolics were extracted with 5 mL methanol/water (60:40, v/v), by shaking for 2 min with an electronic shaker. Hexane and methanol/water phases were separated by centrifugation (at 3500 rpm for 10 min). The methanolic phase (0.2 mL) was diluted with water to a total volume of 5 mL, followed by the addition of 0.5 mL Folin-Ciocalteu reagent (Merck KgaA, Germany). After 3 min, 1mL sodium carbonate solution (35%, w/v) was added to the reaction mixture, which was finally mixed and diluted with water to 10 mL. The absorbance of the solution was measured after 2 h against a blank sample by a Shimadzu Spectrophotometer UV-1700 PharmaSpec (Japan) at 725 nm. Total phenolic content was calculated against the standard curve constructed by known concentrations of caffeic acid (Sigma Chemicals Co.) within the range of 0.05-0.5 mg/mL.

α-Tocopherol content

α-Tocopherol content was determined with the method suggested by IUPAC [15]. Oil sample (1 g) was dissolved in 10 ml hexane, filtered through 0.45-µm cellulose filter and injected into the Agilent technologies HPLC (1100 series) system which was eluted with hexane/2-propanol (99:1; v/v) at the flow rate of 1ml/min. The injection volume was 20 µl. The UV detector (HP 1100) was set to 292 nm wavelength. Analysis was carried out at 25°C. The total run time was 10 min [15]. Waters µ Porasil column (300 mm x 3.9 mm x 10 µm, Ireland) was used [16]. Results were expressed as milligrams per kilogram of oil calculated against a standard curve of α-tocopherol (Calbiochem, U.S.) for quantification.

DPPH· radical scavenging activity (RSA)

DPPH· RSA was determined according to Lavelli [17] and Jiang et al. [18]. Oil sample (1 g) was dissolved in 5 mL methanol and

Harvest Seasons			Storage Period (Months)				*P* value	LSD*
			0	6	12	18		
2008/09	Ayvalık	0%	1.24^{s}	1.03^{t}	0.96^{t}	0,76^{u}	<0.001	0.15
		1%	3.43^{n}	3.22^{o}	3.06^{p}	2.80^{r}		
		3%	7.93^{f}	6.70^{g}	5.78^{i}	4.84^{l}		
	Memecik	0%	5.00^{k}	4.71^{lm}	4.60^{m}	3.40^{n}		
		1%	9.65^{e}	6.82^{g}	6.16^{h}	5.52^{j}		
		3%	14.48^{a}	12.06^{b}	11.84^{c}	10.16^{d}		
	Mean	0%	3.12^{i}	2.87^{j}	2.78^{k}	2.08^{l}	<0.001	0.08
		1%	6.54^{e}	5.02^{f}	4.61^{g}	4.16^{h}		
		3%	11.20^{a}	9.38^{b}	8.81^{c}	7.50^{d}		
		Ayvalık	4.20^{e}	3.65^{f}	3.27^{g}	2.80^{h}	<0.001	0.09
		Memecik	9.71^{a}	7.86^{b}	7.53^{c}	6.36^{d}		
	Mean		6.967^{a}	5.76^{b}	5.40^{c}	4.58^{d}	<0.001	0.07
		0%	2.71^{C}				<0.001	0.06
		1%	5.08^{B}					
		3%	9.22^{A}					
		Ayvalık	3.48^{B}				<0.001	0.05
		Memecik	7.87^{A}					
2009/10	Ayvalık	0%	0.58^{u}	0.48^{v}	0.41^{y}	0.24^{z}	<0.001	0.04
		1%	1.57^{p}	1.45^{r}	1.37^{s}	1.18^{t}		
		3%	4.48^{i}	4.08^{k}	3.79^{l}	3.02^{n}		
	Memecik	0%	4.88^{h}	4.43^{j}	3.46^{m}	2.86^{o}		
		1%	6.86^{b}	5.85^{e}	5.64^{f}	4.89^{h}		
		3%	7.50^{a}	6.69^{c}	6.13^{d}	5.57^{g}		
	Mean	0%	2.73^{i}	2.46^{j}	1.94^{k}	1.55^{l}	<0.001	0.02
		1%	4.21^{e}	3.65^{f}	3.51^{g}	3.03^{h}		
		3%	5.99^{a}	5.38^{b}	4.96^{c}	4.30^{d}		
		Ayvalık	2.21^{e}	2.00^{f}	1.86^{g}	1.48^{h}	<0.001	0.03
		Memecik	6.41^{a}	5.65^{b}	5.08^{c}	4.44^{d}		
	Mean		4.31^{a}	3.83^{b}	3.47^{c}	2.96^{d}	<0.001	0.03
		0%	2.17^{C}				<0.001	0.01
		1%	3.60^{B}					
		3%	5.15^{A}					
		Ayvalık	1.89^{B}				<0.001	0.01
		Memecik	5.40^{A}					

*Least Significant Difference (α=0.05); Values in the same column with different uppercase letters, and in the same row with lowercase letters show statistically significant differences.

Table 1: Changes in chlorophyll (mg/kg) content of Ayvalık and Memecik olive oils obtained with different leaf additions during 2008/09 and 2009/10 harvest seasons.

Harvest Seasons			Storage Period (Months)				*P value*	LSD*
			0	6	12	18		
2008/09	Ayvalık	0%	170.40	166.74	73.65	42.24	-	n.s.
		1%	176.14	169.87	76.16	52.65		
		3%	186.19	175.66	79.79	60.50		
	Memecik	0%	231.33	225.31	199.83	156.81		
		1%	235.24	231.09	205.42	167.23		
		3%	249.29	234.95	211.00	186.80		
	Mean	0%	200.87	196.02	136.74	99.53	-	n.s.
		1%	205.69	200.48	140.79	109.94		
		3%	217.74	205.3	145.39	123.65		
		Ayvalık	177.58^{d}	170.76^{e}	76.53^{f}	51.80^{g}	<0.001	6.67
		Memecik	238.62^{a}	230.45^{b}	205.42^{c}	170.28^{e}		
	Mean		208.10^{a}	200.60^{b}	140.97^{c}	111.04^{d}	<0.001	4.82
		0%	158.29^{C}				<0.001	3.9
		1%	164.22^{B}					
		3%	173.02^{A}					
		Ayvalık	119.17^{B}				<0.001	3.27
		Memecik	211.19^{A}					
2009/10	Ayvalık	0%	133.95	125.88	111.08	51.59	-	n.s.
		1%	166.61	157.31	122.86	57.18		
		3%	170.52	161.31	141.43	68.34		
	Memecik	0%	182.52	173.87	141.90	75.60		
		1%	188.11	178.56	152.68	93.75		
		3%	206.25	192.77	167.39	106.31		
	Mean	0%	158.24	149.87	126.49	63.6	-	n.s.
		1%	177.36	167.94	137.77	75.46		
		3%	188.39	177.04	154.41	87.33		
		Ayvalık	157.03	148.17	125.13	59.04	-	n.s.
		Memecik	192.3	181.73	153.99	91.89		
	Mean		174.66^{a}	164.95^{b}	139.56^{c}	75.46^{d}	<0.001	6.06
		0%	124.55^{C}				<0.001	3.02
		1%	139.63^{B}					
		3%	151.79^{A}					
		Ayvalık	122.34^{B}				<0.001	3.89
		Memecik	154.98^{A}					

* Least Significant Difference (α=0.05); Values in the same column with different uppercase letters. and in the same row with lowercase letters show statistically significant differences.
n. s.: not significant.

Table 2: Changes in total phenol content (mg caffeic acid eq./kg oil) of Ayvalık and Memecik olive oils obtained with different leaf additions during 2008/09 and 2009/10 harvest seasons.

vigorously shaken for 1 h at room temperature and then centrifuged (at 3500 rpm for 10 min) to seperate polar and lipid fractions [17]. DPPH$^{\bullet}$ RSA determination was made at the methanol phase. The extracts (100 µl) were mixed with either 100 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) solution (1900 µl) or methanol (1900 µl) for blanks and left to stand for 15 min at room temperature in the dark [18]. The absorbance values were measured at 517 nm with a spectrophotometer against blanks which were prepared with methanol. Trolox equivalent of the DPPH$^{\bullet}$ RSA was calculated against the standard curve prepared with known concentrations of Trolox (Sigma Aldrich Chemicals Co. USA). The data are expressed as µmol Trolox equivalent of 100 g of each sample (R^2 = 0.9972).

ABTS$^{\bullet+}$ radical scavenging activity (RSA)

ABTS$^{\bullet+}$ RSA was determined spectrophotometrically. Oil sample (0.5 g) was dissolved in 5 mL hexane [19]. ABTS (2,2-azinobis-(3-ethylbensothiazoline)-6-sulfonic acid) was dissolved in water to a 7 mM concentration. ABTS$^{\bullet+}$ was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and mixture was left in darkness at room temperature for 12-16 h before use and diluted with ethanol to an absorbance of 0.70 (± 0.020) at 734 nm [20]. 150 µl of either extracts or standards was mixed with ABTS$^{\bullet+}$ (2000 µl) and the mixture was kept at room temperature in darkness for 15 min. The absorbance of ABTS$^{\bullet+}$ mixtures was measured at 734 nm with a spectrophotometer. Trolox equivalent of the ABTS$^{\bullet+}$ RSA was calculated against the standard curve prepared with known concentrations of Trolox. The data are expressed as µmol Trolox equivalent of 100 g of each sample (R^2=0.9963).

Statistical analysis

All data for each year were subjected to analysis of variance (ANOVA) with SPSS for Windows v 11 (SPSS Inc., USA) separately. The experimental design was completely randomized split plots with storage period as the main, cultivar as the sub, and leaf ratio as the micro plots with three replications. The differences between the means were determined with the Fischer's Least Significant Difference (LSD) test.

Harvest Seasons			Storage Period (Months)				*P value*	LSD*
			0	6	12	18		
2008/09	Ayvalık	0%	157.19^{h}	147.45^{i}	135.75^{j}	124.02^{k}	<0.05	12.18
		1%	158.34^{h}	150.50^{i}	140.21^{j}	133.05^{jk}		
		3%	178.38^{g}	164.08^{h}	153.24^{hi}	143.77^{ij}		
	Memecik	0%	367.92^{a}	342.76^{d}	321.99^{e}	279.89^{f}		
		1%	368.04^{a}	345.22^{cd}	336.98^{d}	281.69^{f}		
		3%	369.21^{a}	364.40^{ab}	356.06b^{c}	291.32^{f}		
	Mean	0%	262.55	245.1	228.87	201.95	-	n.s.
		1%	263.19	247.86	238.6	207.37		
		3%	273.8	264.24	254.65	217.54		
		Ayvalık	164.64^{e}	154.01^{f}	143.07^{g}	133.61^{h}	<0.001	8.26
		Memecik	368.39^{a}	350.79^{b}	338.34^{c}	284.30^{d}		
	Mean		266.51^{a}	252.40^{b}	240.70^{c}	208.95^{d}	<0.001	7.21
		0%	234.62^{C}				<0.001	3.88
		1%	239.25^{B}					
		3%	252.56^{A}					
		Ayvalık	148.83^{B}				<0.001	2.86
		Memecik	335.46^{A}					
2009/10	Ayvalık	0%	171.84^{j}	151.86^{l}	94.56^{n}	62.98^{p}	<0.001	5.50
		1%	178.06^{i}	156.42^{l}	99.54^{n}	66.39^{p}		
		3%	183.08^{i}	162.86^{k}	116.87^{m}	83.10^{o}		
	Memecik	0%	373.37^{b}	301.36^{e}	234.92^{g}	182.23^{i}		
		1%	378.47^{b}	311.96^{d}	260.43^{f}	204.86^{h}		
		3%	384.63^{a}	331.86^{c}	303.98^{e}	255.24^{f}		
	Mean	0%	272.62^{c}	226.61^{f}	164.74^{j}	122.61^{l}	<0.001	2.65
		1%	278.26^{b}	234.19^{e}	179.98^{h}	135.63^{k}		
		3%	283.85^{a}	247.36^{d}	210.42^{g}	169.17^{i}		
		Ayvalık	177.66^{e}	157.05^{f}	103.66^{g}	70.82^{h}	<0.001	3.01
		Memecik	378.82^{a}	315.06^{b}	266.44^{c}	214.11^{d}		
	Mean		278.24^{a}	236.05^{b}	185.05^{c}	142.47^{d}	<0.001	2.02
		0%	196.64^{C}				<0.001	1.94
		1%	207.01^{B}					
		3%	227.70^{A}					
		Ayvalık	127.30^{B}				<0.001	1.58
		Memecik	293.61^{A}					

*Least Significant Difference (α=0.05); Values in the same column with different uppercase letters. and in the same row with lowercase letters show statistically significant differences.
n. s.: not significant.

Table 3: Changes in α-tocopherol content (mg/kg) of Ayvalık and Memecik olive oils obtained with different leaf additions during 2008/09 and 2009/10 harvest seasons.

Results and Discussion

Chlorophyll content

Since chlorophylls act as an antioxidant in darkness and act as a prooxidant under light, presence of chlorophylls in oils are very important [6]. The color of olive oil affects the consumer's preference and sensation of quality. In our study, chlorophyll content of Memecik olive oils were significantly higher than Ayvalık olive oil, 126 % in 2008 and 185 % in 2009 (P<0.001) (Table 1). Compared to the oils of other varieties, the oils of Ayvalık and Memecik contain medium levels [21-25] of chlorophyll. Chlorophyll content of oils were significantly increased with leaf addition before extraction in both years (P<0.001). The results confirm those previously reported by Giovacchino et al. [26]. Giovacchino et al. [26] also reported that by adding 3 % leaf prior to crushing, chlorophyll content increased by 84 %. In our study, 3 % leaf addition before extraction, increased chlorophyll content by 240 % and 137 %, in 2008 and 2009, respectively. The chlorophyll content of both varieties' oils significantly decreased during storage (P<0.001), in both crop seasons. These results are in agreement with Morello et al. [27] and Gomez-Alonso et al. [28]. When the effect of interaction between storage period and leaf addition was considered, a declining trend observed for chlorophyll content (P<0.001). It can be seen in table 1 that although a loss of chlorophyll can be observed in all the oils, the losses were less in leaf added oils compared to control at the end of 18 months storage in 2009. The chlorophyll content of 3 % leaf added oils was higher than the other oils' initial content at the end of 18 months.

Total phenolic content

The content of phenolic compounds in virgin olive oil is affected many factors such as cultivar, climatic and environmental conditions, ripeness index, extraction technologies and storage conditions [4,29]. The amount of phenolic content in olive oil is an important factor for quality and organoleptic evaluation. Polyphenols are especially potent antioxidants, they play an important role in human diet and health [30]. Also, the shelf-life of oil is correlated with the natural antioxidant

Harvest Seasons			Storage Period (Months)				*P value*	LSD*
			0	6	12	18		
2008/09	Ayvalık	0%	55.33^{h}	27.63^{j}	19.19^{kl}	17.06^{l}	<0.001	7.47
		1%	59.34^{gh}	31.73^{j}	23.26^{k}	19.51^{k}		
		3%	66.24^{fg}	32.21^{j}	25.86^{jk}	23.00^{k}		
	Memecik	0%	95.10^{b}	76.23^{de}	70.62^{ef}	41.51^{i}		
		1%	97.36^{b}	92.14^{b}	88.25^{c}	54.82^{h}		
		3%	106.02^{a}	95.52^{b}	90.24^{bc}	78.34^{d}		
	Mean	0%	75.22^{c}	51.93^{h}	44.90^{j}	29.29^{j}	<0.01	4.32
		1%	78.35^{b}	61.94^{e}	55.75^{g}	37.17^{k}		
		3%	86.13^{a}	63.86^{d}	58.05^{f}	50.67^{i}		
		Ayvalık	60.30^{c}	30.52^{d}	22.77^{e}	19.86^{e}	<0.001	5.29
		Memecik	99.50^{a}	87.96^{b}	83.04^{b}	58.22^{c}		
	Mean		79.90^{a}	59.24^{b}	52.90^{c}	39.04^{d}	<0.001	4.76
		0%	50.33^{C}				<0.001	2.36
		1%	58.30^{B}					
		3%	64.68^{A}					
		Ayvalık	33.36^{B}				<0.001	1.64
		Memecik	82.18^{A}					
2009/10	Ayvalık	0%	38.91	21.73	18.33	13.79	-	n.s.
		1%	48.09	25.87	21.01	16.93		
		3%	59.46	31.56	26.26	19.69		
	Memecik	0%	88.25	71.67	69.79	42.74		
		1%	94.93	84.24	73.62	44.26		
		3%	117.57	91.90	84.55	51.97		
	Mean	0%	63.58^{c}	46.70^{e}	44.06^{e}	28.26^{g}	≤0.001	3.72
		1%	71.51^{b}	55.06^{d}	47.31^{e}	30.60^{g}		
		3%	88.51^{a}	61.73^{c}	55.41^{d}	35.83^{f}		
		Ayvalık	48.82^{d}	26.39^{e}	21.87^{e}	16.80^{f}	<0.001	4.71
		Memecik	100.25^{a}	82.61^{b}	75.98^{c}	46.32^{d}		
	Mean		74.53^{a}	54.50^{b}	48.93^{c}	31.56^{d}	<0.001	3.86
		0%	45.65^{C}				<0.001	2.19
		1%	51.12^{B}					
		3%	60.37^{A}					
		Ayvalık	28.47^{B}				<0.001	1.91
		Memecik	76.29^{A}					

*Least Significant Difference (α=0.05); Values in the same column with different uppercase letters. and in the same row with lowercase letters show statistically significant differences

n. s.: not significant.

Table 4: Changes in DPPH• RSA (μ mol TE/100 g) Ayvalık and Memecik olive oils obtained with different leaf additions during 2008/09 and 2009/10 harvest seasons.

content [5]. Phenolic compounds delay the oxidative degradation process, thus extending the product shelf-life [4,30]. In our research, total phenolic content of Memecik olive oils were significantly higher than Ayvalık olive oil (P<0.001). The total phenolic content of Ayvalık olive oil was 119.17 and 122.34 mg CAE/kg oil and of Memecik olive oil was 211.19 and 154.98 mg CAE/kg oil, in 2008 and 2009, respectively (Table 2). Previous studies reported that total phenolic content of Ayvalık olive oils changes between 67.04 and 329.75 mg CAE/kg oil [2,31,32], and of Memecik olive oils between 106.89 and 330.29 mg CAE/kg oil [2,31]. In both years, total phenolic content increased with leaf addition (P<0.001), which is in agreement with previously reported by Giovacchino et al. [26]. Adding 1% and 3% leaves to the olives before extraction increased the total phenolic content by 4% and 9% in 2008 and, 12% and 22% in 2009 compared to control, respectively. Paiva-Martins et al. [11] suggested the addition of olive leaf extract to refined olive oil to improve the stability which was lost during refining. Olive leaves can also be added to fruits directly during crushing to increase the total phenolic content of oils [33,34]. The total phenolic content of oils was significantly decreased during 18 months storage, in both years (P<0.001). The total phenolic content decreased 4-6% in 6 months, 20-32% in 12 months and 47-57% in 18 months. Several authors reported that during storage period total phenolic content decreased because of degradation [27,28,35]. When the effect of interaction between storage period and leaf addition was considered, in both years a declining trend was observed for total phenolic content, although this was not statistically significant.

α-Tocopherol content

Tocopherols are lipophylic phenolic compounds with strong antioxidants properties found in olive oil [2]. In addition to phenols, tocopherols also contribute to the oxidative stability of olive oil [4]. In this research, α-tocopherol content of Ayvalık and Memecik olive oils were found to differ. The α-tocopherol content of Ayvalık olive oil was 148.83 and 127.30 mg/kg, whereas of Memecik olive oil was 335.46 and 293.61 mg/kg, in 2008 and 2009, respectively. The α-tocopherol content of Memecik olive oils were significantly (P<0.001) higher -more than two fold- than of Ayvalık olive oil (Table 3). Results are in agreement

Harvest Seasons			Storage Period (Months)				*P value*	LSD*
			0	6	12	18		
2008/09	Ayvalık	0%	69.27	40.90	34.41	21.70	-	n.s.
		1%	71.22	42.62	36.64	26.50		
		3%	75.64	45.51	44.03	31.37		
	Memecik	0%	109.29	71.52	53.47	44.77		
		1%	112.92	79.85	57.16	49.67		
		3%	114.51	82.34	58.10	51.19		
	Mean	0%	89.28	56.21	43.94	33.23	-	n.s.
		1%	92.07	61.24	46.9	38.09		
		3%	95.08	63.92	51.06	41.28		
		Ayvalık	72.04^{c}	43.01^{f}	38.36^{g}	26.52^{h}	<0.001	2.52
		Memecik	112.24^{a}	77.90^{b}	56.24^{d}	48.54^{e}		
	Mean		92.14^{a}	60.46^{b}	47.30^{c}	37.53^{d}	<0.001	1.9
		0%	55.67^{C}				<0.001	1.8
		1%	59.57^{B}					
		3%	62.84^{A}					
		Ayvalık	44.99^{B}				<0.001	1.17
		Memecik	73.73^{A}					
2009/10	Ayvalık	0%	44.73	35.94	22.30	15.89	-	n.s.
		1%	47.08	37.18	29.36	20.83		
		3%	53.12	45.22	36.64	29.85		
	Memecik	0%	65.96	57.77	48.41	33.98		
		1%	72.35	62.55	53.94	42.65		
		3%	74.34	68.56	57.55	48.58		
	Mean	0%	55.34	46.86	35.36	24.93	-	n.s.
		1%	59.71	49.87	41.65	31.74		
		3%	63.73	56.89	47.09	39.22		
		Ayvalık	48.31	39.45	29.43	22.19	-	n.s.
		Memecik	70.88	62.96	53.3	41.74		
	Mean		59.60^{a}	51.20^{b}	41.37^{c}	31.96^{d}	<0.001	1.61
		0%	40.62^{C}				<0.001	1.34
		1%	45.74^{B}					
		3%	51.73^{A}					
		Ayvalık	34.84^{B}				<0.001	1.67
		Memecik	57.22^{A}					

*Least Significant Difference (α=0.05); Values in the same column with different uppercase letters. and in the same row with lowercase letters show statistically significant differences.
n. s.: not significant.

Table 5: Changes in ABTS$^{\bullet+}$ RSA (μ mol TE/100 g) Ayvalık and Memecik olive oils obtained with different leaf additions during 2008/09 and 2009/10 harvest seasons.

with different researchers [2,31,32]. Leaf addition significantly increased α-tocopherol content in both years (P<0.001). By 3% olive leaf addition, α-tocopherol content increased 8% and 16%, in 2008 and 2009, respectively. Malheiro et al. [6] reported that α-tocopherol content increased nearly 15% in 2009 and 30% in 2010 with 10% leaf addition. This increase is because of the α-tocopherol content of olive leaves. Due to its nutritional value, olive leaves are considered as an alternative source of antioxidant. A significant decrease observed in α-tocopherol content of oils during storage period in both years (P<0.001). Tocopherols are known to act as antioxidants and protect the oils from oxidation [6,35], therefore the loss of tocopherols during storage is expected. When the interaction between storage period and leaf percentage is considered, it can be seen that the α-tocopherol content of all oils decreased significantly during the storage period, in 2009. The α-tocopherol content of 3% leaf added oils was 38% higher than the control oil at the end of 18 months (P<0.001) in 2009. The antioxidant compounds in olive leaf probably prevented the loss of α-tocopherol in oil, hence the highest α-tocopherol content at the end of storage period was obtained in 3% leaf added oils, in 2009. Some researchers have estimated the contribution of minor components to olive oil stability is estimated as follows; phenolic content being about 30%, fatty acids 27%, α-tocopherol content 11% and carotenoids 6% [27].

DPPH$^{\bullet}$ radical scavenging activity (RSA)

In our study, DPPH$^{\bullet}$ radical scavenging activity (RSA) of Memecik olive oils were significantly higher than Ayvalık olive oil (P<0.001). DPPH$^{\bullet}$ radical scavenging activity of Ayvalık olive oils were 33.36 and 28.47 μ mol TE/100 g, Memecik olive oils were 82.18 and 76.29 μ mol TE/100 g, in 2008 and 2009, respectively (Table 4). Kıralan and Bayrak [33] reported similar results regarding the DPPH$^{\bullet}$ RSA of Memecik olive oils. With the increasing amount of olive leaves added DPPH$^{\bullet}$ RSA increased significantly in both years (P<0.001). This increase may have been the results of the increased chlorophyll, phenolic compounds and tocopherol content (Tables 1-3). Different researchers reported that DPPH antioxidant activity of olive oils influenced by chlorophyll, total phenols and α-tocopherol content of oils [18,36,37]. During storage, a significiant decrease was determined in DPPH$^{\bullet}$ RSA

of oils, from 79.90 to 39.40 µ mol TE/100 g and from 74.53 to 31.56 µ mol TE/100 g, in 2008 and 2009, respectively (P<0.001). This reduction is expected because of the decrease in antioxidant compounds, such as chlorophylls, phenols and α-tocopherol as a result of degradation (Table 1-3). Lavelli [17] reported taht the antioxidant activity is related to the degradation level of oils, fresh oils were 3-5 times more efficient than old oils. When we look at the storage period and leaf percentage interaction, we determined that the DPPH• RSA of oils was decreased significantly in 2008 and 2009 (P<0.01 and $P \leq 0.001$). However, at the end of the 18-months storage the highest DPPH• RSA was observed in olive oils with 3% leaves as 50.67 and 35.83 µ mol TE/100 g, in 2008 and 2009, respectively. Although total phenolics in both years and tocopherol content in 2008 did not declined significantly during storage, the greater reduction in chlorophyll content may account for the lower DPPH• RSA (Table 1-3). Endo et al. [36] reported that chlorophyll did not decompose the hydroperoxides, but decreased free radicals such as DPPH. Therefore olive leaves can be considered as a potential antioxidant of natural origin to prolong the shelf life of food products [7].

ABTS•+ radical scavenging activity (RSA)

In this research, ABTS•+ radical scavenging activity (RSA) of Memecik olive oils were significantly higher than Ayvalık olive oil, 64 % in both years (P<0.001) (Table 5). With the addition of olive leaves ABTS•+ RSA was significantly increased both years (P<0.001). By adding 3 % leaf, ABTS•+ RSA increased 13 % and 27 %, in 2008 and 2009, respectively, due to the increase in chlorophyll, total phenol and α-tocopherol contents. ABTS antioxidant activity was positively affected from total phenol content [19,37,38] and α-tocopherol content [38] of oils. Our results suggests that ABTS•+ RSA is affected by chlorophyll content more compared to α-tocopherol and total phenolic content. During storage, a significiant decrease was determined in ABTS•+ RSA of oils, from 92.14 to 37.53 µ mol TE/100 g and from 59.60 to 31.96 µ mol TE/100 g, in 2008 and 2009, respectively (P<0.001). The storage period leaf percentage interaction was not found statistically significant.

Conclusions

In our research, antioxidant content and antioxidant activity of Memecik olive oil was found to be higher than Ayvalık olive oil. Memecik olive oils had better antioxidant properties than Ayvalık olive oil. Differences between years were observed in the minor components of olive oils. The arid conditions during in 2008 season, which probably increased the antioxidant content, may be responsible for this difference. Leaf addition improved the chlorophyll content, total phenolic content, α-tocopherol content, and DPPH• and ABTS•+ radical scavenging activity. During storage, chlorophyll content, total phenolic content, α-tocopherol content, and DPPH• and ABTS•+ radical scavenging activity of olive oils decreased. At the end of 18-month storage period, 3% leaf added oils had considerably higher chlorophyll content and DPPH• radical scavenging activity compared to control (no leaf-added) oils. The obtained results showed that it is possible to increase the antioxidant properties of olive oil by adding olive leaf addition.

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