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Comparative study of phenolic compounds in olive oils from different geographic regions

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Abstract: In this study, the effect of cultivar, harvest year, and the geographic regions (Mediterranean, Aegean, Southeastern Anatolia, Marmara, and the Black Sea) on phenolic compounds of virgin olive oils were investigated. Tyrosol was the major phenolic compound in the Mediterranean, Southeastern, and Black Sea regions. Hydroxytyrosol showed the highest value (2.07 mg/kg) in the Southeastern Anatolia region and the lowest value (0.75 mg/kg) in the Marmara region. Among the phenolic acids, vanillic acid had an important place in phenolic compounds ($p < 0.01$). This phenolic acid gave higher values in the Marmara region compared to other regions. Flavonoids were determined as important groups of phenolic compounds in olive oils. The oils from the Southeastern Anatolia region separated from other oils with the highest levels of flavonoids such as quercetin, luteolin, and apigenin. All phenolic compounds, except transcinnamic acid and quercetin, increased in the second harvest year. Vanillic acid was the highest phenolic compound in the first year, in contrast, tyrosol had higher values than vanillic acid in the second year. Many phenolic compounds were affected by olive cultivar. Memecik has a higher content of tyrosol, vanillic acid, p-coumaric acid, ferulic acid, and quercetin in the Aegean region. However, this variety showed higher values for hydroxytyrosol, transcinnamic acid, luteolin, and apigenin in the Marmara region. Gemlik, Ayvalık, Çelebi, Domat and Memecik from Marmara regions had higher vanillic acid. Butko, Otur and Kızıl Satı showed higher tyrosol. Principal component analysis showed that phenolic compounds could play an important role in the separation of regions and cultivars.

Key words: Olive oil, phenolic compound, geographic region, cultivar

1. Introduction

Olive oil is an important type of oil obtained by pressing the olive fruit without using any chemicals (Cicerale et al., 2009), consisting of 90%–99% triacylglycerols and 0.5%–1% nonglyceride compounds (Tuck and Hayball, 2002). In recent years, there has been an increasing number of studies into the beneficial properties of olive oil and it is stated that olive oil is used as the main source of oil, particularly in the Mediterranean diet. These beneficial properties of olive oil are generally thought to be due to the high percentage (70%–80%) of monounsaturated fatty acids (Tripoli et al., 2005) and the content of many minor compounds such as tocopherols, sterols, and phenolic compounds (Kristakis, 1998; Cicerale et al., 2009).

Olive oil contains approximately 36 phenolic compounds and these compounds are divided into different groups as phenolic acids, phenolic alcohols, secoiridoids, hydroxy-isochromans, flavonoids, and lignans according to their chemical structures (Cicerale et al.,2009). These phenolic compounds protect olive oil from oxidative rancidity due to their strong antioxidant activity and radical scavenging properties (Tuck and Hayball, 2002). The phenolic content of the olive oil varies depending on many factors such as cultivar of olive (Caravaca et al., 2005; Vinha et al., 2005; Cicerale et al., 2010; Arslan and Schreiner, 2012; Del Monaco et al., 2015; Uluata et al., 2016), olives are grown in (Vinha et al. 2005; İlyasoğlu et al., 2010; Alkan et al., 2012; Yorulmaz et al., 2012; Kesen et al., 2014), olive growing technique (Romero et al., 2002; Gomez-Rico et al., 2006), the degree of ripening of the olive (Esti et al., 1998; Brenes et al. 1999; Kalua et al., 2005) and olive oil processing methods (Brenes et al., 2001; Gimeno et al. 2002; Fregapane et al., 2006).

Turkey, an important producer of olive oil in the world with ranks fifth in the world in the 2019/2020 season, with 225,000 tons of olive oil production (International Olive Council, 2021), has about 80 olive varieties and some of which are used in table olives and some are used in olive oil production. Although it is a major olive oil producer with wide geography where different olive cultivars are grown, there is little information about the characterization of olive oil in Turkey. Olive growing is concentrated around

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the Aegean, Marmara and South-eastern Anatolia and Black Sea regions of Turkey . Edremit (Ayvalık), Memecik and Gemlik are the predominant variety in this regions and are followed by other Turkish varietes such as Büyük Topak, Ulak, Çakır, Çekişte, Çelebi, Çilli, Domat, Edincik Su, Eğriburun, Erkence, Halhalı, İzmir Sofralık, Kalembezi, Kan Çelebi, Karamürsel Su, Kilis Yağlık, Kiraz, Manzanilla, Memeli, Nizip Yağlık, Samanlı, Sarı Haşebi, Sarı Ulak, Saurani, Taşan Yüreği, Uslu and Yağ Celebi (IOC, 2012). In the Black Sea region, there are local varieties with unique characteristics such as Butko, Görvele, Marantelli, Pastos, Otur, Satı, Salamuralık, Tuzlamalık and Yağlık (Şeker, 2012). The studies generally focused on quality parameters such as fatty acid composition, total phenol content, free fatty acid, peroxide number, and K values. On the other hand, the studies performed on phenolic compounds, which play an important role in the characterization of olive oil, have been generally focused on certain regions (Alkan et al., 2012; Yorulmaz et al., 2012; Kesen et al., 2014;) and varieties (Ocakoğlu et al., 2009; Kelebek et al., 2015; Arslan et al., 2013; Dağdelen et al., 2013; Kesen et al., 2013; Arslan and Schreiner, 2012; İlyasoğlu et al., 2010). This study aimed to characterize phenolic compounds in virgin olive oils from 5 different geographic regions of Turkey.

2. Materials and methods

2.1. Materials

In this study, the fruits of five olive cultivars were harvested from five different geographic regions (Black Sea, Southeastern Anatolia, Mediterranean, Aegean, and Marmara Regions) (Table 1). This experiment was repeated for two years, 2010 and 2011 in November. Climatic characteristics of studied area were given in Table 2. The olive fruits were processed to virgin olive oil in agricultural facilities of the regions, where the samples were taken. Virgin olive oil samples were produced by the three phase extraction system. A total of 500 mL of oil was taken from each sample, put into brown glass bottles, and kept in dark until the analysis.

Table 1. Olive cultivars from different geographic regions.

Geographic regions					
Cultivar	**Mediterranean**	**Aegean**	**Southeastern Anatolia**	**Marmara**	**Black Sea**
	Gemlik	Memecik	Nizip Yağlık	Gemlik	Butko
	Ayvalık	Domat	Ayvalık	Ayvalık	Otur
	Saurani	Uslu	Kilis Yağlık	Çelebi	Görvele
	Haşebi	Gemlik	Halhalı	Domat	Satı
	Sarı Ulak	Erkence	Karamani	Memecik	Kızıl Satı

Table 2. Climatic characteristics of studied area (MGM, 2022).

			The yearly mean of rainfall (mm)		**The yearly mean of temperature (°C)**		**The yearly mean of humidity (%)**	
Geographic regions	**Cultivar**	**City**	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**
Mediterranean	Gemlik	Antalya	74.16	77.16	19.57	19.93	57.01	58.60
	Ayvalık	Osmaniye	75.75	74.89	17.95	18.85	65.00	62.26
	Saurani, Haşebi	Antakya	87.81	120.38	18.16	18.95	65.97	64.83
	Sarı Ulak	Adana	59.97	88.78	18.95	19.45	63.08	64.04
Aegean	Memecik, Erkence	İzmir	46.80	66.58	17.72	18.63	55.43	58.54
	Domat, Uslu	Manisa	51.63	57.08	15.92	17.48	54.33	52.45
	Gemlik	Aydın	45.23	67.51	17.02	18.36	62.17	60.61
Southeastern Anatolia	Nizip Yağlık, Kilis Yağlık, Ayvalık	Gaziantep	52.94	72.07	15.39	15.94	52.96	53.48
	Halhalı, Karamani	Adıyaman	64.79	96.50	16.64	17.46	54.94	54.43
Marmara	Gemlik, Çelebi, Memecik	Bursa	48.22	67.54	13.89	15.28	71.9	69.34
	Ayvalık, Domat	Balıkesir	46.38	54.21	13.43	14.93	68.19	69.12
Black Sea	Butko, Otur, Görvele, Satı, Kızıl Satı	Artvin	74.32	57.28	11.39	13.02	69.6	63.59

2.2. Phenolic compound analysis

The extraction procedure of the phenolic compounds from olive oil samples was performed according to the Brenes et al. (1999). A total of 14 g of olive oil sample was weighed and 14 mL of methanol/water mixing (80:20) was added into. Gallic acid was added as an internal standard. After that, the sample was mixed and centrifuged. After the addition of 14 mL of methanol/water solution, homogenization and centrifugation was repeated three times. The supernatant was evaporated to remove the methanol and taken up to 15 mL of acetonitrile. After that, the residue was washed three times with 20 mL each of hexane. Acetonitrile was evaporated under a vacuum. The extract was dissolved in 1 mL of methanol/water. The sample was filtered through a 0.45-µm membrane filter and transposed into a vial. The extract was stored at –20 °C until HPLC analysis.

Phenolic compounds of samples were determined by HPLC (Agilent 1100 series, Germany) with an Ace 5 C18 (5 µm, 25 cm × 4.6mm, Aberdeen, Scotland) column. A total of 20 µL of the extract was injected into the HPLC system. Separation was achieved by elution gradient using a composition of 90% water and 10% methanol. The concentration of methanol was increased to 30% in 10 min and maintained for 20 min. Subsequently, the methanol percentage was raised to 40% in 10 min, maintained for 5 min, increased to 50% in 5 min. Finally, the methanol percentage was increased to 60%, 70%, 80%, and 90% in 5 min periods at the end of 55 min. The flow rate was 1 mL/min. The column temperature was kept at 35 °C. Chromatograms were determined at 254 nm. Standards of phenolic compounds were used for the preparation of a standard curve. A series of various concentrations of 1–30 mg/L standard solutions containing standard mix were spiked into samples, six replicates at each level. Mean recoveries, relative standard deviations (RSD), coefficient of regression line (R2), the limits of detection (LOD) and limit of quantification (LOQ) were given in Table 3. The limits of detection (LOD) and limit of quantification (LOQ) were calculated by applying different dilutions (1, 3, 5, 10, 20, and 30 mg/kg) of standard solution and calculated using formula: LOD = 3.3 × Sy/s and LOQ = 10 × Sy/s (Sy: the standard deviation of the response of the curve, s: the slope of the calibration curve). Phenolic compound contents were expressed as mg/kg.

2.3. Statistical analysis

The research was established and conducted based on the nested classification model considering 5 different regions, 5 varieties from each region, and 2 harvest seasons. The research data were subjected to two-way ANOVA through packaged software (IBM SPSS Statistics 20) and the variation sources that were found important were compared with Duncan's multiple comparison test. The variation sources that were found important were compared with Duncan's multiple comparison test. The relationship between cultivar and phenolic compounds as regions was also evaluated by principal component analysis (PCA) using Unscrambler v10.01 (Como Process, A. S., Norway).

3. Results and discussion

The effect of geographic region on phenolic compounds were given in Table 4. Significant differences between geographic regions were determined in terms of phenolic compounds of olive oils. Tyrosol was the major phenolic compound in the Mediterranean, Southeastern, and Black Sea regions. This compound also showed higher values than many phenolic compounds in other regions (Table 4). Another important phenolic alcohol, hydroxytyrosol, which is a characteristic olive oil phenolic compound with

Table 3. Validation parameters of HPLC method for phenolic compounds.

Phenolic compound	RT	LOD (mg/L)	LOQ (mg/L)	RSD% (n = 6) (1 mg/kg)	Recovery	R^2
Hydroxytyrosol	8.15	0.57	1.72	4.88	99.50	0.9999
Tyrosol	11.14	0.92	2.79	4.46	103.33	0.9998
Vanillic acid	13.29	0.45	1.35	4.61	100.67	0.0999
p-coumaric acid	15.06	0.59	1.81	4.11	100.83	0.9999
Ferulic acid	17.46	0.69	1.77	4.04	101.00	0.9999
Transcinnamic acid	20.00	0.68	2.05	3.57	99.33	0.9999
Quercetin	34.40	0.64	1.95	5.10	100.83	0.9999
Luteolin	35.78	0.53	1.62	3.44	97.50	0.9999
Apigenin	38.89	0.69	2.08	1.46	94.33	0.9999

RT; retention time (min), LOD; limit of detection, LOQ; limit of quantification; RSD%; % relative standard deviation.

Table 4. The comparison of phenolic compounds of olive oil (mg/kg) which growing in different regions (mean ± SD).

	Geographic region					
Phenolic compound(mg/kg)	**Mediterranean**	**Aegean**	**Southeastern Anatolia**	**Marmara**	**Black Sea**	**p value**
Hydroxytyrosol	1.74 ± 1.51a	1.81 ± 1.33a	2.07 ± 1.09a	0.75 ± 0.45b	2.02 ± 1.31a	< 0.01
Tyrosol	4.68 ± 5.88a	3.13 ± 2.31a	3.80 ± 2.37a	2.51 ± 1.30a	2.81 ± 0.67a	> 0.05
Vanillic acid	1.88 ± 1.15c	3.96 ± 3.01b	3.68 ± 3.11b	7.01 ± 3.01a	1.87 ± 1.13c	< 0.01
p-coumaric acid	0.20 ± 0.36a	0.13 ± 0.27a	0.11 ± 0.22a	0.13 ± 0.15a	0.00 ± 0.00a	> 0.05
Ferulic acid	1.11 ± 0.13abc	1.08 ± 0.07bc	1.04 ± 0.04c	1.20 ± 0.33a	1.18 ± 0.21ab	< 0.05
Transcinnamic acid	0.41 ± 0.20c	0.55 ± 0.54c	1.36 ± 0.73a	0.44 ± 0.12c	0.99 ± 0.76b	< 0.01
Quercetin	1.07 ± 0.17a	1.06 ± 0.26a	1.09 ± 0.18a	0.92 ± 0.06b	1.04 ± 0.21a	< 0.05
Luteolin	1.83 ± 0.75b	2.23 ± 0.78b	3.30 ± 1.38a	2.93 ± 0.97a	1.95 ± 0.91b	< 0.01
Apigenin	0.77 ± 0.43b	0.25 ± 0.29cd	1.17 ± 0.75a	0.41 ± 0.57c	0.09 ± 0.12d	< 0.01

a–b: Any means in the same line having the same letters are not significantly different ($p > 0.05$).
SD; standard deviation, $p < 0.01$; very significant, $p < 0.05$; significant, $p > 0.05$; not significant.

tyrosol present in olive oils as free or conjugated forms as secoroids or aglycones (E Miro´-Casas et al., 2003), showed the highest value (2.07 mg/kg) in Southeastern Anatolia region and lowest value (0.75 mg/kg) in Marmara region. In previously studies, tyrosol and hyroxytyrosol were found as major phenolic compounds in olive oils from different cultivars of the Aegean region (İlyasoğlu et al., 2010; Alkan et al., 2012; Uncu and Ozen, 2016). Vanillic, p-coumaric, ferulic, and transcinnamic acid were detected as phenolic acids in all olive oil samples. Among these phenolic acids, vanillic acid had an important place in phenolic compounds ($p < 0.01$), changing between 1.88 and 7.01 mg/kg. This phenolic acid gave higher values in the Marmara region compared to other regions, which also made up the highest value of the total phenol content in all regions. In addition, the results showed that among the 4 phenolic acids, transcinnamic acid was identified as another important compounds after vanillic acid and had the highest value in the region of Southeast Anatolia as hydroxytyrosol. Flavonoids such as quercetin, luteolin, and apigenin are important groups of phenolic compounds found in olive oils. The oils from the Southeastern Anatolia region separated from other oils with the highest levels of these flavonoids. These differences between olive oil phenolic compounds could be related to the degree of olive ripening, olive cultivar, climatic conditions, harvesting time, and extraction system (Condelli et al., 2015).

Harvest year is another factor that affects some quality characteristics of olive oil. As seen in Table 5, the harvest year factor had very significant effects ($p < 0.01$) on tyrosol, hydroxytyrosol, vanillic acid, and ferulic acid content of oils. All phenolic compounds, except transcinnamic acid and quercetin, increased in the second harvest year. In the second year, the temperature and rainfall in the studied regions were at higher levels compared to the first year. It is estimated that the increase in phenolic compounds in the second year may be due to these differences (Table 2). In the first year, vanillic acid was the highest phenolic compound with 3.08 mg/kg followed by tyrosol with 2.45 mg/kg, in contrast in the second year tyrosol had higher values than vanillic acid with 4.32 and 4.28 mg/kg respectively. Similar results were also found by Kelebek et al., (2015) in the same harvest years. In studies to determine the changes in phenolic compounds in different

Table 5. The effect of the harvest year on the phenolic compounds of olive oil (mean ± SD).

Phenolic compound (mg/kg)	**Harvest year***		
	First	**Second**	**p value**
Hydroxytyrosol	1.35 ± 1.11b	2.01 ± 1.38a	< 0.01
Tyrosol	2.45 ± 11.14b	4.32 ± 4.08a	< 0.01
Vanillic acid	3.08 ± 2.29b	4.28 ± 3.60a	< 0.01
p-coumaric acid	0.09 ± 0.17a	0.13 ± 0.29a	> 0.05
Ferulic acid	1.06 ± 0.08b	1.18 ± 0.25a	< 0.01
Transcinnamic acid	0.78 ± 0.71a	0.72 ± 0.59a	> 0.05
Quercetin	1.05 ± 0.24a	1.03 ± 0.14a	> 0.05
Luteolin	2.29 ± 1.12a	2.60 ± 1.12a	> 0.05
Apigenin	0.46 ± 0.51a	0.62 ± 0.69a	> 0.05

*First: 2010 harvest year, Second: 2011 harvest year.
a–b: Any means in the same line having the same letters are not significantly different ($p > 0.05$).
SD; standard deviation, $p < 0.01$; very significant, $p < 0.05$; significant, $p > 0.05$; not significant.

harvest years in oils of the Ayvalık cultivar (Andjelkovic et al., 2009) and in oil from the Aegean Sea (Alkan et al., 2012), the researchers found higher levels of tyrosol and hydroxytyrosol in samples from 2005 compared to 2006. However, Ocakoğlu et al. (2009) found that some olive oils from different cultivars showed higher values in the 2006 harvest year than in 2005.

The sensory properties and stability of olive oil are influenced by phenolic compounds, and cultivars of olives differ in terms of phenolic compounds (Kelebek et al., 2015). The influence of cultivar on the determination of phenolic compounds detected in olive oils obtained from different regions from Turkey was given in Table 6. According to the results (Table 6), there were wide variations and significant differences between the phenolic compounds of olive oils from different varieties in different regions. In the Mediterranean region, extra virgin olive oils from cultivar Gemlik (Mediterranean), which was the important olive cultivar of Turkey, showed the highest quantities of tyrosol (10.23 mg/kg) and hydroxytyrosol (3.53 mg/kg). However, this variety showed lower values for these two compounds in the Aegean and Marmara regions than in the Mediterranean. It is thought that this result is due to climatic parameters such as temperature and rainfall in the cities where the Gemlik variety was examined (Table 2). As can be seen from Table 2, in both harvest years, Antalya province was higher than Bursa and Aydın provinces in terms of temperature and rainfall parameters, but the opposite was true in terms of humidity level. Besides, cultivar Gemlik from the Aegean and Marmara regions showed the highest value for vanillic acid. The results showed that among regions vanillic acid had higher values than other phenolic compounds in most of the cultivars (Table 6). The highest phenolic compound in oils from Ayvalık, another important olive cultivar, was tyrosol in the Mediterranean region, while it was vanillic acid in South-eastern Anatolia and Marmara regions. The tyrosol value of Ayvalık samples was followed by luteolin in Southeastern Anatolia and Marmara regions. In the previous studies, it was reported that the contents of these two compounds, hydroxytyrosol, and tyrosol were between 0.21 and 21.39 mg/kg, 0.4 and 9.13 mg/kg (Alkan et al., 2012; Andjelkovic et al., 2009; İlyasoğlu et al., 2010; Ocakoğlu et al., 2009; Yorulmaz et al., 2012) for Ayvalık cultivar. In our study, the contents of these compounds ranged from 0.93–2.44 to 1.42–7.19 mg/kg. Compared to the previous studies, differences were observed in phenolic compounds from the different cultivars from different geographic regions. Andjelkovic et al. (2009) found that the major phenolic compound was pinoresinol followed by luteolin in oils of Ayvalık cultivar from Çanakkale similar to the contents determined in the present study. Moreover, tyrosol, hydroxytyrosol, and elenolic acids were dominant phenolic compounds in all virgin olive oils from Gemlik, Ayvalık, and Memecik cultivar (Kelebek et al., 2015) while pinoresinol was found as a major phenolic compound in Sarıulak cultivar by Arslan et al. (2013). In another study, luteolin and apigenin were determined as important phenolics after pinoresinol in all years and samples from Ayvalık variety (Andjelkovic et al., 2009). In Italian virgin olive oils from different cultivars, oleuropein, tyrosol and hydroxytyrosol were found as important phenolic compounds (del Monaco et al., 2015). On the other hand, tyrosol (4.45–155 mg/kg), pinoresinol (2.9–23 mg/kg), hydroxytyrosol (5.7–38 mg/kg), and luteolin (1.8–2.2 mg/kg) were determined as phenolic compounds in virgin olive oils from important olive cultivars of Brazil (Ballus et al., 2014).

In cultivars from the Aegean region, hydroxytyrosol was determined as an important phenolic compound and the highest value was determined in Uslu (3.02 mg/kg) followed by Domat (2.14 mg/kg) cultivar. Memecik has a higher content of tyrosol, vanillic acid, p-coumaric acid, ferulic acid, and quercetin in the Aegean region, in contrast, this variety showed higher values for hydroxytyrosol, transcinnamic acid, luteolin, and apigenin in the Marmara region. In Aegean olive oils, this cultivar was characterized with a high content of luteolin (Yorulmaz et al., 2012), tyrosol (Ocakoğlu et al., 2009; İlyasoğlu et al., 2010) and elenolic acid (Kelebek et al., 2015).

Kilis Yağlık and Nizip Yağlık are important olive cultivars belonging to the South Eastern Anatolia region with their oils balanced and fruity aroma (Kesen et al., 2014). Amounts of three flavonoids (luteolin, apigenin, and quercetin) were different in each oil sample from different cultivars. Luteolin was higher in Kilis yağlık (4.54 mg/kg) and Nizip Yağlık (4.37 mg/kg) than other cultivars (Table 6) in the Southeastern Anatolia region, while Karamani showed the lowest value (1.76 mg/kg). Yorulmaz et al. (2012) found luteolin as a major phenolic compound for Kilis Yağlık and Nizip Yağlık and higher values with 52.29 and 81.62 mg/kg than this research. The Halhalı variety showed higher values for tyrosol and hydroxytyrosol, in agreement with the results of Kesen et al. (2013). However, olive oils from Halhalı showed higher values for luteolin than other phenolic compounds (Yorulmaz et al., 2012).

Gemlik, Ayvalık, Çelebi, Domat and Memecik were important olive cultivars of Marmara regions. All oils from these cultivars had higher vanillic acid. While the virgin olive oils of Çelebi cultivar showed the lowest tyrosol and hydroxytyrosol value, the ferulic acid and quercetin were found higher than other cultivars in this region. In a study of the distribution of phenolic compounds in Turkish olive oils, the Çelebi variety had higher luteolin levels (504.46 mg/kg) than other varieties that changed luteolin levels between 0.57 and 372.12 mg/kg (Yorulmaz et al., 2012).

Table 6. The interaction effect of geographic regions and cultivars on fatty acid compositions (mean ± SD).

Region	Cultivar	Phenolic compound (mg/kg)								
		Hydroxytyrosol	Tyrosol	Vanillic acid	p-coumaric acid	Ferulic acid	Transcinnamic acid	Quercetin	Luteolin	Apigenin
Mediterraean	Gemlik	3.51 ± 1.64a	10.23 ± 9.45a	1.05 ± 0.10c	0.61 ± 0.48a	1.11 ± 0.06a	0.61 ± 0.25a	1.03 ± 0.09b	1.52 ± 0.56a	0.58 ± 0.49a
	Ayvalık	2.44 ± 1.69a	7.19 ± 7.02ab	1.01 ± 0.17c	0.00 ± 0.00b	1.16 ± 0.21a	0.42 ± 0.06a	0.91 ± 0.06b	1.65 ± 0.64a	0.81 ± 0.78a
	Saurani	0.87 ± 0.31b	2.06 ± 1.17b	1.61 ± 0.76bc	0.00 ± 0.00b	1.00 ± 0.02a	0.36 ± 0.01a	0.95 ± 0.03b	1.54 ± 0.28a	0.65 ± 0.22a
	Haşebi	1.27 ± 0.94b	1.80 ± 0.36b	2.52 ± 1.44ab	0.37 ± 0.43ab	1.12 ± 0.13a	0.30 ± 0.35a	1.24 ± 0.10a	2.40 ± 1.22a	0.91 ± 0.35a
	Sarı Ulak	0.62 ± 0.23b	2.12 ± 0.49b	3.20 ± 0.86a	0.00 ± 0.00b	1.18 ± 0.13a	0.37 ± 0.01a	1.23 ± 0.21a	2.03 ± 0.74a	0.92 ± 0.22a
	p value	< 0.01	< 0.05	< 0.01	< 0.01	> 0.05	> 0.05	< 0.01	> 0.05	> 0.05
Aegean	Memecik	0.56 ± 0.28c	2.37 ± 2.04a	4.43 ± 3.70ab	0.41 ± 0.47a	1.15 ± 0.10a	0.38 ± 0.01b	0.97 ± 0.19a	2.24 ± 1.42ab	0.04 ± 0.02b
	Domat	2.14 ± 1.68b	4.65 ± 4.05a	2.28 ± 0.25b	0.02 ± 0.03b	1.05 ± 0.06b	0.37 ± 0.01b	0.98 ± 0.05a	1.62 ± 0.17b	0.22 ± 0.15b
	Uslu	3.02 ± 1.35a	3.19 ± 1.83a	3.44 ± 1.77ab	0.00 ± 0.00b	1.10 ± 0.04ab	0.39 ± 0.03b	1.24 ± 0.22a	2.44 ± 0.43ab	0.19 ± 0.02b
	Gemlik	1.71 ± 0.94b	3.14 ± 2.95a	6.90 ± 4.72a	0.00 ± 0.00b	1.04 ± 0.02b	0.37 ± 0.01b	0.98 ± 0.07a	2.96 ± 0.25a	0.58 ± 0.54a
	Erkence	1.65 ± 1.17b	2.28 ± 0.73a	2.73 ± 0.32ab	0.20 ± 0.23ab	1.05 ± 0.05b	1.24 ± 1.02a	1.20 ± 0.53a	1.87 ± 0.33b	0.23 ± 0.04b
	p value	< 0.01	> 0.05	> 0.05	< 0.05	< 0.05	< 0.05	> 0.05	< 0.05	< 0.05
Southeastern Anatolia	Nizip Yağlık	2.40 ± 0.95a	4.89 ± 2.43a	6.44 ± 5.54a	0.34 ± 0.40a	1.07 ± 0.05a	2.10 ± 0.08a	0.99 ± 0.18bc	4.37 ± 1.46a	1.61 ± 0.80a
	Ayvalık	2.42 ± 0.30a	1.42 ± 0.16b	5.26 ± 1.56ab	0.01 ± 0.02a	1.02 ± 0.01a	2.26 ± 0.08a	1.25 ± 0.20a	2.64 ± 0.83b	1.48 ± 0.86a
	Kilis Yağlık	2.01 ± 0.70a	4.62 ± 2.95a	1.51 ± 0.20c	0.17 ± 0.19a	1.00 ± 0.01a	1.03 ± 0.09b	1.09 ± 0.11abc	4.54 ± 1.11a	1.12 ± 0.87a
	Halhalı	2.70 ± 1.74a	5.31 ± 2.40a	2.31 ± 0.82bc	0.05 ± 0.11a	1.03 ± 0.01a	0.63 ± 0.17c	1.20 ± 0.05ab	3.20 ± 0.72ab	0.82 ± 0.74a
	Karamani	0.83 ± 0.40b	2.78 ± 0.93ab	2.89 ± 2.05bc	0.00 ± 0.00a	1.06 ± 0.07a	0.78 ± 0.48bc	0.91 ± 0.11c	1.76 ± 0.28b	0.81 ± 0.34a
	p value	< 0.01	< 0.05	< 0.05	> 0.05	> 0.05	< 0.01	< 0.05	< 0.01	> 0.05
Marmara	Gemlik	0.39 ± 0.06c	3.11 ± 1.95a	8.01 ± 0.61b	0.07 ± 0.01a	1.12 ± 0.11a	0.37 ± 0.02a	0.84 ± 0.06b	1.84 ± 0.51c	0.39 ± 0.35a
	Ayvalık	0.93 ± 0.45a	2.67 ± 1.52a	11.37 ± 1.64a	0.12 ± 0.14a	1.12 ± 0.06a	0.46 ± 0.09a	0.95 ± 0.03a	3.92 ± 1.41a	0.91 ± 1.18a
	Çelebi	0.51 ± 0.36bc	2.05 ± 0.70a	5.34 ± 2.17cd	0.10 ± 0.12a	1.58 ± 0.62a	0.37 ± 0.01a	0.96 ± 0.05a	2.75 ± 0.28b	0.30 ± 0.16a
	Domat	1.14 ± 0.67a	2.52 ± 1.45a	6.39 ± 2.27c	0.18 ± 0.21a	1.06 ± 0.09a	0.42 ± 0.04a	0.92 ± 0.04ab	3.07 ± 0.66b	0.21 ± 0.14a
	Memecik	0.78 ± 0.18ab	2.22 ± 1.09a	3.94 ± 1.00d	0.17 ± 0.20a	1.14 ± 0.16a	0.57 ± 0.21a	0.92 ± 0.06ab	3.05 ± 0.44b	0.26 ± 0.13a
	p value	< 0.01	> 0.05	< 0.01	> 0.05	> 0.05	> 0.05	< 0.05	< 0.01	> 0.05
Black Sea	Butko	3.57 ± 0.99a	3.77 ± 0.61a	1.15 ± 0.11b	0.00 ± 0.00a	1.21 ± 0.19a	1.10 ± 0.85ab	0.90 ± 0.16b	1.22 ± 0.14b	0.04 ± 0.09a
	Otur	2.63 ± 1.50ab	2.74 ± 0.70bc	1.56 ± 0.65b	0.00 ± 0.00a	1.01 ± 0.02a	1.52 ± 0.73a	0.90 ± 0.10b	2.11 ± 1.34a	0.03 ± 0.05a
	Görvele	2.05 ± 0.74bc	2.42 ± 0.16bc	2.34 ± 0.53ab	0.00 ± 0.00a	1.19 ± 0.10a	1.49 ± 0.90a	1.21 ± 0.04a	2.70 ± 0.15a	0.04 ± 0.04a
	Satı	0.77 ± 0.43d	2.30 ± 0.31c	2.81 ± 2.16a	0.00 ± 0.00a	1.19 ± 0.13a	0.48 ± 0.14b	1.27 ± 0.25a	2.48 ± 0.91a	0.18 ± 0.18a
	Kızıl satı	1.07 ± 0.11cd	2.80 ± 0.21b	1.51 ± 0.57b	0.00 ± 0.00a	1.30 ± 0.41a	0.38 ± 0.03b	0.95 ± 0.03b	1.25 ± 0.08b	0.17 ± 0.13a
	p value	< 0.01	< 0.01	< 0.05	> 0.05	> 0.05	< 0.01	< 0.01	< 0.01	> 0.05

a–d: Any means in the same column having the same letters are not significantly different ($p > 0.05$).
SD; standard deviation, $p < 0.01$; very significant, $p < 0.05$; significant, $p > 0.05$; not significant.

The Black Sea region, especially the Çoruh Valley, is an important area for olive cultivation, as varieties such as Butko, Satı, Kızıl Satı, Görvele, and Otur are rarely found (Ercişli, 2009). Phenolic compounds of these cultivars were firstly researched in the present study. Butko, Otur, Kızıl Satı showed higher tyrosol, in contrast, Görvele showed the highest value of luteolin. Satı was discriminated from other cultivars with the highest value of vanillic acid (28.11 mg/kg), quercetin (12.67 mg/kg), and apigenin (1.82 mg/kg).

Principal component analysis (PCA) was used to qualitatively differentiate between factors region and phenolic compounds (Figure 1) as well as cultivar and phenolic compounds (Figure 2). The PC1 explained 82% of the variation, while PC2 provided 11% of the variation (Supplementary material 1a and 1b). Marmara and Aegean regions were positively correlated with PC1 and placed at the same side with luteolin, vanillic acid, and p-coumaric acid. In contrast, the Mediterranean, Black Sea, and Southeastern Anatolia regions showed a negative

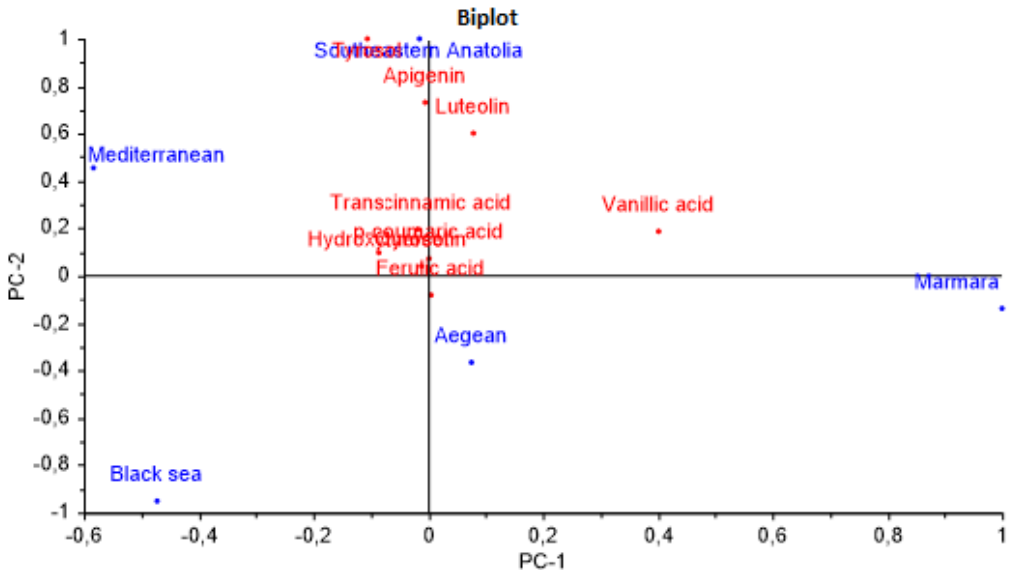

Figure 1. Biplot obtained from PCA of the relationships between the regions and phenolic compounds.

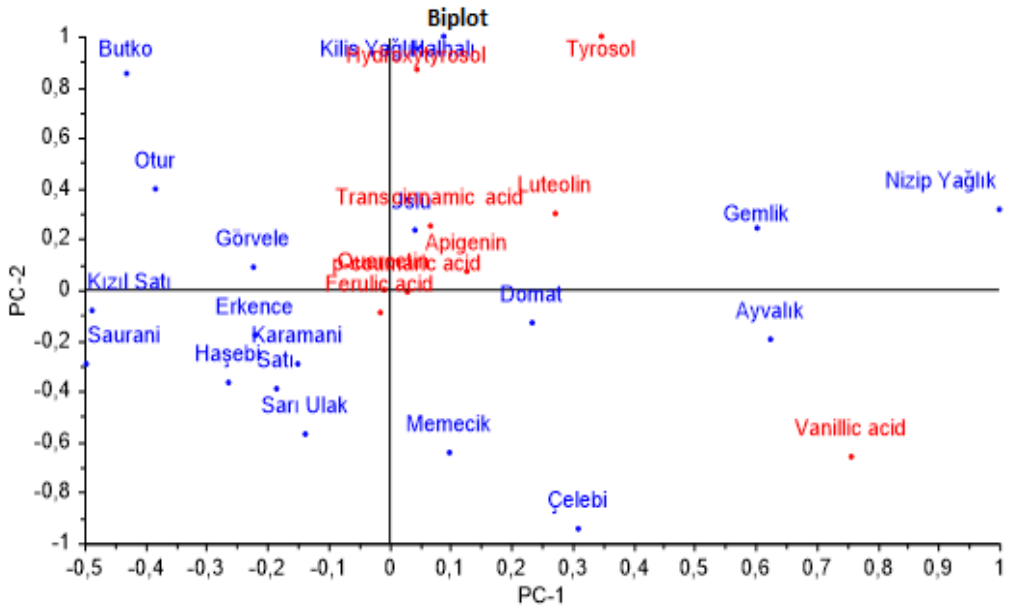

Figure 2. Biplot obtained from PCA of the relationships between the cultivars and phenolic compounds.

correlation with PC1 and located in PC2. On the other hand, in PC2 Southeastern Anatolia and the Black Sea placed positive side and Marmara and Aegean regions placed negative side. However, phenolic compounds except ferulic acid were placed on the positive side of PC2.

In PCA used to explain the relation between cultivar and phenolic compounds, PC1 explained 50% of the variation, 29% of the variation in PC2 (Supplementary material 2a and 2b). In the study, 15 olive oil of different cultivars from five different regions were investigated in terms of phenolic compounds. As can be seen in Figure 2, phenolic compounds except for ferulic acid and quercetin placed positive side of PC1 and PC1 provided a good separation. The significant differences were determined between types. Halhalı, Uslu, Gemlik, and Nizip Yağlık had a closer relationship with luteolin, apigenin, hydroxytyrosol, tyrosol, transcinnamic acid, while Domat, Ayvalık, Memecik, and Çelebi showed a closer relationship with vanillic acid and p-coumaric acid than other groups in PC1 (Figure 2). Phenolic compounds except for ferulic acid, quercetin, p-coumaric acid, and vanillic acid were placed positive side of PC2. Similarly, to PC1, Halhalı, Uslu, Gemlik, and Nizip Yağlık types showed a positive correlation with luteolin, apigenin, hydroxytyrosol, tyrosol, transcinnamic acid. Thus, it can be said that there is a closer relationship between luteolin, apigenin, hydroxytyrosol, tyrosol, transcinnamic acid, and these cultivars.

4. Conclusion

Among phenolic compounds, tyrosol was generally found higher values in different geographic regions. Moreover, this compound was the major phenolic compound in the Mediterranean, Southeastern, and Black Sea regions. Vanillic acid was important phenolic acid in the Marmara region flavonoids were determined as important groups of phenolic compounds in olive oils. On the other hand, Marmara and Aegean regions were positively correlated with PC1 and placed at the same side with luteolin, vanillic acid, and p-coumaric acid. Many phenolic compounds were affected by harvest year, and vanillic acid was the highest phenolic compound in the first year. Similarly, cultivar had significant effects on many phenolic compounds. Moreover, there is a closer relationship between luteolin, apigenin, hydroxytyrosol, tyrosol, transcinnamic acid, and these cultivars. In conclusion, all of the factors caused important changes in phenolic compounds of olive oils, and PCA was an effective way to separate these differences.

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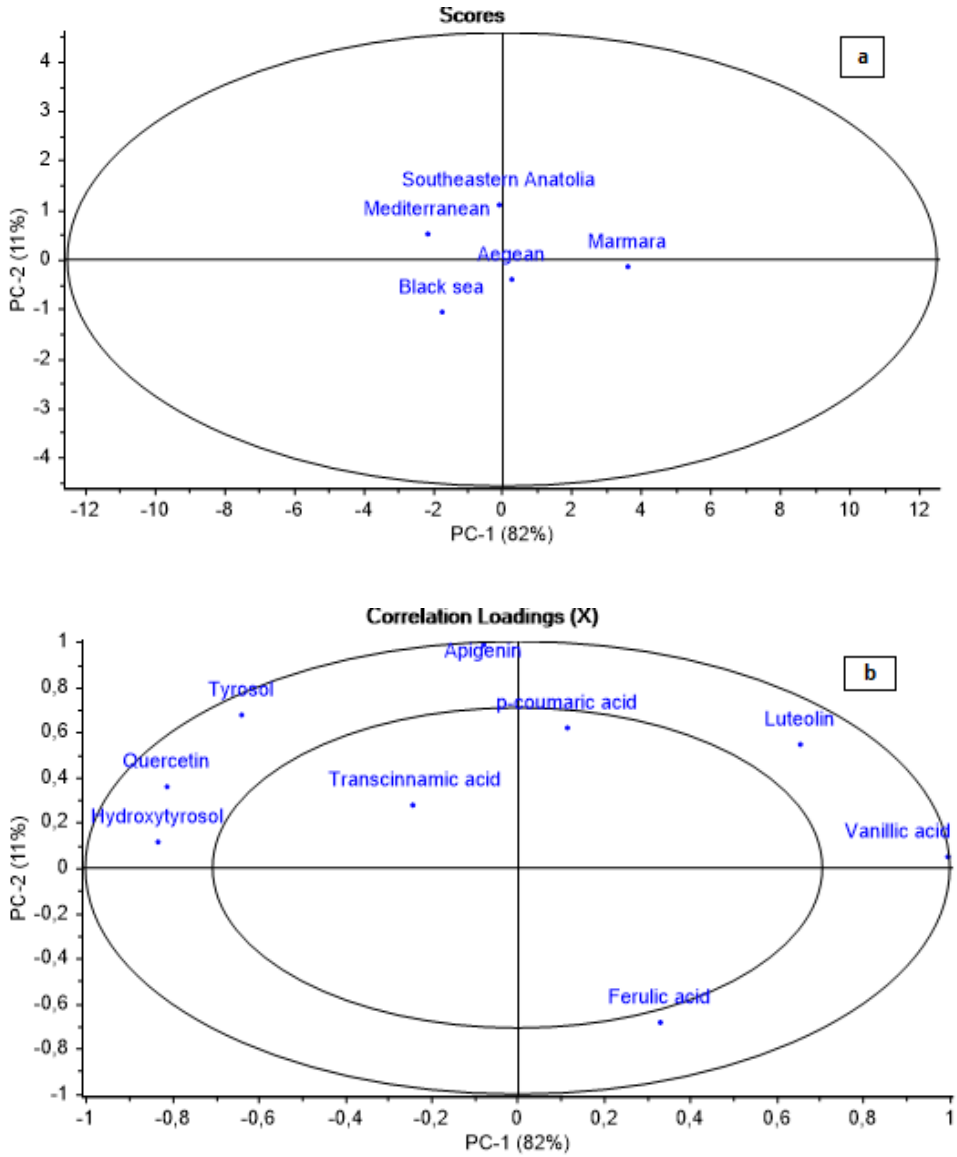
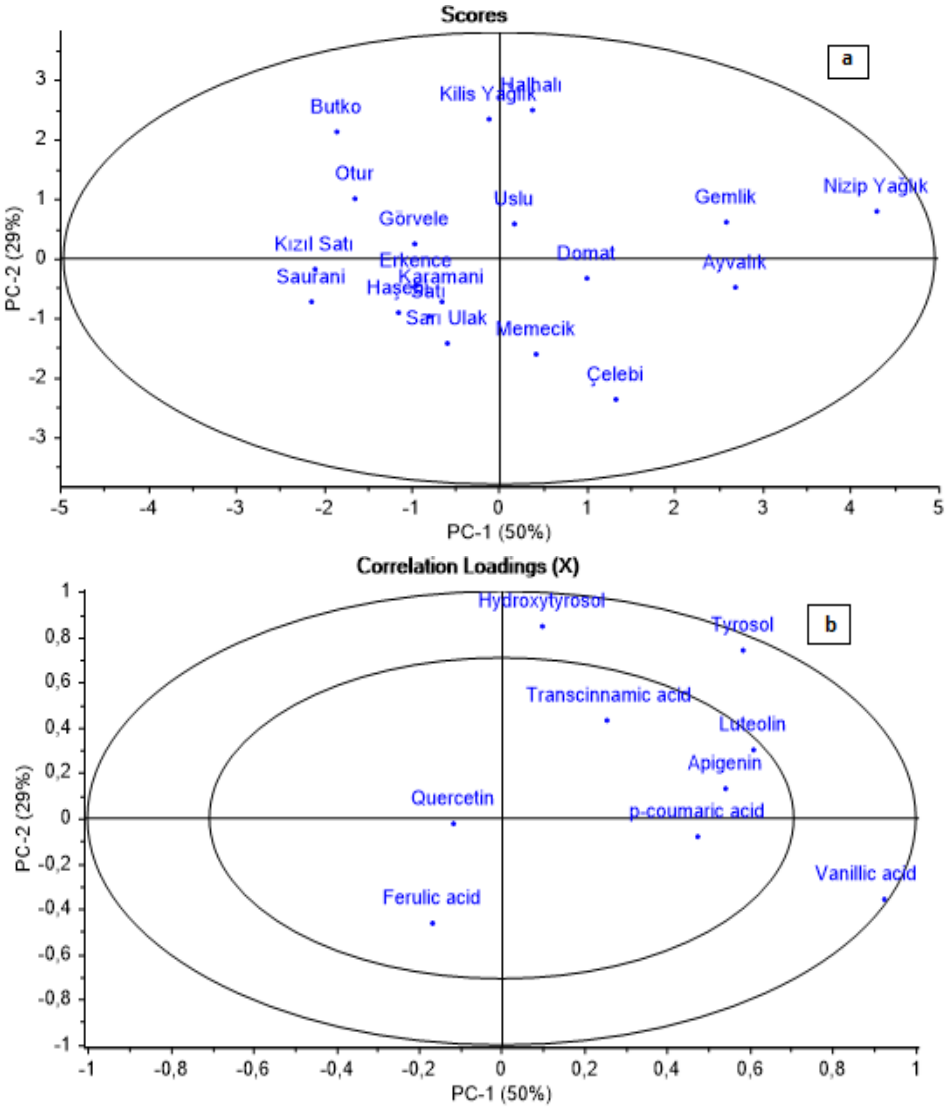
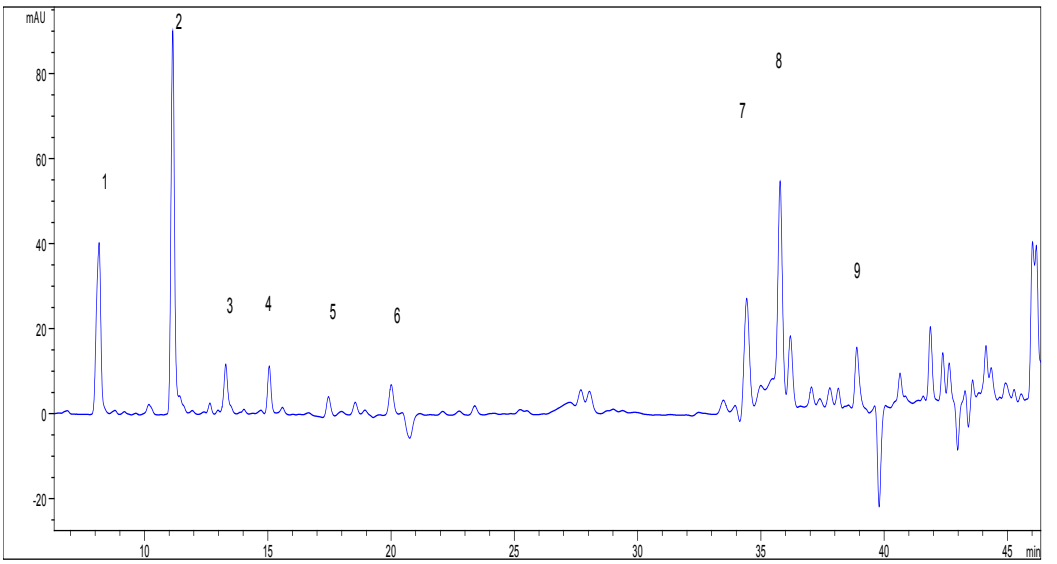


Supplementary material 1. PCA results for the regions (a) and phenolic compounds (b).



Supplementary material 2. PCA results for the cultivars (a) and phenolic compounds (b).



Supplementary material 3. Representative chromatogram of an olive oil.