

LC–ESI–MS Characterization of Phenolic Profiles Turkish Olive Oils as Influenced by Geographic Origin and Harvest Year

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Abstract The purpose of this investigation was to evaluate and compare the differences in the phenolic fractions and antioxidant properties of virgin olive oils from the Nizip yaglik and Kilis yaglik olive varieties cultivated in native and different olive growing areas of Turkey. The phenolic composition of olive oils was carried out by HPLC–DAD and identifications were made by LC–MS. Fourteen phenolic compounds were identified and among these compounds elenolic acid, tyrosol and hydroxytyrosol were the most dominant. Based on the results, there was no difference in distribution of phenolic compounds, but the total phenolic content in oil from native regions was higher than in oil from Bornova regions. The antioxidant capacity of olive oil extracts was determined by two different methods, including DPPH and ABTS. In both methods, antioxidant capacity values were higher in oil from native regions.

Keywords Olive oil · Phenolic compounds · Antioxidant capacity · Harvest year · DPPH · ABTS

Introduction

Virgin olive oil (VOO) quality is related to a large number of factors such as cultivar, geographical production area, climatic conditions in the production year, agronomic techniques, harvesting systems, and processing technology [1]. VOO is the main edible oil of the Mediterranean diet. It is directly obtained from ripe olive fruits without any further refining process. Among the other vegetable oils, VOO provides beneficial effects to human health because of its antioxidant capacity (due to its content of phenols, tocopherols, carotenoids, and other constituents) [2, 3]. Phenolics are important minor components in olive oil which, due to the powerful antioxidant effect, contribute to shelf life stability of olive oil [2, 4]. The most important phenolic compounds in VOO are phenolic acids, phenolic alcohol, secoiridoids, lignans, and flavonoids. The phenolic composition and sensory properties of olive oil vary significantly depending on the olive variety and ripening stage. Among all polyphenols, hydroxytyrosol, tyrosol, caffeic acid, coumaric acid, and *p*-hydroxybenzoic acid exhibit the greatest effect on the sensory characteristics of olive oil. Bitterness, pungency and astringency are sensory attributes of extra virgin olive oils (EVOO), often positively linked to the presence of phenolic compounds in the medium. Bitterness is generally considered as a positive sensorial attribute of the oil and enhances the overall flavor with notes related to unripe olive fruit. The secoiridoid derivatives are the main compounds responsible for the bitter taste of EVOO, such as oleuropein and ligstroside derivatives [3]. These compounds are part of the polar fraction of virgin olive oils. There is evidence that the presence of these compounds especially orthodiphenols in higher amounts decreases the risk of autoxidation and increases the stability of olive oils [5]. In the last few years,

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the health benefits of olive oil have been demonstrated, in particular, the prevention of cardiovascular diseases and cancer. It is generally recognized that the minor components such as phenols have a biological relevance. Among the olive oil hydrophilic phenols, hydroxytyrosol (3,4-DHPEA) has been extensively investigated. Numerous *in vitro* and *in vivo* studies suggest that the health properties of 3,4-DHPEA are mediated by its strong antioxidant activity [6, 7].

Turkey is one of the most important olive oil producing countries in the world, following Spain, Italy and Greece. The olive oil production of Turkey was 206,300 tonnes in 2012 [8]. In Turkey, the 'Aegean' region is the major olive producing area and followed by the 'Marmara', 'Mediterranean' and 'Southeast Anatolia' regions [9]. Nizip yaglik and Kilis yaglik are the major types of olives in South Eastern Anatolia and grow in Gaziantep, Kahramanmaras and Mardin provinces. Fruits are small sized with large seeds and have an oil content of 27–31 %. They are used in oil production. Their oils have a well-balanced and fruity aroma. However, little research has been performed on the phenolic composition and antioxidant properties of olive oils obtained from Nizip yaglik and Kilis yaglik.

The purpose of this study was to investigate the effects of growing region, cultivar and harvest years (2010 and 2011) on phenolic compounds, general chemical properties and antioxidant capacity of olive oils obtained from Nizip yaglik and Kilis yaglik which are two common Turkish olive varieties.

Materials and Methods

Reagents

Deionized water (resistivity over 18 M Ω cm) from a Millipore Q (Millipore Corp., Saint-Quentin, France) water purification system was used in all experiments. Phenolic compounds (caffeic, vanillic, syringic, *p*-coumaric, ferulic, and cinnamic acids, vanillin, tyrosol, apigenin and luteolin) were obtained from Sigma-Aldrich (Steinheim, Germany). Sodium chloride, acetonitrile, formic acid and sodium sulphate, 2,2'-azino-bis-(3-ethyl-benzothiazoline-6-sulphonic acid) diammonium salt (ABTS), potassium persulfate, 2,2-diphenyl-1-picryl hydrazyl (DPPH) were obtained from (Merck, Gernsheim, Germany).

Olives

Olive varieties (Nizip yaglik, NY; Kilis yaglik, KY; Nizip yaglik-Bornova, NY-B; Kilis yaglik-Bornova, KY-B) averaging 50–70 kg were bought from their growing area. NY and KY olives were cultivated in Gaziantep (38°27'N,

27°14'E) and Kilis (36°43'N, 37°7'E), respectively. NY-B and KY-B olives were cultivated at the Bornova Olive Research Station in Izmir province (38°27'N, 27°14'E). The orchards were primarily selected in order to represent the major soil and climate types of the region and to include what appeared to be distinctive differences in fruit ripening capacity, and fruit quality potential. Olives were harvested at the optimum maturity stage in 2010 and 2011 seasons. The olive-maturity index (MI) was determined as described by Kesen et al. [10]. This method was based on the assessment of the color of the olive skin and pulp. MI values range from 0 (very green skin 100 %) to 7 (100 % purple flesh and black skin) [10, 11]. In our samples, maturity indexes were found between 3.9 and 4.2 in both crop seasons.

Extraction of the Olive Oils

Olive oils were extracted by cold-press with a dual phase centrifuge (Oliomio mini, Italy). Fresh olive oils were put into glass bottles and were preserved in a dark and cool place until analysis. Cold press has been a widely used method for the last few years to produce high quality olive oils. If high temperature is applied during the extraction process, volatile compounds are transformed and fat oxidation is likely to be increased. On the other hand, decreasing levels of phenolic compounds, antioxidant activity and vitamin content are observed in olive oils [12].

Standard Chemical Analysis

Moisture and oil content of the olives were evaluated according to Dabbou et al. [13]. Free fatty acids and the peroxide value of the samples were calculated following the methods described in Regulation European Economic Commission [14]. The color of the oil samples was measured using a Hunter colorimeter (HunterLab, Color QuestXE-USA). Results for the L^* , a^* and b^* color system profile were recorded using CIE. Measurements were made at room temperature. Olive oil samples were placed in A sample cell (6 cm diameter) 2 cm deep and the data were read.

Extraction of the Phenolic Compounds

The extraction was performed according to the procedure described by Ouni et al. [4]. Briefly, 2 g of oil was weighed into a centrifuge tube, 5 mL of *n*-hexane and 10 mL of methanol–water (60:40, v/v) were added. The mixture was stirred for 10 min in a vortex apparatus, and the tube was centrifuged at 3,000 rpm for 5 min. The methanol layer was separated and the extraction was repeated twice. The methanolic extracts were combined and evaporated to

dryness under reduced pressure at a temperature not exceeding 35 °C. Samples were dissolved in 1 mL of methanol–water (1:1, v/v) and filtered through a 0.45- μm nylon filter (Whatman Inc., Clinton, NJ, USA) before analysis. Agilent 1100 HPLC system (CA, USA) operated by ChemStation software was used. The HPLC equipment was used with a diode array detector (DAD). The column used was a Beckman C18 ODS (Roissy, France): 4.6 \times 250 mm, 5 μm equipped with a precolumn (4.6 \times 10 mm, 5 μm). The mobile phase consisted of water with 5 % formic acid (solvent A; v/v) and acetonitrile with 40 % solvent A (solvent B; v/v). The elution program was performed as previously described [15]. The identification and assignation of each compounds was performed by comparing their retention times and UV spectra to authentic standards and also confirmed by tandem mass spectrometry (HPLC-ESI/MS) using a Thermo-Finnigan LCQ Advantage spectrometer equipped with an electrospray ionization source and an ion trap mass analyzer. The electrospray ionization mass spectrometry detection was performed in negative ion mode with the following optimized parameters: capillary temperature 400 °C, capillary voltage -3 V, nebulizer gas flow 1.75 L min⁻¹, desolvation gas flow 1 L min⁻¹, and spray voltage 5 kV. The quantification of each of the identified compounds was performed on each collected oil sample using an external standard calibration curve for each compound [4, 15]. The curves were obtained using the commercial standards of the concentrations normally present in olive oil extracts (approximately 1–100 mg kg⁻¹), obtaining regression coefficients (R^2) above 0.995 in all cases.

Measurement of Antioxidant Activity

DPPH Assay. The DPPH assay was performed according to the method developed by Ozkan et al. [16] but slightly modified. Briefly, 0.1 mL of diluted olive extract was mixed with 3.9 mL of DPPH solution (2.36 mg/100 mL methanol) and vigorously vortexed. The solution was kept in the dark at room temperature for 15 min. The absorbance was monitored at 517 nm by a UV–Visible spectrophotometer (Shimadzu UV-1201, Kyoto-Japan). The Trolox calibration curve was used to calculate the antioxidant activity of oil extracts and to express the antioxidant capacity in mM Trolox equivalent per kg of olive oil. The mean and standard deviation were calculated for three replicates.

ABTS Assay. Antioxidant activity of oil extracts was also measured by the ABTS method as described by Nakbi et al. [17]. The ABTS radical cation solution was prepared at a concentration of 7 mM and mixed with 2.5 mM of potassium persulfate, and used after incubation at 23 °C in the dark for 12–16 h. The prepared solution was then diluted with 80 % methanol to obtain an absorbance of

0.7 ± 0.01 at 734 nm. Then, 3.9 mL of ABTS solution was added to 0.1 mL of the test samples and mixed vigorously. The reaction mixture was incubated for 10 min and the absorbance at 734 nm was recorded.

The calibration curve equations related to standard of trolox were $y = 0.0004x + 0.0089$ with $R^2 = 0.9996$ for ABTS and $y = 0.0004x + 0.0082$ with $R^2 = 0.9995$ for DPPH within a concentration range from 5 to 150 $\mu\text{mol L}^{-1}$.

Statistical Analysis

The findings of this study were subjected to analysis of variance using SPSS 17 software package and Duncan's multiple comparison test were examined to find significant differences at the 0.05 level [18]. Also, principal component analysis (PCA) was carried out using XLStat-Pro7.5 (2007) for Windows (Addinsoft, New York, USA).

Results and Discussion

General Properties of Olive and Olive Oils

The general properties of the olive fruits and olive oils for the years 2010 and 2011 are shown in Table 1. As can be seen in Table 1, oil and moisture contents of olives were found between 20.4–30.50 and 43.62–52.48 %, respectively. In the 2011 harvest year, oil contents of all samples were higher than in the 2010 year. Regarding moisture content, in the oils of the 2010 year it was higher than in those of 2011.

Free fatty acids, peroxide values and color values are usually used to evaluate the initial quality of edible oils [19]. The free fatty acid contents of olive oils of 2010 did not exceed the maximum allowable limit set for virgin olive oils (2 %), while olive oils of 2011 had free fatty acid contents lower than 0.8 % (maximum legal limit for extra virgin olive oils). Peroxide values also were below the limit of 20 mequiv oxygen/kg of oil, which is accepted as the limit for extra quality of virgin olive oil (Table 1). The color properties of olive oils influence consumer's preference. Therefore, these parameters should be considered for inclusion in testing the oil [20]. L^* , a^* and b^* values of each year were statistically compared within themselves and were statistically different at the 0.05 level ($p < 0.05$). Olive oils obtained from 2011 had higher L^* , a^* and b^* values than those obtained from 2010 except L^* values of NY. Color values varied depending on the growing years.

Phenolic Compounds of Olive Oils

A total of 14 phenolic compounds including, hydroxytyrosol, 4-hydroxybenzoic acid, tyrosol, 2,3-dihydroxybenzoic acid, caffeic acid, vanillic acid, vanillin, syringic acid, *p*-

Table 1 Physico-chemical characteristics of olive fruits and olive oils

	2010				2011			
	NY	NY-B	KY	KY-B	NY	NY-B	KY	KY-B
Olive								
Maturity index	4.2 ± 0.00 ^a	3.9 ± 0.00 ^b	4.1 ± 0.00 ^a	3.9 ± 0.00 ^b	4.1 ± 0.00 ^a	3.9 ± 0.00 ^b	4.2 ± 0.00 ^a	3.9 ± 0.00 ^b
Oil content (%)	25.11 ± 0.26 ^b	20.40 ± 0.34 ^d	29.40 ± 0.27 ^a	22.31 ± 0.09 ^c	26.30 ± 0.12 ^b	21.80 ± 0.10 ^d	30.50 ± 0.17 ^a	24.50 ± 0.14 ^c
Moisture content (%)	52.48 ± 0.85 ^a	48.78 ± 1.96 ^c	50.42 ± 2.09 ^{ab}	46.20 ± 1.44 ^{bc}	50.33 ± 0.62 ^a	47.46 ± 0.75 ^b	47.04 ± 1.07 ^b	43.62 ± 0.42 ^c
Olive oil								
Free fatty acids (oleic acid %)	1.70 ± 0.10 ^a	1.41 ± 0.28 ^c	1.20 ± 0.26 ^d	0.51 ± 0.10 ^b	0.68 ± 0.08 ^a	0.77 ± 0.18 ^a	0.67 ± 0.14 ^a	0.69 ± 0.08 ^a
Peroxide value (mequiv oxygen kg ⁻¹ oil)	9.40 ± 0.24 ^a	9.90 ± 0.24 ^a	10.00 ± 0.25 ^a	9.30 ± 0.26 ^a	6.40 ± 0.16 ^c	8.02 ± 0.13 ^a	7.40 ± 0.18 ^b	8.30 ± 0.16 ^a
L*	70.01 ± 0.01 ^a	62.30 ± 0.09 ^c	38.71 ± 0.08 ^d	64.64 ± 0.06 ^b	60.64 ± 0.06 ^c	64.86 ± 0.04 ^b	51.70 ± 0.05 ^d	68.60 ± 0.03 ^a
a*	-4.11 ± 0.00 ^d	-3.05 ± 0.02 ^b	-2.32 ± 0.01 ^a	-3.15 ± 0.01 ^c	-1.87 ± 0.03 ^c	2.37 ± 0.02 ^a	-2.11 ± 0.02 ^d	1.17 ± 0.01 ^b
b*	50.16 ± 0.02 ^b	60.89 ± 0.08 ^a	35.29 ± 0.12 ^d	49.31 ± 0.05 ^c	73.05 ± 1.69 ^b	102.21 ± 0.13 ^a	74.38 ± 0.10 ^b	101.13 ± 0.10 ^a

Results are the means of three replications. ± standard deviations

Each year is statistically compared within themselves

NY Nizip yaglik, NY-B Nizip yaglik-Bornova, KY Kilis yaglik, KY-B Kilis yaglik-Bornova

^{a-d} Different superscripts in the same row indicate statistical differences at the 0.05 level ($p < 0.05$)

coumaric acid, ferulic acid, cinnamic acid, elenolic acid, apigenin and luteolin, were identified and quantified in olive oil samples of both harvest seasons (Tables 2, 3). The total phenolic content in oil from native regions was significantly higher than in oil from Bornova in both the 2010 and 2011 years, but distribution of phenolic compounds were similar in all oil samples. In the first year, the highest amount of phenolic compounds was found in KY (91.45 mg kg⁻¹) followed by KY-B (89.40 mg kg⁻¹), NY (79.65 mg kg⁻¹) and NY-B (74.71 mg kg⁻¹). In the second year, NY (104.80 mg kg⁻¹) had the highest amount of phenolic compounds and then NY-B (96.97 mg kg⁻¹), KY (95.30 mg kg⁻¹) and KY-B (92.01 mg kg⁻¹) were aligned. There were significant differences in the mean values of all of the phenolic compounds of the VOO in relation to regions and years. In the literature, it is reported that the total phenolic content of olive oil varies from 50 to 1,000 mg kg⁻¹ [21], depending on cultivars, place of origin, agronomic techniques, olive ripening, possible infestation by the olive fly *Bactrocera oleae* [22], extraction methods, and storage conditions but results obtained by different researchers are hardly comparable because of the variety of methods proposed for their determination. Tanilgan et al. [23] reported that the total phenolic content of Turkish olive oils ranged from 22.5 to 97.1 mg of GA/kg of oil. Also, Bakhouche et al. [24] studied 32 Arbequina EVOOs from different locations in Spain in terms of their phenolic profile, to show the classification of oil samples with respect to geographical area. In this research, quantitative differences were observed in a wide number of phenolic compounds. They said that phenolic content of EVOOs was found to depend highly on the geographical region. Gomez-Alonzo et al. [25] examined the phenolic compounds of four Spanish virgin olive oils (Arbequina, Hojiblanca, Picual and Cornicabra) to determine the effect of cultivar. They reported that the total phenolic content of the four virgin olive oil varieties were as follows: Arbequina 25.5 mg kg⁻¹, Hojiblanca 33.6 mg kg⁻¹, Picual 37.9 mg kg⁻¹ and Cornicabra 39.2 mg kg⁻¹.

With regard to individual phenolics, the most common phenolic compounds that were identified in both years were hydroxytyrosol, tyrosol, elenolic acid, apigenin and luteolin (Table 3). Elenolic acid was determined as the major compound in all olive oils in both harvest seasons, as it accounted for the largest proportion of the total phenolic content. Elenolic acid was reported for the first time in a virgin olive oil by Montedoro et al. [21] and subsequently Mateos et al. [26] proved that the presence of this compound could not be attributed to hydrolysis during the analysis of secoiridoids derivatives containing it. The 2010 and 2011 olive oils contained 52.10–62.04 mg kg⁻¹ and 60.10–68.22 mg kg⁻¹ of elenolic acid, respectively. Elenolic acid content in olive oils of different Italian varieties

Table 2 Retention times, mass spectral details and UV data of phenolic compounds identified in olive oils

Phenolic compounds	Rt (min)	λ max (nm)	[M-H] ⁻ (m/z)
Hydroxytyrosol	2.1	230, 280	153
4-Hydroxybenzoic acid	2.6	254	137
Tyrosol	4.2	230, 275	137
2,3-Dihydroxybenzoic acid	10.1	320	153
Caffeic acid	12.2	320, 300	179
Vanillic acid	20.1	262	167
Vanillin	24.3	309, 284	151
Syringic acid	29.1	275	197
<i>p</i> -Coumaric acid	33.3	308	163
Ferulic acid	36.7	323, 293	193
Cinnamic acid	40.5	278	147
Elenolic acid	45.2	240	241
Apigenin	46.8	230, 270, 340	269
Luteolin	50.2	255, 350	285

ranges from 32 to 184 mg kg⁻¹ [27], and in Spain olive oil it is between 12 and 65 mg kg⁻¹ [28].

Tyrosol is a derivative of phenylethyl alcohol and can protect cells against injury due to oxidation. Although, it is not as potent as other antioxidants present in olive oil, its higher concentration and good bioavailability indicate that it may have an important overall effect [2]. The content of tyrosol varied between 12.45–15.84 mg kg⁻¹ for 2010; and 12.67–18.73 mg kg⁻¹ for 2011. The tyrosol level observed in our olive oil is in accordance with previously reported data such as those from Godoy-Caballero et al. [29] who reported that the contents of tyrosol in olive oils were 10.4 mg kg⁻¹ for Morisca, 11.5 mg kg⁻¹ for Arbequina, 13.2 mg kg⁻¹ for Picual and 16.3 mg kg⁻¹ for Manzanilla Cacereña as well as the results obtained from Tunisian virgin olive oils from different locations [4]. The study of Ilyasoglu et al. [30] found that tyrosol was the main phenolic compound in Ayvalik (1.64–13.91 mg kg⁻¹) and Memecik (7.41–20.08 mg kg⁻¹) olive oils. Another phenolic alcohol at relatively high concentrations is hydroxytyrosol. Its amount changed between 1.56–4.26 mg kg⁻¹ (2010) and 3.54–5.68 mg kg⁻¹ (2011). These results are similar to those reported by several authors for other olive oil varieties [2]. In similar studies, hydroxytyrosol and tyrosol were reported as the predominant phenolic compounds [31].

Luteolin and apigenin are the main flavonoids present in olive oils, which originate from their corresponding glucosides present in the drupe [32]. Concentrations of luteolin and apigenin in VOO ranged from 1.51 to 7.57 mg kg⁻¹ and from 1.17 to 4.96 mg kg⁻¹, respectively. As can be seen in Table 3, the concentrations of

luteolin and apigenin in VOO from native region were slightly higher than in VOO from the Bornova region and the amounts of these compounds in VOO were significantly higher in 2011 season in both studied region. García-Villalba et al. [33] found values between 1.6–6.3 mg kg⁻¹ for luteolin, and 0.5–3.1 mg kg⁻¹ for apigenin in olive olives from Spain.

With regard to phenolic acids, cinnamic acid (0.51–1.86 mg kg⁻¹), caffeic acid (0.02–0.08 mg kg⁻¹), *p*-coumaric acid (0.63–2.01 mg kg⁻¹), 2,3-dihydroxybenzoic acid (0.19–0.41 mg kg⁻¹), vanillic acid (0.03–0.07 mg kg⁻¹) and ferulic acid (0.06–0.38 mg kg⁻¹) were identified and quantified. Amounts of phenolic compounds in the olive oils studied obtained from different varieties were similar to our study [4, 34].

When we compare the amount of individual phenolic compounds of olive oils with the olive oils produced from other varieties under similar conditions, we see that the olive oil made from NY to KY varieties had higher phenolic contents than the olive oils from Tunisian cvs. Oueslati, Chétoui and Chemlali [4, 17] while they had lower phenolics than the olive oils from French cvs. Aglandau and Tanche; Spanish cvs. Cornicabra, Picual, and Verdial [35]; Italian cvs. La Pepa and Severini [36]; Greece cvs. Mavrolia and Koroneiki [37]. In addition to phenolic contents of the olive oil samples were quite similar other olive oils produced in Turkey such as Memecik, Erkence, Gemlik, Ayvalik and Domat [34].

To evaluate the possibility of differentiating the samples taking into account the phenolic fraction, we applied a multivariate statistical analysis for the results of the LC–ESI–MS analyses of the quantitative-phenolic profile. All the phenolic compounds quantified were considered to identify the two principal factors. The variables were selected for the PCA and the explained variance was 67.60 % (Factor 1: 40.81 %; Factor 2: 26.78 %). Figure 1a represents the projection of the variables with regard to the single factor (PC1 or PC2) on the factor plane (PC1 × PC2). We can observe that each quadrant contains, at least, one of the variables. A map of samples (score plot) for the two principal components is shown in Fig. 1b. A distinct categorization according to the concentration of phenolics expressed in the olive oil extracts was observed along PC 1. The variables which were more decisive to discriminate among geographical area were elenolic acid, 4-hydroxybenzoic acid, tyrosol, hydroxytyrosol, luteolin, ferulic acid, 2,3-dihydroxybenzoic acid, apigenin, vanillic acid, syringic acid and *p*-coumaric acid; a finding which is in good agreement with Ouni et al. [4].

The application of the principal component analysis algorithm (PCA) showed two distinct groups (Fig. 1b). The first group represents the result of both years of NY and NY-B and also KY-B 2010 oils. The second one was

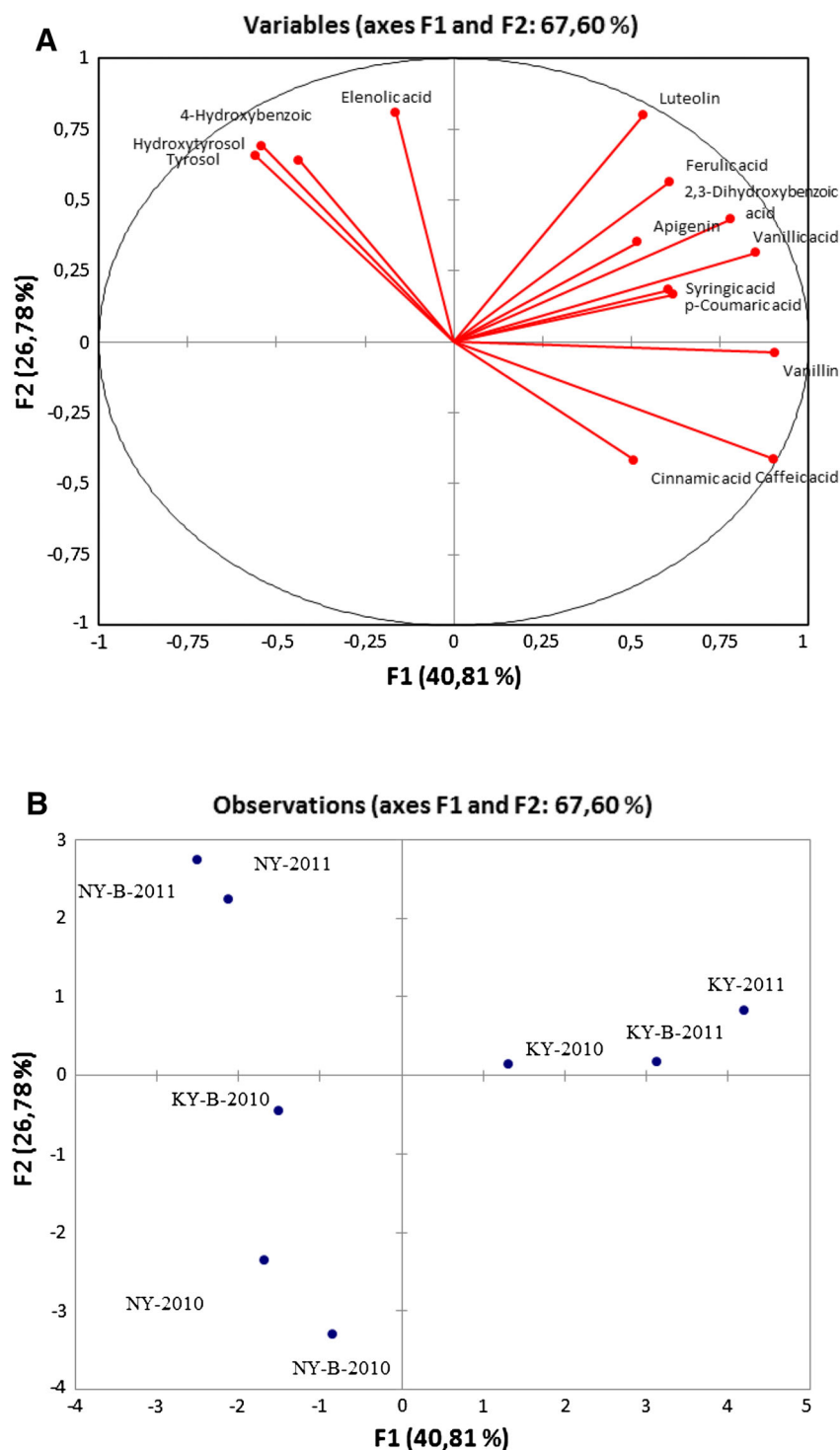
Table 3 Phenolic compounds of olive oils

Phenolic compounds (mg kg ⁻¹)	Olive oil varieties											
	2010						2011					
	NY	NY-B	KY	KY-B	NY	NY-B	NY	NY-B	KY	KY-B	NY	NY-B
Hydroxytyrosol	4.14 ± 0.16 ^b	1.81 ± 0.04 ^c	1.56 ± 0.02 ^d	4.26 ± 0.06 ^a	5.68 ± 0.06 ^a	5.29 ± 0.28 ^b	3.74 ± 0.05 ^c	3.54 ± 0.17 ^c				
4-Hydroxybenzoic acid	0.44 ± 0.00 ^a	0.07 ± 0.00 ^d	0.23 ± 0.00 ^c	0.28 ± 0.00 ^b	0.62 ± 0.00 ^a	0.57 ± 0.04 ^b	0.26 ± 0.01 ^c	0.24 ± 0.02 ^c				
Tyrosol	12.45 ± 0.44 ^c	14.68 ± 0.11 ^b	15.84 ± 0.11 ^a	13.25 ± 0.13 ^b	18.73 ± 0.38 ^a	17.50 ± 0.78 ^b	13.39 ± 0.09 ^c	12.67 ± 0.62 ^c				
2,3-Dihydroxybenzoic acid	0.21 ± 0.01 ^c	0.27 ± 0.00 ^b	0.29 ± 0.00 ^a	0.19 ± 0.01 ^d	0.31 ± 0.01 ^b	0.28 ± 0.02 ^b	0.41 ± 0.00 ^a	0.38 ± 0.04 ^a				
Caffeic acid	0.05 ± 0.00 ^b	0.06 ± 0.01 ^a	0.06 ± 0.02 ^a	0.04 ± 0.00 ^c	0.02 ± 0.00 ^b	0.02 ± 0.01 ^b	0.08 ± 0.00 ^a	0.08 ± 0.01 ^a				
Vanillic acid	0.04 ± 0.01 ^{bc}	0.05 ± 0.00 ^b	0.06 ± 0.01 ^a	0.03 ± 0.00 ^c	0.05 ± 0.00 ^b	0.05 ± 0.01 ^b	0.07 ± 0.02 ^a	0.06 ± 0.01 ^{ab}				
Vanillin	0.02 ± 0.01 ^a	0.02 ± 0.00 ^a	0.03 ± 0.00 ^b	0.02 ± 0.01 ^a	0.02 ± 0.00 ^b	0.02 ± 0.00 ^b	0.03 ± 0.00 ^a	0.03 ± 0.01 ^a				
Syringic acid	0.46 ± 0.02 ^d	0.58 ± 0.01 ^c	0.91 ± 0.07 ^b	1.40 ± 0.03 ^a	0.66 ± 0.03 ^b	0.62 ± 0.02 ^b	1.28 ± 0.09 ^a	1.16 ± 0.14 ^a				
<i>p</i> -Coumaric acid	0.63 ± 0.07 ^d	0.82 ± 0.01 ^c	1.30 ± 0.09 ^b	2.01 ± 0.01 ^a	0.90 ± 0.10 ^b	0.86 ± 0.01 ^b	1.83 ± 0.12 ^a	1.69 ± 0.15 ^a				
Ferulic acid	0.09 ± 0.00 ^b	0.06 ± 0.00 ^c	0.26 ± 0.00 ^a	0.09 ± 0.00 ^b	0.12 ± 0.01 ^b	0.38 ± 0.45 ^a	0.36 ± 0.01 ^a	0.34 ± 0.02 ^a				
Cinnamic acid	1.74 ± 0.28 ^a	1.25 ± 0.03 ^b	0.51 ± 0.01 ^c	1.17 ± 0.03 ^b	0.77 ± 0.01 ^b	0.71 ± 0.04 ^b	1.86 ± 0.02 ^a	1.74 ± 0.12 ^a				
Elenolic acid	56.04 ± 1.06 ^c	52.10 ± 0.50 ^d	61.07 ± 0.94 ^b	62.04 ± 0.94 ^a	68.22 ± 0.29	62.52 ± 1.26	61.42 ± 0.54	60.10 ± 1.10				
Apigenin	1.83 ± 0.03 ^b	1.17 ± 0.01 ^c	4.96 ± 0.06 ^a	1.86 ± 0.02 ^b	2.31 ± 0.11 ^b	2.18 ± 0.07 ^c	2.99 ± 0.03 ^a	2.81 ± 0.16 ^a				
Luteolin	1.51 ± 0.31 ^d	1.77 ± 0.02 ^c	4.38 ± 0.02 ^a	2.74 ± 0.01 ^b	6.38 ± 0.17 ^c	5.98 ± 0.24 ^c	7.57 ± 0.14 ^a	7.19 ± 0.31 ^b				
Total concentration	79.65 ± 0.78 ^c	74.71 ± 1.11 ^d	91.45 ± 1.04 ^a	89.40 ± 0.63 ^b	104.80 ± 0.45 ^a	96.97 ± 2.42 ^b	95.30 ± 0.52 ^b	92.01 ± 1.40 ^c				

Results are the means of three replication. ± standard deviations; Each year is statistically compared within themselves

^{a-d} Different superscripts in the same row indicate statistical differences at the 0.05 level ($p < 0.05$)

Fig. 1 **a** Projection of the variables on the factor-plane (PC1 × PC2) considering the phenolic compounds quantified. **b** Score plot for the two principal components showing the two geographical origins studied



characterized by the KY-2010, KY-2011 and KY-B-2011. The first group was negatively associated with PC1 while the second group was positively associated with PC1. PC1 was dominated by luteolin, ferulic acid, apigenin, vanillic acid, syringic acid and *p*-coumaric acid. However, PC2 was dominated by the following variables: elenolic acid, 4-hydroxybenzoic acid, tyrosol and hydroxytyrosol.

Antioxidant Capacity of Olive Oils

Two methods, ABTS and DPPH assays, were used to measure antioxidant capacity. The results of the antioxidant activities measurements of the olive oils are presented in Table 4. As can be seen, the antioxidant capacities of phenolic compounds were better reflected by the ABTS

Table 4 Antioxidant activity values of olive oils (mM Trolox kg⁻¹)

	NY	NY-B	KY	KY-B
2010 year				
ABTS	1.16 ± 0.04 ^c	1.03 ± 0.13 ^c	1.59 ± 0.22 ^a	1.34 ± 0.04 ^b
DPPH	0.43 ± 0.01 ^c	0.35 ± 0.05 ^d	0.75 ± 0.02 ^a	0.51 ± 0.00 ^b
2011 year				
ABTS	1.90 ± 0.05 ^a	1.77 ± 0.08 ^b	1.64 ± 0.05 ^c	1.54 ± 0.05 ^c
DPPH	0.71 ± 0.00 ^a	0.65 ± 0.04 ^b	0.59 ± 0.01 ^c	0.55 ± 0.02 ^c

Results are means of three replication. ± standard deviations

^{a-c} Different superscripts in the same row indicate statistical differences at the 0.05 level ($p < 0.05$)

assay than the DPPH assay and the ABTS assay produced higher values than DPPH assays. In both methods, antioxidant capacity values were higher in oils from native regions (ABTS 1.16–1.90 mM Trolox kg⁻¹; DPPH 0.43–0.75 mM Trolox kg⁻¹) than in oils from Bornova (ABTS 1.03–1.77 mM Trolox kg⁻¹; DPPH 0.35–0.65 mM Trolox kg⁻¹). This situation was observed in both years. According to results of the first year, the antioxidant activities of olive oils by using ABTS and DPPH methods were 1.03–1.59 and 0.35–0.75 mM Trolox kg⁻¹, respectively, in the second year they ranged from 1.54–1.90 to 0.55–0.71 mM Trolox kg⁻¹. The antioxidant capacity in several oils obtained from different raw materials in the ABTS assay ranged from 0.61 mM Trolox kg⁻¹ (peanut) to 2.20 mM Trolox kg⁻¹ (soybean) [38]. In that study, it was also reported that the antioxidant capacity of olive oil was 0.63 mM Trolox kg⁻¹.

The total phenolic contents and antioxidant capacities as measured by DPPH and ABTS assays were compared. In statistical evaluation between the total phenolic contents and antioxidant capacity values (DPPH and ABTS), good correlations were found in both years. Olive oils had higher phenolic compounds showed higher antioxidant activity. Among the phenolic compounds, luteolin had the highest correlation and followed by caffeic acid, elenolic acid and tyrosol, respectively (Table 5).

Also, our results showed strong correlations between antioxidant capacity and total phenolic contents ($R^2 = 0.956$ with ABTS assay and $R^2 = 0.858$ with DPPH assay) (Table 5). Correlations between the ABTS and DPPH assays were also evaluated. We found significant correlations ($p < 0.01$) between ABTS and DPPH ($R^2 = 0.867$).

Conclusions

In this study, phenolic compounds and antioxidant potential of cvs. NY, KY, NY-B and KY-B olive oils were investigated. A total of 14 phenolic compounds were

Table 5 Correlations between phenolic compounds and antioxidant capacity

	ABTS	DPPH
Hydroxytyrosol	0.558**	0.258
4-Hydroxybenzoic acid	0.633**	0.493*
Tyrosol	0.625**	0.618**
2,3-Dihydroxybenzoic acid	0.513**	0.354*
Caffeic acid	0.650**	0.881**
Vanillic acid	0.327	0.326
Vanillin	0.047	0.140
Syringic acid	0.154	0.158
<i>p</i> -Coumaric acid	0.129	0.142
Ferulic acid	0.363	0.309
Cinnamic acid	-0.391	-0.593**
Elenolic acid	0.707**	0.728**
Apigenin	0.452*	0.738**
Luteolin	0.823**	0.643**
Total concentration	0.956**	0.858**
DPPH	0.867**	1
ABTS	1	0.867**

Correlation values between phenolic compounds and antioxidant capacity are given as the averages of 2010 and 2011 values

* Correlation is significant at the 0.05 level

** Correlation is significant at the 0.01 level

identified and quantified in the phenolic extracts of these cultivars. Elenolic acid was found as the most abundant phenolic compound in all cultivars and in both years followed by tyrosol, hydroxytyrosol and luteolin depending on the year. Based on the results, the total amount of phenolic compounds in oils from native regions was slightly higher than oil from the Bornova region. On the other hand, the content of phenolic compounds in the year 2011 was slightly higher than in the year 2010.

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