Scientia Horticulturae 144 (2012) 141–152

Contents lists available at SciVerse ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Chemical characteristics and antioxidant activity of olive oils from Turkish varieties grown in Hatay province

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ARTICLE INFO

Article history:
Received 12 March 2012
Received in revised form 29 June 2012
Accepted 4 July 2012

Keywords:
Virgin olive oil
Harvest date
Secoiridoids
Tocopherols
Antioxidant activity
PCA

ABSTRACT

Monovarietal virgin olive oil from five different Turkish varieties (Eğriburun, Karamani, Halhalı, Saurani and Haşebi) from Hatay province which were harvested on three different occasions have been analyzed to evaluate minor components (phenolic compounds, tocopherols and pigments), fatty acid profiles and antioxidant activity in order to determine the effects of both the nature of the variety and the time of harvest. The high total phenolics, secoiridoid aglycones, α-tocopherol and antioxidant activity of Saurani variety makes the oil of this variety superior to other varieties analyzed in this study. After evaluating the correlation matrix of the data set, the variables such as secoiridoids (3,4-DHPEA-EDA; 3,4-DHPEA-EA; *p*-HPEA-EDA and *p*-HPEA-EA), some of the phenolic compounds (hydroxytyrosol, vanillic acid and luteolin) and oxidation parameters (beta carotene bleaching activity, peroxide value and free fatty acids) were finally decided as the three factors which led to explore the underlying pattern of relationships by using principal component analysis.

1. Introduction

The three major olive oil producing countries in the world are Spain (providing 40% of the World production), Italy (24%) and Greece (12%), followed by Tunisia (7%) and Turkey (4%). Annually, 112,000 tons of olive oil is produced in Turkey, and approximately 70% of it is exported to other countries. Most of the production occurs in the Aegean, Marmara, Mediterranean and Southeastern Anatolia regions of the country. The Aegean region yields 80.5% of olive production, followed by 11.8% in Mediterranean, 6.1% in Marmara and 1.6% in Southeastern Anatolia regions of Turkey. Furthermore, in the Hatay province, located in the south of Turkey, growths cover 7% of table olive and 10% of olive oil requirements of Turkey (DİE, 2008).

Recently, the increase in table olive and olive oil consumption has resulted in a higher demand and to a rapid establishment of new olive tree orchards in countries such as Turkey where olive trees are widely grown (Toplu et al., 2009). The most important olive varieties cultivated in Hatay are Halhalı, Saurani, Hasebi, Karamani, Sariulak and Gemlik (Dıraman, 2007). Eğriburun is consumed generally as table olives. Saurani and Karamani are generally processed to olive oil and Halhalı and Haşebi varieties are used for both oil extraction and table olive purposes (Anon., 1991). These cultivars are characterized by low frost resistance, medium vigor, and small-sized fruit (except Halhalı), and show alternate and high productivity. The trees produce a large amount of fruit and with a relatively high oil yield (more than 45% in dry matter) (ZAE, 2007). Gemlik variety comprises 80% of olive tree number cultivated in Marmara region of Turkey and covers 11% of total olive tree number in Turkey. The geographical origin of this variety is Gemlik and is being cultivated in a very broad area. It is characterized by medium vigor, is partially resistant to frost, produces medium-sized fruit and with high productivity. Gemlik is generally used in producing green or black table olive (Canozer, 1991). Gemlik variety was reported to contain higher protein, oil, sugar content and higher pH value and a lesser acidity than the other variety of olives which are desired for quality characteristics in black table olive production. For that reason, the Gemlik variety is one of the most suitable varieties for table olive production. The fruits of this variety contain approximately 30% oil. The fatty acid composition of this variety of oil is characterized with its higher palmitic and palmitoleic acids, lower linoleic and linolenic acids when compared to the other domestic cultivars (Dıraman, 2010).

Abbreviations: 3,4-DHPEA-EDA, dialdehydic form of elenolic acid linked with 3,4-dihydroxyphenylethanol or elenolic acid linked to hydroxytyrosol; 3,4-DHPEA-EA, an isomer of oleuropein aglycone (3,4 dihydroxyphenylethanol elenoicacid) or ligostroside aglycon; *p*-HPEA-EDA, the dialdehydic form of elenolic acid linked with *p*-hydroxyphenylethanol or elenolic acid linked to tyrosol; *p*-HPEA-EA, oleuropeine aglycon.

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http://dx.doi.org/10.1016/j.scienta.2012.07.006

During the last few years new cultivars have been introduced in Hatay being mostly the cultivar "Gemlik" which is widespread in Aegean and Marmara Regions of the country and occupies a great part of table olive and olive oil production with its high fruit and oil yield, early maturation, low periodicity and appreciated organoleptic quality by consumers. It is the most easily available cultivar for the local olive growers as 80% of seedlings produced by governmental and private companies belong to this cultivar. This led to a decline in the number of autochthonous cultivars in the province. However, before promoting new cultivars, their behavior in different environments should be evaluated (Dabbou et al., 2010). It is now realized that Gemlik with its generally good plantation characteristics did not adapt well to the environmental conditions of Hatay. This undesirable outcome was a result of wrong decision-making by governmental bodies, such as the omission of necessary scientific evaluation of the consequences.

Little is known about the nature and/or concentrations of minor components and the chemical composition of the oils from the monocultivars grown in Hatay province. Thus, this study was undertaken to evaluate the oil chemical composition of five minor Turkish olive varieties by analyzing several quality-related parameters (e.g., tocopherols, phenolic compounds, pigments, fatty acids and antioxidant activity) and to investigate the effect of the harvesting time on the tested parameters in order to identify the optimum harvest dates (HD) for these varieties cultivated in the Hatay province.

2. Materials and methods

2.1. Olive origin

The present work was carried out on monovarietal virgin olive oils from Turkish varieties; Halhalı, Eğriburun, Haşebi, Karamani and Saurani which were harvested in Altınözü in Hatay, Turkey.

The olive samples were collected from the olive trees in triplicate of all located in close orchards and which benefited from the same cultural practices. Climatic data (temperature, rainfall and humidity) for the experimental year 2008 were obtained from the Turkish State Meteorological Service (Anon., 2008). Olive fruits were hand picked at three different HDs, corresponding to between the 15th of September–1st of October (first HD), the 20th October–1st of November (second HD) and the 20th of November–10th of December (third HD) in the year 2008. 5 kg of olives per variety were collected from the same trees for each HD. After harvesting, the olive fruit samples were immediately transported to the laboratory, where the oil was extracted within 24 h.

The olive ripening index (RI) was determined according to the method developed by Boskou (1996) based on the evaluation of the olive skin and pulp colors. RI values range from 0 (100% intense green skin) to 7 (100% purple flesh and black skin).

2.2. Oil extraction

The olives were washed and deleafed, crushed with a hammer crusher and the paste was mixed at 25 °C for 20 min. The paste was pressed with a stainless steel manual press and the oil was extracted by means of a laboratory basket centrifuge (6000 × *g* for over 5 min) without addition of warm water and then transferred into dark glass bottle. All samples were stored at 4 °C using amber glass bottles without headspace until analysis.

2.3. Oil sample analysis

Free fatty acids and peroxide value of olive oils were determined following the methods described in Regulation European Economic Commission (EEC, 1992).

2.4. Determination of tocopherols

Tocopherols were evaluated according to IUPAC 2432 method (IUPAC, 1992): 1.5 g oil was dissolved in 10 mL hexane and injected into the HPLC system with a LiChroCART, Si 60 column (25 cm × 4 mm × 5 μm) (Merck, Darmstadt, Germany). The chromatographic separation was performed using a Shimadzu liquid chromatograph equipped with an isocratic pump LC-20AT prominence, a CTO-10AS VP heater (column temperature 22 °C), a SIL-20A prominence autosampler and a SPD-M20A Prominence diode-array detector (Shimadzu, Kyoto, Japan). The mobile phase was 0.5% isopropanol in *n*-hexane. The total run time was 40 min and the injection volume was 20 μL. The detector was a DAD operated at a fixed wavelength of 295 nm. Tocopherols were quantified by an external standard method; α-, β-, γ- and δ-tocopherol standards were obtained from Sigma–Aldrich (St. Louis, MO).

2.5. Fatty acid analysis

For the determination of fatty acid composition of the oils, fatty acid methyl esters were prepared from olive oil, using a cold transmethylation (Stefanoudaki et al., 1999). The fatty acids were converted to fatty acid methyl esters before analysis by shaking a solution of 0.2 g oil and 3 mL of hexane with 0.4 mL of 2 N methanolic potassium hydroxide. A Shimadzu (Kyoto, Japan) gas chromatograph, equipped with a flame ionization detector and a split/splitless injector, was employed. Separations were made on a Teknokroma TR-CN100 (Barcelona, Spain) fused-silica capillary column (60 m × 0.25 mm i.d. × 0.20 μm film thickness). The carrier gas was nitrogen, with a flow rate of 1 mL/min. The temperatures of the injector and the detector were held at 220 and 250 °C, respectively. The initial oven temperature of 90 °C was maintained for 7 min, raised to 240 °C at a rate of 5 °C/min, where it was maintained for 15 min. The injection volume was 1 μL. Peaks were identified by comparison of their retention times with those of authentic reference compounds (Sigma–Aldrich, St. Louis, MO, USA).

2.6. Carotenoids and chlorophylls

Carotenoids and chlorophylls (mg/kg oil) were determined at 470 and 670 nm, respectively, in cyclohexane using the specific extinction values, according to the method of Minguez-Mosquera et al. (1991).

2.7. Extraction of phenolic compounds

The extraction was performed according to the procedure described by Pirisi et al. (2000). Briefly, 2 g of oil was weighed into a centrifuge tube, added with 1 mL of *n*-hexane and 2.0 mL of methanol–water (60:40, v/v). Gallic acid (0.5 mL, 100 mg/L) was added to the oil as an internal standard (Baiano et al., 2009). The mixture was stirred for 2 min in a vortex apparatus, and the tube was centrifuged at 3000 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 to be used for HPLC analysis as well as for the determination of total phenols and antioxidant activity assays.

2.8. HPLC analysis of phenolic compounds

The extracted phenolic fractions were analyzed by HPLC. The HPLC system included a LC 10A VP, an LC-20AT prominence pump, a CTO-10AS VP heater (column temperature 22 °C), a SIL-20A prominence autosampler and a SPD-M20A Prominence diode-array

detector (Shimadzu, Kyoto, Japan). The column was an Inertsil ODS-3 (5 μm, 25 cm × 4.6 mm i.d.) (GL Sciences, Tokyo, Japan). PC running Class VP chromatography manager software (Shimadzu, Japan) was used and chromatograms were obtained at 240, 280 and 320 nm. The eluents were a 2% aqueous formic acid solution and methanol, the flow rate was 0.85 mL/min, and the injection volume 40 μL. The total run time was 76 min.

Quantification was carried out by a four-point regression curve on the basis of standards obtained from commercial suppliers with the exception of secoiridoid aglycones such as 3,4-DHPEA-EDA, *p*-HPEA-EDA, 3,4-DHPEA-EA and *p*-HPEA-EA which were identified based on earlier reports and references chromatograms (Garcia et al., 2001; Mateos et al., 2001) and were quantified using gallic acid as internal standard. Hydroxytyrosol (h-tyrosol) was obtained from Extrasynthēse (Genay, France) and cinnamic acid, vanillic acid, *p*-coumaric acid, syringic acid, caffeic acid, apigenin and tyrosol from Fluka (Steinheim, Germany).

2.9. Total phenol content

The total phenol content of methanolic extracts was analyzed using the method described by Vasquez Roncero et al. (1973). The concentration of total polyphenols was estimated with Folin–Ciocalteau reagent at 725 nm. The results were expressed as mg of gallic acid per kg of olive oil.

2.10. β-Carotene bleaching (BCB) inhibition assay

The antioxidant activity of the olive oil extracts was determined using the β-carotene bleaching inhibition assay. The procedure of Cheung et al. (2003) was applied with only one modification using 100.0 μL of methyl linoleate (Larodan AB, MALMÖ, Sweden) instead of 50.0 μL of linoleic acid. The antioxidant activity was calculated using the formula: $AA = 100\,[1 - (A_0 - A_t)/(A^\circ_0 - A^\circ_t)]$, where A_0 and A°_0 are the absorbance values measured at zero time of the incubation for test sample and control, respectively, and A_t and A°_t are the absorbance measured in the test sample and control, respectively, after incubation for 180 min.

2.11. Trolox equivalent antioxidant capacity (TEAC) assay

This assay, measuring the reduction of the ABTS (2,2′-azinobis(3-ethylbenzthiazoline-6-sulphonic acid)) radical cation by antioxidants, was derived from the method previously described by Miller and Rice-Evans (1997).

2.12. Statistical analysis

All parameters analyzed were determined in triplicate and reported as mean values of the three replicates and standard deviations. One-way analysis of variance (one-way ANOVA) was used to evaluate variety and harvest time depended differences regarding the parameters analyzed. In case of significance, differences between mean values of specific varieties and harvest times were evaluated using the Duncan's new multiple range test (Zolman, 1993).

Furthermore, in an attempt to explore whether some underlying pattern of relationships exists, such that the data may be reduced to a smaller set of components that may be taken as source variables accounting for the observed interrelations in the data, an exploratory factor analysis and especially the principal component analysis (PCA) were applied to the variable set of phenolics and antioxidant activity and oxidation status of oils (Jolliffe, 2002). The Kasiser–Meyer–Olkin (KMO) measure of sampling adequacy was used for testing whether the variables were adequate to correlate and the Bartlett's test of sphericity was used for testing if there was a relationship between the variables used. In addition, the varimax rotation method was used to make components/factors of PCA interpretable. In case of skewed distributions, symmetrizing transformations were applied ($\log_{10}$ or sqrt) to avoid distortion of results (Hinton et al., 2004).

Regression factor scores produced by PCA were further used as input variables and statistically analyzed using one-way ANOVA in an attempt to group the varieties/harvest combinations SPSS (1999). The Duncan's new multiple range test was used to evaluate mean values of regression factor scores of specific varieties/harvests.

All analyses were conducted using the statistical software program SPSS for Windows (v. 16). Significance was declared at $P \leq 0.05$.

3. Results

3.1. Physical and chemical parameters

The ripening indexes of olives for the three HD's were 2.5, 4.0, 5.8 for Eğriburun; 2.0, 4.0, 6.0 for Karamani; 2.2, 4.5, 6.1 for Halhalı; 1.4, 3.2, 5.3 for Saurani and 1.5, 3.5, 5.0 for Haşebi varieties, respectively.

The acidity of the oils from varieties used in this study varied between 0.52% and 0.85%, which was below the limit required for extra virgin oils (EEC, 1992) (Table 1). The free acidity values were apparently not affected by the nature of variety on the third HD. As regard to first and second HD's, the oil from Saurani variety had higher acidity values (average ~0.80%) when compared to other variety oils, while it showed lowest peroxide values together with Karamani oils among the five varieties.

Peroxide values of the samples were in the range of 2.01–7.08 mequiv. O_2/kg oil. The highest peroxide values were found in samples of Eğriburun and Halhalı oils 6.75 and 7.08 mequiv. O_2/kg oil, respectively; in the first HD. On the second HD, Haşebi oils showed the highest peroxide values with an average value of 5.67 mequiv. O_2/kg oil.

Free acidity increased towards late harvest times in most of the varieties studied. However, there was no significant change for Halhalı and Karamani oils.

3.2. Chlorophyll and carotenoids

Chlorophyll and carotenoid pigments of all varieties were high at the beginning, followed by a rapid decrease until the end of last HD, from an average value (mg/kg) of 15.38–8.36 and 11.21–5.92, respectively (Table 1). The oils of Eğriburun variety had the highest chlorophyll (9.34–17.73 mg/kg) and carotenoids (7.22–12.57 mg/kg) contents, the chlorophyll and carotenoids were similar in the rest of the varieties analyzed, although there were slight differences for Saurani and Haşebi oils on the first HD and Karamani oils on the second HD, which presents lower carotenoid contents (Table 1).

3.3. Tocopherols

In olive oil samples, four different tocopherols have been described: α-, β-, γ- and δ-tocopherols. The contents of these tocopherols are shown in Table 1. Karamani and Saurani oils showed higher values (224–343 mg/kg) of α-tocopherol than the other variety oils, whereas Haşebi oils contained the lowest levels of this tocopherol. Karamani oils showed also higher values of β-, γ- and δ-tocopherols on the first and second HD's, while Saurani oils showed the lowest values of β- and δ-tocopherols.

Concerning β-, γ- and δ-tocopherols, the highest values were determined in Karamani oils on the first HD's (1.79, 5.88, 0.04; respectively) and in Halhalı oils on the further HD's (3.42, 7.20,

Table 1
Some quality parameters, pigments and tocopherol contents of monovarietal virgin olive oil samples.

Parameters	HD[b]	Olive varieties				
		Eğriburun	Karamani	Halhalı	Saurani	Haşebi
Free fatty acids	1	0.5 ± 0.1B[a] c[‡]	0.6 ± 0.11abc	0.7 ± 0.1 ab	0.7 ± 0.03B a	0.6 ± 0.04B bc
	2	0.8 ± 0.1A ab	0.7 ± 0.03 b	0.7 ± 0.1 b	0.9 ± 0.04A a	0.7 ± 0.1AB b
	3	0.8 ± 0.1A	0.8 ± 0.04	0.8 ± 0.04	0.8 ± 0.02B	0.8 ± 0.1A
Peroxide value	1	6.8 ± 0.6A a	3.9 ± 0.4A b	7.1 ± 0.5A a	4.4 ± 0.5A b	3.8 ± 0.9B b
	2	3.9 ± 0.2B b	2.0 ± 0.2B c	3.9 ± 0.2B b	3.8 ± 0.1A b	5.7 ± 0.8A a
	3	2.5 ± 0.6C b	2.4 ± 0.4A b	4.5 ± 0.5B a	2.7 ± 0.4B b	2.9 ± 0.2B b
Chlorophylls	1	15.9 ± 1.2A ab	15.0 ± 0.9A b	14.1 ± 1.3A b	17.7 ± 1.0A a	14.2 ± 0.6A b
	2	11.9 ± 0.7B b	9.0 ± 0.6B c	8.8 ± 0.5B c	15.4 ± 0.9B a	11.1 ± 0.8B b
	3	8.2 ± 0.8C ab	8.6 ± 1.3B ab	7.0 ± 1.8B b	9.3 ± 0.8C a	8.6 ± 0.5C ab
Carotenoids	1	9.9 ± 0.2A d	12.1 ± 0.1A b	11.5 ± 0.3A c	12.6 ± 0.2A a	9.9 ± 0.4A d
	2	8.3 ± 0.2B bc	6.7 ± 0.3B d	8.9 ± 0.2B b	9.9 ± 0.7B a	7.7 ± 0.5B c
	3	5.3 ± 0.4C b	5.4 ± 0.4C b	6.0 ± 0.6C b	9.2 ± 0.8B a	5.7 ± 0.4C b
α-Tocopherol	1	298.3 ± 32.1A ab	313.1 ± 23.7A a	282.9 ± 72.1 bc	343.0 ± 44.4A a	240.7 ± 29.6A c
	2	133.2 ± 23.6B c	318.5 ± 24.7A a	184.7 ± 33.1 b	224.4 ± 21.2B b	195.6 ± 24.5B b
	3	177.8 ± 21.5B c	285.2 ± 19.4B ab	209.5 ± 66.7 b	304.7 ± 71.7A a	141.9 ± 47.1B c
β-Tocopherol	1	0.3 ± 0.1B c	1.8 ± 0.4 b	1.1 ± 0.2C bc	3.4 ± 1.1A a	0.4 ± 0.2B c
	2	0.1 ± 0.03B d	1.1 ± 0.1 c	2.2 ± 0.3B a	0.1 ± 0.02B c	1.6 ± 0.4A b
	3	2.6 ± 0.7A a	1.5 ± 0.4 b	3.4 ± 0.4A a	0.8 ± 0.2B b	1.0 ± 0.3B b
γ-Tocopherol	1	3.6 ± 0.5B b	5.9 ± 0.5B a	1.1 ± 0.2C c	1.1 ± 0.1B c	1.0 ± 0.2C c
	2	9.8 ± 2.8A b	8.3 ± 0.4A b	12.5 ± 1.1B a	14.8 ± 3.1A a	7.7 ± 0.8A c
	3	0.8 ± 0.2B c	2.0 ± 0.4C b	7.2 ± 0.7A a	2.3 ± 0.4B b	2.4 ± 0.3B b
δ-Tocopherol	1	nd[d]	tr[c]	tr	tr	tr
	2	nd	tr	tr	tr	tr
	3	nd	tr	tr	tr	nd

[a] Mean value ± standard deviation.
[b] HD: harvest date.
[c] Trace.
[d] Not determined.
[‡] Significant differences in the same row are shown by different lowercase letters [comparison between varieties] ($P \leq 0.05$).
Significant differences in the same column are shown by different uppercase letters (A–C) [comparison between harvest dates] ($P \leq 0.05$).

0.12; respectively) whereas the lowest γ-tocopherol levels were found in Eğriburun (third HD) and Haşebi oils (first and second HD). β-Tocopherol was higher at the late harvest than those of earlier samples in Eğriburun, Halhalı and Haşebi oils, but there was a decrease for Saurani oils and a steady level for Karamani oils in β-tocopherol level when fruits harvested later. δ-Tocopherol contents of the oils were generally low, for instance δ-tocopherol was not detected in Eğriburun oils at the three HDs and were determined at trace levels in the other variety oils. In general, γ-tocopherol contents of oils reached to the highest levels on the second HD.

3.4. Fatty acid composition

It is well known that the fatty acid composition of olive oil is quantitatively affected by two main factors: the olive variety used in the production of the oil and the ripening stage at which the olives are harvested (Baccouri et al., 2007).

Linoleic acid, is especially in the first two HD's, was high in Halhalı and Karamani oils as the average ratios of three HD's were 10.55% and 9.67%, respectively. For the other fatty acids, palmitoleic (0.6–2.7%), stearic (1.9–5.0%), linolenic (0.67–1.0%) and arachidic (0.37–0.73%), although their contents changed from one olive oil to another, they were fairly small. Oils of Eğriburun and Karamani varieties had the highest stearic acid content (average 4.47%) (Table 2).

The highest oleic acid ratio was determined in Saurani variety on the second HD, in Eğriburun variety on the first and third HD's and in Karamani variety on the first HD. As compared to the oils of Saurani, Karamani and Eğriburun varieties, Halhalı and Haşebi varieties produced oils with lower levels of oleic acid around 68%. The remaining oils showed high oleic acid levels rising to 75% for Eğriburun variety in the first HD. Although Eğriburun and Karamani oils showed high oleic acid levels, higher stearic acid level in these two varieties make Saurani oils superior in terms of fatty acid composition. Oleic acid levels of Halhalı and Haşebi oils (average 67.74% and 69.44%, respectively) were lower than that in the other variety oils; nevertheless their palmitic acid percentages were high.

On comparing the results by harvest time, significant differences in the fatty acid compositions of the oils can be seen regarding the HD. There was a steady decline in stearic acid ratio of Haşebi oils when HD goes further, whereas no statistically significant decline was observed for Karamani and Halhalı varieties and for Eğriburun and Saurani varieties stearic acid reached the highest levels on the second HD, but then decreased on the third harvest. Palmitic acid level steadily decreased along the fruit maturation or harvest time continuum in all varieties, except Eğriburun variety whose palmitic acid level steadily increased with regard to harvest time. Oleic acid levels also decreased when harvest time progressed, where the decrease was more significant for Karamani and Eğriburun varieties. There were increases in the linoleic and linolenic acid ratios, except for Karamani and Halhalı oils for linoleic acid and Eğriburun and Saurani oils for linolenic acid in which these fatty acid levels did not show significant changes when harvest time progresses. The fatty acid composition of Halhalı variety was more stable regarding the changes due to HD's.

3.5. Phenolic compounds

The calculated amounts of phenolic compounds are given in Table 3. The main phenolic components found in the olive oils studied were h-tyrosol, tyrosol, 3,4-DHPEA-EDA, *p*-HPEA-EDA,

Table 2
Fatty acid compositions of monovarietal virgin olive oil samples.

Fatty acids	HD[b]	Olive varieties				
		Eğriburun	Karamani	Halhalı	Saurani	Haşebi
Palmitic	1	8.9 ± 1.4[a]B b[‡]	9.1 ± 0.3A b	14.8 ± 0.6 a	13.1 ± 0.4A a	14.2 ± 1.5A a
	2	12.8 ± 1.5A a	9.4 ± 0.6A b	14.5 ± 1.2 a	13.7 ± 0.5A a	13.6 ± 0.5AB a
	3	11.6 ± 0.5A b	8.4 ± 0.3B c	13.3 ± 0.7 a	11.4 ± 1.1B b	12.2 ± 0.3B ab
Palmitoleic	1	2.7 ± 0.6 a	1.1 ± 0.2A b	2.6 ± 0.5 a	1.1 ± 0.5A b	0.7 ± 0.1A b
	2	1.7 ± 0.1 a	0.7 ± 0.2B b	1.4 ± 0.5 a	0.8 ± 0.8B b	0.7 ± 0.1AB b
	3	2.4 ± 0.6 a	0.6 ± 0.1B b	1.1 ± 0.1 b	0.7 ± 0.3B b	0.6 ± 0.04B b
Stearic	1	4.5 ± 0.7AB ab	5.0 ± 0.6 a	2.51 ± 0.4 c	2.9 ± 0.5AB c	3.8 ± 0.2A b
	2	5.0 ± 0.9A a	4.1 ± 0.3 ab	2.3 ± 1.2 c	3.5 ± 0.8A bc	2.9 ± 0.3B bc
	3	3.7 ± 0.5B a	4.2 ± 0.4 a	2.3 ± 0.8 b	1.9 ± 0.3B b	2.4 ± 0.4B b
Oleic	1	75.6 ± 1.7A a	73.1 ± 0.9A ab	68.4 ± 2.0 d	71.2 ± 1.1 bc	70.2 ± 1.2 cd
	2	69.3 ± 0.9B b	69.7 ± 0.6B b	67.3 ± 0.4 c	72.5 ± 1.3 a	69.5 ± 0.3 b
	3	71.1 ± 0.9B a	70.6 ± 2.1AB ab	67.5 ± 1.2 c	70.7 ± 0.6 ab	68.7 ± 0.5 bc
Linoleic	1	3.9 ± 0.2B d	9.4 ± 1.2 a	9.9 ± 0.7 a	5.4 ± 0.6A c	7.8 ± 0.6B b
	2	6.8 ± 0.5A c	10.6 ± 0.3 a	10.6 ± 1.0 a	6.3 ± 1.3B c	8.4 ± 0.2B b
	3	7.1 ± 0.2A c	9.1 ± 0.6 b	11.1 ± 0.2 a	11.3 ± 1.1B a	10.2 ± 0.5A ab
Linolenic	1	1.0 ± 0.2 a	0.8 ± 0.2B ab	0.7 ± 0.1B b	1.0 ± 0.2 a	0.7 ± 0.1B b
	2	1.0 ± 0.1 a	1.0 ± 0.2A a	0.8 ± 0.1A b	0.9 ± 0.04 b	0.9 ± 0.1A b
	3	0.9 ± 0.1 ab	1.0 ± 0.1A ab	1.0 ± 0.1A ab	1.0 ± 0.2 a	0.9 ± 0.1AB b
Arachidic	1	0.5 ± 0.04B a	0.5 ± 0.1 ab	0.5 ± 0.04AB a	0.5 ± 0.1 a	0.4 ± 0.1B b
	2	0.6 ± 0.07A a	0.6 ± 0.03 a	0.4 ± 0.1B b	0.5 ± 0.1 b	0.5 ± 0.04A b
	3	0.5 ± 0.03B bc	0.5 ± 0.1 b	0.6 ± 0.08A a	0.4 ± 0.02 bc	0.4 ± 0.03B c

[a] Mean value ± standard deviation.
[b] HD, harvest date.
[‡] Significant differences in the same row are shown by different lowercase letters [comparison between varieties] ($P \leq 0.05$).
Significant differences in the same column are shown by different uppercase letters (A–C) [comparison between harvest dates] ($P \leq 0.05$).

p-HPEA-EA and 3,4-DHPEA-EA. For all the varieties studied, 3,4-DHPEA-EDA was the most abundant phenolic substance ranging from 33.26 to 136.44 mg/kg. Significant differences between the four varieties studied as concerning their phenolic constituent levels were observed. Saurani and Haşebi oils had higher levels of 3,4-DHPEA-EDA, *p*-HPEA-EDA, *p*-HPEA-EA and 3,4-DHPEA-EA, while Haşebi oils showed the lowest levels of luteolin, apigenin and vanillic acid. Karamani oils showed lower levels of *p*-HPEA-EA and *p*-HPEA-EDA, while oils of this variety contained higher levels of luteolin than those of other varieties. The content of h-tyrosol was significantly higher in the oil from Karamani, with the values varied between 31.99 and 74.15 mg/kg. Oils of Eğriburun variety contained remarkable higher levels of tyrosol rising up to 12.87 mg/kg on the second HD, whereas the remaining four varieties contained this compound with values ranging between 0.16 and 4.50 mg/kg.

Some of the phenolic compounds such as 3,4-DHPEA-EA were present at low levels on the first HD then their levels in olive oils increased on the second HD and eventually decreased on the last HD, except the oils from Karamani variety (Table 3). This phenomenon was more often observed for Halhalı, Eğriburun and Saurani varieties for phenolic compounds, such that these varieties contained the highest level of phenolic compounds on the second HD when compared to first and third HD's. Concerning 3,4-DHPEA-EDA, there were reductions in its concentration when the HD moved on, except Saurani variety which had the highest level of this compound on the second HD. The difference was more marked for the Halhalı variety, which was three-times lower than the levels detected in oils extracted from earlier harvested fruits. The changes in some phenolic compounds such as luteolin, apigenin, cinnamic, caffeic, vanillic acids and tyrosol according to harvest time appeared to be more dependent on the varietal origin of the oils. Tyrosol showed decreasing trends in Halhalı and Haşebi oils when the harvest retarded. However, tyrosol in Saurani and Karamani oils was higher at the late harvests then those of earlier samples. The amounts of phenolic acids namely vanillic acid (0.10–0.79), syringic (<0.10), caffeic (0.01–0.31), cinnamic (0.02–0.19) and *p*-coumaric (0.01–0.15) acids were not exceeding 1 mg/kg in the virgin olive oils tested in this study. The contents of caffeic and syringic acids were significantly higher in the oil from Eğriburun and syringic acid levels of Saurani oils were also higher especially in the first and third HD's, whereas the lowest amounts of this compound were recorded in Karamani oils.

Phenolic acids generally decreased as HD moved on except for Halhalı oils as phenolic acid levels in this variety oils increased with harvest time. In all the variety of oils *p*-coumaric acid content presented decreases, while caffeic acid level remained constant or showed increasing trend along the further HD's.

3.6. Total phenolics

The total phenolics in the olive oils ranged between 64.09 and 320.66 mg gallic acid/kg oil (Fig. 1). Even the oils from Eğriburun variety had the highest total phenolic content on the first HD, in general the total phenolic contents of Haşebi and Saurani oils were higher than those of other varieties especially on the second and third HD's. Halhalı oils were the poorest samples in terms of total phenolics.

The total phenolics in the oils generally decreased along the further HD's, whereas the highest values were determined on the second HD for Saurani and Haşebi varieties. This behavior of Saurani and Haşebi varieties may be attributed to their late maturation compared to other varieties.

3.7. Antioxidant activity

Phenolic extracts of Saurani oils showed the highest antioxidant activity measured according to BCB and TEAC assays as well as high total phenolic content (Fig. 1). Together with Saurani oils, TEAC of oils from Karamani variety was higher than those of other variety oils, but this variety did not exhibit such high levels of antioxidant activity in terms of BCB assay. The oils from Eğriburun and Halhalı

Table 3
Content of individual phenols [mg/kg of oil] determined by HPLC in the monovarietal virgin olive oils.

Phenolic compounds	HD[b]	Olive varieties				
		Eğriburun	Karamani	Halhalı	Saurani	Haşebi
Hydroxytyrosol	1	68.6 ± 5.9[a]A a,[‡]	74.2 ± 5.9A a	4.1 ± 0.3B d	16.4 ± 3.2B c	30.0 ± 3.1A b
	2	10.9 ± 1.3B c	31.9 ± 3.9C b	55.3 ± 8.1A a	37.9 ± 5.4A b	5.0 ± 1.1B c
	3	2.3 ± 0.3C b	46.9 ± 4.4B a	3.7 ± 0.5B b	6.1 ± 0.6C b	2.4 ± 0.3B b
Tyrosol	1	1.7 ± 0.2B a	0.8 ± 0.1B b	2.2 ± 0.8A a	0.2 ± 0.0B b	2.3 ± 0.1A a
	2	12.9 ± 3.3A a	2.7 ± 0.9A b	2.4 ± 0.7A b	1.9 ± 0.8B b	1.7 ± 0.3B b
	3	0.9 ± 0.1B b	3.8 ± 0.9A a	0.4 ± 0.1B b	4.5 ± 1.4A a	0.4 ± 0.1C b
Vanillic acid	1	0.4 ± 0.1A b	0.8 ± 0.1A a	0.1 ± 0.01C c	0.2 ± 0.01B c	0.1 ± 0.01B c
	2	0.5 ± 0.1A b	0.2 ± 0.01C cd	0.6 ± 0.1A a	0.3 ± 0.10B c	0.1 ± 0.03C d
	3	0.2 ± 0.01B b	0.3 ± 0.03B b	0.4 ± 0.1B a	0.4 ± 0.05A a	0.2 ± 0.02A c
Caffeic acid	1	0.1 ± 0.01B a	tr c[c]	0.04 ± 0.00B b	0.04 ± 0.00B b	tr c
	2	0.3 ± 0.06A a	tr c	0.19 ± 0.06A b	0.04 ± 0.00B c	tr c
	3	0.3 ± 0.00A a	tr c	0.18 ± 0.04A a	0.08 ± 0.02A b	tr c
Syringic acid	1	0.03 ± 0.01B ab	0.10 ± 0.01A b	ndB b[d]	0.06 ± 0.01A a	0.04 ± 0.03 ab
	2	0.05 ± 0.01A a	nd B b	0.04 ± 0.01A a	tr B b	tr b
	3	tr C b	nd B b	0.04 ± 0.00A b	tr B a	tr b
p-Coumaric acid	1	tr B c	0.15 ± 0.02A a	0.13 ± 0.02A ab	0.11 ± 0.02A b	0.11 ± 0.02A b
	2	tr B b	0.07 ± 0.02B a	tr C b	0.05 ± 0.00B a	tr B b
	3	0.14 ± 0.04A a	0.03 ± 0.00C bc	0.04 ± 0.00B b	tr C c	tr B c
3,4-DHPEA-EDA	1	72.7 ± 17.2A bc	52.2 ± 7.3AB bc	105.2 ± 7.2A b	33.3 ± 7.8B c	136.4 ± 15.8A a
	2	89.6 ± 16.7A ab	65.8 ± 11.1A b	74.8 ± 6.4B b	134.6 ± 5.2A a	110.7 ± 10.5B ab
	3	37.0 ± 7.9Bc	38.2 ± 7.2B c	35.7 ± 4.8C c	54.5 ± 10.5B b	62.8 ± 4.2C a
p-HPEA-EDA	1	24.4 ± 6.17 b	14.1 ± 3.5A bc	6.8 ± 1.5B c	45.3 ± 4.5A a	57.3 ± 9.0A a
	2	23.1 ± 9.16	16.9 ± 2.2A	14.8 ± 3.6A	19.1 ± 7.1B	12.0 ± 1.9B
	3	20.0 ± 10.81 a	2.9 ± 0.4B b	9.4 ± 2.1B b	15.6 ± 4.7B ab	10.2 ± 2.9B ab
Cinnamic acid	1	0.05 ± 0.01B b	0.19 ± 0.04A a	tr C b	0.07 ± 0.07 b	0.06 ± 0.02B b
	2	0.08 ± 0.02B b	0.04 ± 0.00B c	0.04 ± 0.01B c	tr c	0.14 ± 0.04A a
	3	0.15 ± 0.04A a	tr B d	0.08 ± 0.02A bc	0.09 ± 0.01 b	0.05 ± 0.01B cd
3,4-DHPEA-EA	1	9.4 ± 1.4B bc	10.8 ± 2.2A b	8.9 ± 1.8B bc	6.9 ± 1.2B c	19.2 ± 1.4B a
	2	17.8 ± 3.0A b	12.1 ± 1.5A c	13.8 ± 3.0A bc	13.7 ± 0.7A bc	23.8 ± 1.6A a
	3	4.3 ± 0.3C bc	5.1 ± 0.5B b	5.6 ± 0.9B b	3.6 ± 1.0C c	7.3 ± 0.8C a
p-HPEA-EA	1	5.5 ± 1.9 abc	3.1 ± 1.2 bc	5.6 ± 0.2Aabc	4.5 ± 0.9 bc	7.7 ± 0.6A a
	2	3.7 ± 1.2 ab	2.3 ± 1.2 b	5.7 ± 0.5A a	5.9 ± 1.6 a	3.6 ± 0.6B ab
	3	1.2 ± 0.2 c	2.2 ± 0.6 b	2.4 ± 0.1Bcb	6.1 ± 1.8 a	1.9 ± 0.2C b
Luteolin	1	1.4 ± 0.3A a	0.8 ± 0.1B b	0.2 ± 0.1B c	0.2 ± 0.03C c	0.8 ± 0.1A b
	2	0.8 ± 0.1B c	1.9 ± 0.1A a	1.1 ± 0.1A b	0.4 ± 0.1B d	0.3 ± 0.02B d
	3	0.5 ± 0.1B c	0.2 ± 0.01C d	0.9 ± 0.2A b	1.1 ± 0.1A a	0.1 ± 0.01B d
Apigenin	1	0.1 ± 0.00AB b	0.04 ± 0.01 c	0.04 ± 0.00B bc	0.20 ± 0.06A a	nd c
	2	0.2 ± 0.00A a	0.04 ± 0.01 b	0.04 ± 0.00B b	0.20 ± 0.07A a	nd b
	3	tr B b	0.04 ± 0.00 b	0.18 ± 0.05A a	0.05 ± 0.02B b	nd b

[a] Mean value ± standard deviation.
[b] HD, harvest date.
[c] Trace.
[d] Not determined.
[‡] Significant differences in the same row are shown by different lowercase letters [comparison between varieties] ($P \leq 0.05$).
Significant differences in the same coloumn are shown by different uppercase letters (A–C) [comparison between harvest dates] ($P \leq 0.05$).

varieties showed lower antioxidant activity measured in terms of both BCB and TEAC assays.

Olive oil phenolic extracts possessed highest antioxidant activity (BCB) on the second harvest, except Saurani and Haşebi oils which did not exhibit significant change in antioxidant activity among three HD's. The change in antioxidant activity (TEAC) by harvest time was not significant for Saurani and Haşebi oils, but for the rest of varieties it was higher at later harvest dates.

3.8. Principal component analysis (PCA)

Furthermore, the data set for different cultivars and harvest periods regarding the variables of phenolics and antioxidant activity and peroxide values of oils was subjected to exploratory factor analysis and especially to PCA, in an attempt to explore possible underlying pattern of relationships. After evaluating the correlation matrix of the data set, ten variables expressing phenolics and antioxidant activity/oxidation status of oils were finally used for the PCA ($n = 45$).

With the implementation of varimax rotation, three factors were produced, explaining 66.8% of the total variance in the data set used (Table 4). Factor 1 has an eigenvalue of 3.062, accounting after rotation for 25.997% of the total variance in the data set; factor 2 has an eigenvalue of 2, accounting for 20.593% of the total variance, while factor 3 has an eigenvalue of 1.616, accounting for 20.185% of the total variance. The cumulative column shows the after rotation amount of variance (%) accounted for by each consecutive factor added together.

Table 5 displays the coefficients of the ten variables load onto the three factors extracted with PCA and varimax rotation method with Kaiser normalization. A high absolute value of a loading indicates strong correlation between the variable and the factor. Results in

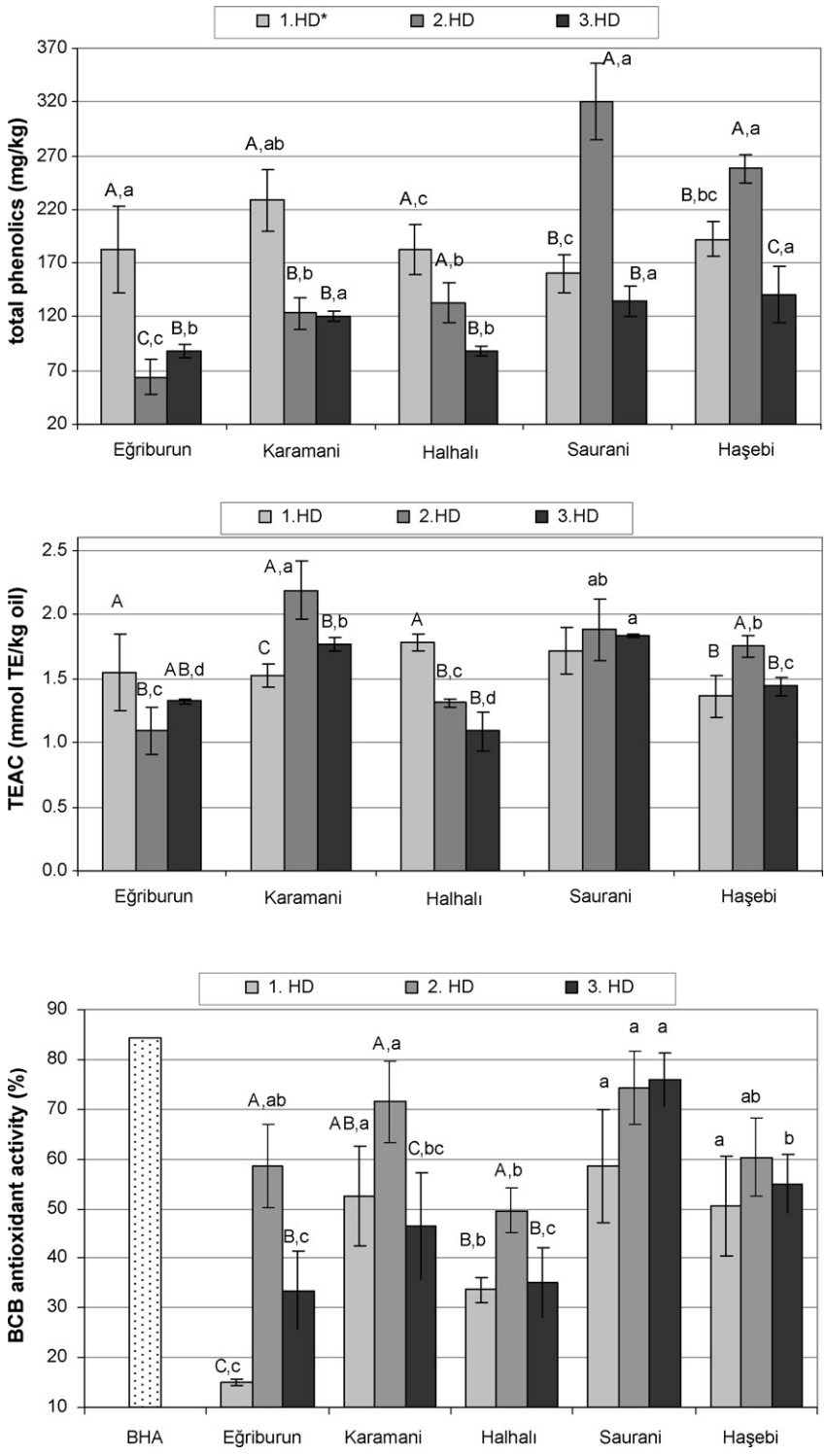

Fig. 1. Total phenolics and antioxidant activity in monovarietal olive oils.

Table 4 indicate that factor 1 is related to DHPEA-EDA-3,4, DHPEA-EA-3,4, *p*-HPEA-EA and LOG_*p*-HPEA-EDA, and can be named as "Secoiridoids", factor 2 is related to LOG_luteolin, LOG_vanillic and LOG_htyrosol and can be called as "Phenolics", while factor 3 is related to BCB, LOG_peroxide and free fatty acids and can be called "Oxidation".

In order to have a clearer picture of which variables significantly "load" onto each one of the three factors extracted by PCA, this correlation can be presented in two plots (Fig. 2 – factor 1 against factor 2 and Fig. 3 – factor 2 against factor 3). Variables at the ends of the zero lines have high loadings on only to that factor, while variables near the intersection (the point 0,0) are associated with neither factor.

Studying Fig. 2, it can be seen that loadings for factor 1 fall into one distinct clump with values close to +1, while the other loadings are less than ±0.5. Similarly, the loadings for factor 2 fall into one distinct clump with values close to +1, while the other loadings are less than ±0.5 denoting no association with the factor. Fig. 3 shows

Table 4
Rotated component matrix (varimax rotation method).

Variable	Component		
	1	2	3
3,4-DHPEA-EDA	0.844	–0.155	–0.090
3,4-DHPEA-EA	0.790	–0.032	–0.129
p-HPEA-EA	0.697	0.245	–0.211
LOG_*p*-HPEA-EDA	0.584	0.327	0.068
LOG_luteolin	0.137	0.836	0.059
LOG_vanilic	–0.386	0.751	–0.152
LOG_htyrosol	0.240	0.695	–0.128
BCB	0.174	–0.085	0.890
LOG_peroxide value	0.306	–0.135	–0.832
Free fatty acids	–0.295	–0.312	0.646

Table 5
Mean values of variables loading strongly the factor 1.

	Variety/harvest	3,4-DHPEA-EA	3,4-DHPEA-EDA	*p*-HPEA-EA	LOG_*p*-HPEA-EDA
Mean	Overall sample	10.8042	73.6196	4.1082	1.17900
	Hasebi/Oct	19.1567	136.4367	7.6533	1.75418

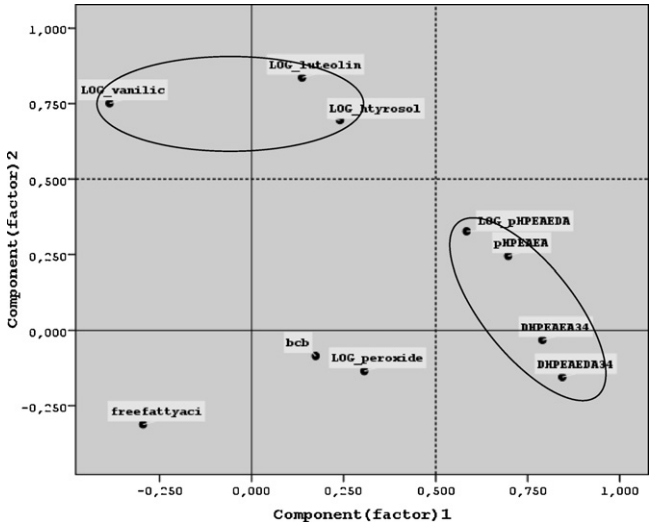

Fig. 2. Component plot of factors 1 and 2 in rotated space.

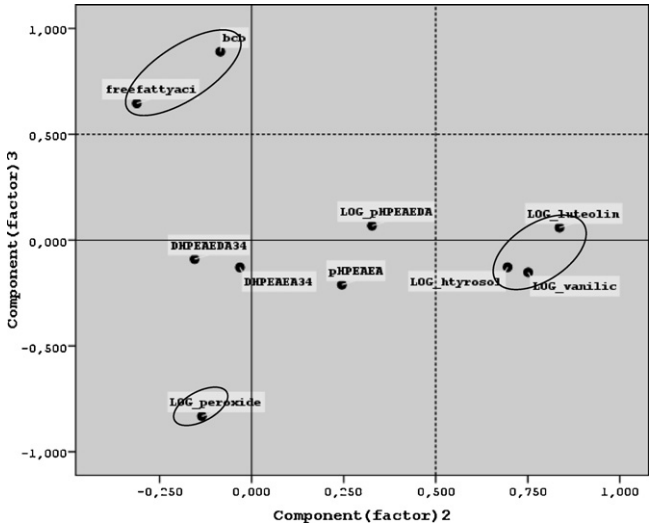

Fig. 3. Component plot of factors 2 and 3 in rotated space.

that loadings for factor 3 fall into two distinct clumps with values close to ±1, while the other loadings are less than ±0.5.

Expressed in their sample mean, REGR factor scores produced by PCA for each factor were plotted in two charts (Figs. 4 and 5) to somehow identify different distinctive variety/harvest groups or/and possible outliers. Note that scores outside ±2 may be considered as extreme.

Fig. 4 shows that factor score 1 (Secoiridoids), Saurani/HD2 and Hasebi/HD2 form one distinct group, while Halhali/HD3, Karamani/HD3 and Eğriburun/HD3 form another one. The other variety/harvest combinations seem to belong more or less to the same group, except Hasebi/HD1 which exhibits a different behavior and has the most extreme score. It is around to 2.2, so it is 2.2 standard deviations larger than the mean of the factor score 1.

To understand why this variety/harvest is extreme, the data were compared with the mean of the overall sample. For all variables that load strongly on factor 1, Hasebi/HD1 has higher values than average of overall sample.

For factor score 2 (Phenolics), Hasebi/HD3, Hasebi/HD2 and Halhali/HD1 seem to form a distinct group, while Karamani/HD1, Halhali/HD2 and Eğriburun/HD1 form another one.

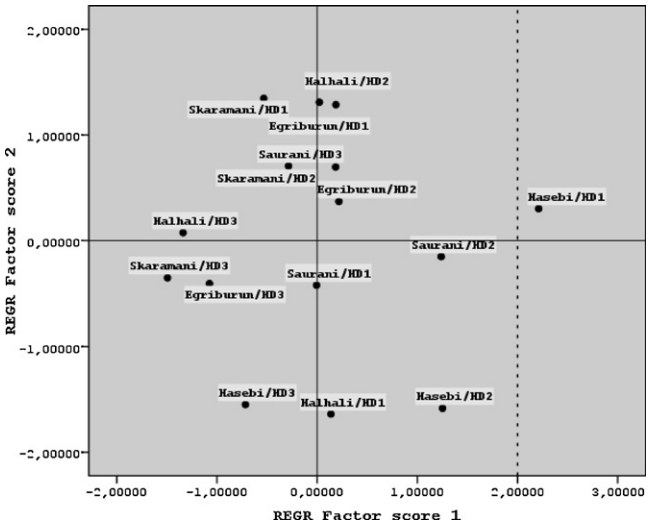

Fig. 4. REGR factor scope plot of factors 1 and 2.

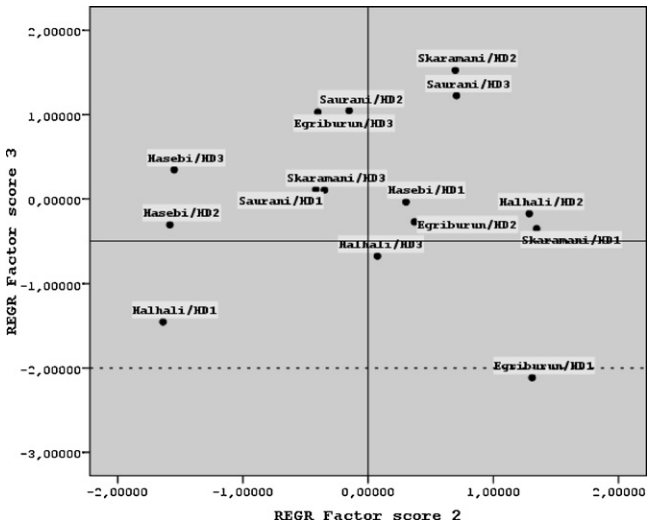

Fig. 5. REGR factor scope plot of factors 2 and 3.

Fig. 5, shows that for factor score 3 (Oxidation), Karamani/HD2, Saurani/HD3, Saurani/HD2 and Eğriburun/HD3 form one distinct group. Halhali/HD1 forms another one, while the other variety/harvest combinations seem more or less to belong to the same group, except Eğriburun/HD1 which exhibits a different behavior. For factor score 3, Eğriburun/HD1 seems to have the most extreme score. It is around to 2.1, so it is 2.1 standard deviations larger than the mean of the factor score 3. To understand why this variety/harvest is extreme we compare its data with the mean of the overall sample (Table 6). As it can be seen, for the variables that load strongly on factor 3, Eğriburun/HD1 has lower free fatty acid and BCB values, while its peroxide is higher than average of overall samples.

In an attempt to group the varieties/harvests more precisely than with the graphical displays, REGR factor scores produced from PCA were further statistically analyzed using one-way analysis of variance (one-way ANOVA). The Duncan's new multiple range tests were used to evaluate mean values of regression factor scores of specific varieties or harvests (Table 7).

4. Discussion

Olives at a later stage of ripening yield oils with higher levels of free acidity since they undergo an increase in enzymatic activity, especially lipolytic enzymes, and are more sensitive to pathogenic infections and mechanical damage (Salvador et al., 2001). This increase in acidity could be due to the activity of an internal olive lipase in the intact fruit cells during fruit maturation or in the milling mass during the process of oil extraction. The level of activity of this enzyme could depend on multiple variables such as variety, the level of maturity, temperature, or humidity and it does not require previous biotic or abiotic fruit damage (Yousfi et al., 2006).

The peroxide values of all varieties decreased when harvest time retarded, except Haşebi variety which showed higher values on the second HD. These ambiguous behaviors have been described by other authors, who reported either increasing (Finotti et al., 2001) or decreasing trends (Baccouri et al., 2007) in peroxide index during maturity process and/or progressing harvest time. Yousfi et al. (2006) did not observe significant influence of ripening on the peroxide index. Apart from cultivation area or cultivar, factors causing damage to the fruits, such as olive fly attacks, improper systems of harvesting, and transport and storage of the olives, and by technological treatments, which may favor the hydrolysis of triglycerides, resulting in an increase of the free fatty acid concentration are reported to have the most significant influence on these quality parameters (Dabbou et al., 2010).

Table 6
Mean values of variables loading strongly the factor 3.

	Variety/harvest	Free fatty acids	BCB	LOG_peroxide value
Mean	Overall sample	0.7218	51.3929	0.57357
	Ergiburun/Oct	0.5167	15.0133	0.82830

The color change in olive oils during maturity and progressing harvest time can be due to the reduction of the pigment concentration, a natural breakdown, which parallels that of phenols and formation of other colored compounds such as anthocyanins (Ben Youssef et al., 2010). Previously published studies on different olive varieties report drops in chlorophyll pigments in oils (mg/kg) with ripening and harvest time such as 12.4–6.0 for Arbequina variety and 26.1–12.3 for Picual variety (Cerretani et al., 2006), 26–2 for Portuguese varieties (Matos et al., 2007), 27–2 for Cornicabra variety (Salvador et al., 2001), 22.01–1.37 for Tunusian varieties (Krichene et al., 2007). Carotenoids (mg/kg) have been reported to show a decreasing trend too, during ripening and harvest time such as 12.9–6.8 for Arbequina and for 13.9–9.8 for Picual variety (Yousfi et al., 2006), 28–2 for Cobrançosa, Madural and Verdeal varieties (Matos et al., 2007), 6.24–0.36 for mature fruits of Tunisian varieties (Krichene et al., 2007), 7.45–1.66 for Chètoui and 11.33–1.23 for Chemlali variety (Baccouri et al., 2008). The five Turkish variety oils can be considered to contain middle levels of chlorophyll and carotenoid pigments when compared to the literature data given above.

The present study and some previous studies also suggest that tocopherol content was highly variety-dependent (Salvador et al., 2001; Krichene et al., 2007). However, Cerretani et al. (2006) reported that the α-tocopherol content did not appear to be dependent on the varietal origin of the oils. The changes in β- and γ-tocopherols according to harvest time appeared to be more dependent on the varietal origin of the oils when compared to α-tocopherol. This assumption could also explain the ambiguous observations in other studies (Yousfi et al., 2006; Finotti et al., 2001; Baccouri et al., 2008; Patumi et al., 2002) in α-tocopherol content of olive oil by harvest time or ripening. Similarly, the β- and γ-tocopherols in olive oils were mostly reported not to show a regular change by ripening or harvest time (Matos et al., 2007; Baccouri et al., 2008). Previous studies reported the α-tocopherol contents of olive oils from many cultivars of Mediterranean countries as 132–261 μg/g (Matos et al., 2007), 141.94–364.23 mg/kg (Finotti et al., 2001), 193.7–349.7 mg/kg (Salvador et al., 2001), 240–480 mg/kg (Dabbou et al., 2009). Baccouri et al. (2007) reported the α-tocopherol contents of some Tunisian varieties at three different ripening stages as 121, 274 and 250 for Chetoi and as 321, 329 and 214 for Chemlali. α-Tocopherol contents of Ayvalık oils from Çanakkale in north west of Turkey were reported as 141.5 and 162.9 mg/kg in the years 2005 and 2006, respectively (Andjelkovic et al., 2009). Thus, the α-tocopherol content of the Karamani and Saurani oils can be considered medium-high (within 224–343 mg/kg). When compared to the β-tocopherol values (mg/kg) reported before for different cultivars from other Mediterranean countries as 2.20–12.83 (Baccouri et al., 2008), 6.32–19.71 (Cerretani et al., 2006), β-tocopherol contents of the oils used in this assay were low. γ-Tocopherol contents of oils in the present study (0.80–14.75 mg/kg) were also lower than the values previously reported as 4.83–18.15 mg/kg (Baccouri et al., 2008), 4.69–63.16 (Cerretani et al., 2006).

There are studies on the fatty acid compositions of olive oils which report that the oleic acid percentage increases (Cossignani

Table 7
Mean values of REGR factor scores produced by PCA (varimax rotation method).

Harvest	Variety	REGR factor scores 1	REGR factor Scores 2	REGR factor scores 3
HD1	1 = Eğriburun	0.0215170B a*	1.3102155D a	−2.1136740A a
	2 = Karamani	−0.5328913A a	1.3455420D a	−0.3534458B a
	3 = Halhali	0.1385652B a	−1.6383439A a	−1.4528761A a
	4 = Saurani	−0.0071421B a	−0.4176136B a	0.1120688B a
	5 = Hasebi	2.210095C a	0.302450C a	−0.0368005B a
HD2	1 = Eğriburun	0.2174748A a	0.3701090A b	−0.2732076A b
	2 = Karamani	0.1845150A b	0.6963095B b	1.5263339B b
	3 = Halhali	0.1865271A a	1.286401C b	−0.1740453A b
	4 = Saurani	1.2377976B b	−0.1522717D a	1.0472468B b
	5 = Hasebi	1.2514220B b	−1.5834497E b	−0.3073739A a
HD3	1 = Eğriburun	−1.0745013B b	−0.4024788A c	1.028508C c
	2 = Karamani	−1.4938515A c	−0.3486968A c	0.1046790B a
	3 = Halhali	−1.3376167A b	0.0743770B c	−0.6779407A c
	4 = Saurani	−0.285519C a	0.706120C b	1.225054C b
	5 = Hasebi	−0.7163919D c	−1.5486700D b	0.3454727B a

* A, B, C: mean values in the same column, for the same harvest time in common do not significantly differ ($P > 0.05$) [comparison between varieties].
a, b, c: mean value in the same column, for the same variety in common do not significantly differ ($P > 0.05$) [comparison between harvests].

et al., 2001; Shibasaki, 2005) as well as some of them report decreases (Baccouri et al., 2008) in the level of oleic acid with ripeness or harvest time. Nonetheless, Finotti et al. (2001) reported that there was a decrease in oleic acid level in Lastorka variety, while there was not any significant change for Buza variety in Croatia. Ranalli et al. (1998) reported that the oil fatty acid composition was affected by the olive ripening process; in particular oleic acid tended to increase, whilst palmitic acid showed a tendency to diminish. In general, the total amount of saturated fatty acids was lower in Karamani oils in all the HD's. Karamani variety is remarkable in terms of fatty acid composition which showed the highest stearic and the lowest palmitic acid ratios almost in all HD's. Matos et al. (2007) reported different trends in the stearic acid composition of virgin olive oils from several Portuguese varieties, such as Verdeal Transmontana and Madural, appear as a very homogeneous group where the maturation has almost no influence, while for Cobrançosa variety there was an increase in stearic acid as ripening proceeds which showed a sinusoidal behavior by the cultivars under this study.

It can be summarized that Saurani and Haşebi oils contained higher levels of the analyzed secoiridoid aglycones and Halhalı and Karamani oils had lower concentration of these compounds. When the values are considered in total, Halhalı oils contained the lowest levels of individual phenolic compounds analyzed in this study. This variety showed higher levels of h-tyrosol, apigenin, caffeic acid and vanillic acids on the second and third HDs. In general the phenolic constituents showed a decreasing trend when the harvesting date moved on. In a previous study (Morello et al., 2004) authors have also confirmed a decrease in the secoiridoid derivatives fraction throughout the ripening process and 3,4-DHPEA-EDA isomer being the most affected compound. Similar to our results, Brenes et al. (1999) reported that both the dialdehydic form and elenolic acid linked to tyrosol or h-tyrosol and the oleuropein or ligstroside aglycons diminished their concentrations in oils with olive maturation and on the contrary the free form of tyrosol and h-tyrosol tended to increase with maturation, as they attributed these changes to an enzymatic hydrolysis such as glucosidases or esterases. Generally the lowest contents of these phenolic acids were detected in the oils from Haşebi variety. In previous studies the amounts of these phenolic acids in virgin olive oils were also reported to be under 1 mg/kg, such as vanillic and *p*-coumaric acids (mg/kg) was reported to be 0.2 and 0.3 for Picual variety from Spain (Garcia et al., 2001), 0.85 and 0.14 for Arbosana variety grown in Tunisia (Allalout et al., 2009), 0.13 and 0.24 for Megaritiki variety from Greece (Iconomou et al., 2010) and 0.31 and 0.35 for Carotina variety from Italy (Caponio et al., 2001). Caponio et al. (2001) also reported steady decreases in the phenolic acids of olive oil of Carotina variety from Italy except ferulic acid which showed an increase by ripening. The decrease in phenolic content during maturation of olive fruit was reported before (Shibasaki, 2005). Baccouri et al. (2008) reported the total phenolic contents of (mg/kg) olive oils at different ripening stages as 138, 292 and 263 for Chetoi and as 331, 444 and 224 for Chemlali. The mean values (mg/kg) for total polyphenols contents of some Portuguese cultivars were reported between 124 and 203 (Matos et al., 2007), and Italian cultivars Carotina and O. salentina as 535, 396, 311 and 308, 257, 198 for oils extracted from green, partial purple and totally purple olives, respectively (Caponio et al., 2001). Again from Italy, Ranalli et al. (1998) reported the total phenolic content of virgin olive oils harvest at the end of October, November and December as 352, 293, 184 for Frantoio and 263, 144, 99 for Leccino varieties. The Turkish varieties analyzed in this study contained close amounts of total phenolics previously reported for the varieties from other Mediterranean countries, while not being as high as the values of Carotina and Chetoi varieties.

Some of the previous studies reported that the correlation between total phenols and antioxidant capacity was very high (Fogliano et al., 1999; Galvano et al., 2007). The higher antioxidant activity of phenolic extracts from Saurani oils can be attributed to the higher total phenolics and secoiridoids such as *p*-HPEA and *p*-HPEA-EDA, however the previous studies emphasize the greatest antioxidant influence exerted by 3,4-DHPEA-EDA than the other phenolic compounds (Morello et al., 2005; Galvano et al., 2007) and its greater efficiency in the micellar system in comparison with 3,4-DHPEA was attributed to its greater lipophilicity by Fogliano et al. (1999). Moreover, Fogliano et al. (1999) demonstrated that 3,4-DHPEA had a higher antioxidant activity than *p*-HPEA and that 3,4-DHPEA-EDA had a higher antioxidant activity than *p*-HPEA-EDA. The higher TEAC of Karamani oils can be due to its higher α-tocopherol, h-tyrosol and luteolin content as well, as Morello et al. (2005) included luteolin next to 3,4-DHPEA-EDA and its derivatives as being one of the most antioxidative phenolic compound. Regarding correlation between TEAC and "α-tocopherol, 3,4-DHPEA-EDA and luteolin", Pearson correlation was found significant only between TEAC and α-tocopherol ($r = 0.486$). No significant correlation was found between TEAC and luteolin or h-tyrosol.

Studying the information of the statistical evaluations appeared in Table 4 regarding varieties, it can be concluded that a group of Halhalı and Eğriburun takes attention which was defined by factor 1 (Secoiridoids) and factor 3 (Oxidation) for HD1 and HD2. As for HD's, regarding factor 1, Eğriburun and Halhalı varieties show

similar characteristics, as this factor forms a group for HD1 and HD2, and another group for HD3, while Karamani and Haşebi form three groups, each for every HD. Regarding factor 2, Karamani, Eğriburun and Halhalı form three groups, each for every HD, too. Regarding factor 3, Eğriburun and Halhalı again form three groups, each for every HD. So, Eğriburun and Halhalı varieties seem to show quite similar trends for the three factors in terms of varieties and HD's. The results of the analyses discussed above provide quite clear profiles/patterns of the olive oil samples influenced by their cultivar and HD regarding phenolics and antioxidant activity and oxidation status of oils.

5. Conclusions

The present study has shown that, the high total phenolics, secoiridoid aglycones, α-tocopherol and antioxidant activity of Saurani variety makes the oil of this variety superior to other varieties analyzed in this study. Halhalı variety oils were the poorest in terms of phenolic constituents, antioxidant activity and oleic acid level and showed the highest peroxide value among the varieties tested. Karamani showed good levels of antioxidant activity in terms of TEAC which could be attributed to its high levels of α-tocopherol, luteolin and h-tyrosol. Oils of Eğriburun variety contained remarkable higher levels of tyrosol on the second HD, which should be taken into account in terms of bitterness as tyrosol influences the flavor of the olive oil (Artajo et al., 2006). Saurani and Haşebi varieties seem to have characteristic phenolic profiles: Saurani for its high amount of *p*-HPEA-EDA, *p*-HPEA-EA and Kargaburun for its high level of 3,4-DHPEA-EDA, and 3,4-DHPEA-EA.

Regarding the effect of HD, it seems advisable not to delay olive harvest due to the decreases in the levels of minor components (phenolic compounds, a-tocopherols and pigments), and antioxidant capacity. Olive fruit harvest of the varieties Saurani and Haşebi can be retarded when compared to the other varieties studied in this research.

According to principal component analysis, the variables such as secoiridoids (3,4-DHPEA-EDA; 3,4-DHPEA-EA; *p*-HPEA-EDA and *p*-HPEA-EA) some of the phenolic compounds (h-tyrosol, vanillic acid and luteolin) and oxidation parameters (beta carotene bleaching activity, peroxide value and free fatty acids) were found to be the most convenient parameters in grouping the varieties for their similarity. Halhalı, Karamani and Eğriburun formed a distinct group at the late harvest, and another group at their earlier harvests in terms of their secoiridoid contents.

The results of the present study may be of particular interest in the introduction of new cultivars to the regions outside their traditional growing regions and are expected to attract the attention of the appropriate officials.

Acknowledgement

The research was supported by the postdoctorate research scholarship from the Turkish Council of Higher Education (YÖK).

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