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Full Length Research Paper

Influence of the seasonal conditions on phenolic composition and antioxidant activity of *Vitis vinifera* L.

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The aim of this study is determining the phenolic acid composition and antioxidant activity potential of *Vitis vinifera* collected from Osmaniye-Turkey. For determining the phenolic acid composition of the grapes collected in different seasons, samples were screened via a high performance liquid chromatography-diode array detection (HPLC-DAD) instrument. By this way, amounts of gallic acid, 2,3-dihydroxybenzoic acid, vanillic acid, caffeic acid, *p*-coumaric acid, ferulic acid, cinnamic acid, luteolin and apigenin were determined quantitatively. September sample was the riches one in terms of phenolic acids and flavonoids studied. This is followed by August and October samples, respectively. Total phenolic and flavonoid contents of the samples were also evaluated, using the Folin–Ciocalteu method. October sample was found as the richest one among the samples tested. Antioxidant activities of the samples were evaluated by using four complementary test systems. Results obtained from these samples showed a strong correlation with their phytochemical contents. The most active sample was found as the grape collected in October.

Key words: *Vitis vinifera,* high performance liquid chromatography (HPLC), extract, antioxidant activity, phenolic, flavonoid.

INTRODUCTION

Phenolic acids are a group of natural products found in many plants (Baublis et al., 2002; Baublis et al., 2000a, b; Saadi et al., 1998). These phenolic acids may vary in structure due to difference in number and position of the hydroxyl groups on the aromatic ring. As a group, these naturally occurring compounds have been found to be strong antioxidants against free radicals and other reactive oxygen species (ROS), the major cause of many chronic human diseases such as cancer and cardiovascular diseases (Andreasen et al., 2001a; Yu et al., 2002, 2003). There are mainly two groups of phenolic acids in plants: benzoic and cinnamic acid derivatives.

Abbreviations: ROS, Reactive oxygen species; **GC-MS,** gas chromatography mass spectrometry; **HPLC-DAD**, high performance liquid chromatography-diode array detection; **MS,** mass spectrometry; **PDA,** photodiode array; **FCR,** Folin–Ciocalteu's reagent; **TCA,** trichloroacetic acid; **DPPH**, 1,1-Diphenyl-2-picrylhydrazyl; **BHT,** Butylated hydroxytoluene; **BHA**, Butylated hydroxyanisole; **AA,** antioxidant activity.

Ferulic acid and other hydroxycinnamic acids (caffeic and *p*-coumaric acid derivatives) have been found to have good antioxidant activities (Andreasen et al., 2001b; Emmons et al., 1999). The presence of the CH=CH–COOH group in the hydroxycinnamic acids is considered to be key for the significantly higher antioxidative efficiency than the COOH in the hydroxybenzoic acids (White and Xing, 1997).

Natural antioxidants, especially plant phenolics, flavonoids, tannins and anthocyanidins, are safe and are also bioactive (Mohsen and Ammar, 2009). Putative therapeutic effects of many traditional medicines may be ascribed to the presence of these natural antioxidants, which can scavenge oxygen radicals and inhibit peroxidation (Maksimovic et al., 2005). Therefore, in recent years, considerable attention has been paid to explore the potential antioxidant property of plant extracts or isolated products of plant origin, which may be used for human consumption (Chua et al., 2008). Numerous crude extracts, from plant materials rich in phenolics, are increasingly of interest in the food industry, because

they can retard oxidative degradation of lipids and thereby improve the quality and nutritive value of food (Kahkonen et al., 1999; RiceEvans et al., 1995). Some studies have shown that the increased dietary intake of natural antioxidants such as flavonoids and other phenolic compounds, present in most plants, may act as potent candidates in preventing diseases related to oxidative stress, such as cancer, atherosclerosis, aging and rheumatoid arthritis (Rios et al., 2009; Behera et al., 2008; Halliwell, 2007).

Grapes are one of the most widely grown fruit crops throughout the world, and their composition and properties have been extensively investigated, with several reports of the presence of large amounts of phenolic compounds. Most phenolic compounds found in wine can act as antioxidants. Likewise, the residues of wine production are also characterized by high contents of phenolic compounds due to their incomplete extraction during wine production (Macheix et al., 1990; Oszmianski and Lee, 1990).

The composition of grape seeds is basically (w/w) 40% fiber, 16% essential oil, 11% protein, 7% complex phenolic compounds like tannins, sugars, minerals, and other substances (Campos et al., 2008). Grape skin is a source of anthocyanidins and anthocyanins, natural pigments with antioxidant properties acting through inhibition of lipoperoxidation and which also have antimutagenic activities (Pedreschi and Cisneros-Zevallos, 2006). Brazilian wine production generates approximately 59.4 million kg of pomace or 18 kg pomace/100 l wine, which is treated as a residue with low profit uses such as in animal feed and manure. Therefore, studies on the use of this residue as a valuable winery byproduct may lead to significant economical gains and prevent or decrease environmental problems caused by grape pomace accumulation (Campos et al., 2008).

The extraction methodologies and analyzing techniques of phenolic acids were summarized in a recent review (Robbins, 2003). Besides the use of GC-MS to measure the content of phenolic acids in foods (Zadernowski et al., 2005; Plessi et al., 2006), more studies employed high performance liquid chromatography (HPLC) coupled with photodiode array (PDA) detector (Mattila and Kumpulainen, 2002; Robbins and Bean, 2004; Wen et al., 2005; Luthria and Pastor-Corrales, 2006) or mass spectrometry (MS) (Ayaz et al., 2005) to isolate and quantify phenolic acids, and the latter became more popular for its efficiency and convenience with no need of derivatization before analyzing (Robbins, 2003).

As far as literature survey could ascertain, there are many reports in the literature concerning the phytochemical composition of *Vitis vinifera* L. from all over the world. However, grape is very special foodstuff for many people and industries. As with other plants, climatic conditions, soil type, altitude, humidity etc. affect the quality and phenolic composition of grape. In parallel to these differences, quality of products produced from grape changes. Wine is one of the most important examples of this. According to our literature search, phenolic acid composition of the grape samples collected from the East Mediterranean region of Turkey have not been reported before. The aim of this study is determining the phenolic acid composition and antioxidant activity potential of *V. vinifera* collected from Osmaniye-Turkey.

MATERIALS AND METHODS

Plant material

V. vinifera L. (barry colour: Adana black) was collected in August, September and October 2008 from Duzici-Osmaniye, Turkey. The voucher specimen has been deposited at the Herbarium of the Department of Biology, Cumhuriyet University, Sivas, Turkey (CUFH-Voucher No: AA3419).

Preparation of the extracts

Air-dried fruits of *V. vinifera* (250 g) were soaked in water for one day with intermittent shaking and the supernatant decanted. The entire process was repeated three times, using a fresh batch of solvent each day. The individual combined extracts were filtered through a nylon mesh, evaporated in vacuo and lyophilised to obtain the respective extracts that were stored in a vacuum desiccator's. Extract yields of the samples were determined as following; August (Au) 12.7%, September (Se) 14.9% and October (Oc) 11.1% (w/w), respectively.

HPLC-DAD analysis of phenolic compounds

The phenolic compound profiles were determined according to the procedure proposed by Chirinos et al. (2008). Spectral data were recorded from 200 to 700 nm during the whole run. An X-terra RP18 (5 mm, 250 × 4.6 mm) column (Waters, Milford, MA) and a 4.6 × 2.0 mm guard column were used for phenolic separation at 30°C. The mobile phase was composed of solvent (A) water: acetic acid (94:6, v/v, pH 2.27) and solvent (B) acetonitrile. The solvent gradient was as follows: 0–15% B in 40 min, 15–45% B in 40 min and 45–100% B in 10 min. A flow rate of 0.5 ml/min was used and 20 ml of sample were injected. Samples and mobile phases were filtered through a 0.22 mm Millipore filter, type GV (Millipore, Bedford, MA) prior to HPLC injection. Each fraction was analysed in duplicate. Phenolic compounds were identified and quantified by comparing their retention time and UV–Vis spectral data to known previously injected standards.

Antioxidant activity

Chemicals

Potassium ferricyanide, ferrous chloride, ferric chloride, Folin–Ciocalteu's reagent (FCR), methanol, and trichloroacetic acid (TCA) were obtained from E. Merck (Darmstadt, Germany). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and butylated hydroxytoluene (BHT) were obtained from Sigma Chemical Co. (Sigma–Aldrich GmbH, Sternheim, Germany). All other chemicals and solvents were of analytical grade.

Total antioxidant activity by β-carotene–linoleic acid method

In this assay antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation (Dapkevicius et al., 1998). A stock solution of β-carotene–linoleic acid mixture was prepared as following: 0.5 mg β-carotene was dissolved in 1 ml of chloroform (HPLC grade). 25 µl linoleic acid and 200 mg Tween 40 was added. Chloroform was completely evaporated using a vacuum evaporator. Then 100 ml of oxygenated distilled water was added with vigorous shaking; 2.5 ml of this reaction mixture was dispersed to test tubes and 0.5 ml of various concentrations (0.4-2.0 mg ml^{-1}) of the extracts in methanol and water were added and the emulsion system was incubated for up to 2 h at 50°C. The same procedure was repeated with the positive control BHT, BHA and a blank. After this incubation period, absorbance of the mixtures was measured at 490 nm. Measurement of absorbance was continued until the color of *β*-carotene disappeared. The bleaching rate (R) of *β*-carotene was calculated according to Equation 1.

$$R = \ln (a/b)/t \quad (1)$$

Where, ln=natural log, *a*=absorbance at time 0, *b*=absorbance at time *t* (30, 60, 90, 120 min) (Cheung et al., 2003). The antioxidant activity (AA) was calculated in terms of percent inhibition relative to the control using Equation 2.

$$AA = [(R_{Control} - R_{Sample}) / R_{Control}] \times 100 \quad (2)$$

Antioxidative activities of the extracts were compared with those of BHT and BHA at 0.4 mg ml^{-1} and blank consisting of only 0.5 ml methanol and water.

Scavenging effect on 1,1-Diphenyl-2-picrylhydrazyl

The hydrogen atoms or electrons donation ability of the corresponding extracts and some pure compounds were measured from the bleaching of purple colored methanol solution of DPPH. The effect of methanolic extracts on DPPH radical was estimated according to Hatano et al. (1988). 1 ml of various concentrations (0.2-0.8 mg ml^{-1}) of the extracts in methanol and water was added to a 1 ml of DPPH radical solution in methanol (final concentration of DPPH was 0.2 mM). The mixture was shaken vigorously and allowed standing for 30 min; the absorbance of the resulting solution was measured at 517 nm with a spectrophotometer (Shimadzu UV-1601, Kyoto, Japan). Inhibition of free radical DPPH in percent (I %) was calculated in following way:

$$I\% = 100 \times (A_{Control} - A_{Sample}) / A_{Control}$$

Where, $A_{Control}$ is the absorbance of the control reaction (containing all reagents except the test compound), and A_{Sample} is the absorbance of the test compound. BHT and BHA were used as a control.

Reducing power

The reducing power was determined according to the method of Oyaizu (1986). Each extract (0.2-1.0 mg ml^{-1}) in methanol and water (2.5 ml) was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricynide and the mixture was incubated at 50°C for 20 min. Then, 2.5 ml of 10% trichloroacetic acid were added, and the mixture was centrifuged at 200 g (MSE Mistral 2000, London, UK) for 10 min. The upper layer (2.5 ml) was mixed with 2.5 ml of deionized water and 0.5 ml of 0.1% ferric chloride. Finally the absorbance was measured at 700 nm against a blank. BHT and BHA were used as a control.

Chelating effects on ferrous ions

The chelating effect was determined according to the method of Dinis et al. (1994). Briefly, 2 ml of various concentrations (0.25-1.00 mg ml^{-1}) of the extracts in methanol was added to a solution of 2 mM $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml). Then, the mixture was shaken vigorously and left at room temperature for 10 min. Absorption readings at 562 nm were taken after 10 min against a blank sample consisting of a 2 ml extract solution with 2 mM $FeCl_2$ (0.05 ml) and water (0.2 ml) without ferrozine. The inhibition percentage of ferrozine–Fe^{2+} complex formation was calculated by using the formula given below:

$$\text{Metal chelating effect (\%)} = [(A_{Control} - A_{Sample})/A_{Control}] \times 100$$

Where $A_{Control}$ is the absorbance of control (The control contains $FeCl_2$ and ferrozine, complex formation molecules) and A_{sample} is the absorbance of the test compound. EDTA was used as a control.

Assay for total phenolics

Total phenolic constituent of the extracts were determined by employing the methods given in the literature (Chandler and Dodds, 1983; Slinkard and Singleton, 1977) involving Folin-Ciocalteu reagent and gallic acid as standard. 1 ml of extract solution containing 2000 µg extract was added to a volumetric flask. 45 ml distilled water and 1 ml Folin-Ciocalteu reagent was added and flask was shaken vigorously. After 3 min, a 3 ml of Na_2CO_3 (2%) solution was added and the mixture was allowed to stand for 2 h by intermittent shaking. Absorbance was measured at 760 nm. The concentrations of phenolic compounds were calculated according to the following equation that was obtained from the standard gallic acid graph:

$$A = 0.0241 \text{ gallic acid}(\mu g) - 0.0307 \quad (R^2 : 0.9997)$$

Assay for total flavonoids

Total flavonoid content was determined using the Dowd method as adapted by Arvouet-Grand et al. (1994). Briefly, 1 ml of 2% aluminium trichloride ($AlCl_3$) in methanol was mixed with the same volume of the solvent extracts (2000 µg). Absorption readings at 415 nm were taken after 10 min against a blank sample consisting of 1 ml extract solution with 1 ml methanol without $AlCl_3$. The concentrations of flavonoid compounds were calculated according to the following equation that was obtained from the standard quercetin graph:

$$\text{Absorbance} = 0.0273 \text{ quercetin } (\mu g) - 0.0125 \quad (R^2: 0.9984)$$

RESULTS AND DISCUSSION

Composition of phenolic compounds

For determining the phenolic acid composition of the

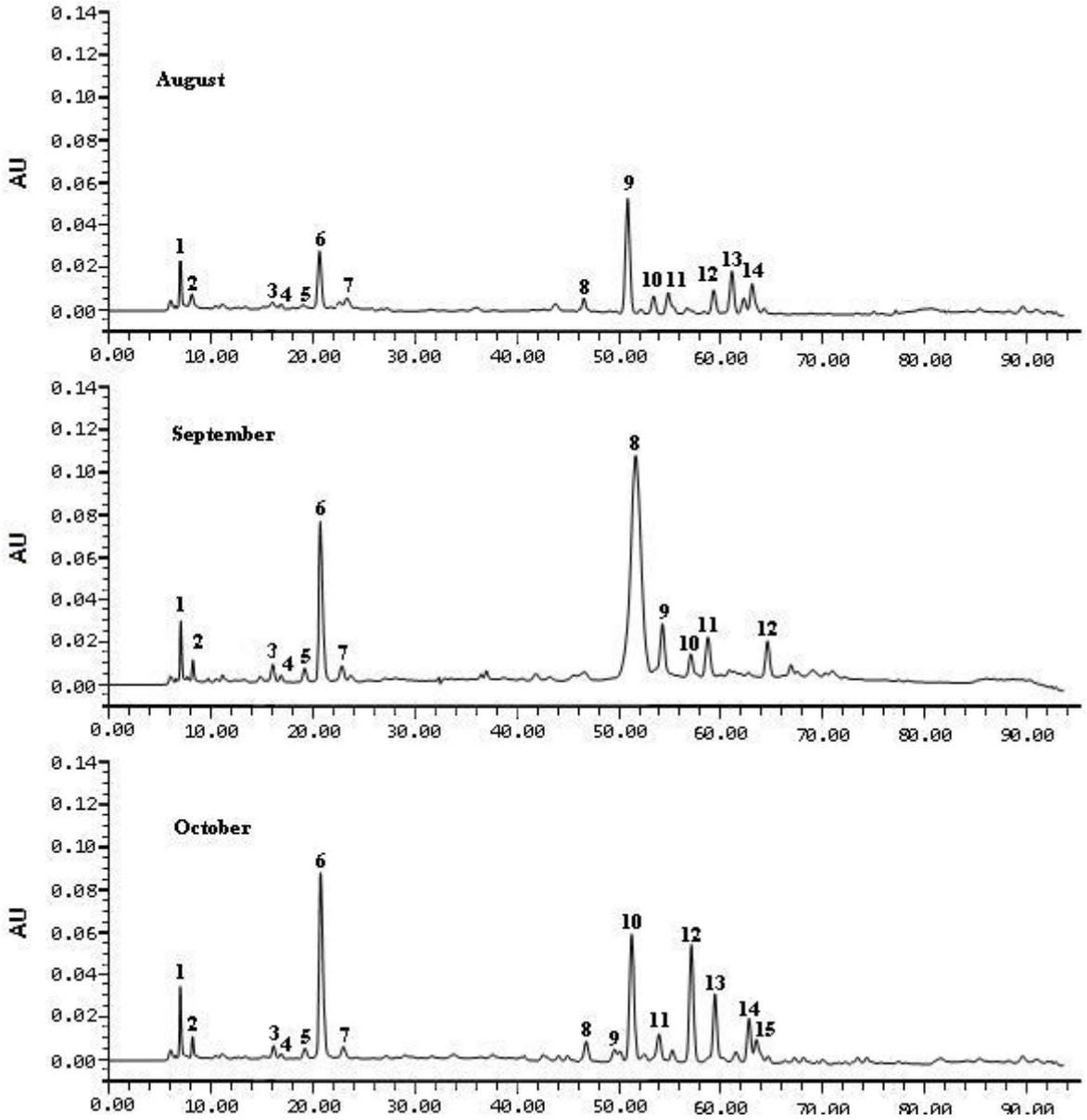

Figure 1. Chromatographic profiles of *V. vinifera* samples. (Peak numbers corresponding to the phytochemicals: 1: gallic acid, 2: 2,3-dihydroxybenzoic acid, 3: vanillic acid, 4: caffeic acid, 5: unidentified, 6: *p*-coumaric acid, 7: ferulic acid, 8: unidentified, 9: cinnamic acid, 10: unidentified, 11: unidentified, 12: luteolin, 13: unidentified, 14: apigenin).

grapes collected in different seasons, samples were screened via an HPLC-DAD instrument. By this way, amounts of gallic acid, 2,3-dihydroxybenzoic acid, vanillic acid, caffeic acid, *p*-coumaric acid, ferulic acid, cinnamic acid, luteolin and apigenin were determined quantitatively. Table 1 shows the amounts of the identified phenolic compounds together with the unidentified phytochemicals. Chromatograms obtained from the HPLC analysis were also presented in Figure 1.

As can be seen from the Table 1, for comparing the effect of season on the phenolic composition, samples were collected in August, September and October, respectively. When compared, September sample was the riches one in terms of phenolic acids and flavonoids studied. This is followed by August and October samples, respectively. In August and September samples, *p*-coumaric and cinnamic acids were determined as the major phenolic acids. In October sample, luteolin was also determined as the major compound (6.52 mg.100 ml^{-1}) in addition to *p*-coumaric and cinnamic acids. In September sample, amounts of *p*-coumaric and cinnamic acids were determined as 13.60 and 22.37 mg.100 g^{-1}, respectively.

Phenolic compounds of *V. vinifera* have been

Table 1. Chromatographic and spectral characteristics of *V. vinifera* samples.

Grape varieties	Peak number	Retention time (min)	Phenolic compound	Amount (mg/100 g fw)
August	1	7.2	Gallic acid	0.18
	2	8.4	2,3-dihydroxybenzoic acid	0.05
	3	16.0	Vanillic acid	0.03
	4	16.9	Caffeic acid	0.02
	5	19.6	Unidentified	0.02
	6	21.8	*P*-coumaric acid	3.62
	7	23.4	Ferulic acid	0.08
	8	46.4	Unidentified	0.12
	9	50.9	Cinnamic acid	6.82
	10	53.6	Unidentified	0.19
	11	55.3	Unidentified	0.24
	12	59.4	Luteolin	0.22
	13	61.1	Unidentified	0.46
	14	63.3	Apigenin	0.28
September	1	7.2	Gallic acid	0.43
	2	8.5	2,3-dihydroxybenzoic acid	0.18
	3	16.0	Vanillic acid	0.20
	4	16.8	Caffeic acid	0.06
	5	19.5	Unidentified	0.16
	6	21.5	*P*-coumaric acid	13.60
	7	22.6	Ferulic acid	0.16
	8	51.6	Cinnamic acid	22.37
	9	54.4	Unidentified	0.76
	10	56.4	Unidentified	0.57
	11	58.8	Luteolin	0.68
	12	64.6	Apigenin	0.64
October	1	7.3	Gallic acid	0.39
	2	8.6	2,3-dihydroxybenzoic acid	0.17
	3	16.4	Vanillic acid	0.13
	4	16.9	Caffeic acid	0.03
	5	19.4	Unidentified	0.14
	6	21.7	*P*-coumaric acid	10.88
	7	22.8	Ferulic acid	0.15
	8	46.4	Unidentified	0.26
	9	49.5	Unidentified	0.22
	10	51.4	Cinnamic acid	7.25
	11	54.0	Unidentified	0.29
	12	57.3	Luteolin	6.52
	13	59.6	Unidentified	3.42
	14	62.8	Apigenin	0.78
	15	63.7	Unidentified	0.28

Vanillic acid, cinnamic acid were quantified at 280 nm using their corresponding standard aglycons, Apigenin and luteolin (flavones) were quantified at 320 nm using their corresponding standard aglycons.

evaluated many times (Rockenbach et al., 2011; Chiou et al., 2007; Santos et al., 2011; Casazza et al., 2010; Bozan et al., 2008; Orak, 2007). However, as mentioned within the first section of this study, grape is an important foodstuff for many industries. Products produced from the grape may vary in terms of phenolic compound content. Grape can be produced in any land of the world. However, some grape varieties grown in certain areas

Table 2. Antioxidant activity (%) of *V. vinifera* samples at different concentrations measured by β-carotene–linoleic acid method[a].

Sample	Sample concentration (mg ml^{-1})		
	0.4	1.0	2.0
August	78.54 ± 1.26	83.14 ± 1.66	88.45 ± 2.72
September	81.21 ± 0.36	87.23 ± 1.16	92.12 ± 0.33
October	86.13 ± 0.72	93.42 ± 0.27	97.25 ± 0.21
BHT	94.56 ± 0.14	-	-
BHA	94.05 ± 0.54	-	-

[a] Values expressed are means ± S.D. of three parallel measurements.

Table 3. Scavenging effect (%) on 1.1-Diphenyl-2-picrylhydrazyl of *V. vinifera* samples at different concentrations[a].

Sample	Sample concentration (mg ml^{-1})		
	0.2	0.4	1.0
August	67.82 ± 0.62	86.98 ± 1.74	82.78 ± 2.19
September	34.84 ± 0.51	66.65 ± 0.42	84.17 ± 1.18
October	37.80 ± 1.43	66.07 ± 1.63	89.94 ± 0.14
BHT	95.46 ± 0.19	-	-
BHA	95.89 ± 0.26	-	-

[a] Values expressed are means ± S.D. of three parallel measurements.

have a special importance due to their phenolic composition. In this respect, grape varieties grown in Turkey are highly valuable and the wines produced from these varieties are very famous. As far as our literature survey could ascertain, phenolic compound composition of the grape species collected from the East Mediterranean region of Turkey have not previously been reported.

Antioxidant activity

There are several ways of determining the antioxidant activities of the plant samples. By using two or more complementary test systems, researchers can obtain different activity profiles even in a single plant species. These differences may result from differences in defatting process before extraction, moisture content of material, solvent used for extraction, time and temperature for extraction, method for determination of antioxidant activity.

Lipid oxidation has been hypothesized a major consequence of free radical cell damage. It may alter intrinsic membrane properties, due to physicochemical changes of oxidized lipids or, consequently, to cross-linking and polymerization of membrane components (Esterbauer et al., 1991). Lipid oxidation may also indirectly contribute to other deleterious effects of ischemia/reperfusion, because it enhances phospholipid susceptibility to degradation by phospholipase (Sevanian et al., 1981; Weglicki et al., 1984) and increases membrane calcium permeability (Bagchi et al., 1997).

According to the results obtained from β-carotene/linoleic acid method, the most active sample was the October (Table 2). It showed great activity potential at 2.0 mg ml^{-1} concentration (97.25%). This is closely followed by the September sample. Antioxidant activity of the synthetic agents BHT and BHA were found superior to the samples tested.

Various extracts might react with free radicals, particularly the peroxy radicals, which are the major propagators of the autoxidation chain of fat, thereby terminating the chain reaction. Antioxidant activity of natural antioxidants has been shown to be involved in termination of free radical reaction (Shimada et al., 1992). Furthermore, Herraiz et al. (2003) found that an essential amino acid L-tryptophan, which is one of the precursors of many secondary metabolites, could react with phenolic aldehydes in food to form phenolic tetrahydro-β-carboline alkaloids that scavenged 2,2-azinobis (3-ethylbenzothiazoline)-6-sulfonic acid effectively. Therefore, the presence of L-tryptophan in various extracts might most likely account for the scavenging activity on DPPH radicals.

The DPPH radical scavenging activity values of the samples are presented in Table 3. From the analysis of Table 3, we can conclude that the scavenging effects of the extracts on DPPH radical increased dose

Table 4. Reducing power (absorbance at 700 nm) of *V. vinifera* samples at different concentrations[a].

Sample	Sample concentration (mg ml^{-1})		
	0.2	0.4	1.0
August	0.248 ± 0.011	0.513 ± 0.037	1.081 ± 0.046
September	0.243 ± 0.015	0.488 ± 0.001	1.110 ± 0.028
October	0.263 ± 0.004	0.553 ± 0.024	1.299 ± 0.041
BHT	2.016 ± 0.013	-	-
BHA	2.410 ± 0.027	-	-

[a] Values expressed are means ± S.D. of three parallel measurements.

Table 5. Chelating effect (%) of *V. vinifera* samples at different concentrations[a].

Samples	Sample concentration (mg ml^{-1})		
	0.25	0.50	1.00
August	81.37 ± 0.55	84.80 ± 0.55	88.24 ± 1.11
September	85.39 ± 1.66	90.05 ± 0.76	94.26 ± 2.43
October	87.21 ± 0.49	93.68 ± 0.21	96.81 ± 0.21
EDTA	97.89 ± 0.69	-	-

[a] Values expressed are means ± S.D. of three parallel measurements, na, not active.

dependently. Free radical scavenging capacity of the October sample was determined as 89.98% at 1.0 mg/ml concentration value. DPPH free radical scavenging potentials of the synthetic antioxidants BHT and BHA at 0.2 mg/ml concentration were found as 95.46 and 95.89%, respectively.

It was reported that the reducing power properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chains by donating a hydrogen atom (Barros et al., 2007; Shimada et al., 1992). In the present study, assay of reducing activity was based on the reduction of Fe^{3+}/ferricyanide complex to the ferrous form in presence of reductants (antioxidants) in the tested samples. The Fe^{2+} was then monitored by measuring the formation of Perl's Prussian blue at 700 nm (Oyaizu, 1986).

Table 4 shows the reducing power of the extracts as a function of their concentration. The reducing power of the samples was increased with concentration. Reducing power of October sample was determined as 1.299 ± 0.041 nm at 1.0 mg/ml concentration. Data obtained from the synthetic antioxidants BHT and BHA were also recorded as 2.016 ± 0.013 and 2.410 ± 0.027 nm, respectively at 0.2 mg/ml concentration.

Chelating agents may serve as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ions. Metal ions can initiate lipid peroxidation and start a chain reaction that leads to the deterioration of food (Gordon, 1990). The catalysis of metal ions also correlates with incidents of cancer and arthritis (Halliwell et al., 1995). Ferrous ions, the most effective pro-oxidants, are commonly found in food systems (Yamaguchi et al., 1998).

In the present study, the chelating ability of the grape samples toward ferrous ions was also investigated. Table 5 shows the chelating effects of grapes. In this study, EDTA was also used as standard on ferrous ions. Among the extracts tested, frappe collected in October showed the most powerful chelating capacity at 1.0 mg ml^{-1} concentration (96.81 ± 0.21%). Chelating effect of EDTA was also determined as 97.89 ± 0.69% at 0.25 mg ml^{-1} concentration.

Phenolic compounds such as flavonoids, phenolic acids, and tannins are considered to be major contributors to the antioxidant capacity of plants. These antioxidants also possess diverse biological activities, such as anti-inflammatory, anti-atherosclerotic and anti-carcinogenic activities. These activities may be related to their antioxidant activity (Chung et al., 1998). Thus, the total phenolic content of the samples was also evaluated, using the Folin–Ciocalteu method. As can be seen from the Table 6, amounts of phenolics and flavonoids were determined as 201.68 ± 1.66 and 16.54 ± 0.04 µg mg^{-1} for the October samples which is the richest one among the others, respectively.

In a conclusion, phenolics are one of the major groups of nonessential dietary components that have been associated with the inhibition of atherosclerosis and cancer, so it is important to consider the effect of the phenolic content on the biological activities of plant samples. The bioactivity of phenolics may be related to their ability to chelate metals, inhibit lipoxygenase and scavenge free radicals. The food industry should take a

Table 6. Total phenolics and flavonoids *V. vinifera* samples [a].

Sample	Phenolic content (µg GAEs/mg extract)[b]	Flavonoid content (µg QEs/mg extract)[c]
August	115.16 ± 1.88	15.98 ± 0.25
September	148.50 ± 0.44	8.07 ± 0.94
October	201.68 ± 1.66	16.54 ± 0.04

[a] Values expressed are means ± S.D. of three parallel measurements, [b] GAEs. gallic acid equivalents, [c] QEs. quercetin equivalents.

note to use various plant extracts as a potential source of phenolics. Plant extracts can be used instead of synthetic antioxidants. Antioxidant properties of plant species are among the major research topics for the many scientists. By these studies, thousands of plant species have been identified for their antioxidant and antimicrobial potentials. As mentioned above, grape varieties grown in certain areas have a special importance due to their phenolic composition. In this respect, grape varieties grown in Turkey are highly valuable and the wines produced from these varieties are very famous. This could be assumed as the first report on the phenolic compound composition of the grape species collected from the East Mediterranean region of Turkey.

REFERENCES

Andreasen AF, Kroon PA, Williamson G, Garcia-Conesa MT (2001b). Esterase activity able to hydrolyze dietary antiox- idant hydroxycinnamates is distributed along the intestine of mammals. J. Agric. Food Chem. 49:5679-5684.

Andreasen MF, Kroon PA, Williamson G, Garcia-Conesa MT (2001a). Intestinal release and uptake of phenolic antioxidant diferulic acids. Free Rad. Biol. Med. 31:304-314.

Arvouet-Grand A, Vennat B, Pourrat A, Legret P (1994). Standardisation d'un extrait de propolis et identification des principaux constituants. J. Pharm. Belg. 49:462-468.

Ayaz FA, Hayirlioglu-Ayaz S, Gruz J, Novak O, Strnad M (2005). Separation, characterization, and quantitation of phenolic acids in a little-known blue- berry (*Vaccinium arctostaphylos* L.) fruit by HPLC-MS. J. Agric. Food Chem. 53:8116-8122.

Bagchi D, Wetscher GJ, Bagchi M, Hinder PR, Perdikis G, Stohs SJ, Hinder DK (1997). Interrelationship between cellular calcium homeostasis and free radical generation in myocardial reperfusion injury. Chem. Biol. Interact. 104:65-85.

Barros L, Ferreira MJ, Queiros B, Ferreira IC, Bapista P (2007). Total phenols, ascorbic acid, β- carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. Food Chem. 103:413-419.

Baublis AJ, Clydesdale FM, Decker EA (2000a). Antioxidants in wheat-based breakfast cereals. Cereal Food World 45:71-74.

Baublis AJ, Decker EA, Clydesdale FM (2000b). Antioxidant effect of aqueous extracts from wheat based ready-to-eat breakfast cereals. Food Chem. 68:1-6.

Baublis AJ, Lu C, Clydesdale FM, Decker EA (2002). Potential of wheat-based breakfast cereals as a source of dietary antioxidants. J. Am. Coll. Nutr. 19:308S-311S.

Behera BC, Verma N, Sonone A, Makhija U (2008). Antioxidant, antibacterial properties of some cultured lichens (Retracted Article). Bioresour. Technol. 99(4):776-784.

Bozan B, Tosun G, Ozcan D (2008). Study of polyphenol content in the seeds of red grape (*Vitis vinifera* L.) varieties cultivated in Turkey and their antiradical activity. Food Chem. 109:426-430.

Campos LMAS, Leimann FV, Pedrosa RC, Ferreira SRS (2008). Free radical scavenging of grape pomace extracts from Cabernet Sauvingnon (*Vitis vinifera*). Bioresour. Technol. 99:8413−8420.

Casazza AA, Aliakbarian B, Mantegna S, Cravotto G, Perego P (2010). Extraction of phenolics from *Vitis vinifera* wastes using non-conventional techniques. J. Food Eng. 100:50-55.

Chandler SF, Dodds JH (1983). The effect of phosphate. Nitrogen and sucrose on the production of phenolics and solasidine in callus cultures of *Solanum lacinitum*. Plant Cell Rep. 2:105.

Cheung LM, Cheung PCK, Ooi VEC (2003). Antioxidant activity and total phenolics of edible mushroom extracts. Food Chem. 81:249-255.

Chiou A, Karathanos VT, Mylona A, Salta FN, Preventi F, Andrikopoulos NK (2007). Currants (*Vitis vinifera* L.) content of simple phenolics and antioxidant activity. Food Chem. 102:516-522.

Chirinos R, Campos D, Costa N, Arbizu C, Pedreschi R, Larondelle Y (2008). Phenolic profiles of andean mashua (*Tropaeolum tuberosum* Ruíz and Pavón) tubers: Identification and evaluation of their antioxidant capacity contribution. Food Chem. 106:1285-1298.

Chua MT, Tung YT, Chang ST (2008). Antioxidant activities of ethanolic extracts from the twigs of *Cinnamomum osmophloeum*. Bioresour. Technol. 99(6):1918-1925.

Chung KT, Wong TY, Huang YW, Lin Y (1998). Tannins and human health: a review. Crit. Rev. Food Sci. Nutr. 38:421-464.

Dapkevicius A, Venskutonis R, Van Beek TA, Linssen PH (1998). Antioxidant activity of extracts obtained by different isolation procedures from some aromatic herbs grown in Lithuania. J. Sci. Food Agric. 77:140-146.

Dinis TCP, Madeira VMC, Almeida LM (1994). Action of phenolic derivates (acetoaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophys. 315:161-169.

Emmons CL, Peterson DM, Paul GL (1999). Antioxidant capacity of oat (*Avena sativa* L.) extracts. 2. In vitro antioxidant activity and contents of phenolic and tocol antioxidants. J. Agric. Food Chem. 47:4894-4898.

Esterbauer H, Shauer RJ, Zollner H (1991). Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Rad. Biol. Med. 11:81-128.

Gordon MH (1990). The mechanism of antioxidant action *in vitro*. In: Hudson, B.J.F. (Ed.), Antioxidants. Elsevier Applied Science, London, New York, pp. 1-18.

Halliwell B (2007). Dietary polyphenols: good, bad, or indifferent for your health? Cardiovasc. Res. 73(2):341-347.

Halliwell B, Murcia HA, Chirco S, Aruoma OI (1995). Free radicals and antioxidants in food an in vivo: what they do and how they work. CRC Crit. Rev. Food Sci. Nutr. 35:7-20.

Hatano T, Kagawa H, Yasuhara T, Okuda T (1988). Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. Chem. Pharm. Bull. 36:1090-2097.

Herraiz T, Galisteo J, Chamorro C (2003). L-tryptophan reacts with naturally occurring and foodoccurring phenolic aldehydes to give phenolic tetrahydro-β-caroline alkaloids: Activity as antioxidants and free radical scavengers. J. Agric. Food Chem. 51:2168-2173.

Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS (1999). Antioxidant activity of plant extracts containing phenolic compounds. J. Agric. Food Chem. 47(10):3954-3962.

Luthria DL, Pastor-Corrales MA (2006). Phenolic acids content of fifteen

dry edible bean (*Phaseolus vulgaris* L.) varieties. J. Food Comp. Anal. 19:205-211.
Maksimovic Z, Malencic D, Kovacevic N (2005). Polyphenol contents and antioxidant activity of Maydis stigma extracts. Bioresour. Technol. 96(8):873-877.
Mattila P, Kumpulainen J (2002). Determination of free and total phenolic acids in plant-derived foods by HPLC with diode-array detection. J. Agric. Food Chem. 50:3660-3667.
Mohsen SM, Ammar ASM (2009). Total phenolic contents and antioxidant activity of corn tassel extracts. Food Chem. 112(3):595-598.
Orak HH (2007). Total antioxidant activities, phenolics, anthocyanins, polyphenoloxidase activities of selected red grape cultivars and their correlations. Sci. Hortic. 111:235-241.
Oszmianski J, Lee CY (1990). Isolation and HPLC determination of phenolic compounds in red grapes. Am. J. Enol. Vitic. 39:259-262.
Oyaizu M (1986). Studies on products of browning reactions: antioxidative activities of browning reaction prepared from glucosamine. Jpn. J. Nutr. 44:307-315.
Pedreschi R, Cisneros-Zevallos L (2006). Antimutagenic and antioxidant properties of phenolic fractions from Andean purple corn (Zea mays L.). J. Agric. Food Chem. 54:4557−4567.
Plessi M, Bertelli D, Miglietta F (2006). Extraction and identification by GC-MS of phenolic acids in traditional balsamic vinegar from Modena. J. Food Comp. Anal. 19:49-54.
RiceEvans CA, Miller NJ, Bolwell GP, Bramley PM, Pridham JB (1995). The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free Rad. Res. 22(4):375-383.
Rios AD, Antunes LMG, Bianchi MDP (2009). Bixin and lycopene modulation of free radical generation induced by cisplatin–DNA interaction. Food Chem. 113(4):1113-1118.
Robbins RJ (2003). Phenolic acids in foods: an overview of analytical methodology. J. Agric. Food Chem. 51:2866-2887.
Robbins RJ, Bean SR (2004). Development of a quantitative high-performance liquid chromatography-photodiode array detection measurement system for phenolic acids. J. Chromatog. A, 1038:97-105.
Rockenbach II, Gonzaga VL, Rizelio VM, Goncalves AESS, Genovese MI, Fett R (2011). Phenolic compounds and antioxidant activity of seed and skin extracts of red grape (*Vitis vinifera* and *Vitis labrusca*) pomace from Brazilian winemaking. Food Res. Int. 44:897-901.
Saadi A, Lempereur I, Sharonov S, Autran JC, Manfait M (1998). Spatial distribution of phenolic materials in durum wheat grain as probed by confocal fluorescence spectral imaging. J. Cer. Sci. 28:107-114.
Santos LP, Morais DR, Souza NE, Cottica SM, Boroski M, Visentainer JV (2011). Phenolic compounds and fatty acids in different parts of *Vitis labrusca* and *V. vinifera* grapes. Food Res. Int. 44:1414-1418.
Sevanian A, Stein RA, Mead JF (1981). Metabolism of epoxidized phosphatidylcholine by phospholipase A2 and epoxide hydrolase. Lipids 16:781-792.
Shimada K, Fujikawa K, Yahara K, Nakamura T (1992). Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J. Agric. Food Chem. 40:945-948.
Slinkard K, Singleton VL (1977). Total phenol analyses: Automation and comparison with manual methods. Am. J. Enol. Vitic. 28:49-55.
Weglicki WB, Dickens BF, Mak IT (1984). Enhanced lysosomal phospholipid degradation and lysophospholipid production due to free radicals. Biochem. Biophys. Res. Commun. 124:229-235.
Wen D, Li C, Di H, Liao Y, Liu H (2005). A universal HPLC method for the determination of phenolic acids in compound herbal medicines. J. Agric. Food Chem. 53: 6624-6629.
White PJ, Xing Y (1997). Antioxidants from cereals and legumes. In F. Shahidi (Ed.), Natural antioxidants, chemistry, health effects, and application. Champaign IL: AOCC press, pp. 25-63.
Yamaguchi T, Takamura H, Matoba T, Terao J (1998). HPLC method for evalution of the free radical-scavenging activity of foods by using 1,1-dicrylhydrazyl. Biosci. Biotechnol. Biochem. 62:1201-1204.
Yu L, Haley S, Perret J, Harris M, Wilson J, Qian M (2002). Free radical scavenging properties of wheat extracts. J. Agric. Food Chem. 5:1619-1624.
Yu L, Perret J, Harris M, Wilson J, Haley S (2003). Antioxidant properties of bran extracts from ‘‘Akron’’ wheat grown at different locations. J. Agric. Food Chem. 51:1566-1570.
Zadernowski R, Naczk M, Nesterowicz J (2005). Phenolic acid profiles in some small berries. J. Agric. Food Chem. 53:2118-2124.