Tarım Bilimleri Dergisi
Tar. Bil. Der.
Dergi web sayfası:
www.agri.ankara.edu.tr/dergi

Journal of Agricultural Sciences
Journal homepage:
www.agri.ankara.edu.tr/journal

Phenolic Composition and Antioxidant Activities of Wines and Extracts of Some Grape Varieties Grown in Turkey

Nilgün Göktürk BAYDAR[a], Zehra BABALIK[b], Filiz Hallaç TÜRK[a], E. Sema ÇETİN[a]

[a] *Süleyman Demirel University, Faculty of Agriculture, Department of Horticulture, 32260, Isparta, TURKEY*

[b] *Horticultural Research Institute, 32500, Eğirdir, Isparta, TURKEY*

ARTICLE INFO

Research Article — Agricultural Food
Corresponding author: E. Sema ÇETİN, e-mail: esemacetin@gmail.com, Tel: +90(246) 211 46 69
Received: 14 September 2010, Received in revised form: 30 March 2011, Accepted: 03 April 2011

ABSTRACT

Grape seed and skin extracts and wines from Cabernet Sauvignon, Kalecik Karası and Narince grape cultivars were assayed for their antioxidant properties and phenolic compositions. Total phenolic contents of the samples were determined by the Folin Ciocalteu method and compositions of the phenolics were separated by HPLC. Antioxidant activities of the samples were evaluated using the 1, 1-diphenyl-2- picrylhydrazyl ($DPPH^{\cdot}$) radical scavenging and reducing power methods. Total phenolic contents varied from 522.49 to 546.50 mg GAE g^{-1} in seed extracts; from 22.73 to 43.75 mg GAE g^{-1} in skin extracts and from 217.06 to 1336.21 GAE mg l^{-1} in wines. Radical scavenging activities and reducing powers of the samples changed depending on the grape cultivars and the different parts of grape and wine types.

Keywords: Grape; Seed; Skin; Wine; Phenolics; Antioxidant activity

Türkiye'de Yetiştirilen Bazı Üzüm Çeşitlerinin Ekstraktları ve Şaraplarının Fenolik Kompozisyonları ve Antioksidan Aktiviteleri

ESER BİLGİSİ

Araştırma Makalesi — Tarımsal Gıda
Sorumlu Yazar: E. Sema ÇETİN, e-posta: esemacetin@gmail.com, Tel: +90(246) 211 46 69
Geliş tarihi: 14 Eylül 2010, Düzeltmelerin gelişi: 30 Mart 2011, Kabul: 03 Nisan 2011

ÖZET

Bu araştırmada Cabernet Sauvignon, Kalecik Karası ve Narince üzüm çeşitlerine ait üzüm çekirdeği ve kabuk ekstraktları ile şarapların antioksidan özellikleri ile fenolik bileşik içerikleri tespit edilmiştir. Toplam fenolik bileşik içeriği Folin Ciocalteu metodu ile; fenolik bileşiklerin tanımlanması ise HPLC de gerçekleştirilmiştir. Antioksidan aktivite 1,1-difenil -2- pikrilhidrazil ($DPPH^{\cdot}$) radikal bağlama aktivitesi ve indirgeme gücü metotları ile belirlenmiştir. Toplam fenolik bileşik içeriğinin çekirdek ekstraktlarında 522.49 ile 546.50 mg GAE g^{-1}; kabuk ekstraktlarında 22.73 ile 43.75 mg GAE g^{-1} ve şaraplarda 217.06 ile 1336.21 mg l^{-1} arasında değiştiği belirlenmiştir. Örneklerin radikal bağlama aktivitelerinin ve indirgeme gücünün üzüm çeşitlerine, üzümün kısımlarına ve şarabın tipine bağlı olarak değişiklik göstermiştir.

Anahtar sözcükler: Üzüm; Çekirdek; Kabuk; Şarap; Fenolik; Antioksidan etki

TARIM BİLİMLERİ DERGİSİ — JOURNAL OF AGRICULTURAL SCIENCES 17 (2011) 67-76

1. Introduction

Antioxidants are defined as substances acting to scavenge and stabilize of free radicals. Because of the toxic, carcinogenic effects and hazardous impact on enzyme system of synthetic antioxidants (Ito et al 1986), the interest in natural antioxidants, especially of plant origin, has greatly increased in recent years. Natural antioxidants can protect the human body free radicals that may cause some chronic diseases including cancer, cardiovascular diseases and cataract (Kinsella et al 1993). A lot of studies show that the antioxidant properties of plant extracts were attributed to their polyphenol contents (Ricardo da Silva et al 1990). Phenolic compounds are defined as organic metabolites containing benzene ring and they have a large and complex family. These compounds are directly related to some quality characteristics such as colour, taste and odour of fruit, vegetables and their products. They have also antiradical and antioxidant properties. The antioxidant activities of phenolic compounds are determined by their molecular structure and more specially, by the position and degree of hydroxylation of the ring structure (Gadow et al 1997). So, plants containing high-level of phenolics have a great importance as natural antioxidants. Grape (*Vitis vinifera* L.) is among the fruits with the highest content of phenolic compounds. Phenolic compounds of wine and wine by-products have attracted much interest due to their antioxidant and antimicrobial properties and their potentially beneficial effects for human health (Sun et al 2002; Baydar et al 2006). It is well known that the grape skins and seeds, waste products generated during wine and grape juice processing, are rich sources of polyphenols (Murthy et al 2002). For these reason, seed and skin produced in large quantities by the winemaking industry have become valuable raw materials for extraction of polyphenols. Wine, a fermented grape product, is rich source of flavonoids and other phenolics in the human diet (Rice Evans et al 1996). Protective health effects of wines have been attributed to their phenolic contents (Li et al 2009). Therefore grape seed, skin and wine have a growing interest in recent years as nutritional supplements and easily accessible sources of natural antioxidants (Ricardo da Silva et al 1990). Although the literature abounds with reports about phenolic composition and antiradical activity of wine or grape seed samples, there are very few papers that report data about grape seeds and skin of the same sample (Iacopini et al 2008). The objectives of this study were to determine the phenolic composition and the antiradical and antioxidant activities of grape seed, skin and wines from Cabernet Sauvignon, Kalecik Karası and Narince winemaking grape cultivars.

2. Materials and Methods

2.1. Materials

Grapes from Cabernet Sauvignon (red), Kalecik Karası (red) and Narince (white) were collected at optimal maturity from the experimental vineyard of the Agricultural Faculty of Süleyman Demirel University (Isparta, Turkey) in 2008. Row spacing for the vines was 3 m and vine spacing was 1.5 m. Berries were harvested randomly from both the outer and internal canopy of selected vines trained to bilateral cordons in order to obtain a homogeneous sample. Each treatment consisted of three replicates for a total of 25 vines and it was used 2 kg of grape in each replication. Three bottles of three different brands of Cabernet Sauvignon, Kalecik Karası and Narince wines were purchased from a local supermarkets. Before analyses, different brands of each wine were combined in equal volumes. Each determination was carried out in triplicate.

2.2. Sample preparation and phenolic extraction

After harvest, undamaged and disease-free berries were snipped from clusters. After seeds and skin were manually separated from whole berries, seeds and skin were dried at room temperature and then were crushed in a grinder for two min. In order to remove the fatty materials from seeds, the powdered grape seeds (100 g) were extracted in a Soxhlet extractor for 6 h with 150 m*l* of petroleum ether at 60°C. The defatted grape seed powder and also powdered skin were extracted in a Soxhlet apparatus for 8 h with 200 m*l* of acetone: water: acetic acid (90:9.5:0.5) at 60°C as described by Jayaprakasha et al (2003). The extracts were

concentrated by rotary evaporator at 70°C to get crude extracts and stored in a desiccators. Wines were dealcoholized in rotary evaporator and then diluted to the original volume with distilled water.

2.3. Determination of total phenolic content

Total phenolic contents of the grape seed and skin extracts and the diluted wine samples were determined spectrophotometrically using a PG Instruments T70 Plus Dual Beam Spectrophotometer (Arlington, MA, USA) according to the Folin-Ciocalteu colorimetric method (Singleton & Rossi 1965), calibrating against gallic acid standards and expressing the results as mg gallic acid equivalents (GAE g^{-1}) extract for seed and skin extracts and mg gallic acid equivalents (GAE l^{-1}) for wines. Data presented are average of three measurements.

2.4. HPLC determination of phenolic compounds

Chromatographic analyses were carried out on a Shimadzu model HPLC system (Shimadzu Corp., Kyoto, Japan). Separation of phenolics was performed by the modified method of Caponio et al (1999). Reversed phase (RP)-HPLC analysis was done using a SCL-10Avp system controller, a SIL-10AD vp autosampler, a LC-10AD vp pump, a DGU-14a degasser, a CTO-10 A vp column heater, and a Diode Array Detector with wavelengths set at 278 nm. The 250 x 4.6 mm i.d. 5 µm column used was filled with Agilent Eclipse XDB-C18 (Wallborn,Germany). The flow rate was 0.8 m*l* min^{-1}, the injection volume was 20 µ*l*, and the column temperature was set at 30°C. For gradient elution, mobile phase A contained 3% acetic acid in water; solvent B contained methanol. The following gradient was used: 0-3 min, from 100% A to 95% A; 3-20 min, from 95% A to 80% A; 20-30 min, from 80% A to 75% A; 30-40 min, from 75% A to 70% A; 40-50 min 70% A to 60% A; 50-55 min, 60% A to 50% AB; 55-65 min, 50% A to 0% A. The data were integrated and analyzed using the Shimadzu Class-VP Chromatography Laboratory Automated Software system. The grape samples, standard solutions and mobile phases were filtered by a 0.45 µm pore size membrane filter (Millipore Co. Bedford, MA). The amount of phenolic compounds in the seed and skin extracts and wine samples were calculated as mg 100 g^{-1} extract and mg l^{-1} wine, separately, using external calibration curves obtained for each phenolic standard. The standarts, caffeic acid, (+)-catechin, chlorogenic acid, *o*-coumaric acid, *p*-coumaric acid, (-)-epicatechin, ferulic acid, gallic acid, kaempherol, *trans*-resveratrol, quercetin, syringic acid and vanillin acquired from Sigma (St. Louis, MO, USA) were determined in the samples.

2.5. Determination of antiradical activity

The free radical scavenging activity of extracts and wines were examined by comparing to those of known antioxidants such as BHT (butylated hydroxytoluene) BHA (Butylated hydroxyanisole) and trolox by 1, 1-diphenyl-2- picrylhydrazyl ($DPPH^{\cdot}$) from Sigma (St. Louis, MO, USA) using the method of Shimada et al (1992). Briefly, a 1.0 ml solution of the samples (seed and skin extracts and standard*s* at 100 µg m*l* in methanol and wines diluted with distilled water as 1:99 (wine:distilled water) was mixed with 1.0 m*l* of methanolic solution of DPPH (0,2 mM). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm against methanol as the blank in a PG Instruments T70 Plus Dual Beam Spectrophotometer (Arlington, MA, USA). The addition of the samples to the DPPH solution caused a rapid decrease in the optical density at 517 nm. The degrees of discoloration indicate the scavenging capacity of the samples. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The effect of antioxidant on $DPPH^{\cdot}$ radical scavenging was thought to be due to their hydrogen donating ability or radical scavenging activity (Baumann et al 1979). Antioxidants break the free radical chain of oxidation and to donate hydrogen from the phenolic hydroxyl groups. Therefore formed stable end-product does not permit further oxidation of the lipid (Sherwin 1978).

All determinations were done in triplicate and the percent of $DPPH^{\cdot}$ decolouration of the samples were calculated according to the formula:

Antiradical activity (%) = 100x[(absorbance of control-absorbance of sample)/ absorbance of control]

2.6. Determination of reducing power

The reducing power of samples was determined according to the method of Oyaizu (1986). Absorbance of supernatant was measured at 700 nm and compared to three standards, BHA, BHT and trolox; any increase in absorbance is synonymous of an increase in reducing power.

2.7. Statistical analysis

Data were subjected to analysis of variance with mean separation by Duncan's multiple range test. Differences were considered statistically significant at the $P \leq 0.05$ levels. Statistical analysis was performed using SPSS 16.0 for Windows.

3. Results and Discussion

3.1. Yield and total phenolic contents of the samples

The yields (dry weight) of grape seed and skin extracts changed significantly according to varieties ($P \leq 0.05$) and data are given in Table 1. Yields of the extracts ranged from 7.58% (Narince) to 9.84 % (Kalecik Karası) for seeds and from 7.21 % (Narince) to 9.68% (Kalecik Karası) for skins. Kalecik Karası and Cabernet Sauvignon had the highest seed extract yields while Narince had the lowest values not only seed but also skin.

Total phenolic contents of the samples were estimated with Folin-Ciocalteu colorimetric method. When total phenolic contents of seeds extracts were calculated as mg GAE g^{-1}, there were not important differences among genotypes ($P \leq 0.05$) (Table 1). Otherwise genotype seemed to be an important factor on the total phenolic contents of grape skin extracts and wines ($P \leq 0.05$). The contents of total phenolic compounds were found 522.49 (Cabernet Sauvignon), 526.55 (Kalecik Karası) and 546.50(Narince) mg GAE per g in grape seed extracts. Baydar et al (2006) also reported that total phenolic content of seed extract from Kalecik Karası was found as 549.54 mg (GAE) g^{-1}. Total phenolic contents of skin extracts were lower than those of seeds as reported before by Iacopini et al (2008). Total phenolic contents of grape skin extracts varied from 22.73 mg GAE g^{-1} (Narince) to 43.75 mg GAE g^{-1} (Kalecik Karası). The contents of total phenolic compounds were found 589.09±10.14 (Hasandede), 506.60±19.78 (Emir) and 549.54±7.10 (Kalecik Karası) mg GAE per g in grape seed extracts. Data obtained from the present study were similar to the findings of Baydar et al (2006). The mean amounts of total phenolics in wine were 1216.17 mg GAE l^{-1} for Cabernet Sauvignon, 1336.21 mg GAE l^{-1} for Kalecik Karası and 217.06 mg GAE l^{-1} for Narince (Table 1). The content of total phenolics has been extensively studied and was found between 1000 and 4000 mg l^{-1} in red wines and 50-2000 mg l^{-1} in white wines (Shadidi & Nazck 1995). Li et al (2009) determined that the content of total phenols varied from 1402 to 3130 mg l^{-1}, averaging 2068 mg l^{-1}, for the red wines and from 189 to 495 mg L^{-1}, averaging 302 mg l^{-1}, for the white wines. These results are similar with the results of this study. Kelebek et al (2010b) studying on the effects of cold maceration on some quality parameters of Öküzgözü wines found that total phenol index (OD280) was 56.4 for control wines and 79.3 for cold maceration wines. Red wines had more phenolic contents than white one as reported by Shadidi & Nazck (1995). Phenolic compounds are important substances of wine. They contribute to sensory characteristics such as colour, flavour, astringency and hardness of it. In addition, antioxidant activities of red wine were reported to be related their measurements of total phenols (Katalinic et al 2004). In this study, it was determined that not only the genotypes but also the different grape samples affected to the total phenolic contents as reported before by Shadidi & Nazck (1995) and Baydar et al (2006).

3.2. HPLC phenolic composition of the samples

HPLC method for analyzing phenolics in the samples has some advantages such as easy and time consuming procedure for preparation of the samples, possibilities of quantification of a great amount of diverse phenolics, the precision, accuracy and detection limits obtained for the phenolics quantified by this method enable its application to grape and wine (Gomez Alonso et al 2007).

The amounts and variations of phenolic compounds in the seed and skin extracts were determined by HPLC and presented in Table 2 and 3. Some phenolic compounds except (+) – catechin,

Table 1-Yield and total phenolic contents (TPC) of the samples
Çizelge 1-Örneklerin ürün ve toplam fenolik içerikleri

	Yield, %		*TPC, mg g^{-1} extract*		*TPC, mg l^{-1}*
Cultivars	*seed*	*skin*	*seed*	*skin*	*wine*
Cabernet Sauvignon	9.74±0.264 a	8.25±0.475 b	522.49±4.26	41.98±1.16 a	1216.17±10.5 b
Kalecik Karası	9.84±0.359 a	9.68±0.486 a	526.55±9.97	43.75±1.48 a	1336.21±12.1 a
Narince	7.58±0.174 b	7.21±0.385 c	546.50±7.32	22.73±0.692 b	217.06±7.15 c
P values	0.002	0.023	0.132	<0.001	<0.001

[a-c]; Within each column, means with the same letter are not significantly different ($P \leq 0.05$)

Table 2-Phenolic composition of seed extracts (mg 100 g^{-1} seed extract)
Çizele 2-Çekirdek ekstraktlarının fenolik kompozisyonu (mg 100 g^{-1}çekirdek ekstrakt)

Phenolics	*Cabernet Sauvignon*	*Kalecik Karası*	*Narince*	*P values*
Phenolic acids				
o-Coumaric acid	4.75±0.315 a	3.10±0.00 b	0.00 c	<0.001
Gallic acid	154.32±1.88 b	242.53±9.12 a	120.64±3.47 c	<0.001
Flavonoids				
(+)-Catechin	970.70±7.39 a	517.13±7.85 b	526.30±13.6 b	<0.001
(-)-Epicatechin	296.90±10.80 b	390.25±5.57 a	320.60±15.9 b	0.003
Quercetin	11.12±0.124 b	14.95±0.202 a	3.16±0.055c	<0.001

[a-c]; Within each line means with the same letter are not significantly different ($P \leq 0.05$)

(-) -epicatechin, gallic acid, *o*-coumaric acid and quercetin in seed extracts and (-)-epicatechin, *o*-coumaric and kaempherol in skin extracts were not found. The genotype as well as the different parts of grape seemed to be the major factors influencing the relative concentrations of the various phenolic compounds. Contents of phenolic compounds determined in seed extracts were changed depending on the genotypes. (+)-Catechin and (-)-epicatechin were the most abundant phenolic compounds in the seed extracts, and this result confirmed by Revilla & Ryan (2000). Rockenbach et al (2011) also reported that catechin was the most abundant monomeric flavanol compound (88.45 mg 100 g^{-1}) identified in the seeds of Cabernet Sauvignon Bakkalbaşı et al (2005) studying on phenolic compositions of seeds taken from some table and wine grape cultivars, found 121-845 mg 100 g^{-1} catechin, 85-893 mg 100 g^{-1} epicatechin and 18-101 mg 100 g^{-1}gallic acid. In addition to these phenolics, quercetin was found in the seed extracts with respective values of 3.16-14.95 mg 100 g^{-1} in our study. *o-C*oumaric acid was the other phenolic compound detected in Kalecik Karası and Cabernet Sauvignon seed extracts but not in Narince seed extract. Otherwise quercetin and *o*-coumaric acid concentrations were extremely low compared with the major phenolics including gallic acid, (+)-catechin and (-)-epicatechin.

Phenolic compositions of grape skin extracts are presented in Table 3. As regards to grapes, the concentrations of these substances seem to vary considerably, since it depends on the genotypes ($P \leq 0.05$). The phenolic acids, including gallic, chlorogenic, ferulic, caffeic, *p*-coumaric and syringic acids showed differences according to the genotypes. The most abundant phenolic acid was syringic acid in red grape skin extracts but not detected in white grape cultivar Narince. Narince being the most abundant chlorogenic acid had the lowest phenolic acid contents when compared to the red grape cultivars. It is an expected result because red grape skin had more phenolic contents according to the white grape skin (Baydar 2006). The most abundant flavonoids were (+)-catechin and quercetin in grape skin extracts. The highest (+)-catechin content was found in Kalecik Karası followed by Cabernet Sauvignon and Narince,

respectively. As regards to the presence of catechin in skin and seeds, it is commonly known that flavan-3-ols are located in both grape skin and seeds; however, skin contains much lower concentrations of flavan-3-ols than seeds (Revilla & Ryan 2000). This agrees with the results obtained by this study. Vanillin was not found in Kalecik Karası and Narince while Cabernet Sauvignon had vanillin in small quantities, 1.13 mg $100g^{-1}$.

trans-Resveratrol, a phytoalexin that belongs to the group of compounds known as stilbenes, is known to occur in grapes and consequently in grape products and in wine. *trans*-Resveratrol was found in the skin extracts between 1.85 and 4.02 mg 100 g^{-1}. The latter confirmed that stilbene content is largely depended on grape cultivars. Careri et al (2003) also found 2.75 mg 100 g^{-1} of *trans*-resveratrol in grape skin extract. Revilla & Ryan (2000) reported that *trans*-resveratrol contents of grape skin were extremely low. Iacopini et al (2008) explained this result as the consequence of the fact that grapes produce stilbenes in response to mold infections and physiological stresses. If these stresses are not present, the levels of stilbenes in grapes remain low.

Phenolic contents of wines are shown in Table 4. Levels of phenolic compounds statistically changed depending on the grape cultivars ($P \leq 0.05$). Some phenolic conpounds including *o*-coumaric acid, ferulic acid and syringic acid were not detected in wine samples. The most abundant phenolic substances were (+)-catechin and gallic acid. The values ranged from 25.98 to 37.23 mg l^{-1} for (+)-catechin and from 9.54 to 17.88 mg l^{-1} for gallic acid. Proestos et al (2005) also found the most abundant compound in Greek wines as (+)-catechin (11.80-40.00 mg l^{-1}). Kelebek et al (2010a) studying on Öküzgözü wine were similarly determined that +(-) catechin was the most abundant flavanol in wines obtained from two different regions (Elazığ and Denizli). (-)-Epicatechin and caffeic acid were the other most abundant phenolics found in wines. (-) - Epicatechin and caffeic acid changed between 5.72 and 16.74 mg l^{-1} and between 2.40 and 12.62 mg l^{-1}, respectively. *p*-Coumaric acid was found in concentrations between 0.45 mg l^{-1} (Cabernet Sauvignon) and 0.50 mg l^{-1} (Kalecik Karası) in red wines whereas *p*-coumaric acid was not detected in white wine. *p*-Coumaric acid, ranging between 0.23 and 7.07 mg l^{-1}, were obtained in the analysis of 23 commercial Italian wines (Lante et al 2004). Chlorogenic acid was found only in white wine cultivar Narince, otherwise this phenolic was not found in red wines.

In this study *trans*-resveratrol in red wines was found higher than in the white one. *trans*-Resveratrol values changed as 0.45-0.68 mg l^{-1} in red wines and 0.27 mg l^{-1} in white wine. *trans*-Resveratrol is abundant in grape skin and present in higher concentration in red grape varieties compared with white varieties. The results obtained in this investigation are in agreement with previous research on *trans*-resveratrol composition in red wines. Lopez et al (2001) and Careri et al (2003) found 0-1.34 μg ml^{-1} and 0.56-2.86 μg ml^{-1} *trans*-resveratrol in commercial red wines, respectively. Similarly quercetin and kaempherol as flavanoids and vanillin as a non-flavanoid were found higher values in red wines compared to the white wine. The results of our analysis indicate that the level of flavonoids was dependent on the colours of wine. Flavonoids are present mainly in grape skin. Skin-contact maceration is either avoided or allowed for a very short period in the production of white wines. Thus absence or very low concentration of flavonoids in the white wines was expected (Makris et al 2006). Quercetin content of red wines varied from 4.80 to 5.24 mg l^{-1} and white wine had 1.11 mg l^{-1} of quercetin. Lopez et al (2001) determined that red wines had 0-4.66 μg ml^{-1} of quercetin. In another study (Careri et al 2003) conducted on the quercetin contents in red wines, it was determined that red wines had 0.89-8.84 μg ml^{-1} quercetin. Kelebek et al (2010a) studying on wines from Öküzgözü, a Turkish variety, found 0.76-2.01 mg l^{-1} quercetin in wines from different vineyard locations. These results are in agreement with our data.

3.3. Radical scavenging activities of the samples

Radical scavenging activities of grape extracts, wines and standards were tested by the DPPH·method and important differences were found among the genotypes and the samples ($P \leq 0.05$). For the seed and skin extracts,

Table 3-Phenolic composition of skin extracts (mg 100 g^{-1} skin extract)
Çizelge 3-Kabuk ekstraktlarının fenolik kompozisyonu (mg 100 g^{-1}kabuk ekstrakt)

Phenolics	*Cabernet Sauvignon*	*Kalecik Karası*	*Narince*	*P values*
Phenolic acids				
Caffeic acid	29.82±0.583 b	35.44±1.44 a	1.79±0.086 c	<0.001
Chlorogenic acid	24.13±0.657 a	22.83±0.638 a	19.24±1.21 b	0.019
p-Coumaric acid	3.46±0.552 b	4.38±0.317 a	0.27±0.029 c	0.001
Ferulic acid	0.92±0.0361 b	1.53±0.115 a	0.49±0.124 c	0.001
Gallic acid	9.80±0.286 a	10.43±0.492 a	5.03±1.13 b	0.004
Syringic acid	35.47±2.15 b	85.70±10.1 a	0.00 c	<0.001
Flavonoids				
(+)-Catechin	3.64±0.261 b	5.40±1.85 a	0.76±0.144 c	0.059
Quercetin	1.85±0.035 b	2.63±0.317 a	1.58±0.144 c	0.025
Non-Flavonoids				
Vanillin	1.13±0.027 a	0.00 b	0.00 b	<0.001
Stilbenes				
trans-Resveratrol	3.68±0.127 b	4.02±0.692 a	1.85±0.202 c	0.023

[a-c]*: Within each line, means with the same letter are not significantly different ($P \leq 0.05$)

Table 4-Phenolic composition of wine samples (mg l^{-1})
Çizelge 4-Şarap örneklerinin fenolik kompozisyonu (mg l^{-1})

Phenolics	*Cabernet Sauvignon*	*Kalecik Karası*	*Narince*	*P values*
Phenolic acids				
Caffeic acid	7.38±0.006 b	12.62±0.053 a	2.40±0.027 c	<0.001
Chlorogenic acid	0.00 b	0.00 b	0.46±0.012 a	<0.001
p-Coumaric acid	0.45±0.006 b	0.50±0.003 a	0.00 c	<0.001
Gallic acid	14.86±0.029 b	17.88±0.042 a	9.54±0.026 c	<0.001
Flavonoids				
(+)- Catechin	37.23±0.222 a	35.89±0.009 a	25.98±0.046 b	<0.001
(-)-Epicatechin	9.88±0.000 b	16.74±0.094 a	5.72±0.003 c	<0.001
Kaempherol	0.82±0.007 b	1.18±0.003 a	0.38±0.003 c	<0.001
Quercetin	4.80±0.008 b	5.24±0.020 a	1.11±0.003 c	<0.001
Non Flavonoids				
Vanillin	2.91±0.008 a	1.14±0.003 b	0.90±0.006 c	<0.001
Stilbenes				
trans-Resveratrol	0.68±0.006 a	0.45±0.007 b	0.27±0.006 c	<0.001

[a-c];Within each line, means with the same letter are not significantly different ($P \leq 0.05$)

wines and the some standard antioxidants including Trolox, BHA and BHT, the DPPH· scavenging activity increased in the following order: Narince skin extract<Narince wine< Cabernet Sauvignon skin extract<Kalecik Karası skin extract<BHT<Cabernet Sauvignon wine<Kalecik Karası wine<BHA<Kalecik Karası seed extract<Narince seed extract<Cabernet Sauvignon seed extract< Trolox (Figure 1). The radical scavenging activities of the seed extracts were considerably better than those of skin extracts. Grape seed extracts almost completely inhibited DPPH· absorption. Otherwise skin extract contained remarkably lower amounts of radical scavenging compounds. In several studies significant correlation was found between DPPH scavenging activity and the total phenolic content of a number of grape seed extracts from different cultivars

(Bakkalbaşı et al 2005). Similarly, it was determined that seed extracts having higher phenolic contents than skin extracts showed higher free radical scavenging activity in this study. Trolox showed higher radical scavenging activity than the grape extracts. BHA and BHT exhibited lower activity compared to grape seed extracts as reported before by Baydar et al (2007). Red wines showed more antiradical activities than the white one. The percentage inhibition for wines was 84.01% for Kalecik Karası, 81.34% for Cabernet Sauvignon and 19.16% for Narince. Similarly, Katalinic et al (2004) found that the percentage inhibition for wines diluted with water was 54.6-82.2% for red wines and 10.7-16.2% for white wines. The model of scavenging the stable DPPH radical is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods.

3.4. Reducing power of the samples

The reducing powers of seed and skin extracts, wines and synthetic antioxidant standards (BHA, BHT and trolox) are presented in Figure 2. As shown in Figure 2 statistically differences were detected in not only genotypes but also the samples ($P \leq 0.05$). Within the seed and skin extracts at 250 μg ml^{-1}, the best reducing powers were obtained

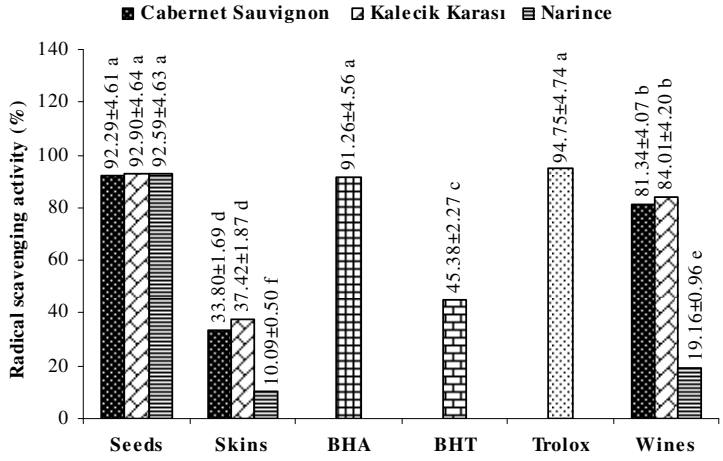

Figure 1-Radical scavenging activities of seeds, skins and wines and standards
Şekil 1-Çekirdek, kabuk, şarap ve standartların radikal bağlama aktiviteleri

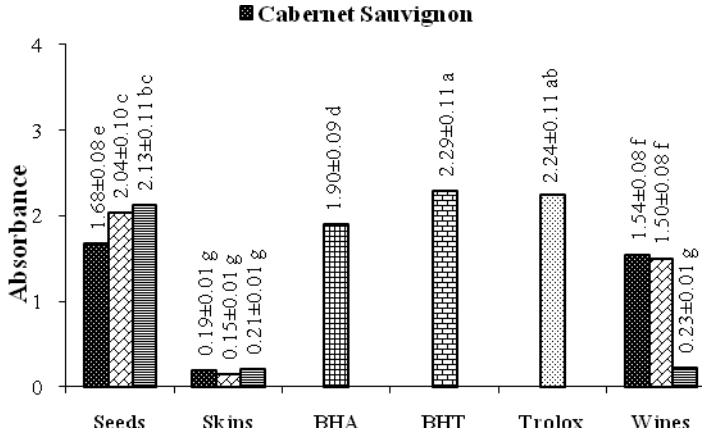

Figure 2-Absorbance of seeds, skins, wines and standards for reducing power
Şekil 2-Çekirdek, kabuk, şarap ve standartların demir ingirgeme gücü için absorbans değerleri

from the seed extracts. Jayaprakasha et al (2001) determined that grape seed extracts extracted with different solvent mixtures showed absorbance between 0.52 and 2.59 at 0.5 mg ml^{-1} and reducing powers of grape seed extracts changed depending on the extraction solvents and the extract concentrations. In this study, the reducing power of skin extracts at the same concentration is generally poor compared to the grape seed extracts and standards. They had low absorbances and low reducing powers. The reducing powers of trolox, BHA and BHT were higher than those of skin extracts but almost similar to those of seed extracts. Red wines showed higher absorbance and stronger reducing power than white wine. These results are in agreement with the results of Katalinic et al (2004).

4. Conclusions

The results obtained in this study showed that large differences were found among the cultivars, wines and grape parts in relation to the phenolics composition. Grape seeds, skins and wines contained different phenolics with different levels and these variations affected the antioxidant capacity of the samples. Total phenolic contents, antiradical activities and reducing powers of grape seed extracts are higher than those of grape skin extracts; and red wines are more total phenolic contents, antiradical activities and reducing powers compared with white wine. Depending on the results, it can be said that there is a positive relationship between phenolic contents and the antioxidant activities of the samples. The results of the present study also indicate that grape seed extracts of these Turkish cultivars can be used as easily accessible source of natural antioxidants. Otherwise it will be extremely useful to utilize the wastes of wine-making process as alternative natural antioxidants to the synthetic antioxidants used in food industry to prolong the shelf life of food. Moreover grape skin and wine are good food products and they can be used an integredient in dietary supplements as a good antioxidant source.

References

Bakkalbaşı E, Yemis O¸ Aslanova D & Artık N (2005). Major flavan-3-ol composition and antioxidant activity of seeds from different grape cultivars grown in Turkey. *European Food Research Technology* **221**: 792-797

Baumann J, Wurm G & Bruchhausen F (1979). Prostaglandin synthetase inhibiting O_2-radical scavenging properties of some flavonoids and related phenolic compounds. *Naunyn-Schmiedebergs Archives of Pharmacology* **30**: 27-32

Baydar N G, Özkan G & Yaşar S (2007). Evaluation of the antiradical and antioxidant potential of grape extracts. *Food Control* **18**: 1131-1136

Baydar N G (2006). Organic acids, tocopherols and phenolic compositions of some Turkish grape cultivars. *Chemistry of Natural Compounds* **42**(2): 156-159

Baydar N G, Sağdıç O, Özkan G & Çetin E S (2006). Determination of antibacterial effects and total phenolic contents of grape (*Vitis vinifera* L.) seed extracts. *International Journal of Food Science and Technology* **41**: 799-804

Caponio F, Alloggio V & Gomes T (1999). Phenolic compounds of virgin olive oil:Influence of paste preparation techniques. *Food Chemistry* **64**: 203-209

Careri M, Corradini C, Elviri L, Nicoletti L & Zagnoni I (2003). Direct HPLC analysis of quercetin and trans-resveratrol in red wine, grape, and winemaking byproducts. *Journal of Agricultural Food Chemistry* **51**: 5226-5231

Crozier A. Jaganath I B & Clifford M N (2007). Phenols, Polyphenols and Tannins: An Overview. In *Plant Secondary Metabolites*, edited by M. N. C. H. A. Alan Crozier.

Gadow A, Joubert E & Hansmann C F (1997). Comparasion of the antioxidant activity of aspalathin with that of other plant phenols of rooibos tea (*Aspalathus linearis*), α-tocopherol, BHT, and BHA. *Journal of Agricultural Food Chemistry* **45**: 632-638

Gomez-Alonso S, Garcia Romero E & Hermosin Gutierrez I (2007). HPLC analysis of diverse grape and wine phenolics using direct injection and multidetection by DAD and fluorescence. *Journal of Food Composition and Analysis* **20**: 618-626

Iacopini P, Baldi M, Storchi P & Sebastiani L (2008). Catechin, epicatechin, quercetin, rutin and resveratrol in red grape: Content, *in vitro* antioxidant activity and interactions. *Journal of Food Composition and Analysis* **21**:589-598

Ito N, Hiroze M, Fukushima G, Tauda H, Shira T & Tatematsu M (1986). Studies on antioxidant; their carcinogenic and modifying effects on chemical carcinogenesis. *Food Chemistry Toxicology* **24**: 1071-1081

Jayaprakasha G K, Selvi T & Sakariah K K (2003). Antibacterial and antioxidant activities of grape (*Vitis vinifera*) seed extracts. *Food Research International* **36**: 117-122

Jayaprakasha G K, Signh R P & Sakariah K K (2001). Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models *in vitro*. *Food Chemistry* **73**: 285-290

Katalinic V, Milos M, Modun D, Music I & Boban M (2004). Antioxidant effectiveness of selected wines in comparison with (+)-catechin. *Food Chemistry* **86**: 593-600

Kelebek H, Canbaş A, Jourdes, M & Teissedre P L (2010a). Characterization of colored and colorless phenolic compounds in Öküzgözü wines from Denizli and Elazig regions using HPLC-DAD–MS. *Industrial Crops and Products* **31**: 499-508

Kelebek H, Selli S, & Canbaş A (2010b). Öküzgözü üzümlerinden kırmızı şarap üretiminde soğuk maserasyon uygulamasının antosiyaninler üzerine etkisi. *Tarım Bilimleri Dergisi* **16**(4): 287-294

Kinsella J E, Frankel E, German B *&* Kanner J (1993). Possible mechanisms for the protective role of antioxidants in wine and plant foods. *Food Technology* **47**: 85-89

Lante A, Crapisi A, Lomolino G & Spettoli P (2004). Chemical parameters, biologically active polyphenols and sensory characteristics of some Italian organic wines. *Journal of Wine Research* **15**(3): 203-209

Li H, Wang X, Li Y, Li P & Wang H (2009). Polyphenolic compounds and antioxidant properties of selected China wines. *Food Chemistry* **112**: 454-460

Lopez M, Martinez F, Del Valle C, Orte C & Miro M (2001). Analysis of phenolics constituents of biological interest in red wines by HPLC. *Journal of Chromatography* **922**: 359-363

Makris D P, Kallithraka S, Kefalas P (2006). Flavonols in grapes, grape products and wines: Burden, profile and influential parameters. *Journal of Food Composition and Analysis* **19**: 396-404

Murthy K N C, Singh R P & Jayaprakasha G K (2002). Antioxidant activity of grape (*Vitis vinifera*) pomace extracts. *Journal of Agricultural and Food Chemistry* **50**: 5909-5914

Oyaizu M (1986). Studies on products of browning reaction: antioxidative activities of products of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition* **44**: 307-315

Özkan G & Baydar N G (2006). A direct RP-HPLC determination of phenolic compounds in Turkish red wines. *Akdeniz Üniversitesi Ziraat Fakültesi Dergisi* **19**(2): 229-234

Proestos C, Bakogiannis A, Psarianos C, Koutinas A A, Kanellaki M & Komaitis M (2005). High performance liquid chromatography analyses of phenolic substances in Greek Wines. *Food Control* **16**: 319-323

Revilla E & Ryan J M (2000). Analysis of several phenolic compounds with potential antioxidant properties in grape extracts and wines by HPLC-photodiode array detection without sample preparation. *Journal of Chromatography A* **881**: 461-469

Ricardo da Silva J M, Rosec J P, Bourzeix M & Heredia N (1990). Separation and quantitative determination of grape and wine procyanidins by HPLC. *Journal of the Science of Food and Agriculture* **53**: 85–92

Rice Evans C A, Miller N J & Paganga G (1996). Structure antioxidant activity relationship of flavonoids and phenolic acids. *Free Radical Biology and Medicine* **20**: 933-956

Rockenbach I V, Gonzaga L V, Rizelio V M, Schmidt Gonçalves A E S, Genovese M I & 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 Research International* **44**(4): 897-901

Shadidi F & Naczk M (1995). Food Phenols: Source, Chemistry, Effects and Applications. *Technomic Publishing Company* (USA). pp. 331

Sherwin E R (1978). Oxidation and antioxidants in fat and oil processing. *Journal of American Oil Chemical Society* **55**: 809-814

Shimada K, Fujikawa K, Yahara K & Nakamura T (1992). Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *Journal of Agricultural and Food Chemistry* **40**: 945-948

Singleton VL & Rossi J R (1965). Colorimetry of total phenolics with Phospho molybdic-phosphotungstic acid. *American Journal of Enology and Viticulture* **16**: 144-158

Sun J, Chu Y F, Wu X & Liu R H (2002). Antioxidant and antiproliferative activities of common fruits. *Journal of Agricultural Food Chemistry* **50**: 7449-7454

Yu J Vasanthan T & Temelli F (2001). Analysis of phenolic acids in barley by high performance liquid chromatography. *Journal of Agricultural Food Chemistry* **49:** 4352-4358