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Determination of Biochemical Characteristics, Antioxidant Activities, and Individual Phenolic Compounds of 13 Native Turkish Grape Juices

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Abstract

Physicochemical parameters, total phenolic contents, phenolic compositions, and antioxidant activities of Turkish native grape juices as well as the correlations among these parameters were investigated in this study. The total phenolic contents ranged from 99 mg GAE (gallic acid equivalent) to 607 mg GAE per 100 g dry weight (DW). The antioxidant activities of the samples were detected as 0.14–4.97 mM Trolox equivalents per 100 g DW using the cupric reducing antioxidant capacity (CUPRAC), ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) methods. The highest antioxidant activity was found in Erkenci Dimrit grape juice. It was determined that caftaric acid was the most common compound among phenolics and (+)-catechin in flavanols. The presence of *t*-resveratrol and (–)-epigallocatechin gallate in all samples was especially important considering their beneficial effects on human health. There were positive correlations between antioxidant activity and caffeic acid, p-coumaric acid, ferulic acid, sinapic acid, quercetin hydrate, kaempferol, *t*-resveratrol, oenin chloride, (–)-epigallocatechin gallate, and total phenolic content. The highest correlation was found between ABTS and CUPRAC (r = 0.99), and the lowest was between FRAP and DPPH (r = 0.89) among the antioxidant capacity methods.

Keywords Grape juices · Polyphenols · *t*-resveratrol · Antioxidant · Correlations

Introduction

Vine has been cultivated for many years in Anatolia, which has an important native grape variety richness. Turkey is one of the most important grape producers in the world. According to data from 2019, it has a total vineyard area of 405,439 ha and produces 4.1 million tons of grape annually (FAO 2021). Most of the grape produced is dried or used as table grape, while the remaining is utilized to make molasses, grape juice, and wine. In the traditional production of grape juice, harvested grapes are crushed and pressed, and the resulting product is consumed either fresh or pasteurized. In recent years, the emergence of positive effects of grapes, especially colored varieties, on health has increased the demand for grape products. This has also resulted in an increase in grape juice production.

Grape juice is a rich and natural source of antioxidants and polyphenols. These polyphenols can be classified into two groups: nonflavonoids, including hydroxycinnamates, hydroxybenzoates, and stilbenes, and flavonoids, consisting of flavan-3-ols, flavonols, and anthocyanin (Garrido and Borges 2013; Aleixandre-Tudo et al. 2018). Hydroxycinnamates and flavonoids are found in greater quantities than other phenolic compounds in grapes and grape products (Kennedy et al. 2006; Teixeira et al. 2013; Aleixandre-Tudo et al. 2018). Many previous studies have shown that these compounds have a preventive effect on diseases related to oxidative stress, such as cancer, as well as cardiovascular and neurodegenerative diseases (Soundararajan et al. 2008; Fraga et al. 2010; Yamagata et al. 2015; Cosme et al. 2018), and that they improve endothelial functions, inhibit platelet aggregation, and decrease plasma protein oxidization and low-density lipoprotein oxidization (Krikorian et al. 2012; Stalmach et al. 2012; Macedo et al. 2013; Natividade et al. 2013; Toaldo et al. 2014).

Because of the high antioxidant capacity of phenolic compounds found in grape and grape products, numerous studies have been conducted to determine the relationship between these phenolic substances and antioxidant activity,

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and important relationships have been revealed (Burin et al. 2010; Büyüktuncel et al. 2014; Lima et al. 2014; Öncül and Karabiyikli 2015; Xu et al. 2015; Cosme et al. 2018; Guler et al. 2018). Analysis methods, such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging, ferric reducing antioxidant power (FRAP), oxygen radical absorbance capacity (ORAC), and cupric reducing antioxidant capacity (CUPRAC) have been frequently used by researchers to determine the antioxidant capacity of grape products (Callaghan et al. 2013; Lima et al. 2014; Yamamoto et al. 2015; Margraf et al. 2016; Fabani et al. 2017; Padilha et al. 2017; Samoticha et al. 2017; Zhang et al. 2017; Guler et al. 2018).

Grape juice quality is affected by several parameters, such as the grapes' physicochemical features, aroma, phenolic compounds, organic acid, and sugar composition. Margraf et al. (2016) stated that functional features of grape juice products sold in various countries are directly related to their bioactive compound profiles and ingredients, especially phenolic acids. Various studies suggest that these bioactive phenolic compounds vary depending on the grape juice production process (Piva et al. 2008; Gollücke et al. 2009; Capanoglu et al. 2013; Lima et al. 2015; Toaldo et al. 2015; Guler et al. 2018), as well as on growing conditions and location, agricultural applications, climate characteristics, and the maturity level and variety of the grapes (Sabir et al. 2010; Granato et al. 2015; Yamamoto et al. 2015). Despite the availability of studies to determine the attributes of grape juice products produced in Turkey (Canbaş et al. 1996; Sabir et al. 2010; Capanoglu et al. 2013; Soyer et al. 2003; Karaoğlan et al. 2015; Kaya and Unluturk 2016; Coskun 2017; Gülcü and Dağlıoğlu 2018), they are limited in number.

The aim of this study was to perform an in-depth examination of the total phenolic content, phenolic compounds, and antioxidant activities of 13 native grape juice samples. In addition, the study explored the relationships among four different antioxidant capacities and between phenolic compounds and these four different antioxidant capacities. For this purpose, 13 different grape juice samples were investigated in this current work. Unclarified grape juice of some traditional grape varieties has been manufactured in Turkey for a long time. For this reason, traditional grape juice production methods were used for these native grapes.

Material and Methods

Chemicals

Folin–Ciocalteu reagent, formic acid, ethyl alcohol, hydrochloric acid, iron (III) chloride hexahydrate, and tris (hydroxymethyl) aminomethane were purchased from Merck (Darmstadt, Germany). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), DPPH, potassium persulfate, sodium acetate trihydrate, acetic acid, phosphoric acid, cupper (II) chloride dihydrate, neocuproine, ammonium acetate, potassium phosphate monobasic, acetonitrile, and methanol were obtained from Sigma–Aldrich (St. Louis, MI, USA). ABTS was purchased from Amresco (Radnor, PA, USA), Waters RS for HPLC Plus from Carlo Erba (Carlo Erba Reagents, Val de Reuil, France), and TPTZ (2,4,6-Tri[-pyridyl]-1,3,5-triazine) from Alfa Asear (Karlsruhe, Germany).

Malvidin chloride was obtained from Extrasynthese (Lyon, France). Gallic acid, caffeic acid, p-coumaric acid, chlorogenic acid, epicatechin, trans-resveratrol, quercetin, kaempferol, and oenin chloride were purchased from Sigma-Aldrich; (+)-catechin (CA), (–)-epigallocatechin gallate (EGCG), vanillic acid, and caftaric acid were obtained from Fluka (St. Louis, MI, USA); and (–)-epicatechin gallate (ECG) and sinapic acid were purchased from Alfa Asear. Ferulic acid and myricetin were purchased from Merck and Cayman Chemical (Ann Arbor, MI, USA), respectively.

Grape Samples and Juice Processing

The grapes (V. *vinifera* L.) used in this study were harvested from the vineyards of Manisa Viticulture Research Institute. The physicochemical properties and their harvest dates are listed in Table 1. The grapes were processed into blurred grape juice by using traditional methods at the grape processing pilot unit of the institute. For this purpose, the harvested grapes were immediately transferred to the pilot processing unit and separated from dust, soil, and other impurities by washing. Then they were passed through an automatic destemmer–crusher machine (Türköz Metal Makine, Denizli, Turkey), and the stems were discarded. The grape mash was heated to 50 °C and kept at this temperature for 60 min for red and 30 min for white varieties. Then the heated mash was pressed using a hydraulic press (Türköz Metal Makine, Denizli, Turkey). The blurred grape juice obtained from the press was poured into 1000-ml glass bottles (colorless) to produce unclarified grape juice. These bottles were kept in 85 °C water for 20 min for the pasteurization. The samples were then immediately cooled to room temperature. The grape juice production was replicated three times.

Physicochemical Properties

Titratable acidity was determined by titrating a 10-ml sample with 0.1 N NaOH to pH 8.1, and the results were expressed as tartaric acid percentage. The soluble solid con-

Table 1 Properties of grape juices

No.	Code	Variety	Color	Harvest date	SS°Brix	TA, %	pH
1	M1	Bulama	White	7 Aug 2017	17.75 ± 0.07^{c}	0.39 ± 0.01^{ef}	3.75 ± 0.01^{i}
2	M2	Exalta	White	7 Aug 2017	18.45 ± 0.07^{c}	0.39 ± 0.01^{ef}	3.85 ± 0.01^{g}
3	M3	Kanon Harabı	White	6 Aug 2017	16.15 ± 0.01^{d}	0.47 ± 0.01^{cde}	3.66 ± 0.01^{l}
4	M4	Köy Yeri	White	6 Aug 2017	18.25 ± 0.07	0.36 ± 0.01^{f}	3.83 ± 0.01^{h}
5	M5	Tergöynek	White	12 Aug 2017	17.85 ± 0.07^{c}	0.42 ± 0.01^{def}	3.68 ± 0.01^{k}
6	M6	Koca Osman	Red	11 Aug 2017	18.45 ± 0.07^{c}	0.39 ± 0.01^{ef}	3.88 ± 0.03^{f}
7	M7	Çilek Üzümü	Red	10 Aug 2017	20.15 ± 0.07^{b}	0.47 ± 0.01^{cde}	3.99 ± 0.05^{c}
8	M8	Yerli Dimrit	Red	4 Aug 2017	18.45 ± 0.07^{c}	0.71 ± 0.01^{a}	3.90 ± 0.01^{e}
9	M9	Balçova Karası	Red	7 Aug 2017	21.8 ± 0.57^{a}	0.46 ± 0.01^{cde}	3.93 ± 0.01^{d}
10	M10	Erkenci Dimrit	Red	4 Aug 2017	19.7 ± 0.07^{b}	0.61 ± 0.01^{b}	3.65 ± 0.01^{l}
11	M11	Kara Erik	Red	8 Aug 2017	21.9 ± 0.07^{a}	0.53 ± 0.01^{c}	3.72 ± 0.01^{j}
12	M12	Denizli Karası	Red	3 Aug 2017	20.55 ± 1.76^{b}	0.49 ± 0.1^{cd}	4.01 ± 0.02^{b}
13	M13	Katı Kara	Red	7 Aug 2017	21.8 ± 0.01^{a}	0.38 ± 0.01^{f}	4.14 ± 0.01^{a}

Values indicated with different letters within each group and column are significantly different for $p < 0.05$
SS soluble solid content, *TA* titratable acidity tartaric acid equivalent

tent (SS) was detected by using a refractometer as degrees Brix. The pH value was measured with a pH meter (Hanna Instrument HI 221, Romania) (Ough and Amerine 1988).

Determination of Total Polyphenols

The total phenolic content (TP) of the samples was determined using the Folin–Ciocalteu colorimetric method (Singleton and Rossi 1965). The absorbance was measured using a Multiskan GO spectrophotometer (Thermo Fisher Scientific, Vantaa, Finland) at 760 nm. Gallic acid was used as standard, and results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g dry weight (DW). Standard concentrations of 5–50 mg/l were used for the calibration curve.

Antioxidant Activities of the Native Grape Juices

Four antioxidant activity analyses were performed to determine the antioxidant properties of the samples and their correlations in the samples. These methods are presented below.

DPPH• Assay

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was performed according to previous studies (Kim et al. 2002; Lima et al. 2014). First, 1.0 mM DPPH• radical solution was prepared in methanol and diluted to the absorbance until 0.900 ± 0.05. Each 0.1 ml of diluted sample was added to 2.9 ml of radical solution, and the absorbance of the DPPH solution was measured at times t = 0 and t = 30 min. The DPPH solution was incubated for 30 min in the dark after addition of the samples. The absorbance measurements were undertaken using an ultraviolet-visible (UV-Vis) spectrophotometer (Multiskan GO, Thermo Fisher Scientific, Vantaa, Finland) at 517 nm. The Trolox analytical standard was used for the calibration curve ($y = 0.1507x + 0.5494$; $R^2 = 0.9977$), and the results were expressed as mM Trolox equivalent (TE) in 100 g DW.

ABTS•+ Assay

This method (2,2′-azinobis-[3-ethylbenzothiazoline-6-sulfonic acid]) was used as previously described by Re et al. (1999). To prepare the ABTS stock solution, 7 mM ABTS and 2.45 mM potassium persulfate were mixed and kept at room temperature for 12–16 h. Then the obtained ABTS•+ solution was diluted with ethanol to 0.700 (±0.02) until absorbance (734 nm). Each 60-µl diluted sample was added to 940 µl of ABTS•+ solution, and the absorbance of these solutions was measured at t = 0 and t = 6 min using a UV-Vis spectrophotometer (Multiskan GO, Thermo Fisher Scientific, Vantaa, Finland). The results were calculated from the calibration graphic ($y = 0.2045x - 0.0681$; $R^2 = 0.9986$) and expressed as mM TE in 100 g DW.

FRAP Assay

The FRAP analysis of the samples was carried out using the method described by Benzie and Strain (1999) and modified by Wern et al. (2016). First, 300 mM acetate buffer (3.1 g $C_2H_3NaO_2 \cdot 3H_2O$ and 16 ml $C_2H_4O_2$), 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ were prepared for the FRAP reagent. Then 25 ml of acetate buffer (3.6 pH), 2.5 ml of TPTZ, and 2.5 ml of $FeCl_3 \cdot 6H_2O$ were mixed to obtain a fresh reagent. For analysis, the FRAP reagent, distilled water, and the samples were warmed to 37 °C. Then a 50-µl sample and FRAP reagent were added to 2 ml of distilled water

at 37 °C, and the mixture was incubated for 4 min at the same temperature in the dark. The mixture absorbance was measured by a UV-Vis spectrophotometer at 593 nm for 8 min. For the calibration graphic ($y = 0.0036x + 0.0417$; $R^2 = 0.9907$), 50–1000 µM standard concentrations were used, and the results were expressed as mM TE in 100 g DW.

CUPRAC Assay

The CUPRAC of the samples was determined according to Apak et al. (2004), with modifications of Callaghan et al. (2013) for the adaptation of the assay to grape samples. In this method, 150 µl of 1 M ammonium acetate, 7.5 mM neocuproine, and 10 mM copper (II) chloride dehydrate were added to 150-µl diluted samples with 0.05 M Tris buffer (pH 7.6) or analytical standard. The mixtures were then incubated for 30 min at room temperature, and their absorbance was measured using a UV-Vis spectrophotometer at 450 nm. Tris buffer was used as blank. The standard concentrations ranged from 50 µM to 1000 µM for the calibration graphic ($y = 0.0038x + 0.01$; $R^2 = 0.9995$), and the results were given as mM TE in 100 g DW.

Individual Phenolic Compounds of the Samples

The phenolic compounds of the samples were determined according to the high-performance liquid chromatography (HPLC; Agilent 1260 Infinity, Germany) method described by Caponio et al. (1999) and modified by Özkan and Baydar (2006), with slight modifications to the analysis and elution conditions. An ultraviolet diode-array detector (UV-DAD; Agilent 1260 Infinity) was used for the detection of the phenolic compounds. A C18 ODS 250 × 4.6 mm, 5 µm (Agilent) column was utilized for the analytical separation. The following phenolic compounds were detected: gallic acid (LOD = 0.009; $R^2 = 0.9999$), CA (LOD = 0.019; $R^2 = 0.9999$), EGCG (LOD = 0.01; $R^2 = 0.9996$), and ECG (LOD = 0.002; $R^2 = 0.9999$) at 280 nm; caftaric acid (LOD = 0.013; $R^2 = 0.9999$), cholorogenic acid (LOD = 0.007; $R^2 = 0.9999$), caffeic acid (LOD = 0.002; $R^2 = 0.9999$), ferulic acid (LOD = 0.004; $R^2 = 0.9999$), sinapic acid (LOD = 0.003; $R^2 = 0.9999$), p-coumaric acid (LOD = 0.002; $R^2 = 0.9999$), and trans-resveratrol (LOD = 0.002; $R^2 = 0.9999$) at 320 nm; myricetin (LOD = 0.015; $R^2 = 0.9998$), quercetin (LOD = 0.002; $R^2 = 0.9999$), and kaempferol (LOD = 0.006; $R^2 = 0.9999$) at 360 nm; and oenin chloride (LOD = 0.037; $R^2 = 0.9999$) and malvidin chloride (LOD = 0.175; $R^2 = 0.9992$) at 520 nm. The samples were first diluted with distilled water and then filtered using a syringe filter (PTFE, 0.45 µm, Sartorius). The injection volume was 10 µ, the flow rate was 1 ml min–1, and the column temperature was 30 °C. The mobile phase consisted of ultrapure water: formic acid (99.8:0.2 v/v) (A) and methanol (B). Gradient elution was 100% A at min 0, 95% A and 5% B at min 3, 80% A and 20% B at min 18, 80% A and 20% B at min 20 (isocratic step), 75% A and 25% B at min 30, 70% A and 30% B at min 40, 60% A and 40% B at min 50, 50% A and 50% B at min 55, and 100% B at min 65. Then 100% A elution was performed for 5 min to return to the initial condition. The data were analyzed using the Agilent ChemStation OpenLAB software program. The individual polyphenols were identified according to the retention times and spectra of analytical standards. The phenolic compound concentrations were calculated using calibration curves. The results were expressed as milligrams per 100 g DW.

Statistical Analysis

In this study, all analyses were performed in triplicate, and the results were given with standard deviations. The obtained results were subjected to an analysis of variance, and the Duncan multiple comparison test was used to determine the differences between the samples. Additionally, the Pearson correlation coefficients were calculated to examine the relationships between the four different antioxidant activities, as well as between the phenolic compounds and different antioxidant activities.

Results and Discussion

The harvest times and SS, TA, and pH values of the investigated five white and eight red grapes are presented in Table 1. All grapes were harvested in August, and SS values ranged from 16.15 °Brix to 21.90 °Brix. Acidity of the grape samples changed between 0.38% and 0.71% at harvest. The pH measurements were between 3.65 and 4.14. Grape juice quality is affected by SS, acidity, and pH. In particular, taste balance is one of the most important quality parameters in fruit juice, and it directly depends on grape SS and acidity. Additionally, these parameters, especially pH values, are affected by processing conditions and thermal applications. It was found that the 13 grapes are appropriate for juice processing in terms of physicochemical parameters.

Total Polyphenols and Antioxidant Activities of the Samples

Phenolic contents in grape products are frequently investigated because of their high antioxidant effect. In addition, phenolic amounts of the grapes are important for juice processing conditions. The TP contents of the 13 grape juice samples are shown in Fig. 1. The differences between the TP values of the samples were statistically significant

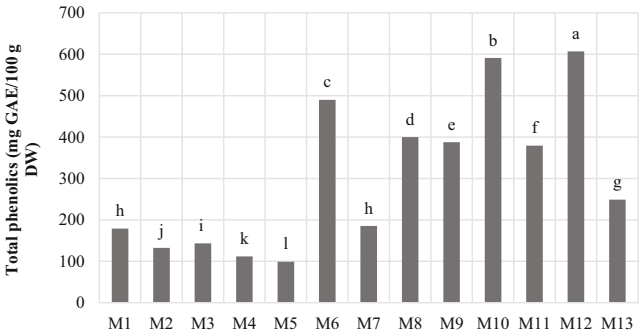

Fig. 1 Total phenolic contents of the samples (mg GAE/100 g DW). Different letters indicate statistically significant differences among the samples ($p < 0.05$)

($p \leq 0.05$). The TP values ranged from 99 mg GAE/100 g DW to 607 mg GAE/100 g DW, with the highest value being observed in the M12 sample and the lowest in M5. Previous studies reported TP contents of 1110–1580 mg GAE/l in Turkish grape juice samples and 2253–2847 mg GAE/l in grapes (Sabir et al. 2010; Pirinççioğlu et al. 2012; Gülcü and Dağlıoğlu 2018). In other studies, these values in grape juices were determined to be 550–3433 mg GAE/l (Burin et al. 2010; Mahdavi et al. 2011; Lima et al. 2014, 2015; Granato et al. 2015; Moreno-Montoro et al. 2015; Toaldo et al. 2015; Margraf et al. 2016; Padilha et al. 2017). The TP values obtained from the grape juice samples in the current study were similar.

To reveal the comparative antioxidant properties of grape juice samples in detail, four different methods (CUPRAC, FRAP, DPPH, and ABTS) were used, and the results are shown in Table 2. For each studied method, there were statistically significant differences ($p \leq 0.05$) between the samples. The highest antioxidant capacity was observed in the M10 sample according to the CUPRAC, FRAP, and ABTS methods at 4.97, 3.99, and 2.84 mM/100 g, respectively, and in the M12 sample according to the DPPH method at 2.71 mM/100 g. The lowest values were determined as 0.59 mM/100 g and 0.52 mM/100 g for the M5 sample according to the CUPRAC and FRAP methods, respectively; 0.14 mM/100 g in the M7 sample according to the DPPH method; and 0.31 mM/100 g in the M3 sample according to the ABTS method.

Studies on Turkish grape juices revealed that the antioxidant capacity of clarified grape juice was 378 µmol/100 g DW for DPPH and 165 µmol/100 g DW for FRAP (Capanoglu et al. 2013). In addition, in red grape juice samples, the antioxidant capacity changed during the process, ranging from 1.14 to 1.21 µmol/ml for DPPH and 7.97 to 11.86 µmol/ml for ABTS (Gülcü and Dağlıoğlu 2018). When the results of this study were compared with the previous work, there were similarities for the FRAP and DPPH methods. On the other hand, the values we obtained from ABTS were lower. This might be due to many parameters, such as grape variety, growth conditions, or grape processing.

In a study conducted on grape juice products obtained from supermarkets in Spain, the antioxidant capacities were determined to be 2.83 mM/l and 15.1 mM/l for white grape juice and 9.16 mM/l and 27.1 mM/l for red grape juice for FRAP and ABTS methods, respectively (Moreno-Montoro et al. 2015). Granato et al. (2015) reported that the antioxidant capacities determined by FRAP in European biodynamic, organic, and conventional *V. labrusca* L. grape juice samples were 6.75, 5.75, and 5.20 mM/l, respectively. Furthermore, many researchers reported antioxidant capacities of *V. labrusca* L. grape juice to vary between 2.51 mM/l and 51.60 mM/l for the DPPH method and 11.05 mM/l and 54.60 mM/l for the ABTS method (Burin et al. 2010; Lima et al. 2014, 2015; Toaldo et al. 2014; Padilha et al. 2017). Ryan and Prescott (2010) determined that the antioxidant

Table 2 Antioxidant activities of the samples (mM Trolox equivalent/100 g dry weight)

Sample	CUPRAC	FRAP	DPPH	ABTS
M1	1.15 ± 0.03^{h}	1.02 ± 0.14^{g}	0.32 ± 0.05^{g}	0.52 ± 0.02^{h}
M2	0.94 ± 0.06^{j}	0.92 ± 0.10^{g}	0.28 ± 0.08^{g}	0.58 ± 0.03^{g}
M3	1.06 ± 0.02^{i}	1.29 ± 0.06^{f}	0.36 ± 0.09^{fg}	0.42 ± 0.03^{i}
M4	0.79 ± 0.01^{k}	0.70 ± 0.05^{h}	0.56 ± 0.02^{e}	0.44 ± 0.03^{i}
M5	0.59 ± 0.02^{l}	0.52 ± 0.10^{i}	0.55 ± 0.09^{e}	0.39 ± 0.07^{lmn}
M6	4.30 ± 0.16^{c}	2.66 ± 0.11^{c}	2.44 ± 0.14^{b}	2.73 ± 0.04^{b}
M7	0.93 ± 0.03^{j}	0.94 ± 0.07^{g}	0.14 ± 0.06^{h}	0.54 ± 0.02^{gh}
M8	3.02 ± 0.12^{e}	2.70 ± 0.17^{c}	0.79 ± 0.10^{d}	1.72 ± 0.04^{d}
M9	3.16 ± 0.06^{d}	1.82 ± 0.10^{e}	0.88 ± 0.06^{d}	1.94 ± 0.02^{c}
M10	4.97 ± 0.10^{a}	3.99 ± 0.31^{a}	2.54 ± 0.10^{b}	2.84 ± 0.05^{a}
M11	2.35 ± 0.07^{f}	1.99 ± 0.05^{d}	1.34 ± 0.13^{c}	1.56 ± 0.04^{e}
M12	4.80 ± 0.12^{b}	3.44 ± 0.08^{b}	2.71 ± 0.11^{a}	2.83 ± 0.02^{a}
M13	1.40 ± 0.03^{g}	1.18 ± 0.08^{f}	0.48 ± 0.13^{ef}	0.80 ± 0.01^{f}

Values indicated with different letters within each group and column are significantly different for $p < 0.05$

CUPRAC cupric reducing antioxidant capacity, *FRAP* ferric reducing antioxidant power, *DPPH* 2,2-diphenyl-1-picrylhydrazyl, *ABTS* 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)

capacity of red V. *labrusca* L. grape juice and concentrate was 5.65 mM/l and 6.70 mM/l, respectively, for the FRAP method. In the current study, the values obtained from the DPPH and ABTS methods were lower compared to previous studies. However, our results were similar to those of Yamamoto et al. (2015), who reported the antioxidant capacities of *V. labrusca* × *V. vinifera* grape juice samples to vary between 2.57 mM/l and 3.66 mM/l for the DPPH method. Grape species and varieties mostly affect the composition and compounds in grape and grape-based products. Their amounts and compositions can also change depending on conditions such as grape-growing applications, climatic differences, soil, and maturity.

Phenolic Compounds of the Samples

Phenolic compounds that have antioxidant properties and are found abundantly in grape and grape products are important for human health, and therefore they are investigated in many studies undertaken in this field. In this study, the phenolic composition of grape juice samples was investigated in detail.

The phenolic acid profiles of the samples are shown in Table 3. The differences between the samples with respect to all investigated phenolic acids were found to be statistically significant ($p \le 0.05$). Caftaric acid was determined as the major acid of all seven phenolic acids, ranging between 0.60 mg/100 g and 74.46 mg/100 g in the samples, with the highest amount in M10 and the lowest in M5. Higher amounts of caftaric acid compared to other phenolic acids in grape juice and wine samples were previously found in other studies (Yamamoto et al. 2015; Toaldo et al. 2015; Karaoğlan et al. 2015; Padilha et al. 2017; Aleixandre-Tudo et al. 2018).

In grape juice samples, the amount of gallic acid was determined as 0.07–7.60 mg/100 g, with the highest amount being found in M10 and the lowest in M5. The contents of chlorogenic, caffeic, p-coumaric, ferulic, and sinapic acids in the grape juice samples ranged from 0.43 mg/100 g to 5.36 mg/100 g, 0.10 mg/100 g to 12.66 mg/100 g, 0.03 mg/100 g to 0.51 mg/100 g, 0.03 mg/100 g to 0.22 mg/100 g, and 0.03 mg/100 g to 1.88 mg/100 g, respectively. The highest amount of chlorogenic acid was found in M7 and the lowest in M5. The highest amounts of caffeic, p-coumaric, and ferulic acid were found in M6, M11, and M12 and the lowest in the M5, M4, and M5 samples.

In previous studies, the amounts of gallic, chlorogenic, caffeic, p-coumaric, and ferulic acids were detected in the grape juices (Natividade et al. 2013; Lima et al. 2014, 2015; Padilha et al. 2017), wines (Özkan and Baydar 2006; Anli and Vural 2009; Karaoğlan et al. 2015; Padilha et al. 2017, Zhang et al. 2017), and grapes (Sensoy 2012; Eyduran et al. 2015; Fabani et al. 2017). In general, the amounts of gallic acid were similar to the results for grape juice but were lower than wine and higher than grape compared to the literature. The amounts of chlorogenic acid were within the ranges reported in the literature but were higher than the results for wine and grape. The amounts of caffeic acid were similar to the previous results for grape juice and wine, but higher for grape. The amounts of ferulic and p-coumaric acids were very similar to the results in grape juice and wine in the literature. The results obtained for sinapic acid were similar to those reported by Zhang et al. (2017).

The results of flavanols, stilbenes, and anthocyanins are shown in Table 4, indicating statistically significant differences ($p \le 0.05$) between the samples. Epigallocatechin gallate (EGCG) is one of the most significant compounds for human health and prevents metabolic syndrome. In

Table 3 Phenolic acid compositions of the samples (mg/100 g DW)

Sample	Gallic acid	Caftaric acid	Chlorogenic acid	Caffeic acid	p-Coumaric acid	Ferulic acid	Sinapic acid
M1	0.13 ± 0.05^{ik}	33.43 ± 0.04^{e}	2.23 ± 0.02^{f}	0.58 ± 0.06^{i}	0.11 ± 0.01^{g}	0.04 ± 0.01^{ef}	0.09 ± 0.01^{e}
M2	0.30 ± 0.01^{i}	6.11 ± 0.01^{k}	1.00 ± 0.01^{i}	0.87 ± 0.08^{h}	0.06 ± 0.01^{i}	0.05 ± 0.01^{e}	0.04 ± 0.01^{e}
M3	0.34 ± 0.01^{h}	21.65 ± 0.02^{h}	1.08 ± 0.01^{h}	0.31 ± 0.01^{j}	0.05 ± 0.01^{j}	0.04 ± 0.01^{f}	0.06 ± 0.01^{e}
M4	0.11 ± 0.01^{k}	16.42 ± 0.03^{i}	0.68 ± 0.01^{j}	0.23 ± 0.01^{k}	0.03 ± 0.01^{k}	0.05 ± 0.01^{e}	0.03 ± 0.01^{e}
M5	0.07 ± 0.01^{l}	0.60 ± 0.01^{m}	0.43 ± 0.06^{k}	0.10 ± 0.01^{l}	nd	0.03 ± 0.01^{g}	nd
M6	2.37 ± 0.01^{d}	69.72 ± 0.29^{b}	4.91 ± 0.01^{c}	12.66 ± 0.01^{a}	0.17 ± 0.01^{f}	0.18 ± 0.01^{b}	1.68 ± 0.01^{b}
M7	0.15 ± 0.01^{j}	8.64 ± 0.02^{j}	5.36 ± 0.02^{a}	2.17 ± 0.05^{f}	0.07 ± 0.01^{h}	0.05 ± 0.01^{e}	0.08 ± 0.01^{e}
M8	2.47 ± 0.01^{c}	58.15 ± 0.43^{c}	5.04 ± 0.01^{b}	10.41 ± 0.01^{b}	0.51 ± 0.01^{a}	0.13 ± 0.01^{c}	0.81 ± 0.01^{c}
M9	3.57 ± 0.01^{b}	49.64 ± 0.21^{d}	3.76 ± 0.06^{d}	8.68 ± 0.01^{c}	0.25 ± 0.01^{e}	0.09 ± 0.01^{d}	0.69 ± 0.01^{e}
M10	7.60 ± 0.01^{a}	74.46 ± 0.07^{a}	3.75 ± 0.05^{d}	10.37 ± 0.01^{b}	0.37 ± 0.01^{c}	0.17 ± 0.01^{b}	1.66 ± 0.01^{b}
M11	1.13 ± 0.01^{f}	22.97 ± 0.02^{g}	3.43 ± 0.01^{e}	7.17 ± 0.01^{e}	0.44 ± 0.01^{b}	0.17 ± 0.01^{b}	0.44 ± 0.01^{d}
M12	1.92 ± 0.01^{e}	23.64 ± 0.01^{f}	3.75 ± 0.01^{d}	7.96 ± 0.01^{d}	0.24 ± 0.01^{e}	0.22 ± 0.01^{a}	1.88 ± 0.04^{a}
M13	0.52 ± 0.01^{g}	5.13 ± 0.05^{l}	1.92 ± 0.03^{g}	1.01 ± 0.02^{g}	0.28 ± 0.01^{d}	0.09 ± 0.01^{d}	0.43 ± 0.22^{d}

Values indicated with different letters within each group and column are significantly different for $p < 0.05$
nd not detected, DW *dry weight*

Table 4 Phenolic compounds of the samples (mg/100 g DW)

Sample	(+)-Catechin	(−)-Epigallocatechin gallate	(−)-Epicatechin gallate	Myricetin	Quercetin	Kaempferol	*t*-Resveratrol	Oenin chloride	Malvidin chloride
M1	4.19 ± 0.02^{ef}	0.05 ± 0.01^{i}	0.24 ± 0.01^{cdefg}	0.27 ± 0.02^{i}	0.07 ± 0.01^{j}	0.03 ± 0.01^{cd}	0.04 ± 0.01^{f}	W	W
M2	0.82 ± 0.25^{g}	0.14 ± 0.01^{gh}	0.13 ± 0.02^{f}	0.05 ± 0.01^{k}	0.13 ± 0.01^{g}	0.05 ± 0.01^{c}	0.04 ± 0.01^{f}	W	W
M3	3.98 ± 0.02^{f}	0.11 ± 0.02^{gh}	0.27 ± 0.12^{cde}	0.40 ± 0.01^{h}	0.08 ± 0.01^{i}	0.04 ± 0.01^{cd}	0.01 ± 0.01^{g}	W	W
M4	4.90 ± 0.02^{d}	0.14 ± 0.02^{g}	0.13 ± 0.01^{ef}	0.25 ± 0.02^{i}	0.05 ± 0.01^{k}	n.d.	0.04 ± 0.01^{f}	W	W
M5	1.31 ± 0.01^{g}	0.08 ± 0.01^{hi}	0.25 ± 0.01^{cdef}	0.49 ± 0.02^{g}	0.04 ± 0.01^{k}	n.d.	0.05 ± 0.01^{ef}	W	W
M6	19.06 ± 0.23^{a}	0.63 ± 0.06^{c}	0.45 ± 0.03^{b}	5.50 ± 0.04^{a}	0.09 ± 0.01^{h}	0.07 ± 0.03^{b}	0.42 ± 0.02^{b}	0.08 ± 0.01^{e}	nd
M7	4.63 ± 0.01^{de}	0.40 ± 0.01^{e}	0.15 ± 0.01^{efg}	0.14 ± 0.01^{j}	0.05 ± 0.01^{k}	0.03 ± 0.01^{cd}	0.03 ± 0.02^{fg}	0.40 ± 0.26^{de}	$2.41 \pm 0.04^{*}$
M8	5.77 ± 0.58^{c}	0.12 ± 0.03^{gh}	0.19 ± 0.02^{defg}	4.01 ± 0.02^{b}	0.33 ± 0.01^{c}	0.11 ± 0.07^{a}	0.41 ± 0.04^{b}	nd	$2.40 \pm 0.02^{*}$
M9	4.73 ± 0.49^{de}	0.15 ± 0.02^{g}	0.28 ± 0.17^{cd}	2.77 ± 0.01^{d}	0.17 ± 0.01^{f}	0.04 ± 0.01^{cd}	0.26 ± 0.01^{c}	nd	nd
M10	11.01 ± 0.49^{b}	0.25 ± 0.01^{f}	0.25 ± 0.03^{cde}	3.01 ± 0.01^{c}	0.19 ± 0.01^{e}	0.03 ± 0.01^{cd}	0.58 ± 0.01^{a}	nd	nd
M11	3.83 ± 0.57^{f}	0.91 ± 0.01^{a}	0.33 ± 0.02^{c}	<LQD	0.25 ± 0.01^{d}	0.06 ± 0.01^{c}	0.20 ± 0.01^{d}	2.51 ± 0.22^{a}	$2.24 \pm 0.09^{*}$
M12	4.30 ± 0.09^{ef}	0.77 ± 0.08^{b}	0.21 ± 0.05^{cdefg}	0.90 ± 0.06^{f}	0.52 ± 0.01^{a}	0.06 ± 0.01^{c}	0.20 ± 0.01^{d}	1.29 ± 0.37^{b}	nd
M13	0.70 ± 0.04^{h}	0.55 ± 0.02^{d}	0.48 ± 0.06^{a}	1.19 ± 0.06^{e}	0.39 ± 0.02^{b}	0.05 ± 0.01^{c}	0.07 ± 0.01^{e}	0.81 ± 0.12^{d}	nd

Values indicated with different letters within each group and column are significantly different for $p < 0.05$

nd not detected, *LQD* least quantitative detection, *W* white grapes, *DW* dry weight

*Values are not significantly different in the column for $p < 0.05$

addition, it has more antioxidant capacity than other catechins (Rice-Evans 1999; Legeay et al. 2015). Among flavonols, CA, EGCG, and ECG were studied and determined to be 0.70–19.06 mg/100 g, 0.05–0.91 mg/100 g, and 0.13–0.48 mg/100 g, respectively. The highest amount of CA was found in M6 and the lowest in M13. For ECG, the highest amount was observed in M11 and the lowest in M1. For ECG, the highest and lowest values were found in the M13 and the M2 and M4 samples. The CA, EGCG, and ECG contents of the grape juice samples were in agreement with previous studies (Natividade et al. 2013; Lima et al. 2014, 2015; Toaldo et al. 2015; Padilha et al. 2017).

In grape juice samples, the amounts of myricetin, quercetin, and kaempferol were measured as 0.05–5.50 mg/100 g, 0.04–0.52 mg/100 g, and 0.03–0.11 mg/100 g, respectively. Kaempferol was not detected in M15 or M16 samples. The highest amount of myricetin was determined in M6, and quercetin and kaempferol in the M12 and M8 samples, respectively. The results obtained from this study for these three flavonols were similar to previous reports on grape juice (Natividade et al. 2013; Lima et al. 2015; Toaldo et al. 2015), wine (Pirinççioğlu et al. 2012; Capanoglu et al. 2013; Eyduran et al. 2015; Kaya and Unluturk 2016), grapes (Sensoy 2012; Eyduran et al. 2015; Ünal et al. 2015; Fabani et al. 2017), and sour grape juices (Nikfardjam 2008; Guler et al. 2018).

The t-resveratrol in samples were between 0.01 mg/100 g DW and 0.58 mg/100 g DW. The highest amount was observed in M10 and the lowest in M3. Our findings related to t-resveratrol were compatible with the results of Gülcü and Dağlıoğlu (2018), Natividade et al. (2013), Yamamoto et al. (2015), but they were lower compared to other research (Lima et al. 2015; Toaldo et al. 2015). Despite the low amounts of resveratrol in grape, grape juice, and wine, it has important potential for human health with its antifungal and antimicrobial properties and its role in inhibiting platelet aggregation, oxidizing low-density lipoproteins, and preventing lipid peroxidation in the lung (Lamikanra et al. 1996; Romero-Pérez et al. 1999; Zhang et al. 2017). In the current study, determination of t-resveratrol in all samples reveals the importance of grape juice for human health.

In this study, oenin and malvidin chloride anthocyanins were also investigated in samples. Oenin chloride was found to be between 0.08 mg/100 g DW and 2.51 mg/100 g DW, but it was not detected in M8, M9, or M10. The highest amount was determined in M11 and the lowest in M6. In all analyzed samples, the amounts of malvidin chloride ranged from 2.24 mg/100 g DW to 2.41 mg/100 g DW, but it was not detected in M6, M9, M10, M12, or M13. In a previous study, the amounts of malvidin-3,5-di-O-glucoside-chloride were given as 0.40–5.08 mg/l and the amounts of malvidin-3-O-glucoside-chloride as 18.84–24.30 mg/l in

Table 5 Correlations between individual polyphenols and antioxidant activities

	CUPRAC	FRAP	DPPH	ABTS
CUPRAC	1	–	–	–
FRAP	0.96**	1	–	–
DPPH	0.93**	0.89**	1	–
ABTS	0.99**	0.94**	0.93**	1
Gallic acid	0.81**	0.83**	0.68*	0.78**
Caftaric acid	0.78**	0.76**	0.62*	0.76**
Chlorogenic acid	0.64*	0.62*	0.45	0.65*
Caffeic acid	0.91**	0.87**	0.78**	0.93**
p-Coumaric acid	0.61**	0.69**	0.42	0.61*
Ferulic acid	0.90**	0.89**	0.89**	0.92**
Sinapic acid	0.98**	0.93**	0.95**	0.97**
(+)-Catechin	0.65*	0.58*	0.66*	0.66*
(–)-Epigallocatechin gallate	0.44*	0.42*	0.56*	0.50*
(–)-Epicatechin gallate	0.27	0.20	0.30	0.30
Myricetin	0.69**	0.62*	0.54	0.70**
Quercetin hydrate	0.53	0.57*	0.49	0.52
Kaempferol	0.48	0.52	0.28	0.48
t-Resveratrol	0.87**	0.88**	0.75**	0.86**
Oenin chloride	0.17	0.20	0.27	0.21
Malvidin chloride	–0.06	0.06	–0.18	–0.034
Total () polyphenols	0.84**	0.81	0.70**	0.83**
TP (Folin–Ciocalteu)	0.98**	0.96**	0.91**	0.98**

CUPRAC cupric reducing antioxidant capacity, *FRAP* ferric reducing antioxidant power, *DPPH* 2,2-diphenyl-1-picrylhydrazyl, *ABTS* 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), *TP* total phenolic contents

*Correlations are significant at $p \le 0.05$

**Correlations are significant at $p \le 0.01$

V. *vinifera* L. (Tempranillo, Syrah, and Alicante Bouschet) grape juice samples (Natividade et al. 2013). The values obtained from the current study were slightly lower than previously reported, probably due to the different varieties and processes. In fact, in another study, it was reported that the amount of total anthocyanin was reduced by 50% during the production of grape juice (Gülcü and Dağlıoğlu 2018).

Results of Correlation Analysis Between Individual Polyphenols and Antioxidant Activities

In this study, the correlations between the antioxidant capacity methods and those between phenolic compounds and antioxidant activities of the samples were determined. The findings are shown in Table 5. Significant correlations were determined between the antioxidant analysis methods used ($p \le 0.01$). The highest correlation (r = 0.99) was found between ABTS and CUPRAC and the lowest (r = 0.87) between FRAP and DPPH. Margraf et al. (2016) reported a correlation between the ABTS and FRAP methods at the r = 0.5914 ($p = 0.001$) level. Another study found a strong correlation (r = 0.88; $p < 0.01$) between the DPPH and ABTS methods in grape juice samples (Lima et al. 2014). Furthermore, Guler et al. (2018), monitoring variations in antioxidant activity, determined a strong correlation (r = 0.998; $p \le 0.01$) between the DPPH and ABTS methods. In the current study, the correlation between the ABTS and DPPH methods was similar to the results of previous studies, but correlation between the ABTS and FRAP methods was higher. This can be attributed to the different samples and methodological variations.

Positive correlations ($p \le 0.01$) were determined between the CUPRAC results and ferulic acid (r = 0.90), gallic acid (r = 0.81), caftaric acid (r = 0.78), caffeic acid (r = 0.91), p-coumaric acid (r = 0.61), sinapic acid (r = 0.98), myricetin (r = 0.69), t-resveratrol (r = 0.87) and TP (r = 0.98). There were also significant positive correlations between the FRAP results and gallic acid (r = 0.83), caftaric acid (r = 0.76), caffeic acid (r = 0.87), p-coumaric acid (r = 0.66), ferulic acid (r = 0.89), sinapic acid (r = 0.93), t-resveratrol (r = 0.88) and TP (r = 0.96) at $p \le 0.01$ level. Similarly, significant positive correlations were found at the $p \le 0.01$ level between the DPPH method results and caffeic acid (r = 0.78), ferulic acid (r = 0.89), sinapic acid (r = 0.95), t-resveratrol (r = 0.75) and TP (r = 0.91). Additionally, positive correlations were revealed ($p \le 0.01$) between the ABTS results and gallic acid (r = 0.78), caftaric acid (r = 0.76), caffeic acid (r = 0.93), ferulic acid (r = 0.92), sinapic acid

(r = 0.97), myricetin (r = 0.70), t-resveratrol (r = 0.86) and TP (r = 0.98). Furthermore, positive correlations were found between gallic acid, caftaric acid, CA, EGCG, and the DPPH results, chlorogenic acid, p-coumaric acid, CA, EGCG, and the ABTS results at the level of $p \leq 0.05$.

There were also positive correlations at the $p \leq 0.05$ level between EGCG and the results of all four methods used to determine antioxidant activity. This is one of the most important findings of this study because high antioxidant effects of EGCG were revealed with the four methods.

Lima et al. (2014) investigated the correlation (Pearson) between phenolic compounds and antioxidant activities determined by the DPPH and ABTS methods in grape juice samples. The antioxidant activities were reported to be positively correlated with gallic acid, caffeic acid, p-coumaric acid, myricetin, ECG, TP, and CA, and negatively correlated with quercetin (for $p \leq 0.05$ and $p \leq 0.01$), whereas there was no correlation with kaempferol, t-resveratrol, or chlorogenic acid (Lima et al. 2014). In another study analyzing grape juice samples, the results of FRAP and ABTS methods had a positive correlation with ferulic acid but no correlation with gallic, chlorogenic, caffeic, and p-coumaric acids (Margraf et al. 2016). When we compared our correlation findings to previous studies, they were in agreement with those of Lima et al. (2014), demonstrating the presence of a correlation between the results of DPPH and ABTS and kaempferol, gallic acid, caffeic acid, p-coumaric acid, CA, myricetin, and TP. However, no similarities were observed for the correlations of antioxidant activity with chlorogenic acid, quercetin hydrate, ECG and t-resveratrol. On the other hand, the correlations reported by Margraf et al. (2016) between the ABTS and FRAP results and ferulic acid were similar to our results, while there were differences in relation to gallic, chlorogenic, p-coumaric and caffeic acids.

Conclusions

In the current study, the total phenolic contents, antioxidant activities, and phenolic compounds of Turkish native grape juices were revealed. The correlations between the phenolic compounds and antioxidant activities as well as those between the results of antioxidant activity methods were also determined. There were positive correlations between antioxidant activity and caffeic acid, p-coumaric acid, ferulic acid, sinapic acid, quercetin hydrate, kaempferol, t-resveratrol, EGCG, and TP. In addition, significant positive correlations were found between the results of four different methods used to determine antioxidant activity. Caftaric acid was the most common compound among phenolic acids, and CA was most common among flavonols. Another remarkable finding concerned the detection of t-resveratrol and EGCG, which have positive effects on human health, in all grape juice samples. More research is needed to characterize the grape and grape-based products produced from Turkish native grape varieties regarding their physicochemical parameters; mineral, organic acid, and sugar compositions; phenolic profiles; and antioxidant activities.

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Conflict of interest A. Guler, A. Candemir, K.E. Ozaltin, F.B. Asiklar, and S. Saygac declare that they have no competing interests.

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