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ORIGINAL ARTICLE

Comparison of Phenolic Profile and Antioxidant Properties of Pulp and Seeds of Two Different Grapes Types (*Vitis vinifera* L. and *Vitis labrusca* L.) Grown in Anatolia

The Amount of Resveratrol of Grape Samples

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Abstract

Background Grape is the most widely cultivated fruit all over the World and it is both food and a good source of antioxidants. The fruit is rich in phytochemicals and its seed is especially a source for resveratrol, a stilbene that shows health promoting effect.

Objective In this study, phenolic profile and antioxidant properties of eight grape cultivars with two species cultivated in Turkey were compared. Isabella, Razaki, Alphonse Lavalleé, Red Globe and Emir cultivars were used.

Methods Total phenolic substance (TPS), total flavonoid substance (TFS), total antioxidant capacity (FRAP) and phenolic compositions of the pulp and the seeds were determined. Phenolic composition of grapes were found by using HPLC-UV.

Results The results showed that total phenolic substances and total antioxidant capacities were richer in the seeds than in the pulps. Catechin and epicatechin are major phenolic compounds in the grape seeds, and chrysin, pinocembrin, resveratrol, luteolin and caffeic acid phenethyl ester (CAPE) were detected in the pulp as major. Resveratrol varied from 4.50 to 101.89 mg/100 g, while was equally in both the seed and pulp. It was found that all grape samples were rich in phenolic compounds and had antioxidant activity.

Conclusion It was clear that antioxidant capacity and phenolic profile of samples changes according to grape species and cultivars.

Keywords Isabella · Karaerik · Emir · Alphonse Lavalleé · Red globe · Resveratrol

1 Introduction

Grape is the most widely cultivated fruit all over the world. It is used both fresh and dried. It is a balanced food in terms of water, sugar, vitamins, minerals and polyphenols [1]. Grapes are utilized for various purposes such as table grapes, vine, vinegar, marmalades and grape juice in industry [2]. Its composition, aromatic, sensory properties, and bioactive properties depends on the variety, characteristics of the region where it is grown, maturity and harvesting time [3, 4].

There are 1600 grape varieties in Turkey and they are reported to be protected in the national grape gene bank in Tekirdağ [5]. For example, Isabella grape (*V. labrusca* L.) is a wild grape type that is very common in the East Black Sea Region. It is grown naturally without any pesticides or fertilizers. However, studies are limited on the East Black Sea

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Region of Isabella grapes. In another example is Karaerik grape (*V. vinifera* cv. *Cimin*). It is an endemic grape species and grown in Üzümlü district of Erzincan of Eastern Anatolian Region of Turkey. It is very popular with its flavor, sensory and aromatic properties [6]. Emir grape is a white variety, mostly grown in Cappadocia (Nevsehir), Turkey. It is also an endemic grape with light greenish and yellowish in color and mostly used in white wine production. It is suitable for sparkling wine production.

Grape is both food and a good source of antioxidants. This fruit is rich in phytochemicals, its seed is especially a source for resveratrol, a stilbene that shows health promoting effect. It is known that grapes contain resveratrol more than many fruits. Phytochemicals, which are biologically active substances, have an effective role in the formation of colors, odors and flavors unique to plants. Terpenes, phenolic compounds, phytosterols and phytostanols are included in the phytochemical family. Hydroxybenzoic acids, hydroxycinnamic acids, flavanones, flavones, stilbenes are important phenolic components. Resveratrol (trans-3, 5, 4'-trihydroxy-stilbene) is one of the widely studied phytochemicals with different medicinal value due to its antioxidative, anti-inflammatory, antitumor, antiviral, antidiabetes, cardioprotective, protective against eye diseases, phytoestrogen and life-prolonging properties [7]. Today, interest in resveratrol has increased greatly. It is known that resveratrol has an antioxidant and anti-inflammatory effect, which plays a role in the basis of many diseases. It has proven in vitro and in vivo its potential importance in the prophylaxis/treatment of chronic diseases such as cancer, heart disease, diabetes, obesity, and aging-related eye diseases [8, 9]. Thus, resveratrol is an important component that found especially in grapes. It does not show significant toxicity at low doses and has significant *in vitro/in vivo* biological activities.

Table 1 The names, the regions and physical characteristics of grape samples

Code	Common mane	Region	Scientific name	Properties
G1	Isabella	Trabzon	*V. labruska*	Black-purple-fine grained, hard shell
G2	Isabella	Eynesil		Black-purple-fine grained, hard shell
G3	Isabella	Tirebolu		Black-purple-fine grained, hard shell
G4	Razaki	Denizli	*V. vinifera*	Green- pinky -long oval shaped, coarse-grained
G5	Alphonso lavalleé	Yalova		Black-blue-purple, coarse-grained
G6	Red Globe	Denizli		Pink-red, coarse-grained, thick-skinned
G7	Kara erik	Erzincan		Black- blue-purple, thin-shelled, medium coarse
G8	Emir	Nevşehir		White, light greenish and yellowish

In this study, grape samples harvested from different regions of Turkey were classified according to their species and physical properties. Then, extracts were prepared from different parts of the grape samples (seed and pulp) and the total phenolic content of the prepared extracts and their antioxidant properties were compared. In addition, the phenolic profile of each grape samples was determined. The amount of resveratrol, the most important bioactive component of grapes, was determined and the highest resveratrol amount was determined.

2 Materials and Methods

2.1 Chemicals and Grape Samples

All chemicals were used analytical grade and obtained from Sigma-Aldrich (Chemie, Germany) and Merck (Darmstadt, Germany). The matured grapes were harvested in September and October in 2018. The grape samples were harvested randomly. The physical properties of the grapes are given in Table 1. The fresh grapes were washed with water and then dried at 40ºC and weighted. The seeds were removed and dried at also 40 °C and the remaining pulp separated. The pulp was blended to obtained homogenize mixture, filtered with a cloth, then the filtrate was centrifuged. The clear supernatant was used for the study. After the seeds and pulp parts of the grapes were separated and dried, methanol extracts of the powdered samples were prepared. For this, 10 g of sample was mixed with 100 mL of methanol in a shaker for 2 days and then filtered. The filtrates were kept at +4 °C until they were used.

2.2 Determination of Total Phenolic Substance (TPS)

Total phenolic substances of the grape sample extracts were measured by means of Folin-Ciocalteu's method [10], using gallic acid (GAE) as standard. A standard graph of gallic acid was drawn with the measured absorbance values of gallic acid against methanol solutions at different concentrations (1.0; 0.5; 0.25; 0.125; 0.0625 and 0.03125 mg/mL). The total phenolic content of the extracts was calculated according to the drawn graph and TPS is expressed as mg GAE/100 g of fresh grape sample in the pulp and mg GAE/100 g dried seed sample using a standard curve. All analyses were performed triplicate.

2.3 Determination of Total Flavonoid Substance (TFS)

Total flavonoid substances (TFS) of the grapes sample extracts were measured using a spectrophotometric method with quercetin as standard [11]. As a standard, different concentrations (0.125 – 0.0078 mg/mL) of quercetin (QE) were used. TFS was expressed as mg QUE/100 g fresh grape sample in the pulp and mg QUE/100 g dried seed sample by the curve. All analyses were performed triplicate.

2.4 Determination Ferric Reducing/Antioxidant Power (FRAP)

The total antioxidant capacity of the grape sample extracts were measured by the ferric reducing/antioxidant power assay (FRAP) method [12]. FRAP values were expressed as µmol $FeSO_4.7H_2O$ equivalent/g fresh grape sample and µmol $FeSO_4.7H_2O$ equivalents/g dried seed sample using the curve. All analyses were performed triplicate.

2.5 Analyses of Phenolic Composition by HPLC-UV

In order to prepare the grape extracts for phenolic composition analyses, 15 mL extract was evaporated at 40 °C, the residue was then dissolved with 15 mL distilled water (pH: 2), and the aqueous solution was extracted with 5 mL diethyl ether (15 min, 200 rpm, room temperature). The organic phase was collected three times after each extraction and the aqueous solution was then extracted three times with 5 mL ethyl acetate (15 min, 200 rpm, room temperature). After these extractions, the organic phases were mixed and evaporated. The residue was dissolved in 2 mL of methanol, filtered using 0.45 µm filters, and given to the HPLC device for analysis.

Calibrations were also performed at HPLC using nineteen standard phenolic compounds at 280 and 340 nm (Elite La Chrome; Hitachi, Tokyo, Japan) on a device fitted out with a reverse phase C18 column (150 mm, 4.6 mm, 5 µm; Fortis). The calibration curves for each compound were between 0.998 and 1.000. The program employed was as described in previous studies with acetic acid, water and acetonitrile being used as the mobile phase [13]. The mobile phase consisted of (A) 2% acetic acid in water and (B) acetonitrile: water (70:30). Finally, 20 µL of sample was injected individually at 25 °C, and the flow rate was set to 0.75 mL/ min. All analyses were performed triplicate.

2.6 Statistical Analyses

Statistical analyses were performed on SPSS version 11.5 software (IBM SPSS Statistics, Armonk, New York, USA). Descriptive statistics were expressed as mean ± SD. Correlation analysis was performed using the Mann–Whitney U-test. Significance was set at $p < 0.05$.

3 Results and Discussion

In this study, 8 different grape samples with two grape types were examined in terms of polyphenol content and antioxidant properties. There were differences between color, morphological and characteristics properties of eight kinds of grape samples used in this study (Table 1). The biochemical properties of grape samples were presented in Table 2. According to results, the grape seeds were found to be richer in terms of TPS, especially the Razaki grape seed was the richest grape sample (3580.44 ± 20.32 mg GAE/100 g). The pulps of the Isabella grapes were found to be richer in terms of TPS than the others (479.60 ± 5.80 mg GAE/ 100 mg). It has been determined that the antioxidant activity measured according to the FRAP method is higher in the seeds than the pulps. While Isabella grape showed the highest activity in the pulp of the grapes, whereas Razaki grape showed the lowest activity. The phenolic profile analysis of the pulp and seeds were given in Tables 3 and 4, respectively. It can be seen from these two tables that the grape seeds are richer about phenolic substances than the pulps. Resveratrol was

Table 2 Biochemical properties of the grape samples

Code	Total Phenolic Content (mg GAE/ 100g)		Total Flavonoid Content (mg QUE/ 100 g)		Antioxidant Capacity (µmol $FeSO_4.7H_2O$/g)	
	Pulp	Seed	Pulp	Seed	Pulp	Seed
G1	357.00 ± 4.10^{a}	2014.10 ± 70.30^{c}	35.40 ± 1.80^{b}	30.80 ± 2.00^{a}	31.17 ± 1.24^{c}	102.00 ± 1.29^{b}
G2	251.60 ± 6.50^{b}	2110.40 ± 10.92^{c}	24.50 ± 3.00^{a}	31.80 ± 3.40^{a}	21.00 ± 2.39^{b}	108.79 ± 2.73^{b}
G3	479.60 ± 5.80^{d}	2392.10 ± 10.29^{d}	47.80 ± 1.90	60.50 ± 1.30^{d}	32.39 ± 0.59^{c}	141.07 ± 5.24^{c}
G4	242.80 ± 4.20^{b}	$3580.44 \pm 20.32^{d.e}$	48.60 ± 4.90^{d}	54.70 ± 3.10^{c}	12.40 ± 0.25^{a}	174.90 ± 0.19^{d}
G5	324.30 ± 7.20^{c}	2800.05 ± 10.10^{d}	34.20 ± 5.60^{b}	47.90 ± 5.70^{c}	31.54 ± 1.6^{c}	124.06 ± 1.10^{c}
G6	256.00 ± 3.40^{b}	1592.70 ± 2.40^{a}	26.40 ± 3.20^{a}	41.70 ± 1.40^{c}	18.85 ± 0.65^{b}	236.00 ± 8.93^{e}
G7	380.00 ± 4.20^{a}	1783.20 ± 11.14^{b}	39.00 ± 0.10^{c}	37.80 ± 1.20^{b}	28.45 ± 1.15^{c}	130.256 ± 2.15^{c}
G8	289.50 ± 6.00^{c}	1523.10 ± 14.20^{b}	33.30 ± 0.30^{b}	30.00 ± 2.05^{a}	24.88 ± 0.39^{b}	134.364 ± 1.77^{c}

Different letters in the same columns show statistically differences between means ($p<0.05$)

Table 3 Phenolic profiles in the pulp of the grapes by HPLC (mg/100 g)

Phenolic compounds	G1	G2	G3	G4	G5	G6	G7	G8
Hydroxybenzoic acids								
Gallic acid	-	-	-	220.48	-	-	0.46	-
p-OH benzoic acid	20.94	5.82	2.41	-	-	-	-	-
p-Coumaric acid	-	6.46	-	-	-	-	-	-
Syringic acid	26.06	3.42	1.574	1.20	2.30	39.54	-	-
Hydroxycinnamic acids								
t-cinnamic acid	3.52	1.46	1.22	4.76	2.43	3.83	67.74	12.19
Caffeic acid	-	11.26	10.62	-	-	-	-	-
CAPE	11.82	4.33	3.33	17.01	10.94	10.94	16.24	2.23
Ferulic acid	-	11.29	-	-	-	-	-	-
Flavan-3-ols								
Epicatechin	3.55	-	-	-	-	3.59	0.91	-
Flavanones								
Pinocembrin	9.424	3.66	3.86	8.55	5.86	5.86	233.36	-
Flavones								
Chrysin	112.86	47.46	46.64	138.28	73.85	177.21	233.36	-
Luteolin	34.59	1.20	14.51	1.05	23.60	54.29	7.31	22.84
Daidzein	-	-	-	-	-	-	-	15.60
Stilbenes								
Resveratrol	28.85	11.86	12.30	35.94	18.48	42.25	101.89	79.64

Table 4 Phenolic profiles in the seeds of the grapes by HPLC (mg/ 100 g)

Phenolic Compounds	G1	G2	G3	G4	G5	G6	G7	G8
Hydroxybenzoic acids								
Gallic acid	161.13	5.30	9.16	5.20	37.62	4.03	8667.41	278.24
Protocathequic acid	-	-	-	-	-	-	-	-
p-Coumaric acid	-	1.07	-	-	-	-	-	-
Hydroxycinnamic acids								
t-cinnamic acid	-	-	2.29	0.29	-	1.95	-	2.88
Caffeic acid	37.79	-	-	-	14.74	-	7.26	16.29
CAPE	13.82	2.10	1.80	1.34	-	7.49	-	-
Ferulic acid	-	-	-	-	22.03	-	4.43	8.90
Flavan-3-ols								
Catechin	24.30	8.20	14.10	8.62	356.19	5.00	778.78	325.12
Epicatechin	707.96	4.02	8.04	9.67	495.60	15.15	3.50	2.88
Flavonols								
Rutin	2121.64	-	-	-	4911.95	-	19.57	40.90
Flavanones								
Pinocembrin	12.82	-	-	6.40	-	6.03	-	-
Flavones								
Chrysin	139.58	24.50	10.80	8.72	-	7.19	-	-
Luteolin	-	-	2.36	-	-	-	-	-
Stilbenes								
Resveratrol	18.77	12.00	37.50	4.50	35.66	18.00	34.60	3.60

detected in both pulps and seeds. While gallic acid was detected in all grape seeds, it was detected only pulps of Razaki and Karaerik grapes. While resveratrol was detected in all grapes both of the parts, the highest value was measured in the Karaerik and the Alphonse Lavalleé seeds, and Karaerik pulps. In addition, TPS, TFS, total antioxidant capacity and phenolic compositions of the tree Isabella grapes grown from different areas of the East Black Sea region were also found to differ slightly. It is clear that the seeds of the samples were contained higher phenolic matters than the pulp.

Studies in the literature support that the grape seeds are richer in polyphenols than the pulps [4]. However, in a study conducted on Cardinal grape, TPS 72.266 mg GAE/ 100 g in the pulp and 461.563 mg GAE/100 g in the seed was reported. Contrary to the total phenolic substance amount,

it was determined that the total amount of flavonoid substance was approximately the same in the seed and pulp parts. The total amount of polyphenol substance in both parts of the grape samples was found to be much higher than the total amount of flavonoid substance. The data show that very little of the polyphenols found in the seeds as flavonoids, they are thought to be composed of non-flavonoid polyphenols such as tannins, stilbenes, anthocyanin etc. As a matter of fact, the excess of non-flavonoid polyphenols such as resveratrol in grapes confirm this situation. However, resveratrol is a polyphenol in the stilbenes group and it has been reported that grape contain resveratrol with highest amount [3, 14, 15]. The grape seed parts had higher antioxidant value than their pulp parts. It was determined that the highest antioxidant activity in the pulp part was in the Isabella grape (G3 and G1) and the seed was Razaki and Red Globe (G4 and G6) grapes. This is confirmed by the fact that the antioxidant capacity of the grapes varies according to the amount of polyphenol substance that the seed with the highest TPS is Razaki grape. The differences in polyphenol content among grape varieties vary according to both the grape type and the climatic and soil characteristics of the region where it is grown. There are many studies that support this, and the time of harvest, that is ripening, of grapes has been shown as an important criterion [16, 17]. In a previous study conducted on white grapes of Narince variety collected from Tokat in Turkey, the amount of total phenolic was found 73.378 mg/L in 2014 and 147.156 mg/L, and the amount of flavonoids was found in 18.22 mg/L in 2014 and 31.489 mg/L. It was stated that the amount of total phenolic content changes according to the year [18]. In a study, the antioxidant activity of Izabella grape samples was found 117.79 µmol/g [19]. It was determined that the highest polyphenol in the pulp parts of the samples was chrysin, and luteolin, followed by resveratrol. Chrysin and luteolin are important flavone derivatives, chrysin was maximum in the Karaerik grape, while luteolin was maximum in the Red Globe [20]. Unlike these two flavone derivative in the pulp, very few varieties contained in the grape seeds such as Isabella grape seed seems to be rich in chrysin. Rutin is an important water-soluble flavonol and was not detected in the grape pulps, while it was found high ratio in Alphonse Lavalleé, and Isabella (G1) seeds. Smaller amounts were also detected in Karaerik and Emir grapes. It was reported that rutin was 51.107 mg/ 100 g in Cardinal grape seed and 140.033 mg/100 g in Razaki grape seed [4]. Epicatechin, like catechin, was also detected in all sample's seed, and the highest amount was found in Isabella and Alphonse Lavalleé. Whereas gallic acid was detected in all grape seeds in high amount, the pulps contained lower. It was the highest in the variety of Karaerik and Emir, followed by Isabella grape. In previous studies, gallic acid was measured in both parts of Cardinal, Müşküle, Razaki varieties and the highest level was found in Cardinal seeds [4]. In an early study, it was reported that phenolic composition in different grape samples grown in Argentina was depended on the varieties [21]. In the same study, it was also reported that the red grapes are rich in anthocyanins which are color pigments, but caffeic acid and gallic acid were found under limit of detection (LOQ) and trans-resveratrol was detected in Merlot variety at 7 mg/L by HPLC-UV measurement [21]. Resveratrol was detected in varying amounts in both parts of the grape samples, but the pulp parts were contained higher than the seeds. Although the grape seeds contain higher polyphenols than the pulps, the result was found interesting. When compared the values with the other studies in the literature, it has been reported that the Razaki contained 12.245 mg/100 g resveratrol in the seed and 1.11 mg /100 g in the pulp, in Cardinal grape seed contained 46 mg/100 g resveratrol [4]. In a study of Prokupac variety grape berries (*Vitis vinifera* L.) in Serbia was analyzed with UHPLC-DAD MS /MS and results were reported that the grapes contained catechin as major level followed by ellagic and gallic acid. It was also reported in the same study that caffeic acid, pinosembrin, hesperetin and phlorizin were detected in minor level and resveratrol was not detected [22]. In another study, phenolic compounds in Australian grape samples were determined [23]. Total phenolic content of grape samples ranged between 1.73 and 4.31 mg GAE/g and antioxidant activity of ranged between 2.11 and 4.91 mg AAE/ g. It was reported that the phenolic content of the pulp had a significant positive correlation with the antioxidant activity.Golden Globe type grape samples had phenolic concentration in HPLC-PDA quantification, particularly for phenolic acids (gallic acid and chlorogenic acid) and flavonoids (kaempferol) [23]. In a study, different drying techniques were used to dry fresh Isabella grape samples [24]. It was reported that TPC, TFC ferric reducing capacity of fresh grape were 1101.61 ± 35.12 mg GAE, 460.18 ± 23.48 mg CE and 57.03 ± 1.68 per/100 g dw. It was reported that Isabella grape samples were rich in chlorogenic acid and protocatechuic acid. They also contained gallic acid (5.51 ± 0.17 mg/100 g dw) [24]. In another study, relationship between phenolic compounds, antioxidant activity and color was determined [25]. The lightness and hue angle (h) are the color coordinates strongly related to some phenolic compounds and ACN, whereas a* was related to DPPH and TPC. It was indicated that the simple color measurements could be a useful tool for rapid screening of the quality of grapes [25].

4 Conclusion

Grape is a fruit rich in phenolic components and it contains secondary metabolites. It is an important nutrition with its phenolic compounds and anthocyanin. There are different types of grapes over the world. But studies about grapes are mostly concentrated about wine products. The studies about biochemical properties of grape types are limited. In this study, antioxidant capacity, the amount of total phenolic and phenolic compounds of different grape samples determined and the results were compared. The results were showed that the antioxidant activity of seeds were higher than the pulps. In contrary, the amount of resveratrol of pulps were higher than the seeds. It is suggested that consuming pulp and seeds together will create a synergistic effect in the prevention of diseases such as cancer. The further studies should be performed by animal researches to find out health benefits of grapes.

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Declarations

Conflict of interest The authors declare no competing interests.

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