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DETERMINATION OF PHENOLIC SUBSTANCES AND ANTIOXIDANT ACTIVITIES IN SOME GRAPE CULTIVARS BY HPLC

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ABSTRACT

Every day that passes, both researchers and consumers are ever more interested to explore phenolic compounds in foods, their antioxidant activities and the impact on human health. This study was carried out to determine the phenolic substances and antioxidant activities of juices of five grapes cultivars (Silfoni, Agin Beyazi, Kis Kirmizisi, Okuzgozu and Ercis) grown in Van province of Turkey by the means of High Performance Liquid Chromatography (HPLC). While different values of phenolic substances were detected for different cultivars, the highest antioxidant activities (5.74 ± 2.38 TEAC mmol l^{-1}) were identified in cv. Kis Kirmizisi. Gallic acid was only detected in cv. Ercis (2.78 ±0.09 mg l^{-1}).

Key words: Grape, Phenolic substances, Antioxidant activity.

INTRODUCTION

Cultivated plants are originated from the eight major gene centers present in different localities: four in America, one in Europe-Africa, and three in Asia (Vavilov, 1926). *Vitis vinifera* (Common Grape Vine) (Vitaceae) is a species of *Vitis*, native to the Mediterranean region, central Europe, and southwestern Asia, from Morocco and Portugal north to southern Germany and east to northern Iran (Vavilov, 1926; Celik *et al.*, 1998). The gene center covering the Mediterranean region, Anatolia and the Caucasus is also the establishment place of viticulture. *Vitis vinifera* covers more than 90% of grapes production in the world and distributed into various subspecies like *V. vinifera sativa* D.C., *V. vinifera sylvestris* Gmelin and *V. vinifera caucasina* Vavilov (Celik *et al.,* 1998; Agaoglu, 1999). Celik *et al.* (1998) stated that Anatolia (Turkey) has a great potential for viticulture because of its intersection with gene centers, besides primitive, deep-rooted and rich viticulture exists in Anatolia; along with culturing of grapes in Anatolia since approximately 7-8 thousand years ago.

The grapes are grown in several parts of the world, and are using as fresh, dried or in fruit processing industry. Grapes are a non-climacteric fruit, specifically a berry that grows on the perennial and deciduous woody vines. Grapes can be eaten raw or they can be used for making jam, juice, jelly, vinegar, wine, grape seed extracts, raisins, molasses and grape seed oil. Moreover, in Turkey, grapes are used in traditional food-processing industry as pekmez (syrup), kofter, and bastık etc. (Celik *et al.,* 1998; Agaoglu, 1999).

Grapes are among important fruits both worldwide and in Turkey 67 million tons and 4.8 million tons of production on 7.4 million and 480 thousands ha area, respectively (FAOSTAT, 2009). Grapes, which have been consumed with great pleasure since ancient times, have become more and more important fruit species because of nutritional composition and beneficial role in human health (Cemeroglu *et al*., 2004).

Most phytochemicals have antioxidant activity and protect our cells against oxidative damage and reduce the risk of developing certain types of cancer (Park and Pezzuto, 2002). Phytochemicals with antioxidant activity are allyl sulfides (onions, leeks, and garlic), carotenoids (fruits, carrots), flavonoids (fruits, vegetables), polyphenols (tea, grapes)**.**

Grapes composition revealed that phenolic compounds are the most widely distributed component within the plant secondary metabolites. Thousands of phenolic compounds with the different characteristics, amounts, and functions have been identified (Saldamli, 2007; Kafkas *et al.,* 2006). Phenolic compounds in foods cause variation in various organoleptic qualities like taste, bitterness and sourness; and color properties are also altered with phenolic compounds and anthocyanins. Moreover, attributable to beneficial effects on human health in particular, importance of phenolic compounds has increased with each passing day (Nizamlioglu and Nas, 2010). Phenolic compounds from plants source are categorized into two groups as "phenolic acids" and "flavanoids" while phenolic acids are further sub-categorized into two groups as cinamic (hydroxycinamics) and benzoic (hydroxybenzoic) acids (Cemeroglu *et al*., 2004).

Various studies are conducted in the world to determine the phenolic compounds and antioxidant activities of different grape varieties (Negro *et al.*, 2003; Kedage *et al.*, 2007). Conversely, the Turkey has great biodiversity in grapes especially *Van* province (located in eastern Anatolia of Turkey) and limited studies are conducted on phenolic substances and antioxidant

contents of the grapes cultivars grown in this region. Accordingly, this study was carried out to determine the phenolic compounds (gallic, catechin, caffeic, chlorogenic, *o*-coumaric, *p*-coumaric, ferulic, ciringic, vanillic, quercetin, and rutin acids), and total antioxidant capacity (TEAC) of the grapes genotypes (Silfoni, Agin Beyazi, Kis Kirmizisi, Okuzgozu and Ercis) grown in *Van* province of the Turkey by the means of HPLC.

MATERIALS AND METHODS

Material: The city of *Van* is located between 38° 28′ north longitude and 43° 21′ east latitude and at an altitude of 1725 m above sea level. It has a continental climate, with the highest average temperature in July (22.1 ºC) and the lowest average temperature in January (-3.7 ºC). The oldest grape samples were discovered in the ruins of tombs belonging to the Early Iron Age (Belli, 2000).

Among the grape cultivars (Celik, 2002) that have been evaluated, cv. Silfoni has medium sized grapes green-yellow in color and 3-4 seeds in each grape. Its clusters have conical-cylindrical structure, and it is the medium-sized and plump, a late maturing cultivar. The cv. Agin Beyazı has medium sized grapes green-yellow in color and 2-3 seeds in each grape. Its clusters have conical-cylindrical structure, and it is the large-sized and plump, a mid-season maturing cultivar. The cv. Kis Kirmizisi has medium sized grapes red in color and 2-3 seeds in each grape. Its clusters have winged-conic structure, and it is the medium-sized and plump, a late maturing cultivar. The cv. Okuzgozu has large sized grapes gray misty black in color and 2-3 seeds in each grape. Its clusters have winged-conic structure, and it is the large-sized and plump, a late maturing cultivar. The cv. Ercis has small sized grapes purplish black in color and approximately 2 seeds in each grape. Its clusters have winged structure, and it is the small-sized and plump, a late maturing cultivar.

Determination of phenolic compounds: The phenolic compounds (gallic, catechin, caffeic, chlorogenic, *o*-coumaric, *p*-coumaric, ferulic, ciringic, vanillic, quercetin, and rutin acids) were determined using the HPLC separation method described by Rodriguez-Delgado *et al*. (2001). In three replicas, about 100g of samples was fragmented and 5mL fruit juice from each sample was transferred to centrifuge tubes. The samples were mixed homogenously then diluted 1:1 with distilled water and centrifuged at 15000*g* for 15 minutes. The supernatant were passed through 0.45 mm membrane filter (Millipore Millex-HV Hydrophilic PVDF, Millipore, USA), then injected into HPLC system. The Chromatographic separation in Agilent 1100 series HPLC was took placed in DAD detector (Agilent. USA) with 250 x 4.6 mm, 4µm ODS column (HiChrom, USA). The following solvents in water with a flow rate of 1mL min^{-1} and 20 µL injection volume were used for spectral measurements at 254 and 280 nm: as mobile phase solvent A, methanol-acetic acid-water (10:2:88) and Solvent B, methanol-acetic acid-water (90:2:8) (Table 1.).

Determination of total antioxidant activity: For the standard trolox equivalent antioxidant capacity (TEAC) assay, ABTS [2,29-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] was dissolved in acetate buffer and prepared with potassium persulfate, as described by Rice-Evans *et al.* (1995) and Ozgen *et al*. (2006). The mixture was diluted in acidic medium of 20 mM sodium acetate buffer (pH 4.5) to an absorbance of 0.700 ± 0.01 at 734 nm for longer stability (Ozgen *et al*., 2006). For the spectrophotometric assay, 3 mL of the $ABTS^{+}$ solution and 20 µL of fruit extract were mixed and incubated for 10 min and the absorbance was determined at 734 nm.

RESULTS AND DISCUSSION

Based on the antioxidant activities of cultivars in terms of Trolox Equivalent Antioxidant Capacity (TEAC), the average values of the cultivars ranged from 2.29 ± 0.41 mmol L^{-1} to 5.74 ± 2.38 mmol L^{-1} (Table 2). The highest TEAC value was obtained from cv. Kis Kirmizisi, whereas the lowest TEAC value was found in cv. Ercis.

Some phenolic substances (Flavanoids and Phenolic acids) in the contents of grapes cultivars were detected (Table 3). Among the flavanoids, cv. Okuzgozu had the highest content of cathesin acid (1.80±0.08 mg L^{-1}) and clorogenic acid (3.01±0.16 mg L^{-1}); cv. Agin Beyazi had the highest content (3.77 ±0.64 mg L^{-1}) of rutin acid; cv. Silfoni had the highest content (0.44±0.007 mg L^{-1}) of quersetin acid. Among the hydroxycinamic acid derivatives in phenolic acids, while cv. Kis Kirmizisi had the highest content (1.58 ±0.05 mg L^{-1}) of ferulic acid, but none was detected in cv. Ercis and cv. Silfoni; cv. Agin Beyazi had the highest content of *o*-cumaric acid (1.87 ±0.07 mg L^{-1}) and cafeic acid (2.77 ±0.04 mg L^{-1}); cv. Silfoni had the highest content (0.14 ±0.07 mg L^{-1}) of *p*-cumaric acid. Among the hydroxybenzoic acid derivatives in phenolic acids, while cv. Okuzgozu had the highest content (2.02 ±0.05 mg L^{-1}) of ciringic acid, but none was detected in cv. Agin Beyazi; while cv. Kis Kirmizisi had the highest content (0.46 ±0.007 mg L^{-1}) of vanilic acid, but none was detected in cv. Ercis and Agin Beyazi; and Gallic acid was only detected in cv. Ercis (2.78 ±0.09 mg L^{-1}).

Kedage *et al*. (2007) determined that antioxidant activity of eleven grapes cultivars grown in India and surrounding Asian countries ranged from 0.3 to 0.7 µmol Trolox g^{-1}. In another study, TEAC values in black and white grapes were determined as 3.85 and 2.29 mmol L^{-1},

respectively (Pellegrini *et al*., 2003). In the present study, TEAC values of the cultivars varied from 2.29 ± 0.41 mmol L^{-1} to 5.74 ± 2.38 mmol L^{-1}. TEAC values of the colored grapes were similar to the second mentioned study, but TEAC values of the white grapes were higher than those of them.

In a study of three raisins, total polyphenol content of these cultivars ranged from 151 to 269 mg $100g^{-1}$ (Chiou *et al*., 2007). These researchers found that the most common phenolic substance was vanilic acid (1.21±0.23 mg $100g^{-1}$) followed by caffeic acid, gallic acid, ciringic acid, p-coumaric acid, pyrocateshic acid, ferulic acid, and quersetin, respectively. In the present study, the most common phenolic substance was rutin acid, while vanilic acid was detected in small amounts in three cultivars, but it was not detected in two cultivars.

Pastrana-Bonilla *et al.* (2003) determined the phenolic contents and antioxidant capacities of 10 muscadin grapes (five bronze and five purple skin) grown in Georgia. Phenolics in seed, skin, pulp and leaves were determined as 2178.8, 374.6, 23.8 and 351.6 mg gallic acid g^{-1} equivalents, respectively. Antioxidants in seed, skin, pulp and leaves were determined as 281.3, 12.8, 2.4 and 236.1 µmol TEAC g^{-1} equivalents, respectively.

Breksa *et al.* (2010) investigated the phenolic contents and antioxidant capacities of 6 raisin cultivars and 10 new raisin selections. Antioxidant capacities ranged from 7.7 to 60.9 µmol Trolox g^{-1} DW (Dry Weight), and total phenolic content of these genotypes which were highly correlated with antioxidant capacities varied from 316.3 to 1141.3 to 269 mg gallic acid $100g^{-1}$ equivalent. While A95-15 selection had the lowest content (153.5 µg g^{-1}) of trans-caftaric acid, cv. Fiesta had the highest content (598.7 µg g^{-1}) of trans-caftaric acid. In the present study, antioxidant capacities of the cultivars ranged from 2.29 to 5.74 mmol L^{-1}. Moreover, gallic acid was only detected in cv. Ercis.

Hogan *et al.* (2009) studied antioxidant properties and bioactive components in cv. Norton (*V. aestivalis*) and two different clones of cv. Cabarnet Frank (*V. vinifera*), and found that the antioxidant values of these cultivars were very high (in terms of ORAC (Oxygen Radical Absorbance Capacity): ranged from 22.9 to 26.7 µmol Trolox g^{-1}). In all three cultivars, hydroxybenzoic acids (especially gallic acid) were determined as the main phenolic acid. Coumaric and chlorogenic acids were not detected in cv. Norton, but gallic, vanilic, caffeic, and feluric acids were determined as72.6, 49.4, 9.2, and 0.6 mg g^{-1}, respectively. Gallic, chlorogenic, vanilic, caffeic, coumaric, and ferulic acid in cv. Cabarnet Frank determined as 16.8, 2.7, 2.4, 1.3, 3.5 and 0.2 mg g^{-1}, respectively. In the present study, a single cultivar contained gallic acid; three cultivars contained feluric and vanilic acid; and four cultivars contained cirincic acid. Other phenolics ranged from high to low as rutin, caffeic, chlorogenic, catechin, and chlorogenic acids.

Table 1. Gradient elution program

Time (min)	Dissolvent A (%)	Dissolvent B (%)
0	100	0
15	85	15
25	50	50
35	15	85
45	0	100

Table 2. Antioxidant capacities of the grape cultivars.

Antioxidant Activities (TEAC) (mmol l^{-1})		
Cultivar		**Mean**
Dark Colored Cultivars	Okuzgozu	3.61 ±1.31
	Kis Kirmizisi	5.74 ± 2.38
	Ercis	2.29 ± 0.41
Light Colored Cultivars	Silfoni	4.19 ± 0.38
	Agin Beyazi	4.59 ± 1.29

Table 3. Fruit phenolic matter contents of grape cultivars

		PHENOLIC COMPOUNDS										
Cultivars		FLAVANOIDS				PHENOLIC ACIDS						
						HYDROXYCINAMIC ACIDS				HYDROXYBENZOIC ACIDS		
		Catechin (mg l^{-1})	Rutin (mg l^{-1})	Quercetin (mg l^{-1})	Chlorogenic (mg l^{-1})	Ferulic (mg l^{-1})	*o*-Coumaric (mg l^{-1})	*p*-Coumaric asit (mg l^{-1})	Caffeic (mg l^{-1})	Ciringic (mg l^{-1})	Vanilic (mg l^{-1})	Gallic (mg l^{-1})
Dark colored	Okuzgozu	1.80 ± 0.08	1.43 ± 0.16	0.30 ± 0.007	3.01 ± 0.16	1.24 ± 0.15	0.89 ± 0.10	0.05 ± 0.007	1.45 ± 0.42	2.02 ± 0.05	0.27 ± 0.02	0.0
	Kis Kirmizisi	1.07± 0.08	2.28 ± 0.16	0.03 ± 0.006	1.21 ± 0.11	1.58 ± 0.05	1.69 ± 0.06	0.06 ± 0.007	2.23 ± 0.12	0.88 ± 0.02	0.46 ± 0.007	0.0
	Ercis	0.81 ± 0.04	1.36 ± 0.10	0.39 ± 0.030	1.37 ± 0.08	0.0	1.28 ± 0.10	0.07 ± 0.007	0.97 ± 0.02	1.70 ± 0.05	0.0	2.78 ± 0.09
Light	Silfoni	0.38 ± 0.08	2.11 ± 0.61	0.44 ± 0.007	0.42 ± 0.05	0.0	0.95 ± 0.007	0.14 ± 0.07	1.75 ± 0.07	0.27 ± 0.06	0.24 ± 0.04	0.0
	Agin Beyazi	0.43 ± 0.007	3.77 ± 0.64	0.12 ± 0.030	1.23 ± 0.07	1.15 ± 0.10	1.87 ± 0.07	0.01 ± 0.0007	2.77 ± 0.04	0.0	0.0	0.0

Conclusion: The present study was the first comprehensive investigations to determine the phenolic contents and antioxidants in grapes grown in *Van* ecological conditions. Therefore, this initial study would be more convenient to organize further researches in the coming periods. Based on the results obtained from the present study, employment of the cultivars (having more phenolic substances) in fruit juice processing technology might contribute to the effective outcomes. It was seen that the cv. Ercis was in the forefront because gallic acid which has analgesic, anti-allergenic, anti-asthmatic, antibacterial, immunostimulant, antiviral, antiseptic and cancer-preventive effects in terms of health. Moreover, cv. Kis Kirmizisi had higher values in terms of antioxidant activity.

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