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Determination of Some Antioxidant Activity Values in Wines of *Vitis vinifera* L. Karalahna, Karasakız and Çavuş Grape Varieties Produced in Bozcaada

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Abstract
Çanakkale Bozcaada region, which has witnessed different cultures in different periods for centuries due to its strategic location and wines obtained from grapes belonging to the *Vitis vinifera* L. species, whose traditional names are Karalahna, Çavuş and Karasakız, have been consumed by the local people for years. Wine is a traditional fermented beverage rich in phenolic substances and antioxidant properties, due to the production process, especially during maceration stage, where grape berries are processed with their skins. The geographical structure, plant flora and climate of the region where the grapes grow can create different quality characteristics in grapes, and this affects the quality of the wine through wine processing methods. In this study, to determine some antioxidant activity values of wines produced from Karalahna, Karasakız, and Çavuş grapes grown in Bozcaada. 2, 2-Diphenyl-1-picryl-hydrazyl (DPPH·) free radical scavenging activity, total phenolic substance (Folin-Ciocalteu), reducing power, total flavonoid, anthocyanin, and condensed tannin amounts were measured. DPPH·% Inhibition value of Karalahna wine was found to be 90.6±1.14, condensed tannin was 54.22±0.612 µg CE/g, anthocyanin was found to be 2.46±0.09 mg cyanidin-3-glucoside/g. The results obtained were examined by comparing them with other wine samples.

Keywords: Wine, flavonoids antioxidant, reducing power, phenolic substance.

Bozcaada'da Üretilen *Vitis vinifera* L.'nin Karalahna, Karasakız ve Çavuş üzüm Çeşitlerine Ait Şaraplarda Bazı Antioksidan Aktivite Değerlerinin Belirlenmesi

Öz
Stratejik konumu nedeniyle yüzyıllardır farklı dönemlerde farklı kültürlere tanıklık eden Çanakkale Bozcaada bölgesi, geleneksel isimleri Karalahna, Çavuş ve Karasakız olan *Vitis vinifera* L. türüne ait üzümlerden elde edilen şaraplar yöre halkı tarafından yıllarca tüketilmektedir. Şarap, üretim süreci nedeniyle ve özellikle de maserasyon aşamasında üzüm tanelerinin kabuklarıyla birlikte işleme alındığı, fenolik maddeler ve antioksidan özellikler açısından zengin, geleneksel fermente bir içecektir. Yetiştiği bölgenin coğrafi yapısı, bitki florası ve iklimi üzümlerde farklı kalite özellikleri oluşturabilmekte ve bu durum şarap işleme yöntemleri ile şarapta kaliteyi etkilemektedir. Bu çalışmada, Bozcaada'da yetiştirilen Karalahna, Karasakız ve Çavuş üzümlerinden üretilen şarapların bazı antioksidan aktivite değerlerinin belirlenmesi amacıyla 2,2-Difenil-1-pikril-hidrazil (DPPH•) serbest radikal temizleme aktivitesi, toplam fenolik madde (Folin-Ciocalteu), indirgeme gücü, toplam flavonoid, antosiyanin, yoğunlaştırılmış tanen miktarları belirlenmiştir. Karalahna şarabının DPPH % inhibisyon değeri 90.6±1.14, kondense tanin 54.22±0.612 µg CE/g ve antosiyanin 2.46±0.09 mg siyanidin-3-glikozit/g olarak bulunmuştur. Elde edilen sonuçlar diğer şarap örnekleri ile karşılaştırılarak incelenmiştir.

Anahtar Kelimeler: Şarap, flavonoid, antioksidan, indirgeme gücü, fenolik madde.

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1. Introduction

Wine is a traditional beverage consumed by human beings for centuries. Spontaneous alcohol fermentation in grape must is defined as a complex process in which different yeast genera and species, other than *Saccharomyces cerevisiae*, which is considered the main wine yeast, are present in grapes, must, and wine and contribute to wine aroma, sequentially participate [1] and sulfurization process is one of them [2]. Terroir, which is an important factor in the quality and style of wine, defines the geographical, topographical, and climatic structure of the region where the grape grows and its relationship with the sun [3]. The amount of phenolic compounds in grapes is important for wine quality. Grape grows region can be effective in determining the phenolic compound and aroma level of the grape [4].

Wine is also rich in phenolic compounds, which contain alcohol, polysaccharides, polyphenols, proteins, organic acids, and minerals [5, 6, 7, 8], and grape skins, pulp, and seeds are partially removed from the must [9]. During fermentation, total phenolic compound, total anthocyanin, and antioxidant values in red grape varieties can reach their maximum level when the maceration is completed on the 10^{th} day [10]. However, maceration is not applied to white grape wines and the must has a low amount of phenolic compounds [11]. Polyphenolic composition varies in wine production depending on grape variety, weather, viticulture, and wine process techniques [12, 13]. Phenolic compounds are among the most important quality criteria for wine and create a unique taste in wine [14]. Around the world, the main determining factor of polyphenolic amount variation in red wine may have a period of exposure to sunlight during planting. Additionally, considering the type and geographical origin of the wine sample, differentiation, and classification may occur depending on flavanols, and trans-resveratrol concentrations [15]. Polyphenols can positively affect our health with their antioxidant activity [16], and polyphenol antioxidants such as resveratrol, catechin, epicatechin and quercetin, especially found in red wines, may have an inhibitory effect on cell growth in the fight against prostate cancer [17]. Resveratrol, found in high concentrations in grape skins [18] and it has been analyzed in over 70 plant species [19]. Diets containing high amounts of grapes and resveratrol containing grape powder have been observed to reduce the pathogenesis of heart failure in Dahl-Salt-Sensitive mice, a model of hypertension and diastolic dysfunction [20]. In addition, resveratrol polyphenol has positive effects in preventing cancer and protecting the heart [21]. In France, high consumption of saturated fat and moderate consumption of red wine positively improved coronary heart disease [22].

Karalahna grape, one of the famous local grape varieties from Bozcaada (Tenedos) in Çanakkale, is used for wine production which is a unique dark color [23]. Çavuş grape is grown almost everywhere in Turkey, especially in Bozcaada Çanakkale, Marmara, Central Anatolia, Central Western Black Sea, and Aegean Regions [24,25,26,27,28]. However, it is known as Bozcaada Çavuşu and has grown most intensively in Bozcaada [29,30]. The effect of the island's soil, climate, and topography characteristics (terroir elements) and differences in pollinator varieties have caused Bozcaada Çavuş Grape to gain distinctive characteristics compared to the Çavuş grape variety grown in other regions and regions [30]. Karasakız grape has become a variety whose cultivation is given more importance due to the Çanakkale Tekel

Wine and Brandy Factory established in 1960 [31]. Even though separate studies have been conducted for Karasakız, Karalahna, and Çavuş grapes, no study has been found targeting the region in terms of wine specific to the Bozcaada region.

In this study, total flavonoids, total anthocyanins, reducing power, chelating capacity, and total phenolic properties were determined to determine some antioxidant properties of red and white wines obtained from local grape species grown in Bozcaada.

2. Materials and Methods

In the study, wines obtained from Karalahna, Çavuş, and Karasakız grapes from the 2020-2021 Bozcaada harvest period were purchased commercially from the winery in Bozcaada, Turkey. Sodium thiocyanate, butylated hydroxytoluene (BHT), routine, gallic acid, and catechin were from Fluka Chemical Co. (Buchs, Switzerland), 2,2-diphenyl-1-picryl-hydrazyl (DPPH) was obtained from Sigma Chemical Co. (St. Louis, MO). Potassium ferricyanide, trichloroacetic acid (TCA), $AlCl_3$, $FeCl_3$, Vanillin, Der. Hydrochloric acid was obtained from Merck and all other reagents were analytical grade. In the quality analysis of antioxidant activity values, 30 wine samples were taken for each wine type.

2.1. Determination of total phenolic compounds

Total phenolic compounds were determined according to the Folin-Ciocalteu method according to the method of Slinkard and Singleton (1977) with some modifications [32]. Absorbances were measured using a Shimadzu 1208 UV–Vis spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The amount of total phenolic compounds was calculated as mg gallic acid equivalents (GAE) from the calibration curve of the gallic acid standard solution and expressed as mg GAE/ml.

2.2. Determination of total flavonoid content

Total flavonoid content was determined using a modified version of a method described by Whiskey and Salatino (1998) [33] Wine samples were diluted 1/20. Mean (±SD) results of triplicate analyzes were expressed as mg Rutin/ml of Rutin equivalents of total extractable compounds.

2.3. DPPH radical scavenging activity

Wine samples were diluted 1/20, then 2.2-diphenyl-1-picrylhydrazyl (DPPH) was used, a modified version of method used by Brand-Williams et al. in 1995 [34]. Samples prepared at different concentrations were calculated using 6. 10^{-5} DPPH•, according to following formula, after measuring absorbance at 515 nm after a waiting period of 30 minutes:

$\%Inh = A_0 – (A - A_b) / A_0 \times 100$

A_0: absorbance of DPPH•
A: Absorbance of the substance

A_b: Blank absorbance
IC_{50} for sample extracts, expressed as micrograms of material equivalents per milliliter (extract concentrations providing inhibition values are the concentration of compounds capable of inhibiting 50% of total DPPH radicals) were calculated by non-linear regression via graph plotting and BHT was the positive control. Lower IC_{50} values indicate higher antioxidant activity and vice versa.

2.4. Reducing power

The Reducing powers of wines at different concentrations were determined according to method described by Oyaizu et al. [35]. From each sample (0.25;0.5;1 mg/ml), BHT (0.25;0.5;1 mg/ml) was mixed with an equal volume of 0.2 M phosphate buffer (pH 6.6) and 1% potassium ferricyanide followed by incubation at 50 ºC was incubated for 30 minutes. To stop reaction, 1% trichloroacetic acid (TCA) was added to mixture than mixture was centrifuged at 1000 rpm. Supernatant, distilled water and 0.1% $FeCl_3$ were added, then reducing powers of tested samples were measured by absorbance values by reading at 700 nm (Table 1).

2.5. Amount of condensed tannin

Condensed tannin content was determined using 4% vanillin solution [36]. After adding 4% vanillin solution and 1% HCl to samples prepared at different concentrations, they were placed in a water bath at 30 °C with constant shaking for 20 minutes. After incubation, samples were centrifuged. Absorbance was then measured at 500 nm using a UV-Vis spectrophotometer (Thermo Aquamate). Results were expressed as microgram catechin equivalents (µg CE/g).

2.6. Determination of total anthocyanin

Anthocyanin content was calculated using technique developed by Padmawati, Sakthivel, Thara and Reddy in 1997 [37], modified by Chung et al. 2005 [38]. The total anthocyanin content was estimated as cyanidin-3-glucoside equivalents in milligrams per gram wine sample. The sample was kept in the dark at room temperature for 2 hours with methanol acidified with 1% HCl, then centrifuged at 1000 rpm and measured at 653 nm and 530 nm, and then it was calculated according to following formula;

Anthocyanin content (mg cyanidin-3-glycoside/ g wine) = $A_{530} – (0{,}24 \times A_{653})$

2.7. Statistical analysis

The SPSS version 18 software [Statistical Packages for the Social Sciences (SPSS) version 18 commercial software (IBM Corp.; Armonk, NY, USA)] was utilized for data analysis in the study. Descriptive analyses were conducted to provide information about the general characteristics of the groups. Data pertaining to continuous variables were summarized as Mean ± Standard deviation. Normality tests for numerical variables were performed using the Kolmogorov-Smirnov test and examined through measures of kurtosis and skewness. Differences between means were compared using one-way analysis of variance (ANOVA).

Following the analysis of variance, DUNCAN multiple comparison tests (Post-hoc tests) were applied. Values with $p < 0.05$ were considered statistically significant.

3. Result and Discussion

Table 1. Reducing power of grape samples at different concentrations

	Wines				
	Karasakız	**Karalahna**	**Çavuş**	**F**	**p**
Concentration (mg/ml)	X̄ ± SD	X̄ ± SD	X̄ ± SD		
0.25	0.83±0.011[a]	0.99±0.012[b]	0.34±0.005[c]	30372.90	**<.001**
0.5	1.28±0.008[a]	1.26±0.013[b]	0.39±0.002[c]	133074.61	**<.001**
1	1.55±0.010[a]	1.81±0.001[b]	0.49±0.009[c]	212229.83	**<.001**

X̄: *Mean* SD*: standard deviation* [a-c]*: There is no difference between values with the same letter.*

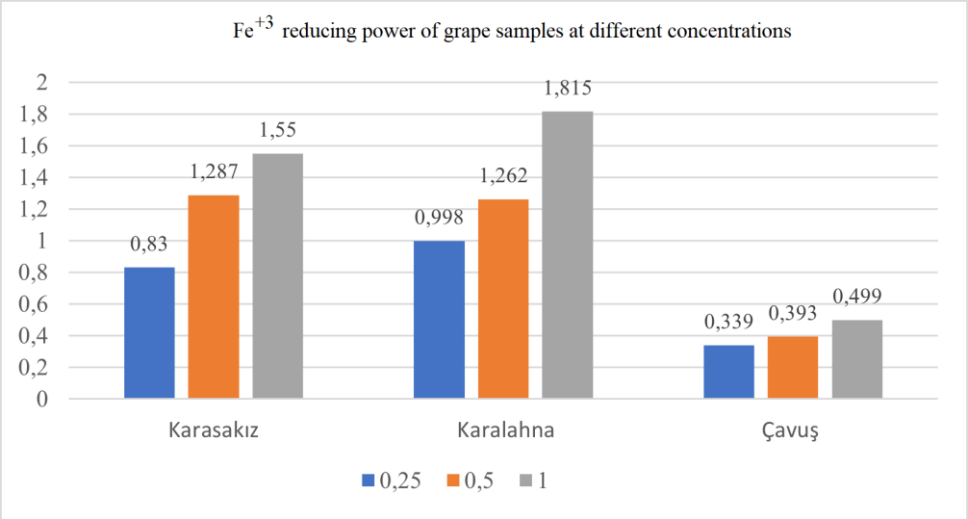

Figure 1. Reducing power of grape samples at different concentrations

Table 2. Spectrophotometric properties wines of the Karasakız, Karalahna and Çavuş grapes

	Wines					
	Karasakız	**Karalahna**	**Çavuş**			**BHT**
	X̄ ± SD	X̄ ± SD	X̄ ± SD	**F**	**p**	X̄ ± SD
Condensed tannin (µg CE/g)	48.44±0.483[a]	54.22±0.612[b]	15.58±0.179[c]	67359.28	**<.001**	
DPPH· IC_{50} (µg/ml)	1.7±0.125[a]	1.6±0.157[a]	2.07±0.142[b]	77.96	**<.001**	**1.34±0.710**
DPPH· % inhibition	89.6±1.14[a]	90.6±1.14[b]	84.8±0.837[c]	196.85	**<.001**	**96.5±0.930**
Total flavonoids (µg rutin/ml)	2927.58±0.531[a]	2887.3±8.367[b]	2714.64±2.016[c]	11918.19	**<.001**	
Anthocyanin (mg cyanidin-3 - glucoside/g wine)	1.98±0.007[a]	2.46±0.09[b]	0.74±0.005[c]	396731.76	**<.001**	
Total phenolics (µg GAE /ml)	2519.34±0.786[a]	2026.4±0.894[b]	434.52±0.705[c]	45790710.4	**<.001**	

X̄: *Mean* SD*: standard deviation* [a-c]*: There is no difference between values with the same letter.*

The amount of condensed tannin in Karalahna grape wine was measured as 54.62%, and the free radical scavenging activity was measured as 97% anthocyanin value is 2.46 (mg cyanidin-3-glucoside/g wine) (Table 2). According to these data, the antioxidant power of Karalahna grape wine is higher than Çavuş, and Karasakız grape wines. The high values in Karasakız and Karalahna grape wines, which have antioxidant effect values close to vitamin C, are related to the amount of anthocyanins and condensed tannins. Additionally, anthocyanin, condensed tannin and DPPH free radical scavenging activity in Karalahna grape wine is higher than Karasakız and Çavuş grape wines. In previous studies, the total phenolic contents of Karasakız and Karalahna wines were 1497.45 mg GAE/L, 1702.91 mg GAE/L [40], and 3052.42 mg GAE/L [41]. In this study, the total phenolic contents of Karasakız grape wines were 2519.34 µg GAE /ml and the high reducing power observed in Karalahna grape wine depends on the total amount of polyphenolics and anthocyanins (Table 1). On the other hand, when the results are evaluated, a statistically significant difference was observed between the antioxidant values of all samples of wine.

In addition, the total phenolic substance in Vasilaki grape wine grown in Bozcaada is 478 mg/L in terms of gallic acid [42], and Çavuş grape wine was content approximately 434.52 (µg GAE / ml). The total amount of phenolic substances in white grape wines produced from the Narince grape variety in Tokat is 345 mg GAE/L [43] and 1090 mg GAE/L in Muscatel grape wines was determined [44]. While wines made from Riesling grapes had the lowest total phenol content with 250 mg GAE/L, wines produced from Cabernet Sauvignon grapes reached 2005 mg GAE/L [45]. In the analyses conducted on white and red wine produced in the Czech Republic in 2006, the total phenolic compound amount was found to be in the range of 90–118 mg GAE/L in white wines and in the range of 874–2262 mg GAE/L in red wines [46]. In addition, the total amount of phenolic compounds is 406.9 mg GAE/L in white wine and 1787 mg GAE/L in red wine [47]. Cabernet Sauvignon, Boğazkere, Öküzgözü, Papazkarası, Shiraz, Merlot, Kalecik Karası, Kuntra (Karasakız), and Karalahna grape varieties are 1412-3183 mg GAE/L (2007) and between 1119-4285 mg GAE/L (2008) [48].

The total amount of flavonoids in white wine, in terms of catechin, is 33.12 mg/L for Narince grape wine and 43.84 mg/L for Emir grape wine [49]. Just as the total phenolic compound value is higher than the wines made from black grapes grown in the Tokat region when compared to total phenolic values of Kuntra (Karasakız) and Karalahna wines grown in the Tokat region, Bozcaada Karalahna and Karasakız grape wines were found to have a higher phenolic substance content. The total amount of flavonoids in Çavuş grape wine, also grown in Bozcaada, is approximately Emir and Narince grape wines. Öküzgözü grape is a quality wine grape variety. The anthocyanin amount in Öküzgözü grape wine was determined to be 2.17 (cyanidin-3-glycosides) in previous studies [50]. In this study, Karalahna wine was measured as 2.46 (cyanidin-3-glycosides), a higher value than Özküzgözü grape wine. The phenolic content of grapes and wines varies depending on the region and may vary from year to year [51], however, it is also known that phenolic compounds are affected by terroir properties [52, 53].

4. Conclusion

In this study, some spectrophotometric quality characteristics of factory wines obtained from Karasakız, Karalahna and Çavuş grape varieties in Bozcaada were determined to investigate the antioxidant capacity of wine.

The amount of condensed tannin in Karalahna grape wine was measured as 54.22 µg CE/g, and the total flavonoid amount in Karasakız grape wine was determined as 2927.58 µg routine/ml. In free radical scavenging activity (DPPH·), the lower the IC_{50} values, the higher the % inhibition value. According to the results, the free radical scavenging activity and antioxidant capacity of Karalahna grape wine was higher. The total phenolic amount in Çavuş grape variety white wine was lower than in red wines. The antioxidant effect in the factory red wine obtained from the Karalahna grape variety grown in the Bozcaada region is stronger and the anthocyanin value is higher than in the Karasakız and Çavuş grape wines of the same region.

Ethics in Publishing

There are no ethical issues regarding the publication of this study.

Author Contributions

Designing study, evaluating results, writing article and performing analysis: Tuğba Güngör Ertuğral; Conducting analyses: Gülen Türker

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