Research Journal of Biology Sciences
E-ISSN: 1308-0262 14(1): 30-38, 2021
Research Article

THE EFFECTS OF DIFFERENT ALTITUDES ON THE PHENOLIC CONTENTS AND ANTIOXIDANT ACTIVITIES OF SOME WHITE GRAPE (*Vitis Vinifera* L.) VARIETIES

Sevinç Aydın[1,a,*], Nesrin Karaca Sanyürek[2,b], Atilla Çakır[3,c], Ökkeş Yılmaz[4,c]

[1]*Çemişgezek Vocational School, Munzur University, Tunceli, Turkey*
[2]*Tunceli Vocational School, Munzur University, Tunceli, Turkey*
[3]*Bingol University, Agriculture Faculty, Horticulturae Department, Turkey*
[4]*Fırat University, Science Faculty, Biology Department, Elazığ, Turkey*

**Corresponding Author:*
E-mail: sevincaydin2380@gmail.com

(Received 14th May 2021; accepted 02th June 2021)

a: ORCID 0000-0001-8597-8064, b: ORCID 0000-0003-3362-1973, c: ORCID 0000-0001-9732-9272
d: ORCID 0000-0002-8276-4498

ABSTRACT. Turkey is one of the most suitable places for viticulture in the economic sense with its climate conditions, soil properties and location. This study aimed to present the phenolic profiles of the seeds of 5 different table grape varieties grown at different altitudes (800 m, 1000 m and 1200 m) and the relationship of these phenolic contents with antioxidant activity, as well as determining the effects of the different altitudes on the values obtained at the end of the study. A high-performance liquid chromatography device (HPLC-UV, Shimadzu) was used to analyze the phenolic compounds of the grape seeds, while antioxidant activity (radical scavenging activity (DPPH)) was measured with a spectrophotometer. According to the results we obtained, as altitude increased, phenolic acid quantities increased, while it was determined that, among the studied phenolic compounds, the quantity of vanillic acid was found to be higher than the quantities of caffeic and ferulic acids ($p<0.001$). DPPH, which is a marker of antioxidant activity, was directly proportional to the increase in altitude ($p<0.001$). In this study, it was shown that the phenolic contents of the seeds of grape varieties grown at different altitudes varied based on the varieties and the altitudes, and based on this chance, antioxidant activity was also positively affected.

Keywords: *phenolic, DPPH, altitudes, Vitis vinifera L., antioxidant*

INTRODUCTION

The grape species *Vitis vinifera* L., which is a prevalently grown and economically significant species in the world, constitutes a rich resource due to the climate conditions of Turkey. Because of their importance, grape products have attracted interest throughout history, and the chemical compounds in these products have been investigated in detail.

There are several studies providing information that grapeseed is rich in phenolic compounds, phytosterols, vitamins and unsaturated fatty acids [1, 2, 3]. Phenolic compounds are secondary metabolites that are produced by plants for protecting these plants against stress conditions. These conditions may be infections, injuries or UV radiation [4]. They not only play functional roles such as protection against ultraviolet (UV) radiation, pigmentation and pathogen prevention [5] but also contribute to the quality of various grape products [6]. Due to their positive effects on human health, they continue to be a subject of research without losing their significance.

The chemical composition of grapes and grape products shows significant differences for each variety. The concentrations and contents of primary and secondary metabolites

synthesized in grapes differ at different maturation stages. It is also a known fact that these are influenced by genotype, environmental factors, as well as viticulture practices [7, 8].

Phenolic compound accumulation is largely dependent on environmental factors [9, 10, 11]. It is influenced by vineyard soil quality, temperature, humidity, sunlight, UV radiation and altitude [12]. Certain climate conditions in a region with low latitude and high altitude provide grape plants with unique quality characteristics [11]. That is, each variety is a characteristic potential against environmental factors, and determining different levels of compound accumulation in plants on different levels, that is, the interaction between genetics and environment, results in different behaviors. For this reason, climate diversity and growth techniques constitute factors that determine significant differences in grape contents [13]. With its compounds carrying antioxidant properties, grapeseed acts as a good alternative to be used against free radicals. Furthermore, the effects of grapeseed extracts on health inspired by these properties have been proven in several studies [14-20].

Phenolic acids, which were investigated in our study, are a significant group of phenolic compounds where hydroxycinnamic acids are prevalent. Caffeic acid, p-coumaric acid and ferulic acid are among the main hydroxycinnamic acids. Previous studies have reported various pharmacological effects of these compounds [21, 22]. With this study, it was aimed to not only contribute to the improvement of the phenolic profiles and antioxidant effects of grapes that are grown in regions with altitudes that have different climate conditions but also provide guidance in terms of the production of high-quality grapes and grape-derived products.

MATERIALS AND METHODS

Plant Material

The Ağın Beyazı, Tahannebi, Kabarcık, Şilfoni and Besni grape varieties collected in the harvesting period from vineyard areas at the altitudes of 800 m, 1000 m and 1200 m in the province of Malatya in Turkey constituted the plant material of this study. The grapes that were harvested at commercial maturity (22° Brix) were brought to the laboratory, and their seeds were separated by hand, washed with water and dried. After the dried seeds were labeled based on their varieties, they were made ready for analysis.

Determining Some Flavonoid Types with the HPLC Device

This analysis was carried out by modifying the method reported by Zu et al. [23]. Onto 1 gr of the sample, 10 mL of methanol was added, and the mixture was homogenized for 60 sec. The homogenized samples were then centrifuged and taken into vials. For the chromatographic analysis of flavonoids, a PREVAIL C18 (15x4.6 mm) column with an internal diameter of 5 µm and a mobile phase containing a methanol/water/acetonitrile (46/46/8, v/v/v) with 1% acetic acid were used. A DAD detector was used for the flavonoid analyses, and in these analyses, the wavelengths of 280 nm for vanillic acid and ROS and 320 nm for caffeic and ferulic acids were used. All chromatographic procedures were carried out at 25 °C.

DPPH Radical Scavenging Activity

The free radical scavenging activities of the grapeseed samples were analyzed by using the method reported by Brand-Williams et al. [24] (based on the disruption of the

color of a methanolic solution containing 2,2-diphenyl-1-picrylhydrazyl (DPPH)). A 25 mg/L DPPH solution was prepared inside methanol, and 4.0 ml of this solution was mixed with 25, 50, 100, 250, 500 and 1000 µL of the sample. The reaction mixture was kept at room temperature and in the dark for 30 min, and the absorbance values of the mixture were measured in a spectrophotometer at 517 nm.

The DPPH radical scavenging capacity of the samples was calculated with the following equation:

DPPH radical scavenging activity (%) = [(Abs control – Abs sample)] / (Abs control)] × 100. Here, Abs control is the absorbance of the DPPH radical + methanol, and Abs sample is the absorbance of the DPPH radical + sample / standard.

Eqn. 1

Statistical Analysis

The SPSS 18.0 software was used for the statistical analysis. The comparison between experiment groups was carried out by using analysis of variance (ANOVA) and an LSD (least significant difference) test. The values of $p<0.05$, $p<0.01$ and $p<0.001$ were used for the differences between the groups (comparisons are made according to 800 altitude).

RESULTS AND DISCUSSION

Composition of Phenolic Compounds

The composition of phenolic compounds in Ağın Beyazı grape seed samples is shown in Table 1. In this study, it was observed that the phenolic compound concentrations (Caffeic acid,vanilic acid and ferrulic acid) in Ağın Beyazı sample were significantly higher in the sample grown at 1200 altitude than the sample grown at 800 and 1000 altitude. Especially, the amount of vanillic acid in the sample 1200 altitude was obtained to be 100,11 µg/g while the amount of that in the sample 800 altitude as 30,79 µg/g ($p<0.01$).

Table 1. *Phenolic Composition (µg/g) of Ağın Beyazı grape seeds*

Phenolic compounds	800 altitude	1000 altitude	1200 altitude
Caffeic acid	0,15±0,01	0,30± 0,05^{d}	0,68±0,01^{d}
Vanillic acid	30,79±0,01	59,62±0,05^{d}	100,11±0,01^{d}
Ferrulic acid	0,10±0,01	0,14±0,01^{b}	0,33±0,01^{d}

$p<0.001$, $p<0.05$

Table 2 shows the composition of phenolic compounds in Tahannebi grape seed samples. The results of this study showed that phenolic compound concentrations in Tahennebi sample were higher in samples grown at 1200 altitude than in samples grown at altitudes of 800 and 1000 altitudes (Table 2).

Table 2. *Phenolic Composition (µg/g) of Tahennebi grape seeds*

Phenolic compounds	800 m altitude	1000 m altitude	1200 m altitude
Caffeic acid	0,30±0,01	0,58±0,01^{d}	0,75±0,01 ^{d}
Vanillic acid	89,86±0,08	112,06±0,01^{d}	123,20±0,85^{d}
Ferrulic acid	0,11±0,01	0,30±0,01^{d}	0,43±0,01 ^{d}

$p<0.001$

Table 3 shows the composition of phenolic compounds in Kabarcık grape seed samples. The results we obtained in this experiment showed that phenolic compound concentrations were higher in samples grown at 1200 altitude than in samples grown at altitudes of 800 and 1000 altitudes. However, the amount of flavonoid in the sample grown at 800 altitude is higher than that in 1000 altitude.

Table 3. *Phenolic Composition (µg/g) of Kabarcık grape seeds*

Phenolic compounds	800 m altitude	1000 m altitude	1200 m altitude
Caffeic acid	0,58±0,01	0,38±0,01^{d}	0,80±0,01^{c}
Vanillic acid	65,15±0,01	58,14±0,01^{d}	109,08±0,01^{d}
Ferrulic acid	0,51±0,01	0,30±0,01^{d}	0,63±0,01^{d}

p<0.001, p<0.01

Table 4 shows the composition of phenolic compounds in Şilfoni grape seed samples. The results of this experiment showed that phenolic concentrations were higher in samples grown at 1200 altitude than in samples grown at altitudes of 800 and 1000 altitudes. However, the phenolic amount of the sample grown at 800 altitude was determined more than that at 1000 altitude.

Table 4. *Phenolic Composition (µg/g) of Şilfoni grape seeds*

Phenolic compounds	800 m altitude	1000 m altitude	1200 m altitude
Caffeic acid	0,41±0,01	0,20±0,01^{d}	0,55±0,01^{d}
Vanillic acid	84,25±0,01	75,67±0,30^{d}	92,93±0,05^{c}
Ferrulic acid	0,20±0,01	0,13±0,01^{d}	0,31±0,01^{d}

p<0.001, p<0.01

Table 5 shows the composition of the phenolic compounds in the Besni grape seed samples. As a result of this experiment, the total amount of flavonoid was determined less than the other 4 cultivars.

Table 5. *Phenolic Composition (µg/g) of Besni grape seeds*

Phenolic compounds	800 m altitude	1000 m altitude	1200 m altitude
Caffeic acid	0,13±0,01	0,43±0,01d^{c}	0,60±0,01^{d}
Vanillic acid	33,45±0,01	38,65±0,01^{c}	53,11±0,01^{d}
Ferrulic acid	0,08±0,01	0,14±0,01^{c}	0,20±0,01^{d}

p<0.001, p<0.01

Antioxidant Capacity of Grape Seeds

DPPH radical scavenging rates of Ağın Beyazı grape seeds are shown in Table 6. In the Ağın Beyazı sample, it was observed that the DPPH radical scavenging effect was highest at 500 µL and at 1200 altitudes (p <0.001). On the other hand, in the Ağın Beyazı sample, the highest DPPH radical scavenging effect was observed at 1200 altitude than in samples grown at altitudes of 800 and 1000 altitudes (p <0.001) (Table 6).

Table 6. *Antioxidant Activity of Ağın Beyazı grape seeds (%)*

DPPH values	800 m altitude	1000 m altitude	1200 m altitude
50 µL	10,01±0,16	17,24±0,17[d]	63,07±0,02[d]
250 µL	30,33±0,02	41,70±0,02[d]	72,94±0,01[d]
500 µL	60,33±0,07	77,55±0,02[d]	186,11±0,02[d]
1000 µL	57,51±0,01	74,73±0,01[d]	161,43±0,01[d]

p<0.001

Table 7 shows the DPPH radical scavenging rates of Tahannebi grape seeds. In the Tahannebi sample, similar to Ağın Beyazı, it was observed that the DPPH radical scavenging effect was highest at 500 µL and at 1200 altitudes (p <0.001) (Table 7).

Table 7. *Antioxidant Activity of Tahannebi grape seeds (%)*

DPPH values	800 m altitude	1000 m altitude	1200 m altitude
50 µL	33,53±0,03	64,09±0,13[d]	85,72±0,15[d]
250 µL	31,71±0,08	39,32±0,01[d]	82,43±0,29[d]
500 µL	52,18±0,11	102,01±0,01[d]	197,08±0,02[d]
1000 µL	47,79±0,12	98,46±0,01[d]	150,54±0,26[d]

p<0,001, c: p<0,01

DPPH radical scavenging rates of Kabarcık grape seeds are shown in Table 8. And, it was observed that the DPPH radical scavenging effect was highest at 1000 µL and at 1200 altitudes (p <0.001). But differently, it was observed that the DPPH radical scavenging effect was higher at 800 altitude samples compared to the samples grown at 1000 altitude sample (Table 8).

Table 8. *Antioxidant Activity of Kabarcık grape seeds (%)*

DPPH values	800 m altitude	1000 m altitude	1200 m altitude
50 µL	47,51±0,03	40,06±0,01[d]	52,24±0,05[d]
250 µL	53,38±0,08	44,77±0,0,15[d]	78,99±0,07[d]
500 µL	76,05±0,09	69,52±0,01[d]	91,66±0,14
1000 µL	102,37±0,11	91,96±0,11[d]	130,07±0,01[d]

p<0,001

DPPH radical scavenging rates of Şilfoni grape seeds are shown in Table 6. According to the data obtained, DPPH radical scavenging effect was the highest at 500 µL (p <0.001). And it was observed that the DPPH effect was higher in samples grown at 1200 altitudes compared to the samples grown at 800 and 1000 altitudes (p <0.001) (Table 9).

Table 9. *Antioxidant Activity of Şilfoni grape seeds (%)*

DPPH values	800 m altitude	1000 m altitude	1200 m altitude
50 µL	15,72±0,09	14,13±0,15[d]	42,67±0,16[d]
250 µL	36,76±0,20	22,79±0,03[d]	58,53±0,01[d]
500 µL	63,34±0,06	57,55±0,02[d]	85,48±0,01[d]
1000 µL	43,75±0,06	40,62±0,60[d]	77,16±0,02[d]

p<0,001

DPPH radical scavenging rates of Besni grape seed samples are shown in Table 10. Similar to Kabarcık variety, DPPH effect in the Besni samples was found to be highest at 1000 µL and 1200 altitudes ($p < 0.001$) (Table 10).

Table 10. *Antioxidant Activity of Besni grape seeds (%)*

DPPH values	800 m altitude	1000 m altitude	1200 m altitude
50 µL	13,69±0,13	25,23±0,16[d]	35,95±0,16[d]
250 µL	17,00±0,14	40,24±0,16[d]	47,78±0,10[d]
500 µL	33,96±0,11	40,31±0,14[d]	64,72±0,47[d]
1000 µL	41,79±0,07	52,79±0,05[d]	72,88±0,07[d]

p<0,001

Grapeseed is a highly rich source of phenolic compounds, phytosterols, vitamins (vitamin E, vitamin C and beta-carotene) and fatty acids [1, 2]. It was reported that grapeseed contains catechin, epicatechin, procyanidins and anthocyanidins as flavonoid contents, gallic acid among phenolic acids, and it is a significant source of resveratrol [25]. Likewise, in other studies, the total phenolic contents of the fruits, seeds and skins of different grape varieties have been determined. Studies have reported that the total phenolic content in *Vitis vinifera* L. seeds is higher in comparison to that of skins [26].

A previous study examined the phenolic contents and antiradical activities of the seeds of the *Vitis vinifera* L. grape species, and it showed that two varieties among the examined varieties were the richest in terms of both total and individual flavanol contents, while they also had the highest antioxidant activity levels [27]. Coklar [26] associated grape phenolics changing based on years with climate changes and reported that altitude has a very strong effect on the phenolics in grape. They also determined that phenolic accumulation, and alongside it, antioxidant activity increased when altitude rose from 1000 m to 1500 m.

In a study conducted on the *Vitis vinifera* L. *cv.* Cabernet Sauvignon variety grown at different altitudes of highlands in southwestern China, significant changes were determined among the quantities of the detected anthocyanins, and it was reported that the production of especially cyanidin-type anthocyanins and quercetin-type flavanols was largely promoted in fruits grown at higher altitudes [11]. Ristic et al. [28] investigated the effects of shading, i.e., the amount of light changing by altitude, on the Shiraz grape variety and showed that excessive shading may reduce the color of wine, anthocyanins and tannins, while it could also change sensory qualities. According to the results of their study, the total phenolic content of the grapes grown in shading was lower in comparison to those subjected to normal sunlight. Although it is known that colored fruits of grape have richer phenolic contents, it was reported that the skins and seeds of white grape varieties are quantitatively and qualitatively close to colored grape varieties in terms of their non-anthocyanin polyphenol contents [29].

Previous studies have investigated the antioxidant effect and free radical scavenging activity of grapeseed extracts and reported the degree of these activities [30, 31]. Similarly, a previous study on the same topic demonstrated that the extract of proanthocyanidins obtained from grapeseed provided protection against free radicals based on their free radical scavenging experiment, and this effect was superior to even the effects of vitamins C and E [32]. In another study conducted on the antioxidant effects of grape contents, Du et al. [33] examined the polyphenol and total antioxidant activities of four wine grape and four table grape varieties, and as a result of their study, they

reported that the phenolic contents of the fruits were largely associated with the antioxidant capacities of wines. Again, in a similar study, the authors stated that, independent of the method of analysis, grapeseed had the best antioxidant activity, and grapes with darker skins had higher antioxidant activity than that of grapes with lighter skins [34].

In this study, changes in the phenolic acid contents of the seeds of 5 white table grape varieties based on varying altitude values and the relationship of these changes to antioxidant activity were investigated. The obtained results showed that, as in the case of previous studies, phenolic acid quantities increased as altitude increased, and the quantity of especially vanillic acid was higher than those of caffeic and ferulic acid. Considering the antioxidant activity results, it was observed that an increase in altitude was directly proportional to DPPH effects. Moreover, it was determined that the samples collected from the altitude of 1000 m in the Kabarcık and Şilfoni grape varieties had lower antioxidant activity values than those collected from the altitude of 800 m. It was thought that this difference may have originated from varieties, environmental factors, climate, altitude, culture procedures or a combination of two or more of these factors.

CONCLUSION

In light of all these studies and the obtained results, it may be stated that high-altitude regions may help the growth of *Vitis vinifera* L. varieties that differ in terms of characteristics like the intensity of their color, flavor and acidity based on the longer phenological cycles of grape varieties in these regions, as well as higher levels of sunlight availability. In addition to these aforementioned benefits to be provided by growing grape varieties based on the analysis results of different altitudes along with other factors, the importance of this contribution will also be undeniably great for industrial applications, the food sector and medical improvements.

REFERENCES

[1] Bail, S., Stuebiger, G., Krist, S., Untrwager, H., Buchbaner, G. (2008): Characterization of Various Grape Seed Oils by Volatile Compounds, Triacylglycerol Composition, Total Phenols and Antioxidant Capacity. Food Chemistry 108: 1122-1132.

[2] Crews, C., Hough, P., Godward, J., Brereton, P., Lees, M., Guiet, S., Winkelmann, W. (2006): Quantitation of the Main Constituents of Some Authentic Grape-seed Oils of Different Origin. Journal of Agricultural and Food Chemistry 54: 6261-6265.

[3] Rombaut, N., Savoire, R., Thomasset, B., Belliard, T., Castello, J., Van Hecke, E., Lanoiselle, J. L. (2014): Grape Seed Oil Extraction: Interest of Supercritical Fluid Extraction and Gas-assisted Mechanical Extraction for Enhancing Polyphenol Co-extraction in Oil. Comptes Rendus Chimie 17: 284-292.

[4] Alcantara, M. A., de Lima Brito Polari, I., de Albuquerque Meireles, B. R. L., de Lima, A.E.A., da Silva Junior, J. C., de Andrade Vieira, E., Dos Santos, N. A., de Magalhaes Cordeiro, A. M. T. (2019): Effect of the Solvent Composition on the Profile of Phenolic Compounds Extracted from Chia Seeds. Food Chemistry 275: 489–496.

[5] Gómez-Míguez, M., González-Manzano, S., Escribano-Bailón, M. T., Heredia, F. J., Santos-Buelga, C. (2006): Influence of Different Phenolic Copigments on the Color of Malvidin 3-Glucoside. Journal of Agricultural and Food Chemistry 54: 5422-5429.

[6] Gawel, R. (1998): Red Wine Astringency: A Review. Australian Journal of Grape and Wine Research 4: 74-95.

[7] Dai, Z. W, Vivin, P., Ollat, N., Barrieu, F., Delrot, S. (2010): Physiological and Modelling Approaches to Understand Water and Carbon Fluxes in Relation with Grape Berry Growth and Quality. Australian Journal of Grape and Wine Research 16: 70-85.

[8] Kumšta, M., Pavloušek, P., Kárník, P. (2014): Use of anthocyanin profiles when differentiating individual varietal wines and terroirs. Food technology and biotechnology 52(4), 383-390.

[9] Castellarin, S. D., Matthews, M. A., Di Gaspero, G., Gambetta, G. A. (2007): Water deficits accelerate ripening and induce changes in gene expression regulating flavonoid biosynthesis in grape berries. Planta 227(1): 101-112.

[10] Schultz, H. (2000): Climate change and viticulture: a European perspective on climatology, carbon dioxide and UV-B effects. Australian Journal of grape and wine research 6(1), 2-12.

[11] Xing, R. R., He, F., Xiao, H. L., Duan, C. Q., Pan, Q. H. (2015): Accumulation Pattern of Flavonoids in Cabernet Sauvignon Grapes Grown in a Low-Latitude and High-Altitude Region. South African Journal of Enology and Viticulture 36(1): 32-43.

[12] Gladstones, J. (2011): Wine, Terroir and Climate Change. Wakefield Press, Kent Town, South Australia.

[13] Malinovski, L. I., Brighenti, A. F., Borghezan, M., Guerra, M. P., Silva, A. L., Porro, D., ... & Vieira, H. J. (2014): Viticultural performance of Italian grapevines in high altitude regions of Santa Catarina State, Brazil. In XXIX International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes (IHC2014): IV 1115 (pp. 203-210).

[14] Valli Kanagarla, N.S.S.A., Kuppast, I.J., Veerashekar, T., Reddy Chindala Laxman. (2013): A Review on Benefits and Uses of *Vitis vinifera* (Grape). Research& Reviews Biosciences 7(5): 175-180.

[15] Nassiri-Asl, M., Hosseinzadeh, H. (2009): Review of the Pharmacological Effects of *Vitis vinifera* (Grape) and Its Bioactive Compounds. Phytotherapy Research 23(9): 1197-1204.

[16] Gargari, B., Abedini, S., Babaei, H., Aliasgarzadehand, A., Pourabdollahi, P. (2011): Effect of Supplementation with Grape Seed (*Vitis vinifera*) Extract on Antioxidant Status and Lipid Peroxidation in Patient with Type II Diabetes. Journal of Medicinal Plants Research 5(10): 2029-2034.

[17] Hemmati, A., Nazari, Z., Samei, M. (2008): A Comparative Study of Grape Seed Extract and Vitamin E Effects on Silica-Induced Pulmonary Fibrosis in Rats. Pulmonary Pharmacology & Therapeutics 21(4): 668-674.

[18] Morin, B., Narbonne, J. F., Ribera, D., Badouard, C., Ravanat, J. L. (2008): Effect of Dietary Fat-Soluble Vitamins A and E and Proanthocyanidin-Rich Extract from Grape Seeds on Oxidative DNA Damage in Rats. Food and Chemical Toxicology 46(2): 787-796.

[19] Enginar, H., Cemek, M., Karaca, T., Unak, P. (2007): Effect of Grape Seed Extract on Lipid Peroxidation, Antioxidant Activity and Peripheral Blood Lymphocytes in Rats Exposed to X-Radiation. Phytotherapy Research 21(11): 1029-1035.

[20] Erdman, J., Balentine, D., Arab, L., Beecher, G., Dwyer, J. T., Folts, J., Harnly, J., Hollman, P., Keen, C. L., Mazza, G., Messina, M., Scalbert, A., Vita, J., Williamson, G., Burrowes, J. (2007): In Flavonoids and Heart Health: Proceedings of the ILSI North America Flavonoids Workshop may 31-june 1, 2005, Washington DC. The Journal of Nutrition 137(3): 718-737.

[21] Khan, F. A., Maalik, A., Murtaza, G. (2016): Inhibitory Mechanism Against Oxidative Stress of Caffeic Acid. Journal of Food and Drug Analysis 24(4): 695-702.

[22] Langland, J., Jacobs, B., Wagner, C. E., Ruiz, G., Cahill, T. M. (2018): Antiviral Activity of Metal Chelates of Caffeic Acid and Similar Compounds Towards Herpes Simplex, VSV-Ebola Pseudotyped and Vaccinia Viruses. Antiviral Research 160: 143-150.

[23] Zu, Y. G., Fu, Y. J., Liu, W., Hou, C. L., Kong, Y. (2006): Simultaneous Determination of Four Flavonoids in Pigeonpea [*Cajanus cajan* (L.) Millsp.] Leaves Using RP-LC-DAD. Chromatographia 63(9): 499-505.

[24] Brand-Williams, W., Cuvelier, M. E., Berset, C. L. W. T. (1995): Use of a free radical method to evaluate antioxidant activity. LWT-Food science and Technology 28(1): 25-30.
[25] Yilmaz, Y., Toledo, R. T. (2004): Major flavonoids in grape seeds and skins: antioxidant capacity of catechin, epicatechin, and gallic acid. Journal of agricultural and food chemistry 52(2): 255-260.
[26] Coklar, H. (2017): Antioxidant capacity and phenolic profile of berry, seed, and skin of Ekşikara (*Vitis vinifera* L.) grape: Influence of harvest year and altitude. International Journal of Food Properties 20(9): 2071-2087.
[27] Bozan, B., Tosun, G., Özcan, D. (2008): Study of Polyphenol Content in the Seeds of Red Grape (*Vitis vinifera* L.) Varieties Cultivated in Turkey and Their Antiradical Activity. Food Chemistry 109(2): 426-430.
[28] Ristic, R., Downey, M. O., Iland, P. G., Bindon, K., Francis, I. L., Herderich, M., Robinson, S. P. (2007): Exclusion of sunlight from Shiraz grapes alters wine colour, tannin and sensory properties. Australian Journal of Grape and Wine Research 13(2): 53-65.
[29] Rockenbach, I. I., Rodrigues, E., Gonzaga, L. V., Caliari, V., Genovese, M. I., Gonçalves, A. E. D. S. S., Fett, R. (2011): Phenolic compounds content and antioxidant activity in pomace from selected red grapes (*Vitis vinifera* L. and *Vitis labrusca* L.) widely produced in Brazil. Food Chemistry 127(1): 174-179.
[30] Jayaprakasha, G. K., Selvi, T., Sakariah, K. K. (2003): Antibacterial and antioxidant activities of grape (*Vitis vinifera*) seed extracts. Food research international 36(2): 117-122.
[31] Caillet, S., Salmiéri, S., Lacroix, M. (2006): Evaluation of free radical-scavenging properties of commercial grape phenol extracts by a fast colorimetric method. Food Chemistry 95(1): 1-8.
[32] Bagchi, D., Bagchi, M., Stohs, S. J., Das, D. K., Ray, S. D., Kuszynski, C. A., ... & Pruess, H. G. (2000): Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention. Toxicology 148(2-3), 187-197.
[33] Du, B., He, B. J., Shi, P. B., Li, F. Y., Li, J., Zhu, M. (2012): Phenolic content and antioxidant activity of wine grapes and table grapes. Journal of Medicinal Plants Research 6(17): 3381-3387.
[34] Anastasiadi, M., Pratsinis, H., Kletsas, D., Skaltsounis, A. L., Haroutounian, S. A. (2010): Bioactive non-coloured polyphenols content of grapes, wines and vinification by-products: Evaluation of the antioxidant activities of their extracts. Food research international 43(3): 805-813.