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RESEARCH ARTICLE

Vitamin content and MDA levels of certain white grape varieties from different altitudes in Turkey

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ABSTRACT

Grapevine (*Vitis vinifera* L.) is regarded as one of the world's most important fruit crops in terms of acreage and commercial worth. Grapes are used to make not only wine but also fresh fruit, dried fruit, and juice. The quality of grapes or wine is significantly affected by environmental factors such as altitude, soil type, and climate. In this study, the 5 white grape varieties (Ağın beyazı, Tahannebi, Kabarcık, Şilfoni, and Besni) grown at different altitudes (800 m, 1000 m and 1200 m) in Turkey were investigated for their vitamin, and phytosterol concentrations as well as lipid peroxidation content. Şilfoni grape harvested at 800 m was found to possess the lowest lipid peroxidation content. High altitudes, were found to reduce lipid peroxidation contents in the Ağın beyazı, Tahannebi, and Kabarcık strains. The highest stigmasterol (98.34 μg/g), and beta-sitosterol (86.90 μg/g) concentrations were found within Şilfoni grape variety harvested at 800 m. The highest vitamin D-2 and D-3 levels were found in Ağın beyazı (1.39 μg/g) and Kabarcık (4.13 μg/g) grape varieties, both harvested at 1200 m. Kabarcık grape, however, revealed the highest concentrations of R-tocopherol (63.09 μg/g) and alpha-tocopherol (24.13 μg/g) at 1200 m within the samples. The highest vitamin K-2 level was determined within the Tahannebi grape (15.44 μg/g) from 1200 m. It was observed that the vitamin and phytosterol concentrations of white grape varieties tended to increase, while the lipid peroxidation values tended to decrease at high altitudes, especially at 1200 m. This study also exhibited a strong relationship among Vitamin D3, R-tocopherol, and alpha-tocopherol in the studied white grape cultivars.

Keywords: *Vitis vinifera*; White grape; Vitamin; MDA; Altitude

INTRODUCTION

Grapes (*Vitis vinifera* L.) are one of the most cultivated fruits throughout the world. In 2019, about 77 million tonnes of grapes were produced in the World. In Europe, on the other hand, about 27 million tonnes of grapes were produced, consisting 34.6% of the total grape production in the World. After Italy, Spain, and France, Turkey is the fourth largest grape producer in Europe with more than 4 million tonnes in a year (FAOSTAT, 2021). Almost half of the produced grapes are consumed as wine, while 33.3% are consumed as fresh fruits, and the rest of the grapes are used to make dried fruits, grape juice, or grape must (FAO and OIV, 2016).

Viticulture is a significant socioeconomic activity in many parts of the world. Therefore, the nutritional properties of raw or dried grapes have been extensively studied. Grapes have been known for their enormous antioxidant potential through their phenolic composition. Certain grape products, such as grape seed extract, have long been reported to possess anti-inflammatory, anti-cancer, and anti-atherosclerotic properties (Garavaglia et al., 2016). Indeed, the protective effects of grapes and grape products against many diseases, such as obesity, inflammatory bowel disease, neurodegenerative diseases, cancer, and autoimmune diseases, have been suggested (Magrone et al., 2020).

Vitamin E has long been known to promote antioxidant activity through polyunsaturated lipid deoxidation (Zhao et al., 2017). Vitamin E activity may be generated from four structural isomers of tocopherol (α-, β-, γ-, and δ-) and the corresponding four tocotrienols. Among the structural isomers, only -tocopherol can meet human vitamin E requirements. α-Tocopherol is generally recognized to play an essential role in preserving membrane-bound unsaturated fatty acids from oxidation through its strong lipid-soluble, chain-breaking antioxidant action. Plants produce naturally occurring α-tocopherol, which occurs

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as a single stereoisomer, RRR- α-tocopherol, and is often referred to as natural vitamin E (Kuchan et al., 2018). The content of α-tocopherol in grapes has lately acquired significance for its availability to be substituted with synthetic antioxidants and to create cost-effective solutions for the present limitations of cosmetic and food sectors (Gouvinhas et al., 2020).

The quality of grapes or wine is significantly affected by environmental factors such as altitude, soil type, and climate (sunlight, temperature, and rainfall) (Perestrelo et al., 2014). Altitude, especially, has been reported as an important factor to affect grape and wine quality. The content of phenolic compounds which significantly affect the quality in red wines has been shown to be increased with altitude (Jin et al., 2017) (Gouvinhas et al., 2020) (Tarara et al., 2008). Many studies reported the effect of altitude on the antioxidant capacity and phytochemical content of grape varieties. However, no studies reported the vitamin and phytosterol contents of white grape varieties harvested from different altitudes. In this study, the 5 white grape varieties (Ağın beyazı, Tahannebi, Kabarcık, Şilfoni, and Besni) harvested from different altitudes as 800 m, 1000 m, and 1200 m in Turkey were evaluated by means of vitamin, and phytosterol concentrations as well as lipid peroxidation contents.

METHODOLOGY AND MATERIALS

Chemicals and standards

Sigma Aldrich supplied the hexane, isopropanol, methanol, sulfuric acid, sodium chloride, KOH, and KHCO3 utilized in this research (Steinheim, Germany). Merck and VWR supplied the acetonitrile and acetic acid, respectively.

Plant materials and sample preparation

The grape varieties (*V. vinifera* L.) used in the study were determined as Ağın Beyazı, Tahannebi, Kabarcık, Şilfoni, and Besni. Short ampelographic definitions were made and local evaluation methods were determined (Table 1).

The grapevines were collected in Malatya, Turkey, at altitudes of 800 m, 1000 m, and 1200 m. The grapefruit seeds were removed and rinsed in double-distilled water. After that, the seeds were dried in an oven at 40°C for 3 hours to remove excess moisture. The seeds were then pulverized using an Agat mortar to make grapeseed powder, which was then stored at -20°C for further investigations. Before each test, the samples were homogenized individually.

Analysis of vitamins and phytosterols

1 g of grape seed powder was homogenized in a hexane/isopropanol solution (v/v, 3:2). The tubes were then treated with 5% KOH for 15 minutes, vortexed, and left at 85 °C for hydrolysis. After allowing the tubes to cool to room temperature, 5 mL of distilled water was added. 2x5 mL hexane was used to extract the non-soaping lipophilic compounds. Under the nitrogen stream, the hexane phase evaporated. It was diluted in a 1 mL (v/v, 3:2) acetonitrile/methanol solution and transferred to autosampler vials for HPLC analysis (HPLC-UV, Shimadzu). The device used the SPD-10AVP as the LC-10 ADVP UV-visible detector, the CTO-10ASVP as the column furnace, the SIL-10ADVP as the autosampler, and the DGU-14A and Class VP software as the degasser units (Shimadzu, Kyoto, Japan). As a mobile phase, a solution of acetonitrile and methanol (v/v, 3:2) was employed. The flow rate was set to 1 milliliter per minute. The analysis was performed using a Supelcosil LC TM 18 column (15x4.6 mm, 5 μm; Sigma, USA) and a UV detector. The detection wavelengths utilized were 202 nm. The findings are expressed as μg/g (Aydın, 2020).

Lipid peroxidation (MDA) assay

0.5 g of grape seed powder was homogenized in 0.1% trichloroacetic acid (TCA), and the homogenate was centrifuged at 10.000 rpm. 2 mL thiobarbituric acid (TBA) at a concentration of 0.5% was added to 2 mL supernatant, and the combination was heated for 30 minutes in a water bath at 95 °C (TBA was prepared in 20% TCA). Following that, the samples were chilled in an ice bath. The cooled mixture was centrifuged once again at 10.000 rpm. At 532 and 600 nm, the absorbance of the supernatant was determined. The Abs = ε.C.L formula was used to calculate the MDA concentration, and then the MDA concentration in 1 g of tissue was measured. The molar absorption coefficient used to determine the MDA concentration is 155 mM^{-1} cm^{-1} (Heath and Packer, 1968). The findings are expressed in nmol/g.

Statistical analysis

SPSS software (18.0) was used for statistical analysis. The experimental findings are expressed as the mean ± SEM

Table 1: Some ampelographic characteristics of the studied cultivars

Variety	Skin Color	Purpose of the use	Flower	Seed count	Maturity Time
Ağın Beyazı	Yellow	Table	Hermaphrodite	2-3	Mid Season
Tahannebi	Green-Yellow	Table	Hermaphrodite	1-2	Mid Season
Kabarcık	Green-Yellow	Table- juice	Hermaphrodite	1-3	Mid Season
Şilfoni	Green-Yellow	Table	Hermaphrodite	3-4	Late Season
Besni	Green-Yellow	Raisin	Hermaphrodite	1-4	Mid Season

(standard error of the mean). $P<0.05$ was considered as statistical significance. To compare the findings across groups, an analysis of variance (ANOVA) and an LSD (least significant difference) test were used. Pearson correlation and principle component analysis (PCA) assays were carried out via XLSTAT 2016 software.

RESULTS AND DISCUSSION

Grape quality may be influenced by both internal variables such as variety and external ones such as climate, soil, and production methods. Climate, exposure to the sun, and altitude are all variables that affect grape and wine quality and are included in the French word "terroir" which has been shown to have a significant impact on the quality of grapes and wine. Altitude, in particular, has the potential to have a significant impact on the mesoclimate as it is directly linked with the resultant temperature, humidity, sunshine exposure, and other environmental variables that influence the development of grapes (Alessandrini et al., 2017). At high altitudes, the environments are characterized by high thermal amplitudes and great solar radiations, especially ultraviolet-B (Arias et al., 2022). The altitude of a growing site also affects humidity, sunlight hours, water deficits, and other environmental factors, therefore altitude can strongly influence climatic conditions. Growing grapevines at high altitudes has been one of the most effective new viticultural strategies for mitigating the negative impacts of global warming on grape and wine quality, particularly because it delays grape ripening Therefore, high-altitude viticulture has been acquiring great importance in the last decades due to its potential to produce high-quality wines mostly due to a lower mean air temperature (Gutiérrez-Gamboa et al., 2021).

In this study, vitamin D-2, D-3, K-2, R-tocopherol, and alpha-tocopherol levels as well as lipid peroxidation content of 5 white grape varieties harvested from Turkey at different altitudes (800 m, 1000 m, and 1200 m) were evaluated. Lipid peroxidation contents of the grapes were assessed by malondialdehyde (MDA) concentrations that is a marker of oxidative stress. MDA quantity is one of the products of membrane lipid peroxide, and its production can also aggravate the damage to the membrane (Hu et al., 2019).

Table 2 shows the MDA levels of 5 white grape varieties harvested at altitudes of 800 m, 1000 m, and 1200 m. Şilfoni grapes harvested at 800 m had the lowest lipid peroxidation content in the samples studied. It was observed that the lipid peroxidation content of Şilfoni grape increased with increasing altitudes. However, the amount of MDA was found to be decreased gradually from low to high altitudes in Ağın beyazı, Tahannebi, and Kabarcık cultivars, suggesting that high altitudes decrease lipid peroxidation content of Ağın beyazı, Tahannebi and Kabarcık strains. Furthermore, in Besni grape the lipid peroxidation content increased from 800 m to 1000 m, then decreased at 1200 m. The highest MDA value in the studied samples was determined on the Besni grape harvested at 1000 m. Our results showed that although altitude affects the lipid peroxidation content of white grape varieties mostly similarly, the differences that occur could be related to other factors such as chemical content of grape varieties.

Table 2: Lipid peroxidation content (MDA)(nmol/g) in 5 white grape varieties grown in different altitudes of Malatya, Turkey

Grape types	800 m	1000 m	1200 m
Ağın Beyazı	243.50±0.01	232.00±0.012^{b}	214.00±0.012^{d}
Tahannebi	248.52±0.02	228.00±0.01^{c}	216.03±0.02^{d}
Kabarcık	414.51±0.01	392.00±0.01^{c}	327.00±0.01^{d}
Şilfoni	208.01±0.01	222.03±0.02^{c}	310.51±0.01^{d}
Besni	317.00±0.01	462.01±0.02^{d}	324.51±0.02^{c}

Data presented as mean±SEM of triplicate analysis b: $P<0.05$, c: $P<0.01$, d: $P<0.001$

*Comparisons and lettering were made according to 800 m altitude within the species.

High altitude was reported to change the antioxidant capacities of grapes. Many factors including UV-B radiation which is variable at different altitudes could change the levels of antioxidative substances (Berli et al., 2013). In a study, the phenolic composition and antioxidant activity of Ekşikara which is a native Turkish grape cultivar were shown to be increased as altitude arised from 1000 to 1500 m (Coklar, 2017). Similarly, 2 different grape cultivars from China were reported to possess increased levels of total phenolic compounds, and greater antioxidant capacities with increasing altitudes (Jin et al., 2017). However, the antioxidant activity of Kara erik grape variety from Turkey was reported to be decreased gradually with increasing altitudes as the highest antioxidant activity at 1200 m, and lowest at 1500 m (Özel et al., 2022). Likewise, in a Brasilian Mascotel grape cultivar, higher polyphenolic content and higher antioxidant activities were reported in low altitudes (Gouvinhas et al., 2020). These studies suggest that high altitudes does not always increase the antioxidant activities of grape cultivars.

The effects of altitude on phytochemicals such as anthocyanin levels were also widely reported. For example in a study, higher altitude cultivation was reported to greatly promote the production of anthocyanins and flavonols in two consecutive vintages (Xing et al., 2016). Similarly, in another study, a high level of anthocyanins was shown in grapes harvested from the western region, with high altitude and low annual rainfall, as compared to the eastern region, with low altitude and high annual rainfall (Liang et al., 2014).

Possibly, altitude affects the biochemical properties of grape cultivars along with many other factors, such as sunlight and rainfall, since these factors are also changed at different altitudes. With this regard, Ristic et al., (2007) reported the effects of shading, and the amount of light at different altitudes on wine color, anthocyanin and tannin composition.

Tocopherols are within natural antioxidants found in plants as different types of which the molecular structure and antioxidant profiles vary. A variety of methylated phenol rings are found in tocopherols creating different types. Different variations of vitamin E are also known as tocopherols. In humans, the most active form of vitamin E is alpha-tocopherol. As a fat-soluble compound, alpha-tocopherol acts on a cellular membrane (Tangolar et al., 2011).

Vitamin and phytosterol concentrations of Ağın beyazı are shown in the Table 3. Vitamin D2 was found to be increased from low to high altitudes, as 1.39 μg/g at 1200 m in Ağın beyazı. However, Vitamin D3 concentration was significantly decreased from 800 m (1.04 μg/g) to 1000 m (0.11 μg/g), then increased again at 1200 m (1.08 μg/g, $p<0.001$). While the R-tocopherol level was decreasing, the alpha-tocopherol level in Ağın beyazı was increased from low to high altitudes ($p<0.001$). K-2 levels, on the other hand, were decreased from 800 m (0.89 μg/g) to 1000 m (0.65 μg/g, $p<0.01$), then increased to the highest level at 1200 m (1.49 μg/g, $p<0.001$). In this study, phytosterol concentrations were also evaluated in white grape varieties. Stigmasterol level of Ağın beyazı was significantly increased from low to high altitudes, as 12.95 μg/g at 800 m, 36.48 μg/g at 1000 m, and 54.06 μg/g at 1200 m ($p<0.001$). β-Sitosterol level of Ağın beyazı also increased from low to high altitude, as 10.00 μg/g at 1000 m, and 11.77 μg/g at 1200 m ($p<0.05$). Therefore, in this study, the altitude of 1200 m was determined to be the optimum one in order to obtain high levels of vitamins and phytosterols, in general.

The concentrations of vitamins and phytosterols of Tahannebi grape variety is shown in Table 4. Vitamin D-2, D-3 and K-2 concentrations increased from low altitude to higher altitudes ($p<0.001$) in Tahannebi grape. The same phenomenon was observed in vitamin E concentrations in this study, resulting the highest R-tocopherol (0.95 μg/g, $p<0.001$) and alpha-tocopherol (5.87 μg/g, $p<0.001$) levels in Tahannebi variety at 1200 m. Therefore, the studied vitamins were found to be higher in concentrations within the samples harvested from higher altitudes. In phytosterols, stigmasterol and β-sitosterol levels significantly increased from low altitude to higher altitudes.

Vitamin and phytosterol levels of Kabarcık grape harvested from 3 different altitudes are shown in the Table 5. Evaluating all of the studied vitamins and phytosterols in Kabarcık grape, the lowest concentrations were found in the samples harvested at 1000 m ($p<0.001$). The highest concentrations of vitamins D-2, D-3, R-tocopherol, alpha-tocopherol, β-sitosterol and stigmasterol were found in the samples harvested at 1200 m ($p<0.001$). The concentration of R-tocopherol, especially, was significantly high (63.09 μg/g, $p<0.001$) in the sample of 1200 m as compared to the one at 800 m (0.53 μg/g) and 1000 m (0.28 μg/g, $p<0.001$). The alpha-tocopherol concentration, likewise, was found to be significantly high in the sample of 1200 m as 24.13 μg/g ($p<0.001$), while decreasing in the sample of 800 m to 21.89 μg/g ($p<0.001$), and even more decreased

Table 3: Vitamin and phytosterol concentrations of Ağın beyazı (μg/g)

Vitamins and phytosterols	800 m	1000 m	1200 m
D-2	1.03±0.01	1.25±0.01[b]	1.39±0.01[d]
D-3	1.04±0.01	0.11±0.01[d]	1.08±0.01[d]
R-tocopherol	12.04±0.01	0.59±0.01[d]	0.41±0.01[d]
alpha-tocopherol	4.29±0.01	8.12±0.01[b]	16.95±0.01[d]
K-2	0.89±0.01	0.65±0.01[c]	1.49±0.01[d]
Stigmasterol	12.95±0.35	36.48±0.01[d]	54.06±0.03[d]
β-sitosterol	2.05±0.57	10.00±0.01[b]	11.77±0.01[b]

Data presented as mean±SEM of triplicate analysis b: $P<0.05$, c: $P<0.01$, d: $P<0.001$
*Comparisons and lettering were made according to 800 m altitude within the species.

Table 4: Vitamin and phytosterol concentrations of Tahannebi (μg/g)

Vitamins and phytosterols	800 m	1000 m	1200 m
D-2	0.25±0.01	0.54±0.01[d]	0.61±0.01[d]
D-3	0.17±0.01	0.24±0.01[c]	0.30±0.01[d]
R-tocopherol	0.48±0.01	0.61±0.01[c]	0.95±0.01[d]
alpha-tocopherol	2.74±0.01	5.57±0.01[d]	5.87±0.01[d]
K-2	0.44±0.01	13.56±0.01[d]	15.44±0.01[d]
Stigmasterol	33.02±0.02	41.16±0.01[d]	53.49±0.01[d]
β-sitosterol	8.70±0.01	11.24±0.01[d]	13.21±0.02[d]

Data presented as mean±SEM of triplicate analysis c: $P<0.01$, d: $P<0.001$
*Comparisons and lettering were made according to 800 m altitude within the species.

Table 5: Vitamin and phytosterol concentrations of Kabarcık (μg/g)

Vitamins and phytosterols	800 m	1000 m	1200 m
D-2	0.44±0.01	0.33±0.01[d]	0.85±0.01[d]
D-3	0.18±0.01	0.11±0.01[d]	4.13±0.01[d]
R-tocopherol	0.53±0.01	0.28±0.01[d]	63.09±0.01[d]
alpha-tocopherol	21.89±0.01	5.24±0.01[d]	24.13±0.01[d]
K-2	1.52±0.01	1.01±0.02[c]	1.20±0.01[d]
Stigmasterol	11.21±0.01	10.78±0.01[b]	12.67±0.01[c]
β-sitosterol	14.27±0.01	10.24±0.01[a]	19.09±0.01[d]

Data presented as mean±SEM of triplicate analysis a: $P>0.05$, b: $P<0.05$, c: $P<0.01$, d: $P<0.001$
*Comparisons and lettering were made according to 800 m altitude within the species.

to 5.24 μg/g ($p<0.001$) in the sample of 1000 m. Therefore, the highest concentration of alpha-tocopherol was found in the sample harvested at 1200 m. This phenomenon was also seen in the vitamin K-2, stigmasterol, and β-sitosterol concentrations in Kabarcık grape. Indeed, the highest stigmasterol and β-sitosterol concentrations were found in the samples of 1200 m ($p<0.001$), as 12.67 μg/g, and 19.09 μg/g, respectively.

Table 6 shows the vitamin and phytosterol concentrations of Şilfoni grape. The highest vitamin D-2 level (0.89 μg/g, $p<0.001$) in Şilfoni grape was shown at 1200 m, while the highest vitamin D-3 (0.73 μg/g) concentration was determined at 800 m. Furthermore, the highest R-tocopherol (1.64 μg/g, $p<001$), alpha-tocopherol (7.30 μg/g, $p<001$), and K-2 (1.30 μg/g, $p>0.05$) concentrations were found in Şilfoni grape harvested at 1200 m, Therefore, the optimum altitude for the viticulture of Şilfoni grape is found as 1200 m in this study. However, to obtain the highest stigmasterol (98.34 μg/g) and β-sitosterol (86.90 μg/g) concentrations, the altitude of 800 m was determined as the optimum altitude.

The vitamin and phytosterol concentrations of Besni grape is shown in Table 7. The vitamin D-2 and D-3 concentrations were increased from low to high altitudes as 0.38 μg/g and 0.08 μg/g at 800 m, while they were obtained to be 0.79 μg/g and 0.35 μg/g at 1200 m, respectively

Table 6: Vitamin and phytosterol concentrations of Şilfoni (μg/g)

Vitamins and phytosterols	800 m	1000 m	1200 m
D-2	0.83±0.01	0.72±0.01[b]	0.89±0.01[d]
D-3	0.73±0.01	0.61±0.01[a]	0.51±0.01[d]
R-tocopherol	0.29±0.01	0.52±0.01[d]	1.64±0.01[d]
alpha-tocopherol	3.90±0.01	3.73±0.02 [c]	7.3±0.01[d]
K-2	1.29±0.01	1.23±0.01[a]	1.30±0.01[a]
Stigmasterol	98.34±0.01	67.48±0.01[d]	68.24±0.01[d]
β-sitosterol	86.90±0.01	15.09±0.01[d]	20.49±0.01[d]

Data presented as mean±SEM of triplicate analysis a: P>0.05, d: P<0.001
*Comparisons and lettering were made according to 800 m altitude within the species.

Table 7: Vitamin and phytosterol concentrations of Besni (μg/g)

Vitamins and phytosterols	800 m	1000 m	1200 m
D-2	0.38±0.01	0.63±0.01[b]	0.79±0.01[d]
D-3	0.08±0.01	0.16±0.01[d]	0.35±0.01[d]
R-tocopherol	1.04±0.01	1.21±0.01[d]	1.37±0.01[c]
alpha-tocopherol	3.17±0.01	3.29±0.01[c]	3.90±0.01[d]
K-2	1.65±0.02	0.24±0.01[d]	1.08±0.01[d]
Stigmasterol	14.04±0.01	37.06±0.02[d]	75.33±0.01[d]
β-sitosterol	11.11±0.01	22.61±0.02[d]	23.14±0.01[d]

Data presented as mean±SEM of triplicate analysis a: *P*>0.05, c: *P*<0.01, d: *P*<0.001
*Comparisons and lettering were made according to 800 m altitude within the species.

($p<0.001$). This phenomenon was also seen in R-tocopherol and alpha-tocopherol levels in Besni grape, to be the highest level at 1200 m, as 1.37 μg/g and 3.90 μg/g, respectively ($p<0.01$, $p<0.001$). Vitamin K-2 concentration, on the other hand, was determined to be the highest level in the samples at 800 m, however, the value was decreased at 1000 m, then significantly increased at 1200 m ($p<0.001$). The highest concentrations of stigmasterol and β-sitosterol were achieved at 1200 m, as 75.33 μg/g, and 23.14 μg/g, respectively ($p<0.001$). Therefore, in this study, 1200 m was found to be the optimum altitude for Besni grape to obtain high levels of selected vitamins and phytosterols.

Vitamin E concentrations in grape and grape products were widely studied. However, the change in vitamin E contents in grape cultivars from different altitudes has not been reported. Tangolar et al (2011) studied 15 grape varieties of Turkey, and reported the alpha-tocopherol, found as the predominant tocopherol fraction, ranged 4.692- 24.32 μg/g in seeds, 18.37- 50.61 μg/g in pomace, 23.09- 89.20 μg/g in bagasse, and 32.17-108.5 μg/g in stalks. In another study, alpha-tocopherol contents within the seeds of 21 grape varieties were reported as between 2.31-36.42 μg/g of which 36.42 μg/g belonged to the Besni grape (Seker et al., 2012). However, in our study alpha-tocopherol content of Besni grape cultivar was found to be 3.90 μg/g in 1200 m, which is much lower than the above-mentioned study. The highest alpha-tocopherol level in our study was shown in Kabarcik grape cultivar (24.13 μg/g).

The phytosterol content of grapes and grape seeds has been extensively studied. Simchuer and Srihanam reported mature grape seeds to possess 1980 μg/g β-sitosterol, and 690 μg/g stigmasterol, while immature grape seeds to contain 460 μg/g β-sitosterol, and 30 μg/g stigmasterol (Simchuer and Srihanam, 2018). The maturity of the grapes affected the content of phytosterols in that study. β-sitosterol and stigmasterol concentrations of grapes were also reported as 600-1120 μg/g and 20-120 μg/g, depending on the treatment and phenological stage (Ruggiero et al., 2013). The effects of altitude on the phytosterol composition of grape varieties have not been reported so far. In our study, the highest β-sitosterol and stigmasterol concentrations were obtained from Şilfoni grape at 800 m as 86 μg/g and 98 μg/g with no treatments. The high β-sitosterol and stigmasterol values may also be associated with the low lipid peroxidation content at this altitude.

Table 8 shows Pearson's correlation matrix of the results. It is clear from Table 8 that there is a positive correlation between vitamin D-3 levels and R-tocopherol (0.961) and alpha-tocopherol (0.640) levels. Also, a positive correlation between R-tocopherol and alpha-tocopherol levels (0.602).

Table 8: Pearson's Correlation matrix

Variables	D-2	D-3	R-tocop	alpha-tocop	K-2	Stigmast	β-sitost
D-2	**1**						
D-3	0,312	**1**					
R-tocop	0,149	**0,961**	**1**				
alpha-tocop	0,265	**0,640**	**0,602**	**1**			
K-2	-0,179	-0,137	-0,117	-0,098	**1**		
Stigmasterol	0,313	-0,168	-0,342	-0,339	0,096	**1**	
β-sitosterol	0,079	0,069	-0,039	-0,112	-0,118	**0,665**	**1**
MDA	-0,414	-0,043	0,103	0,198	-0,355	-0,452	-0,134

*Values in bold are different from 0 with a significance level alpha=0,05.

Priciple component analysis also revealed the same results (Fig. 1). Positive correlation between stigmasterol and beta-sitosterol levels (0.665) was also obtained via Pearson's correlation analysis (PCA). In PCA biplot results, Ağın beyazı variety harvested from different altitudes clustered together. Tahannebi variety samples also clusted together in biplot PCA analysis. Great differences in Kabarcık grape samples were revealed in PCA. On the other hand, Besni grape samples harvested at 800 m and 1000 m were clusted together, but not with the 1200 m sample. Also, Şilfoni grape harvested at 1000 m and 1200 m were clustered together, not correlated with Şilfoni 800 m Kabarcık grape from 800 m was clustered with MDA value, revealing a low antioxidant profile.

Numerous studies have shown a strong link between the use of medicinal plants and the prevention and treatment of a wide range of health issues, including cancer, cardiovascular disease, and neurodegenerative illness. Phenolic substances found in medicinal plants and foods have been shown to reduce the negative effects of chemically reactive species on normal physiological functioning in humans (Akbaba, 2021). This study shows a high antioxidant profile of grape seeds belonging to 5 different grape varieties harvested from different altitudes. Therefore, it shows the significance of environmental as well as genetic factors on the antioxidant activity of grape products.

Grape seeds are a rich source of phenolic compounds, phytosterols, vitamin E, vitamin C and, beta-carotene,and fatty acids (Bail et al., 2008) (Crews et al., 2006). Polyphenols, which are plentiful in grapes and their derivatives, have been identified as the chemical group responsible for the grape's health-promoting characteristics (Akbaba et al., 2021). Within grape products, grape seeds especially have been shown to possess high antioxidant activity (Anastasiadi et al., 2010). The chemical composition of the products may differ significantly within the variety, that is, each variety has a characteristic potential against environmental factors. This concentration can also vary according to the ripening rate of the grape, environmental factors (altitude, temperature, humidity, etc.), as well as viticulture practices (Dai et al., 2010) (Kumšta et al., 2014). In our

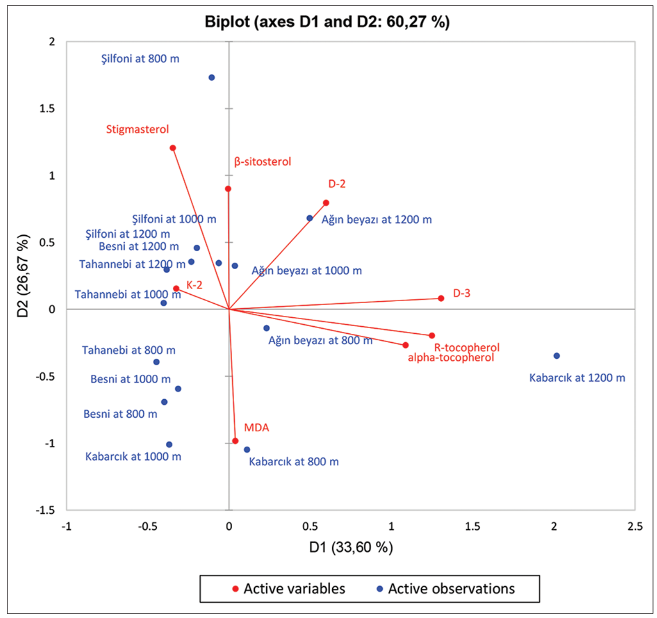

Fig 1. PCA biplot. *Active variables are MDA, vit K-2, D-2, D-3, T-tocopherol, alpha-tocopherol, stigmasterol, and beta-sitosterol. Active observations are grape varieties harvested at 800 m, 1000 m and 1200 m.

study, positive but weak correlations between vitamin E and MDA activity have been observed. Apart from these components, other important compounds such as phenolic compounds add antioxidant activity. In our previous study, the relationship of phenolic compounds in these 5 white grape species with DPPH analysis, which is an indicator of antioxidant activity was examined (Aydin et al., 2021). Therefore, antioxidant activity may be affected by multiple environmental factors (altitude, temperature, humidity, etc.), and the effects of many chemical compounds may be different. This difference can also be changed with the harvest time of the white table grapes, and environmental differences can also change the density of these chemical compounds.

CONCLUSION

In this study, the changes in the amounts of vitamins and phytosterols of 5 white table grape varieties depending on the altitude and the effect of this change on the lipid peroxidation content were investigated. The altitude affected lipid peroxidation contents, and vitamin and phytosterol concentrations of White grape varieties in different ways in this study.

In light of these results, grape seeds are suggested as good alternatives to synthetic antioxidants. Inspired by these properties, grape seed extracts are recommended to be used as a supplement or preservative in the food industry as well as in the health field, and also as a feed supplement in the livestock sector.

Authors' contrıbutions

In this study, N. Karaca Sanyürek and A. Çakır collected the plant materials and prepared the samples. S. Aydin and F. Erdem Erişir performed the analysis of vitamins and phytosterols. S. Aydın and E. Akbaba performed the MDA assay. Statistical analysis was performed by S. Aydin and E. Akbaba. The results were evaluated by all authors. The article was written by E Akbaba and S. Aydin.

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