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Araştırma Makalesi

Flavonoid and Phenolic Properties of Dried Seedless and Seeded Grape Cultivars (*Vitis vinifera* L.)

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ABSTRACT

In raisin cultivation, seeded (cvs. Gök Üzüm and Eksi Kara), parthenocarpic (cv. Black Corinth), and stenospermocarpic (cvs. Black Kishmish and Sultani Çekirdeksiz) grape varieties are used. Very little is known about the metabolic properties of raisins derived from these cultivars. According to the existing literature, certain metabolites including flavonoids and phenolic acids were studied in different raisin varieties. In this study, anthocyanidin, flavonol, flavone, flavanone, catechin, hydroxycinnamic acid, and hydroxybenzoic acid contents of 5 different grape cultivars were examined. As a result of the study, it was determined that phenolic acid and flavonoid contents were found to be significantly different among all varieties. Flavonol, catechin, and anthocyanin concentrations were higher in the cv. Gök Üzüm compared to other cultivars. As a result, when compared to seedless varieties, raisin varieties with seeds had a higher concentration of flavonoid capacity. In contrast, higher levels of most phenolic acids were detected in raisins produced from seedless varieties than in raisins produced from seeded varieties.

Key words: *raisins, flavonoids, phenolic acids, stenospermocarpic, seeded, parthenocarpic*

Kurutulmuş Çekirdeksiz ve Çekirdekli Üzüm Çeşitlerinin (*Vitis vinifera* L.) Flavonoid ve Fenolik Özellikleri

ÖZ

Kuru üzüm yetiştiriciliğinde çekirdekli (Gök Üzüm ve Ekşi Kara), partenokarpik (Black Corinth) ve stenospermokarpik (Black Kishmish ve Sultani Çekirdeksiz) üzüm çeşitleri kullanılmaktadır. Bu üzüm çeşitlerinden elde edilen kuru üzümlerin metabolik özellikleri hakkında çok az şey bilinmektedir. Mevcut literature göre farklı kuru üzüm çeşitlerinde flavonoidler ve fenolik asitler dahil olmak üzere belirli metabolitler üzerinde çalışılmıştır. Bu çalışmada, 5 farklı üzüm çeşidinde antosiyanidin, flavonol, flavon, flavanon, kateşin, hidroksisinamik asit ve hidroksibenzoik asit içerikleri analiz edilmiştir. Araştırmanın sonucunda, Fenolik asit ve flavonoid içeriğinin tüm çeşitler arasında önemli ölçüde farklılık oluşturduğu saptanmıştır. Gök Üzüm çeşidinin flavonol, kateşin ve antosiyanin konsantrasyonları diğer çeşitlere kıyasla daha yüksek bulunmuştur. Sonuç olarak, çekirdeksiz çeşitlerle karşılaştırıldığında, çekirdekli kuru üzüm çeşitleri daha yüksek konsantrasyonda flavonoid kapasiteye sahip olduğu bulunmuştur. Bunun aksine, çekirdekli çeşitlerden üretilen kuru üzümlere göre, çekirdeksiz çeşitlerde, fenolik asitlerin çoğunun daha yüksek seviyeleri belirlenmiştir.

Anahtar kelimeler: *kuru üzüm, flavonoidler, fenolik asitler, stenospermokarpik, çekirdekli, partenokarpi*

INTRODUCTION

In crop plants whose fruit is their commercial product, such as in the case of grape production, the parthenocarpy characteristic is crucial (Varoquaux et al., 2000). Pollination and fertilization are required for fruit set to succeed, however, the existence of seeds and their impact on hormone production stimulate fruit growth and cell expansion (Varoquaux et al., 2000). Cell division terminates due to ovary abscission, which is triggered by the lack of fertilization and pollination (Dauelsberg et al., 2011). Despite this, fertilization is not necessary for the ovary to mature, resulting in the appearance of seedless berries (Costantini et al., 2021). In seedless grapevine types, parthenocarpy and stenospermocarpy phenomena have been described (Pratt, 1971). Parthenocarpic kinds yield seedless berries, while stenospermocarpic variants complete ovule fertilization. Still, as the ovule integuments expand before ceasing, the embryo and endosperm abort, leaving tiny, primitive seed remnants in the ripe berry (Costantini et al., 2021). Parthenocarpic cultivars, such as Black Corinth or Black Currant, are characterized by small, spherical berries absent of seeds (Vargas, 2007; Royo, 2016; Costantini et al., 2021). Raisin growers are attracted to parthenocarpic grapevine varieties as they yield small-sized fruits that are suitable for raisin production while tackling environmental issues with fertilization and pollination (Lahogue et al., 1998).

In table grape breeding processes, the stenospermocarpic variety Sultanina, which is Thompson Seedless or called Sultani Çekirdeksiz in Türkiye, has been exploited as the main source of seedlessness (Adam et al., 2001; Ibáñez et al., 2009; Costantini et al., 2021). Berries from stenospermocarpic types have partially developed seeds; for commercial purposes, they are usually regarded as seedless (Costantini et al., 2021), though applying hormones to the berries may occasionally boost their size (Pérez & Gómez, 2000). To produce seedless table grape types, stenospermocarpic grapevine varieties are increasingly widely used (Rahman et al., 2021).

In Türkiye, grapes are grown for a variety of uses, including table grapes, wine grapes, grape juice, concentrated must, and other local products including vinegar. Türkiye has surpassed Uzbekistan, Brazil, Egypt, the United States, and Chile to rank as the world's third-largest producer of fresh table grapes, after China and India. In Türkiye, fresh grape cultivation accounts for most of the grape output, with the remaining third being dried for raisin manufacturing. Sultani Çekirdeksiz grapes are used to create most raisins in Türkiye. Other local grape types, such as cvs. Gök Üzüm, Eksi Kara, Black Kishmish, and Black Corinth grapes, are additionally used. Due to their great nutritional and physiological benefits, as well as their readily digested phenolic compounds and flavonoids, raisins are a popular dried fruit (Keskin et al., 2022). A comparative study conducted about the drying process with seeded and seedless cultivars showed that when compared to raisins made from seeded kinds, seedless varieties showed a higher quantity of several minerals, vitamins, and hormones as well as antioxidant capability. In contrast, the raisins made from seedless cultivars had lower amounts of most measured carbohydrates and amino acids than did the varieties of raisins with seeds (Kaya et al., 2022).

However, not much research has been done on how the fertilization of parthenocarpic and stenospermocarpic types affects the variations in biochemical components. To better understand these variations, this study examined the phenolic compounds and flavonoids of five raisin varieties: Black Corinth, a parthenocarpic variety; Sultani Çekirdeksiz and Black Kishmish, two stenospermocarpic varieties; and Eksi Kara and Gök Üzüm, two seeded varieties.

MATERIALS and METHODS

Plant Resources and Research Location

During the 2020 season, the study involved two stenospermocarpy cultivars (*Vitis vinifera* L. cv; Sultani Çekirdeksiz; Prime name: Sultanina, Variety number VIVC; 12051 and cv. Black Kishmish; Prime name: Kishmish Chernyi, Variety number VIVC; 6256); one parthenocarpic cultivar (*Vitis vinifera* L. cv; Black Corinth); and two seeded cultivars (*Vitis vinifera* L. cvs; Eksi Kara, Prime name: Eksi Kara, Variety number VIVC; 3852 and Gök Üzüm, Prime name: Goek Uezuem, Variety number VIVC; 4847). A vineyard established within the Manisa Viticulture Research Institute in Manisa provided the cvs. Sultani Çekirdeksiz, Black Kishmish, and Black Corinth grapes. On the other hand, the grapes for cvs. Gök Üzüm and Eksi Kara came from a vineyard at Selçuk University in Konya. The vines selected for this experiment ranged in age from 8 to 12 years, and they were placed 2.0 meters apart within rows and 3.0 meters between rows, yielding a plant density of about 1600 vines. Viticulture procedures including fertilization, pruning management, vine disease control, and irrigation were applied to both chosen vineyards. In summary, agricultural pesticides were sprayed while assessing the maximum residual levels and advised preharvest interval values. Vine pests and diseases were also managed. Based on the results of the soil analysis, fertilization was applied to the vineyards, and each vineyard's soil underwent four rounds of plowing.

The Desiccation Procedure

At a comparable technological maturity, the study's grapes were picked when their soluble solids concentration approached 22 °Brix. For every grapevine type, about 12 kg of fresh grapes were collected at their optimal maturity; three replications weighing 4 kg each were identified. The grape drying procedure followed commercial concerns and was comparable to that described by other authors (Keskin et al., 2022). The Manisa Viticulture Research Institute in Manisa, Türkiye, dried the grapes to make sure that every type of raisins was made using the same method as commercial raisins. Grapes from cvs. Eksi Kara, Black Corinth, and Black Kishmish were sun-dried without any prior preparations. After being soaked in a potassium carbonate solution, cv. Sultani Çekirdeksiz grapes were sun-dried. After being immersed in a wood ash solution, cv. Gök Üzüm grapes were dried under shade (Keskin et al., 2022). The trial's grapes were dried until they had a 13–15 % moisture level. Following, 500 g of each kind and replication of raisins were put into polyethylene bags and kept at -20 °C.

Analytical techniques for evaluating the phenolic acids and flavonoids in grape berries

The method devised by Pantelić et al. (2017) was slightly modified to determine phenolic acids. The measurement of polyphenols required the application of sophisticated analytical tools and exact separation methods. For this purpose, a mass spectrometer (TSQ Quantum Access Max triple-quadrupole with heated electrospray ionization (HESI) by ThermoFisher Scientific (Basel, Switzerland) was used in conjunction with an ultra-high performance liquid chromatography (UHPLC) system, specifically the Dionex Ultimate 3000 UHPLC equipped with a diode array detector (DAD). A ThermoFisher Scientific Syncronis C18 column from Bremen, Germany was used to accomplish the analytical separation. Two components made up the mobile phase for gradient elution were 100% acetonitrile and a 0.1% aqueous formic acid solution. A precise gradient pattern was followed during the elution process, which began at 5% for 2.0 min and increased over the next 10 min (from 2.0 to 12.0 min) from 5% to 95%. Then, in 0.1 minutes (from 12.0 to 12.1 min), there was a quick change from 95% to 5%, and the mobile phase stayed at 5% for the next three minutes. Every distinct phenolic component was measured by directly contrasting it with standards that are readily available in the market. The analysis's conclusions were given as the berry samples' amounts in milligrams per kilogram (mg/kg).

With few adjustments, the flavonoid analysis from berries was conducted using the methodology described by Brossa et al. (2009). In summary, 10 µL of each painstakingly filtered extract was added to the Waters Acquity UPLC (Milford, MA, USA) liquid chromatography (LC) equipment. A C18 column (3.5 µm; 100 × 2.1 Waters, Milford, MA, USA) operating at a steady temperature of 42 °C and a flow rate of 0.3 mL/min was used for the chromatographic separation. The Electrospray Ionization (ESI) mode was used for flavonoid detection and quantification. Anthocyanins were detected using positive ionization (UPLC-(ESI+)-MS/MS), while all other flavonoids were detected using negative ionization (UPLC-(ESI-)-MS/MS). A range of 100 to 900 atomic mass units per charge (amu/z) was used to record the spectra. Furthermore, flavonols were detected at 365 nm and 520 nm, respectively, in the UV/visible spectrum.

Statistical Analysis

The statistical package included with R Studio was used for all the descriptive analyses in this study. An extensive analysis of variance (ANOVA) was conducted, once more utilizing the capabilities of the statistical package within R Studio, to thoroughly evaluate the impact of the cultivar (five levels) on the levels of phenolic acids and flavonoids. Effects were included in the statistical model, and the data were then checked for compliance with normality assumptions. Two separate models were painstakingly built to assess the main influences of cultivars on the levels of flavonoids and phenolic acids. When an ANOVA revealed statistical significance, post hoc analysis was carried out with Tukey's test, which is a well-used and accepted technique for the thorough investigation of differences between several groups. Principal Component Analyses (PCAs) were used to provide further understanding and depict the correlations between different variables. This analysis method was performed for flavonoids and phenolic acids using ggplot2 in R Studio. By using PCA, multidimensional data can be reduced to a more understandable format, making it possible to find underlying patterns and trends in even the most complicated datasets. The heatmap produced by the pheatmap package made it easier to visually investigate any correlations and variations (R Core, 2013).

FINDINGS and DISCUSSION

The content of flavonoids and phenolic acids in the cvs. Black Corinth, Eksi Kara, Sultani Çekirdeksiz, Gök Üzüm, and Black Kishmish for the season 2020 were evaluated. The content of anthocyanidins in raisin grapes was aligned from 16.67 mg/kg in cv. Gök Üzüm to 4.66 mg/kg in cv. Sultani Çekirdeksiz. The most flavonols content was in cv. Black Kishmish (312.00 mg/kg) while the least flavonols content was in cv. Sultani Çekirdeksiz

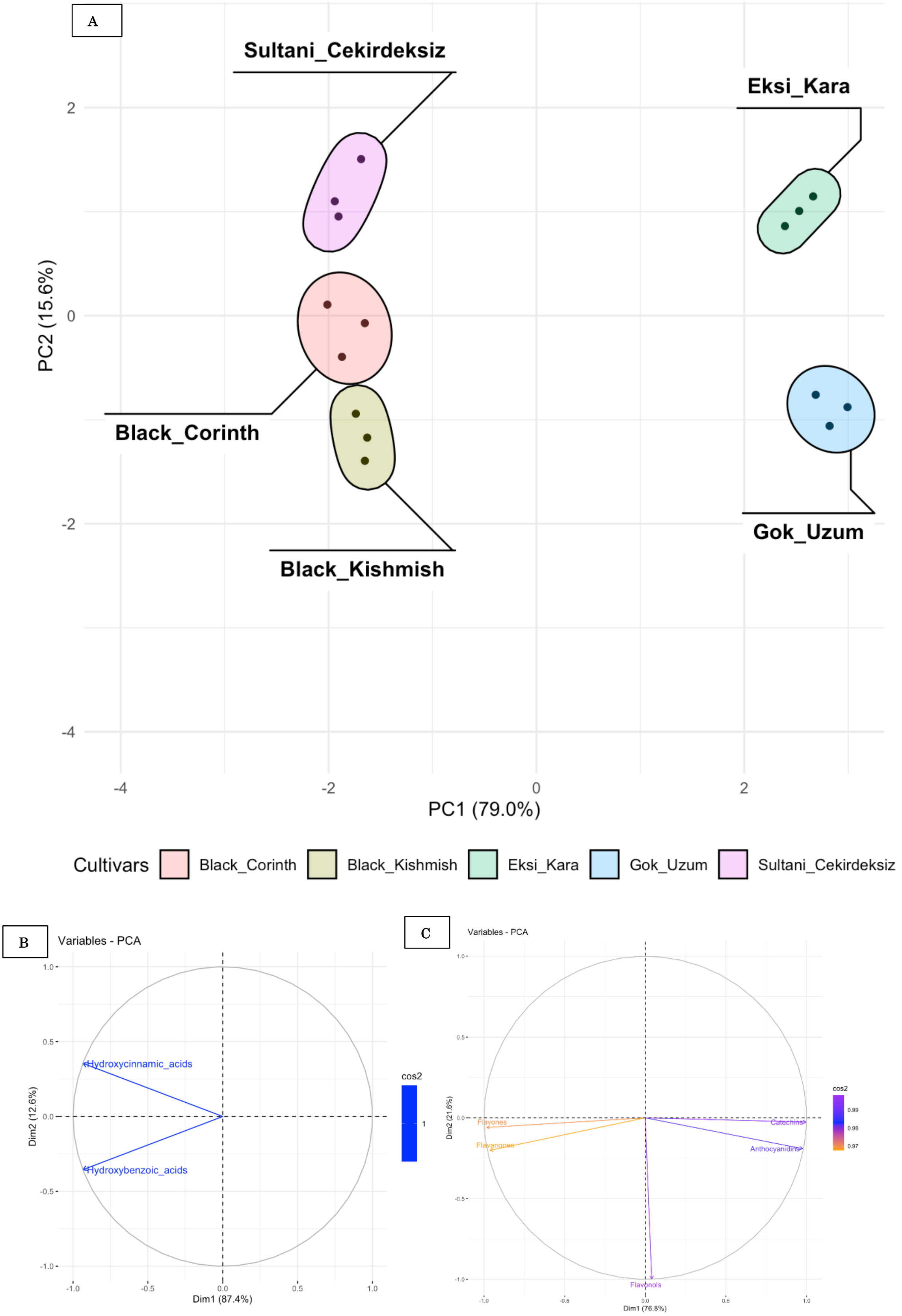
(225.00 mg/kg). Flavone content varied from 147.00 to 19.70 mg/kg (cvs. Black Kishmish and Ekşi Kara, respectively). Flavanones content aligned from 75.00 mg/kg in cv. Black Corinth to 21.80 mg/kg in cv. Ekşi Kara. Catechins content varied from 2321.00 to 98.00 mg/kg (cvs. Gök Üzüm and Black Kishmish, respectively). Hydroxycinnamic acids content varied from 6.22 mg/kg in cv. Black Kishmish to 1.25 mg/kg cv. Eksi Kara. Hydroxybenzoic acid content aligned from 4.30 mg/kg in cv. Black Corinth to 0.67 mg/kg in cv. Eksi Kara (Table 1). Regarding cultivars, Dim1 and Dim2 mention 79.0% and 15.6% of the variance, respectively. Two seeded cultivars (Eksi Kara and Gök Üzüm) are close to each other and on the positive axis of Dim1 while three seedless ones (Black Corinth, Sultani Çekirdeksiz, and Black Kishmish) were located at the axis side of Dim1, were illustrated. The latter leans at the positive axis of Dim2 while the third one is at the negative axis of Dim2 (Figure 1A). For phenolic acids, Dim1 and Dim2 states for 87.4% and 12.6% of the variance, respectively. Hydroxycinnamic acids and Hydroxybenzoic acids, the two dominant phenolic acids, were illustrated. The latter leans at the positive side of Dim2 while the former is at the positive side of Dim1 (Figure 1B). For flavonoids, Dim1 expresses 76.8% of the variance while Dim2 states 21.6% of the variance. Flavonoids like anthocyanidin and catechins are located on the positive side of Dim1 while flavonoids and Flavonols are located on the negative side of Dim1 (Figure 1C). Figure 1D shows the hierarchically clustered heatmap in different kinds of grape berry components by cultivars. The heatmap demonstrates the relative content of phytochemicals which are phenolic acids and flavonoids. They are clustered at the bottom of the heatmap. The differences and similarities in the components of phenolic acids and flavonoids resulted from the distancing clusters. At the bottom of the heatmap, anthocyanidins, flavonols, flavones, and flavanones had one group while anthocyanidins, hydroxycinnamic acids, and hydroxybenzoic acids had another group. Two different groups were conducted among genotypes, which is one group is cvs. Gök Üzüm and Eksi Kara, and the other group cvs. Black Corinth, Sultani Çekirdeksiz, and Black Kishmish suggested similar phytochemical compositions because of exhibited their close clusters (Figure 1D).

Table 1. Flavonoids (mg/kg) and Phenolic acids (mg/kg) Ekşi Kara, Gök Üzüm, Black Corinth, Sultani Çekirdeksiz and Black Kishmish in 2020 season.

	Ekşi Kara	**Gök Üzüm**	**Black Corinth**	**Sultani Çekirdeksiz**	**Black Kishmish**	**p-values**	**Significance**
Flavonoids (mg/kg)							
Anthocyanidins	13.29±0.55b	16.67±0.63a	6.44±0.07c	4.66±0.03c	6.74±0.10c	2.1e-08	***
Flavonols	234.00±6.55c	305.00±5.30a	276.00±4.50b	225.00±5.70c	312.00±6.32a	1.18e-06	***
Flavones	19.70±3.50c	22.40±4.20c	123.00±5.40b	144.70±5.32a	147.00±4.60a	1.29e-10	***
Flavanones	21.80±2.30c	25.00±2.45c	75.00±3.65a	60.70±3.55b	69.70±3.33ab	2.54e-08	***
Catechins	1893.00±15.60b	2321.00±13.55a	127.00±8.32c	107.00±3.50c	98.00±3.30ac	<2e-16	***
Phenolic acids (mg/kg)							
Hydroxycinnamic acids	1.25±0.25c	1.54±0.30c	4.63±0.21b	5.63±0.34a	6.22±0.54a	1.23e-08	***
Hydroxybenzoic acids	0.67±0.08d	0.82±0.09d	4.30±0.02a	3.29±0.08 b	2.30±0.07 c	1.83e-10	***

* Data are stated as averages of the data and their standard deviations. Different letters within a row indicate significant differences (Tukey test, ***, Significant at p-value < 0.001).

The phenolic acids and flavonoids of grapes from the cvs. Eksi Kara, Gök Üzüm, Black Corinth, Sultani Çekirdeksiz, and Black Kishmish varieties are thoroughly analyzed in this study. The analysis of different flavonoids and phenolic acids provides insight into the complex interactions between cultivar types (Table 1). We discussed how our results aligned and differed from earlier studies to critically analyze our findings and determine their importance and consequences. The current study's most notable conclusion is that the cultivar has a significant impact on the amount of these flavonoid components. This observation is consistent with earlier studies on the subject. For instance, research conducted by Ali et al. (2011) and by Roxana et al. (2020) examined the phytochemical profiles of various grape cultivars and discovered that notable differences in flavonoids were present among the kinds. This is in line with the results of the current study, which showed that cultivar variations in flavonoids and phenolic acids were significantly different. These variances are a result of a variety of factors, including genetic diversity, environmental conditions, and the activation of particular metabolic pathways within distinct grapevine kinds.



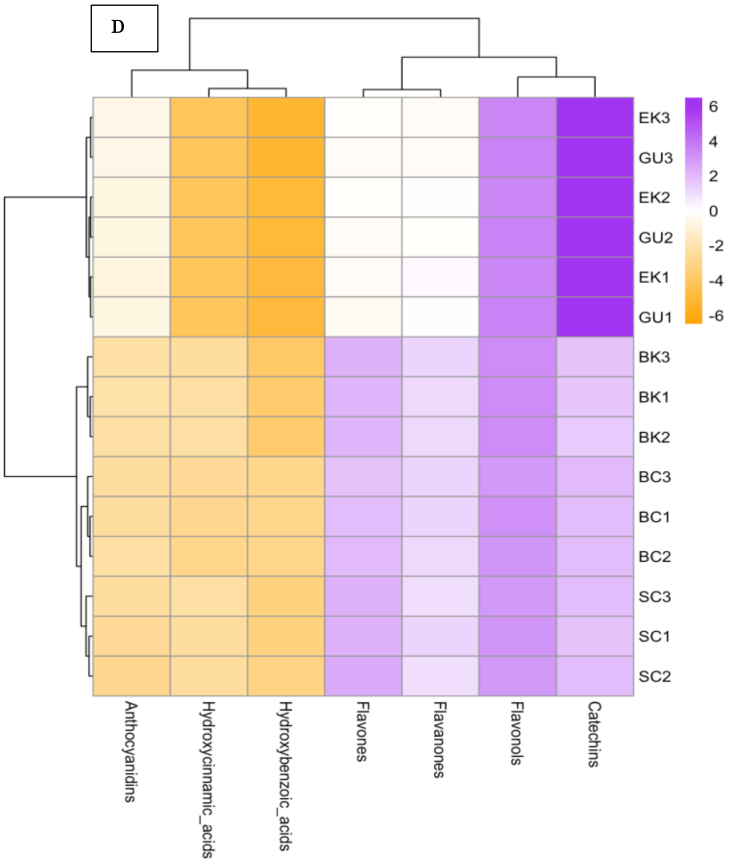


Figure 1. PCAs biplots are colored by Cultivars including Phenolic acids and flavonoids (Figure 1A). All Phenolic acids (Figure 1B) and Flavonoids (Figure 1C) content are demonstrated. Performed a heatmap analysis that scrutinizes the components of Phenolic acids and Flavonoids are illustrated (Figure 1D).

Numerous research in the field have focused on the intricate interactions between these variables. The reported variations in grape flavonoids are also influenced by environmental factors, including soil composition, climate, and vineyard management techniques. The impact of climate on the production of flavonoids is highlighted in the study by Harb et al. (2015), which also emphasizes how different grape types respond to environmental conditions differently, resulting in variations in compound levels. This emphasizes how crucial it is to consider the grape-growing environment when figuring out these chemicals' makeup. On the other hand, the flavonoid and phenolic acid profiles of different grapevine species are greatly influenced by genetic variation. Distinct genetic traits among cultivars influence the production and accumulation of different flavonoid and phenolic acid molecules. This is consistent with the findings of He et al. (2010) and Jeandet et al. (2012), who highlighted the genetic foundations of these variances by reporting considerable variability in flavonoids and phenolic acids among various grape varieties. The current study's most notable discovery is the cultivar's significant influence on the amount of these flavonoids and phenolic acid components. This observation is consistent with earlier studies on the subject. According to studies conducted by Yang et al. (2009) and Katalinić et al. (2010), for instance, diverse grape cultivars showed notable differences in their flavonoid profiles when their profiles were examined. This agrees with the results of the current investigation, which showed that there are substantial variations in phenolic acids and flavonoids amongst the current cultivars. These variances are a result of a variety of factors, including genetic diversity, environmental conditions, and the activation of metabolic pathways within distinct grapevine kinds. The current study shows substantial differences in anthocyanidin content between the grape types when cultivar differences are considered. Cv. Gök Üzüm regularly shows a greater level of anthocyanins (16.67 mg/kg), flavonols (305.00 mg/kg), and catechins (2321.00 mg/kg) (Table 1) without considering their age and ecologies. This result is consistent with earlier studies. For instance, Costa et al. (2014) found that there are differences in the anthocyanin concentration of grape cultivars, with some having a higher anthocyanin content than others. Given that different grape types have distinct pathways for the manufacture of anthocyanins, the observed variances can be explained by genetic variables. These results highlight how crucial it is to take cultivar selection into account when trying to increase the amount of a particular flavonoid in grape products. The present study employed sophisticated multivariate analytical techniques, including PCA biplots and hierarchical clustering heatmaps, to effectively visualize the complex relationships and distribution of flavonoids and phenolic acid components among various grape cultivars. A thorough understanding of the interrelationships between the different flavonoid and phenolic acid components in the dataset was offered by the PCA biplots. According to the PCA biplots, several chemicals have a substantial association with the principal components in this study, which indicates how important these compounds are for describing the flavonoid and phenolic acid composition of grapes (Figure 1A, B). This implies that these substances have a major role in the observed variations and parallels among grape samples. Conversely, heatmaps including hierarchical clustering provided an extra level of information about the data (Figure 1C). Heatmaps were used to visually arrange similar samples based on components of phenolic acids and flavonoids and find clusters of samples with similar makeup. In the end, this technique helped identify patterns and trends in the dataset, leading to a better comprehension of the connections between the cultivars, or parts of berries. Pietrafesa et al. (2023) underlined the significance of PCA and hierarchical clustering heatmaps in their study of multivariate analysis of flavonoid data in plant sciences, supporting its use in examining complex datasets. Their study emphasizes how useful these methods are for clarifying links and patterns in intricate biological information.

CONCLUSION

This extensive study carefully monitored the changes in flavonoids and phenolic acids in the grape cultivars Black Kishmish, Black Corinth, Black Corinth, Sultani Çekirdeksiz, and Ekşi Kara. Numerous phenolic acid and flavonoid molecules were analyzed, which helped to clarify the complex chemistry involved in grape development. The study found that the phenolic acid and flavonoid content of all grape varietals changed significantly, with notable variations observed in the components of catechins, flavones, flavanones, anthocyanidins, flavonols, hydroxycinnamic acid, and hydroxybenzoic acid. The grape cultivar has an impact on these substances, which are well-known for their contributions to grape color, quality, possible health advantages, and nutritional value. Cv. Gök Üzüm continuously showed larger levels of anthocyanins, flavonols, and catechins, highlighting the cultivar's significance. This study essentially highlighted the complex interactions between grape cultivar selection, flavonoid composition, and phenolic acid content. Although the ecological conditions and ages of the varieties were not taken into account in this study, in future studies, both the ages of the varieties and their similar ecology will yield more distinct results for comparison. To maximize grape-based products and deepen their understanding of grape phytochemistry, researchers, winemakers, and grape producers can greatly benefit from these results, which will further the continuous progress of the agricultural and viticultural sectors.

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