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Changes in Biochemical and Bioactive Compounds in Two Red Grape Cultivars during Ripening and Cold Storage

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Abstract: The market value of grapes (*Vitis vinifera* L.) is affected by their quality and harvesting time. Veraison and stage of ripening are the most important factors determining table grape quality. Therefore, the present research was performed to determine the effects of various ripening stages on the combination and postharvest quality of two red grape cultivars. Based on color change, fruits were harvested at the veraison stage, two weeks after the veraison stage, and at the full ripening stage. Fruits harvested at the fully ripe stage were stored for 7, 14, and 21 days (at 1 °C with 85–90% relative humidity). The greatest soluble solids content (SSC) (16.1%) and SSC/TA ratio were observed in 'Khoshnav', while the greatest titratable acidity (TA) (0.45%) and pH (3.60) were observed in 'Rashah'. The results of the present study showed that regardless of the storage period, fruits harvested at time V (veraison time) and two weeks after veraison (2WAV) had significantly higher firmness and vitamin C content. 'Khoshnav' had the lowest weight loss (2.05%), and 'Khoshnav' and 'Rashah' had the greatest firmness (5.95 N) and vitamin C content (89.48 mg 100 g^{-1} FW). The greatest anthocyanin content was observed on day 7 of storage in 'Rashah'. Total phenol (TP) and total flavonoid (TF) contents and antioxidant capacity (AC) increased significantly until harvest and decreased thereafter during cold storage. 'Rashah' showed the greatest AC, TP, and TF values. Based on the findings of this study, the 'Rashah' grape cultivar is shown to be rich in vitamin C, anthocyanins, flavonoids, phenolics, and antioxidants. It holds potential for utilization in both processing and breeding programs as a functional food ingredient.

Keywords: table grapes; harvest; veraison; phenolic compounds; vitamin C; firmness

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1. Introduction

Fruits like grapes need to be harvested at a specific stage of ripeness, which also affects their quality [1,2]. Soluble solids content (SSC) and titratable acidity (TA) are parameters commonly used to assess the internal quality of table grapes. The ratio of these values, known as the ripeness index, is widely used in the industry to determine the appropriate time for fruit harvesting and marketing [2]. However, the practical effectiveness of this index is limited. The color of fruits, particularly red table grapes, strongly influences consumer acceptance [3].

The veraison period is a critical stage in the growth and maturation of grape berries, marking the beginning of the important ripening processes. During this phase, various transformative changes occur, including sugar accumulation, acidity reduction, pigment emergence, and volatile aroma synthesis [4]. One significant transformation during veraison is the synthesis of anthocyanins, pigments responsible for the vibrant colors observed in grape skins. These compounds are initially produced during veraison and continue to increase throughout ripening [5]. The development of anthocyanins contributes to the visual appearance of grapes, with hues ranging from pale green or yellow to deep red, purple, or even blue, depending on the grape variety. Beyond color changes, veraison leads to complex modifications within the grape berries themselves [5]. Alterations in cell walls become apparent, accompanied by the accumulation of various secondary metabolites in the pulp and peel. These changes play a pivotal role in shaping the texture, flavor, and nutritional composition of grapes.

Fruit ripening occurs in the later stages of maturity and involves a series of physiological, biochemical, and sensory changes that lead to the development of an edible ripe fruit with desirable quality parameters [2]. The accumulation of sugars and the reduction in acidity during veraison have a profound impact on the grape's taste profile. As the berries progress through this phase, the sugar content steadily rises, lending a pleasant sweetness to the fully ripened fruit. Simultaneously, the levels of acidity decrease, resulting in a more balanced and mellow flavor profile. In addition, grapes contain bioactive antioxidants such as vitamins and phenolic compounds that have beneficial effects on human health [6]. Anthocyanins, a type of phenolic compound, play an important role in the color of all red, purple, and dark purple varieties of *Vitis vinifera* intended either for fresh consumption or for wine production [6,7]. During ripening, chlorophyll degradation and anthocyanin accumulation result in red, purple, and black grapes [8]. In red grapes, it is important to consider their color- and flavor-providing components. Phenolic compounds, especially flavonoids, are well known as they are responsible for the color of wines.

In the field of food production, there is a growing interest in developing products with health-promoting ingredients, such as those with strong antioxidant capacity, to meet consumer demand for the relationship between nutrition and health [9]. Studies have shown that free radicals cause oxidative damage to lipids, proteins, and nucleic acids [10]. Antioxidants play a crucial role in neutralizing these free radicals to prevent diseases, as reported by Chaudhary et al. (2023) [11]. The increasing interest in antioxidants and their impact on human health has led to extensive research in the field of food science. The concentration of various compounds in grape berries depends on factors such as developmental stage, vegetative vigor, genetics, the environment, and viticulture practices [2,5,12].

Understanding the intricate processes that unfold during veraison is of paramount importance to grape growers, viticulturists, and winemakers. This knowledge empowers them to make informed decisions regarding the optimal timing of harvest, ensuring that the grapes are harvested at their peak of flavor and quality. Table grape is a non-climacteric fruit with a low rate of physiological activity but which is subject to serious physiological deformations after harvest and during long-term storage. Forms of quality loss include weight loss, color change, berry softening, and panicle browning, resulting in reduced shelf life and overall quality [13]. Winemakers, viticulturists, and grape growers must comprehend the complex processes that take place during veraison. With this information, they can decide when to harvest grapes most effectively, bringing the grapes to their fullest flavor and quality. Although grapes are not climate-sensitive and have a modest rate of physiological activity, they can develop severe physiological issues both after harvesting and during prolonged storage. Weight loss, color shift, berry softening, and panicle browning are examples of forms of quality loss that lower grapes' shelf life and overall quality [8,13]. Various antioxidant chemicals, including flavonoids, phenolics, and anthocyanins, are involved in fruit ripening and postharvest life. However, several variables, like ventilation, atmospheric gas composition, temperature, relative humidity during storage, etc., affect how quickly quality degrades. Furthermore, there is a lack of information regarding the biochemical and bioactive behavior of the red grape varieties examined in this research at different ripening stages and during cold storage. Thus, the primary goal of this study was to ascertain the alterations in biochemical and bioactive components in two red grape cultivars, "Khoshnav" and "Rashah," picked at various phases of color development, both during cold storage and upon harvest.

2. Material and Methods

2.1. Plant Source and Sampling

Grapevines (*Vitis vinifera* L.) of two cultivars, 'Rashah' and 'Khoshnav', grown in a commercial vineyard (Sanandaj, Iran) received ordinary horticultural care in terms of fertilization, irrigation, and soil management. In this area, the average temperature in spring and summer is 15–20 °C and 25–30 °C, respectively. The average annual temperature is 15 °C, and the average annual rainfall is 445 mm. The minimum temperature in winter is −26 °C, and the maximum temperature in summer is 46 °C.

Sampling was conducted at the stage of veraison, two weeks after veraison (2WAV), and at harvest. The fruits were immediately taken to Kurdistan University, Sanandaj, and graded to ensure that the fruits were of uniform size and had no defects and were then used for further assessments. After the last round of sampling, the fruit of each cultivar was divided into 12 groups of five clusters, packed in boxes, and stored in cold storage at 2 °C and 90% relative humidity. Stored fruit were sampled at 7, 14, and 21 days and used for similar evaluations.

2.2. SSC, TA, and pH Measurements

The pH of fruit juice was measured using a pH meter (Metrohm 827, Herisau, Switzerland). TA was measured using a composite sample of 10 berries, which were juiced and analyzed together. Aliquots of 10 mL were titrated to pH 8.1 with 0.1 N NaOH and expressed as % tartaric acid [14]. The juice was also used to measure SSC using an Atago digital refractometer (Brix 0–32%, Atago, Tokyo, Japan) [15].

2.3. Weight Loss (WL)

The weight of all fruits was measured before storage (W0) and at each sampling time (Wt). WL was expressed as a percentage of fresh weight using the following equation:

$$WL\ (\%) = (W0 - Wt)/(W0) \times 100$$

2.4. Berry Firmness

The firmness of the pulp was measured on the peeled sides of each fruit using a penetrometer (Santam STM-1, KM-1; Fujiwara Scientific Company, Ltd., Tokyo, Japan) equipped with a 3 mm probe, and the fruit size was about 20 mm in both diameter and length for 'Rashah' cultivar and 22 mm in diameter and 23 mm in length for 'Khoshnav'. Values were expressed in Newtons (N).

2.5. Vitamin C Assay

The same extracted juice used in Section 2.2 was used to assay vitamin C and anthocyanin content. Vitamin C content was determined by titration with 2,6 dichlorophenolindophenol (DCPIP) [16] and expressed as mg of vitamin C per 100 g fresh weight (FW). Ascorbic acid reacts with DCPIP, and the grape juice turns pink.

2.6. Anthocyanin Content

Anthocyanin content was determined by the pH differential method. Absorbance was measured with a spectrophotometer (UV-S2100) at 510 nm and 700 nm in buffers of pH 1.0 and 4.5 [17]. Results were expressed as milligrams of pelargonidin-3-glucoside equivalent in grape extract per 100 g FW. The difference in absorption between buffer systems was calculated according to Equation (1):

$$A = (A510 - A700\ nm)\ pH1.0 - (A510 - A700\ nm)\ pH4.5 \quad (1)$$

Then, the concentration of total anthocyanin (mg/L) was calculated according to Equation (2):

$$A\ (mg/L) = A \times MW \times DF \times 1000/\varepsilon \quad (2)$$

where:

MW: Molecular weight of pelargonidin 3-glucoside = 433.39 g/mol;
DF: Dilution factor = 10;
ε: Coefficient of molar absorptivity = 22,400.

2.7. Total Phenol (TP) Assay

TP concentrations were measured by homogenizing 0.7 g of frozen tissue from each replicate with 2 mL of ice-cold 1% HCl methanol solution and then centrifuging at 12,000$\times$ g for 10 min at 4 °C. The supernatant was collected and used to determine total phenols and antioxidant capacity [17]. The TP content in the extracts was determined by the Folin–Ciocalteu assay and using gallic acid (range of 0.05 to 0.25 mg mL^{-1}) as a standard curve. Results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g^{-1} FW.

2.8. Total Flavonoid (TF) Assay

TF content was measured as previously described [18]. Briefly, 1 mL of diluted methanol extract was mixed with 1 mL of reagent (2% $AlCl_3$ $6H_2O$ in methanol), and absorbance was measured 10 min later at 430 nm. Quercetin (Sigma, Burlington, MA, USA) was used as a standard, and results were expressed as milligrams of quercetin equivalents (QE) per 100 g^{-1} FW.

2.9. Antioxidant Capacity (AC) Assay

Antioxidants were extracted from the grape sample using the same protocol described above for phenol extraction. AC was determined using the 2,2-diphenyl-1-picryl-hidrazil (DPPH) radical scavenging method, as described by Koushesh Saba et al. (2012) [17]. Absorbance was measured at 517 nm using a spectrophotometer (UV-S2100). Total antioxidant activity was expressed as percent inhibition of DPPH radical and determined according to the following equation:

$$AC = \frac{\text{ABS Sample} - \text{ABS Control}}{\text{ABS Control}} \times 100$$

where AC is the total antioxidant activity and ABS is the absorbance.

2.10. Statistical Analysis

Data for analytical determinations were subjected to analysis of variance (ANOVA). Sources of variation were storage time (days) and cultivars. A least significant difference (LSD) test with $p \leq 0.05$ was used to compare the means between cultivars at each sampling. Pearson correlation was used to evaluate the relationship between the measured parameters. All analyses were performed using SAS software (version 9.4).

3. Results and Discussion

3.1. Biochemical Quality Attributes

A linear increase in SSC and pH was observed in both grape cultivars with ripening and during cold storage (Figure 1A,B). The SSC was significantly different in the two cultivars, with 'Khoshnav' showing a higher SSC value (19.37 °Brix). The 'Khoshnav' cultivar indicated the highest pH on S2 but was not significantly different from S3 during cold storage.

A decreasing trend in TA was found in both cultivars during ripening and cold storage (Figure 1C). The two grape cultivars showed fluctuations in TA, which initially reduced from veraison to harvest time and then stayed constant up to last day of storage. In contrast, the SSC: TA ratio increased during ripening and cold storage (Figure 1D).

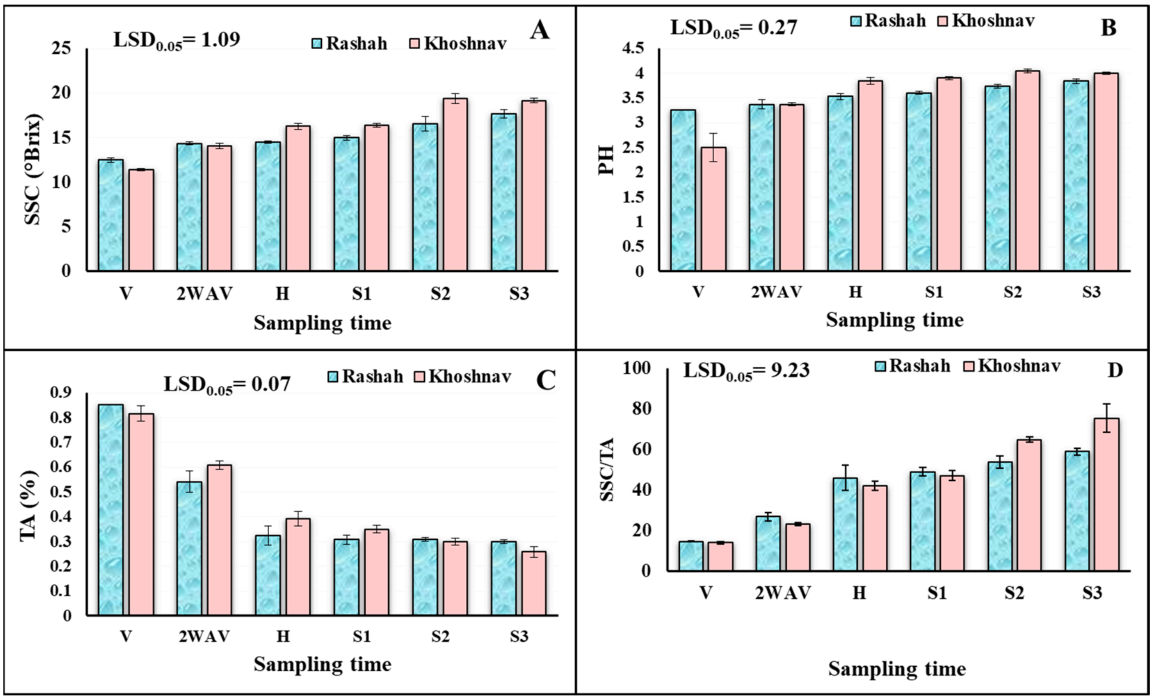

Figure 1. Changes in SSC (**A**), pH (**B**), TA (**C**), and SSC/TA (**D**) in grape cultivars 'Rashah' and 'Khoshnav' during fruit ripening and cold storage. V: veraison time; 2WAV: two weeks after veraison; H: harvest; S1, S2, and S3: 7, 14, and 21 days at 2 °C, respectively. Data are means ± SE (n = 3).

The combination of sugars in grapes is primarily determined by genotype, while sugar concentration is strongly influenced by environmental factors and crop management [2]. After veraison, high concentrations of glucose and fructose accumulate, while organic acid content decreases and berries soften [18]. Our findings align with previous studies that reported a gradual increase in SSC and pH, as well as a decrease in TA, in table grapes of various cultivars during various maturity stages and during cold storage [19]. The acidity of grape berries at harvest depends on the concentration ratio between free organic acids and their potassium salt forms, which increases during ripening and is an important quality characteristic [2]. The acid–sugar ratio, commonly known as the ripeness index, is widely used for commercial purposes as a quality criterion for proper fruit harvesting and marketing. During cold storage, the increase in SSC is likely due to water loss from the berries, while the reduction in TA may be attributed to malate and pyruvate decarboxylation.

3.2. WL and Firmness

A significant linear increase in fruit WL was observed in both grape cultivars during cold storage, with 'Khoshnav' exhibiting a lower increase (2.05%) compared to 'Rashah' (2.46%) (Figure 2A). Previous studies have also reported an increase in fruit WL in table grapes at different maturity stages [19]. Crisosto et al. (2001) [20] highlighted substantial water loss in table grapes during postharvest handling. Fruit WL continues to increase linearly with extended storage time due to water loss and respiration [21]. Ripe berries are protected by a waxy cuticle layer on their surface, which helps prevent moisture loss [22]. Visible fruit quality is affected by moisture loss, leading to loss of turgidity and subsequent fruit softening [20]. In our study, fruit harvested at the third stage displayed more withering and shriveling, likely due to higher water loss. Similar findings regarding berry WL have been reported in different studies [23,24].

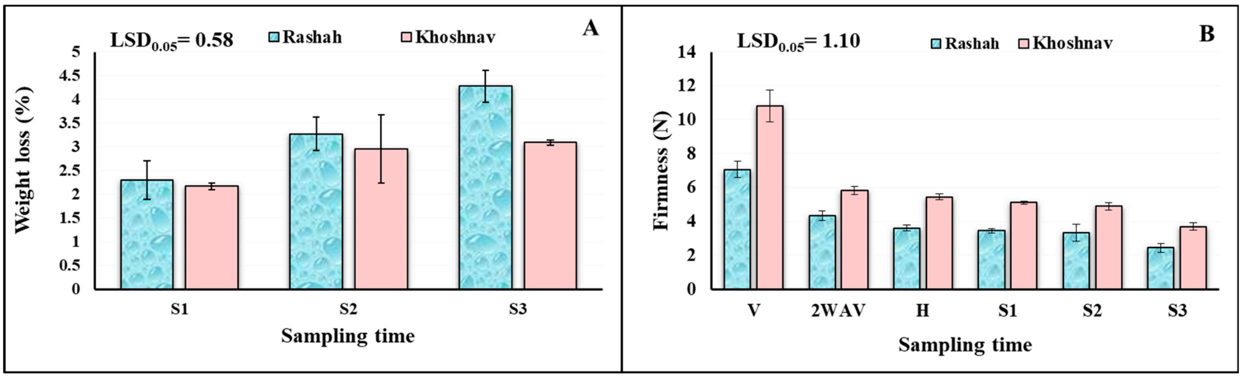

Figure 2. Changes in weight loss (**A**) and firmness (**B**) in grape cultivars 'Rashah' and 'Khoshnav' during fruit ripening and cold storage. V: veraison time; 2WAV: two weeks after veraison; H: harvest; S1, S2, and S3: 7, 14, and 21 days at 2 °C, respectively. Data are means ± SE (n = 3).

The study also found that the highest berry firmness was observed during veraison, followed by a decrease after 2WAV. After this initial decrease, the firmness remained stable in both cultivars throughout the sampling periods. The 'Khoshnav' cultivar consistently exhibited higher fruit firmness (5.95 N) compared to the 'Rashah' cultivar (4.03 N) at all sampling times (Figure 2B).

A previous study by Khalil et al. (2023) [19] supports these findings, reporting that the firmness of berries harvested at different ripeness stages did not significantly differ but showed a significant linearly decreasing trend during cold storage. Firmness is an important quality parameter used to determine fruit maturity, optimal harvest timing, and postharvest fruit quality grading. It plays a crucial role in fruit evaluation. Another study conducted by Lo'ay et al. (2017) [24] observed a negative correlation between fruit firmness and moisture loss. In line with their findings, the present study also demonstrated that 'Khoshnav' grapes had greater firmness, while the WL was lower. Interestingly, in our study, grapes exhibited an increase in WL from harvest to the end of storage, while firmness decreased. Similar trends of decreased grape firmness during storage have been reported in other studies [24,25]. These findings highlight the importance of firmness as a quality parameter for assessing fruit maturity, determining harvest timing, and evaluating postharvest fruit quality. The 'Khoshnav' cultivar consistently displayed higher firmness compared to 'Rashah', while the firmness of grapes decreased over time during storage, accompanied by an increase in weight loss. These findings contribute to our understanding of fruit quality dynamics and provide insights for the cultivation, harvesting, and postharvest handling of table grapes.

3.3. Vitamin C

The highest vitamin C content was observed at the veraison stage, and then it decreased significantly during ripening and cold storage (Figure 3). However, the rate of decline was influenced by cultivar, with the 'Rashah' grape showing significantly higher vitamin C content. These findings are consistent with reports on raspberry and blackberry cultivars [26], which are considered excellent sources of vitamin C. The decrease in vitamin C levels may be attributed to its chemical properties, as it is readily oxidized in aqueous medium and in the presence of heavy metals. Generally, fresh fruits contain more vitamin C than cold-stored ones, and the extent of vitamin C loss varies greatly across different fruits and vegetables. Prolonged storage, higher temperatures, low relative humidity, physical damage, and exposure to cold can exacerbate the loss of vitamin C [27].

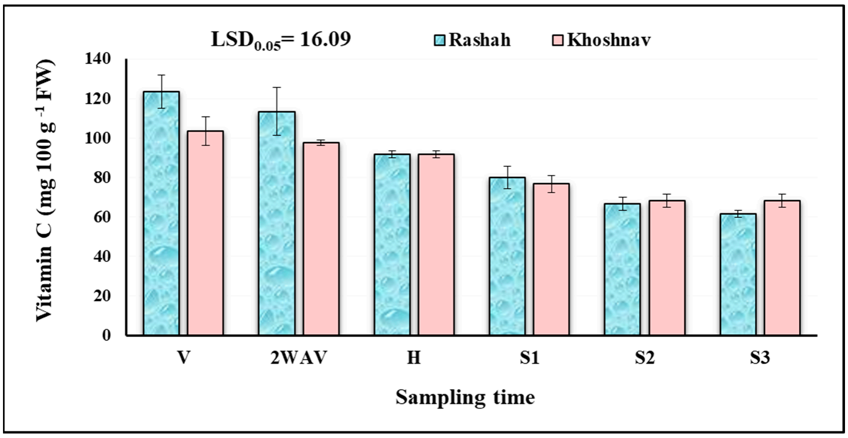

Figure 3. Changes in vitamin C in grape cultivars 'Rashah' and 'Khoshnav' during fruit ripening and cold storage. V: veraison time; 2WAV: two weeks after veraison; H: harvest; S1, S2, and S3: 7, 14, and 21 days at 2 °C, respectively. Data are means ± SE (n = 3).

3.4. Anthocyanin Content

The lowest anthocyanin content was observed at the veraison stage, which then increased sharply at harvest and gradually continued to increase until two weeks of storage, after which it decreased in both cultivars (Figure 4A). These observations are in line with the results for 'Cardinal' table grapes, which showed an initial increase in total anthocyanin content after 3 days of storage, followed by a decrease until the end of a 33-day storage period [28]. In this study, the 'Rashah' grape cultivar exhibited the highest anthocyanin content at 18.43 mg 100 g^{-1} FW (Figure 4A). Previous studies by Khalil et al. (2023) [19] have also reported significant differences in anthocyanin content between ripening stages and cultivars, suggesting that these differences may be responsible for the red color of the fruits [29]. Additionally, Xi et al. (2017) [30] reported an increasing trend of anthocyanins with advanced maturity. Similar results have been reported for anthocyanin content in raspberry [26] and blackberry [31] cultivars, highlighting the importance of anthocyanins as antioxidants in berry fruits [32].

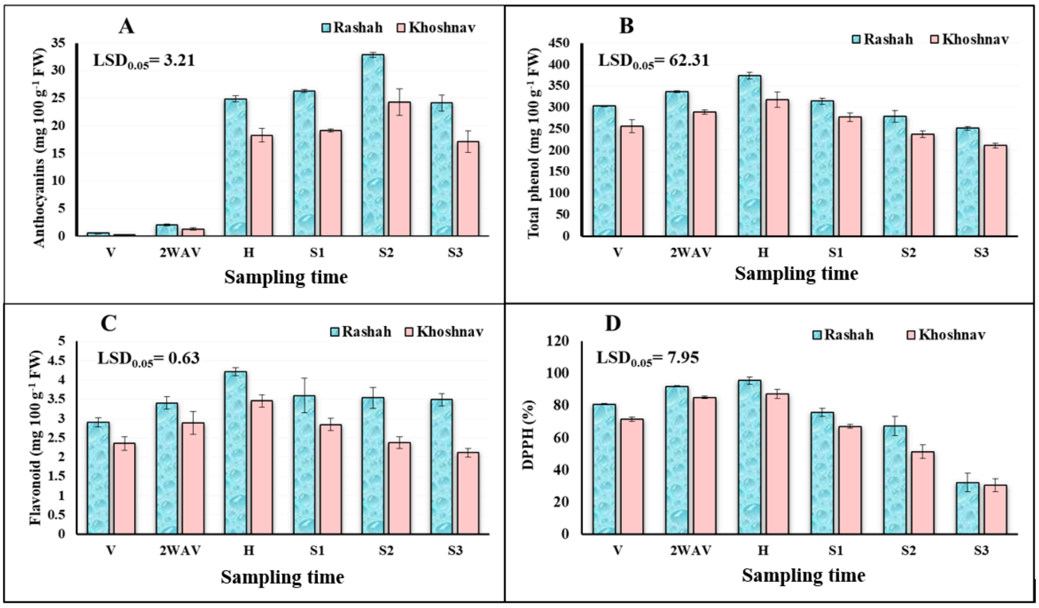

Figure 4. Changes in anthocyanins (**A**), total phenolics (**B**), total flavonoids (**C**) and DPPH (**D**) in grape cultivars 'Rashah' and 'Khoshnav' during fruit ripening and cold storage. V: veraison time; 2WAV: two weeks after veraison; H: harvest; S1, S2, and S3: 7, 14, and 21 days at 2 °C, respectively. Data are means ± SE (n = 3).

The higher antioxidant activity of these cultivars is mainly attributed to anthocyanins (R = 0.33), which are found in the skins of grapes and are mainly responsible for their intense red color [29]. Anthocyanins, a glycosylated form of anthocyanidins, are a group of flavonoids mainly responsible for fruit color ranging from red to purple to blue. The accumulation of anthocyanins in grape skins begins with veraison and reaches a maximum around harvest time [33]. Berries are rich in anthocyanins [5], and strong antioxidant activities from anthocyanins in various model systems have been reported [17,34].

3.5. Total Phenols

The total phenol (TP) content increased significantly from veraison to harvest time and then gradually decreased during cold storage in both grape cultivars (Figure 4B). In this study, the 'Rashah' grape cultivar had a higher TP value of 309.71 mg GAE 100 g^{-1} FW compared to 'Khoshnav' with 264.72 mg GAE 100 g^{-1} FW (Figure 4B). Similar reductions in total phenolic content after harvest have been observed in other studies [25,35,36]. The reduction in total phenolic content can be attributed to the activity of the PPO enzyme, which leads to the oxidation of phenolic compounds [30]. Jediyi et al. (2019) [37] reported that different grape varieties exhibit varying phenolic content during ripening. In the present study, the higher TP value observed in 'Rashah', which also had higher anthocyanin content, suggests a positive correlation between these two compounds. Overall, the different trends in phenolics and flavonoids may be due to factors such as plant species, growing conditions, ripening stages, storage conditions, and determination methods [38]. In the current study, the different antioxidant activities of fruits can be attributed to their total phenolic concentrations, as a positive correlation was found between TP and AC (R= 0.68). Similar results were reported by other researchers [31], who found a linear correlation between total antioxidant capacity and phenolic content in both blackberries (R = 0.96) and raspberries (R = 0.91). The present study highlights the potential of 'Rashah' grape cultivar as a valuable source of TP content, which could be utilized as a functional food ingredient in processing and breeding programs.

3.6. Total Flavonoid

Total TF concentration increased until harvest and then decreased during storage (Figure 4C). TF content was highest at harvest time in the 'Rashah' grape cultivar. It was reported that the average flavonoid content varied between fruits [39]. An increase in flavonoid content with ripening was also reported by Livani et al. (2013) [40] in blackberry fruit with leaves and by Zuhair et al. (2013) [38] in papaya and from the white to red ripening stages in watermelon [41]. Phenolic compounds, especially flavonoids, possess various biological activities, but the most important of these properties are antioxidant activity and anti-cancer activity [42]. Flavonoids, a large group of phenolic compounds, have been found to have antioxidant activity. It can be hypothesized that differential genetic control over berries' physical changes occurs during ripening and/or differences among cultivars exist in the nonlinear accumulation of TF during ripening [43].

It is important to note that while flavonoids have been associated with various health benefits, such as antioxidant and anti-cancer activities, their specific effects may vary depending on the type and amount of flavonoids consumed, as well as individual factors. Further research is needed to fully understand the potential health effects of flavonoids from table grapes and other dietary sources.

3.7. Antioxidant Capacity

The AC increased from veraison to harvest time in both grape cultivars, and then reduced up to the last day of storage (Figure 4D). The AC differed between the two cultivars during ripening and the greatest AC (73.95%) was associated with the 'Rashah' cultivar. These reductions in AC could be related to the decrease in TP. A difference in antioxidant activity was also observed between fruit cultivars [39]. Karadeniz et al. (2005) [39] reported the average antioxidant activities of 'Muskule' (37.6%) and 'Seedless' (15.6%) table grape cultivars, which are lower than the values for the grape varieties studied in this work. However, the differences between the reports and the present results could be due to the species or genotype and fruit stage [41]. AC had a positive correlation with TP, TF, and vitamin C (Table 1). Howard et al. (2003) [44] found a positive correlation between AC and TP, total anthocyanins, tartaric acid esters, and flavonoids in two growing seasons for different highbush blueberry cultivars. Ding et al. [45] and Pantelidis et al. [46] found that AC positively correlated with TP and total anthocyanins in cultivated berries but not in wild berries. Considering the influence of environmental conditions at the growing site, different authors reported a variation in the AC of berries between different locations and years for a single growing site [44]. It has been observed that fruit antioxidant activity is also correlated with flavonoid content [39]. These findings emphasize the relationship between antioxidant capacity and the presence of phenolic compounds, such as TP and flavonoids, as well as vitamin C in table grapes. The variations in antioxidant capacity among cultivars and the influence of environmental factors highlight the importance of considering these factors in breeding programs and cultivation practices to maximize the antioxidant potential of table grapes.

Table 1. Pearson's simple correlation coefficient between fruit characteristics of the two grape cultivars.

	TP	AC	TF	Vitamin C	TA
TP	1				
AC	0.68 **	1			
TF	0.66 **	0.44 **	1		
Vitamin C	0.38 *	0.65 **	0.03	1	
TA	−0.05	0.33 *	0.36 *	−0.76 **	1

* = significant at $p < 0.05$.; ** = significant at $p \leq 0.01$.

4. Conclusions

Grapes are a widely consumed fruit species worldwide, available in various forms such as fresh fruit, dried raisins, and wine. The quality and composition of grapes depend on the ripening processes, which involve biochemical, physiological, and anatomical changes. In table grapes, the maturity stage significantly influences the fruit's quality and shelf life. The results of this study demonstrated that the highest content of bioactive compounds was achieved between veraison and harvest, with a subsequent linear decrease during cold storage. Moreover, a stronger correlation was observed between antioxidant activity and phenolic content compared to the correlation between antioxidant activity and anthocyanin content. These findings highlight the 'Rashah' grape cultivar as an exceptionally rich source of vitamin C, anthocyanins, phenolics, flavonoids, and antioxidants. As such, it holds great potential as a functional food ingredient in both processing and breeding programs. In conclusion, understanding the ripening processes and their impact on grape quality is crucial for optimizing production and developing high-quality grape products. Further research and utilization of grape cultivars like 'Rashah' can contribute to the development of functional foods with enhanced nutritional value and health benefits.

Author Contributions: M.K.S. and S.M.: Conceptualization and design of the study, methodology. M.K.S.: Supervision of the research, financial preparation. S.M. and S.S.: Performing the experiments, collection of data. S.M., M.K.S., P.I. and G.L.: Contribution to literature research and original draft preparation. S.M., M.K.S., P.I. and G.L.: Analysis and interpretation of data and visualization. M.K.S., P.I. and G.L.: Revising the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

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