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Color, phenolic composition, and antioxidant properties of hardaliye (fermented grape beverage) under different storage conditions

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Abstract: This study was conducted to determine the effects of storage temperature and time on color and antioxidant properties of hardaliye. Hardaliye was obtained from papazkarası blue-black grapes as a potential source of anthocyanin pigment and high antioxidant activity. Grape variety plays a significant role in color, flavor, and chemical properties. However, the effects of storage conditions on products and especially on phenolic composition and antioxidant properties are not known because there is not any research about storage effects at different temperatures. For this purpose, hardaliye was produced by the conventional method. After having been exposed to lactic acid fermentation, it was bottled and then stored at 4 °C and 20 °C for 60 days. The analyses were carried out on prepared beverage at 15, 30, 45, and 60 days of storage. The results of color parameters obtained showed the highest proportion of red color in the samples at the beginning (dA % = 94.87%). As expected, the brown color increased with storage time and the highest value was determined at 60 days depending on the storage temperature. Storage at 4 °C and 20 °C resulted in 60% and 78% losses in anthocyanin content, respectively. The losses of anthocyanin during storage at the higher temperature were accompanied by increased polymeric color values and other color parameters, which indicates that anthocyanins were also extensively polymerized. Phenol compositions were also analyzed using an LC-MS-MS system. It was revealed that malvidin-3-O-glucoside was the predominant anthocyanin and also that hardaliye has high amounts of resveratrol. A high content of total phenolic (1743 ± 8.67 mg of gallic acid equivalents L^{-1}) and antioxidant activity value (8.53 mM Trolox mL^{-1}) is present in the fresh beverage. There was a significant decrease in total phenols during storage, irrespective of temperature.

Key words: Anthocyanin, color, grape juice, hardaliye

1. Introduction

Fermentation is one of the oldest methods used in food preservation. Essentially, such fermented foods are thought to have certain values over their normal nutritional content (Naidu et al., 1999). Therefore, there is growing interest in traditional fermented products. Hardaliye is an example of traditional fermented beverages produced in Thrace, the European part of Turkey. The process is carried out with lactic acid fermentation. It is produced from grape juice and pomace with the addition of different concentrations of mustard seeds and sour cherry leaves. The color of hardaliye reflects the original color of the grapes and it has a characteristic aroma (Kılıç and Çopur, 1988; Prado et al., 2008, Ünal et al., 2015).

It is known that color intensities of hardaliye change widely depending on grape varieties and production methods. There has been great interest in grape beverages and their products for their potential health benefits due to their impressive antioxidative properties, which are highly correlated with their polyphenol content, including anthocyanins (ACNs) (Gil et al. 2000). However, there are no studies describing the effect on the specifications of the storage conditions and time. This is a serious industrial problem in the production of hardaliye. The color and taste properties have great importance for consumer acceptance of hardaliye.

The attractive red-violet color of grape juice arises from its ACN contents, which vary greatly according to variety. ACNs are unstable and susceptible to degradation, leading to a brownish color during processing and storage as a result of the oxidation of ACNs due to their antioxidant properties (Liang et al., 2008; Tiwari et al., 2010; Karasu et al., 2016). The polymerization of ACNs with tannins is associated with both color deterioration as well as original color (Rommel et al., 1992). These include the type of method and storage time, as well as temperature (Frankel et al., 1998; Burin et al., 2010). All these changes affect both the quality parameters and sensorial properties of hardaliye.

The major objective of our investigation was to evaluate the effects of storage temperature and time

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on the physiochemical changes, spectrophotometric and chromatographic color changes, and antioxidant properties of hardaliye.

2. Materials and methods

2.1. Chemicals and reagents

Analytical grade chemicals, potassium sorbate, sodium benzoate, Folin–Ciocalteu reagent, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid), and potassium persulfate were obtained from Sigma Aldrich (Milwaukee, WI, USA). Ammonium formate, formic acid, and methanol of HPLC grade, purchased from Merck (Darmstadt, Germany), were used.

2.2. Material

Hardaliye beverages were produced using papazkarası blue-black grapes, which are grown in the Thrace Region, Kırklareli. Papazkarası is a both table and wine grape variety. It no longer has high production in Turkey. However, the Thrace Region has vineyards for papazkarası because it has high quality and is preferred for hardaliye production. Ripe grape fruits (100 kg) were purchased from local farmers in September 2013. The fruit was frozen at –18 °C after harvest and was stored at this temperature until analysis. Black mustard seeds (*Brassica nigra* L.) and daily picked leaves of sour cherry tree (*Prunus cerasus* L.) were added to the hardaliye composition, and sodium benzoate and potassium sorbate were used as preservatives.

2.3. Production of hardaliye

Hardaliye was produced from the papazkarası grape variety in the laboratory. For this purpose, two plastic containers (20 L) containing a tap were used for fermentation. Primarily, grapes were washed, separated to the stalks, crushed slightly, and added to the containers as crushed grapes, black mustard seeds (1%), preservative (0.1%, sodium benzoate, and potassium sorbate), and leaves of sour cherry tree (1%), respectively. The samples were allowed to ferment at 22 °C for 30 days. After the fermentation process, the samples were filtered through several layers of cheesecloth to remove grape seeds and skins, then filtered through a coarser filter paper and finally added to amber-colored bottles with volumes of 250 mL and stored at 4 °C (under refrigerated conditions) and 20 °C (in a cupboard with no sunlight) for 60 days. Physicochemical and spectrophotometric analyses of samples were conducted at days 0, 15, 30, 45, and 60 of the storage period. Phenolic composition and resveratrol and ACN contents were determined at the beginning and the end of the storage period. Analyses were carried out as 3 parallel sets and 2 replications.

2.4. Compositional analysis

The total soluble content (°Brix) of samples was determined with a portable refractometer (SOIF VBR90A). Measurements were carried out at 20 °C. pH was measured potentiometrically with a pH meter (WTW Inolab Level 1, Weilheim, Germany). Total acidity (TA) was determined according to the method outlined by the International Fruit and Vegetable Juice Association (IFU, 1968) and it was expressed as g anhydrous lactic acid/100 mL sample.

2.5. Spectrophotometric analysis

2.5.1. Monomeric ACN analysis

The total ACN content was determined using the pH differential method described by Giusti and Wrolstad (2005). Prior to absorbance measurements, the solutions were filtered through a 0.45-mm polyvinylidene fluoride (PVDF) filter (Millipore, Bedford, MA, USA) to remove the haze. The absorbance of equilibrated solutions was measured at 513 nm (λmax) for ACN content and 700 nm for haze correction on a UV-Vis double-beam spectrophotometer (Shimadzu 2600, Shimadzu, Kyoto, Japan) with disposable cuvettes with 1-cm path lengths (Brand GmbH, Wertheim, Germany). Absorbance measurements were carried out at room temperature and made against distilled water as a blank. Pigment content was calculated as malvidin-3-O-glucoside equivalents with a molecular weight of 493.5 g mol^{-1} and extinction coefficient of 28,000 L cm^{-1} mol^{-1}. The difference in absorbance values at pH 1.0 and 4.5 was directly proportional to ACN concentration. ACN measurements were replicated three times.

2.5.2. Polymeric color content

The percentage of polymeric color content was determined using the bisulfite bleaching method described by Giusti and Wrolstad (2005). The absorbances of bisulfite-treated and nontreated solutions were measured at 420 nm for brown pigments, 512 nm (λmax) for monomeric ACNs, and 700 nm for haze correction. Disposable cuvettes of 1 cm in path length were used. Absorbance measurements were carried out at room temperature and made against distilled water as a blank. Polymeric color measurements were replicated three times.

2.5.3. Color parameters

The color parameters, absorbances at 420 (d420), 520 (d520), and 620 nm (d620), were measured under a 1-mm optical path. The color intensity (CI, sum of the three absorbances) (Glories, 1984), the color nuances (d420/d520) (Sudraud, 1958), and the yellow (d420 %), red (d520 %), and blue (d620 %) components were also calculated.

2.5.4. Determination of total phenols

The amounts of total phenols in the grape juices were determined according to the Folin–Ciocalteu colorimetric juice method (Singleton and Rossi, 1965), and the results were expressed as gallic acid equivalent (mg GAE L^{-1}).

2.5.5. The evaluation of antioxidant activity

For $ABTS^{\cdot+}$ assay, the procedure followed the method described by Miller and Rice-Evans (1997) and Arts et

al. (2001), with some modifications. The stock solutions included 7.4 mM $ABTS^{\bullet+}$ solution and 2.6 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and then it was left to react for 12 h at room temperature in the dark. Afterwards the solution was diluted by mixing 1 mL of $ABTS^{\bullet+}$ solution with 60 mL of methanol to obtain an absorbance value of 1.1 ± 0.02 units at 734 nm using the spectrophotometer. Fresh $ABTS^{\bullet+}$ solution was prepared for each assay. After mixing of 10, 20, or 30 µL of sample into diluted $ABTS^{\bullet+}$ solution, respectively, the reaction mixture was incubated for 5 min at 30 °C. The decrease in the absorbance reflected the $ABTS^{\bullet+}$ radical scavenging capacity of the antioxidant. The standard curve was obtained by Trolox assay and the results were expressed in mM Trolox mL^{-1} as Trolox equivalent antioxidant capacity (TEAC).

2.6. LC-MS/MS analysis of phenols

Phenol analyses were performed on the Agilent 1200 Infinity LC in combination with the Agilent 6460 Triple Quadrupole MS/MS System equipped with a Jet Stream Electrospray ionization source (Agilent Technologies, Palo Alto, CA, USA). The analytical column was the Agilent Poroshell 120 EC-C18 (4.6 × 50 mm, 2.7 µm particle size) and set at 25 °C. Mobile phase A consisted of ultrapure water (UPW), 0.2% ammonium formate (v/v), and 0.2% formic acid (v/v). Mobile phase B consisted of methanol, 0.2% ammonium formate (v/v), and 0.2% formic acid (v/v). The flow rate was 0.3 mL/min under ambient temperature. The injection volume was 1 µL and the LC gradient conditions were as follows: 0 to 1 min, 70% A, 30% B; 3 to 7 min, 30% A, 70% B; 9 to 10 min, 50% A, 50% B; 11 to 12 min, 70% A, 30% B. The run time was 12 min.

The optimized MS parameters of analysis were as follows: gas temperature was set at 325 °C, the nebulizer gas pressure was set at 45 psi, the nozzle voltage was set at 500 V, the capillary at 3000 V, sheath gas temperature at 400 °C, and sheath gas flow at 12 L min^{-1}. Multiple reaction monitoring was performed in the positive and negative ion mode.

Data acquisition was carried out with Mass Hunter (version B.06.01) software. Collision energy, fragmentor voltage, precursor ion, production, and polarity values are presented in Table 1. Nitrogen (N_2) was used as the collision gas at 1.12 mTorr.

Calibration standard mixes were prepared in 50% UPW, 25% methanol, and 25% isopropanol at calibration concentrations ranging from 1 to 200 ng mL^{-1}.

2.7. Statistical analyses

Data were collected with three replicates. First, for availability, homogeneity of variance was examined and variances were found to be homogeneous. Data were suitable for ANOVA and t-tests. Variance analysis and Duncan's multiple range tests were applied to compare the means of time and group factors. The effects of hardaliye beverage storage time and temperature on color changes were analyzed by one-way ANOVA (SPSS 17.0). Correlation coefficients were also estimated for the already studied parameters. Statistical differences between means were determined by analysis of Duncan's multiple range test at the 5% significant level ($P < 0.05$) (Orhan et al., 2004).

Table 1. Collision energy, fragmentor voltage, precursor ion, production, and polarity properties for phenolic compounds of hardaliye.

Phenolic compound	R^2	Retention time (min)	Precursor (*m*/*z*)	Product (*m*/*z*)	Fragmentor voltage	Collision energy	Polarity
Gallic acid	0.99	2.286	227.2	143.1	127	24	Negative
Resveratrol	0.99	5.966	169.1	125.1	88	8	Negative
Trans-caffeic acid	0.99	4.951	291.28	139.1	93	12	Positive
Catechin	0.99	2.519	193.17	134.1	83	32	Negative
Salicylic acid	0.99	6.756	153.1	109.1	83	12	Negative
Trans-*p*-coumaric acid	0.99	5.703	179.2	135.1	93	12	Negative
Protocatechuic acid	0.99	2.687	137.1	93.1	83	12	Negative
2,5-Dihydroxybenzoic acid	0.99	2.463	223.2	208	101	16	Negative
Syringic acid	0.99	5.023	163.2	119.1	86	12	Negative
Trans-ferulic acid	0.99	5.772	153.1	108	91	20	Negative
Trans-sinapic acid	0.99	5.668	197.2	182	86	8	Negative
Malvidin-3-O-glucoside (oenin chloride)	0.99	15.17	494.44	331.9	109	20	Positive

3. Results and discussion

3.1. Changes in compositional measurement of hardaliye during storage

Table 2 presents values of pH, TA, and Brix parameters for hardaliye beverages under different storage conditions. The pH values and acidity of all the samples were recorded throughout storage in order to follow pH changes, since they have a significant impact on pigment stability (Heredia et al., 1998; Cabriata et al., 2000; Cevallos and Cisneros-Zaevallos, 2004).

The pH values decreased to 3.60 and 3.56 at 4 °C and 20 °C respectively, while the TA fluctuated between 0.70 and 0.66 g/100 mL of lactic acid. The Brix value also decreased with storage. There were minor changes for all compositional values during storage for all the samples. The pH value and acidity decreases were observed in samples within expected values. During the fermentation process, some of the flesh of grapes and the pigments in the grape skins go into the juice. These materials show buffering features, so an important decrease in pH was not observed (Cemeroğlu, 2007; Coşkun et al., 2012).

3.2. Anthocyanins, polymeric color, and color parameters

The aim of this research was to investigate the stability of total monomeric ACNs and individual ACN (malvidin-3-O-glucoside) of hardaliye at different storage temperatures (4 and 20 °C). Changes in monomeric and individual ACNs were determined during 60 days of storage. Values concerning the stability of hardaliye beverage ACNs during storage were compared with each other.

The determination of total ACNs was performed by the pH differential method and the results are shown in Table 3. Total monomeric ACNs were significantly lower in samples stored for 60 days compared to the beginning beverages for both temperatures ($P < 0.05$). As expected, a greater loss was observed at 20 °C.

While monomeric ACN content of hardaliye had decreased 60% after 60 days of storage at 4 °C, over 77% loss was observed for storage at 20 °C. The major individual ACN of hardaliye was identified by HPLC-

Table 2. Changes in compositional measurements of hardaliye beverage samples during storage at different conditions.

Days	pH		Total acidity (g 100 mL^{-1})		Brix (°)	
	4 °C	20 °C	4 °C	20 °C	4 °C	20 °C
0	3.96 ± 0.01	3.96 ± 0.01	0.70 ± 0.01	0.70 ± 0.03	21.40 ± 0.01	21.40 ± 0.00
15	3.60 ± 0.03	3.40 ± 0.01	0.76 ± 0.03	0.74 ± 0.02	21.20 ± 0.00	22.20 ± 0.01
30	3.50 ± 0.01	3.60 ± 0.03	0.75 ± 0.03	0.74 ± 0.04	20.20 ± 0.01	21.50 ± 0.01
45	3.70 ± 0.02	3.60 ± 0.01	0.75 ± 0.02	0.74 ± 0.02	20.60 ± 0.02	21.10 ± 0.00
60	3.60 ± 0.01	3.53 ± 0.02	0.69 ± 0.03	0.66 ± 0.04	21.00 ± 0.00	21.15 ± 0.00

Total acidity is expressed as g anhydrous lactic acid/100 mL sample.

Table 3. The effect of storage temperatures and storage period on total ACN content of hardaliye beverage.

Days	Total monomeric anthocyanin values (mg L^{-1})	
	4 °C	20 °C
0	86^{a} ± 0.58	86^{a} ± 0.58
15	47^{c} ± 1.15	52^{b} ± 2.65
30	50^{b} ± 1.73	40^{c} ± 6.81
45	47^{c} ± 0.00	16^{d} ± 0.58
60	34^{d} ± 0.58	19^{d} ± 0.58

Differences between means as indicated by the same letters for the same column are not statistically significant for different days ($P \geq 0.05$; one-way ANOVA followed by Duncan's test).

MS-MS as malvidin-3-O-glucoside, at 24,230 ppb for beginning samples. After storage at 4 and 20 °C, malvidin-3-O-glucoside content in hardaliye had decreased 12.5% and 25%, respectively.

The determining factors in the variation of ACN concentrations in grape juice are the techniques used in the processing and the different storage conditions (Frankel et al., 1998). Hardaliye is often packed in transparent bottles and can be exposed to light, which affects the phenolic content, and especially the ACNs, which could lead to a reduction in its color level (Morris et al., 1986). The total ACN content of different hardaliye samples was determined different by similar studies (Gucer et al., 2009; Amoutzopoulos et al., 2013; Aydoğdu et al., 2014). These differences may be due to the type of grape used, the juice processing, and storage methods. The temperature during processing and storage of juices can cause changes in the color of the product. This is due to the formation of chalcones causing loss of color due to ACN degradation (Jackman and Smith, 1996).

The monomeric ACN content decreased with increasing time as a function of storage temperature, whereas the percentage of polymeric color increased. The percentage of polymeric color increased significantly in all samples stored at 4 and 20 °C, as shown in Table 4. As expected, the degradation of ACNs and formation of a brown color occurred, which caused an increase in the percentage of polymeric color at a faster rate with increasing storage temperature. Statistically, storage had a significant impact ($P < 0.05$) on the percentage of polymeric color, while no significant difference ($P > 0.05$) was determined between 4 and 20 °C, but faster changes were observed at 20 °C. These results were in agreement with the literature data (Calvi, 1978; Bassa and Francis, 1987; Iyer and Dubash, 1993; Baublis et al., 1994; Heredia et al., 1998; Durst and Wrolstad, 2001; Dyrby et al., 2001; Marti et al., 2001; Turker et al., 2004).

The color of red-colored fruit juices and wines is expressed by the optical density values at 420, 520, and 620 nm. The sum of these three absorbances is called color intensity (CI) and the rate of them is called dA % and color nuance. The yellow (d420 %), red (d520 %), and blue (d620 %) components were also calculated (Ribéreau-Gayon et al., 2000). The color intensity indicates the red color density depending on the grape varieties (Ribéreau-Gayon et al., 2000). Color nuance is obtained by dividing the absorbance value at 420 nm by the absorbance value at 520 nm.

Tables 5a and 5b present the results concerning color intensity, color nuances, d420, d520, d620, and dA %. An increase in color nuances and a decrease in color intensity for the samples stored at both temperatures were observed. It is known that increasing red color causes a decrease in color nuance. The obtained results showed that red color components, especially ACNs, were significantly lower at the end of the storage ($P < 0.05$) and so there was a higher content of browning components. ACN concentration decline led to the formation of reddish-orange color reaction products, which is consistent with the observed color nuance increase. Moreover, degradation of coloring

Table 4. The effect of storage temperatures and storage period on polymeric color of hardaliye beverage.

Temperature (°C)	Days	Polymeric color	Percent polymeric color (%)
4 °C	0	$2.37^{b} \pm 0.12$	$50.56^{b} \pm 7.24$
	15	$1.74^{c} \pm 0.13$	$56.13^{b} \pm 4.80$
	30	$2.29^{b} \pm 0.07$	$55.09^{b} \pm 3.94$
	45	$6.18^{a} \pm 0.07$	$81.94^{a} \pm 2.17$
	60	$6.11^{a} \pm 0.13$	$81.62^{a} \pm 3.27$
20 °C	0	$2.37^{b} \pm 0.14$	$50.56^{b} \pm 7.24$
	15	$3.90^{c} \pm 0.07$	$73.61^{a} \pm 3.88$
	30	$3.53^{b} \pm 0.08$	$80.00^{a} \pm 0.69$
	45	$3.28^{a} \pm 0.08$	$80.49^{a} \pm 5.97$
	60	$2.07^{a} \pm 0.09$	$83.71^{a} \pm 5.94$

Differences between means as indicated by the same letters are not statistically significant ($P \geq 0.05$; one-way ANOVA followed by Duncan's test).

Table 5a. The effect of storage period on spectrophotometric color parameters of hardaliye beverage stored at 4 °C .

Days (4 °C)	d420	d520	d620	dA	Color intensity	Color nuances
0	$31.47^{d} \pm 0.01$	$39.06^{a} \pm 0.42$	$29.47^{a} \pm 0.05$	$94.87^{a} \pm 0.10$	$4.18^{b} \pm 0.12$	$0.80^{d} \pm 0.00$
15	$43.14^{a} \pm 0.34$	$35.69^{c} \pm 0.24$	$21.17^{d} \pm 0.52$	$38.44^{d} \pm 2.07$	$0.81^{d} \pm 0.01$	$1.21^{b} \pm 0.01$
30	$39.08^{c} \pm 0.31$	$38.15^{b} \pm 0.19$	$22.77^{c} \pm 0.23$	$57.24^{b} \pm 1.45$	$1.26^{c} \pm 0.01$	$1.02^{c} \pm 0.01$
45	$42.80^{a} \pm 0.17$	$35.63^{a} \pm 0.09$	$21.5^{d} \pm 0.08$	$40.42^{d} \pm 0.84$	$1.25^{c} \pm 0.01$	$1.20^{b} \pm 0.01$
60	$41.31^{b} \pm 0.04$	$33.40^{b} \pm 0.05$	$25.29^{b} \pm 0.04$	$52.03^{c} \pm 0.19$	$1.32^{a} \pm 0.01$	$1.24^{a} \pm 0.17$

Differences between means as indicated by the same letters are not statistically significant ($P \geq 0.05$; one-way ANOVA followed by Duncan's test).

Table 5b. The effect of storage period on spectrophotometric color parameters of hardaliye beverage stored at 20 °C .

Days (20 °C)	d420	d520	d620	dA	Color intensity	Color nuances
0	$31.47^{d} \pm 0.01$	$39.06^{a} \pm 0.04$	$29.47^{a} \pm 0.05$	$94.87^{a} \pm 0.10$	$4.18^{a} \pm 0.01$	$0.80^{c} \pm 0.00$
15	$42.41^{a} \pm 0.12$	$34.60^{b} \pm 0.21$	$23.00^{e} \pm 0.09$	$43.90^{d} \pm 0.42$	$2.86^{c} \pm 0.01$	$1.23^{b} \pm 0.01$
30	$40.98^{c} \pm 0.29$	$32.91^{e} \pm 0.03$	$26.10^{b} \pm 0.03$	$54.80^{b} \pm 0.17$	$3.90^{b} \pm 0.01$	$1.25^{a} \pm 0.00$
45	$42.45^{a} \pm 0.17$	$33.85^{d} \pm 0.08$	$23.70^{c} \pm 0.08$	$44.58^{cd} \pm 0.87$	$2.09^{d} \pm 0.01$	$1.23^{b} \pm 0.01$
60	$42.16^{b} \pm 0.19$	$34.36^{c} \pm 0.16$	$23.47^{d} \pm 0.13$	$45.61^{c} \pm 0.95$	$1.35^{e} \pm 0.01$	$1.25^{a} \pm 0.01$

Differences between means as indicated by the same letters are not statistically significant ($P \geq 0.05$; one-way ANOVA followed by Duncan's test).

matter during storage may also explain the observed decrease of both phenolic compounds and CI (Chira et al., 2012). Furthermore, regarding tonality (color nuances), the normal values of a young red wine are between 0.5 and 0.7, but they increase during aging up to values of 1.3 (Ribéreau-Gayon et al., 1998).

3.3. Total polyphenols and phenolic compositions

The quantity and composition of phenolic compounds vary according to the species, variety, maturity of the grapes, weather, viticultural practices, the region where the grapes are grown, and beverage processing methods (Mazza, 1995; Baustista-Ortin et al., 2007; Burin et al., 2010). This includes the type of extraction, additives, fermentation method, and contact time, as well as heat and enzymatic treatments (Frankel et al., 1998; Burin et al., 2010). High temperatures during extraction, storage, or pasteurization lead to the degradation of ACNs and consequently, a decrease in the color and total phenolic content (Morris et al., 1986; Burin et al., 2010). Table 6 shows the changes of phenolic contents in the storage period.

Although there is not enough research about hardaliye, it is possible to compare the results with red grape beverage research. Total phenolic values, using the Folin–Ciocalteu method as carried out by Frankel et al. (1998), in grape beverage ranged between 2600 mg GAE L^{-1} and 1400 mg GAE L^{-1} (Burin et al., 2010). The same authors mentioned that the phenolic content of grape juice may be influenced by the procedures employed in the juice production and reactions occurring during storage. Red wine, the other grape product similar to hardaliye, was analyzed for total phenolic content, showing values ranging from 1.008 to 1.597 mg GAE L^{-1} (Falcão, 2007; Burin et al., 2010). These values are lower than those of grape juice samples analyzed in different studies. This shows that grape juice can be considered as a source of phenolic compounds without being exposed to alcoholic fermentation.

In this research, the total phenolic content of hardaliye was determined as 1743 mg GAE L^{-1} at the beginning of storage and ranged between 1370 and 1324 mg GAE L^{-1} at the end of the storage period at 4 °C and 20 °C, respectively, as shown in Table 7. Both beverage samples showed significant differences ($P < 0.05$). These results have similarity with total phenolic content of red grape juices according to various studies (Soleas et al., 1997; Gil et al., 2000; Burin et al., 2010).

Polyphenolic compounds of grapes and wines usually include derivatives of hydroxybenzoic (syringic, gentisic, salicylic, gallic, and protocatechuic acid) and

Table 6. The phenolic composition at the end of the storage period at different temperatures (ppb).

Components	Temperature		
	0 °C	4 °C	20 °C
Gallic acid	1513 ± 24	1793 ± 19	2411 ± 22
2,5-Dihydroxybenzoic acid	1030 ± 18	989 ± 26	1486 ± 14
Protocatechuic acid	1024 ± 15	998 ± 41	1486 ± 14
Catechin	108 ± 09	95 ± 12	185 ± 23
Trans-p-coumaric acid	186 ± 11	219 ± 17	391 ± 16
Trans-ferulic acid	17 ± 0.22	22 ± 1.6	40 ± 1.9
Syringic acid	20 ± 0.15	53 ± 2.7	56 ± 1.7
Trans-caffeic acid	18 ± 0.33	35 ± 2.2	40 ± 2.1
ABA	471 ± 21	650 ± 25	759 ± 11
Trans-sinapic acid	6 ± 0.07	9 ± 0.12	22 ± 0.12
Salicylic acid	5 ± 0.09	9 ± 0.23	7 ± 0.12
Trans-resveratrol	2731 ± 38	3463 ± 34	4646 ± 39
Malvidin-3-O-glucoside (oenin chloride)	24,230 ± 47	20,944 ± 67	18,564 ± 64

Table 7. The effect of storage temperatures on total phenol content and antioxidant activity of hardaliye beverage samples.

Sampling day	Total phenol content (mg L^{-1})		Antioxidant activity (mM Trolox mL^{-1})	
	4 °C	20 °C	4 °C	20 °C
0	1743^{a} ± 08.67	1743^{a} ± 08.67	8.53 ± 0.05	8.53 ± 0.05
15	1321^{d} ± 19.81	1631^{b} ± 01.76	8.21 ± 0.02	8.33 ± 0.05
30	1314^{d} ± 03.52	1471^{c} ± 01.33	7.90^{b} ± 0.07	7.85 ± 0.06
45	1421^{b} ± 01.76	1267^{d} ± 06.56	7.95 ± 0.03	7.49 ± 0.03
60	1370^{c} ± 03.06	1324^{d} ± 11.02	7.45 ± 0.08	7.22 ± 0.04

Differences between means as indicated by the same letters are not statistically significant ($P \geq 0.05$; one-way ANOVA followed by Duncan's test).

hydroxycinnamic acid (p-coumaric, cinnamic, and caffeic acid), trihydroxystilbene (resveratrol and its glucoside (piceid)), flavonoids including flavonols (e.g., quercetin), flavan-3-ols (e.g., catechin, epicatechin), and polymers of the latter, defined as procyanidin and ACNs (Estruch, 2000; Stupans et al., 2002; Sun et al., 2002).

It is known that the phenolic compounds in fermentative beverages derived from grapes have different reactions during fermentation. Moreover, both the composition of phenolic compounds and the reactions vary depending on harvest time, variety, process technology, and other factors (Soleas et al., 1997). There is not much research about phenolic changes in the processing and storage of hardaliye. However, it is certainly known that caffeic, p-coumaric, and ferulic acids have low concentrations after pressing in processing, and the amounts are increased by the addition of additives containing sulfide and continue to increase thereafter (Cheynier et al., 1986). This is similar to our results about these phenolic compounds.

Resveratrol, another phenolic compound, is the most mentioned compound found in hardaliye. It exists in trans (t) and cis (c) isomeric forms, and both t- and c-resveratrol also occur as glucosides called piceid. However, the c-form is a byproduct of fermentation and it is rarely found in

grapes (Siemann and Creasey, 1992). Although some factors such as climate, growing conditions, and variety affect the amount of resveratrol, the most effective one is processing conditions. Resveratrol is present in the skin but not in the flesh of grapes. Grape skins contain various important polyphenols, especially t-resveratrol.

There are many studies about resveratrol in beverages and wine. Resveratrol content could not be determined in fermentation without skin. The pectolytic enzymes that are used in wine and fruit juice processes cause an increase in the amount of t-resveratrol. In addition, an inverse relationship was identified between the total monomeric ACN and t-resveratrol content (Wightman et al., 1997). It is known that maceration increases the amount of t-resveratrol before fermentation and there will be a decrease in t-resveratrol when the increase in c-resveratrol continues with alcohol fermentation. In hardaliye production, the process begins with maceration and ends with an increase in the t-resveratrol concentration with the skin and flesh. Some authors have found that vinification techniques have marked effects on resveratrol and piceid levels in wine, particularly maceration with skins (Jeandet et al., 1995; Mattivi et al., 1995). More sulfur-containing additives, added after maceration, lead to an increase in the amount of t-resveratrol (Castellari et al., 1998).

3.4. Antioxidant activity

The antioxidant capacity (TEAC) evaluated using the ABTS method ranged from 8.53 to 7.22 mM Trolox mL^{-1} for hardaliye stored at 20 °C and to 7.45 mM Trolox mL^{-1} for hardaliye stored at 4 °C (Table 7). No significant differences between the samples stored at different temperatures ($P > 0.05$) were observed.

It is known that the antioxidant activity of grape products is influenced by their phenolic composition (Dávalos et al., 2005). In order to investigate the contribution of phenolic constituents to the antioxidant activity in hardaliye beverages, a linear regression was obtained between the TEAC values, total phenolic content, and total monomeric anthocyanin (TMA) in samples (Table 8). Higher correlations were found between total phenolic content and TEAC for 20 °C ($r = 0.9205$) when compared with 4 °C ($r = 0.4386$). A correlation coefficient of 0.8686 was determined between TMA and TEAC values for 20 °C, which is more than the values obtained for samples stored at 4 °C ($r = 0.8122$).

The highest phenolic compound in hardaliye is estimated to be resveratrol. Numerous studies on the benefits for health and antioxidant activity are related to t-resveratrol. It is known that t-resveratrol, as an antioxidant, is more effective than butylhydroxytoluene, quercetin, or tocopherol on lipid peroxidation in liposomes and rat liver (Frankel et al., 1993; Blond et al., 1995; Belguendouz et al., 1997, 1998; Fremont et al., 1997). However, contrary to expectations, it was determined that hardaliye does not have very high antioxidant activity.

3.5. Conclusions

The definite color properties of hardaliye can be an indicator of the product quality. Moreover, the degradation pattern of the ACN profile could serve as a good indicator of storage time. The contents of total phenols, TMA, and antioxidant activity have a strong correlation, which indicates that grape juice is a source of these compounds, providing an alternative option for artificial and fizzy drinks. Hardaliye has a short shelf life, like other fermented

Table 8. The relation between the TEAC values, total phenolic content (TP), and total monomeric anthocyanin (TMA) in hardaliye samples.

Variables		TEAC[a]	TP[b]	TMA[c]
4 °C	TEAC	1	0.4357	0.7604
	TP		1	0.8122*
	TMA			1
20 °C	TEAC	1	0.9205**	0.8686*
	TP		1	0.9480**
	TMA			1

[a] TEAC: Antioxidant activity was determined by ABTS method and the related values are expressed as mM Trolox mL^{-1}.
[b] TP: Total phenolic contents were estimated by the Folin–Ciocalteu assay of Singleton and Rossi (1965). Values are expressed as mg L^{-1}.
[c] TMA: Total monomeric anthocyanins were determined by the pH differential method of Giusti and Wrolstad (2005). Values are expressed as mg L^{-1}.
*Significance at 0.05, **significance at 0.01.

products. However, it was obvious that there were no important differences between storage under cold or room temperature. The results suggest that the total polyphenols of grape juice can be used to determine the characteristics of this product, and that it can also be used as a parameter for monitoring the productive process, or even for studies on quality control.

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References

Amoutzopoulos B, Löker GB, Samur G, Çevikkalp SA, Yaman M, Timur K, Pelvan E (2013). Effects of a traditional fermented grape-based drink "hardaliye" on antioxidant status of healthy adults: a randomized controlled clinical trial. J Sci Food Agr 93: 3604-3610.

Arts IC, Hollman PC, Feskens EJ (2001). Catechin intake might explain the inverse relation between tea consumption and ischemic heart disease: the Zutphen Elderly Study. Am J Clin Nutr 74: 227-232.

Aydoğdu H, Yıldırım Ş, Halkman AK, Durgun T (2014). A study on production and quality criteria of hardaliye; a traditional drink from Thrace Region of Turkey. Gıda 39: 139-145.

Bassa LA, Francis FJ (1987). Stability of anthocyanins from sweet potatoes in a model beverage. J Food Sci 52: 1753-1754.

Baublis A, Spomer A, Berber-Jimnez MD (1994). Anthocyanin pigments: comparison of extracts stability. J Food Sci 59: 1219-1221.

Baustista-Ortin AB, Fernandez-Fernandez JI, Lopez Roca JM, Gomez Plaza E (2007). The effects of enological practices in anthocyanins, phenolic compounds and wine colour and their dependence on grape characteristics. J Food Compost Anal 20: 546-552.

Belguendouz L, Fremont L, Gozzelino MT (1998). Interaction of transresveratrol with plasma lipoproteins. Biochem Pharmacol 55: 811-816.

Belguendouz L, Fremont L, Linard A (1997). resveratrol inhibits metal ion-dependent and independent peroxidation of porcine low-density lipoproteins. Biochem Pharmacol 53: 1347-1355.

Blond J, Denis M, Bezard J (1995). Antioxidant action of resveratrol in lipid peroxidation. Sci Aliments 15: 347-358.

Burin VM, Falcao LD, Gonzaga LV, Fett R, Rosier JP, Bordignon-Luiz MT (2010). Colour, phenolic content and antioxidant activity of grape juice. Cienc Tecnol Aliment 30: 1027-1032.

Cabriata L, Fossen T, Andersen OM (2000). Colour and stability of the six common anthocyanidin 3-glocosides in aqueous solutions. Food Chem 68: 101-107.

Calvi JP (1978). Stability of Concord grape (*V. labrusca*) anthocyanins in model systems. J Food Sci 43: 1448-1456.

Castellari M, Spinabelli U, Riponi C, Amati A (1998). Influence of some technological practices on the quantity of resveratrol in wine. Z Lebensm Unters Forsch A 206: 151-155.

Cemeroğlu B (2007). Gıda Analizleri. Ankara, Turkey: Gıda Teknolojisi Derneği Yayınları (in Turkish).

Cevallos BA, Cisneros-Zaevallos L (2004). Stability of anthocyanin-based aqueous extracts of Andean purple corn and red-fleshed sweet potato compared to synthetic and natural colorants. Food Chem 86: 69-77.

Cheynier VF, Trousdale EK, Singleton VL, Salgues MJ, Wylde R (1986). Characterization of 2-S-glutathionyl caftaric acid and its hydrolysis in relation to grape wines. J Agric Food Chem 34: 217-221.

Chira K, Jourdes M, Teissedre PL (2012). Cabernet sauvignon red wine astringency quality control by tannin characterization and polymerization during storage. Eur Food Res Technol 234: 253-261.

Coşkun F, Arıcı M, Çelikyurt G, Gülcü M (2012). Changes occurring at the end of storage in some properties of hardaliye produced by using different methods. Journal of Tekirdağ Agricultural Faculty 9: 62-66 (in Turkish with English abstract).

Dávalos A, Bartolomé B, Gómez-Cordovés C (2005). Antioxidant properties of commercial grape juice and vinegars. Food Chem 93: 325-330.

Durst RW, Wrolstad RE (2001). Separation and characterization of anthocyanins by HPLC. In: Wrolstad RE, Acree TE, Decker EA, Penner MH, Reid DS, Schwartz SJ, Shoemaker CF, Smith D, Sporns P, editors. Current Protocols in Food Analytical Chemistry. Hoboken, NJ, USA: Wiley-Interscience, pp. F1.3.1-F1.3.13.

Dyrby M, Westergaard N, Stapelfeldt H (2001). Light and heat sensitivity of red cabbage extract in soft drink model systems. Food Chem 72: 431-437.

Estruch R (2000). Wine and cardiovascular disease. Food Res Int 33: 219-226.

Falcão LD (2007). Relation between scavenging properties with total polyphenol, anthocyanins content and color characteristics in Cabernet Sauvignon wines. In: Symposium International D'oenologie De Bordeaux, p. 234.

Frankel EN, Bosanek CA, Meyer AS, Silliman K, Kirk LL (1998). Commercial grape juices inhibit the in vitro oxidation of human low-density lipoproteins. J Agric Food Chem 46: 834-838.

Frankel EN, Waterhouse AL, Kinsella JE (1993). Inhibition of human LDL oxidation by resveratrol. Lancet 341: 1103-1104.

Fremont S, Kanny G, Nicolas JP, Moneret-Vautrin DA (1997). Prevalence of lysozyme sensitization in an egg-allergic population. Allergy 52: 224-228.

Gil MI, Tomas-Barberan FA, Hess-Pierce B, Holcroft DM, Kader AA (2000). Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. J Agric Food Chem 48: 4581-4589.

Giusti MM, Wrolstad RE (2005). Characterization and measurement of anthocyanins by UV-visible spectroscopy. In: Wrolstad RE, Acree TE, Decker EA, Penner MH, Reid DS, Schwartz SJ, Shoemaker CF, Smith D, Sporns P, editors. Handbook of Food Analytical Chemistry: Pigments, Colorants, Flavors, Texture, and Bioactive Food Components. Hoboken, NJ, USA: John Wiley and Sons, pp. 19-31.

Glories Y (1984). La couleur des vins rouges. II. Mesure, origine et interpretation. International des Sciences de la Vigne et du Vin Journal 18: 253-271 (in French).

Gucer Y, Aydogdu H, Durgun T (2009). A traditional Thracian beverage; "Hardaliye". Trakia J Sci 7: 208-210.

Heredia FJ, Francia-Aricha EM, Vicario IM, Santos-Buelga C (1998). Chromatic characterization of anthocyanins from red grapes - I. pH effect. Food Chem 63: 491-498.

IFU (1968). Determination of pH-Value, IFU Method No. 11 (1968/1989). Codex Committee on Methods of Analysis and Sampling. Paris, France: IFU.

Iyer CM, Dubash PJ (1993). Anthocyanin of Karwand (*Carissa carandas*) and studies on its stability in model system. Int J Food Sci Tech 30: 246-248.

Jackman RL, Smith JL (1996). Anthocyanins and betalains. In: Hendry GAF, Houghton JD, editors. Natural Food Colourants. 2nd ed. London, UK: Chapman & Hall, 1996, pp. 245-309.

Jeandet P, Bessis R, Maume BF, Meunier P, Trollat P (1995). Effect of enological practices on the resveratrol isomer content of wine. J Agric Food Chem 43: 316-319.

Karasu S, Başlar M, Karaman S, Kılıçlı M, Us AA, Yaman H, Sağdıç O (2016). Characterization of some bioactive compounds and physicochemical properties of grape varieties grown in Turkey: thermal degradation kinetics of anthocyanin. Turk J Agric For 40: 177-185.

Kılıç O, Çopur ÖU (1988). Quality criteria of hardaliye produced from different grape varieties. In: Turkey 3rd Vineyard Symposium, 31 May–3 June 1988, Uludağ University, Bursa, Turkey, pp. 1-12 (in Turkish).

Liang Z, Wu B, Fan P, Yang C, Duan W, Zheng X, Liu C, Li S (2008). Anthocyanin composition and content in grape berry skin in *Vitis* germplasm. Food Chem 111: 837-844.

Marti N, Perez-Vicente A, Garcia-Viguera, C (2001). Influence of storage temperature and ascorbic acid addition on pomegranate juice. J Sci Food Agric 82: 217-221.

Mattivi F, Reniero F, Korhammer S (1995). Isolation, characterization, and evolution in red wine vinification of resveratrol monomers. J Agric Food Chem 43: 1820-1823.

Mazza G (1995). Anthocyanins in grape and grape products. Crit Rev Food Sci Nutr 35: 341-371.

Miller NJ, Rice-Evans CA (1997). Factors influencing the antioxidant activity determined by the ABTS radical cation assay. Free Radical Res 26: 195-199.

Morris JR, Sistrunk WA, Sims CA (1986). Effects of fruit maturity, juice storage, and juice extraction temperature on quality of 'Concord' grape juice. J Amer Soc Hort Sci 111: 742-746.

Naidu AS, Bidlack WR, Clemens RA (1999). Probiotic spectra of lactic acid bacteria (LAB). Crit Rev Food Sci Nutr 39: 13-126.

Orhan H, Efe E, Şahin M (2004). SAS yazılımı ile istatistiksel analizler. Isparta, Turkey: Süleyman Demirel University (in Turkish).

Prado F, Parada JL, Pandey A, Soccol CR (2008). Trends in non-dairy probiotic beverages. Food Res Int 41: 111-123.

Ribéreau-Gayon P, Glories Y, Maujean A, Dubourdieu D (1998). Handbook of Enology. New York, NY, USA: John Wiley and Sons.

Ribéreau-Gayon P, Glories Y, Maujean A, Dubourdieau D (2000). Handbook of Enology, Volume 2: The Chemistry of Wine and Stabilization and Treatments. London, UK: John Wiley and Sons.

Rommel A, Wrolstad RE, Heatherbell DA (1992). Blackberry juice and wine: Processing and storage effects on anthocyanin composition, color and appearance. J Food Sci 57: 385-391.

Siemann EH, Creasey LL (1992). Concentration of the phytoalexin resveratrol in wine. Am J Enol Vitic 43: 49-52.

Singleton VL, Rossi JA (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagent. Am J Enol Vitic 16: 144-158.

Soleas GJ, Dam J, Carcey M, Goldberg DM (1997). Determination of polyphenolic antioxidants of wine by gas chromatography with mass-selective detection. J Agric Food Chem 45: 3871-3880.

Stupans I, Kirlich A, Tuck KL, Hayball PJ (2002). Comparison of radical scavenging effect, inhibition of microsomal oxygen free radical generation, and serum lipoprotein oxidation of several natural antioxidants. J Agric Food Chem 50: 2464-2469.

Sudraud P (1958). Interpetation Des Courbes D'absorption Des Vins Rouges. Ann Technol Agr 7: 203-208 (in French).

Sun AY, Simonyi A, Sun GY (2002). The "French paradox" and beyond: neuroprotective effects of polyphenols. Free Radical Biol Med 32: 314-318.

Tiwari B, Brunton N, Cullen PJ, O'Donnell CP (2010). Effect of ultrasound processing on anthocyanins and color of red grape juice. Ultrason Sonochem 17: 598-604.

Turker N, Aksay S, Ekiz IH (2004). Effect storage temperature on the stability of anthocyanins of a fermented black carrot (*Daucus carota* var. L.) beverage: shalgam. J Agric Food Chem 52: 3807-3813.

Ünal MÜ, Şener A, Şen K, Yılmaztekin, M (2015). Seasonal variation in amino acid and phenolic compound profiles of three Turkish white wine grapes. Turk J Agric For 39: 984-991.

Wightman JD, Price SF, Watson BT, Wrolstad RE (1997). Some effects of processing enzymes on anthocyanins and phenolics of Pinot noir and Cabernet Sauvignon wines. Am J Enol Vitic 48: 39-48.