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## Physicochemical, Functional and Microbiological Properties of Hardaliye Beverages Produced from Different Grapes and Collected from Different Households

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### ABSTRACT

Twenty-three hardaliye beverage samples were collected from different regions of Kırklareli city in Turkey and five hardaliye samples were produced under laboratory conditions according to traditional methods. Total phenolic compounds, monomeric anthocyanins (malvidin-3-glucoside), volatile acid, ethyl alcohol, total sugar contents, total acidity (lactic acid), pH, color values (*L* (brightness), *a* (red-green), *b* (yellow-blue)), total mesophilic aerobic bacteria, lactic acid bacteria, yeast and mold and coliform counts were determined. Acidity between 0.38 and 0.91%, pH from 3.54 and 4.33, color (*L.a.b.*) values from 7.57 to 13.74, from 0.71 to 7.68, from 0.73 to 4.50, respectively were determined. Total phenolics from 368.8 to 2647.5 mg L<sup>-1</sup>, ethanol between 0.4 and 6.0% in 7 out of 23 examples, TMAB number 3x10<sup>1</sup>-3.2x10<sup>6</sup> cfu mL<sup>-1</sup>, LAB 1x10<sup>2</sup>-3x10<sup>5</sup> cfu mL<sup>-1</sup> in 16 out of 23 samples, yeast and mold count 1x10<sup>1</sup>-2,3x10<sup>4</sup> cfu mL<sup>-1</sup> in 18 out of 23 samples were found. According to the results obtained, coliforms and *Escherichia coli* were not found in the analyzed samples. Statistical analysis results revealed that differences between samples were significant (P≤0.01). Given its bioactive characteristics, hardaliye beverage should be produced at large-scale and be recognized by large communities.

Keywords: Fermentation; Grape juice; Hardaliye; Mustard seeds; Traditional beverage

## Farklı Evlerden Toplanan ve Farklı Üzümlerden Üretilen Hardaliye Örneklerinin Fizikokimyasal, Fonksiyonel ve Mikrobiyolojik Özellikleri

### ESER BİLGİSİ

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**ÖZET**

Bu arařtırmada, Kırklareli'nin farklı bölgelerinden yirmi üç hardaliye örneđi toplanmıř ve laboratuvar řartlarında geleneksel metotlara göre beř adet hardaliye üretilmiřtir. Bu örneklere toplam fenolik madde, monomerik antosiyanin (malvidin-3-glikozit), uçucu asit, etil alkol, invert řeker, toplam asitlik (laktik asit cinsinden), pH, renk deđerleri (*L* (parlaklık), *a* (kırmızı-yeřil), *b* (sarı-mavi)), toplam aerobik mezofil bakteri, laktik asit bakterileri, maya-küf ve koliform bakteri sayıları belirlenmiřtir. Asitlik % 0.38-0.91 arasında, pH 3.54-4.33 arasında, renk (*L.a.b.*) deđerleri sırasıyla 7.57-13.74; 0.71-7.68; 0.73-4.50 olarak tespit edilmiřtir. Toplam fenolik madde 368.8-2647.5 mg L<sup>-1</sup> arasında, etanol 23 örneđin 7'sinde % 0.4-6.0 arasında, TMAB sayısı 3x10<sup>1</sup>-3.2x10<sup>6</sup> kob mL<sup>-1</sup> arasında, LAB sayısı 23 örneđin 16'sında (16 out of 23 samples) 1x10<sup>2</sup>-3x10<sup>5</sup> kob mL<sup>-1</sup> arasında, maya-küf sayısı 23 örneđin 18'inde (18 out of 23 samples) 1x10<sup>1</sup>-2.3x10<sup>4</sup> kob mL<sup>-1</sup> arasında bulunmuřtur. Elde edilen sonuçlara göre, analiz edilen hiç bir örnekte koliform bakteri ve *Escherichia coli* tespit edilmemiřtir. Analizler sonuçlarına göre örneklere arasındaki farklılıklar istatistiksel olarak önemli bulunmuřtur (P≤0.01). Biyoaktif özellikleri dikkate alındığında, hardaliyenin büyük ölçekli üretimini yapılması ve toplumun tanınması sađlanmalıdır.

Anahtar Kelimeler: Fermantasyon; Üzüm suyu; Hardaliye; Hardal tohumu; Geleneksel iecek

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**1. Introduction**

Hardaliye is a lactic acid fermented beverage that is produced from red grape or grape juice with the addition of crushed mustard seeds and benzoic acid and it is widely consumed in the Thrace region of Turkey (Arici & Coskun 2001). As well known, grape is rich fruit in terms of phenolic compounds (Shahidi & Naczka 1995). In vitro experimental systems also showed that flavonoids possess antiinflammatory, antiallergic, antiviral, and anticarcinogenic properties (Middleton 1998). Kanemaru & Miyamoto (1990) detected that 0.8% mustard extract showed bacteriostatic action against *Staphylococcus aureus* and *Escherichia coli* in culture medium. When essential oil of mustard was used, total mesophilic aerobic bacteria and lactic acid bacteria were significantly lower than the control after 2 days of storage (Lemay et al 2002). In a research conducted by Nielsen & Rios (2000), allyl isothiocyanate, a bioactive substance of mustard, was shown to have an inhibitory effect against *Penicillium commune*, *P. roqueforti*, *Aspergillus flavus* and *Endomyces fibuliger*. They also determined minimum inhibitory concentration to range between 1.8 and 3.5 mg mL<sup>-1</sup>. Therefore, it is possible to say that the beneficial effects of hardaliye are not only related with grape which includes bioactive components but also with

mustard which gained antibacterial and antifungal effects to hardaliye beverage.

The present study is composed of two parts. In the first part, 23 hardaliye beverage samples collected from local producers were analyzed in terms of their physicochemical, functional and microbiological properties. In the second part, fresh hardaliye beverage samples were produced from different grape types and their physicochemical and microbiological properties were investigated.

**2. Material and Methods****2.1. Material**

In this research, twenty-three hardaliye samples were collected from different households of Kırklareli city in Turkey. In the second stage, hardaliye samples were produced in the laboratory using different types of grapes. These grape types were Dingil Kara, Kara Uzum, Carbernet Sauvignon, Yerli Izabella and Siyah Uzum and procured from Tekirdag Viticulture Research Institute.

**2.2. Methods**

Grapes are washed and pressed in oak barrels which have a tap at the position of 10 cm above the bottom of the barrel. Then 0.2% of crushed raw mustard

seed and 0.1% of sodium benzoate were added and the solution was left to fermentation at room temperature (22 °C) for 7 days.

The pH of the samples was measured using WTW 330 model pH meter with composite electrodes. Total acidity was determined according to the titration method reported by Cemeroğlu (2007). Total sugar contents of the samples were determined using Lane Eynon method (Cemeroğlu 2007). In order to determine total phenolic content of the samples, Folin-Ciocalteu spectrophotometric method was used (Singleton & Rossi 1965). Monomeric anthocyanins were determined using the method reported by Cemeroğlu (2007). Alcohol contents of the samples were determined using ebulliometer (Jacobson 2006). Total volatile acid contents were determined using the method reported by Fidan (1975). The color of the samples was measured using a Hunter Lab colorimeter (Model D25 LT).

Standard methods defined by FDA/Bacteriological Analytical Manual for microbiological analysis were used for preparation of the samples for analysis. 25 mL hardaliye sample was added into Maximum Recovery Diluent (MRD) and it was homogenization. Other serial dilutions ( $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$  and  $10^{-5}$ ) were prepared using the initial dilution. These dilutions prepared were used for all microbiological analysis. Total bacteria were enumerated using plate count agar (PCA). For counting coliform bacteria, violet red bile agar (VRBA) and for counting *E. coli*, eosin methylene blue (EMB) agar were used. For counting yeast-mold, potato dextrose agar (PDA), was used. Numbers of total mesophilic aerobic bacteria, coliform bacteria, *E. coli* and yeast and mold were determined by providing the appropriate incubation temperature and duration (Anonymous 2001a; 2001b; 2001c; 2002). De Man Rogosa Sharpe (MRS) agar was used for determination of lactic acid bacteria (Baumgart 1993).

In order to determine differences between samples, one-way ANOVA was performed using SPSS 18.0 statistical package. All statistical

analyses were performed according to completely randomized design (SPSS 2009).

### 3. Results and Discussion

Some physicochemical properties of hardaliye samples collected from different households are shown in Table 1. Some chemical properties of hardaliye samples collected from different households are shown in Table 2. As can be seen from the tables, remarkable differences between the values of total phenolic, monomeric anthocyanins and total sugar contents were observed. Differences between the samples were found to be significant ( $P < 0.01$ ). Some microbiological properties of hardaliye samples collected from different households are shown in Table 3.

In the second part of the study, the fresh hardaliye samples were produced from different grape types according to the traditional production method. Their physicochemical and chemical properties are presented in Tables 4 and 5. Differences between the samples were found to be significant ( $P < 0.01$ ). Total bacteria, lactic acid bacteria and yeast-mold counts of the samples are shown in Table 6. Coliforms and *E. coli* were not observed.

In this study, pH values of hardaliye samples collected from producers are between 3.54 and 4.33. Arici & Coskun (2001) collected twenty-six hardaliye samples from different spots of the Kirklareli city and the pH values determined in this study was similar to those determined in their study. pH values of samples produced in the laboratory are between 3.33 and 3.73. These values were lower than pH value (4.42) reported by Coşkun et al (2009). Total acidity values obtained in these two studies are similar. Volatile acid values of all samples were similar or slightly higher than value ( $0.25 \text{ g L}^{-1}$ ) reported by Coşkun et al (2009).

Total phenolics of anthocyanins in hardaliye samples collected from houses are generally higher than those produced in the laboratory. Diversity of grape varieties used in the production might have caused different anthocyanin and total phenolic contents in the samples. Unsuitable storage conditions, time and temperature causes

**Table 1- Some physicochemical properties of the hardaliye samples collected from different households**

Sample no	Acidity (%)	pH	Color		
			L	a	b
1	0.84±0.02	3.62±0.02	7.78±0.02	2.88±0.03	2.59±0.01
2	0.49±0.01	4.11±0.01	7.79±0.04	1.62±0.02	2.10±0.00
3	0.63±0.02	4.02±0.00	7.86±0.01	1.78±0.02	0.47±0.00
4	0.59±0.02	4.01±0.01	8.46±0.01	2.21±0.01	1.27±0.02
5	0.49±0.06	3.80±0.02	8.67±0.02	3.48±0.02	1.79±0.01
6	0.42±0.01	3.90±0.01	8.57±0.02	2.65±0.05	1.44±0.04
7	0.56±0.04	3.81±0.01	7.57±0.02	1.11±0.01	0.85±0.05
8	0.70±0.03	3.56±0.02	9.80±0.02	4.49±0.01	1.86±0.01
9	0.56±0.01	4.02±0.02	12.09±0.01	3.45±0.02	4.33±0.01
10	0.42±0.02	4.25±0.02	10.77±0.02	4.71±0.01	2.36±0.01
11	0.35±0.00	4.12±0.02	11.45±0.02	2.09±0.01	1.96±0.01
12	0.38±0.02	4.03±0.03	11.81±0.01	2.46±0.01	3.77±0.02
13	0.42±0.01	4.33±0.03	12.49±0.02	5.09±0.01	4.50±0.05
14	0.49±0.02	3.88±0.02	13.74±0.02	4.19±0.02	4.48±0.03
15	0.70±0.01	3.99±0.01	8.35±0.02	0.71±0.01	0.73±0.01
16	0.45±0.02	4.13±0.03	7.99±0.01	1.76±0.01	0.99±0.01
17	0.56±0.01	3.87±0.02	11.06±0.01	3.82±0.02	2.45±0.05
18	0.56±0.02	4.00±0.01	11.82±0.02	1.81±0.01	1.91±0.01
19	0.88±0.01	3.60±0.02	10.42±0.02	3.55±0.05	3.82±0.02
20	0.77±0.03	3.54±0.02	13.25±0.05	5.23±0.03	1.30±0.02
21	0.56±0.01	3.97±0.02	7.91±0.01	4.07±0.02	1.99±0.01
22	0.38±0.00	3.80±0.01	13.69±0.04	7.68±0.03	1.53±0.03
23	0.91±0.01	4.01±0.01	9.23±0.03	3.95±0.05	0.92±0.02
Mean	0.58±0.018	3.93±0.017	10.11±0.020	3.25±0.021	2.15±0.020

**Table 2- Chemical properties of the hardaliye samples collected from different households**

Sample no	Total phenolic content (mg L <sup>-1</sup> )	Monomeric anthocyanin <sup>+</sup> (mg L <sup>-1</sup> )	Volatile acids (g L <sup>-1</sup> )	Ethanol (%)	Total sugar (%)
1	1067.5±0.50	54.4±0.03	0.30±0.01	n.d	18.0±0.10
2	1102.5±1.50	44.6±0.10	0.24±0.00	n.d	17.6±0.10
3	2647.5±2.02	118.4±0.11	0.30±0.01	1.6	15.9±0.02
4	2727.5±1.50	109.1±0.10	0.38±0.01	1.3	15.8±0.07
5	1129.6±0.10	29.0±0.01	0.43±0.00	0.9	17.7±0.01
6	861.8±0.08	65.4±0.03	0.26±0.01	n.d	24.8±0.05
7	2318.9±1.53	198.0±0.00	0.24±0.00	n.d	24.5±0.10
8	1693.9±1.00	37.8±0.02	0.50±0.01	n.d	21.3±0.01
9	1111.8±0.10	15.5±0.10	0.42±0.01	n.d	22.3±0.02
10	611.8±0.15	55.0±0.53	0.30±0.01	n.d	24.1±0.02
11	922.50±1.50	12.1±0.02	0.54±0.01	0.4	19.3±0.03
12	1336.8±0.40	6.5±0.00	0.36±0.00	n.d	19.4±0.07
13	870.9±0.10	39.5±0.20	0.60±0.01	n.d	24.3±0.10
14	583.1±0.02	27.1±0.01	0.36±0.01	n.d	15.2±0.02
15	1636.8±0.03	147.2±0.14	0.50±0.01	6.0	13.7±0.01
16	1129.6±0.04	124.6±0.02	0.24±0.01	n.d	25.4±0.06
17	813.1±0.10	4.5±0.03	0.61±0.01	n.d	20.8±0.05
18	576.60±0.18	12.7±0.10	0.48±0.00	n.d	19.50±0.02
19	512.4±0.40	11.6±0.05	0.55±0.01	n.d	23.1±0.03
20	908.8±0.10	27.9±0.10	0.72±0.01	2.1	7.2±0.01
21	1311.8±0.09	71.9±0.04	0.38±0.00	n.d	18.6±0.04
22	368.8±0.10	23.4±0.01	0.30±0.01	n.d	24.4±0.09
23	890.4±0.30	103.2±0.08	0.54±0.01	1.0	12.9±0.02
Mean	1179.7±0.515	55.5±0.080	0.41±0.007		19.4±0.046

<sup>+</sup>, Monomeric anthocyanidin (malvidin-3-glycoside); n.d, not detected. Note: Ethanol values are not included in the statistical analysis

**Table 3- Microbiological properties of the hardaliye samples collected from different households (cfu mL<sup>-1</sup>)**

Sample no	T.M.A.B.	LAB (Lactobacilli)	Yeast-Molds
1	4.00x10 <sup>3</sup> ±1x10 <sup>2</sup>	1.00x10 <sup>2</sup> ±0.00	<10±0.00
2	3.00x10 <sup>1</sup> ±0.6x10 <sup>1</sup>	<10±0.00	2.30x10 <sup>4</sup> ±1.5x10 <sup>3</sup>
3	6.00x10 <sup>3</sup> ±1x10 <sup>3</sup>	4.00x10 <sup>3</sup> ±2x10 <sup>2</sup>	3.30x10 <sup>3</sup> ±5.7x10 <sup>1</sup>
4	1.19x10 <sup>6</sup> ±5x10 <sup>3</sup>	1.00x10 <sup>2</sup> ±0.00	<10±0.00
5	1.15x10 <sup>5</sup> ±1x10 <sup>3</sup>	1.80x10 <sup>4</sup> ±4x10 <sup>2</sup>	1.00x10 <sup>4</sup> ±1x10 <sup>2</sup>
6	3.00x10 <sup>5</sup> ±1x10 <sup>4</sup>	4.10x10 <sup>4</sup> ±1.5x10 <sup>3</sup>	1.00x10 <sup>1</sup> ±0.1x10 <sup>1</sup>
7	3.20x10 <sup>6</sup> ±2x10 <sup>5</sup>	<10±0.00	2.00x10 <sup>1</sup> ±0.1x10 <sup>1</sup>
8	3.00x10 <sup>3</sup> ±0.00	9.00x10 <sup>2</sup> ±1x10 <sup>2</sup>	1.20x10 <sup>3</sup> ±1x10 <sup>2</sup>
9	2.00x10 <sup>3</sup> ±2x10 <sup>2</sup>	1.60x10 <sup>3</sup> ±0.00	1.00x10 <sup>4</sup> ±1x10 <sup>2</sup>
10	4.00x10 <sup>3</sup> ±1x10 <sup>2</sup>	3.00x10 <sup>2</sup> ±1x10 <sup>1</sup>	2.00x10 <sup>2</sup> ±1x10 <sup>1</sup>
11	2.00x10 <sup>3</sup> ±1x10 <sup>2</sup>	<10±0.00	2.00x10 <sup>2</sup> ±1x10 <sup>1</sup>
12	2.00x10 <sup>5</sup> ±3x10 <sup>3</sup>	1.00x10 <sup>5</sup> ±1x10 <sup>1</sup>	7.00x10 <sup>2</sup> ±1x10 <sup>1</sup>
13	2.00x10 <sup>5</sup> ±2x10 <sup>3</sup>	1.70x10 <sup>5</sup> ±3x10 <sup>3</sup>	<10±0.00
14	5.00x10 <sup>5</sup> ±1x10 <sup>4</sup>	3.00x10 <sup>5</sup> ±5x10 <sup>3</sup>	4.00x10 <sup>1</sup> ±0.1x10 <sup>1</sup>
15	1.00x10 <sup>5</sup> ±0.00	3.20x10 <sup>3</sup> ±1x10 <sup>2</sup>	<10±0.00
16	1.00x10 <sup>5</sup> ±1x10 <sup>3</sup>	1.00x10 <sup>3</sup> ±1x10 <sup>2</sup>	1.00x10 <sup>1</sup> ±0.1x10 <sup>1</sup>
17	3.00x10 <sup>4</sup> ±3x10 <sup>3</sup>	1.00x10 <sup>4</sup> ±1x10 <sup>2</sup>	6.60x10 <sup>2</sup> ±1x10 <sup>1</sup>
18	2.00x10 <sup>4</sup> ±4x10 <sup>2</sup>	1.20x10 <sup>4</sup> ±1x10 <sup>3</sup>	7.00x10 <sup>2</sup> ±6.1x10 <sup>1</sup>
19	1.00x10 <sup>5</sup> ±2x10 <sup>3</sup>	<10±0.00	2.50x10 <sup>3</sup> ±1x10 <sup>2</sup>
20	3.00x10 <sup>4</sup> ±1x10 <sup>3</sup>	<10±0.00	3.80x10 <sup>3</sup> ±1x10 <sup>2</sup>
21	3.00x10 <sup>4</sup> ±1x10 <sup>3</sup>	<10±0.00	<10±0.00
22	4.00x10 <sup>4</sup> ±3x10 <sup>3</sup>	<10±0.00	4.00x10 <sup>2</sup> ±1x10 <sup>1</sup>
23	2.60x10 <sup>4</sup> ±1x10 <sup>3</sup>	1.50x10 <sup>2</sup> ±1x10 <sup>1</sup>	1.90x10 <sup>4</sup> ±5x10 <sup>2</sup>
Mean	2.70x10 <sup>5</sup> ±1.07x10 <sup>4</sup>	2.90x10 <sup>4</sup> ±5.46x10 <sup>2</sup>	3.30x10 <sup>3</sup> ±1.17x10 <sup>2</sup>

cfu, colony forming units

**Table 4- Physicochemical properties of the hardaliye samples produced in laboratory**

Trials	Grape variety	Acidity (%)	pH	Color		
				L	a	b
1	Dingil Kara	0.73±0.02 <sup>b</sup>	3.66±0.02 <sup>d</sup>	9.24±0.02 <sup>c</sup>	2.89±0.04 <sup>a</sup>	1.27±0.01 <sup>c</sup>
2	Kara Üzüm	1.05±0.04 <sup>c</sup>	3.47±0.32 <sup>b</sup>	9.71±0.01 <sup>d</sup>	3.69±0.02 <sup>c</sup>	1.04±0.03 <sup>b</sup>
3	Cabernet Sauvignon*	0.56±0.01 <sup>a</sup>	3.73±0.03 <sup>c</sup>	8.45±0.05 <sup>a</sup>	4.20±0.04 <sup>d</sup>	1.89±0.01 <sup>d</sup>
4	Yerli İzabella**	0.77±0.01 <sup>c</sup>	3.33±0.03 <sup>a</sup>	9.86±0.02 <sup>c</sup>	3.13±0.03 <sup>b</sup>	1.28±0.01 <sup>c</sup>
5	Siyah Üzüm	0.98±0.02 <sup>d</sup>	3.50±0.06 <sup>c</sup>	8.75±0.05 <sup>b</sup>	5.00±0.03 <sup>c</sup>	0.86±0.02 <sup>a</sup>

Note: The analyzes were carried out at the end of the fermentation. The different letters in the form of upper indices indicate that the averages are different (P<0.01) according to the Duncan Multiple Comparison Test. \*, Cabernet Sauvignon; \*\*, Yerli İzabella

**Table 5- Chemical properties of the hardaliye samples produced in laboratory**

Trials	Grape variety	Total phenolic content (mg L <sup>-1</sup> )	Monomeric anthocyanin <sup>+</sup> (mg L <sup>-1</sup> )	Volatile acids (g L <sup>-1</sup> )	Ethanol (%)	Total sugar (%)
1	Dingil Siyah	2286.8±2.15 <sup>c</sup>	210.8±1.33 <sup>c</sup>	0.43±0.01 <sup>c</sup>	n.d*	16.5±0.4 <sup>b</sup>
2	Kara Üzüm	1858.2±0.20 <sup>d</sup>	116.3±1.3 <sup>a</sup>	0.55±0.02 <sup>d</sup>	n.d	15.2±0.3 <sup>a</sup>
3	Cabernet Sauvignon**	1165.4±1.40 <sup>c</sup>	200.6±0.3 <sup>c</sup>	0.42±0.02 <sup>bc</sup>	n.d	19.7±0.4 <sup>d</sup>
4	Yerli İzabella***	1093.9±0.50 <sup>b</sup>	201.3±1.30 <sup>d</sup>	0.39±0.01 <sup>a</sup>	n.d	21.4±0.3 <sup>c</sup>
5	Siyah Üzüm	961.8±0.40 <sup>a</sup>	161.1±1.00 <sup>b</sup>	0.40±0.02 <sup>ab</sup>	n.d	17.9±0.2 <sup>c</sup>

<sup>+</sup>, Monomeric anthocyanin (malvidin-3-glucoside); \*, n.d, not detected; \*\*, Cabernet Sauvignon; \*\*\*, Yerli İzabella. Note: Ethanol values are not included in the statistical analysis

**Table 6- Microbiological properties of the hardaliye samples produced in laboratory (cfu mL<sup>-1</sup>)**

<i>Trials</i>	<i>Grape variety</i>	<i>TMAB<sup>+</sup></i>	<i>LAB (Lactobacilli)</i>	<i>Yeast-Molds</i>
1	Dingil Kara	7.00x10 <sup>6</sup> ±1x10 <sup>6</sup> b	1.68x10 <sup>6</sup> ±1x10 <sup>4</sup> c	<10±0.00 a
2	Kara üzüm	1.60x10 <sup>7</sup> ±2x10 <sup>6</sup> c	2.20x10 <sup>5</sup> ±2x10 <sup>4</sup> d	1.00x10 <sup>2</sup> ±0.1x10 <sup>1</sup> c
3	Cabernet Sauvignon*	2.00x10 <sup>5</sup> ±1x10 <sup>4</sup> a	1.00x10 <sup>2</sup> ±0.1x10 <sup>1</sup> a	3.50x10 <sup>1</sup> ±0.00 b
4	Yerli İzabella	8.16x10 <sup>5</sup> ±6x10 <sup>3</sup> a	2.76x10 <sup>4</sup> ±3x10 <sup>2</sup> c	<10±0.00 a
5	Siyah üzüm	1.00x10 <sup>5</sup> ±1x10 <sup>3</sup> a	3.00x10 <sup>3</sup> ±3x10 <sup>2</sup> b	<10±0.00 a

<sup>+</sup>TMAB, total mesophilic aerobic bacteria; LAB, Lactic acid bacteria; cfu, colony forming units; \*, Cabernet Sauvignon

the amount of these components to decrease. For this reasons, total phenolics of anthocyanins of hardaliye samples collected from producers may be lower than others. Coşkun et al (2012) determined that the amount of monomeric anthocyanins (malvidin-3-glucoside) and total phenolics of hardaliye samples decreased from 114.1 to 54.4 mg L<sup>-1</sup> and from 1392.5 to 1067.5 mg L<sup>-1</sup> respectively, during 1 year of storage. Zarfilla et al (2003) produced wine from red grapes grown organically and traditionally in Spain and stored in glass bottles in a dark environment for 7 months at 20 °C. They observed a decrease of approximately 65% over 7 months in the total amount of total phenolics. In the same study, the monomeric anthocyanins (malvidin-3-glucoside) decreased from 248.34 mg L<sup>-1</sup> to 32.29 mg L<sup>-1</sup> in the conventional red grape wine, from 228.5 mg L<sup>-1</sup> to 22.45 mg L<sup>-1</sup> in the organic red grape wine. Hardaliye was produced from papazkarası blue-black grapes by conventional method by Aşkın & Atik (2016). 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 in prepared beverage within the 15, 30, 45 and 60 days of storage. The results of color parameters obtained show 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 for 60 days depending on the storage temperature. Storage under 4 °C and 20 °C resulted in 60 and 78% losses in anthocyanin content, respectively.

In the present study, ethyl alcohol was found in some hardaliye samples collected from different

households. The reason might be that preservatives such as benzoic acid and mustard seeds were not used at the sufficient amount. Moreover, improper storage conditions and prolonged storage time increases formation of alcohol.

In the study conducted by Coşkun et al (2009), hardaliye sample was produced using black grape from Malatya region's grape varieties by applying traditional. Total mesophilic aerobic bacteria count in the hardaliye sample was lower (4.7x10<sup>4</sup>) than those in the samples produced in laboratory in this study. This may be caused by harvesting and fermentation conditions.

The results of this study were also in accordance with the study conducted by Arici & Coskun (2001) in which red colour (measured by Hunter Lab) of hardaliye samples ranged from 1.33 to 9.66 and total mesophilic aerobic bacteria, lactic acid bacteria and yeasts-molds counts ranged from 3.5x10<sup>2</sup> to 8x10<sup>5</sup> cfu mL<sup>-1</sup>, 1.0x10<sup>2</sup> to 4.0x10<sup>4</sup> cfu mL<sup>-1</sup> and 1.0x10<sup>2</sup> to 8.1x10<sup>4</sup> cfu mL<sup>-1</sup>, respectively. Furthermore, coliforms and *E. coli* were not found in none of the samples.

Güven & Aksoy (2009) produced hardaliye using verigo variety of grapes. 2.5 g mustard seeds were used. Hardaliye was fermented for 21 day. On the 7<sup>th</sup> and 21<sup>st</sup> days of fermentation, pH and total acidity were detected to range from 4.17 to 3.94 and 3.39 to 10.40%, respectively. They found higher pH value than the present study, however, total acidity was comparable. They did not detect ethyl alcohol which was similar to the hardaliye samples produced by applying traditional method in the present study.

Kılıç & Copur (1988) used Muskule, Razaki and Erenkoy Beyazı as grape varieties in their research. The total sugar content of hardaliye produced from these varieties was determined as 12.92, 14.94 and 11.47 g 100 mL<sup>-1</sup>, respectively. Only the 3 hardaliye samples from the present study were in accordance with Kılıç & Çopur (1988)'s observation. In this study, the total sugar contents of hardaliye samples were detected to range from 7.2% to 25.4%, which may be attributed to different grape varieties and maturities. The total amount of sugar in the grapes increased towards end of the maturity period (Çelik 1998). The grape harvest for hardaliye production usually starts on October or November. The hardaliye samples with high sugar content may be produced from late harvested grapes. The hardaliye samples with low sugar content may be produced from early harvested grapes. The formation of alcohol due to uncontrolled fermentation or improper storage conditions may have caused to decrease of sugar content.

#### 4. Conclusions

There are very few scientific studies about traditional hardaliye beverage although it has been produced traditionally for years. It brings the opportunity of consuming grapes in winter or spring seasons; thus, preventing the excess grapes from being wasted. Just like any other popular traditional food products, industrial-scale production of hardaliye should be realized and this traditional beverage should enjoy the large number of consumers' preference. In addition, more research is needed in order to its adaptation to industry.

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