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Effect of *Lactobacillus plantarum* AK4-11 and Different Grape Varieties on the Properties of Hardaliye

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ABSTRACT

This article reports the effects of using *Lactobacillus plantarum* AK4-11 and different grape varieties on some properties of hardaliye. The results showed that grape variety did not have any effect on pH during fermentation period, but using red grapes resulted in higher pH 4.10 in hardaliye after 90 day storage. On the other hand using white grape resulted in higher brix values ranged from 12.90 to 14.00 at the end of the 14th day of fermentation. The colour results indicated that CI and redness values were higher (2.01-2.90 and 41.84-44.50, respectively) and yellowness values were lower (41.71-43.15) in hardaliye samples produced with red grapes. Using red grapes also increased the amount of phenolic compounds in hardaliye samples. Results of this study indicated that using *L. plantarum* AK4-11 and different grape varieties in hardaliye manufacture affected some quality parameters of hardaliye.

Keywords*:* Hardaliye; Probiotic; Grape; Phenolic compounds

Lactobacillus plantarum AK4-11 ve Farklı Üzüm Çeşitlerinin Hardaliye Üzerine Etkisi

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ÖZET

Bu makale *Lactobacillus plantarum* AK4-11 ve farklı üzüm çeşitlerinin hardaliyenin bazı özellikleri üzerindeki etkisini açıklamaktadır. Elde edilen sonuçlar üzüm çeşitlerinin fermantasyon süresince pH'yı etkilemediğini ancak kırmızı üzüm kullanımının 90 gün depolama sonrasında pH'yı yükselttiğini (pH 4.10) göstermiştir. Diğer taraftan beyaz üzüm kullanımı ile 14 günlük fermantasyon sonunda 12.90 ile 14.00 arasında daha yüksek briks değeri ölçülmüştür. Kırmızı üzümle

üretilen hardaliye örneklerinin renk ölçüm sonuçlarına göre renk yoğunluğu ve kırmızılık değerleri yüksek (sırasıyla 2.01-2.90 ve 41.84-44.50) ve sarılık değeri ise düşük (41.71-43.15) bulunmuştur. Hardaliye örneklerinin kırmızı üzüm ile üretilmesi fenolik bileşenlerde artışa sebep olmuştur. Bu çalışmanın sonuçları, *L. plantarum* AK4-11 ve farklı üzüm çeşitleri kullanılarak yapılan üretimin hardaliyenin bazı kalite parametrelerini etkilediğini ortaya koymuştur.

Anahtar Kelimeler: Hardaliye; Probiyotik; Üzüm; Fenolik bileşikler

1. Introduction

Functional foods are thought to provide benefits beyond basic nutrition and may play a role in reducing or minimizing the risk of some diseases and other health conditions (IFICF 2011). Probiotics can be considered functional foods because they provide health benefits beyond the traditional nutrition function (Lin 2003). A probiotic is a viable microbial dietary supplement that beneficially affects the host through its effects in the intestinal tract (Salminen et al 1998). There is evidence that the oral consumption of probiotics might have beneficial effects on several microbial disorders of the gut and produces a protective effect on the gut flora (Dembele et al 1998). The most commonly used strains belong to the genera *Lactobacillus* and *Bifidobacterium* (Quwehand et al 2002). Lactic acid bacteria (LAB) are generally regarded as safe and widely used in fermentation of a variety of food for the flavor, texture and preservation purposes. Certain strains can be used as probiotic organisms possess some important properties to improve human health (Fuller 1989). It is well documented that probiotic bacteria inhibit the growth of various pathogenic bacteria by producing different organic acids such as lactic and acetic acid, hydrogen peroxide, bacteriocins, bacteriocin like substances and possibility biosurfactants (Gilliand & Speck 1977; Chang et al 2001). In addition, probiotic bacteria could prevent the attachment of pathogens and stimulate their removal from the infected intestinal tract (Lee et al 2000). The mechanisms of these beneficial effects are related to exclusion of pathogenic bacteria by direct antagonism, competition for nutrients, adhesion receptors and stimulation of host immunity (Elmer et al 1996).

In the last decades there is a growing interest in traditional foods all over the world. Traditional fermented foods are essential for the well-being of many people of the world (Hesseltine & Wang 1980). Therefore many studies on traditional foods have been focused on improving health benefits, quality, safety and processing methods of these products. "Hardaliye" is also a traditional fermented beverage that has been produced and consumed since ancient times in Thrace region of Turkey. It is manufactured by lactic acid fermentation of red grape or grape juice (Arici & Coskun 2001). Due to the LAB flora of hardaliye; it has been classified as non-dairy probiotic beverage (Prado et al 2008). In hardaliye production, the grapes are washed and crushed in a jar or barrels and 0.3-0.4% of crushed mustard seeds and/or 0.1% of benzoic acid is added, the solution is left to fermentation at room temperature for 10 days. After fermentation, hardaliye is removed from mustard seeds, vine leaves and grape residues by filtration (Arici & Coskun 2001; Prado et al 2008; Gucer et al 2009). The color of hardaliye reflects the original color of the grapes and has a characteristic aroma (Arici & Coskun 2001; Coskun & Arici 2006; Prado et al 2008). Mustard seeds, K-benzoate or Na-benzoate are used as preservative agents. Mustard seeds and K-benzoate mixture inhibits the yeast growth and prevents the alcohol fermentation (Coşkun 2012).

It has already been reported that a moderate intake of grape products like wines or grape juices have health protection effects (Dani et al 2009). Because of the production technique and potentially high grape polyphenol content, hardaliye is hypothesized to provide antioxidative effects (Amoutzopoulos et al 2013). Grapes and grape juice contain many of the same biologically active phenolic compounds such

as catechins, epicatechins, epicatechin-3-O-gallate and dimeric, trimeric and tetrameric procyanidins, all of which are antimutagenic and antiviral agents (Saito et al 1998). The health benefits of catechins and procyanidins have led to the use of grape seed extract as a dietary supplement (Soleas et al 1997).

The objective of this study was to investigate differences between traditional hardaliye production with natural fermentation, and controlled fermentation with probiotic *L. plantarum* AK4-11 in two different grape varieties, red (R) and white (W). Moreover, we determined the chemical, microbiological, sensory, and phenolic characteristics of hardaliye samples.

2. Material and Methods

2.1. Probiotic culture

A probiotic strain, *L. plantarum* AK4-11, was used as a starter culture in the production of hardaliye. The strain was isolated from feces samples and some probiotic properties of the strain were determined (Başyiğit 2004). This strain was also identified by 16S rRNA analysis (Başyiğit Kılıç & Karahan 2010). The strain was inoculated in de Man, Rogosa and Sharpe (MRS) broth and incubated at 37 °C for 24 h until the cell number reached 10^9 CFU mL^{-1}. The cells were pelleted by centrifugation at 5000 x *g* for 10 minutes at 20 °C, and the pellets were washed in phosphate-buffered saline solution (PBS, pH 7.4) twice. Finally, the probiotic bacterium was added in grape juices at the level of 10^6 CFU mL^{-1}.

2.2. Hardaliye production

In this study, red (Demre) (R) and white (Gimrik) (W) grape varieties were used. Hardaliye production was carried out with three different groups for each grape variety. The fresh grapes were collected from local markets in Isparta, Turkey during autumn season. The control group was produced using grape juice, crushed mustard seeds and cherry leaves, the first group was produced using grape juice, *L. plantarum* AK4-11, crushed mustard seeds and cherry leaves and the second group was produced using grape juice, *L. plantarum* AK4-11, crushed mustard seeds, cherry leaves and cloves. Groups were titled as CW: Control group produced with white grape; 1W: 1st group produced with white grape; 2W: 2nd group produced with white grape; CR: Control group produced with red grape; 1R: 1st group produced with red grape; 2R: 2nd group produced with red grape. Hardaliye production method is presented in Figure 1. Hardaliye was obtained 25 days after.

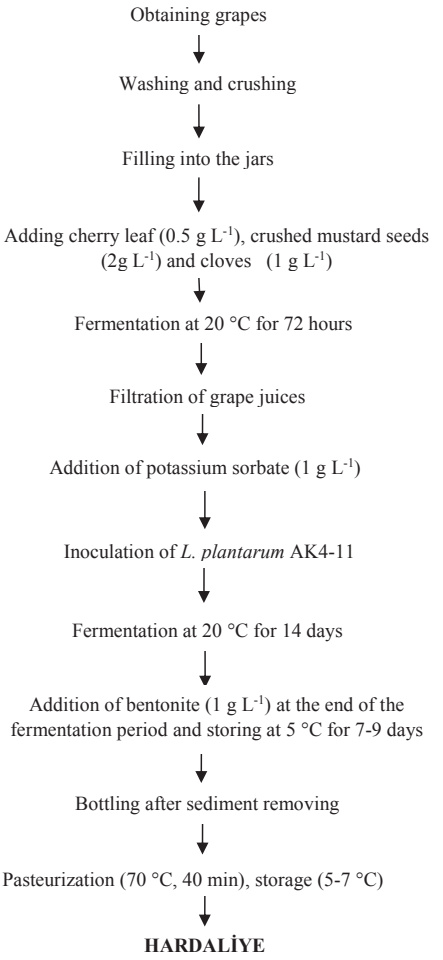

Figure 1- Hardaliye production process with grape juice, *L. plantarum* AK4-11, crushed mustard seeds, cherry leaves and cloves

Şekil 1- Üzüm suyu, L. plantarum AK4-11, ezilmiş hardal tohumu, kiraz yaprağı ve karanfilden hardaliye üretim işlemi

2.3. Chemical and microbiological analyses

The chemical analyses were performed after the addition of *L. plantarum* AK4-11, 7th and 14th days of the fermentation and 3 months of the storage. The pH of the hardaliye samples was measured with a pH-meter (InoLab WTW-537, Germany) and soluble solids (°Brix) was measured by using a hand refractometer (Atago, Japan) at 20 °C. The average values of two measurements for pH and soluble solids were recorded.

Microbiological analyses were carried out at the 1st, 7th and 14th days of fermentation. The preparation of the samples and dilutions for microbiological examinations was performed according to IDF standard 122C (Anonymous 1996; Karahan et al 2002). Ten (10) mL of hardaliye samples suspended in 90 mL of sterile 1/4 ringer solution. Decimal dilutions in ringer solution were made and plated on MRS agar (Merck, Germany) for lactobacilli counts. MRS plates were incubated for 48 h at 37 °C. Potato Dextrose agar pH 3.5 (Merck, Germany) was used to determine the yeast and molds. PCA agar was used for total mesophilic aerobic bacteria (Anonymous 1998a; APHA 2002). All analyses were performed in duplicate.

2.4. Color analyses

Spectrophotometric measurements of color were carried out by measuring the absorbance with a quartz cell of 1 mm path length at 420, 520 and 620 nm (SHIMADZU, UV-1601, Japan) at the end of the 90-day storage period. The color intensity (CI), proportions of red (R%), yellow (Y%) and blue (B%) were determined according to the Glories procedure (Glories 1984) by using Equations 1-4, respectively. All samples were analyzed in duplicate.

$$CI = A_{420} + A_{520} + A_{620} \quad (1)$$

$$R\% = A_{520} * 100/CI \quad (2)$$

$$Y\% = A_{420} * 100/CI \quad (3)$$

$$B\% = A_{620} * 100/CI \quad (4)$$

2.5. Determination of phenolic compounds

At the end of the 90-day storage period, phenolic compounds were evaluated by high performance liquid chromatography (RP-HPLC) (Shimadzu, Japan) with direct injection. Detection and quantification was carried out with a SCL-10Avp System controller, a SIL-10AD vp Autosampler, a LC-10AD vp pump, a DGU-14a degasser, a CTO-10 A vp column heater and a diode array detector with wavelengths set at 278 nm. The 250 x 4.6 mm i.d., 5 µm column was used (Agilent Eclipse XDB-C18). The flow rate was 0.8 mL min^{-1}, injection volume was 20 µL and the column temperature was 30 °C. Gradient elution of two solvents was used. Solvent A consisted of acetic acid-water (3:100 v v^{-1}) and solvent B consisted of methanol. The data were integrated and analyzed using the Shimadzu Class-VP Chromatography Laboratory Automated Software system. The hardaliye samples, standard solutions and mobile phases were filtered by a 0.45-µm pour size membrane filter. The amount of phenolic compounds in the extracts was calculated as µg L^{-1} wine using external calibration curves, which were obtained for each phenolic standard. Standards were purchased from Sigma-Aldrich (Steinheim, Germany). Phenolic compositions of wines were determined by the modified method of Caponio et al (1999).

2.6. Sensory analyses

Sensory analyses was performed at the end of the storage period at the Department of Food Engineering at the Suleyman Demirel University by a group of eighteen non-smoker panellists experienced in the sensory evaluation of fruit juice. Hardaliye samples from each treatment was randomly chosen and served to the panelists. The taste (the taste of grape, clove taste, bitterness), smell, appearance (clarity), acidity and the overall acceptability of hardaliye samples were evaluated. Hardaliye attribute intensities were rated on 5 point scale.

2.7. Statistical analysis

The entire experiment was replicated two times on separate production days. Data collected for microbiological level, physicochemical properties and sensory attributes were analyzed by the statistical analysis system (Anonymous 1998b). The

generated data was analyzed by analysis of variance (ANOVA). Differences among mean values were established using the Duncan's multiple range test and were considered significant at P<0.05.

3. Results and Discussion

In this study, two varieties of grapes were used and three different combinations of hardaliye were produced for each grape variety. The results for the chemical and microbiological properties of hardaliye samples are shown in Table 1 and 2. The pH and brix values of the hardaliye samples produced by two different grapes decreased until the 14th day of the fermentation. However, the values increased at the 90th days of the storage (P<0.05). There were not any significant differences at pH values between the groups during the 1st and 14th days of fermentation. At the end of the 90 days of storage, the pH of hardaliye samples produced with red grapes was higher than that of hardaliye samples produced with white grapes (P>0.05). Similar to our results, Güven & Aksoy (2009) reported that the pH values of the hardaliye produced with only mustard seeds, and mustard seed and clove were 4.02-3.94 and 4.06-3.91, respectively, during period between 3rd and 21st day of fermentation. In another study conducted by Aydoğdu et al (2014), the pH value of hardaliye produced from the Alphonse Lavallée grapes was 4.27 on 1st day of fermentation and 3.96 on 10th day.

The initial brix values in hardaliye samples produced with red grapes were higher than those produced with white grapes. However, the brix values in hardaliye samples produced with white grapes were found to be higher than those produced with red grapes at the 14th day of fermentation (P<0.05). In this study, even though there was no significant difference in LAB counts among the groups of hardaliye samples produced with white grapes (P>0.05), LAB counts were approximately 1 log CFU mL^{-1} higher in 1R and 2R groups compared to CR group (P<0.05). The activity of the LAB makes this beverage safe in terms of pathogenic microorganisms (Aydoğdu et al 2014). In this study the number of yeast in all groups was found to be around 6 log CFU mL^{-1} and mold growth were not observed in the hardaliye samples. No significant changes were determined in all R and W hardaliye groups for the number of total

Table 1- Changes in pH and solid content of hardaliye samples during storage days (5 ºC)

Çizelge 1- Depolama süresince (5 ºC) hardaliye örneklerinde meydana gelen pH ve kurumadde değişimi

Groups	*Day*	*pH*	*Brix*
CW	1	3.7±0.15^{a*}	16.3±2.12^{ab}
CW	7	3.6±0.32^{ab}	15.7±2.51^{ab}
CW	14	3.3±0.19^{b}	14.0±0.84^{b}
CW	90	3.8±0.10^{a}	15.2±3.64^{ab}
1W	1	3.7±0.14^{a}	16.2±4.06^{ab}
1W	7	3.5±0.14^{ab}	15.6±2.27^{ab}
1W	14	3.3±0.30^{b}	13.1±0.98^{b}
1W	90	3.8±0.19^{a}	15.4±4.87^{abc}
2W	1	3.7±0.08^{a}	16.9±2.89^{ab}
2W	7	3.6±0.20^{a}	16.7±3.71^{ab}
2W	14	3.3±0.30^{b}	12.9±0.42^{b}
2W	90	3.8±0.14^{a}	16.2±3.71^{ab}
CR	1	3.8±0.03^{a}	18.6±1.62^{a}
CR	7	3.7±0.03^{a}	17.5±0.70^{a}
CR	14	3.5±0.42^{ab}	9.7±0.63^{c}
CR	90	4.1±0.36^{c}	11.9±6.36^{abc}
1R	1	3.8±0.06^{a}	18.7±1.94^{a}
1R	7	3.7±0.05^{a}	17.4±0.98^{a}
1R	14	3.5±0.45^{ab}	9.7±0.84^{c}
1R	90	4.1±0.39^{c}	11.5±7.03^{abc}
2R	1	3.7±0.04^{a}	19.8±1.37^{a}
2R	7	3.7±0.02^{a}	19.2±1.14^{a}
2R	14	3.5±0.35^{ab}	9.1±0.91^{c}
2R	90	4.1±0.30^{c}	13.2±9.82^{abc}

CW, control group produced with white grape juice, crushed mustard seeds and cherry leaves; 1W, 1st group produced with white grape juice, *L. plantarum* AK4-11, crushed mustard seeds and cherry leaves; 2W, 2nd group produced with white grape juice, *L. plantarum* AK4-11, crushed mustard seeds, cherry leaves and cloves; CR, control group produced with red grape juice, crushed mustard seeds and cherry leaves; 1R, 1st group produced with red grape juice, *L. plantarum* AK4-11, crushed mustard seeds and cherry leaves; 2R, 2nd group produced with red grape juice, *L. plantarum* AK4-11, crushed mustard seeds, cherry leaves and cloves; *, values within columns with different superscript letter are significantly different (P<0.05) (n= 4)

mesophilic aerobic bacteria throughout the 14th days of storage (P>0.05). Contrary to the expectations of this study, the addition of mustard seeds, cloves and potassium sorbate did not show a significant inhibitory effect on the microbial count. Arıcı & Coşkun (2001) investigated physicochemical and microbiological properties of 26 days aged hardaliye samples collected from Kırklareli region in Turkey and researchers reported that the LAB count ranged $1.0x10^2$ and $4.0x10^4$ CFU mL^{-1}. The same researchers reported that pH of hardaliye samples manufactured in laboratory conditions dropped from 3.86 to 3.39 during the 7 day fermentation period. Coşkun & Arıcı (2011) reported that there were no significant difference in total mesophilic aerobic bacteria and LAB counts between hardaliye samples containing white (*Brassica alba* (L.) Boiss) or black (*Brassica nigra* (L.) Koch) mustard seeds. On the other hand the researchers observed lower yeast and mold counts in hardaliye samples containing black mustard seeds. As a result of different varieties, the year, region, and juice content in relevant studies it is natural to see different microbiological/chemical results (Aydoğdu et al 2014). Aydoğdu et al (2014) observed a progressive reduction/increase/reduction pattern for the aerobic mesophilic bacteria and lactic acid bacteria colony counts.

Table 2- Microbiological changes in hardaliye samples (log CFU g^{-1}) during storage days (5 ºC)

Çizelge 2- Depolama süresince hardaliye örneklerinde meydana gelen mikrobiyolojik değişimler (log KOB g^{-1}) (5 ºC)

Groups	*Day*	*TMAB*	*LAB*	*Y*
CW	1	6.6±0.53^{b*}	6.5±0.41^{a}	6.4±0.37^{a}
CW	7	7.3±0.56^{a}	6.9±0.19^{a}	6.0±0.03^{a}
CW	14	7.2±0.44^{ab}	6.6±0.08^{a}	5.9±0.74^{a}
1W	1	6.7±0.002^{ab}	6.8±0.49^{ab}	6.4±0.03^{a}
1W	7	6.9±0.27^{ab}	6.4±0.94^{ab}	6.5±0.55^{a}
1W	14	7.1±0.30^{ab}	6.1±0.79^{ab}	6.9±0.62^{a}
2W	1	6.8±0.22^{ab}	6.8±0.53^{ab}	6.3±0.12^{a}
2W	7	7.2±0.46^{ab}	7.3±0.42^{a}	6.0±0.56^{a}
2W	14	7.3±0.24^{ab}	6.7±0.47^{a}	5.3±0.93^{a}
CR	1	6.6±0.11^{ab}	5.8±0.79^{ab}	6.3±0.22^{a}
CR	7	6.5±0.40^{ab}	5.5±0.92^{b}	6.4±0.35^{a}
CR	14	6.5±0.12^{ab}	5.8±0.18^{ab}	6.6±0.03^{a}
1R	1	6.8±0.24^{ab}	6.7±0.23^{ab}	6.4±0.24^{a}
1R	7	6.8±0.01^{ab}	6.8±0.04^{ab}	6.7±0.12^{a}
1R	14	6.4±0.12^{b}	6.5±0.25^{ab}	6.2±0.07^{a}
2R	1	6.7±0.399^{ab}	6.7±0.37^{ab}	6.4±0.28^{a}
2R	7	6.8±0.38^{ab}	6.6±0.61^{ab}	6.3±0.56^{a}
2R	14	6.8±0.15^{ab}	6.6±0.11^{ab}	6.3±1.82^{a}

CW, control group produced with white grape juice, crushed mustard seeds and cherry leaves; 1W, 1st group produced with white grape juice, *L. plantarum* AK4-11, crushed mustard seeds and cherry leaves; 2W, 2nd group produced with white grape juice, *L. plantarum* AK4-11, crushed mustard seeds, cherry leaves and cloves; CR, control group produced with red grape juice, crushed mustard seeds and cherry leaves; 1R, 1st group produced with red grape juice, *L. plantarum* AK4-11, crushed mustard seeds and cherry leaves, 2R, 2nd group produced with red grape juice, *L. plantarum* AK4-11, crushed mustard seeds, cherry leaves and cloves; TMAB, total mesophilic aerobic bacteria; LAB, lactic acid bacteria; Y, yeast; *, values within columns with different superscript letter are significantly different (P<0.05) (n= 4)

The color measurement results of hardaliye samples are presented in Table 3. Results indicated that different grape varieties and treatments affected the CI values of hardaliye samples (P<0.05). The CI of R groups was higher than that of W groups (P<0.05). However, these values did not show any

Table 3- Colour changes in hardaliye samples

Çizelge 3- Hardaliye örneklerinde meydana gelen renk değişimi

Groups	*CI*	*R%*	*Y%*	*B%*
CW	1.1±0.08^{c*}	29.1±0.38^{b}	51.6±1.06^{a}	19.2±0.68^{a}
1W	0.7±0.02^{c}	29.2±0.58^{b}	51.3±1.51^{a}	19.4±0.93^{a}
2W	0.8±0.09^{c}	27.8±0.56^{b}	54.4±2.50^{a}	17.7±1.93^{ab}
CR	2.0±0.16^{b}	44.5±3.39^{a}	42.5±2.81^{b}	12.9±0.57^{b}
1R	2.9±0.36^{a}	41.8±1.89^{a}	41.7±0.53^{b}	16.4±1.36^{ab}
2R	2.4±0.22^{a}	43.1±1.24^{a}	43.1±0.07^{b}	13.6±1.31^{b}

CW, control group produced with white grape juice, crushed mustard seeds and cherry leaves; 1W, 1st group produced with white grape juice, *L. plantarum* AK4-11, crushed mustard seeds and cherry leaves; 2W, 2nd group produced with white grape juice, *L. plantarum* AK4-11, crushed mustard seeds, cherry leaves and cloves; CR, control group produced with red grape juice, crushed mustard seeds and cherry leaves; 1R, 1st group produced with red grape juice, *L. plantarum* AK4-11, crushed mustard seeds and cherry leaves; 2R, 2nd group produced with red grape juice, *L. plantarum* AK4–11, crushed mustard seeds, cherry leaves and cloves; CI, colour intensity; R%, proportions of red values; Y%, proportions of yellow values; B%, proportions of blue values; *, values within columns with different superscript letter are significantly different (P<0.05) (n= 4)

significant difference among treatment groups for each grape variety. As expected yellowness value was clearly higher in W groups and red and yellow components were also higher in R groups.

In this study, separation was achieved for 19 components including phenolic acids and flavonoids. A previous study revealed that dark fruit products such as juice or red wine have, on the average, several-fold greater concentration of polyphenols than light-coloured juices or white wines (Makris et al 2003). Phenolic contents of six different hardaliye (mg L^{-1}) were shown in Table 4. In our study, as expected, phenolic compounds in the sample groups from red grape juice (R) were higher than in the sample groups from white grape juice (W) ($P<0.05$). The bioavailability of phenolic compounds can also be affected by differences in cell wall structures, location of glycosides in cells and binding of phenolic compounds within the food matrix (Hollman et al 1997). While the major compounds in R groups were gallic, cafeic, syringic, and coumaric acid. Gallic acid and syringic acid were observed in all R groups and coumaric acid was also observed in all W groups. On the other hand, catechin, chlorogenic acid, epicatechin, rutin, resveratrol, hesperidin, apigenin-7-glucoside, rosmarinic acid, eriodictyol, quercetin, naringenin, luteolin, apigenin, ferulic acid and acacetin were not detected in any of the hardaliye samples.

Since there has not been any information in the literature for the phenolic compounds of hardaliye, it is difficult to compare the results for the phenolic content determined in our study. Therefore, results of phenolic content are compared with other beverages. The amount of phenolic compounds of hardaliye produced with red grapes in our study was lower than the phenolic compounds of red wine samples reported by Del Alamo et al (2004). Balasundram et al (2006) mentioned in his review article that red wines contain more than 1000 mg gallic acid equivalents (GAE) L^{-1} of total phenolics, compared to less than 500 mg GAE L^{-1} for most white wines. Anthocyanins from grape skins are the major component responsible for the color and the higher phenolic content of red wines compared to white wines (Mazza et al 1999). Özkan & Baydar (2006) mentioned that the most abundant phenolic were catechin (17.82-33.59 mg L^{-1}) as flavonoid and gallic acid (13.25-16.39 mg L^{-1}) as phenolic acid in red wines from four different Turkish grape cultivars. There are wide variations between the total phenolic contents of the different fruits or vegetables, or even for the same fruits or vegetables reported by different authors (Balasundram et al 2006). These differences may be due to the complexity of these groups of compounds, and the methods of extraction and analysis (Kalt 2001).

Table 4- Phenolic compounds of hardaliye groups (mg L^{-1})

Çizelge 4- Hardaliye gruplarının fenolik bileşenleri (mg L^{-1})

Groups	*Gallic acid*	*Cafeic acid*	*Syringic acid*	*Coumaric acid*
CW	nd[¥]	nd	nd	0.02±0.002[a]
1W	nd	0.1±0.04[a]	0.1±0.07[a]	0.02±0.003[a]
2W	nd	nd	nd	0.03±0.003[a]
CR	0.5±0.25[a*]	nd	1.9±0.69[b]	0.07±0.006[a]
1R	0.3±0.12[a]	0.7±0.35[b]	1.7±0.54[b]	0.02±0.005[a]
2R	0.3±0.17[a]	0.5±0.33[b]	1.2±0.74[b]	nd

CW, control group produced with white grape juice, crushed mustard seeds and cherry leaves; 1W, 1st group produced with white grape juice, *L. plantarum* AK4-11, crushed mustard seeds and cherry leaves; 2W, 2nd group produced with white grape juice, *L. plantarum* AK4-11, crushed mustard seeds, cherry leaves and cloves; CR, control group produced with red grape juice, crushed mustard seeds and cherry leaves; 1R, 1st group produced with red grape juice, *L. plantarum* AK4-11, crushed mustard seeds and cherry leaves; 2R, 2nd group produced with red grape juice, *L. plantarum* AK4-11, crushed mustard seeds, cherry leaves and cloves; [¥]nd, not detected; *, values within columns with different superscript letter are significantly different ($P<0.05$) (n= 4)

Data of sensory evaluation is presented in Table 5. The results showed that the addition of clove affected some of the sensory attributes compared with the C group. The odour acceptance of the groups was not different among C and 1st groups, but a 2nd group has higher odor intensity compared with the other groups. This may be explained by the addition of cloves into these groups. The CW and 2R groups were received the higher taste scores and the acidity was also higher in these groups. The overall

Table 5- Sensory quality of hardaliye groups

Çizelge 5- Hardaliye örneklerinin duyusal özellikleri

Sensory quality	*CW*	*1W*	*2W*	*CR*	*1R*	*2R*
Clarity	3.3±1.2^{ab*}	3.6±0.9^{b}	3.6±1.4^{b}	2.6±0.8^{a}	2.5±1.2^{a}	3.0±0.7^{ab}
Odor	3.3±0.9^{b}	3.1±1.1^{ab}	3.6±0.9^{c}	2.8±0.7^{a}	3.1±0.8^{ab}	3.8± 1.2^{c}
Taste	4.3±1.2^{a}	3.8±1.4^{b}	3.8±1.1^{b}	2.6±1.1^{c}	3.0±1.3^{c}	4.0±1.2^{a}
Bitterness	1.8±0.5^{ab}	2.1±0.9^{b}	2.0±0.7^{b}	1.1±0.8^{a}	2.0±0.9^{b}	1.1±0.9^{a}
Acidity	3.3±1.1^{ab}	3.1±1.3^{ab}	2.8±1.0^{a}	3.0±1.3^{ab}	3.3±1.2^{b}	3.6±1.4^{b}
Clove taste	2.5±1.1^{a}	2.3±0.9^{a}	3.5±1.4^{b}	2.5±0.9^{a}	2.3±1.1^{a}	3.3±1.3^{b}
Grape taste	3.8±1.3^{a}	3.0±1.2^{b}	3.5±1.3^{a}	3.6±1.5^{a}	3.0±1.1^{b}	4.0±0.9^{a}
Appearance	3.6±1.3^{a}	3.6±1.4^{a}	4.0±1.3^{a}	3.8±1.2^{a}	3.8±0.9^{a}	4.6±0.8^{b}
The overall acceptability	3.1±1.1^{ab}	2.8±1.1^{a}	3.8±0.9^{b}	3.8±1.3^{b}	2.8±0.8^{a}	4.6±0.9^{c}

CW, control group produced with white grape juice, crushed mustard seeds and cherry leaves; 1W, 1st group produced with white grape juice, *L. plantarum* AK4-11, crushed mustard seeds and cherry leaves; 2W, 2nd group produced with white grape juice, *L. plantarum* AK4-11, crushed mustard seeds, cherry leaves and cloves; CR, control group produced with red grape juice, crushed mustard seeds and cherry leaves; 1R, 1st group produced with red grape juice, *L. plantarum* AK4-11, crushed mustard seeds and cherry leaves; 2R, 2nd group produced with red grape juice, *L. plantarum* AK4-11, crushed mustard seeds, cherry leaves and cloves; *, values within rows with different superscript letter are significantly different (P<0.05)

acceptability scores of 1st groups were lower than that of groups C and 2. Panelists indicated that in both W and R groups, the clove added (2nd) groups received the highest scores for overall acceptability. The results of sensory evaluation revealed that 2R group had the best acceptability.

4. Conclusions

The effects of *L. plantarum* AK4-11 and grape varieties on some properties of hardaliye were investigated. The results of this study showed that using red grape in hardaliye production resulted in higher phenolic compounds, CI and redness values and lower brix and yellowness values. The addition of *L. plantarum* AK4-11 and clove caused one log unit increase on LAB counts in hardaliye produced with red grapes. No significant differences were determined among groups for total mesophilic bacteria and yeast during the fermentation and storage period.

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