

A STUDY ON PRODUCTION AND QUALITY CRITERIA OF HARDALIYE; A TRADITIONAL DRINK FROM THRACE REGION OF TURKEY

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ABSTRACT

This study reports on an assessment of the production methodology of hardaliye; a traditional lactic fermented beverage specific to the Thrace region. Microbiological and chemical analyses were performed on the laboratory scale production using Alphonse Lavallée and Papazkarası grapes. There were some differences in the microbiological and chemical analyses due to use of different concentrations of mustard seed. Coliform bacteria, *E. coli*, yeasts and molds were either absent or their numbers were significantly reduced during the fermentation process. In general, a progressive reduction/ increase/ reduction pattern was observed for the aerobic mesophilic bacteria and lactic acid bacteria colony counts. This progressive pattern of bacterial numbers was considered significant ($P<0.05$) when the colony counts were assessed together with the increase of acidity. Due to the low amount of yeasts, alcohol was not produced and the reducing sugar content did not significantly change during the production of hardaliye. In contrast, depending on the increase in the numbers of lactic acid bacteria, the acidity increased as expected. According to the results, due to the low numbers of coliform bacteria and adequate lactic acid fermentation, it is suggested that hardaliye is a microbiologically safe beverage. However, further research is needed for production on an industrial scale.

Anahtar kelimeler: Hardaliye, mustard seed, grape juice, LAB, yeast, mold

TRAKYA BÖLGESİ GELENEKSEL İÇECEĐİ HARDALIYENİN ÜRETİMİ VE KALİTE KRİTERLERİ ÜZERİNE BİR ARAŞTIRMA

ÖZET

Bu çalışmada, Trakya Bölgesi'ne özgü geleneksel bir içecek olan hardaliyenin laboratuvar koşullarında üretimi yapılarak mikrobiyolojik ve kimyasal özellikleri belirlenmiş, hardaliye üretim yöntemi değerlendirilmiştir. Laboratuvar ölçekli üretimde Alphonse Lavallée ve Papazkarası üzüm çeşitleri kullanılmıştır. Denemelerde, farklı konsantrasyonlarda hardal tohumu kullanılmış, buna bağlı olarak mikrobiyolojik ve kimyasal analizlerde beklenen şekilde farklı sonuçlar alınmıştır. Elde edilen hardaliyelerde koliform bakteri, *E. coli*, maya ve küf sayıları yok ya da fermantasyon boyunca kayda değer ölçüde azalırken, toplam aerobik mezofil koloni sayısı ve laktik asit bakterileri sayılarında genel olarak azalma/ artma/ azalma şeklinde bir seyir izlenmiştir. Bu seyir, asitlik artışı ile birlikte değerlendirildiğinde anlamlı bulunmuştur ($P<0.05$). Aerobik mezofilik koloni sayısı ile laktik asit bakteri sayısının çok yakın olması, bu bakteriyel floranın önemli ölçüde laktik asit bakterilerinden geldiğini göstermektedir. Maya sayısının düşük olması nedeni ile hardaliyelerde alkol oluşması gerçekleşmemiş ve şeker içeriğinde bir değişiklik olmamıştır. Tersine olarak laktik asit bakteri sayısındaki artışa bağlı olarak asitlikte beklediği şekilde artış olmuştur. Elde edilen sonuçlara göre, geleneksel bir üzüm değerlendirme şekli olan hardaliyenin, koliform grup bakteri sayısının düşüklüğü ve laktik asit fermantasyonuna bağlı olan yüksek asitliği nedeni ile mikrobiyolojik açıdan güvenli bir gıda olduğu söylenebilir. Endüstriyel ölçekli üretime geçiş için bu konuda daha fazla araştırmaya gerek vardır.

Keywords: Hardaliye, hardal tohumu, üzüm suyu, LAB, maya, küf

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INTRODUCTION

All over the world there is an increasing interest in traditional fermented products. In many regions of Turkey there are many different traditional fermented foods produced from products of a particular region. Hardaliye is a fermented beverage produced in a traditional way in Thrace, the European part of Turkey.

Hardaliye is a lactic acid fermented traditional beverage produced from grape juice and pomace with the addition of different concentrations of whole/ground or heat-treated mustard seeds and, of sour cherry leaves. The color of hardaliye reflects the original color of the grapes and has a characteristic aroma (1-4).

Lactic acid bacteria play a significant role in the production of many fermented products. As a result of lactic acid fermentation, the acidity, aroma and texture of the product changes making it more durable and healthier since it prevents the development of pathogenic microorganisms (2, 5, 6).

Since small scale local technologies are still effectively used in the production technology of hardaliye, the current related literature presents the traditional production techniques and also different methods aiming to develop the current technology. Some studies have been conducted on the production of various types of hardaliye using different varieties of grape and different concentrations of mustard seed and chemicals, aromatized with various additives such as sour cherry tree leaves, cloves and ginger as well as starter culture additives (1-3, 7-12).

This study reports on the assessment of the microbiological and chemical quality criteria of hardaliye that was produced from a modification of the traditional production process, and offers suggestions for industrial scale production.

MATERIAL AND METHOD

Material

Two different hardaliye beverages were produced using two different grape varieties; Alphonse Lavallée, a red aromatic grape variety and Papazkarası blue black grapes both grown in the Thrace Region. To the grapes were added; wild

black mustard seeds (*Brassica nigra* L.), daily picked leaves of the sour cherry tree (*Prunus cerasus* L.), and sulfur, sodium benzoate and potassium sorbate as preservatives.

Method

Hardaliye Production; Alphonse Lavallée

Hardaliye were produced from the Alphonse Lavallée grape variety under laboratory conditions using 10L glass jars. Bunches of grapes were washed under running water, the rotted grapes were removed. Then the grapes were processed using a crushing and destemming machine, pressed using a basket press and filtered to obtain the clear grape juice.

In order to inhibition of microorganisms, 25 mg/L sulfur as potassium metabisulfite was added to the grape juice. Ground mustard seeds were weighed and put into cheesecloth bags, each containing 2% concentration of mustard seeds. The bags were then heated to 50 °C in a small amount of grape juice to reveal the active substance, and then left for cooling. This mixture (bags and juice), 500 g grape pomace for colorization, 25 g sour cherry tree leaves for aroma, and 0.25 g sodium benzoate and 0.25 g potassium sorbate as preservatives were added into 10 L jars and topped up with juice and left to fermentation at room temperature. The microbiological and chemical analysis were carried out during the 29 days and the tests were repeated three times.

Hardaliye Production; Papazkarası

The same method as with Alphonse Lavallée grapes was used with the Papazkarası grapes except that the potassium metabisulfite was not added and the mustard seed concentration was 1%. The tests for this production were also repeated three times.

Microbiological Analysis

The juice and hardaliye samples were analyzed in terms of the aerobic mesophilic colony (Aerobic Plate Count; APC), lactic acid bacteria (LAB), coliform bacteria, *E. coli*, and the yeast and mold counts. For the microbiological analysis, the juice and hardaliye samples were diluted using the Maximum Recovery Diluent (MRD; Merck

1.12535) at standard ratio of 1:9 to obtain suspensions within the range of 10^0 and 10^{-6} using. For the analysis of the APC, Plate Count Agar (PCA; Merck 1.05463; at 28-30°C, 48 hours) was used. The LAB analysis was undertaken using De Man-Rogosa-Sharpe Agar (MRS; Merck 1.10660; at 28-30°C, 48 hours) and the analysis of coliform group bacteria and *E. coli* was carried out using VRB Agar and Chromocult TBX Agar (Merck 1.16122; at 37°C, 24 hours). For the analysis of the yeast-mold, Rose Bengal Chloramphenicol Agar (RBC; Merck 1.00467; at 28-30°C, 5 days) was used (13). The average values were calculated from the three repeats of the tests and the results were given as log CFU/mL.

Chemical Analysis

The chemical analysis of juice and hardaliye samples consisted of determining; the titratable acidity (using titration method and g/L as tartaric acid), pH (using Hanna HI 221 model pH meter), soluble solid (Brix) (using Soif WYA Abbe refractometer), ash (14), reducing sugar (with DNS method using Mecasys Optizen POP UV/VIS spectrophotometer at 522 nm) (15) and alcohol (using EON Trading electronic Ebulliometer). The results for the chemical analysis were also given as the average of the three repetitions for each grape type.

Statistical Analysis

The Minitab 15.0 software was utilized for the statistical analysis using a two-factor analysis of variance.

RESULTS and DISCUSSION

Results of the Microbiological Analysis

For the tests conducted with the samples containing the Alphonse Lavallée grape variety; the yeast count of 4.7 CFU/mL in the juice was reduced to below the limit (<1 log CFU/mL) following 1st day of the fermentation. The mold count was 4.6 log CFU/mL in the juice, 3.3 log CFU/mL on 1st day, 2.3 log CFU/mL on 2nd day and <1 log CFU/mL beginning from 3rd day of fermentation. *E. coli* was not detected in any of the analyzed samples (<1 log CFU/mL). Although coliform group bacteria were found in the juice samples (3.3 log CFU/mL), none of this bacteria was

observed beginning from 1st day of fermentation (<1 log CFU/mL).

Although there was a difference between the APC and LAB counts of hardaliye samples on 1st day of fermentation, these two values showed a nearly parallel increase and decrease beginning from 3rd day.

Figure 1 shows the results of APC and LAB counts throughout the fermentation period.

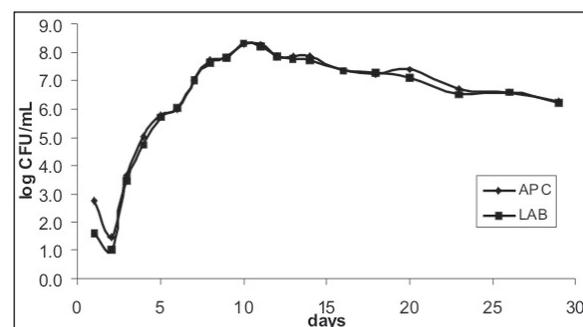


Figure 1. APC and LAB counts in Hardaliye produced from Alphonse Lavallée Grape Variety (log CFU/mL)

The lowest APC and LAB counts were found on 2nd day (APC; 1.48 log CFU/mL and LAB; 1.03 log CFU/mL). Increasing after 2nd day, the APC count reached its maximum value on 11th day (8.31 log CFU/mL) and the highest LAB count was seen on 10th day (8.30 log CFU/mL). The number of bacteria started to decrease following the days when the maximum values were obtained.

No coliform group bacteria and as a result no *E. coli* was found (<1 log CFU/mL) in the juice and hardaliye samples produced from Papazkarası grape variety throughout the fermentation period. In contrast to the samples containing Alphonse Lavallée grape variety, the total yeast count in the juice samples was 6.6 log CFU/mL, which decreased to 5.7 on 1st day of fermentation and 4.5 log CFU/mL on 2nd day, and no yeast was found after this point in time (<1 log CFU/mL). The total mold count juice, of 5.1 log CFU/mL, decreased regularly until 14th day of fermentation and dropped below the counting limit (<1 log CFU/mL) in the successive days.

As in the Alphonse Lavallée grape variety samples, the APC and LAB counts for the Papazkarası grape variety also showed a parallel increase and decrease (Figure 2). The APC and LAB counts were 6.1 log CFU/mL in the juice, decreased to

their lowest value on 2nd day of fermentation. Then these values started to increase and reached the highest value on 12th day with the APC being 7.5 log CFU/mL and the LAB being 7.4 log CFU/mL. Following 12th day, the values decreased until 29th day when they were the same as the values obtained from juice.

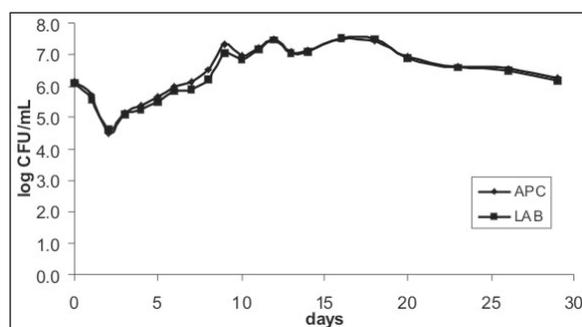


Figure 2. APC and LAB counts in Hardaliye samples produced from Papazkarası Grape Variety (log CFU/mL)

In another study, in which hardaliye samples were produced under laboratory conditions and monitored for 7 days, the highest values of the APC and LAB counts were obtained on 1st day of fermentation (8.0×10^5 and 2.3×10^5). These values then started to decrease until 7th day, when they were at their lowest (1.3×10^2 and 1.2×10^3). The pH value was found to be 3.46 on 1st day and 3.39 on 7th day (2). In the current study, 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, when LAB count was at its highest. The decrease in LAB and APC counts after 10th and 11th days respectively can be explained by the increase in acidity and the decrease in pH, which may have inhibited the bacteria. In addition, according to the results of the current study, the APC and LAB counts in the hardaliye produced from the two grape varieties were very high compared with the results of the above mentioned study (2). This can be explained by the low pH value of hardaliye in their study, which further decreased during fermentation. In the same study an analysis was conducted on the 26 hardaliye samples collected from small shops and street market and it was reported that the APC and LAB counts of these samples were also under the expected value due to the low pH level (2).

In another study, the traditional method was modified and the researchers added *L. sanfrancisco*, *L. acetotolerans*, *L. pontis*, *L. paracasei* subsp. *paracasei* species isolated from traditional hardaliye

samples to pasteurized grape juice as the starter culture. In this study, the hardaliye variety containing *L. paracasei* subsp. *paracasei*, and white and black mustard seeds reached the minimum pH value, and the LAB counts for this variety were higher than the other varieties, and reduced following 5th day in all hardaliye varieties (3). In the current study, the LAB counts were found to be higher in both hardaliye varieties even though no starter culture was used; however, the LAB counts started to decrease on 11th day. These results suggest that when hardaliye is fermented for a longer time, the amount of lactic acid bacteria decreases and the increased acidity during fermentation might have contributed to this decrease.

When ground mustard seeds used in hardaliye production are contact with the grape juice, depending on the pH and temperature, the myrosinase (EC 3.2.1.147) enzyme naturally found in seeds hydrolyze the sinigrin to allyl isothiocyanates. The pungent and characteristic aroma and the taste of the final product come from the allyl isothiocyanates. This compound also has an antimicrobial effect on many microorganisms and thus protects the products. There are multiple studies investigating the use of mustard seeds, the contained sinigrin, its compounds and the effect of allyl isothiocyanates in particular (8, 10, 18-24).

In the current study, the mustard seed concentrations used for the production of the hardaliye was 2% for the Alphonse Lavallée grape variety and 1% for the Papazkarası. This value ranged between 0.2% and 2.5% in other studies (1-3, 8, 10, 11 and 25). Another study reported that the myrosinase enzyme activity of *Brassica nigra* was optimum at pH 7 and 55 °C, and ground mustard seeds have a higher enzymatic activity (26). In other studies, enzyme activity was found to have increased after ground mustard seeds with a small amount of juice were heated to 40-50 °C, cooled and then added to the juice (1, 10, 11).

Although coliform group bacteria were found in the juice, they were not detected in any of the samples during the production process of the hardaliye. Moreover, *E. coli* was not observed in the juice or hardaliye samples. This finding is similar to the results of the study by Arici and Coskun (2). The disappearance of coliform

bacteria after the addition of mustard seeds shows that they have an antibacterial effect on coliforms. The antibacterial effect of mustard seeds on the *E. coli* O157:H7 serotype, a food borne pathogen, has also been reported in other studies (16, 27), one of which showed that the antibacterial effect of allyl isothiocyanate on *E. coli* O157:H7 occurs at low pH values (27). Having a low pH value, hardaliye is a suitable product for allyl isothiocyanates to reveal their antibacterial effect.

The yeast and mold in juice (Alphonse Lavallée) could not be detected following 3rd days of fermentation. In contrast to the hardaliye produced from the Alphonse Lavallée grapes, the amount of yeasts and molds was higher in the Papazkarası grape variety and they survived for a longer time. In a study (8) where the traditional method was used to produce pasteurized and non-pasteurized hardaliye samples, an analysis carried out one week after fermentation showed that the total of the total yeast-mold was 1.7×10^3 and 1.2×10^4 CFU/mL, respectively. In the same study even though the concentration of mustard seeds (0.2%) was much lower than in the current study and the concentration of preservative substances (potassium benzoate) was higher (0.1%) with yeast and mold being detected even 10 days after fermentation. This suggests that the protective effect of mustard seeds is more important. In addition, it is also important to take into consideration the amount of essential oil and sinigrin contained in the mustard seeds (1, 20).

There are also studies investigating the inhibitory effect of myrosinase enzyme activity on the development of certain yeast and mold species (19, 21, 28, and 29). Allyl isothiocyanates have been found to completely inhibit the development of certain mold varieties; such as *Aspergillus flavus*, *Penicillium commune*, *Penicillium corylophilum*, *Penicillium discolor*, *Penicillium polonicum*, and *Penicillium roqueforti* (28). In addition, preservatives used in production; such as sulfur (25 mg/L), sodium benzoate (25 mg/L) and potassium sorbate (25 mg/L) prevent the development of yeasts and molds, and alcohol fermentation during the fermentation process (30-32).

The amount of sorbate-benzoate used in the traditional hardaliye production and in certain

other studies is reported as 1 g/L (2, 8, 25); however, according to the Turkish Food Codex Regulation of Food Additives Section of Aromatized Drinks excluding milk-based drinks, the highest concentrations to be used are given as; 300 mg/L for sorbate and derivatives, 150 mg/L for benzoate and derivatives, and 250+150 mg/L for using both (33).

Results of the Chemical Analysis

In the hardaliye sample produced from Alphonse Lavallée grapes, the pH value in the juice was 4.24 and total acidity was 2.80 g/L. These values were 3.79 and 11.93 g/L on 29th day of fermentation, respectively. There was no significant change in the Brix (17.0) and the reducing sugar content (13.11%) measured in the juice ($P > 0.5$). In the Papazkarası hardaliye, the total acidity in the juice was higher (5.93g/L) and pH was 3.82. On 29th day, the pH and the total acidity were measured as 11.4 g/L and 3.73, respectively. Similar to the Alphonse Lavallée hardaliye samples, there was no significant difference ($P > 0.05$) in the Papazkarası samples in terms of the Brix in the juice (18.0) and the reducing sugar content throughout the fermentation process (~16%). For both grape varieties' hardaliye samples, alcohol was below the detection limit of 0.01% v/v.

The pH values found in the current study were similar to the results of other studies; 3.45-3.59 (1), 3.21-3.90 (2), 3.48-3.95 (8) and 3.91-4.38 (10). The pH values measured during the production process ranged between the optimum and minimum values for the development of lactic acid bacteria, and the activity of the lactic acid bacteria makes this beverage safe in terms of pathogenic microorganisms. The level of total acidity was also in agreement with the results of other studies (1, 11).

During hardaliye processing, LAB added as the starter culture and mustard seeds have been reported to have no significant effect on the reduction of the total sugar content (3). This finding also explains why the reducing sugar content did not change significantly during the fermentation process despite the high number of LAB.

In studies investigating hardaliye production as a vintage technique to preserve grapes in

their ripest state (including the current study); the Brix and sugar content are usually below 20%; however, "the sugar content of the juice ranges between 120-320 g/L and is not usually less than 200 g/L in warm areas. The sugar content of the juice obtained from overripe grapes is generally more than 250 g/L" (30). This suggests that ripening status of the grapes should be taken into consideration in the production technology of hardaliye.

Even though there are studies that did not find a significant amount of alcohol in hardaliye (8, 10), some studies reported low concentrations of alcohol, such as 0.28%; 0.32%; 0.35% and 595.50 mg/dL (1, 2). The difference between these findings could be the result of using chemical preservatives and different concentrations of mustard seeds. In the current study, the absence of yeast development after addition of sulfur, mustard seeds and chemical preservatives also explains the absence of alcohol.

CONCLUSION

Hardaliye produced for the domestic economy using local techniques is consumed fresh within 1-2 months. In order to extend the shelf life of hardaliye for industrial production, a starter culture appropriate for a controlled fermentation, microbiological and technological potentials and grape juice techniques (such as clarifying and filtration) should be further explored and assessed. For the management of the product color, the pomace should be macerated and standardization should be achieved. Also, changing of phenolic compounds should be investigated for suggestions in industrial production.

As a result of different varieties, the year, region, and juice content in relevant studies it is natural to see different microbiological/ chemical results. It is necessary to determine the appropriate harvest time and ripening status of the grapes and the quality norms of hardaliye.

Further research is also needed to investigate the optimum concentrations of mustard seeds and its sinigrin content, the concentration of chemical preservatives that are subject to limits, aromatic additives such as sour cherry tree leaf, cloves and ginger; sugar/ acid balance and product diversification using CO₂.

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