INVESTIGATION OF THE EFFECT OF BULGUR ADDITION IN FERMENTED POMEGRANATE VINEGAR PRODUCTION*

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ABSTRACT

In this study, microbiological, physicochemical and sensory properties of pomegranate vinegar produced from pomegranate grains were examined in the laboratory. Pomegranate vinegars produced in the study were made in two ways: bulgur added and bulgur free. Total acidity, pH, color, Brix values were measured in pomegranate vinegars produced. Coliform, yeast-mold, and lactic acid bacteria were studied in chromogenic coliform agar (CCA), Potato dextrose agar (PDA), and Man Rogosa Sharp (MRS) media, respectively. It has been observed that the pH of bulgur added vinegars varies between 3.10-3.47 during fermentation and between 2.90-3.32 for bulgur free vinegars. Dry matter values were determined as 2.2-4.0 Brix in bulgur added samples and 2.1-4.2 Brix in bulgur free samples. The total acidity of bulgur added samples ranged from 8.10-22.05 % during the fermentation period and from 6.30-13.5 0% for bulgur free samples. It has been observed that bulgur free pomegranate vinegars obtained as a result of fermentation are clearer, deteriorate later and receive more sensory acclaim. It has been determined that the addition of bulgur during vinegar production does not provide an advantage.

Keywords: *Pomegranate vinegar, Bulgur added vinegar, Fermentation, Sensory properties*

INTRODUCTION

Pomegranate (*Punica granatum* L.) fruit, one of the oldest known edible fruits is also widely consumed both fresh and in processed forms such as juice, wine, jam, vinegar **[1, 2, 3, 4].** Pomegranate fruits are known to contain phenolic ingredients known to be natural antioxidants such as gallotannins, ellagic acid and anthocyanins, as well as active ingredients such as carbohydrates, minerals and vitamin C **[5, 6, 7, 8].** Some studies have shown that natural antioxidants found in pomegranate fruit can reduce the risk of chronic disease and prevent disease progression by protecting people against oxidative stress **[9, 10].** Recently, pomegranate vinegar has been produced by applying various technologies, with the preservation of the functional properties

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of pomegranate fruit and improving the sensory properties with new aroma components **[11].** Vinegar is a special product produced from various foods since very old years, used as a flavoring agent and preservative. Various vinegars are produced by using different raw materials and production methods all over the world, especially in the Far East and European countries **[12].**

According to TSE 1880 EN 13188 vinegar standard, vinegar is defined as "a unique product produced biologically from agricultural liquids or other substances by two-stage fermentation of alcohol and acetic acid". In this standard vinegar varieties, according to the raw materials used in the production of vinegars is classified as: wine vinegar, fruit vinegar, fruit wine vinegar, cider vinegar, alcohol vinegar, cereal vinegar, malt vinegar, flavored vinegar and others. Of these, wine (grape) vinegar has been defined as" vinegar obtained only from wine (only from fresh grapes) by fermentation of acetic acid by biological pathways" **[13].** Vinegar production is a batch process consisting of two stages as ethyl alcohol and acetic acid fermentation. The first stage is ethyl alcohol fermentation, and yeasts break down sugar into ethyl alcohol by anaerobic pathway. In acetic acid fermentation, which is the second stage, ethyl alcohol is oxidized to acetic acid under aerobic conditions by vinegar (acetic acid) bacteria such as *Acetobacter* and *Gluconobacter* **[14].** Vinegar production can be produced in different ways, such as slow (traditional) method, orleans (French) method, pastor method, generator method and immersion methods. In these methods, alcohol fermentation is carried out in the raw material first in the slow method. When the alcohol level rises to 13%, acetic acid bacteria increase on the liquid surface, forming a membrane (mother of vinegar). The mother of vinegar formed on the surface allows ethyl alcohol to be converted into acetic acid. This method produces vinegar quite slowly. Some of the vinegars produced in our country are also produced by generator method. In order to provide a wide surface for vinegar bacteria, wood chipper, grain-removed corn cob parts and similar materials are used in the fermenter. In submerge method, acetic acid bacteria multiply inside the substrate without fillers. Because the filling material is not used, there are no problems caused by the filling material. In this method, vinegar production is 30 times faster than generator method. Fermentation is carried out at 24-29 °C with *Acetobacter* culture by continuous mixing in an environment containing 8-12% alcohol. Fermentation occurs on the inside of the liquid, not on the surface. During fermentation, oxygen is delivered to the environment in a controlled manner. With this method, very high proportions of vinegar can be produced in a short time. 5-10 tons of vinegar with an acidity of 4-6% in 24 hours can be produced by this method **[15].**

Vinegar quality depends primarily on raw material. The chemical composition of vinegar is directly related to the raw material. The second factor affecting quality is production technique. The production technique is determined after many years of research. Other parameters that affect quality are microorganisms, added ethanol concentration and vinegar concentration (in commercial production), O_2 amount, fermentation temperature and duration (aging), storage, bottling and pasteurization. 80% of vinegar is water, 20% is organic acids, alcohols, polyphenols, amino acids **[14].**

Vinegar can be produced naturally and artificially.

Natural vinegars are obtained by fermentation, artificial vinegars are obtained by the addition of acetic acid. Artificial vinegar is largely colorless because it contains no substance other than water and acetic acid, and artificial vinegar and natural vinegar can be easily distinguished as it does not contain vitamins and other fermentation byproducts formed during acetic acid fermentation. Artificial vinegar is not allowed in many countries.

The beneficial effects of vinegar on health are caused by bioactive compounds such as organic acids, amino acids, phenolic compounds and melanoidins. These compounds found in vinegar have been reported to have antimicrobial, antioxidant, antidiabetic, anticarcinogenic, antitumor, antiinfection effects **[12].** For instance, black rice vinegar (kurosu), traditionally produced in Japan, was found to be richer in phenolic substances than wine and apple vinegar, and its antioxidant activity was also higher compared to other vinegars. Vinegar is a fermented product produced by obtaining raw materials from a variety of different ways. In this study, positive effects on health are scientifically proven pomegranate fruit is used. Various characteristics of vinegars were studied which were produced in 2 different types as bulgur added and bulgur free.

MATERIALS AND METHODS

Material

Pomegranate that purchased from a local market as raw material for the production of pomegranate vinegar used in research. In this study 2 different vinegar were produced, one of them was produced as bulgur free and the other one was produced with bulgur.

Method

Pomegranate Vinegar Made on a Laboratory Scale

The traditional method was used in the production of pomegranate vinegar. 780 grams of pomegranate seeds separated from their shells were added into 3-liter glass jars, after crushing the grains, water was added until 400 mL of space remained on it. After adding 11 grams of sugar to both jars, bulgur (1 tablespoon) wrapped in muslin was added to a jar. All vinegars made are kept in the study at 22 ˚C. Mixing was carried out every day until the pomegranate seeds collapsed to the bottom. When pomegranate seeds collapsed to the bottom and mother of vinegar formed on it, fermentation was stopped (fermentation lasted 28 days) and then filtered. After the preparation of pomegranate vinegar, samples were taken and analyzed in 1, 7, 14, 21 and 28 days.

Determination of Total Acidity

Determination of total acidity in vinegar samples was made by titrimetric method. After 20 mL vinegar sample was completed to 100 mL with distilled water, 20 mL of mixture was taken to erlenmeyer, 1-2 drops of phenolphthalein were dripped on it and titrated with 0.1 N NaOH solution until pH 8.1. The total acidity was calculated in % acetic acid (g/ 100 mL) according to the amount which used in titration **[16].**

pH Measurement

A sam   ple of vinegar was taken into the beaker and pH was measured at room temperature using a pH meter (Mettler Toledo S220) probe.

Color Measurement

Color measurement of vinegars was performed with Tindometer (Lovibond PFX880) device. Before reading the device, the device is calibrated. Vinegar samples were placed on the lovibond tintometer and CIE L*a*b* values were read. L* value indicating lightness (L*: 0 black, L*: 100 white), a* value indicating redness and greenness (+a: red, 0: gray, -a: green), and b* values indicating yellowness and blueness (+b: yellow, 0: gray, -b: blue) degree of pomegranate vinegar. Measurements were made in 2 parallel and averages were taken.

Determination of Water-Soluble Dry Matter (Brix)

The determination of Brix was made using the Abbe refractometer (Leica Reichert Abbe AO Mark II). 2-3 drops of vinegar were dripped on the refractometer. Results are expressed in ° Bx.

Microbiological analysis

10 mL of bulgur added and bulgur free vinegars were added to sterile stomacher bags separately, homogenized by mixing with peptone water in a ratio of 1:10. Samples for microbiological analysis were taken in 7., 14., 21. and 28. days. Potato dextrose agar (PDA) for yeast and mold, Man Rogosa Sharp (MRS) for lactic acid bacteria and Chromogenic coliform agar (CCA) for coliform were used as media. The petries were left incubation for 48 hours at 22˚C, 37˚C and 37˚C respectively. Colonies formed at the end of 48 hours were counted and the number of microorganisms in 1 mL was determined.

RESULTS AND DISCUSSION

Determination of Total Acidity

Change in total acidity values (acetic acid %) in samples taken in 1, 7, 14, 21 and 28 days in bulgur added and bulgur free pomegranate vinegars produced is shown in Figure 1. The total acidity of bulgur added and bulgur free pomegranate vinegar was found to be between 8.1%-22.05% and 6.3% and 13.5%, respectively (Figure 1). In a study, it was observed that the acidity value of pomegranate vinegar is between 9% and 10% **[17]**. In another study, the acidity of pomegranate vinegar was found to be 4.63 % **[18]**. It is observed that the acidity level changes according to the production method of pomegranate vinegar. It has been observed that the addition of bulgur to pomegranate vinegar increases the acidity level of vinegar and negatively affects its sensory properties.

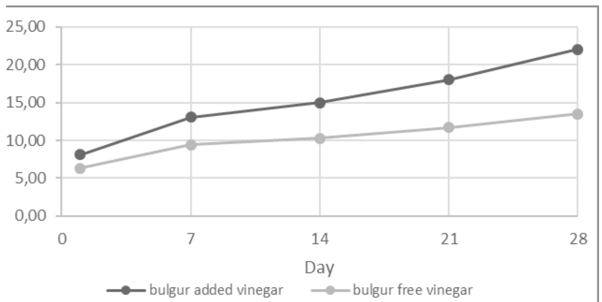

Figure 1. Change in total acidity values of pomegranate vinegars in fermentation process (acetic acid %)

pH Measurement

The change in pH during the fermentation period in bulgur added and bulgur free pomegranate vinegars were examined in samples taken at 1, 7, 14, 21 and 28 days and given in Figure 2. In bulgur added pomegranate vinegar, the pH value was 3.47 at the beginning of fermentation and 3.10 at the end of the fermentation. The change in pH in bulgur free pomegranate vinegar was found to be 3.32-2.90. In a study, it was observed that the pH of pomegranate vinegar during the 5-day fermentation period ranged from 3.41-3.24 **[19].** In another study, it was observed that the pH of pomegranate vinegar during the fermentation period was 2.98 on average **[18].** It was determined that the pH values measured in the study were compatible with the literature.

A decrease in pH was observed due to an increase in acidity in both vinegar. It was determined that the acidity in bulgur vinegar was very high in addition to this the decrease in pH was also excessive. In vinegar samples, mother of vinegar formation began to be observed after the 14th day (Figure 2). It is thought that the sudden increase in pH is associated with the formation of mother of vinegar (Figure 3).

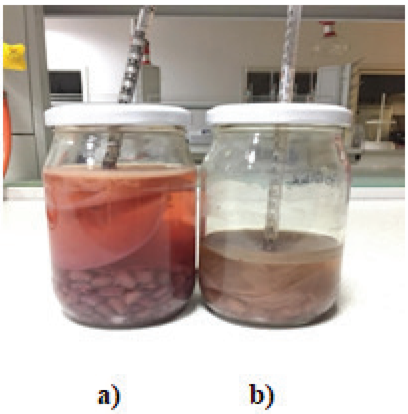

Figure 2. Mother of vinegar formed in pomegranate vinegars; a) bulgur free vinegar, b) bulgur added vinegar

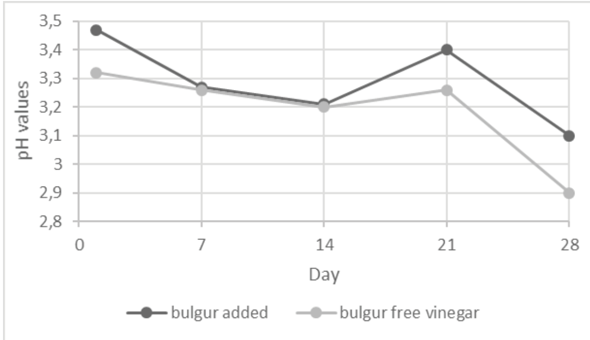

Figure 3. pH values of pomegranate vinegars

Determination of Water-Soluble Dry Matter (Brix)

Water-soluble dry matter values (Brix, °Bx) in bulgur added and bulgur free pomegranate vinegars are given in Figure 4. During the fermentation, the values of water-soluble dry matter in bulgur added and bulgur free pomegranate vinegar were measured as 4-2.2 °Bx, 4.2-2.1 °Bx, respectively. It was thought that the decrease in Brix value was due to the decrease in the rate of sugar by microorganisms using the sugar found in the environment. It was found that the values of water-soluble dry matter determined in vinegars are similar to the literature **[20].**

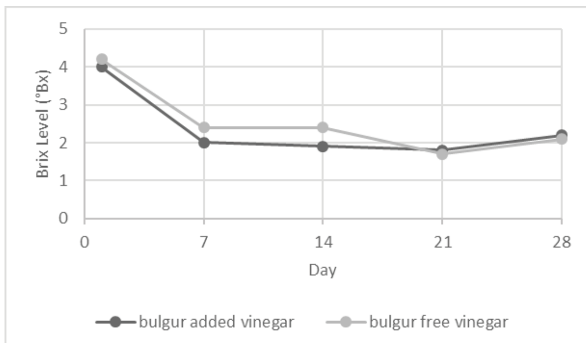

Figure 4. Water-soluble dry matter values of vinegar samples (°Bx)

Color Measurement

The values of color measurements made with lovibond tintometer in bulgur added and bulgur free pomegranate vinegars are given in Table 1.

L* value indicating lightness, a* value indicating red and green, and b* values indicating yellow and blue in bulgur added vinegar compared to a sample of bulgur free vinegar it was found higher up to the 21st day. But in bulgur free vinegar a sudden increase in values measured on the 28th day has been observed. Color is one of the important parameters that affect the consumer's purchase of vinegar **[21].** In a study, it was determined that L* values ranged from 0.28 to 20.15, a* values ranged from 0.09 to 14.88 and b* values ranged from 0.43 to 14.11 in grape and apple vinegars produced by traditional methods **[22].** The data in the literature coincides with the data in our study.

Table 1. L*a*b* values in bulgur added and bulgur free pomegranate vinegars

Day	Color Parameter	Bulgur Added	Bulgur Free
1	L*	9.87	7.025
	a*	16.70	12.98
	b*	12.73	7.84
7	L*	17.49	2.19
	a*	11.18	3.76
	b*	12.62	2.29
14	L*	15.8	3.42
	a*	7.36	4.65
	b*	8.27	3.15
21	L*	1.86	1.60
	a*	1.26	2.00
	b*	1.46	1.34
28	L*	3.13	15.77
	a*	1.96	9.93
	b*	2.23	11.25

Microbiological Analysis

E. coli coliform was not found in the samples during the 4-week fermentation period in bulgur added and bulgur free vinegar. The changes in the number of lactic acid bacteria and yeast in vinegars are given in Table 2.

Table 2. The number of lactic acid bacteria and yeast in the fermentation process in bulgur added and bulgur free vinegar

Days	Number of lactic acid bacteria Log_{10}CFU/mL		Number of yeasts Log_{10}CFU/mL	
	Bulgur added pomegranate vinegar	Bulgur free pomegranate vinegar	Bulgur added pomegranate vinegar	Bulgur free pomegranate vinegar
7	6,209	6,149	6,110	6,041
14	5,257	5,875	6,354	6,320
21	-nd	-	-	-
28	-	5,531	5,447	5,875

nd: not determined

Both the number of lactic acid bacteria and the number of yeast were found to be similar at the beginning of fermentation in bulgur added and bulgur free vinegars. But after the 2nd week, the analysis was not carried out, because bulgur added vinegar was deteriorated. In bulgur free vinegar, the number of LABs decreased by about 1 log at the end of the 4-week fermentation period. Similarly, the number of yeast has decreased.

At the beginning of fermentation in pomegranate vinegar, the pH of vinegar was also found to be low (approximately pH: 3.5). A further decrease in pH during the fermentation period was expected to prevent the development of lactic acid bacteria. But bulgur added vinegars were removed from the trial because they deteriorated at the end of 2 weeks. In bulgur free vinegar, it has been observed that they maintain LAB vitality, although the pH drops to 2.9 at the end of fermentation. In fact, they are generally undesirable to be present in vinegar, as LAB and other rod-shaped bacteria cause the formation of sensory undesirable compounds **[23].** But in our study, it was determined that lactic acid bacteria maintain their viability despite the decrease in pH. It is believed that this condition does not lead to an undesirable development in bulgur free vinegar, but lactic acid bacteria also play a role in the disfavor and degradation of bulgur added vinegar.

CONCLUSION

Vinegar is a special product that has been produced all over the world using different methods with different fruits and vegetables since very old years. The vinegar microbiota consists mainly of acetic acid bacteria, lactic acid bacteria and yeasts. Acetic acid bacteria produce acetic acid, on the one hand, improving the flavoring property of vinegar, and on the other hand, allowing it to be used as a natural antimicrobial. The bioactive components in its composition contain many different functional properties. Pomegranate fruit is rich in phenolic compounds such as flavonoids (anthocyanin, catechins and other complex flavonoids), polyphenols, fatty acids, aromatic compounds, amino acids, tocopherols, sterols, phenolic alkaloids. Due to these phytochemicals in its content, the medicinal and economic importance of pomegranate vinegar is quite high. In our study, it was determined that bulgur used in the production of many vinegars is not compatible with pomegranate vinegar, which leads to the deterioration of the vinegar in a short time, so the addition of bulgur in the production of pomegranate vinegar is not recommended.

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