

EFFECT OF THE ADDITION OF DIFFERENT SPICES ON SOME CHARACTERISTICS OF BOZA DURING STORAGE

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Abstract

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The purpose of this study is to investigate the effect of cinnamon, sage, lemon and clove on some properties of boza during storage. Crude boza s with spices was fermented for 1 day, and they were stored in the refrigerator for 5 days (the end of fermentation was the first day of storage). Both a decrease in the amount of total sugar (%), pH and viscosity, and a steady increase in ethyl alcohol (%) and acidity (%) values of all boza were observed during storage. Properties in lemon boza changed most. The highest reduction of total mezophilic aerobic bacteria and lactic acid bacteria counts was obtained in clove boza and sage boza, respectively. However, the highest reduction of yeast count was detected in clove, cinnamon and sage boza. In this study, coliform bacteria, mould and *Staphylococcus aureus* were not found. According to the sensory analysis, on the first day of storage, the highest score was in lemon boza in terms of appearance, in cinnamon boza in terms of flavors and in sage boza in terms of consistency. On the fourth day, the highest score was in plain boza in terms of appearance, in cinnamon boza in terms of flavor and consistency. There was a positive impact of adding spices on the shelf life and aroma of boza. Modifications are not enough in boza studies. Therefore, this study is important in terms of contributing to studies in this direction.

Key words: fermentation, cinnamon, clove, sage, boza

Introduction

Fermented foods have had great significance for people's nutrition. They have been produced and consumed for centuries (Velitchka et al., 2001). The yeasts, lactic acid bacteria, fungi and sometimes the activities of mixed populations, which move accordantly, generally stimulate natural, grain-based fermentations. While bacteria are proteolytic, yeasts first decrease the amount of carbohydrates (Chavan and Kadam, 1989). Fermentation by yeasts and lactobacillus, influence the amount of oil, mineral substances and vitamin in foods (Khetarpaul and Chauhan, 1991). Digestion of food products increases (Steinkraus, 1994). Nutritious and organoleptic features of fermented foods are better when compared to the raw material used (Campbell-Platt, 1994). Fermentation enables a great variety in tastes and textures of foods. It helps protect the food (Velitchka et al., 2001). Bosa is one of these fermented foods. Boza is considered as an old beverage with its 8000 – 9000 years of history (Arici and Daglioglu, 2002). 'Bosa' in

Turkish means 'millet' and it was derived from a Persian word 'buze'. Turks living in the Middle Asia call this beverage 'bas-soi'. This beverage has been known in Central Asia for centuries. It was introduced to Europe and Anatolia after migrating from Central Asia (Uylaser et al., 1998). In Eastern European countries and in the Balkans, this beverage is called 'braga' or 'brascha'. It is named as 'bosa' in Bulgaria, 'bosan', 'bousa' or 'bouza' in Nigeria and 'bouza' in Egypt (Arici and Daglioglu, 2002). Boza is a product, which is prepared by cooking one or more mixtures of pulps or flours of refined grains such as millet, rice, wheat, corn etc. in drinking water; adding white sugar, and subjecting it to alcohol and lactic acid fermentations according to the Turkish Standards Institute (TS 9778, 1992). Barley, oat, potatoes and cracked wheat (bulgur) can be used to produce boza, in combination with the mentioned grains at the Turkish standards. Millet (boza) is defined as the most preferred product owing to its sensorial properties. Boza is classified as sweet or sour boza depending on its acid content. It is a kind of dense liquid with its sweet-sour taste. It is a kind

of favorite beverage of the winter nights with pale-yellow color and characteristic acidic – alcohol smell (Akpinar-Bayizid et al., 2010). Raw material, microflora, fermentation process and storage conditions influence the sensorial quality of boza (Zorba et al., 2003). Cooling effect of lactic acid makes it possible to consume boza in summer. High temperature causes a fast increase in the existing microflora. As a result, dramatic changes happen in its sensorial properties. So, boza is an ideal beverage in winter (Yucel and Otlas, 1998). Boza can be consumed four seasons with additives. Importance of using spices and their extracts to protect foods has increased today because people tend to consume minimally processed foods without any chemical additives (Aran, 1998).

Antimicrobial effects of spices usually result from the essential oils in spices (Akgul and Kivanc, 1989; Akgul, 1993). Many kinds of spices are known as effective protectors. Sage, because of its intense etheric oil (*á*-tujon, *amphora*, *viridiflorol*, *borneol*, *1,8-sineol*, *â*-tujon) and phenolic (*rosmarinic acid*, *carnosol*, *carnosic acid*, *methyl carnosate*) content, shows antimicrobial activity on many bacteria and fungi species (Biljana et al., 2007). Eugenol (80-90%) is one of the main essential oil components, which is obtained (with an efficiency of 17%) from dried sprouts of *clovesis*. Eugenol (2 methoxy-4-allyl phenol) is a highly effective antimicrobial compound, which gives its typical odor and flavor to the spices. It is stated that cinnamic aldehyde at the rate of 65-80% and eugenol at the rate of 5-10% in the essential oil of cinnamon causes strong antimicrobial effect (Akgul, 1993). Some environmental conditions such as pH, salt and various chemical protectors have great importance on the antimicrobial effect of spices (Coskun, 2006). Spices and their extracts are influential in every stage of microbial development. With the use of them; lag phase gets longer, reproduction rate at the logarithmic phase slows down and total cell number decreases. Some organic acids show antimicrobial activity by decreasing intracellular or environmental pH rate or by changing the cell membrane permeability and infecting substrate transportation, or chelating with some metals necessary for microorganisms to live. Citric acid, which is found intensely in lemon, is one of them (Ova, 2001). In this study, adding new tastes with spice addition to boza, enhancing variety, addressing a larger number of people, and analyzing the effect of some antimicrobial effective spices on the storage period of boza were aimed.

Material and Methods

Crude boza, used in this study, was produced only from millet at the Vefa Food Industry and Trade Limited Company institutions. Millet was first cleaned of all contaminants. Then it was broken into semolina-sized pieces, and it was sieved to

refine it from bran pieces. It was boiled in 4 – 6 times larger volumes of drinkable water by stirring continually. For this aim, stainless steel vessels were preferred. While boiling, hot water was added a few times until the boiling process ended because the mixture absorbed the water. Boiling was stopped when a homogeneous mixture was obtained. Boza masscuite, which was obtained, was cooled after it had boiled. After cooling process, crude boza was rarefied and filtered by stirring continually in 2.5 times larger volume of water. 20% sugars and 2-3% fermented bread-dough were added to the sugar-free crude boza. Boza was bottled into 500 mL (580 g) plastic bottles. Cinnamon, sage and clove, which were taken from Harman Trade Limited Company, and had been irradiated earlier, were powdered by a grinder. Then 1.5 g was added to each 580 grams of boza. 1.5 g of lemon zest and lemon juice was added to boza. Boza was left for fermentation at 25°C for 24 hours. The physicochemical, microbiological and sensory analysis of plain and spicy boza, which was stored in conditions of refrigerator for 5 days, was done.

Physicochemical and microbiological analysis of boza was done on each day while it was being stored at 4°C for 5 days. The pH values of boza samples were directly found out with the help of a jointed electrode digital pH-meter (Hana Instruments pH 211 microprocessor pH-meter). Titratable acidity was calculated according to Anonymous (1983) in terms of lactic acid percentage. Determination of total sugar was done in Luff School method (Cemeroglu, 1992). For the ethanol determination, distillation and titration were applied, and ethyl alcohol percentage was calculated (TS 1594, 1998). Boza samples, which were stored at 4°C, reached room temperature, and they were homogenized. Then viscosity measurements were done with AND SV10 vibro-viscometer (Anonymous, 2005). A total mesophilic aerobic bacteria count was done in pour plate method, and PCA (Plate Count Agar) was used as a medium (48 hours at 35°C) (Anonymous, 1995). The number of lactic acid bacteria was determined by using the planting on surface method at MRS agar (de Man, Rogosa and Sharpe) (30 ± 1°C, 72 hours) (Baumgart et al., 1986) and at M17 agar (30 ± 1°C, 48 hours) (Gilliand et al., 1984). The number of coliform group bacteria was determined by using planting on surface method, and VRBA (Violet Red Bile Agar) was used as a medium (35°C, 24 hours). The number of yeast and mould was determined by using planting on surface method, and PDA (Potato Dekstrose Agar) was used as a medium (25°C, 5-7 days) (Marshall, 1992). BPA was used as a medium (37°C, 48 hours) while determining the number of *S. aureus* (Speck, 1976), and planting on surface method was applied. A sensorial analysis on boza was done with a panelist group of 9 people from the lecturers of Food Engineering Department, on the first and the fourth days of the storage

process. Sensorial analysis was done according to the preference test (Hedonic Scale)(7-point; Like Extremely, Like Moderately, Like Slightly, Neither Like Nor Dislike, Dislike Slightly, Dislike Moderately, Dislike Extremely) (Anonymous, 1988). The results of physicochemical and microbiological analysis were statistically assessed according to randomized blocks experimental design. Variance analysis was done by using SPSS 10.0 packaged software. Duncan test was applied in order to find out the importance levels of variant considered significant in variance analysis (Sosyal, 1992).

Results and Discussion

Physicochemical Analysis Results

The pH of boza samples decreased steadily from the first day to the fifth day (Table 1). The greatest decrease in pH

from the first day to the fifth day occurred (after the third day) in lemon boza because yeasts grow better in acidic environment. At the end of the fifth day, the highest pH was in sage boza, and the subsequent was cinnamon boza. The variance analysis was done to find out whether the differences between kinds of boza and storage period were significant or not. According to the results, the difference was found important in $P < 0.01$ level of significance in terms of boza types, and in $P < 0.01$ level of significance in terms of period. Acidity (%) values of boza samples increased regularly from the first day to the fifth day (Table 1). At the end of the fifth day, acidity increase was the highest in lemon boza, the least in sage boza, and the second to the least in cinnamon boza. According to the results of variance analysis, the difference between boza types was in $P < 0.05$ level of significance, and the difference between the period was in $P < 0.01$ level of significance.

Table 1
The change of physico chemical properties of boza samples during 5-day storage(4°C) period

Samples	Days	pH	Acidity, %	Total sugar, %	Ethyle alcohole, %	Viscosity mPa	TMAB kob g ⁻¹	LAB kob ⁻¹	Yeast kob ⁻¹
Plain boza	1	4.02	0.4	16.2	0.55	1000	1.0x10 ⁸	6.0x10 ⁷	2.6x10 ⁶
	2	3.8	0.46	15.71	0.8	630	8.0x10 ⁷	7.2x10 ⁷	4.0x10 ⁶
	3	3.62	0.53	15.02	1.6	510	9.0x10 ⁷	8.6x10 ⁷	7.5x10 ⁶
	4	3.41	0.59	14.9	1.81	530	2.0x10 ⁸	8.0x10 ⁷	8.0x10 ⁶
	5	3.2	0.63	14.89	1.95	600	1.4x10 ⁸	6.0x10 ⁷	2.0x10 ⁶
Boza with cinnamon	1	3.91	0.44	17.11	0.32	880	1.1x10 ⁸	6.0x10 ⁷	1.2x10 ⁶
	2	3.8	0.47	16.81	0.58	650	9.0x10 ⁷	8.0x10 ⁷	3.0x10 ⁶
	3	3.62	0.54	15.52	0.75	620	6.5x10 ⁷	8.8x10 ⁷	2.7x10 ⁶
	4	3.45	0.57	15.06	0.91	560	1.1x10 ⁸	7.3x10 ⁷	2.0x10 ⁶
	5	3.27	0.61	14.78	1.03	580	8.0x10 ⁷	5.3x10 ⁷	5.0x10 ⁵
Boza with sage	1	4.02	0.41	16.45	0.35	970	1.5x10 ⁸	7.0x10 ⁷	2.0x10 ⁶
	2	3.98	0.44	15.38	0.62	600	7.3x10 ⁷	5.6x10 ⁷	3.4x10 ⁶
	3	3.75	0.51	15.1	0.79	510	7.9x10 ⁷	7.3x10 ⁷	3.8x10 ⁶
	4	3.52	0.53	14.95	0.98	540	1.2x10 ⁸	6.5x10 ⁷	4.0x10 ⁶
	5	2.41	0.57	14.91	1.11	540	1.0x10 ⁸	5.2x10 ⁷	3.3x10 ⁵
Boza with lemon	1	3.76	0.48	17.11	0.61	1160	8.0x10 ⁷	5.0x10 ⁷	2.4x10 ⁶
	2	3.6	0.54	16.2	0.75	610	4.0x10 ⁷	3.8x10 ⁷	3.0x10 ⁶
	3	3.42	0.58	15.08	1.6	460	4.5x10 ⁷	6.8x10 ⁷	4.9 x10 ⁶
	4	2.98	0.65	14.75	1.92	480	1.1x10 ⁸	6.0x10 ⁷	5.7x10 ⁶
	5	2.81	0.73	14.1	2.1	650	9.6x10 ⁷	6.0x10 ⁷	2.3x10 ⁶
Boza with clove	1	3.89	0.43	16.31	0.23	860	1.5x10 ⁸	4.6x10 ⁷	1.4x10 ⁶
	2	3.61	0.52	15.81	0.59	620	9.0x10 ⁷	4.5x10 ⁷	1.8x10 ⁶
	3	3.45	0.58	15.6	0.71	490	9.0x10 ⁷	7.2x10 ⁷	2.6x10 ⁶
	4	3.32	0.59	15.48	0.85	590	1.8x10 ⁸	8.0x10 ⁷	2.9x10 ⁶
	5	3.1	0.68	14.44	0.95	640	7.0x10 ⁷	6.0x10 ⁷	6.0x10 ⁵

TMAB: Total Mesophilic Aerobic Bacteria

LAB: Lactic Acid Bacteria

The total sugar (%) decreased during the storage period (Table 1). The highest decrease was in lemon boza because of the increase in yeast activities. The lowest decrease was observed in sage boza. The difference between boza types and period in variance analysis of the total sugar was in $P < 0.01$ level of significance. Ethyl alcohol increased during storage (Table 1). The highest increase in the level of ethyl alcohol was in lemon boza on the fifth day and the subsequent were in plain boza. The amount of ethyl alcohol (%) of lemon boza and plain boza increased fast after the second day because of the increase in the yeast activity. The lowest increase in the amount of ethyl alcohol level at the end of the fifth day was in clove boza, and the subsequent was in cinnamon boza and sage boza. In variance analysis, the difference between boza types and period was found important in $P < 0.01$ level of significance. The highest decrease in the viscosity of boza samples was from the first day to the second day (Table 1). The lowest viscosity value was in lemon boza on the third day. In variance analysis of viscosity values, the difference between boza types was found insignificant in $p < 0.05$ level of significance, and the difference between period was found significant in $P < 0.01$ level of significance. According to the Turkish Boza Standard, rate of total acid (lactic acid) is 0.2-0.5% in sweet boza, and 0.5-1.0% in sour boza. Total sugar (saccharose) ought to be at least 10%. Rate of ethyl alcohol ought to be at most 2% (TS 9778, 1992). In this study, all kinds of boza can be classified as sweet boza after fermentation (the 1st day). On the second day; while plain boza, sage boza and cinnamon boza are classified as sweet boza; lemon boza and clove boza are classified as sour boza. On the third, fourth and fifth days, all kinds of boza are classified as sour boza. In this study, total sugar of boza samples are in accordance with the standards. Ethyl alcohol values are in accordance with the standards except lemon boza, ethyl alcohol level of which is 2.1% on the fifth day. Level of ethyl alcohol in sage boza, clove boza and cinnamon boza is low, and it is the result of the antifungal effect of these spices. The number of yeast is less in cinnamon boza, sage boza and cloveboza than the others. Plain boza and lemon boza became sour faster than sage boza and clove boza. Natural acidity in lemon increased the acidity in boza, and decreased pH level of the environment. The increase in the number of yeast in lemon boza increased alcohol rate; thus accelerated its deterioration.

Yucel and Kose (2002) did a research to investigate the suitability of 9 different types of boza, which were taken from different selling areas in Izmir for the Turkish Standards Institute. In this research, they found pH level between 3.22 and 3.82, the acidity between 0.15% and 0.5, total amount of sugar between 16.11% and 22.59%, and ethyl alcohol between 0.03% and 0.39%. When this study is compared with

the study of Yucel and Kose (2002), it can be understood that the pH values are similar, the acidity (%) and ethyl alcohol are usually higher, and the total amount of sugar (%) are lower than their study. Hancioglu and Karapinar (1999) studied the changes which occurred during 24-hour fermentation of boza, which they made by mixing cooked rice, corn and wheat flour in traditional method. According to the results they obtained, the pH level decreased from 6.13 to 3.48 during fermentation. Acidity rate increased from 0.02% to 0.27%, and the rate of alcohol increased from 0.02% to 0.79%. In this study, the pH and acidity (%) levels are higher than the pH and acidity (%) levels in the study of Hancioglu and Karapinar (1999), and the rate of alcohol is lower than the rate in the study mentioned after the 24-hour fermentation. Ustun and Evren (1998) found pH level between 2.93 and 3.72, the acidity (lactic acid) between 0.24% and 0.45, total amount of sugar between 7.33% and 21.89%, ethyl alcohol between 0.14% and 0.53% in boza, which they made by mixing cracked wheat (bulgur), bread, millet, potatoes, rice and wheat. In this study, the pH level was higher than the pH level in the study of Ustun and Evren (1998), rate of acidity (%) and ethyl alcohol (%) was lower than the rate in the same study, and total sugar (%) was similar in the study mentioned after fermentation. Topal and Yazicioglu (1986) stated that the general amount of acid in boza was 0.3-0.5%. In this study, acidity values are between 0.40% and 0.48 after the 24-hour fermentation, and they are totally between the mentioned values. They are between the values mentioned on the second day, and they exceed those mentioned values on the third day.

Microbiological Analysis Results

In this study, total mesophilic aerobic bacteria (TMAB) number was the highest in plain boza, and it was the lowest in clove boza and the subsequent cinnamon boza on the fifth day of storage (Table 2). According to the results of variance analysis, the difference between boza types and storage period on the number of TMAB was $P < 0.01$ and it was significant. Lactic acid bacteria (LAB) number on the fifth day was the highest in plain boza, and the lowest in sage boza and cinnamon boza. According to the results of variance analysis, the difference between boza types and storage period was $P < 0.01$ and it was significant. The number of yeast in boza samples on the fifth day was the highest in lemon boza, then in plain boza, and it was the lowest in sage boza, the subsequent in cinnamon boza and clove boza. According to the results of variance analysis, the difference between boza types and storage period was $P < 0.01$ and it was significant. In the study, no mould, no coliform group bacteria and no *S. aureus* were detected.

Table 2
The sensory properties of boza samples on 1 and 4th day of storage(4°C)

Days	Sensory Analysis	Plain Boza	Boza with cinnamon	Boza with sage	Boza with lemon	Boza with clove
1	Appearance	4.77	4.88	4.22	5.33	3.88
	Taste	4.33	5.88	4.22	4.22	2
	Consistency	4.77	5.77	5.88	5.33	5.66
4	Appearance	5.88	4.66	3.77	5	3.33
	Taste	3.77	5.33	3.77	2.88	3.33
	Consistency	5.55	5.66	4.88	5	4.55

Tuncer et al. (2008) analyzed the microbiological characteristics of 15 boza samples collected from four different provinces of Turkey: Isparta, Antalya, Istanbul and Ankara. The number of TMAB was between 2.4×10^4 and 3.2×10^4 kob mL⁻¹, the average of LAB number was found 9.3×10^4 kob mL⁻¹, the average of ferment-mould number was found 1.9×10^6 kob mL⁻¹. In this study, the numbers of TMAB, LAB and yeast in boza samples are similar with the numbers Tuncer et al. (2008) obtained in their research. Meric (2010) detected TMAB number between 8.3×10^5 and 3.1×10^8 kob mL⁻¹; LAB number between 5.9×10^6 and 4.5×10^8 kob mL⁻¹; yeast-mould number between 1.3×10^5 and 6.2×10^8 kob mL⁻¹ in 27 boza samples from different production places and shops, produced commercially in Thrace region. The results of this study are similar with the results Meric (2010) obtained. In the study of Hancioglu and Karapinar (1999), they reported that LAB number in mL increased from 7.6×10^6 kob mL⁻¹ to 4.6×10^8 kob mL⁻¹; and the number of yeast increased from 2.25×10^5 kob mL⁻¹ to 8.1×10^6 kob mL⁻¹ in boza during the 24-hour fermentation. LAB and yeast numbers of boza samples, which were analyzed in this study, after fermentation (1st day) were lower than the numbers in the study of Hancioglu and Karapinar (1999). This might be because of spices used.

Sensory Analysis Results

Lemon boza got the highest point, and clove boza got the lowest point in appearance; cinnamon boza got the highest point, and clove boza got the lowest point in taste; and sage boza got the highest point, and plain boza got the lowest point in consistency in sensorial analysis on the first day. Plain boza got the highest point, and clove boza got the lowest point in appearance; cinnamon boza got the highest point, and lemon boza got the lowest point in taste; and cinnamon boza got the highest point, and clove boza got the lowest point in consistency on the fourth day. According to the results of variance analysis; the difference between boza types was in P<0.01 level of significance in the sensorial analysis of appearance, thus it was important; and the difference between the period was

in P<0.05 level of significance, thus it was unimportant. In the sensorial analysis of taste, the difference between boza types was in P<0.01 level of significance, thus it was important, and the difference between the period was in P<0.05 level of significance, thus it was unimportant. In the sensorial analysis of consistency, the difference between boza types and the period was in P<0.05 level of significance, thus it was unimportant. The results of the sensorial analysis were shown in Table 2. In an additional study; in which the amount of clove was reduced by half and only sensorial analysis of taste was done, clove boza was preferred more than lemon boza and sage boza.

Conclusion

In this study, it was concluded that adding spices to boza might influence consumers' preferences positively in bringing new tastes in terms of sensorial taste. The most preferred kind was cinnamon boza. Adding spices contributed to the shelf life as well as to the aroma of boza. The number of yeast, which is the most effective factor in storage period, was determined lower in sage boza, cinnamon boza and clove boza than in other kinds of boza during storage period. With the increase in the studies on kinds of spices that may influence storage period, it might be possible to consume well-loved boza four seasons, instead of consuming it only in winter. Modifications are quite rare in the boza studies, so this study is important in contributing to the future researches on this subject.

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