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The effect of different essential oils on antioxidant and shelf life of traditional fermented grain beverage Turkish boza

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Abstract

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Aromatic essential oils are used as natural preservatives due to their antibacterial properties and increased awareness of flavor, aroma and health benefits. The target was to provide new flavors and functional properties to boza, without changing the original appearance of this traditional drink. Boza samples obtained by adding sage, fennel, and ginger essential oils were compared with control boza in terms of some physicochemical features, microbiological, phenolic, antioxidant, color, and sensory properties. Boza samples were observed to have a fall in pH during 5 days of storage and the alcohol and ash values were appropriate for boza standards. The highest phenolic amounts and the greatest antioxidant activity were found in the sage and ginger boza samples, where the phenolic content was 148.11 and 122.88 mg/kg, respectively, and the antioxidant amounts were 108.6 and 96.32 mg/L, respectively. In terms of taste and aroma, the highest appreciation was seen in boza samples containing ginger on the 1st day, and boza samples containing sage on the 5th day.

Keywords: boza; essential oils; antioxidant; antimicrobial

Introduction

Boza is a pale yellow, traditional product with bittersweet flavor obtained by boiling and fermenting cereals or flours like millet, rice, wheat, or corn, alone or in combination, and water (Akpinar-Bayizit et al., 2010; Gotcheva et al., 2001; Yegin & Fernández-Lahore, 2012). With mild acidity and high viscosity, boza has a characteristic flavor and yeasts (Li et al., 2010) and lactic acid bacteria play the main roles (Botes et al., 2007; Gotcheva et al., 2000) in increasing shelf life. Boza has a gradual production process, and factors such as raw material selection and/or uncontrolled fermentation cause differences in boza varieties (Arici & Daglioglu, 2002). Fermentation duration and temperature have significant effects on physicochemical properties. Boza may be consumed in all stages of fermentation until pH drops to nearly 3.5. Boza is a product that easily degrades with short shelf-life of 15 days when stored at +45°C (Caputo et al., 2012; Gotcheva et al., 2001). The microbial viability of boza (homo- or hetero-fermentative LAB and yeasts (Gotcheva et al., 2000) assist in forming the typical features of the product, while pathogenic and/or degrading bacteria (Kabak & Dobson, 2011) cause degradation of boza. In recent times, natural antimicrobials and derivatives like their essential oils, e.g., spices, have attracted attention for food preservation (Xu et al., 2007). Aromatic essential oils have antibacterial and antioxidant features and are increasingly used as natural preservatives in terms of giving flavor and aroma to

foods (Karpińska et al., 2001). Ginger, fennel and sage may be used in food and drink due to their characteristic spicy aroma and flavor. Additionally, they are perfect sources of many bioactive compounds (Abdellaoui et al., 2020; Aman et al., 2020; Yazgan, 2020). The aim of this study was to benefit from the antimicrobial and antioxidant effect of essential oils in the traditional drink of boza to increase the shelf life and add new flavors to boza without changing the original structure.

Material and Methods

Raw boza was produced using 1:1 ratio of bulgur wheat and rice. The raw material was continuous mixed and boiled with up to 5 times the amount of water. Up to 20% (w/v) sugar was added and it was left to cool for 1 hour and raw unfermented boza was obtained. Sage, fennel and ginger essential oil obtained by hydrodistillation method with Clevenger device was added to boza at a rate of 100 µL/mL (1:10 v/v). Then 2-3% commercial boza yeast added and were left to fermentation at 30ºC for 48 hours. Boza samples were named BZ1 control boza, BZ2 boza containing sage essential oil, BZ3 boza containing fennel essential oil and BZ4 boza containing ginger essential oil (Figure 1).

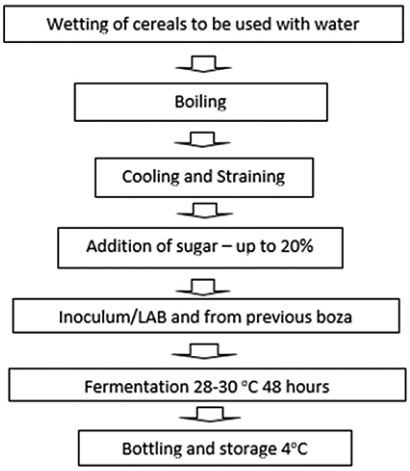

Fig. 1. Boza production flow scheme

Preparation of plant hydrosols

Dried leaf samples from *Salvia* species in the sage family (Lamiaceae), ginger (*Zingiber officinale)* from the ginger family and fennel (*Foeniculum vulgare)* from the parsley (Apiaceae) family were used. Hydrosols were obtained as following the method of Sagdic (2003). The ground plant materials were separately placed in bottles containing 1 L hydrodistilled water (1:10 w/v) for 2 hours in a Clevenger apparatus (Ildam, 125 Türkiye). After hydrodistillation, essential oil was separated through the cooling tunnels.

pH, Acidity, Brix, Ash and Alcohol Analysis

The pH values of the samples were determined by using a pH meter (Nel Mod 821). Ash analysis was completed using the incineration method at 550°C according to TS-1511. Alcohol content of the sample was measured by refractometer index (Gerogiannaki-Christopoulou et al., 2003). Firstly, ethyl alcohol standard solutions were prepared from 1%, 2%, 3%, 5%, 10% to 100% with the aid of a micropipette with drinking water, with the refraction index of each sample measured at room temperature. The boza samples to be analyzed were measured and recorded in a similar way and at similar temperature. The diluted solutions were prepared and measured. Standards were mixed well and dropped as film on the measurement window of an abbe refractometer. Each measurement was repeated 3 times and the mean was calculated. A calibration graph was created from the measurement results and the % concentration of alcohol was found from the measured refractive indices based on the equation $y = 0.025x + 1.333$ obtained from the graph.

Microbiological analysis

After 1st, 3rd and 5th day of fermentation, 10 ml of boza was removed under aseptic conditions and transferred to 90 ml 0.1% peptone water. From the appropriate ten-fold dilutions, pour plate counts were made using the following media and incubation conditions: de man, Rogosa and Sharpe Man (MRS, Merck) agar overlaid with the same medium for lactic acid bacteria (Collins, 1967) 37°C 48 h incubation; Plate Count (PCA, Merck) agar for TMAB 30°C 48 h incubation; and dechlorane rose Bengal chloramphenicol (DRBC, Oxoid CM 727) agar for yeasts, 30°C 48–72 h incubation (Marshall, 1992).

Phenolic Analysis

Extraction

Extraction from boza and raw material samples was completed in accordance with the method applied by Li et al. (2010). According to this method, phenolic matter is extracted using methanol/1 M HCl (85:15, v/v) as solvent. Samples were centrifuged at 4000 rpm at 15°C for 30 min. The uppermost supernatant was concentrated under vacuum and dissolved in 4 mL methanol (80%).

Total Antioxidant Activity Tests

Total antioxidant activity was determined according to the method of Brand-Williams et al. (1995) and Li et al.

(2010). DPPH solution of 60 µM was prepared using 95% ethanol. Then 200 µl of extract solution and 3.8 mL DPPH solution were mixed and incubated for 60 minutes. The absorbance was measured at 515 nm for phenolic activity and 765 nm for antioxidant activity (Shimadzu UV-Visible 1700, Tokyo, Japan) using a spectrophotometer (Shimadzu UV-Visible 1700, Tokyo, Japan) against a blank, consisting of pure solvent. Results are expressed as µmol Trolox equivalent (TE)/100 g sample.

Color Test

Color measurements of boza samples were taken with a Konica Minolta Chroma Meter (CR-400, Minolta Co. Ltd., Osaka, Japan) device. As a result of measurements, the L value light value or degree of lightness (100 fully white, 0 black), a value for redness and greenness (+red, 0 gray, − green), and b value for yellowness and blueness (+yellow, 0 gray, − blue) were measured. The C* value is a value between 0 and 100 showing color saturation. Another parameter of h* provides information about the deviation of a color from yellow, red, green or blue with the unit in degrees and values from 0° (red), 90° (yellow), 180° (green) and 270° (blue).

Sensory Analysis

Sensory analysis of the samples was carried out by 14 semi-trained panelists who were undergraduate or graduate students in the Food Engineering Department of Yildiz Technical University, Turkey. Appearance, aroma, texture, taste and general acceptance were evaluated using a hedonic sensory analysis form with scale of 1–6 points where "1" is not preferred and "6" is most preferred by panelists.

Statistical Analysis

Data values, three repeats of samples and results of three parallel analyses had means calculated. Values are given with standard deviation. Significant differences in statistical terms between results ($p < 0.05$) were determined using the one-way or two-way ANOVA test. Analyses used the JMP 9 statistical program.

Results

The physicochemical, microbiological and sensory features were researched for a 5-day interval in the study. The initial pH of boza was determined as 6.1±0.3, pH changes on the 1st, 3rd and 5th days during storage were determined and the values are shown in Table 1. The unfermented boza had pH value from 4.1–6.7, while it dropped to 4.0 or lower with fermentation (Arici & Daglioglu, 2002; Botes et al., 2007; Caputo et al., 2012; Gotcheva et al., 2000; Kabak & Dobson, 2011). There were differences in the pH values for boza produced using different essential oil acids ($p < 0.05$). The standard BZ1 had pH values from 3.73–3.54, BZ2 boza containing sage essential oil had pH 3.59–3.40, BZ3 boza containing fennel essential oil had pH 3.69–3.47 and BZ4 containing ginger essential oil had pH 3.57–3.39. Different rates of pH decrease were observed in all boza samples during 5 days ($p < 0.05$). On the 1st day, the pH for BZ1 and BZ3 boza and for BZ2 and BZ4 boza were statistically similar and were lower than for BZ1 boza. On the 3rd day, BZ1 had the highest pH with other varieties statistically similar but lower. On the 5th day, the highest value for BZ1 and lowest for BZ4.

Ash tests results identified the ash amounts in boza from 0.17 to 0.21% the values are shown in Table 1. The lowest ash amount was determined in the fermented plain boza sample, while the samples containing different essential oils were statistically similar but higher than plain boza. According to the Turkish standards (Türk Standartları Enstitüsü, 1992), the maximum ash amount allowed in boza is 0.2%. Our results are within these values, and it was identified that fermentation of boza containing essential oils did not cause a negative situation in terms of ash values. According to the results obtained, the alcohol rates in fermented boza were examined on the 1st, 3rd and 5th days and an increase was observed; the values are shown in Table 1. Values on the 1st day were 0.62–0.74% with highest values for BZ1 and lowest value for BZ2 boza containing sage essential oil. On the 3rd day, values were 0.63–0.90% with highest alcohol for BZ1 and lowest for BZ2. On the 5th day,

Table 1. Some physico-chemical analysis results of boza samples

Boza samples	Essential oil type	pH			Alcohol, %			Ash, %
		1.day	3.day	5.day	1.day	3.day	5.day	
BZ1	Control	3.73±0.02^{a}	3.64±0.03^{a}	3.54±0.02^{a}	0.74^{a}	0.90^{a}	0.94^{a}	0.17±0.02^{b}
BZ2	Sage	3.59±0.02^{b}	3.53±0.03^{b}	3.40±0.03^{c}	0.62^{c}	0.63^{c}	0.66^{d}	0.21±0.05^{a}
BZ3	Fennel	3.69±0.01^{a}	3.52±0.02^{b}	3.47±0.02^{b}	0.66^{b}	0.68^{b}	0.81^{b}	0.20±0.02^{a}
BZ4	Ginger	3.57±0.01^{b}	3.54±0.02^{b}	3.39±0.00^{c}	0.67^{b}	0.69^{b}	0.71^{c}	0.21±0.0^{a}

Note: Different letters in the same column show statistically significant degree of difference. Capital letters in the same row are different for 1st, 3rd and 5th days). The level of significance was preset at $p < 0.05$ compared to each other

the interval was 0.66-0.94% and all varieties were statistically different ($p < 0.05$). The alcohol rate in BZ3 and BZ4 samples were statistically similar on the 1st and 3rd days, while BZ3 had higher values than BZ4 on the 5th day. The lowest alcohol value during storage was determined in the BZ2 sample. In this study, alcohol values were determined to be in accordance with boza standards (Türk Standartları Enstitüsü, 1992).

Phenolic and Antioxidant Activity

Phenolic matter in samples results for boza samples are presented in Table 2. It was observed that the phenolic matter content in boza obtained from bulgur wheat and rice varied according to the type of essential oil added. The fennel, sage and ginger boza had higher values compared to plain boza, with highest value obtained for sage boza. The phenolic amount in BZ3 sample was lower than for BZ2 and higher than BZ1. BZ4 boza was determined to be closest to BZ2.

The total antioxidant activity results for boza samples are presented as Trolox equivalent in Table 2. There were statistically significant differences observed for total antioxidant activity linked to the type of essential oil added to boza produced using wheat and rice raw material ($p < 0.05$). The lowest antioxidant value was determined for BZ1, with highest for BZ2, BZ4 sample had higher values than BZ3 sample.

Table 2. Phenolic and DPPH antioxidant activity of boza samples

Boza samples	Phenolic gallic acid, mg/kg	DPPH trolox equivalent, mg/L
BZ1	74.23±0.64^{d}	50.81±2.28^{d}
BZ2	148.11±1.91^{a}	108.6±10.28^{a}
BZ3	88.20±1.27^{c}	89.48±0.11^{c}
BZ4	122.88±1.91^{b}	96.32±0.57^{b}

Note: Different letters in the same column show statistically significant degree of difference.
The level of significance was preset at $p < 0.05$ compared to each other

Microbiological Analysis Results

In this study performed with the aim of determining the effect of essential oils on boza quality, the total mesophilic aerobic bacteria, lactic acid bacteria and yeast-mold counts were performed on the 1st, 3rd and 5th days of the 5-day storage period. The results for total mesophilic aerobic bacteria, lactic acid bacteria and yeast-mold counts are shown in Figure 2. The essential oil varieties in boza were found to be statistically significant for total mesophilic aerobic bacteria, lactic acid bacteria and yeast-mold counts. According to microbiological analysis, change in the total mesophilic aerobic bacterial count during the storage period (1., 3. and 5. day) was 8.07–8.68 log CFU/g for BZ1, 6.63–7.56 log CFU/g for BZ2, 7.92–8.19 log CFU/g for BZ3, and 6.75–7.49 log CFU/g for BZ4. For lactic acid bacteria counts, the values were 7.76–7.82 log CFU/g for BZ1, 8.17–8.13 log CFU/g for BZ2, 7.35–7.58 log CFU/g for BZ3, and 8.21–8.35 log CFU/g for BZ4. For the total yeast-mold counts were 5.55-6.81 log CFU/g for BZ1, 4.22–4.54 log CFU/g for BZ2, 4.44-6.08 log CFU/g for BZ3, and 3.86–4.56 log CFU/g for BZ4.

As a result of the 5-day evaluation, the highest value in the total number of mesophilic aerobic bacteria was in BZ1 sample, while the lowest value in BZ2 and BZ4 samples, the highest value in the amount of lactic acid bacteria in BZ4 samples, the lowest value in BZ3 samples, and the highest value in yeast-mold control samples was determined in BZ2 and BZ4 samples. In terms of yeast-mold count, the highest was observed for BZ1 with lower values for boza containing essential oils. The lowest values were observed for BZ4 and BZ2 boza.

Color and Sensory Features of Boza

The results of the analysis found significant correlations between viscosity with L* (whiteness, sheen), a* (redness, greenness) and b* (yellowness, blueness) color values at $p < 0.05$ level. The results of the study were assessed for L*, a*, b*, C* and h* color values and are given in the Table 3.

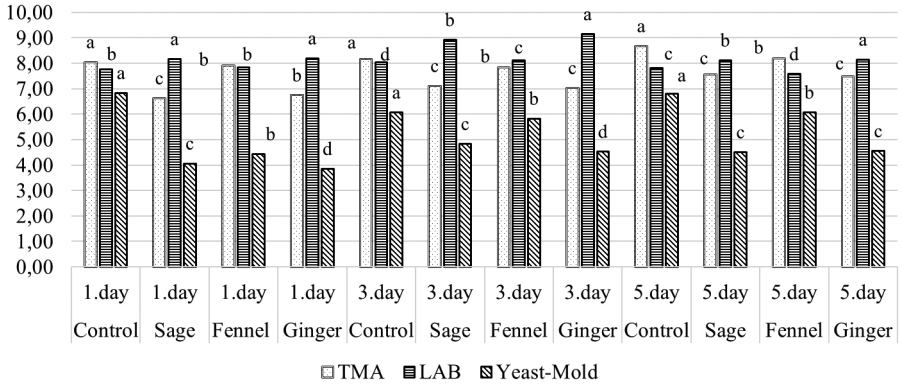

Fig. 2. Microbiological count results for boza samples (log CFU g^{-1})

Note: The total mesophilic aerobic bacteria count, lactic acid bacteria and yeast-mold counts for 1st, 3rd and 5th days are given as log CFU/g. Different letters show statistically significant degree of difference. The level of significance was preset at $p < 0.05$ compared to each other

Table 3. Color analysis results of boza

Boza samples	L*	a*	b*	C*	h*
BZ1	58.94±0.1[a]	2.49±0.0[b]	1.05±0.1[b]	2.66±0.0[b]	160.3±0.1[a]
BZ2	60.41±0.1[a]	2.25±0.1[c]	1.96±0.7[a]	2.44±0.0[c]	157.5±1.2[b]
BZ3	58.25±0.3[a]	2.64±0.1[a]	2.15±0.1[a]	3.61±0.1[a]	141.3±1.3[c]
BZ4	60.61±0.1[a]	2.60±0.0[a]	1.88±0.2[a]	2.77±0.0[b]	153.1±1.5[b]

Note: Different letters in the same column show statistically significant degree of difference.
The level of significance was preset at $p < 0.05$ compared to each other

Table 4. Sensory analysis results of boza

Boza Samples	1. Day				5. Day			
	Color	Taste	Consistency	Aroma	Color	Taste	Consistency	Aroma
BZ1	5.55±1.0[a]	5.35±0.7[a]	5.10±0.8[a]	5.35±0.5[b]	4.88±0.12[a]	2.43±0.3[d]	3.14±0.4[a]	2.92±0.09[d]
BZ2	4.85±1.1[d]	4.55±1.0[b]	4.71±0.8[b]	5.40±0.9[a]	4.84±0.17[a]	4.29±0.5[a]	3.07±0.5[a]	4.39±0.06[a]
BZ3	5.25±0.8[b]	3.20±1.4[c]	4.88±1.1[b]	4.29±1.1[c]	4.80±0.16[a]	2.93±0.8[c]	3.00±0.0[a]	4.23±0.07[c]
BZ4	5.05±0.7[c]	5.15±1.0[a]	5.25±0.8[a]	5.38±1.2[a]	4.75±0.13[a]	4.21±0.2[b]	3.14±0.2[a]	4.32±0.16[b]

Note: Different letters in the same column are statistically significantly different. The capital letters are different for the 1st, 3rd and 5th days on the same line.
The level of significance was preset at $p < 0.05$ compared to each other

When compared in terms of L* values, all boza were not observed to have statistically significant differences in terms of sheen. For the a* value, the highest value was observed for the fennel and ginger boza samples, with lowest for the sage boza. For the b* value, the boza samples containing essential oil were statistically similar, with higher values compared to BZ1. In terms of the C* parameter, the differences between the samples were significant ($p < 0.05$). The BZ1 and BZ4 boza samples were similar in terms of C*, with highest values for BZ3. The highest h* values for boza were for plain and BZ2 with lowest for BZ3 boza containing essential oil. The results for in the evaluation of the of sensory characteristics boza are shown in Table 4. Panelists assessed color, taste, texture, flavor on days 1 and 5 with points from 1-6. According to the results obtained, in terms of color all boza samples were found to be statistically significant. In terms of color on the 1st day, BZ1 was the most liked, while BZ2 was liked least.

According to the panelists, the highest approval for taste was control and BZ4 on day 1st, and sage and BZ2 and BZ4 on day 5st. In terms of aroma, the highest taste was the boza containing BZ1 and BZ4 on the 1st day, and the boza containing BZ2 on the 5th day. In the sensory tests performed on the 1st day, the consistency of the boza was similar to BZ1 and BZ4, and scored higher than BZ2 and BZ3. On the 5th day, the boza types were found to be statistically similar in terms of color and consistency.

Conclusion

It was determined that the total number of mesophilic aerobic bacteria and yeast molds of the boza samples containing essential oil were lower than the control boza samples during storage. Essential oils increased the shelf life of boza. Boza samples containing different essential oils had higher phenolic and antioxidant values compared to control boza. In terms of color, all boza types had sheen, while the boza containing essential oil were more yellow in color. In sensory terms, the ginger boza was liked the most in terms of taste, texture and aroma compared to control boza on the 1st day, while sage boza was highly appreciated for the 5th day. The use of essential oils obtained from different plants in boza samples provided positive contributions in terms of developing the microbiological, antioxidant and sensory properties of boza.

Conflict of interest

Authors declare no conflict of interest.

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