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THE EFFECT OF THE USE OF FRESH AND LYOPHILIZED BOZA ON BREAD QUALITY PROPERTIES

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ABSTRACT
Boza, a functional traditional beverage, is a product obtained by fermenting grains. In this study, the use of different proportions of boza (25%, 50%, 60% on flour basis) and lyophilized boza (5%, 8%, 10% on flour basis) as a starter and their effects on the microbiological, chemical, textural and sensory properties of bread were investigated. The addition of boza was found to increase dough extensibility and resistance to extension compared to control bread. The proportions of fresh boza or lyophilized boza were significantly correlated with the hardness of breads. In addition, the L value in boza added breads were significantly decreased compared with the control bread. It was determined that the lyophilization caused a significant decrease in lactic acid bacteria compared with yeast counts. The most acceptable group after the control bread was determined to be 25% fresh boza added bread and 5% lyophilized boza added bread.
Keywords: Boza, bread, lyophilization, starter culture

TAZE VE LİYOFİLİZE BOZA KULLANIMININ EKMEK KALİTE ÖZELLİKLERİNE ETKİSİ

ÖZ
Fonksiyonel bir geleneksel içecek olan boza, tahılların fermente edilmesiyle elde edilen bir üründür. Bu çalışmada farklı oranlarda boza (un bazında %25, %50, %60) ve liyofilize boza (un bazında %5, %8, %10) ilavelerinin starter olarak kullanımı ve ekmeğin mikrobiyolojik, kimyasal, tekstürel ve duyusal özellikleri üzerine etkileri araştırılmıştır. Kontrol ekmeğine kıyasla boza ilavesinin hamur uzayabilirliği ve uzamaya karşı direnci arttırdığı görülmüştür. Taze boza veya liyofilize boza oranları, ekmeklerin sertliği ile önemli ölçüde ilişkilidir. Ayrıca boza ilave edilen ekmeklerde L değeri, kontrol ekmeğine göre önemli ölçüde azalmıştır. Liyofilizasyonun maya sayısına göre laktik asit bakterilerinde önemli bir düşüşe neden olduğu belirlenmiştir. Kontrol ekmeğinden sonra en kabul edilebilir grup %25 taze boza katkılı ekmek ve %5 liyofilize boza katkılı ekmek olarak belirlenmiştir.
Anahtar kelimeler: Boza, ekmek, liyofilizasyon, starter kültür

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INTRODUCTION

With the development of technology, people want to consume healthier and higher quality food. Functional foods have come to the fore recently due to increasing the life span of people, maintaining their well-being, and improving health (Roberfroid, 2000). One of the first methods that comes to mind when it comes to improving the quality of food is fermentation technology. Bread, yoghurt, cheese, tarhana, beer, wine, kefir, pickles, vinegar, turnip, and boza are examples of fermented foods produced (Uçok and Tosun, 2012). Among these products, bread is the most consumed traditional, grain-based fermented product. For this reason, many studies are carried out on bread. Among these, the use of different flour and additives, the use of different fermentation technologies and cooking methods, and finally, the starter culture studies for bread with different types of yeast and bacteria combinations draw attention.

Boza is a valuable beverage with a high nutritive value, consumed especially in winter, produced by the fermentation of many grains, slightly sour, and has a unique smell (Altay et al., 2013; Bayat and Yıldız, 2019). The microbial flora involved in the fermentation of boza consists of lactic acid bacteria and yeasts (Zorba et al., 2003). Studies have shown that yeast isolated from boza belong to *Candida*, *Geotrichum*, *Saccharomyces* genera, and bacteria belong to *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Pediococcus*, *Weisella*, and *Leuconostoc* genera. As a result of the co-operation of lactic acid bacteria and yeasts, many metabolites are released into the product that improve its sensory, functional, and nutritional properties. These include lactic acid, acetic acid, aroma compounds, exopolysaccharides (EPS), bacteriocin, various enzymes, and vitamins. In addition, boza has positive effects on health as a product with rich fiber content and high digestibility.

Heperkan et al. (2014) evaluated the enzyme activities and EPS production of 13 lactic acid bacteria isolated from boza and aimed to find the best starter for boza. *L. Paracasei*, *Lc. Lactis*, *L. Coyniformis*, *L. Plantarum*, and *P. Parvulus* have been proposed as potential starter cultures. Another study evaluating the enzyme activity of boza is aimed at reducing the amount of phytic acid by the phytase activity of lactic acid bacteria. The bacterium with the highest phytase activity was determined to be *Pediococcus pentosaceus*. As a result of the study, it has been shown that traditionally produced boza has high phytase activity and offers the opportunity to reduce antinutritional substances such as phytate. Boza has been reported to be a potential source of starter culture for sourdough fermentation (Doğan and Tekiner, 2020; Milanovic et al., 2020). There have no many studies investigating the use of boza as a starter in bakery products. Bircan et al. (2017) examined the sensory and technological effects of yoghurt, boza, and traditional sourdough on bread. It was reported that the best result in the sensory evaluation was in the bread with yoghurt, and the best result in the shelf life evaluation was in the bread with boza. In another study, the physical, microbiological, and rheological properties of bread dough and the effect of boza powder obtained by drying and grinding boza under vacuum and air circulation, on bread quality were investigated. It has been concluded that up to 8% of boza powder can be used in bread production for both drying methods in terms of all its properties (Pala, 2012).

When the literature was examined, it was seen that no study has been found on investigating the effect of adding fresh boza and lyophilized boza at different rates on the quality of bread. In this direction, the aim of this study is to evaluate the use of boza as an alternative starter in bread production and to examine how it affects bread quality.

MATERIAL AND METHOD

Material

Wheat and millet for boza production, boza culture (*L. bulgaricus*, *S. thermophilus*, *L. plantarum, Lc. lactis* ssp *cremocis*, *Lc. lactis* ssp *diacetylactis*, *Lc. lactics* ssp. *lactis*, *Leuconostoc mesentorides* ssp *cremocetils*, *S. diacetilactis*, Dogadanbizim maya, Istanbul, Türkiye), instant yeast (Dr. Oetker, Izmir, Türkiye), wheat flour (Soke, Aydın, Türkiye) and salt (Billur, Izmir, Türkiye) were obtained from local stores.

Method

Boza production

Boza production was carried out according to the method of Arıcı and Daglioglu (2002) (Figure 1). A mixture of 1 kg of wheat (80%) and millet (20%) was used as raw material. Fermented boza was stored at 4 °C until analysis.

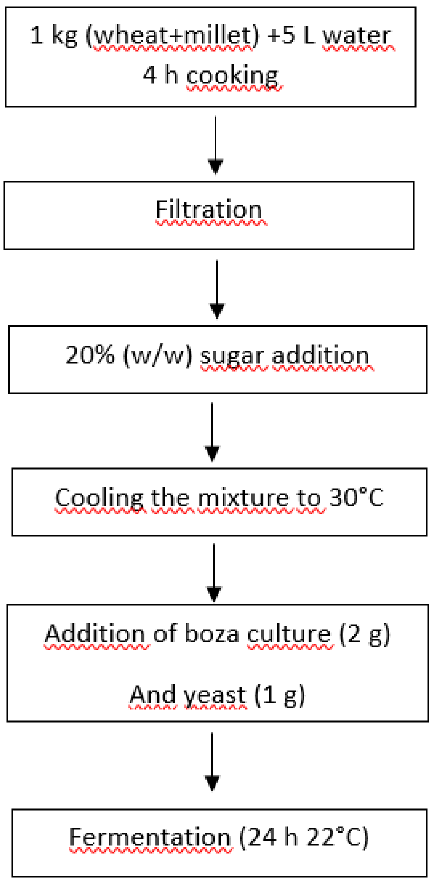

Figure 1. Boza Production Stages

Determination of chemical properties of boza

Total acidity and pH analysis

The pH value of the samples was measured by immersing the probe of the pH meter (OHAUS ST 300 Portable pH Meter, USA) calibrated with standard buffer solutions into the sample brought to 20±2 °C. The total acidity was determined by the volumetric analysis method using 0.1 N NaOH solution and phenolphthalein solution as an indicator, and the total acidity value was calculated as g/100 mL in terms of lactic acid according to Equation 1 (AOAC, 1990; Cemeroğlu, 2018). Total acidity and pH analyzes were carried out at 0^{th} and 24^{th} h.

% Total acidity=0.009* titration consumption (mL)*100/ sample volume (mL) (Equation 1)

Total dry matter analysis

The total dry matter of boza was determined following the AOAC method (AOAC, 1990; Cemeroğlu, 2018). Total dry matter analyzes were carried out at 0^{th} and 24^{th} h.

Total ash analysis

Total ash content of boza was detected by incineration method (AOAC, 1990). Total ash analyzes were carried out at 0^{th} and 24^{th} h.

Production of lyophilized boza

The produced fresh boza was pre-frozen at -40 °C for 2 h. It was then placed in a freeze-dryer (CoolerMed, Türkiye) and dried for 24 h. Lyophilization was carried out at a vacuum of 0.04 mbar and at -50 °C. Dried boza was ground and stored in airtight containers (Boyacı-Gündüz et al., 2018).

Microbiological analysis

The spread plate method was used for total yeast and bacteria counts in fresh and lyophilized boza. The boza samples were diluted 1:10 in physiological saline (0.85% NaCl), and 100 µL was spread-plated on agar. YPDA (Yeast Peptone Dextrose Agar) was used for yeast count and MRS (de MAN, ROGOSA and SHARPE) agar was used for lactic acid bacteria count. The plates were enumerated after incubation at 30 °C and 37 °C for 48 h, respectively. Results were given as cfu/mL (Heperken et al., 2014; Arslan-Tontul & Erbas, 2020).

Bread production

Bread dough was prepared using wheat flour, salt, and drinkable water. Bread formulations are shown in Table 1. After mixing all the components for 7 min, they were fermented at 30 °C for 1 h. After they were shaped, it was kept at 30 °C /30 min for the final fermentation. At the end of fermentation, they were baked for 30 min in a oven (Venarro, Türkiye) (bottom temperature of 220 °C and upper temperature of 210 °C) (Pala, 2012; Alkay et al., 2020). Breads were subjected to a 2 h cooling period at room temperature. The breads for analyses stored in polyethylene bags at 21 °C.

Table 1. Ingredients Used in Bread Production

	Wheat Flour (g)	Lyophilized Boza (g)	Fresh Boza (g)	Water (mL)	Yeast (g)	Salt (g)
Control	250.00	0.00	0.00	160.00	5.00	3.75
YB1	250.00	0.00	62.50	110.00	0.00	3.75
YB2	250.00	0.00	125.00	100.00	0.00	3.75
YB3	250.00	0.00	150.00	65.00	0.00	3.75
LB1	250.00	12.50	0.00	190.00	0.00	3.75
LB2	250.00	20.00	0.00	185.00	0.00	3.75
LB3	250.00	25.00	0.00	120.00	0.00	3.75

Control: Bread without Boza, YB: Bread with Fresh Boza, LB: Bread with Lyophilized Boza

Extensibility properties of dough

To determine the extensibility (mm) and resistance to extension (g) of the dough, the method given by Kieffer et al. (1998) was used with some modifications. After weighing 10 g of fermented dough, it was compressed between greased hard plastic plates at 30 °C for 40 min. Then, the dough pieces divided between the plates were carefully removed with the help of a thin spatula and placed in the device. The device parameters were determined as test speed of 3.3 mm/s and target distance of 75 mm. Analysis was performed on TA, XT Plus C Texture Analyzer (Stable Micro Systems, England) device using SMS/Kieffer Dough and Gluten Extensibility Rig (Buresova et al., 2014).

Texture analysis of bread

Texture Profile Analysis (TPA) of all breads was carried out using a 36 mm cylinder probe in TA, XT Plus C Texture Analyzer (Stable Micro Systems, England). After baking, the breads were cooled and tested by cutting them at a height of 2.5 cm. Analysis parameters of the device; test speed is 5 mm/s, pre-test speed is 1 mm/s, post-test speed is 5 mm/s, target distance is 10 mm, trigger force is 5 g and load cell with 5 kg capacity. The results are given over the hardness, springiness, cohesiveness, gumminess, chewiness, and resilience of breads (Kadan et al., 2001; Su et al., 2005; Alkay et al., 2020).

Color analysis of bread

L, a, b values, which are a criterion for determining the color characteristics of all breads, were determined with a colorimeter device (PCE-CSM 4 Colorimeter, Türkiye). L value indicates brightness, a value indicates red-green, and b value indicates yellow-blue. Inner color measurements were made 1 h after the breads were baked (Su et al., 2005).

Sensory analysis

Descriptive analysis was applied to determine the sensory properties of all breads. For this purpose, 15 semi-trained panelists evaluated the breads in terms of taste, odour, firmness, color, and general acceptability 1 h after baking. A scoring scale of 7 was used (1 the minimum and 7 the maximum) (Feili et al., 2013).

Statistical analysis

The significance of the difference (P <0.05) between the data to be obtained in these whole analysis were calculated using the Tukey test and revealed by one-way analysis of variance (ANOVA). All analyzes were repeated three times and data were expressed as mean ± standard deviation. SPSS Statistic 22 (SPSS INC., Chicago, IL, USA) software package was used for statistical analysis.

RESULT AND DISCUSSION

The results of the chemical analyzes of boza samples are shown in Table 2. The pH values were determined as 6.73 at the 0th h and as 4.99 at the end of the 24th h. Akpınar-Bayizit et al. (2010), reported that the fermentation was terminated because pH change less after the 24th h in boza production. The total acidity was determined as 0.31% at 0th h. In parallel with the decrease in pH, the total acidity increased at the 24th h and reached the acidity value of 0.83%. With the lyophilization, the total acidity decreased from 0.83% to 0.4%. Boyacı Gündüz et al. (2018) reported that the total acidity of fermented instant turnip decreased by approximately 45% after the lyophilization process. According to TS 9778 Turkish Boza Standard (TSE, 2017), the total acidity for boza is determined as 0.5-1% for sour boza and 0.2-0.5% for sweet boza in terms of lactic acid. Furthermore, it was requested that the total dry matter content should be at least 20%. As a result of the analysis, the total dry matter content for fresh boza at the end of the 0th and 24th h was found to be 13.21% and 10.55%, respectively. The chemical composition of 9 different boza samples were evaluated in the study of Yücel and Köse (2002) and it was reported that the dry matter content varied between 17-22%. The evaluation of different grains and the use of different amounts of sugar in boza samples affect the viscosity and dry matter content of the boza (Gotcheva, 2000). When the results of the ash analyzes were examined, the ash content for the 0th and 24th h were calculated as 1.28% and 0.18%, respectively. After the lyophilization process, there was no change in the ash content of the boza and it was calculated as 0.19%.

Table 2. Chemical Properties of Fresh and Lyophilized Boza

	pH	Total acidity (%)	Total Dry Matter (%)	Ash(%)
Fresh boza (T_o)	6.73	0.31±0.01[a]	13.21±0.65[b]	1.28±0.03[b]
Fresh Boza (T_{24})	4.99	0.83±0.05[b]	10.55±0.23[a]	0.18±0.01[a]
Lyophilized Boza		0.40±0.04[a]	97.46±0.15[c]	0.19±0.01[a]

T_0: 0th h of fermentation, T_{24}: 24th h of fermentation

Different letters within the same column indicate significant differences (P <0.05) according to Tukey multiple comparison test.

When the production of boza is examined, it is stated that the fermentation occurs in two stages. First, yeasts perform alcohol fermentation using sucrose. Then, lactic acid bacteria convert the formed metabolites into lactic acid by lactic acid fermentation. In these fermentations, the most significant microbial activities take place within 24 h. As a result of the 24 h fermentation of boza, the yeast and lactic acid bacteria ratios increased (Table 3) and were similar to other studies in the literature (Gotcheva et al., 2001; Moncheva et al., 2003; Arici and Daglioglu, 2002; Hancioğlu and Karapinar, 1997; Gotcheva et al., 2000).

Table 3. Microbial Counts of Fresh and Lyophilized Boza

	Yeast (log cfu/mL)	Bacteria (log cfu/mL)
Fresh boza (T_o)	4.62±0.36[ab]	5.23±0.25[b]
Fresh Boza (T_{24})	5.09±0.03[b]	6.10±0.02[c]
Lyophilized Boza	4.37±0.14[a]	3.24±0.12[a]

Different letters within the same column indicate significant differences (P <0.05) according to Tukey multiple comparison test.

T_0: 0th h of fermentation, T_{24}: 24th h of fermentation

The resistance to extension and extensibility values of all breads are given in Table 4. The properties of the doughs evaluated in this study were determined by the SMS/Kieffer Dough and Gluten Extensibility Rig method, which is an important method that uses a small amount of dough and shows a high correlation with the extensograph results (Kieffer et al., 1998; Yue et al., 2020). It is stated that for a good quality bread, the dough should have strong extensibility and good resistance to extension. In addition, bread hardness is also an important parameter for quality along with sensory analysis (Buresova et al., 2014). While YB1 has the strongest resistance to extension and extensibility among breads with fresh boza, it is LB3 in breads with lyophilized boza. The results indicate that the addition of boza changes the dough and bread structure and can create values close to wheat bread.

Table 4. Extensibility Properties of Doughs and Hardness of Breads

	Resistance to Extension (g)	Extensibility (mm)	Hardness (g)
Control	16.69±1.31[a]	15.71±1.36[c]	196.63±26.18[a]
YB1	23.95±1.23[b]	20.59±2.05[bc]	3549.64±345.45[b]
YB2	17.42±0.87[a]	18.34±0.52[bc]	4177.83±324.94[b]
YB3	18.11±1.00[a]	17.92±0.38[c]	4004.75±314.71[b]
LB1	15.57±0.47[a]	23.23±1.88[ab]	3461.12±223.83[b]
LB2	18.39±0.44[a]	19.70±1.08[bc]	3498.64±227.39[b]
LB3	32.65±0.83[c]	25.70±0.99[a]	3467.67±321.88[b]

Different letters within the same column indicate significant differences (P <0.05) according to Tukey multiple comparison test. Control: Bread without Boza, YB: Bread with Fresh Boza, LB: Bread with Lyophilized Boza

The results of TPA analysis are given in Table 4. Compared to the control bread, the amount of hardness increased in all breads regardless of the substitution rate, and this increase was found to be statistically significant (P <0.05). But, it is stated that breads enriched with 5% millet flour exhibit significantly lower stickiness, firmness and chewiness than wheat bread (Maktauf et al., 2016). In a study examining the effect of boza powder on bread, it is seen that as the amount of added boza powder increases, the hardness of the bread increases, and the results of this study are similar (Pala, 2012). When the breads prepared with the same amount of fresh boza and lyophilized boza were compared, it was determined that the lyophilized boza breads had less hardness values, but this difference was not statistically significant (P >0.05). Arendt et al. (2007) reported that the starter culture content and applied dough technology affect the texture of the bread in the production of sourdough bread. This effect changes as a result of gas formation created by microorganisms.

Color measurements of the crumbs of all breads are shown in Table 5. It was determined that L(brightness), a, and b values of breads added to both fresh boza and lyophilized boza. In the study conducted by Pala (2012), it was reported that the a and b values increased, but the L values decreased, depending on the substitution rate, in the breads produced with the addition of dry boza. When the brightness, a, b values are examined, it was observed that the breads with 10% lyophilized boza added were similar to the control group. Bircan et al. (2017) reported that crust color was lower in sourdough breads added to fresh boza compared to traditional sourdough bread. It has been reported that the addition of millet flour to wheat flour in bread production causes lower L, a and b values in bread crumb color (Mannuramath et al., 2015).

Sensory analysis results are given in Figure 2. After control bread, the most favorite breads were the ones with 25% added of fresh boza, and the ones with 5% added of lyophilized boza. When the results are evaluated, it can be said that the increase in the substitution rate in breads added to both wet and lyophilized boza negatively affects the sensory acceptability. Pala (2012) reported that the color, odor and texture scores of breads containing 8% boza powder were the lowest in sensory analysis. In addition, it is state that, bread added millet flour up to 30% has sensory results similar to wheat bread (Mannuramath et al., 2015). In the study, it was stated that it would be appropriate to use up to 2% of boza powder in breads, where volume is an important criterion.

Table 5. Crumb Color Characteristic of Breads

	L	a	b
Control	62.09±1.48^{c}	1.89±0.19^{b}	14.48±0.79^{d}
YB1	46.38±0.16^{a}	1.00±0.04^{a}	10.46±0.06^{a}
YB2	49.63±3.24^{ab}	1.26±0.03^{a}	11.31±0.44^{abc}
YB3	51.96±0.39^{b}	1.28±0.01^{a}	12.27±0.23^{c}
LB1	46.45±1.03^{a}	1.31±0.06^{a}	11.06±0.11^{ab}
LB2	48.14±2.66^{ab}	1.21±0.22^{a}	11.82±0.28^{bc}
LB3	52.06±0.42^{b}	2.19±0.06^{b}	14.49±0.08^{d}

Different letters within the same column indicate significant differences (P <0.05) according to Tukey multiple comparison test. Control: Bread without Boza, YB: Bread with Fresh Boza, LB: Bread with Lyophilized Boza

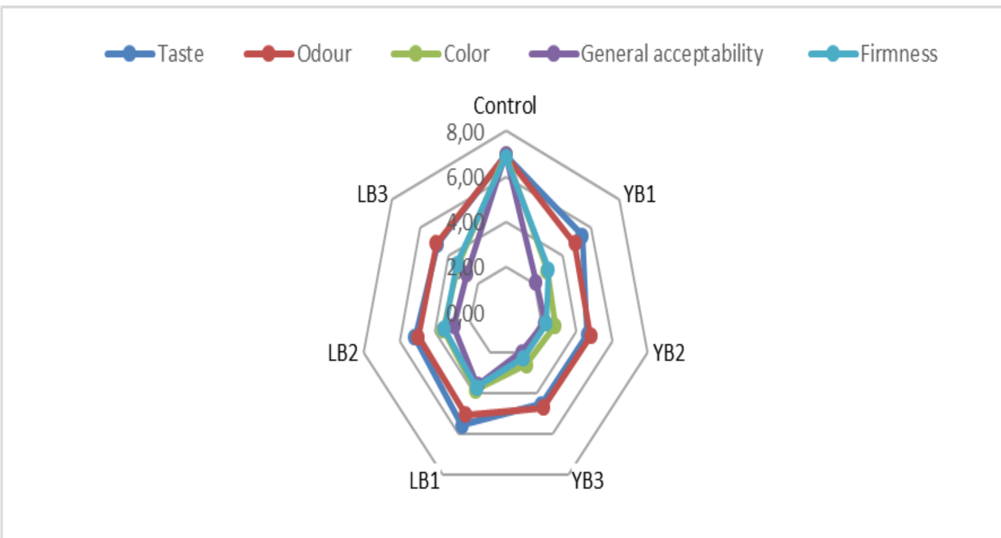

Figure 2. Spider Diagram for Descriptive Characteristics of Breads

CONCLUSION

Within the scope of the study, the effects of fresh and lyophilized boza use on the quality of bread and its use as a starter were investigated. It was observed that the lyophilization process caused a decrease of approximately 1 log in yeast count and 2.5 log in bacteria count. The addition of boza caused an increase in bread hardness and a decrease in brightness values. However, breads with boza were found acceptable in terms of taste. Among the breads with boza, the most liked ones were 25% fresh boza and 5% lyophilized boza added bread. As a result, boza can be used in bread production as a starter, but it can be suggested to improve the bread structure that adding boza at different rates, prolonging the fermentation time or the use of different raw materials in the production of boza. It is thought that the study is important in order to increase the consumption of boza, which is not consumed by everyone due to its unique sour taste.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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