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ORIGINAL RESEARCH PAPER

EFFECTS OF WHEAT BULGHUR, MAIZE FLOUR AND STARCH ON FERMENTATIVE CHARACTERISTICS OF BOZA

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Abstract
Fermented products play a significant role in the global market of ready-to-eat foods and beverages. In this study, the physicochemical, microbial, and sensory properties of boza, a traditional fermented beverage, prepared using wheat bulgur, maize flour, and pregelatinized waxy maize starch were investigated. After 24h fermentation, the highest total titratable acidity and viscosity, and the lowest pH, dry matters, and total sugars were measured for wheat bulgur boza ($p<0.05$). The protein content range of samples was 3.12-3.23%. The total aerobic bacteria and yeast content of all samples were detected to be statistically similar ($p<0.05$). The combined boza sample containing 5% pregelatinized waxy maize starch was more appreciated in terms of overall acceptance than other samples ($p<0.05$). Nevertheless, no significant differences were observed between the samples in terms of other sensory evaluation attributes. Replacing wheat bulgur with maize flour in the formulation of boza could result in a product that meets consumer preferences sufficiently, while potentially offering a more cost-effective and health-friendly alternative for individuals with conditions such as celiac disease.

Keywords: Boza, maize flour, wheat bulgur, cereal beverage, fermentation

Introduction

Cereals, including maize, wheat, rice, barley, sorghum, millet, teff, oats, rye, and triticale, are members of the monocot *Gramineae* family (Basinskiene and Cizeikiene, 2020). Cereal grains are an important part of nutrition in most cultures and are adopted as a source of protein and calories. More than 50% of energy and protein requirements of human population is provided by wheat, maize, rice, barley,

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sorghum, oats, rye, and millet (Cordain, 1999). Most of the nutrients needed by the human body, such as carbohydrates, fats, proteins, vitamins, minerals, and bioactive compounds are found in cereal grains. According to past studies, the consumption of cereal grains is associated with a reduction in cardiovascular disease (Mellen *et al.*, 2008) and type 2 diabetes (Kyrø *et al.*, 2018; Basinskiene and Cizeikiene, 2020).

Cereals, unlike many edible plants, cannot be consumed raw and need to be transformed. The production of the final edible product from cereals comprises several steps. A variety of grain-based food and beverage formulations have been created throughout history. Grain-based non-alcoholic drinks have recently attracted a lot of attention. These drinks may be stimulating like tea and coffee, refreshing like water and soft drinks, or nutritious like milk. The preparation step can involve non-microbial processing (such as using physicochemical methods), enzymatic clarification, or microbial fermentation (Basinskiene and Cizeikiene, 2020).

Fermentation is an old and economical way to preserve food (Altay *et al.*, 2013). In the fermentation process, unique flavors and benefits are obtained in a completely natural way (Irkin, 2019). This process improves the nutritional value, level of digestibility, shelf life, functional properties, texture, taste, and flavor of beverages (Pasqualone and Summo, 2021).

An approved class of fermented drinks are cereal-based ones. Due to the dominance of lactic acid bacteria (LAB) in the fermentation process, the pH of the product is reduced and by preventing the growth of pathogenic bacteria, it increases the durability and safety of the product. Fermented beverages contain useful microorganisms that have important health functions. In addition, fermentation has been found to improve protein digestibility and bioavailability of minerals and other micronutrients. By consuming these drinks, the harmful outcomes and economic effects of improper diets may be reduced. Also, these drinks can be consumed by vegetarians, vegans, and lactose-intolerant persons (Pasqualone and Summo, 2021).

Boza is a conventional non-alcoholic colloid suspension, prepared from millet, maize, wheat, rice by fermentation (Altay *et al.*, 2013; Pasqualone and Summo, 2021). Boza is a viscous beverage with a pale yellow color and sweet or sour taste. This highly nutritious easily digestible drink is a great source of vitamins, essential amino acids, and fatty acids. Boza's rich microflora is beneficial for digestion and intestinal flora. People of Turkey, Bulgaria, and some other countries of the Balkan Peninsula consume it widely (Akpinar-Bayizit *et al.*, 2010; Altay *et al.*, 2013; Bayat and Yildiz, 2019). The technological processes of boza production may have some differences while the cereal grains are always the main constituent. Fermented boza, sourdough, or yogurt can be used as a starter culture for fermentation. Boza's microflora mainly consists of LAB and yeasts, and alcoholic fermentation and lactic acid fermentation occur simultaneously. Uncontrolled interactions between LAB and yeasts during the process lead to variability in product quality (Irkin, 2019). The use of diverse kinds of cereals and cereal-based products as primary constituents, as also different fermentation and storage conditions affect the composition of boza samples (Gotcheva *et al.*, 2001; Altay *et al.*, 2013). Therefore, in this research, the physicochemical, microbial, and sensorial attributes of boza formulated with wheat bulgur, maize flour, and pregelatinized waxy maize starch were compared to evaluate the possibility of production of a cost-effective nutritious beverage for gluten-sensitive populations.

Materials and methods

Materials

Raw materials including sugar, wheat bulgur, maize, and pregelatinized waxy maize starch were obtained from local markets in Kermanshah (Iran). All chemicals were analytical reagents and were acquired from Sigma-Aldrich (Merck, Darmstadt, Germany) and DRM-Chem Co. (Tehran, Iran). Plate Count Agar (PCA) medium and Yeast Glucose Chloramphenicol Agar (YGC) medium were obtained from Merck (Darmstadt, Germany).

Boza production

The production process consisted of several steps and was performed following the method previously described, with modifications (Zorba *et al.*, 2003; Akpinar-Bayizit *et al.*, 2010; ÇelİK *et al.*, 2016; Bayat and Yildiz, 2019). At first, the combination of wheat bulgur and water with a ratio of 6:1 (wt.) was boiled for 60 min under continuous stirring. Then the mixture was allowed to cool and diluted with water with a ratio of 1:1(wt.). The diluted mixture was mixed in a household blender for 3 minutes and then strained with a fine strainer to separate the starchy part from barks, gums, and ungelatinized coarse particles. Then 20% (wt.) sucrose was added and the sample was fermented at 23°C for 56h. This product was used as inoculum for further preparing the boza samples.

Boza samples were prepared using maize flour (420 μm), wheat bulgur (1200 μm), and pregelatinized waxy maize starch mixed in different ratios (Table 1). The process of preparing unsweetened raw boza was done as before, but at the end, 15% (wt.) sucrose was added and samples were inoculated with 3% (wt.) boza from the previous batch and kept at 23˚C for 24h. Finally, boza samples were stored in glass containers at 4˚C until analysis

Table 1. The ingredients used in unsweetened raw boza samples.

Sample	Wheat bulgur (wt.%)	Maize flour (wt.%)	Pregelatinized waxy maize starch (wt.%)
W	100	-	-
M	-	100	-
WM	50	50	-
WMS	47.5	47.5	5

Physicochemical analysis

The pH was detected using a digital pH meter (WTW model 720, Weilheim, Germany). Total protein content was determined using Kjeldahl method according to AOAC Official Method (991.20, AOAC, 2000). The titratable acidity expressed as percentage of lactic acid, was determined by the titration method, according to AOAC Official Method (942.15, AOAC, 2000). The dry matter of the samples was measured by the gravimetric method according to AOAC Official Method (985.26, AOAC, 2000). Total sugars in terms of glucose equivalent were estimated by Lane and Eynon method as described by AOAC Official Method (925.50, AOAC, 2000).

Viscosity analysis

The viscosity of the samples was determined at 20°C using a Brookfield viscometer (Model DV-II+ Pro, Brookfield Engineering Laboratories, Inc, Middleboro, Massachusetts, USA) instrument equipped with a spindle numbered 63 at 30 rpm. Three readings were recorded after 30 s (Hayta *et al.*, 2001; Zorba *et al.*, 2003; Göncü *et al.*, 2023).

Microbial analysis

To count total mesophilic aerobic bacteria, 10 ml of boza samples were added to 90 ml of 0.1% peptone water under aseptic conditions. From appropriate tenfold dilutions pour plate counts were made using the PCA medium at 30°C for 72h incubation. The YGC medium was used for the determination of yeasts and molds at 25°C after 120h incubation and the results were declared as log cfu/ml (Zorba *et al.*, 2003; Göncü *et al.*, 2023).

Sensory analysis

Sensory evaluations were applied using a 5-point hedonic scale questionnaire (Scale: 1-dislike extremely; 2-dislike slightly; 3-neither like nor dislike; 4-like slightly; 5-like extremely) for five characteristics of taste, odor, color, consistency, and overall acceptance. Ten panelists (male and female, ranging in age from 19–27 years, laboratory staff) participated in the descriptive study. They all had experience in sensory descriptive analysis but none of them had participated in sensory tests with boza before. However, they were very interested in boza consumption. The study was conducted in accordance with the Declaration of Helsinki, and all panelists signed the informed consent form. Samples were coded by randomly chosen numbers. Approximately 50 mL boza samples with random cod numbers were presented to each subject. To give similar oral conditions, the panelists were asked to drink a cup of water at ambient temperature before each evaluation. Sensory analysis was performed in separate booths with air conditioning and white light (Teramoto *et al.*, 2001; Akpinar-Bayizit *et al.*, 2010; Göncü *et al.*, 2023).

Statistical analysis

The SPSS Statistics 19.0 software was used for data analysis. One-way ANOVA and Duncan’s post hoc tests were used to compare parametric data, and Friedman test and Wilcoxon’s post hoc were used to compare nonparametric data and a P-value less than 0.05 was considered statistically significant.

Results and discussion

Physiochemical analysis

For boza and other fermented beverages, the pH value depends on the fermentation duration and estimates the LAB content (Pulatsu *et al.*, 2023). Results of the physicochemical analysis are presented in Table 2. According to these results, after 24h of fermentation, the total titratable acidity level was found to be lowest in maize flour sample, at about 0.35%, and highest in wheat bulgur sample (0.52 %). The pH ranged between 3.97 and 4.37 for wheat bulgur and maize flour boza, respectively. The same trend was observed in previous research which was attributed to the probable higher fermentable carbohydrate content of wheat compared to other raw materials such as rice, maize, and millet (Akpinar-Bayizit *et al.*, 2010). Also in another study fermentation parameters were greater in wheat than in barley (Agyekum *et al.*, 2020). The pH value dropping from 5.1 to 3.4 has been observed after 24h in boza samples produced from whole wheat grain and whole wheat flour and it has been attributed mainly to the acids produced by LAB involved (Gotcheva *et al.*, 2001). Also, a pH value of 3.48 and total titratable acidity of 0.27% were reported for boza made from a combine of maize, wheat, and rice flour (2:1:1) after 24h fermentation (Hancioğlu and Karapinar, 1997). In our study, the addition of 5% pre-gelatinized waxy maize starch led to a significant decrease in pH value of WMS sample in comparison to the WM sample ($p<0.05$). In another study, addition of 10% non-fat dry milk significantly increased the initial pH values from 3.95 to about 6 ($p<0.05$); however, further increase of the addition level from 10 to 40% had no affect the pH values significantly ($p<0.05$). Lactic acid bacteria grow readily in nutrient media and rapidly lowers the pH until other competing organisms are unable to grow (Pulatsu *et al.*, 2023). Based on the Turkish Boza Standard, the range of total acid (lactic acid) is 0.2-0.5% in sweet boza, and 0.5-1.0% in sour boza, and also total sugar must be at least 10% (Akpinar-Bayizit *et al.*, 2010; Ilgaz, 2014).

Table 2. Physicochemical characteristics of boza samples.

Sample	pH	Acidity (%)	Protein (%)	Dry matter (%)	Total sugars (%)	Viscosity (cP)
W	3.97±0.01^{d}	0.54±0.02^{a}	3.20±0.02^{a}	21.52±0.11^{d}	12.09±0.17^{d}	2232.0±76.21^{a}
M	4.37±0.02^{a}	0.35±0.01^{d}	3.21±0.02^{a}	23.61±0.12^{a}	14.28±0.19^{b}	423.33±15.94^{c}
WM	4.22±0.02^{b}	0.42±0.00^{c}	3.23±0.02^{a}	22.46±0.13^{b}	13.36±0.13^{c}	582.00±19.00^{b}
WMS	4.13±0.03^{c}	0.46±0.02^{b}	3.12±0.01^{b}	23.12±0.02^{c}	15.30±0.11^{a}	647.66±28.02^{b}

* Different letters on the same column indicate significant differences among results ($p<0.05$)

In our study, wheat bulgur boza showed the least dry matter and total sugar amount (21.52 and 12.09%, respectively), after 24h fermentation, ($p<0.05$). The protein content range of samples was 3.12-3.23%. Although wheat generally has more protein than maize, but due to the coarser particles, part of the wheat bulgur substrate remained behind the sieve, which can be used as animal feed. This could be the reason for less dry matter and no significant amount of protein between the final products ($p<0.05$). Earlier, a dry matter content range of 23-28% was reported for traditional boza samples prepared from local markets (Genç *et al.*, 2002). In another study, using of quinoa and chia flours combinations did not lead to a significant effect in the moisture content of boza samples, however higher protein and ash content in chia flour caused to increase in these parameters in final product ($p>0.05$) (Göncü *et al.*, 2023). Also, water-soluble proteins of 4.58 mg/ml and pH 3.5 have been reported for boza prepared with corn, wheat, and rice flour (1:1:1), after 24h fermentation (Hayta *et al.*, 2001). In another study, boza made from a mixture of maize, wheat, and rice flours (2:1:1) was found to have a total sugar content of 12.3% (Zorba *et al.*, 2003). The decrease in total sugar levels in this study was attributed to the microorganisms' preference for utilizing available sugars as a primary energy source. This utilization led to the breakdown of sugars by the enzymes of the microorganisms, resulting in the production of alcohol, CO_2, and other products (Zorba *et al.*, 2003).

Viscosity analysis

The type and amount of substrate used during fermentation showed significant effects on the rheological characteristics (p <0.05), and a more viscous structure was obtained in wheat bulgur boza sample. Previously the viscosity value of 0.5 Pa·s was reported for boza prepared with corn, wheat, and rice flour (1:1:1) at 30 rpm at 20 ºC (Hayta *et al.*, 2001). Also, the apparent viscosity below 3 Pa·s was detected for shear rates more than 8 (1/sec) at 10 ˚C for boza prepared by maize, wheat, and rice flours (2:1:1) (Genç *et al.*, 2002). In another study, a more viscous boza was obtained from chia, which had higher fiber content (Göncü *et al.*, 2023). Also, significantly higher viscosity values were observed in boza with whole-wheat flour (2650 cP at 30 ˚C, 24 h fermentation) than in boza with whole-wheat grains (630 cP, 30 ºC, 24 h fermentation) and it was attributed to flour bran particles (Gotcheva *et al.*, 2001). It has been established that the boza sample with leblebi flour alone had a higher amount of consistency coefficient than different ratios of maize, wheat, rice, and leblebi flour, however, no clear trend was seen between the combined samples (ÇelİK *et al.*, 2016). Earlier, the addition of 20–40% non-fat dry milk to the boza formulation yielded very thick slurries, which were significantly different from the consistency of the control sample (Pulatsu *et al.*, 2023). In another study, although no clear trend was observed among the dry matter concentration and the rheological properties of market samples possibly due to a variety of constituents, the laboratory samples showed a clear trend (Genç *et al.*, 2002). In this regard, when studying of the effects of dietary fiber on dough characteristics, some authors stated that, depending on the type, structure, size and amount of added dietary fiber, the gluten network structure may be improved or deteriorated (Liu *et al.*, 2019). It was also announced that the interaction between protein and other components (fat and sugar) determines the strength of the three-dimensional network in almond yogurt (Zhao *et al.*, 2021). Other researchers have also declared that determining the contribution of the components in the final viscosity is complicated only based on mechanical considerations (Lie-Piang *et al.*, 2022).

Microbial analysis

Alcoholic fermentation, which increases the volume due to the production of carbon dioxide bubbles, and lactic acid fermentation, which forms the acidic characteristics of boza by producing lactic acid, occurs simultaneously in boza. These microorganisms engage in a competitive process for the available sugar source, leading to the production of fermentation metabolites such as alcohol, organic acids, and free acids based on their activity levels and cell counts. So, their activity during fermentation affects the final product composition (Akpinar-Bayizit *et al.*, 2010). The microbial analysis results are presented in Table 3. The total aerobic bacteria, yeast, and mold content of all boza samples were statistically similar (P<0.05). Earlier, total mesophilic aerobic bacteria numbers between 7.90 and 8.17 cfu/ml and yeast number between 6.07 and 6.41 cfu/ml were measured in boza containing various spices (Coskun and Cakır, 2014). Also, total aerobic bacteria ranged from 8.02 to 8.71 log cfu/ml, and mold counts ranged from 0 to 2.72 log cfu/ml were reported for 24h fermented boza samples were purchased from the local market in Turkey (Uysal *et al.*, 2009). In another study of the microflora of boza from three local producers in Bulgaria, total bacteria and yeasts count of 7.93 to 8.10 log cfu/ml, and 7.41 to 7.59 log cfu/ml were reported, respectively. Boza is a good source of probiotics. Variation and population of LAB and yeast are affected by raw materials, fermentation, and storage conditions (Göncü *et al.*, 2023).

Table 3. Microbial analysis of boza samples.

Sample	Total aerobic bacteria (log cfu/ml)	Yeast (log cfu/ml)	Molds (log cfu/ml)
W	8.70±0.19[a]	6.13±0.11[a]	1.77±0.12[a]
M	8.46±0.23[a]	6.53±0.36[a]	1.30±0.51[a]
WM	8.53±0.31[a]	6.52±0.47[a]	1.40±0.45[a]
WMS	8.58±0.30[a]	6.35±0.38[a]	1.35±0.38[a]

* Different letters on the same column indicate significant differences among results ($p<0.05$)

Sensory analysis

The results of the sensorial analysis of samples are presented in Table 4. Although the sensorial results were not within a wide range, WMS sample was more appreciated in terms of overall acceptance than other samples ($p<0.05$).

Table 4. Sensory analysis of boza samples.

Sample	Taste	Odor	Color	Consistency	Overall Acceptance
W	3.10±0.56[a]	3.20±0.63[a]	3.60±0.51[a]	3.20±0.63[a]	3.40±0.69[ab]
M	3.00±0.66[a]	2.80±0.63[a]	3.20±0.63[a]	3.00±0.81[a]	2.80±0.63[b]
WM	3.20±0.42[a]	3.40±0.51[a]	3.40±0.51[a]	3.30±0.67[a]	3.30±0.67[ab]
WMS	3.30±0.48[a]	3.30±0.48[a]	3.50±0.52[a]	3.80±0.42[a]	3.70±0.67[a]

* Different letters on the same column indicate significant differences among results ($p<0.05$)

It was declared that the sensory qualities, alcohol content, and organic acid levels of boza are considerably influenced by the present microorganisms present and their metabolic activities. In a separate study, maize boza scored higher than rice, millet, and wheat boza, in terms of all sensory characteristics, likely due to its similarity in color and flavor to commercially available boza (Akpinar-Bayizit *et al.*, 2010). It was stated that quinoa boza scored higher in all sensory attributes than chia boza or combined samples, because of its slightly yellowish color, slightly acidic taste and smell, and less viscous consistency (Göncü *et al.*, 2023). In another study, only the partial replacement of cereals by leblebi flour in boza formulation was accepted by panelists, due to its high viscosity and distinct leblebi flavor (ÇelİK *et al.*, 2016). In the case of boza samples prepared from maize, whole wheat, rice flours, and two different bacterial cocktails, it has been reported that the increase in acidity during storage had a negative effect on the taste of boza samples (Öztürk *et al.*, 2013).

Conclusion

The results showed the wheat bulgur fermentation in boza caused changes in physico-chemical properties compared to maize flour and starch, by increasing in beverage viscosity and total titritable acidity, and also by decreasing in dry matter and total sugar content. The total aerobic bacteria, yeast and mold contents of all samples were statistically similar. The lack of significant differences between the samples in terms of most sensory attributes indicated that replacing wheat bulgur with maize flour in the boza formula could result in a product that meets consumer preferences sufficiently, while potentially offering a more cost-effective and health-friendly alternative for individuals with conditions such as celiac disease.

Conflict of interest: No conflict of interest exists.

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