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Cereal source and microbial consortia of the starter culture influence the chemical composition and physicochemical characteristics of boza

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Abstract: Boza is a popular cereal-based traditional beverage particularly consumed during winter months in Balkan countries and Turkic societies. The present study aims to investigate the influence of different cereal sources and starter cultures used in the commercial production of boza on its chemical and microbiota compositions and physicochemical characteristics. Detailed experimentation has revealed that chemical compositions, i.e. carbohydrates (beta-glucans, starches), organic acids, vitamins, dietary fibers, proteins, fat, and minerals, along with physicochemical characteristics, are subject to significant differentiation depending upon the cereal types and microbial consortia of the starter culture used in boza production. These variations also influence the physicochemical characteristics and the nutritive value of the final product. In the course of the study, various lactic acid bacteria [*Lactococcus lactis*, *Leuconostoc* (*Leuconostoc pseudomesenteroides*, *Lc. lactis*, *Lc. citreum*), *Lactobacillus* spp. (*L. plantarum*, *L. paracasei*, *L. brevis*, *L. delbrueckii* subsp. *delbrueckii*)] and yeasts (*Pichia fermentans*, *Candida colliculosa*, *Geotrichum* sp., and *Galactomyces geotrichum*) were identified as the members of the microbial community in three boza samples. Results of the present study open a new research line for better understanding of the contribution of the cereal source and microbiota composition of boza beverage to its nutritive value as well as biological effects on human health.

Key words: Boza, lactic acid bacteria, yeast, wheat, maize, millet

1. Introduction

Among fermented health products, boza is a popular traditional beverage among the Turkic and Balkan societies. It is produced by the fermentation of various cereals such as wheat, oat, barley, maize, rice semolina, and/or millet or their combinations with mixed cultures of yeasts and lactic acid bacteria (LAB) (Arıcı and Dağlıoğlu, 2002). Selected grains are first cooked in water under pressurized or nonpressurized conditions and then the viscous extract obtained is submitted to fermentation after addition of sugar. The microbiota composition of boza production has been reported by several studies (Gotcheva et al., 2001; Kivanç et al., 2011). During the fermentation process yeasts help to decompose the carbohydrates, while bacteria degrade proteins due to their proteolytic activity and both also have an impact on the fat, vitamin, and mineral composition of boza. Eventually the final product gains a higher nutritional value with higher bioavailability (Gotcheva et al., 2001). Moreover, LAB convert sugars in the fermentation medium into organic acids, would further contributes to the pleasant taste and flavor of boza,

as well as to its rheological characteristics (Öztürk et al., 2013).

In spite of its long historical background, physicochemical and compositional analyses, as well as characteristics of boza types obtained from different cereal sources, have only recently attracted the attention of the scientific community. The effect of fermentation on the viscosity and protein solubility in boza was studied by Hayta et al. (2001) and they hypothesized that during the heating process the starch content of cereals undergoes gelatinization by the swelling of starch granules and then viscosity increases through leaching of amylose. Upon cooling of the starch, molecules align themselves back into a crystalline structure, forcing out water, and the viscosity decreases. A significant increase in resistant starch content was also reported by Quintieri et al. (2012).

Investigations have revealed that the composition of boza shows remarkable variations due to the use of distinct types of cereals as well as microbiota composition and fermentation conditions (Altay et al., 2013). Today boza

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production in Turkey is mostly carried out at an industrial scale under controlled processes and hygienic conditions.

This study aims to elucidate the diversifications in the chemical and microbial compositions, physicochemical characteristics, and nutritive values of three commercial boza beverages produced industrially from different cereals and starter culture sources during processing and storage.

2. Materials and methods

2.1. Production and preservation of boza samples

Boza samples were collected from 3 boza manufacturers located in different geographical regions of Turkey, where production was carried out following traditional boza manufacturing techniques. The main criteria for selection of the three boza samples for this study were their cereal sources. The compositions of the boza samples were as follows: 'MaWMi', 40% maize, 20% wheat, 40% millet; 'MaW', 70% maize, 30% wheat; 'Mi', 100% millet (brand names of the companies were kept anonymous).

Boza production was carried out in 4 steps. First the cereals were comminuted and boiled in water for 1–2 h, then poured onto shallow trays. When cooled, they were reconstituted and sieved (size 0.1–1.0 mm), and then saccharose (12%–16%) was added and left for spontaneous fermentation at 12 °C with natural microbiota for 16–24 h. Then the three boza samples were transported to the MRC Food Institute under cold-chain, where they were stored at 12 °C for 2 weeks.

2.2. Sampling of boza and storage

Four samples from each boza type were taken: 1) before fermentation; 2) following the 16 h of fermentation at the production units; and 3) after 1 week and 4) after 2 weeks of storage at 12 ± 1 °C at the MRC Food Institute. After microbiological plating and water content analyses, each boza sample was freeze-dried in a pilot freeze-dryer (Telstar, LyoBeta 35, Terassa, Spain), enclosed under vacuum in low oxygen barrier packaging, and conserved in a refrigerator at 4 ± 1 °C for further analyses. All analyses (except microbiological and moisture analyses) were conducted on freeze-dried samples to obtain more coherent results.

2.3. Microbiological characterization of boza samples

2.3.1. Isolation and preservation of lactic acid bacteria and yeasts

Each boza sample (25 mL) was diluted with 225 mL of a sterile saline solution (0.85% NaCl + Tween 80) before lyophilization and homogenized through a stomacher (Easy Mix, AES Labs, France) for 60 s. The homogenate was then serially diluted in saline solution and plated in duplicate by using a spread plate technique onto the following agar media:

1) Man Rogosa Sharp agar (MRS agar, Difco Labs, USA)

and neutral red chalk agar (NRC agar) were used for lactobacilli/leuconostocs and lactococci/leuconostocs, respectively, and for almost all boza microbiota, consisting of mesophilic bacteria, plates were incubated at 30 ± 1 °C for 2 days under anaerobic conditions using gas-generating Anaerocult A kits and jars (Merck, Germany) for the evaluation of LAB composition.

2) Yeast glucose chloramphenicol agar (YGC agar, Difco Labs, USA) incubated at 25 ± 1 °C for 2–4 days were used for the evaluation of yeast content.

The LAB colonies on MRS agar and NRC agar were observed for their cell morphology by immersed microscopy and Gram reaction. After checking the negative catalase activity of LAB colonies using 30% H₂O₂, 10 colonies were randomly selected from MRS agar and NRC agar dilution plates and single colonies were transferred to MRS broths (Difco Labs, USA). Yeast colonies were transferred to YGC broths from YGC agar dilution plates. LAB and yeasts were subcultured many times by streaking on MRS agar and YGC agar plates, respectively, to obtain purified colonies. Isolates were preserved by lyophilization in skimmed milk and were stored for further analyses.

2.3.2. Identification of LAB

All isolates were examined for their growth at 10 °C and 45 °C when inoculated in MRS broth medium. Litmus milk reaction was assessed in sterilized BBL litmus milk (Becton, Dickinson & Co., USA) at 30 ± 1 °C for 2 days. Ammonia production from arginine test using Nessler reagents (Merck, Germany), CO₂ production in MRS-glucose broth, and EPS-positive colonies displaying slimy morphology on sucrose-MRS agar were observed according to Schillinger and Lücke (1987).

Carbohydrate fermentation patterns were obtained using the API 50 CH (BioMérieux, France) kit, containing 49 different compounds and carried out according to the manufacturer's instructions. The colonies were suspended in API 50 CHL medium, then inoculated onto the strips, covered with mineral oil, and incubated at 30 ± 1 °C for 2 days. Results were evaluated observing the color change in each well visually. Identification of each microorganism was done with the MiniAPI (BioMérieux) Automatic Bacteriological Analyzer.

2.3.3. Identification of yeasts

Yeast identification was carried out following colonial morphological, physiological, and culture characterization of isolated strains. Carbohydrate fermentation patterns were obtained using the API ID 32C (BioMérieux) kit containing 31 different compounds and carried out according to the manufacturer's instruction. The colonies were suspended in API C medium, then inoculated onto strips and incubated at 25 ± 1 °C for 5 days. Plates were evaluated automatically in the MiniAPI. Yeasts capable of utilizing soluble starch (Merck, Germany) as a sole carbon

source were tested on Yeast Nitrogen Base Medium (Difco Labs, USA) and evaluated turbidimetrically considering the cell concentration.

2.3.4. Identification by molecular techniques

Genomic DNA was isolated as described by the Wizard Genomic DNA Purification Kit (Promega Corp., USA). DNA concentration was determined by NanoDrop (Implen, Germany). Bacteria isolates were identified by amplifying and sequencing 16S rDNA as described by Baruzzi et al. (2000). Yeast isolates were identified by amplifying and sequencing the 26S rDNA D1/D2 domain as described by Kurtzman and Robnett (1998). Colonies were identified by comparing sequences with bacterial 16S rDNA sequences available in the NCBI BLAST database (USA).

2.4. Physicochemical characterization of boza samples

2.4.1. pH characterization

The pH values of boza samples were determined by pH-meter (Orion Star A211, Thermo Scientific, USA).

2.4.2. Resistant starch contents

The amount of starch resistant to digestion was determined enzymatically with the K-RSTAR 08/11 kit (Megazyme Int., Ireland) following the manufacturer's instructions. Initial D-glucose content was determined quantitatively prior to analysis. Starch prone to digestion was first hydrolyzed to D-glucose by the combined action of α -amylase and amyloglucosidase for 16 h at 37 ± 1 °C. Initial D-glucose content was subtracted from final D-glucose to determine the total starch content in boza samples. Then the undigested starch portion was harvested by centrifugation and the resulting pellet was washed with 50% (v/v) ethanol and dissolved in 2 M KOH in an ice-bath. This was further hydrolyzed enzymatically to D-glucose representing that from resistant starch. D-glucose contents in samples were determined spectrophotometrically by monitoring the color change resulting from the reaction between glucose oxidase/peroxidase reagent and D-glucose. The assay was further confirmed by Mega-Calc software (Megazyme, Ireland).

2.4.3. Ethanol contents

Ethanol was determined as described by AOAC International Official Method 983.13. The ethanol concentration in each boza sample was determined by measuring its concentration in headspace using GC-MS (Thermo Scientific, Italy).

2.4.4. Moisture, ash, protein, fat, dietary fiber, and carbohydrate contents

Moisture content was determined gravimetrically as described by AOAC International Official Method 934.06. A preweighed sample was placed in a drying oven and the amount of loss was evaluated as moisture content.

Ash contents of boza samples were determined by ignition as described by AOAC International Official

Method 900.02. Each sample was placed in an ash-oven at 550 °C and the residual ash after ignition was determined gravimetrically.

Protein content was determined by the Kjeldahl method (Kjeltech Analyzer Unit 2300, Foss Tecator AB, Sweden). The nitrogen amount was determined by distillation after digestion of each sample. This value was used to estimate protein content.

Fat contents were assayed as described by AOAC International Official Method 991.36. Each sample was first subjected to acid hydrolysis with approximately 8.3 M HCl and then free fatty acids were extracted with diethyl ether and petroleum ether. After removal of the solvent, the fat content of each sample was determined gravimetrically.

Dietary fiber content of samples was determined by enzymatic-gravimetric method (AOAC International Official Method 991.43). The boza samples were briefly treated with α -amylase, protease, and amyloglucosidase to hydrolyze protein and starch. Unhydrolyzed beverage constituents were precipitated and washed with ethanol and dried. Dietary fiber content of each sample was determined gravimetrically. Carbohydrate and energy contents were determined by the Atwater method as described by Watt and Merrill (1975); the carbohydrate amount was determined by subtracting the moisture, ash, protein, fat, and dietary fiber concentrations from total sample amount. Energy values of the boza samples were calculated by multiplying protein and carbohydrate amounts by 4 kcal/g and total fat by 9 kcal/g.

2.4.5. Total sugars and sugar composition

Total sugars were determined according to the Lane-Eynon method (AOAC International Official Method 923.09). Sugars in each sample were converted to invert sugars and their concentrations were determined by titration.

Sugars were extracted from the boza samples with ethanol as described by Zorba et al. (2003). Then the extract was filtered through a 0.45- μ m syringe filter before injection into the HPLC system. Chromatographic analysis was performed with the Shimadzu Class 10AVP Series HPLC system consisting of an NH_2 column (25 cm \times 4.6 mm i.d.; 5- μ m particle size) at 30 °C and an RI detector. The mobile phase was acetonitrile-water (80/20, v/v) at 1.3 mL/min. The injection amount was 10 μ L with a total elution time of 18 min.

2.4.6. Organic acid contents

Boza samples were extracted and then subjected to HPLC analysis (Shimadzu Class 10AVP Series, Japan) consisting of an ACE C8 column (25 cm \times 4.6 i.d.; 5- μ m particle size) at 40 °C and a UV detector (214 nm). A mobile phase composed of 0.2 M KH_2PO_4 solution adjusted to pH 2.4 with phosphoric acid was used with the following adjustments: flow rate, 0.8 mL/min; injection volume, 10 μ L; total elution time, 35 min. The organic acid standards

were prepared in the mobile phase and the chromatograph was calibrated using the standard solution (AOAC International Official Method 986.13). All determinations were performed from two separate extractions of each sample and each was injected in duplicate.

2.4.7. Mineral contents

Boza samples were subjected to microwave digestion with nitric acid and hydrogen peroxide as described in the Milestone Application notes. Mineral contents in digested solutions were determined by atomic absorption spectroscopy (PerkinElmer AAS, USA) according to the AOAC method for Zn, Cu, Fe (Digestion Official Method 999.10) and for Na, K, P, Mn, Ca, and Mg (Official Method 985.35), and the Environmental Protection Agency (EPA) methods (Official Method 7741A) for Se. The mineral solution was ignited at a colorless argon hydrogen flame and the mineral concentration was measured by the spectrum at the predetermined wavelength.

2.4.8. Vitamin contents

Vitamins concentrations in boza samples were determined by HPLC. The protocols of previous studies were used for vitamins B₁ and B₂ (Finglas and Faulks, 1984), vitamin B₃ (Ndaw et al., 2002), vitamin B₆ (Kall, 2003), and vitamin K₂ (Koivu-Tikkanen et al., 2000). Vitamin B₁₂ concentration was determined microbiologically with the R-Biopharm P1002 kit (Germany).

3. Results and discussion

3.1. Microbial consortia of boza types

Boza is a fermented beverage and the diversity in fermentation flora and variations in numbers of LAB and yeasts define its overall properties. Boza microbiota composition exhibits large variations depending on the grain type used, the production season, the temperatures, the manufacturing processes, and the storage conditions (Arıcı and Dağlıoğlu, 2002; Petrova and Petrov, 2017). Viable counts for LAB and yeasts reached 9.84 in 1 week and 6.47 log cfu/mL in 2 weeks of storage. These values were found to be higher than those reported previously for Turkish (Hancıoğlu and Karapınar, 1997; Caputo et al., 2012) and Bulgarian (Botes et al., 2007) boza, possibly due to the longer storage periods of samples. Viable counts and compositions of microbiota in boza samples are tabulated in Table 1. Confirming the previous studies (Özteber et al., 2011; Caputo et al., 2012), a complex microbial consortium was identified, including diverse LAB and yeasts species during the production and storage stages of boza manufacturing.

A total of 200 strains of LAB and yeasts from 12 boza samples were isolated and identified with culture-based and nonculture-based methods from these three boza samples. In MaWMI leuconostocs (*Lc. mesenteroides* subsp. *mesenteroides*, *Lc. mesenteroides* subsp. *dextranicum*, *Lc. pseudomesenteroides*, *Lc. lactis*, *Lc. citreum*, and *Lc.*

holzapfelii) were the only predominating members of the lactic acid population, while in MaW and Mi lactococci and lactobacilli were found in higher frequencies (Cholakov et al., 2016).

Mi samples demonstrated the richest biodiversity in terms of heterofermentative and homofermentative lactobacilli (*L. plantarum*, *L. paracasei* subsp. *paracasei*, *L. delbrueckii* subsp. *delbrueckii*, *L. brevis*) and leuconostocs (*Lc. lactis*, *Lc. citreum*, *Lc. mesenteroides* subsp. *mesenteroides*, *Lc. pseudomesenteroides*, *Lc. mesenteroides* subsp. *dextranicum*). *Lc. holzapfelii* was not isolated in Mi, but *Lactococcus lactis* was found in high frequencies.

L. plantarum strains were the only lactobacilli recovered in MaW, and previously they were identified as the predominant species exhibiting antimicrobial activity in Turkish boza (Kivanç et al., 2011; Borcaklı et al., 2014). Other species found in these studies were *Lac. lactis* subsp. *lactis*, *Lc. citreum*, *L. brevis*, and *L. paracasei* subsp. *paracasei*. Hancıoğlu and Karapınar (1997) also reported the presence of two common leuconostocs, *Lc. mesenteroides* subsp. *mesenteroides* and *Lc. mesenteroides* subsp. *dextranicum*, in Turkish boza.

Osimani et al. (2015) investigated the microbial composition of boza samples produced from durum wheat flour with acid and sweeteners addition, collected from three local retailers in Bulgaria during the storage steps of production. Diversity in microbiota members of Turkish and Bulgarian boza is evident due to the use of different raw materials in production. Botes et al. (2007) identified *L. paracasei* subsp. *paracasei*, *L. pentosus*, *L. plantarum*, *L. brevis*, *L. rhamnosus*, and *L. fermentum* in Bulgarian boza samples, but not the strains common in Turkish boza: *Lc. mesenteroides* and *Lc. mesenteroides* subsp. *dextranicum*.

In Turkey boza is manufactured and consumed during winter, when the average temperature is about 12 °C. Therefore, all LAB strains under investigation were incubated at 10 °C. In accordance with the literature (Osimani et al., 2015), no thermophilic LAB strains growing at 45 °C were isolated from Turkish boza samples.

The yeast populations first started to occur after 16 h of fermentation or under the storage conditions. Viable counts of yeasts were found lower than those of LAB, ranging from 1.69 to 4.39 log cfu/mL.

Among the yeast populations in MaWMI and MaW, *Pichia fermentans* (syn. *Candida lambica*) and *Geotrichum* species were found as the dominant species. The former was also reported by many authors in boza samples (Botes et al., 2007; Caputo et al., 2012; Osimani et al., 2015). In contrast to the report of Hancıoğlu and Karapınar (1997), *Saccharomyces* strains were not detected in our samples, in accordance with the findings of Botes et al. (2007). *Candida colliculosa* (syn. *Torulaspora delbrueckii*) identified in MaWMI was previously identified in sugary foods (Şenses-Ergül et al., 2012; Jooyandeh, 2013) and was reported for

Table 1. Viable counts of lactic acid bacteria and yeasts and identification of the 3 boza beverages prepared from different cereal sources with different starter cultures.

	MaW Mi				MaW				Mi			
	0 h	16 h	1 week	2 weeks	0 h	16 h	1 week	2 weeks	0 h	16 h	1 week	2 weeks
Microorganism counts (log ₁₀ cfu/mL ⁻¹)												
MRSA	7.11	7.41	6.20	5.54	3.30	6.96	6.9	5.30	3.47	8.41	5.30	3.69
NRCLA	5.51	6.60	7.47	5.32	3.07	8.54	9.84	5.11	2	8.69	8.90	7.98
YGCA	<1	3.47	2.88	4.39	<1	<1	2.71	3.66	<1	1.69	1.69	6.47
Identification of lactic acid bacteria and yeasts ^f												
Lactic acid bacteria	<i>Lc. mes.</i> ⁱ <i>Lc. dex.</i> ⁱⁱ	<i>Lc. mes.</i> <i>Lc. lac.</i> ⁱⁱⁱ <i>Lc. dex.</i>	<i>Lc. mes.</i> <i>Lc. dex.</i> <i>Lc. holz.</i> ^{iv} <i>Lc. cit.</i> ^v <i>Lc. pse</i>	<i>Lc. mes.</i> <i>Lc. lac.</i> <i>Lc. dex.</i> <i>Lc. pse</i>	<i>Lc. lac.</i> <i>Lc. holz.</i> <i>Lc. dex.</i> <i>Lc. pse</i> <i>L. plan.</i> ^{viii}	<i>Lc. lac.</i> <i>Lc. holz.</i> <i>Lc. cit.</i> <i>Lc. pse</i> <i>L. plan.</i>	<i>Lc. lac.</i> <i>Lc. holz.</i> <i>Lc. cit.</i> <i>Lc. dex.</i> <i>L. plan.</i>	<i>Lc. lac.</i> <i>Lac. lac.</i> ^{vii} <i>L. plan.</i>	<i>Lc. lac.</i> <i>Lc. pse</i> <i>L. plan.</i>	<i>Lc. mes.</i> <i>Lc. dex.</i> <i>Lac. lac.</i> <i>L. plan.</i> <i>L. para.</i> ^{ix} <i>L. bre.</i> ^{xi}	<i>Lac. lac.</i> <i>L. plan.</i> <i>L. para.</i> <i>L. del.</i> ^x <i>L. bre.</i> ^{xi}	<i>Lc. mes.</i> <i>Lc. cit.</i> <i>L. plan.</i> <i>L. para.</i> <i>L. del.</i>
Yeasts	-	<i>P. fer.</i> ^a <i>C. col.</i> ^b	<i>P. fer.</i> <i>C. col.</i>	<i>P. fer.</i> <i>G. sp.</i> ^c	-	-	<i>P. fer.</i> <i>G. sp.</i> <i>Gl. geo.</i> ^d	<i>P. fer.</i> <i>G. sp.</i> <i>Gl. geo.</i>	-	<i>G. spp.</i> <i>Tri. spp.</i> ^e	<i>G. spp.</i> <i>P. fer.</i> <i>Tri. sp.</i>	<i>G. spp.</i> <i>P. fer.</i> <i>Tri. sp.</i>

*Lc. mes.*ⁱ *Leuconostoc mesenteroides* subsp. *mesenteroides*; *Lc. dex.*ⁱⁱ *Leuconostoc mesenteroides* subsp. *dextranicum*; *Lc. lac.*ⁱⁱⁱ *Leuconostoc lactis*; *Lc. holz.*^{iv} *Leuconostoc holzapfelii*; *Lc. cit.*^v *Leuconostoc citreum*;

Lc. pse.^{vi} *Leuconostoc pseudomesenteroides*; *Lac. lac.*^{vii} *Lactococcus lactis*.

L. plan.^{viii} *Lactobacillus plantarum*; *L. para.*^{ix} *Lactobacillus paracasei* subsp. *paracasei*; *L. del.*^x *Lactobacillus delbrueckii* subsp. *delbrueckii*; *L. bre.*^{xi} *Lactobacillus brevis*.

P. fer.^a *Pichia fermentans*; *C. col.*^b *Candida colliculosa*; *G. sp.*^c *Geotrichum* sp.; *Gl. geo.*^d *Galactomyces geotrichum*; *Tri. sp.*^e *Trichosporon* sp. ^f (% ident. is between 98.9%–100% according to culture based and nonculture based methods)

its functional properties in some Nigerian traditional fermented foods (Alakeji et al., 20015). *Galactomyces geotrichum* was also identified by molecular methods in MaW with 99% sequence identity. A *Trichosporon* species, *T. otae*, was identified in Mi, which is known to be a part of the normal human flora of the skin and gastrointestinal tract (dos Santos et al., 2016).

3.2. Protein, fat, dietary fiber, carbohydrate, and alcohol contents of boza samples

Chemical compositions of the Turkish boza samples manufactured with various raw materials were previously reported by several authors (Arıcı and Dağlıoğlu, 2002; Çelik et al., 2016). In this study, the chemical compositions of the samples were investigated comparatively based on their raw material compositions. Chemical profiles of the freeze-dried boza samples obtained during the production stage and storage conditions are tabulated in Table 2.

It is evident that protein and fat contents vary depending on the cultivar/variety and the cultivation conditions of the cereals. In terms of protein content of fermented boza samples, Mi was shown to contain twice higher protein as compared to MaW Mi, and less fat (Table 2). Average protein contents of wheat (12.6%) and millet

flours (12.2%) were reported to be very close, while in maize a lower protein content (3.3%) was noted (Petrova and Petrov, 2017). Thus, due to the high ratio (40%) of maize in the raw materials of MaW Mi and MaW, the protein values were found to be lower. Millet contains higher fat (4.2%) than wheat (1.5%) and maize (1.4%), and hence the fat value in fermented Mi was found lower than in MaW Mi and MaW. Moreover, the total glucose rate of Mi was found to be the lowest.

Various lactobacilli and yeast species in boza microbiota, i.e. *L. plantarum*, *L. casei*, *L. brevis*, *Pichia fermentans*, and *Geotrichum* species, were shown to have lipid-degrading activities (Chander, 1973; Rashmi and Gayathri, 2014; Esteban-Torres et al., 2015). Lipid stability of cereals is highly susceptible to decrease with the activity of their endogenous lipases. In an earlier study it was demonstrated that the lipase activity of wheat was usually low enough to avoid the degradation of lipids (Gerits et al., 2014), but considerable lipid changes were observed in millet in terms of oxidation and lipolytic activity during storage (Lai and Varriano-Marston, 1980). The enzyme activities of microbiota during fermentation and inside cereals could explain the discrepancy in protein and fat

Table 2. Chemical characterization of the 3 freeze-dried boza samples and their caloric values.

	MaW Mi				MaW				Mi			
	0 h	16 h	1 week	2 weeks	0 h	16 h	1 week	2 weeks	0 h	16 h	1 week	2 weeks
Energy (kcal/100 g)	373.42	-	-	364.78	373.42	-	-	364.78	373.42	-	-	364.78
Carbohydrate	89.66	-	-	87.5	87.08	-	-	79.82	86.49	-	-	79.15
Moisture (g/100 g)	4.71	-	-	4.87	4.67	-	-	5.41	5.2	-	-	5.42
Ash (g/100 g)	0.35	-	-	0.37	0.5	-	-	0.53	0.38	-	-	0.38
Protein (g/100 g, N × 6.25)	1.75	-	-	1.94	2.38	-	-	2.31	3.94	-	-	3.5
Total fat (g/100 g)	0.85	-	-	0.78	0.94	-	-	0.86	0.52	-	-	0.56
Moisture (boza 100 mL)	76.75	-	-	-	79.98	-	-	-	83.40	-	-	-
Vitamins (per 100 g)												
Thiamine, B ₁ (µg)	2.5	4	14.5	2	10	20.5	18	26	22	19.5	16.5	28
Riboflavin, B ₂ (µg)	28.5	33.5	35	47	53.5	47.5	62	67.5	41.5	37	47.5	43.5
Niacin, B ₃ (mg)	600	700	1000	600	1100	800	1000	1000	1000	900	800	800
Pyridoxine, B ₆ (µg)	51	55.5	52.5	128	86.5	66	88	79	34	44.5	52.3	52
Cyanocobalamin, B ₁₂ (µg)	0.041	0.032	0.052	0.067	0.034	0.049	0.052	0.047	0.032	0.066	0.093	0.173
Menaquinones, K ₂ (µg)	1.68	1.46	2.33	1.99	1.1	2.05	2	2.66	1.7	1.63	1.49	5.5
Minerals (mg/kg)												
Cu	<0.11	-	-	-	<0.11	-	-	-	<0.11	-	-	-
Ca	77.6	-	-	-	136	-	-	-	198	-	-	-
Na	146	-	-	-	454	-	-	-	144	-	-	-
Se	0.028	-	-	-	0,017	-	-	-	0.018	-	-	-
K	684	-	-	-	1090	-	-	-	870	-	-	-
Mg	193	-	-	-	255	-	-	-	176	-	-	-
Zn	14.3	-	-	-	13.	-	-	-	14.2	-	-	-
Mn	1.36	-	-	-	3.75	-	-	-	2.86	-	-	-
P	1352	-	-	-	1278	-	-	-	1400	-	-	-
Fe	1.891	-	-	-	1.864	-	-	-	1.341	-	-	-
Sugars (g/100)												
Total sugars	77.20	73.94	66.46	66.46	66.46	64.02	55.26	50	59.66	53.03	44.87	44.87
Dietary fiber	2.52	2.41	4.28	4.68	4.11	5.32	9.51	11.37	3.56	3.94	11.84	10.9
Raw cellulose	0.16	0.58	0.44	0.54	0.6	0.58	0.7	0.46	0.58	0.36	0.8	0.36
Fructose	0.15	0.91	2.78	2.88	0.11	2.36	7,8	8.82	0.06	2.31	9.02	11.31
Glucose	0.34	0.75	6.18	7.25	0.11	0.57	2.76	5.47	0.19	0.93	4.37	7.01
Saccharose	72.91	70.89	65.21	62.91	65.78	64.07	47.55	37.40	58.02	50.82	33.26	26.69
Maltose	ND*	ND	ND	ND	ND	ND	2.59	3.87	ND	ND	2.22	2.74
Alcohol												
Alcohol (mg/kg)	10.1	47.8	449.9	257.3	30.6	79	99.5	ND	88.2	332.5	272.5	423.6
Organic acids (mg/100 g)												
Lactic acid	11.19	163.56	965.62	1156.71	42.03	675.09	1274.08	1508.70	65.04	930.96	1872.73	2635.45
Acetic acid	50.02	146.51	524.57	504.06	88.51	116.12	306.17	394.41	353.36	463.24	490.61	711.36
Formic acid	17.92	12.69	44.61	58.67	66.00	76.19	66.03	14.65	38.38	43.84	52.73	16.50
Malic acid	43.71	15.71	31.90	23.09	29.55	ND	ND	ND	48.49	ND	ND	ND
Citric acid	11.94	7.52	10.82	53.54	41.16	ND	ND	ND	11.94	ND	ND	ND
Fumaric acid	1.00	0.14	ND	ND	2.66	ND	ND	ND	7.14	0.39	ND	ND

*ND: Not detected.

values in boza samples. On the other hand, both MaW and Mi were determined to have higher dietary fiber contents as compared to MaWMi despite its multiple cereal contents. This divergence might be due to the different composition ratio, dietary fiber content of cereals (maize 2%, millet 8.5%, wheat 12.2%), and microbial enzyme activity in boza types. Saccharose fermentation is initiated by its cleavage with sucrose hydrolase enzyme to glucose and fructose. Consequently, during the storage period the simple sugar content in all boza samples increased sharply, while the saccharose concentration in MaW and Mi reduced by almost 50%. Moreover, dietary fiber contents increased two or three times during the 2-week storage period. Dietary fibers are water-soluble or swelling viscous oligo- and polysaccharides, including resistant starch, arabinoxylan, inulin, pectin, dextrin, and beta-glucans. Increase in dietary fibers can be explained with the increased ratios of components such as resistant starch, beta-glucans, and glucose. Dietary fibers are also important due to their prebiotic effects, provoking the growth of lactic bacteria in the colon (Petrova and Petrov, 2017). The alcohol content of all boza samples was low, being in conformity with the Turkish Food Codex, due to the low levels of yeast populations compared to the competitive lactic acid bacteria. The variation and number of yeast species in the three boza samples play an essential role in their alcohol contents.

3.3. Changes in vitamin contents

The vitamin content of boza mainly originates from its raw materials; however, certain LAB strains are known to have the capability to synthesize vitamins such as water-soluble B-group (folates, riboflavin, thiamin, cyanocobalamin) and fat-soluble K₂ (menaquinones) vitamins (Saulnier et al., 2009; Masuda et al., 2012; Da Silva et al., 2016). Similarly, yeasts are rich sources of vitamin and interestingly all yeasts are able to synthesize riboflavin and folic acid (Halász and Lásztity, 1991). Microbial community members in boza may also have the ability to synthesize vitamins, but they require and consume several vitamins to grow (van Niel and Hahn-Haègerdal, 1999). Vitamin requirements of LAB can be classified into three categories: essential, stimulatory, and nonessential vitamins. Shortages of these groups of vitamins in fermentation media may lead to 67%, 34% to 66%, and up to 67% reduction in microbial growth, respectively. A severe inhibition of *L. plantarum* growth was reported with the reduced concentration of riboflavin (B₂) in the medium by Wegkamp et al. (2009). Thiamine and biotin are also defined as stimulatory factors for the growth of some LAB strains. On the other hand, yeasts need a number of vitamins (biotin, folic acid, inositol, niacin, pantothenate, pyridoxine, riboflavin, and thiamin) to be able to grow (Reed, 2012). Data in the literature in terms of production and consumption of vitamins by LAB and yeasts during fermentation may explain the fluctuations

in the vitamin content of boza samples. Nevertheless, there is a trend of increase in vitamin contents during the fermentation and 2-week storage period (Table 2). Overall, boza samples were found to contain mainly B-group vitamins in diverse concentrations. Among the boza samples, in terms of B-group vitamin contents, MaW was found to be superior to the others, while K₂ in Mi and B₆ in MaWMi reached remarkably higher values after 2 weeks of storage. Our analytical data demonstrated that 100 g of lyophilized boza may provide the following vitamins: 0.1%–1.9% of B₁, 1.7%–4.0% of B₂, 3%–5.5% of B₃, 1.7%–6.4% of B₆, 0.5%–2.9% of B₁₂, and 1.4%–6.9% of K₂, depending on the cereal compositions and storage periods, according to the recommended daily allowance values issued by the FDA.

3.4. Mineral contents of boza types

Among the minerals, MaW was found to be rich in sodium, potassium, and manganese, and Mi in calcium, while somewhat higher contents of iron and selenium were determined in MaWMi (Table 2). Since the mineral contents of boza directly originate from its cereal source, fermentation processes would not be expected to influence this content. However, the mineral presence in boza would play a key role in the growth and metabolic activity of microbiota and further be involved in optimization and control of the enzymatic activity. For example, *Lc. mesenteroides* in boza cannot grow in the absence of metal ions and Mn²⁺ is stimulatory for its growth. Mg²⁺, Mn²⁺, Na⁺, K⁺, and Cl⁻ were found to be essential components in minimal growth media for *L. plantarum* (Hayek and Ibrahim, 2013). On the other hand, absorption of the minerals is hindered by phytic acid in the cereals (de Valdez et al., 2010). However, during boza fermentation, phytic acid is catalyzed by the activation of phytase enzyme in LAB and consequently this would cause and upsurge of mineral absorption. Overall, 100 g of lyophilized boza may supply the official daily intake levels: for manganese 6.8%–28.8%, for phosphate 12.8%–14.0%, for iron 7.5%–10.4%, and for zinc 8.8%–9.5%. Eventually, boza might be useful to support iron and zinc in the case of deficiency.

3.5. Changes in organic acid compositions

Organic acids are known to contribute to the aroma and taste of boza. As shown in Table 2, somewhat different organic acid profiles were observed for the boza samples. In all samples no propionic and quinic acids were detected, while lactic, acetic, and formic acids were in lower ratios. Differently from MaW and Mi, MaWMi was found to contain citric and malic acids. Under suitable conditions certain yeast species are able to synthesize citric acid by using carbohydrate sources like starchy substrates in boza (Yalçın et al., 2010). Rapid citric acid consumptions were observed in MaW and Mi, while an increase was seen in MaWMi at 2 weeks of storage, possibly due to the citric acid production of *Candida colliculosa*, a member

of its microorganism consortium. Angumeenal and Venkappayya (2013) stated that *Candida* species are ideal candidates for the biosynthesis of citric acid. On the other hand, in all 3 boza types a remarkable reduction was detected in malic acid concentrations during the fermentation and storage stages, possibly due to consumption of malic acid by *Leuconostoc* species and *L. plantarum*. A similar observation was also reported by Xiong et al. (2014) during Chinese sauerkraut fermentation. Citric acid is first converted to acetic acid and oxaloacetate; the latter is then decarboxylated to pyruvate. During fermentation, LAB and yeasts concomitantly utilize citric acid and other organic acids (Yalçın et al., 2010). Citric acid levels in the boza samples were probably sufficient to stimulate acetic acid biosynthesis by bacteria. On the contrary, Akpınar-Bayızıt et al. (2010) determined citric acid only in boza prepared from rice.

Clinical investigations have revealed that lactic acid or acetic acid addition to the diet reduced the glycemic index and insulin level significantly (Östman et al., 2002). Although factors involved in such a process are yet unknown, the effect of boza on the blood sugar and insulin levels may be the subject of further detailed investigations in healthy and diabetic volunteers.

3.6. Exopolysaccharide production

Certain LAB are able to produce exopolysaccharide (EPS), either attached to the cell wall (capsular EPS) or released into the extracellular environment (Torino et al., 2015). LAB can synthesize cell-wall structural polysaccharides such as peptidoglycan and lipoteichoic acids and exocellular polymers. The latter include both capsular polysaccharides, covalently bound to the cell surface, and EPS, which may form a loosely bound layer that can be secreted into the environment (Chapot-Chartier et al., 2011) and can easily be observed on agar plates. Several cereal-based fermented foods, such as tarhana powder and sourdough, are also rich sources of dextran-like polymer-producing LAB strains. It has been observed that EPS production is strain-dependent and is strongly affected by the processing conditions of fermented beverages, such as composition, pH, temperature, or fermentation time (Torino et al., 2005). *Lc. mesenteroides* and *Lc. pseudomesenteroides* are the most widely found LAB in boza types (Arıcı and Dağlıoğlu, 2002). They mostly can produce dextran, a very linear polysaccharide, containing α -1,6 linkages (95%), by dextransucrase (Monsan et al., 2001). The straight chain consists of α -1,6 glycosidic linkages between glucose molecules, while branches begin from α -1,3 linkages. The ability to synthesize exopolysaccharides from sucrose was screened on MRS-sucrose agar among LAB strains exhibiting a ropy/mucoid colony character recovered from 3 boza types. *L. plantarum*, *L. paracasei* subsp. *paracasei*, *Lc. lactis*, and *Lc. pseudomesenteroides* in Mi and *L. plantarum*, *Lc. citreum*, *Lc. pseudomesenteroides*, and *Lc.*

lactis in MaWMi and MaW were found to produce cell-bound capsular and cell-released polysaccharides. The differences between these two types of polysaccharides were obvious from the volume of the material surrounding the colonies (Chapot-Chartier et al., 2011). Cell-released polysaccharides were spread on the whole surface of the petri dishes. In Table 3A, EPS-producing LAB isolated from boza types are listed, either attached to the cell wall (capsular EPS) or released into the extracellular environment (EPS).

3.7. Changes in starch and resistant starch contents

From the view point of the cereal content and the production process, boza is a suitable milieu for fermentation. Fermentation starts just after the cooling of the cereal mush and continues with saccharose addition. Then simple sugars are accumulated through enzymatic hydrolysis of both saccharose and cereal starch that are utilized by the LAB and yeasts during the fermentation. Some LAB hydrolyze the starch first into dextrans through extracellular amylase enzyme and then to maltose. Maltose was detected only after 1 week of fermentation in MaW and Mi and increased slightly after a 2-week storage period. Microbiota of all boza samples were found to be composed of LAB consuming maltose. In MaWMi, starch hydrolysis probably took place slowly and the maltose thus formed was rapidly consumed by the microbiota (Petrova et al., 2013). The study reported by Turpin et al. (2011) demonstrated that the type strain of *Lc. mesenteroides* subsp. *mesenteroides* ATCC 8293 lacks genes encoding amylases. It synthesized only oligo-1,6-glucosidase, able to degrade short chains of dextrans. Confirming this finding, none of the *Leuconostoc* strains isolated from the 3 boza types in the present study degraded the sole source of starch. Maltose produced in MaW and Mi might have been due to the rapid degradation of starch by the yeast population after its concentration increased during the 1-week storage period. As shown in Table 3B, a high rate of starch utilization was observed by the yeasts in Mi, particularly during the fermentation (16 h) and storage (2 weeks) periods.

Changes in the starch content of boza types during the production and storage steps are shown in the Figure. All 3 boza types were mainly composed of nonresistant starch, ranging between 8.8% and 26.8%, while the resistant starch ratios were low. The starch content was found to be highest in Mi. Although the starch content of MaW and Mi did not fluctuate significantly from the initial step during the production and storage conditions, its ratio reduced sharply in MaWMi. This might be possibly due to the production technique or the activity of its yeast population. On the other hand, the ratio of resistant starch in MaW and Mi increased during the storage conditions. This point was also stated by Bird et al. (2000). Resistant starch serves as a carbon source for the growth of intestinal microbiota

Table 3. Biosynthesis capability of LAB and yeast strains isolated from boza samples.

Storage period	MaWMi	MaW	Mi
A. A- Exopolysaccharide-producing LAB (MRS-sucrose agar)			
0 h	—*	<i>Leuconostoc pseudomesenteroides</i>	<i>Leuconostoc pseudomesenteroides</i>
16 h	<i>Leuconostoc lactis</i>	<i>Leuconostoc lactis</i>	<i>Lactobacillus paracasei</i> subsp. <i>paracasei</i>
1 week	<i>Leuconostoc citreum</i>	<i>Leuconostoc plantarum</i>	<i>Lactococcus lactis</i> <i>Lactobacillus plantarum</i>
2 weeks	<i>Leuconostoc pseudomesenteroides</i>	<i>Leuconostoc lactis</i>	—
B. B- Starch utilization rates of yeast strains (API ID 32C kit)			
16 h	<i>Pichia fermentans</i> (+) <i>Candida colliculosa</i> (+)	—*	<i>Geotrichum</i> sp. (+++) <i>Trichosporon</i> sp. (+++)
1 week	<i>Pichia fermentans</i> (+) <i>Candida colliculosa</i> (+)	<i>Pichia fermentans</i> (+) <i>Geotrichum</i> sp. (+) <i>Galactomyces geotrichum</i> (+++)	<i>Geotrichum</i> sp. (+) <i>Pichia fermentans</i> (+) <i>Trichosporon</i> sp. (+++)
2 weeks	<i>Pichia fermentans</i> (+) <i>Geotrichum</i> sp. (+)	<i>Pichia fermentans</i> (+) <i>Geotrichum</i> sp. (+) <i>Galactomyces geotrichum</i> (+)	<i>Geotrichum</i> sp. (+) <i>Pichia fermentans</i> (+) <i>Trichosporon</i> sp. (+++)

* No exopolysaccharide production or starch utilizing capacity was determined.
(+) Low starch utilization rate, (+++) high starch utilization rate.

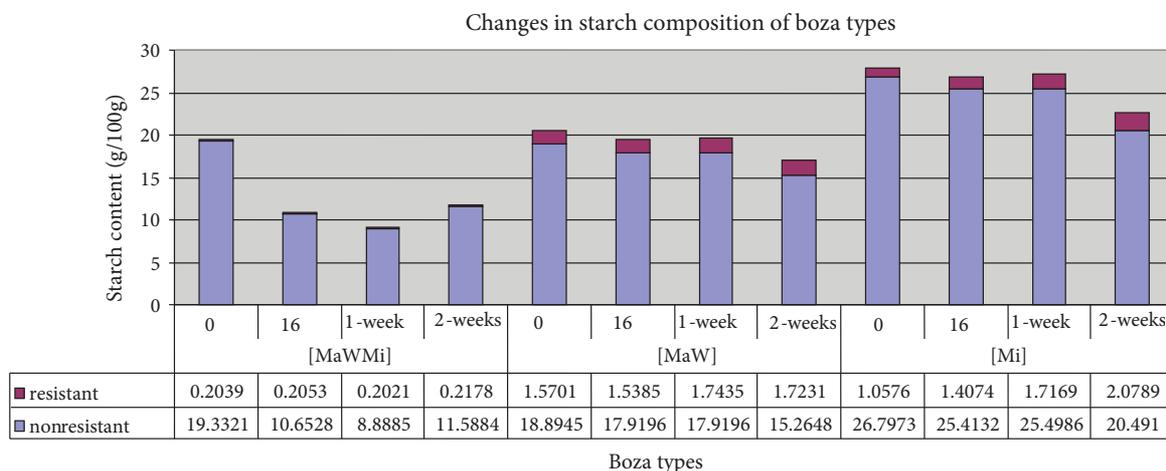


Figure. Changes in starch content of boza types during fermentation progress.

to produce short-chain fatty acids. These metabolites help to lower the intestinal pH and thus inhibit growth of pH-sensitive pathogens, increasing intestinal tonus and food absorption (Bird et al., 2000).

3.8. Conclusions

In this comprehensive study 3 different boza samples obtained from various industrial boza producers from diverse locations in Turkey were comparatively investigated. Results have demonstrated that composition of microbial consortia and types of raw materials utilized in boza production would have remarkable impacts on

its physicochemical characteristics, nutritive value, and possible beneficial properties during the processing steps and 2 weeks of storage. Further studies are needed to elucidate the role of individual microorganisms in the consortia with regards to their beneficial metabolites, probiotic properties, and effects on the nutritive components of boza.

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