Journal of Food Safety and Food Quality 72, Heft 5 (2021), Seiten 139–176



Arch Lebensmittelhyg 72,
158–163 (2021)
DOI 10.2376/0003-925X-72-158


ISSN 0003-925X

Korrespondenzadresse:
tgumus@nku.edu.tr

Tekirdağ Namık Kemal University, Agricultural Faculty, Department of Food Engineering, Tekirdağ, Turkey

# Isolation and identification of lactic acid bacteria from boza produced traditionally in the thrace region of Turkey

## *Isolierung und Identifizierung von Milchsäurebakterien aus Boza, die traditionell in der Region Thrakien der Türkei hergestellt wurden*

Melda Yağmur Tortum, Tuncay Gümüş, Deniz Damla Altan Kamer

**Summary**

Boza, a traditional beverage, has a microflora rich in lactic acid bacteria and yeast. In this study, 112 lactic acid bacteria (LAB) isolates from five different boza brands (samples A–E) produced in the Trakya region of Turkey were identified by a Polimerase Chain Reaction (PCR) based method. In addition, the samples were examined for microbiological properties. The most common bacterial species and their distribution in the five boza samples were as follows: *Lactobacillus fermentum* (25%), *Lactobacillus plantarum* (25%) in sample A, *L. plantarum* (100%) in sample B, *L. plantarum* (16.67%), in sample C, *Lactobacillus pentosus* (15%), *Lactobacillus brevis* (25%), *Leuconostoc lactis* (25%), *Leuconostoc citreum* (25%), *Lactobacillus paracasei* (10%) in sample D and *L. brevis* (9.09%), *Ln. citreum* (27.27%), *L. plantarum* (9.09%), *Lactococcus lactis* (47.22%), *Micrococcus yunnanensis* (9.09%) in sample E. Especially, Boza samples from the Velimeşe district (samples D and E) showed higher number of varieties of LAB compared to the other boza samples. However, determination of the technological properties of the identified microorganisms and production with a standard starter culture may be needed for standardization of boza production.

**Keywords:** Velimese Boza, Lactic acid bacteria, PCR

## Introduction

Due to its fat, protein, carbohydrate, fiber, vitamin, amino acid and lactic acid content, boza is a healthy and nutritious beverage with good sensory properties (Arici and Daglioglu, 2002). Boza is thought to have originated over 8000–9000 years ago in Mesopotamia (Caputo et al., 2012). It is generally accepted that boza was discovered and produced in Asia and spread to other countries as a result of migration (Uylaser, 1998). Todorov et al. (2008), stated that boza production methods differed by region, and was particularly popular in Balkan countries. According to the Turkish Standards Institute, for the preparation of boza, cereals like millet, rice, wheat, corn etc. are picked clean and crushed. Next, one or more of the flours generated from these cereals are mixed with potable water and cooked with white sugar. The mixture is then subjected to alcohol and lactic acid fermentation prior to consumption (Anonymous, 1992). Botes et al., (2007) reported that it was possible to develop a starter culture with LAB and yeast for the generation of boza; moreover, specific probiotic species found in the starter culture were effective against several different human pathogens via the production of bacteroicins. Morea, (2008) reported that probiotic LAB that are enriched during the production and storage of boza inhibited pathogenic bacteria such as *Escherichia coli, Pseudomonas auruginosa* and *Enterecoccus faecelis* through the increased production of lactic acid and acetic acid.

These studies highlighted the importance of accurately defining the starter culture for the production of boza. The fermentation process that widely involves the use of LAB, is a natural process to preserve and maintain raw materials for longer periods (Douillard and de Vos, 2014). In addition, the World Health Organization recommends the consumption of probiotics such as boza that provide live microorganisms in the diet.

LAB and yeast strains that are effective in the fermentation of boza have been reported to be homofermentative and heterofermentative (Altay et al., 2013). Several studies have reported specific species that form the microflora of boza. In general, most of the isolated bacteria belong to the genus *Leuconostoc, Lactobacillus* and *Lactococcus,* which are members of LAB (Pamir 1961, Hancıoğlu and Karapınar, 1997, Gotcheva et al., 2000, Kabadjova et al., 2000, Zorba et al., 2003, Todorov and Dicks, 2006). Numerous subspecies belonging to the genus *Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Weissella* are also genera of LAB have been isolated from boza. Careful selection of characteristics of the microbial species and their use in controlled fermentation, industrial applications, probiotics, or as potential starter culture may improve the quality attributes of fermented cereals and affect the stability, safety and overall quality of the product (Manini et al., 2016; Ogunraremi, Banwo, and Sanni, 2017). LAB strains with probiotic properties are suitable to enhance the quality and production of indigenous fermented foods with beneficial health effects since these strains are generally regarded as safe (GRAS) (Adesulu-Duhunsi et al., 2017).

As an energy dense beverage, boza is primarily consumed during the winter months and is widely produced in the Thrace region of Turkey (Anonymous, 2017). There is limited statistical data on Boza production in Turkey. This is primarily because apart from a handful of companies, most of the boza is made by small family-type businesses or by families for the direct consumption of the household. Although product optimization via the use of specific starter cultures has been successfully applied to other fermented foods including cheese, pickles, yoghurt and beer, to the best of our knowledge there are no systematic studies related to starter culture improvement for the production of boza (Zorba et al., 2003).

During the production of boza, the fermentation technique requires the use of a starter culture taken from the previous batch. However, Velimeşe boza is produced by a natural fermentation method without the addition of a starter culture. The properties of the boza therefore can change because of variations in raw material and seasonal changes. It is very important that the rich microflora of Velimeşe boza is standardized to ensure that the boza is continuously presented to the market with the same taste and quality. In the current study, LAB were isolated and identified from boza samples produced by 5 different companies by traditional methods in the Thrace region of Turkey with the aim to generate an optimal starter culture.

## Material and methods

### Material

Boza samples produced by five different companies in the Thrace region of Turkey were collected by random sampling. Two of these five samples (D and E) were boza samples produced in Velimeşe, a town in the Thrace region, by traditional methods. The production scheme of traditional Velimeşe boza is shown in Figure 1.

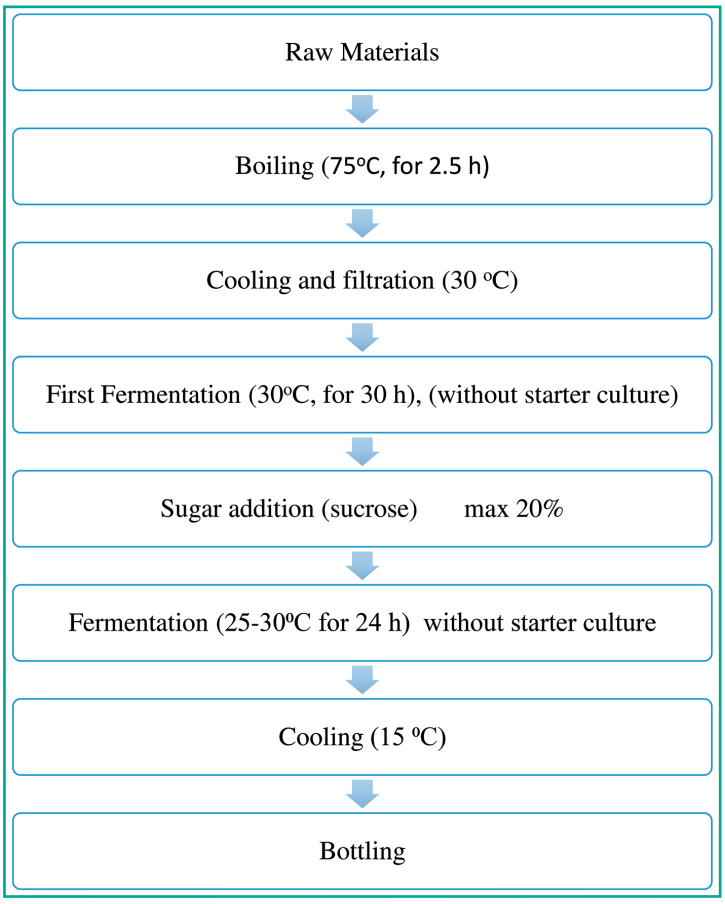


**FIGURE 1:** *Production stages for traditional Velimeşe boza.*

Journal of Food Safety and Food Quality 72, Heft 5 (2021), Seiten 139–176



## Methods

### *Microbial analyses*

Coliform count and yeast-mold count were carried out for the five boza samples as described by Marshall (1992). *Staphylococcus aureus* and coliform count was carried out as described by Ünlütürk and Turantaş (2002). Ten grams from each sample was weighed out aseptically, diluted 1:10 (w/v) with 90 mL of sterile peptone water and mixed by vortexing for 2 min. Serial dilutions were prepared by mixing 1 mL of the sample with 9 mL of sterile peptone water. The samples were incubated in Violet Red Bile Agar (VRBA, Merck, Darmstadt, Germany) at 35 ± 2°C for 48 h for coliform bacteria counts and in Baird Parker Agar (BPA, Merck) at 37°C for 48 h for *S. aureus* counts. Microbial counts were expressed as the number of viable bacterial colonies per gram (log CFU/g).

For the enrichment of *Salmonella* spp, 25 g of each sample was mixed with buffered peptone water (BPW, Merck, Darmstadt, Germany) at 37°C for 20 h and grown in Selenite Cystine Broth (SCB, Oxoid, Hampshire, UK) at 35°C for 24 h; following this, the cultures were streaked onto Bismuth Sulfite Agar (BSA, Oxoid, Hampshire, UK) and incubated at 35°C for 24 h. Typical *Salmonella* colonies were subjected to subsequent biochemical tests by using Triple Sugar Iron Agar (TSIA, Oxoid, Hampshire, UK) slants. Due to technical constraints, the presumptive *Salmonella* cultures from the agar slants could not be subjected to serological tests for final confirmation. Therefore, the survival of these bacteria was expressed as presence or absence of viable colonies (positive/negative), rather than as plate counts.

Yeast counts were determined on Potato Dextrose Agar (PDA, Oxoid, Hampshire, UK) (adjusted pH to 3.5 with 10% tartaric acid) and incubated at 25°C for 5 days. Yeast counts were expressed as the number of viable yeast colonies per gram (log CFU/g). All analyses were carried out in duplicate.

### *Isolation and identification of LAB*

For the isolation of LAB strains, 10 g of boza samples were taken aseptically and transferred to separate sterile bags. The samples were mixed with 90 mL of sterile saline solution (0.85%). Serial decimal dilutions were prepared and aliquots of these dilutions were plated on MRS agar (De Man, Rogosa and Sharpe, Darmstadt, Germany) and incubated at 30°C for 72 h under aerobic conditions. The samples plated on M17 agar plates were incubated at 30ºC for 48 h under anaerobic conditions (Collins and Layne, 1984). Colonies with typical characteristics were randomly selected from MRS and M17 agar plates and tested for Gram staining, cell morphology and catalase reaction. Gram-positive, catalase-negative isolates were considered as presumptive LAB. In total, 112 LAB isolated were further investigated for genotypic characterization.

### *DNA isolation from bacteria and amplification of the 16S rDNA region by PCR*

To identify the specific LAB strains isolated from boza samples, polymerase chain reaction (PCR) was conducted as described by Beasley and Saris (2004). Genomic DNA from bacteria was isolated from overnight (18h) cultures using a commercial DNA isolation kit according to the manufacturer's protocol (Genomic DNA Purification KIT, Fermentas, Vantaa, Finland) and used as a template for PCR amplification. General primers were used for the amplification and identification of bacteria was carried out by the 16S rDNA method. The sequences of the primers used are as follows: Universal primer pairs 16S forward (5-GCAAACAGGATTAGATAC CC-3) and 16S reverse (5'-AGGAGGTGATCCAACCGCA-3'), 16S forward 1492r (5'- CCCGGGATCCAGCTTTACCTTGTTAC-GACTT-3'), 16S reverse primer 27f (5'-CCGAATTC-GTCGACAACAGAGTTTGATCMTGGA-3'). fD1(5'-AGAGTTTGATCCCTGGCTCAG-3') and reverse primer rD1 (5'- CCGTCAATTCCTTTGAGTTT – 3') (Beasley and Saris 2004). The PCR mix (total volume of 50 µl) consisted of 2.5 µl Buffer (not containing $MgCl_2$), 0.5 µl (deoxynucleotide triphosphate) dNTP mix (dATP, dCTP, dGTP, dTTPs mixture prepared at a concentration of 200 µM), 0.5 µl 16S forward and 0.5 µl 16S reverse primers, 2 µl $MgCl_2$ and 0.5 µl Taq DNA polymerase enzyme, 1 µl DNA and (for the no template control, 1 µl of sterile water was used) and 17.5 µl of molecular biology grade water. The tubes were placed in a thermal cycler and PCR was carried out using the following program: 5 min initial denaturation at 94°C, 45 sec denaturation at 94°C, 1 min annealing at 53 °C, and 1 min extension at 72°C and this process was repeated 30 times. A final extension step was carried out at 72°C for 2 min (Blaiotta et al. 2002). The PCR products were separated with electrophoresis on 2% (wt/vol) agorose gels at 100 V, 325 mA for 60 min. Visualization of the bands was carried out after incubating the gel in 0.2 µg/ml of ethidium bromide for 30 min. The gels were imaged in a Kodak Gel Logic 200 Imaging System (Kodak, New York, NY, USA) under UV light at 366 nm wavelength (Macrina et al. 1982).

### *Purification of PCR products and DNA sequence analysis*

The PCR amplification products were purified from the agarose gel using a Qiagen kit (Cat. No. 28104) and DNA sequence analysis was carried out at the Namık Kemal University Scientific and Technological Research Application and Research Center (NABILTEM).

### *BLAST scanning*

Sequences obtained were interrogated by using Ribosomal Database and the identities of isolates were determined by aligning the sequences with the National Center for Biotechnological Information (NCBI) database (http://www.ncbi.nlm.nih.gov./BLAST/) using the BLAST algorithm. A similarity criterion of 97–100% and matching score of 0.97 or higher were considered acceptable (Ely and Chen, 2001; Altschul et al., 1997).

## Results and discussion

### Microbiological properties of boza samples

Yeast, coliform bacteria, *S. aureus* and LAB counts as well as data regarding the presence/absence of *Salmonella* in the five different boza samples are shown in Table 1. While LAB grown in MRS agar were found as minimum 7.71 and maximum 9.41, the number of LAB grown in M17 agar was found as minimum 7.61 and maximum 9.14 log CFU/g.

Coliform, *S. aureus* and *Salmonella* species remained below detection limits in all five boza samples. In addition, yeast species were found as minimum 6.69 and maximum 58.69 log CFU/g. According to the Turkish Boza Standards, a maximum 10 CFU/g coliform bacteria and 20 CFU/g of yeast are allowed in boza. In addition, *Salmonella, S. aureus* and fecal coliform bacteria should not be present in

**TABLE 1:** Microbiological analysis results of five different boza samples (log CFU/g).

| Samples | *S. auerus* | *Salmonella* spp. | Coliform bacteria | Yeast | LAB (MRS Agar) | LAB (M17 Agar) |
|---|---|---|---|---|---|---|
| A | –[a] | –[b] | – | 6.69±0.04 | 7.71±0.05 | 7.63±0.01 |
| B | – | – | – | 8.38±0.01 | 8.88±0.05 | 7.82±0.01 |
| C | – | – | – | 8.69±0.01 | 9.41±0.01 | 9,14±0.05 |
| D | – | – | – | 8.20±0.04 | 8.82±0.04 | 8.32±0.01 |
| E | – | – | – | 8.36±0.05 | 8.86±0.05 | 8.50±0.04 |

± Standard deviation, [a]: >10 ; [b]: not determined

boza. In the samples examined in the current study, the number of yeast samples exceeded the guidelines of this standard.

Genomic DNA from LAB isolated from five different boza samples was amplified by using PCR and primer pairs indicated in Materials and Methods and the products were visualized using a 2% agarose gel.

The most represented species is *Gluconocebacter liquefaciens* (50%) in sample C, and *Lc. lactis* (47.22%) in sample E, and least are represented the species *L. brevis, L. plantarum* and *M. yunnanensis* in sample E, 9.09% each (Table 2).

As shown in Table 2, *L plantarum, L. brevis, Ln. citreum, Ln. lactis* and *Lc. lactis* were found to be the dominant species. The presence of *L. plantarum* in the boza samples examined in the current study corroborate the results of Hancıoğlu and Karapınar (1997) and Gotcheva et al (2000). In addition, corroborating the data of Bayram (2005), *L. paracasei, Ln. citreum, Ln. lactis* and *Lc. lactis* were also detected in the current study. In addition to established probiotic properties of *L. plantarum, L. paracasei* and *L. fermentum* (Bayram, 2005), *L. plantarum* and *L. fermentum* reportedly have immune system regulating activities, while the presence of *Lactobacillus plantarum* can enhance the ω-3 fatty acids content of food during storage. These bacterial species may contribute towards biological protection by inhibiting the growth of pathogenic microorganisms. Additionally, these bacteria are known to protect the intestinal mucosa and contribute to the formation of short-chain fatty acids, which are used by the colon cells as an energy source (Bengmark, 1998; Tomasik and Tomasik, 2003).

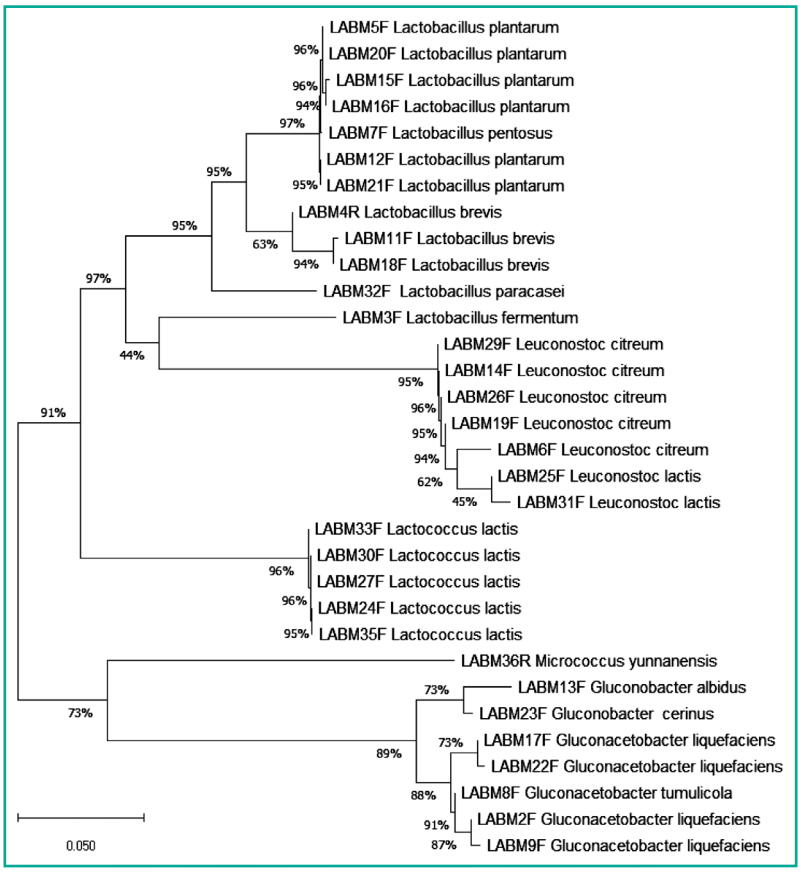


**FIGURE 2:** *Phylogenetic analysis of representative isolates based on 16S rRNA gene sequences.*

**TABLE 2:** Percent distribution according to the identification result of bacteria isolated from boza.

| Samples | Number of isolates | LAB | Similarity with NCBI | Percent (%) of isolates |
|---|---|---|---|---|
| A | 16 | *Gluconocetobacter liquefaciens* | 99% | 25 (4/16) |
| | | *L. fermentum* | 99% | 25 (4/16) |
| | | *Gluconocetobacter tumulicola* | 99% | 25 (4/16) |
| | | *L. plantarum* | 100% | 25 (4/16) |
| B | 10 | *L. plantarum* | 99% | 100 (10) |
| | | *Gluconocetobacter liquefaciens* | 99% | 50 (15/30) |
| C | 30 | *Gluconobacter albidus* | 99% | 16.67 (5/30) |
| | | *Gluconobacter cerinus* | 99% | 16.67 (5/30) |
| | | *L. plantarum* | 100% | 16.67 (5/30) |
| D | 20 | *Lactobacillus pentosus* | 99% | 15 (3/20) |
| | | *L. brevis* | 99% | 25 (5/20) |
| | | *Ln. lactis* | 99% | 25 (5/20) |
| | | *Ln. citreum* | 100% | 25 (5/20) |
| | | *Lactobacillus paracasei* | 99% | 10 (2/20) |
| E | 36 | *L. brevis* | 99% | 9.09 (3/36) |
| | | *L. citreum* | 100% | 27 (10/36) |
| | | *L. plantarum* | 99% | 9.09 (3/36) |
| | | *Lc. lactis* | 99% | 47.22 (17/36) |
| | | *Micrococcus yunnanensis* | 97% | 9.09 (3/36) |

Phylogenetic analysis of representative isolates based on 16S rRNA gene sequences is shown in Figure 2. The isolated strains LABM 3, 5, 7, 11,12, 15, 16, 18, 20, 32F and 4R were clustered with standard strains of *Lactobacillus* and while LABM 24, 27, 30, 33, and 35F were clustered with standard strains of *Lactococcus.* In addition, the isolated strains LABM 6, 14, 19, 25, 26, 29 and 31F were clustered with standards strains of *Leuconostoc.* Phylogenetic similarities were observed between these four isolate groups. The genera *Lactobacillus, Lactococcus* and *Leuconostoc* are usually found in boza, and to the best of our knowledge, the most frequently reported dominant LAB were the genus *Lactobacillus, Leuconostoc* and *Lactococcus.*

Hancıoğlu and Karapınar (1997) reported the presence and prevalence of *Leuconostoc* paramesenteroides (25.6%), *Lactobacillus sanfrancisco* (21.9%), *Leuconostoc mesenteroides* subsp. *mesenteroides* (18.6%), *Lactobacillus coryniformis* (9.1%), *Lactobacillus confusus* (7.8%), *Ln. mesen-*

Journal of Food Safety and Food Quality 72, Heft 5 (2021), Seiten 139–176



*teroides* subsp. *dextranicum* (7.3%), *L. fermentum* (6.5%) and *Leuconostoc oenos* (3.7%) from boza produced in Turkey. Gotcheva et al. (2000), reported the presence and prevalence of *L. plantarum* (24%), *Lactobacillus acidophilus* (23%) and *L. fermentum* as the dominant microflora in boza from Bulgaria. These authors reported lower prevalence of *Lactobacillus coprophilus* (11.0%), *L. brevis* (15.0%), *Ln. raffinolactis* (9.0%) and *Leuconostoc mesenteroides.* Bayram (2005) reported the presence and prevalence of *L. plantarum* (19.2%), *L. paracasei* (15.4%), *L. fermentum* (9.6%), *Ln mesenteroides* subsp. *mesenteroides/dextranicum* (9.6%), *Lc. lactis* (9.6%), *Ln. citreum* (7.7%), *Ln. lactis* (7.7%), *Lactobacillus delbrueckii* (7.7%), *Lactococcus raffinolactis* (5.8%), *Lactobacillus salivarus* (1.9%), *L. coprophilus* (1.9%), *L. brevis* (1.9%), *Pediococcus* spp. (1.9%) in shop bought boza from Turkey.

In addition to LAB isolated from boza, acetic acid bacteria such as *G. liquefaciens, G. tumulicola, G. albidus, G. cerinus* were also detected. These bacteria are normally Gram negative strains, but are known to be Gram variable in that some cells can be converted to Gram positive strains (Kadere et al., 2008). Gram-negative bacteria are surrounded by a thin peptidoglycan cell wall, which itself is surrounded by an outer membrane containing lipopolysaccharide. Gram-positive bacteria lack an outer membrane but are surrounded by much thicker layers of peptidoglycan compared to Gram-negatives (Roh et al., 2008). Acetic acid bacteria do not contribute to fermentation as they carry out oxidative metabolism; however, they can degrade ethanol to acetic acid by oxidation. Specific properties of vegetable, fruit, yeast, beer, wine and vinegar are known to be disrupted in the presence of these bacteria; in some cases these bacteria are added as fermentation inhibitory agents (Doleib, 2008). In our study, acetic acid bacteria were detected especially in the samples A and C. But, no acetic acid bacteria were detected in the traditionally produced Velimeşe boza (samples D and E).

Üner (2012) investigated the antifungal substances produced by LAB against yeast strains and reported that the strain with the highest antifungal feature was *L. pentosus* via the release of organic acids. *L. brevis* is known to be a GRAS, is a potential probiotic bacteria and is used in the fermentation of many foods. This strain was also reported to inhibit the growth of pathogenic bacteria such as *Escherichia coli, Staphylococcus aureus* and *Streptococcus thermophilus* (Rönka et al., 2003). Todorov et al., (2008) reported that *L. plantarum, L. paracasei, L. rhamnosus* and *L. pentotus* isolated from boza had probiotic properties; boza can therefore be considered as a functional food. A bacteriocin produced by *Lc. lactis* subsp. *lactis* known as nisin has been used commercially as a food preservative for many years due to its antimicrobial properties (Thomas and Delves 2005). The antimicrobial compounds produced by LAB are natural preservatives and could be used in formulations to increase the shelf-life and safety of minimally processed foods (Kıvanç et al., 2011).

## Conclusion

Because of probiotic, antimicrobial and, antioxidant properties of boza, it has positive effects on human health. The substantial differences in LAB content of the boza samples from the different regions may be attributed to differences in the geographic location of the raw materials and operational parameters. Boza from the Velimeşe district (samples D and E) showed more varieties of LAB compared to the other boza samples. LAB isolates are known to have both probiotic properties as well as bactericidal properties through the production of bacteriocin. This high diversity in the microflora of Velimeşe boza may have positive effects on human health and can be a source of novel LAB for further studies. In order to understand the fermentation of boza, it is necessary to identify the microflora and check the fermentation conditions. The implementation of carefully selected strains as starter cultures and/or co-cultures during fermentation processes provides better quality to the boza. Therefore, it is important to investigate of probiotic, anti-bacterisidal and, etc. features of the selected isolated strains from Boza.

## Conflict of interest

The authors declare that they have no conflict of interest.

## References

**Adesulu-Duhunsi AT, Sanni AI, Jeyeram K, Banwo K (2017):** Genetic diversity of *Lactabacillus plantarum* strains from some indigenous fermented foods in Nigeria. LWT-Food Sci Tech 82: 199–206.

**Altay F, Güler FK, Dikmen CD, Heperkan D (2013):** A review on traditional Turkish fermented non-alcoholic beverages: Microbiata, fermentation process and quality characteristics. Int J Food Microbiol 167: 44–56.

**Altschul SF, Madden TL, Schaffer AA, Zhang JZZ, Miller W, Lipman DJ (1997):** GappedBlastandPsi-Blast: a newgeneration of protein databasesearchprograms 3389–3402, Oxford University Press.

**Anonymous (1992):** Boza Standardı. T.S. 9778. Türk Standartları Enstitüsü, Necatibey Cad.112, Ankara. 6 s.

**Anonymous (2017):** Trakya Turizm Rotası (2017). Lezzet Rotası. http://trakyaturizmrotasi.com/wp-content/uploads/2016/11/TrakyaLezzet-Rotası.pdf. Erişim Tarihi: 10 Haziran 2017.

**Arici M, Daglioglu O (2002):** Boza: a lactic fermented cereal beverage as a traditional Turkish food. Food Rev Int 18: 39–48.

**Bayram B (2005):** Laktik Asit Bakterilerinin Sakkaroz Parçalama Aktivitelerinin Bozada İncelenmesi, Yüksek Lisans Tezi, İstanbul Teknik Üniversitesi, Fen Bilimleri Enstitüsü, İstanbul

**Beasley SS, Saris PEJ (2004):** Nisin-producing *Lactococcus lactis* strains from human milk. Appl Environ Microbiol 70: 5051–5053.

**Bengmark S (1998):** Immunonutrition: role of biosurfactans, fiber and probiotic bacteria. Nutr 14: 585–594.

**Blaiotta G Pepe O Mauriello G Villani F Andolfi R Moschetti G (2002):** 16S-23S rDNA İntergenic spacer region polymorphism of *Lactococcus garviae, Lactococcus raffinolactis* and *Lactococcus lactis* as revealed by PCR and nucleotide sequence analysis. Syst Appl Microbiol 25: 520–527.

**Botes A, Todorov SD, Mollendorff JW, Botha A, Dicks LMT (2007):** Identification of lactic acid bacteria and yeast from Boza. Process Biochem 42: 267–270.

**Chen J, Ely RL, (2001):** Comparison of artificialneural network, genetic programming and mechanistic modeling of complex biological processes. Environ Eng Sci 18: 267–278.

**Collins CH, Layne PM (1984):** Microbiological Methods. Butterworthand Co Ltd., 450 p, London.

**Caputo L, Quinteri L, Baruzzi F, Borcakli M, Morea M (2012):** Molecular and phenotypic characterization pf *Pichia fermentans* strains found among Boza yeasts. Food Res Int 48: 755–762.

**Doleib NH (2008):** Microbiology and Biochemistry of Duma: Fermented Honey Drink of Southern Sudan A Thesis Submitted to the University of Khartoum in Fulfillment of The Requirements for the Degree of Doctor of Philosophy (Ph.D.).

**Douillard FP, de Vos WM (2014):** Functional genomics of lactic acid bacteria: From food to health. Microbial Cell Factories, 13 (suppl 1), S8. doi.org/10.1186/1475-2859-13-S1-S8

**Gotcheva V, Pandiella SS, Angelov A, Roshkova ZG, Webb C (2000):** Microflora identification of the Bulgarian cereal-based fermented beverage Boza. Process Biochem 36: 127–130.

**Hancıoğlu Ö, Karapınar M (1997):** Microflora of boza, a traditional fermented Turkish beverage. Int J Food Microbiol 35: 271–274.

**Kabadjova P, Gotcheva I, Ivanova I, Dousset X (2000):** Investigation of bacteriocin activity of lactic acid bacteria isolated from boza. Biotec Biotech Equip 14: 56–59.

**Kadere TT, Miyomoto T, Oniong K, Kutima PM, Njoroge SM (2008):** Isolation and identification of the genera *Acetobacter* and *Gluconobacter* in coconut toddy (mnazi). Afr J Biotechnol 7: 2963–2971.

**Kıvanç M, Yılmaz M, Çakır E (2011):** Isolation and identification of lactic acid bacteria from boza, and their microbial activity against several reporter strains. Türk J Biol 35: 313–324.

**Macrina FL, Tobian JA, Jones KR, Evans RP, Clewell DB (1982):** A cloning vectorable to replicate in *Escherichia coli* and *Streptococcus sangius.* Gene 19: 345–353.

**Manini F, Casiraghi MC, Poutanen K, Brasca M, Erba D, Plumed-Ferrer C (2016):** Characterization of lactic acid bacteria isolated from wheat bran sourdough. LWT-Food Sci Technol 66: 275–283.

**Marshall RT (1992):** Standard Methods for the Exmination of Dairy Products. (16th ed.), American Public Health Association, Washington, DC.

**Morea M (2008):** Searcing for functional beverages produced in Eastern European contries. J Food Sci Nutr 4: 33–44.

**Ogunremi OR, Banwo K, Sanni AI (2017):** Starter-culture to improve the quality of cereal based fermented foods: Trends in selection and application. Curr Opin Food Sci 13: 38–43.

**Pamir H (1961):** Boza Üzerinde Mikrobiyolojik ve Kimyasal Araştırmalar. A.Ü. Ziraat Fakültesi Yayınları No:176. Çalışmalar No:109. A.Ü. Basımevi, Ankara. 60 s.

**Roh S, Nam Y, Chang H, Kim K, Kim M, Ryu J, Kim S, Lee W, Bee J (2008):** Phylogenic characterization of two novel commensal bacteria involved with innate immune homeostasis in *Drosophila melanogaster.* Appl Environ Microbiol 74: 6171–6177.

**Rönka E, Malinen E, Saarela M, Rinta-Koski M, Aarnikunnas J, Palva A (2003):** Probiotic and milk technological properties of *Lactobacillus brevis.* Int J Food Microbiol 83: 63–74.

**Thomas LV, Delves B (2005):** Nisin. In: Antimicrobials in Food. Davidson P.M. Sofos JN, Branen AL. (chief eds), Taylor & Francis Group, New York, pp 237–275.

**Todorov SD, Dicks LMT (2006):** Screening for bacteriocin-producing lactic acid bacteria from boza, a traditional cereal beverage from Bulgaria comparison of the bacteriocins. Process Biochem 41: 11–19.

**Todorov SD, Botes M, Guigas C, Schillinger U, Wiid I, Wachsman MB, Holzapfel WH, Dicks LMT (2008):** Boza, a natural source of probiotic lactic acid bacteria. J Appl Microbiol 104: 465–477.

**Tomasik PJ , Tomasik P (2003):** Probiotics and prebiotics, Cereal Chem 80: 113–117.

**Uylaşer V, Korukluoğlu M, Göçmen D, Şahin İ (1998):** Bursa'da Satışa Sunulan Bozaların Bileşimi ve Kalitelerinin Araştırılması. Gıda Mühendisliği Kongresi, 16–18 Eylül, Gaziantep, 135-130.

**Üner A (2012):** Laktik Asit Bakterileri Tarafından Üretilen Antimikrobiyal Maddelerin Gıda Patojeni Olan Mayalar Üzerine Etkisinin İncelenmesi, Yüksek Lisans Tezi, İstanbul Teknik Üniversitesi, Fen Bilimleri Enstitüsü, 2012.

**Ünlütürk A, Turantaş F (2002):** İndikatör ve Patojen Mikroorganizmaların Aranması ve Sayımı, Gıdaların Mikrobiyolojik Analizi (2. Baskı). Meta Basım Matbaacılık Hizmetleri Bornova-İZMİR

**Zorba M, Hancıoğlu Ö, Genç M, Karapınar M, Ova G (2003):** The use of starter cultures in the fermentation of boza, a traditional Turkish beverage. Process Biochem 38: 1405–1411.

**Address of corresponding author:**
Dr. Tuncay Gümüş
Food Engineering Department
Agriculture Faculty
Tekirdağ Namık Kemal University
TR-59030, Tekirdağ
Turkey
tgumus@nku.edu.tr