Extracellular phytase activities of lactic acid bacteria in sourdough mix prepared from traditionally produced boza as starter culture

Murat Doğan¹, İsmail Hakkı Tekiner²

Cite this article as:
Doğan, M., Tekiner İ.H. (2020). Extracellular phytase activities of lactic acid bacteria in sourdough mix prepared from traditionally produced Boza as starter culture. Food and Health, 6(2), 117-127. https://doi.org/10.3153/FH20013

ABSTRACT

Fermentation using Lactic Acid Bacteria (LAB) and LAB species can exhibit extracellular activities such as decreasing of antinutritional factors, in particular phytic acid (PA) or phytate. The objective of this study was to assess extracellular phytase activities of LAB in sourdough mix prepared from traditionally produced boza as starter culture. To do this, thirty-five boza samples were collected from Central Anatolia, Marmara and Eastern Anatolia regions in Turkey to be used as starter culture for preparing sourdough mix. In each mixture, LAB strains and phytase (+) ones were screened by culture-based examination, characterized by VITEK® MS, and extracellular phytase activity of each LAB strain was determined by spectrophotometry. Overall, 29 presumptive strains of LAB were isolated. Of them, 21 were found to be phytase (+). The average extracellular phytase activity was 656.8±188.1 U/mL, and a Pediococcus pentosaceus EK1 isolate showed the highest activity as 1285.5 U/mL. In conclusion, the traditionally produced bozas have been found as potential starter culture reservoirs for sourdough fermentation with significantly higher extracellular phytase activities, thus challenging opportunities to lower antinutritional factors, in particular phytic acid (PA) or phytate in the foods for the consumers.

Keywords: Boza, Fermentation, Health, Lactic Acid Bacteria, Phytic acid, Phytase, Sourdough
Introduction

Cereals and cereal-based products are a good source of phenolic compounds, lignans, phytosterols, phytic acid, fiber, vitamins, minerals and other biologically active compounds. However, they are rich in phytic acid (myo-Inositol (1, 2, 3, 4, 5, 6)-hexakisphosphate, InsP6) or salts, also known as phytates. Phytic acid (PA) is a naturally occurring compound found in all seeds and cells of plants. It accumulates up to seed ripening during development, and phosphorus is its main form of storage accounting for 60% of total phosphorus content in cereals, legumes, nuts and oil seeds (Lotti et al., 2000; Grases et al., 2017).

Many studies show that a diet based on foods with high phytate content may cause anemia and deficiencies in mineral absorption. Phytate levels can be reduced by phytases, which are the valuable enzymes by phytate hydrolysis. Phytate hydrolysis produces low myo-inositol phosphates by enzymatic degradation. This enzymatic degradation can be achieved by increasing activity of phytase, or adding phytase active microorganisms (Hurrell et al., 2003; Shi et al., 2004; Noobarie et al., 2015; Moll & Davis, 2017).

Traditional cereal-fermented products are widely consumed all over the world, in particular in Asia and Africa. For instance, boza is one of the well-known fermented cereal-based beverages. To make boza, a ground amount of different cereals such as millet, corn, rice, rye, oats, and wheat is cooked with water, and the mixture is allowed for fermentation by adding sugar. There exist diverse microorganisms in the boza occurring from raw materials, production process and storage conditions. On the other hand, the dominant microflora mainly include LAB (Osimani et al., 2015; Petrova & Petrov, 2017).

The food industries and scientific related areas are emphasizing the capacity of fermentation using LAB species to improve the nutritive quality of cereals and cereal-based foods by decreasing of some antinutritional factors such as PA or phytate, tannins and enzyme inhibitors. The activities of LAB species during cereal fermentation produce a broad range of metabolites and compounds, including organic acids, expopolysaccharides, antimicrobial compounds, and useful enzymes LAB species encoding phytases may be utilized as starter culture suitable for legume and cereal fermentations (Sumengen et al., 2013; Rollán, Gerez, & LeBlanc, 2019).

In this study, we aimed to assess extracellular phytase activities of LAB in sourdough mix prepared from traditionally produced boza as starter culture.

Materials and Methods

Collection of Boza Samples

During the year 2019, thirty-five traditionally produced boza samples were collected from the boza producers located in the Regions of Marmara (n=15), Central Anatolia (n=10) and Eastern Anatolia (n=10) in Turkey. All the collected samples were taken to the laboratory under sterile conditions at 4°C until further analysis.

Chemicals and Reagents

The chemicals and reagents used in this study were DeMan, Rogosa and Sharpe (MRS) agar (Merck 1.10660, Germany), MRS Broth (Merck 1.10661), M17 agar (1.15108 Merck) and M17 broth (Merck 1.15029) for cultural examination, pre-identification and storage of LAB strains from sourdoughs; crystal violet, safranin and lugol dyes for biochemical and morphological tests; physiological saline solution (PSS) (8.5 g NaCl dissolved in water, autoclaved 15 minutes at 121°C, and cooled to room temperature) for dilution, and 20% glycerol (Merck 10494) for store of culture, respectively. 0.1% sodium phytate (Sigma Aldrich 68388, Germany) and 0.2% glucose to MRS/M17 Broth medium (52.2 g/L) was used for phytase (+) LAB strains (Media with a pH of 6.2 sterilized at 121°C for 15 minutes at 1.2 atm. In the identification of phytase (+) LAB strains, Escherichia (E.) coli ATCC® 25922™ for positive testing control, and 1 μL alpha-cyano-4-hydroxycinnamic acid (CHCA) matrix solution for crystallization of the strain to be tested according to the instructions by VITEK® MS (bioMerieux, Marcy l’Etoile, France). Finally, 100 mM sodium acetate (Sigma Aldrich W302406) - acetic acid (Sigma Aldrich W200603) buffer, and 500 μl of 10% (w/v) trichloroacetic acid solution (TCA) (Sigma Aldrich T3399) for determination of extracellular phytase activities of the phytase (+) LAB. All the chemicals and reagents were prepared according to the Instructions by ISO 11133 (2014), Songré-Ouatattara et al. (2008), Raghavendra & Halami (2009), and Dubois et al. (2012).

Preparation of Sourdough

Ten grams of boza sample were initially mixed with 150 g of whole-wheat flour, 2 g of table salt and 350 mL of drinking water in a mixer for 5 minutes. Subsequently, the blend was allowed for fermentation at 35°C for 24 hours. At the end of the duration, 50 g of whole-wheat flour and 25 mL of drinking water more were added to the dough, the dough was kneaded for 1 minute, refreshed, and left to ferment again at...
35°C during 10 days. At every 24 hours, 50 g whole-wheat flour and 25 mL of drinking water were added to the dough, and the dough was kneaded for 1 minute as previously suggested by Menteş et al. (2007).

**Culture-Based Analysis and Isolation of LAB Strains**

The cultural examination of the suspected LAB strains were made according to the Instructions by ISO 11133 (2014) and ISO 6887-6 (2013). Ninety mL of PSS was added to 10 grams of the homogenized and fermented sample to prepare serial dilutions of $10^{-2}$ and $10^{-3}$, respectively. After that, 1 mL of the diluted suspension was transferred to MRS agar or M17 agar, allowed for incubation at 37°C for 24-48 hours (NÜVE EN-500, Ankara, Turkey). At the end of the incubation, suspected LAB colonies were examined morphologically under microscope. To ensure the purity of the suspected colonies, MRS and/or M17 were inoculated into broth tubes, and activated at 37°C for 24 hours under aerobic/anaerobic conditions. Then, the matte-cream colored colonies were evaluated as LAB strains. Dyeing was performed for pure cultures; Gram (+), cocci and rods were determined under the light microscope, and followed by the catalase test. Those negative for catalase test were selected.

**Detection and Enumeration of Phytase (+) LAB Strains**

To detect phytase (+) LAB strains, sodium phytate MRS/M17 broths were prepared to inoculate the suspected LAB strains with 200 μl active cultures. Then, the suspensions were allowed for incubation at 37°C for 24 hours (NÜVE EN-500, Ankara, Turkey). After incubation, 100 μl of the incubated culture were inoculated into MRS/M17 agar containing sodium phytate, and left for incubation at 37°C for 24 hours under aerobic/anaerobic conditions. Then, the matte-cream colored colonies were evaluated as LAB strains. Dyeing was performed for pure cultures; Gram (+), cocci and rods were determined under the light microscope, and followed by the catalase test. Those negative for catalase test were selected.

**Characterization of Phytase (+) LAB Strains Using MS**

The phytase (+) LAB strains were characterized using VI-TEK® MS according to the manufacturer's instructions. A reference strain of *E. coli ATCC® 25922™* was used for the positive test control (Dubois et al., 2012).

**Determination of Extracellular Phytase Activity**

One unit of phytase activity (U) is defined as the amount of enzyme producing one nmol of inorganic phosphorus per minute at 50°C. Phytase enzyme activity was calculated by incubating the sourdough mix prepared with 250 μl cell suspensions and 250 μl of 2 mM substrate in 100 mM sodium acetate-acetic acid buffer for 15 minutes at 50°C (NÜVE EN-500, Ankara, Turkey). A blind tube was prepared by adding 10% TCA solution before adding the substrate. Then, reaction was stopped by adding 500 μl of 10% (w/v) TCA. Finally, inorganic phosphate was calculated at 700 nm using iron sulfate-ammonium molybdate method by a UV-VIS spectrophotometer (Shimadzu UV-1280, Kyoto, Japan) (Raghavendra & Halami, 2009).

**Results and Discussion**

In this study, the extracellular phytase activity of the LAB strains isolated from the sourdough mix prepared from the traditionally produced boza samples as starter culture were assessed. Our study showed that 29 presumptive strains of LAB were isolated. Of them, 21 (1 *Enterococcus faecium*, 5 *Lactobacillus casei*, 1 *Lactobacillus fermentum*, 4 *Lactobacillus pentosus*, 3 *Leuconostoc lactis*, and 7 *Pediococcus pentosaceus*) were found to be phytase (+). The average extracellular phytase activity was 468.2 U/mL and 1285.5 U/mL, and a *Pediococcus (P.) pentosaceus* EK1 strain showed the highest activity as 1285.5 U/mL.

Sourdough has been produced since 3.000 BC by fermentation method. Since the 19th century, its use has decreased due to faster production and faster consumption habits, and replaced with commercial baker’s yeasts, i.e., *Saccharomyces (S.) cerevisiae*. However, the use of sourdough has started increasing in the recent years due to public interest in healthy eating and artisanal products. Sourdough is a specific ecosystem inhabited by mainly heterofermentative LAB species such as *L. fermentum, L. paralimentarius, L. plantarum*, and *L. sanfranciscensis* and yeasts. The diverse compositions of sourdough microbiota is affected by the diversity of fermentation processes. Sourdough has diverse contributions to the foods, such as improvement of nutritional properties, extension of shelf life, and enhancement of sensory characteristics (De Vuyst et al., 2014; Gänzle & Ripari, 2016; De Vuyst et al., 2017; Kourkouta et al., 2017; Papadimitriou et al., 2019; Catzeddu, 2019). In this study, we prepared the sourdough...
mix using the traditionally produced boza as starter culture, instead of utilizing a starter culture such as *S. cerevisiae*, or other sourdough food. This way of fermentation is one of the most widely preferred approaches to making fermented food. The distributions of the collected boza samples based on the geographical region in Turkey were 28.6% Central Anatolia, 42.9% Marmara, and 28.6% Eastern Anatolia.

Boza is one of the most well known cereal-based fermented drinks. Its pleasant taste, flavor, and nutritional value have made it a very popular beverage among the people of all ages. It is normally produced by fermentation involving mixed cultures of LAB and yeasts. However, LAB is always the basic microflora in the boza with an average LAB/yeasts ratio of 2.4 (Erkmen & Bozoğlu, 2016). Differences between the microflora of boza are related to production processes, storage temperature and period, and raw materials. The lactic acid fermentation is one of the two different simultaneously occurring types of fermentation in the boza production, which produces lactic acid, and determines the acidic character of this traditional beverage. Vast majority (96.3%) of the strains common in boza were the multiple LAB species (25.6% *Leuconostoc (L.) paramesenteroides*, 21.9% *L. sanfrancisco* and 18.6% *L. mesenteroides*) (Hancioglu & Karapinar, 1997; Petrova & Petrov, 2017; Irkin, 2019). On the other hand, *L. plantarum* (24%), *L. acidophilus* (23) and *L. fermentum* (19%) were dominant in the Bulgarian boza, whereas *L. plantarum* was the major species isolated from Turkish boza samples (Gotcheva et al., 2001; Kivanc et al., 2011; Lokumcu Altay et al., 2013). A recent study in Turkey by Borcaklı et al. (2018) showed that various LAB involving *Lactococcus lactis*, *leuconostoces* (*L. pseudomesenteroides, Lc. lactis, Lc. citreum*), and *Lactobacillus* spp. (*L. plantarum, L. paracasei, L. brevis, L. delbrueckii subsp. delbrueckii*) were identified as the common members of the microbial community in the boza samples (Borcaklı, Öztürk, & Yeşilada, 2018). In our study, initial cultural examination revealed 29 presumptive LAB strains (1 *E. faecium*, 11 *L. casei*, 1 *L. fermentum*, 6 *L. pentosus*, 3 *L. lactis* and 7 *P. pentosaceus*) from the fermented sourdoughs. Of them, 21 (1 *E. faecium*, 5 *L. casei*, 1 *L. fermentum*, 4 *L. pentosus*, 3 *L. lactis* and 7 *P. pentosaceus*) were found to be phytase (+), whereas 8 (6 *L. casei* and 2 *L. pentosus*) were phytase (-). Our results showed that multiple LAB strains were common in the sourdoughs, and similar to those previously conducted national and international works (Table 1 & Figure 1).

### Table 1. Results of phytase screening in culturally isolated presumptive LAB strains

| No | Name of strain | Result of phytase screening (n) | Origin of boza * |
|----|---------------|-------------------------------|-----------------|
|    |               | Phytase (+) | Phytase (-) |          |
| 1  | *E. faecium*  | 1     | 0    | CA  |
| 2  | *L. lactis*   | 3     | 0    | CA  |
| 3  | *P. pentosaceus* | 7 | 0 | CA, M, EA |
| 4  | *L. casei*    | 5     | 6    | M, EA |
| 5  | *L. fermentum* | 1 | 0 | M  |
| 6  | *L. pentosus* | 4     | 2    | M, EA |
|    | Total         | 21    | 8    |      |

*CA: Central Anatolia, M: Marmara, EA: Eastern Anatolia*

![Figure 1](https://example.com/image.png)

**Figure 1.** Distribution of phytase (+) and phytase (-) LAB strains based on the origins of the collected boza samples
Phytic acid is an antinutrient because of its ability to bind nutrients such as minerals and proteins, either directly or indirectly, and thus adversely affect their solubility, functionality, absorption, and digestibility (Damayanti et al., 2017). The organisms, including plants, microorganisms, and animal cells have the ability to synthesize phytases. Generally, fungi produce extracellular phytases, whereas bacteria produce the cell-associated enzymes mostly. In the literature, only bacteria exhibiting extracellular phytase activity are those of the genera Bacillus and Enterobacter. LAB were within the first bacteria to be evaluated because of their involvement in food fermentations and in the human health. However, not all LAB are linked to food fermentations (Papadimitriou et al., 2016). Phytases have gained great interest for biotechnological applications, in particular for the reduction of phytate content in feed and food (Konietzny & Greiner, 2004). Sumengen et al. (2013) studied phytase produced from L. plantarum isolated from a fermented food (Shalgam), and determined extracellular and intracellular enzyme activities of L. plantarum to be 984.50 U/mL and 494 U/g, respectively (Sumengen et al., 2013). Metabolism of sourdough microbiota and the activity of cereal enzymes are interdependent (Gänzle, 2014). According to Reale et al. (2007), the extent of phytate degradation is mostly independent from LAB strain used for fermentation, and phytate degradation during cereal dough fermentation is positively correlated with endogenous plant phytase activity. Lactic acid fermentation significantly decreases phytate content in plant-based foods. It is widely believed that this reduction is because of the activity of the intrinsic plant phytases, and LAB strains provide suitable conditions for the endogenous cereal phytases by lowering pH value in the medium. So far, only L. amylovorus and L. plantarum were reported to produce significant extracellular phytase activities. On the other hand, Reale et al. (2007) claims that if a wild-type LAB strain produces extracellular phytase activity, its production can be sufficient for the phytate dephosphorylation during fermentation (Reale et al., 2007). Similarly, Leenhardt et al. (2005) reported that a moderate drop of the dough pH (around 5.5) was sufficient to lower significantly the phytate content of a wholemeal flour (Leenhardt et al., 2005). However, a few strains of LAB have shown consistent phytase activity to degrade phytate by producing extracellular phytases (Anastasio et al., 2010). Therefore, there has been a growing interest in deriving alternate strategies of phytate utilization by probiotics in the human, as they are capable of producing phytase to combat mineral deficiency of zinc and iron (Priyodip, Prakash, & Balaji, 2017). During boza fermentation, phytic acid is catalyzed by the activation of phytase enzyme in LAB, resulting in cause and upsurge of mineral absorption (Borçakli, Öztürk, & Yeşilada, 2018). Zamudio et al. (2001) investigated the intracellular and extracellular phytase activities of six LAB (Ped. pentosaceus, Leuc. mesenteroides, Lact. casei, Lact. fermentum, Lact. delbrueckii and Lact. plantarum). There was no intracellular phytase activity, whereas L. plantarum showed the highest extracellular phytase activity (6.3 mU/mL) (Zamudio et al., 2001). Khodaii et al. (2013) reported that L. casei from dairy products exhibited higher phytase activity (> 0.004 U) than those isolates from pharmaceutical products (40% versus 27%) (Khodaii et al., 2013). Cizeikiene et al. (2015) showed that the highest extracellular phytase activity produces Pediococcus pentosaceus strains from rye sourdough with 32 to 54 U/mL, respectively, under conditions similar to leavening of bread dough (Cizeikiene et al., 2015). On the other hand, a study by Goswami et al. (2017) did not show phytases activity of the LAB strains in the extracellular medium. The specific activities of the studied lactobacilli against phytate varied from 0.03 U/mg to 0.43 U/mg proteins, being the lowest in L. fermentum and the highest in L. Plantarum (Goswami et al., 2017). In this study, we detected 21 phytase isolates out of 29 presumptive LAB strains in the prepared sourdoughs. At the end of 24 hours, the vitabilities of the phytase (+) isolates varied between 8.52 log cfu/g (P. pentosaceus EK1 from Marmara Region) and 3.60 log cfu/g (P. pentosaceus NB32 from Central Anatolia region). Phytase production of each strain was mainly determined by production of clear zones around the colonies on the sodium phytate containing medium (Sümengen, Dinçer, & Kaya, 2012). Phytase activity of each strain at the end of 24 hours were changed from 6 mm (P. pentosaceus EK1 from Marmara) down to 3 mm (L. casei strains from Marmara and Eastern Anatolia, and P. pentosaceus from Central Anatolia), respectively. Accordingly, the average extracellular phytase activity was found to be 656.8±188.1 U/mL, and a P. pentosaceus EK1 isolated from the sourdough prepared using the boza from Marmara region as starter culture showed the highest activity as 1285.5 U/mL among them, as similar to that reported by Cizeikiene et al. (2015) (Table 2 & Figure 2). Our results showed that the a LAB strain, P. pentosaceus EK1, isolated from sourdough mix prepared using traditionally produced boza from Marmara Region as starter culture yielded a performance of extracellular phytase activity better than the previously identical strains isolated from different sources of foods.
Table 2. Viability and phytase activities of LAB strains

| Isolate type / code | Origin* | V(log cfu/g)** | PA (mm)*** | EPA (U/mL)**** |
|---------------------|---------|---------------|------------|----------------|
| 1 E. faecium NB32A  | CA      | 4.30          | 5.93       | 3.5            | 4.4            | 548.2        |
| 2 L. casei B21      | M       | 3.54          | 5.69       | 3              | 4              | 44            | 594.6        |
| 3 L. casei B31A     | M       | 3.99          | 5.99       | 4              | 5              | 682.7        |
| 4 L. casei K11      | EA      | 4.41          | 5.51       | 3              | 3              | 44            | 487.3        |
| 5 L. casei K22      | EA      | 3.92          | 5.11       | 3              | 4              | 44            | 506.4        |
| 6 L. casei K32      | EA      | 3.72          | 5.97       | 3              | 4              | 44            | 635.3        |
| 7 L. fermentum B1A  | M       | 3.57          | 6.93       | 5              | 5.5            | 64            | 743.7        |
| 8 L. pentosus B1    | M       | 4.68          | 5.54       | 4.5            | 5              | 54            | 678.5        |
| 9 L. pentosus B31   | M       | 4.30          | 7.69       | 4              | 5              | 54            | 763.0        |
| 10 L. pentosus B33  | M       | 3.96          | 6.62       | 5              | 5.5            | 74            | 634.4        |
| 11 L. pentosus B33A | M       | 4.53          | 6.86       | 5              | 7              | 94            | 714.7        |
| 12 L. lactis B11    | CA      | 4.96          | 5.46       | 4              | 5              | 44            | 463.6        |
| 13 L. lactis B12    | CA      | 4.93          | 5.48       | 5              | 6              | 64            | 943.1        |
| 14 L. lactis B32    | CA      | 4.46          | 6.92       | 5.5            | 6              | 84            | 810.5        |
| 15 P. pentosaceus EK1 | M   | 3.80         | 8.52       | 6              | 7.5            | 11            | 1285.5       |
| 16 P. pentosaceus EK2 | M   | 3.94         | 5.40       | 3              | 3.5            | 44            | 559.4        |
| 17 P. pentosaceus EK3 | M   | 4.36         | 5.71       | 3              | 4              | 44            | 576.3        |
| 18 P. pentosaceus K33 | EA  | 3.89         | 4.90       | 4              | 4              | 54            | 603.6        |
| 19 P. pentosaceus NB1 | CA  | 4.46         | 5.98       | 3.5            | 4              | 44            | 497.6        |
| 20 P. pentosaceus NB32 | CA  | 3.56         | 3.60       | 3              | 3              | 44            | 521.7        |
| 21 P. pentosaceus NB34 | CA  | 4.81         | 6.00       | 4              | 4              | 44            | 532.8        |

*CA: Central Anatolia, M: Marmara, EA: Eastern Anatolia, **V: Viability, ***PA: Phytase Activity, ****EPA: Extracellular Phytase Activity
Figure 2. Extracellular phytase activity (EPA) of phytase (+) LAB isolates
Conclusion

In conclusion, the traditionally produced bozas have been found as potential starter culture reservoirs for sourdough fermentation with significantly higher extracellular phytase activities, thus challenging opportunities to lower antinutritional factors, in particular phytic acid (PA) or phytate in the foods for the consumers.

Compliance with Ethical Standard

Conflict of interests: The authors declare that for this article they have no actual, potential or perceived the conflict of interests.

References

Anastasio, M., Pepe, O., Cirillo, T., Palomba, S., Blaiotta, G., Villani, F. (2010). Selection and use of phytate-degrading LAB to improve cereal-based products by mineral solubilization during dough fermentation. Journal of Food Science, 75, M28-35. https://doi.org/10.1111/j.1750-3841.2009.01402.x

Bae, H. D., Yanke, L. J., Cheng, K. J., Selinger, L. B. (1999). A novel staining method for detecting phytase activity. Journal of Microbiological Methods, 39(1), 17-22. https://doi.org/10.1016/S0167-7012(99)00096-2

Borcaklı, M., Öztürk, T., Yeşilada, E. (2018). Cereal source and microbial consortia of the starter culture influence the chemical composition and physicochemical characteristics of boza. Turkish Journal of Agriculture and Forestry, 42, 412-422. https://doi.org/10.3906/tar-1802-3

Catzeddu, P. (2019). Sourdough breads. In Flour and breads and their fortification in health and disease prevention (pp. 177-188). Academic Press. https://doi.org/10.1016/B978-0-12-814639-2.00014-9

Cizeikiene, D., Juodeikiene, G., Bartkiene, E., Damasius, J., Paskevicius, A. (2015). Phytase activity of lactic acid bacteria and their impact on the solubility of minerals from wholemeal wheat bread. International Journal of Food Sciences and Nutrition, 66(7), 736-742. https://doi.org/10.3109/09637486.2015.1088939

Damayanti, E., Ratisiwi, F., Istiqomah, L., Sembiring, L., Febrisiantos, A. (2017). Phytate degrading activities of lactic acid bacteria isolated from traditional fermented food. AIP Conference Proceedings 1823, 020053. https://doi.org/10.1063/1.4978126

De Angelis, M., Gallo, G., Corbo, M. R., McSweeney, P. L., Faccia, M., Giovine, M., Gobbetti, M. (2003). Phytase activity in sourdough lactic acid bacteria: purification and characterization of a phytase from Lactobacillus sanfranciscensis CB1. International Journal of Food Microbiology, 87(3), 259-270. https://doi.org/10.1016/S0168-1605(03)00072-2

De Vuyst, L., Van Kerrebroeck, S., Harth, H., Huys, G., Daniel, H. M., Weckx, S. (2014). Microbial ecology of sourdough fermentations: diverse or uniform?. Food Microbiology, 37, 11-29. https://doi.org/10.1016/j.fm.2013.06.002

De Vuyst, L., Van Kerrebroeck, S., Leroy, F. (2017). Microbial ecology and process technology of sourdough fermentation. Advances in Applied Microbiology, 100, 49-160. https://doi.org/10.1016/bs.aambs.2017.02.003

Dubois, D., Grare, M., Prere, M. F., Segonds, C., Marty, N., Oswald, E. (2012). Performances of the Vitek MS matrix-assisted laser desorption ionization-time of flight mass spectrometry system for rapid identification of bacteria in routine clinical microbiology. Journal of Clinical Microbiology, 50(8), 2568-2576. https://doi.org/10.1128/JCM.00343-12

Erkmen, O., Bozoğlu, T. F. (2016). Food Microbiology: Principles into Practice. New Jersey: Wiley, p. 366, ISBN 9781119237761 https://doi.org/10.1002/9781119237860

Gänzle, M. G. (2014). Enzymatic and bacterial conversions during sourdough fermentation. Food Microbiology, 37, 2-10. https://doi.org/10.1016/j.fm.2013.04.007

Gänzle, M., Ripari, V. (2016). Composition and function of sourdough microbiota: From ecological theory to bread quality. International Journal of Food Microbiology, 239, 19-25.
Goswami, G., Bora, S. S., Parveen, A., Boro, R. C., Barooah, M. (2017). Identification and functional properties of dominant lactic acid bacteria isolated from Kahudi, a traditional rapeseed fermented food product of Assam, India. *Journal of Ethnic Foods*, 4(3), 187-197. https://doi.org/10.1016/j.jef.2017.08.008

Gotcheva, V., Pendiella, S.S., Angelov, A., Roshkova, Z., Webb, C. (2001). Monitoring the fermentation of the traditional Bulgarian beverage boza. *International Journal of Food Science and Technology*, 36, 129-134. https://doi.org/10.1046/j.1365-2621.2001.00429.x

Grases, F., Prieto, R. M., Costa-Bauza, A. (2017). Dietary phytate and interactions with mineral nutrients. In Clinical Aspects of Natural and Added Phosphorus in Foods (pp. 175-183). Springer, New York. https://doi.org/10.1007/978-1-4939-6566-3_12

Hancioglu, O., Karapinar, M. (1997). Microflora of Boza, a traditional fermented Turkish beverage. *International Journal of Food Microbiology*, 35, 271-274. https://doi.org/10.1016/S0168-1605(96)01230-5

Hurrell, R. F., Reddy, M. B., Juillerat, M. A., Cook, J. D. (2003). Degradation of phytic acid in cereal porridges improves iron absorption by human subjects. *The American Journal of Clinical Nutrition*, 77(5), 1213-1219. https://doi.org/10.1093/ajcn/77.5.1213

Irkin, R. (2019). Natural Fermented Beverages. In A. M. Grumezescu & A. M. Holban (Eds.), Natural Beverages (p. 399-425). Cambridge MA: Woodhead Publishing Elsevier. ISBN: 9780128166895 https://doi.org/10.1016/B978-0-12-816689-5.00014-6

ISO 6887-6. (2013). Microbiology of food and animal feed - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 6: Specific rules for the preparation of samples taken at the primary production stage.

ISO, E. 11133: 2014. Microbiology of food, animal feed and water—Preparation, production, storage and performance testing of culture media.

Karaman, K., Sagdic, O., Durak, M. Z. (2018). Use of phytase active yeasts and lactic acid bacteria isolated from sourdough in the production of whole wheat bread. *LWT-Food Science and Technology*, 91, 557-567. https://doi.org/10.1016/j.lwt.2018.01.055

Khodaii, Z., Natanzi, M., Naseri, M., Goudarzvand, M., Dodson, H., Snelling, A. (2013). Phytase activity of lactic acid bacteria isolated from dairy and pharmaceutical probiotic products. *International Journal of Enteric Pathogens*, 1, 12-16. https://doi.org/10.17795/ijep9359

Kivanc, M., Yilmaz, M., Cakir, E. (2011). Isolation and identification of lactic acid bacteria from boza, and their microbial activity against several reporter strains. *Turkish Journal of Biology*, 35, 313-324.

Konietzny, U., Greiner, R. (2004). Bacterial phytase: potential application, in vivo function and regulation of its synthesis. *Brazilian Journal of Microbiology*, 35(1), 11-18. https://doi.org/10.1590/S1517-83822004000100002

Kourkouta, L., Koukourikos, K., Iliadis, C., Ouzounakis, P., Monios, A., Tsaloglidou, A. (2017). Bread and health. *Journal of Pharmacy and Pharmacology*, 5, 821-826. https://doi.org/10.17265/2328-2150/2017.11.005

Leenhardt, F., Levrat-Verny, M.A., Chanliaud, E., Réméy, C. (2005). Moderate decrease of pH by sourdough fermentation is sufficient to reduce phytate content of whole wheat flour through endogenous phytase activity. *Journal of Agricultural and Food Chemistry*, 53(1), 98-102. https://doi.org/10.1021/jf049193q

Lokumcu Altay, F., Karbanciou-Guler, F., Daskaya Dikmen, C., Heperkan, Z.D. (2013). A review on traditional Turkish fermented non-alcoholic beverages: Microbiota, fermentation process and quality characteristics. *International Journal of Food Microbiology*, 167, 44-56. https://doi.org/10.1016/j.ijfoodmicro.2013.06.016

ISO 6887-6. (2013). Microbiology of food and animal feed - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 6: Specific rules for the preparation of samples taken at the primary production stage.

ISO, E. 11133: 2014. Microbiology of food, animal feed and water—Preparation, production, storage and performance testing of culture media.

Karaman, K., Sagdic, O., Durak, M. Z. (2018). Use of phytase active yeasts and lactic acid bacteria isolated from sourdough in the production of whole wheat bread. *LWT-Food Science and Technology*, 91, 557-567. https://doi.org/10.1016/j.lwt.2018.01.055

Khodaii, Z., Natanzi, M., Naseri, M., Goudarzvand, M., Dodson, H., Snelling, A. (2013). Phytase activity of lactic acid bacteria isolated from dairy and pharmaceutical probiotic products. *International Journal of Enteric Pathogens*, 1, 12-16. https://doi.org/10.17795/ijep9359

Kivanc, M., Yilmaz, M., Cakir, E. (2011). Isolation and identification of lactic acid bacteria from boza, and their microbial activity against several reporter strains. *Turkish Journal of Biology*, 35, 313-324.

Konietzny, U., Greiner, R. (2004). Bacterial phytase: potential application, in vivo function and regulation of its synthesis. *Brazilian Journal of Microbiology*, 35(1), 11-18. https://doi.org/10.1590/S1517-83822004000100002

Kourkouta, L., Koukourikos, K., Iliadis, C., Ouzounakis, P., Monios, A., Tsaloglidou, A. (2017). Bread and health. *Journal of Pharmacy and Pharmacology*, 5, 821-826. https://doi.org/10.17265/2328-2150/2017.11.005

Leenhardt, F., Levrat-Verny, M.A., Chanliaud, E., Réméy, C. (2005). Moderate decrease of pH by sourdough fermentation is sufficient to reduce phytate content of whole wheat flour through endogenous phytase activity. *Journal of Agricultural and Food Chemistry*, 53(1), 98-102. https://doi.org/10.1021/jf049193q

Lokumcu Altay, F., Karbanciou-Guler, F., Daskaya Dikmen, C., Heperkan, Z.D. (2013). A review on traditional Turkish fermented non-alcoholic beverages: Microbiota, fermentation process and quality characteristics. *International Journal of Food Microbiology*, 167, 44-56. https://doi.org/10.1016/j.ijfoodmicro.2013.06.016
Lopez, H. W., Ouvry, A., Bervas, E., Guy, C., Messager, A., Demigne, C., Remesy, C. (2000). Strains of lactic acid bacteria isolated from sour doughs degrade phytic acid and improve calcium and magnesium solubility from whole-wheat flour. *Journal of Agricultural and Food Chemistry*, 48(6), 2281-2285. https://doi.org/10.1021/jf000061g

Lott, J. N., Ockenden, I., Raboy, V., Batten, G.D. (2000). Phytic acid and phosphorus in crop seeds and fruits: a global estimate. *Seed Science Research*, 10(1), 11-33. https://doi.org/10.1017/S0960258500000039

Menteş, Ö., Ercan, R., Akçelik, M. (2007). Inhibitor activities of two Lactobacillus strains, isolated from sourdough, against rope-forming Bacillus strains. *Food Control*, 18(4), 359-363. https://doi.org/10.1016/j.foodcont.2005.10.020

Moll, R., Davis, B. (2017). Iron, vitamin B12 and folate. *Medicine*, 45(4), 198-203. https://doi.org/10.1016/j.mpmed.2017.01.007

Nuobariene, L., Cizeikiene, D., Gradzeviciute, E., Hansen, Å.S., Rasmussen, S.K., Juodeikiene, G., Vogensen, F. K. (2015). Phytase-active lactic acid bacteria from sourdoughs: Isolation and identification. *LWT-Food Science and Technology*, 63(1), 766-772. https://doi.org/10.1016/j.lwt.2015.03.018

Osimani, A., Garofalo, C., Aquilanti, L., Milanović, V., Clementi, F. (2015). Unpasteurised commercial boza as a source of microbial diversity. *International Journal of Food Microbiology*, 194, 62-70. https://doi.org/10.1016/j.ijfoodmicro.2014.11.011

Papadimitriou, K., Alegría, Á., Bron, P.A., de Angelis, M., Gobbetti, M., Kleerebezem, M., Lemos, J.A., Linares, D.M., Ross, P., Stanton, C., Turroni, F., van Sinderen, D., Varmanen, P., Ventura, M., Zúñiga, M., Tsakalidou, E., Kok, J. (2016). Stress physiology of lactic acid bacteria. *Microbiology and Molecular Biology Reviews: MMBR*, 80(3), 837-890. https://doi.org/10.1128/MMBR.00076-15

Papadimitriou, K., Zoumpopoulou, G., Georgalaki, M., Alexandraki, V., Kazou, M., Anastasiou, R., Tsakalidou, E. (2019). Sourdough Bread. In Innovations in Traditional Foods (pp. 127-158). Woodhead Publishing. https://doi.org/10.1016/B978-0-12-814887-7.00006-X

Petrova, P., Petrov, K. (2017). Traditional Cereal Beverage Boza Fermentation Technology, Microbial Content and Healthy Effects. In Fermented Foods, Part II (pp. 284-305). CRC Press. ISBN: 978-1-1386-3784-9

Priyodip, P., Prakash, P. Y., Balaji, S. (2017). Phytases of probiotic bacteria: Characteristics and beneficial aspects. *Indian Journal of Microbiology*, 57(2), 148-154. https://doi.org/10.10107/s12088-017-0647-3

Raghavendra, P., Halami, P. M. (2009). Screening, selection and characterization of phytic acid degrading lactic acid bacteria from chicken intestine. *International Journal of Food Microbiology*, 133(1-2), 129-134. https://doi.org/10.1016/j.ijfoodmicro.2009.05.006

Reale, A., Mannina, L., Tremonte, P., Sobolev, A.P., Succi, M., Sorrentino, E., Coppola, R. (2004). Phytate degradation by lactic acid bacteria and yeasts during the whole-meal dough fermentation: a 31P NMR study. *Journal of Agricultural and Food Chemistry*, 52(20), 6300-6305. https://doi.org/10.1021/jf049551p

Reale, A., Konietzny, U., Coppola, R., Sorrentino, E., Greiner, R. (2007). The importance of lactic acid bacteria for phytate degradation during cereal dough fermentation. *Journal of Agricultural and Food Chemistry*, 55(8), 2993-7. https://doi.org/10.1021/jf063507n

Rollán, G.C., Gerez, C.L., LeBlanc, J.G. (2019). Lactic fermentation as a strategy to improve the nutritional and functional values of pseudocereals. *Frontiers in Nutrition*, 6, 1-16.

Shi, J., Arunasalam, K., Yeung, D., Kakuda, Y., Mittal, G. (2004). Phytate from edible beans: chemistry, processing and health benefits. *Journal of Food Agriculture and Environment*, 2, 49-58. https://doi.org/10.3389/fnut.2019.00098
Songré-Ouattara, L.T., Mouquet-Rivier, C., Icard-Verrière, C., Humblot, C., Diawara, B., Guyot, J.P. (2008). Enzyme activities of lactic acid bacteria from a pearl millet fermented gruel (ben-saalga) of functional interest in nutrition. International Journal of Food Microbiology, 128(2), 395-400. https://doi.org/10.1016/j.ijfoodmicro.2008.09.004

Sumengen, M., Dincer, S., Kaya, A. (2013). Production and characterization of phytase from Lactobacillus plantarum. Food Biotechnology, 27(2), 105-118. https://doi.org/10.1080/08905436.2013.781507

Sümengen, M., Dincer, S., Kaya, A. (2012). Phytase production from Lactobacillus brevis. Turkish Journal of Biology, 36, 533-541.

Tharmaraj, N., Shah, N.P. (2003). Selective enumeration of Lactobacillus delbrueckii ssp. bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus, bifidobacteria, Lactobacillus casei, Lactobacillus rhamnosus, and propionibacteria. Journal of Dairy Science, 86(7), 2288-2296. https://doi.org/10.3168/jds.S0022-0302(03)73821-1

Yıldırım, R.M., Arıcı, M. (2019). Effect of the fermentation temperature on the degradation of phytic acid in whole-wheat sourdough bread. LWT - Food Science and Technology, 112, 108224. https://doi.org/10.1016/j.lwt.2019.05.122

Zamudio, M., González, A., Medina, J.A. (2001). Lactobacillus plantarum phytase activity is due to non-specific acid phosphatase. Letters in Applied Microbiology, 32, 181-184. https://doi.org/10.1046/j.1472-765x.2001.00890.x