Journal of Food Biosciences and Technology,
Islamic Azad University, Science and Research Branch, Vol. 9, No. 1, 11-18, 2019

Effect of Using *Lactobacillus Brevis* IBRC-M10790 and Baker's Yeast on Phytate Degradation of Sourdough and Barbari Bread

H. Hadaegh [a], S. M. Seyyedain Ardabili [b]*, M. Tajabadi Ebrahimi [c], M. Chamani [d], R. Azizi Nezhad [e]

[a] Ph. D Student of the Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran.
[b] Associate Professor of the Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran.
[c] Associate Professor of the Department of Biology, Central Tehran Branch, Islamic Azad University, Tehran, Iran.
[d] Professor of the Department of Animal Science, Science and Research Branch, Islamic Azad University, Tehran, Iran.
[e] Assistant Professor of the Plant Breeding Department, Science and Research Branch, Islamic Azad University, Tehran, Iran.

Received: 10 September 2018 Accepted: 8 October 2018

ABSTRACT: The possible use of *Lactobacillus brevis* IBRC-M10790 with degrading phytase activity was investigated in sourdough and Barbari bread –using 20 and 30% of sourdough in the formulation- in order to enhance the nutritional bioavailability, and the efficiency of the bacteria in decreasing phytate via using in sourdough was compared to yeast. Results showed that *L. brevis* IBRC-M10790 has better efficiency in phytate reduction than yeast alone or the combination of yeast and bacteria, and can be effectively used for improving the nutritional properties of Barbari bread. Use of 30% sourdough in bread formulation gave the highest percentage of phytate reduction in breads. It seems that not only the percentage of sourdough (the amount of used yeast), but also fermentation time is important in reducing the level of phytate in Barbari bread. Statistical analysis revealed that pH is not the major factor for phytate reduction in Barbari breads with different percentages of sourdough.

Keywords: *Barbari Bread, Lactobacillus brevis, Phytate Reduction, Sourdough, Yeast.*

Introduction

Phytic acid (myoinositol 1, 2, 3, 4, 5, 6-hexakisdihydrogen phosphate, phytate or IP6), is the major storage form of phosphorous, comprising 1–5% by weight in cereals, legumes, oil seeds and nuts (Gupta *et al*., 2015; García-Estepa *et al.*, 1999). It has the ability to chelate multivalent metal ions, especially zinc, calcium and iron, as well as resulting in poor bioavailability of minerals (García-Estepa *et al*., 1999; Gupta *et al*., 2015). Degradation of phytate occurs via the enzyme phytase (EC 3.1.3.8). This enzyme can be derived from different sources, such as animals, plants and microorganisms (De Angelis *et al*., 2003; Leenhardt *et al*., 2005; Matsuo *et al*., 2012). Considering fermented bakery products, phytase activity has been shown in different strains of commercial baker`s yeast (Türk *et al*., 2000; Lambrechts *et al*., 1992), however, it seems not to be sufficient for the degradation of phytate. Thus, the addition of phytase or micro-organisms producing

*Corresponding Author: mahdi_seyedain@yahoo.com

phytase was proposed by various researchers (Lopez *et al*., 2000; Sandberg and Andlid, 2002; De Angelis *et al*., 2003; Reale *et al*., 2007; Palacios *et al*., 2008; Didar and Haddad Khodaparast, 2011). Nevertheless, different reports have revealed that phytase activity in lactic acid bacteria seems to be strain-specific (Reale *et al*., 2007; Lopez *et a*l., 2000).

Bread made from wheat is a staple food in many countries, especially in Iran. Its consumption is 140-160 kg per year (more than twice of what is consumed in Europe) (Abtahi *et al*., 2014). Since bread is consumed at almost every meal, it is of utmost importance in the case of nutrition bioavailability. Thus, reducing phytate should be given great attention. Among four types of bread mainly consumed in Iran, Barbari is the most popular one, and its taste becomes unique when produced by sourdough (Pourafshar *et al*., 2015).

The use of sourdough in bread processing has a long tradition and plays an important role in the quality and nutritional value of different breads (Diowksz and Kordialik-Bogacka, 2017), especially in the case of IP6 reduction (Najafi *et al*., 2012; Poutanen *et al*., 2009; Gobbetti *et al*., 2005; Gobbetti *et al*., 2014). In addition, using lactic acid bacteria (LAB) in sourdough has received extensive attention, due to their impact on different characteristics of the dough and bread. Phytase is one of the metabolites produced by some species of LAB (Reale *et al*., 2007; De Angelis *et al*., 2003; Didar and Haddad Khodaparast, 2011). According to different published reports, there is still a challenge between the role of yeast and LAB, regarding IP6 degradation. Leenhardt *et al*. (2005) illustrated the effect of dough acidification in the phytate breakdown either by LAB or lactic acid. However, Reale *et al*. (2007) have reported that LAB do not have a direct effect on phytate degradation, and they carry out their function by lowering pH, in order to provide favorable conditions for endogenous cereal phytase. On the other hand, Palacios *et al*. (2008) stated that in the presence of certain bifidobacterial strains, reducing pH is not the main factor for hydrolyzing phytase.

Several studies have been carried out on the purification and characterization of phytase from different LAB or evaluating its activity in various foods (Sumengen *et al*., 2013; Zamudio *et al*., 2001; Reale *et al*., 2007; De Angelis *et al*., 2003; Lopez *et al*., 2000; Tang *et al*., 2010), however, this study aims to use *Lactobacillus brevis* IBRC-M10790 in sourdough, to improve the nutritional value of bread by producing phytase, and comparing its effect with yeast on phytate degradation, as well as evaluating the effect of pH for providing an optimum condition. To the best of our knowledge, this novel strain of LAB isolated from Tarhana (Tafvizi and Tajabadi Ebrahimi, 2015), has not been used in any food matrix, for verifying its phytase production.

Materials and Methods

- *Materials*

The flour utilized in this study for measuring IP6 quantity, producing sourdough and Barbari bread was commercial-type wheat flour (Tehran Bakhtar Co., Tehran, Iran) with an extraction rate of 79%. Flour characteristics are listed in Table 1. Salt was obtained from the local market. A typical fresh baker`s yeast (*Saccharomyces cerevisiae*) was purchased from Iran Mayeh Co., Tabriz, Iran. *L. brevis* IBRC-M10790, was used as a sourdough starter, obtained from Tak Gen Zist Co. (Tehran, Iran).

Table 1. Chemical and rheological characteristics of the flour

Characteristics	
Chemical	
Moisture (%)	14.18
Wet Gluten (%)	30.2
Protein (% of dry weight basis)	12.5
Ash (% of dry weight basis)	0.66
Rheological	
Farinograph water absorption (%)	54.1
Dough development time (min)	2.06
Dough stability (min)	18.03
Falling Numbers	364

Mixing properties of the flour were determined using a Farinograph according to AACC method (54.21 on 50.0 g of the flour) (AACC, 2000). Values represent the average of three replications.

- *Methods*

- *Starter Preparation*

L. brevis IBRC-M10790 was received in a lyophilized form, and then grown in a sterile industrial broth culture for 12 h in a 37°C incubator. Prior to this step, bacteria were incubated for 5, 12, 24 and 48 h, and results showed that 12 h is the best time for incubation, as some derivatives were consumed by the bacteria after this time. After incubation, all broth medium within the cells were utilized in the preparation of sourdough with the cell density of 1.0×10^9 CFU/ml.

- *Sourdough preparation*

Sourdoughs were prepared in a spiral mixer (Diosna Co., model SP12-SP160, Germany), by mixing 500 g wheat flour with 310 g tap water, 10 g salt, 20 g yeast (in the trials which yeast was included) and the inoculum of the sourdough starter (1.0×10^9 CFU/ml) for 2 min in slow speed and 6 min fast speed. For the preparation of sourdough starter, *L. brevis* IBRC-M10790 was incubated for 12 h in MRS broth (De Man *et al*., 1960) at 37°C in an aerobic condition. Prepared sourdoughs were thereafter put in the bowel, covered with a plastic cover and held for 24 h in room temperature.

- *Determination of pH*

pH measurements were carried out according to AACC method (02-52) in triplicate order.

- *Bread production*

For the preparation of Barbari bread, 600 g of flour, 10.8 g of salt, 24 g of fresh yeast, and 420 ml of water were utilized (control bread). For evaluating the effect of sourdough on phytate reduction in bread, Barbari samples were prepared using two different amounts of sourdough: 20 g/100 g of dough (formulation 1), and 30 g/100 g of dough (formulation 2). All ingredients were mixed and kneaded in a spiral mixer (Diosna, model SP12-SP160, Germany) for 2 min slow speed and 6 min fast speed. The prepared dough was divided into 500 g pieces and put into proofer (38◦C, RH=85%) for 20 min. Thereafter, semi-proofed dough pieces were brought out from the proofer, formed to the flattened shape by hands, punched by fingers, and continued proofing for 6 min. Baking was carried out in a Miwe rack oven (Germany) in 250°C for 11 min. Breads were cooled after 30 min and packed in plastic bags for further analysis.

- *Sample preparation*

Phytate was extracted from the samples

based on the method described by Dost and Tokul (2006). The extracts were centrifuged at 12000×g for 15 min (Micro 200R, Hettich Co., UK), while the supernatants were filtered through a 0.22 μm filter (Sartorius No. 17593Q, Germany).

- *Phytate analysis by HPLC*

HPLC analysis was conducted following the chromatographic method described by Lehrfeld (1994) with some modifications. Briefly, a PLATINblue UHPLC-System (Knauer, Germany) was used, equipped with an L-7100 pump. Samples (10 μl) were loaded onto a Hamilton anion exchange PRP-X 100 column (PRP-X100 5 µm 4.1 x 150 mm, Kenauer, Germany) with an automatic injection system, a Photodiode array detector (PDA) set at 460 nm, operated using EZchrom software. Elution was performed at a flow rate of 1 ml/min, at 45°C using an isocratic mobile phase consisting of water phase A: 0.5 ml formic acid, 8 ml of 0.04M Tetrabutylammonium hydroxide solution (TBAOH), and adjusting the pH to 4.1 with 5 M sulphuric acid; Phase B: acetonitrile. All chemicals were of chromatographic and analytical grade, obtained from Merk Millipore Co., Germany. Commercially available (Sigma-Aldrich) D-myo-inositol 1, 2, 3, 4, 5, 6-Hexakisphosphate, and dodecasodium salt (Cat. No. 407125, CAS No. 14306-25-3) were used as standards. Samples were run in duplicate.

- *Statistical analysis*

All the statistical analyses were carried out using the SPSS software, version 23 (IBM, USA). Values are given as the means ± standard error. Significant differences among means were determined by one-way ANOVA. The level of statistical significance was set at $P < 0.05$.

Results and Discussion

Phytate content of the flour was obtained as 300.494 ± 0.01 mg/ 100 g of dry weight basis. After 24 h fermentation in the control sourdough (prepared by yeast), it decreased to 121.272 ± 0.11 mg/ 100 g of dry weight basis (60% reduction) ($P < 0.05$). GarcõÂa-Estepa *et al*. (1999). reported almost the same amount of phytate for commercial white wheat flour, and 50% reduction of phytic acid for commercial breads. By using both yeast and *L. brevis* in the formulation of sourdough, phytate content was reduced to 95.927 ± 0.01 mg/ 100 g of dry weight basis ($P < 0.05$), and when using *L. brevis* in sourdough without any added yeast, the amount of phytate was obtained as 48.125 ± 0.11 mg/ 100 g of dry weight basis (68 and 84% loss of phytate, respectively) ($P < 0.05$).

In control Barbari bread (without any added sourdough), phytate content was obtained as 217.587± 0.12 mg/100 g of dry weight basis (28% reduction) ($P < 0.05$). This loss may be the result of yeast activation or intrinsic phytases in flour in optimum condition of fermentation, as well as high temperature during baking (García-Estepa *et al*., 1999). The use of sourdoughs in breads, either 20 or 30% of the formulation, resulted in further reduction of phytate content (Table 2). In other words, phytate reduction was significantly higher in Barbari breads with added sourdough ($P < 0.05$). This might be attributed to the activity of both yeast and bacteria in sourdough and also their extra time for activity during the proofing of breads. Other reports also confirmed the effect of fermentation and sourdough in phytate reduction (Arendt *et al*., 2007, Najafi *et al*., 2012, Leenhardt *et al*., 2005, Kasprowicz-Potocka *et al*., 2017, Mohammed *et al*., 2017) as well as the time dependency of phytase degradation (Palacios *et al*., 2008, Türk and Sandberg, 1992, Mohammed *et al*., 2017). All breads including 20 or 30% sourdough in the formulation (without *L. brevis*) showed approximately 96% phytate

reduction. Phytate content was obtained as 11.335 ± 0.08 and 11.563 ± 0.08 mg/100 g of dry weight basis, respectively ($P < 0.05$). Adding *L. brevis* to the sourdough, showed a little more, but significant reduction of phytate in all breads. Phytate content for such Barbari breads with 20 or 30% sourdough was 10.656 ± 0.14 and 10.269± 0.07 mg/100 g of dry weight basis, respectively (about 96.5% reduction) ($P < 0.05$). All data are summarized in Table 2.

- *pH and Phytase Activity*

pH of the flour and control bread without any sourdough, was measured as 6.1 and 5.9, respectively (Table 2). Adding sourdough to the bread formulation reduced the pH significantly ($P < 0.05$). The most reduction was observed in breads with both yeast and bacteria sourdough. Comparing sourdoughs with each other, pH in the sourdoughs including *L. brevis* was lower (pH=4.52) perhaps due to the metabolites they produced. Sourdoughs with both yeast and bacteria (pH=5.11) and sourdoughs with yeast without bacteria (pH=5.39) were the next. Leenhardt *et al*. (2005) reported that pH of the dough was the main determining factor for phytate hydrolysis in whole bread, and by using sourdough in the formulation, this optimum condition can be achieved. Požrl *et al.* (2009) also confirmed the influence of pH on phytic acid reduction. They stated that samples of lower pH had lower phytic acid content (Simčič, 2009). Didar and Haddad Khodaparast (2011) reported that pH and phytic acid content of breads follow nearly the same pattern. On the contrary, Palacios *et al.* (2008) stated that, reducing pH did not play any major role in phytate reduction, mainly in the presence of different strains of bifidobacterial species.

Regarding phytate degradation in sourdoughs, it can be seen that all three kinds of sourdoughs had phytate reduction. This confirms the previous reports that both yeast and bacteria have phytase activity (Reale *et al*., 2004). Gupta *et al.* (2015) stated that the properties of different phytases (such as optimum pH, temperature, etc.) obtained from various sources exhibited differently. They reported the optimum temperature and pH of phytases in the range of 25 to 80°C, and 4.5 to 6, respectively. Regarding this, all the sourdoughs in this

Table 2. Phytate content, its residue and reduction percentage, and pH changes in flour, sourdoughs after 24 hours' fermentation, and Barbari bread

Samples	Phytate (mg/100g, dry weight basis)	Residue (%)	Reduction (%)	pH
Flour	300.494 ± 0.01 a	99.997 ± 0.003 a	0.003 ± 0.003 i	6.10 ± 0.005 a
C0	217.587 ± 0.12 b	72.410 ± 0.04 b	27.59 ± 0.04 h	5.90 ± 0.010 b
C20	11.335 ± 0.08 g	3.772 ± 0.03 f	96.23 ± 0.02 c	5.81 ± 0.005 c
C30	11.563 ± 0.08 f	3.848 ± 0.03 g	96.15 ± 0.02 d	5.78 ± 0.000 d
SDY	121.272 ± 0.11 c	40.356 ± 0.07 c	59.64 ± 0.07 g	5.39 ± 0.005 g
SDB	48.125 ± 0.11 e	16.015 ± 0.03 e	83.99 ± 0.03 e	4.52 ± 0.030 i
SDYB	95.927 ± 0.01 d	31.922 ± 0.01 d	68.08 ± 0.01 f	5.11 ± 0.010 h
TD4Y20	10.656 ± 0.14 h	3.546 ± 0.05 h	96.45 ± 0.05 b	5.60 ± 0.010 f
TD4Y30	10.269 ± 0.07 i	3.417 ± 0.02 i	96.58 ± 0.02 a	5.66 ± 0.005 e

TD4Y20: Barbari breads with 20% sourdough including yeast and *L. brevis*, *TD4Y30:* Barbari breads with 30% sourdough including yeast and *L. brevis*, *C0*: Barbari bread without sourdough, *C20*: Barbari bread with 20% sourdough including yeast, *C30*: Barbari bread with 30% sourdough including yeast, *SDB*: sourdough with *L. brevis* without yeast, *SDY*: sourdough with yeast without *L. brevis. SDYB*: sourdough with yeast and *L. brevis.*
Mean and standard error of two determinations, expressed on a dry weight basis.
Different letters in each column indicate statistically significant differences ($P < 0.05$)

study had such an optimum condition during fermentation. Sourdoughs containing *L. brevis* and not yeast, had the highest phytate reduction and the lowest pH=4.52. This may be as a result of the activity of lactic and acetic acids that are from the most produced metabolites of *L. brevis* IBRC-M10790 (data are not shown), and are effective in decreasing the pH of the dough. Although Türk *et al.* (1996) stated that organic acids such as lactic and acetic acids do not have a significant effect on phytate reduction, in the viewpoint of pH. This result is in line with previous works carried out by different researchers which had reported that the highest phytate degradation in baking studies is around 4.5 (Türk *et al*., 1996, Larsson and Sandberg, 1991). Microbial phytases have been reported to be active over the wider range of pH than plant phytases (Türk and Sandberg, 1992), however, lower pH may have favored the activity of endogenous phytase (Türk *et al*., 1996). The optimum pH for plant seeds phytases have been reported between 4 and 5.6 (Gupta *et al*., 2015). As the pH for all sourdoughs in this study was in this range, intrinsic phytase of the flour may have also participated in the degradation of phytate in all samples.

Comparison of the pH of Barbari breads containing yeast with those containing both yeast and bacteria, showed that the pH of latter breads was significantly lower ($P < 0.05$). Results of previous works showed that depending on the starter culture used, the pH of the sourdough varies (Palacios *et al*., 2008, Esteve *et al*., 1994). Esteve *et al.* (1994) also reported that the presence of yeast in the sourdough results in increased pH of breads. They also enumerated the percentage of sourdough as the most important factor affecting acidity. In our study, increasing the percentage of sourdough from 20 to 30 in breads containing yeast and not bacteria, reduced the pH of the breads significantly ($P < 0.05$), although phytate reduction did not increased. Nevertheless, using 30% sourdough containing *L. brevis* and yeast in breads significantly decreased the pH and also reduced the phytate of the bread ($P < 0.05$). By achieving such a result, it can be concluded that in agreement with Palacios *et al.* (2008), pH may be helpful in speeding up the reduction of phytate, but is not the major factor for phytate reduction in Barbari breads with different sourdough percentage.

Conclusion

In most bakery products such as bread, fermentation time is approximately 2 h, which is not enough for sufficient hydrolysis of phytate. Using sourdough in bread production with or without LAB can lead to phytate degradation, as there would be more time available for the activity of both yeast and bacteria. It seems that not only the percentage of sourdough (the amount of used yeast), but also fermentation time is important in reducing phytate level in Barbari bread. Results of this study also revealed that *L. brevis*IBRC-M10790 has better efficiency in phytate reduction than yeast alone, as well as the combination of yeast and bacteria, and can be effectively utilized for enhancing the nutritional properties of Barbari breads. Statistical analysis did not show a strong correlation between pH and phytate reduction. Perhaps pH was the minor factor for phytate reduction.

References

AACC. (2000). Approved Methods of the American Association of Cereal Chemists, methods 54-21, 02-52

Abtahi, M., Neyestani, T. R., Pouraram, H., Siassi, F., Dorosty, A. R., Elmadfa, I. & Doustmohammadian, A. (2014). Iron-fortified flour: can it induce lipid peroxidation? International journal of food sciences and nutrition, 65**,** 649-654.

Arendt, E. K., Ryan, L. A. & Dal Bello, F. (2007). Impact of sourdough on the texture of

bread. Food microbiology, 24**,** 165-174.

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**,** 259-70.

De Man, J. C., Rogosa, M. & Sharpe, M. E. (1960). A Medium For The Cultivation Of Lactobacilli. Journal of Applied Bacteriology, 23**,** 130-135.

Didar, Z. & Haddad Khodaparast, M. H. (2011). Effect of different lactic acid bacteria on phytic acid content and quality of whole wheat Toast bread. Journal of food bioscience and technology, 1, 1-10.

Diowksz, A. & Kordialik-Bogacka, E. (2014). Stimulation of gluten-free sourdough fermentation. Acta Alimentaria, 43(2), 225-231.

Dost, K. & Tokul, O. (2006). Determination of phytic acid in wheat and wheat products by reverse phase high performance liquid chromatography. Analytica Chimica Acta, 558**,** 22-27.

Esteve, C. C., De Barber, C. B. & Martínez-Anaya, M. A. (1994). Microbial Sour Doughs Influence Acidification Properties and Breadmaking Potential of Wheat Dough. Journal of Food Science, 59**,** 629-633.

García-Estepa, R. M., Guerra-Hernández, E. & García-Villanova, B. (1999). Phytic acid content in milled cereal products and breads. Food Research International, 32**,** 217-221.

Gobbetti, M., De Angelis, M., Corsetti, A. & Di Cagno, R. (2005). Biochemistry and physiology of sourdough lactic acid bacteria. Trends in Food Science & Technology, 16**,** 57-69.

Gobbetti, M., Rizzello, C. G., Di Cagno, R. & De Angelis, M. (2014). How the sourdough may affect the functional features of leavened baked goods. Food Microbiology, 37**,** 30-40.

Gupta, R. K., Gangoliya, S. S. & Singh, N. K. (2015). Reduction of phytic acid and enhancement of bioavailable micronutrients in food grains. Journal of Food Science and Technology, 52**,** 676-684.

Kasprowicz-Potocka, M., Zaworska, A., Gulewicz, P., Nowak, P. & Frankiewicz, A. (2017). The effect of fermentation of high alkaloid seeds of Lupinus angustifolius var. Karo by *Saccharomyces cerevisieae*, *Kluyveromyces lactis*, and *Candida utilis* on the chemical and microbial composition of products. Journal of Food Processing and Preservation**,** e13487.

Lambrechts, C., Boze, H., Moulin, G. & Galzy, P. (1992). Utilization of phytate by some yeasts. Biotechnology Letters, 14**,** 61-66.

Larsson, M. & Sandberg, A. S. (1991). Phytate reduction in bread containing oat flour, oat bran or rye bran. Journal of Cereal Science, 14**,** 141-149.

Leenhardt, F., Levrat-Verny, M. A., Chanliaud, E. & Remesy, 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**,** 98-102.

Lehrfeld, J. (1994). HPLC separation and quantification of phytic acid and some inositol phosphates in foods: problems and solutions. Journal of Agricultural and Food Chemistry, 42**,** 2726-2731.

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**,** 2281-5.

Matsuo, A., Sato, K., Park, E. Y., Nakamura, Y. & Ohtsuki, K. (2012). Control of amylase and protease activities in a phytase preparation by ampholyte-free preparative isoelectric focusing for unrefined cereal-containing bread. Journal of Functional Foods, 4**,** 513-519.

Mohammed, M. A., Mohamed, E. A., Yagoub, A. E. A., Mohamed, A. R. & Babiker, E. E. (2017). Effect of Processing Methods on Alkaloids, Phytate, Phenolics, Antioxidants Activity and Minerals of Newly

Developed Lupin (*Lupinus albus L.*) Cultivar. Journal of Food Processing and Preservation, 41**,** e12960.

Najafi, M. A., Rezaei, K., Safari, M. & Razavi, S. H. (2012). Use of sourdough to reduce phytic acid and improve zinc bioavailability of a traditional flat bread (sangak) from Iran. Food Science and Biotechnology, 21**,** 51-57.

Palacios, M. C., Haros, M., Sanz, Y. & Rosell, C. M. (2008). Phytate degradation by Bifidobacterium on whole wheat fermentation. European Food Research and Technology, 226**,** 825-831.

Pourafshar, S., Rosentrater, K. A. & Krishnan, P. G. (2015). Using alternative flours as partial replacement of barbari bread formulation (traditional Iranian bread). Journal of Food Science and Technology, 52**,** 5691-5699.

Poutanen, K., Flander, L. & Katina, K. (2009). Sourdough and cereal fermentation in a nutritional perspective. Food Microbiology, 26**,** 693-699.

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**,** 2993-2997.

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 wholemeal dough fermentation: a 31P NMR study. Journal of Agricultural and Food Chemistry, 52**,** 6300-6305.

Sandberg, A. S. & Andlid, T. (2002). Phytogenic and microbial phytases in human nutrition. International Journal of Food Science and Technology, 37**,** 823-833.

Simčič, M. (2009). Phytate degradation during breadmaking: the influence of flour type and breadmaking procedures. Czech Journal of Food Sciences, 27**,** 29-38.

Sumengen, M., Dincer, S. & Kaya, A. (2013). Production and Characterization of Phytase from Lactobacillus plantarum. Food Biotechnology, 27**,** 105-118.

Tafvizi, F. & Tajabadi Ebrahimi, M. (2015). Application of Repetitive Extragenic Palindromic Elements Based on PCR in Detection of Genetic Relationship of Lactic Acid Bacteria Species Isolated from Traditional Fermented Food Products. Journal of Agricultural Science and Technology 17**,** 87-98.

Tang, A. L., Wilcox, G., Walker, K. Z., Shah, N. P., Ashton, J. F. & Stojanovska, L. (2010). Phytase Activity from Lactobacillus spp. in Calcium- Fortified Soymilk. Journal of Food Science, 75**,** 373-376.

Türk, M., Carlsson, N. G. & Sandberg, A. S. (1996). Reduction in the levels of phytate during wholemeal bread making; effect of yeast and wheat phytases. Journal of Cereal Science, 23**,** 257-264.

Türk, M. & Sandberg, A.-S. (1992). Phytate degradation during breadmaking: effect of phytase addition. Journal of Cereal Science, 15**,** 281-294.

Türk, M., Sandberg, A. S., Carlsson, N. G. & Andlid, T. (2000). Inositol hexaphosphate hydrolysis by Baker's yeast. Capacity, kinetics, and degradation products. Journal of Agricultural and Food Chemistry, 48**,** 100-4.

Zamudio, M., Gonzalez, A. & Medina, J. (2001). Lactobacillus plantarum phytase activity is due to non-specific acid phosphatase. Letters in Applied Microbiology, 32**,** 181-184.