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Effect of Different Lactic Acid Bacteria on Phytic Acid Content and Quality of Whole Wheat Toast Bread

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ABSTRACT: Nowadays, consumption of whole flours and flours with high extraction rate is recommended, because of their large amount of fiber, vitamins and minerals. Despite nutritional benefits of whole flours, concentration of some undesirable components such as phytic acid is higher than white flour. In this study, the effect of several sourdough lactic acid bacteria on toast bread was investigated. Sourdough was made from lactic acid bacteria (*Lb. plantarum*, *Lb. reuteri*) with different dough yields (Dy) and incubated at 30˚C for 20 hours, then added to dough in the ratios of 10, 20 and 30% replacement. Breads supplemented with *Lb. plantarum* sourdough had lower phytic acid. Higher replacement of sourdough and higher DY caused higher decrease in phytic acid content. Sourdough made from *Lb. plantarum*, DY = 300 and 30% replacement caused the highest decrease in phytic acid content (49.63 mg/100g). As indicated by the panelists, *Lb. reuteri* sourdough can present the greatest effect on overall quality score of the breads. DY reduction caused a decrease in bread quality score. Sensory score of toast bread was 81.71 in the samples treated with *Lb. reuteri* sourdough with DY = 250 and 20% replacement.

Keywords: *Lactic Acid Bacteria, Phytic Acid, Sourdough, Toast Bread, Whole Wheat Flour.*

Introduction

bread is one of the most important and basic foods all over the world (Rajabzadeh, 2001). Bread consumption is predicted about 10-12 million tons per year in Iran (Payan, 1998). Nowadays, consumption of whole flours and flours with high extraction rate is recommended, because of their large amount of fiber, vitamins and minerals. Despite the nutritional benefits of whole flour, some undesirable components such as phytic acid have higher concentration than white flour (Faridi, 1980) Bread making with high extraction flour has several limitations namely, reduction in loaf volume, fermentation tolerance, non-elastic and condensed crumb in bread. These limitations resulted in lower consumption of whole flour in bread making than white flour (Screeramulu *et al*., 1996). Phytic acid (*myo*-Inositol-1,2,3,4,5,6-hexa*kis*phosphate) is an abundant form of phosphorus in plant seeds and other plant tissues. Phytate works in a broad pH-region as a high negatively charged ion and therefore, its presence in the diet has a negative impact on the bioavailability of divalent and trivalent mineral ions such as Zn^{2+}, $Fe^{2+/3+}$, Ca^{2+}, Mg^{2+}, Mn^{2+} and Cu^{2+} (Angel *et al*., 2004). According to FAO/WHO [1992] report, more than 20 billion people have iron deficiency in the world. It has been reported that 30% and 31% of Iranians are suffering from iron and zinc deficiency, respectively (Sheykholeslam, 1997; Shirai *et al*., 1994).

There are several methods for phytic acid reduction in bread that one of these methods is use of sourdough. Use of different types of sourdough for phytic acid reduction has been proposed by some researchers. Shirai *et al* (1994) reported that some of lactic acid bacteria isolated from sourdough were able

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to hydrolyze phytic acid by phytase production (Shirai *et al*., 1994). Fretzdorff *et al* (1992) proposed that acid production and accordingly pH decrease by sourdough resulted in phytic acid degradation increase (Brummer & Fretzdorff, 1992). Akhavipoor (1998) pronounced that Barbari and Taftoon bread made with sourdough, since it has higher acidity content, caused 8-10% higher decrease in phytic acid content than liquid fermentation (Akhavipoor, 1997). Angelis *et al* (2003) reported that 8 hours incubation of *Lb. sanfranciscensis* CB_1 caused 64-74% decrease in sodium phytate concentration (Angelis *et al*., 2003).

The consumption of toast bread is steadily increasing. Most of the toast bread being consumed in Iran is made from white flour, which is depleted of natural dietary fiber. The total dietary fiber content of whole wheat flour is about 10.2% compared with 2.5% for white flour. On the other hand, the values for total dietary fiber in wheat bran have a range between 40 and 44%, thus they make it an ideal natural supplement for producing high fiber baked products (Ranhotra *et al*., 1990). Keeping in view the necessity of increasing dietary fiber content in Iranian diet, this research work was focused mainly on developing a toast bread containing a large amount of dietary fiber, but, at the same time, having less phytic acid content and consequently superior eating qualities than the whole wheat flour bread, therefore bread consumption could be increased. In this study, we used whole wheat flour for production of toast bread to investigate the effect of lactic acid bacteria on phytic acid content and the quality of bread.

Materials and Methods

Materials

Alvand wheat was purchased from the Agricultural Research Center of Neyshabour and it was milled in the laboratory mill AQC 109 after being cleaned and conditioned with extraction rate of 98%. The strains used throughout this study were *Lactobacillus plantarum* (PTCC 1058) and *Lactobacillus reuteri* (PTCC 1655) that were purchased from Iranian Research Organization for Science and Technology in a lyophilized form.

Methods

Moisture, ash, wet gluten and gluten index were determined according to the standard Procedures 46–16A, 08–01 and 38–12 of AACC, respectively (AACC, 1995; AACC, 1995; AACC, 1995). Protein content and phytic acid were determined by ISIRI 2863 and Garcia-Estepa methods, respectively (Garcia – Estepa *et al*., 1999; Gibson *et al*., 1998). pH of the samples was measured immediately after removal from the production by diluting 5 g samples with 30 ml water according to the standard method (Garcia – Estepa *et al*., 1999).

Preferment preparation

Both lactic acid bacteria strains were transferred to MRS broth medium in sterile condition, incubated at 37°C for 18 hours, then centrifuged (4000 rpm for 10 min) and microbial cells were harvested. Different dilution (10^{-1}-10^{-7}) of mother culture was prepared, transferred to MRS agar and cultured by pour plate method. The number of each bacterial strain was nearly 10^7cfu/g. Sourdough was prepared with dough yields (DY) 250 and 300. From each bacterial strain, 10 ml of mother culture was centrifuged and mixed for l min, transferred to a large beaker ,covered with aluminum foil and then incubated at 37°C for 20 h. Biomass was mixed with wheat flour until dough formation.

Bread production

The bread formula used for this kind of bread was consisted of flour (80 kg); wet baking yeast (400g); sugar (900g); dry baking yeast (100 g); salt (300g) and water (about 40 liter based on water absorption). Sourdough was replaced in the ratios of 10, 20 and 30% instead of flour in dough formulation. A baking technique, similar in principle to that of commercial procedure, was used for baking experimental loaves having almost equal volumes. In this procedure, the ingredients were mixed to optimum dough development. The dough samples were fermented in sealed containers at 30 °C and 75–85% RH for 40 min, then divided into 450 g pieces and moulded. The pieces were allowed to proof for 20 min in a sealed container placed in the proofing cabinet. The dough pieces were then baked for 15 min at 260 °C to obtain the proper thickness acceptable color and texture.

Sensory evaluation

Sensory analysis was carried out using a 5-point hedonic scale, scoring 1 (lowest) to 5 (highest). Sensory evaluation was performed by 10 trained panelists. Three attributes of bread, i.e., internal properties, external properties and overall quality score were selected according to the bread evaluation method described by American Institute of baking. For each of the attributes, the average of the panelist scores was calculated.

Statistical analysis

In order to assess significant differences among samples, a completely randomized design was performed using the MSTATC program (version 1.41). Duncan's new multiple range test was used to describe means with 99% confidence.

Results and Discussion

Chemical Characteristics of Toast Bread Flour

The chemical compositions of wheat flours are presented in Table 1. The characteristics of the wheat flour are in the range of typical values of medium strong flour.

Table 1. Quality characteristics of Toast bread flour

Attribute	Value
Protein (g/100 g, d.b.)	11.54
Moisture (g/100g)	7.52
Ash (g/100 g, d.b.)	1.74
Phytic acid (mg/100g)	894.66
Wet gluten (g/100g)	30.1
Gluten index	73.42

Phytic acid content of flours depends on several factors such as wheat cultivar, weather condition and milling parameters such as bran content and extraction rate (Clarke *et al*., 2004). The flour used in this study had high phytic acid content, and this is only because of high extraction rate of flour (98%).

Measurement of dough pH

The pH of dough samples supplemented with sourdough is described in Fig. 1. According to Fig. 1, sourdough from Lb. plantarum with DY=300 and 30% replacement in dough formulation showed the most marked effect on reducing pH of dough. Lb.plantarum was more effective in decreasing pH of dough in comparison with Lb. reuteri because Lb. plantarum is a facultative homofermentative strain and Lb. reuteri is a heterofermentative strain (Spicher & Rabe, 1980). Higher DY of sourdough resulted in higher decrease in pH of dough. This is probably because of better diffusion of organic acids present in the environment (Spicher & Rabe, 1980). Acid production is related to temperature and time of fermentation and DY (Stainer *et al*., 1989). Dalbello *et al* (2007) showed that pH of dough supplemented with Lb. plantarum and Lb. sanfranciscensis after 48 hours in 30 °C is 4.43 and 4.13, in dough after 80 minutes is 5.58 and 5.37 and in final bread is 5.72 and 5.48 (Dal Belloa *et al*., 2007).

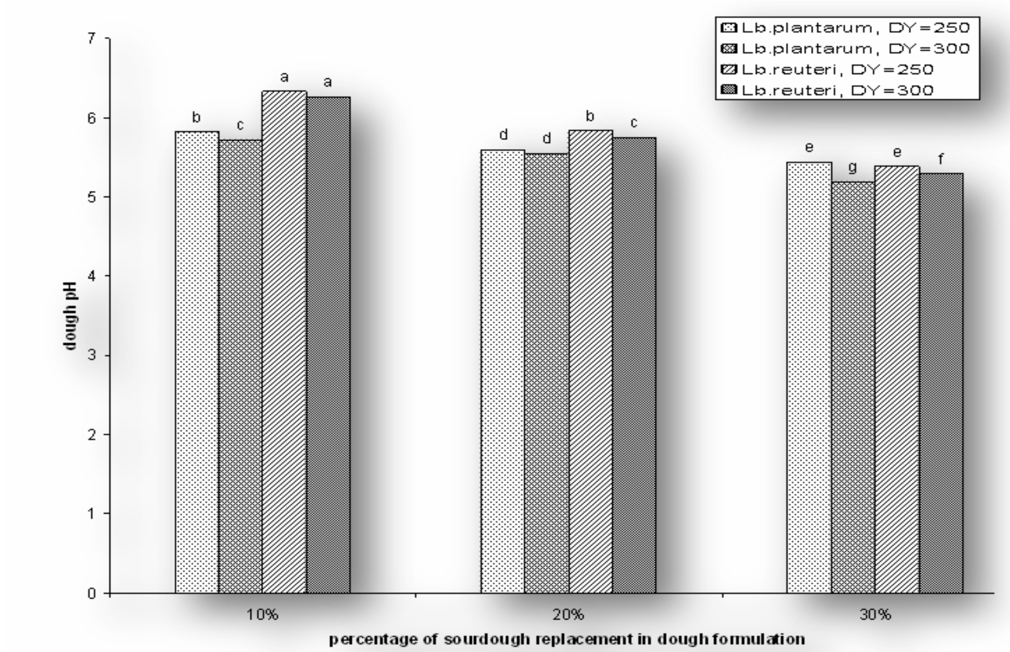

Fig. 1. Effect of bacterial strain, DY and percentage of sourdough replacement on pH of Toast dough; Columns marked by the same letter are not statistically different at P < 0.01

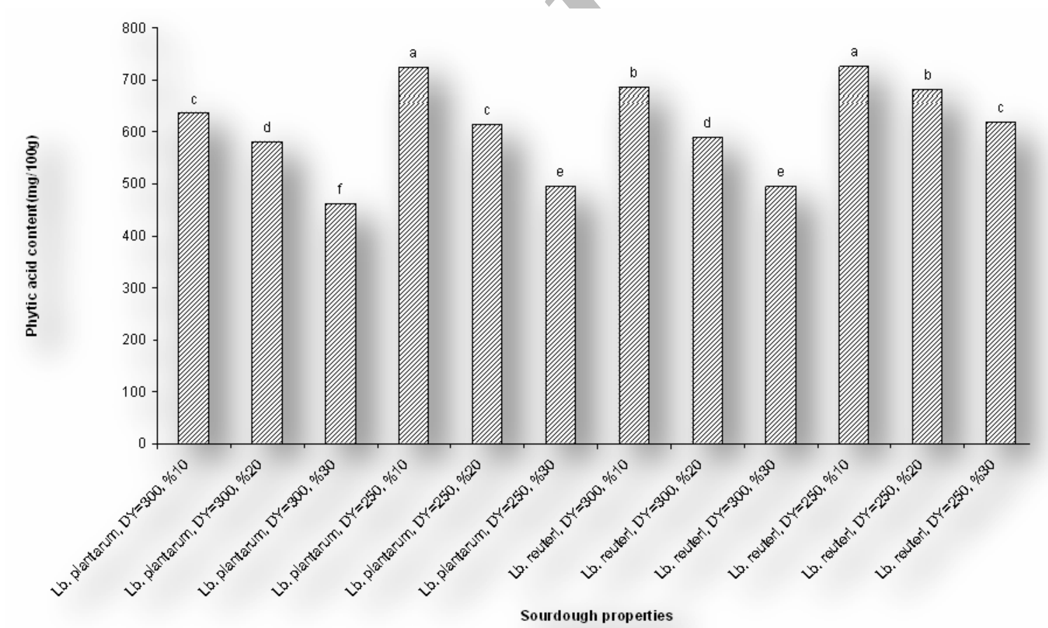

Fig. 2. Effect of bacterial strain, DY and percentage of sourdough replacement on phytic acid content (mg/100g) of Toast dough; Columns marked by the same letter are not statistically different at P < 0.01

Phytic acid measurement

The phytic acid content in the samples from the three different bread preparations (Fig. 2) followed nearly the same pattern as pH.

The phytic acid in bread samples supplemented with sourdough from *Lb. plantarum* was lower than samples treated with sourdough from *Lb. reuteri*. In addition, higher DY of sourdough resulted in higher decrease in phytic acid content of bread samples. Toast bread made with sourdough from *Lb. plantarum* with DY=300 and 30% replacement in dough formulation has 461.7 mg/100g phytic acid. This is probably because of microbial phytase enzyme and dough acidification that provided suitable condition for endogenic and microbial phytase activity and solubility increase of phytate complexes. Chaoui *et al.,* (2003) showed that bread making with sourdough from *Lb. plantarum* and *Leu. mesenteriodes* resulted in 76.5% and 67% decrease in phytic acid content, respectively (Chaoui *et al*., 2003). Lopez *et al* (2000) reported high phytase activity of *Lb. plantarum*, *Lb. acidophilus* and *Leu. mesenterisoes* in whole flour medium (Lopez *et al*., 2001). Palacios *et al*., (2008) detected high phytase activity by *Lb. reuteri (LM-15)*. Bread from 24h-old sourdough of this strain has lower phytic acid than breads from other strains. According to this study, this bacterial strain is able to complete phytic acid degradation in bread (Palacios *et al*., 2008). Lopez *et al.,* (2001) reported that fermentation with sourdough from *Lb. plantarum* and *Leuc. mesenteroides* caused 10 and 25% decrease in phytic acid content, respectively. This decrease was 38 and 62% at the end of fermentation (5 hours). Phytic acid degradation is because of phytase production, acid production and pH decrease (Lopez *et al*., 2000). Citric, lactic, acetic, butyric and formic acids formation during fermentation will cause an increase in mineral absorption because of soluble ligands with complexes and prevent from formation of insoluble complexes (Gibson *et al*., 1998). pH decrease prepares suitable condition for endogenic phytase because optimum pH for endogenic phytase is 4.8-5.5 (Lopez *et al*., 2000).

Bread quality

Table 2 shows that all sensory attributes of toast bread are influenced by sourdough addition.

DalBello *et al.,* (2007) compared the effect of sourdough from *Lactobacillus plantarum* and *Lactobacillus sanfranciscensis* on dough specific volume which were 4 and 3.5, respectively (Dal Belloa *et al*., 2007). Clarke *et al.,* (2004) also reported that breads treated with sourdough had a more specific volume than those chemically acidified (Clarke *et al*., 2004). Thiele *et al.,* (2004) showed that sourdough caused an increase in dough volume. Bread texture parameters were influenced by microbial acidification and extent of substrate breakdown in dough that resulted in microbial activity. Extent of acidification affected dough components such as gluten, starch and arabinoxylan (Thiele *et al*., 2004). Swell of gluten in acidic conditions is a well known effect (Zeleny, 1947). Direct effect of organic acids on rheological properties of dough is proved. Organic acids cause weakening of dough and reduction of mixing time (Wehrle *et al*., 1997).Takeda *et al.,* (2001) proposed that in acidic pH, solubility of gluten proteins is increased. Studies on weak gluten showed that modification in bread volume should be dependent on other factors (Takeda *et al*., 2001). Positive effects of sourdough on volume are because of different factors including:

1- Heterofermentative lactic acid bacteria that caused an increase in yeast's metabolic activity that resulted in higher CO_2 production.

2- Sufficient acidity caused an increase in gluten ability to gas holding (Gobbetti *et al*., 1995; Gobbetti *et al*., 1995).

3- Accumulation of water soluble pentosans that caused the volume increase that resulted from changes in water distribution (Corsetti *et al*., 2000).

4- Positive effects of sourdough on volume were due to exopolysaccharides formation during fermentation that affected the volume and staling (Korakli *et al*., 2001).

Table 2. Sensory characteristics of fresh Toast bread containing selected sourdoughs
Values are the average of ten replicates from three different bread making samples; Different letters in the same column indicate significant differences, (P < 0.01); All scores were from 0 to 5, with 5 being the highest value

Treatment	Characteristic		
	Internal properties	External properties	Overall Quality score
Lb. plantarum, DY=250, 10%	[ab] 4.38	[abc] 4.07	4.28[abcd]
Lb. plantarum, DY=250, 20%	[ab] 4.46	[a] 4.29	4.29 [abcd]
Lb. plantarum, DY=250, 30%	[a] 4.62	[ab] 4.17	4.47[ab]
Lb. plantarum, DY=300, 10%	[ab] 4.24	[de] 3.74	4.07[bcde]
Lb. plantarum, DY=300, 20%	[ab] 4.52	[bcd] 3.98	4.34[abcde]
Lb. plantarum, DY=300, 30%	[a] 4.70	[bcd] 4	4.47[ab]
Lb. reuteri, DY=250, 10%	[ab] 4.24	[e] 3.54	4[de]
Lb. reuteri, DY=250, 20%	[b] 3.98	[bcd] 3.94	3.96[e]
Lb. reuteri, DY=250, 30%	[a] 4.64	[a] 4.35	4.54 [a]
Lb. reuteri, DY=300, 10%	[ab] 4.14	[cd] 3.81	4.03[cde]
Lb. reuteri, DY=300, 20%	[ab] 4.32	[ab] 3.72	4.29[abcde]
Lb. reuteri, DY=300, 30%	[a] 4.66	[bcd] 4	4[abc]

Lb. plantarum and *Lb. reuteri* are exopolysaccharide producers (Desai *et al*., 2006; Tiekling & Ganzle, 2005). Katina *et al.,* (2006) reported that bran addition caused a more decrease in the specific volume in comparison with white bread, while if the bran was fermented, *Lb. brevis* caused an increase in whole flour bread volume and the same occurred when bran was fermented with α- amylase, xylanase, lypase and the volume was the same as white bread volume in all cases (Katina *et al*., 2006). According to the report of Clarke *et al.,* (2004), sourdough from *Lb. brevis*, *Lb. plantarum* alone or mixed rather than control caused an increase in bread volume and also caused an increase in air cell number and air cells with diameter lower than 4 mm^2 (Clarke *et al*., 2004). Salim–Ur–Rehman *et al.,* (2007) showed that sourdough from *Lb. bulgaricus* caused an increase in bread volume more than control bread (with 1% yeast), and also other bread properties such as form and shape, crust color, crumb, aroma, taste and texture were significantly modified (Salim – Ur – Rehman *et al*., 2007). Different enzyme produced from lactic acid bacteria such as xylanase, peroxidase and glucose oxidase caused modification in bread volume and crumb structure (Pointillart, 1993) therefore defined mixture of them caused a modification in gas holding in dough, extensibility and fermentation time (Collar *et al*., 2000). According to Lacanzen *et al.,* (2007), dextrin produced from *Leuc. mesenteroides*, caused an increase in bread volume, texture modification and softening in rye bread (Lacanze *et al*., 2007). Salmenkallio – Marttila *et al* (2001) showed that primary fermentation of bran by yeast or yeast combined with *Lb. brevis* caused a modification in crumb structure, bread volume and shelf life of breads that bran was added to them (Salim – Ur – Rehman *et al*., 2007). Robert *et al.,* (2006) reported that bread making with sourdough from *Lb. plantarum* and *Leucnostoc* caused an increase in bread score in viewpoint of external properties in comparison with control bread. Crumb was modified and sensory properties were significantly increased. According to this article, total score of bread was increased more than control and there was no difference between two strains(Robert *et al*., 2006).The effect of sourdough on fiber amount (soluble and insoluble) was important and related to technological effects. Soluble arabinoxylans with high molecular weight caused a modification in volume and softening of bread (Courtin & Delcour, 2002). Boskov *et al.,* (2002) reported a reduction in dietary fiber and an increase in solubility of arabinoxylans during fermentation with rye sourdough (Boskov *et al*., 2002). Fermentation with sourdough in rye caused an increase in soluble pentosans and a decrease in molecular size of pentosans that is an important result of the decrease in pH (Härkönen *et al*., 1995). Addition of pentosans extracted from wheat bran caused the modification of bread volume (Zhen *et al*., 2003). Highest score in toast bread is from bread made with *Lb. reuteri*, DY = 250 and 30% replacement. Proteolysis caused amino acid release that acted as flavor precursors (Gobbetti et al., 1995; Gobbetti *et al*., 1995). According to Salim-ur- Rehman *et al.,* (2007), bread that made with *Lb. bulgaricus* and bread made with yeast and bacteria had the highest score concerned sensory evaluation (Salim – Ur – Rehman *et al*., 2007).

Conclusion

In this study, the significant effect of sourdough on phytic acid content and quality of Iranian toast bread was clarified. Dough yield (DY), strain type and the percentage of sourdough addition affected pH, phytic acid content and quality of bread. Based on these results, higher dough yield and higher sourdough addition decreased phytic acid content. Organoleptic analysis showed that *Lb. plantarum* sourdough with dough yield of 250 and 30% addition can present the greatest effect on overall quality score of the breads.

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