

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

Z. Didar, A. Pourfarzad, M. H. Haddad Khodaparast

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

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

I. INTRODUCTION

BREAD is one of the most important and basic foods all over the world [36]. Bread consumption is predicted about 10-12 million tons per year in Iran [34]. Nowadays, consumption of whole flours and flours with high extraction rate is recommended, because of their high amount of fibers, vitamins and minerals. Despite nutritional benefits of whole flour, concentration of some undesirable components such as phytic acid is higher than white flour [17]. Bread making with high extraction flour has several limitations such as reduction in loaf volume and fermentation tolerance, non elastic and condense crumb in bread. These defects resulted in lower

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consumption of whole flour in bread making than white flour [40]. Phytic acid (*myo*-Inositol-1,2,3,4,5,6-hexakisphosphate) is an abundant form of phosphorus in plant seeds and other plant tissues. Phytate works in a broad pH-region as a highly 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+} [6]. According to FAO/WHO [1992] report, more than 20 billion people have iron deficiency in the world [50]. It has been reported that 30% and 31% of Iranians are suffering from iron and zinc deficiency, respectively [42, 32].

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 is proposed by some researchers. Shirai *et al* (1994) reported that some of lactic acid bacteria that isolated from sourdough are able to phytic acid hydrolysis by phytase production [43]. Fretzdorff *et al* (1992) proposed that acid production and accordingly pH decrease by sourdough resulted in phytic acid degradation increase [18]. Akhavi-poor (1998) pronounced that Barbari and Taftoon bread making with sourdough, since of higher acidity content, cause 8-10% higher decrease in phytic acid content than liquid fermentation [4]. Angelis *et al* (2003) reported that 8 hour incubation of *Lb. sanfranciscensis* CB₁ cause 64-74% decrease in sodium phytate concentration [7].

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 10.2% compared with 2.5% for white flour. On the other hand, the values for total dietary fiber in wheat bran range between 40 and 44%, thus making it an ideal natural supplement for producing high fiber baked products [53]. 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 higher amounts of dietary fiber, but, at the same time, having fewer phytic acid content and superior eating qualities than the whole wheat flour bread, so that bread consumption could be increased. In this study we used whole wheat flour for production of Toast bread to investigation effect of lactic acid bacteria on phytic acid content and bread quality.

II. MATERIALS AND METHODS

Materials

Alvand wheat was purchased from the Agricultural Research Center of Neyshabour and it was milled on the laboratory mill AQC 109 after being cleaned and conditioned to extraction rate of 98%. The strains used throughout this study were *Lactobacillus plantarum* (PTCC 1058) and *Lactobacillus reuteri* (PTCC 1655) that 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 [1, 2, 3]. Protein content and phytic acid were determined by ISIRI 2863 and Garcia-Esteva methods, respectively [20, 19]. PH of the samples was measured immediately after removal from the production by diluting 5 g samples with 30 ml water according to standard method [19].

Preferment preparation

Both lactic acid bacteria strains transferred to MRS broth medium in sterile condition and incubated at 37°C for 18 hour, and then centrifuged (4000 rpm for 10 min) and microbial cells harvested. Different dilution (10^{-1} - 10^{-7}) of mother culture prepared and transferred to MRS agar and cultured by pour plate method. The number of each bacterial strain was nearly 10^7 cfu/g. Sour dough was prepared with dough yield (DY) 250 and 300. From each bacterial strain, 10 ml of mother culture was centrifuged and mixed for 1 min, transferred to a large beaker and 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 consisted of flour (80 kg); wet baking yeast (400g); sugar (900g); dry baking yeast (100 g); salt (300g); water (about 40 liter based on water absorption). Sourdough was replaced in the ratio 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% R.H. for 40 min, and 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 and 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 [5].

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.

III. RESULTS AND DISCUSSION

Chemical Characteristics of Wheat and Soy Flours

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 I
QUALITY CHARACTERISTICS OF 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 [11]. The flour used in this study had high phytic acid amount, 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 [44]. Higher DY of sourdough resulted in higher decrease in pH of dough. This is probably because of better diffusion of organic acids that is present in environment [44]. Acid production is related to temperature and time of fermentation and DY [45]. Dalbello *et al* (2007) showed that pH of dough supplemented with *Lb. plantarum* and *Lb. sanfranciscensis* after 48 hour in 30 °C is 4.43 and 4.13, in dough after 80 minute is 5.58 and 5.37 and in final bread is 5.72 and 5.48 [15].

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.

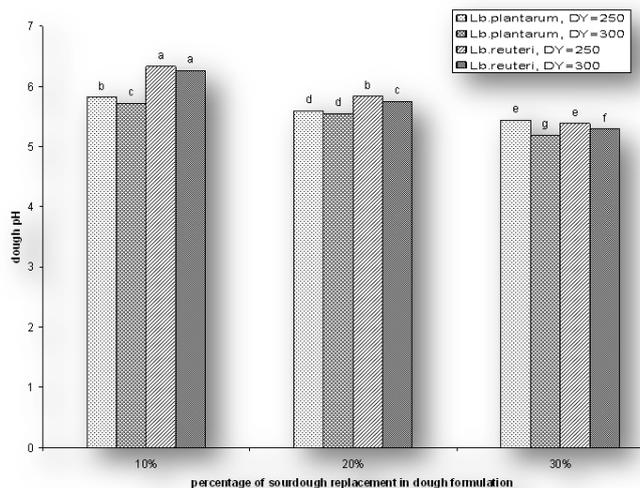


Fig. 1 Effect of bacterial strain, DY and percentage of sourdough replacement on pH of Sangak dough; Columns marked by the same letter are not statistically different at $P < 0.01$.

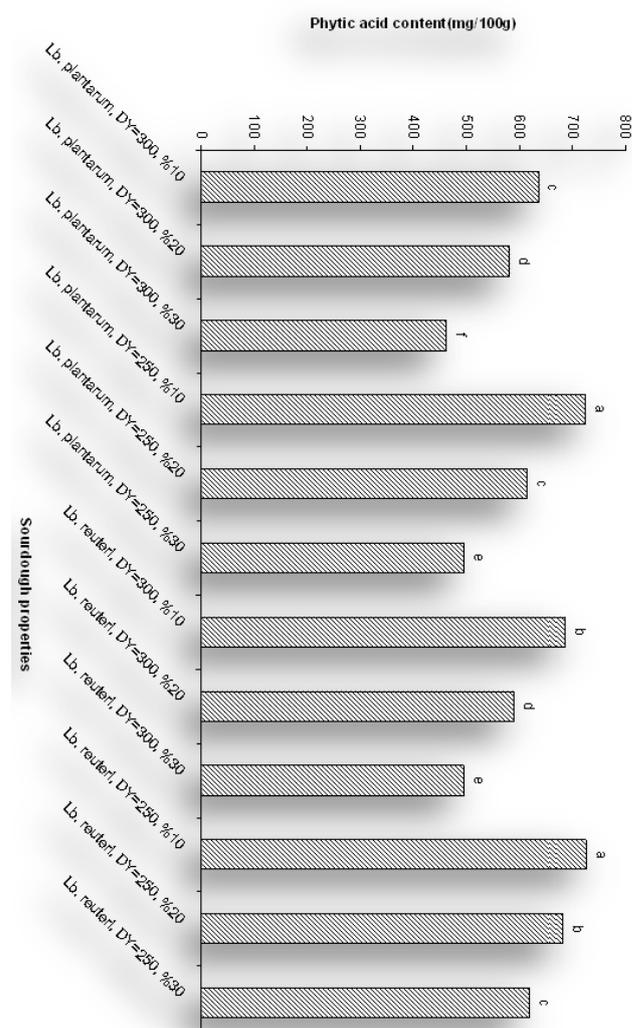


Fig. 2 Effect of bacterial strain, DY and percentage of sourdough replacement on phytic acid content (mg/100g) of Sangak dough; Columns marked by the same letter are not statistically different at $P < 0.01$.

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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 endogenous 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 [10]. Lopez *et al* (2000) reported high phytase activity of *Lb. plantarum*, *Lb. acidophilus* and *Leu. mesenterisoes* in whole flour medium [30]. 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 [33]. Lopez *et al* (2001) reported that fermentation with sourdough from *Lb. plantarum* and *Leuc. mesenteroides* cause 10 and 25% decrease in phytic acid content, respectively. This decrease was 38 and 62% at the end of fermentation (5 hour). Phytic acid degradation is because of phytase production, acid production and pH decrease [31]. Citric, lactic, acetic, butyric and formic acids formation during fermentation cause increase in mineral absorption because of soluble ligands with complexes and prevent from formation of insoluble complexes [20]. PH decrease prepares suitable condition for endogenous phytase because optimum pH for endogenous phytase is 4.8-5.5 [31].

Bread quality

Table II 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 that is 4 and 3.5, respectively [15]. Clarke *et al* (2004) also reported that breads treated with sourdough have more specific volume than those chemically acidified [11]. Thiele *et al* (2004) showed that sour dough cause increase in dough volume. Bread texture parameters are 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, arabinoxylan [47]. Swell of gluten in acidic conditions is a well known effect [51]. Direct effect of organic acids on rheological properties of dough is proved. Organic acids cause weakening of dough and reduction of mixing time [49]. Takeda *et al* (2001) proposed that in acidic pH, solubility of gluten proteins increases. Studies on weak gluten showed that modification in bread volume should be dependent on other factors than gluten network stability that led to better gas holding [46]. Positive

effects of sourdough on volume are because of different factors including:

1- Heterofermentative lactic acid bacteria cause increase in yeast's metabolic activity that resulted in higher CO₂ production.

2- Sufficient acidity causes increase in gluten ability to gas holding [21, 22].

3- Accumulation of water soluble pentosans causes volume increase that results from change in water distribution [13].

4- Positive effects of sourdough on volume because of exopolysaccharides formation during fermentation that cause volume increase and staling retarding [28].

TABLE II
SENSORY CHARACTERISTICS OF FRESH TOAST BREAD CONTAINING SELECTED SOURDOUGHS

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

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.

Lb. plantarum and *Lb. reuteri* are exopolysaccharide producers [16, 48]. Katina *et al* (2006) reported that bran addition caused more decrease in specific volume in comparison with white bread, while if the bran is fermented with *Lb. brevis* cause an increase in whole flour bread volume

and the same occurred when bran is fermented with α -amylase, xylanase and lipase that volume is same to white bread volume in all cases [26]. According to report of Clarke *et al* (2004), sourdough from *Lb. brevis*, *Lb. plantarum* alone or mixed rather than control cause an increase in bread volume and also cause an increase in air cell number and increase in air cells with diameter lower than 4 mm² [11]. Salim-Ur-Rehman *et al* (2007) showed that sourdough from *Lb. bulgaricus* cause 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 are significantly modified [38]. Different enzyme that produced from lactic acid bacteria such as xylanase, peroxidase and glucose oxidase cause modification in bread volume and crumb structure [35] and defined mixture of them cause modification in gas holding in dough, extensibility and fermentation time [12]. According to Lacanzen *et al* (2007), produced dextrin from *Leuc. mesenteroides*, cause increase in bread volume and texture modification and softening in rye bread [29]. Salmenkallio – Marttila *et al* (2001) showed that primary fermentation of bran by yeast or yeast combined with *Lb. brevis* cause modification in crumb structure, bread volume and shelf life of breads that bran is added to them [38]. Robert *et al* (2006) reported that bread making with sourdough from *Lb. plantarum* and *Leucnostonoc* cause increase in bread score in the viewpoint of external properties in comparison to control bread. Crumb is modified and sensory properties significantly increased. According to this article, total score of bread increased than control and no difference was between two strains [37]. Effect of sourdough on fiber amount (soluble and insoluble) is important and is related to technological effects. Soluble arabinoxylans with high molecular weight causes modification in volume and softening of bread [14]. Boskov *et al* (2002) reported reduction in dietary fiber and increase in solubility of arabinoxylans during fermentation with rye sourdough [9]. Fermentation with sourdough in rye causes an increase in soluble pentosans and decrease in molecular size of pentosans that is important result of decrease in pH [23]. Addition of pentosans that extracted from wheat bran causes modification of bread volume [52]. Highest score in Toast bread is from bread that made with *Lb. reuteri*, DY = 250 and 30% replacement. Lavash bread from *Lb. reuteri* sourdough with DY = 160 and 20% replacement, gain 71.86 score. Proteolysis cause amino acid release that act as flavor precursors [21, 22]. According to Salim-ur- Rehman *et al* (2007), bread that made with *Lb. bulgaricus* and bread that is made with yeast and bacteria has the highest score of sensory evaluation [38].

IV. CONCLUSION

In this study, 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=250 and 30% addition can present the

greatest effect on overall quality score of the breads.

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