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Moderate Decrease of pH by Sourdough Fermentation Is Sufficient To Reduce Phytate Content of Whole Wheat Flour through Endogenous Phytase Activity

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Whole wheat bread is an important source of minerals but also contains considerable amounts of phytic acid, which is known to impair their absorption. An in vitro trial was performed to assess the effect of a moderate drop of the dough pH (around 5.5) by way of sourdough fermentation or by exogenous organic acid addition on phytate hydrolysis. It was shown that a slight acidification of the dough (pH 5.5) with either sourdough or lactic acid addition allowed a significant phytate breakdown (70% of the initial flour content compared to 40% without any leavening agent or acidification). This result highlights the predominance of wheat phytase activity over sourdough microflora phytase activity during moderate sourdough fermentation and shows that a slight drop of the pH (pH value around 5.5) is sufficient to reduce significantly the phytate content of a wholemeal flour. Mg "bioaccessibility" of whole wheat dough was improved by direct solubilization of the cation and by phytate hydrolysis.

KEYWORDS: Whole wheat bread; bioavailability; magnesium; phytic acid; pH; vegetal phytase

INTRODUCTION

Whole grain products and wheat bran in particular are important sources of minerals such as potassium, magnesium, iron, and zinc but also contain considerable amounts of phytic acid (PA). By chelating and precipitating multivalent cationic minerals with its six anionic phosphate groups, PA or *myo*-inositol hexakisphosphate (IP_6) has been found to lower the absorption of minerals Ca and Mg as well as trace oligolelements such as Fe and Zn (*1*, *2*). Some mineral deficiencies are common in developing countries, but marginal mineral deficiencies also occur in developed countries. In France, the SU-VI-MAX study showed that 72% of men and 77% of women had magnesium intakes lower than the French recommended dietary allowances (*3*). Bread made from wheat (*Triticum aestivum*) is a staple food in many countries, and whole wheat bread ranks within the highest foods for its Mg contribution. Therefore, increasing bread mineral contribution by using wholemeal flour and lowering the PA content to a level that does not affect the mineral bioavailability would be necessary to prevent metabolic disorders associated with mineral deficiencies.

Phytate-degrading enzymes are present in cereals (*4*), yeast (*5*), and lactic acid bacteria isolated from sourdough (*6*). During breadmaking, these phytases are activated and may hydrolyze PA into IP_5 and then into lower *myo*-inositol phosphate esters (IP_4-IP_1), which are less likely to bind minerals and form weaker mineral complexes (*7*, *8*).

A previous work has shown that sourdough fermentation was more efficient than yeast fermentation in reducing PA content in bread and that only sourdough fermentation reduces the pH of the dough (*9*). It was supposed that the lower pH due to organic acid production by the sourdough microflora may be optimal for endogenous phytase activity present in the whole grain flour and that PA destruction in sourdough bread may be the resulting effect of intrinsic plant phytase and extrinsic microbial phytases.

In the present study, it was hypothesized that during a moderate sourdough fermentation, which induces a dough pH value of not lower than 5.5, corresponding to the optimal pH of intrinsic wheat phytase, phytate was principally degraded by the phytase of the whole wheat flour. The following work was therefore designed to determine if phytate disappearance during sourdough fermentation could be reproduced by the addition of lactic acid only, without lactic acid bacteria. Thus, the kinetics of PA disappearance during breadmaking was studied in different conditions of dough treatment, with yeast, sourdough, or lactic acid addition. The repercussions of acidity and phytate destruction on magnesium solubility were also assessed.

MATERIALS AND METHODS

Raw Material. Wholemeal flour was obtained by milling a mixture of the main commercialized wheat varieties in France (Apache, Soissons, and Caphorn) with a standard roller mill and by blending the total milling fractions. Fresh compressed yeast (purchased at the

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Table 1. Preparation of Whole Wheat Doughs[a]

	control	yeast	sourdough	control + lactic acid
wholemeal flour	1500	1500	1350	1500
water	1050	1050	900	1050
salt	22.5	22.5	22.5	22.5
yeast		37.5		
sourdough			300	
lactic acid (mL) 6.88 mmol/L				7.4

[a] Values are expressed in mg (wet weight).

local bakery), acetic acid, and L-(+)-lactic acid (>99%, Prolabo, VWR) were used in the preparation of doughs.

Sourdough was obtained by mixing whole wheat flour (1 weight), distilled water (1 weight), and a commercial single strain starter containing *Lactobacillus brevis* (Saf Levain LV2, kindly provided by Lesaffre; 0.25% of the total mixture). The resultant dough was incubated for 24 h at 30 °C before it was incorporated into the bread dough.

Fermentation Procedure. Four types of bread were prepared, the recipes being described in **Table 1**. The ingredients were mixed, kneaded for 5 min at room temperature, and left to proof at 30 °C at a relative humidity of 80%. Quadruplet samples of dough were taken every 30 min during the 4-h fermentation and were immediately stored at −80 °C for subsequent analytical procedures. The pH was immediately determined on each dough with a Sentron pH System 1001. Supernatant fractions of the dough were obtained by ultracentrifugation at 20000*g* for 10 min at 4 °C.

Analytical Procedures. Dough moisture was determined as the difference between wet weight and dry weight on aliquots of dough that were dried to constant weight.

The amount of lactic acid was determined spectrophotometrically on neutralized perchloric acid extracts of supernatant fractions of dough by using an enzymatic method (*10*). The amount of acetic acid was measured by GLC on supernatant fractions of dough.

Phytic acid was determined using HPLC (Dionex, Sunnyvale, CA) as described previously (*11*); the instrumentation consisted of a gradient pump equipped with a 25-μL injector loop and an anion-exchange Dionex HPIC AS-11 analytical column (0.5 cm i.d. × 25 cm). An anion-exchange Dionex HPIC AG-11 guard column was used. An anion micromembrane suppressor (AMMS) was used to minimize basal conductivity for detection. Dough samples (~300 mg) were extracted with 10 mL of 0.65 mol/L HCl under vigorous mechanical agitation for 3 h at room temperature. The extracts were centrifuged at 5000*g*, and 2 mL of the supernatant was evaporated to dryness in a centrifugal evaporator (Jouan SA, St. Herblain, France). The residue was resuspended in 2 mL of deionized water (Millipore water system) and passed through a 0.45-μm filter. The filtrate was then diluted in deionized water (1:10) and injected into the liquid chromatograph. Sodium phytate (Sigma Chemical Co., St. Louis, MO) was used as the standard for external calibration. A wholemeal flour, referenced for IP_6 quantification, was submitted to the same procedure to check the reproducibility.

Magnesium was determined on the supernatant fractions (soluble Mg) and on the doughs and flour (total Mg) after dry-ashing (10 h at 500 °C) and extraction at 130 °C in HNO_3/H_2O_2 (2:1; v/v) (Merck, Suprapur, Darmstadt, Germany) until decoloration. Final dilutions were made in 1% HNO_3. Mg concentrations were determined by atomic absorption spectrophotometry (Perkin-Elmer 420, Norwalk, CT) in an acetylene−air flame at 285 nm (*12*). Appropriate quality controls (certified wholemeal flour BCR-189) were analyzed with each set of measurements.

Wheat Phytase Activity. Ten milligrams of commercial wheat phytase (EC 3.1.3.26, 0.04 unit/mg; Sigma Chemical Co., 2 mL, suspension in deionized water) was added to 2 g of white flour (type 45) supplemented with 1.5 mmol/L sodium phytate solution (10 mL). In the experiment with wholemeal flour phytase, 2 g of the wholemeal flour used in the fermentation procedure was supplemented with 1.5 mmol/L sodium phytate solution (10 mL). No buffer was used because we have found that the pH in these systems is stable throughout incubation. The pH was adjusted with 0.2 M acetic acid to 4.5, 5, 5.5, 6, and 6.5 (final volume = 14−15 mL). Each pH experiment was incubated at 30 or 55 °C for 1 h. The pH was measured again after incubation and remained unchanged. The reaction was stopped by adding 4 mol/L HCl (2.5 mL) and centrifugation. The released phosphate was determined on the supernatant fractions by using a Biotrol kit (Merck, Nogent sur Marne, France). The increase of the unreduced phosphomolybdate complex measured at 340 nm is directly proportional to the amount of inorganic orthophosphate in the sample.

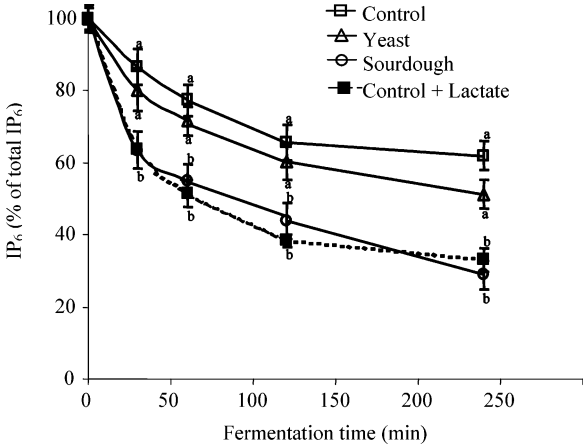

Figure 1. Kinetics of phytic acid (PA) decline in unfermented dough (□) and in dough fermented with yeast (△) or sourdough (○) during 240 min of incubation at 30 °C. An acid control dough was realized with the addition of lactic acid (■). Values are means ± SEM for four determinations at each experimental point. Different letters indicate significant differences ($p < 0.05$).

Calculations and Statistical Analysis. The percentage of Mg solubility was calculated using the following formula: % soluble/total = [soluble concentration (mg/mL) × dough moisture (mL)/dough content (g)] × 100/[total concentration (mg/g)].

Values are given as the means ± SEM; when appropriate, the significance of the differences among means was determined by one-way ANOVA coupled with the Bonferroni test (Instat, San Diego, CA). Differences with $P < 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

To evaluate the different impacts of the leavening agent on phytate degradation and on mineral bioavailability during dough fermentation, two different methods of French breadmaking (with yeast and with sourdough) were compared to a control condition without the addition of a leavening agent. As it has been shown that cooking deactivates the phytases (*6*), only the fermentation stage was studied.

Phytate Content. Figure 1 shows the changes in PA content of doughs during 4 h of fermentation. Phytic acid content decreased from the start of fermentation, as soon as the flour was hydrated. At $t = 0$, the PA contents in the different doughs were identical. After only 120 min, ~35% of PA had disappeared in the control unfermented dough, and the PA content tended to stabilize ~60% of its initial value at the end of the 4-h proofing. Because there was no addition of exogenous microorganism likely to provide phytase activity to the control dough, the degradation of PA may be the result of the activity of the endogenous phytase present in the whole wheat flour. The kinetics of PA disappearance in the yeast fermentation was not significantly different from the control condition, but PA degradation tended to be slightly more pronounced with the addition of yeast, indicating that yeast had some phytase activity. After only 30 min of fermentation, 13 and 20% of PA disappeared in control and yeast doughs, respectively, and PA

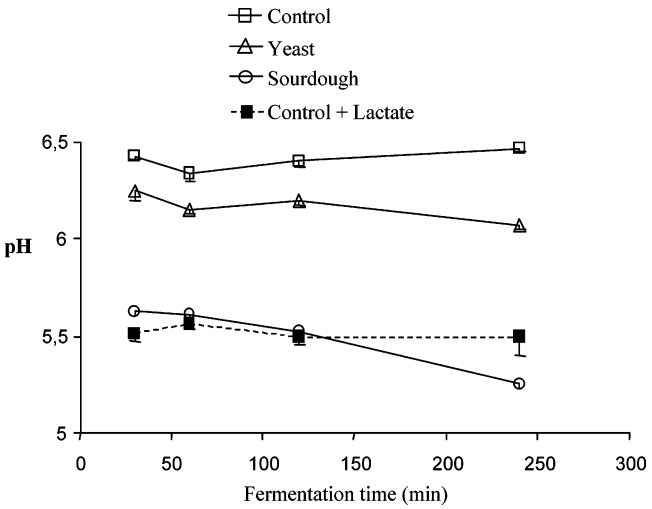

Figure 2. Changes in pH during fermentation without exogenous leavening agent (□) or in the presence of yeast (△) or sourdough (○). An acid control dough was realized with the addition of lactic acid (■).

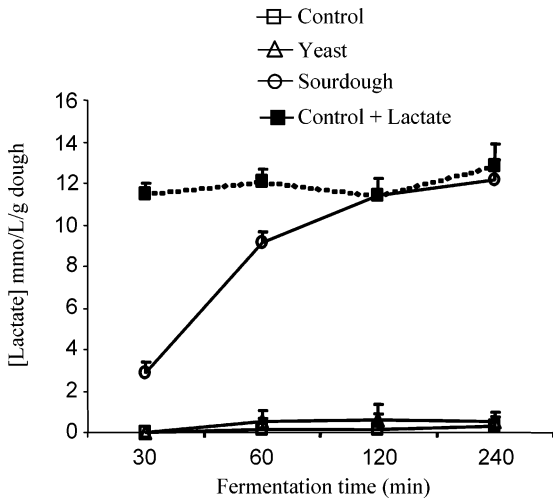

Figure 3. Changes in lactic acid concentration during fermentation without exogenous leavening agent (□) or in the presence of yeast (△) or sourdough (○). An acid control dough was realized with the addition of lactic acid (■). Values are means ± SEM ($n = 4$).

contents were stabilized during the first 4 h of incubation at around 60 and 50% of the initial content. This is in agreement with recent research indicating that the contribution of baker's yeast to phytate degradation is extremely small (*5*). The pH of the doughs was followed throughout fermentation (**Figure 2**). The initial pH of the unfermented control dough was maintained at a value of ~6.4 throughout proofing, whereas in yeast-fermented dough, the pH dropped slightly from 6.25 to 6.0. The lower pH may have favored endogenous phytase activity, which could explain the limited change of phytate content in this dough. However, we could not exclude the fact that baker's yeast slightly contributes to PA breakdown through its own phytase activity.

Whereas ~40% of initial PA flour content was hydrolyzed after 4 h of incubation without the addition of leavening agent, a similar degree of PA degradation (36%) was obtained after only 30 min of sourdough fermentation, which is significantly greater as PA degradation in control condition at the same time. At the end of the proofing period (4-h fermentation), the percentage value of remaining PA was 2 times lower in the sourdough condition than in the control condition. These results

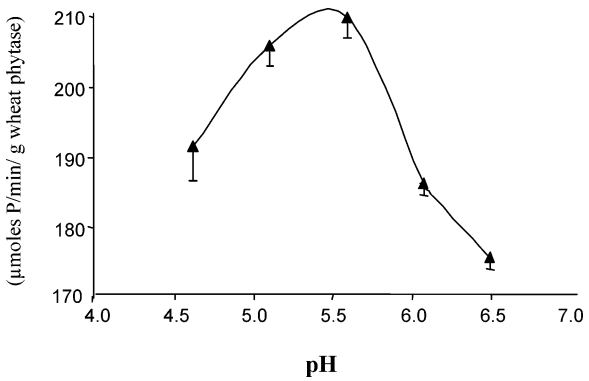

Figure 4. Effect of pH on wheat phytase activity. Sodium phytate (1.5 μmol/mL) was incubated at 55 °C (▲) for 1 h with commercial wheat phytase. The pH was adjusted with 0.2 M acetic acid. Enzymatic activity was expressed as micromoles of phosphate liberated per minute and per gram of enzyme.

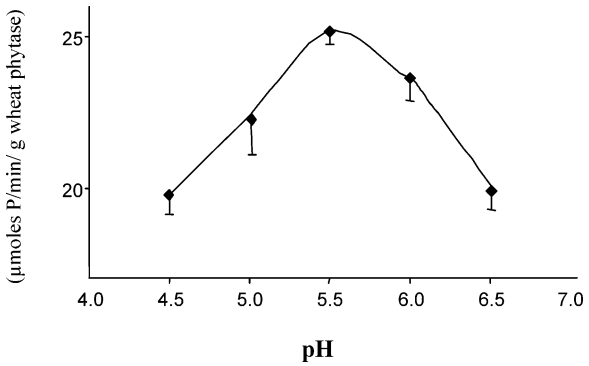

Figure 5. Effect of pH on wheat phytase activity. Sodium phytate (1.5 μmol/mL) was incubated at 30 °C (◆) for 1 h with commercial wheat phytase. The pH was adjusted with 0.2 M acetic acid. Enzymatic activity was expressed as micromoles of phosphate liberated per minute and per gram of enzyme.

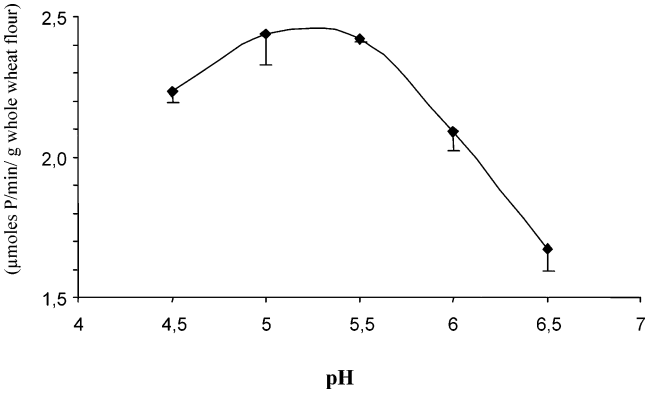

Figure 6. Effect of pH on whole wheat phytase activity. Sodium phytate (1.5 μmol/mL) was incubated at 30 °C for 1 h with whole wheat flour. The pH was adjusted with 0.2 M acetic acid. Enzymatic activity was expressed as micromoles of phosphate liberated per minute and per gram of flour.

confirm a previous work from our laboratory which showed that sourdough fermentation was more efficient than yeast fermentation in reducing the PA content of wholemeal flour (*9*). The significant difference in phytate degradation between baker's yeast and sourdough fermentation could be explained by the phytase activity of these two types of leavening agents or by their pH-lowering effect. Lactic acid bacteria isolated from sourdough have been found to express significant phytase

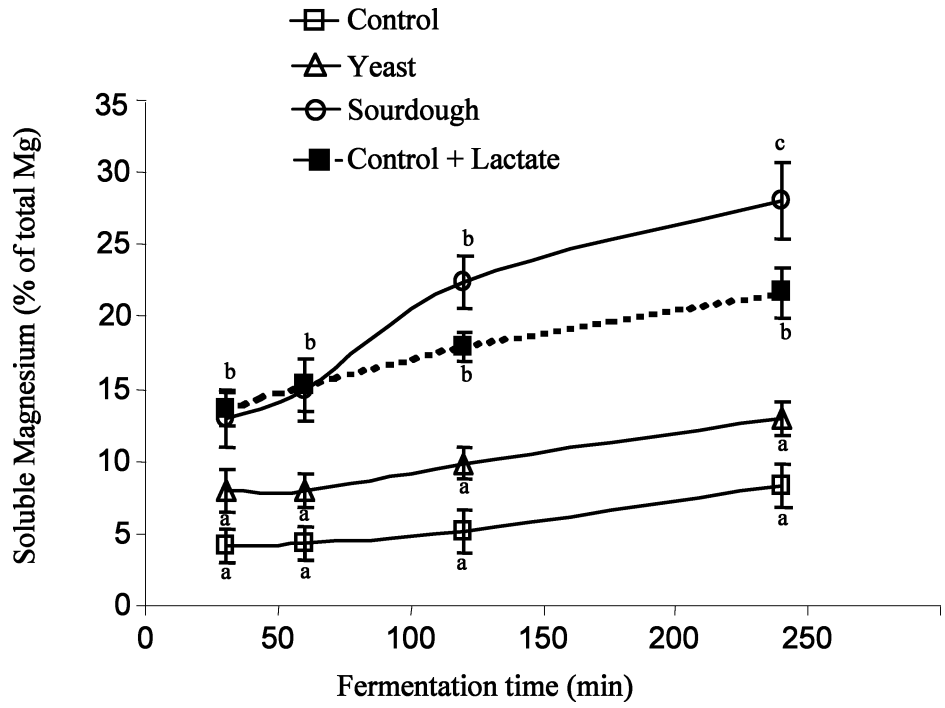

Figure 7. Kinetics of Mg solubilization in unfermented dough (□) and in dough fermented with yeast (△) or sourdough (○) during 240 min of incubation at 30 °C. An acid control dough was realized with the addition of lactic acid (■). Values are means ± SEM for four determinations at each experimental point. Different letters indicate significant differences ($p < 0.05$).

activity (*11*, *13*); on the other hand, it was suggested by an earlier study that dough pH was the main determining factor to increase PA hydrolysis in whole bread (*14*). Indeed, a pH optimum of 5.2 at 55 °C has been reported for wheat phytase (*4*). **Figure 4** demonstrates the effect of pH variation on wheat phytase activity at 55 °C. Activity was high over a fairly broad pH range of 5.0−6.0 with an optimal value at 5.5. Sodium phytate solution was also incubated with wheat phytase at 30 °C, the temperature of dough fermentation (**Figure 5**): phytase activity was 10 times lower than at 55 °C but was again maximal over the same pH range with an optimal value at 5.5. Therefore, it could be assumed that PA hydrolysis is more pronounced as the pH of the dough is closer to this critical value. The same experiment was done with endogenous whole wheat phytase at 30 °C (**Figure 6**): optimal activity occurred in a pH interval of 5−5.5. In our experiment, the pH of the sourdough varied from 5.6 to 5.5 during the first 2-h fermentation and dropped to 5.25 after 4 h (**Figure 2**). This decrease of pH is mainly due to the development of lactic acid bacteria from sourdough. This was reflected by lactic acid concentrations (**Figure 3**), whereas the acetic acid level remained low after 4 h of sourdough fermentation (2 mmol of acetate/g of dough, result not shown).

All of these data support the hypothesis that a sourdough fermentation process providing a pH value of ∼5.5 was the optimal condition for endogenous phytase activity.

To distinguish the respective impact of endogenous wheat phytase and microbial phytases on PA disappearance during sourdough fermentation, a new condition of fermentation was tested: control dough was directly acidified with organic acid and left to proof at 30 °C. **Figure 1** shows the kinetics of PA degradation after acidification of the wholemeal dough by exogenous organic acid. Because lactobacilli are the predominant microorganisms in sourdough and are mostly responsible for the dough acidification, lactic acid was chosen to decrease the pH. The amount of acid added in control unfermented dough was adjusted to the lactic acid concentration of the sourdough sample at the end of the fermentation and induced a dough pH value corresponding to the optimal pH of the wheat phytase. From the beginning of the incubation, the extent of PA hydrolysis was significantly higher in the lactic-acidified dough compared with the control dough: after only 30 min, PA reduction in the acidified dough was ∼20% higher than in the control dough, and after 4 h of incubation, acidification resulted in a 67% PA destruction, whereas in the control condition, only 38% PA was degraded. Moreover, the kinetics of PA disappearance during natural lactic fermentation (sourdough condition) and during incubation of the chemically acidified dough were not significantly different. The PA content of wholemeal flour diminished in an equal proportion in both conditions from the beginning (after 30 min) to the end (4 h) of the incubation period. Thus, the same PA degradation that was observed during sourdough fermentation can be reproduced with an exogenous acidification. It must be noted that the use of another organic acid (acetic acid) gave the same effect as lactic acid on PA breakdown (data not shown). It can be concluded that a moderate acidification of the dough is sufficient to induce a significant phytate breakdown. Because a simple addition of organic acid largely reproduced the effect of sourdough, it seems to be important to select wheat varieties for their level of phytase activity.

Magnesium Solubilization. Phytic acid is principally localized in aleurone cells, where it is mainly present as a mixed magnesium−potassium salt of PA. **Figure 7** shows the kinetics of magnesium (Mg) solubilization in the different conditions of fermentation studied. The proportion of soluble Mg was significantly higher after the sourdough fermentation in comparison with the yeast and the control conditions. These results are in coherence with the kinetics of PA breakdown and confirm that the PA content of wholemeal breads affects mineral availability. However, it must be emphaszied that two factors can contribute to solubilize magnesium: the decrease of pH, which weakens the ionic bonds between the cation magnesium

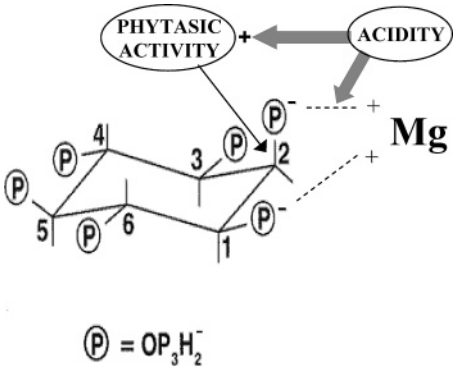

Figure 8. Possible mechanism for Mg solubilization.

and the anion phosphate of the PA molecule, and the hydrolysis of phytic acid into lower inositol phosphates by phytases (**Figure 8**). The effect of the pH on magnesium solubilization was immediately observed in unfermented dough after the addition of exogenous acid (**Figure 7**). During the first hour of incubation, acidification gave the same results on Mg solubilization as the use of sourdough, for similar dough pH values. After 4 h of fermentation, there was a greater decrease of pH with the sourdough fermentation, which induced a greater solubilization of Mg, whereas PA hydrolysis remained the same in both conditions. As Mg absorption is mainly influenced by Mg solubility (*15*), it can be concluded that sourdough fermentation improves Mg bioavailability both by direct solubilization of the cation and by phytate hydrolysis.

Conclusions. The current trend in the baking industry to produce bread without chemical additives has created an interest in using wheat sourdough to improve bread texture and flavor. The present work confirms that sourdough fermentation also improves the nutritional properties of wheat bread by reducing amounts of phytates and shows that phytate breakdown is mainly explained by the acidity level in the sourdough process, which promotes the greater wheat phytase activity. As it is mainly colocalized with PA in aleurone cells, endogenous phytase could hydrolyze phytates in only intact bran structure. However, it cannot be ruled out that some aleurone cells have been damaged or that phytates have been partly solubilized at acid pH, becoming accessible to exogenous microbial phytases.

Previous studies have shown that sourdough fermentation resulting in a whole wheat dough pH of ~4.5 led to an almost complete PA hydrolysis (*14*). However, most consumers dislike the acidic taste of sourdough breads. The present work shows that a slight acidification of the dough, which does not alter the sensory properties of the bread (pH 5.5), was also optimal for the activity of the wheat phytase at the temperature of dough fermentation, and effectively reduced the phytic acid content. As mixed starters with both lactic acid bacteria and yeast are recommended for an aromatic and pleasant sourdough bread flavor, an efficient fermentation procedure combining a minimal proportion of sourdough (or dough from the precedent breadmaking) with baker's yeast could be a good compromise to achieve breads with high mineral bioavailability.

ABREVIATIONS USED

PA, phytic acid; IP_6, inositol hexakisphosphate; SEM, standard error of the mean.

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