Prolonged Fermentation of Whole Wheat Sourdough Reduces Phytate Level and Increases Soluble Magnesium

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This work was designed to compare the effects of different leavens (yeast, sourdough, and a mixture of both) on phytic acid (PA) degradation and to assess the repercussions of PA breakdown on phosphorus and magnesium solubility during bread-making. Sourdough fermentation was more efficient than yeast fermentation in reducing the phytate content in whole wheat bread (−62 and −38%, respectively). Furthermore, lactic acid bacteria present in sourdough enhanced acidification, leading to increased magnesium and phosphorus solubility. To intensify phytate breakdown, bran was incubated with microorganisms (yeast or sourdough) before bread-making. Using this new method, the percentage of phytate breakdown was near 90%, whereas 40% of phytate remained in traditional French bread. In conclusion, a prolonged fermentation with sourdough still leads to improved Mg and P solubility by decreasing phytate content and through acidification.

Keywords: *Bioavailability; lactic acid bacteria; magnesium; phosphorus; phytic acid*

INTRODUCTION

The consumption of whole grain products has become more popular due to increasing awareness of their nutritional benefits. Whole cereal products can protect against lipid peroxidation (*1*), colon cancer (*2*), and an accumulation of hepatic lipids (*3*) thanks to fibers and phytic acid (PA). Moreover, the intake of PA relates negatively with the glycemic index of normal individuals (*4*). Even if whole cereals are also important sources of minerals such as K, P, Mg, Fe, or Zn, mineral utilization is limited by the presence of PA (*5*). PA is highly charged with six phosphate groups, and it forms insoluble complexes with dietary cations, thus hindering their intestinal absorption (*6*). Mineral bioavailability in whole grain wheat can be improved by the action of phytase, a phosphomonoesterase capable of hydrolyzing PA to free inorganic phosphate and low *myo*-inositol phosphate esters. Phytate-degrading enzymes exist in cereals (*7*), yeast (*8*, *9*), and lactic acid bacteria isolated from sourdough (*10*−*12*). During bread-making, PA is hydrolyzed by phytases (*13*, *14*), and the reduction of PA contents in different bread types may vary between 13 and 100%. The highest levels of PA remained in unleavened breads (*15*). Various factors contribute to phytate destruction in bread, including temperature, pH, water, and fermentation time (*16*). Different additives (organic acids or calcium salts) can also influence the extent of phytate hydrolysis by altering the pH of the dough or precipitating insoluble phytate complexes (*17*, *18*). Because the consumption of whole grain breads is increasing in France and whole grain flours are high in phytate, a whole wheat bread with increased mineral bioavailability would be beneficial. This can be accomplished by decreasing the PA content of French bread to levels that do not affect mineral absorption.

This work was designed to compare the effects of different leavens (yeast, sourdough, and a mixture of both) on PA degradation and to assess the repercussions of phytate breakdown on phosphorus and magnesium solubility during bread-making. In addition, a new method for making French bread was used to intensify PA degradation in bread-making.

MATERIALS AND METHODS

Starter Culture. Doughs were purchased from regional bakers known for their sourdough bread taste. For each sourdough, a 10% suspension of dough was prepared in trypton salt (Merck, Darmstadt, Germany). Then different lactic acid strains were isolated in MRS medium plus maltose (20 g/L) and cycloheximide (200 mg/L) and in Mayeux medium (only for dextrane-producing *Leuconostoc mesenteroides*) salt (Merck). These media were inoculated with 1 mL of the 10% suspension and the mixtures held for 48 h at 30 °C. After several purifications on MRS agar plus maltose, gram coloration, catalase test, and 45 °C growth were performed in each strain. Pure strains were identified by API 50CH kits (BioMérieux, Charbonnières-les-Bains, France),and the profiles obtained were analyzed by BioMérieux for identification. Pure strains were maintained in MRS agar; these were *Lactobacillus plantarum* S18 (from rye sourdough) and *Leuconostoc mesenteroides* subsp. *mesenteroides* S50 (from rye sourdough).

Bread-Making Procedure. Sourdoughs were prepared by mixing white wheat flour (2 volumes), distilled water (1 volume), and a mixture of *La. plantarum* S18 and *Le. mesenteroides* subsp. *mesenteroides* S50 (10^9 colony forming units/g of flour for each bacterium). The resultant dough was incubated for 18 h at 30 °C before it was incorporated to bread-making.

Six varieties of breads were prepared, following recipes described in Table 1. For the first three conditions (yeast,

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Table 1. Recipes Used To Prepare Whole Wheat Breads[a]

	yeast	sour-dough	sourdough + yeast	4 h water[b]	4 h yeast[b]	4 h sour-dough[b]
white flour	1500	1100	1100	1500	1500	1100
bran	450	450	450	450	450	450
water	1200	1000	1000	1200	1200	1000
salt	40	40	40	40	40	40
yeast	50		4	50	50	
sourdough		600	600			600

[a] Milled grain was separated into bran and flour. [b] In these breads, 450 g of bran was incubated at 30 °C for 4 h in the presence of water (500 mL), water (500 mL) + yeast (50 g), or sourdough (600 g). After this period, wet bran was incorporated into the process and mixed with other ingredients.

sourdough, and sourdough plus yeast), the ingredients were mixed for 5 min (first sample). Then, the resultant dough was left at 30 °C to start fermenting (second sample). It was then kneaded again to develop gluten, and it was left to proof at 30 °C. The dough was baked after 5 h of fermentation. During the 5 h fermentation, quadruplicate samples of dough were taken every 30 min and were immediately stored at −80 °C. Supernatant fractions on the doughs were obtained by centrifugation at 20000*g* for 10 min at 4 °C.

For the other conditions (4 h water, 4 h yeast, and 4 h sourdough), a new method for French bread making was tried: 350 g of coarse bran was incubated at 30 °C for 4 h in the presence of water (500 mL), water (500 mL) plus yeast (50 g), or sourdough (600 g). After this soaking, bran dough was incorporated into the process and mixed with other ingredients (white wheat flour, remaining water, and salt) for 5 min (first sample). The resultant dough was left at 30 °C to start fermenting (second sample). It was then kneaded again to develop gluten and left to proof at 30 °C. The dough was baked after 5 h of fermentation. During the 5 h fermentation, quadruplicate samples of dough were taken every 30 min and were immediately stored at −80 °C. Supernatant fractions on the doughs were obtained by centrifugation at 20000*g* for 10 min at 4 °C.

Determination of pH and Total Amount of Titratable Acids (TTA). The pH and the TTA were determined every 30 min with a Delta 320 pH meter (Mettler, France). A sample (10 g) was mixed with distilled water (90 mL). The mixture was then titrated with NaOH (0.1 M) with stirring to pH 8.5. The total titratable acidity was expressed as the amount of NaOH consumed, in milliliters.

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.

Phytic acid was determined using HPLC (Dionex, Sunnyvale, CA) as described previously (*11*). The HPLC system consisted of a gradient Dionex 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 for conductivity detection. Samples (routinely 2000 mg) were extracted with 40 mL of HCl (0.65 mol/L) under vigorous mechanical agitation (Ika-Werk HS 500, Staufen, Germany) for 4 h at room temperature. The extracts were centrifuged at 5000*g*, and 2 mL supernatant was diluted to 10 mL with deionized water (Millipore water system). The diluted supernatant was passed through a 200−400 mesh AG 1-X8 chloride anion-exchange column (Bio-Rad, Richmond, CA). The columns were washed with 15 mL of HCl (0.025 mol/L), and phytic acid was eluted from the resin with 15 mL of HCl (2 mol/L). The eluates were evaporated under vacuum at 50 °C (evaporator concentrator, Jouan SA, St Herblain, France) and resuspended in deionized water. Potassium phytate (Sigma, St. Louis, MO) was used as the reference calibration standard.

Mg and P were determined on the supernatant fractions (soluble) and on the untreated doughs (total) after dry-ashing (10 h at 500 °C) and extraction at 130 °C in HNO_3/H_2O_2 (2:1) (Merck, Suprapur) 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. Appropriate quality controls were analyzed with each set of measuments. Phosphorus was determined by using a Biotrol kit (Merck, Nogent sur Marne, France). The increase in formation of the unreduced phosphomolybdate complex measured at 340 nm is directly proportional to the amount of inorganic orthophosphate in the sample.

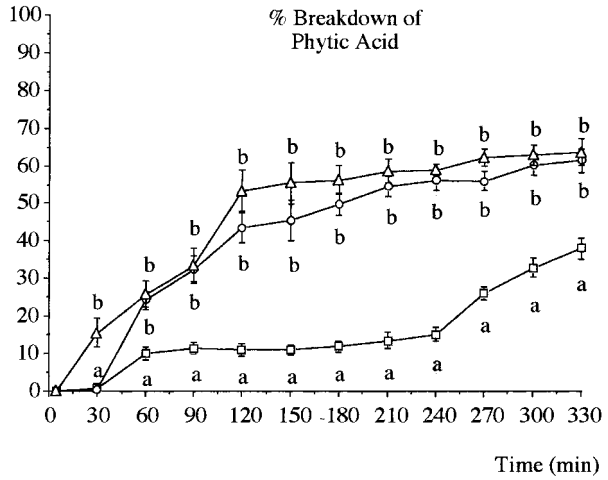

Figure 1. Decline of PA during conventional fermentation (from 5 to 300 min) and cooking (330 min): (□) yeast; (○) sourdough; (△) sourdough plus yeast. Values are means ± SEM for four determinations at each experimental point. Different letters indicate significant differences ($p < 0.05$).

Calculations and Statistical Analysis. For Mg and P, the percentage of solubility was calculated using the following formula:

$$\%\ \text{soluble} = [\text{soluble concentration (g/L)} \times \text{dough moisture (L)}] \times [\text{total concentration (g/kg)} \times \text{dough content (kg)}]$$

Values are given as the means ± SEM; when appropriate, significance of the differences among means was determined by one-way ANOVA coupled with the Student−Newman−Keuls test (StatView, Abacus, Berkeley, CA). Differences between groups were considered to be significant if $p < 0.05$.

RESULTS AND DISCUSSION

To compare the effects of different types of leavening agent on phytate degradation and on mineral bioavailability (P and Mg) during bread-making, three different conditions, yeast, sourdough, and sourdough plus yeast, were performed.

PA Breakdown, Dough Acidification, and Mineral Solubility during Classical Bread-Making. Changes in PA contents in doughs were determined during fermentation (from 5 to 300 min) and baking (330 min). Figure 1 shows that the loss of phytate was relatively rapid at the beginning of the fermentation. At time 0, the PA contents in the different doughs were identical. After only 60 min of fermentation, 10% of PA had disappeared in yeast fermentation, whereas the PA breakdown in sourdough was near 25%. At the end of the fermentation (5 h of fermentation), the destruction of PA remained greater after sourdough fermentation than that observed in yeast fermentation (62 and 38%, respectively). Therefore, sourdough fermentation was more efficient than yeast fermentation in reducing the PA content in bread. It must be noted that baking after

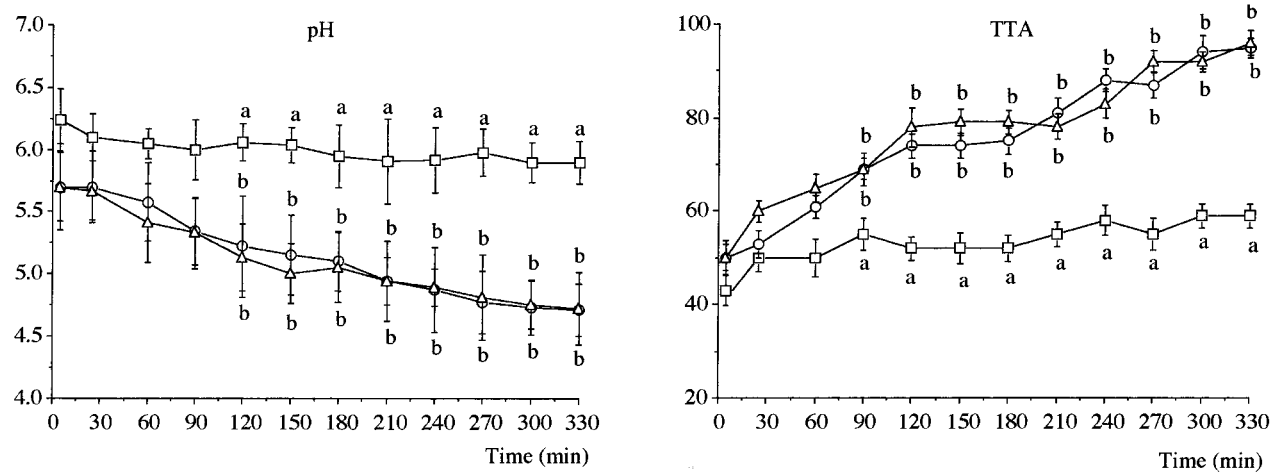

Figure 2. Changes in pH and TTA, expressed in milliliters of NaOH added to obtain pH 8.5, during conventional fermentation (from 5 to 300 min) and cooking (330 min): (□) yeast; (○) sourdough; (△) sourdough plus yeast. Values are means ± SEM for four determinations at each experimental point. Different letters indicate significant differences ($p < 0.05$).

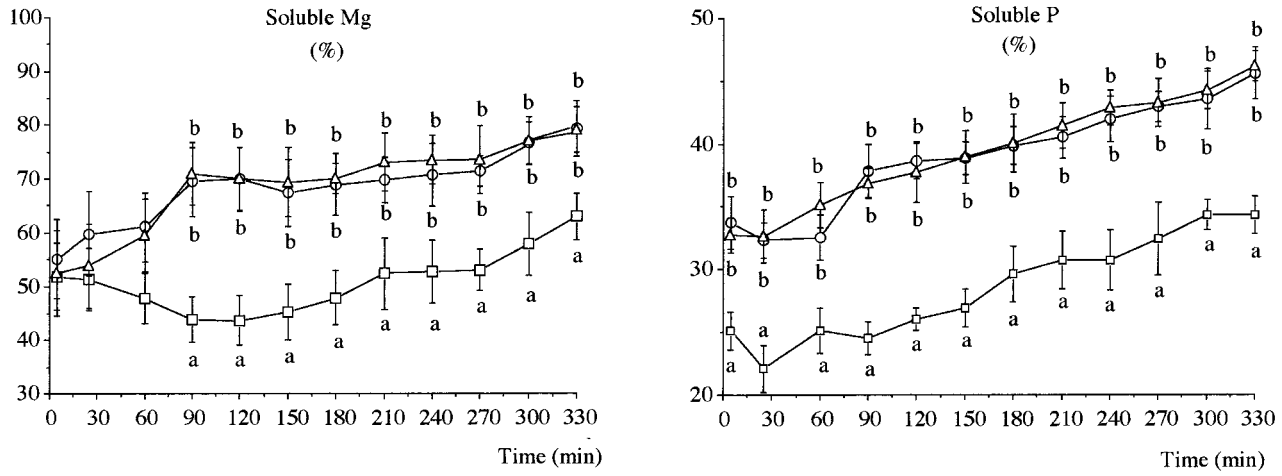

Figure 3. Changes in soluble Mg and P during conventional fermentation (from 5 to 300 min) and cooking (330 min): (□) yeast; (○) sourdough; (△) sourdough plus yeast. Values are means ± SEM for four determinations at each experimental point. Different letters indicate significant differences ($p < 0.05$).

fermentation (data at 330 min) did not affect the PA content in yeast and sourdough fermentations. Therefore, in the present conditions of bread-making, no phytate degradation took place after fermentation. The third fermentation was characterized by the presence of 0.2% yeast in bread made with sourdough. This condition was tested because French legislation allows the maximum addition of 0.2% yeast in sourdough bread-making. It was also interesting to assess the relative importance of the two microorganisms in the same dough. The kinetics of PA disappearance were similar in sourdough and in sourdough plus yeast fermentations. The addition of yeast into sourdough appeared to have no influence on phytate degradation. Thus, in the same dough containing yeast and lactic acid bacteria, the sourdough fermentation seemed to be predominant in PA reduction. The pH of dough and bread is of great importance for phytate degradation. Solubilities of the PA chelates with cations depend on the pH and the amounts and kinds of cations (*6*). The barrier against phytate destruction in whole wheat dough above pH 6 is the insolubility of its Mg salt, whereas at pH 5 the limiting factor appears to be the level of phytase (*19*). Because the binding properties of PA are pH-dependent, pH and TTA during fermentation are reported in Figure 2. The incorporation of sourdough in bread-making caused a pH decrease, whereas the pH of yeast fermentation remained constant: after only 60 min, a significant difference between these two conditions was observed. At the end of fermentation, the pH was significantly higher for yeast fermentation than after sourdough fermentation (6 and 4.7, respectively). In parallel, the dough TTA at time 0 varied between 40 and 50 mL of NaOH added to pH 8.5. If the TTA in the yeast fermentation was still near 55 mL of NaOH, TTA increased to 90 mL of NaOH in the lactic acid bacteria fermentation. Both acidification and destruction of PA led to increased Mg and P solubilities (Figure 3). The proportions of soluble P and Mg were significantly higher after the sourdough fermentation in comparison to the yeast fermentation.

PA Breakdown, Dough Acidification, and Mineral Solubility during a New Method of Bread-Making. To intensify PA degradation in bread-making, a new method for French bread was performed: bran was incubated at 30 °C for 4 h in the presence of water, water with yeast, or sourdough. After this period, the bran dough was mixed with the other bread-making ingredients (white flour, remaining water, and salt) for 5 min. It must be noted that any significant difference between the 4 h fermentation of bran with water alone and with yeast had no observable effect on PA content

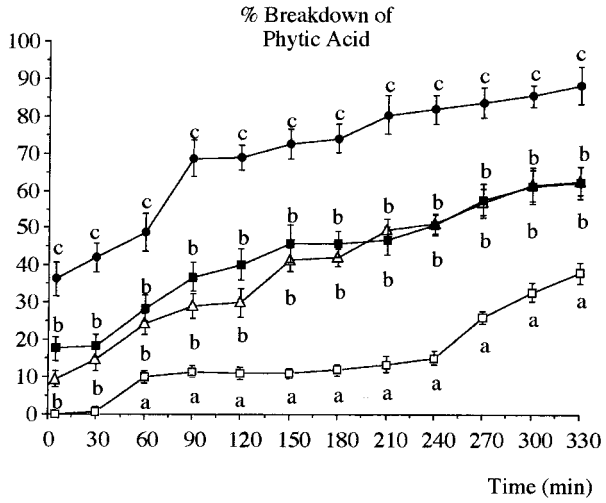

Figure 4. Decline of PA during new method for bread-making: (□) yeast; (△) 4 h water; (■) 4 h yeast; (●) 4 h sourdough. Values are means ± SEM for four determinations at each experimental point. Different letters indicate significant differences ($p < 0.05$).

(Figure 4) Thus, under these new conditions, the addition of baker's yeast in bread-making did not play a major role in PA breakdown. These data are in agreement with Harland and Frölich (*20*), who found no activity in Swedish yeast and only a small effect on phytate level for Norwegian yeast. Recent research indicates that baker's yeast does contribute to phytate degradation during bread-making, but the contribution is evidently extremely small (*9*). Because the phytase activity from baker's yeast was low, the main degradation of PA was a result of activity by endogenous phytase present in the flour. However, vegetal phytase seemed to be minor, especially compared to the phytase activity of the lactic acid bacteria. Indeed, the best PA destruction of the six conditions tried in the present study was the 4 h fermentation of bran with sourdough before the start of the bread-making. At 300 min, the percentage of PA destruction was close to 90% in this condition, whereas 40% of PA remained in 4 h water and 4 h yeast doughs. Our recent results show that fermentation of whole wheat flour using *La. plantarum* S18 (from rye sourdough) or *Le. mesenteroides* subsp. *mesenteroides* S50 (from rye sourdough) improves PA hydrolysis (*11*). Bread-making experiments confirm that these two strains have the ability to degrade PA in whole wheat products. The changes in pH and TTA show that only sourdough fermentation reduces the pH. Other conditions were far less effective (Figure 5). The low pH induced by lactic acid bacteria may actually be

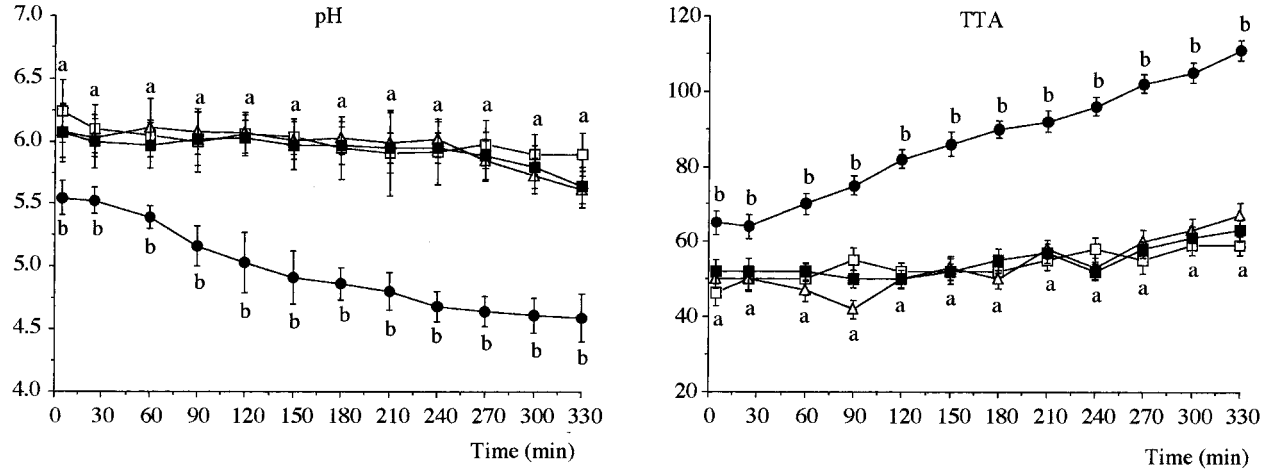

Figure 5. Changes in pH and TTA, expressed in milliliters of NaOH added to obtain pH 8.5, during new method for bread-making: (□) yeast; (△) 4 h water; (■) 4 h yeast; (●) 4 h sourdough. Values are means ± SEM for four determinations at each experimental point. Different letters indicate significant differences ($p < 0.05$).

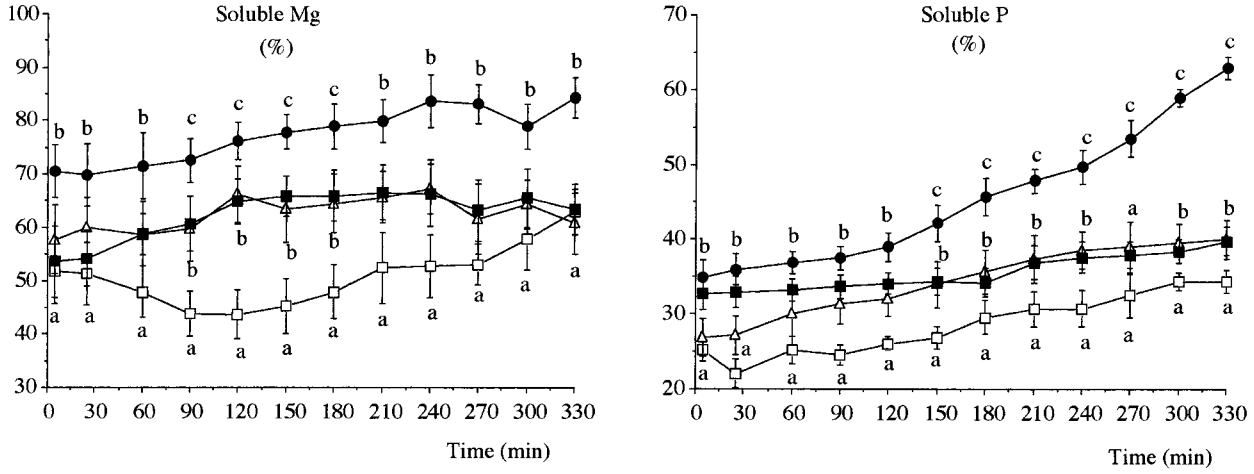

Figure 6. Changes in soluble Mg and P during new method for bread-making: (□) yeast; (△) 4 h water; (■) 4 h yeast; (●) 4 h sourdough. Values are means ± SEM for four determinations at each experimental point. Different letters indicate significant differences ($p < 0.05$).

the optimum pH for the endogenous phytase. Indeed, plant phytases have pH optima at 4.8−5.5, and their activities diminish markedly as the pH is varied from its optimal value (*21*). Even if microbial phytases seem to be less sensitive to pH and are active over a wider pH range than endogenous phytases (*22*), PA destruction observed in sourdough bread appears to be due to synergic effects of both kinds of phytase. An additional factor, the low Ca content of the bread, also may have contributed to the optimal conditions for phytase. Enzyme activity is more efficient when phytic acid is in free form, that is, not bound to divalent cations. The formation of PA−metal complexes is favored by the binding of Ca to PA (*23*). Thus, the lack of added Ca in French bread enhanced PA degradation and the availability of phytate-P as well as mineral solubility. The low level of PA as well as the acidification of dough increased the solubility of Mg and P in 4 h sourdough conditions, compared to other conditions (Figure 6). The increased solubility of P was due to PA hydrolysis in dough. Indeed, during bread-making, phytate breakdown leads to less phosphorylated inositol phosphates and free P that can be absorbed. In many developed countries, the consumption of foods that are practically devoid of minerals has increased. For this reason, the usual diets may no longer meet the recommended dietary Mg allowances of numerous subjects. Breads made with wholemeal or high extraction flour are rich in both Mg and PA. Some authors found an inhibitory effect of PA on Mg bioavailability (*24*). By degrading PA and through acidification, sourdough bread-making improves Mg solubility. As mineral solubility is one of the principal determinants of Mg absorption, Mg bioavailability is high in fermented cereal products. As for Mg, trace element bioavailability from whole wheat products may be changed by the new method of bread-making. Indeed, the chelating effects of PA on Zn or Fe utilization have often been reported (*25*, *26*). Although the beneficial effects of organic acids on Fe absorption are well established (*27*), a prolonged fermentation with sourdough eliminates PA and produces lactic acid, improving mineral absorption.

Even if the highest rate of phytate reduction is at the beginning of fermentation, the results of PA determinations show that its hydrolysis during bread-making by rapid processes is indeed less extensive than after a long fermentation process. Unfortunately, the duration of the normal French bread fermentation (between 90 and 120 min) was insufficient to improve PA degradation. To eliminate PA in whole breads, a 4 h fermentation of bran before bread-making would be more effective, especially when bran is incubated with sourdough. Moreover, the choice of *Saccharomyces cerevisiae* rather than sourdough or a mixture of both by bakers should be reassessed with the goal of improving mineral bioavailability.

Conclusion. In conclusion, effective reduction of PA in bread-making can be obtained via sourdough fermentation or prolonged fermentation time. This reduction of phytate content to very low levels would make the wholemeal breads a good source of soluble phosphorus and magnesium. Future animal or human experiments should confirm if this higher mineral solubility observed in breads has repercussions on P and Mg utilization by the body.

ABBREVIATIONS USED

MRS; PA, phytic acid; TTA, total titratable acidity.

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