Phytic Acid. III. Two Barriers to the Loss of Phytate During Breadmaking[1]

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ABSTRACT

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Phytate phosphorus (Pp) decreased linearly with time in a 2% yeasted, whole wheat dough at pH 4 during 0–6 hr of fermentation. After 6 hr, 70% Pp was lost. During fermentation at pH 6, Pp decreased linearly for 3 hr; then its rate of loss declined. During the first 3-hr fermentation period at pH 6, 34% Pp was lost, but only 9% more was lost after an additional 3 hr. In a fermenting dough, Pp was lost almost twice as rapidly at pH 5 as at pH 4 or 6. The solubility of sodium phytate in aqueous solution containing magnesium, calcium, iron, and zinc ions depended strongly on pH; 75% of phytate was insoluble between pH 6 and 7, whereas 15% was insoluble at pH 5. The barrier to destroying phytate in whole wheat dough above pH 6 is the insolubility of its magnesium salt, whereas at pH 5 the limiting factor appears to be the level of phytase.

We previously reported (Tangkongchitr et al 1981a) an analytical scheme to measure phytate phosphorus (Pp), inorganic phosphorus (Pi) and phosphorus not precipitated by ferric ion (Ps) in flour, dough, and bread. The cumulative loss of Pp in pup loaves made from freshly milled whole wheat was ~16, 19, and 22% after fermentation, proofing, and baking, respectively (Tangkongchitr et al 1981b). We also found that the loss of Pp was equal to the gain in inorganic phosphate; this indicated that no intermediate phosphate esters of inositol accumulated in the dough or bread.

Our data on the rate of loss of Pp during yeast fermentation and baking led us to speculate that the limited destruction of phytate in whole wheat bread is due to insolubility of phytate in a dough at ~pH 6.

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In the work we report here, we studied the effect of pH on the rate of destruction of phytate in whole wheat dough. In addition, we used a model system to mimic the aqueous phase of a dough and examined the solubility of phytate in the presence of the major divalent and trivalent mineral ions that occur in whole wheat flour. We also determined whether phytate is deesterified in the presence of fermenting yeast cells in a liquid brew containing no flour. Finally, we have proposed a mechanism for the destruction of phytic acid in wheat dough.

MATERIALS AND METHODS

General

Whole wheat flour (WW) was pin milled from a commercial blend of hard winter wheats. Magnesium chloride, calcium chloride, zinc sulfate, and ferric chloride were analytical reagent grade. Pp and Ps were determined as described by Tangkongchitr et al (1980a). All analyses were done in duplicate. The pH of a dough or bread was determined by a standard procedure (AACC 1961).

Effect of pH on Loss of Phytate Phosphorus in 2% Yeasted Doughs

Seven doughs were prepared by optimally mixing, for each dough, 100.0 g (14% mb) of WW, 2.0 g of compressed yeast, and 70.0 ml of water in a pin mixer. The doughs were fermented at pH 6 for 0–6 hr at 30° C and 90% rh. At 1-hr intervals, a dough was removed from the cabinet, quickly frozen, and freeze-dried. The pH of each dough was measured, and Pp and Ps were also determined.

A second set of three doughs was prepared using 65.0 ml of water instead of 70.0 ml. The three doughs were mixed to optimum and fermented 3 hr at pH 6.0. The three doughs were removed from the fermentation cabinet, and the pH of each dough was adjusted to 4.0 by remixing for 2 min with 5.0 ml of 34% aqueous lactic acid. The doughs were fermented for an additional 1, 2, or 3 hr; then, a dough was freeze-dried and analyzed for Pp, Ps, and pH.

A third set of three doughs was mixed to optimum, using 67.0 ml of water instead of 70.0 ml. These doughs were fermented for 3 hr at pH 6 and then were remixed to pH 5 after adding 3.0 ml of 17.2% aqueous lactic acid. The doughs were fermented an additional 1, 2, or 3 hr, freeze-dried, and assayed for Pp, Ps, and pH.

Loss of Phytate Phosphorus

From Doughs Fermented at pH 4. Seven whole wheat doughs were mixed initially to pH 4.0, using 100.0 g (14% mb) of whole wheat flour, 2.0 g of compressed yeast, 65.0 ml of water, and 5.0 ml of 34% aqueous lactic acid. The doughs were fermented 0–6 hr, and after each hour a dough was removed, freeze-dried, and assayed for Pp and Ps. The pH of each dough was also recorded.

From Doughs Fermented 5 Hr at pH 3.7–6.0. Seven whole wheat doughs were mixed at pH 3.7–6.0, using various amounts of 17% aqueous lactic acid. Adding 0, 2.0, 4.0, 6.0, 8.0, 10.0, and 13.0 ml of 17% lactic acid with 70.0, 68.0, 66.0, 64.0, 62.0, 60.0, and 57.0 ml of water, respectively, gave doughs with pH 6.0, 5.5, 4.8, 4.5, 4.2, 4.0, and 3.7, respectively. After 5 hr of fermentation, doughs were freeze-dried, and the pH, Pp, and Ps of each were measured.

Precipitation of Phytate from Aqueous Solution by Minerals that Occur in Dough

The precipitation of phytate by magnesium, calcium, zinc, and ferric ions was examined in water 25 ± 2° C between pH 1 and 9. Ten milliliters of sodium phytate solution (3.55×10^{-2} *M*) and 10.0 ml of solution containing one of the following metallic salts was added to preweighed 40-ml centrifuge tubes at the concentrations given: magnesium chloride, 1.36×10^{-1} *M*; calcium chloride, 2.78×10^{-2} *M*; zinc sulfate, 1.03×10^{-1} *M*; and ferric chloride, 2.6×10^{-4} *M*. Each mixture was stirred, and its pH was adjusted between 1 and 9 by adding either 36 *M* hydrochloric acid or 10 *M* sodium hydroxide solution. Adding acid or alkali never changed the volume of the mixture more than 2.5%. Blank solutions of pH 1–9 were also prepared without phytate. After a mixture stood for 24 hr at 25 ± 2° C, any precipitated salt was collected by centrifuging for 30 min at 2,600 × *g*. The supernatant was decanted, and the precipitate was freeze-dried and weighed. Each metallic ion and Pp in the precipitate was measured after wet digestion with a mixture of concentrated sulfuric acid (1 ml) and nitric acid (3 ml).

A second precipitation experiment on phytate was done as described, except at ~1.6 times the concentration of Pp and 1.6 times the concentration of the four metallic ions used in the more dilute system.

In a third precipitation experiment, done between pH 1 and 9, we used sodium phytate in the combined presence of Mg, Ca, Zn, and Fe. In this experiment, the concentration of sodium phytate in the final reaction mixture (2.14×10^{-2} *M*) was slightly higher than the concentration of 1.77×10^{-2} *M* used in the first precipitation experiment. In this third experiment, 4.0 ml of 1.07×10^{-1} *M* phytate was mixed with 4.0 ml of each of the following salt solutions: magnesium chloride, 3.39×10^{-1} *M*; calcium chloride, 6.95×10^{-2} *M*; zinc sulfate, 2.58×10^{-3} *M*; and ferric chloride, 6.49×10^{-3} *M*. The total volume in each tube was 20 ml, and pH was adjusted using 36 *M* hydrochloric acid and 10 *M* sodium hydroxide. Blank solutions, also mixed between pH 1 and 9, consisted of the four salt solutions but no sodium phytate. All reaction mixtures were allowed to stand for 24 hr, and precipitates were collected as described.

Composition of Phytate Precipitated with Magnesium or Calcium Ion

An accurately weighed sample of water-insoluble magnesium or calcium phytate (7–10 mg) was wet-digested in a mixture of concentrated sulfuric acid (1 ml) and nitric acid (3 ml), and the digest was made to volume (100 ml) with water. Pp was determined as described previously, and magnesium and calcium were determined by atomic absorption spectroscopy. The standard solutions of magnesium (0.1–2.0 ppm) were prepared as follows. Dodecasodium phytate (~15% H_2O) (~15.0 g) (Sigma Chemical Co., St. Louis, MO) was dissolved in 100 ml of water, converted to phytic acid by passage through a strongly acidic ion-exchange resin in the hydrogen ion form, and the effluent made to volume (250 ml) with water. The solution was assayed for Pp and was found to be 25.3m*M* in phytic acid (0.152*M* in Pp). An aliquot (1 ml) of the phytic acid solution was digested in a mixture of concentrated sulfuric acid (1 ml) and nitric acid (3 ml), and the digest was dissolved in water (50 ml) and added to a known amount of magnesium chloride. The mixture was made to volume (100 ml) with water, and the solution used as a standard solution of magnesium ion. In a similar manner, standard solutions of calcium (1.0–7.0 ppm) were prepared using calcium chloride. To nullify the effect of orthophosphate on calcium during measurement in the spectrometer, 0.5% of lanthanum oxide was added to the standard solutions and to the unknowns (Schrenk 1975).

Phytate Hydrolysis in Fermenting Brew at pH 5

A premix solution of yeast nutrients, prepared as described by Ling and Hoseney (1977), contained ammonium acetate, 3.36×10^{-2} *M*; magnesium sulfate, 6.29×10^{-3} *M*; potassium chloride, 8.94×10^{-3} *M*; thiamine hydrochloride, 8.6×10^{-4} *M*; pyridoxine hydrochloride, 1.62×10^{-5} *M*; and citrate buffer (pH 5) at 2.93×10^{-3} *M*. Three identical brews were mixed; each contained the premix solution (9.0 ml), 0.128*M* dodecasodium phytate (1.5 ml), sucrose (0.75 g), and compressed yeast (1.0 g). The initial quantity of Pp in the brew mixture was 35.7 mg. Immediately after mixing, each brew was placed in the Gasograph 12 apparatus (D & S Instrument Ltd., Pullman, WA) to monitor carbon dioxide production. A brew was removed from the Gasograph after 0, 3, and 5 hr of fermentation at 30° C, held 3 hr at −78° C, and stored 8 hr at −20° C. After thawing, a brew was centrifuged at 2,000 × *g*, and the supernatant was decanted and filtered by gravity through Whatman No. 2 filter paper. The clear supernatant was made to volume (100 ml) with water, and an aliquot (2.0 ml) was analyzed for Pp (Tangkongchitr 1981a).

RESULTS AND DISCUSSION

When a yeasted dough (2%) made from whole wheat flour and water is allowed to ferment, Pp is lost from the dough, principally by enzymic hydrolysis of its phosphate groups. The hydrolysis is mediated by phytase, which is present in wheat flour (Tomlinson and Ballou 1962; Lim and Tate 1971, 1973). No phytase enzyme is associated with yeast cells as proposed by Reinhold et al (1974) and Harland and Harland (1980). The data in Table I shows no hydrolysis of sodium phytate in an actively fermenting brew.

TABLE I
Loss of Phytate Phosphorus (Pp) in Fermenting Brew

Fermentation Time (hr)	Gassing power (mm/Hg)	Pp (mg)	Loss of Pp (%)
Control	0	33.7	···
3	438	33.3	1.2
5	511	33.7	0

Effect of pH on Loss of Phytate in Yeasted Dough

Our whole wheat dough, which initially contained 280 mg of Pp per dough piece, had a pH of 6.0 after mixing. The pH of the dough remained essentially constant during fermentation, declining to pH 5.8–5.9 after 6 hr of fermentation. At pH 6, the curve in Fig. 1 shows that Pp was lost linearly with time during the first three hours of fermentation. But after the first three hours, when the loss of Pp had reached ~34%, the rate of loss of phytate declined, so that the loss of Pp after 6 hr of fermentation was ~43%. The increased stability of phytate after 3 hr of fermentation in a dough at pH 6 could be due to one or more of three causes: heat denaturation of phytase, product inhibition of phytase, or inaccessibility of phytate.

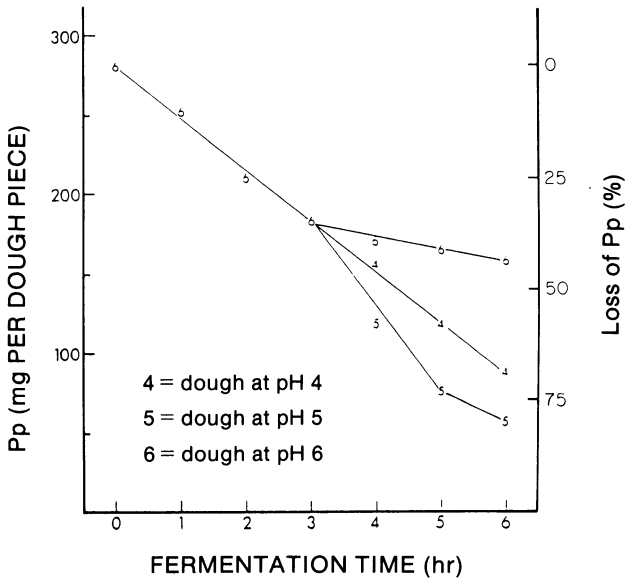

Fig. 1. Loss of phytate phosphorus **(Pp)** during fermentation of a 2% yeasted whole wheat, flour-water dough. All doughs were prepared from 86 g of dry flour and were fermented at pH 6 from 0 to 3 hr and at pH 4, 5, or 6 from 3 to 6 hr.

The whole wheat flour used in this phase of our work on phytate had been stored at ~5° C for two years. During that time, the Pp content decreased from 299 to 286 mg/86 g of dry flour. In our earlier work (Tangkongchitr et al 1981b), we found the loss of Pp from doughs made with the same flour (which at that time had been stored for three months at 5° C) to be 25% in 3 hr and 27% in 5 hr.

Peers (1953) showed that the pH optimum of wheat phytase at 55° C is 5.2, and that the enzymic activity is approximately half at pH 4 and 6. When we adjusted the pH of the fermenting dough from 6 to 4 after 3 hr of initial fermentation at pH 6, the dough at pH 4 then began to lose Pp linearly between 3 and 6 hr of additional fermentation (Fig. 1, curve 4). Loss of Pp after 3 hr of fermentation at pH 6 plus three more hours of fermentation at pH 4 was 68%. Furthermore, the rate of loss of Pp in the dough at pH 4 was equal to the initial rate of loss of Pp at pH 6 (Fig. 1).

These results indicate that inaccessibility of phytate is the cause of the decelerating rate of loss of Pp beginning after 3 hr of fermentation at pH 6. The rate of loss of Pp at pH 4 is independent of substrate concentration up to a loss of approximately 70% (Fig. 2), which indicates that the enzymic reaction is occurring with an excess of substrate and that the reaction rate is controlled by the concentration of the enzyme. Because the initial rate of loss of Pp at pH 6 is equal to that observed at pH 4 and because the enzymatic activity of phytase is equal at pH 4 and 6 (Peers 1953), an excess of substrate is also initially present in a dough at pH 6. As fermentation proceeds beyond 3 hr at pH 6, however, the substrate concentration appears to limit the reaction rate. Thus, for some reason, phytate begins to be inaccessible to the enzyme after 3 hr of fermentation at pH 6.

The rate of loss of Pp is most rapid in dough at pH 5 (Fig. 1, curve 5). The slopes of the lines in Fig. 1 show that loss of Pp was 1.7 times more rapid at pH 5 than at pH 4 or 6, which agrees well with the factor of two predicted by the work of Peers (1953).

The results shown in Fig. 3 also verify that Pp is lost most rapidly from a dough at pH of ~5. The curves in Fig. 3 are not of equal slope on both sides of pH 5.1, probably because of the inaccessibility of phytate at pH 6 and 5.5 after 3 hr of fermentation. If the doughs reported in Fig. 3 had been fermented 1 hr instead of 5 hr, the slope of the line to the right of pH 5.1 presumably would

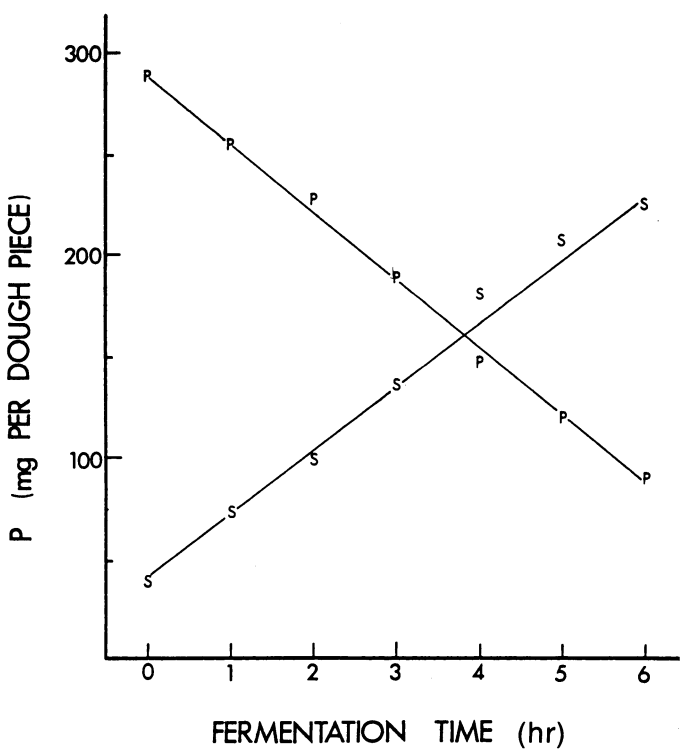

Fig. 2. Change of phytate phosphorus **(P)** and supernatant phosphorus **(S)** in a 2% yeasted whole wheat, flour-water dough at pH 4 at different fermentation times.

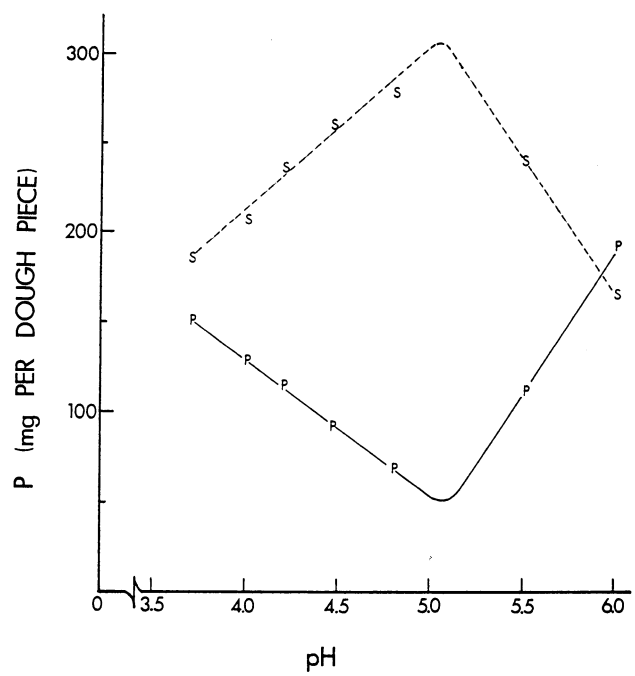

Fig. 3. Change in phytate phosphorus **(P)** and supernatant phosphorus **(S)** in 2% yeasted whole wheat, flour-water doughs after 5 hr of fermentation at different pH values.

equal that of the other line. Of course, the slopes are opposite in sign.

In addition to following Pp in all fermenting doughs, we analyzed for Ps, which includes the phosphorus compounds that are extracted along with Pp from a dough but that do not precipitate with ferric ion. We previously showed that in a fermenting dough, Ps was almost exactly equal to Pi (Tangkongchitr et al 1981b). The data in Fig. 4 show that increases in Ps during fermentation were as expected from the losses of Pp, as shown in Fig. 1. The same was true for Ps, as depicted in the curves in Figs. 2 and 3. In all doughs, the sum of Pp and Ps was consistent, with values ranging from 320 to 335 mg per dough piece.

Ranhotra (1973) proposed that Pi might inhibit the action of phytase in fermenting doughs. Lim and Tate (1973) showed that the purified F_1 fraction of wheat phytase is rather strongly inhibited by Pi. From the calculated concentration of Pi in dough, we speculated that the F_1 fraction is competitively inhibited in dough (Tangkongchitr et al 1981b). The activity of the F_2 phytase fraction or of other unknown fractions, however, must be constant during dough fermentation. Figure 2 shows that at pH 4, the rate of loss of phytate from 0 to 70% is linear, and no inhibition of the enzymic reaction occurs in dough with increasing time. However, in full-formula doughs containing milk or other concentrated sources of Pi, the results might differ. Ranhotra (1973) found that adding 6.5 mmol of Pi to a dough made from 70 g of wheat flour and 30 g of wheat-protein concentrate reduced the loss of phytate in the bread by approximately one third. That dough initially contained 281 mg (9.1 mmol) of Pp and 246 mg (7.6 mmol) of Pi. The whole wheat dough we used initially contained 286 mg (9.2 mmol) of Pp, but only 26 mg (0.8 mmol) of Pi.

The dramatic decline in hydrolytic cleavage of phytate during fermentation (Fig. 1) could be due to some other effect on phytase, such as heat denaturation. Wheat phytase probably is not thermally denatured during fermentation at 30° C, because phytase isolated from whole wheat has a temperature optimum at 55° C and a pH optimum of ~5.2 (Peers 1953). Even at pH 6, which is the approximate pH of fermenting whole wheat dough, phytase retains 50% of its optimum activity at pH 5.2. The linear loss of phytate during fermentation for up to 5 hr at pH 4 shows that little, if any, heat denaturation of phytase occurs during fermentation.

Data from other investigations on breadmaking also support solubilization of phytate as the rate-limiting factor in the destruction of phytate (Tangkongchitr 1981b).

Precipitation Studies on Phytate

What causes the inaccessibility of phytate during fermentation of doughs at pH 6? The principal barrier appears to be the insolubility of most of the phytate in the dough, which is principally due to the presence of magnesium ion. Fulcher et al (1981) recently used transmission electron microscopy to show that in the aleurone cells of wheat, phytic acid is occluded as globoids inside the aleurone grains. From the mineral content of the aleurone layer in wheat, we estimated (Tangkongchitr et al 1981b) that 63% of the phytate is present as the magnesium salt, 7% as the calcium salt, and the remainder as the potassium salt. In rice bran (Dikeman et al 1981) and in barley (Pomeranz 1973), phytic acid occurs in the aleurone grains as a mixture of the Mg-K salts, not the Mg-Ca salt.

To test our hypothesis, we examined the precipitation of phytate in an aqueous system. We chose concentrations of phytate, magnesium, calcium, zinc, and iron to mimic the concentrations of those divalent ions (iron is trivalent) in the aqueous phase of a dough. The levels of those minerals in the whole wheat flour used in this work were reported previously (Tangkongchitr et al 1981b).

The volume of water in a dough made from 100 g (14% mb) of flour was assumed to be 80 or 50 ml. The larger volume represents the upper limit of water in a dough having an absorption of 65%, whereas the smaller volume is the "free" water in the dough. The free water was calculated as the difference between the total water in a dough at 65% absorption and the amount of bound water. Davis and Webb (1969) reported that the bound water in a dough is 0.33 g of water per gram of dry flour, whereas Bushuk and Mehrotra (1977) reported a value of ~0.58 g per gram of dry flour. For our purpose, we used the value reported by Davis and Webb (1969). Thus, for our model precipitation studies, we used a dilute and a concentrated aqueous system with 80 and 50 ml total volume, respectively. The concentrations of Pp, Mg, Ca, Zn, and Fe are given in Table II.

When the individual metallic ions as chloride salts (zinc as sulfate salt) were mixed with an aqueous solution of sodium phytate at various pHs, only the magnesium ion gave precipitation of phytate in both the dilute and the more concentrated model systems. Furthermore, the blank solutions containing magnesium at pH 1–8

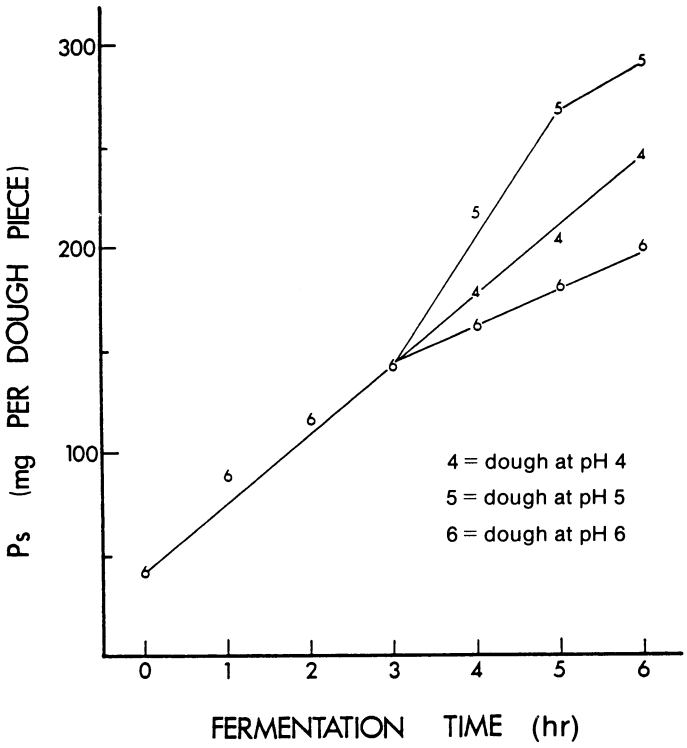

Fig. 4. Change in supernatant phosphorus (**Ps**) in 2% yeasted whole wheat, flour-water doughs at pH 4, 5, and 6 at different fermentation times.

TABLE II
Concentrations of Phytate Phosphorus (Pp) and Mineral Ions in the Reaction Mixture in the Precipitation Experiments

System and Ions	Concentration in Reaction Mixture (*M*)	Amount[a] of Mineral in Reaction Mixture (mg)
Dilute system, with individual metal ions		
Phytate	1.77×10^{-2}	225.0 (Pp)
Mg	6.78×10^{-2}	132.0
Ca	1.39×10^{-2}	46.6
Zn	5.16×10^{-4}	2.7
Fe	1.30×10^{-4}	5.8
Concentrated system, with individual metal ions		
Phytate	2.79×10^{-2}	225.0 (Pp)
Mg	1.09×10^{-1}	132.0
Ca	2.23×10^{-2}	46.6
Zn	8.25×10^{-4}	2.7
Fe	2.08×10^{-4}	5.8
Dilute system, with combined metal ions		
Phytate	2.14×10^{-2}	306.0 (Pp)
Mg	6.78×10^{-2}	132.0
Ca	1.39×10^{-2}	46.6
Zn	5.16×10^{-4}	2.7
Fe	1.30×10^{-3}	5.8

[a]The amount of mineral ions in a reaction mixture (80 or 50 ml total volume) equals the average amount of mineral in 100 g (14% m.b.) of the whole wheat flour (Tangkongchitr et al 1981b).

gave no precipitate. Figure 5 shows that the amount of phytate precipitated by magnesium was strongly influenced by pH but was not much influenced by the difference in concentrations of the ions in the model systems. At pH 7 or 8, 80–90% of the phytate phosphorus was precipitated by Mg. However, the solubility of phytate increased sharply as the pH of the medium decreased below pH 6.5; at pH 5.0, 95% of the Pp was soluble in the presence of Mg.

Calcium ion was the only other single metallic ion that caused

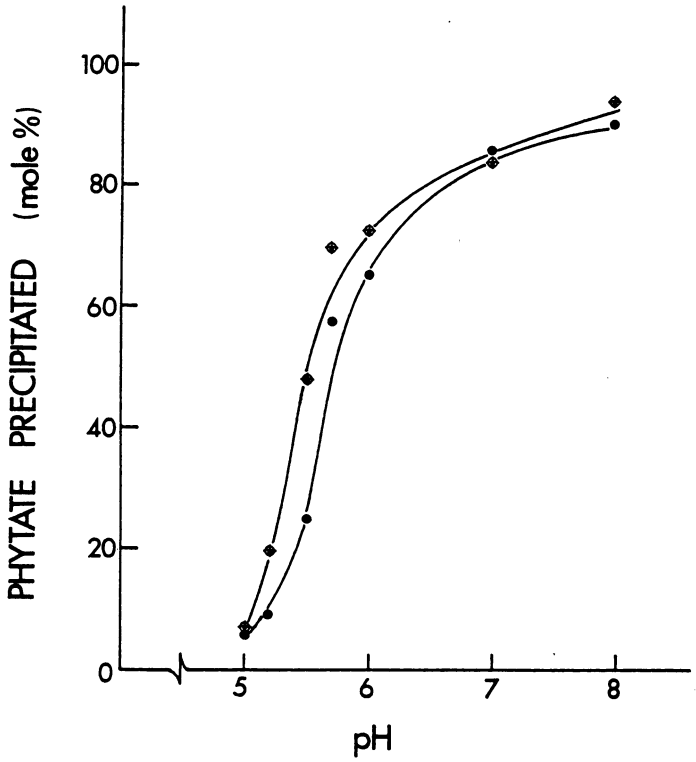

Fig. 5. Precipitation of phytate phosphorus **(Pp)** by magnesium ion at various pH values in water. Two initial concentrations of magnesium and Pp were examined: • = $1.77 \times 10^{-2} M$ in Pp and 6.78×10^{-2} in Mg; ⊙ = $2.79 \times 10^{-2} M$ in Pp and $1.09 \times 10^{-1} M$ in Mg.

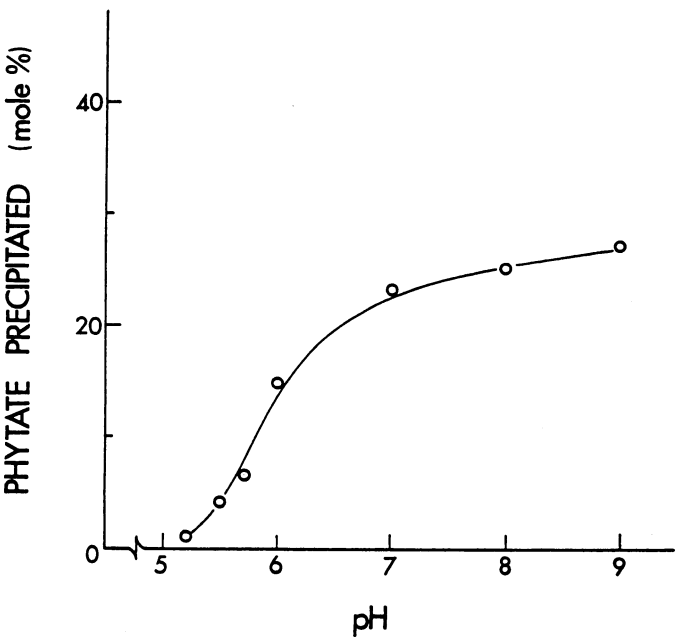

Fig. 6. Precipitation of phytate phosphorus by calcium ion at various pH values. The concentration of phytate was $2.79 \times 10^{-2} M$ and calcium $2.23 \times 10^{-2} M$.

phytate to precipitate, and then it did so only in the more concentrated model system. The curve in Fig. 6 shows that at pH 6.0, only ~15% of the phytate phosphorus would be insoluble, because of the native levels of calcium ion in a dough. Once again, calcium phytate was soluble below pH 5.0, and no precipitate formed in the blank solutions at pH 1–9.

In our third precipitation experiment, sodium phytate was precipitated under conditions that closely resemble those in a dough. Sodium phytate was mixed with all four minerals (Mg, Ca, Zn, and Fe) at once, between pH 1 and 9. The concentrations of the phytate and minerals were calculated from those in a dough containing 80 ml of total water per 86 g of dry flour (dilute system). The phytate concentration of $2.14 \times 10^{-2} M$ used in this experiment was equal to the concentration predicted in the dough, whereas the concentration of phytate ($1.77 \times 10^{-2} M$) used in the first precipitation experiment was slightly lower. This unintentional difference in concentration was due to a difference in the purity of the sodium phytate used in the two precipitation experiments.

The curve in Fig. 7 shows that when sodium phytate was mixed with the same divalent ions present in a dough, 70–75% of the Pp was insoluble between pH 6 and 7, whereas ~97% was soluble at pH 4. Furthermore, as expected, the solubility of phytate increased

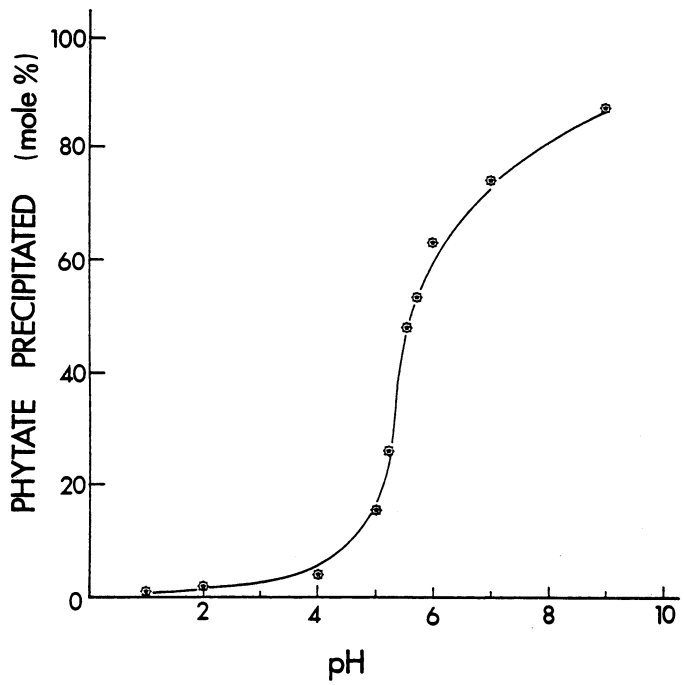

Fig. 7. Precipitation of phytase phosphorus (⊙) in a mixture containing phytate ($2.14 \times 10^{-2} M$), magnesium ($6.78 \times 10^{-2} M$), calcium ($1.39 \times 10^{-2} M$), zinc ($5.16 \times 10^{-4} M$), and ferric ions ($1.30 \times 10^{-3} M$) at different pH values.

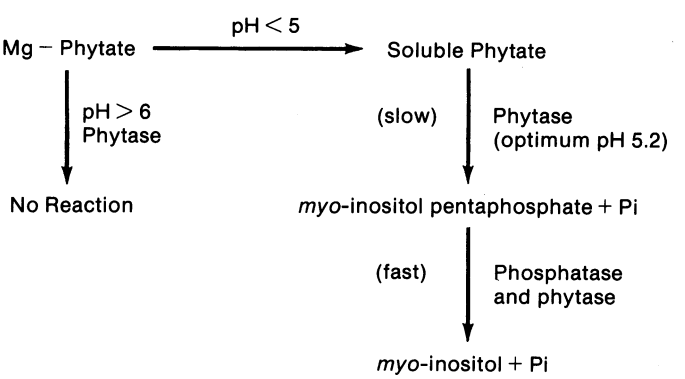

Fig. 8. Proposed mechanism for destruction of magnesium phytate during breadmaking. Pi = inorganic phorphorus.

TABLE III
Molar Ratio of Magnesium to Phytate Phosphorus (Pp) in Magnesium-Phytate Precipitates

pH of Precipitation Medium	Molar Ratio of Mg to Pp	
	Dilute System	Concentrated System
5.0	0.670	0.714
5.2	0.705	0.665
5.5	0.722	0.724
5.7	0.735	0.759
6.0	0.776	0.726
7.0	0.783	0.770
8.0	0.789	0.739
Mean	0.740	0.726

TABLE IV
Molar Ratio of Calcium to Phytate Phosphorus (Pp) in the Calcium-Phytate Precipitates

pH of Precipitation Medium	Molar Ratio of Ca to Pp in Concentrated System
5.5	0.321
5.7	0.437
6.0	0.540
7.0	0.427
8.0	0.425
9.0	0.350
Average	0.420

approximately fivefold as the pH of the system decreased from pH 6 to pH 5 (Fig. 7). The elemental composition of the precipitated salt at pH 7 and 5 showed that the precipitates were predominantly magnesium phytate. The blank solutions of the mixture of metal ions gave 3–5% precipitate at pH 7 and 9.

Assuming that phytate is the strongest metallic ion chelator in a dough, the results of the model precipitation study provide good evidence that the solubility of phytate limits its destruction in a dough at pH 6 or above. However, the potassium salt (~25%) of the phytate in a dough can be destroyed above pH 6.

The rate of destruction of phytate at pH 6 in a dough is initially controlled by the level of phytase present in a flour, but the rate slows after the soluble portion has been destroyed. Thus, during extended fermentation periods at pH 6, the rate of destruction of phytate is limited by both the activity of phytase and the availability of substrate (Fig. 8).

The most rapid rate of phytate destruction in a dough occurs when it is fermented at pH ~5.0. At or near that pH, the activity of phytase is maximal, and the substrate phytate is almost totally accessible (Fig. 8). Decreasing the pH of a dough below pH 5 decreases the rate of destruction of phytate, because phytase activity is halved when going from pH 5 to 4. However, at long fermentation periods of 4–6 hr, phytate could be more completely destroyed at pH 4 (sour doughs) than at pH 5. Figure 7 predicts that ~18% of phytate is insoluble in whole wheat doughs at pH 5, compared with 5% insoluble at pH 4. In fact, Ter-Sarkissian et al (1974) found complete destruction of phytate in a sour dough that was fermented for 8 hr.

Metal-to-Phosphorus Ratio in the Metallic-Phytate Precipitate

Analyzing the minerals and Pp of the metallic-phytate precipitates showed that for the magnesium salt of phytate, the molar ratio of magnesium to phosphorus (averaged from the magnesium phytate precipitate formed at all pHs in both the dilute and concentrated model systems) was 0.73 (Table III). Thus, the ratio of Mg to P in the magnesium phytate is 4.4:6, or approximately 4:6, and the precipitate probably has the elemental formula of $C_6H_{10}Mg_4O_{24}P_6$.

In regard to the calcium salt of phytate, the precipitate was formed only in the more concentrated system, and the molar ratio of calcium to phosphorus, which was averaged from the calcium phytate precipitate formed at all pHs, was 0.42 (Table IV). The calcium-to-phosphorus ratio in the calcium phytate precipitate probably was ~3 to 6, indicating that three calcium molecules are bound to each *myo*-inositol in the calcium phytate complex. The elemental formula of calcium phytate would probably be $C_6H_{12}Ca_3O_{24}P_6$. The metallic-phytate salts were analyzed immediately after precipitation without further purification. The elemental formulas should therefore be regarded as tentative.

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