Phytic Acid. II. Its Fate During Breadmaking[1]

U. TANGKONGCHITR, P. A. SEIB, and R. C. HOSENEY[2]

ABSTRACT

Cereal Chem. 58(3):229–234

The analytical scheme developed to measure phytate phosphorus, inorganic phosphorus, and phosphorus not precipitated by ferric ions was used to follow the loss of phytate during breadmaking. In whole wheat pup loaves, the cumulative loss of phytate phosphorus after fermentation, proofing, and baking was ~16, 19, and 22%, respectively. The phytate phosphorus loss was almost totally accounted for by an increase in inorganic phosphorus. Phytate phosphorus in seven commercial whole grain breads ranged from 218 to 808 mg per pound loaf "as is," whereas that in white bread ranged from 83 to 173 mg per pound. The principal barrier to destruction of phytate in breadmaking appears to be the insolubility of magnesium phytate in dough.

Phytic acid, *myo*-inositol hexaphosphate, hydrolyzes during the breadmaking process. However, the degree of its destruction depends on many variables, some of which are not well understood. In general, the following factors have been reported to increase destruction: 1) yeast fermentation—the more vigorous the fermentation, the greater the destruction—(Widdowson 1941), 2) increasing compressed yeast from 0 to 15 g per pound loaf (Ranhotra et al 1974), 3) sour-dough fermentation (Ter-Sarkissian et al 1974), 4) adding acetic acid to decrease the pH of dough to 5.2 (de Lange et al 1961, Pringle and Moran 1942), and 5) adding citric acid (de Lange et al 1961).

On the other hand, the following factors protect phytate against destruction during breadmaking: 1) adding calcium, especially calcium acetate and monocalcium phosphate (de Lange et al 1961, Pringle and Moran 1942, Ranhotra 1972), 2) adding magnesium acetate or sodium bicarbonate (de Lange et al 1961), 3) adding ferrous sulfate (Ranhotra 1972), and 4) increasing the extraction rate of flour (Reinhold 1975, Widdowson 1941). Loss of phytate during breadmaking has been reported from 100% for white bread (Pringle and Moran 1942, Ranhotra et al 1974) to 13% in village flat breads made in Iran (Reinhold 1972). About 40–50% of phytate was lost (de Lange et al 1961, Pringle and Moran 1942) from an American-style, whole wheat bread produced using a 1%-yeasted dough after 4 hr of fermentation, although recently Harland and Harland (1980) found that loss to be 21–25%.

Our objective was to follow phytate phosphorus (Pp) through fermentation, proofing, and baking in western-style, whole wheat and white breads.

MATERIALS AND METHODS

Flour

Three flours were used in this work. Two whole wheat flours were prepared by pin milling commercial blends of hard wheats. Whole wheat A (WWA) was from a commercial blend of hard winter wheats and whole wheat B (WWB) from a commercial blend mostly of hard winter wheats but containing ~10% hard spring wheat. White flour (WF) was the regional baking standard for the 1977 crop year, obtained from K. Finney of the U.S. Grain Marketing Research Center, Manhattan, KS. The regional baking standard is a straight grade flour from a mixture of hard winter wheats grown in the Great Plains. Protein and moisture were determined by AACC methods 46-11 and 44-15A, respectively. After wet combustion of the flour, the calcium, magnesium, zinc, and iron were determined by atomic absorption spectroscopy. Total phosphorus (Pt), Pp, and inorganic phosphorus (Pi) were determined as described previously (Tangkongchitr et al 1981). The results are given in Table I.

Full-Formula Bread

Bread was baked as pup loaves according to the procedure of Finney and Barmore (1943). The full-formula dough contained the following ingredients: flour, 100 g (14% mb); optimum water, which was 72 g for the whole wheat flours and 66 g for WF; sucrose, 6 g; nonfat dry milk, 4 g; shortening, 3 g; compressed yeast, 2 g; sodium chloride, 1.5 g; enzyme-active malt (240° L), 0.5 g; and potassium bromate, 2 mg. The doughs were mixed to optimum in a verticle pin mixer, fermented (90% rh) for 180 min at 30° C with 55 min proofing at 30° C, and baked 24 min at 218° C.

Whole Wheat Doughs

WWA (100 g, 14% mb) was mixed to optimum with water and various other ingredients, depending upon the experiment. Doughs were fermented at 30° C and 90% rh (or, if unyeasted, given a lay time) for various times (0–300 min). Dough was removed from the fermentation cabinet, placed in jars, and frozen at −15° C in a freezer. The doughs were then freeze-dried and ground in a Waring Blendor before analysis. The moisture contents of the freeze-dried doughs were determined by drying an additional 4 hr at 105° C.

Measurement of Phosphorus

All doughs or breads were analyzed for phosphorus according to the scheme described by Tangkongchitr et al (1981), using 1.2% HCl/10% Na_2SO_4 as extraction medium.

Values for phosphorus are reported per dough piece or per loaf, each made with 100 g of flour containing 14.0% moisture. Calculations were based on the concentrations of the various forms of phosphorus and the bone-dry weights of dough before and after fermentation.

Measurement of pH

The pH of doughs and breads was measured in duplicate with a pH meter on a mixture of ground dry sample (5.0 g) in distilled water (100 ml).

Experiments

Preliminary. Doughs, made as described, contained 2 g of yeast and 66 g of water. They were fermented for 0, 60, 120, 180, 240, or 300 min. In this preliminary experiment, supernatant phosphorus was not measured.

Variation of Yeast. Doughs were made with 70 g of water and either 2 or 4 g of yeast. They were fermented for 0, 180, or 300 min.

Unyeasted Dough. Doughs were made with 70 g of water. Lay times were 0, 180, and 300 min.

Rapidly Fermenting Dough. Doughs were made with 70 g of water, 6 g of sucrose, 2 g of yeast, and 0.2 g of ammonium acetate. They were fermented for 0, 180, or 300 min.

Full-Formula Doughs and Breads. Full-formula doughs and breads were prepared from WWA, WWB, and WF. Analysis for phosphorus was made after mixing, fermentation, proofing, and baking steps.

Commercial Breads. Seven whole-grain and six white breads were assayed for Pt and Pp.

[1]Contribution 81-287-J, Department of Grain Science and Industry, Kansas Agricultural Experiment Station, Kansas State University, Manhattan 66506.
[2]Graduate research assistant, professor, and professor, respectively, Department of Grain Science and Industry, Kansas State University, Manhattan 66506.

0009-0352/81/03022906/$03.00/0

RESULTS AND DISCUSSION

Fate of Pp During Fermentation or Lay Periods

Preliminary Experiment. Losses of Pp were followed in a fermenting dough (Fig. 1). The extractable phosphorus (Pe) in the extraction medium accounted for 87–96% of the Pt in the solid sample. During the first 3 hr of fermentation, approximately 25% of Pp was lost, with an almost equal increase in Pi. Loss of Pp increased slightly to 27% (86 mg) after 5 hr of fermentation. This is equivalent to 304.9 mg of phytic acid. (Because phytic acid contains 185.82 g of phosphorus per mole, the formula

$$(\text{Pp, mg}) \times (3.546) = \text{phytic acid, mg}$$

was used.) During that same fermentation period, the dough piece gained 92 mg of Pi. The pHs of the fermenting doughs after various minutes of fermentation were: 0, 6.34; 60, 6.28; 120, 6.23; 180, 6.20; 240, 6.17; and 300, 6.16.

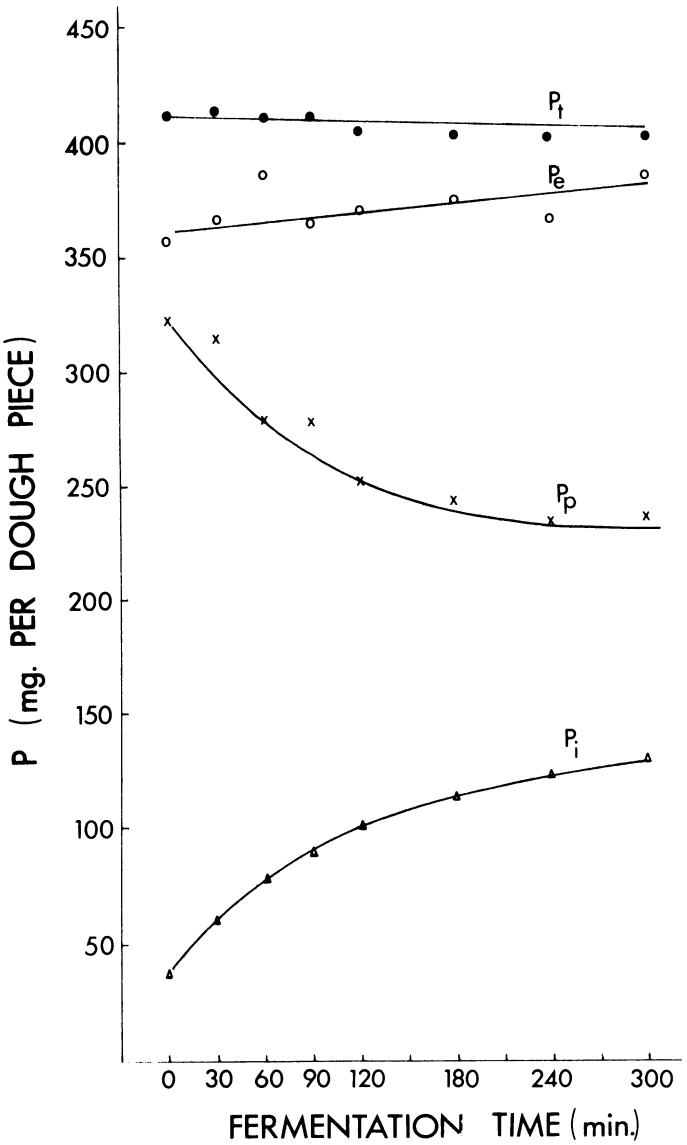

Fig. 1. Total phosphorus (**Pt**), extracted phosphorus (**Pe**), phytate phosphorus (**Pp**), and inorganic phosphorus (**Pi**) in a 2%-yeasted whole wheat flour-water dough at different fermentation periods.

Effect of pH Level. Many investigators (Björn-Rasmussen 1974, Harland and Harland 1980, de Lange et al 1961, Pringle and Moran 1942, Ranhotra 1972, Ranhotra et al 1974, Reinhold 1975, Widdowson 1941) have reported that yeast fermentation promotes loss of phytate in breadmaking. Whether the loss stems from decreasing the pH of the dough towards the pH optimum (5.2–5.5) of wheat phytase (Peers 1953, Pringle and Moran 1942) or to the possible phosphatase on the surface of yeast (Harland and Harland 1980, Reinhold et al 1974) is not known. We found that doubling the yeast from 2 to 4% in a whole wheat dough had little effect on the loss of Pp (Fig. 2).

The pH of the 2%-yeasted dough was 6.34 (0 min), 6.20 (180 min), and 6.16 (300 min) and of the 4%-yeasted dough, 6.28 (0 min), 6.13 (180 min), and 6.04 (300 min). Because the pH of those doughs remained almost constant throughout fermentation, we speculate that the change in the pH of the dough is the main mechanism whereby yeast fermentation accelerates loss of phytate.

That speculation is further supported by changes in Pp in a nonyeasted dough and in a rapidly fermenting dough (Fig. 3). The pH of the rapidly fermenting dough changed from 6.29 to 5.65 in 5 hr of fermentation, whereas the pH of the nonyeasted dough remained nearly constant at 6.39–6.41.

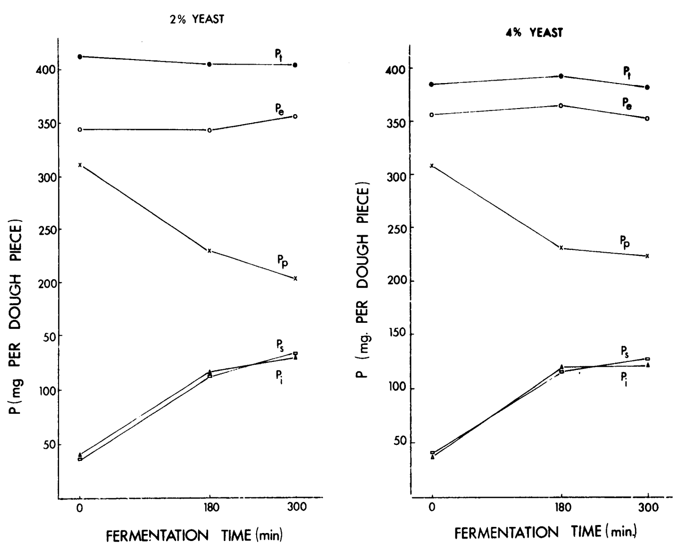

Fig. 2. Various forms of phosphorus in 2 and 4%-yeasted whole wheat flour-water doughs at 0, 3, and 5-hr fermentations. **Pt** = Total phosphorus, **Pe** = extracted phosphorus, **Pp** = phytate phosphorus, **Ps** = supernatant phosphorus, **Pi** = inorganic phosphorus.

TABLE I
Moisture, Protein, Phosphorus, and Minerals in Flours

Type of Flour	Moisture (%)	Protein[a] (%)	Phosphorus[b] Pt[c]	Pp[d]	Pi[e]	Minerals[b] Ca	Mg	Zn	Fe
Whole wheat A	14.0	12.6	440.8	348.4	26.55	54.2	153.5	3.1	6.7
Whole wheat B	13.0	13.3	439.6	370.3	21.46	55.1	157.5	4.1	6.5
White	12.9	12.4	109.3	37.1	7.32	30.9	2.5	1.4	3.5

[a] Protein reported on 14% mb.
[b] In milligrams per 100 g of dry flour.
[c] Total phosphorus.
[d] Phytate phosphorus.
[e] Inorganic phosphorus.

Loss of Pp. In 5 hr of fermentation, the following losses of Pp were observed from the various whole wheat doughs: nonyeasted, 15%; 2%-yeasted, 29%; 4%-yeasted, 28%; and rapidly fermenting, 35%. A summary of Pp losses is shown in Fig. 4.

An example of our mass-balance calculations, which show almost quantitative conversion of Pp to Pi in dough, is given in Table II. If all the Pp lost during fermentation ended up as Pi, then the figures in the last column would read 100%. The experimental values ranged from 94 to 101%.

The results in Figs. 2 and 3 show the importance of accounting for phosphorus during fermentation. Pe generally accounted for ~95% of the Pt in a sample. Furthermore, the sum of Pp and Pi in all doughs equaled 94–101% of Pe. Finally, Pi in all doughs was practically equal to the supernatant phosphorus obtained after ferric phytate was removed. Thus, one can tentatively conclude that at no time during fermentation of whole wheat dough do any intermediary hydrolysis products accumulate from phytate.

Fate of Pp During Fermentation, Proofing, and Baking

We did another series of experiments to determine how much Pp was lost during various stages of breadmaking. Table III shows that practically all the loss of Pp occurs during fermentation and proofing. Figure 5 shows cumulative losses of Pp in various stages of making whole wheat bread. The 23% loss of phytate is approximately half that observed by de Lange et al (1961) and Pringle and Moran (1942) but equal to that observed by Harland and Harland (1980).

The Pp in white dough and bread is only 5–10% of that in whole wheat dough and bread (Table III). The absolute loss of Pp was less in bread made from WF (21 mg per loaf) than in bread from WWA or WWB (~68 mg per loaf), but on a percentage basis, the loss was 66% from white bread compared with 23% from whole wheat bread.

The Pe from a dough piece or bread loaf was always 43–74 mg less than its Pt content (Table III). The nonextractable phosphorus was probably in the form of phospholipid, more of which is in the endosperm region of wheat (Colborne and Laidman 1975, Mecham 1971). Thus, of the 133–135 mg of Pt per white bread loaf or dough piece, 53–54 mg was nonextractable.

Pp and Pt in Commercial Breads

The contents of Pp in three commercial whole wheat breads ranged from 235 to 297 mg per 100 g of dry bread (Table IV), or 59–66% of the Pt in the breads (398–451 mg of Pt per 100 g of dry bread). The average phytic acid content was 2.6 g in 1 lb of whole wheat on an "as is" moisture basis. In six commercial white breads (Table V), Pp ranged from 30 to 63 mg per 100 g of dry bread, or 16–33% of Pt in the breads (151–195 mg of Pt per 100 g of dry bread). The average phytic acid content was 0.48 g in 1 lb ("as is" mb) of fresh bread.

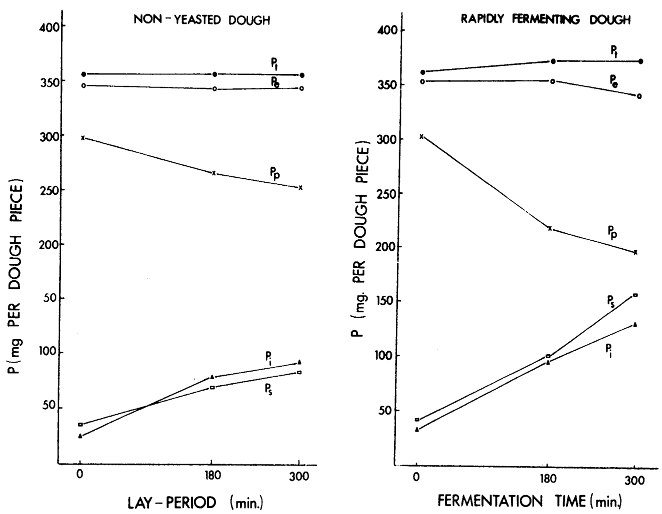

Fig. 3. Left, phosphorus components in a whole wheat flour-water dough vs lay time. **Right,** phosphorus components vs fermentation time for a yeasted (2%) whole wheat flour-water dough containing added ammonium acetate and sucrose. **Pt** = Total phosphorus, **Pe** = extracted phosphorus, **Pp** = phytate phosphorus, **Ps** = supernatant phosphorus, **Pi** = inorganic phosphorus.

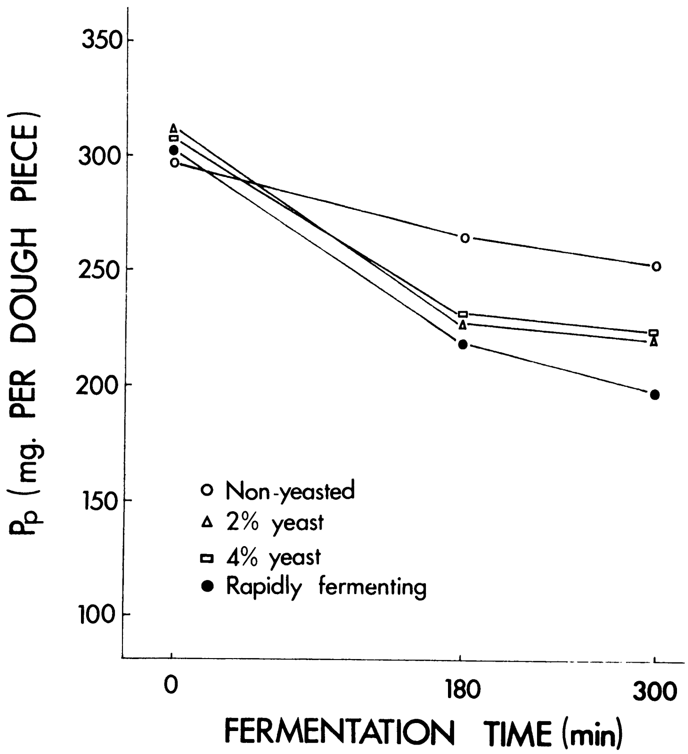

Fig. 4. Comparison of phytate phosphorus (Pp) levels in different kinds of doughs at different fermentation times.

TABLE II
Conversion of Phytate Phosphorus[a] (Pp) to Inorganic Phosphorus (Pi) During Fermentation of Whole Wheat Flour-Water Doughs With 2% Yeast and Without Yeast

Type of Dough	Lay Period or Fermentation Time (min)	Pe[b] (mg)	Pp + Ps[c] Amount (mg)	Pp + Ps[c] Percent of Pe	Pp + Pi Amount (mg)	Pp + Pi Percent of Pe
Nonyeasted	0	345.44	334.17	96.7	323.91	93.8
	180	342.75	334.67	97.6	344.47	100.4
	300	345.44	336.11	97.3	344.81	99.8
2% yeasted	0	344.09	346.97	100.8	349.02	101.4
	180	342.63	341.14	99.6	342.72	100.0
	300	355.82	353.45	99.3	351.88	98.9

[a] All phosphorus levels are given in milligrams of phosphorus per dough piece made from 100 g of flour (14% mb).
[b] Extracted phosphorus.
[c] Supernatant phosphorus.

Significance of Results

The primary barrier to destruction of phytate in moistened flour appears to be solubilization of magnesium phytate. Ferrel (1978) has shown that 80% of wheat phytate is *myo*-inositol hexaphosphate, and O'Dell et al (1972) that more than 90% of phytate is in the aleurone layer. The aleurone layer contains, in each 100 g of tissue, 3.74×10^{-2} moles of Pp, 2.4×10^{-2} moles of magnesium, 0.18×10^{-2} moles of calcium, 2.9×10^{-2} moles of potassium, and lesser amounts (total of 0.05×10^{-2} moles) of iron and zinc. Thus, ~72% of the phytate in the aleurone cells probably exists as a 13:1 mixture of magnesium-calcium salts, which are least soluble under slightly alkaline conditions (Brown et al 1961, Oberleas 1973). The remaining ~28% of phytate in the aleurone probably is the readily soluble potassium salt. Similar calculations on the contents of the entire wheat kernel show that it also contains ~70% of the relatively insoluble magnesium phytate and 30% of the soluble potassium salt (O'Dell et al 1972).

In model studies ~86% of the phytate in ground whole wheat was

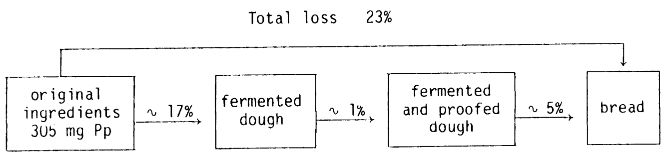

Fig. 5. Loss of phytic acid during whole wheat breadmaking. All percentage losses are calculated on the basis of the initial amount of phytate phosphorus (Pp).

hydrolyzed when one part of the ground wheat was shaken 1 hr at 35° C in 10 parts of 0.1 *M* acetate buffer at pH 5.2 (Ferrel 1978). At pH 5.2, five of the ionizing hydroxyls on phytic acids, which have pKa's between 6.85 and 12.0, are monoprotonated, and one other phosphate hydroxyl (pKa 5.7) is partially protonated. The remaining six hydroxyls, one on each phosphate group, remain ionized at pH 5.2 because their pKa values are 1.5 (Costello et al 1976). Apparently, the protonation of one hydroxyl on each phosphate group solubilizes magnesium phytate so that it can be attacked by phytase, either inside or outside the aleurone cells. Therefore, the thick cell walls of the aleurone cells are not a barrier to the enzymolysis of phytate, provided the pH of the medium is low enough for phytate solubilization. Aleurone cell walls are tough and rubbery, and few are torn apart during milling. One may thus conclude that the type of milling used to produce whole wheat flour would have little effect on losses of phytate during breadmaking. That conclusion has not been tested.

In this work we found the loss of phytate in 2%-yeasted whole wheat flour-water dough (Figs. 1 and 2) to be ~25% during 3 hr of fermentation at 30° C. The graph in Fig. 1 shows a relatively steep loss of phytate during the first 120 min, followed by little additional loss up to 300 min. Harland and Harland (1980) observed the same phenomenon. Why does the enzymatic reaction cease? One of two explanations seems possible: 1) phytase is somehow being denatured or inhibited, or 2) the soluble potassium salt of phytate is hydrolyzed during the first 2 hr of fermentation but the insoluble form remains after that time. We favor the latter explanation.

Wheat phytase has a pH optimum of 5.2 (Peers 1953), so its activity should increase, not decrease, as fermentation time

TABLE III
Changes in the Forms of Phosphorus After Mixing, Fermenting, Proofing, and Baking Full-Formula Breads

Type of Flour[a]	Step After Which Analyzed	Bone-Dry Weight of Dough Piece or Loaf (g)	pH	Phosphorus,[b] mg per piece Pt	Pe	Pp	Pi	Ps	Loss of Pp (mg)	Destruction of Phytate (%)
WWA	Mixing	103.1	6.31	418	366	305	58	68	...	...
	Fermentation	102.3	6.00	415	370	253	114	111	52	17
	Proofing	98.7	5.91	421	378	250	119	129	55	18
	Baking	97.7	5.74	420	374	236	125	135	69	23
WWB	Mixing	106.3	6.22	444	370	335	36	46	...	...
	Fermentation	104.7	5.98	442	378	288	83	84	52	15
	Proofing	102.2	5.89	424	382	271	89	95	65	19
	Baking	102.2	5.78	440	382	269	99	100	67	20
WF	Mixing	104.9	6.13	133	80	32	29	32	...	...
	Fermentation	104.9	5.72	133	79	13	35	39	19	60
	Proofing	103.4	5.59	134	81	12	40	42	20	63
	Baking	99.8	5.43	135	81	11	60	60	21	66

[a] WWA and WWB are whole wheat flours and WF is a straight-grade white flour.
[b] Pt = Total phosphorus, Pe = extracted phosphorus, Pp = phytate phosphorus, Pi = inorganic phosphorus, Ps = supernatant phosphorus.

TABLE IV
Phosphorus and Phytic Acid Contents of Commercial Breads Containing Whole Grains

Manufacturer	Name of Bread	Phosphorus[a] Pt	Pp Amount	Pp Percent of Pt	Phytic Acid[b] in 100 g of Dry Bread	Phytic Acid[b] in 1-lb Fresh Loaf[c]
Balanced Foods Inc.	Flat Whole Wheat	398.31	234.63	58.91	0.83	2.26
Akin Southwest Distributing Co.	Wheat Bread	320.90	152.02	47.37	0.54	1.47[d]
Food For Life Baking Co., Inc.	Honey Wheat Berry Bread	397.30	205.98	51.84	0.73	1.99
Dillon's Bakery	Dillon's Whole Wheat	436.81	281.37	64.41	1.00	2.72
Safeway Store, Inc.	Safeway's Whole Wheat	451.29	296.77	65.76	1.05	2.86
Alexander Bros. Baking Co.	Roman Meal	315.23	127.36	40.40	0.45	1.22[d]
Betts Baking Co.	Rainbow Wheat Bread	209.81	80.41	38.33	0.29	0.79[d]

[a] Pt = Total phosphorus, Pp = phytate phosphorus; in milligrams per 100 g of dry bread.
[b] Calculated as Pp × 3.546, in grams.
[c] Calculated on the assumption that fresh bread contains 60% solids.
[d] Breads with wheat flour listed in their ingredient statements.

TABLE V
Phosphorus and Phytic Acid Contents of Commercial White Breads

Manufacturer	Name of Bread	Phosphorus[a] Pt	Pp Amount	Pp Percent of Pt	Phytic Acid[b] in 100 g of Dry Bread	Phytic Acid[b] in 1-lb Fresh Loaf[c]
ITT Continental Baking Co., Inc.	Wonder	151.24	43.81	28.97	0.16	0.43
Betts Baking Co.	Rainbow	191.29	30.46	15.92	0.11	0.30
Dillon's Bakery	Top Fresh	187.85	44.69	23.79	0.16	0.45
Safeway Store, Inc.	Sky Lark	195.07	63.59	32.60	0.23	0.63
Dillon's Bakery	Red Label	188.23	47.05	24.99	0.17	0.47
Safeway Store, Inc.	Mrs. Wright's	192.84	59.83	31.03	0.21	0.60

[a] Pt = Total phosphorus, Pp = phytate phosphorus; in milligrams per 100 g of dry bread.
[b] Calculated as Pp × 3.546, in grams.
[c] Calculated on the assumption that fresh bread contains 62% solids.

increases and the pH of the dough moves nearer the pH optimum. On the other hand, we estimate the soluble phytate in wheat flour at pH 6–7 to be ~30%, which nearly equals the percentage of phytate lost from whole wheat in breadmaking. The pH of the 2 and 4%-yeasted doughs (Fig. 4) fell from 6.3 to 6.0–6.2 after 5 hr of fermentation, whereas the pH of the nonyeasted control remained constant at pH 6.4, and the pH of the 2%-yeasted dough containing 0.2% ammonium acetate declined to pH 5.65 after 5 hr of fermentation. The curves in Fig. 4 show that destruction of phytate increases as dough acidity increases. Loss of phytate during 3 hr of fermentation was lower (~16%) in fully formulated whole wheat dough (Table III) than in yeasted flour-water dough (~26%). The pH of the fully formulated dough was 6.0 after fermentation. Calcium in the nonfat dry milk may have precipitated more phytate in the fully formulated dough.

Data from other investigations on breadmaking also support solubilization of phytate as the rate-limiting factor in destruction of phytate. Pringle and Moran (1942), who made bread with 0.5–1% yeasted doughs with flour of 85% extraction containing 137 mg (4.4×10^{-3} moles) of Pp per 100 g of dry flour, reported 50–64% destruction of phytate after 3–5 hr of fermentation of the dough, which gave bread crumb with pH 5.6–5.7. When those workers added acetic acid to the dough so that the bread crumb had pH 5.4 and 5.2, the destruction of phytate during a 5-hr fermentation was 91 and 99%, respectively. Furthermore, adding 0.5×10^{-3} moles of monocalcium acid phosphate per 100 g of dry flour reduced phytate loss to 21%, whereas adding 3.6×10^{-3} moles of calcium or magnesium acetate completely stopped hydrolytic loss of phytate. Apparently, the added calcium or magnesium precipitated all the phytate.

de Lange et al (1961) baked whole wheat bread from 1%-yeasted whole wheat doughs using a 3-hr fermentation with and without added vinegar or citric acid. Again, loss of phytate in the breads was proportional to the acidity of the bread crumb: ~50% loss at pH 6.1 and ~77% loss at pH 5.8.

Ter-Sarkissian et al (1974) reported losses of phytate in a flat bread made in Iranian villages, starting with flours of 99.6% extraction. Using a sour dough fermentation period of only 1/2 hr, they found an 18% loss of phytate after fermentation and a 39% loss after baking 3–4 min. They also compared fermentative losses of phytate in yeasted and sour doughs made from four samples of 75%-extraction flour. In 2 hr at 23°C, sour doughs lost 37–50% of phytate, whereas the yeasted doughs lost only 12–34%.

The alternate explanation for the dramatic decline in hydrolytic cleavage of phytate during fermentation, as shown in Figs. 1 and 2, would be impairment of the phytase enzyme. We consider it unlikely that wheat phytase is 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.

Phytase enzymes may be inhibited by some factor in dough that increases with decreasing pH. For example, fraction F_1 of phytase in wheat bran is competitively inhibited rather strongly by Pi, whereas fraction F_2 is not inhibited up to a phosphate concentration of 0.01 *M* (Lim and Tate 1973). Figure 1 shows that the concentration of Pi tripled during the first 3 hr of fermentation. Knowing that a dough contains 40 mg of Pi (Fig. 1) and ~90 ml of water, one can estimate the initial concentration of Pi to be 15×10^{-3} *M*. Phytase F_1 has a K_i equal to 0.3×10^{-3} at pH 5 and therefore is probably completely inhibited when the dough piece is mixed. The final concentration of Pi (~0.04 *M*) could possibly begin to inhibit phytase F_2 and other phytase enzymes in dough.

Finally, the destruction of phytate in bread dough probably involves the action of two enzymes, both a phytase (Lim and Tate 1971, 1973; Nagai and Funahashi 1962; Peers 1953; Tomlinson and Ballou 1962) and a phosphomonoesterase (Booth 1944, Peers 1953). The evidence for the action of two enzymes is that our phosphorus mass balance on fermenting dough indicates the absence of any intermediary breakdown products of phytate. That result is unexpected for phytase action alone because several investigators have reported that isolated wheat phytase attacks phytate in a step-wise manner (Ferrel 1978, Lim and Tate 1973, Tomlinson and Ballou 1962). Ferrel (1978), for example, treated sodium phytate at 55°C and pH 5.2 with a crude phytase isolated from wheat. After 16 hr, 86 mole-percent of the phytate was hydrolyzed, with the release of penta- (3%), tetra- (43%), tri- (51%), di- (9%), and monophosphate esters (2%) of *myo*-inositol. Our results (Figs. 1 and 2, Table III) show that the loss of phytate in fermenting bread doughs is equal to the gain in Pi in the dough. Apparently, the intermediary phosphate esters of *myo*-inositol are rapidly hydrolyzed by a phosphomonoesterase, which is lost during isolation of crude wheat phytase. Ferrel (1978) also showed that phytate hydrolyzed during autolysis of ground whole wheat gave no lower phosphate esters of *myo*-inositol. In his experiments, shaking one part ground whole wheat in 10 parts 0.1 *M* acetate buffer (pH 5.2) for 4–16 hr at 35°C gave a 90% loss of phytate, with a release of 5–8% *myo*-inositol monophosphate, 0–2.7% diphosphates and triphosphates, and the remainder as Pi.

Peers (1953) estimated that ~40% of the total phytase activity in wheat is in the aleurone and ~34% in the endosperm region. Ranhotra (1972) found only 3% loss of phytate from bread made with wheat protein concentrate. Apparently the pH of the dough was too high to obtain solubilization and enzymolysis of phytate.

ACKNOWLEDGMENT

We thank the U.S. Department of Agriculture for financial support under Grant 89-106.

LITERATURE CITED

AMERICAN ASSOCIATION OF CEREAL CHEMISTS. Approved Methods of the AACC. Method 46-11, approved April 1961; Method 44-15A, approved April 1967. The Association: St. Paul, MN.

BJÖRN-RASMUSSEN, E. 1974. Iron absorption from wheat bread. Nutr. Metab. 16:101.

BOOTH, R. G. 1944. Cereal phosphatases. I. The assay of free wheat phosphomonoesterase and characterization of the free phosphatases of wheat. Biochem. J. 38:355.

BROWN, E. C., HEIT, M. L., and RYAN, D. E. 1961. Phytic acid: An

analytical investigation. Can. J. Chem. 39:1290.
COLBORNE, A. J., and LAIDMAN, D. L. 1975. The extraction and analysis of wheat phospholipids. Phytochemistry 14:2639.
COSTELLO, A. J. R., GLONEK, T., and MEYERS, T. C. 1976. ^{31}P Nuclear magnetic resonance-pH titration of *myo*-inositol hexaphosphate. Carb. Res. 46:159.
DE LANGE, D. J., JOUBERT, C. P., and DU PREEZ, S. F. M. 1961. The determination of phytic acid and factors which influence its hydrolysis in bread. Proc. Nutr. Soc. South Africa 2:69.
FERREL, R. E. 1978. Distribution of bean and wheat inositol phosphate esters during autolysis and germination. J. Food Sci. 43:563.
FINNEY, K. F., and BARMORE, M. A. 1943. Yeast variability in wheat variety test baking. Cereal Chem. 20:194.
HARLAND, B. F., and HARLAND, J. 1980. Fermentative reduction of phytate in rye, white, and whole wheat breads. Cereal Chem. 57:226.
LIM, P. E., and TATE, M. E. 1971. The phytases. I. Lysolecithin-activated phytase from wheat bran. Biochem. Biophys. Acta 250:155.
LIM, P. E., and TATE, M. E. 1973. The phytases. II. Properties of phytase fractions F_1 and F_2 from wheat bran and the *myo*-inositol phosphates produced by fraction F_2. Biochem. Biophys. Acta 302:316.
MECHAM, D. K. 1971. Lipids. Page 393 in: Pomeranz, Y., ed. Wheat Chemistry and Technology, 2nd ed. Am. Assoc. Cereal Chem., Inc.: St. Paul, MN.
NAGAI, Y., and FUNAHASHI, S. 1962. Phytase (myoinositolhexaphosphate phosphohydrolase) from wheat bran. Agric. Biol. Chem. 26:794.
OBERLEAS, D. 1973. Phytates. Page 363 in: Toxicants Occurring Naturally in Foods, 2nd ed. Nat. Acad. Sci.: Washington, DC.
O'DELL, B. L., DE BOLAND, A. R., and KOIRTYOHANN, S. R. 1972. Distribution of phytate and nutritionally important elements among the morphological components of cereal grains. J. Agric. Food Chem. 20:718.
PEERS, F. G. 1953. The phytase of wheat. Biochem. J. 53:102.
PRINGLE, W. J. S., and MORAN, T. 1942. Phytic acid and its destruction in baking. J. Soc. Chem. Ind. 61:108.
RANHOTRA, G. S. 1972. Hydrolysis during breadmaking of phytic acid in wheat protein concentrate. J. Food Sci. 37:12.
RANHOTRA, G. S., LOEWE, R. J., and PUYAT, L. V. 1974. Phytic acid in soy and its hydrolysis during breadmaking. J. Food Sci. 39:1023.
REINHOLD, J. G. 1972. Phytate concentrations of leavened and unleavened Iranian breads. J. Food Nutr. 1:187.
REINHOLD, J. G., PARSA, A., KARIMIAN, N., HAMMICK, J. W., and ISMAIL-BEIGI, F. 1974. Availability of zinc in leavened and unleavened whole meal wheat breads as measured by solubility and uptake by rat intestine in vitro. J. Nutr. 104:976.
REINHOLD, J. G. 1975. Phytate destruction by yeast fermentation in whole wheat meals. J. Am. Diet. Assoc. 66:38.
TANGKONGCHITR. U., SEIB, P. A., HOSENEY, R. C. 1981. Phytic acid. I. Determination of three forms of phosphorus in flour, dough, and bread. Cereal Chem. 58:226.
TER-SARKISSIAN, N., AZAR, M., GHAVIFEKR, H., FERGUSON, T., and HEDAYAT, H. 1974. High phytic acid in Iranian breads. J. Am. Diet. Assoc. 65:651.
TOMLINSON, R. V., and BALLOU, C. E. 1962. Myoinositol polyphosphate intermediates in the dephosphorylation of phytic acid by phytase. Biochem. J. 1:166.
WIDDOWSON, E. M. 1941. Phytic acid and the preparation of food. Nature 148:219.

[Received June 23, 1980. Accepted September 8, 1980]