Fermentative Reduction of Phytate in Rye, White, and Whole Wheat Breads

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ABSTRACT

In experiments performed with three breads (rye, white, and whole wheat) phytate content was reduced by doubling the yeast in each recipe and extending the fermentation time. The phytate content of each of the breads is presented, as are the concentrations of the following minerals: calcium, phosphorus, magnesium, copper, iron, manganese, and zinc. Phytate/zinc molar ratios are presented to give an estimation of the bioavailability of the zinc in the breads.

Cereal Chem. 57(3):226–229

Increased consumption of whole grain bread as a reasonable way to increase dietary nutrient and fiber intake has been accepted by the scientific community and consumers. The nutritive contribution of these whole grain breads could be increased if the phytate in the products were reduced (Ranhotra 1972, Reinhold 1975, Ter-Sarkissian et al 1974). Phytate, an anion, myoinositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate) (IUPAC-IUB 1968) has been shown to bind zinc and other minerals (O'Dell and Savage 1960).

Phytate is the storage form of phosphorus and is found in plant seeds and in many roots and tubers. The phytate of plant seeds is contained primarily in the germ and bran of cereal grains. Essentially no phytate is found in bananas, celery, citrus fruits, lettuce, mushrooms, onions, and prunes, and only traces in apples, broccoli, carrots, and green beans. Moderate amounts of phytate exist in artichokes, figs, potatoes, and strawberries; the greatest amounts are found in cereals, legumes, and nuts. Table I shows the percentage of phytate in grains and breads.

Only eight of the 12 dissociable protons of phytate are available for metal binding (Brown et al 1961). The final combination of metals complexed with phytate when mixed in a solution depends upon the concentration of the metal, the pH of the solution, and the concentration of secondary cations. Most of the cations of physiological importance are least soluble at a pH between 4 and 8 (normal gastrointestinal pH). Phytate thus combines with calcium, iron, zinc, and other divalent metals to form compounds with low solubility that are not readily absorbed from the intestine.

Research concerning the effects of phytate on mineral absorption in humans is sparse. The interaction of phytate with the essential trace mineral zinc has been studied more thoroughly than its interaction with other trace minerals. High phytate content of a food may decrease the absorption of zinc (Reinhold et al 1973). Thus the phytate/zinc molar ratio may serve as an indicator of the availability of zinc from a specific food. Other phytate/divalent cation ratios may be similarly evaluated.

According to Davies and Olpin (1979), a phytate/zinc molar ratio greater than 10 may produce zinc deficiency in rats; the maximum phytate/zinc molar ratio needed to avoid zinc deficiency in humans has not been established. Typical hospital diets contain a phytate/zinc molar ratio between 3 and 6 (Harland and Peterson 1978). Unleavened breads with a phytate/zinc molar ratio of greater than 20 comprise over 90% of the diet in the Middle East (Reinhold 1975), where clinical zinc deficiency was first described. The higher phytate content of the bread was believed to contribute to the development of human zinc deficiency (Ronaghy 1970).

Yeast contains a phosphatase which can hydrolyze phytate to orthophosphate and inositol, thereby eliminating the available binding sites. In the dry, dormant seed, the enzyme appears to be

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inactive. Inorganic phosphate increases and phytate decreases when stored wheat becomes wet at warm temperatures (Glass and Geddes 1959). An active phytase has been purified from wheat bran (P 1259, Sigma Chemical Co., St. Louis, MO 63178). Among the cereal grains, rye appears to contain the most active phytase (Hoff-Jorgensen et al 1946).

Human studies have indicated that phytate may be hydrolyzed in the gastrointestinal tract (Bitar and Reinhold 1972). Although enzymes effecting this action are present in the intestinal mucosa, they are not consistent in bringing about the degradation of phytate (Reinhold 1975).

Few processes are available for removing phytate from the food chain. Phytate can be removed from soybean isolates, but this requires extensive processing (Okubo et al 1975). The practicality of the removal of phytate from bread flours has yet to be determined. Because the consumption of whole grain breads, both homemade and commercial, is increasing and because whole grain flours are high in phytate, a decrease in the phytate content of bread would be beneficial.

The experiment reported here was designed to evaluate the effects of increased yeast and extended rising time on the reduction of phytate in three varieties of breads.

MATERIALS AND METHODS

Three varieties of breads were prepared, following recipes that are typically used in the home Table II. Each of the three breads was subjected to one rising period of either 0, 2, 4, or 8 hr. A plug of dough (5 g) was dispersed in deionized water at 26°C and the pH was measured just before baking. Breads were baked in greased loaf tins at 191°C (375°F), oven-dried at 79°C (200°F), and blended to a coarse powder in a Waring Blendor. Aliquots were taken for phytate determination (Harland and Oberleas 1977); mineral analyses were performed on bread prepared with one or two packages of yeast and 2 hr of rising time by inductively coupled argon plasma spectrophotometry (Fassel and Kniseley 1974). All baking trials and analytical determinations were performed in quadruplicate.

The phytase activity of the baker's yeast (Fleischmann's) was measured [3] as described below. A phytase standard, isolated from wheat, was purchased (Sigma Chemical Co.). It had an activity of 0.04 Sigma unit/mg of solid. One unit of this standard liberated 1.0 μmol of inorganic phosphorus from a $1.5 \times 10^{-3} M$ phytate solution per minute at pH 5.15 at 55°C. Ten milliliters of $0.2 M$ sodium acetate buffer (pH 5.15), 0.4 ml of $0.1 M$ $MgSO_4$, 5 ml of water, 4.4 ml of $0.00682 M$ sodium phytate, and 0.2 ml of the wheat phytase dissolved in water (10 mg/ml) were mixed (total volume 20 ml) and incubated for 1 hr at 55°C. Aliquots (2-ml) were removed at 0, 10, 20, 30, 40, 50, and 60-min intervals, and the reaction was stopped by the addition of 1 ml of 10% trichloroacetic acid. Color was

[3] Private communications Sigma Chemical Co., St. Louis, MO.

TABLE I
Phytate (% Dry Weight) in Grains and Breads

Source	Phytate
Grains	
Wheat bran	3.70[a]
Soybeans	2.58[b]
Wild rice	2.20[d]
Barley	1.19[c]
Wheat flour	0.96[c]
Oats	0.77[c]
Corn	0.12[a]
Breads	
Cornbread	1.36[a]
Whole wheat	0.56[c]
Rye	0.41[c]
Pumpernickel	0.16[a]
Raisin	0.09[a]
French	0.03[a]
White	0.03[c]

[a] D. Oberleas and B. F. Harland, unpublished.
[b] Oberleas et al (1966).
[c] B. F. Harland, unpublished.
[d] D. Oberleas, unpublished.
[e] Averill and King (1926).

TABLE II
Recipes Used to Prepare Rye, White, and Whole Wheat Breads[a]

Ingredients	Amounts: By Measure	Amounts: By Weight, g	Percent of Dry Ingredients
Rye Bread[b,e]			
Rye flour	4 cups	408	52.4
Whole wheat flour	2 cups	240	30.9
Brown sugar	1/3 cup	73	9.4
Hydrogenated shortening	2 tbsp	25	3.2
Salt, iodized	1½ tbsp	25	3.2
Yeast, dry	1 pkg (1 tbsp)	7	0.9
White Bread[c,e]			
All-purpose flour	6 cups	822	92.4
Sugar, granulated	2 tbsp	25	2.8
Hydrogenated shortening	2 tbsp	25	2.8
Salt, iodized	2 tsp	11	1.2
Yeast, dry	1 pkg (1 tbsp)	7	0.8
Whole Wheat Bread[d,e]			
Whole wheat flour	6 cups	720	81.4
Sugar, granulated	3/4 cup	150	16.9
Salt, iodized	1½ tsp	8	0.9
Yeast, dry	1 pkg (1 tbsp)	7	0.8

[a] Adapted from Rombauer 1946.
[b] Dissolve yeast in 1/2 cup (120 ml) of lukewarm tap water (27°C, 80°F). Combine ingredients. Add 1¾ cups (420 ml) of lukewarm tap water. Add dissolved yeast. Stir. Spoon mixture into two greased loaf pans.
[c] Dissolve yeast in 1/4 cup (60 ml) of lukewarm tap water (27°C, 80°F). Pour one cup (240 ml) of scalded milk (92°C, 198°F) over the sugar, shortening, and salt. Stir. Add dissolved yeast. Stir. Add flour gradually with stirring. Knead for 4 min. Divide dough into two equal sections. Shape loaves and place into two greased loaf pans.
[d] Dissolve yeast in 1/2 cup (120 ml) of lukewarm tap water (27°C, 80°F). Combine ingredients. Add dissolved yeast. Stir. Add 2½ cups (600 ml) of lukewarm tap water. Stir. Spoon mixture into two greased loaf pans.
[e] Let rise at 27°C (80°F). (Fermentation time was dictated by experimental design.) Bake at 191°C (375°F) for 15–35 min, until inserted cake tester comes out clean.

TABLE III
Mean Percent ± Standard Error[a] of Phytate in Breads Prepared with Increased Yeast and Fermentation[b] Time

Rising Time (hr)	No Yeast	One Package of Yeast	Two Packages of Yeast
	Rye Bread		
0	0.78 ± 0.005	0.80 ± 0 abc	0.43 ± 0.001 abc
2	0.77 ± 0.002	0.41 ± 0 ade	0.28 ± 0.002 ade
4	0.76 ± 0	0.34 ± 0.001 bdf	0.23 ± 0.001 bdf
8	0.76 ± 0.003	0.37 ± 0.001 cef	0.21 ± 0.001 cef
	White Bread		
0	0.03 ± 0.003	0.04 ± 0.002	0.03 ± 0
2	0.03 ± 0.001	0.03 ± 0.001	0.02 ± 0.001
4	0.03 ± 0.001	0.02 ± 0.001	0.02 ± 0.001
8	0.03 ± 0.001	0.01 ± 0.001	0.02 ± 0
	Whole Wheat Bread		
0	0.64 ± 0.001 abc	0.64 ± 0 abc	0.60 ± 0.001 abc
2	0.59 ± 0 a	0.56 ± 0.001 ade	0.57 ± 0.002 ade
4	0.59 ± 0 b	0.48 ± 0.001 bdf	0.47 ± 0.001 bdf
8	0.59 ± 0.001 c	0.42 ± 0.001 cef	0.43 ± 0.001 cef

[a] Values with the same letters are significantly different ($P < 0.05$).
[b] Dry weight basis.

TABLE IV
Mineral Analyses of Three Types of Breads[a]

Bread	Minerals						
	Calcium	Phosphorus	Magnesium	Copper	Iron	Manganese	Zinc
Rye	10.4 ± 0.50	68.8 ± 0.33	20.8 ± 1.36	0.14 ± 0.009	0.78 ± 0.044	0.60 ± 0.016	0.57 ± 0.008
White	12.4 ± 0.10	31.1 ± 0.45	6.1 ± 0.16	0.05 ± 0.013	0.80 ± 0.071	0.08 ± 0.000	0.14 ± 0.004
Whole wheat	7.6 ± 0.23	92.4 ± 2.42	34.7 ± 1.03	0.12 ± 0.013	0.95 ± 0.032	0.86 ± 0.029	0.72 ± 0.024

[a]Mean ± standard error (mg/28-g slice) of four determinations taken after 2 hr of fermentation (two analyses each of each bread containing one and two packages of yeast).

TABLE V
Phytate/Zinc Molar Ratios[a] in Breads Prepared with Increased Yeast and Rising Time

Rising Time (hr)	No Yeast	One Package of Yeast	Two Packages of Yeast
		Rye Bread	
0	39	40	21
2	38	20	14
4	38	17	11
8	38	18	10
		White Bread	
0	6	8	6
2	6	6	4
4	6	4	4
8	6	2	4
		Whole Wheat Bread	
0	24	24	23
2	22	21	22
4	22	18	18
8	22	16	16

[a]Based on a formula weight for phytate of 660.

developed by first adding 2 ml of water and then 5 ml of Taussky-Schoor reagent. After the contents were mixed, absorbance was read immediately at 660 nm. The Taussky-Schoor reagent was prepared as follows: 10 g of ammonium molybdate was placed in a 100-ml volumetric flask and diluted to volume with 10*N* H_2SO_4. Ten milliliters of the H_2SO_4-NH_4Mo solution was poured into a 100-ml volumetric flask and diluted with 70 ml of deionized water. To this solution, 5 g of ferrous sulfate (heptahydrate) was added and the solution was diluted to volume with deionized water.

A taste panel of 20 volunteers tested the acceptability of the rye, white, and whole wheat breads. All samples presented to the taste panel had a fermentation time of 2 hr. Each panel member tasted 6 samples—2 rye, 2 white, and 2 whole wheat—one of which had been baked using either 0.8% (white and whole wheat breads) or 0.9% (rye bread) yeast in the recipe (Table II). Rye, white, and whole wheat breads were identified for the panel members, who were asked to state a preference (if one existed) between the 0.8 and 0.9% or the 1.6 and 1.8% yeast breads. The panel members were to base their judgments on overall acceptability. An opportunity was provided for written comments.

Phytate/zinc molar ratios were calculated on a formula weight of 660 for the acid form of phytate. Differences in phytate content of the breads with increased yeast and fermentation time were calculated for significance (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION

Phytate analysis of the breads at both yeast levels and all fermentation times revealed that with increasing yeast (Ranhotra et al 1974) and fermentation periods (Mellanby 1944, Widdowson 1941), phytate in the breads was reduced. Because the binding properties of phytate are pH-dependent, the pH changes in the three breads were measured as rising time progressed. Previous experiments have shown that the optimum pH for the activity of wheat phytase is 5.15.[3] During the fermentation period, the carbon dioxide produced may have contributed to a lower pH. Our experiments produced no consistent pH trends with increased yeast or extended rising periods; nor was any pH range characteristic of any one bread. The lowest pH (5.56) occurred in white bread containing two packages of yeast after 4 hr of rising. The highest pH (6.52) was found in the rye dough containing no yeast at zero rising time. The composition of the rye and whole wheat bread recipes tested here may have acted to suppress pH changes in the doughs. A greater decrease in phytate may occur if recipes could be adjusted to achieve optimum pH for phytase activity.

The percentage pf phytate was reduced from 0.78 to 0.21 in rye bread, from 0.03 to 0.02 in white bread, and from 0.64 to 0.43 in whole wheat bread (Table III) (Harland and Prosky 1979). The major reduction of phytate in the three breads occurred during the first 2 hr of rising. Only a small decrease was observed in the period between 2 and 8 hr of rising time. Although the percentages of phytate in rye and whole wheat bread are similar (0.78 and 0.64, respectively), phytate decreased more in rye bread after yeast was doubled than it did in whole wheat bread. This may be attributed to the fact that more phytase is normally present in rye flour than in whole wheat flour. Sugar enhances and salt interferes with yeast cell multiplication, so differences in amounts of sugar and salt in the three recipes may have been factors influencing phytase activity. The decrease in the percentage of phytate in the white bread was less than in the other two breads, probably because of lower initial phytate and phytase contents.

Table IV shows the mineral composition of the breads. No significant differences were found in the mineral content of breads when yeast was doubled from 0.8 or 0.9% to 1.6 or 1.8% of the dry ingredients. The whole wheat bread contained the highest concentrations of P, Mg, Fe, Mn, and Zn. The enriched white bread contained the highest concentration of calcium (milk was an ingredient in the white bread), and rye bread contained the highest concentration of copper.

A diet with a high phytate/zinc molar ratio (approximately 10) has been shown to jeopardize zinc status in rats (Davies and Olpin 1979). In the present experiment, all rye and whole wheat breads made with no yeast or one package of yeast had phytate/zinc molar ratios above 15. Table V shows the changes in the phytate/zinc molar ratios in the breads as yeast was doubled and rising times were extended. With these two treatments, the phytate/zinc molar ratio was decreased in rye bread from 39 to 10, in white bread from 6 to 4, and in whole wheat bread from 24 to 16. These treatments lowered the phytate/zinc molar ratios of the rye and whole wheat breads to a level more favorable than those associated with zinc deficiency in rats (Davies and Olpin 1979) and humans (Reinhold 1975).

The taste panel did not detect a yeast flavor nor reject any of the breads at either yeast level.

When activities of the two sources of phytase were compared, that from wheat, purchased from Sigma Co., was found to be 1.2 times greater than that from the yeast used in this experiment.

If bread has a high phytate or a low zinc content, increasing the yeast or the rising time or both may result in a considerable improvement in mineral availability. This may be of great significance in a diet containing bread as the staple.

In summary, the results of this experiment show that phytate may be effectively reduced by increasing the yeast or extending the fermentation time. The practical application of these two methods is to encourage people to consume leavened breads and, when baking, to increase the yeast or the rising time or both to reduce phytate content and thereby increase mineral availablity in the baked products.

ACKNOWLEDGMENTS

We appreciate the technical assistance of John Jones, Division of Chemistry and Physics, who performed the mineral analyses of the breads by inductively coupled plasma spectrophotometry. Clarisse Jones and Philip Dzugan contributed valuable laboratory assistance. Donald Oberleas, Department of Nutrition and Food Science, University of Kentucky, Lexington 40506, was consultant for the project.

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[Received October 30, 1979. Accepted January 28, 1980]