Interactions of Iron, Alone and in Combination with Calcium, Zinc, and Copper, with a Phytate-Rich, Fiber-Rich Fraction of Wheat Bran Under Gastrointestinal pH Conditions[1]

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ABSTRACT

Cereal Chem. 64(2):102–105

The effects of a simulated gastrointestinal pH treatment on the mineral, phytic acid, and protein solubility of a phytate-rich, fiber-rich fraction of wheat bran in the presence of Fe, alone and in combination with Ca, Zn, and Cu were determined. It was found that 98% of the phytic acid in the wheat bran fraction was soluble after a simulated gastrointestinal pH treatment. When this fraction underwent a similar treatment in the presence of 0.025 mmol of added Fe, most of the Fe was bound to the insoluble fiber fraction rather than the soluble phytic acid. Repetition of this experiment with 0.5 mmol of Ca or 0.025 mmol of Zn in the presence of 0.025 mmol of Fe indicated a decrease in the solubility of both Fe and phytic acid. However, when the experiment was repeated with 0.025 mmol of Cu in the presence of 0.025 mmol of Fe, there was no effect on Fe or phytic acid solubility but there was an increase in protein solubility; this was the only system that affected protein solubility significantly. Under the same experimental conditions with the same mineral combinations but using only Na phytate at levels equivalent to the phytic acid content of the wheat bran fraction, it was found that only Ca affected Fe and phytic acid solubility.

Birdsall (1985) stated that

> adding to the difficulty of assessing amounts of available nutrients in wheat are problems with methodologies on bioavailability, as well as effects of other dietary constituents such as phytate and the competitive interplay among the minerals. Much more knowledge is needed to determine what factors enhance or inhibit the absorption of those micronutrients present intrinsically in the foods, those added through fortification, and how other foods in the meal affect absorption.

A number of recent studies show that the iron-binding capacity of wheat bran, components of wheat bran, and chemically related substances are all affected by other minerals commonly found in the diet (Berner and Hood 1983; Rao and Narasinga Rao 1983; Champagne et al 1985b; Garcia-Lopez and Lee 1985; Platt and Clydesdale 1985, 1986).

It has also been shown that iron binding to wheat bran (Thompson and Weber 1979), fiber components (Platt and Clydesdale 1984, Reinhold et al 1981), and phytate (Jackman and Black 1951) are affected by pH. Furthermore, phytic acid solubility in various brans is also affected by pH (Hill and Tyler 1954).

Little research has been conducted to study the effects of a gastrointestinal pH treatment on the solubility of food components such as phytic acid in wheat bran in the presence of minerals. This type of research may be significant because food is subjected to an acid environment in the stomach before entering the duodenum. The purpose of this research was to investigate the effects of a gastrointestinal pH treatment on the mineral and phytic acid solubility of a phytate-rich, fiber-rich fraction (PRFR) of wheat bran in the presence of added Fe and other minerals.

MATERIALS AND METHODS

Reagents

All reagents were of analytical grade, and solutions were prepared with double-distilled deionized water (DDW). All glassware was acid washed with concentrated HCl and then thoroughly rinsed with DDW.

Materials

Sodium phytate (corn crystalline) was purchased from Sigma Chemical Co., St. Louis, MO. Soft white wheat bran was obtained from the American Association of Cereal Chemists, Medallion Labs, St. Paul, MN, and held under refrigeration (4° C) until use.

Phytic Acid, Phosphorous, Neutral Detergent Fiber, and Protein Analyses

Phytic acid was determined using a modification of the ion-exchange method of Harland and Oberleas (1977) as described by Ellis and Morris (1983). Phosphorus was determined using the method of Fiske and Subbarow (1925). Neutral detergent fiber (NDF) was determined using a modification of the method of Goering and Van Soest (1970) as described by Mongeau and Brassard (1982), and soluble protein was determined by the Lowry reaction for protein determination using bovine serum albumin as the reference protein (Cooper 1977).

Apparatus

A Perkin-Elmer model 372 atomic absorption spectrophotometer (AAS) was used for measuring mineral concentrations; A Perkin-Elmer Lambda 3 UV/VIS spectrophotometer was used for absorption measurements; and a Fibotec system M was used for NDF determinations.

Analysis of Minerals

Atomic absorption standards and reagents used for the analysis of minerals have been described in an earlier report (Platt and Clydesdale 1985). The endogenous mineral content of the wheat bran samples was determined by AAS after digesting 100 g with 20 ml of concentrated HNO_3 for 15 min. The Ca content of the systems was determined in the presence of 1.0% lanthanum oxide.

The total and soluble mineral content of each sample was determined by AAS after digesting with 20 ml of HNO_3 for 15 min.

Ionic iron was determined using a modification of the bathophenanthroline procedure of Lee and Clydesdale (1979) as described by Platt and Clydesdale (1984). It should be noted that ionic iron is defined as bathophenanthroline-reactive iron and as such may represent more than ionic iron alone (Clydesdale and Nadeau 1984). Complexed iron was obtained by the difference of soluble minus ionic iron.

Statistics

All statistics were carried out according to methods described by Steel and Torrie (1980).

Preparation of the PRFR Fraction of Wheat Bran

Soft white wheat bran (SWWB) (100 g) was suspended in 6 L of

TABLE I
Phytic Acid, NDF, and Content of Selected Minerals in the PRFR and PDFR Fractions of Wheat Bran (per g dry wt)[a]

Sample	Phytate (mg)	NDF (%)	P (mg)	Ca (mg)	Fe (μg)	Zn (μg)	Cu (μg)
PRFR fraction	34.97 ± 0.74	60.1 ± 0.7	12.72 ± 0.01	1.02 ± 0.03	115.0 ± 1.6	94.8 ± 3.3	17.6 ± 0.3
PDFR fraction	5.94 ± 0.41	82.3 ± 2.5	...[b]	...	...	...	...

[a] NDF = Neutral detergent fiber, PRFR = protein-rich, fiber-rich fraction, PDFR = protein-depleted, fiber-rich fraction.
[b] Not detected by atomic absorption spectrophotometry.

DDW and stored under refrigerated conditions for 60 hr. The mixture was then vacuum filtered through a coarse glass filter, and the residue (PRFR-fraction) washed three times with DDW and twice with acetone. Finally the residue was dried overnight in an oven at 60° C.

Preparation of the Phytate-Depleted, Fiber-Rich (PDFR) Fraction of Wheat Bran

SWWB (100 g) was suspended in 6 L of DDW in the presence of 500 mg of wheat phytase. The mixture was incubated for 60 hr at room temperature with stirring. The insoluble fraction was filtered, washed three times with DDW and then twice with acetone. Finally the residue (PDFR-fraction) was dried overnight in an oven at 60° C.

Binding Capacity of Insoluble Fiber Versus Phytate

It was initially proposed that comparisons be made between the mineral binding characteristics of the PRFR and PDFR fractions of wheat bran. However, only 83% of the total phytic acid was destroyed after mixing SWWB (100 g) and 500 mg of phytase in 6 L of DDW at room temperature for 60 hr (Table I). The inability of phytase to completely dephytinize wheat bran has also been demonstrated by a number of other investigators (Andersson et al 1983, Frolich and Asp 1985). Furthermore, the NDF content of the PDFR fraction (82.3 ± 2.5%) was shown to be significantly higher than the NDF content of the PRFR fraction (60.1 ± 0.7%), as shown in Table I.

Because of these difficulties, it was decided to compare the effects of phytate versus insoluble fiber in the PRFR fraction, with a Na phytate control. During the preparation of the PRFR fraction from wheat bran, any water-soluble ligands, including water-soluble fibers, were removed. Thus, a more direct comparison of the mineral binding capacity of insoluble fibers plus phytate versus phytate alone was obtained than if wheat bran or phytase-treated wheat bran (PDFR) were used. Therefore, the following procedure was followed:

Samples (1.00 g wet wt) of the PRFR fraction were suspended in triplicate in 100 ml of DDW. The pH of the samples was reduced to pH 2.00 ± 0.01 with 12N HCl and then 5 ml of various mineral solutions, acidified with 1% v/v 12N HCl for Zn(ZnSO$_4$ · 7H$_2$O, 5mM), Fe(FeSO$_4$ · 7H$_2$O, 5mM), Cu(CuSO$_4$ · 5H$_2$O, 5mM), and 5% v/v 12N HCl for Ca(CaCO$_3$), were added in the amounts and combinations shown in Table II. The systems were equilibrated at pH 2 for 20 min. At the end of this period the pH was adjusted to 5.00 ± 0.01 first with 10N, w/v, followed by 1.0N, and then 0.1N NaOH, and equilibrated for 30 min. After the final equilibration period 40 ml aliquots of each system were centrifuged at a relative centrifugal force of 2,335 g for 10 min. The supernatant was decanted from the insoluble fraction and filtered through ashless Whatman No. 41 filter paper, then analyzed for phytic acid, soluble protein, soluble P, Ca, Fe, Zn, Cu, and ionic iron as outlined in the previous sections.

Phytic acid controls, at the same concentration as those in the PRFR fraction, were also performed using the above procedure.

RESULTS AND DISCUSSION

Effects of pH on the Solubility of Phosphorus and Phytic Acid in the PRFR Fraction of Wheat Bran

The PRFR fraction of wheat bran was shown to contain 12.72 mg phosphorus per gram dry weight (Table I). Of this, only 6.7 ± 0.3% was solubilized when 1.00 g of the wheat bran fraction was mixed in 100 ml of DDW (pH 7.01) for 60 min. This observation

TABLE II
Combinations of Minerals Added to the Phytate-Rich, Fiber-Rich Fractions of Wheat Bran and Na Phytate Controls[a]

System	Mineral Added (mmol)[b]			
	Fe (0.025)	Cu (0.025)	Zn (0.025)	Ca (0.50)
1	−[c]	−	−	−
2	+	−	−	−
3	+	+	−	−
4	+	−	+	−
5	+	−	−	+

[a] Wheat bran and Na phytate each provided 0.05 mmol of phytic acid.
[b] As determined by atomic absorption spectrophotometry.
[c] + = Mineral added, − = not added.

supports the previous findings of Hill and Tyler (1954) who showed that phosphorus solubility in wheat bran decreases above pH 5.

Hill and Tyler (1954) also demonstrated an increase in phosphorus solubility under acidic conditions. However, no research has been conducted to study the effect of a simulated gastric and sequential intestinal pH treatment on phytic acid and phosphorus solubility. Figure 1 shows that 62.4 ± 4.1% of the total phytic acid was solubilized from the wheat bran fraction after incubation at pH 2 for 20 min and 97.9 ± 6.0% was solubilized when the pH was sequentially increased to 5. Furthermore, it was found that 91.3 ± 2.5% of the total phosphorus was solubilized after a similar simulated gastrointestinal pH treatment.

From these results, it is apparent that mineral solubility data for wheat bran fractions containing phytic acid should be conducted after a simulated gastrointestinal pH treatment in order to
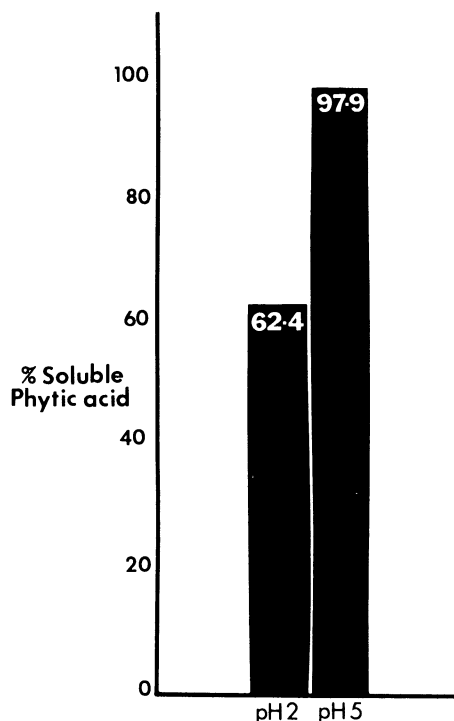

Fig. 1. Solubilization of phytic acid from the protein-rich, fiber-rich fraction of wheat bran after incubation at pH 2 for 20 min and after the pH was sequentially increased to 5.

realistically predict the effect that phytic acid may have on mineral availability. Therefore, this treatment was incorporated throughout the remainder of this study.

Effects of a Gastrointestinal pH Treatment on the PRFR Fraction of Wheat Bran in the Presence of Various Mineral Combinations

Table III shows that when Fe was added to the PRFR fraction of wheat bran and subjected to a gastrointestinal pH treatment, $31.2 \pm 2.5\%$ of the total iron became soluble. The addition of Fe also resulted in a significant decrease ($P < 0.05$) of 10.5% in soluble phosphorus compared to the endogenous system. In a separate study it was also shown that phytic acid solubility was decreased by 13.6%, however, this decrease was not statistically significant. When Fe was added to a phytic acid control (i.e., an amount of Na phytate equal to the phytic acid in the PRFR fraction of wheat bran), it was shown that 100% of the iron and phosphorus remained in a soluble state, with 85.6% of the total Fe complexed with phytic acid as shown in Table IV. These results suggest that the insoluble fiber fraction in wheat bran contributed significantly to iron binding in the presence of phytate. Coincident with this, studies in this laboratory have shown that insoluble fibers, such as lignin, have a high affinity for iron under duodenal conditions (Platt and Clydesdale 1986, *unpublished*).

It can be seen in Table III that when Cu as well as Fe was added to the PRFR fraction of wheat bran, it had no significant effect ($P > 0.05$) on phosphorus, phytic acid, endogenous Ca, or iron solubility, but significantly increased ($P < 0.05$) protein solubility compared to the PRFR fraction in the presence of Fe. As well, copper solubility was shown to be significantly greater than Fe solubility ($65.9 \pm 1.2\%$ compared to $29.1 \pm 1.3\%$) when equimolar concentrations of the two minerals were added to the PRFR fraction. When equimolar concentrations of Fe and Cu were added to Na phytate controls, 100% of the Cu, Fe, and phosphorus was found to be soluble, and 98.0% of the total Fe was complexed with the soluble phytic acid (Table IV).

When both Zn and Fe were added to the PRFR fraction, there was a significant decrease ($P < 0.05$) in phytic acid (22.8%), phosphorus (19.0%), endogenous Ca (0.9 mg), and soluble Fe (23.0%), compared to the PRFR fraction in the presence of Fe alone (Table IV). A decrease in protein solubility was also observed; however, this effect was not statistically significant.

When Zn and Fe were added to the exogenous Na phytate control, 100% of the Fe, Zn, and phytic acid were soluble, and 91.4% of the total iron was complexed with the soluble phytic acid (Table IV). These observations indicate that it is not solely the presence of added minerals that decreases solubility and causes precipitation. The factors responsible for precipitation may either be the presence of endogenous minerals—because a decrease in the soluble endogenous Ca content was observed—or the formation of an insoluble protein-phytate complex with zinc. In fact, it was shown that zinc forms insoluble complexes with albumins in the pH range 5–6.7 above a temperature of 5° C (Gurd 1954).

Several studies indicate that phytic acid has a negative effect on zinc absorption in humans but has no effect on Cu absorption (Obizoba 1981; Turnlund et al 1984, 1985). Turnlund and co-workers (1985) suggest that these complexes are pH dependent, with zinc-phytate precipitating at intestinal pH while copper-phytate remains soluble. The results of the present investigation with Na phytate alone do not support this hypothesis, because it was shown to form soluble complexes with both Cu and Zn in the presence of Fe. However, the addition of Zn to the PRFR fraction reduced the solubility of phytate, and only 10.6% of the Zn remained soluble. When Cu was added to the PRFR fraction there was no effect on the solubility of the phytic acid, and 65.9% of the Cu remained soluble. This suggests that Zn has a greater tendency than Cu to form insoluble phytate complexes in the presence of other dietary constituents, such as the protein and endogenous Ca of wheat bran, thus explaining the findings of Obizoba (1981) and Turnlund et al (1984, 1985).

When both Ca and Fe were added to the PRFR fraction, there was a significant decrease in phytic acid (49.4%), phosphorus (28.3%), and Fe (26.8%) solubility compared with the PRFR fraction in the presence of Fe alone (Table III). However, Ca had no effect on protein solubility and it reduced soluble Fe and phosphorus in the Na phytate controls (Table IV). Phytate has been shown to bind protein via divalent cation bridges at a neutral pH in soy protein (DeRham and Jost 1979). However, when the calcium concentration was increased, the complex between soy protein and phytate was destroyed and calcium phytate precipitated.

The findings in this study indicating that Ca forms an insoluble complex with Na phytate in both the wheat bran system and the control (Tables III and IV) are supported by Champagne and

TABLE III
Percent Soluble Phytic Acid, Minerals and Soluble Protein in a PRFR Fraction of Wheat Bran, in the Presence of Various Mineral Combinations, After a Gastrointestinal pH Treatment[a]

	Percent Soluble						Percent Ionic Fe	Soluble Protein[b] (μg/ml)
System	Phytate[b]	P	Ca[c]	Zn	Cu	Fe		
PRFR fraction	97.9 ± 6.0	81.3 ± 2.5	0.87 ± 0.03 mg	...[d]	...	...	...	33.49 ± 3.33
PRFR fraction + Fe	84.3 ± 12.2	80.8 ± 2.8	0.89 ± 0.01 mg	...	...	31.2 ± 2.5	2.0 ± 0.9	29.57 ± 2.44
PRFR fraction + Fe + Cu	79.8 ± 4.2	78.8 ± 2.5	0.90 ± 0.05 mg	...	65.9 ± 1.2	29.1 ± 1.3	1.3 ± 0.3	36.04 ± 4.42
PRFR fraction + Fe + Zn	61.5 ± 9.1	61.8 ± 2.3	0.80 ± 0.02 mg	10.6 ± 0.3	...	8.2 ± 1.6	1.8 ± 0.7	23.78 ± 5.34
PRFR fraction + Fe + Ca	34.9 ± 7.2	52.5 ± 5.5	$71.0 \pm 1.3\%$	...	...	4.4 ± 1.1	0	31.43 ± 0.77

[a] PRFR = Protein-rich, fiber-rich fraction. Means ± standard deviation for three readings.
[b] Soluble phytate and protein were determined in independent studies.
[c] Values for Ca are expressed in mg, except when Ca was added to the wheat bran systems.
[d] Not detectable by atomic absorption spectrophotometry.

TABLE IV
Percent Soluble P, Ca, Zn, Cu, Fe, and Ionic Fe in Na Phytate Controls, in the Presence of Various Mineral Combinations, After a Gastrointestinal pH Treatment[a]

	Percent Soluble					Percent Ionic Fe
System	P	Ca	Zn	Cu	Fe	
Na phytate + Fe	102.3 ± 0.2	...[b]	...	...	98.7 ± 1.2	13.1 ± 6.7
Na phytate + Fe + Cu	99.9 ± 0.4	...	...	93.6 ± 0.6	98.0 ± 0.7	0
Na phytate + Fe + Zn	101.2 ± 0.6	...	101.5 ± 1.3	...	95.0 ± 2.8	4.6 ± 4.2
Na phytate + Fe + Ca	45.3 ± 10.2	66.5 ± 4.6	...	...	6.3 ± 2.9	2.9 ± 1.9

[a] Means and standard deviations for three readings.
[b] Not detected by atomic absorption spectrophotometry.

co-workers (1985a), who observed that the solubility of Ca in rice bran decreases above pH 5 and suggested that this results from the formation of insoluble Ca phytate complexes. Furthermore, Jackman and Black (1951) showed a rapid drop in the solubility of Ca phytate at pH 5.5–6.0. That Ca was shown to decrease the solubility of Fe after a gastrointestinal pH treatment in the present study, and that it previously has been shown to affect solubilities of Fe (Rao and Narasinga Rao 1983), Zn (Oberleas et al 1966), and Cu (Oberleas 1973) in the presence of phytate has significance when considering the bioavailability of these minerals. There is no doubt that Ca salts of phytate are unabsorbable (Allen 1982), but there is evidence that phytate is digested in the large intestine to varying degrees and Ca may be absorbed at this point. However, there is no evidence to suggest that copper, zinc, and iron are. Therefore the synergistic effect noted between the transition metals (Fe, Zn, Cu) and Ca with phytate may have a greater effect on Fe, Zn, and Cu availability than on Ca availability.

CONCLUSIONS

This study suggests that correlations between in vitro and in vivo studies for phytate-rich products should be made after a sequential pH treatment simulating gastrointestinal pH conditions because many of the reactions are pH dependent.

When Fe was added to the PRFR fraction of wheat bran it was bound to the insoluble fiber fraction rather than phytic acid after a gastrointestinal pH treatment and had no effect on either protein or phytic acid solubility.

When Cu was added to the PRFR fraction of wheat bran in the presence of Fe, it was shown to have no effect on either phytic acid or Fe solubility after a sequential pH treatment. However, when Zn or Ca were added to the PRFR fraction of wheat bran in the presence of Fe, they were found to affect both phytic acid and Fe solubility. The effects of Cu, Zn, and Ca on Fe solubility found in this study are in agreement with our earlier study (Platt and Clydesdale 1986a) with soft white and hard red spring wheat bran conducted at an endogenous pH of approximately 7.

There is some evidence in the literature (Champagne et al 1985a,b) to suggest that the effect of added minerals on phytic acid solubility found in this investigation may not hold in the case of endogenous minerals. In studies with rice bran, no relationship was found between endogenous Fe, Zn, and Cu with phytic acid solubility (Champagne et al 1985a), but there was a relationship when the minerals were exogenous (Champagne et al 1985b).

With the Na phytate controls in this study, only Ca was shown to affect Fe and phytic acid solublity, which suggests that model Na phytate-mineral systems may not be good predictors of more complex food systems. Because solubility is regarded as a primary prerequisite of availability (Van Campen 1983) these results illustrate the importance of considering the effects that minerals added to fortified foods containing phytic acid may have on the potential availability of other essential minerals in the diet.

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