In Vitro Interaction of Rice Hemicellulose with Trace Minerals and Their Release by Digestive Enzymes[1,2]

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ABSTRACT

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In vitro binding of calcium, magnesium, and manganese by water- and alkali-soluble hemicelluloses and hemicellulose molecular weight fractions above 100,000 were determined after incubation at 37° C for ½, 1, and 2 hr. Binding was complete within 30 min in the model system. Effects of digestive enzymes (hemicellulase, pepsin, and trypsin) and pH on minerals bound to hemicelluloses were examined in 16-hr incubation tests. Both enzymes and changes in pH released considerable amounts of bound minerals to different degrees, suggesting that such released minerals should be available for resorption in vivo. Data on calcium and magnesium in combination for binding studies with water-soluble hemicelluloses showed that both are bound to the hemicellulose in essentially the same amounts, indicating that neither should interfere with the other for absorption and/or binding by hemicellulose in vivo. However, calcium and magnesium used in combination for binding studies with alkali-soluble hemicellulose indicated that calcium intake may affect dietary magnesium binding and release by this class of hemicellulose.

Although considerable research has been done, evidence that dietary fiber components may promote good health, prevent diverticulosis, and prevent colonic cancer is incomplete. Many important unsolved problems still exist on the exact nature of dietary fiber and its possible significance in health and disease. Because the exact chemical structure of the constituents in plant fibers often vary among plant species, variations in experimental results are possible.

Mineral metabolism in relation to dietary fiber is of particular interest to food technologists and nutritionists. The bioavailability of essential minerals is affected by the body's need for the minerals, digestibility of the food that supplies the minerals, and interaction of the minerals with other dietary components. The property of fiber to act as a cation-exchange resin could produce undesirable effects, such as reducing the absorption of several mineral elements (Branch et al 1975, James et al 1978). High fiber intake has been reported to impair the absorption of several minerals, including calcium, phosphorus, magnesium, and zinc (Reinhold et al 1976). Hemicellulose and cellulose supplements to diets inhibit absorption of calcium and magnesium (McHale et al 1979). Hemicellulose and cellulose isolated from grass Yorkshire Fog have little ability to bind either calcium or magnesium (Molloy and Richards 1971). Dietary phytate is known to reduce the availability of calcium, zinc, magnesium, iron, manganese, and copper (Davies and Nightingale 1975, Oberleas 1973). Reinhold et al (1975) reported that fiber rather than phytate determines bioavailability of calcium, zinc, and iron, whereas, Davies et al (1977) concluded that phytate rather than fiber is the major determinant of zinc availability. Hunter (1981) suggested that reported differences on effects of phytate on iron absorption may be due to differences in experimental conditions or to the presence of dietary fiber in the diet. Minerals are known competitors for absorption, as are copper and zinc or magnesium and calcium (McChance et al 1942), and addition of dietary fiber may have undesirable results.

A recent in vitro study showed that copper, zinc, and iron are bound to water- and alkali-soluble hemicelluloses, but considerable amounts of the bound minerals can be released (Mod et al 1981) and should be available for resorption in vivo.

This study reports the in vitro binding of calcium, magnesium, and manganese to water- and alkali-soluble rice bran hemicelluloses and their release by digestive enzymes, as well as the effects of pH on release of the minerals.

MATERIALS AND METHODS

Defatted rice bran was obtained from Riviana Foods, Inc. The metal sources were calcium sulfate ($CaSO_4{\cdot}2H_2O$), magnesium sulfate ($MgSO_4{\cdot}7H_2O$), and manganous sulfate ($MnSO_4{\cdot}H_2O$) (J. T. Baker Chemical Company). The three enzymes were hemicellulase (Miles Laboratories), pepsin (Nutritional Biochemicals Corporation), and trypsin (Calbiochem). Sodium phytate was obtained from Sigma Chemical Company.

Isolation of Water- and Alkali-Soluble Hemicelluloses and Arabinogalactan

Water-soluble-1 bran hemicelluloses were extracted and purified according to Cartano and Juliano (1970); alkali-soluble bran hemicelluloses were isolated and purified according to Gremli and Juliano (1970). Water-soluble-2 hemicellulose was isolated as described for the water-soluble hemicellulose, except that the soluble starch and protein were not removed. The arabinogalactan was isolated from the water-soluble bran hemicellulose by ammonium sulfate fractionation as described by Neukom and Markwalder (1975) and separated into molecular weight fractions by ultrafiltration through a Diaflo membrane filter.

Gas-Liquid Chromatography (GLC)

Polysaccharides were hydrolyzed by the procedure of Roberts et al (1976). The hydrolyzed sugars were identified qualitatively and quantitatively by GLC analysis on a Hewlett-Packard model 5750 equipped with a flame-ionization detector. The column was a 10-ft long stainless-steel tube with a ⅛-in. o.d. and was packed with 5.8% OV-1 on Chromosorb W, 60–80 mesh. The column was operated isothermally at 190° C with a carrier gas flow of 18 ml/min of nitrogen. The sugars were equilibrated overnight in pyridine and silylated with trimethylchlorosilane and hexamethyldisilane according to Sweeley et al (1963). The sugars were identified by comparison of retention times and peak enhancement with known sugars. Quantitative estimates of each sugar were made by comparison of peak areas with sorbitol as internal standard.

Analytical Methods

Nitrogen was determined by micro-Kjeldahl and protein as nitrogen × 5.95.

Conditions for Hemicellulose/Mineral Binding

Hemicellulose and the metal salt were dissolved in 25 ml of deionized water to yield the equivalent of 10 mg of metal to 1 g of hemicellulose (for example, 5.4 mg of calcium sulfate to 126 mg of hemicellulose). Each container was incubated in a constant-temperature bath at 37° C, and aliquots were removed after ½, 1, and 2 hr for a 2-hr time study. If then used for further enzymatic treatment, the total reaction time was 2 hr. The aliquots were

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[1] Presented at the 66th Annual Meeting, Denver, CO, Oct. 1981.

[2] Names of companies or commercial products are given solely for the purpose of providing specific information; their mention does not imply recommendation or endorsement by the U.S. Department of Agriculture over others not mentioned.

placed in a dialysis bag with a 3,500 molecular weight cut off and dialyzed for 48 hr in a continuous dialysis apparatus (Fig. 1). The metal-hemicellulose complexes were lyophilized, and metal contents were determined by atomic absorption spectrophotometry.

Conditions for Enzymatic Release of Minerals

Twenty-two milligrams of metal-hemicellulose complex and 1.3 mg of trypsin were placed in a container, then 10 ml of 0.1*M* Tris buffer, pH 8.1 was added, and the mixture was incubated at 37° C for 16 hr. The samples were dialyzed, lyophilized, and analyzed as described. The same procedure and hemicellulose-enzyme ratio were used for pepsin and hemicellulase, but 0.1*M* acetate buffer, pH 5.5, was used for pepsin and pH 4.0 acetate for hemicellulase in place of the Tris buffer.

Conditions for Hemicellulose-Phytic Acid-Mineral Binding

A 1:1 ratio of hemicellulose-phytic acid and the metal salt were dissolved in 25 ml of deionized water to yield the equivalent of 10 mg of metal to 1 g of hemicellulose or 1 g of phytic acid (for example 5.4 mg of calcium sulfate to 126 mg of hemicellulose and 126 mg of phytic acid). The mixture was incubated at 37° C for 2 hr, after which the samples were dialyzed, lyophilized, and analyzed as described. The same procedure was used for the 2:1 ratio of hemicellulose-phytic acid and the metal salt, except that 63 mg of phytic acid was used instead of the 126 mg.

Atomic Absorption Spectrophotometry

The atomic absorption spectrometer used for analysis of metals was a Perkin-Elmer model 380. Approximately 10 mg of dried hemicellulose was weighed on a semimicro balance into a 10-ml volumetric flask. The flask was partially filled with deionized water and agitated in an ultrasonic bath until all solids appeared to be uniformly dispersed (about 10 min). The flasks were allowed to cool to room temperature and brought to volume with deionized water. The solutions were analyzed at the most sensitive wavelength for each element, at optimum conditions described in the Perkin-Elmer manual "Analytical Methods for Atomic Absorption Spectrophotometry." Standards were prepared fresh daily from 1,000-ppm stock solutions. Standard curves, calculated by the method of least squares for four observations, each on a blank, and solutions of the metal at three concentration levels, gave a correlation coefficient of 0.99. Five replicate measurements were made on a minimum of two samples of each mineral, and the average values and their correlation coefficients were calculated. Coefficient of variation for calcium, magnesium, and manganese were 1.3, 1.5, and 1.7%, respectively. The density of each "solution" was assumed to be 1, and the ppm (μg/g) of each element was calculated from the weight of the sample. To check the possibility of interferences, each element was analyzed by the method of additions on several samples. The results agreed with those obtained by comparison with aqueous standards.

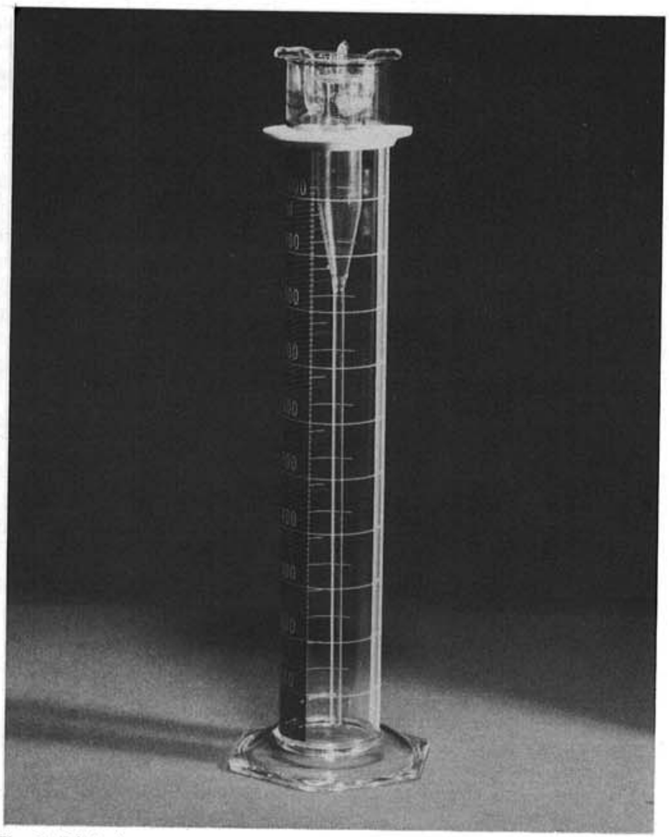

Fig. 1. Dialysis apparatus.

RESULTS AND DISCUSSION

Compositions of water- and alkali-soluble rice bran hemicelluloses shown in Table I indicate that both contain the same sugars—rhamnose, arabinose, xylose, mannose, galactose, and glucose—as well as some associated protein. Arabinose and xylose are the predominant sugars for the alkali-soluble hemicellulose, but arabinose and galactose are the predominant ones for the water-soluble hemicellulose. The alkali-soluble hemicellulose contains the least protein. Fractionation of the alkali-soluble hemicellulose by ultrafiltration indicates that the molecular weight fraction above 100,000 is the major one (90%) and consists of 58.6% arabinose, 37.4% xylose, and 4.0% galactose.

An arabinogalactan that was isolated from the water-soluble hemicellulose by ammonium sulfate fractionation by the procedure of Neukom and Markwalder (1975) is similar in composition to an arabinogalactan isolated from wheat hemicellulose. The composition of the arabinogalactan and the two molecular weight fractions separated from the arabinogalactan by ultrafiltration are shown in Table II. The arabinogalactan and each of the molecular weight fractions have essentially the same arabinose and galactose contents. However, the two molecular weight fractions differ in composition because xylose is the third sugar in the 50,000–100,000 fraction, and glucose is the third sugar in the fraction above 100,000.

Both water- and alkali-soluble hemicelluloses approached the maximum amount of calcium, magnesium, and manganese bound to the hemicellulose within 30 min of incubation. Analysis for calcium, magnesium, and manganese in the hemicellulose fractions before binding studies were as follows: alkali-soluble hemicellulose was 2,542 ppm calcium, 3,267 ppm magnesium, and 170 ppm of manganese; water-soluble hemicellulose (9.82% protein) was 1,457

TABLE I
Composition of Rice Bran Hemicelluloses

	Composition (wt%)						
Hemicellulose	**Rham**	**Ara**	**Xyl**	**Mann**	**Gal**	**Glu**	**Protein (N × 5.95)**
Alkali-soluble	3.1	45.7	32.9	0.7	6.0	7.3	4.28
Water-soluble-1	trace	26.6	6.0	4.9	30.7	21.9	9.82
Water-soluble-2	trace	12.4	4.0	1.6	19.3	12.6	50.34

TABLE II
Composition of Arabinogalactan and Molecular Weight Fractions

Arabinogalactan Composition (%)		**Molecular Weight Fractions**	
		50,000–100,000	**>100,000**
Arabinose	40.5	39.03	39.09
Xylose	3.0	5.26	···
Galactose	56.5	55.71	56.40
Glucose	trace	···	4.51
Galactose/ arabinose	1.4/1	1.43/1	1.44/1

TABLE III
Binding of Three Minerals by Hemicellulose and Release by pH Effect and Enzymes

Original Hemicellulose (ppm)		pH 4.0	Hemicellulase	pH 5.5	Pepsin	pH 8.1	Trypsin
		Alkali-Soluble Hemicellulose After Release by Enzymes (ppm)					
Calcium	9,294	6,147 (34)[a]	5,096 (45)	6,520 (30)	4,499 (52)	8,914 (4)	4,049 (56)
Magnesium	6,868	2,563 (67)	2,939 (76)	1,796 (73)	0 (100)	394 (98)	0 (100)
Manganese	10,786	7,938 (26)	8,510 (21)	8,128 (24)	8,521 (21)	3,986 (63)	3,478 (68)
		Water-Soluble Hemicellulose					
Calcium	7,330	1,611 (78)	683 (91)	1,678 (75)	1,512 (79)	3,759 (49)	2,692 (63)
Magnesium	3,832	0 (100)	0 (100)	0 (100)	33 (99)	720 (81)	265 (93)
Manganese	6,834	506 (92)	1,114 (84)	1,212 (82)	1,596 (77)	1,190 (82)	1,904 (72)

[a] ( ) = percent released mineral.

TABLE IV
Effects of Protein on Interaction of Three Minerals with Water-Soluble Hemicellulose

Original Hemicelluloses (ppm)	Protein Content (%)	pH 4.0	After Release by pH and Enzymes (ppm)				
			Hemicellulase	pH 5.5	Pepsin	pH 8.1	Trypsin
			Calcium				
7,330	9.82	1,611 (78)[a]	680 (91)	1,678 (75)	1,512 (79)	3,759 (49)	2,692 (62)
2,058	50.34	1,128 (45)	828 (60)	2,507 (0)	1,543 (25)	3,241 (0)	1,143 (44)
			Magnesium				
3,832	9.82	0 (100)	0 (100)	0 (100)	33 (99)	720 (81)	265 (93)
349	50.34	0 (100)	0 (100)	0 (100)	0 (00)	0 (100)	0 (100)
			Manganese				
6,834	9.84	506 (92)	1,114 (84)	1,212 (82)	1,596 (77)	1,190 (82)	1,904 (72)
2,469	50.34	94 (96)	81 (97)	1,213 (51)	155 (93)	1,261 (49)	1,130 (54)

[a] ( ) = percent released mineral.

TABLE V
Binding of Calcium, Magnesium, and Manganese

	By Arabinogalactan[a] (bound mineral, ppm)[b]	By Alkali-Soluble Hemicellulose[a] (bound mineral, ppm)[c]
Calcium	5,277 (7,330)	4,946 (9,294)
Magnesium	3,234 (3,932)	3,080 (6,868)
Manganese	6,437 (6,834)	7,086 (10,786)

[a] Molecular weight > 100,000.
[b] ( ) = bound by water-soluble hemicellulose.
[c] ( ) = bound by alkali-soluble hemicellulose.

ppm calcium, 742 ppm magnesium, and 98 ppm manganese; water-soluble hemicellulose (50.47% protein) was 1,286 ppm calcium, 1,869 ppm magnesium, and 40 ppm manganese; arabinogalactan fraction greater than 100,000 mol wt was 319 ppm calcium, 106 ppm magnesium, and 72 ppm manganese; alkali-soluble fraction greater than 100,000 mol wt was 1,107 ppm calcium, 1,547 ppm magnesium, and 31 ppm manganese. Although 2,142 ppm of calcium was found in trypsin; this should not have any effect on the release of the minerals examined because dialysis did not remove this calcium from the trypsin. Alkali-soluble hemicellulose bound almost 100% of the available calcium and manganese and about 70% of the available magnesium. Water-soluble hemicellulose bound about 70% of the available calcium and manganese and about 40% of the available magnesium. Manganese had maximum binding to alkali-soluble hemicellulose, followed by calcium and magnesium. Calcium had maximum binding to water-soluble hemicellulose, followed by manganese and magnesium.

The binding of calcium to water- and alkali-soluble hemicelluloses and its release by both pH and the enzymes, hemicellulase, pepsin, and trypsin, are shown in Table III. Although considerable calcium is bound to the alkali-soluble hemicellulose, a large portion is released by acid (30–34%). The combination of pH and enzymes (as would be found in vivo) released slightly more (45–56%). This should be available for resorption during passage through the gastrointestinal tract in vivo. With the exception of trypsin, which indicates a larger release of calcium due to enzyme activity rather than pH, the remaining pH effect (4 and 5.5) showed a larger release of calcium than did the two enzymes, hemicellulase and pepsin. Similarly, considerable amounts of calcium were bound to the water-soluble hemicellulose, and a larger percentage of calcium is released than is released from the alkali-soluble hemicellulose. pH effects released 49–78% of the bound calcium, with the greatest amount released at pH 4. Digestion by the enzymes released 63–91% calcium, with hemicellulase releasing the most, followed by pepsin and trypsin.

The binding of magnesium to water- and alkali-soluble hemicellulose and its release by pH effects and the three enzymes are also shown in Table III. The data indicate substantial binding of magnesium (6,868 ppm) to the alkali-soluble hemicellulose, with a larger percent of bound metal (67–100%) released for magnesium than was released for calcium (4–56%). Of the three minerals examined, magnesium showed the least amount of binding (3,832 ppm) to water-soluble hemicellulose. It also showed the highest percentage of metal released, ranging from 81 to 100%. Like that found for calcium, the amount of magnesium released is influenced more by pH than be digestive enzymes.

The binding of manganese by water- and alkali-soluble hemicelluloses and their release by pH effects and the digestive enzymes are also shown in Table III. Although manganese binds more than the other two minerals to alkali-soluble hemicellulose, very little is released by acid pH (4–5.5), or by hemicellulose and pepsin. Trypsin and pH effects at 8.1, on the other hand, release 63–68%. Although the water-soluble hemicellulose binds less manganese than does the alkali-soluble hemicellulose, more manganese is also released by pH effects rather than by enzymatic activity.

Because protein may be involved in the binding and release of calcium, magnesium, and manganese to water-soluble hemicellulose, their binding to water-soluble hemicellulose containing different protein contents was investigated.

The effects of protein on calcium-water-soluble hemicellulose interaction and its release by pH effects and hemicellulase, pepsin and trypsin are shown in Table IV. Calcium shows an inverse

TABLE VI

Binding of Calcium and Magnesium Mixture and Release by pH and Enzymes

	Original Hemicellulose (ppm)		Original Hemicellulose Mixture (ppm)	After Release by pH and Enzymes		
				Hemicellulase 4.0	Pepsin 5.5	Trypsin 8.1
By water-soluble hemicellulose	Calcium	7,330	4,316 (59)[a]	778 (82)	1,110 (74)	888 (80)
	Magnesium	3,832	2,123 (55)	0 (100)	0 (100)	0 (100)
By alkali-soluble hemicellulose	Calcium	9,294	8,366 (90)[a]	451 (95)	3,483 (58)	6,587 (21)
	Magnesium	6,868	5,321 (77)	1,060 (80)	124 (98)	220 (96)

[a] ( ) = percent released calcium and magnesium by pH and enzyme.

TABLE VII

Effect of Phytic Acid on Hemicellulose Interaction

Phytic Acid, Sodium Salt Content (%)	Bound Calcium (ppm)	Percent Lost Calcium
	Calcium-Alkali-Soluble	
0	9,294	...
33.3	7,503	19
50.0	5,525	41
	Magnesium-Alkali-Soluble	
0	6,868	...
33.3	5,948	13
50.0	5,222	23

relationship to protein content, binding more to the hemicellulose containing the least amount of protein. As protein content increased, the effects of both pH and enzyme on calcium released decreased. All three enzymes released more bound calcium than was released by pH effects, with hemicellulase releasing the most, followed by trypsin and pepsin.

The effect of protein on magnesium-water-soluble hemicellulose interaction and its release by pH effects and the enzymes, hemicellulase, pepsin and trypsin, are also shown in Table IV. Magnesium resembles calcium in that it binds more to the hemicellulose containing the least protein. The effect of increased protein content on the binding of magnesium was more pronounced for magnesium (349 ppm) than for the other metals examined. Not surprisingly, therefore, both pH and enzyme effects release 100% of the bound magnesium.

The effects of protein on manganese-water-soluble hemicellulose interaction and its subsequent release by pH and enzymes are also shown in Table IV. Manganese also shows maximum binding to the hemicellulose containing the least protein. Unlike calcium, which showed a decrease in the percentage of mineral released with increased protein content, or magnesium, which showed an increase in percent of released mineral with increased protein content, the percent of bound manganese released by the three enzymes differed. Hemicellulase and pepsin increased the release of manganese as the protein content of the hemicellulose increased. Trypsin, on the other hand, showed a decrease in released manganese with increased protein content.

An arabinogalactan was isolated from the water-soluble hemicellulose by ammonium sulfate fractionation and separated into molecular weight fractions by ultrafiltration.

The amounts of calcium, magnesium, and manganese bound by the arabinogalactan molecular weight fraction about 100,000 are shown in Table V. All three minerals bind less to the arabinogalactan than to the composite water-soluble hemicellulose. However, manganese and magnesium show a difference of only 397 and 598 ppm, respectively. Alkali-soluble hemicellulose was also separated into molecular weight fractions by ultrafiltration. The major fraction greater than 100,000 molecular weight was examined in metal binding studies (Table V). Like the arabinogalactan, all three minerals, calcium, magnesium, and manganese, bind less to this fraction than to the composite hemicellulose.

Several minerals are competitors for binding, such as copper and zinc or calcium and magnesium. The binding of calcium and magnesium alone and in mixtures to water-soluble hemicellulose and their release by pH and hemicellulase, pepsin or trypsin are shown in Table VI. The data indicate that 4,316 ppm (59%) of calcium and 2,123 (55%) of magnesium were bound to the hemicellulose. Therefore, it appears that calcium and magnesium do not compete for binding sites to water-soluble hemicellulose. Treatment of the combined calcium/magnesium/hemicellulose complex with hemicellulase, pepsin and trypsin indicated that 100% of the magnesium was released by all three enzymes, whereas, hemicellulase released 82% calcium, 74% pepsin, and 80% trypsin.

The binding of calcium and magnesium alone and in mixture to alkali-soluble hemicellulose and their release by hemicellulase, pepsin, and trypsin are also shown in Tavle VI. The data show that 90% of calcium and 77% of magnesium were bound to the alkali-soluble hemicellulose. Thus, with alkali-soluble hemicelluloses, calcium intake may influence dietary magnesium binding/release by pH and enzymes. Treatment of the calcium/magnesium/hemicellulose complex with hemicellulase, pepsin, and trypsin showed that hemicellulase released the most calcium (95%), followed by pepsin (58%) and trypsin (21%), whereas, pepsin released the most magnesium (98%) followed by trypsin (96%) and hemicellulase (80%).

Since the report that phytic acid binds essential minerals, rendering them unavailable for absorption during digestion (Davies and Nightingale 1975, Oberleas 1973), we investigated the effects of phytic acid on calcium-alkali-soluble hemicellulose interaction. Table VII indicates that, at the 33.3% phytic acid level, 7,503 ppm of calcium was bound to the hemicellulose or a loss of 19% calcium, and at the 50% level 5,525 ppm of calcium was bound, showing a loss of 41% calcium. Although considerable calcium was bound to phytic acid rather than to hemicellulose and may be unavailable for resorption, the hemicellulose binds more calcium than does the phytic acid. The hemicellulose may have more binding sites than are present in phytic acid.

The effect of phytic acid on magnesium-alkali-soluble hemicellulose interaction (Table VII) was less than that found for calcium. At the 33.3% phytic acid level, 5,948 ppm of magnesium was bound to the hemicellulose, a loss of 13%, and at the 50% level, 5,222 ppm of magnesium was bound, showing a loss of 23%.

These results show that both water- and alkali-soluble hemicellulose of rice bind substantial amounts of free calcium, magnesium, and manganese alone and in the presence of other minerals. Protein associated with hemicelluloses (as in a balanced meal) can affect the binding of these minerals. As protein contents increased, binding of all three minerals decreased. Release of bound minerals is also affected by pH (as in the stomach and GI tract) and the digestive enzymes hemicellulase, pepsin, and trypsin. Acid pH seemed to release more bound minerals than the digestive enzymes do, but the combination of pH and enzymes releases slightly more. Hemicelluloses, therefore, do bind essential minerals, but conceivably the minerals that are released in vivo should be available for resorption. Phytic acid appears to bind less calcium and magnesium than does the hemicellulose, possibly because of more potential binding sites on the hemicelluloses than are present in phytic acid.

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