Chemical Properties and Interactions of Rice Hemicellulose with Trace Minerals in Vitro

Robert R. Mod,* Robert L. Ory, Nancy M. Morris, and Floyd L. Normand

In vitro binding of copper, zinc, and iron by water- and alkali-soluble hemicelluloses and molecular weight fractions of the hemicellulose above 100 000 was determined after incubation at 37 °C for 0.5, 1, and 2 h. Binding was complete within 30-min incubation in a model system. The sequence of mineral binding to alkali-soluble hemicellulose was copper > zinc > iron, and for the water-soluble hemicellulose it was copper > iron > zinc. Copper bound the most to both hemicelluloses. Binding of copper and zinc in combination by hemicellulose suggests that zinc may affect dietary copper binding and release. Effects of digestive enzymes (hemicellulase, pepsin, and trypsin) on minerals bound to hemicellulose were examined in 16-h incubation tests. These enzymes released over half of the bound minerals, suggesting that released minerals should be available for resorption in vivo.

The possible beneficial effects of dietary fiber are well documented, with much of the current interest attributed to observations by Burkitt (1971) and Trowell (1972). They suggested an inverse relationship between the consumption of fiber and the incidence of some diseases. However, in many instances, experimental evidence and the chemical basis for these effects are still lacking (Leveille, 1977). Apparently, not all dietary fibers are equally effective.

Dietary fiber is frequently defined as all food components that are not broken down by enzymes in the digestive tract, including hemicelluloses, cellulose, pectic substances, gums, mucilages, certain carbohydrates, and lignin. Few studies have focused on the effects of specific components of dietary fiber. The main fibers in which nutritionists and health scientists are interested are cellulose, hemicellulose, lignin, and pectins. Fiber properties that contribute to current interest in its components are the capacity to absorb water and to act as a cation-exchange resin (Kelsay, 1978).

Evidence presented thus far suggests that increased fiber consumption is desirable, but it is important to consider any possible adverse effects of fiber intake. The property of fiber to act as a cation-exchange resin could result in some undesirable effects, such as reducing the absorption of several mineral elements. Also, phytic acid binds a number of minerals, and zinc deficiencies have resulted from high phytic acid intakes (Oberleas et al., 1966). Evidence has been presented suggesting that high fiber intakes may impair the absorption of a number of minerals, including calcium, phosphorus, magnesium, and zinc (Reinhold et al., 1976). Because intakes of some mineral elements seem to be marginal in certain segments of the population, high fiber intakes might induce clinical deficiencies of these minerals. Thompson and Weber (1979) reported that changes in pH affect mineral-binding capacities of various fiber sources and that when iron and copper were present in the residues, their levels did not correlate to any of the constituents examined. Zinc, however, did correlate to protein and phytic acid. They also concluded that the binding of trace minerals by fiber sources is a complex mechanism and that understanding this mechanism will require further elucidation of the influence of physicochemical properties.

We isolated, purified, and characterized the water- and alkali-soluble rice bran hemicelluloses as to sugar composition, protein contents, amino acid profiles, as well as arabinose:xylose and arabinose:galactose ratios, and hexuronic acid content (Mod et al., 1978, 1979). Normand et al. (1979) reported the binding of bile acids to water-soluble rice bran hemicellulose. In continuation of these studies, the effects of water- and alkali-soluble bran hemicelluloses and selected molecular weight fractions of these hemicelluloses on binding of three essential metals (copper, zinc, and iron) have been investigated. In vitro studies were performed before and after treatment with digestive enzymes, simulating conditions that exist in vivo, to determine if these essential minerals could be available for resorption during passage through the gastrointestinal tract (GI tract). The results of these investigations and possible mechanisms for binding of the metals are presented.

MATERIALS AND METHODS

Defatted rice bran was obtained from Riviana Foods, Inc. The metal sources were as follows: ferrous sulfate ($FeSO_4 \cdot 7H_2O$) and cupric sulfate ($CuSO_4 \cdot 5H_2O$), Fisher Scientific Co.; zinc sulfate ($ZnSO_4 \cdot H_2O$), J. T. Baker Chemical Co. The three enzymes were hemicellulase (Miles Laboratories), pepsin (Nutritional Biochemicals Corp.), and trypsin (Calbiochem).

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 above for the water-soluble hemicellulose; however, soluble starch and protein were not removed. The arabinogalactan was isolated from the

Southern Regional Research Center, Science and Education Administration, U.S. Department of Agriculture, New Orleans, Louisiana 70179.

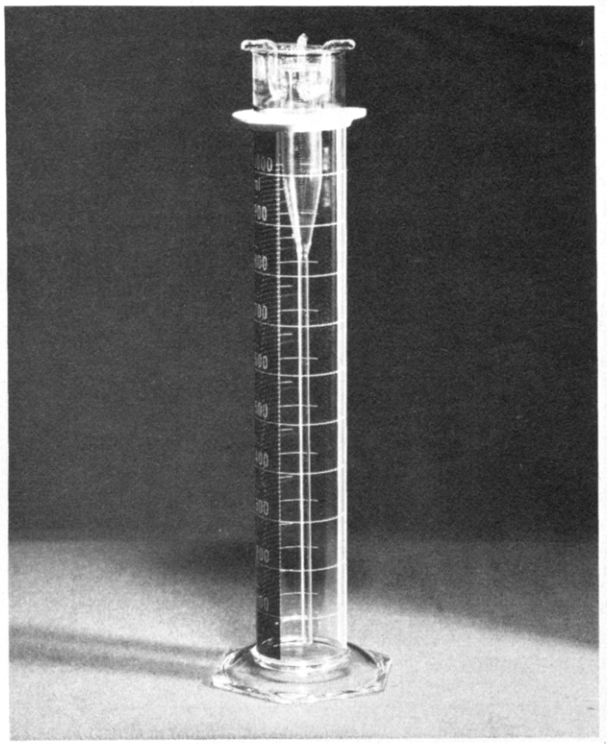

Figure 1. Dialysis apparatus.

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 (GC). Polysaccharides were hydrolyzed by the procedure of Roberts et al. (1976). The hydrolyzed sugars were identified qualitatively and quantitatively by GC analysis on a Hewlett-Packard Model 5750 equipped with a flame ionization detector. The column was a stainless steel tube, $^1/_8$ in. o.d., 10-ft long, 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. The sugars were equilibrated overnight in pyridine and silylated with trimethylchlorosilane and hexamethyldisilane according to Sweeley et al. (1963). The derivatized sugars were identified by comparing retention times and peak enhancement with known sugars. Quantitative estimates of each sugar were made by comparison of peak areas with sorbitol as the internal standard.

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, 6.2 mg of ferrous sulfate to 126 mg of hemicellulose). Each container was incubated in a constant-temperature bath at 37 °C, and aliquots were removed after 0.5, 1, and 2 h for a time study of 2 h. If then used for further enzymatic treatment, the reaction time was 2 h (total). The aliquots were placed in a dialysis bag with a 3500 molecular weight cutoff and dialyzed for 48 h in a dialysis apparatus (Figure 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 the metal–hemicellulose complex and 1.3 mg of trypsin were placed in a container and then 10 mL of Tris buffer, pH 8.1 (0.01 M $CaCl_2$), was added and the mixture was incubated at 37 °C for 16 h. The samples were dialyzed, lyophilized, and analyzed as described above. The same procedure and hemicellulose:enzyme ratio were used for pepsin and hemicellulase, but acetate buffer, pH 5.5, was used for pepsin and pH 4.0 acetate for hemicellulase in place of the Tris buffer.

Titrations for Hydrogen Ion Buffering Capacity. Hemicelluloses were titrated before and after enzymatic treatment with trypsin. The same enzymatic treatment was used as described above, but unreacted hemicelluloses were used instead of metal-bound ones. In order to determine if the small amount of trypsin left in the treated hemicellulose would have any effect on the buffering capacity of the treated hemicellulose, we determined the corresponding titration curves with untreated hemicellulose containing trypsin. Untreated hemicelluloses with and without added trypsin showed the same titration curves. Thus, the small amount of trypsin left with the treated hemicellulose does not affect the buffering capacity of the hemicellulose.

Titrations were performed according to Thompson and Weber (1979). One gram of hemicellulose was suspended in 30 mL of deionized water and stirred. The initial pH was determined after 2 min. Increments of 0.1065 N HCl or 0.1026 N NaOH were added at 3-min intervals, and the pH was recorded after it had stabilized. Hemicellulose samples were titrated with HCl to an end point of pH 2.0 and with NaOH to an end point of pH 10.0.

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 (~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 1000-ppm stock solutions. Standard curves, calculated by the method of least squares, gave a correlation coefficient of 0.99. Five replicate determinations were made on each sample, and the average values and their correlation coefficients were calculated. The coefficients of variation for copper, zinc, and iron were 2.5, 3.1, and 2.3%, respectively. The density of each "solution" was assumed to be 1 g/cm^3, and the ppm (microgram per gram) of each element was calculated from the weight of the sample. To check the possibility for error due to aspiration of dispersed samples rather than solutions, we analyzed each element by the method of additions on several samples. The results agreed well with those obtained by comparison with aqueous standards.

RESULTS AND DISCUSSION

Compositions of the 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 amount of 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.

Table I. Composition of Rice Bran Hemicelluloses

hemicellulose	composition, wt %						
	Rham	Ara	Xyl	Mann	Gal	Glu	protein
alkali soluble	3.1	45.7	32.9	0.7	6.0	7.3	4.28
water soluble 1	Tr[a]	18.4	4.1	3.4	21.2	15.2	37.66
water soluble 2	Tr	12.4	4.0	1.6	19.3	12.6	50.34

[a] Tr = trace.

Table II. Composition of Arabinogalactan and Molecular Weight (M_r) Fractions

	arabinogalactan composition, %	M_r 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	Tr[a]		4.51
galactose/ arabinose	1.4:1	1.43:1	1.44:1

[a] Tr = trace.

Table III. Binding of Cupric Copper by Hemicellulose and Enzymatic Release

original hemicelluose, ppm	after release by enzymes, ppm		
	hemi-cellulase	pepsin	trypsin
	Alkali-Soluble Hemicellulose		
9949 ± 120	7323 ± 203 (26)[a]	6294 ± 171 (37)	4100 ± 82 (59)
	Water-Soluble Hemicellulose		
7179 ± 136	5698 ± 153 (21)	3943 ± 174 (45)	4206 ± 191 (41)

[a] Percent released copper by enzyme is indicated in parentheses.

Table IV. Lysine and Arginine Contents of Rice Bran Hemicelluloses

amino acid	contents, wt %,[a] for rice variety[b]			
	S (LA)	S (AR)	S (TX)	C (CA)
	Alkali Soluble			
Lys	6.6	5.7	5.4	5.3
Arg	6.0	4.2	7.1	6.3
	Water Soluble			
Lys	3.3	2.7	1.7	3.4
Arg	1.1	1.7	1.7	3.4

[a] Weight percent of recovered amino acids. [b] S = Starbonnet; C = Calrose.

Table V. Binding of Zinc by Hemicellulose and Enzymatic Release

original hemicellu-lose, ppm	after release by enzymes, ppm		
	hemicellulase	pepsin	trypsin
	Alkali-Soluble Hemicellulose		
9399 ± 70	3409 ± 106 (64)[a]	8630 ± 264 (8)	9356 ± 336 (0)
	Water-Soluble Hemicellulose		
3000	245 (92)	810 (73)	2378 (21)

[a] Percent released zinc by enzyme is indicated in parentheses.

An arabinogalactan was isolated from the water-soluble hemicellulose by ammonium sulfate fractionation by the procedure of Neukom and Markwalder (1975) and 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 content. 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 cupric copper, zinc, and ferrous iron bound to the hemicelluloses, within 30 min of incubation time. There was only a slight increase in binding as incubation time increased. Analysis for copper, zinc and iron in alkali-soluble hemicellulose before binding studies indicated the presence of 23 ppm of copper, 113 ppm of zinc, and 31 ppm of iron. Water-soluble hemicellulose, on the other hand, showed 62 ppm of zinc, and only one enzyme, pepsin, indicated the presence of 10 ppm of zinc. Although 2142 ppm of calcium was found in trypsin, this should not have any effect on the release of the metals examined since dialysis did not remove this calcium from the trypsin. Maximum binding to alkali-soluble hemicelluloses was copper > zinc > iron, and for the water-soluble hemicellulose it was copper > iron > zinc. Copper binds the most to both hemicelluloses.

The binding of copper to water- and alkali-soluble hemicelluloses and its release after enzymatic treatment with hemicellulase, pepsin, and trypsin are shown in Table III. This indicates that a considerable amount of the bound metal is released and should be available for resorption during passage through the GI tract in vivo. Trypsin released the most copper (59%), followed by pepsin (37%) and hemicellulase (26%) for the alkali-soluble hemicellulose, but pepsin (45%) released the most copper from water-soluble hemicellulose, followed by trypsin (41%) and hemicellulase (21%). The amounts of metals released under these conditions is probably due to a combination of enzyme action (as shown here) and to enzyme and pH effects as suggested by Nelson and Potter (1980). Trypsin is a pancreatic proteolytic enzyme that preferentially catalyzes the hydrolysis of peptide bonds between arginine or lysine and their adjacent amino acids. Pepsin is a endopeptidase that catalyzes the hydrolysis of a variety of peptide bonds, depending upon the amino acids involved and the nature of the peptides possessing the bonds. It, therefore, seems reasonable to assume that copper is bound mostly to the protein or glycoprotein portion of the hemicellulose, probably through a chelate-type bond, rather than to the carbohydrate moiety. This assumption if further substantiated by the lysine and arginine contents of the protein associated with both hemicelluloses, shown in Table IV. The alkali-soluble hemicellulose contains about twice the amount of lysine and arginine than does the water-soluble hemicellulose. Enzymatic hydrolysis of the hemicellulose with hemicellulase, pepsin, and trypsin, followed by GC analysis for sugars, revealed, as expected, that only hemicellulase hydrolyzed

Table VI. Binding of Ferrous Iron by Hemicellulose and Enzymatic Release

original hemicellulose, ppm	after release by enzymes, ppm		
	hemicellulase	pepsin	trypsin
	Alkali-Soluble Hemicellulose		
5088 ± 68	5164 ± 125 (0)[a]	5045 ± 244 (0)	4726 ± 37 (7)
	Water-Soluble Hemicellulose		
5047	3207 (36)	2024 (60)	3184 (37)

[a] Percent released iron by enzyme is indicated in parentheses.

Table VII. Effects of Protein on Cupric Copper-Water-Soluble Hemicellulose Interaction

original hemicellulose, ppm	after release by enzymes, ppm			protein content, %
	hemicellulase	pepsin	trypsin	
7922	2910 (63)[a]	4744 (40)	3991 (49)	9.82
6220	1129 (82)	3459 (44)	3449 (45)	22.84
7179	5698 (21)	3943 (45)	4206 (41)	37.66

[a] Percent released copper by enzyme is indicated in parentheses.

the polysaccharide and confirmed the presence of arabinose, xylose, and glucose.

The binding of zinc to water- and alkali-soluble hemicelluloses and its release after treatment with hemicellulase, pepsin, and trypsin are shown in Table V. Like that shown for copper, a considerable amount of zinc is released for possible resorption. Hemicellulase (64%) released the most, followed by pepsin (8%), for the alkali-soluble hemicellulose. Trypsin did not release any zinc. Apparently, zinc bound to the alkali-soluble hemicellulose is primarily bound to the polysaccharide portion rather than to the protein or glycoprotein moiety. Water-soluble hemicellulose, on the other hand, releases zinc after treatment with all three enzymes. Hemicellulase (92%) releases the most, followed by pepsin (73.0%) and trypsin (21%). This finding suggests that both carbohydrate and protein or glycoprotein moieties are probably involved in the binding of zinc in this case.

The binding of iron to alkali-soluble hemicellulose (Table VI) shows the least amount of binding and subsequent release of the three minerals examined. The data suggest that iron may be tightly bound to the alkali-soluble hemicellulose. However, iron bound to water-soluble hemicellulose is released by pepsin (60%), hemicellulose (36%), and trypsin (37%). This finding also suggests that carbohydrate and protein or glycoprotein are probably involved in the binding.

Because copper, zinc, and iron bound to water-soluble hemicellulose seem to be bound to both protein and polysaccharide moities of the hemicellulose, the binding of the minerals to water-soluble hemicellulose containing different protein contents was investigated.

The effects of protein on copper and water-soluble hemicellulose interaction and its subsequent release by hemicellulase, pepsin, and trypsin are shown in Table VII. The values in parentheses show the percentage of bound copper released by enzymes. Although copper seemed to bind the most to hemicellulose containing the least protein, 7922 ppm, there does not seem to be any correlation between the protein content of the hemicellulose preparations and the amount of copper that is bound nor with the amount of copper released by hemicellulase. Treatment of the copper–hemicellulose complex with hemicellulose, pepsin, and trypsin indicates that hemicellulase releases the most copper, followed by trypsin and pepsin. However, there does seem to be a correlation between the amount of copper released by pepsin and trypsin and the protein content of the hemicellulose preparation. Pepsin increases the amount of bound copper released as the protein content increases, but trypsin shows a slight decrease in the release of copper with increasing protein content.

Table VIII. Effects of Protein on Zinc-Water-Soluble Hemicellulose Interaction

original hemicellulose, ppm	after release by enzymes, ppm			protein content, %
	hemicellulase	pepsin	trypsin	
6244	1645 (74)[a]	4092 (34)	3829 (38)	9.82
5921	741 (88)	1758 (71)	2363 (61)	22.84
3000	245 (92)	810 (73)	2378 (21)	37.66

[a] Percent released zinc by enzyme is indicated parentheses.

Table IX. Effects of Protein on Ferrous Iron-Water-Soluble Hemicellulose Interaction

original hemicellulose, ppm	after release by enzymes, ppm			protein content, %
	hemicellulase	pepsin	trypsin	
7488	6674 (11)[a]	7013 (6)	7400 (0)	9.82
6634	5067 (24)	5459 (18)	6612 (0)	22.84
5047	3207 (37)	2024 (60)	3124 (37)	37.66

[a] Percent released iron by enzyme is indicated in parentheses.

The effects of protein on zinc–hemicellulose interaction and its subsequent release by hemicellulase, pepsin, and trypsin are shown in Table VIII. Like copper, zinc also binds most to hemicellulose containing the least amount of protein. However, unlike copper, which showed no correlation in the amount bound with change in protein content, zinc shows a decrease in amounts bound with increasing protein content. Enzymatic treatment of the zinc–hemicellulose complex shows that considerable amounts of zinc are released by hemicellulase and pepsin as the protein content of the hemicellulose increases. The percentage of bound zinc released by these two enzymes also increases with the protein content of the preparation. The amount of zinc released by trypsin does not seem to be related the protein content.

The effects of protein on iron–hemicellulose interaction and its subsequent release by hemicellulase, pepsin, and trypsin are shown in Table IX. Iron shows maximum binding to the hemicellulose containing the least amount of protein, the same as for copper and zinc, but enzymatic treatments show less release of iron compared to the effects on copper and zinc. The percentage of iron released by hemicellulase and pepsin seems to be correlated with protein content; trypsin is not. These data suggest that as the protein content of the iron–hemicellulose complex increases, the amount of iron released by hemicellulase and pepsin also increases.

For summarization of these results, increasing the amount of protein associated with rice hemicellulose (as one would expect in a normal meal containing whole rice rather than purified hemicelluloses) seems to have different effects on the binding and the subsequent enzymatic release of bound copper, zinc, and iron. Binding of copper does not seem to correlate with protein contents, but release of bound copper by trypsin seems to be inversely related to increasing contents. Binding of zinc and iron seems to be inversely related to increasing protein contents,

Table X. Binding of Cupric Copper and Zinc Mixture by Hemicellulose and Enzymatic Release for Water-Soluble-1 Hemicellulose

	original hemicellulose, ppm	original hemicellulose mixture, ppm	after release by enzymes, ppm	
			hemicellulase	trypsin
Cu	7178	4128 (58)[a]	3822 (7)	3286 (20)
Zn	3000	2852 (95)	822 (69)	800 (72)

[a] Percent released metal by enzyme is indicated in parentheses.

Table XI. Binding of Cupric Copper and Zinc Mixture by Hemicellulose and Enzymatic Release for Alkali-Soluble Hemicellulose

	original hemicellulose, ppm	original hemicellulose mixture, ppm	after release by enzymes	
			hemicellulase	trypsin
Cu	9949	8702 (87)[a]	2670 (69)	4914 (44)
Zn	9358	9360 (100)	1728 (82)	5578 (40)

[a] Percent released metal by enzyme is indicated in parentheses.

Table XII. Binding of Cupric Copper, Zinc, and Ferrous Iron by Arabinogalactan from Water-Soluble Hemicellulose

element	Cu	Zn	Fe
arabinogalactan, $M_r > 100\,000$, bound mineral, ppm	4318	5227	1469

but the percentage of these bound minerals released by hemicellulase and pepsin activities (especially hemicellulase) are both increased with increasing amounts of protein. This result suggests that, in vivo, in a normal diet containing sufficient protein, one might expect a good percentage of the minerals bound by hemicelluloses to be released for resorption as they pass through the GI tract.

Theoretically, certain minerals affect the availability of zinc and copper, but there are differing opinions on interactions between zinc and copper. One reports that copper is antagonistic to zinc (*Consum. Res. Mag.*, 1979), and another reports that increased zinc intake will reduce dietary copper absorption (*Food Prod. Dev.*, 1979). Table X indicates that the latter may be correct. Comparison of the total copper and zinc bound to water-soluble hemicellulose alone and in combination shows that 58% of the available copper and 95% of zinc were bound to the hemicellulose. Treatment of the combined copper–zinc–hemicellulose complex with hemicellulase indicates essentially no effect on copper, but 69% of the zinc is released and should be available for possible resorption under in vivo conditions. Treatment with trypsin, on the other hand, releases 20% of the copper and 72% of the zinc.

The binding of copper and zinc together by alkali-soluble hemicellulose and the amounts released by enzymatic treatment (Table XI) suggest that 87% of the copper and 100% of available zinc are bound to the hemicellulose. Like that found for the water-soluble hemicellulose, results with the alkali-soluble hemicellulose also indicate that zinc intake may reduce dietary copper absorption, but to a lesser extent than from the water-soluble hemicellulose. Treatment of the complex with hemicellulase and trypsin shows that considerable amounts of copper and zinc are released for possible resorption. Hemicellulase releases more copper (69%) and zinc (82%) than trypsin, which releases 44% copper and 40% zinc.

After determination of the amounts of copper, zinc, and iron bound by the hemicellulose, binding of metals to specific components of the hemicellulose was undertaken. An arabinogalactan was isolated from the water-soluble hemicellulose by ammonium sulfate fractionation and separated into molecular weight fractions by ultrafiltration. The binding of copper, zinc, and iron by the arabinogalactan molecular weight fraction above 100 000 is shown in Table XII. Copper and iron bound less to the arabinogalactan than to the complete water-soluble hemicellulose, but zinc binds more to the arabinogalactan than it does to the composite hemicellulose.

Table XIII. Binding of Cupric Copper, Zinc, and Ferrous Iron by the Alkali-Soluble Hemicellulose Fraction

element	Cu	Zn	Fe
$M_r > 100\,000$, bound mineral, ppm	8728	5283	7233

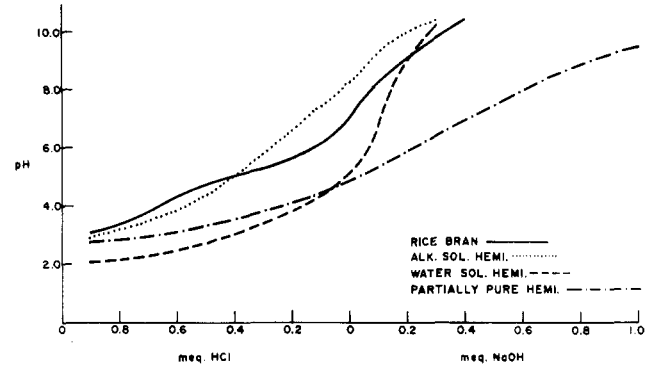

Figure 2. Buffering capacity of hydrogen ions by hemicelluloses.

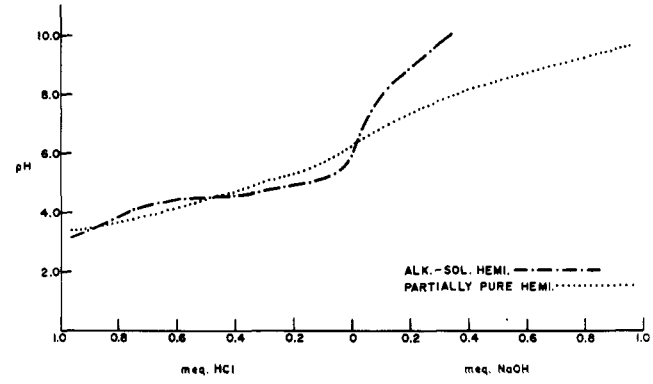

Figure 3. Buffering capacity of hydrogen ions by trypsin-treated hemicelluloses.

Alkali-soluble hemicellulose was also separated into molecular weight fractions by ultrafiltration. The major fraction (90%) of the hemicellulose greater than 100 000 molecular weight was also investigated for metal binding. The binding of cupric copper, zinc, and ferrous iron by the fraction above 100 000 molecular weight is shown in Table XIII. Copper and zinc bind less to this fraction than to the complete hemicellulose, but iron binds more to this fraction than it does to the composite hemicellulose.

These data suggest that copper, zinc, and iron may be bound to the protein or glycoprotein moiety of the hemicellulose. Because protein has good buffering capacity of hydrogen ions, untreated and trypsin-treated hemicellulose were titrated from pH 2 to pH 10. The titration curves for untreated hemicelluloses, water-soluble-1 and water-soluble-2, alkali-soluble hemicellulose, and whole rice bran (for comparison purposes) are shown in Figure 2. Water-soluble-2 hemicellulose typifies the best buffering capacity, as indicated by the slope of the line, followed by rice bran, alkali-soluble hemicellulose, and water-soluble-1 hemicellulose. This hemicellulose also has the highest

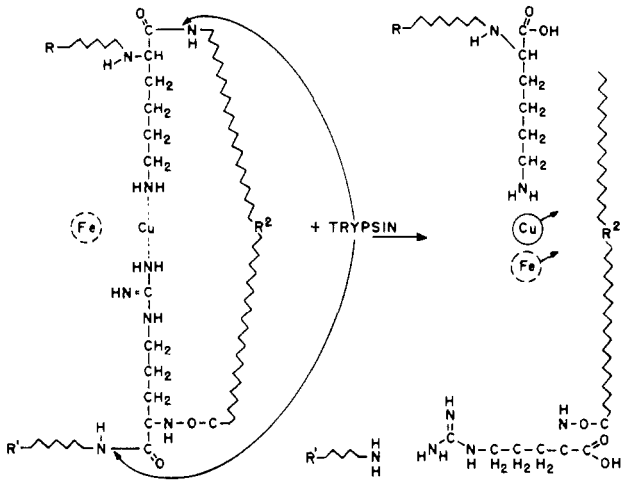

Figure 4. A proposed mechanism for copper–fiber interaction.

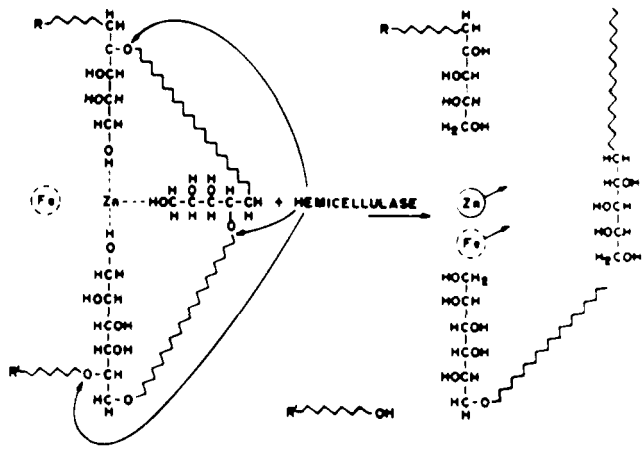

Figure 5. A proposed mechanism for zinc–fiber interaction.

protein content, particularly the albumin fracton of the rice bran (50.3%). However, water-soluble-1 hemicellulose, which has a protein content of 37.7%, showed a poorer buffering capacity than did the whole rice bran that contained only 18.3% of protein.

The buffering capacities of purified alkali-soluble hemicellulose and water-soluble-2 hemicellulose after treatment with trypsin are shown in Figure 3. Although the protein content of the alkali-soluble hemicellulose (4.3%) was not reduced after treatment with trypsin (in fact, it was raised slightly: 5.8%), probably because of trypsin and the inability of the large hydrolyzed peptides to migrate from the dialysis bag with a molecular weight cutoff of 3500, buffering capacity increased. However, the buffering capacity of the water-soluble-2 hemicellulose did not change after trypsin treatment, even though the protein content was reduced from 50 to 37%. Other physicochemical properties may influence its buffering capacity.

From the results of this study, a hypothetical mechanism is propsed for copper–fiber, zinc–fiber, and iron–fiber interactions and their subsequent release by enzymatic activity in the intestional tract. Figure 4 shows a possible mechanism for copper–fiber interaction and its subsequent release by trypsin. In the binding of copper to hemicellulose, a chelate can be formed between the terminal amino groups of lysine and arginine of the protein moiety of hemicellulose and the copper. Treatment with trypsin cleaves the peptide linkages between arginine and lysine and their adjacent amino acids. This cleavage would destroy the chelate bond and release the copper for possible resorption during passage through the upper intestinal tract.

A proposed mechanism for zinc–fiber interaction and its subsequent release by hemicellulase activity is shown in Figure 5. Like that for copper, the binding of zinc to hemicellulose seems to be by a chelate-type bond. However, in this case, the zinc seems to bind to the polysaccharide moiety of the hemicellulose, particularly the arabinose, xylose, and glucose sugars. Treatment with hemicellulase hydrolyzes the polysaccharide, which destroys the chelate, releasing the zinc for possible resorption. This resorption would take place in the large intestine, where hemicellulase activity is present in the intestinal microflora.

Unlike the mechanisms for copper and zinc, in which either protein or polysaccharide groups seem to be involved, iron–fiber interactions may involve a combination of both protein and polysaccharide. Therefore, both mechanisms for enzymatic release by trypsin and hemicellulase may be involved in the release of chemically bound iron for resorption, some in the upper GI tract and some in the lower.

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