

# ***In Vitro* Binding Capacity of Wheat Bran, Rice Bran, and Oat Fiber for Ca, Mg, Cu, and Zn Alone and in Different Combinations<sup>†</sup>**

A. Idouraine,<sup>‡</sup> M. J. Khan,<sup>§</sup> and C. W. Weber\*

Department of Nutrition and Food Science, University of Arizona, Tucson, Arizona 85721

Wheat bran (WB), rice bran (RB), and oat fiber (OF) were analyzed for their proximate constituents and mineral content and evaluated for their binding capacity for calcium (Ca), magnesium (Mg), copper (Cu), and zinc (Zn). Protein, soluble and insoluble dietary fiber, and endogenous mineral contents varied significantly among the three dietary fiber sources. With the exception of Mg, in higher concentration in RB, WB contained significantly ( $P < 0.05$ ) more endogenous Ca, Cu, and Zn than RB or OF. Overall, WB bound significantly more Ca and Mg (alone or combined with other minerals) than RB and OF, respectively. OF bound more Cu than RB or WB. Zn (alone or in the presence of other minerals) was bound more strongly in WB and RB than OF. Re-acid washing stripped most of the bound minerals from the dietary fiber sources.

**Keywords:** Mineral binding; wheat bran; rice bran; oat fiber; dietary fiber

## INTRODUCTION

Dietary fibers include cellulose, hemicellulose, pectins, gums, and mucilages, which are biosynthesized from pentose, hexose, and uronic acid, and lignin built from phenylpropane units such as cinamyl and couamyl alcohols. Proportions of these constituents vary generally among dietary fiber sources and can markedly influence the absorption of minerals. Associated with dietary fiber are antinutrients, such as phytic acid and oxalic acid, and proteins which could affect to a certain extent mineral bioavailability. Minerals can also interact and influence their absorption. Dietary fiber might limit mineral bioavailability by binding, diluting, and trapping minerals within dietary fiber particles or shortening the transit time of nutrients through the intestine. There are several *in vivo* and *in vitro* studies which indicate that dietary fiber might have important impacts on mineral balance or binding.

Frølich (1983) reviewed more than 180 *in vivo* and *in vitro* studies on the effects of dietary fiber from cereals on mineral bioavailability and mineral binding. The author concluded that dietary fibers from cereals have different binding properties and seem to influence mineral bioavailability due to their components and recommended more studies in the field.

Reinhold et al. (1976) reported a negative balance of calcium, magnesium, zinc, and phosphorus with increased consumption of dietary fiber by humans. Similarly, Kivistö et al. (1986) showed that when consumed at high level, extruded dietary fiber from cereals had negative effects on zinc, calcium, and magnesium but not on iron. More recently, Moak et al. (1988) reported that the addition of oat bran and wheat bran to the diet of adult men significantly lowered zinc, calcium, magnesium, and zinc absorption.

Contrary to these studies in humans, Bagheri and Guegun (1981) found no adverse effects on zinc, calcium, magnesium, and phosphorus absorption in rats fed 10 and 15% wheat bran diets for 41 days. Sandberg et al. (1982) also reported no effect of wheat bran on phosphorus, calcium, zinc, magnesium, and iron absorption in humans consuming 16 g of dietary fiber per day.

*In vitro* studies showed that binding of minerals to dietary fiber might vary depending on the source and type of the fiber, the methodology used, and the conditions of the experiment (mineral concentration and pH of the solution). The presence or absence of other factors such as phytic acid, oxalic acid, and proteins might enhance or reduce mineral binding making the distinction between the effects of dietary fiber and other factors more difficult. Thompson and Weber (1981) studied the binding of copper, zinc, and iron in wheat bran, corn bran, soy bran, oat hulls, rice bran, and cellulose at pH 0.65 and pH 6.8. The authors found that pH as well as the fiber type affect mineral binding. Copper was reported to bind more than zinc to dietary fiber. Camire and Clydesdale (1981) indicated that wheat bran and other fractions of dietary fiber (cellulose and lignin) had high binding capacity for calcium, magnesium, zinc, and iron. Cellulose had low binding capacity while lignin and pectin had strong binding capacities. Mineral binding to fiber was found pH dependent. In more recent studies, Persson et al. (1987) showed that soluble fiber fractions of wheat bran interact strongly with copper. Casterline and Ku (1993) studied the effects of pH, mineral concentration, and incubation time on zinc binding in wheat bran and apple fiber. These workers indicated that maximum binding occurred at pH 7.2 at an initial concentration of 220  $\mu\text{g}$  zinc/g dietary fiber incubated for 24 h. A few studies have examined the binding capacity of dietary fibers for minerals in combination (Mod et al., 1981; Garcia-Lopez et al., 1985; Platt and Clydesdale, 1986; Elhardallou and Walker, 1993).

The purpose of this work was, therefore, to study the binding capacity of wheat bran, rice bran, and oat fiber for calcium, magnesium, copper, and zinc alone and in different combinations at pH 7.0.

\* Author to whom correspondence should be addressed.

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<sup>‡</sup> Present address: Harrington Arthritis Research Center, 300 N. 18th St., Phoenix, AZ 85006.

<sup>§</sup> Soil Micro-Biology Section, ARI Tarnab Peshawar, Pakistan.

**Table 1. Proximate Composition of Wheat Bran, Rice Bran, and Oat Fiber<sup>d</sup>**

sample	moisture (%)	protein <sup>e</sup> (%)	crude fat (%)	ADF <sup>f</sup> (%)	CHO <sup>g</sup> (%)	ash (%)	energy <sup>h</sup> (kcal/100 g)
wheat bran	7.8 ± 0.0 <sup>a</sup>	19.0 ± 0.1 <sup>a</sup>	4.5 ± 0.4 <sup>b</sup>	12.1 ± 0.1 <sup>c</sup>	57.6 ± 0.5 <sup>a</sup>	6.8 ± 0.0 <sup>a</sup>	347 ± 2 <sup>b</sup>
rice bran	4.0 ± 0.0 <sup>c</sup>	14.0 ± 0.2 <sup>b</sup>	19.8 ± 0.2 <sup>a</sup>	12.3 ± 0.1 <sup>b</sup>	48.0 ± 0.2 <sup>c</sup>	6.0 ± 0.0 <sup>b</sup>	426 ± 1 <sup>a</sup>
oat fiber	4.9 ± 0.0 <sup>b</sup>	4.4 ± 0.1 <sup>c</sup>	1.5 ± 0.0 <sup>c</sup>	39.8 ± 0.2 <sup>a</sup>	50.1 ± 0.0 <sup>b</sup>	4.2 ± 0.1 <sup>c</sup>	231 ± 0 <sup>c</sup>

<sup>a-c</sup> Mean values with the same superscript within a column are not significantly different. <sup>d</sup> Determined in duplicate dry samples (mean ± SD). <sup>e</sup> Protein = N × 6.25. <sup>f</sup> ADF = acid detergent fiber. <sup>g</sup> CHO = available carbohydrate calculated by difference: 100 - (protein + lipid + ADF + ash). <sup>h</sup> Calculated (protein and CHO by 4; crude fat by 9).

## MATERIALS AND METHODS

**Sample Preparation.** Hard Red Spring wheat bran (American Association of Cereal Chemists, St. Paul, MN) was ground in a Model No. 2 Wiley mill until all samples passed through a 60 mesh screen. Rice bran (California Natural Products, Lathrop, CA) and oat fiber (D. D. Williamson, Louisville, KY) were received as fine particles and used as is. Aliquots of the samples were saved for proximate analysis while the remaining fiber sources were defatted for 8 h using hexane (5 mL/g sample) and used for mineral binding.

**Proximate Chemical Composition.** Moisture, protein, crude fat, and ash were analyzed according to AOAC (1990) Methods Nos. 925.08, 984.13, 920.39, and 923.03, respectively. Acid detergent fiber was determined according to the method of Robertson and Van Soest (1981). Carbohydrate contents were determined by difference [100 - (protein + fat + acid detergent fiber + ash)]. Energy levels were calculated by multiplying protein and carbohydrate contents by a factor of 4 and fat by 9. All analyses were carried out in duplicate dried samples.

**Dietary Fiber Determination.** Soluble (SF), insoluble (IF), and total dietary (TDF) fiber were determined in duplicate fat-free dried samples following the 32-07 AACC (1991) method. Duplicate samples of each fiber were weighed into 400 mL beakers, treated with enzymes as described in the method, and filtered through sintered glass filters. The residue (insoluble fiber) was washed with ethanol and then acetone, and dried. The soluble portion (SF) was precipitated with 4 volumes of ethanol and filtered through sintered glass filters. After correction for ash and protein, SF and IF were calculated by weight difference. TDF was calculated by adding the values for soluble and insoluble fibers.

**Mineral Binding.** Defatted samples were acid washed with a HCl solution (pH 1, ratio 7:1 w/v) by shaking the slurry overnight and filtering through a 0.0017 in. nylon screen (Monodur Screen Fabrics No. 42, Industrial Fabrics Corporation, Minneapolis, MN) using a porcelain filter funnel as a support. The residue was washed several times with distilled deionized water until the filtrate tested neutral (pH 7), then air-dried on a bench top, and stored frozen. Before binding, samples were dried overnight in a vacuum oven at 50 °C. Aliquots (2 g) were saved for mineral analysis while the remaining samples (6 g) were shaken for 3 h with calcium, magnesium, copper, and zinc solutions alone (8 mL of 1000 ppm standard mineral solutions/g of sample) and in four different combinations (8 mL of each standard mineral solution/g of sample). Standard mineral solutions (Atomic Absorption Standard) were bought from Fisher Scientific (Pittsburgh, PA). The slurry was centrifuged at 2500g for 15 min. Supernatants were discarded while the residues were washed three times with deionized water (pH 7), transferred to weighing dishes, freeze-dried, and stored until analysis. Portions of fiber with bound mineral (2 g) were further re-acid washed with HCl solution (pH 1, 7:1 ratio, w/v) overnight, and centrifuged at 2500g for 15 min. The procedure was repeated three times. Supernatants were discarded while the residues were freeze-dried and saved for mineral analysis.

**Mineral Analysis.** Duplicate 0.5 g samples of defatted, acid-washed, mineral bound, and re-acid-washed materials were wet ashed as described previously (Idouraine et al., 1996). Samples (0.5 g) were weighed, transferred to 25 mL volumetric tubes, mixed with 5 mL of concentrated nitric acid, and then predigested at 60 °C in a water bath for 1 h. After predigestion, the temperature was raised to about 100 °C and five drops of 30% hydrogen peroxide were added into each tube every 30

min for 2 h. Before the tubes were removed from the water bath, another drop of hydrogen peroxide was added to bring the solution to a light green color (indication of complete digestion). The solution was adjusted to 10 mL with deionized water, transferred to centrifuge tubes, and centrifuged at 1000g for 10 min. Supernatants were either measured directly or diluted to the appropriate concentrations for elemental analysis by flame atomic absorption. Minerals were measured at the appropriate wavelength using a Hitachi Model 180-70 spectrophotometer and quantified by reference to standard curves made from standard mineral solutions (Fisher Scientific, Pittsburgh, PA). Ca and Mg were determined in the presence of 1% lanthanum oxide to avoid interferences with other compounds.

**Statistical Analysis.** Data were statistically analyzed using one-way analysis of variance with means separated and least significance difference set at  $P < 0.05$  (Steel and Torrie, 1960).

## RESULTS AND DISCUSSION

### Proximate Composition of Dietary Fiber Sources.

Data on proximate analysis of wheat bran, rice bran, and oat fiber are listed in Table 1. Moisture contents varied significantly ( $P < 0.05$ ) among the three fiber sources. Wheat bran had significantly the highest moisture levels followed by oat fiber and rice bran. Conditions of processing and storage might explain these differences. Protein contents of wheat and rice bran were significantly higher ( $P < 0.05$ ) than that of oat fiber. Our values are in agreement with those reported by Polizzoto et al. (1983) and Weber et al. (1993) but lower than that indicated by Kahlon et al. (1990) for oat bran. Fat values were significantly different among the samples, rice bran showing the highest fat value and oat fiber the lowest. Our fat contents for wheat bran and oat fiber were close to those listed by Polizzoto et al. (1983), Seibert (1987), and Kahlon et al. (1990). Rice bran had a fat content in the range of that reported by Seibert (1987) but much lower than that indicated by Polizzoto et al. (1983). Acid detergent fiber contents were 2.6–3.2-fold higher in oat fiber than in wheat and rice bran, respectively. These values agree with those found by Saunders (1990) and Idouraine et al. (1995). The carbohydrate contents, calculated by difference, were significantly ( $P < 0.05$ ) different among the three fiber sources, wheat bran having the highest level and rice bran the lowest. Values are in the range of those reported by Polizzoto et al. (1983). Ash values were significantly different among the fiber sources studied and similar to those indicated in the literature (Polizzoto et al., 1983; Seibert, 1987; Saunders, 1990; Idouraine et al., 1995). Energy levels were significantly different among the three fiber sources, wheat, and rice bran containing more calories per gram than oat fiber.

**Soluble, Insoluble, and Total Dietary Fiber.** Results on insoluble (IF), soluble (SF), and total (TDF) dietary fiber of wheat bran, rice bran, and oat fiber are reported in Table 2. Levels of IF, SF, and TDF varied significantly among the three fiber sources. Oat fiber

**Table 2. Soluble, Insoluble, and Total Dietary Fiber (TDF) of Wheat Bran, Rice Bran, and Oat Fiber<sup>d</sup>**

sample	insoluble (%)	soluble (%)	TDF <sup>e</sup> (%)
wheat bran	49.3 ± 2.7 <sup>b</sup>	1.5 ± 0.1 <sup>b</sup>	50.8 ± 2.6 <sup>b</sup>
rice bran	23.6 ± 0.4 <sup>c</sup>	2.7 ± 0.4 <sup>a</sup>	26.3 ± 0.8 <sup>c</sup>
oat fiber	78.2 ± 0.1 <sup>a</sup>	0.2 ± 0.2 <sup>c</sup>	78.4 ± 0.1 <sup>a</sup>

<sup>a-c</sup> Mean values with the same superscript within a column are not significantly different. <sup>d</sup> Determined in duplicate, fat-free dry samples (mean ± SD). <sup>e</sup> TDF (total dietary fiber) = soluble + insoluble dietary fiber.

had significantly ( $P < 0.05$ ) higher IF and TDF content than wheat and rice bran. Rice bran showed the highest SF content among the three fiber sources. Our values for wheat and rice bran agree with those reported by Dreher (1987), Kahlon et al. (1990), and Weber et al. (1993). Fiber contents of oat fiber were similar to those cited by Weber et al. (1993) but much lower than those of oat bran (Dreher 1987; Kahlon et al., 1990).

**Mineral Contents of Raw and Acid-Washed Dietary Fiber.** Calcium (Ca), magnesium (Mg), copper (Cu), and zinc (Zn) contents of defatted and acid-washed wheat bran, rice bran, and oat fiber are listed in Table 3. Defatted dietary fibers contained significantly ( $P < 0.05$ ) more Mg than Ca, Zn, and Cu, respectively. The levels of these four minerals varied significantly among the three fiber sources. Ca, Cu, and Zn were at significantly higher concentrations in wheat bran than in rice bran and oat fiber, while Mg was higher in rice bran. Acid washing stripped significant amounts of the minerals studied from dietary fiber sources. Ca and Mg appeared more efficiently removed with the acid solution (93–100% reduction) than Cu and Zn (27–86% reduction). This might suggest that Ca and Mg have lower energy bonds than Cu and Zn or that these last two minerals are more tightly bound to the dietary fibers. Phytic acid, carboxylic acid, and protein content may also have a direct effect on the degree of binding. Our values for endogenous minerals were in the range of those previously reported (Thompson and Weber, 1981; Platt and Clydesdale, 1986; Saunders, 1990; Weber et al., 1993; Idouraine et al., 1995). Mineral

contents of the acid-washed dietary fibers listed in this study were not always consistent with those cited by Weber et al. (1993) and Idouraine et al. (1995). Differences in the origin and particle size of the dietary fiber might explain these variations.

#### Binding Capacity of Dietary Fiber Sources for Ca.

The binding capacity of wheat bran, rice bran, and oat fiber for Ca alone and in combination with other minerals varied significantly ( $P < 0.05$ ) (Table 4). Fiber sources bound significantly more Ca when it was added alone than when it was combined with other minerals. Wheat bran bound significantly more Ca than rice bran and oat fiber, suggesting that wheat bran might have more specific sites for Ca than other fiber sources or it might have the same number of sites but they might have greater affinity for Ca than the binding sites on other fibers. The binding capacity of fiber sources for Ca was significantly affected by the presence of other minerals. With the exception of rice bran which bound more Ca in (Ca + Zn) combination, wheat bran bound significantly more Ca than rice bran and oat fiber in all other combinations. This might be due to the higher protein content of wheat bran compared to that of rice bran and oat fiber and/or to their difference in chemical composition. Phytic acid, oxalic acid, and other fiber components have been implicated in mineral binding. Torre et al. (1990) reported that cellulose had no ability in binding Fe and Ca while lignin interacts strongly with both elements. Wheat bran bound significantly more Ca in the presence of Cu than in the presence of Mg or Zn or the three minerals combined. In rice bran, Ca combined with Zn showed significantly higher amount of binding than when it was combined with Mg or Cu, or when the three minerals were mixed together. In oat fiber, the highest amount of Ca bound in the presence of other minerals was when it was combined with Mg, and the lowest when the four minerals were combined together. This might suggest that the dietary fibers studied have specific binding sites for Ca. Platt and Clydesdale (1986) reported a similar trend in wheat bran bound with iron in the presence of calcium. Washing the bound dietary fiber with a HCl solution

**Table 3. Mineral Content in Defatted and Acid-Washed Wheat Bran, Rice Bran, and Oat Fiber<sup>j</sup>**

mineral	mineral (μg/g)					
	defatted			acid washed		
	wheat bran	rice bran	oat fiber	wheat bran	rice bran	oat fiber
Ca	1186 ± 48 <sup>a,g</sup>	710 ± 25 <sup>b,g</sup>	761 ± 1 <sup>b,g</sup>	20 ± 5 <sup>c,g</sup> (98) <sup>l</sup>	47 ± 9 <sup>c,f</sup> (93)	16 ± 3 <sup>c,f</sup> (98)
Mg	5792 ± 3 <sup>b,f</sup>	5817 ± 6 <sup>a,f</sup>	5219 ± 4 <sup>c,f</sup>	23 ± 1 <sup>d,g</sup> (100)	26 ± 0 <sup>d,g</sup> (100)	4 ± 0 <sup>e,h</sup> (100)
Cu	24 ± 3 <sup>a,i</sup>	15 ± 0 <sup>b,i</sup>	8 ± 0 <sup>c,i</sup>	3 ± 0 <sup>d,h</sup> (88)	3 ± 1 <sup>d,h</sup> (80)	1 ± 0 <sup>d,h</sup> (88)
Zn	135 ± 5 <sup>a,h</sup>	87 ± 3 <sup>b,h</sup>	15 ± 1 <sup>d,h</sup>	42 ± 1 <sup>c,f</sup> (69)	12 ± 1 <sup>d,h</sup> (86)	11 ± 0 <sup>d,g</sup> (27)

<sup>a-e</sup> Mean values with the same superscript within a row are not significantly different ( $P < 0.05$ ). <sup>f-i</sup> Mean values with the same superscript within a column are significantly different ( $P < 0.05$ ). <sup>j</sup> Expressed as μg of minerals per g of sample. <sup>k</sup> Mean ± SD in duplicate fat-free acid-washed dry samples. <sup>l</sup> Numbers in parentheses indicate percent reduction after acid washing.

**Table 4. Binding Capacity of Wheat Bran, Rice Bran, and Oat Fiber for Calcium Alone and in Combination with Other Minerals<sup>k</sup>**

combination	Ca (μg/g)					
	wheat bran		rice bran		oat fiber	
	bound	re-acid washed	bound	re-acid washed	bound	re-acid washed
Ca alone	6075 ± 8 <sup>a,f,l</sup>	99 ± 21 <sup>d,f</sup>	3134 ± 6 <sup>b,f</sup>	92 ± 23 <sup>d,f</sup>	1116 ± 54 <sup>c,f</sup>	5 ± 1 <sup>e,f</sup>
Ca + Mg	3019 ± 4 <sup>a,h</sup>	23 ± 1 <sup>d,g</sup>	2420 ± 75 <sup>b,h</sup>	7 ± 0 <sup>d,g</sup>	579 ± 17 <sup>c,g</sup>	3 ± 0 <sup>d,g</sup>
Ca + Cu	4316 ± 59 <sup>a,g</sup>	11 ± 2 <sup>d,g</sup>	2410 ± 58 <sup>b,h</sup>	12 ± 1 <sup>d,g</sup>	337 ± 0 <sup>c,h</sup>	1 ± 0 <sup>d,h</sup>
Ca + Zn	2467 ± 42 <sup>b,i</sup>	19 ± 1 <sup>d,g</sup>	2667 ± 44 <sup>a,g</sup>	4 ± 1 <sup>d,g</sup>	337 ± 14 <sup>c,h</sup>	2 ± 0 <sup>d,g,h</sup>
Ca + Mg + Cu + Zn	2145 ± 126 <sup>a,j</sup>	4 ± 0 <sup>d,g</sup>	837 ± 4 <sup>b,j</sup>	1 ± 1 <sup>d,g</sup>	306 ± 32 <sup>c,h</sup>	3 ± 0 <sup>d,g</sup>

<sup>a-e</sup> Mean values with the same superscript within a row are not significantly different ( $P < 0.05$ ). <sup>f-j</sup> Mean values with the same superscript within a column are not significantly different. <sup>k</sup> Expressed as μg of calcium per g of sample in duplicate fat-free acid-washed dry samples. <sup>l</sup> Mean ± SD.

**Table 5. Binding Capacity of Wheat Bran, Rice Bran, and Oat Fiber for Magnesium Alone and in Combination with Other Minerals<sup>k</sup>**

combination	Mg ( $\mu\text{g/g}$ )					
	wheat bran		rice bran		oat fiber	
	bound	re-acid washed	bound	re-acid washed	bound	re-acid washed
Mg alone	2135 $\pm$ 8 <sup>a,f,l</sup>	37 $\pm$ 3 <sup>d,f</sup>	1698 $\pm$ 127 <sup>b,f</sup>	37 $\pm$ 6 <sup>d,f</sup>	328 $\pm$ 15 <sup>c,g</sup>	7 $\pm$ 3 <sup>d,f</sup>
Mg + Ca	1295 $\pm$ 6 <sup>b,h</sup>	4 $\pm$ 0 <sup>d,g</sup>	925 $\pm$ 2 <sup>a,h</sup>	3 $\pm$ 0 <sup>d,g</sup>	180 $\pm$ 3 <sup>c,i</sup>	0 <sup>d,g</sup>
Mg + Cu	1619 $\pm$ 10 <sup>a,g</sup>	3 $\pm$ 0 <sup>d,h</sup>	1336 $\pm$ 20 <sup>b,g</sup>	1 $\pm$ 0 <sup>d,g</sup>	188 $\pm$ 5 <sup>c,i</sup>	0 <sup>d,g</sup>
Mg + Zn	1087 $\pm$ 4 <sup>a,i</sup>	6 $\pm$ 0 <sup>d,g</sup>	838 $\pm$ 3 <sup>b,h</sup>	1 $\pm$ 0 <sup>d,e,g</sup>	215 $\pm$ 2 <sup>c,h</sup>	0 <sup>e,g</sup>
Mg + Ca + Cu + Zn	411 $\pm$ 3 <sup>b,j</sup>	0 <sup>d,i</sup>	145 $\pm$ 7 <sup>c,i</sup>	0 <sup>d,g</sup>	551 $\pm$ 7 <sup>a,f</sup>	0 <sup>d,g</sup>

<sup>a-e</sup> Mean values with the same superscript within a row are not significantly different ( $P < 0.05$ ). <sup>f-j</sup> Mean values with the same superscript within a column are not significantly different. <sup>k</sup> Expressed as  $\mu\text{g}$  of magnesium per g of sample in duplicate fat-free acid-washed dry samples. <sup>l</sup> Mean  $\pm$  SD.

**Table 6. Binding Capacity of Wheat Bran, Rice Bran, and Oat Fiber for Copper Alone and in Combination with Other Minerals<sup>k</sup>**

combination	Cu ( $\mu\text{g/g}$ )					
	wheat bran		rice bran		oat fiber	
	bound	re-acid washed	bound	re-acid washed	bound	re-acid washed
Cu alone	1212 $\pm$ 95 <sup>b,f,l</sup>	45 $\pm$ 6 <sup>d,f</sup>	2416 $\pm$ 95 <sup>b,f</sup>	90 $\pm$ 7 <sup>d,f</sup>	3737 $\pm$ 155 <sup>a,j</sup>	21 $\pm$ 7 <sup>d,f</sup>
Cu + Zn	1151 $\pm$ 11 <sup>c,f,g</sup>	46 $\pm$ 14 <sup>d,f</sup>	2469 $\pm$ 99 <sup>b,f</sup>	21 $\pm$ 1 <sup>d,g</sup>	5457 $\pm$ 38 <sup>a,h</sup>	3 $\pm$ 0 <sup>d,g</sup>
Cu + Ca	1055 $\pm$ 42 <sup>c,g</sup>	10 $\pm$ 0 <sup>d,g</sup>	1399 $\pm$ 126 <sup>b,g</sup>	8 $\pm$ 1 <sup>d,h</sup>	6604 $\pm$ 33 <sup>a,f</sup>	2 $\pm$ 0 <sup>d,g</sup>
Cu + Mg	751 $\pm$ 31 <sup>c,h</sup>	11 $\pm$ 0 <sup>d,g</sup>	1227 $\pm$ 35 <sup>b,g</sup>	7 $\pm$ 0 <sup>d,h</sup>	6146 $\pm$ 7 <sup>a,g</sup>	2 $\pm$ 0 <sup>d,g</sup>
Cu + Mg + Zn + Ca	1082 $\pm$ 18 <sup>c,g</sup>	13 $\pm$ 0 <sup>d,g</sup>	2623 $\pm$ 26 <sup>b,f</sup>	15 $\pm$ 0 <sup>d,g,h</sup>	5234 $\pm$ 0 <sup>a,i</sup>	2 $\pm$ 0 <sup>d,g</sup>

<sup>a-e</sup> Mean values with the same superscript within a row are not significantly different ( $P < 0.05$ ). <sup>f-j</sup> Mean values with the same superscript within a column are not significantly different. <sup>k</sup> Expressed as  $\mu\text{g}$  of copper per g of sample in duplicate fat-free acid-washed dry samples. <sup>l</sup> Mean  $\pm$  SD.

**Table 7. Binding Capacity of Wheat Bran, Rice Bran, and Oat Fiber for Zinc Alone and in Combination with Other Minerals<sup>k</sup>**

combination	Zn ( $\mu\text{g/g}$ )					
	wheat bran		rice bran		oat fiber	
	bound	re-acid washed	bound	re-acid washed	bound	re-acid washed
Zn alone	6056 $\pm$ 50 <sup>b,f,2</sup>	156 $\pm$ 9 <sup>d,f</sup>	6493 $\pm$ 196 <sup>a,f</sup>	6 $\pm$ 0 <sup>d,h</sup>	3078 $\pm$ 60 <sup>c,i</sup>	18 $\pm$ 3 <sup>d,f</sup>
Zn + Cu	4457 $\pm$ 38 <sup>a,f</sup>	34 $\pm$ 0 <sup>d,g</sup>	4302 $\pm$ 15 <sup>b,h</sup>	13 $\pm$ 0 <sup>d,f</sup>	2255 $\pm$ 19 <sup>c,j</sup>	2 $\pm$ 1 <sup>d,g</sup>
Zn + Ca	5604 $\pm$ 33 <sup>b,g</sup>	10 $\pm$ 0 <sup>d,h</sup>	6582 $\pm$ 10 <sup>a,f</sup>	11 $\pm$ 0 <sup>d,g</sup>	4259 $\pm$ 17 <sup>c,g</sup>	3 $\pm$ 0 <sup>d,g</sup>
Zn + Mg	5146 $\pm$ 7 <sup>b,h</sup>	32 $\pm$ 1 <sup>d,g</sup>	5857 $\pm$ 19 <sup>a,g</sup>	5 $\pm$ 0 <sup>d,h</sup>	4714 $\pm$ 79 <sup>c,f</sup>	2 $\pm$ 1 <sup>d,g</sup>
Zn + Ca + Mg + Cu	4234 $\pm$ 10 <sup>a,j</sup>	7 $\pm$ 1 <sup>d,h</sup>	3627 $\pm$ 27 <sup>b,f</sup>	6 $\pm$ 1 <sup>d,h</sup>	3513 $\pm$ 11 <sup>c,h</sup>	4 $\pm$ 1 <sup>d,g</sup>

<sup>a-e</sup> Mean values with the same superscript within a row are not significantly different ( $P < 0.05$ ). <sup>f-j</sup> Mean values with the same superscript within a column are not significantly different. <sup>k</sup> Expressed as  $\mu\text{g}$  of zinc per g of sample in duplicate fat free acid washed dry samples. <sup>l</sup> Mean  $\pm$  SD.

(pH 1) removed significant amounts of Ca. The reduction was more important with Ca combined with other minerals than with Ca alone.

**Binding Capacity of Dietary Fiber Sources for Mg.** The binding capacity of wheat bran, rice bran, and oat fiber for Mg was significantly affected by the presence of other minerals and varied significantly among the fiber sources studied (Table 5). Overall, wheat bran bound significantly more Mg when it was alone or combined with other minerals than rice bran and oat fiber, suggesting that wheat bran might have more specific binding sites for Mg than rice bran or oat fiber. This also could be related to their difference in protein content or other components which might have more affinity for Mg. In wheat bran, Mg in the presence of Cu was bound significantly more followed by combinations with Ca, Zn, and the three minerals together. The presence of other minerals appeared to affect Mg binding. In rice bran, more Mg was significantly bound in the presence of Cu than with Ca, Zn, or the three minerals together. In oat, however, the highest Mg binding occurred when the three minerals were mixed, and the lowest when Mg was combined with Ca or Cu. Little has been reported in the literature on the *in vitro* binding of Mg by dietary fiber. Camire and Clydesdale (1981) found no significant effect of cellulose on Mg

binding but a significant effect of lignin and wheat bran on the binding of this mineral. *In vivo* studies reported a trend toward a negative balance of Mg when high dietary fiber levels were consumed (Ismail-Beigi et al., 1977; Ink, 1988). Re-acid washing stripped most of the Mg from fiber sources, particularly in samples where Mg was combined with other minerals.

**Binding Capacity of Dietary Fiber Sources for Cu.** The binding capacity of wheat bran, rice bran, and oat fiber for Cu differed significantly from that observed for Ca and Mg. While Mg and Ca were bound in wheat and rice bran, Cu was bound especially well in oat fiber suggesting that this fiber source might have a considerable number of binding sites for this mineral (Table 6). Oat fiber bound significantly ( $P < 0.05$ ) less Cu when it was added alone than when it was combined with other minerals. The binding may occur not only with fiber sources but also with other minerals, which could lead to the formation of larger complexes. Persson et al. (1987) reported a strong binding affinity of Cu for wheat bran. Rice bran bound significantly more Cu than wheat bran but 1.6–5.0-fold less than oat fiber. Cu in combination with Ca, Mg, or Zn, and with these minerals together resulted in a higher binding capacity for Cu than when Cu was added alone. In rice bran, Cu in combination with Zn bound significantly more

than when it was mixed with Mg or Ca. When Cu and Zn were combined in equimolar concentrations, Thompson and Weber (1981) showed that the binding of both minerals was reduced from the level bound when added singly. They attributed this reduction to the increase of ionic strength. Re-acid washing removed considerable amounts of bound Cu from dietary fibers.

**Binding Capacity of Fiber Sources for Zn.** The binding capacity of wheat bran, rice bran, and oat fiber for Zn alone and combined with other minerals was high in all three fiber sources (Table 7). Although the binding capacity values for the three fiber sources were similar, wheat and rice bran had significantly higher binding values than those of oat fiber. As in our study, Casterline and Ku (1993) indicated a higher binding capacity for wheat bran than oat fiber. Their data, however, were 1.6–3.5 times lower than those indicated in this study. The source of dietary fiber, method of analysis, and conditions of processing might explain these variations. When a single mineral or multiple minerals were combined with Zn, the highest binding occurred in wheat and rice bran and in the presence of Ca, Mg, Cu, and the three minerals together, respectively. In oat fiber, this happened sequentially in Mg, Ca, Cu, and the three minerals combined. Elhardallou and Walker (1993) studied the binding capacity of fiber-rich fractions of butter beans, broad beans, and lentils for Zn alone and in combination with Fe, Ca, Mg, and Cu. They found that this mineral was bound more significantly in the presence of other minerals than when alone. Re-acid washing removed almost all Zn bound to these dietary fiber sources.

**Conclusion.** Ca, Mg, Cu, and Zn content varied significantly among the samples of wheat bran, rice bran, and oat fiber. With the exception of Mg, which was bound in greater amounts in rice bran, all minerals studied were bound in a significantly higher concentration in wheat bran than in rice bran or oat fiber. The binding capacity of wheat bran, rice bran, and oat fiber for Ca, Mg, Cu, and Zn either alone or in combination varied greatly among the dietary fiber sources. Overall, wheat bran bound significantly more Ca and Mg either singly or in combination than rice bran and oat fiber, respectively. Oat fiber bound markedly more Cu than rice bran and wheat bran, respectively. Although binding was high in all three fiber sources, Zn alone or in combination was bound more in wheat bran and rice bran than oat fiber. As reported in this study and others (Garcia-Lopez et al., 1985; Platt and Clydesdale, 1986; Elhardallou and Walker, 1993), the binding capacity of dietary fibers for a mineral alone or in combination with others might vary considerably depending on the dietary fiber source, the type of minerals and their number in combination, and the presence or absence of other factors such as antinutrients and protein. More research on the binding capacity of dietary fiber sources for single minerals or mineral combinations is needed to understand more fully the influence of dietary fiber on minerals and eventually predict their bioavailability in the human gastrointestinal tract.

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