An in Vitro Study of Wheat Bran Binding Capacity for Hg, Cd, and Pb

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Water-soluble dietary fiber (WSDF), water-insoluble dietary fiber (WIDF) from wheat bran, and the carboxymethylated product of WIDF (CIDF), all having low contents of protein, ash, and phytic acid, were evaluated for their scavenging capacity for three heavy metals, Hg, Cd, and Pb. The results showed that WIDF had higher BC_{max} (maximum amount of bound heavy metal ions) and BC_{min} values (minimum concentration of heavy metal ions below which the ions cannot be bound by dietary fibers) than WSDF at two pH conditions (pH 2.0 and 7.0). Carboxymethylation of WIDF improved its binding capacity for heavy metals (increase in BC_{max} and decrease in BC_{min}). The pH value significantly affected the binding capacity for heavy metals; BC_{max} sharply increased and BC_{min} sharply decreased for each heavy metal ion for all of the dietary fibers when the pH was raised from 2.0 to 7.0. The binding capacity of dietary fibers for heavy metals was slightly affected by amino acids, calcium, iron, and zinc but significantly affected by copper. Colon fermentation released part of the heavy metal ions from dietary fibers. From the results it can be concluded that dietary fibers from wheat bran can effectively bind all three tested metal ions to prevent the body from being affected by their toxicity.

Keywords: *Wheat bran; dietary fiber; heavy metals; scavenging*

INTRODUCTION

Positive and adverse effects have been reported with an increase in fiber intakes. Positive effects include a reduction of constipation, ischemic heart disease, diverticular disease of colon, diverticulosis, breast cancer, colorectal cancer, appendicitis, varicose veins, obesity, and gallstones (Baghurst et al., 1996). Adverse effects are concentrated on the fiber's adsorption of minerals and fat-soluble vitamins (Claye et al., 1996, 1998; Idouraine et al., 1996; Kelsay, 1990). Because dietary fiber can bind minerals, such as Ca, Mg, Fe, Zn, and Cu, it may also bind some toxic heavy metals such as Hg, Cd, or Pb, which, in modern society, enter our bodies through polluted water, foods, and air (Dickman and Leung, 1998; Harnly et al., 1998; Stefnov et al., 1995).

Wheat bran dietary fiber shows higher binding capacities for Ca, Mg, Zn, and Fe than rice bran or oat bran dietary fibers (Idouraine et al., 1996), and it is convenient to add to foods because of its soft texture. Thus, the aim of the present study was to investigate the possibility of using dietary fiber from wheat bran to scavenge Hg, Cd, and Pb after they enter our body and to elucidate the dietetic factors that affect the heavy metal binding capacity, the endogenous factors such as protein, phytic acid, or phenolic acids (Idouraine et al., 1996; Torre et al., 1995) and the exogenous factors such as pH (gastric and intestinal), protein in foods (casein as a whole), and interaction with other minerals (Ca, Fe, Zn, and Cu). Measures were taken to remove phytic acid and protein and to maintain the phenolic acids, which were covalently bound to the carbohydrates, and the effects of pH and the hydrolysate of protein on the binding capacity for heavy metals were also observed. An in vitro fermentation model was carried out to investigate the extent of heavy metals released from dietary fibers by the colon inoculate from fresh feces.

MATERIALS AND METHODS

Preparation of Dietary Fiber. Wheat bran was soaked in 3 volumes of citric buffer (pH 5.5) that contained 1 mmol/L $MgSO_4$, continuously stirred for 2 h on a water bath at 55 °C, washed with deionized water, dried at 105 °C, ground to pass through 100 mesh, and defatted with hexane. Water-soluble dietary fiber (WSDF) and water-insoluble dietary fiber (WIDF) were prepared. Five kilograms of deffated wheat bran was soaked with 50 L of deionized water and treated with α-amylase, proteinase, and amyloglucosidase according to the method of Prosky et al. (1985). After filtration (10 μM), the residue was washed with deionized water and alcohol (95%) and dried at 105 °C, providing WIDF. The filtrate was concentrated to 2.5 L by ultrafiltration using a 10 kDa molecular cutoff membrane and was treated by 2.5% trichloroacetic acid (62.5 g of trichlorocetic acid gradually added to the filtrate with constant stirring) and centrifuged at 4000*g* for 20 min. The supernatant pH was adjusted to 7.0 with NaOH, and the supernatant was mixed with 12.5 L of 95% alcohol with constant stirring, centrifuged at 8000*g* for 20 min; the residue was washed with 80% alcohol and lyophilized to obtain WSDF. Carboxymethyl-WIDF (CIDF) was prepared as described by Miyamoto et al. (1996). WIDF and CIDF were acid-washed with HCl solution (pH 1, ratio = 1:5 w/v) by shaking the slurry overnight and filtered in a nylon screen (10 μM), and the residue was rinsed with deionized water several times until the filtrate tested neutral (pH 7.0 ± 0.1). WSDF was washed as the method described above in a dialysis bag of 10 kDa molecular cutoff.

Determination of the Maximum Binding Capacity for Heavy Metal (BC_{max}). Two samples (1.0 g of WIDF, CIDF,

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and WSDF) were mixed with three heavy metal standard solutions [10 mmol/L $HgCl_2$, $CdCl_2$, and $Pb(NO_3)_2$] at a ratio of 1:100 (w/v), and the pH was adjusted, respectively, to 2.0 and 7.0. Slurries were shaken (120 rpm) at 37 °C for 3 h in acid-washed bottles. WIDF and CIDF slurries were centrifuged (4000*g*) for 20 min, heavy metal contents determined in the supernatant, and the residues (heavy metal containing WIDF and CIDF) washed with 80% alcohol and dried. Four hundred milliliters of 95% alcohol was added to the slurry of WSDF under constant stirring and centrifuged (4000*g*) for 20 min. Heavy metal concentration was determined in the supernatant and the residue (heavy metal containing WSDF precipitated by alcohol) washed with 80% alcohol and dried. The maximum binding capacity for heavy metal ions (BC_{max}) was calculated as

$$BC_{max} = [(IC - EC) \times V]/W$$

where IC is the concentration (μmol/L) of heavy metal ions added (10000 μmol/L), EC is the concentration (μmol/L) of heavy metal ions in the supernatant (for WSDF, EC is equal to the determined value × 5), *V* is the volume of the solution (mL), and *W* is weight of dietary fiber added (g).

Determination of Minimum Binding Concentration (BC_{min}). Two and a half grams of WIDF, CIDF, and WSDF was mixed with 0.5 mmol/L of the standard solutions [$HgCl_2$, $CdCl_2$, and $Pb(NO_3)_2$] at a ratio of 1:40 (w/v), and the pH was adjusted, respectively, to 2.0 or 7.0. The slurries were shaken (120 rpm) for 3 h in acid-washed bottles at 37 °C, time enough to reach the equilibrium concentration (when the amount of ions bound by dietary fiber equals the amount of ions released from dietary fiber), which is BC_{min}.

Competition between Some Essential Minerals and Heavy Metals. Two grams of WIDF, CIDF, and WSDF was mixed with 10 mmol/L $CaCl_2$, $FeSO_4 \cdot 7H_2O$, $ZnSO_4 \cdot 7H_2O$, and $CuSO_4 \cdot 5H_2O$ standard solutions, respectively, at a ratio of 1:50 (w/v), and the pH was adjusted to 7.0. Slurries were shaken (120 rpm) for 3 h in acid-washed bottles at 37 °C. The complexes of dietary fiber and minerals (residues) were separated and dried. One gram of the complexes was used to determine their BC_{max} and BC_{min} values for Hg^{2+}, Cd^{2+}, and Pb^{2+} at pH 7.0 and a temperature of 37 °C.

Competition between Dietary Fiber and Amino Acids for Binding Heavy Metal Ions. One gram (for BC_{max}) and 2.5 g (for BC_{min}) of the same dietary fibers were mixed with casein (1.0 g) and papain (0.1 g) and 100 mL of $CdCl_2$ standard solutions (10 mmol/L for BC_{max} and 0.5 mmol/L for BC_{min}, respectively), and the pH was adjusted to 7.0. Slurries were shaken in acid-washed bottles for 4 h at 37 °C, and Cd BC_{max} and BC_{min} were determined as described previously.

Release of Heavy Metals from Dietary Fiber by Fermentation. An in vitro fermentation system was designed according to the guidelines of Monsma and Marlett (1995). It was performed in buffer flasks that contained 100 mL of the following mixed solutions: 5.0 mL of human feces solution, 0.1 mol/L $NaH_2PO_4-Na_2HPO_4$ buffer (pH 6.5), 0.63 mmol/L cysteine−HCl, and 1.0 g of the dietary fiber and heavy metals complexes. Flasks were placed in an anaerobic cabinet at 37 °C for 24 h. Fermentation containing purified fibers (no heavy metal-binding dietary fibers) was performed as a control. To prepare fresh fecal solution, 20 g of feces was prepared from healthy people, who did not take antibiotics for at least 6 months before the test, and diluted into 200 mL of 0.1 mol/L $NaH_2PO_4-Na_2HPO_4$ buffer (pH 6.5), blended for 1 min, and squeezed through a 100 μM nylon sieve prior to use. Feces, solutions, and containers were kept under constant flow of nitrogen during inoculate preparation. After fermentation, samples were collected to determine the contents of water-soluble and water-insoluble dietary fiber and the concentration of released heavy metal ions.

Chemicals and Analysis. Mineral salts (atomic absorption standard), enzymes, casein, and phytic acid were from Sigma Chemical Co., St. Louis, MO. Protein, ash, and phytic acid were analyzed according to AOAC (1990) Methods 984.13, 920.03, and 986.11, respectively. CEC was determined by titration using sodium hydroxide. Cd and Pb were determined using a Hitachi Model 180-70 atomic absorption spectrophotometer at 228.8 and 283.4 nm wavelength, respectively. Hg was determined by using a vapor generator (CG-CH) adapted to the same absorption spectrometer, and reads were made at 253.7 nm wavelength. A $SnCl_2$ solution was used as reducing agent. Water-soluble dietary fiber and water-insoluble dietary fiber were determined following the method of Prosky et al. (1985).

Table 1. Physicochemical Properties of Dietary Fibers

dietary fiber	protein[d] (%)	ash (%)	phytic acid (μg/g)	CEC[e] (mequiv/g)
WIDF[f]	0.5 ± 0[a]	0.2 ± 0[c]	17.4 ± 0.3[a]	0.42 ± 0.01[c]
CIDF[g]	0.3 ± 0[b]	0.8 ± 0.1[a]	13.2 ± 0.1[b]	1.44 ± 0.02[a]
WSDF[h]	0.3 ± 0[b]	0.3 ± 0[b]	10.8 ± 0.1[c]	0.46 ± 0.01[b]

[a−c] Mean values (mean ± SD, determined in duplicate) with the same superscript within a column are not significantly different at 5% level. [d] Protein = N × 6.25. [e] CEC, cation exchange capacity. [f] WIDF, water-insoluble dietary fiber. [g] CIDF, carboxymethyl-WIDF. [h] WSDF, water-soluble dietary fiber.

Statistical Analysis. Data, determined in duplicate, 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

Physicochemical Properties of Dietary Fiber. Protein, ash, and phytic acid, although their contents varied significantly among the three dietary fiber fractions (Table 1), were reduced if compared with their raw material wheat bran values (Idouraine et al., 1996), the influence of which on heavy metal binding capacity can be ignored. CEC (cation exchange capacity) is an important parameter for judging the binding capacity for cations. In our experiment, a moderate preparation procedure of dietary fiber (by enzymes instead of by alkaline) was used to avoid the loss of phenolic acids and amino acids, which are covalently attached to dietary fiber (Torre et al., 1995), the main components (besides uronic acids) contributing to CEC. Also, carboxylmethylation of WIDF was introduced to increase its CEC.

Heavy Metal Binding Capacity of Dietary Fibers at Acid and Alkaline pH. Water-insoluble dietary fiber (WIDF) from wheat bran bound significantly more Hg, Cd, and Pb than water-soluble dietary fiber (WSDF) under pH conditions similar to those found in the human stomach (Table 2). Carboxymethylation of WIDF (CIDF) improved this binding capacity toward the three heavy metals studied, with 2 times BC_{max} values. However, the fixed negative charges on dietary fibers were not saturated by the heavy metal ions according to their CEC values. From the BC_{min} value it can be judged that WSDF showed higher affinity for all heavy metal ions than WIDF. Improved also by carboxymethylation, although, BC_{min} values of CIDFs were not below toxic levels for humans (Reilly, 1980). Therefore, dietary fiber in the stomach is not enough for scavenging heavy metal contents reduction not to be harmful for our bodies.

Our intake of dietary fiber remains for only a short time (1−2 h) in the stomach, which is not the main organ to absorb minerals. It is unsuitable to judge the detoxifying capacity of dietary fibers merely by their detoxifying ability in the stomach. In contrast, to the stomach is the small intestine, which is the main organ

Table 2. In Vitro Binding Capacity of Dietary Fiber Fraction for Hg^{2+}, Cd^{2+}, and Pb^{2+} at pH 2.0

	BC_{max} (μmol/g)			BC_{min} (μmol/L)		
	Hg^{2+}	Cd^{2+}	Pb^{2+}	Hg^{2+}	Cd^{2+}	Pb^{2+}
WIDF[d]	57.8 ± 0.8^b	59.3 ± 0.8^b	58.2 ± 0.1^b	166.2 ± 0.8^a	185.6 ± 0.7^a	174.2 ± 1.1^a
CIDF[e]	124.3 ± 0.8^a	127.8 ± 0.6^a	128.2 ± 0.6^a	123.6 ± 1.1^c	138.4 ± 1.3^b	126.1 ± 0.7^c
WSDF[f]	43.1 ± 1.0^c	42.3 ± 0.3^c	42.3 ± 0.4^c	155.4 ± 1.1^b	138.6 ± 0.7^b	142.5 ± 1.8^b

[a–c] Mean values (mean ± SD, determined in duplicate) with the same superscript within a column are not significantly different at 5% level. [d] WIDF, water-insoluble dietary fiber. [e] CIDF, carboxymethyl-WIDF. [f] WSDF, water-soluble dietary fiber.

Table 3. In Vitro Binding Capacity of Dietary Fiber Fraction for Hg^{2+}, Cd^{2+}, and Pb^{2+} at pH 7.0

	BC_{max} (μmol/g)			BC_{min} (μmol/L)		
	Hg^{2+}	Cd^{2+}	Pb^{2+}	Hg^{2+}	Cd^{2+}	Pb^{2+}
WIDF[d]	193.6 ± 2.1^b	226.4 ± 2.0^b	214.8 ± 2.5^b	7.8 ± 0.1	9.4 ± 0.1	10.8 ± 0.3
CIDF[e]	615.6 ± 4.7^a	623.5 ± 3.7^b	621.6 ± 4.5^a	4.3 ± 0	6.5 ± 0.1	6.2 ± 0
WSDF[f]	154.6 ± 0.8^c	138.3 ± 0.3^c	149.8 ± 2.5^c	1.2 ± 0	1.8 ± 0	1.4 ± 0

[a–c] Mean values (mean ± SD, determined in duplicate) with the same superscript within a column are not significantly different at 5% level. [d] WIDF, water-insoluble dietary fiber. [e] CIDF, carboxymethyl-WIDF. [f] WSDF, water-soluble dietary fiber.

Table 4. Effect of Ca, Fe, Zn, and Cu on Maximum Binding Capacity (BC_{max}) of Heavy Metal Ions at pH 7.0

	BC_{max} (μmol/g)								
	Hg^{2+}			Cd^{2+}			Pb^{2+}		
	WIDF[f]	CIDF[g]	WSDF[h]	WIDF	CIDF	WSDF	WIDF	CIDF	WSDF
control	193.6 ± 2.1^a	615.6 ± 4.7^a	154.6 ± 0.8^a	226.4 ± 2.0^a	623.5 ± 3.7^a	138.3 ± 0.3^a	214.8 ± 2.5^a	621.6 ± 4.5^a	149.8 ± 2.5^a
Ca	158.7 ± 1.0^c	544.7 ± 1.6^c	134.5 ± 0.6^c	170.6 ± 1.8^b	511.8 ± 2.8^c	106.8 ± 0.8^c	178.5 ± 0.8^c	568.9 ± 3.0^b	112.5 ± 1.0^d
Fe	178.5 ± 0.6^b	562.4 ± 1.3^b	151.6 ± 0.8^b	198.7 ± 1.8^c	552.4 ± 2.1^b	114.8 ± 1.0^b	189.6 ± 1.1^b	578.2 ± 2.0^b	126.5 ± 0.7^b
Zn	180.8 ± 1.0^b	558.2 ± 1.3^b	154.3 ± 0.8^b	192.5 ± 2.3^c	542.9 ± 3.7^b	108.4 ± 0.8^c	182.5 ± 1.3^b	560.8 ± 2.5^c	120.6 ± 1.6^c
Cu	82.4 ± 0.4^d	256.6 ± 0.8^d	62.3 ± 0.4^d	58.4 ± 0.6^d	150.3 ± 0.4^d	36.1 ± 0.3^d	85.6 ± 0.7^d	250.7 ± 0.8^d	55.3 ± 0.4^e

[a–e] Mean values (mean ± SD, determined in duplicate) with the same superscript within a column are not significantly different at 5% level. [f] WIDF, water-insoluble dietary fiber. [g] CIDF, carboxymethyl-WIDF. [h] WSDF, water-soluble dietary fiber.

Table 5. Effect of Casein and Its Hydrolysate on the Binding Capacity of Dietary Fibers for Cadmium

	no addition of casein		addition of casein	
dietary fiber	BC_{max} (μmol/g)	C_{min} (μmol/L)	BC_{max} (μmol/g)	C_{min} (μmol/L)
WIDF[b]	226.4 ± 1.5	9.4 ± 0.6	187.8 ± 1.4^a	13.4 ± 0.8^a
CIDF[c]	623.5 ± 3.5	6.5 ± 0.6	527.0 ± 2.1^a	9.5 ± 0.6^a
WSDF[d]	138.3 ± 1.4	1.8 ± 0.3	132.5 ± 1.0	3.4 ± 0.4

[a] Significantly different at 5% level comparing with BC_{max} or C_{min} of "no addition of casein". [b] WIDF, water-insoluble dietary fiber. [c] CIDF, carboxymethyl-WIDF. [d] WSDF, water-soluble dietary fiber.

to absorb minerals, and foods and toxic materials remain there much longer than in stomach; therefore, the small intestine is a more important organ in the judgment of the detoxifying capacity of dietary fibers. Under pH conditions similar to those found in the small intestine (pH 7.0), BC_{max} values were 3−5 times higher than at acid pH (Table 2) and BC_{min} values sharply decreased for all of the dietary fibers and heavy metals (Table 3); these values were so reduced that the heavy metals would not cause any damage to the body (Reilly, 1980).

Effect of Some Essential Minerals on the Binding Capacity for Heavy Metals. Dietary fibers also bind other divalent minerals such as Ca^{2+}, Fe^{2+}, Zn^{2+}, and Cu^{2+}(Claye et al., 1996, 1998; Idouraine et al., 1996; Torre et al., 1995). This chelation affects their bioavailability, but this adverse effect could be eliminated by supplementation of these essential minerals (Ou et al., 1997); the chelation also has a detoxicant effect on heavy metals that should be studied. The results of this effect show that BC_{max} values of dietary fibers for Hg^{2+}, Cd^{2+}, and Pb^{2+} (Table 2) decreased significantly as an effect of Ca^{2+}, Fe^{2+}, Zn^{2+}, and Cu^{2+} (Table 4). The most effective element that interferes with heavy metals was copper. Zn, Fe, and Ca supplementation would not affect the expression of heavy metal scavenging capacity of dietary fibers, because its effectiveness was reduced if compared with Cu.

Effect of Amino Acids on the Binding Capacity of Dietary Diber for Heavy Metals. Proteins are one of the main food components that we intake in large amounts in the diet and which are gradually hydrolyzed in the small intestine by proteinase. A complex system with the addition of protein, proteinase (papain), dietary fiber, and $CdCl_2$ was designed in our experiment to test how the hydyolysate of casein would affect the scavenging capacity of heavy metals by dietary fiber. The results in Table 5 showed that BC_{max} and BC_{min} values of WIDF and CIDF were significantly affected by the hydrolysate of casein, reducing BC_{max} and increasing BC_{min}, but those of WSDF were not.

Table 6. Degradation of Dietary Fibers and the Release of Bound Heavy Metals by Fecal Inoculate Fermentation in Vitro

dietary fiber	control	Hg-DF[d]	Cd-DF	Pb-DF	Hg-DF	Cd-DF	Pb-DF
WIDF[e]	49.2 ± 2.6^b	38.5 ± 3.3^b	40.8 ± 2.8^b	42.7 ± 3.1^c	33.5 ± 1.0^b	36.4 ± 1.1^b	38.6 ± 2.5^b
CIDF[f]	54.8 ± 1.8^b	46.4 ± 2.7^b	48.3 ± 3.4^b	51.2 ± 2.5^b	35.8 ± 1.4^b	38.4 ± 0.8^b	39.2 ± 2.3^b
WSDF[g]	86.4 ± 2.3^a	78.2 ± 3.0^a	80.6 ± 3.4^a	81.5 ± 2.1^a	69.2 ± 3.0^a	71.3 ± 3.3^a	71.2 ± 2.8^a

[a–c] Mean values with the same superscript in a column are not significantly different at 5% level. [d] DF, dietary fiber. [e] WIDF, water-insoluble dietary fiber. [f] CIDF, carboxymethyl-WIDF. [g] WSDF, water-soluble dietary fiber.

Release of Heavy Metals from Dietary Fiber by Fermentation. Heavy metals bound by dietary fibers could be partly released by colon fermentation (Table 6). The percentage of heavy metals released was related to the fermentation rate of dietary fibers—the more the dietary fiber was fermented, the more heavy metals were released. Water-soluble dietary fiber released much more heavy metals than water-insoluble dietary fiber, including WIDF and CIDF. The degradation rates of heavy metal-bound dietary fibers (especially Hg) were much lower than those of their pure forms, possibly due to the toxicity of these elements to the colon microorganisms.

Conclusion. Water-soluble dietary fiber and water-insoluble dietary fiber from wheat bran can bind large amounts of Hg, Cd, and Pb and decrease the concentration of these metal ions in the ambient solution to such an extent that they would not cause damage to the human body under pH conditions similar to those of the small intestine. This was not the case under pH conditions similar to those of the stomach. WIDF showed a higher binding capacity for heavy metal ions than WSDF, and the capacity can be further increased by carboxymethylation. However, WSDF had a higher affinity for all three metal ions than WIDF. Heavy metal scavenging capacities of dietary fibers were slightly affected by Ca, Fe, Zn, and casein hydrolysate and extensively affected by copper. Colon fermentation can release >30% of bound heavy metals from dietary fiber. It is not absolutely safe to use dietary fiber from wheat bran as a heavy metal scavenger.

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