Journal of Hazardous Materials 186 (2011) 236–239

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

In vitro binding capacities of three dietary fibers and their mixture for four toxic elements, cholesterol, and bile acid

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ARTICLE INFO

Article history:
Received 9 September 2010
Received in revised form 28 October 2010
Accepted 29 October 2010
Available online 9 November 2010

Keywords:
Dietary fibers
Binding capacity
Toxic elements
Cholesterol
Sodium cholate

ABSTRACT

Water-soluble dietary fibers from apple peels and water-insoluble dietary fibers from wheat bran and soybean-seed hull were used to evaluate their binding capacities for four toxic elements (Pb, Hg, Cd, and As), lard, cholesterol, and bile acids. The water-soluble dietary fibers showed a higher binding capacity for three toxic cations, cholesterol, and sodium cholate; and a lower binding capacity for lard, compared to the water-insoluble ones. A mixture of the dietary fibers from all samples – apple peels, wheat bran, and soybean-seed hull – in the ratio 2:4:4 (w/w) significantly increased the binding capacity of water-insoluble dietary fibers for the three toxic cations, cholesterol, and sodium cholate; moreover, the mixture could lower the concentrations of Pb^{2+} and Cd^{+} in the tested solutions to levels lower than those occurring in rice and vegetables grown in polluted soils. However, all the tested fibers showed a low binding capacity for the toxic anion, AsO_3^{3-}.

1. Introduction

Intake of foods containing high levels of heavy metals and cholesterol harms human health, and an increase in intake of dietary fiber may mitigate these negative effects.

Humans can be exposed to heavy metals through various pathways. Wastewater irrigation, solid-waste disposal, sludge applications, vehicular exhaust, and industrial activities are the major sources of contamination of soil with heavy metals [1]. There are many reports concerning the transfer of heavy metals from polluted soils to various sources of food, such as vegetables, rice, wheat, chicken, and so on, in urban and mining regions, resulting in pollutant levels higher than those declared permissible for human consumption by the Food and Agriculture Organization and the World Health Organization [2–8]. Adjustments in the diet may help in reducing the toxicity of pollutants.

Cardiovascular diseases (CVDs) are among the most common causes of death and disability worldwide. An estimated 17.5 million people died from CVDs in 2005, representing 30% of all global deaths [9]. In the United States, about 59% of young adults have coronary heart disease or its equivalents [10]. In China, CVDs accounted for 32% of the deaths in 2005, ranking second among the leading causes of death in China [11]. High dietary intake of fat, cholesterol, and sodium; and low intake of fruits, vegetables, and fish are linked to cardiovascular risk [11].

Dietary fibers can adsorb heavy metals [12,13] and act as a potential “functional food” that reduces the incidence of CVDs [9] by reducing the risk of type-2 diabetes, body weight, and serum low-density lipoprotein–cholesterol levels [14] and adsorbing bile acids. Bile acids, derived from cholesterol, are necessary for the digestion of lipids in the small intestine. Several dietary fibers have been reported to interact with bile acids in the small intestine, resulting in a lower level of reabsorption and a higher level of excretion of bile acids, thus increasing the hepatic synthesis of bile acids from blood cholesterol [15,16]. However, there are no reports on the direct adsorption of cholesterol from the diet, possibly because of the difficulty in designing a model system.

To reduce the harmful effects of food-derived heavy metals and cholesterol on humans, the capacities of the dietary fibers obtained from apple peels, wheat bran, and soybean-seed hull to adsorb heavy metals and bile acids were investigated; moreover, an egg yolk-adsorption system was designed to test their binding capacity for cholesterol.

2. Materials and methods

2.1. Reagents and chemicals

Heat-stable α-amylase Termamyl 120 L (EC 3.2.1.1 from Bacillus licheniformis, 120 Kilo Novo units/g), protease Alcalase 2.4 L (EC 3.4.21.62, from B. licheniformis, 2.4 Anson units/g), and amyloglucosidase AMG 300 L (EC 3.2.1.3, from Aspergillus niger, 300 amyloglucosidase units/g) were purchased from Novo Nordisk (Bagsværd, Denmark). Sodium cholate and cholesterol were pur-

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0304-3894/$ – see front matter © 2010 Published by Elsevier B.V.
doi:10.1016/j.jhazmat.2010.10.120

chased from Sigma Company (St. Louis, MO). Pork (for preparation of lard), eggs, and apples were purchased from the local market. All other chemicals used were of analytical grade.

2.2. Preparation of dietary fibers

Wheat bran and soybean-seed hull were respectively obtained from Guangdong Nanfang Flour Group (Guangzhou, Guangdong Province, China) and China Grains and Oils Group Corporation (Dongguan, Guangdong Province, China). They were milled and passed through a 200-mesh sieve, followed by removal of starch (using α-amylase and amyloglucosidase) and protein (using protease) according to the methods described in our previous work [17]. The residues obtained as the dietary fibers, were sequentially air-dried and dried in an oven at 105 °C for 4 h.

Water-soluble dietary fibers were prepared according to the following procedure. Fresh apple peels, homogenized in five volumes of deionized water, were extracted twice with 5 volume of deionized water at 80 °C under constant stirring. The residue was removed using a 200-mesh nylon cloth, and the filtrates were combined and concentrated using an NUF model hollow-fiber ultrafiltrator (Wuxi Ultrafiltrating Equipment Company, Jiangsu Province, China) with a 5000-Dalton molecular cutoff. The retentate was centrifuged at 2000 × *g* for 20 min. Subsequently, 5 parts of 95% ethanol were mixed with 1 part of the supernatant to precipitate the water-soluble dietary fibers; the precipitates were collected, air-dried for 60 min, and then dried in an oven at 60 °C for 8 h.

A combination of the dietary fibers, namely, the fiber powders from wheat bran, soybean-seed hull, and apple peels, was prepared in the ratio 4:4:2.

The cation-exchange capacity (CEC) of the fibers was determined by titration using 0.1 mol/L of sodium hydroxide.

2.3. Adsorption capacity of dietary fibers for toxic ions

The maximum binding capacity (BC_{max}) and the minimum binding concentration (BC_{min}) of the fibers for toxic ions were determined according to the methods published in our previous work [17], with some modifications. When determining BC_{max}, 1.0 g of dietary fiber was suspended in 100 mL of a solution containing 10 mmol/L of the following solutions, $HgCl_2$, $CdCl_2$, $Pb(NO_3)_2$, and $NaAsO_2$, in a 250-mL conic flask; and the pH was adjusted to 2.0 and 7.0 to duplicate the conditions in the stomach and small intestine, respectively. The slurries were shaken (120 rpm) for 3 h in a water-bath incubator maintained at 37 °C. At the end of adsorption, a 2-mL volume of the sample was collected, and absolute ethanol (8 mL) was added to precipitate the water-soluble dietary fibers. The mixture was centrifuged at 4000 × *g* for 20 min. The concentrations of Hg, Cd, Pb, and As in the supernatant were determined by inductively coupled plasma-atomic emission spectroscopy (Optima 2000 DV, Perkin-Elmer, Norwalk, CT, USA). When determining BC_{min}, 2.5 g of dietary fibers and 500 μmol/L of $HgCl_2$, $CdCl_2$, $Pb(NO_3)_2$, and $NaAsO_2$ were used; all other conditions were the same as for BC_{max}.

2.4. Binding capacity of dietary fibers for sodium cholate

In vitro bile acid-binding test was carried out using the method of Kahlon et al. [16], with some modifications. Two grams of dietary fibers were incubated with 10 mmol/L sodium cholate in 100 mL of phosphate buffer (pH 7.0). The slurry was shaken (120 rpm) for 3 h in a 250-mL flask maintained at 37 °C. The water-soluble dietary fibers were precipitated as mentioned in Section 2.3. The concentration of cholate in the supernatant was determined using high-performance liquid chromatography (Agilent 1100 Series, Waldbronn, Germany) with a C-18 column (YMC-Pack ODS-A, 5 μm, 4.6 mm × 150 mm). The mobile phase was acetonitrile: water: phosphoric acid (35:65:0.1), with a flow rate of 1.1 mL/min. Cholate was detected at 192 nm against a standard curve.

2.5. Binding capacity of dietary fibers for cholesterol in egg yolk

As cholesterol is difficult to dissolve in water even after addition of emulsifiers, egg yolk was used as a model system in this research. Fresh egg yolk (16.4 g/egg, on average), naturally containing 14.26 mg/g of cholesterol, was whipped with 9 volumes of deionized water. Mixtures of 2.0 g dietary fibers with 50 mL of the diluted yolk at pH 7.0 and 2.0, respectively (similar to the pH conditions prevailing in the stomach and small intestine, respectively), were shaken at 80 rpm for 2 h in a water-bath incubator maintained at 37 °C, diluted yolk without dietary fiber being the blank. At the end of adsorption, 4 mL of the sample were collected, and 16 mL of absolute ethanol were added to precipitate the water-soluble dietary fibers. The mixture was centrifuged at 4000 × *g* for 20 min; ethanol in the supernatant was removed using vacuum at 40 °C. one mL of the concentrate was diluted 5 times with 90% acetic acid. Color was developed by adding 0.1 mL of o-phthaladehyde reagent and 2 mL of concentrated H_2SO_4, according to the method of Park [18]. Absorbance was read 20 min after addition of H_2SO_4 at 550 nm against a reagent blank. The cholesterol concentration in the samples was determined against a standard curve generated using a standard cholesterol solution.

The binding capacity (BC) was calculated as follows:

$$BC = [C_{yolk} - (C_{blank} - C_d) \times F] \times \frac{50}{w}$$

where C_{yolk}, C_{blank}, and C_d are the concentrations of cholesterol in the yolk, the yolk without dietary fiber, and the yolk mixed with dietary fibers, respectively; F is the dilution factor (10); 50 is the adsorption volume (mL); and w is the weight of the dietary fibers.

2.6. The binding capacity of dietary fibers for lard

The binding capacity of dietary fibers for oil was determined according to the method proposed by Sangnark and Noomhorm [19]. Three grams of dietary fibers were mixed with melted lard (prepared from fresh pork) in a centrifugal tube and left undisturbed for 1 h at 37 °C. The mixture was then centrifuged at 1500 × *g* for 10 min. The supernatant was decanted, and the pellet was recovered by filtration through a nylon cloth. The binding capacity (BOC) was calculated as follows:

$$BOC = \frac{W_2 - W_1}{W_1}$$

where W_1 and W_2 were the weights of the dietary fibers before and after adsorbing lard, respectively.

3. Experimental results

3.1. Binding of the four toxic ions to dietary fibers

The maximum binding capacity (BC_{max}) is the level at which all the binding sites (including the physical and chemical binding sites) of the dietary fibers are saturated by the toxic ions. This parameter can be used to evaluate the binding capacity of the dietary fibers. BC_{min} is the equilibrium concentration at which the amount of ions bound by the dietary fibers equals the amount of ions released from the dietary fibers, provided that the binding sites of the dietary fibers are not saturated by the toxic ions; it could be used to evaluate the affinity of the dietary fibers for the toxic ions.

Table 1
The values of the maximum binding capacity (BC_{max}) and the minimum binding concentration (BC_{min}) of the four dietary fibers for Hg, Pb, Cd, and As at pH 2.0.

Fiber source	CEC (mmol/g)	BC_{max} (μmol/g)				BC_{min} (μmol/L)			
		Hg	Pb	Cd	As	Hg	Pb	Cd	As
Wheat bran	0.48 ± 0.04[a]	12.4 ± 1.3[a]	15.6 ± 1.9[a]	14.3 ± 2.3[a]	6.7 ± 1.5[b]	362.8 ± 7.5[d]	478.7 ± 6.4[d]	385.4 ± 10.6[d]	402.4 ± 5.9[a]
Soybean hull	0.52 ± 0.03[a]	17.5 ± 1.7[b]	17.8 ± 3.0[a]	162.4 ± 1.8[c]	2.1 ± 0.3[a]	332.6 ± 5.7[c]	244.9 ± 6.1[b]	318.6 ± 12.3[c]	412.7 ± 2.6[b]
Apple peel	1.52 ± 0.07[c]	247.8 ± 10.2[d]	324.1 ± 14.4[c]	286.3 ± 12.2[d]	47.6 ± 5.7[d]	231.3 ± 6.3[b]	273.8 ± 9.2[c]	251.2 ± 13.6[b]	427.0 ± 8.5[c]
Mixed fiber	0.84 ± 0.08[b]	112.6 ± 5.9[c]	134.5 ± 6.8[b]	126.5 ± 6.7[b]	26.9 ± 0.5[c]	189.6 ± 6.4[a]	213.4 ± 8.1[a]	192.6 ± 7.8[a]	424.3 ± 6.0[c]

Mean value ± SD (n = 3) with different letters within a column are significantly different at 5% level.

Table 2
The values of the maximum binding capacity (BC_{max}) and the minimum binding concentration (BC_{min}) of the four types of dietary fibers for Hg, Pb, Cd, and As at pH 7.0.

Fiber source	BC_{max} (μmol/g)				BC_{min} (mg/L)			
	Hg	Pb	Cd	As	Hg	Pb	Cd	As
Wheat bran	197.7 ± 11.0[a]	280.6 ± 9.2[a]	180.3 ± 6.3[a]	4.9 ± 0.1[b]	1.44 ± 0.05[c]	1.97 ± 0.04[c]	1.29 ± 0.06[d]	35.67 ± 3.1[c]
Soybean hull	395.4 ± 17.1[c]	402.6 ± 6.2[c]	382.4 ± 7.8[c]	1.8 ± 0.3[a]	1.12 ± 0.04[b]	1.12 ± 0.09[b]	0.85 ± 0.03[c]	35.65 ± 4.2[c]
Apple peel	398.4 ± 12.1[c]	422.2 ± 14.4[c]	402.3 ± 10.6[d]	40.6 ± 3.6[d]	0.86 ± 0.03[a]	0.99 ± 0.04[a]	0.47 ± 0.06[a]	30.37 ± 4.6[a]
Mixed fiber	378.3 ± 10.6[b]	342.4 ± 9.4[b]	289.6 ± 6.7[b]	24.3 ± 3.5[c]	1.38 ± 0.05[c]	1.16 ± 0.06[b]	0.63 ± 0.05[b]	32.22 ± 4.4[b]

Mean value ± SD (n = 3) with different letters within a column are significantly different at 5% level. For later discussion, the unit was transformed to mg/L.

Table 3
Adsorption capacity of dietary fibers for lard, cholesterol, and bile acid.

Fiber source	Lard (g/g)	Cholesterol (pH 2.0, mg/g)	Cholesterol (pH 7.0, mg/g)	Sodium cholate (mg/g)
Wheat bran	5.64 ± 0.28[d]	2.17 ± 0.07[a]	3.48 ± 0.03[a]	3.16 ± 0.03[a]
Soybean hull	5.14 ± 0.05[b]	5.89 ± 0.10[b]	7.40 ± 0.22[b]	3.46 ± 0.42[a]
Apple peel	4.57 ± 0.04[a]	10.75 ± 0.14[d]	11.34 ± 0.15[d]	6.37 ± 0.12[c]
Mixed fiber	5.37 ± 0.27[c]	6.37 ± 0.04[c]	9.23 ± 0.10[c]	4.53 ± 0.14[b]

Mean value ± SD (n = 3) with different letters within a column are significantly different at 5% level.

Dietary fibers prepared from apple peels, which have higher CEC, showed a much higher binding capacity for all the toxic cations considered here under pH conditions similar to those in the stomach and the small intestine (Tables 1 and 2). Compared to the dietary fibers prepared from wheat bran and soybean-seed hull, a mixture of the three types of dietary fibers could significantly increase the binding capacity for the toxic cations at acidic pH values. All the dietary fibers had high BC_{min} for the toxic cations at pH 2.0, indicating that they had low affinity for the toxic cations under conditions similar to those in the stomach; however, when the pH was neutral, similar to that in the small intestine, all the dietary fibers could bind the toxic cations at very low concentrations (BC_{min} in Table 2). These findings indicate that dietary fibers, especially the water-soluble ones from apple peels and the mixture of the different types, could effectively reduce the toxicity of the tested cations.

The binding capacity of all the tested dietary fibers for $AsO_3{}^{3-}$ was very low under both pH conditions, although a significant difference existed between the individual dietary fibers (Tables 1 and 2). Their BC_{min} values were high at both the pH values, and a change from an acidic to neutral pH increased the BC_{min} value, indicating that these dietary fibers could not effectively detoxify the ingested arsenic.

3.2. *Binding capacity of dietary fibers for fat, cholesterol, and cholic acid*

The results in Table 3 show that the dietary fibers from wheat bran had the highest, and the water-soluble dietary fibers from apple peels (high CEC value), had the lowest binding capacity for lard. A mixture of the three types of dietary fibers ranked second. On the contrary, the fibers from apple peels had a much higher binding capacity for cholesterol than the fibers from wheat bran and soybean-seed hull at both the pH values. Furthermore, mixing these two types of fibers with the fibers from apple peels significantly increased their binding capacity (Table 3). Fibers from apple peel also had the highest binding capacity for sodium cholate, followed by the mixture of dietary fibers, the dietary fiber from soybean-seed hull, and that from wheat bran (Table 3).

4. Discussion

Dietary fibers are classified into two categories according to their solubility in water: insoluble and soluble. Water-insoluble fiber is responsible for the increased bulk of the stools and helps to regulate bowel movements [9]. Soluble dietary fibers play a significant role in the reduction of cholesterol level and blood pressure, prevention of gastrointestinal problems, and protection against the onset of several cancers, which include colorectal, prostate, and breast cancer [20]. However, ingestion of soluble fibers at high doses is unpalatable, giving a viscous texture to food and an undesirable aftertaste. Thus, a ratio of one part of soluble to three or four parts of insoluble dietary fibers has been recommended [22] for daily intake. Because cereals mainly contain insoluble dietary fibers [20], a mixture of fibers from apple peels and dietary fibers from wheat bran and soybean-seed hull was used to test their binding capacity for toxic ions, cholesterol, and bile acids.

Table 4
Reported contents of toxic elements (mg/kg) in cereals and vegetables.

Crops	Pb	Hg	Cd	As	Reference
Rice	2.042	0.022	0.224	0.154	[3]
Wheat	0.177	0.003	0.055	0.038	[4]
Cauliflower	1.56	–	2.57		[5]
Chinese cabbage (DM)	17.36	–	1.31	1.85	[6]
Mustard	0.21	–	0.19	–	[21]

4.1. Detoxification of four toxic ions by dietary fibers

The parameter BC_{min} was used in this research to evaluate the affinity of dietary fibers for toxic ions; it represents the concentration below which the dietary fibers cannot bind toxic ions [17]. As shown in Tables 2 and 4, a mixture of dietary fibers can adsorb lead from rice and dried Chinese cabbage; and cadmium from dried cauliflower and Chinese cabbage, all grown on polluted soil. All the tested dietary fibers could not reduce arsenic toxicity, because they showed a low binding capacity for As.

Three possible mechanisms were proposed for binding capacity of dietary fibers for heavy metals: chemisorption, physical sorption [13]. Of which, chemisorption is the main one; it is connected with the presence in the fiber matrix of phenolic groups from lignin and carboxyl groups from uronic acids [13]. Our results indicated that the dietary fibers with higher CEC value showed higher binding capacity for toxic cations (Tables 1 and 2); when the pH value increased, the carboxyl groups were dissociated to carboxyl anions ($RCOO^-$) which showed stronger interaction with the toxic cations than that of undissociated carboxyl groups, resulting in higher binding capacity of the dietary fibers for them (Table 2).

As to the toxic anion, AsO_2^-, the negative charges of carboxyl groups (especially at higher pH) would reject its adsorption to the dietary fibers, making the dietary fibers showing much lower binding capacity for the anion than that for cations. However, the tested dietary fibers still adsorbed part of As, we propose it mainly through physical sorption.

4.2. Fat- and cholesterol-lowering effects of the mixture of dietary fibers

The link between dietary fat, blood-lipid profile, and heart disease has been clearly established. Saturated fatty acids and cholesterol are considered atherogenic fats [23]. Bile acids injure the gastric mucosal epithelial cells [24], cause DNA damage, and act as carcinogens in humans [25]; thus, detoxification of harmful metabolites (and cancer prevention) by dietary fibers is very important and often evaluated in vitro by measuring their binding capacities for bile acids [16]. Dietary sources, such as wheat bran and soybean-seed hull, containing a large proportion of water-insoluble dietary fibers showed a higher binding capacity for saturated fat and lard, and addition of water-soluble dietary fibers from apple peels significantly increased their binding capacity for cholesterol and sodium cholate (Tables 3 and 4). From Table 3, we can calculate that a daily intake of 25 g of a mixture of soluble and insoluble dietary fibers helps remove 230.75 mg (0.597 mmol) of cholesterol (the approximate content of cholesterol in the yolk of one egg) from foods; moreover, 113.25 mg (0.263 mmol) of sodium cholate would be removed, meaning that 0.86 mmol of cholesterol would be excreted from the body through the feces.

5. Conclusions

Water-soluble dietary fibers prepared from apple peels showed a higher binding capacity for three toxic cations, cholesterol, and sodium cholate, but a lower capacity for lard, compared to the water-insoluble dietary fibers from wheat bran and soybean-seed hull. The binding capacities of the water-insoluble dietary fibers from wheat bran and soybean-seed hull for the toxic cations Hg, Pb, and Cd; cholesterol; and sodium cholate could be significantly increased when water-soluble dietary fibers from apple peels (20% of total concentration) was mixed with them.

Acknowledgments

We would like to thank the Department of Education of Guangdong Province (CGZHZD0709) and Guangzhou Municipal Government (2003Z3-E0061) for supporting this research project.

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