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Binding of cholesterol and bile acid to hemicelluloses from rice bran

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Abstract
The objective of this study was to investigate the possibility of using hemicellulose from rice bran to scavenge cholesterol and bile acid *in vitro* study. This paper demonstrates that rice bran hemicellulose A (RBHA), rice bran hemicellulose B (RBHB) and rice bran hemicellulose C (RBHC) have the potential for binding cholesterol and bile acid. The quantity of cholesterol and bile acid bound varies from one rice bran fibre to another. As it can be inferred from the results of the study, RBHB was characterized by the highest capacity for cholesterol binding, followed by RBHC and RBHA. Binding of cholesterol and bile acid to rice bran insoluble dietary fibre (RBDF) and cellulose from rice bran was found to be poor. Lignin from rice bran was the least active fraction for binding cholesterol and bile acid. This confirms that the RBHB preparation from defatted rice bran has great potential in food applications, especially in the development of functional foods.

Keywords: *rice bran, hemicelluloses, dietary fibre, cholesterol, bile acid*

Introduction

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 (Viuda-Martos et al. 2010). In the USA, about 59% of young adults have coronary heart disease or its equivalents (Kuklina et al. 2010). In China, CVDs accounted for 32% of the deaths in 2005, ranking second among the leading causes of death in China (Lu and Li 2010). High dietary intake of fat, cholesterol and sodium and low intake of fruits, vegetables and fish are linked to cardiovascular risk (Lu and Li 2010).

Rice bran is a by-product obtained from outer rice layers and is a good source of protein, mineral and fatty acids and dietary fibre content (Mccaskill and Zhang 1999). Also rice bran is used for the enrichment of some foods, due to its high dietary fibre content. Since the middle of the 1970s, the role of dietary fibre in health and nutrition has stimulated a wide range of research activities and caught public attention. It was evidenced that increased intake of dietary fibre can have beneficial effects against diseases, such as CVDs, gastrointestinal disease, decreasing blood cholesterol, diverticulosis, diabetes and colon cancer (Spiller et al. 1980; Wrick et al. 1983; Cummings 1985; Chen and Anderson 1986; Dukehart et al. 1989; Cara et al. 1992; Burton 2000). In view of the therapeutic potential of dietary fibre, more fibre incorporated into food products are being developed. The addition of dietary fibre to a wide range of products will contribute to the development of value-added foods or functional foods that currently are in high demand. In addition to the physiological benefits provided by high fibre foods, studies have shown that fibre components can give texture, gelling, thickening, emulsifying and stabilizing properties to certain foods (Sharma 1981; Dreher 1987).

Not much work has been done on rice bran and its dietary fibre in China. Rice bran is mostly burnt off at the rice mills and very little is used in animal feed. By understanding the functional properties of dietary fibre, one can increase its use in food applications and aid in developing food products with high consumer acceptance. We systematically studied the chemical constituents and functional properties of dietary fibre from rice bran (Hu and Huang 1998; Hu 2001;

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Hu and Zhai 2002; Hu and Huang 2003; Hu et al. 2007, 2008, 2009). Rice bran hemicellulose B (RBHB) had been reported to have many biological activities including decreasing blood cholesterol and preventing colon cancer (Hu et al. 2007). RBHB and rice bran insoluble dietary fibre (RBDF) from defatted rice bran have great potential in food applications, especially in functional foods (Hu et al. 2009).

Thus, the aim of this study was to investigate the possibility of using dietary fibre from rice bran to scavenge cholesterol and bile acid. The objective of the paper was to determine the ability of rice bran hemicellulose A (RBHA), RBHB, rice bran hemicellulose C (RBHC) and RBDF from defatted rice bran to bind cholesterol and bile acid *in vitro* study.

Materials and methods

Materials

Rice bran (Shanghai Minhang Huili Grain-processing Plant, Shanghai, PR China) was milled to pass through a 600 mm sieve. Defatting was immediately carried out in a Soxhlet apparatus using *n*-hexane as a solvent. The dry defatted rice bran was then kept in a sealed container in a desiccator until further treatment was carried out.

Extraction of RBHA, RBHB, RBHC, RBDF, cellulose and lignin preparation

RBHB was prepared from rice bran after lipids were removed with organic solvents and extracted with sodium hydroxide as described by Siegel (1968). The defatted rice bran was digested with protease (60°C, 3 h), followed by digesting with amyloglucosidase (60°C, 2 h) to remove protein and starch. Twenty volumes of 4% NaOH were then added to extract hemicelluloses at room temperature for 18 h under N_2 flow, followed by filtration. The filtrate was then neutralized with 5% acetic acid. After centrifugation, the precipitate was oven-dried overnight (60°C, 16 h) in an air oven and then weighed, providing RBHA (Figure 1). The filtrate was dialysed under running tap water for 3 days. After dialysis, four volumes of 95% ethanol were then added to the filtrate. The precipitate was oven-dried overnight (60°C, 16 h) in an air oven and then weighed providing RBHB (Figure 1). The filtrate was concentrated under vacuum and then oven-dried overnight (60°C, 16 h) in an air oven to obtain RBHC (Figure 1). RBDF was prepared from defatted rice bran after protein and starch were removed with proteinase and amyloglucosidase as described by Prosky et al. (1985) with some modifications. Five kilograms of defatted rice bran was soaked with 50 l of deionized water for 12 h and treated with proteinase, and amyloglucosidase according to the method of Prosky et al. (1985). After filtration, the residue was washed with deionized water and alcohol (95%). The residue was oven-dried overnight (60°C, 16 h) in an air oven and then weighed to obtain RBDF. Cellulose and lignin were prepared from rice bran after lipids were removed with organic solvents and extracted with sodium hydroxide as described by Hu and Huang (1998), Hu et al. (2007). Water-insoluble wheat bran dietary fibre (WIDF, control sample) which was used to evaluating binding capacities was prepared as described by Ou et al. (1999).

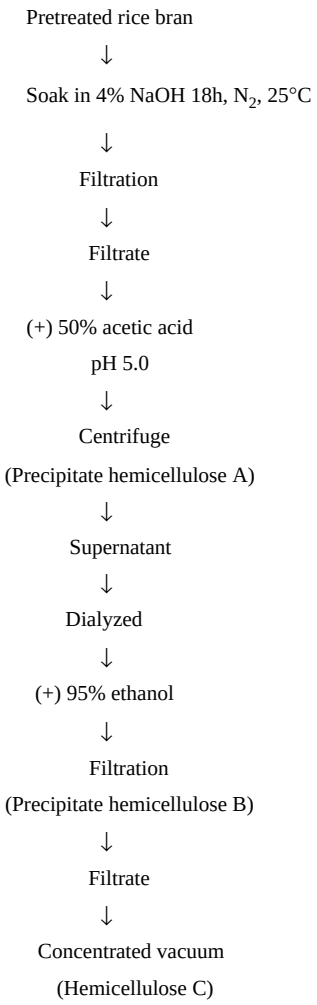

Figure 1. Flow chart of extraction procedure for hemicelluloses A, B and C.

Determination of crude protein, moisture, ash, oil and dietary fibre

The recommended methods of the Association of Official Analytical Chemists (AOAC 1984) were adopted to determine the levels of crude protein, moisture, ash and oil. Nitrogen content was determined using the Kjeldahl method (Kjeldahl 1883) and multiplied by a factor 6.25 to determine the crude protein content. Moisture content was determined by drying the samples at 105°C to a constant weight. Ash was determined by the incineration of 1.0 g samples placed in a muffle furnace, maintained at 550°C for

5 h. Crude fat was determined by the Soxhlet method. Crude fat was obtained by exhaustively extracting 5.0 g of each sample in a Soxhlet apparatus using petroleum ether (boiling point range 40–60°C) as the solvent. Dietary fibre content of the defatted samples was determined by decomposing starches with sulphuric acid and proteins, with sodium hydroxide, and then filtering (Nielsen 1998). All results were expressed on a dry weight basis.

Functional properties of RBHA, RBHB, RBHC, RBDF, cellulose and lignin preparation

The water-binding capacity (WBC) was determined according to the method described by Sosulski et al. (1976) and Auffret et al. (1994), although some modifications were made. Samples (300 mg) were weighed and left to stand for 1 h in distilled water (10 ml) at room temperature (25°C) before being centrifuged for 20 min at 14,000*g*. The residues were left for 30 min, dried overnight at 110°C and weighed. The swelling capacity (SC) measurement was made using 0.15 mol/l NaCl, as described by Guillon et al. (1992). WBC and SC were expressed as millilitre of water held per gram of sample. Fat binding capacity (FBC) was measured using a method adapted from Lin et al. (1974). A 4 g of sample was added to 20 ml of corn oil in a 50 ml centrifuge tube. The content was then stirred for 30 s every 5 min and, after 30 min, the tubes were centrifuged at 1600*g* for 25 min. The free oil was then decanted and absorbed oil was then determined by difference. The FBC was expressed as millilitre of absorbed oil per gram of sample. Viscosity of the dietary fibre was determined using the method of Frost et al. (1984). RBHB solution (2%, w/w) was prepared by slowly adding an appropriate amount of dietary fibre preparation to distilled water and mixing at high speed in a blender for 1 min. The solution was allowed to sit at room temperature for 24 h to come to equilibrium and entrapped air to escape before viscosity measurements were made. The viscosity was measured using a NDJ viscometer (NDJ-1 Model, 60 r/min, Shanghai Hengping Instrument Co., Ltd, Shanghai, PR China). The measurement was performed at room temperature.

Binding capacity of rice bran dietary fibres for sodium cholate

In vitro bile acid-binding test was carried out using the method of Kahlon et al. (2009) and Zhang et al. (2011) with some modifications. One gram of dietary fibres (RBHA, RBHB, RBHC, RBDF, cellulose, lignin and WIDF) was 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 concentration of cholate in the supernatant was determined using high-performance liquid chromatography (Agilent 1100 Series) with a C-18 column (5 μm, 4.6 mm × 150 mm). The mobile phase was acetonitrile:water:phosphoric acid (40:60:0.1), with a flow rate of 1.2 ml/min. Cholate was detected at 192 nm against a standard curve.

Binding capacity of dietary fibres for cholesterol in egg yolk

In vitro cholesterol in egg yolk-binding test was carried out using the method of Zhang et al. (2011) and Park (1999) with some modifications. Fresh egg yolk was whipped with nine volumes of deionized water. Mixtures of 2.0 g dietary fibres with 50 ml of the diluted yolk at pH 7.0 and 4.0, respectively, were shaken at 80 rpm for 2 h in a water-bath incubator maintained at 37°C, diluted yolk without dietary fibre being the blank. The mixture was centrifuged at 4000*g* for 20 min; ethanol in the supernatant was removed using vacuum at 40°C. One millilitre of the concentrate was diluted five times with 90% acetic acid. Colour was developed by adding 0.1 ml of *o*-phthalaldehyde reagent and 2 ml of concentrated H_2SO_4, according to the method of Park. The binding capacity (B) was calculated as follows:

$$B = [C_1 - (C_2 - C_3) \times F] \times \frac{50}{w},$$

where C_1, C_2 and C_3 stand for the concentrations of cholesterol in the yolk, the yolk without dietary fibre and the yolk mixed with dietary fibres, respectively; F is the dilution factor (10); 50 is the adsorption volume (ml) and w is the weight of the dietary fibres.

Statistical analysis

All results were subjected to statistical analyses. Mean and standard deviation (SD) were calculated (Steel and Torrie 1960). Mean values of all data were obtained from triplicate determinations. Values expressed are mean ± SD. Significance of differences between control and treated samples was evaluated using Duncan's multiple range test at 5% level.

Results

Functional properties of RBHA, RBHB, RBHC, RBDF, cellulose and lignin

The chemical composition of RBHA, RBHB, RBHC and RBDF preparations used in this study is presented in Table I. As illustrated, RBHA, RBHB and RBHC preparation contained high amount of dietary fibre (81.36%, 82.94% and 83.20%) and little starch. WIDF preparation contained 83.06% dietary fibre. RBHA preparation contained 0.69% fat, 3.81% protein and 3.29% ash. RBHB preparation contained 0.59% fat, 2.69% protein and 3.17% ash. RBHC preparation contained 0.55% fat, 2.53% protein and 3.18% ash. RBDF preparation contained low content

Table I. Proximate analysis of the rice bran fibres.

Components	Percentage (%)*			
	RBHA	RBHB	RBHC	RBDF
Crude fat	0.69 ± 0.02	0.59 ± 0.01	0.55 ± 0.01	2.88 ± 0.05
Crude protein	3.81 ± 0.05	2.69 ± 0.03	2.53 ± 0.04	8.35 ± 0.09
Moisture	10.37 ± 0.11	10.31 ± 0.12	10.21 ± 0.16	10.98 ± 0.18
Ash	3.29 ± 0.05	3.17 ± 0.04	3.18 ± 0.03	4.21 ± 0.10
Starch	0.5†	0.3†	0.3†	10.9†
Total dietary fibre	81.36 ± 0.41	82.94 ± 0.36	83.20 ± 0.50	62.73 ± 0.45

Note: RBHA, rice bran hemicellulose A; RBHB, rice bran hemicellulose B; RBHC, rice bran hemicellulose C; RBDF, rice bran insoluble dietary fibre; *Values are mean ± SD, $n = 3$; †Values were obtained via gravimetric procedure.

dietary fibre (62.73%), 2.88% fat, 8.35% protein, 10.9% starch and 4.21% ash. These fibres had many desirable properties, including high water-holding capacity and SC. RBHB and RBDF had high WBC and SC, namely 5.20, 6.08 and 5.11, 5.93 ml/g, respectively (Table II). RBHB was found to be less viscous, and the viscosity of 2% solution (w/w) was 7.8 cps at room temperature (NDJ-1 Model, 60 r/min).

Binding capacity of dietary fibres for fat, cholesterol and cholic acid

Table III shows that RBHB and RBDF exhibited FBC (4.96 and 4.35 ml/g, respectively) than FIBREX reported (a commercial fibre from sugar beet, 1.29 ml/g; Azizah and Yu 2000). RBHB exhibited higher FBC than dietary fibre from rice bran reported (4.54 ml/g; Azizah and Yu 2000). They are significantly different from control at 5% level. The results in Table III show that the dietary fibres from RBHB had the highest binding capacity for cholesterol than the RBHA, RBHC and RBDF fibres from rice bran and WIDF at both the pH values. Fibres from RBHB also had the highest binding capacity for sodium cholate, followed by the WIDF, RBHC and RBHA dietary fibres, which are significantly different from control at 5% level (Table III). Binding of cholesterol and cholic acid to RBDF and cellulose from rice bran was found to be poor. Lignin from rice bran was the least active fraction for binding cholesterol and cholic acid.

RBHB has bound the largest amount among fat, cholesterol and cholic acid of different fibres from rice bran. RBHB was the most active fibre with respect to cholesterol (pH 4.0, 5.85 mg cholesterol/g RBHB), cholesterol (pH 7.0, 6.50 mg cholesterol/g RBHB) and sodium cholate (4.28 mg sodium cholate/g RBHB). RBHC showed a high activity towards cholesterol (5.78 mg cholesterol/g RBHC), whereas cellulose displayed a low capacity of binding cholesterol and sodium cholate (pH 4.0, 0.2 mg cholesterol/g cellulose; pH 7.0, 0.78 mg cholesterol/g cellulose and 0.81 mg sodium cholate/g cellulose, respectively). RBHA was capable of binding fat. The quantity of RBHA bound fat 4.63 mg fat/g RBHA. There was a considerable difference at different pH in the quantities of RBHC-bound cholesterol (pH 4.0, 1.76 mg cholesterol/g RBHA; pH 7.0, 3.36 mg cholesterol/g RBHA) and a small difference for RBDF and WIDF bound.

Discussion

As it can be seen from these data, the amount of cholesterol and bile acid bound by rice bran fibres differed from one fibre to another. From the investigations reported in the literature, it can be inferred that the stability of bile acid–dietary fibre (DF) complexes differs according to the metal involved and depends on the fibre source. The capacity of the fibre to bind chemical elements depends on its chemical structure and composition (Borycka and Stachowiak 2008). The mechanism of cholesterol and bile acid binding abilities from many different DFs including RBHB is not clear. Sorption is a complex process and may proceed according to three mechanisms: chemisorption, physical sorption and mechanical sorption (Krejpcio et al. 1997). Chemisorption is connected with the presence in the fibre matrix of phenolic groups from lignin and carboxyl groups from uronic acids. Physical sorption results from van der Waals' forces, which are temperature dependent, whereas mechanical sorption depends on the degree of porosity of the sorbent and its ability to

Table II. Functional properties of dietary fibre from defatted rice bran.

Samples	Water-holding capacity (ml/g)*	SC (ml/g)*	Viscosity* (2%, cps)
RBHA	4.19 ± 0.10	5.57 ± 0.22	
RBHB	5.20 ± 0.12	6.08 ± 0.20	7.8
RBHC	5.17 ± 0.12	6.11 ± 0.28	6.7
RBDF	5.11 ± 0.14	5.93 ± 0.17	
Cellulose	3.78 ± 0.09	5.00 ± 0.16	
Lignin	2.99 ± 0.08	3.13 ± 0.10	
WIDF	5.03 ± 0.11	5.86 ± 0.15	

Note: RBHA, rice bran hemicellulose A; RBHB, rice bran hemicellulose B; RBHC, rice bran hemicellulose C; RBDF, rice bran insoluble dietary fibre; WIDF, water-insoluble wheat bran dietary fibre; *Values are mean ± SD, $n = 3$.

Table III. Adsorption capacity of dietary fibres for fat, cholesterol and bile acid.

Dietary fibre	Control	RBHA	RBHB	RBHC	RBDF	Cellulose	Lignin	WIDF
Fat (ml/g)	0.88 ± 0.06a	4.63 ± 0.11d	4.96 ± 0.08d	4.22 ± 0.12d	4.35 ± 0.09d	1.87 ± 0.09 b	0.90 ± 0.07a	2.74 ± 0.18c
Cholesterol (pH 4.0, mg/g)	0.06 ± 0.01a	1.76 ± 0.62b	5.85 ± 0.93d	3.43 ± 0.44c	0.47 ± 0.05a	0.20 ± 0.04a	0.07 ± 0.02a	3.77 ± 0.90c
Cholesterol (pH 7.0, mg/g)	0.06 ± 0.01a	3.36 ± 0.47c	6.50 ± 0.69d	5.78 ± 0.76d	1.26 ± 0.11b	0.78 ± 0.08a	0.06 ± 0.01a	4.36 ± 0.58d
Sodium cholate (mg/g)	0.11 ± 0.02a	2.04 ± 0.76b	4.28 ± 0.37d	3.17 ± 0.55c	0.82 ± 0.08a	0.81 ± 0.09a	0.12 ± 0.03a	3.48 ± 0.87c

Note: Mean value ± SD ($n = 3$) with different letters within a column are significantly different at 5% level.
RBHA, rice bran hemicellulose A; RBHB, rice bran hemicellulose B; RBHC, rice bran hemicellulose C; RBDF, rice bran insoluble dietary fibre; WIDF, water-insoluble wheat bran dietary fibre.

trap the substances in its spatial structure. The soluble fibres extracted from rice bran with the alkali solutions were mainly composed of arabinose and xylose. These polysaccharides of rice bran hemicelluloses are arabino-xylans (Aoe et al. 1993; Hu and Huang 1998; Hu and Zhai 2002; Hu et al. 2007, 2008). From our results, lignin from rice bran was the least active fraction for cholesterol and bile acid. The mechanism of cholesterol and bile acid binding to rice bran fibre including RBHB, RBHC and RBHA is to be resolved through further research.

Dietary sources, such as wheat bran (WIDF), containing a large proportion of water insoluble dietary fibres showed a higher binding capacity for saturated fat, and the addition of water-soluble dietary fibres from rice bran (RBHB, RBHC and RBHA) significantly increased their binding capacity for cholesterol and sodium cholate (Table III). The link between dietary fat, blood–lipid profile and heart disease has been clearly established. Saturated fatty acids and cholesterol are considered atherogenic fats (Heshmati et al. 2009). From Table III, we can postulate that a daily intake of hemicelluloses from rice bran helps remove cholesterol from foods; moreover, sodium cholate would be removed, meaning that cholesterol would be excreted from the body through the faeces. High dietary intake of fat, cholesterol and sodium and low intake of fruits, vegetables and fish are linked to cardiovascular risk. Detoxification of harmful metabolites (and cancer prevention) by dietary fibres is very important and often evaluated *in vitro* by measuring their binding capacities for bile acids (Kahlon et al. 2009). Hemicelluloses from rice bran can adsorb cholesterol and bile acid and act as a potential 'functional food' that reduces the incidence of CVDs by reducing the risk of type-2 diabetes, body weight and serum low-density lipoprotein–cholesterol levels and adsorbing bile acids. Bile acids, derived from cholesterol, are necessary for the digestion of lipids in the small intestine. This confirms that the RBHB preparation from defatted rice bran has great potential in food applications, especially in the development of functional foods.

Conclusions

The quantity of cholesterol and bile acid bound to rice bran fibres varies. The RBHB from rice bran was characterized by the highest capacity of cholesterol and bile acid binding. The RBHC fraction ranked the second. Binding of cholesterol and bile acid to insoluble dietary fibre (RBDF) from rice bran was poor. Lignin from rice bran was found to be the least active fraction for binding cholesterol and bile acid. The capacity of cholesterol and bile acid binding mainly depends on the fibre source from rice bran. The mechanism of cholesterol and bile acid binding to rice bran fibre including RBHB, RBHC and RBHA is to be resolved through further research.

Declaration of interest

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