Evaluating Healthful Properties of Cereals and Cereal Fractions by Their Bile-Acid-Binding Potential[1]

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- In vitro bile acid binding is a valuable tool for screening food fractions for their healthful potential before animal and human studies are warranted.
- The healthful, cholesterol-lowering or detoxification of harmful metabolites potential of cereals and cereal fractions could be predicted by evaluating their in vitro bile acid binding under physiological conditions.

In vitro bile acid binding without the use of labeled isotopes is an economical method for screening various foods and food fractions to evaluate their healthful potential before initiating time- and cost-intensive animal and human studies. Bile acids are needed for fat absorption, and high-fat diets are implicated in obesity, as well as raising plasma cholesterol. Bound bile acids are excreted and are not available for absorption through enterohepatic circulation. In response to lowered bile acid levels, the liver uses cholesterol to synthesize additional bile acids. Secondary bile acids are known to be carcinogenic. Cholesterol-lowering and cancer-risk-reduction potential of food fractions could be evaluated by their bile-acid-binding potential. Some plant breeding companies have been using an in vitro bile-acid-binding procedure (18) in their selections to propagate more health-promoting crops. Cereals and cereal bran are considered to be desirable for human consumption due to their reported health benefits. Extensive research reviewed by Kahlon and Chow (17) has shown that incorporating rice bran, oat bran, or barley fractions in the diet results in plasma cholesterol reductions, which lower the risk of cardiovascular disease. Milling wheat bran to a smaller particle size has been shown to ameliorate the impaired bioavailability of vitamin E by the coarse wheat bran (21). Extruded wheat bran and other foods have been shown to lower blood cholesterol in humans (36). Extruded wheat bran at low specific mechanical energy (SME) of 120–221 Wh/kg has been reported to significantly lower plasma and liver lipids in hamsters compared with unextruded wheat bran (23,25). Health benefits associated with the consumption of wheat bran include fecal bulking and improved regularity (34). Various processing techniques, such as milling, flaking, rolling, and extrusion, are used in the production of many popular foods, including ready-to-eat (RTE) cereals, snacks, and pasta. Finer particle size whole grain products are being introduced to increase the whole grain consumption as recommended by the USDA Food Guide Pyramid (2005) and to meet the color and texture preferences of the consumers. This feature article highlights in vitro binding studies exploring relative health-promoting potential of cereals and cereal fractions.

Metabolic Role of Bile Acids

Bile acids are acidic steroids synthesized in the liver from cholesterol. After conjugation with glycine or taurine, they are secreted into the duodenum. Bile acids are actively reabsorbed by the terminal ileum and undergo an enterohepatic circulation (13). The bile acids are needed for the absorption of dietary fat from the GI tract. The dietary fat is metabolized to acetate. Acetate is the principal precursor of cholesterol synthesis in the body. Dietary fiber's function of binding bile acids and increasing fecal excretion has been hypothesized as a possible mechanism for lowering cholesterol (2,32,42). By binding bile acids, cereal fibers prevent their reabsorption and stimulate plasma and liver cholesterol conversion to additional bile acids (5,10,29). Secondary bile acids (deoxycholic acid, lithocholic acid) and their metabolite (ursodeoxycholic acid) have been found to be mutagenic and promote tumor growth (8). The healthful, cholesterol-lowering (atherosclerosis amelioration, detoxification of harmful carcinogenic metabolites) potential of cereals and cereal fractions could be predicted by evaluating their in vitro bile acid binding, based on positive correlations found between in vitro and in vivo studies showing that cholestyramine (bile-acid-binding, cholesterol-lowering drug) binds bile acids and cellulose does not (9,18,37,40). Significant bile acid excretion was observed with oat bran diets in metabolic ward studies (3,15,28). Jenkins et al. (14) reported a significant increase in fecal bile acids with soluble fiber from oat bran and psyllium in free-living volunteers. Marlett et al. (34) reported that oat bran lowers serum cholesterol levels by altering bile acid metabolism. Oat bran increased bile acid excretion in ileostomy patients (11,30,45).

Bile-Acid-Binding Procedure

Kritchevsky and Story (29) have described the in vitro bile-acid-binding procedure in which labeled isotopes of bile acids were used. The in vitro bile-acid-binding procedure described here does not use labeled isotopes, thus eliminating the radiation hazards and disposal costs of the isotopes. The in vitro bile-acid-binding procedure has been established by Kahlon and Chow (18) and further fine-tuned by Kahlon and Woodruff (19,20). The stock bile acid mixture was formulated with glycocholic bile acids providing 75% and taurine-conjugated bile acids providing 25% of the bile acids based on the composition of human bile (7,38). This stock solution contained glycocholic acid (9 mmol/L), glycochenocholic acid (9 mmol/L), glycodeoxycholic acid (9 mmol/L), taurocholic acid (3 mmol/L), taurochenocholic acid (3 mmol/L), and taurodeoxycholic acid (3 mmol/L) in a pH 6.3, 0.1*M* phosphate buffer. A stock solution of 36 mmol/L was stored in a freezer maintained at –20°C. Working solutions of 0.72 µmol/mL were prepared from the stock solution just prior to each assay. Cellulose, a non-bile-acid-binding fiber, was the negative control and cholestyramine, a bile-acid-binding anionic resin, was the positive control. Cholestyramine is a drug that lowers cholesterol by binding bile acids. Six replicates rather than three of 100 mg of dry matter of each test substrate, cholestyramine 25 mg and cellulose 25 mg dry matter, are desirable for accurate bile-acid-binding studies. One substrate blank, one positive blank (2.88 µmol of bile acid mixture per incubation), and six treatment replicates were weighed into 16 × 150-mm

[1] *The mention of firm names or trade products does not imply that they are endorsed or recommended by the USDA over other firms or similar products not mentioned.*

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glass, screw-capped tubes. The bile-acid-binding procedure is given in Figure 1. Each supernatant sample was analyzed in triplicate for unbound bile acids. Values were determined from a standard curve obtained by analyzing Trinity Biotech bile acid calibrators (No. 450-11) at 5, 25, 50, 100, and 200 µmol/L. Individual blank substrates were subtracted, and bile acid concentrations were corrected based on the mean recoveries of bile acid mixture (positive blank).

Bile Acid Binding of Cereal Bran

Cholestyramine is an anion exchange resin that binds cholesterol and is recommended for lowering cholesterol. Comparing bile acid binding of cereal bran with cholestyramine is very appropriate. Assigning bile acid binding to cholestyramine at 100%, the relative bile acid binding was rice bran at 13–25%, wheat bran at 16–20%, oat bran at 5%, barley or β-glucan-enriched barley at 5–6%, and corn bran at 3–4% (Tables I and II). There was significantly higher bile acid binding with rice bran and wheat bran than with oat bran and corn bran. Bound bile acids are in the residue after centrifugation and unbound bile acids are determined in the supernatant. However, it could have been possible that soluble fiber could be binding bile acids but would be present in the supernatant. In order to test this possibility, soluble fiber was precipitated out with 70% ethanol and total unbound bile acids were determined for this supernatant. In oat bran treatments, all of the unbound bile acid was found in the supernatant after precipitating soluble fiber with ethanol (19). Very low in vitro bile acid binding by oat bran may possibly be due to hydrolysis of soluble fiber in 1 h of acidic digestion. Minimal binding of bile acids by oat bran is consistent with the low neutral sterol excretion reported with an oat bran diet in hamsters (24). The bound bile acids and neutral sterol excretion may represent two different types of electrostatic bindings; however, these observations appear to have significant positive correlation. Various human studies have shown that oat bran-soluble fiber binds bile acids (3,11,14,15,28,30,34,45). Very low bile acid binding was observed with added β-glucanase to an oat bran diet of ileostomy subjects (30). Wheat bran has limited potential to lower cholesterol (4,41,43). Marcus and Heaton (33) and Alberts et al. (1) have reported that wheat fiber and wheat bran bind bile acids, reduce transit time, and lower bile acid concentration by fecal bulking, thereby lowering the risk of colon cancer. Epidemiological data have shown that increased consumption of whole grains and cereal fiber lowered the risk of cancer (6,35). Bile-acid-binding data of rice bran and wheat (Tables I and II) suggest that rice bran should be tested for its cancer prevention potential. Story and Kritchevsky (39) observed 11% binding (relative to cholestyramine at 100%) of bile acids by wheat bran in contrast to Vahouny (44), who found little or no bile-acid-sequestering activity with wheat bran. The higher bile acid binding with wheat bran (Tables I and II) may be explained by the use of a bile acid mixture with a pH of 6.3, which is similar to that secreted in the human duodenum (7,38), as well as simulated gastric and pancreatin digestion steps.

Bile Acid Binding of Processed Wheat Bran

Extrusion processing is used in the production of many popular foods, including RTE cereals, snacks, and pasta. The extrudates have physical and chemical characteristics different from those of the original food (12,31). These differences depend on the extrusion parameters (e.g., energy, time, and the type of extruder used) and the physical and chemical properties (moisture, fat, and fiber content) of the raw material. Extrusion alters the starch, protein, fat, and fiber components of cereals, forming complexes that may affect their bile acid-binding and cholesterol-lowering properties. In vitro bile acid binding by wheat bran (WB) unextruded and extruded at five SME levels (120–358 Wh/kg of dry matter extrusion energy input) was evaluated (26). Relative to cholestyramine bile-acid-binding values for various wheat bran treatments were 14–23% (Table III). Bile acid binding for 177 Wh/kg of extruded wheat bran was significantly higher than all the other bran treatments. Relative bile acid binding on a dry matter basis was WB-177 > WB-120 > WBU = WB-234 = WB-291 > WB-358. Higher (358 Wh/kg) SME resulted in significantly lowering the bile-acid-binding potential of wheat bran, this may be the result of the production of new complexes that inhibit bile acid binding. In a hamster feeding study, diets containing 120 Wh/kg of SME-extruded wheat bran resulted in significant reduction in total cholesterol and very-low-density lipoprotein cholesterol compared with unextruded wheat bran (25). There was

Substrate (100 mg) + 1 mL, 0.01*N* HCl
Incubate 1 h ↓37°C (shaker bath)
+ 0.1 mL, 0.1*N* NaOH (neutralize)
+ 4 mL of bile acid mixture (0.72 µmol/mL)*
+ 5 mL (5X, 10 mg/mL) of porcine pancreatin (amylase, protease, and lipase)
1 h 37°C (shaker bath) ↓(5X, 10 mg/mL, in 0.1*M* phosphate buffer, pH 6.3)
Transfer contents to 10-mL centrifuge tubes
Centrifuge 99,000 × *g* ↓18 min, 25°C
Remove supernatant (1)
Rinse incubation tubes with 5 mL of phosphate buffer
Centrifuge 99,000 × *g* ↓18 min, 25°C
Remove supernatant (2)
Pool supernatants (1) and (2), Store –20°C
[Analyze for bile acids Trinity Biotech bile acids procedure No. 450-A, using a Ciba-Corning Express Plus analyzer]
* 0.1*M* phosphate buffer, pH 6.3 (phosphate buffer only for blank)

Fig. 1. Diagram of the bile-acid-binding procedure.

Table I. In vitro bile acid binding by rice bran, oat bran, wheat bran, and corn bran relative to cholestyramine[x]

Treatment	Experiments 1 and 2 (%)[y]	Experiments 3 and 4 (%)[z]
Rice bran	25.0 a	13.0 a
Oat bran	5.0 c	4.6 b
Wheat bran	20.0 b	15.9 a
Corn bran	2.9 c	3.9 b

[x] In experiments 1 through 4, incubations were conducted in triplicate and repeated to confirm the findings; therefore, $n = 6$ are pooled values of two separate triplicate incubations. Values within a column that do not share a common letter differ significantly ($P \leq 0.05$), $n = 6$. Values are relative to considering cholestyramine binding bile acids as 100%. Data from Kahlon and Chow (21).

[y] On an equal weight, dry matter basis. All treatments contained 30 mg of dry matter (experiments 1 and 2).

[z] Dry matter (mg) used per incubation: for rice bran, 100; for oat bran, 139; for wheat bran, 52; and for corn bran, 31 (experiments 3 and 4).

Table II. In vitro bile acid binding by rice bran, oat bran, barley, and enriched barley relative to cholestyramine[y]

Treatment	%[z]
Rice bran	12.1 a
Oat bran	4.4 d
Barley (dehulled)	5.2 c
Enriched barley	6.1 b

[y] Rice bran, oat bran, barley, and enriched barley treatments contained 140, 147, 196, and 199 mg of dry matter, respectively, on an equal weight, dry matter basis. Values are relative to considering cholestyramine binding bile acids as 100%. Data from Kahlon and Woodruff (23).

[z] Pooled values within a column that do not share a common letter differ significantly ($P \leq 0.05$), $n = 6$.

significant liver cholesterol reduction in hamsters by all the extruded wheat bran diets compared with the unextruded wheat bran diet. Highest liver cholesterol reductions were with the diet containing 120 Wh/kg of SME (32%) and 177 Wh/kg of SME (22%). These observations support and validate in vitro bile-acid-binding studies.

Milling wheat bran resulted in significant improvement in its bile acid binding compared with the values for the unmilled wheat bran (27). Extruding the milled wheat bran resulted in no further enhancement in its bile-acid-binding capacity; it was even lowered at SME levels of 120, 234, and 291 Wh/kg (Table IV). Data suggest that milling (low-cost processing) wheat bran significantly improved its bile acid binding, which was previously observed by extrusion (high-cost technology) of unmilled wheat bran (26). The bile acid binding appears to be related to the surface area of the wheat bran particles.

Bile Acid Binding of RTE Breakfast Cereals

The in vitro bile-acid-binding capacity of 15 RTE breakfast cereals obtained from a local supermarket was evaluated to determine their health-promoting potential to lower cholesterol, bind toxic metabolites, and reduce the risk of atherosclerosis and cancer (20). The relative bile acid binding for the cereals containing wheat alone or in combination with barley or brown rice was 3–13% and for cereals containing oats was 8–10% (Table V). The proportion of different grain fractions in a RTE cereal could influence their bile-acid-binding potential. Within the rice-containing cereals, the highest relative bile-acid-binding values were observed for rice flakes (4%). Cholesterol-lowering properties of oat, rice, and barley fibers and fractions have been reviewed extensively (16,17). Improvement in cholesterol-lowering potential of wheat bran by low torque extrusion relative to unextruded wheat bran has been reported (23,25). It has been shown that relative to cholestyramine wheat bran bound bile acids at 17–23% (Table III) and this binding could be further enhanced (6–15%) by process technologies. It is possible to enhance the bile-acid-binding capacity in RTE cereals, especially those with 3–7% relative bile acid binding by incorporating additional wheat bran and/or through process technologies.

Relative to cholestyramine, bile acid binding for rice bran (22%) has been reported (18). Bile acid binding observed in rice-containing cereals was only 2–4% (20). It would be desirable to incorporate additional quantities of rice bran into RTE cereals to increase their bile-acid-binding potential. Relative bile acid binding of RTE cereals containing extruded oats or toasted oats and extruded oat bran of (8–10%) is very encouraging. The process technologies and/or fortification (modified corn starch and whole wheat flour) lower degradation of soluble oat fiber and appear to enhance healthfulness of oat RTE cereals, as only 5% bile acid binding by oat bran has been previously reported (18,19). The higher bile acid binding of oat RTE cereals suggests that process technologies and fortification (modified corn starch and fruit pectin) could also enhance the health-promoting potential of other grains, such as corn, rice, and barley, to a level higher than observed for their bran.

Table III. In vitro bile acid binding of unextruded and extruded wheat bran relative to cholestyramine[y]

Treatment	%[z]
WBU (unextruded)	17.7 c
WB-120	20.6 b
WB-177	22.6 a
WB-234	16.5 c
WB-291	17.0 c
WB-358	14.0 d

[y] Dry matter used for incubation for all the bran samples was 100–103 mg. Wheat bran (WB) extruded at a specific mechanical energy of 120, 177, 234, 291, and 358 Wh/kg of dry matter on an equal weight, dry matter basis. Values are relative to considering cholestyramine binding bile acids as 100%. Data from Kahlon et al. (26).

[z] Mean values within a column that do not share a common letter differ significantly ($P \leq 0.05$), $n = 6$.

Table IV. In vitro bile acid binding of milled wheat bran (MWB) and milled extruded wheat bran (MEB) relative to cholestyramine[y]

Treatment	%[z]
MWB	20.8 a
MEB-120	18.8 b
MEB-177	21.0 a
MEB-234	19.2 b
MEB-291	18.0 c
MEB-358	21.4 a

[y] Dry matter used for incubation for all the bran samples was 97–103 mg. Wheat bran extruded at a specific mechanical energy of 120, 177, 234, 291, and 358 Wh/kg of dry matter on an equal weight, dry matter basis. Values are relative to considering cholestyramine binding bile acids as 100%. Data from Kahlon et al. (27).

[z] Mean values within a column that do not share a common letter differ significantly ($P \leq 0.05$), $n = 6$.

Table V. In vitro bile acid binding by various ready-to-eat cereals relative to cellulose and cholestryramine[y]

Treatment	%[z]
Wheat bran extruded	3.2 a
Wheat shredded	9.4 bc
Wheat barley flakes	8.1 cd
Wheat bran flakes	6.9 de
Wheat barley nuggets	5.9 e
Wheat brown rice flakes	2.9 g
Oat bran extruded	9.8 b
Oats toasted	8.00 cd
Oats extruded	7.9 d
Rice flakes	4.4 f
Rice extruded	3.1 g
Rice puffed	2.00 gh
Rice toasted	1.9 gh
Corn extruded	2.4 gh
Corn flakes	1.7 gh

[y] On an equal weight, dry matter basis. Values are relative to considering cholestyramine binding bile acids as 100%. Data from Kahlon and Woodruff (24).

[z] Pooled values within a column that do not share a common letter differ significantly ($P \leq 0.05$), $n = 6$.

Conclusion

The healthful, cholesterol-lowering, and cancer-risk-reduction potential of cereals and cereal fractions could be predicted by evaluating their in vitro bile acid binding under physiological conditions. In vitro bile acid binding of rice bran and wheat bran was significantly higher than oat bran and corn bran. Bile acid binding of wheat bran could be enhanced by milling it to finer particle size or by process technologies. Process technologies of milling, rolling, extrusion, shredding, toasting, flaking, and fortification would enhance the healthful potential of cereals and cereal fractions. For greater health-promoting potential of plant foods, commercial breeding companies have been making use of this in vitro bile-acid-binding methodology in their selections.

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