Health-promoting Potential of Cereals, Grain Fractions, and Beans as Determined by Their in Vitro Bile Acid Binding[1]

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- Whole grains, bran, and legumes, when consumed regularly, can help lower the risk of cardiovascular disease and certain cancers and improve public health.
- In vitro bile acid binding is a cost-effective method for screening foods and food fractions to evaluate their health-promoting potential.
- Study results suggest processing, fortification, and use of different grain fractions in RTE cereals can be used to enhance bile acid binding and improve their potential health benefits.

Atherosclerosis and cancer are the two leading causes of death and disability in the developed world, and the occurrence of both is increasing rapidly in the developing world. Incidence of these major diseases can be decreased through diet and a physically active lifestyle. The health-promoting, cholesterol-lowering (atherosclerosis amelioration) potential of food 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 (a bile acid-binding, cholesterol-lowering drug) binds bile acids and cellulose does not (6,14,25,30).

Bile acids, acidic steroids synthesized in the liver from cholesterol, are required for absorption of dietary fat from the gastrointestinal tract. Dietary fat, which is metabolized to acetate, is the principal precursor of cholesterol synthesis in the body. High-fat diets are implicated in obesity and elevated levels of plasma cholesterol. By binding bile acids, food fractions prevent their reabsorption and stimulate plasma and liver cholesterol conversion to additional bile acids (3,8,22). Toxic metabolites in the gut and secondary bile acids increase the risk of colorectal cancer (5). In response to decreased bile acid levels, the liver uses cholesterol to synthesize additional bile acids. Because excretion of bile acids is the major route for removal of cholesterol from the body (9), binding of bile acids and increasing their fecal excretion have been hypothesized as possible mechanisms by which food fractions lower cholesterol (2,23,31).

In vitro bile acid binding without the use of labeled isotopes is a cost-effective method for screening various foods and food fractions to evaluate their health-promoting potential before initiating time- and cost-intensive studies with animals and humans. The cholesterol-lowering and cancer risk-reduction potential of foods can be evaluated based on their bile acid-binding capacities.

Kahlon and Chow (12) established an in vitro bile acid-binding procedure that has been subsequently refined by Kahlon and Woodruff (13,14). By relating bile acid binding to dry matter, total dietary fiber (insoluble or soluble), proteins, and lipids, dry matter was identified as the most effective measure for determining the health-promoting potential of foods. Some international plant breeding companies have been using this in vitro bile acid-binding procedure (14) in their seed selections to propagate crops with higher health-promoting potentials. This in vitro bile acid-binding procedure has been proposed to AACC International for approval as a screening method to assess bioactive compounds for their health-promoting potential. One of the benefits of the in vitro bile acid-binding procedure is that it does not use labeled isotopes, which eliminates the radiation hazards and disposal costs associated with isotopes. Instead, bile acid-binding values are determined relative to cholestyramine binding.

Bile Acid Binding of Cereals and Grain Fractions

Wheat. Whole-grain and pearled wheat varieties have been evaluated previously for their relative bile acid-binding capacities after cooking (21). Relative to cholestyramine, bile acid-binding values were 7.7% for whole-grain hard red winter wheat, 7.5% for whole-grain hard white winter wheat, 6.3% for pearled hard white winter wheat, 6.0% for pearled hard red winter wheat, 5.5% for whole durum, and 5.4% for pearled durum (Table I). Pearled wheat grain could be considered lightly refined because total dietary fiber is reduced by 1–3%. Binding values for whole-grain hard red winter wheat and hard white winter wheat were similar and significantly ($P \leq 0.05$) higher than those for pearled hard red winter wheat and hard white winter wheat, and whole and pearled durum. A bile acid binding value for whole-grain hard white winter wheat similar to that of whole-grain hard red winter wheat suggests that the red color commonly associated with whole grain may not necessarily indicate a higher health-promoting potential. The data suggest that whole-grain hard red winter and hard white winter wheat have significantly higher health-promoting potentials than their pearled grain counterparts. The health-promoting potentials of

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

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doi:10.1094/CFW-56-4-0151

whole-grain and pearled durum wheat were similar to those of pearled hard white winter wheat and hard red winter wheat.

Consumption of products containing whole-grain hard red winter wheat and hard white winter wheat are recommended. Finely ground whole-grain flours are being introduced to the market to help increase consumer acceptance of whole-grain foods. The USDA Food Guide Pyramid recommends that at least 50% of intake from the grains group should be from whole-grain foods (32).

Cereal Bran. Bile acid binding on an equal dry matter basis was significantly higher with rice and wheat brans than with corn and oat brans. Assigning bile acid binding to cholestyramine as 100%, the relative bile acid binding was 13–28% for rice bran, 16–22% for wheat bran, 5–10% for oat bran, and 3–4% for corn bran (12).

Higher bile acid binding with rice bran suggests a possible mechanism for cholesterol lowering includes binding bile acids and increasing neutral sterol excretion. This agrees with previous animal feeding studies with rice bran (10,11). Minimal binding of bile acids with oat bran is consistent with the low neutral sterol excretion reported for an oat bran diet in hamsters (11). The results do not support the suggestion that the mechanism for cholesterol lowering with oat bran is through the binding of bile acids with soluble fiber (2). The relationship between cholesterol lowering and increased bile acid excretion in rats (29) and increased bile acid excretion in ileostomy patients consuming a high-fiber oat-based diet (33) may be explained as a result of the higher bacterial content found in diets high in fermentable fiber, because bacteria may bind bile acids in the cecum (23). The moderate level of bile acid binding with wheat bran (16–22%) may be associated with health-promoting effects such as dilution of toxic metabolites, improvement in gastrointestinal mucosal health, prevention of constipation, and reduction in the risk of certain cancers. It has been reported (1,24) that wheat fiber and bran bind bile acids, reduce transit time, and lower bile acid concentration through fecal bulking, thereby preventing colon cancer. Wheat bran prevented colon cancer in rats by improving colon health, and binding toxic metabolites, bile acids, and cancer-causing agents (26,28).

Significant differences in bile acid binding among the four cereal fractions (rice, oat, barley, and β-glucan–enriched oat) have been shown. Relative to cholestyramine, bile acid binding on a dry matter basis was 12–13% for rice bran, 4–5% for oat bran, 5% for barley, and 6% for β-glucan–enriched barley (12,14). The variability in bile acid binding among various cereal fractions may be related to differences in anionic, cationic, physical, and chemical structures. Drzikova et al. (7) reported that in vitro bile acid binding increased with increasing proportions of oat bran, total dietary fiber, insoluble dietary fiber, and β-glucan in the extrudates.

Significantly higher bile acid binding by β-glucan–enriched barley than oat bran suggests that β-glucan–enriched barley may have a higher health-promoting potential than oat bran. It is possible that soluble dietary fiber present in the supernatant may have bound bile acids. However, in three supernatants each of oat bran and β-glucan–enriched barley samples, analysis of soluble dietary fiber revealed that all the unbound bile acids were quantitatively present in the clear supernatant (14). The data suggest that bile acid binding in the cereals tested was not related to soluble dietary fiber level. These data confirmed our previous observations (14) that the primary mechanism of cholesterol lowering by cereal bran is not through bile acid binding by soluble dietary fiber. This agrees with studies reporting there was no chemical binding between isolated barley β-glucans and bile acids (4).

The in vitro bile acid-binding potential of wheat bran (WB) extruded (E) at five specific mechanical energy (SME) levels and an unextruded (U) control have been reported (19). Relative bile acid binding on a dry matter basis was as follows: EWB-177 > EWB-120 > UWB = EWB-234 = EWB-291 > EWB-358 (Table II). The significantly lower bile acid-binding potential of wheat bran at higher SME (358 W·hr/kg) may have been caused by deactivation of the bile acid binding sites in fiber, protein, and fat complexes. In a hamster feeding study, diets containing EWB-120 resulted in a significant reduction in total and VLDL (very low-density lipoprotein) cholesterol compared with the UWB control (18). A significant reduction in liver cholesterol in hamsters was observed with all of the diets containing EWB compared with the diet containing UWB. The highest liver cholesterol reductions (35 and 22%) were observed with the diets containing EWB-120 and EWB-177, respectively. These findings support the validity of in vitro bile acid-binding studies.

Wheat bran (WB) was milled (M) before extrusion (E) at five SME input levels, and its bile acid-binding potential was reported (13). Bile acid-binding values for MWB, MEWB-177, and MEWB-358 were similar and significantly higher than those for MEWB-120, MEWB-234, and MEWB-291 (Table III). The bile acid-

Table I. In Vitro Bile Acid Binding with Cooked Whole Grain Versus Refined Wheat (dmb)[a]

Treatment	Bile Acid Binding Relative to Cholestyramine (%)
Whole-grain wheat	
Hard red winter	7.72 b
Hard white winter	7.50 b
Durum	5.50 c
Pearled wheat	
Hard red winter	6.01 c
Hard white winter	6.32 c
Durum	5.44 c
Control	
Cholestyramine	100.00 a
Cellulose	–11.14 d

[a] Values followed by different letters differ significantly ($P \leq 0.05$) ($n = 6$). Dry matter used for all grain samples was 100–101 mg; cholestyramine and cellulose treatments contained 25 mg each. Data from Kahlon et al. (21).

Table II. In Vitro Bile Acid Binding with Unextruded and Extruded Wheat Bran (WB) on an Equal Weight Basis (dm)[a]

Treatment[b]	Bile Acid Binding Relative to Cholestyramine (%)
WB (unextruded)	17.7 d
WB (120 W·hr/kg)	20.6 c
WB (177 W·hr/kg)	22.6 b
WB (234 W·hr/kg)	16.5 d
WB (291 W·hr/kg)	17.0 d
WB (358 W·hr/kg)	14.0 e
Cholestyramine	100 a
Cellulose	0.8 f

[a] Values followed by different letters differ significantly ($P \leq 0.05$) ($n = 6$). Dry matter used for all bran samples was 100–103 mg; cholestyramine and cellulose treatments contained 25 mg each. Data from Kahlon et al. (19).

[b] WB was extruded at five levels of specific mechanical energy (dwb).

Table III. In Vitro Bile Acid Binding with Milled Wheat Bran (MWB) and Milled Extruded Wheat Bran (MEB) on an Equal Weight Basis (dm)[a]

Treatment[b]	Bile Acid Binding Relative to Cholestyramine (%)
WBM (unextruded)	20.8 b
MEB (120 W·hr/kg)	18.8 c
MEB (177 W·hr/kg)	21.0 b
MEB (234 W·hr/kg)	19.2 c
MEB (291 W·hr/kg)	18.0 c
MEB (358 W·hr/kg)	21.4 b
Cholestyramine	100 a
Cellulose	0.8 f

[a] Values followed by different letters differ significantly ($P \leq 0.05$) ($n = 6$). Dry matter used for all bran samples was 97–103 mg; cholestyramine and cellulose treatments contained 25 mg each. Data from Kahlon et al. (20).

[b] MEB was extruded at five levels of specific mechanical energy (dwb).

binding values for MWB observed here were significantly higher than those previously reported for unmilled wheat bran (19). Extruding MWB resulted in no further enhancement in bile acid binding, and it was lowered at SME levels of 120, 234, and 291 W·hr/kg. The data suggest that milling wheat bran significantly improved its bile acid-binding capability compared with unmilled wheat bran (21 versus 18%). However, the expected increase of up to 5% in bile acid binding due to extrusion, as had been reported previously (19), was not observed. Instead, all of the observed improvement in bile acid binding was realized by pin-milling the wheat bran compared with unmilled wheat bran; extruding milled wheat bran resulted in no further increase in bile acid-binding capacity. The data suggest that the low-cost technology of milling wheat bran to a smaller particle size resulted in an improvement in health-promoting potential similar to that observed with high-cost extrusion technology. It could be speculated that a finer particle size whole-grain flour would have a higher health-promoting potential due to inclusion of bran; in addition, as observed in this study (20), a finer particle size bran binds more bile acids than does coarse bran.

Ready-to-Eat Cereals. The bile acid-binding potentials of ready-to-eat (RTE) breakfast cereals have been reported previously (15). Relative to cholestyramine, bile acid binding with RTE cereals containing wheat alone (extruded bran, shredded, and bran flakes) and in combination with barley (flakes and nuggets) was approximately 6–13%. The value for wheat-brown rice flakes was only approximately 3%. Values for cereals containing oats (extruded bran, toasted, and extruded) were approximately 8–10%. Values for cereals containing rice (flakes, extruded, puffed, and toasted) were only approximately 2–5%, and for cereals containing corn (extruded and flakes), values were approximately 2%.

Processing (extruding, shredding, toasting, and flaking) may enhance the bile acid-binding potential of RTE cereals. In addition, the proportion of different grain fractions in an RTE cereal could influence their bile acid binding potential. In this study, bile acid binding observed with cereals containing rice was only approximately 2–5%. It would be desirable to incorporate additional quantities of rice bran into RTE cereals to increase their bile acid-binding potential. Bile acid binding with oat bran (4%) relative to cholestyramine has been reported previously (14). The considerably higher (8–10%) relative bile acid binding observed with extruded and toasted oats and extruded oat bran was very encouraging. It suggests that process technologies and/or fortification (modified corn starch and whole-wheat flour) can be used to increase the health-promoting potential of oat-containing RTE cereals to levels even higher than those observed with the use of oat bran. The higher bile acid binding of oat-containing RTE cereals suggests that process technologies and fortification (modified corn starch and fruit pectin) could also be used to increase the health-promoting potential of other grains such as corn, rice, and barley to levels higher than those observed with their brans.

Bile Acid Binding of Beans

Relative bile acid binding on dry matter basis (13) was 15% for soy protein, 6% for pinto beans, 8% for black beans, and 9% for wheat gluten (Table IV). The variability in bile acid-binding capacity observed between various samples may be related to differences in anionic, cationic, physical, and chemical structures. The significantly higher bile acid binding observed with soy protein suggests that a possible mechanism for its influence on lipid and lipoprotein metabolism and reduction of aortic plaque involves binding bile acids and increasing neutral sterol excretion. This agrees with previous observations showing a significant reduction (–45%) in aortic plaque in hamsters over a 6-week period in which soy protein isolate replaced casein in the diet as a source of protein (11). Relative bile acid binding with wheat gluten (9%) may be associated with its health-promoting effects, such as dilution of toxic metabolites, improvement in gastrointestinal mucosal health, prevention of constipation, and reduction in cancer risk (1,23,24,26). However, the higher bile acid binding observed with wheat bran suggests that in addition to wheat protein there are other components of wheat bran that also bind bile acids.

Relative bile acid binding on dry matter basis (16) was 2% for soybean, 3% for black eye bean, 10% for garbanzo, and 4% for lima bean (Table V). Garbanzo bound significantly more bile acid than black eye bean, lima bean, and soybean. The data suggest that animal and human studies should be conducted to explore the health-promoting potential of garbanzo. The relative bile acid-binding values were 3% for kidney bean, 3% for black gram, 7% for Bengal gram, and 3% for moth bean (17). Binding values for Bengal gram were significantly higher than for kidney bean, black gram, and moth bean (Table VI) (garbanzo and Bengal gram are two varieties of *Cicer arietinum*). The data suggest there are large varietal differences in bile acid binding. Based on these results, it would be desirable to include beans in the formulation of high-protein, health-promoting snacks.

Table IV. In Vitro Bile Acid Binding with Soy Protein, Pinto Bean, Black Bean, and Wheat Gluten on an Equal Weight Basis (dm)[a]

Treatment[b]	Bile Acid Binding Relative to Cholestyramine (%)
Soy protein	14.5 b
Pinto bean	5.5 d
Black bean	8.2 c
Wheat gluten	8.8 c
Cholestyramine	100.0 a
Cellulose	–1.0 e

[a] Pooled values followed by different letters differ significantly ($P \leq 0.05$) ($n = 6$). Data from Kahlon and Woodruff (13).

[b] Soy protein, pinto bean, black bean, wheat gluten, cholestyramine, and cellulose treatments contained 33, 108, 92, 34, 25, and 25 mg, respectively.

Table V. In Vitro Bile Acid Binding with Soybean, Black Eye Bean, Garbanzo, and Lima Bean on an Equal Weight Basis (dm)[a]

Treatment[b]	Bile Acid Binding Relative to Cholestyramine (%)
Soybean	1.9 d
Black eye bean	3.3 c
Garbanzo	10.0 b
Lima bean	3.7 c
Cholestyramine	100.0 a
Cellulose	1.5 d

[a] Values followed by different letters differ significantly ($P \leq 0.05$) ($n = 6$). Data from Kahlon and Shao (16).

[b] Soybean, black eye bean, garbanzo, lima bean, cholestyramine, and cellulose treatments contained 70, 99, 110, 107, 24, and 24 mg, respectively.

Table VI. In Vitro Bile Acid Binding with Kidney Bean, Black Gram, Bengal Gram, and Moth Bean on an Equal Weight Basis (dm)[a]

Treatment[b]	Bile Acid Binding Relative to Cholestyramine (%)
Kidney bean	2.5 cd
Black gram	3.3 c
Bengal gram	6.7 b
Moth bean	3.1 c
Cholestyramine	100.0 a
Cellulose	1.5 d

[a] Pooled values followed by different letters differ significantly ($P \leq 0.05$) ($n = 6$). Data from Kahlon et al. (17).

[b] Kidney bean, black gram, Bengal gram, moth bean, cholestyramine, and cellulose treatments contained 108, 101, 114, 98, 24, and 24 mg, respectively.

Conclusions

In vitro bile acid-binding capacity can indicate the health-promoting potential of foods. Whole grains and cereals made from whole grains and bran (rice, oat, and barley) and legumes (garbanzo and Bengal gram) were found to have higher bile acid-binding capacities. Whole grains, bran, and legumes, when consumed regularly, could help lower the risk of cardiovascular disease and certain cancers and improve public health.

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