# In Vitro Bile Acid Binding Capacity of Wheat Bran Extruded at Five Specific Mechanical Energy Levels

T. S. Kahlon,[1,2] J. de J. Berrios,[1] G. E. Smith,[1] and J. L. Pan[1]

## ABSTRACT

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The in vitro binding of bile acids of extruded wheat bran (EWB) at five specific mechanical energy (SME) levels of 120 (EWB-120), 177 (EWB-177), 234 (EWB-234), 291 (EWB-291), and 358 (EWB-358) Wh/kg on a dry weight basis, was determined using a mixture of bile acids secreted in human bile at a duodenal physiological pH 6.3. Experiments were conducted using six treatments and two blank incubations to test unextruded and extruded wheat bran samples on an equal dry matter (DM) basis. Relative to cholestyramine (bile acid binding, cholesterol-lowering drug), in vitro bile acid binding ability of unextruded and extruded wheat bran treatments on an equal DM basis was 14–23%. The bile acid binding ability of the total dietary fiber (TDF) was 28–51% and insoluble dietary fiber (IDF) was 29–53%. Bile acid binding on DM, TDF, and IDF bases for EWB-177 was significantly ($P \leq 0.05$) higher, and EWB-358 was significantly lower than UWB, EWB-120, EWB-234, and EWB-291. Bile acid binding for EWB-120 was significantly higher than that for UWB, EWB-234, and EWB-291. These results demonstrate the relative health promoting potential of EWB-177 > EWB-120 > UWB = WB-234 = EWB-291 >EWB-358 as indicated by bile acid binding ability on DM, TDF, and IDF bases. The variability in bile acid binding of wheat bran treatments may be due to fragmenting of particles or macromolecules, creating new linkages in proteins, starches, and nonstarch polysaccharides, changes in physical and chemical characteristics, and binding sites.

World wheat production is ≈585 million metric tons per year, resulting in ≈140 million metric tons of wheat bran. Health benefits associated with the consumption of wheat bran include fecal bulking and improved regularity (Marlett et al 2002). Extrusion processing is used in the production of many popular foods including ready-to-eat cereals, snacks, and pasta. The extrudates have physical and chemical characteristics different from those of the original food (Harper and Clark 1979; Linko and Mercier 1981). These differences depend on the extrusion parameters (e.g., energy input, residence time, and the type of extruder used) and the chemical composition (e.g., moisture, fat, and fiber content) of the raw material. Extrusion cooking modifies the starch, protein, fat, and fiber components of the raw material used by forming physical and chemical complexes in the extrudate that may have cholesterol-lowering properties. Extruded wheat bran and other foods have lowered blood cholesterol in humans (Meshcheriakova et al 1995). Extruded wheat bran (EWB) at low specific mechanical energy (SME) of 221 Wh/kg, significantly lowered cholesterol in hamsters compared with unextruded wheat bran (UWB), whereas, wheat bran extruded at high energy (442 Wh/kg) resulted in cholesterol values similar to those of unextruded and low energy (221 Wh/kg) extruded wheat bran (Kahlon et al 1998). 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 (Hofmann 1977). Binding of bile acids and increasing fecal excretion has been hypothesized as a possible mechanism for lowering cholesterol by dietary fiber (Trowell 1975; Lund et al 1989; Anderson and Siesel 1990). By binding bile acids, cereal fibers prevent reabsorption and stimulate plasma and liver cholesterol conversion to additional bile acids (Eastwood and Hamilton 1968; Balmer and Zilversmit 1974; Kritchevsky and Story 1974). Toxic metabolites in the gut and secondary bile acids increase the risk of colorectal cancer (Costarelli et al 2002). The healthful, cholesterol-lowering (atherosclerosis amelioration) or detoxification of harmful metabolites (cancer prevention) potential of food fractions such as wheat bran could be predicted by evaluating 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 (Suckling et al 1991; Nakamura and Matsuzawa 1994; Daggy et al 1997; Kahlon and Chow 2000).

The objective of this study was to evaluate in vitro bile acid binding by UWB and EWB processed at five SME levels of 120 (EWB-120), 177 (EWB-177), 234 (EWB-234), 291 (EWB-291), and 358 (EWB-358) Wh/kg on a dry weight basis, using a bile acid mixture similar to that found in human bile (Carey and Small 1970; Rossi et al 1987) at physiological pH 6.3, approximating that of the duodenum.

## MATERIALS AND METHODS

### Extrusion Cooking Conditions

Hard red winter wheat bran was produced on a Buhler mill (Buhler AG, Switzerland) by a local miller. A twin-screw extruder (Continua 37, Werner and Pfleiderer, Ramsey, NJ) system with co-rotating and closely intermeshing screws was used to process the wheat bran materials. The extruder system was controlled by a programmable controller (Series One Plus, General Electric, Charlottesville, VA). The extruder had eight barrel sections, each with a length of 160 mm. The screw diameter was 37 mm, and the total configured screw length was 1,324 mm, which gave an overall L/D ratio of 35.78. The screw configuration used for extrusion was a combination of right- and left-handed screw elements and kneading blocks: RHSE, 40 PD x 150; RHSE, 26 PD x 60; {RHKB x 40; Spacer (SP) x 1; LHKB x 40}; RHSE, 26 PD x 40; {RHKB x 40; SP x 1; LHKB x 40}; RHSE, 26 PD x 60; {LHKB x 40; SP x 1; RHKB x 40; SP x 1; LHKB x 40}; RHSE, 26 PD x 60; {RHKB x 40; SP x 1; LHKB x 20; SP x 1; LHKB x 20; SP x 1; RHKB x 20; SP x 1; LHKB x 20}; RHSE, 26 PD x 60; {RHSE, 40 PD x 10; SP x 1; LHSE, 40 PD x 10; SP x 1} x 5; RHSE, 26 PD x 100; {RHSE, 40 PD x 10; SP x 1; LHSE, 40 PD x 10; SP x 1} x 8; RHSE, 40 PD x 100. Screws were driven by a 11.2 kW variable speed DC drive (model DC300, General Electric) operated at 400 rpm. The die plate contained two circular openings 2.5 mm in diameter. The temperature of each barrel section was controlled by a recirculating hot oil system (model MK4X06-TI, Mokon Div., Protective Closures, Buffalo, NY). Temperature of the barrel sec-

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[1] Western Regional Research Center, USDA, Agricultural Research Service, 800 Buchanan Street, Albany, CA 94710. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

[2] Corresponding author. Phone: 510-559-5665. Fax: 510-559-5777. E-mail: tsk@pw.usda.gov

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tions was maintained within ±1°C by a cool heat exchanger (packaged chiller model CD-5-A, Edwards Engineering, Pompton Plains, NJ). The heating profile used in this study was no heat, 80, 80, 100, 100, 115, 115, and 130°C corresponding to barrel sections 1–8, respectively. Feed was metered into the feed port by a twin-screw, lost-in-weight gravimetric feeder (model LWFD5-20, K-Tron, Pitman, NJ) at a rate of 15 kg/hr (dwb). Peanut oil (15% dwb) was injected 70 mm downstream from the center of the feed port, using a variable stroke piston pump (model P5-120, Bran and Luebbe, Wheeling, IL). With a similar pump, variable amounts of water 19.6, 15.0, 11.0, 7.45, and 6.0 kg/hr was injected to obtain final percent torque of 31.5, 37.5, 43.5, 49.5, and 55.5, respectively. A computer collected the extruder parameter data at 1-sec intervals for a total of 5 min, using a data acquisition system (LabView, v. 5.0, National Instruments, Austin, TX). Extruded wheat bran (EWB) at five SME levels of 120 (EWB-120), 177 (EWB-177), 234 (EWB-234), 291 (EWB-291), and 358 (EWB-358) Wh/kg, dwb, was collected ≈10 min after the operation conditions of torque and pressure were at steady-state.

Unextruded and extruded wheat bran were analyzed using six replicates for insoluble and soluble dietary fiber (Prosky et al 1988); nitrogen by combustion (FP-428, Leco, St. Joseph, MI); ether extracted crude fat in by method 920.39C (AOAC 1990), and for moisture by method 935.29 (AOAC 1990). Nitrogen, crude fat, and moisture analyses were conducted in triplicate.

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. Eight replicate incubations, six with bile acid mixture and two blanks (one substrate without bile acid mixture and another bile acid mixture without the substrate) were run for each treatment and each control. All wheat bran treatments used 100–103 mg, cholestyramine 25 mg, and cellulose 25 mg dry matter per incubation.

### Bile Acid Binding Procedure

The in vitro bile acid binding procedure was a modification of that by Camire et al (1993) as previously reported (Kahlon and Chow 2000). The stock bile acid mixture was formulated with glycocholic bile acids providing 75% and taurine-conjugated bile acids 25% of the bile acids based on the composition of the human bile (Carey and Small 1970; Rossi et al 1987). This mixture 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 pH 6.3, 0.1*M* phosphate buffer. A stock solution of 36 mmol/L was stored in a freezer at –20°C. Working solutions of 0.72 µmol/mL were prepared from the stock solution just before each assay. Six replicates of 100–103 mg of dry matter of each of the wheat bran treatments were tested. One substrate blank, one positive blank (2.88 µmol of bile acid mixture per incubation), and six treatment replicates were weighed into glass, screw-capped tubes 16 × 150 mm. Samples were digested in 1 mL of 0.01*N* HCl for 1 hr in a water bath maintained at 37°C under continuous agitation. After this acidic incubation simulating gastric digestion, the sample was adjusted to pH 6.3 with 0.1 mL of 0.1*N* NaOH. Bile acid mixture (4 mL) working solution (0.72 µmol/mL) in a 0.1*M* phosphate buffer, pH 6.3, was added to each test and positive blank treatments, while 4 mL of 0.1*M* phosphate buffer, pH 6.3, was added to the substrate blanks. After adding 5 mL of porcine pancreatin (5×, 10 mg/mL, in a 0.1*M* phosphate buffer, pH 6.3), which provided amylase, protease, and lipase for digestion of samples, the reaction tubes were incubated for 1 hr in a water bath maintained at 37°C under continuous agitation. Mixtures were transferred to 10-mL centrifuge tubes (Oak Ridge 3118-0010 Nalgene, Rochester, NY) and centrifuged at 99,000 × *g* in a 75-Ti rotor at 39K for 18 min at 25°C in an ultracentrifuge (model L-60, Beckman, Palo Alto, CA). Supernatant was removed into a second set of labeled tubes. An additional 5 mL of phosphate buffer was used to rinse out the incubation tube and added to the centrifuge tube, which was vortexed and centrifuged as before. This second supernatant was removed and combined with the supernatant previously obtained. Aliquots of pooled supernatant were stored at –20°C for bile acids analysis. Bile acids were analyzed by a bile acids procedure (No. 450, Trinity Biotech Distribution, St. Louis, MO) using an analyzer (Ciba-Corning Express Plus, Polestar Labs, Escondido, CA). Each sample was analyzed in triplicate. Values were determined from a standard curve obtained by analyzing bile acid calibrators (Trinity Biotech 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). The effect of treatment was tested using Lavene's test for homogeneity and least square means were calculated. Dunnett's one-tailed test was used for comparison of cholestyramine and cellulose against all treatments, and differences among wheat bran treatments were tested for significance with Tukey's test for comparison (SAS Institute, Cary, NC). The criterion of significance was $P \leq 0.05$.

## RESULTS AND DISCUSSION

Total dietary fiber composition of the unextruded and extruded wheat bran is given in Table I. The concentration of total dietary fiber (TDF), insoluble dietary fiber (IDF), and soluble dietary fiber (SDF) determined in the UWB was 51, 48, and 3%, respectively. Extrusion cooking did not significantly ($P \leq 0.05$) affect the concentration of TDF, IDF, and SDF. The TDF, IDF, and SDF determined in the EWB were 50–52, 48–50, and 2–3%, respectively.

On an equal dry matter (DM) basis, bile acid binding was significantly higher for cholestyramine and significantly lower for cellulose than all the wheat bran treatments (Table II). Bile acid binding of EWB-177 was significantly ($P \leq 0.05$) higher than that

TABLE I
Dietary Fiber Composition of Unextruded Wheat Bran (UWB) and Extruded Wheat Bran (EWB)[a]

| Diet | Total Dietary Fiber (TDF %) | Insoluble Dietary Fiber (IDF %) | Soluble Dietary Fiber (SDF %) |
|---|---|---|---|
| UWB | 51.33 ± 0.15 | 48.20 ± 0.07 | 3.13 ± 0.11 |
| EWB-120 | 52.23 ± 0.14 | 49.82 ± 0.04 | 2.41 ± 0.12 |
| EWB-177 | 51.43 ± 0.06 | 49.07 ± 0.05 | 2.36 ± 0.06 |
| EWB-234 | 50.70 ± 0.28 | 48.73 ± 0.10 | 1.97 ± 0.32 |
| EWB-291 | 50.93 ± 0.11 | 47.98 ± 0.09 | 2.95 ± 0.04 |
| EWB-358 | 50.21 ± 0.23 | 47.61 ± 0.18 | 2.60 ± 0.10 |

[a] Wheat bran extruded at SME of 120, 177, 234, 291, 358 Wh/kg, dwb; $n = 6$.

TABLE II
In Vitro Bile Acid Binding of Unextruded Wheat Bran (UWB) and Extruded Wheat Bran (EWB), DM Basis[a–c]

| Treatment | Bile Acid Binding (µmol/100 mg of DM) | Bile Acid Binding Relative to Cholestyramine (%) |
|---|---|---|
| UWB | 1.46 ± 0.03d | 17.7 ± 0.4d |
| EWB-120 | 1.70 ± 0.07c | 20.6 ± 0.8c |
| EWB-177 | 2.14 ± 0.13b | 22.6 ± 1.4b |
| EWB-234 | 1.35 ± 0.03d | 16.5 ± 0.3d |
| EWB-291 | 1.40 ± 0.04d | 17.0 ± 0.5d |
| EWB-358 | 1.15 ± 0.02e | 14.0 ± 0.2e |
| Cholestyramine | 10.23 ± 0.06a | 100.0 ± 0.6a |
| Cellulose | 0.06 ± 0.02f | 0.8 ± 0.2f |

[a] On equal weight, dry matter (DM) basis.

[b] All wheat bran treatments used 100–103 mg, cholestyramine 25 mg, and cellulose 25 mg dry matter per incubation. Wheat bran extruded at SME of 120, 177, 234, 291, 358 Wh/kg, dwb.

[c] Mean ± SEM within a column with different letters differ significantly ($P \leq 0.05$); $n = 6$.

for other extruded and unextruded wheat bran. Bile acid binding for UWB, EWB-234, and EWB-291 was similar and their values were significantly lower than EWB-120 and significantly higher than EWB-358. Cholestyramine bound 90% of the bile acids. These experimental values are similar to those previously reported for cholestyramine bile acid binding capacity (Sugano and Goto 1990; Kahlon and Chow 2000). However, Story and Kritchevsky (1976) reported 81% bile acid binding by cholestyramine using 50 mg of substrate and 50 μmol of bile acids. Higher bile acid binding by cholestyramine in our studies may be due to the use of physiological pH or a higher substrate to bile acid ratio.

Assigning a bile acid binding value of 100% to cholestyramine, the relative bile acid binding on dry matter basis for the wheat bran tested was EWB-177 23%; EWB-120 21%; UWB 18%; EWB-234 17%; EWB-291 17% and EWB-358 14%. Bile acid binding for EWB-177 was significantly higher than all the other EWB and UWB treatments. Binding values for UWB, EWB-234, and EWB-291 were similar and their values were significantly lower than those for EWB-120 and significantly higher than those for EWB-358. Relative bile acid binding on a dry matter basis was EWB-177 > EWB-120 > UWB = EWB-234 = EWB-291 > EWB-358. Extrusion processing of wheat bran at SME of 120 and 177 Wh/kg resulted in significant improvement in the health-promoting potential of the final EWB product. EWB obtained at higher SME of 0.358 kWh/kg resulted in a significantly lower bile acid binding potential of wheat bran. This may be caused by deactivating the bile acid binding sites in fiber, protein, and fat complexes. Water was injected during extrusion at the rate of 6, 7.45, 11.0, 15.0, and 19.6 kg/hr to obtain the desired SME input of 358, 291, 234, 177, and 120 Wh/kg, respectively. Injection of water at 15.0 and 19.6 kg/hr to obtain SME of 177 and 120 Wh/kg resulted in most desirable chemical changes in terms of significant increase in the bile acid binding of extruded wheat bran. In a hamster feeding study, diets containing EWB-120 resulted in significant reduction in total cholesterol and very low density lipoprotein cholesterol compared with UWB control (Kahlon et al 2006). There was significant liver cholesterol reduction in hamsters by all the EWB diets compared with the UWB diet. The highest liver cholesterol reduction of 35% and 22% was observed with diets containing EWB-120 and EWB-177, respectively. These observations document validity of in vitro bile acid binding studies. In addition, excretion of toxic metabolites and secondary bile acids could lower the risk of cancer (Costarelli et al 2002).

The bile acid binding on an equal total dietary fiber (TDF) basis is shown in Table III. Cholestyramine bound bile acids significantly higher and cellulose significantly lower than the wheat bran treatments. On TDF basis, considering cholestyramine as 100% bound, bile acid binding values were EWB-177 51%; EWB-120 40%; UWB 35%; EWB-234 33%; EWB-291 33%, and EWB-358 28%. Bile acid binding of EWB-177 was significantly higher and EWB-358 was significantly lower than EWB-120, UWB, EWB-234, and EWB-291. Binding values for EWB-120 were significantly higher than those of UWB, EWB-234, and EWB-291. Relative bile acid binding on a TDF basis was EWB-177 > EWB-120 > UWB = EWB-234 = EWB-291 > EWB-358. This is in agreement with previously reported relative bile acid binding values for unextruded wheat bran of 30–33% on a TDF basis (Kahlon and Chow 2000). Extrusion processing results in dietary fiber and carbohydrate modification (Berrios et al 2002). Extrusion processing could also result in proteins, lipids, starches, and nonstarch polysaccharides fragmentation/modification, which may significantly affect the bile acid binding capacity of EWB product. Extrusion of wheat bran at SME input of 120 and 177 Wh/kg, resulted in a significant improvement in bile acid binding capacity of EWB product, possibly due to changes in the physical and chemical characteristics or binding sites of macromolecules.

The bile acid binding on an equal insoluble dietary fiber (IDF) basis is shown in Table IV. Cholestyramine-bound bile acids were significantly higher and cellulose was significantly lower than the wheat bran treatments. On an IDF basis, considering cholestyramine as 100% bound, bile acid binding values were EWB-177 53%; EWB-120 41%; UWB 37%; EWB-234 34%; EWB-291 36%, and EWB-358 29%. Relative bile acid binding on an IDF basis was EWB-177 > EWB-120 > UWB = EWB-234 = EWB-291 > EWB-358. Extrusion of wheat bran at SME of 120 Wh/kg resulted in significant improvement in bile acid binding over UWB; EWB-177 resulted in significantly higher bile acid binding than EWB-120. The relative significant effects of various wheat bran treatments were the same on DM, TDF, and IDF bases because there were only small differences in DM, TDF, and IDF content between the various wheat bran treatments.

In conclusion, considering cholestyramine (bile acid binding, cholesterol-lowering drug) as 100% bound, the relative in vitro bile acid binding capacity of DM, TDF, and IDF, respectively, was 18, 35, and 37% for UWB; 21, 40, and 41% for EWB-120; 23, 51, and 53% for EWB-177; 17, 33, and 34% for EWB-234; 17, 33, and 36% for EWB-291; 14, 28, and 29% for EWB-358. These results demonstrate the relative health-promoting potential of EWB-177 > EWB-120 > UWB = WB-234 = EWB-291 > EWB-358 as indicated by bile acid binding ability on DM, TDF, and IDF basis. Low SME extrusion processing, under the conditions of this study, demonstrated a significant effect on wheat bran components that was reflected in a significantly higher bile acid binding ability of extruded wheat bran EWB-177 and EWB-120 treatments. Extrusion of wheat bran under the selected SME used in this study may have induced changes in the physical and chemi-

**TABLE III**
**In Vitro Bile Acid Binding of Unextruded Wheat Bran (UWB) and Extruded Wheat Bran (EWB), TDF Basis[a–c]**

| Treatment | Bile Acid Binding (μmol/100 mg of TDF) | Bile Acid Binding Relative to Cholestyramine (%) |
|---|---|---|
| UWB | 2.84 ± 0.06d | 34.6 ± 0.8d |
| EWB-120 | 3.25 ± 0.13c | 39.5 ± 1.6c |
| EWB-177 | 4.17 ± 0.25b | 50.7 ± 3.1b |
| EWB-234 | 2.67 ± 0.05d | 32.5 ± 0.7d |
| EWB-291 | 2.75 ± 0.08d | 33.4 ± 0.9d |
| EWB-358 | 2.29 ± 0.03e | 27.8 ± 0.4e |
| Cholestyramine | 10.23 ± 0.06a | 100.0 ± 0.6a |
| Cellulose | 0.06 ± 0.02f | 0.8 ± 0.2f |

[a] On equal weight, total dietary fiber basis (TDF) basis.

[b] Total dietary fiber used for incubation for all bran samples was 50–53 mg, cholestyramine 25 mg, and cellulose 25 mg dry matter per incubation. Wheat bran extruded at SME of 120, 177, 234, 291, 358 Wh/kg, dwb.

[c] Mean ± SEM within a column with different letters differ significantly ($P \le 0.05$); $n = 6$.

**TABLE IV**
**In Vitro Bile Acid Binding of Unextruded Wheat Bran (UWB) and Extruded Wheat Bran (EWB), IDF Basis[a–c]**

| Treatment | Bile Acid Binding (μmol/100 mg of IDF) | Bile Acid Binding Relative to Cholestyramine (%) |
|---|---|---|
| UWB | 3.03 ± 0.07d[c] | 36.8 ± 0.8d |
| EWB-120 | 3.41 ± 0.14c | 41.4 ± 1.7c |
| EWB-177 | 4.37 ± 0.27b | 53.1 ± 3.2b |
| EWB-234 | 2.78 ± 0.06d | 33.8 ± 0.7d |
| EWB-291 | 2.92 ± 0.08d | 35.5 ± 1.0d |
| EWB-358 | 2.41 ± 0.03e | 29.3 ± 0.5e |
| Cholestyramine | 10.23 ± 0.06a | 100.0 ± 0.6a |
| Cellulose | 0.06 ± 0.02f | 0.8 ± 0.2f |

[a] On equal weight, insoluble dietary fiber basis (IDF) basis.

[b] Insoluble dietary fiber used for incubation for all bran samples was 48-50 mg, cholestyramine 25 mg, and cellulose 25 mg dry matter per incubation. Wheat bran (WB) extruded at SME of 120, 177, 234, 291, 358 Wh/kg, dwb.

[c] Mean ± SEM within a column with different letters differ significantly ($P \le 0.05$), $n = 6$.

cal characteristics of wheat bran or creation of new linkages through the binding sites of the proteins, starches, and nonstarch polysaccharides, which significantly increased the bile acid binding ability of the EWB products. Specific changes in chemical composition as a result of extrusion at various SME levels and different particle sizes as they relate to bile acid binding potential will be explored in subsequent studies.

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