

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

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ABSTRACT

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The in vitro binding of bile acids of milled wheat bran (MWB) and milled extruded wheat bran (MEB) at five specific mechanical energy (SME) levels of 120 (MEB-120), 177 (MEB-177), 234 (MEB-234), 291 (MEB-291), and 358 (MEB-358) Whr/kg on a fat-free dry weight basis was determined using a mixture of bile acids secreted in human bile at duodenal physiological pH 6.3. Relative to cholestyramine (bile acid binding, cholesterol lowering drug) in vitro bile acid binding capacity on dry matter, total dietary fiber (TDF), and insoluble dietary fiber (IDF) basis was for MWB: 21, 43, 45%; the range for MEB was 18–21%, 34–41%, and 36–43%, respectively. MWB resulted in significantly higher bile acid binding than that of MEB at 120, 234, and 291 Whr/kg on a dry matter, TDF, and IDF basis. These results demonstrate the relative health-

promoting potential of MWB = MEB-177 = MEB-358 > MEB-120 = MEB-234 = MEB-291 as indicated by the bile acid binding on a dry matter basis. Data suggest that significant improvement in health-promoting (cholesterol-lowering and cancer-preventing) potential could be obtained in WB by milling (low-cost processing) the bran to finer particle sizes and extruding (high-cost technology). Milling WB to small particle size (weighted mean 0.508 mm) increased surface area, in addition it may have induced changes in the physical and chemical characteristics of WB or created new linkages, binding sites of the proteins, starches, and nonstarch polysaccharides, which significantly increased the bile acid binding ability of the MWB.

World wheat production is ≈585 million metric tons per year, resulting in nearly 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). Various processing techniques such as milling, flaking, rolling, and extrusion are used in the production of many popular foods including ready-to-eat cereals, snacks, and pasta. USDA Food and Nutrition Information Center (2005) Food Guide Pyramid—Steps to a Healthier You (<http://www.mypyramid.gov>) recommends daily active life with 50% of the grain products consumed made from whole grains. Finer particle size whole grain products are being introduced to increase the whole grain consumption and to meet the color and texture preferences of the consumers. Milling wheat bran to smaller particle size ameliorates the impaired bioavailability of vitamin E by the coarse wheat bran (Kahlon et al 1986). Extruded wheat bran and other foods lower blood cholesterol in humans (Meshcheriakova et al 1995). Extruded wheat bran at low specific mechanical energy (SME) of 120–221 Whr/kg, significantly lowers lipids in hamsters compared with unextruded wheat bran (Kahlon et al 1998, 2006a). Extruding wheat bran at 120 and 177 Whr/kg bound significantly higher bile acid than unextruded wheat bran (WB) and that extruded at higher (234, 291, and 358 Whr/kg) SME levels (Kahlon et al 2006b). It was previously reported that bile acid binding of unextruded wheat bran (unmilled) was 18, 35, and 37% on dry matter, TDF, and IDF basis (Kahlon et al 2006b). The bile acid binding capacity of wheat bran may be enhanced by milling to finer particle sizes; milling and extrusion at specific SME levels may offer additional health-promoting benefits. 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 excre-

tion 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 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 (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 milled wheat bran (MWB), milled and extruded WB (MEB) at five SME levels of 120 (MEB-120), 177 (MEB-177), 234 (MEB-234), 291 (MEB-291), and 358 (MEB-358) Whr/kg on dwb, 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 mill (Buhler AG, Switzerland) by a local miller. The wheat bran used in the study reported here was the same as that reported in Kahlon et al (2006b), however it was milled before extrusion under same conditions and SME levels. It was pin-milled (160Z, Alpine, Company, Augsburg, Germany) to produce finer particle sizes. A twin-screw extruder (Continua 37, Werner and Pfleiderer Corp., Ramsey, NJ) system with co-rotating and closely intermeshing screws was used to process the milled wheat bran. The extruder system was controlled by a programmable controller (Series One Plus, General Electric Co., 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 (RHSE and LHSE), kneading blocks (KB), pitch diameter (PD), length in mm (× number), and times repeated { } × number;

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as followed: RHSE, 40 PD × 150; RHSE, 26 PD × 60; {RHKB × 40; Spacer (SP) × 1; LHKB × 40}; RHSE, 26 PD × 40; {RHKB × 40; SP × 1; LHKB × 40}; RHSE, 26 PD × 60; {LHKB × 40; SP × 1; RHKB × 40; SP × 1; LHKB × 40}; RHSE, 26 PD × 60; {RHKB × 40; SP × 1; LHKB × 20; SP × 1; LHKB × 20; SP × 1; RHKB × 20; SP × 1; LHKB × 20}; RHSE, 26 PD × 60; {RHSE, 40 PD × 10; SP × 1; LHSE, 40 PD × 10; SP × 1} × 5; RHSE, 26 PD × 100; {RHSE, 40 PD × 10; SP × 1; LHSE, 40 PD × 10; SP × 1} × 8; RHSE, 40 PD × 100. Screws were driven by a 11.2 kW variable speed DC drive (model DC300, General Electric, Erie, PA) operated at 400 rpm. The die plate length was 11.17 mm and it 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 Co., Buffalo, NY). Temperature of the barrel sections was maintained within ±1°C by a cool heat exchanger (packaged chiller model CD-5-A, Edwards Engineering Corp., 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 Corp., 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 SME input of 120 (MEB-120), 177 (MEB-177), 234 (MEB-234), 291 (MEB-291), and 358 (MEB-358) Whr/kg on a dwb, respectively. The extrusion conditions such as SME levels, addition of peanut oil, water injection rates, feed rate, and temperature profile were the same optimized conditions as reported previously (Kahlon et al 1998, 2006a,b). A computer collected extruder parameter data at 1-sec intervals for a total of 5 min, using LabView data acquisition system (v. 5.0, National Instruments, Austin, TX). The extruded material was collected ≈10 min after the operation conditions of torque and pressure were at steady-state.

Particle size distribution of wheat bran (WB) and milled wheat bran (MWB) was determined in triplicate with a Ro-Tap shaker (WS Tyler, Mentor, OH) using U.S. standard sieves (Table I). MWB and milled extruded wheat bran (MEB) were analyzed using six replicates for insoluble and soluble dietary fiber (Prosky et al 1988); nitrogen by combustion (FP-428, Leco Corp., St. Joseph, MI); ether extracted crude fat and moisture, respectively, by methods 920.39C and 935.29 (AOAC 1990). Nitrogen, crude fat, and moisture analyses were conducted in triplicate.

Cellulose, a nonbile acid binding fiber, was the negative control; 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 97–103 mg (fat free, dmb), cholestyramine 25 mg and cellulose 25 mg of dry matter per incubation. Previously we observed that peanut oil does not bind bile acids (Kahlon et al 2000); therefore fat-free dry matter was compared for bile acid binding in this study.

Bile Acid Binding Procedure:

The in vitro bile acid binding procedure was a modification of Camire et al (1993) as previously reported (Kahlon and Chow 2000). The stock bile acid mixture was formulated with glycocholic bile acids providing 75%; taurine-conjugated bile acids provided 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) pH 6.3, in 0.1M 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, before each assay. All the samples were ground in a mini-mill (Arthur Thomas, Philadelphia, PA) to pass a 0.4-mm screen. Six replicates of 97–103 mg dm (fat-free) 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 16- × 150-mm glass, screw-capped tubes. Samples were digested in 1 mL of 0.01N 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 0.1N NaOH. Bile acid (4 mL) mixture working solution (0.72 μmol/mL) in a 0.1M phosphate buffer, pH 6.3, was added to each test and positive blank treatment, while 4 mL of 0.1M phosphate buffer, pH 6.3, was added to the substrate blanks. After the addition of 5 mL of porcine pancreatin (5×, 10 mg/mL, in a 0.1M 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

TABLE I
Particle Size Distribution of Wheat Bran (WB) and Pin-Milled Wheat Bran (MWB)^{a,b}

US Sieve	Particle Size	WB (%)	Total	MWB (%)	Total
12	>1.70 mm	9.6		–	
14	1.70 ≤ 1.40 mm	13.5		–	
16	1.40 ≥ 1.18 mm	11.5		2.9	
18	1.18 ≥ 1.00 mm	15.5		5.9	
20	1.00 ≥ 0.85 mm	14.4		8.9	
25	850 ≥ 710 μm	10.1	74.6 %	9.9	27.6 %
30	710 ≥ 600 μm	7.1		10.9	
40	600 ≥ 425 μm	7.2		15.7	
60	425 ≥ 250 μm	7.6		17.3	
80	250 ≥ 180 μm	1.5		6.8	
	≤ 180 μm	2.0		–	
100	180 ≥ 150 μm	–		2.8	
120	150 ≥ 125 μm	–		2.0	
140	125 ≥ 106 μm	–		1.7	
200	106 ≥ 75 μm	–		4.9	
>200	≤ 75 μm	–	25.4%	10.3	72.4%

^a Samples were sieved using a Tyler Ro-Tap shaker for 1 hr; n = 3.

^b Weighted mean particle size is 1.018 mm for WB and 0.508 mm for MWB.

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 first supernatant. 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 $\mu\text{mol/L}$.

Statistical Analyses

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 the least square means were calculated. Dunnett's one-tailed test was used for comparison of cholestyramine as well as cellulose against all treatments, and differences among wheat bran treatments were tested for significance with Tukey's test for comparison of all possible pairs of means (SAS Institute, Cary, NC). A value of $P \leq 0.05$ was considered the criterion of significance.

RESULTS AND DISCUSSION

Unmilled WB had a weighted mean particle size of 1.018 mm; its major portion (74.6%) with particle size ≥ 0.710 mm, by pin milling resulted in weighted mean particle size of 0.508 mm; its major portion (72.4%) with particle size of ≤ 0.710 mm (Table I). Total dietary fiber composition of the wheat bran (WB), milled wheat bran (MWB), and milled extruded wheat bran (MEB) is given in Table II. The total dietary fiber (TDF), insoluble dietary fiber (IDF), and soluble dietary fiber (SDF) in the WB was 51, 48, and 3%, respectively. There were significant ($P \leq 0.05$) reductions in TDF, IDF, and SDF by milling the WB and the values were 48, 46, and 2%, respectively. Data suggest that milling resulted in finer particles with higher digestibility of dietary fiber. Extruding MWB at all SME levels resulted in significantly higher TDF and IDF values. The TDF values for MEB-234 and MEB-291 were significantly higher than WB, MWB, MEB-120, MEB-177, and MEB-358. IDF values for MEB-120, MEB-177, MEB-291, and MEB-358 were significantly higher than those of WB, MWB, and MEB-234. SDF values for WB, MEB-234, and MEB-291 were significantly higher than in MWB, MEB-120, MEB-177, and MEB-358. Extruding milled wheat bran at 234 and 291 Whr/kg resulted in significant elevations in TDF and IDF compared with the MWB and WB. The elevation in SDF with these two levels of extrusion resulted in significantly higher

values than those of MWB. This could be attributed to production of indigestible complexes with the combination water addition at 11.0 and 7.45 kg/hr and SME input at 234 and 291 Whr/kg, respectively.

On an equal fat-free dmb, bile acid binding was significantly higher for cholestyramine and significantly lower for cellulose than all the wheat bran treatments (Table III). Bile acid binding values of MWB, MEB-177, and MEB-358 were significantly ($P \leq 0.05$) higher than those of MEB-120, MEB-234, and MEB-291. There was no consistent trend in SME input during extrusion and the bile acid binding of MEB. 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 a dry matter basis for the wheat bran tested was MWB 21%; MEB-177 21%; MEB-358 21%; MEB-120 19%; MEB-234 19%; and MEB-291 18%. Bile acid binding values for MWB, MEB-177, and MEB-358 were similar and significantly higher than those of MEB-120, MEB-234, and MEB-291. No dose response was observed in the extrusion SME input and the bile acid binding of milled extruded wheat bran. Relative bile acid binding on fat-free dmb was MWB = MEB-177 = MEB-358 > MEB-120 = MEB-234 = MEB-291. Kahlon et al (2006b) reported bile acid binding on a dry matter basis for the same wheat bran unmilled (18%) and the values were significantly higher for the extruded unmilled wheat bran at SME of 120 and 177 Whr/kg (21 and 23%). The bile acid binding values for MWB observed here were significantly higher than those previously reported for same wheat bran unmilled under similar conditions (Kahlon et al 2006b). Extruding the MWB resulted in no further enhancement in bile acid binding capacity, it was even lowered at SME levels of 120, 234, and 291 Whr/kg. Higher bile acid binding by MEB-177 observed here is encouraging, and the bile acid binding complexes formed and the mechanisms will be explored in future studies. Previously, the highest bile binding was observed with SME-177 extruded unmilled wheat bran (Kahlon et al 2006b), which was consistent with significant triglyceride lowering in hamsters with diets containing unmilled SME-177 extruded wheat bran (Kahlon et al 2006a). Data suggest that milling WB significantly improved its bile acid binding compared with unmilled wheat bran (21 vs. 18%); however, the expected

TABLE III
In Vitro Bile Acid Binding of Milled Wheat Bran (MWB) and Milled Extruded Wheat Bran (MEB) on Equal Weight, Fat-Free Dry Matter (DM) Basis^{a-c}

Treatment	Bile Acid Binding ($\mu\text{mol}/100$ mg of DM)	Bile Acid Binding Relative to Cholestyramine (%)
MWB	1.71 \pm 0.05b	20.8 \pm 0.6b
MEB-120	1.54 \pm 0.04cd	18.8 \pm 0.5c
MEB-177	1.73 \pm 0.06b	21.0 \pm 0.7b
MEB-234	1.58 \pm 0.03c	19.2 \pm 0.4c
MEB-291	1.48 \pm 0.02cd	18.0 \pm 0.2c
MEB-358	1.77 \pm 0.03b	21.4 \pm 0.3b
Cholestyramine	10.23 \pm 0.06a	100 \pm 0.6a
Cellulose	0.06 \pm 0.02e	0.8 \pm 0.2d

^a Values followed by the same letter in the same column are not significantly different ($P \leq 0.05$); $n = 6$.

^b Fat free dry matter used for incubation for all the bran samples was 97–103 mg, cholestyramine 25 mg, and cellulose 25 mg.

^c Wheat bran extruded at specific mechanical energy of 120, 177, 234, 291, 358 Whr/kg on a dry weight basis.

TABLE II

Dietary Fiber Composition of Wheat Bran (WB), Milled Wheat Bran (MWB), and Milled Extruded Wheat Bran (MEB)^{a,b}

Diet	Total Dietary Fiber TDF %	Insoluble Dietary Fiber IDF %	Soluble Dietary Fiber SDF %
WB	51.34 \pm 0.15bc	48.20 \pm 0.07c	3.13 \pm 0.11a
MWB	48.21 \pm 0.63d	46.16 \pm 0.35d	2.05 \pm 0.46bc
MEB-120	51.35 \pm 0.21bc	49.82 \pm 0.16a	1.54 \pm 0.12c
MEB-177	51.22 \pm 0.23c	49.70 \pm 0.21a	1.52 \pm 0.18c
MEB-234	52.30 \pm 0.34a	49.19 \pm 0.07b	3.11 \pm 0.33a
MEB-291	52.56 \pm 0.11a	49.74 \pm 0.07a	2.82 \pm 0.08a
MEB-358	52.26 \pm 0.18b	49.73 \pm 0.07a	2.53 \pm 0.20b

^a Wheat bran extruded at specific mechanical energy of 120, 177, 234, 291, and 358 Whr/kg on a dry weight basis.

^b Values followed by the same letter in the same column are not significantly different ($P \leq 0.05$); $n = 6$.

increase of up to 5% in bile acid binding by extrusion as reported previously (Kahlon et al 2006b) was not observed in this study. Previously, when the same bran was extruded without milling, significant improvement in bile acid binding was observed by extrusion at SME levels of 120 and 177 Whr/kg compared with unmilled wheat bran (Kahlon et al 2006b). In the present study, all the 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 bile acid binding on equal total dietary fiber (TDF) basis is shown in Table IV. Cholestyramine-bound bile acids were significantly higher and cellulose was significantly lower than the wheat bran treatments. On TDF basis, considering cholestyramine as 100% bound, bile acid binding values were MWB 43%; MEB-177 41%; MEB-358 41%; MEB-120 37%; MEB-234 37%; and MEB-291 34%. Bile acid binding of MWB, MEB-177, and MEB-358 was significantly higher than MEB-120, MEB-234, and MEB-291. Relative bile acid binding on TDF basis was MWB = MEB-177 = MEB-358 > MEB-120 = MEB-234 = MEB-291. The expected higher bile acid binding by extruding the milled wheat bran was not observed as previously reported for unmilled wheat bran (35%) and unmilled extruded at SME-120 and SME-170 (40 and 51%, respectively) on TDF basis (Kahlon et al 2006b). The bile acid binding is not related to the dietary TDF content because milling decreased TDF content and increased bile acid binding observed here compared with previous reported values for the unmilled wheat bran (Kahlon et al 2006b). Data suggest that milling the WB results in significant enhancement in bile acid binding that was previously observed by extrusion under similar conditions where WB was not milled before extrusion.

The bile acid binding on an equal insoluble dietary fiber (IDF) basis is shown in Table V. 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 MWB 45%; MEB-358 43%; MEB-177 42%; MEB-234 39%; MEB-120 38%; and MEB-291 36%. Relative bile acid binding on an IDF basis was MWB = MEB-177 = MEB-358 > MEB-234 = MEB-120 = MEB-291. Using the same wheat bran, Kahlon et al (2006b) reported a significant increase in bile acid binding on an IDF basis where it was extruded unmilled under same conditions at SME-120 (41%) and SME-177 (53%) compared with unmilled wheat bran control (37%). The bile acid binding observed here by MWB (45%) was significantly higher than that reported previously (Kahlon et al 2006b) for unmilled wheat bran (37%). Bile acid binding was not

related to the IDF content as milling the WB lowered IDF content and resulted in higher bile acid binding. The MWB resulted in significantly higher bile acid binding than extruding the MWB at SME of 120, 177, 234, and 291 Whr/kg. It has been reported that milling the wheat bran to finer particle size ($\leq 500 \mu\text{m}$) eliminated the impaired bioavailability of vitamin E, which was observed with coarse ($\leq 2 \text{ mm}$) wheat bran (Kahlon et al 1986). Data reported here suggest that wheat bran with a finer particle size (weighted mean 0.508 mm) is more healthful as indicated by its bile acid binding compared with unmilled wheat bran with a larger particle size (weighted mean 1.018 mm). In addition, simpler processing, such as milling, resulted in similar or higher health-promoting potential as observed with more complex and expensive technology like extrusion (Kahlon et al 2006b).

CONCLUSIONS

Considering cholestyramine (bile acid binding, cholesterol lowering drug) as 100% bound, the relative in vitro bile acid binding capacity on a dry matter, TDF, and IDF basis was for MWB: 21, 43, 45%; MEB-177: 21, 41, 42%; MEB-358: 21, 41, 43%; MEB-120: 19, 37, 38%; MEB-234: 19, 37, 39%, and MEB-291: 18, 34, 36%, respectively. These results demonstrate the relative health promoting potential of MWB = MEB-177 = MEB-358 > MEB-120 = MEB-234 = MEB-291 as indicated by their bile acid binding ability on fat-free dry matter basis. Data suggest that significant improvement in bile acid binding of WB related to its health-promoting (cholesterol-lowering and cancer-preventing) potential could be obtained by milling (low-cost processing) the bran to a finer particle size and extruding (high-cost technology) milled bran did not further enhance its bile acid binding capacity. Milling WB to particle size (weighted mean 0.508 mm) increased its surface area; in addition, it may have introduced physical or chemical characteristics, creation of new linkages, binding sites of the proteins, starches, and nonstarch polysaccharides, which significantly increased the bile acid binding ability of the MWB. Specific changes in the physical and chemical composition as a result of reducing the particle sizes as they relate to bile acid binding potential will be explored in subsequent studies. Positive correlations have been reported in the cholesterol- and lipid-lowering response in hamsters fed extruded wheat bran diets (Kahlon et al 2006a) and bile acid binding of wheat bran used in these diets (Kahlon et al 2006b). Hamster feeding studies are planned to validate the significant improvement in bile acid binding observed here by milling the wheat bran compared with the unmilled wheat bran.

TABLE IV
In Vitro Bile Acid Binding of Milled Wheat Bran (MWB) and Milled Extruded Wheat Bran (MEB) on Equal Weight, Total Dietary Fiber (TDF) Basis^{a-c}

Treatment	Bile Acid Binding ($\mu\text{mol}/100 \text{ mg}$ of TDF)	Bile Acid Binding Relative to Cholestyramine (%)
MWB	3.55 \pm 0.10b	43.2 \pm 1.2b
MEB-120	3.01 \pm 0.08cd	36.6 \pm 1.0cd
MEB-177	3.38 \pm 0.12b	41.1 \pm 1.4b
MEB-234	3.02 \pm 0.06c	36.7 \pm 0.8c
MEB-291	2.81 \pm 0.04d	34.2 \pm 0.4d
MEB-358	3.38 \pm 0.05b	41.1 \pm 0.6b
Cholestyramine	10.23 \pm 0.06a	100 \pm 0.6a
Cellulose	0.06 \pm 0.02e	0.8 \pm 0.2e

^a Values followed by the same letter in the same column are not significantly different ($P \leq 0.05$); $n = 6$.

^b Total dietary fiber used for incubation for all the bran samples was 47–53 mg, cholestyramine 25 mg, and cellulose 25 mg.

^c Wheat bran extruded at specific mechanical energy of 120, 177, 234, 291, 358 Whr/kg on a dry weight basis.

TABLE V
In Vitro Bile Acid Binding of Milled Wheat Bran (MWB) and Milled Extruded Wheat Bran (MEB) on Equal Weight, Insoluble Dietary Fiber (IDF) Basis^{a-c}

Treatment	Bile Acid Binding ($\mu\text{mol}/100 \text{ mg}$ of IDF)	Bile Acid Binding Relative to Cholestyramine (%)
MWB	3.71 \pm 0.10b	45.1 \pm 1.2b
MEB-120	3.10 \pm 0.09de	37.7 \pm 1.0de
MEB-177	3.48 \pm 0.12c	42.3 \pm 1.4c
MEB-234	3.21 \pm 0.07d	39.1 \pm 0.8d
MEB-291	2.97 \pm 0.04e	36.1 \pm 0.5e
MEB-358	3.55 \pm 0.05bc	43.2 \pm 0.6bc
Cholestyramine	10.23 \pm 0.06a	100 \pm 0.6a
Cellulose	0.06 \pm 0.02f	0.8 \pm 0.2f

^a Values followed by the same letter in the same column are not significantly different ($P \leq 0.05$); $n = 6$.

^b Insoluble dietary fiber used for incubation for all the bran samples was 45–50 mg, cholestyramine 25 mg, and cellulose 25 mg.

^c Wheat bran (WB) extruded at specific mechanical energy of 120, 177, 234, 291, 358 Whr/kg on a dry weight basis.

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