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Abstract
Extruded breakfast cereals (EBC), processed from two oat lines, N979-5-2-4 (N979) and "Jim", with β-glucan concentrations of 8.7 and 4.9%, respectively, were used to determine the impact of dry solids (DS) and bile acid (BA) concentrations on in vitro BA binding efficiency. A full fractional factorial design with levels for BA concentrations of 0.20, 0.47, 0.95, 2.37, and 4.73 μmol/g of total EBC slurry and for DS in the slurries of 0.8, 2, 3, and 4% (w/w) was selected. The absolute amount of BA bound (μmol) was measured for each trial in the experiment design. The percentage (%) of BA bound based on the total amount of BA added and BA bound per gram of DS of the EBC (μmol/g) were also presented and discussed. N979 in vitro digestion slurries had greater BA binding (μmol) than Jim slurries at different DS and BA concentrations, with greater differences at DS of 3% or above and at BA concentrations of 2.37 μmol/g or above. No difference in the absolute amount of BA bound (μmol) and percentage (%) BA bound occurred between the EBC slurries made from the two oat types at the lowest DS of 0.8% or the lowest BA concentration of 0.20 μmol/g. The efficiency of BA binding by β-glucan in these two EBC became more distinguishable at 3% DS or above and BA concentrations of 2.37 μmol/g or above, indicating that these two conditions can be employed to measure BA capacities for similar foods. Also, the β-glucan in the EBC produced from the N979 oat line was more soluble than that from the EBC produced from the Jim oat line. Thus, greater BA binding capacity may have been caused by both a greater amount of β-glucan and a greater solubility of β-glucan in N979 than in Jim EBC.

Keywords
β-Glucan, oats, bile acid, dry solids, cereals

Disciplines
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Impact of Dry Solids and Bile Acid Concentrations on Bile Acid Binding Capacity of Extruded Oat Cereals

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Extruded breakfast cereals (EBC), processed from two oat lines, N979-5-2-4 (N979) and "Jim", with β-glucan concentrations of 8.7 and 4.9%, respectively, were used to determine the impact of dry solids (DS) and bile acid (BA) concentrations on in vitro BA binding efficiency. A full fractional factorial design with levels for BA concentrations of 0.20, 0.47, 0.95, 2.37, and 4.73 μmol/g of total EBC slurry and for DS in the slurries of 0.8, 2, 3, and 4% (w/w) was selected. The absolute amount of BA bound (μmol) was measured for each trial in the experiment design. The percentage (%) of BA bound based on the total amount of BA added and BA bound per gram of DS of the EBC (μmol/g) were also presented and discussed. N979 in vitro digestion slurries had greater BA binding (μmol) than Jim slurries at different DS and BA concentrations, with greater differences at DS of 3% or above and at BA concentrations of 2.37 μmol/g or above. No difference in the absolute amount of BA bound (μmol) and percentage (%) BA bound occurred between the EBC slurries made from the two oat types at the lowest DS of 0.8% or the lowest BA concentration of 0.20 μmol/g. The efficiency of BA binding by β-glucan in these two EBC became more distinguishable at 3% DS or above and BA concentrations of 2.37 μmol/g or above, indicating that these two conditions can be employed to measure BA capacities for similar foods. Also, the β-glucan in the EBC produced from the N979 oat line was more soluble than that from the EBC produced from the Jim oat line. Thus, greater BA binding capacity may have been caused by both a greater amount of β-glucan and a greater solubility of β-glucan in N979 than in Jim EBC.

KEYWORDS: **β-Glucan; oats; bile acid; dry solids; cereals**

INTRODUCTION

There is good evidence that the consumption of products made with oat and barley may lead to lower cholesterol levels (*1*−*5*). Hypocholesterolemic effects of oat bran have been attributed to the main soluble fiber components of oats, (1→3)(1→4)-β-D-glucan, as confirmed by numerous clinical studies after the consumption of oats or oat bran (*6*−*13*). Oats provide a palatable source of viscous fiber, a dietary component greatly lacking in Western diets.

Investigations into the binding of bile acids (BA) by β-glucan have sought answers regarding the mechanism for the hypocholesterolemic effects of oats. Both attenuation of blood glucose and insulin levels and a hypocholesterolemic effect were related to viscosity of the bolus when evaluated in rats and in in vitro studies (*14*−*17*) as the viscosity in the terminal ileum can affect the degree of BA loss (*18*). Aqueous extracts or extracts with lower viscosities were less effective in lowering cholesterol than were extracts with higher viscosities (*19*). Increased gut viscosity may contribute to a hypocholesterolemic effect by three separate actions: (i) impede uptake of dietary cholesterol, (ii) inhibit BA reabsorption, and (iii) physically bind BA to the fiber.

BA is formed in the liver from cholesterol following enzymatic reactions in the mitochondria and microsomes of the hepatocytes (*20*). The function of BA lies mainly in their detergent action, which leads to dispersion, solubilization, and absorption of fats and fat-soluble vitamins in the small intestine (*21*). Removing BA from recirculation stimulates the conversion of serum and liver cholesterol to form additional BA, with a concomitant drop in serum and liver cholesterol (*18, 22*). The binding of BA to dietary fiber has been evaluated by many researchers (*21, 23*). Two mechanisms have been proposed for BA binding to dietary fiber. One theory suggests that BA is chemically bound to the fiber, and the other suggests that BA

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may be physically trapped in a β-glucan mesh within the fecal milieu. Chemical binding may involve interactions between some polysaccharides and dyes that have steric requirements (*24*). Physical trapping includes binding caused by hydrophilic or hydrophobic interactions or entrapment of a bile salt in a gel matrix. Heddleson et al. (*25*) proposed molecular modeling depicting native oat β-glucan binding with taurocholate by entrapping the BA in the β-glucan metrix. Studies conducted in vitro generally used BA concentrations well above those required for the formation of multimolecular detergent aggregates, referred to as micelles (*26, 27*). Also, BA concentrations and dry solids (DS) concentrations of oat slurries studied in vivo or in vitro varied greatly among researchers (*20*−*23, 26, 27*), making it difficult to compare studies evaluating BA binding effects of food containing dietary fibers, including β-glucan.

The current study was undertaken to understand the impact of varying the conditions for BA binding tests on the actual data. Furthermore, we are interested in proposing standard conditions for this type of test so studies could be compared to each other. Indeed, the in vitro effects of BA binding efficiency were evaluated at different concentrations of BA and different concentrations of DS. In our case, the DS were ready-to-eat extruded breakfast cereal (EBC) made from oats with high and typical levels of β-glucan. EBC was chosen because it is a popular form of oat food used to deliver β-glucan to consumers and contributes to almost one-third of U.S. whole-grain consumption (*28*).

MATERIALS AND METHODS

Oat EBC Preparation. One experimental oat line, N979-5-2-4 (N979), developed at Iowa State University, and one publicly available cultivar, "Jim", developed at the University of Minnesota, were grown in 2005 at the Agronomy and Agricultural Engineering Field Research Center near Ames, Iowa. The groats had β-glucan concentrations of 8.7% for N979 and 4.9% for Jim. The crops were harvested in August 2005, and the oats were dried and stored in plastic bags at 4 °C with a relative humidity of 40−50% until being processed. Oats were shipped to the Quaker Oats Inc. pilot plant located at Cedar Rapids, Iowa, for milling (*29*), where the hulls of the oats were removed by use of a Buhler Aspirator. The groats were steamed for 1 min at 80 °C and rolled to a flake thickness of 0.61 mm. The flakes were then ground into flour with a Hammer Mill through a 0.56 mm screen.

The formula (db) of directly expanded EBC included 60% whole oat flour, 35.75% wheat starch (Midsol 50, MGP Ingredient Inc. Atchison, KS), 3% sugar, 0.25% salt, and 1% sodium bicarbonate. The oat-based formulation was mixed immediately before extrusion using a Kitchen Aid mixer (Kitchen Aid, St. Joseph, MI) at the lowest speed and then further mixed at speed 2 for 5 min. The moisture content of the formulation was adjusted to 18% (db) during mixing as previously suggested (*29*). An amount of 1 kg of ingredients was mixed at one time to ensure even distribution of water among the ingredients. The EBC was produced at the Department of Grain Science and Industry, Kansas State University, on a laboratory scale, self-wiping, corotating, twin-screw extruder (American Leistritz Extruder Co., Somerville, NJ, model Micro-18, 18 mm barrel diameter and 30:1 length/diameter ratio). The extruder barrel was composed of six programmable temperature zones, which were electrically heated and cooled by water circulation. The feed rate was set at 39.5 g/min with an extrusion shaft speed of 370 rpm. Temperatures of six barrel zones were increased incrementally, starting at 30 °C for the first zone, to a final extrusion temperature of 180 °C near the die exit. The EBC was collected as a continuous strand after torque, barrel temperatures, and die pressure reached a steady state. EBCs were cut manually into lengths of 15 cm and dried for 20 min in a laboratory oven (Thelco 160DM, Precision Scientific, Chicago, IL) at 100 °C. The final product was sealed in plastic bags until needed for analysis.

EBCs were ground on an ultracentrifugal mill (ZM-1, Retch GmbH&Co. Haan, Germany) fitted with a 0.5 mm sieve for further analysis. The moisture content of ground EBC was determined by using a Moisture Analyzer (MB45, Ohaus Corp., Pine Brook, NJ). The total β-glucan (T-βGlu) concentration in the EBC was determined enzymatically by using AACC Method 32-23 (*30*), with the mixed β-glucan linkage kit from Megazyme (Megazyme Ltd. Co., Wicklow, Ireland). The starch content in flour and in the extracts was analyzed by AACC Method 76-13 (*30*), by using a Total Starch Kit from Megazyme. Lipids were analyzed by the gravimetric method after extraction with petroleum ether on a Soxhlet system (AACC Method 30-25) (*30*). Protein in the EBC was determined by using an automatic nitrogen analyzer (elementar, Analyzensysteme GmbH, Germany) and utilizing a protein conversion factor of 6.25. Dietary fiber analyses, including insoluble dietary fiber (IDF) and total dietary fiber (TDF), followed AACC Methods 32-21 and 32-05 (*30*), with the use of a Total Dietary Fiber Kit (TDF 100, Sigma-Aldrich Co., St. Louis, MO). The ash content was determined according to AACC Method 08-01 (*30*).

Insoluble β-glucan (I-βGlu) was separated from water-soluble β-glucan according the method of Åman and Graham (*31*) with modifications and then quantified by using the mixed β-glucan linkage kit from Megazyme. Water-soluble β-glucan was removed from ground EBC (200 ± 20 mg) by extracting with water (12 mL) in bottles arranged horizontally and aligned with the linear motion of the water bath (shake speed of 200 strokes/min) for 3 h at 47 °C as suggested by Skendi (*32*). The content was then centrifuged (1500*g*, 5 min). The supernatant was decanted, and the pellets were resuspended in 12 mL of water for 1 h and then centrifuged. The resuspension step was repeated. The I-βGlu concentration in the residual was analyzed by using the Megazyme Kit. All analyses were done in duplicate, averaged, and reported on a dry basis.

In Vitro Digestion and BA Binding Procedures. An in vitro digestion and a BA binding procedure were conducted by using a method suggested by Beer et al. (*33*) with modifications allowing viscosity and BA binding to be measured from the same sample preparation (**Figure 1**). The effectiveness of enzymes, including human salivary α-amylase, pepsin, and pancreatin had been confirmed in a preliminary study carried out in our laboratory or by previous researchers (*34*). The amount of ground EBC used was based on the desired DS concentration determined by a full fractional factorial design to achieve a final weight of 28 g after adding BA. The BA mixture, containing sodium cholate, sodium deoxycholate, sodium glycocholate, and sodium taurocholate (Sigma-Aldrich Co.), was prepared with proportions as 35, 35, 15, and 15% (w/w), respectively (*35*). Unbound BA in the supernatant after filtering through Whatman #4 filter paper was analyzed by using a BA Diagnostic Kit with appropriate dilutions (Trinity Biotech plc, Bray Co., Wicklow, Ireland).

BA reagents A [containing nicotinamide adenine dinucleotide (NAD), nitro blue tetrazolium salt (NBT), and diaphorase] and B [containing 3α-hydroxysteroid dehydrogenase (3α-HSD)] in the Diagnostic Kit were prepared following the instructions. The test reagent was a mixture of A and B (A:B = 4:1). The blank reagent was a mixture of A and deionized water (A:water = 4:1). After centrifugation, 0.2 mL of appropriately diluted supernatant was pipetted into test tubes. At timed intervals, 0.5 mL of test reagent and 0.5 mL of blank reagent were added to the corresponding tubes, mixed, and incubated at 37 °C for 5 min. The absorbance was read at 530 nm. The concentration of BA was calculated based on a standard curve developed from the BA at different concentrations. A quaternary ammonium anion exchange resin, cholestyramine (Sigma-Aldrich Co.), a component of a drug frequently used to treat high cholesterol, also was included for each set of analyses. Binding of BA was determined as the difference between the amount of BA added and that recovered in the centrifuge supernatant after in vitro digestion. BA binding was not affected by pH in the range of 6.40−8.71. When BA concentrations of 2.37 μmol/g or greater were used, a dilution factor of 10 was generally required for measurement. BA binding can be presented in three ways: (i) absolute amount of BA bound (μmol), (ii) percentage (%) BA bound based on the total amount of BA added, and (iii) BA bound per gram of DS of the EBC (μmol/g) with the total final weight of EBC slurry controlled at 28 g as explained in the experiment design section. The absolute amount of BA bound (μmol) was reported in this study, from which μmol/g BA bound and percentage (%) BA bound generally can be

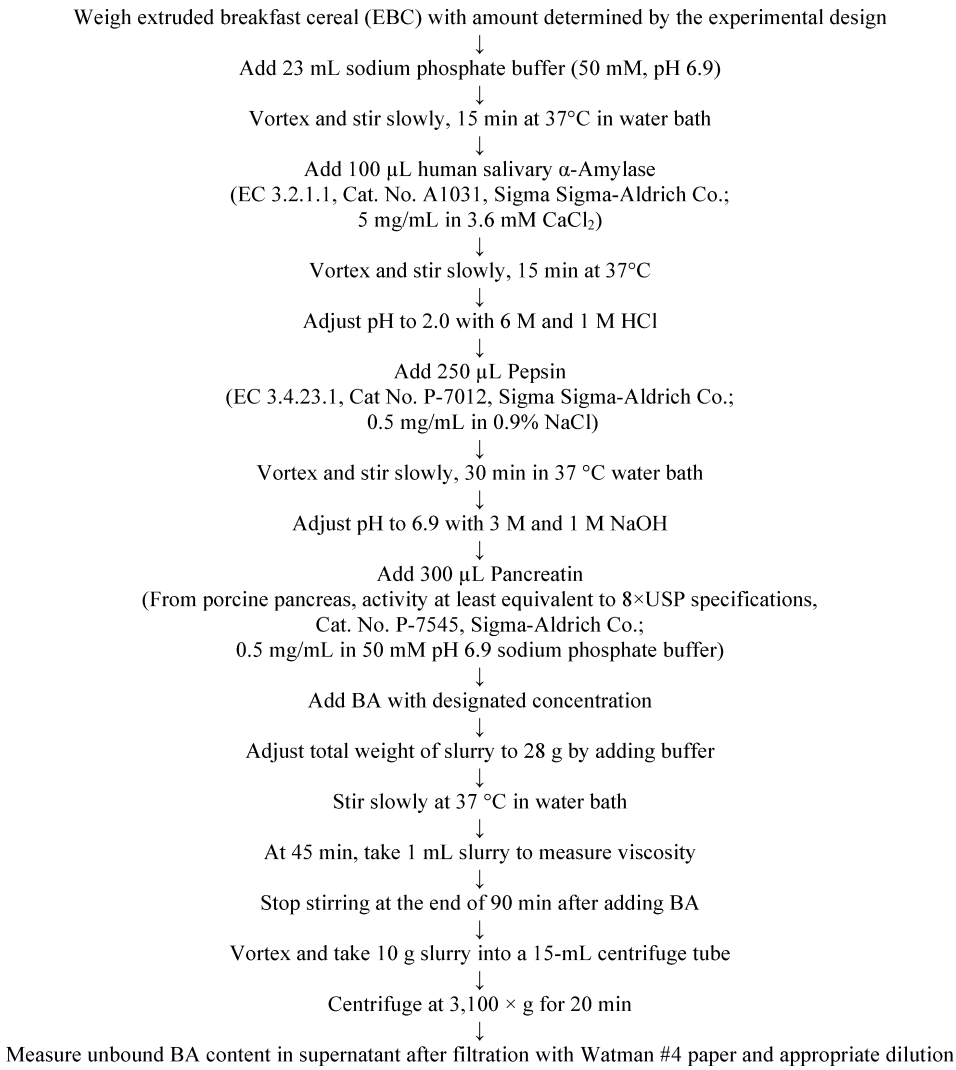

Figure 1. Flow chart of BA binding and viscosity measurements during in vitro digestion.

Table 1. Compositions of Two Groat Types and EBCs

	starch (%, db)[a]	protein (%, db)	lipid (%, db)	ash (%, db)	TDF (%, db)[b]	IDF (%, db)[b]	T-βGlu (%, db)[b]	I-βGlu (%, db)[b]
N979-groat	50.6 ± 0.08 d	17.6 ± 0.07 a	7.4 ± 0.00 a	2.5 ± 0.07 a	17.8 ± 0.41 a	7.4 ± 0.36 a	8.7 ± 0.52 a	0.9 ± 0.07 a
N979-EBC	60.9 ± 0.03 b	10.6 ± 0.04 c	4.4 ± 0.02 b	1.5 ± 0.04 c	14.7 ± 0.71 b	4.4 ± 0.22 bc	4.9 ± 0.04 b	0.6 ± 0.03 b
Jim-groat	57.8 ± 0.14 c	15.8 ± 0.17 b	7.8 ± 0.62 a	2.1 ± 0.08 b	14.7 ± 0.06 b	5.1 ± 0.38 b	4.9 ± 0.34 b	0.6 ± 0.03 b
Jim-EBC	69.2 ± 0.66 a	9.5 ± 0.10 d	4.7 ± 0.37 b	1.3 ± 0.05 d	12.6 ± 0.83 c	3.6 ± 0.17 c	3.1 ± 0.07 c	0.5 ± 0.08 b

[a] Values within a column followed by a common letter are not significantly different ($P > 0.05$). [b] TDF, total dietary fiber; IDF, insoluble dietary fiber; T-βGlu, total β-glucan; I-βGlu, insoluble β-glucan. All reported as a percentage of whole groat or EBC on a dry weight basis.

calculated. In some figures, where average values are presented, one cannot directly calculate these values; thus, the additional values also are provided.

Viscosity Determination. After BA was added, the slurry was incubated in a 37 °C water bath for 45 min before measuring the viscosity with a Brookfield spindle type rotational and programmable viscometer, model DV-II+ with a CPE-42 spindle and CPE-44PY cup (Brookfield Engineering Laboratories Inc., Stoughton, MA). A 1 mL portion of slurry was removed for the viscosity measurement according to instructions for operation of the instrument, and the remaining slurry was put back into the water bath immediately to continue the incubation. The viscosity was measured at various rpm. Although data were valid with torques >10%, all readings were reported at the maximum rpm after stabilization at 37 °C (*36*). The shear rate equaled 3.8 × rpm (*36*).

Statistical Experimental Design and Analysis. A full fractional factorial design with two replications for each level chosen for BA concentration and DS was designated, and the data were analyzed by Minitab v14 (Minitab Inc., State College, PA). The BA concentrations employed were 0.20, 0.47, 0.95, 2.37, and 4.73 μmol/g of total slurry of EBC. The DS levels of EBC were 0.8, 2, 3, and 4% (w/w). Actual amounts of added BA and weights of EBC were calculated based on 28 g as the final weight. Comparisons of means were conducted by least significant difference (LSD) at $\alpha = 0.05$ by using SAS 9.1, where analysis of variance (ANOVA) analysis found the responses significantly different ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Composition and Solubility of β-Glucan in Groats and EBC. As shown in **Table 1**, N979 groats and EBC had greater protein, β-glucan, ash, and TDF but lower starch concentrations than their counterparts of Jim. The lipid content of these two groats or EBC was not different. N979 groats had a higher IDF concentration than Jim groats. The IDF of N979 EBC was slightly higher than that of Jim but not significant.

N979 groats and EBC had a greater I-βGlu concentration than did Jim groats and EBC. However, the percentages of I-βGlu

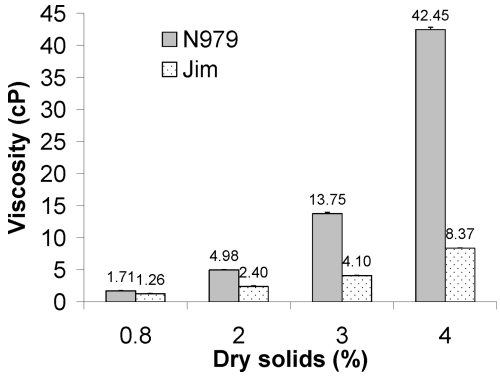

Figure 2. Viscosity of EBC made from two groat types varying in T-βGlu concentration and measured at different percentages of DS.

within the T-βGlu in the groats and EBC, calculated from the data in **Table 1**, were 10.1 and 12.2% for N979 and Jim groats and 12.2 and 16.1% for N979 and Jim EBC, respectively. Thus, the β-glucan from N979 groats or EBC was more soluble than from Jim groats or EBC. The solubility of the β-glucan from both sources of groats and EBC was above 80%, similar to values obtained by Åman and Graham (*31*) who used water as the extraction fluid. These values are greater than the 70% solubility obtained by using acid buffer (pH 1.5) or mild reagents (*37*−*39*). The greater proportion of soluble β-glucan in groats and EBC from N979 than from Jim may arise from structural differences, such as ratios of contiguous cellotriosyl residual blocks separated by β-(1−3)-linkages or in molecular conformation in the two oat types (*40, 41*). Long blocks of adjacent β-(1−4)-linkages in the β-glucan molecule are thought to contribute to insolubilization (*42, 43*). The high solubility of β-glucan in these two EBC is not likely caused by β-glucanase, because previous research in our laboratory confirmed very low β-glucanase activity in these ungerminated oats (*44*).

Similarly, the calculated ratio of I-βGlu to T-βGlu of EBC was greater than that in the original groats. The differences in solubility before and after extrusion may result from changes in β-glucan solubility caused by the extrusion process. However, the ratio of IDF to TDF was higher in groats than in EBC, which meant that extrusion can enhance the solubility of TDF. This was consistent with the findings of Gualberto et al. (*45*) who also reported an increase of soluble dietary fiber in oat bran after extrusion. The lower solubility of β-glucan after extrusion could be attributed to either the method used to define I-βGlu and IDF or the unique characteristics of β-glucan. The latter possibility suggests that β-glucan may tend to combine with other components during high shear and high temperature extrusion, thus becoming insoluble.

Viscosity of EBC Slurries during in Vitro Digestion. Slurries made from N979 EBC had greater viscosity than slurries made from Jim EBC at all four DS (**Figure 2**). The viscosity of slurries with various BA concentrations but with the same DS was not different. Differences between viscosity of N979 and Jim EBC slurries became greater at greater DS, the cause likely being greater β-glucan concentrations in N979 EBC, as starch and proteins had been hydrolyzed by enzymes during in vitro digestion, thus having very limited contribution to viscosity. Viscosity is reported as an exponential function of the concentration of dissolved β-glucan (*46*). In previous findings from our laboratory, the peak molecular weight of β-glucan of these oat types plus two more types positively correlated with slurry viscosity ($r = 0.78$, $P < 0.01$) (*47*).

Shear thinning of the N979 EBC slurry was noticeable at concentrations of 3% DS and above. At 3% DS, there was about 0.14% β-glucan as calculated from the T-βGlu of N979 EBC. The viscosity remained constant when the shear rate increased at lower DS (data not shown). The Jim EBC slurry exhibited slight shear thinning at 4% DS, with about 0.12% β-glucan present, but was identified as a Newtonian fluid at lower DS. When the shear rate increased, N979 slurry viscosity decreased but fully recovered when the shear rate was again decreased (data not shown). The consideration of viscosity is important because it impacts the hypocholesterolemic effect of a food. A high viscosity of the contents in the gastrointestinal (GI) tract could result in a reduced diffusion rate leading to a reduction of the absorption of cholesterol and BA (*48*). Our results showed the occurrence of shear thinning only when β-glucan was above 0.12−0.14% in solution. This finding is fairly consistent with data reported elsewhere, where oat gums showed non-Newtonian shear-thinning behavior above a 0.2% concentration (*49*). Our results also indicated that the Newtonian fluids of the two EBC also had some binding capacity but at a lower level than non-Newtonian EBC fluids obtained at higher DS. Previous researchers reported that greater viscosity of intestinal contents was strongly associated with cholesterol reduction (*50*).

No meaningful correlations between viscosity and BA bound per gram of DS of the EBC (μmol/g) were observed when only the BA concentration was varied (data not shown). The R^2 of the correlation between amount of BA bound (μmol) and viscosity increased from 0.68 for the BA concentration of 0.2 μmol/g slurry to 0.78 for the BA concentration of 2.37 μmol/g and 0.75 for 4.73 μmol/g of BA concentration (**Figure 3**). The relationship between % BA bound and viscosity at different BA concentrations was similar to that of amount of BA bound (μmol) and viscosity.

Effect of DS. ANOVA, as related to the experimental design, indicated that DS, BA concentration, and oat type had significant main effects and interactions on BA binding (**Table 2**). The coefficient of determination value (R^2) of the regression model for BA bound (μmol) by EBC slurries was close to 100% (**Table 2**). The results reported in **Figure 4** are the average of BA bound (μmol) from different BA concentrations at each DS level. The BA bound per gram of DS of EBC (μmol/g) can be calculated from the amount of BA bound (μmol) and the DS level indicated in **Figure 4**.

The BA bound (μmol) by N979 EBC slurries gradually increased when DS increased from 0.8 to 4% (**Figure 4**). On the other hand, the amount of BA bound (μmol) of Jim slurries had the greatest binding at 3% and then decreased at 4%. N979 slurries had greater amounts of BA bound (μmol) than Jim EBC slurries at all DS with the difference being greatest at 3% DS or above.

For % BA bound, the binding of N979 and Jim EBC slurries increased proportionally with greater DS (**Figure 4**). N979 EBC also had greater % BA bound than Jim EBC, with the difference being greater at DS of 3% or above. DS had a greater effect on % BA bound of N979 slurries than Jim slurries. The % BA bound of N979 EBC increased from 20% at 0.8% DS to 60% at 4% DS, whereas that of Jim EBC increased from 14% at 0.8% DS to only 34% at 4% DS. The difference could be explained by the greater amount of β-glucan in N979 than in Jim groats.

BA bound per gram of DS of EBC (μmol/g) for N979 remained unchanged when DS increased, whereas that of Jim EBC was the greatest at DS of 2% and then decreased at greater DS. N979 EBC had greater BA bound per gram of DS (μmol/g) than Jim EBC at all DS, including at 0.8% when both N979 and Jim EBC slurries had similar viscosities. The viscosity of EBC slurries with

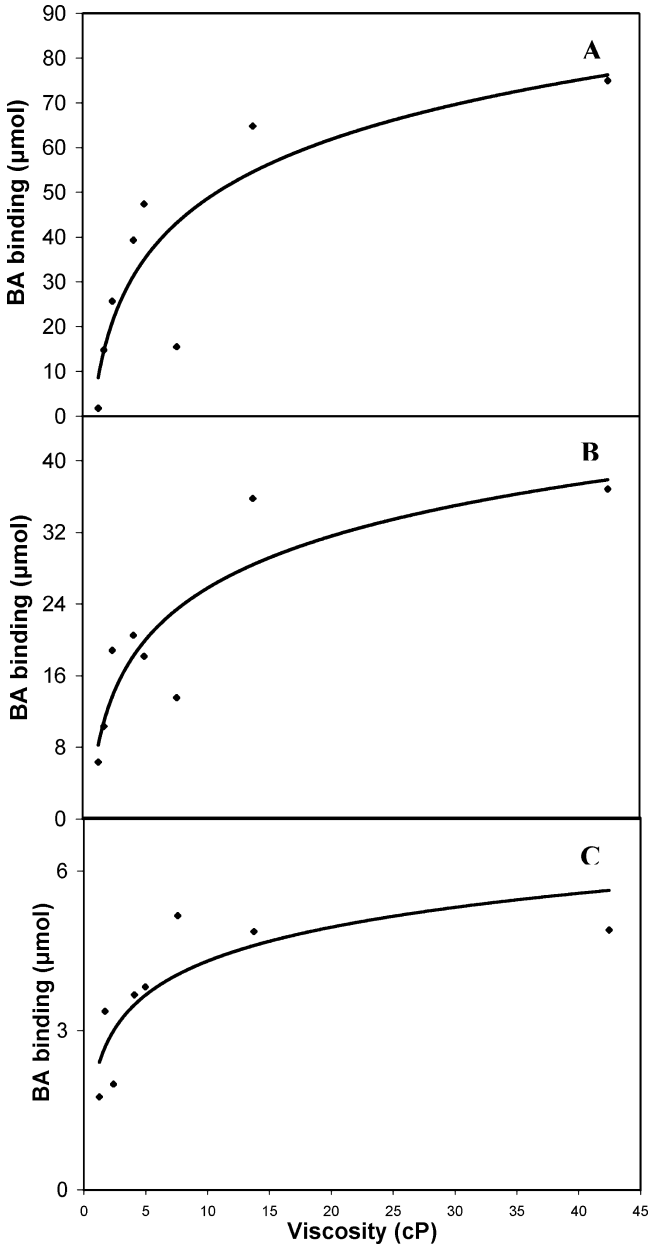

Figure 3. BA binding (μmol) of EBC slurries at different viscosities and BA concentrations. BA concentrations were as follows: (**A**) 4.73 μmol/g, (**B**) 2.37 μmol/g, and (**C**) = 0.2 μmol/g.

Table 2. Analysis of Variance for BA Binding as Related to the Experimental Design

source	DF[a]	mean squares[b] BA binding (μmol)
oat genotype (OG)	1	1674
DS (%)	3	700
BA concentration (μmol/g)	4	3183
OG × DS	3	249
OG × BA concentration	4	645
DS × BA concentration	12	237
OG × DS × BA concentration	12	79
error	40	2
R^2		99.7%

[a] Degrees of freedom. [b] Analysis of variance results are all significant ($P < 0.001$).

various DS differed greatly, thus suggesting that factors other than viscosity affected BA binding. Indeed, lignin, an insoluble fiber in oat, affected BA binding during previous research conducted in our laboratory and might be a factor here (*51*).

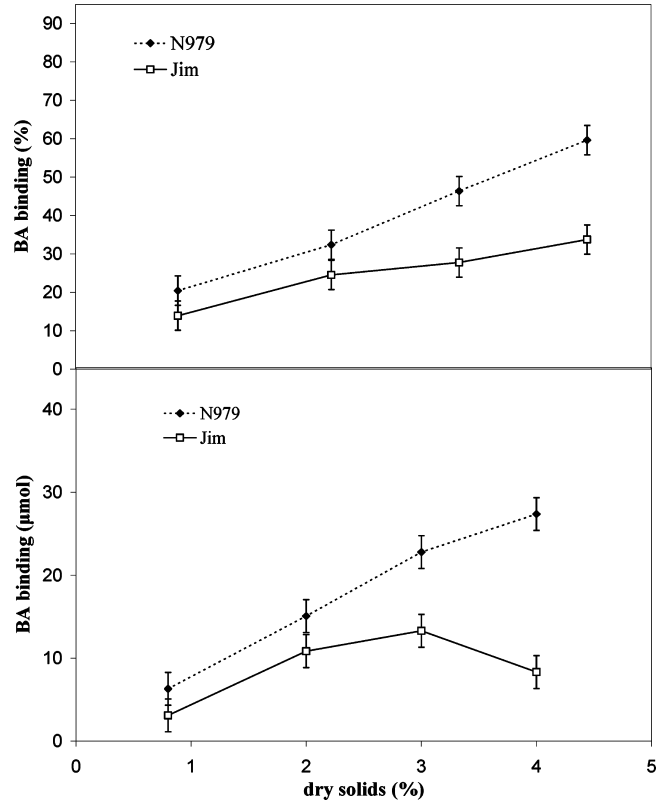

Figure 4. BA binding of EBC made from two groat types varying in β-glucan concentration, Jim and N979, at different DS measurements. Error bars represented the standard deviation among various BA concentrations at each DS level of Jim and N979 groat types.

For both oat types, when DS in the digestion slurries were greater than 2%, greater amounts of β-glucan were precipitated after digestion with BA. These findings suggested that BA was either entrapped in the high viscous matrix as suggested by Heddleson et al. (*25*) or precipitated along with β-glucan. The properties of the binding, either hydrophilic or hydrophobic, between BA and β-glucan are unknown. Congo red and Calcofluor can specifically bind β-glucan, and these components have been used to detect the presence of β-glucan (*24*). The conjugated BA is hydrophilic and is absorbed through an active transport mechanism, whereas the deconjugated lipophilic BA is readily absorbed by passive diffusion (*20*). Previous experiments have indicated that more than 95% of the BA reaching the terminal ileum is reabsorbed from the mixture present in the gut lumen (*20*). Thus, it makes sense that removing BA directly from circulation or reducing the reabsorption of BA, either by forming a complex with β-glucan or entrapping BA in the viscous matrix, would have health benefits. However, the mechanism needs further investigation.

Although the actual BA binding process for any given type of fiber is very complex and cannot be attributed to a single mechanism, our results indicated that viscosity and DS present in the digested slurry play an important role in BA binding efficiency. Considering that some EBC slurries with DS of 4% were more viscous than optimum for BA binding at human digestion conditions, whereas all EBC with 3% DS still greatly impacted BA binding, 3% DS of EBC is recommended for future analysis of BA binding capacity for ground oat EBC. Clearly, however, DS for other types of materials, including uncooked oat products, will need to be optimized to provide appropriate viscosities. In humans, a significant reduction of blood cholesterol can be achieved with a daily dose corresponding to 1.3−8 g of β-glucan (*52*). However, our study indicated

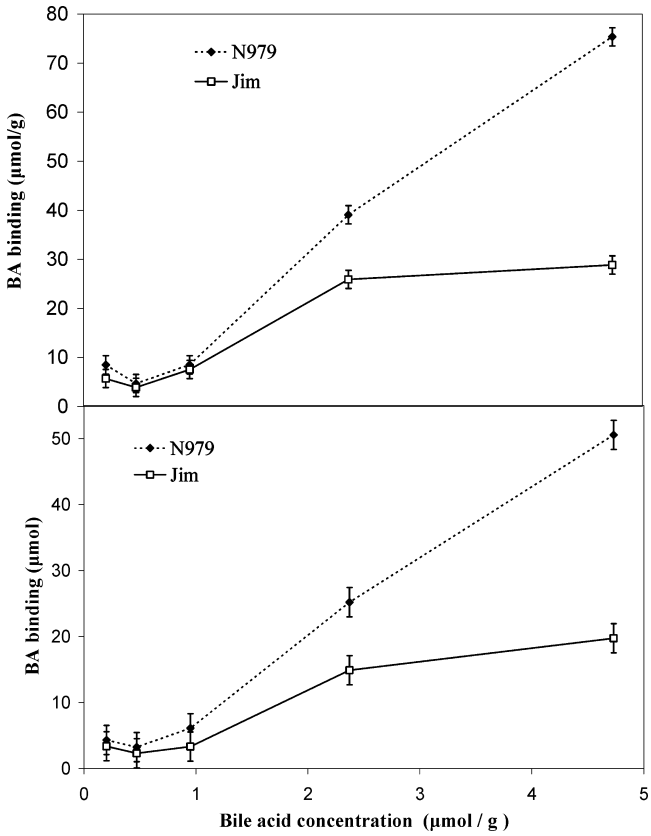

Figure 5. BA binding of EBC made from two groat types varying in T-βGlu concentration, Jim and N979, at different BA concentrations. Error bars represented the standard deviation among various DS levels at each BA concentration for Jim and N979 groat types.

the importance of considering not only the absolute amount of β-glucan intake but also the viscosity caused by the β-glucan in the food consumed, when evaluating the impact on BA binding.

Effect of BA Concentration. The BA concentration also interacted with genotype to impact BA binding (**Table 2**). The results reported in the **Figure 5** are the average of BA bound (μmol) from different DS levels at each BA concentration. % BA bound at each BA concentration level can be calculated from BA bound (μmol).

The amount of BA bound (μmol) of slurries from both oat types increased with BA concentrations greater than 0.95 μmol/g (**Figure 5**). The amounts of BA bound (μmol) of N979 and Jim EBC were not different from each other at the lower BA concentrations of 0.20, 0.47, and 0.95 μmol/g slurry. However, the amount of BA bound (μmol) of N979 EBC was greater than for Jim EBC at BA concentrations of 2.37 and 4.73 μmol/g. The amount of BA bound (μmol) of N979 EBC increased from 4.4 μmol at 0.2 μmol/g BA concentration to 50 μmol at 4.73 μmol/g, whereas that of Jim EBC increased only from 3.4 to 20 μmol at these two BA concentrations.

Similar to the results for the absolute amount of BA bound (μmol), % BA bound of N979 EBC slurries was also greater than that of Jim EBC slurries. Both EBC bound the greatest % BA when the BA concentration was the least, whereas the absolute amount of BA bound (μmol) was also the least for both EBC slurries at this BA concentration. Thus, without indicating the concentration of BA applied, simply comparing % BA binding from different research studies could lead to invalid conclusions.

The amount of BA bound per g DS (μmol/g) of both EBC types increased with an increase in BA concentration, similar

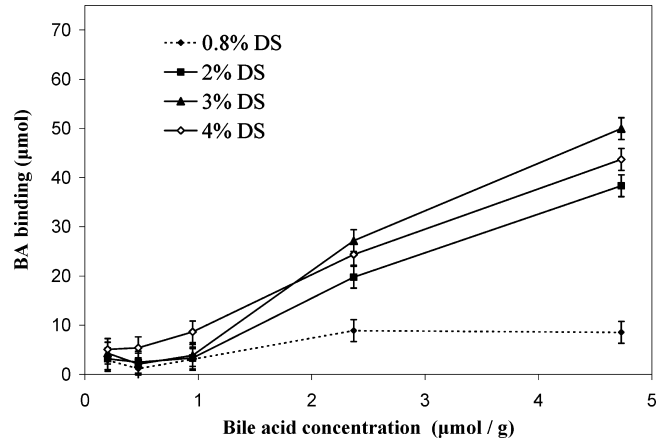

Figure 6. BA binding of EBC made from two groat types varying in β-glucan concentration, Jim and N979, with various DS and BA concentrations. Lines represent the mean of both groat types. Error bars represent the standard deviation between two groat types.

to the findings for the absolute amount of BA bound (μmol) (**Figure 5**). The BAs bound per DS (μmol/g) of the N979 EBC slurries were not different from those of the Jim EBC slurries at lower BA concentrations of 0.2−0.95 μmol/g but were greater than those of the Jim EBC at the two higher BA concentrations. In the current study, the cholestyramine control bound 92.5% of BA when the BA concentration was 2.37 μmol/g, a value within the range of 80−100% reported by other researchers (*19*−*21*). BA binding by dietary fiber, including β-glucan, was not as efficient as binding by cholestyramine, which was affected very little by DS.

Vahouny et al. (*27*) reported that 5 mM bile salts, calculated to be about 4.96 μmol/g, is considered to be within the physiological range of what is found in the jejunum; thus, the BA concentrations used in the current study are relevant. Without specifying BA concentration and DS, it would be difficult to compare the effects of BA binding among different studies. On the basis of the findings in the current study, a value of 2.37 μmol/g was the most useful BA concentration for in vitro studies of BA binding of EBC, because it was close to the reported physiological range of about 4.96 μmol/g yet provided high binding for both EBC types. Values greater than 2.37 μmol/g gave lower values for the Jim EBC. Conducting the tests with a high BA concentration of 4.73 μmol/g would waste BA reagent, because a dilution of about 20 times is required for measurement of BA with the BA kit.

Interaction of BA Concentration and DS. The DS also interacted with BA concentration (**Table 2**) to affect BA binding. The amount of BA bound (μmol) in **Figure 6** was the average of N979 and Jim EBC at each DS and BA concentration level. The BA bound expressed by % and μmol/g for the average of these two EBC can be calculated from the results reported in **Figure 6**. The amount of BA bound (μmol) of EBC generally was greater at greater BA concentrations, except when the DS was 0.8%, at which point the amount of BA bound (μmol) remained at a consistently low level (**Figure 6**). There was no clear trend for the impact of BA concentration at DS greater than 3%, which confirmed our previous observation that DS greater than 3% would not necessarily result in greater amounts of BA bound (μmol).

The % BA bound was the least at a medium BA concentration of 0.95 μmol/g for all DS. The EBC bound the greatest % of BA when the BA concentration was the least. Furthermore, there was no difference in % BA bound when BA concentration was 2.37 μmol/g or greater.

The BA bound per g DS (μmol/g) also generally was greater at BA concentrations of 2.37 μmol/g or more, except for 0.8% DS, where no difference occurred between BA concentrations of 2.37 and 4.73 μmol/g. EBC slurries with 2 and 3% DS had bound more BA (μmol/g) than with 0.8% DS. These data again confirm our suggestion that a BA concentration of greater than 2.37 μmol/g was not necessary to detect differences in binding capacity among EBC types.

Conclusions. The β-glucan in the EBC produced from the N979 oat line (8.7% β-glucan) was more soluble than that from the EBC produced from the Jim oat line (4.9% β-glucan). Also, the former EBC bound significantly greater amounts of BA than the latter. Results from tests in which different DS and BA concentrations were evaluated showed that DS of 3% and a BA concentration of 2.37 μmol/g provided the best conditions for studying the effects of BA binding of EBC in vitro.

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LITERATURE CITED

(1) Anderson, J. W.; Chen, W. L. Cholesterol-lowering properties of oat products. In *Oats: Chemistry and Technology*; Webster, F. H., Ed.; Am. Assoc. Cereal Chem.: St. Paul, MN, 1986; pp 309−333.

(2) Gold, K. V.; Davidson, D. M. Oat bran as a cholesterol-reducing dietary adjunct in a young, healthy population. *West J. Med.* **1988**, *148*, 299–302.

(3) Newman, R. K.; Lewis, S. E.; Newman, C. W.; Boik, R. J.; Ramage, R. T. Hypocholesterolemic effect of barley foods on healthy men. *Nutr. Rep. Int.* **1989**, *39*, 749–760.

(4) Anderson, J. W.; Norman, H. G.; Deakins, D. A.; Smith, S. F.; O'Neal, D. S.; Dillon, D. W.; Oeltgen, P. R. Lipid response of hypercholesterolemic men to oat-bran and wheat-bran intake. *Am. J. Clin. Nutr.* **1991**, *54*, 678–683.

(5) Braaten, J. T.; Wood, P. J.; Scott, F. W.; Wolynetz, M. S.; Lowe, M. K.; Bradley-White, P.; Collins, M. W. Oat β-glucan reduces blood cholesterol concentration in hypercholesterolemic subjects. *Eur. J. Clin. Nutr.* **1994**, *48*, 465–474.

(6) Beer, M. U.; Wood, P. J.; Weisz, J. Molecular weight distribution and (1−3)(1−4)- β-D-glucan content of consecutive extracts of various oat and barley cultivars. *Cereal Chem.* **1997**, *74* (4), 476–480.

(7) Lund, E. K.; Gee, J. M.; Brown, J. C.; Wood, P. J.; Johnson, I. T. The effect of oat gum on the physical properties of the gastrointestinal contents and the uptake of D-galactose and cholesterol by rat small intestine in vitro. *Br. J. Nutr.* **1989**, *62*, 91–101.

(8) Davidson, M. H.; Dugan, L. D.; Burns, J. H.; Bova, J.; Story, K.; Drennan, K. B. The hypocholesterolemic effects of β-glucan in oatmeal and oat bran. *J. Am. Med. Assoc.* **1991**, *265*, 1833–1839.

(9) Judd, P. A.; Truswell, A. S. The effects of rolled oats on blood lipids and fecal steroid excretion in man. *Am. J. Clin. Nutr.* **1981**, *34*, 2061–2067.

(10) Keenan, J. M.; Wenz, J. B.; Myers, S.; Ripsin, C.; Huang, Z. Q. Randomized, controlled, crossover trial of oat bran in hypercholesterolemic subjects. *J. Fam. Pract.* **1991**, *33*, 600–608.

(11) Anderson, J. W.; Spencer, D.; Hamilton, C.; Smith, S.; Tietyen, J.; Bryant, C.; Oeltgen, P. Oat bran cereal lowers serum total and LDL cholesterol in hypercholesterolemic men. *Am. J. Clin. Nutr.* **1990**, *52*, 495–499.

(12) Kestin, M.; Moss, R.; Clifton, P. M.; Nestel, P. J. J. Comparative effects of three cereal grains in plasma lipids, blood pressure, and glucose metabolism in mildly hypercholesterolemic men. *Am. J. Clin. Nutr.* **1990**, *52*, 661–666.

(13) Kirby, R. W.; Anderson, J. W.; Sieling, B.; Rees, E. D.; Chen, W. L.; Milelr, R. E.; Kay, R. M. Oat-bran intake selectively lowers serum low-density lipoprotein cholesterol concentration in hypercholesterolemic men. *Am. J. Clin. Nutr.* **1981**, *34*, 824–829.

(14) Autio, K.; Myllymki, O.; Mlkki, Y. Flow properties of solutions of oat β-glucans. *J. Food Sci.* **1987**, *52*, 1364–1366.

(15) Anderson, J. W.; Bridges, S. R. Hypocholestrolemic effects of oat bran in humans. In *Oat Bran*; Wood, P. J., Ed.; Am.Assoc. Cereal Chem.: St. Paul, MN, 1993; pp 139−157.

(16) Wood, P. J.; Braaten, J. T.; Scott, F. W.; Riedel, D.; Wolynetz, M. S.; Collins, M. W. Effect of dose and modifications of viscous properties of oat gum on plasma glucose and insulin following an oral glucose load. *Br. J. Nutr.* **1994**, *72*, 731–743.

(17) Jenkins, D. J.; Thorne, M. J.; Wolever, T. M.; Jenkins, A. L.; Rao, A. V.; Thompson, L. U. The effect of starch-protein interaction in wheat on the glycemic response and rate of in vitro digestion. *Am. J. Clin. Nutr.* **1987**, *45*, 946–951.

(18) Jenkins, D. J. A.; Marchie, A.; Augustin, L. S. A.; Ros, E.; Kendall, C. W. C. Viscous dietary fibre and metabolic effects. *Clin. Nutr. Supl. J.* **2004**, *1* (2), 39–49.

(19) Doehlert, C. D.; Zhang, D.; McMullen, M. S.; Moore, W. R. Estimation of mixed linkage β-glucan concentration in oat and barley from viscosity of whole grain flour slurry. *Crop Sci.* **1997**, *37*, 235–238.

(20) Salter, J.; Chaplin, M.; Dickerson, J.; Davies, J. Bile acids and health: is fibre the answer. *Nutr. Food Sci.* **1996**, *6*, 29–33.

(21) Elhardallou, S. The bile acid binding of the fibre-rich fractions of three starch legumes. *Plant Foods Hum. Nutr.* **1992**, *42*, 207–218.

(22) Shinnick, F. L.; Marlet, J. A. Physiological responses to dietary oats in animal models. In *Oat Bran*; Wood, P. J., Ed.; American Association of Cereal Chemists: St. Paul, MN, 1993; pp 113−137.

(23) Eastwood, M. A.; Boyd, G. S. The distribution of bile salts along the small intestine of rats. *Biochim. Biophys. Acta* **1967**, *137*, 393–396.

(24) Wood, P. J.; Fulcher, R. G.; Stone, B. A. Studies on the specificity of interaction of cereal cell wall components with congo red and calcofluor. Specific detection and histochemistry of (1→3), (1→−4), -β-D-glucan. *J. Cereal Sci.* **1983**, *1*, 95–110.

(25) Heddleson, R.; Perdon, A.; Marston, B.; Hite, E.; Sung, S. Food products with improved bile acid binding funcationality and method for their preparation. United States Patent 20030147993, 2003.

(26) Story, J. A.; Kritchevsky, D. Comparison of the binding of various bile acids and bile salts in vitro by several types of fiber. *J. Nutr.* **1976**, *106*, 1292–1294.

(27) Vahouny, G. V.; Tombes, R.; Cassady, M. M.; Kritchevsky, D.; Gallo, L. Dietary fibers: V. Binding of bile salts, phospholipids and cholesterol from mixed micelles by bile acid sequestrants and dietary fibers. *Lipids* **1980**, *15*, 1012–1018.

(28) Cleveland, L.; Moshfegh, A.; Albertson, A.; Goldman, J. Dietary intake of whole grains. *J. Am. Coll. Nutr.* **2000**, *19* (3), 331S–338S.

(29) Yao, N.; Jannink, J.-L.; Alavi, S.; White, P. Physical and sensory characteristics of extruded products made from two oat lines with different β-glucan concentrations. *Cereal Chem.* **2006**, *83* (6), 692–699.

(30) AACC International. *Approved Methods of the AACC*, 10th ed.; Methods 08-01, 32-23, 76-13, 30-25, 32-21, 32-05; The Association: St. Paul, MN, 2000.

(31) Åman, P.; Graham, H. Analysis of total and insoluble mixed-linked (1→3) (1→4)-β-D-glucans in barley and oats. *J. Agric. Food Chem.* **1987**, *35*, 704–709.

(32) Skendi, A.; Biliaderis, C. G.; Lazaridou, A.; Izydorczyk, M. S. Structure and rheological properties of water soluble glucans from oat cultivars of *Avena sativa* and *Avena bysantina*. *J. Cereal Sci.* **2003**, *38*, 15–31.

(33) Beer, M. U.; Wood, P. J.; Weisz, J.; Fillion, N. Effect of cooking and storage on the amount and molecular weight of (1−3)(1−4)-β-D-glucan extracted from oat products by an in vitro digestion system. *Cereal Chem.* **1997**, *74* (6), 705–709.

(34) Asp, N. G.; Johansson, C. G.; Hallmer, H.; Siljestrom, M. Rapid enzymatic assay of insoluble and soluble dietary fiber. *J. Agric. Food Chem.* **1983**, *31*, 476–482.
(35) Sayar, S.; Jannink, J.-L.; White, P. J. In vitro bile acid binding of flours from oat lines varying in percentage and molecular weight distribution of β-glucan. *J. Agric. Food Chem.* **2005**, *53*, 8797–8803.
(36) Anonymous. Operating Instructions (Manual No. M/97-164) for Brookfield DV-II+ Programmable Viscometer; Brookfield Engineering Laboratories, Inc.: Stoughton, MA. 2005.
(37) Bhattty, R. S. Total and extractable β-glucan contents of oats and their relationship to viscosity. *J. Cereal Sci.* **1992**, *15*, 185–192.
(38) McCleary, B. V.; Glennie-Holmes, M. Enzymatic quantification of (1→3) (1→4) β-D-glucan in barley and malt. *J. Inst. Brew.* **1985**, *91*, 285–295.
(39) Bhatty, R. S.; MacGregor, A. W.; Rossnagel, B. G. Total and acid-soluble beta-glucan content of hulless barley and its relationship to acid-extract viscosity. *Cereal Chem.* **1991**, *68*, 221–227.
(40) Izawa, M.; Kano, Y.; Koshino, S. Relationship between structure and solubility of (1→3), (1→4)-β-D-glucan from barley. *ASBC J.* **1993**, *51* (3), 123–127.
(41) Westerlund, E.; Andersson, R.; Aman, P. Isolation and chemical characterization of water-soluble mixed-linked β-glucans and arabinoxylans in oat milling fractions. *Carbohydr. Polym.* **1993**, *20* (2), 115–123.
(42) Igarashi, H.; Amaha, M. Studies on frozen beer precipitates II. Chemical structure of the β-glucan isolated from the precipitates. *J. Inst. Brew.* **1969**, *75*, 292–299.
(43) Woodward, J. R.; Fincher, G. B.; Stone, B. A. Water-soluble (1→3), (1→4)-β-D-glucan from barley (*Hordeum vulgare*) endosperm. II. Fine structure. *Carbohydr. Polym.* **1983**, *3*, 207–225.
(44) Colleoni-Sirghie, M.; Jannink, J.; Kovalenko, I. V.; Briggs, J. L.; White, P. J. Prediction of β-glucan concentration based on viscosity evaluations of raw oat flours from high β-glucan and traditional oat lines. *Cereal Chem.* **2004**, *81* (4), 434–443.
(45) Gualberto, D. G.; Bergman, C. J.; Kazemzadeh, M.; Weber, C. W. Effect of extrusion processing on the soluble and insoluble fiber, and phytic acid contents of cereal brans. *Plant Foods Hum. Nutr.* **1997**, *51* (3), 187–198.
(46) Varum, K. M.; Smidsrød, O. Partial chemical and physical characterisation of (1→3)(1→4)-β-glucans from oat (*Avena sativa* L.) aleurone. *Carbohydr. Polym.* **1988**, *9*, 103–117.
(47) Yao, N.; Jannink, J.-L.; White, P. Molecular weight distribution of (1−3)(1−4)-beta-glucan and pasting property of the flour from oat lines with high and typical amounts of beta-glucan. *Cereal Chem.* **2007**, *84* (5), 471–479.
(48) Asp, N. G. Effects of processing on dietary fiber. In *Dietary Fibers*; Amado, R., Schweizer, T. F., Eds.; Academic Press: New York, 1986; pp 177−189.
(49) Autio, K.; Myllymäki, O.; Suortti, T.; Saastamoinen, M.; Poutanen, K. Physical properties of (1→3), (1→4)-β-D-glucan preparates isolated from Finnish oat varieties. *Food Hydrocolloids* **1992**, *5* (6), 513–522.
(50) Gallaher, D. D.; Hassel, C. A.; Lee, K.-J.; Gallaher, C. M. Viscosity and fermentability as attributes of dietary fibre responsible for hypocholesterolemic effect in hamsters. *J. Nutr.* **1993**, *123*, 244–252.
(51) Sayar, S.; Jannink, J.-L.; White, P. J. In vitro bile acid binding activity within flour fractions from oat lines with typical and high β-glucan amounts. *J. Agric. Food Chem.* **2006**, *54* (14), 5142–5148.
(52) Mälkki, Y.; Autio, K.; Hänninen, O.; Mylltmäkki, O.; Pelkonen, K.; Suortti, T.; Törrönen, R. Oat bran concentrates: Physical properties of β-glucan and hypocholesterolemic effects in rats. *Cereal Chem.* **1992**, *69*, 647–653.

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