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## Abstract

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## Keywords

Agronomy,  $\beta$ -Glucan, oats, bile acid, molecular weight

## Disciplines

Agricultural Science | Agriculture | Agronomy and Crop Sciences | Food Chemistry

## Comments

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## In Vitro Bile Acid Binding of Flours from Oat Lines Varying in Percentage and Molecular Weight Distribution of $\beta$ -Glucan

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Two experimental high  $\beta$ -glucan oat (*Avena sativa*) lines (7.64 and 8.05%) and two traditional lines (4.77 and 5.26%  $\beta$ -glucan) were used to evaluate the effect of  $\beta$ -glucan quantity and molecular weight on bile acid (BA) binding. The oat flour samples were digested by an in vitro system that simulated human digestion. No significant differences among oat type were found in the overall  $\beta$ -glucan, starch, and pentosan digestibilities. Considering the standard, cholestyramine, as 100% bound, the relative BA binding for the oat flour samples on a dry matter basis was in the range of 7.5–14.8%, which is higher than the values determined for some other grains and plant materials in the literature. Although the high  $\beta$ -glucan flours bound a high amount of BA, no significant correlations were found between  $\beta$ -glucan content in the flours and BA binding. Significant correlations were found between BA binding and insoluble dietary fiber content. Partial hydrolysis with lichenase of the  $\beta$ -glucan molecules did not affect the BA binding. A summary of all data suggested that BA binding is a multicomponent-dependent process.

**KEYWORDS:**  $\beta$ -Glucan; oats; bile acid; molecular weight

### INTRODUCTION

Health benefits of oat (*Avena sativa*) products are well-recognized (1, 2). There is general agreement that oat products can lower serum cholesterol levels, and the (1 $\rightarrow$ 3),(1 $\rightarrow$ 4)- $\beta$ -D-glucan (hereafter referred to as  $\beta$ -glucan) is believed to be the active component (3). The cascade of events leading to the removal of cholesterol from the serum by  $\beta$ -glucan is briefly described. Cholesterol is removed from circulation by being converted to bile acids (BA) in the liver (4). The BAs make up part of the mixed micelles in the small intestine and are necessary for lipid digestion. Normally, BAs are nearly completely reabsorbed and transported to the liver via the enterohepatic circulation by active and passive mechanisms (5). Physical elimination of BAs from the enterohepatic circulation necessitates increased synthesis of BA, which in turn consumes cholesterol. This elimination can be accomplished by preventing BA reabsorption in the ileum, thus increasing BA excretion in the feces (4, 5). Simply stated, biliary excretion increases the synthesis of BA, and it is the major mechanism by which the body disposes of excess cholesterol (4).

Several dietary components are able to interact in the small intestine with BA (6, 7), resulting in a lower reabsorption rate of BA. Oat  $\beta$ -glucan is one of these components that may lower the blood cholesterol level in this manner when consumed in the daily diet, although its mechanism of interaction with BA

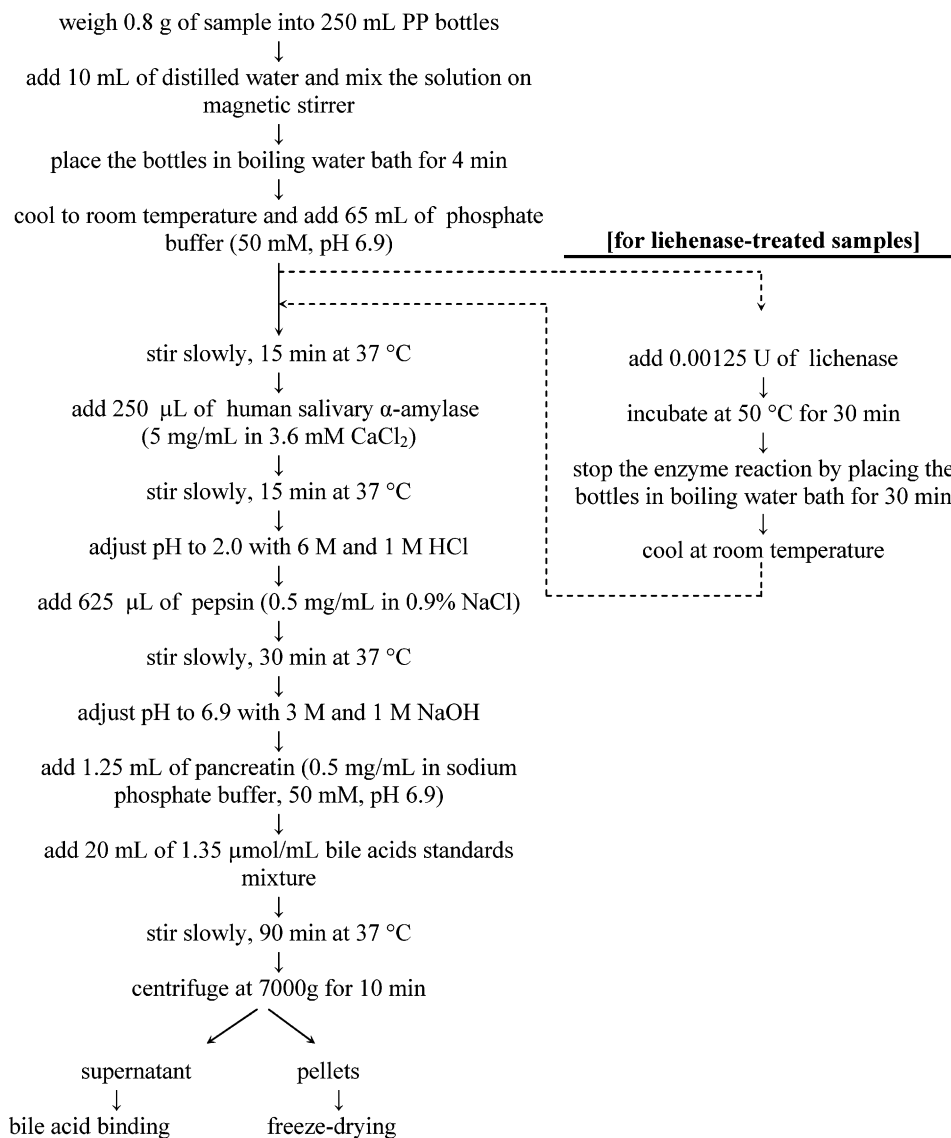
is currently not fully understood. Three competing hypotheses are currently proposed. First,  $\beta$ -glucan may bind BA directly. Second,  $\beta$ -glucan may inhibit the reabsorption of BA by increasing the viscosity of ileum fluids, rather than any specific binding (2, 8, 9). Finally, binding might occur between  $\beta$ -glucan and micelles formed from bile and fatty acids rather than the isolated BA alone (8).

Eastwood and Hamilton (7) were the first authors to show the absorption of bile salts and acids to dietary fiber. They noted that lignin had the highest impact on BA binding of the dry mixture of maize and barley after malting. A major way that soluble fibers, such as  $\beta$ -glucans from oats, lower plasma cholesterol is via their gel-forming properties, which increases the viscosity of intestinal chyme. The increased viscosity also is thought to lower glucose and insulin concentrations in the plasma, a benefit for individuals suffering from diabetes (9). Increased viscosity of the intestinal chyme promotes the excretion of cholesterol and BAs via the intestine and weakens the action of lipase, thus decreasing fat absorption (10). In 1997, the U.S. Food and Drug Administration registered oat bran, and specifically  $\beta$ -glucan, at a level of 3 g/day, as the first cholesterol-reducing food and, therefore, a food that can reduce the risk of heart disease (11). Interference with intestinal cholesterol and BA absorption leading to an increase in fecal neutral sterol and BA excretion is thought to be another mechanism for the hypocholesterolemic effect of nondigestible carbohydrates (10). Oat bran, for example, increased fecal BA loss more than 2-fold and increased loss of one of the BAs, deoxycholic acid, by 240% (12). A few recent studies have

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**Figure 1.** In vitro digestion procedure for oat flour. The scheme on the right-hand side was used for the lichenase-hydrolyzed flours.

demonstrated in vitro the interactions of BA with either soluble or insoluble fibers from different sources. However, the mechanisms for the interactions between dietary fibers and BA in vitro or in vivo are yet unknown (6).

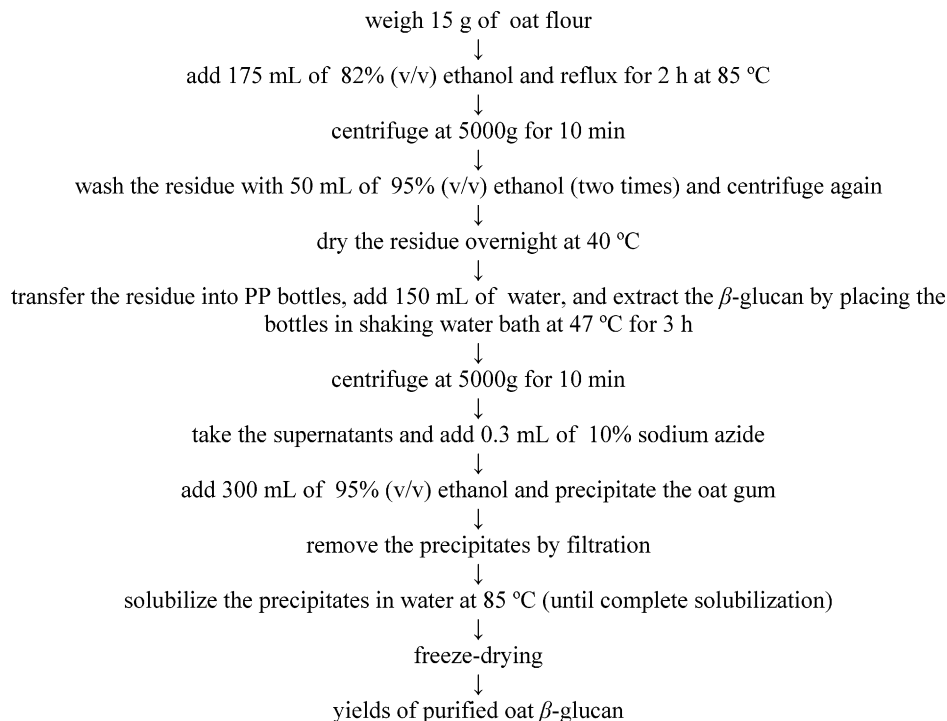
The aim of this work was to examine the impact of  $\beta$ -glucan on the in vitro BA binding properties of oat flours. Two high  $\beta$ -glucan oat lines, developed at Iowa State University, and two traditional lines were investigated. The oat lines differed in their percentage and structure of  $\beta$ -glucan. Therefore, we intended to clarify the effect of  $\beta$ -glucan quantity and molecular weight distribution on BA binding.

## MATERIALS AND METHODS

**Oat Grains.** Two experimental oat (*A. sativa*) lines [IA95111, described in Cervantes-Martinez (13) and N979-5-2-4 (hereafter referred as N979, unpublished data)] and two publicly available cultivars [Jim, developed at the University of Minnesota, and Paul, a naked cultivar (14)] were chosen for this study to span a broad range of  $\beta$ -glucan concentrations (4.77–8.05%  $\beta$ -glucan). Oat grain types were grown in 2003 at the Agronomy and Agricultural Engineering Field Research Center near Ames, IA. Oat samples, except the naked variety, Paul, were dehulled with an air pressure dehuller (Codema, Eden Prairie, MN), and the kernels were ground on an ultra centrifugal mill (ZM-1, Retch GmbH&Co, Haan, Germany) fitted with a 0.5 mm sieve.

**Oat Composition.** All analyses of oat groats were done in triplicate and reported on a dry matter basis. The moisture of oat flours was determined by AACC Method 44-15 A (15). The  $\beta$ -glucan content in flours and in extracts was determined enzymatically by AACC Method 32-23 (16), by using the mixed  $\beta$ -glucan linkage kit from Megazyme (Megazyme Ltd., Co., Wicklow, Ireland). Pentosan in flours was analyzed by the phloroglucinol colorimetric method (17). Oat groat flour proteins were determined by the automatic nitrogen analyzer (elementar, Analyzensysteme GmbH, Germany) with a protein conversion factor of 6.25. The starch content in flour and in the extracts was analyzed by AACC Method 76-13 (16), by using a Total Starch Kit from Megazyme (Megazyme Ltd, Co.). Lipids were analyzed by the gravimetric method after extraction with petroleum ether on a Soxhlet system (Method 30-25) (16). Dietary fiber analyses, including soluble dietary fiber (SDF) and insoluble dietary fiber (IDF), were performed by the AACC Method 32-21 (16), by using a Total Dietary Fiber Kit (TDF 100, Sigma-Aldrich Co., St. Louis, MO).

**In Vitro Digestion and BA Binding.** An in vitro digestion process was applied according to the method given by Beer (18) with some minor modifications (Figure 1). Oat flours were first cooked in boiling water for 4 min and subjected to human salivary  $\alpha$ -amylase (EC 3.2.1.1), porcine pepsin (EC 3.4.23.1), and pancreatin (from porcine pancreas, activity at least equivalent to  $8\times$  USP specifications) enzymes (Sigma-Aldrich Co.), respectively. The BA mixture (20 mL containing 1.35  $\mu$ M), containing sodium cholate, sodium deoxycholate, sodium glycocholate, and sodium taurocholate (Sigma-Aldrich Co.), was added



**Figure 2.** Extraction scheme of  $\beta$ -glucans from whole oat flours.

before the pancreatic digestion step. The BA mixture was made up according to previous studies (19, 20). The *in vitro* digested sample solutions were centrifuged and separated to an insoluble part (pellets) and extract (supernatant). The supernatant was used for BA binding analyses, and the pellets were freeze-dried for other studies. Digestibility of the samples was calculated from the following equation:

$$\text{digestibility (\%)} = \frac{M_S - M_P}{M_S} \times 100 \quad (1)$$

where  $M_S$  is the dry weight of the sample used in the digestion and  $M_P$  is the dry weight of the pellets (undigestible part).

The unbound BAs were analyzed in the extract by using a Bile Acid Diagnostic Kit (Trinity Biotech plc, Bray Co., Wicklow, Ireland) as described elsewhere (19, 20). A non-BA binding negative control, cellulose, and a BA binding anionic resin positive control, cholestyramine (Sigma-Aldrich Co.), were also included for each set of analyses. All of the samples were diluted to fall within the BA concentration range of the test kit.

To further determine the effects of MW of  $\beta$ -glucan on the BA binding properties, the oat flour–phosphate buffer solutions were incubated with 0.00125 U of lichenase (Megazyme Ltd, Co.). After 30 min of incubation, the enzyme was inactivated by placing these solutions in a boiling water bath for 30 min, and the *in vitro* digestion process continued (Figure 1).

**Extraction and Molecular Characterization of  $\beta$ -Glucan.** The  $\beta$ -glucan extraction method given by Skendi (21) was used with minor modifications (Figure 2). The ethanol-refluxing step was applied to inactivate the endogenous  $\beta$ -glucanases, a step that also removes most of the lipids from the oat flours (21). Previously, the water extraction of  $\beta$ -glucans at 47 °C resulted in very little starch solubilization (21). A further protein removal step decreased the MW of  $\beta$ -glucan molecules (21); thus, it was not included in the current study. The MW of the purified  $\beta$ -glucan samples and of the *in vitro* digested extracts were determined by using high-performance size-exclusion chromatography (HPSEC). The chromatography system consisted of a Varian Solvent Delivery Module (model 210, ProStar, Varian Inc., CA), an injection valve (Rheodyne, CA) with a 100  $\mu$ L sample loop, a guard column (Ohpak SB-G, Shodex, Showa Denko K. K., Japan), three serially connected columns (Ohpak SB-806 HQ, Ohpak SB-805 HQ, Ohpak SB-804 HQ, Shodex Showa Denko K. K.), and a Varian Refractive Index (RI, model 350, ProStar, Varian Inc.) detector. The column

temperature was maintained at 40 °C in a column heater (Timberline Instruments Inc., CO). The flow rate of the mobile phase, MiliQ water (Millipore, Bedford, MA), containing 0.02%  $\text{NaN}_3$ , was 0.5 mL/min. The mobile phase was degassed online (DG-700, Viscotek, TX) before entering the system. Samples were prepared in MiliQ water at a concentration of 1 mg/mL and filtered through a 0.45  $\mu\text{m}$  filter (25 mm i.d. nylon syringe filter, Whatman, NY) before analysis. Five pullulan standards (Shodex Std. P-82, Showa Denko K. K. Japan) with known  $M_w$  values ( $2.37 \times 10^4$ ,  $4.80 \times 10^4$ ,  $18.6 \times 10^4$ ,  $38.0 \times 10^4$ , and  $166.0 \times 10^4$  g  $\text{mol}^{-1}$ ) were used to determine the experimental setup and calculations.

**Statistical Analysis.** All analyses were conducted at a minimum in triplicate:  $n$  values are reported. Results were analyzed by using a statistical analysis software system (SPSS Version 12.0, SPSS Inc., IL). Differences among samples and treatments were compared by using a least significant difference (LSD) test with a probability level ( $\alpha$ ) of 0.05.

## RESULTS AND DISCUSSION

**Composition of the Oat Groats.** The oat lines chosen for this study displayed a broad range of  $\beta$ -glucan concentrations with significant differences among oat lines (Table 1). The Jim and Paul lines are traditional varieties with normal  $\beta$ -glucan levels (4.77 and 5.26%). The experimental lines, IA95111 and N979-5-2-4, contained 7.64 and 8.05%  $\beta$ -glucan, respectively, which is greater than typical values reported for domestic *A. sativa* cultivars in the literature (3.7–5.0%) (22). Starch percentages were between 54.4 and 63.3%, with the highest concentration in Jim. There was a negative correlation between starch and  $\beta$ -glucan percentages of the samples ( $R = -0.84$ ) (Table 1). The lipid concentrations of the Paul oats were significantly greater than the other three lines but within the range of values reported for common oat cultivars (5–9%) (23). Small variations were observed in pentosan contents. The SDF and IDF percentages of the oat lines were in the range of 5.57–8.79 and 2.80–5.98%, respectively. As expected, SDF was well-correlated with the  $\beta$ -glucan concentrations of the samples ( $R^2 = 0.92$ ). The greater SDF values than  $\beta$ -glucan content of the sample can be explained by the contribution of some gummy

**Table 1.** Chemical Composition (% db) of Flours from Different Oat Lines<sup>a</sup>

| sample  | $\beta$ -glucan ( $n = 6$ ) | starch ( $n = 6$ ) | fat ( $n = 3$ ) | protein ( $n = 3$ ) | IDF ( $n = 3$ ) | SDF ( $n = 3$ ) | pentosan ( $n = 6$ ) |
|---------|-----------------------------|--------------------|-----------------|---------------------|-----------------|-----------------|----------------------|
| Jim     | 4.8 ± 0.12 a                | 63.3 ± 0.20 c      | 6.8 ± 0.17 a    | 12.1 ± 0.09 a       | 2.8 ± 0.16 a    | 5.6 ± 0.06 a    | 1.6 ± 0.40 a         |
| Paul    | 5.3 ± 0.08 b                | 57.6 ± 0.87 b      | 8.0 ± 0.03 b    | 13.8 ± 0.02 b       | 6.0 ± 0.65 c    | 6.9 ± 0.61 b    | 1.9 ± 0.28 ab        |
| IA95111 | 7.6 ± 0.29 c                | 56.8 ± 1.35 b      | 6.8 ± 0.18 a    | 15.1 ± 0.15 c       | 5.6 ± 1.21 bc   | 8.8 ± 0.00 c    | 2.1 ± 0.42 ab        |
| N979    | 8.1 ± 0.19 d                | 54.4 ± 0.86 a      | 7.2 ± 0.38 a    | 20.1 ± 0.01 d       | 4.4 ± 0.77 b    | 8.8 ± 0.69 c    | 2.4 ± 0.18 b         |

<sup>a</sup> Values are means of  $n$  measurements ± standard deviation. Values within a column followed by a common letter (a–d) are not significantly different ( $P > 0.05$ ).

**Table 2.** Overall and Component in Vitro Digestibility of Flours from Different Oat Lines<sup>a</sup>

| sample  | overall digestibility (%) | component digestibility (%) |              |             |
|---------|---------------------------|-----------------------------|--------------|-------------|
|         |                           | $\beta$ -glucan             | starch       | pentosan    |
| Jim     | 78.6 ± 3.9 a              | 63.1 ± 2.7 a                | 86.9 ± 8.0 a | 7.0 ± 1.7 a |
| Paul    | 80.9 ± 2.1 a              | 69.6 ± 4.4 ab               | 83.4 ± 5.5 a | 5.6 ± 2.9 a |
| IA95111 | 76.9 ± 2.1 a              | 70.8 ± 0.9 b                | 87.6 ± 8.5 a | 7.6 ± 1.5 a |
| N979    | 81.4 ± 1.4 a              | 66.7 ± 3.4 ab               | 85.9 ± 4.4 a | 6.9 ± 2.6 a |

<sup>a</sup> Values are means of  $n = 5$  measurements ± standard deviation. Values within a column followed by a common letter (a–d) are not significantly different ( $P > 0.05$ ).

materials in oats to the SDF values. The IDF was highest for the Paul cultivar. Literature values for SDF and IDF for oat are between 2.5 and 7.1 and 3.9 and 10.6%, respectively (24, 25). The SDF concentrations of the oat lines used in this study were greater than the literature values, which can be explained by the greater  $\beta$ -glucan percentage of our oat lines.

#### In Vitro Digestibility and BA Binding of the Oat Flours.

The in vitro digestion procedure used in this study was designed to mimic aspects of the human digestion system. A slight change was made to the reported in vitro digestion procedures used in other BA binding studies (6, 19, 26). Methods used by Camire (19) and Kahlon (26) included only acidic digestion of the samples at pH 2, without the addition of the pepsin enzyme followed by pancreatin digestion. Drzikova (6) used a treatment with a mixture of only pancreatin and amyloglucosidase enzymes for digestion of the samples. For the current procedure, we used the human salivary  $\alpha$ -amylase, pepsin and pancreatin enzymes for in vitro digestion of the samples to simulate the human digestion systems more appropriately than in previous methods.

Overall digestibility of the oat flour samples, calculated by eq 1, was determined to be in the range of 76.9–81.4% (Table 2) with no significant differences among the samples. Percentages of digested amounts of the individual components, including  $\beta$ -glucan, starch, and pentosan, are given in Table 2. With the same enzyme combination for the in vitro digestion of aleurone-rich wheat bran and purified  $\beta$ -glucan, Wood (2) determined the overall digestibility to be around 60% for both of the samples. Amerin (27) determined overall digestibility values of between 28 and 32% for wheat aleurone. It can be seen that overall digestibilities of the oat flour samples used in this study were higher than wheat aleurone and wheat bran. Because oat flour contains higher amounts of digestible components than wheat aleurone and wheat bran, higher digestibility values were expected. Beer (18) reported that 13–33% of total  $\beta$ -glucan was digestible with noticeable differences in different oat bran sources. Higher digestibility values (63–70%) (Table 2) of the  $\beta$ -glucan in this study might be attributed to the physical nature of the samples. Oat bran likely contains higher amounts of insoluble  $\beta$ -glucans, which are difficult to extract by means of in vitro digestion. Starch digestibility was higher than 80% (Table 2), which was expected, because all

**Table 3.** In Vitro BA Binding by Flours from Different Oat Lines, Cellulose, and Cholestyramine<sup>a</sup>

| sample         | BA bound                          |   |
|----------------|-----------------------------------|---|
|                | $\mu\text{mol}/100 \text{ mg DM}$ | $\mu\text{mol}/100 \text{ mg } \beta\text{-glucan}$ |
| Jim            | 0.84 ± 0.15 b                     | 0.18 ± 0.03 a                                       |
| Paul           | 1.66 ± 0.22 c                     | 0.32 ± 0.04 b                                       |
| IA95111        | 1.50 ± 0.25 c                     | 0.20 ± 0.03 a                                       |
| N979           | 1.51 ± 0.20 c                     | 0.19 ± 0.03 a                                       |
| cellulose      | 0.05 ± 0.02 a                     |   |
| cholestyramine | 11.21 ± 0.10 d                    |   |

<sup>a</sup> Values are means of  $n = 6$  measurements ± standard deviation. Values within a column followed by a common letter (a–d) are not significantly different ( $P > 0.05$ ).

of the samples were digested with human salivary  $\alpha$ -amylase. Amerin (27) and Wood (2) reported starch digestibility values of higher than 90% for wheat aleurone and wheat bran. On the other hand, pentosan digestibility was only between 7 and 8% for the oat flour samples used in this study. Low pentosan extractability also was expected, because the enzymes used in this study were not capable of hydrolyzing the pentosans.

The BA binding of the oat flours, cholestyramine, and cellulose on a dry matter (dmb) and  $\beta$ -glucan content basis is reported (Table 3). Cholestyramine bound 11.21  $\mu\text{mol}$  BA/100 mg dmb, which is equal to 87.6% of the total added BA. The negative control sample, cellulose, bound only 0.05  $\mu\text{mol}$  BA/100 mg dmb or 0.4% of the total added BA. These results are similar to the results given for cholestyramine and cellulose in the literature (28, 29). In vitro values for BA binding of oat flours were between 0.84 and 1.66  $\mu\text{mol}/100 \text{ mg}$  dry matter. Assigning BA binding to cholestyramine as 100%, the relative BA bindings of Jim, Paul, IA95111, and N979 flours were 7.5, 14.8, 13.4, and 13.5%, respectively. A comparison of these results with those from previous studies was performed on the basis of binding according to cholestyramine (assigning BA binding to cholestyramine as 100%), to eliminate the methodological effects. BA binding of various beans, including soybean, lima bean, black gram, bengal gram, moth bean, and kidney bean, was between 1.9 and 8.2% (29, 30). In the case of the bran of rice, oat, wheat, and corn, BA binding values were 12.1, 4.4, 20.0, and 2.9%, respectively (26). The BA bindings of the extruded potato peels and wheat bran were 10.0 and 13.0%, respectively (calculated from the data given in the paper) (19). Comparing the BA binding of the oat flours used in the current study shows that Paul, IA95111, and N979 lines bound greater amounts of BA than did beans, oat bran, corn bran, and extruded potato peels from previous studies. Except for the Jim oat line, our oat flours bound amounts of BA that were similar to or approaching reported values for wheat bran. Drzikova (6) also noted that increased contents of  $\beta$ -glucan in oat meal and oat bran extrudates increased BA binding. The high BA binding of the Paul, IA95111, and N979 oat flours point to their possible health-promoting effects.

On an equal dmb, the high  $\beta$ -glucan flours, IA95111 and N979, bound more BA than did the Jim flour, as expected. The

**Table 4.** BA Binding Calculated on the Basis of Composition of the Flours from Different Oat Lines<sup>a</sup>

| sample  | BA bound                              |                                   |                                   |                                      |  |
|---------|---------------------------------------|-----------------------------------|-----------------------------------|--------------------------------------|--|
|         | $\mu\text{mol}/100\text{ mg protein}$ | $\mu\text{mol}/100\text{ mg SDF}$ | $\mu\text{mol}/100\text{ mg IDF}$ | $\mu\text{mol}/100\text{ mg starch}$ | $\mu\text{mol}/100\text{ mg pentosan}$ |
| Jim     | 0.07 ± 0.01 a                         | 0.15 ± 0.03 a                     | 0.30 ± 0.05 a                     | 0.013 ± 0.002 a                      | 0.54 ± 0.04 a                          |
| Paul    | 0.13 ± 0.01 b                         | 0.24 ± 0.02 b                     | 0.28 ± 0.04 a                     | 0.029 ± 0.004 b                      | 0.88 ± 0.24 b                          |
| IA95111 | 0.10 ± 0.02 ab                        | 0.17 ± 0.03 a                     | 0.27 ± 0.05 a                     | 0.026 ± 0.004 b                      | 0.74 ± 0.25 ab                         |
| N979    | 0.07 ± 0.01 a                         | 0.17 ± 0.02 a                     | 0.34 ± 0.04 a                     | 0.028 ± 0.004 b                      | 0.64 ± 0.11 ab                         |

<sup>a</sup> Values are means of  $n = 3$  measurements ± standard deviation. Values within a column followed by a common letter (a–d) are not significantly different ( $P > 0.05$ ).

BA binding values, when calculated on an equal  $\beta$ -glucan basis, were not statistically different for Jim, IA95111, and N979-5-2-4. However, flour from the Paul line gave an unexpectedly high BA binding value, which, when calculated as BA bound per amount of  $\beta$ -glucan, was greater than the values for the other lines. These findings suggested that other factors, such as  $\beta$ -glucan structure or even other components in the oat flour itself, may impact the flour binding capacity. Thus, further evaluations were conducted to explore these hypotheses.

The BA binding of the oat flours was calculated based on protein, starch, IDF, SDF, and pentosan compositions (Table 4). The BA binding was related only to the IDF. If the IDF were considered as the BA binding component, Jim, Paul, IA95111, and N979 bound 0.30, 0.28, 0.27, and 0.34  $\mu\text{mol BA}/100\text{ mg IDF}$ , respectively. Relative BA binding values on an equal IDF basis were not statistically different ( $P > 0.05$ ), suggesting that BA binding is related to IDF percentage. Pearson's correlation coefficients also showed that BA binding was correlated with the IDF ( $P = 0.929$ ). Correlation of BA binding with the IDF also was determined in previous studies (6, 7, 10, 28). Story and Kritichevsky (28) attributed the BA binding of alfalfa and wheat bran to lignin content. Eastwood and Hamilton (7) also suggested that lignin is the main BA absorbent in dry grain (a mixture of maize and barley after malting). In contrast to Story and Kritichevsky (28) and Eastwood and Hamilton's (7) study, Chen (31) found hemicellulose, rather than lignin, to be the important binding factor in dried vegetables. However, BA binding did not appear to be proportional to the TDF, SDF, protein, starch, or lipid content of various food samples (26, 29, 30). Kahlon and Woodruff (32) speculated that BA binding of rice bran, oat bran, and  $\beta$ -glucan-enriched barley was related to their IDF content. According to Pandolf and Clydesdale (33), binding between BA and fibers is implausible, because both of them are anionic. Studies using <sup>13</sup>C CP/MAS NMR conducted by Bowles (8) suggested that the hypocholesterolemic property of  $\beta$ -glucan did not involve a simple binding of bile salt molecules to specific sites on the  $\beta$ -glucan polymer. This supports the proposition that the ability of  $\beta$ -glucan to inhibit reabsorption of BA is a function of its high viscosity in aqueous solution, rather than any specific binding or complexation (8).

**MW Determination of  $\beta$ -Glucan in Oat Groats and Its Effects on BA Binding.** Several different methods have been reported in the literature for  $\beta$ -glucan extraction from oat and barley (18, 21, 34, 35), with the extractable  $\beta$ -glucan varying from 30 to 80% depending on starting material and extraction conditions (35). Extraction of  $\beta$ -glucans to be used in MW determinations must preserve the natural form of the molecules; thus, the extraction method should minimize any harsh treatments on the  $\beta$ -glucan molecules. Therefore, the aqueous extraction method, given by Skendi (21), was used for this study (Figure 2), resulting in 62–70% of the total  $\beta$ -glucans being extracted (Table 5). Aliquots for MW determination were taken after solubilization of the ethanol-precipitated polymers (before

**Table 5.** Extraction Yield and Peak Molecular Weight of the  $\beta$ -Glucan Extracted from Flours from Different Oat Lines

| sample  | extraction yield <sup>a</sup> (%) | molecular weight ( $\times 10^6$ ) (g mol <sup>-1</sup> ) |                          |   |
|---------|-----------------------------------|---|--------------------------|---|
|         |                                   | from aqueous extraction                                   | after in vitro digestion | after lichenase treatment and in vitro digestion <sup>b</sup> |
| Jim     | 63.1                              | 2.73  | 1.30                     | 0.81  |
| Paul    | 68.1                              | 2.40  | 1.04                     | 0.55  |
| IA95111 | 70.3                              | 3.45  | 1.16                     | 0.71  |
| N979    | 62.4                              | 3.24  | 1.67                     | 0.58  |

<sup>a</sup> Percentages of the  $\beta$ -glucan extracted from the oat flours. <sup>b</sup> Incubated with 0.00125 U of lichenase for 30 min before in vitro digestion.

freeze drying), because freeze drying might decrease the solubility of  $\beta$ -glucan (18). The peak MW of the extracted  $\beta$ -glucans and of the in vitro digested materials determined from the peak retention time of the HPSEC chromatograms is shown (Table 5). The MW values varied from  $2.40 \times 10^6$  to  $3.45 \times 10^6$  g mol<sup>-1</sup> for the purified  $\beta$ -glucans, with values being greater for the MW of the  $\beta$ -glucans from the high  $\beta$ -glucan flours than from the traditional oat flours, a feature that also was reported by Colleoni-Sirghie (34). The MW values determined in this study were generally higher than the values given for oat  $\beta$ -glucan in previous studies. A wide variation of MW ranges ( $0.76$ – $3.23 \times 10^6$  g mol<sup>-1</sup>) for oat  $\beta$ -glucans is reported in the literature (34, 35). We used enzymatically inactivated flours and a simple extraction procedure to protect  $\beta$ -glucan molecules from degradation, likely resulting in greater MW values for oats than the previously measured.

Great decreases in the MW of  $\beta$ -glucan occurred during the in vitro digestion (Table 5). The acidic digestion at pH 2, possible impurities in the enzymes used in the digestion process, and indigenous enzymes in the flours could affect the MW of the  $\beta$ -glucans. There was no inactivation step in the in vitro digestion process. To further evaluate the impact of MW on BA binding, partial hydrolysis of the  $\beta$ -glucan before digestion was accomplished by a lichenase treatment (Figure 2). The MW values of the  $\beta$ -glucan after partial lichenase hydrolysis were markedly decreased by the process (Table 5). Furthermore, the partial hydrolysis significantly ( $P < 0.01$ ) increased the  $\beta$ -glucan digestibility for all oat flour types (Table 6). An association between lower MW and increased digestibility has been noted previously (36). When evaluated further, however, no significant changes occurred ( $P > 0.05$ ) in BA binding characteristics of the flour with partially hydrolyzed  $\beta$ -glucan when compared with the flours before lichenase treatment (Table 6). From these studies, the BA binding was not related to the MW of the  $\beta$ -glucans.

Controversies over the mechanism for the interaction between BA and any dietary fiber components remain. In the current study, high  $\beta$ -glucan oat flours bound high amounts of BA, especially when compared with oat lines from previous studies. Significant correlations between IDF and BA binding were also

**Table 6.** Effects of Partial Lichenase Hydrolysis on the in Vitro Digestibility of  $\beta$ -Glucan from the Flours of Different Oat Lines and on the BA Binding Capacity of the Oat Flours<sup>a</sup>

| sample  | digestibility of the $\beta$ -glucan (%) |                                   | BA binding capacity of the oat flours ( $\mu$ mol/100 mg DM) |                                   |
|---------|--|-----------------------------------|--|-----------------------------------|
|         | natural form <sup>b</sup>                | partially hydrolyzed <sup>c</sup> | natural form   | partially hydrolyzed <sup>c</sup> |
| Jim     | 63.1 $\pm$ 2.7 a                         | 92.3 $\pm$ 3.7 a                  | 0.88 $\pm$ 0.09 a  | 0.85 $\pm$ 0.13 a                 |
| Paul    | 69.6 $\pm$ 4.4 ab                        | 104.1 $\pm$ 6.9 b                 | 1.58 $\pm$ 0.16 b  | 1.68 $\pm$ 0.14 b                 |
| IA95111 | 70.8 $\pm$ 0.9 b                         | 99.4 $\pm$ 2.5 b                  | 1.42 $\pm$ 0.17 b  | 1.48 $\pm$ 0.08 b                 |
| N979    | 66.7 $\pm$ 3.4 ab                        | 95.6 $\pm$ 5.6 ab                 | 1.47 $\pm$ 0.09 b  | 1.40 $\pm$ 0.19 b                 |

<sup>a</sup> Values are means of  $n = 3$  measurements  $\pm$  standard deviation. Values within a column followed by a common letter (a–d) are not significantly different ( $P > 0.05$ ). <sup>b</sup> Data from **Table 2**. <sup>c</sup> Incubated with 0.00125 U of lichenase for 30 min before in vitro digestion.

determined. It is likely that BA binding is a multicomponent-dependent process. Physical conditions of the media or gut, such as viscosity, pH, and temperature, and physical properties of the dietary fiber preparation, such as particle size and cell wall structure, also likely influence the interaction (8, 15). Effects of the physical conditions of the in vitro digestion extracts on BA binding studies are in progress.

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