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Abstract
The impact of β-glucan molecular weight (MW) on *in vitro* bile-acid binding and *in vitro*fermentation with human fecal flora was evaluated. β-Glucan extracted from oat line 'N979-5-4' was treated with lichenase (1,3−1,4-β-d-glucanase) to yield high (6.87 × 105 g/mol), medium (3.71 × 105 g/mol), and low (1.56 × 105 g/mol) MW fractions. The low MW β-glucan bound more bile acid than did the high MW β-glucan ($p < 0.05$). If the positive control, cholestyramine, was considered to bind bile acid at 100%, the relative bile-acid binding of the original oat flour and the extracted β-glucan with high, medium, and low MW was 15, 27, 24, and 21%, respectively. Significant effects of high, medium, and low MW β-glucans on total SCFA were observed compared to the blank without substrate ($p < 0.05$). There were no differences in pH changes and total gas production among high, medium, and low MW β-glucans, and lactulose. The low MW β-glucan produced greater amounts of SCFA than the high MW after 24 h of fermentation. Among the major SCFA, more propionate was produced from all MW fractions of extracted β-glucans than from lactulose. *In vitro* fermentation of extracted β-glucan fractions with different MW lowered pH and produced SCFA, providing potential biological function.

Keywords
β-Glucan, oat, in vitro bile-acid binding, in vitro fermentation

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KEYWORDS: β-Glucan; oat; *in vitro* bile-acid binding; *in vitro* fermentation

INTRODUCTION

Oats are nutritious food materials, having positive physiological effects, including control of blood cholesterol and glucose levels, and of the insulin response, thus decreasing the incidence of obesity, heart disease, cancer, and type-2 diabetes (*1*, *2*). The health benefits of oat-based food products are attributed to the mixed linkage (1→3)(1→4)-β-D-glucan (β-glucan), a soluble dietary fiber. The U.S. Food and Drug Administration (FDA) approved a health claim that oat β-glucan at a level of 3 g per day may reduce cholesterol and lower the risk of coronary heart disease (*3*).

Many mechanisms have been proposed for the cholesterol-lowering effect derived from the consumption of β-glucan (*1*, *2*, *4*). One such mechanism is the ability of β-glucan to lower the reabsorption of bile acids, thus increasing fecal excretion of bile acids (*5*). Bile acids, acidic steroids synthesized in the liver from cholesterol, are actively reabsorbed by the terminal ileum and undergo an enterohepatic circulation. By binding bile acids with β-glucan, cholesterol in the liver can be converted to additional bile acids which then are excreted, thus reducing cholesterol (*5*). In addition, the cholesterol-lowering occurs as a secondary reaction of microbial fermentation of β-glucan in the large intestine (*1*, *6*, *7*). β-Glucans are not digested in the small intestines of humans: they are fermented by the colonic microflora in the large intestine. Fermentation of β-glucan results in the formation of short-chain fatty acids (SCFA), primarily acetate, propionate, and butyrate. Propionate, in particular, was reported to reduce cholesterol by suppressing cholesterol synthesis in the liver (*6*). Also, the low pH in the gut caused by the production of SCFA can prevent the growth of harmful bacteria and aid in the absorption of minerals, such as calcium and magnesium (*8*).

Viscosity is the key physical characteristic contributed by oat β-glucan. Two factors influencing the viscosity of solutions are concentration and MW of β-glucan (*2*), thus contributing to the nutritional effects of β-glucan. Wood et al. (*9*) reported high correlations between β-glucan concentration in oat and the viscosity of the solutions. Butt et al. (*10*) suggested that β-glucan, in order to be physiologically active, should be soluble, the concentration and MW should be sufficiently high, and MW vary between 2.68×10^4 and 3.24×10^6 g/mol; however, the role of β-glucan MW in cholesterol reduction is not well established. The cholesterol-lowering activity of barley β-glucan occurred at both high and low MW (*11*). An animal study showed that the cholesterol-lowering effect was lost after extensive hydrolysis of β-glucans (*12*). Frank et al. (*13*) also reported that β-glucans with low MW (2.17×10^5) and high MW (7.97×10^5 g/mol) had similar cholesterol-lowering effects in human subjects consuming oat breads made with these β-glucan sources. However, some studies showed cholesterol-lowering effects only from low MW (0.7×10^5) β-glucan consumption (*14*, *15*). The inconsistent results of β-glucan MW on physiological effects is still in question and needs further investigation.

The objectives in this study were to yield β-glucan extracts with high, medium, and low MW fractions from an experimental oat

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line developed at Iowa State University, and to determine the impact of β-glucan MW on *in vitro* bile-acid binding, solution viscosity, and *in vitro* fermentation with human fecal flora by using β-glucan extracts with the different MW.

MATERIALS AND METHODS

Oat Grain and Oat Flour Preparation. The experimental oat line 'N979-5-4', developed at Iowa State University, was used to yield high, medium, and low MW β-glucan. Oat grain was grown at the Agronomy and Agricultural Engineering Field Research Center in Ames, IA, and harvested in 2008. Oats were dried and dehulled with an air-pressure dehuller (Codema, Eden Prairie, MN). The kernels were ground in an ultracentrifugal mill (ZM-1, Retch GmbH & Co., Haan, Germany) with a 0.5 mm sieve. Oat flours were then stored in plastic bags at 4 °C until used.

Extraction and Hydrolysis of β-Glucan. Oat flours were refluxed with 82% (v/v) ethanol for 2 h at 85 °C to inactivate endogenous enzymes and to remove fat (*4*) (**Figure 1**). Water-soluble β-glucans were extracted from the treated oat flours by using water with heat-stable α-amylase (Sigma-Aldrich Co., St. Louis, MO) and pancreatin (Sigma-Aldrich Co.) according to the procedure of Yao et al. (*16*). The extracted β-glucan suspension (defined as high MW β-glucan) was hydrolyzed by using lichenase (EC 3.2.1.73, Cat No. E-LICHN, Megazyme International Ltd. Co., Wicklow, Ireland), which is a 1,3−1,4-β-D-glucan-4-glucanohydrolase derived from *Bacillus subtilis* and cleaves the 1,4-linkage of the 3-*O*-substituted glucose residues in β-glucan, to yield medium and low MW β-glucan fractions. Lichenase (0.00125 U/g of oat flour to produce medium-MW β-glucan and 0.01 U/g of oat flour to produce low-MW β-glucan) was added to the extracted β-glucan suspension and incubated at 60 °C for 20 min. The hydrolyzed β-glucan suspensions were heated in a boiling water bath for 10 min to inactivate the lichenase. The β-glucan suspensions with different MW were freeze-dried to produce high, medium, and low MW β-glucan fractions, respectively (BG-High MW, BG-Med MW, and BG-Low MW).

Molecular Weight Determination. Relative MW distribution of the different β-glucan extracts after hydrolysis with lichenase were analyzed by using size-exclusion high-performance liquid chromatography (SE-HPLC) (*16*). The SE-HPLC was composed of a solvent delivery module (model 210, ProStar, Varian Inc., Rheodyne, CA), a 100 μL loop injection valve, a guard column (Ohpak SB-G, Shodex Showa Denko K. K., Tokyo, Japan), three serially connected columns (Ohpak SB-806 HQ, Ohpak SB-805 HQ and Ohpak SB-804 HQ; Shodex Showa Denko K. K.), and a refractive index detector (model 350, ProStar, Varian Inc.). Column and detector were controlled at 40 °C. The flow rate of the mobile phase, Milli-Q water (Millipore, Bedford, MA) containing 0.02% sodium azide (Sigma-Aldrich Co.), was 0.5 mL/min. Samples were filtered through a 0.45 μm nylon syringe filter (25 mm i.d., Whatman, NJ) before the injection. β-Glucan MW standards (Cat No. P-MWBGS, Megazyme) with MW values of 3.59×10^5, 2.45×10^5, 1.83×10^5, 1.23×10^5, and 0.4×10^5 g/mol were used to estimate the actual MW ranges of high, medium, and low MW β-glucans. The peak MW and number-average MW (M_n) were obtained by a first-order polynomial curve of log MW against retention time. The M_n was calculated by the equation $M_n = \sum w_i / \sum(w_i/\mathrm{MW}_i)$, where w_i was the weight fraction of time × height derived from the HPLC chromatogram, and MW_i was the MW of the *i*th species calculated from the standard curve (*16*).

Proximate Composition. Moisture content of oat flour and extracted β-glucans was determined by AACC method 44-15A (*17*). The β-glucan concentrations in oat flour and extracted β-glucans with high, medium, and low MW were analyzed enzymatically by AACC method 32-23 by using a mixed β-glucan linkage kit (Megazyme). Starch content was analyzed by AACC method 76-13 by using a Total Starch Kit (Megazyme). Proteins were determined by using an automatic nitrogen analyzer (Elementar Analzen System GmbH, Germany) with a nitrogen conversion factor of 6.25. All analyses were run in triplicate and the average reported on a dry-weight basis (db).

Water Solubility. Water solubility of high, medium and low MW β-glucan was determined according to the method of Park et al. (*18*). The β-glucan dispersion in water (1%, w/v) was agitated at 37 °C for 24 h and then centrifuged at 1400*g* for 20 min. The supernatant was separated and freeze-dried. The solubility was calculated as the percentage (%) = (weight of β-glucan dissolved in the supernatant)/(initial weight of β-glucan in the dispersion) × 100.

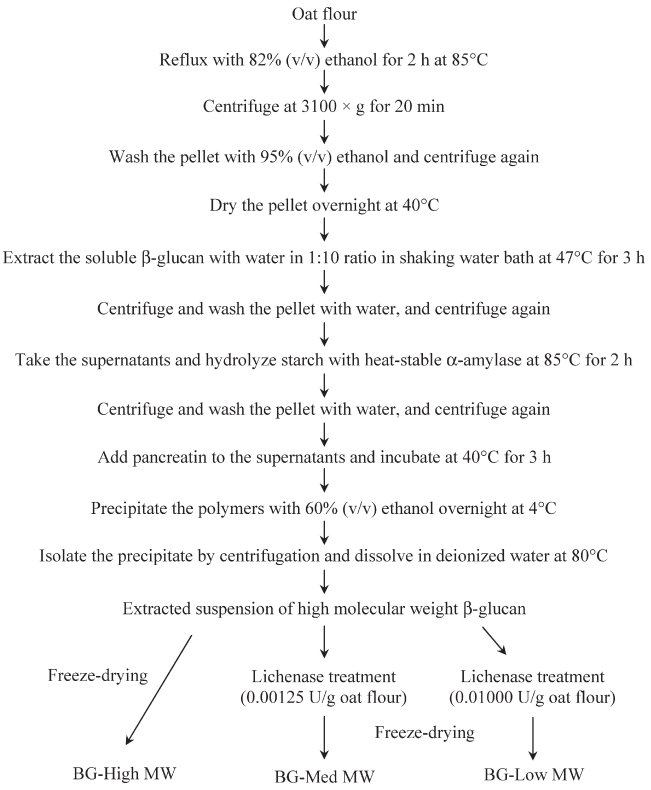

Figure 1. Preparation of extracted β-glucan fractions having high, medium and low MW.

Viscosity Determination. The apparent viscosity of high, medium, and low MW β-glucan solutions was determined by using a Rapid Visco-Analyzer (RVA, Newport Scientific, Warriewood, Australia). Conditions were a stirring speed of 33.03*g* for 10 s, followed by stirring at 0.47*g* for 4 min at 25 °C. The peak viscosity and final viscosity were measured. All pasting curves were collected in duplicate from two slurries, and the average was reported.

***In Vitro* Bile-Acid Binding.** *In vitro* bile-acid binding of high, medium, and low MW β-glucan fractions was measured according to previous procedures with modification (*19*, *20*). The bile acid mixture was prepared with sodium cholate, sodium deoxycholate, sodium glycocholate, and sodium taurocholate (Sigma-Aldrich Co.) with proportions as 35%, 35%, 15%, and 15% (w/w) in 50 mM phosphate buffer at pH 6.9, respectively. The cholesterol-lowering effects of dietary fiber can be predicted by evaluating *in vitro* bile-acid binding compared with cholestyramine, a positive control, and cellulose, a negative control (*5*, *19*). Previous studies showed a positive correlation between *in vitro* and *in vivo* studies and the bile-acid binding impact of these controls (*19*). The high, medium, and low MW β-glucans, oat flour, cholestyramine (Sigma-Aldrich Co.; a bile acid binding anionic resin), and cellulose (Sigma-Aldrich Co.; a non-bile acid binding fiber) were weighed at 50 mg into centrifugal tubes. Samples were digested with 1 mL of 0.01 N HCl in a shaking water bath at 37 °C for 1 h, which simulated gastric digestion. The sample pH was adjusted to 6.9 with 0.1 N NaOH. To each sample, 4 mL of bile acid mixture (1.4 μmol/mL) and 5 mL of porcine pancreatin (activity at least equivalent to 8× USP specifications, 6.25 mg/mL in a 50 mM phosphate buffer, pH 6.9; to provide amylase, protease, and lipase for digestion) were added and incubated at 37 °C for 1 h in a shaking water bath. Sample mixtures were centrifuged at 3100*g* for 10 min. The supernatant was removed. An additional 5 mL of phosphate buffer was used to rinse out the residue, and the mixtures were centrifuged again. Supernatant was removed and combined with the previous supernatant. Unbound bile acid in the supernatant was analyzed by using a Bile Acid Diagnostic Kit (Trinity Biotech plc, Bray Co., Wicklow, Ireland). Samples were diluted to fall within the range of the test kit. The concentration of bile acid was

Table 1. Chemical Composition and Molecular Weight Distributions of the Extracted β-Glucan Fractions with High, Medium, and Low MW

	composition[a] (%, dry wt basis)			MW[a] ($\times 10^5$ g/mol)	
	β-glucan	starch	protein	M_n[b]	peak
oat flour	6.75 ± 0.01 a	54.72 ± 0.43 a	14.35 ± 0.10 a	6.87 ± 0.33 a	9.50 ± 1.14 a
BG-High MW[c]	64.14 ± 2.83 b	5.85 ± 0.09 b	4.53 ± 0.11 b	6.87 ± 0.33 a	9.50 ± 1.14 a
BG-Med MW	65.07 ± 3.59 b	5.05 ± 0.03 c	4.54 ± 0.14 b	3.71 ± 0.18 b	5.13 ± 0.51 b
BG-Low MW	66.60 ± 2.58 b	5.04 ± 0.01 c	4.27 ± 0.24 b	1.56 ± 0.11 c	2.06 ± 0.23 c

[a] Values are means of triplicate determinations. Values with different letters within a column are significantly different ($P < 0.05$). [b] M_n, number-average MW. [c] BG, extracted β-glucan. MW, molecular weight.

calculated based on a standard curve developed from the bile acid at different concentrations.

***In Vitro* Fermentation.** *In vitro* fermentation of high, medium, and low MW β-glucan was conducted by a batch fermentation system under strict anaerobic conditions for 24 h with human fecal flora by following the method of Sayar et al. (*21*). The inoculums were prepared from the fresh feces collected from two healthy volunteers who had not received antibiotics for at least 3 months and had not suffered from indigestion problems within the previous week. It was previously reported that total bacteria counts and number and distribution of species in the feces were sufficiently uniform to produce similar *in vitro* fermentation findings (*22*). Also, the previous work of our laboratory reported that different human feces obtained from three healthy individuals had similar patterns during *in vitro* fermentation (*23*). In this study, the mixture of feces from two individuals was used for all samples including blank, lactulose, and extracted β-glucan.

The anaerobic fermentation medium was prepared with brain heart infusion (Difco Laboratories, Detroit, MI) according to the method of Zheng et al. (*24*). The high, medium, and low MW β-glucans were weighed to 100 mg into 50 mL serum bottles. Fermentation medium (8 mL) was added to each bottle, and the headspace of the bottle was flushed with CO_2. The serum bottles were sealed with PTFE/silicone septa and aluminum caps (Supelco Inc., Bellefonte, PA). The extracted β-glucans with high, medium, and low MW were hydrated overnight at 4 °C. Blank without any substrate and lactulose (Sigma-Aldrich Co.) as a completely fermentable substrate were prepared as controls. The inoculums were prepared from fresh feces collected from the volunteers. Feces from two volunteers were immediately pooled. Each fecal sample was mixed with three parts of the fermentation medium and filtered through four layers of cheesecloth in an Erlenmeyer flask under continuous CO_2 flow. 100 mL of filtrate from two different fecal samples was mixed together. The mixed filtered inoculums (2 mL) were added to each sample bottle and the headspace flushed with CO_2. The recapped bottles were incubated in a shaking water bath at 37 °C for 0, 2, 4, 8, 12, and 24 h. Total gas production was measured by the overpressure in the headspace of the bottle by using a digital manometer (Fisher Scientific, Pittsburgh, PA). Fermentation was terminated by adding 0.1 mL of saturated mercury chloride solution. The sample was transferred to a centrifuge tube, and pH was measured. After centrifugation at 3100*g* for 10 min, 1 mL of aliquot from the supernatant was taken for the SCFA analysis.

Shot-Chain Fatty Acids Analysis. The SCFA, such as acetate, propionate, isobutyrate, butyrate, isobalerate, and valerate, were analyzed as their silyl derivatives by a gas chromatography (*21*). The aliquot of fermentation solution (1 mL) was mixed with 100 μL of 2-ethylbutyric acid as an internal standard. Hydrochloric acid (0.5 mL) to protonize the SCFA and diethyl ether (3 mL) were added and mixed with a vortexer. One milliliter of the ether layer was removed and derivatized by 100 μL of *N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoroacetamide (MTBSTFA; Sigma Co.) at 80 °C for 20 min. After standing at room temperature in the dark for 24 h for complete derivatization, 1 μL of material was injected into a Hewlett-Packard 5890 GC (Hewlett-Packard, Palo Alto, CA). The column was an SPB-5 (30 m × 0.25 mm; Supelco, Inc.), and helium was used as the carrier gas. The oven temperature was kept at 70 °C for 3 min and programmed to increase to 160 at 7 °C/min and stay for 5 min. The injector and detector temperature were 220 and 250 °C, respectively. The SCFA were identified and quantified by comparison with known fatty acid standards (Sigma-Aldrich Co.). The reproducibility of the SCFA analysis by GC was determined by running four replicates of the standards of acetic, propionic, butyric, and valeric acid. The coefficients of variation for measuring fatty acids were 1.3−2.1%. The low coefficients of variations showed that the GC method for the SCFA analysis was reproducible.

Table 2. Viscosity of the Extracted β-Glucan Slurries Made with High, Medium, and Low MW β-Glucan

		viscosity[a] (cP)	
extracted β-glucan	β-glucan (%) in the solution	peak	final
BG-High MW[b]	0.52	726 ± 18 a	700 ± 45 a
BG-Med MW	0.65	451 ± 58 b	363 ± 19 b
BG-Low MW	0.56	121 ± 2 c	59 ± 28 c

[a] Values are means of triplicate determinations. Values with different letters within a column are significantly different ($P < 0.05$). [b] BG, extracted β-glucan. MW, molecular weight.

Statistical Analysis. Results were analyzed by using the analysis of variance (ANOVA), followed by Tukey's test to compare the differences among treatments by using SPSS version 11.0 (SPSS Inc., Chicago, IL) at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Characterization of the Extracted β-Glucans with High, Medium, and Low MW. The extracted β-glucan MW fractions contained 64.1−66.6% of β-glucan, 5−5.8% of starch, and 4−4.5% of protein (**Table 1**). Most of the starch and protein in the oat flours was removed during treatment of β-glucan with α-amylase and pancreatin. The number-average MW (M_n) and peak MW of high, medium, and low MW β-glucan determined from the peak retention time of the SE-HPLC chromatograms after the hydrolysis of β-glucan are shown (**Table 1**). The M_n of high MW β-glucan was greatly decreased from 6.87×10^5 g/mol to 3.71×10^5 g/mol (medium MW) and to 1.56×10^5 g/mol (low MW), respectively ($p < 0.05$), depending on the lichenase amount. The peak MW values of high, medium, and low MW β-glucan differed ($p < 0.05$). Water-soluble β-glucans previously were reported to have MW values in the range from 2×10^4 to 4×10^7 g/mol (*10*). Even though the MW range of three different fractions of β-glucan was not great compared to the one previously reported (*10*), the M_n and peak MW of high, medium, and low β-glucan MW fractions were significantly different. The viscosity (one of the most important characteristics of β-glucan) of three β-glucan fractions was greatly different as shown in **Table 2**. We hypothesized that these ranges of MW of β-glucan could affect the biological function of β-glucan.

The water solubilities of the extracted high, medium, and low MW β-glucan were 82.9 ± 4.61, 88.9 ± 2.34, and 91.8 ± 1.95%, respectively. As the MW of β-glucan decreased, the solubility increased. β-Glucan with a lower MW might be more mobile and diffuse more easily compared to a higher MW. Chang et al. (*25*) suggested that increased water solubility is a desirable property for pharmaceutical applications, because it provides an environment in the gut that enhances physiological activities such as anticoagulation and antitumorigenesis. The increased water solubility can also benefit blending of β-glucan into foods,

because of the enhanced solubility, and provide more stable mixtures (*17*).

Peak and final viscosity of the high, medium, and low MW β-glucan solutions were determined by using the RVA at 25 °C (**Table 2**). Viscosity of the solutions increased greatly with higher MW of β-glucan ($p < 0.05$). In agreement with these findings, a greater MW of β-glucan in some oat types caused greater slurry viscosities than a lower MW in other oat types (*16*). Yao et al. (*16*) also reported that the substantial contributor of peak viscosity in oat flour slurries was β-glucan. The enzymatic degradation by lichenase in medium and low MW β-glucans selectively degraded β-glucan and led to modifications in the viscosity of solutions.

***In Vitro* Bile-Acid Binding.** *In vitro* bile-acid binding of cholestyramine, cellulose, oat flour, and high, medium, and low MW β-glucan on a dry weight basis (db) are shown (**Table 3**). Cholestyramine bound 10.00 μmol bile acid/100 mg db, which was equal to 89.2% of the total added bile acid. Cellulose as a negative control bound only 0.03 μmol bile acid/100 mg db, which was 0.2% of the total added bile acid. These values are similar to the results reported for cholestyramine and cellulose in the literature (*4*, *19*).

In vitro bile-acid binding values of the oat flour and of the extracted β-glucan with high, medium, and low MW were 1.51, 2.12, 2.41, and 2.73 μmol bile acid/100 mg oat material db. When the bile-acid binding value of cholestyramine was 100%, the relative bile-acid binding values of the oat flour and of the high, medium, and low MW β-glucan fractions were 15%, 21%, 24%, and 27%, respectively. All β-glucan fractions bound more bile acid than the oat flour ($p < 0.05$). The low MW and medium MW β-glucan bound more bile acid than did the high MW β-glucan. The bile-acid binding values of the oat flours from different oat lines and oat bran were in the range of 6% to13.5% (*4*, *19*).

Table 3. *In vitro* Bile-Acid Binding by the Extracted β-Glucan Fractions Containing High, Medium, and Low MW β-Glucan

sample	bile-acid binding[a] (μmol/100 mg sample, db)	bile-acid binding to total bile acid (%)
cholestyramine	10.00 ± 0.13 a	89.2
BG-High MW[b]	2.12 ± 0.04 c	18.9
BG-Med MW	2.41 ± 0.12 bc	21.5
BG-Low MW	2.73 ± 0.09 b	24.3
oat flour	1.51 ± 0.04 d	13.5
cellulose	0.03 ± 0.01 e	0.2

[a] Values are means of replicate determinations. Values with different letters within a column are significantly different ($P < 0.05$). [b] BG, extracted β-glucan. MW, molecular weight.

The concentration of β-glucan in the oat flour and the extracted β-glucan fractions with high, medium, and low MW were 6.75%, 64.14%, 65.07%, and 66.60%, respectively (**Table 1**). The oat flour, with the lowest concentration, had the greatest bile-acid binding value per amount of β-glucan. The extracted β-glucans, with about 10-fold higher concentrations of β-glucan than the oat flour, resulted in lower bile-acid binding per amount of β-glucan. These data suggest that the oat flour might have other components besides β-glucan contributing to bile-acid binding, or that β-glucan might bind more optimally in more dilute surroundings. Sayar et al. (*4*) observed no significant correlations between β-glucan content in oat flours differing in β-glucan concentrations and bile-acid binding, but significant correlations between insoluble dietary fiber content from the oat flour and bile-acid binding. Kahlon and Woodruff (*19*) reported that bile-acid binding was related to the insoluble dietary fiber, not total dietary fiber and soluble dietary fiber. They also suggested that the primary mechanism of cholesterol-lowering by oat bran was not caused by the bile-acid binding by its soluble dietary fiber, such as β-glucan (*19*). Bowles et al. (*26*) found that the cholesterol-lowering property of β-glucan did not involve a simple binding of bile salt molecules to specific sites on the β-glucan polymer. The intensity of interactions with bile acid was affected by the fine structure of both the polysaccharides and the steroids, as well as by the pH of the media (*27*, *28*). Thus, further evaluations are needed to explore these hypotheses.

***In Vitro* Fermentation.** The *in vitro* fermentation progress of the blank, lactulose, and high, medium, and low MW β-glucans was monitored by pH changes, gas production, and SCFA formation during 0, 2, 4, 8, 12, and 24 h of fermentation (**Figures 2** and **3**). The pH of all treatments decreased until 4−8 h of fermentation, when the total gas production generally reached its maximum. The pH slightly increased until the end of fermentation after 8 h. These results are consistent with those observed for oat bran and oat flour (*21*, *23*, *29*). The pH of lactulose, which is completely metabolized in the colon by enteric bacteria, dropped greatly during fermentation ($p < 0.05$). The pH of the extracted β-glucan

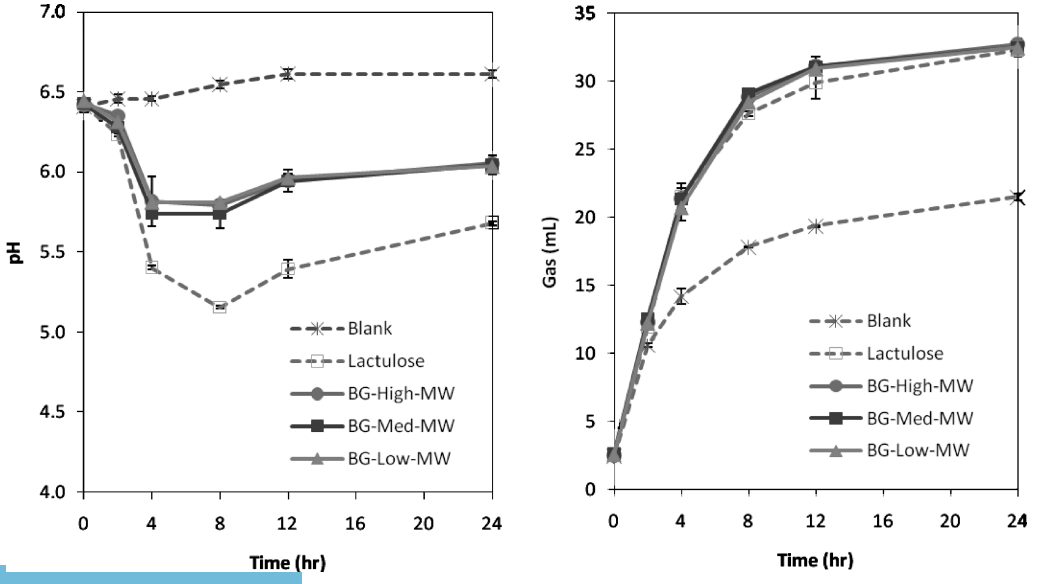

Figure 2. The pH changes and total gas production during *in vitro* fermentation of blank without substrate, lactulose, and the extracted β-glucan fractions having high, medium, and low MW β-glucan.

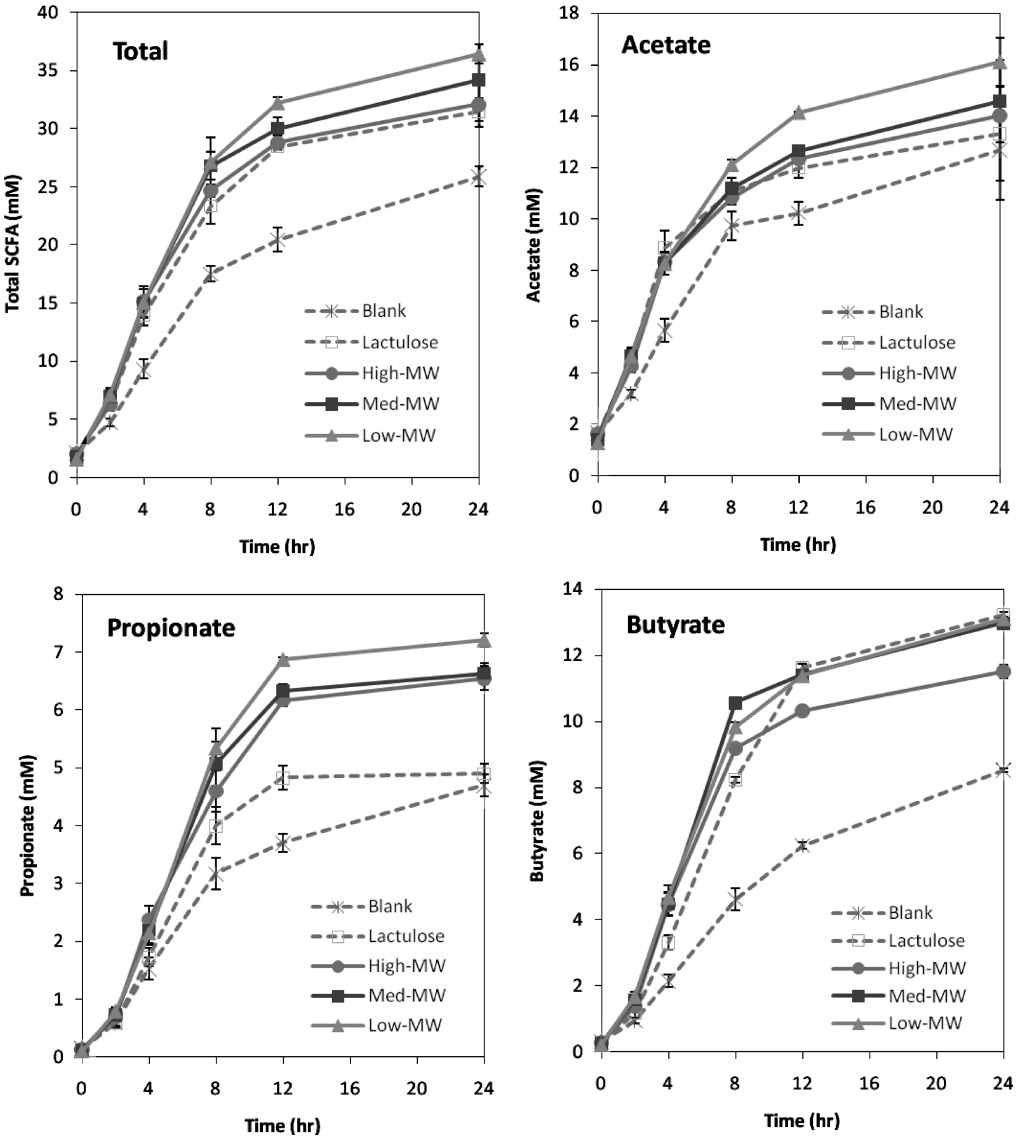

Figure 3. The SCFA production during *in vitro* fermentation of blank without substrate, lactulose, and the extracted β-glucan fraction having high, medium, and low MW β-glucan.

fractions with high, medium and low MW decreased from 6.4 to 5.7−5.8, with no differences among different MW β-glucans ($p > 0.05$).

As the fermentation time increased from 0 to 24 h, the total gas production of the extracted β-glucan fractions with high, medium, and low MW increased (**Figure 2**). Lactulose and the β-glucan fractions produced greater amounts of gas than did the blank ($p < 0.05$). No significant differences among lactulose and the β-glucan fractions were found. Total amounts of gas after 24 h of fermentation were about 32−33 mL for lactulose and β-glucan fractions and 21 mL for the blank. These values were similar to data from other studies with oat flours, oat bran, and purified β-glucan (*21*, *29*).

Total SCFA formation, including acetate, propionate, and butyrate, from the blank, lactulose, and the β-glucan fractions during *in vitro* fermentation are shown (**Figure 3**). The production of SCFA for all treatments continuously increased as fermentation time proceeded. Total gas production and SCFA formation were highly correlated during fermentation ($R^2 = 0.90$). Lactulose, as a standard for complete fermentation in the colon, tended to produce less SCFA than the β-glucan fractions with high, medium, and low MW. In previous work, the production of SCFA from the lactulose was greater than from the digested oat flour and oat bran (*21*, *29*). The extracted β-glucan fractions were highly fermentable, especially compared to lactulose in the current study. The low MW β-glucan produced a greater amount of SCFA than did the high MW fraction after 24 h of fermentation ($p < 0.05$).

Acetate, propionate, and butyrate, the main SCFA formed from *in vitro* fermentation, were typical metabolites for dietary fiber polysaccharide fermentation (*21*, *29*). In addition to the three main SCFA, small amounts of isobutyrate, valerate, and isovalerate were produced (< 5−7% of the total) from all treatments, which are the major products of protein fermentation (*21*). The amount of acetate, propionate, and butyrate increased but by different amounts during fermentation (**Figure 3**). Acetate was produced in the greatest proportions, followed by butyrate and propionate. Acetate is the main metabolite from which the human body obtains energy out of dietary fiber and is the primary substrate for cholesterol synthesis (*1*). The extracted high, medium, and low MW β-glucan produced more propionate than did lactulose. The low MW β-glucan produced more propionate than did high and medium MW during fermentation (**Figure 3**). The production of propionate has been reported to lower blood

glucose and insulin levels (*30*) and increase high density lipoprotein (HDL) cholesterol and triglycerides (*30*, *31*). Butyrate is also physiologically important because of its use for colonic cell growth and differentiation (*1*, *32*).

Different molecular features of the oat β-glucan affected the SCFA profile during *in vitro* fermentation (**Figures 2** and **3**). The low MW β-glucan fraction (shorter chain length) produced more SCFA than the high MW fraction. Several studies suggested that compounds with shorter (DP < 10) chain lengths are fermented more rapidly, with more gas production, than long-chain carbohydrates (*33*, *34*). Fructo-oligosaccharide materials differing in MW gave different SCFA profiles during fermentation (*33*). The water solubility of the extracted β-glucan fractions was in the order of low MW > medium MW > high MW β-glucan (91.8%, 88.9%, and 82.9%, respectively). Perhaps the greater water solubility of the low MW β-glucan over that of the high or medium MW β-glucan resulted in the greater SCFA production. In other work, different physiological mechanisms were observed between cereal β-glucan materials with different MW features. The cholesterol-lowering effect of barley β-glucan in hamsters occurred with both low (1.75×10^5 g/mol) and high MW (1.0×10^6 g/mol), but lower accumulation of cholesterol esters was observed with the low-MW β-glucan (*11*). A low-MW barley β-glucan did not lower serum cholesterol significantly (*35*), whereas 5 g per day of oat β-glucan with a low MW (0.7×10^5 g/mol) in a drink lowered serum cholesterol (*36*). Wood (*2*) determined that dispersion or solubilization of the β-glucan was more important to the physiological effect than was MW.

High (6.87×10^5 g/mol), medium (3.71×10^5 g/mol), and low (1.56×10^5 g/mol) MW fractions of water-extracted β-glucan were successfully produced by using lichenase. The lower the MW of the β-glucan fraction, the lower the viscosity of the solution, and the greater the *in vitro* bile-acid binding. *In vitro* fermentation of the extracted β-glucan with high, medium, and low MW lowered pH as a result of the SCFA production. The low-MW β-glucan produced greater SCFA than the high-MW β-glucan after 24 h of fermentation. The extracted β-glucans produced greater amounts of propionate which has great potential for lowering cholesterol. The impact of the low MW β-glucan on the potential biological function maybe related to the high solubility.

LITERATURE CITED

(1) Malkki, Y.; Virtanen, E. Gastrointestinal effects of oat bran and oat gum: a review. *Lebensm.-Wiss. -Technol.* **2001**, *34*, 337–347.

(2) Wood, P. J. Cereal β-glucans in diet and health. *J. Cereal Sci.* **2007**, *46*, 230–238.

(3) FDA. Food labeling: Health claims; oat and coronary heart disease. Final rule. *Fed. Regist.* **1997**, *62*, 3584−3681.

(4) 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.

(5) Drzikova, B.; Donowski, G.; Gebhardt, E.; Habel, A. The composition of dietary fibre-rich extrudates from oat affects bile acid binding and fermentation in vitro. *Food Chem.* **2005**, *90*, 181–192.

(6) Nishina, P. M.; Freedland, R. A. Effects of propionate on lipid biosynthesis in isolated rat hepatocytes. *J. Nutr.* **1990**, *120*, 668–673.

(7) Queenan, K. M.; Stewart, M. L.; Smith, K. N.; Thomas, W.; Fulcher, R. G.; Slavin, J. L. Concentrated oat β-glucan, a fermentable fiber, lowers serum cholesterol in hypercholesterolemic adults in a randomized controlled trial. *Nutr. J.* **2007**, *6*, 6–13.

(8) Cummings, J. H. Short chain fatty acids in the human colon. *Gut* **1981**, *22*, 763–779.

(9) Wood, P. J.; Beer, M. U.; Butler, G. Evaluation of role of concentration and molecular weight of oat β-glucan in determining effect of viscosity on plasma glucose and insulin following an oral glucose load. *Br. J. Nutr.* **2000**, *84*, 19–23.

(10) Butt, M. S.; Tahir-Nadeem, M.; Khan, M. K. I.; Shabir, R.; Butt, M. S. Oat: unique among the cereals. *Eur. J. Nutr.* **2008**, *47*, 68–79.

(11) Wilson, T. A.; Nicolosi, R. J.; Delaney, B.; Chadwell, K.; Moolchandani, V.; Kotyla, T.; Pondurus, S.; Zheng, G.; Hess, R.; Knutson, N.; Curry, L.; Kolberg, L.; Goulson, M.; Ostergren, K. Reduced and high molecular weight barley β-glucan decrease plasma total and non-HDL-cholesterol in hypercholesterolemic Syrian golden hamsters. *J. Nutr.* **2004**, *123*, 2617–2622.

(12) Bengtsson, S.; Aman, P.; Graha, H.; Newman, C. W.; Newman, R. K. Chemical studies on mixed-linked beta-glucans in hull-less barley cultivars giving different hypocholesterolemic responses in chickens. *J. Sci. Food Agric.* **1990**, *52*, 435–445.

(13) Frank, J.; Sundberg, B.; Kamal-Eldin, A.; Vessby, B.; Aman, P. Yeast-leavened oat breads with high or low molecular weight β-glucan do not differ in their effects on blood concentrations of lipids, insulin, or glucose in humans. *J. Nutr.* **2004**, *134*, 1384–1388.

(14) Naumann, E.; van Ress, A. B.; Onning, G.; Oste, R.; Wydra, M.; Mensink, R. P. β-Glucan incorporated into a fruit drink effectively lowers serum LDL-cholesterol concentration. *Am. J. Clin. Nutr.* **2006**, *83*, 601–605.

(15) Biorklund, M.; van Rees, A.; Mensink, R. P.; Onning, G. Changes in serum lipids and postprandial glucose and insulin concentrations after consumption of beverages with β-glucans from oats or barley, a randomized dose-controlled trial. *Eur. J. Clin. Nutr.* **2005**, *59*, 1272–1281.

(16) Yao, N.; Jannink, J.-L.; White, P. J. Molecular weight distribution of (1−3)(1−4)-β-glucan affects pasting properties of flour from oat lines with high and typical amounts of β-glucan. *Cereal Chem.* **2007**, *84*, 471–479.

(17) AACC International. *Approved Methods of the AACC*, 10th ed.; Methods 30-25, 32-23, 44-15A, 76-13; The Association of Cereal Chemists: St. Paul, MN, 2000.

(18) Park, S. Y.; Bae, I. Y.; Lee, S.; Lee, H. G. Physicochemical and hypocholesterolemic characterization of oxidized oat β-glucan. *J. Agric. Food Chem.* **2009**, *57*, 439–443.

(19) Kahlon, T. S.; Woodruff, C. L. *In vitro* binding of bile acids by rice bran, oat bran, barley and β-glucan enriched barley. *Cereal Chem.* **2003**, *80*, 260–263.

(20) Yao, N.; White, P. J.; Jannink, J.-L.; Alavi, S. Impact of dry solids and bile acid concentrations on bile acid binding capacity of extruded oat cereals. *J. Agric. Food Chem.* **2008**, *56*, 8672–8679.

(21) Sayar, S.; Jannink, J.-L.; White, P. J. Digestion residues of typical and high β-glucan oat flours provide substrates for *in vitro* fermentation. *J. Agric. Food Chem.* **2007**, *55*, 5308–5311.

(22) McBurney, M. I.; Thompson, L. U. Effect of human faecal inoculum on *in vitro* fermentation variables. *Br. J. Nutr.* **1987**, *58*, 233–243.

(23) Kim, H. J.; White, P. J. *In vitro* fermentation of oat flours from typical and high β-glucan oat lines. *J. Agric. Food Chem.* **2009**, *57*, 7529–7536.

(24) Zheng, Y.; Hu, J.; Murphy, P. A.; Alekel, D. L.; Franke, W. D.; Hendrich, S. Rapid gut transit time and slow fecal isoflavone disappearance phenotype are associated with greater genistein bioavailability in women. *J. Nutr.* **2003**, *133*, 3110–3116.

(25) Chang, Y. J.; Lee, S.; Yoo, M. A.; Lee, H. G. Structural and biological characterization of sulfated-derivatized oat β-glucan. *J. Agric. Food Chem.* **2006**, *54*, 3815–3818.

(26) Bowles, R. K.; Morgan, K. R.; Furneaux, R. H.; Coles, G. D. ^{13}C PC/MAS NMR study of the interaction of bile acids with barley β-glucan. *Carbohydr. Polym.* **1996**, *29*, 7–10.

(27) Dongowski, G. Influence of pectin structure on the interaction with bile acids under *in vitro* conditions. *Eur. Food Res. Technol.* **1995**, *201*, 390–398.

(28) Dongowski, G. Effect of pH on the *in vitro* interactions between bile acids and pectin. *Eur. Food Res. Technol.* **1997**, *205*, 185–192.

(29) Wood, P. J.; Arrigoni, E.; Miller, S. S.; Amado, R. Fermentability of oat and wheat fractions enriched in β-glucan using human fecal inoculation. *Cereal Chem.* **2002**, *79*, 445–454.

(30) Todesco, T.; Rao, A. V.; Bosello, O.; Jenkins, D. J. A. Propionate lowers blood glucose and alters lipid metabolism in healthy subjects. *Am. J. Nutr.* **1991**, *54*, 860–865.

(31) Chen, W. J.; Anderson, J. W.; Jennings, D. Propionate may mediate the hypocholesterolemic effects of certain soluble plant fibers in cholesterol-fed rats. *Proc. Soc. Exp. Biol. Med.* **1984**, *175*, 215–218.
(32) McIntyre, A.; Gibson, P. R.; Young, G. P. Butyrate production from dietary fiber and protection from large bowel cancer in a rat model. *Gut* **1993**, *34*, 38.
(33) Stewart, M. L.; Timm, D. A.; Slavin, J. L. Fructooligosaccharides exhibit more rapid fermentation than long-chain inulin in an in vitro fermentation system. *Nutr. Res.* (*N.Y.*) **2008**, 329–334.
(34) Hernot, D. C.; Boileau, T. W.; Bauer, L. L.; Middelbos, I. S.; Murphy, M. R.; Swanson, K. S.; Fahey, G. C., Jr. *In vitro* fermentation profiles, gas production rates, and microbiota modulation as affected by certain fructans, galactooligosaccharides, and Polydextrose. *J. Agric. Food Chem.* **2009**, *57*, 1354–1361.
(35) Keogh, G. F.; Cooper, G. J. S.; Mulvey, T. B.; McArdle, B. H.; Coles, G. D.; Monro, J. A.; Poppitt, S. D. Randomized controlled crossover study of the effect of a highly β-glucan-enriched barley on cardiovascular disease risk factors in mildly hypercholesterolemic men. *Am. J. Clin. Nutr.* **2003**, *78*, 711–718.
(36) Biorklund, M.; van Rees, A.; Mensink, R. P.; Onning, G. Changes in serum lipids and postprandial glucose and insulin concentrations after consumption of beverage with β-glucans from oats or barley, a randomized dose-controlled trial. *Eur. J. Clin. Nutr.* **2005**, *59*, 1272–1281.

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