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Optimizing the Molecular Weight of Oat β-Glucan for in Vitro Bile Acid Binding and Fermentation

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Abstract
A previous study showed β-glucan with low molecular weight (MW, 1.56 × 105 g/mol) bound more bile acid and produced greater amounts of short-chain fatty acids (SCFA) than did β-glucan with high MW (*M*n = 6.87 × 105 g/mol). In the current study, β-glucan extracted from oat flour was fractionated into six different MW levels (high MW, 7.09 × 105; low level 1 (L1), 3.48 × 105; L2, 2.42 × 105; L3, 1.61 × 105; L4, 0.87 × 105; and L5, 0.46 × 105 g/mol) and evaluated to find the optimum MW affecting in vitro bile acid binding and fermentation. The β-glucan fractions with 2.42 × 105–1.61 × 105 g/mol (L2 and L3) bound the greatest amounts of bile acid. After 24 h of fermentation, no differences were found in total SCFA formation among L1, L2, L3, and L4 fractions; however, the high MW and L5 MW fractions produced lower amounts of total SCFA. Thus, the optimum MW of β-glucan to affect both hypocholesterolemic and antitumorigenic in vitro effects was in the range of 2.42 × 105–1.61 × 105 g/mol. This MW range also was the most water-soluble among the MWs evaluated.

Keywords
oat β-glucan, molecular weight, in vitro bile acid binding, in vitro fermentation

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Optimizing the Molecular Weight of Oat β-Glucan for in Vitro Bile Acid Binding and Fermentation

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ABSTRACT: A previous study showed β-glucan with low molecular weight (MW, 1.56×10^5 g/mol) bound more bile acid and produced greater amounts of short-chain fatty acids (SCFA) than did β-glucan with high MW ($M_n = 6.87 \times 10^5$ g/mol). In the current study, β-glucan extracted from oat flour was fractionated into six different MW levels (high MW, 7.09×10^5; low level 1 (L1), 3.48×10^5; L2, 2.42×10^5; L3, 1.61×10^5; L4, 0.87×10^5; and L5, 0.46×10^5 g/mol) and evaluated to find the optimum MW affecting in vitro bile acid binding and fermentation. The β-glucan fractions with $2.42 \times 10^5 - 1.61 \times 10^5$ g/mol (L2 and L3) bound the greatest amounts of bile acid. After 24 h of fermentation, no differences were found in total SCFA formation among L1, L2, L3, and L4 fractions; however, the high MW and L5 MW fractions produced lower amounts of total SCFA. Thus, the optimum MW of β-glucan to affect both hypocholesterolemic and antitumorigenic in vitro effects was in the range of $2.42 \times 10^5 - 1.61 \times 10^5$ g/mol. This MW range also was the most water-soluble among the MWs evaluated.

KEYWORDS: oat β-glucan, molecular weight, in vitro bile acid binding, in vitro fermentation

INTRODUCTION

Oat β-glucan is a soluble dietary fiber found primarily in the endosperm cell walls of oats. It has gained much attention resulting from its health benefits to help regulate blood glucose and insulin levels, reduce blood cholesterol levels, and control weight through prolonged satiety.[1−4] In 1997, the U.S. Food and Drug Administration (FDA) approved a health claim stating that consumption of β-glucan at a level of 0.75 g per serving in a product may reduce cholesterol and lower the risk of coronary heart disease.[5]

β-Glucans are linear homopolysaccharides composed of D-glucopyranosyl units linked via a mixture of β-(1→3) and β-(1→4) linkages. Because β-glucans exhibit consecutive (1→4)-linked β-D-glucose in blocks that are separated by a single (1→3) linkage, they form viscous solutions.[6] The structure and molecular features of β-glucan, such as the ratio of β-(1→3)/β-(1→4) linkages, ratio of trimers and tetramers, amounts of longer cellulosic oligomers, and molecular weight (MW), play important roles in the physical and physiological functions of β-glucan.[7] The MW of β-glucan determines viscosity, solubility, dispersibility, and gelation properties, as well as physiological functions in the gastrointestinal tract.[8−10] The estimated range of MW to be physiologically active varies between 26,800 and 3,240,000 g/mol,[11] depending upon genetic and environmental factors, isolation and purification processes, and the method used for MW estimation.[4] The physiological functions, including cholesterol-lowering and glucose-attenuating effects of β-glucan at different ranges of MW, have been reported.[1−3,7]

Studies show mixed results regarding the impact of β-glucan MW. A clinical study showed that low-density lipoprotein (LDL) cholesterol of human subjects consuming ready-to-eat breakfast cereals with 3 g of high MW β-glucan (22.10×10^5 g/mol) was lower than that of subjects consuming 4 g of low MW β-glucan (2.10×10^5 g/mol), and the efficacy of low MW β-glucan was reduced by 5%.[12] Frank et al.[13] reported that β-glucan with low MW (2.17×10^5 g/mol) 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. Some studies showed cholesterol-lowering effects only from very low MW β-glucan ($(0.70-0.80) \times 10^5$ g/mol) consumption.[14,15] Tosh et al.[16] demonstrated that the glycemic responses of healthy human subjects consuming oat bran muffins containing 4 or 8 g of β-glucan improved as the MW increased. Our previous study showed that β-glucan with low MW (1.56×10^5 g/mol) bound more bile acid and produced greater amounts of short-chain fatty acids (SCFA) after 24 h of in vitro fermentation than that with high MW (6.87×10^5 g/mol).[17] The findings from studies evaluating the impact of β-glucan MW on physiological effects are inconsistent, with further investigations needed. In the current study, β-glucan extracted from an experimental oat line developed at Iowa State University was fractionated to provide six different levels of MW to determine the optimum MW affecting in vitro bile acid binding, water solubility, and in vitro fermentation with human fecal flora by using β-glucan extracts with different levels of MW.

MATERIALS AND METHODS

Preparation of Oat Flour. Experimental oat line (N979-5-4) developed at Iowa State University and grown in 2009 at the Agronomy and Agricultural Engineering Field Research Center in Ames, IA, was chosen for this study because this line had a greater β-glucan concentration (8%) than publicly available cultivars (4−5%). The harvested oat kernels were dried and dehulled with an air-pressure dehuller (Codema, Eden Prairie, MN). The resulting groats were ground in an ultracentrifugal mill (ZM-1, Retch GmbH & Co., Hann, Germany) with a 0.5 mm sieve. Oat flours were then stored at 4 °C until used.

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Extraction of β-Glucan MW Fractions. Water-soluble β-glucans were extracted from oat flours according to the procedure of Yao et al.[18] with modification of the enzyme treatment as described. Oat flours were refluxed with 82% (v/v) ethanol for 2 h at 85 °C to inactivate endogenous enzymes and to remove fat. Starch and protein were removed by heat-stable α-amylase (200 U/g of oat flour; catalog no. E-BLAAM100; Megazyme International, Wicklow, Ireland) and pancreatin (1.3 mg/g of oat flour; catalog no. P-8096; Sigma Aldrich Co., St. Louis, MO). After ethanol precipitation of polymers, the precipitate was isolated by centrifugation at 3100*g* for 20 min and redissolved in deionized water at 80 °C under magnetic stirring. The extracted β-glucan suspension (defined as high MW β-glucan) was hydrolyzed by using lichenase (330 U of lichenase/mg of protein; <0.0001 U of β-glucaosidase, cellulose, and endo-1,3-β-glucanase, and <0.0004U of α-amylase and amyloglucosidases; EC 3.2.1.73, catalog no. E-LICHN, Megazyme), which is a 1,3−1,4-β-D-glucan-4-glucanohydrolase and cleaves the 1,4-linkage of the 3-*O*-substituted glucose residues in β-glucan to yield low MW β-glucan fractions. Lichenase (0.005, 0.025, 0.050, 0.125, and 0.375 U/g of oat flour) was added to the extracted β-glucan suspension and incubated at 50 °C for 20 min to produce different levels of low MW β-glucan. The hydrolyzed β-glucan suspensions were heated in a boiling water bath for 10 min to inactivate the lichenase. After MW determination by HPLC, the β-glucan suspensions with different MW levels were freeze-dried to produce high MW and five different levels of low MW β-glucan fractions. Three replicates of each extracted β-glucan fraction were prepared and analyzed.

Determination of Molecular Weight by High-Performance Liquid Chromatography. The relative MW distribution of the extracted β-glucans with different levels of MW was determined by using size exclusion high-performance liquid chromatography (SE-HPLC).[19] The SE-HPLC consisted of a solvent delivery module (model 210, ProStar, Varian Inc., Harbor City, 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, Ohpak SB-804 HQ; Shodex Showa Denko K.K.), and a refractive index detector (model 350, ProStar, Varian Inc.). The column temperature was 40 °C, and the flow rate of the mobile phase, Milli-Q water (Milipore, Bedford, MA) containing 0.02% sodium azide, was 0.5 mL/min. Aliquots were filtered through a 0.45 μm filter (25 mm i.d., GD/X 25 nylon Syringe Filter, Whatman Inc., Piscataway, NJ) before injection. β-Glucan MW standards (catalog no. P-MWBG, Megazyme) with MW values of 3.59×10^5, 2.45×10^5, 1.83×10^5, 1.23×10^5, and 0.40×10^5 g/mol were used to estimate the actual MW ranges of the extracted β-glucan fractions. The peak MW and number-average MW (M_n) were obtained by a first-order polynomial curve of log MW versus retention time of the HPLC chromatogram.[18]

Proximate Composition. Moisture concentration of the extracted β-glucan was analyzed by using AACC method 44-15A.[20] The concentrations of β-glucan in extracts were measured enzymatically by using AACC method 32-23, with the application of a Mixed β-glucan linkage kit (Megazyme). Starch concentration was determined with AACC method 76-13 by using a Total Starch Kit (Megazyme). Proteins were analyzed by using an automatic nitrogen analyzer (Elementar Analzen System GmbH, Germany) with a nitrogen conversion factor of 6.25. Levels of total, soluble, and insoluble dietary fiber in extracts were determined by using a kit (Megazyme) according to AACC method 32-07.[20] All analyses were run in triplicate and the averages reported on a dry weight basis.

Water Solubility. Water solubility of extracted β-glucan with different MWs was determined according to the method of Park et al.[21] The β-glucan concentration was measured by using AACC method 32-23,[20] with the following minor modification. 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 the concentration of β-glucan in the supernatant was analyzed. The solubility was calculated as follows: water solubility (%) = (β-glucan weight dissolved in the supernatant)/(initial weight of β-glucan in the dispersion) × 100.

In Vitro Bile Acid Binding. In vitro bile acid binding of β-glucan fractions with different MWs was determined by using previously published procedures.[17,22] 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. Cholestyramine (Sigma-Aldrich Co.) was used as a positive and cellulose (Sigma-Aldrich Co.) as a negative control.[22] Fifty milligrams of cholestyramine, cellulose, and β-glucan extracts was digested with 1 mL of 0.01 N hydrochloric acid and incubated in a shaking water bath at 37 °C for 1 h, which simulated gastric digestion. The pH of the materials was then adjusted to 6.9 with 0.1 N sodium hydroxide. Four milliliters of bile acid mixture (1.4 μmol/mL) and 5 mL of porcine pancreatin (Sigma-Aldrich Co.; activity at least equivalent to 8 × USP specifications; 6.25 mg/mL in a 50 mM phosphate buffer, pH 6.9) were added and incubated in a shaking water bath at 37 °C for 1 h to digest the starch and protein. After centrifugation 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. The 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, Bray Co., Wicklow, Ireland). The mixtures were diluted to fall within the range of the test kit. The concentration of bile acid was calculated on the basis of a standard curve developed from the bile acid at different concentrations.

In Vitro Fermentation. In vitro fermentation of β-glucan extracts with different levels of MW was conducted by a batch fermentation system in an anaerobic environment for 24 h with human fecal flora.[17] The inocula 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. The fermentation medium was prepared immediately by diluting feces (1:3, w/v) with brain−heart infusion (BHI; Difco Laboratories, Detroit, MI) medium according to the method of Zheng et al.[23] Homogeneous fecal slurries were filtered through four layers of cheesecloth, and 2 mL of filtered inocula was added to each serum bottle containing 100 mg of β-glucan extracts prehydrated in 8 mL of BHI medium overnight at 4 °C. The headspace of the bottle was flushed with CO_2, and the serum bottles were sealed with PTFE/silicone septa and aluminum caps (Supelco Inc., Bellefonte, PA). The bottles were incubated in a shaking water bath at 37 °C for 0, 2, 4, 8, 12, and 24 h. A blank without any substrate and lactulose (Sigma-Aldrich Co.) as a completely fermentable substrate were prepared as controls. 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 100 μL of saturated mercury chloride ($HgCl_2$) solution, and the pH was measured. The fermented mixture was then centrifuged at 3100*g* for 10 min, and a 1 mL aliquot from the supernatant was removed for SCFA analysis.

Short-Chain Fatty Acid (SCFA) Analysis. SCFAs, including acetate, propionate, butyrate, valerate, isobutyrate, and isovalerate, were analyzed according to the method described in Sayar et al.[19] A 1 mL aliquot of fermentation solution was mixed with 100 μL of 2-ethylbutyric acid as an internal standard and acidified by 0.5 mL of hydrochloric acid. The SCFA was extracted with 3 mL of diethyl ether, and 1 mL of ether layer was transferred and derivatized by 100 μL of *N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoroacetamide (MTBSTFA; Sigma-Aldrich Co.) at 80 °C for 20 min. After 24 h of standing at room temperature to complete derivatization, 1 μL of silylated derivative was injected into a HP 5890 GC (Hewlett-Packard, Palo Alto, CA) equipped with a flame ionization detector. The column was an SPB-5 (30 m × 0.25 mm i.d. × 1 μm d_f; Supelco Inc.), and helium was used as carrier

Table 1. Molecular Weight Distributions and Chemical Composition of the Extracted β-Glucan Fractions with Different Molecular Weights

	MW[a] ($\times 10^5$ g/mol)		composition[a] (%, dry weight basis)				
						dietary fiber	
β-glucan MW fraction	M_n[b]	peak MW	β-glucan	starch	protein	SDF[c]	IDF[d]
high	7.09 ± 0.44 a	8.98 ± 0.50 a	89.0 ± 3.9 ab	1.4 ± 0.3	1.5 ± 0.4	90.9 ± 3.5	3.5 ± 0.8
L1	3.48 ± 0.77 b	4.87 ± 1.07 b	86.3 ± 1.6 ab	1.5 ± 0.2	1.7 ± 0.2	91.0 ± 1.7	3.4 ± 0.6
L2	2.42 ± 0.04 c	2.79 ± 0.07 c	88.7 ± 4.5 ab	1.4 ± 0.5	1.6 ± 0.2	89.4 ± 4.1	3.6 ± 0.2
L3	1.61 ± 0.23 cd	1.80 ± 0.09 cd	84.6 ± 2.2 b	1.6 ± 0.3	1.8 ± 0.3	89.5 ± 5.1	3.3 ± 0.9
L4	0.87 ± 0.06 d	0.91 ± 0.02 d	91.6 ± 3.6 a	1.4 ± 0.4	1.6 ± 0.2	92.2 ± 1.5	3.7 ± 0.2
L5	0.46 ± 0.20 e	0.53 ± 0.28 e	88.5 ± 1.0 ab	1.2 ± 0.2	1.9 ± 0.3	91.0 ± 2.5	3.2 ± 0.5

[a] Values are the mean ± standard deviation. Values followed by different letters within a column are significantly different ($p < 0.05$). [b] Number-average MW. [c] Soluble dietary fiber including β-glucan. [d] Insoluble dietary fiber.

gas. The oven temperature was kept at 70 °C for 3 min and programmed to increase to 160 °C at 7 °C/min and stay for 5 min. The injector and detector temperatures were 220 and 250 °C, respectively. SCFAs were identified by comparison with known fatty acid standards (Sigma-Aldrich Co.) and quantified by using the peak area ratio of the analyte to the internal standard.

Statistical Analysis. Data were analyzed by using the analysis of variance (ANOVA), followed by least significant differences (LSD) for comparison among different MW fractions of β-glucan by using the GLM procedure in SAS version 9.1 (SAS Institute, Cary, NC) at α = 0.05.

RESULTS AND DISCUSSION

Characterization of β-Glucan MW Fractions. The MW) and composition of high and low MW (L1−L5) β-glucan fractions extracted from oat flour are shown (Table 1). The number-average MW (M_n) and peak MW of β-glucan extracts were determined from the peak retention time and area of the SE-HPLC chromatograms after hydrolysis. The M_n and peak MW of the high MW β-glucan fraction were 7.09×10^5 and 8.98×10^5 g/mol, respectively, which were similar to the values of high MW β-glucan (6.87×10^5 and 9.50×10^5 g/mol) in our previous study.[17] The M_n of the high MW β-glucan fraction was greatly decreased to 3.48×10^5, 2.42×10^5, 1.61×10^5, 0.87×10^5, and 0.46×10^5 g/mol for L1, L2, L3, L4, and L5 MW fractions, respectively ($p < 0.05$), by using different amounts of lichenase treatment. The peak MW of β-glucan fractions at different MW levels differed ($p < 0.05$). The β-glucan extracts were prepared to provide more MW divisions than in our previous study to better isolate the optimum MW of β-glucan providing maximum physiological effects.[17] The peak MW of the high MW β-glucan fraction in the current study was lower (8.98×10^5 g/mol) than those from other studies ($10.0-20.0 \times 10^5$ g/mol).[3,24,25] These different values might be attributed to oat type, environmental or growth factors, and methods used for extraction, purification, and determination.[25] In the current study, the extraction procedure with hot water and added thermostable α-amylase may cause the presence of small fractions of β-glucan with lower peak MW in the extracts.[26] The estimated MW also can vary according to the standards used in the SE-HPLC procedure. For example, in the current study, we used β-glucan calibration standards obtained from Megazyme ($(0.4-3.59) \times 10^5$ g/mol), whereas, in our study by Yao et al.,[18] we used pullulan standards ($(0.48-16.6) \times 10^5$ g/mol) to evaluate the same oat line, N979-5-4, grown in the years 2002, 2003, and 2004, obtaining peak MWs of 41.2×10^5, 38.5×10^5, and 31.6×10^5 g/mol instead of the 8.98×10^5 g/mol in the current study. Although the peak MW of the high MW β-glucan fraction in the current study was lower than those of other studies reported, the viscosity of high MW β-glucan suspension (1% w/v) was much greater (peak viscosity = 7986 cP) than those of the other low MW fractions (55−1331 cP, data not shown); thus, the MW range represented in the current study provided appropriate materials for evaluation.

The extracted β-glucan fractions with different MWs contained 85−92% β-glucan, which was greater than in our previous study (64.1−66.6%).[17] In the current study, the amounts of thermostable α-amylase and pancreatin during β-glucan extraction from oat flour were increased from 130 U/g of oat flour and 1 mg/g of oat flour to 200 U/g and 1.3 mg/g, respectively. Likely, the increased amounts of enzymes to remove starch and protein helped to extract and yield greater concentrations of β-glucan from oat flour. Several studies also reported that the extraction yield of β-glucan varies depending on the techniques, such as solvents, temperature, incubation condition, and time.[24,25]

The starch and protein concentrations in the extracts were 1.2−1.6 and 1.5−2.0%, respectively ($p > 0.05$) (Table 1), as most of the starch and protein in the oat flour were removed during treatment with α-amylase and pancreatin. Insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) including β-glucan were 3.2−3.7 and 89.4−92.2%, respectively. Most of the contents in the extracted β-glucan MW fractions were SDF, that is, β-glucan. The differences between SDF and β-glucan concentrations ranged from 0.6 to 4.9%. Other complex carbohydrates, such as resistant oligosaccharides, might not be measured by either of the methods for β-glucan analysis or SDF analysis.[27]

Water Solubility of β-Glucan MW Fractions. The water solubility of the extracted β-glucan fractions with high, L1, L2, L3, L4, and L5 MW were 56.9, 82.2, 83.4, 87.3, 80.8, and 79.8%, respectively (Table 2). The high MW β-glucan fraction dissolved in water at a much lower concentration than the lower MW β-glucan fractions. The high MW β-glucan had a lower diffusion rate in solution,[28] which lowered its solubility. As the MW of β-glucan decreased, the water solubility increased initially but decreased later. The β-glucan with the lowest MW of 0.46×10^5 g/mol had the lowest solubility among the low MW β-glucan fractions. This was also observed in the study of Tosh et al.[16] The tendency of cereal β-glucan molecules to associate in aqueous solutions has been reported.[29,30] Once the molecules are sufficiently low in size to become mobile enough in solution, they

Table 2. Water Solubility and in Vitro Bile Acid Binding of the Extracted β-Glucan Fractions with Different Molecular Weights

		bile acid bound[a]			
β-glucan MW fraction	water solubility[a,b] (%)	μmol/100 mg total,[c] dwb	relative % to cholestyramine[d]	μmol/100 mg β-glucan,[e] dwb	relative % to cholestyramine[d]
cholestyramine		10.77 ± 0.07 a	100.00	10.77 ± 0.07 a	100.00
cellulose		0.04 ± 0.01 d	0.33	0.04 ± 0.01 d	0.33
high	56.9 ± 5.3 b	2.45 ± 0.58 c	22.79	2.76 ± 0.66 c	25.64
L1	82.2 ± 1.3 a	3.03 ± 0.03 bc	28.09	3.51 ± 0.03 bc	32.57
L2	83.4 ± 3.0 a	3.33 ± 0.30 b	30.95	3.77 ± 0.53 b	35.01
L3	87.3 ± 1.4 a	3.32 ± 0.18 b	30.79	3.87 ± 0.14 b	35.90
L4	80.8 ± 2.2 a	2.99 ± 0.52 bc	27.78	3.28 ± 0.71 bc	30.48
L5	79.8 ± 3.4 a	2.34 ± 0.52 c	21.70	2.64 ± 0.57 c	24.50

[a] Values are the mean ± standard deviation. Values with different letters within a column are significantly different ($p < 0.05$). [b] % β-glucan solublized in water. [c] Calculated on the basis of 100 mg total sample weight of cholestyramine, cellulose, and extracted β-glucan with different MWs. [d] Relative bile acid binding percentage (%) when cholestyramine is considered to bind 100% bile acid. [e] Calculated on the basis of 100 mg of β-glucan in the extracted β-glucan.

diffuse into a position where they can aggregate with each other like the formation of a gel.[16] Likely, the β-glucan molecules of L4 and L5 MW fractions aggregated together, resulting in lower water solubility.

In Vitro Bile Acid Binding. The total amount of bile acid initially added in tests of in vitro bile acid binding of cholestryramine, cellulose, and high and low MW β-glucan fractions was 11.2 μmol/100 mg of treatment (Table 2). The positive control, cholestyramine, a component of a drug frequently used to treat hyperlipidemia, bound 10.77 μmol of bile acid/100 mg of cholestyramine. The negative control, cellulose, a nonbinding bile acid fiber, bound only 0.04 μmol of bile acid/100 mg of cellulose. If the amount bound by cholestyramine were considered to bind bile acid at 100%, then the amount bound by cellulose would be calculated as 0.4% (= 0.04 μmol/10.77 μmol × 100%). These values of both controls are similar to the results given for cholestyramine and cellulose in other studies.[22,31]

The relative bile acid binding values of high and low MW β-glucan fractions were calculated on the basis of 100% bile acid bound to cholestyramine to eliminate methodological effects. Values were 22.8, 28.1, 31.0, 30.8, 27.8, and 21.7% for high, L1, L2, L3, L4, and L5 MW β-glucan, respectively (Table 2). The bile acid binding values also were calculated on the basis of 100 mg of β-glucan (Table 2) to evaluate the effect of MW on bile acid binding to β-glucan. The high MW and the lowest MW (L5) β-glucan fractions bound the least amount of bile acid per 100 mg of total weight and per 100 mg of β-glucan among all fractions ($p < 0.05$). The L2 (2.42×10^5 g/mol) and L3 MW (1.61×10^5 g/mol) β-glucan fractions bound greater amounts of bile acid per 100 mg of total weight and per 100 mg of β-glucan than the high MW and L5 MW fractions. The high MW and the lowest MW (L5) β-glucan fractions bound the least amount of bile acid among all fractions ($p < 0.05$). The L2 (2.42×10^5 g/mol) and L3 MW (1.61×10^5 g/mol) β-glucan fractions bound greater amounts of bile acid than the rest of the β-glucan fractions. The major component of β-glucan fractions with different MW levels was β-glucan, a SDF (Table 1); thus, the different bile acid binding values of β-glucan extracts could be mostly attributed to the β-glucan component.

The relative bile acid binding values (converted on the basis of 100% bile acid binding to cholestyramine) per 100 mg of β-glucan at different average MWs (M_n) obtained from the current study and our previous study results[17] were combined (Figure 1) to demonstrate the optimum MW of β-glucan for bile acid binding. As the MW of β-glucan decreased, the bile acid binding increased initially and decreased later in a pattern similar to that of water solubility (Table 2). These data suggest that the low solubility of β-glucan resulted in low bile acid binding. The low mobility of the high MW β-glucan fraction and the high self-aggregation of the lowest MW β-glucan (L5) likely reduced the bile acid binding to β-glucan. Our results indicated that the optimum MW of β-glucan providing the greatest bile acid binding, under the conditions presented, ranged from 1.61×10^5 to 2.42×10^5 g/mol, demonstrating the importance of determining solubility, in addition to MW, for the formulation and development of oat-containing food products to obtain a desired physiological effect.

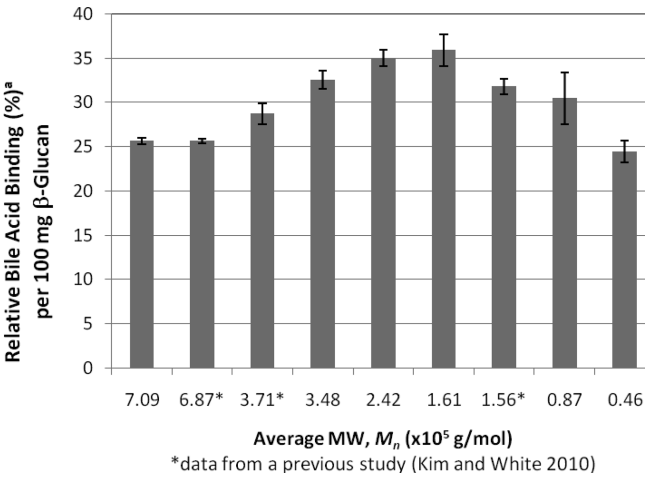

Figure 1. Relative bile acid binding percentage of β-glucan extracts with different average MWs. Relative bile acid binding values (%) were calculated by assuming a bile acid binding value of cholestyramine of 100%.

Bile acids synthesized in the liver from cholesterol are reabsorbed and transported to the liver, where they are extracted efficiently and again secreted into the bile, a process described as enterohepatic circulation.[32] Therefore, eliminating bile acids from the circulation or reducing the reabsorption of bile acids consumes cholesterol and lowers the serum cholesterol level.

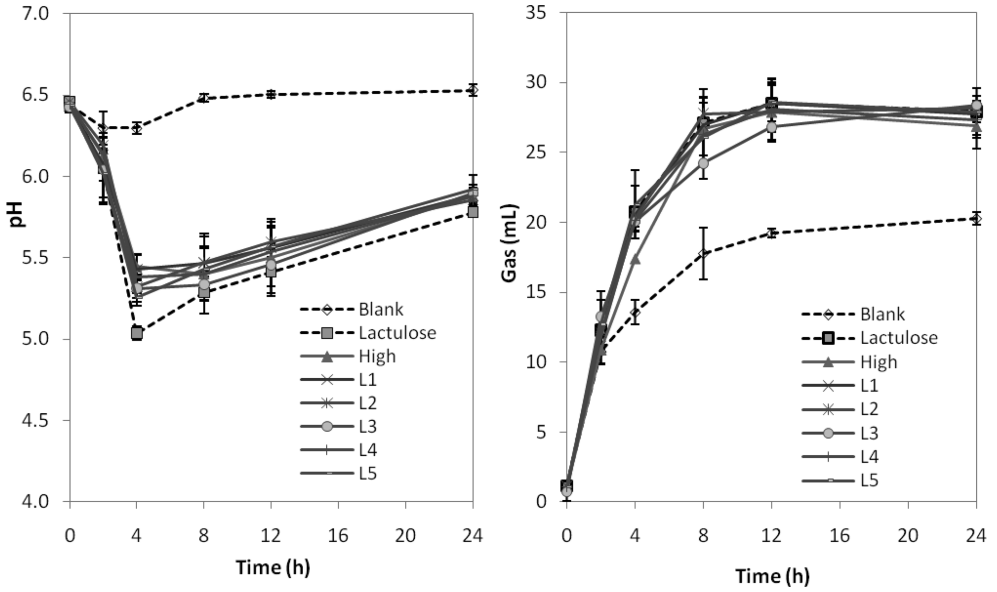

Figure 2. pH changes and total gas production during in vitro fermentation of the blank, lactulose, and the extracted β-glucan fractions with different MWs.

Table 3. Total Short-Chain Fatty Acid (SCFA) Production during in Vitro Fermentation of the Extracted β-Glucan with Different Molecular Weights

	total SCFA[a] (mM) at a fermentation time of					
β-glucan MW fraction	0 h	2 h	4 h	8 h	12 h	24 h
blank	2.1 ± 0.2	8.3 ± 0.2 b	15.2 ± 0.2 b	18.5 ± 0.2 b	24.4 ± 0.2 d	29.9 ± 2.1 c
lactulose	2.0 ± 0.1	11.8 ± 0.9 a	21.3 ± 2.4 a	24.1 ± 0.1 ab	30.5 ± 0.1 bc	34.8 ± 1.0 b
high	2.1 ± 0.2	10.4 ± 1.3 ab	20.6 ± 2.3 a	23.4 ± 0.2 ab	32.3 ± 1.2 abc	34.0 ± 2.6 b
L1	2.1 ± 0.1	9.7 ± 1.9 ab	20.3 ± 0.9 a	27.0 ± 0.2 a	33.5 ± 1.2 ab	36.9 ± 1.4 a
L2	1.9 ± 0.3	9.7 ± 0.8 ab	21.0 ± 0.4 a	23.3 ± 0.2 ab	34.8 ± 1.9 a	36.8 ± 1.9 a
L3	2.0 ± 0.1	10.1 ± 2.0 ab	21.4 ± 0.6 a	26.6 ± 0.5 a	35.2 ± 1.3 a	36.5 ± 2.8 a
L4	2.2 ± 0.1	9.7 ± 1.0 ab	21.2 ± 0.9 a	24.4 ± 0.2 ab	32.3 ± 1.2 abc	37.3 ± 1.6 a
L5	2.0 ± 0.1	9.7 ± 1.4 ab	18.2 ± 0.1 ab	20.8 ± 0.1 ab	28.2 ± 1.0 cd	33.0 ± 2.0 bc

[a] Values are the mean ± standard deviation. Values with different letters within a column are significantly different ($p < 0.05$).

Likely, β-glucan lowers the cholesterol level by preventing bile acid reabsorption in the ileum, thus increasing bile acid excretion in the feces,[31,32] although its mechanism of interaction in bile acid binding still needs further investigation.

In Vitro Fermentation. Progress of in vitro fermentation of the blank, lactulose, and β-glucan MW fractions (L1−L5) measured by pH changes, gas production, and total SCFA formation during 0, 2, 4, 8, 12, and 24 h of fermentation is shown (Figure 2 and Table 3). Included were a blank with no substrates as a negative standard, and lactulose, a positive standard for complete fermentation in the colon. The pH of all treatments decreased rapidly between 0 and 4 h of fermentation, followed by a slight increase toward the end of fermentation (24 h, Figure 2). The pH of lactulose dropped sharply to pH 5.0. The pH of β-glucan extracts with different MWs decreased from 6.4−6.5 to 5.3−5.4, with no differences among MW fractions. This pH drop resulted from SCFA formation during fermentation.[33] The slight increase in the pH after 4 h of fermentation may be attributed to the production of different metabolites in the media, such as ammonia.[34] The low pH caused by the production of SCFA can promote positive physiological effects in the colon, such as preventing colon cancer and protecting against pathogenic bacteria.[35] Total gas production of all treatments increased as fermentation proceeded from 0 to 24 h (Figure 2). Lactulose and β-glucan MW fractions produced greater amounts of gas than did the blank, but did not differ from each other. These results of pH and gas production are consistent with those observed for whole oat flour and purified β-glucan with high MW in other studies.[17,19]

Total SCFA formation from the blank, lactulose, and β-glucan MW fractions continuously increased as fermentation time increased from 0 to 24 h (Table 3). The production of SCFA lowered pH and was highly correlated (R = 0.9) to total gas production during in vitro fermentation. Lactulose tended to produce fewer SCFA than did the β-glucan MW fractions, except for the L5 MW β-glucan fraction. After 24 h of fermentation, there were no differences in total SCFA formation among L1, L2, L3, and L4 MW fractions; however, the high MW and L5 MW fractions produced lower amounts of total SCFA than the other MW β-glucan fractions (Table 3). Therefore, β-glucan fractions in the range of 0.87×10^5−3.48×10^5 g/mol MW produced

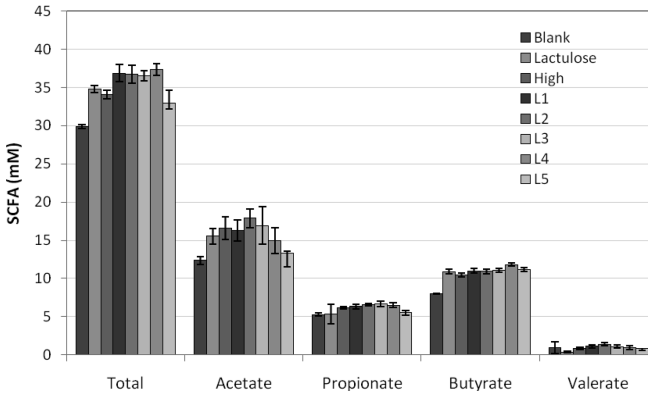

Figure 3. Effect of molecular weight on the total and individual short-chain fatty acid production after 24 h of in vitro fermentation (values are the mean ± standard deviation).

greater amounts of SCFA than did those at high or low MW after 24 h of fermentation. Acetate, propionate, and butyrate, among all SCFAs, were the main SCFAs, and small amounts of isobutyrate, valerate, and isovalerate were produced from all treatments during in vitro fermentation (Figure 3). The total of the three main SCFAs accounted for 90−94% of the total SCFAs produced. Acetate was produced in the greatest amount, followed by butyrate and propionate. The β-glucan extracts tended to produce more propionate than did lactulose, and the L2, L3, and L4 MW fractions produced greater amounts than the high and L5 MW β-glucan fractions (Figure 3).

Some health benefits of β-glucan are attributed to the production of SCFAs.[4,9,35] The SCFAs taken up from the colon are metabolized to contribute to energy needs. Acetate is known as the main metabolite and the primary substrate for cholesterol synthesis.[9] The production of propionate has been reported to lower blood glucose and insulin levels and raise high-density lipoprotein (HDL) cholesterol and triglycerides.[36−38] Rectal infusions of propionate reduced serum cholesterol, whereas acetate alone increased serum cholesterol.[39] Butyrate also has gained attention for its potential to promote colon health, as it is the preferred metabolic substance for colonocytes.[4] Health benefits of β-glucans suggested by the SCFA profiles during in vitro fermentation would likely be affected by the molecular features of the oat β-glucans (Table 3 and Figure 3). Our previous study showed that the low MW β-glucan fraction (1.56×10^5 g/mol) produced greater quantities of SCFA than the high MW (6.87×10^5 g/mol) after 24 h of fermentation.[17] In the current study, the low MW β-glucan fractions (0.87×10^5−3.48×10^5 g/mol) produced greater SCFAs than the high MW β-glucan; however, the lowest MW β-glucan fraction (0.46×10^5 g/mol) produced less total SCFAs than fractions having slightly greater MW. These SCFA results positively correlated with water solubility and in vitro bile acid binding (Table 2). The low solubility of the lowest MW β-glucan fraction was related to both in vitro bile acid binding ($R = 0.8$) and SCFA production during in vitro fermentation ($R = 0.7$). The solubility (or dispersion) of β-glucan has been reported to be responsible for the biological activity of β-glucan.[8,40] Cheng et al.[41] noted that increased water solubility can enhance the physiological activities of β-glucan.

β-Glucan fractions, containing 85−92% of β-glucan from oat, were treated with lichenase to contain different MWs. The highest (7.09×10^5 g/mol) and lowest MW (0.46×10^5 g/mol) β-glucans bound less bile acid than did the L2 (2.42×10^5 g/mol) and L3 (1.61×10^5 g/mol) β-glucan MW fractions. No differences were found in total SCFA formation among L1, L2, L3, and L4 fractions, having MWs of 0.87×10^5−3.48×10^5 g/mol, respectively, after 24 h of in vitro fermentation. The high and L5 MW fractions produced the lowest amounts of SCFAs. These physiological effects of β-glucan fractions were related to the water solubility of β-glucan: the high MW fraction had the lowest water solubility values. The optimum MW of β-glucan to provide potential biological function, under the conditions of this study, ranged from 2.42×10^5 to 1.61×10^5 g/mol, lower than the high MW, that is, the original MW of β-glucan in oat. This study demonstrated the importance of knowing both the solubility and MW of β-glucan in the development and formulation of food products to obtain a desired physiological effect.

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