Molecular Weight of β-Glucan Affects Physical Characteristics, In Vitro Bile Acid Binding, and Fermentation of Muffins

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ABSTRACT

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Muffins containing different amounts and molecular weights (MW) of β-glucan were evaluated for the effect of β-glucan on the physical characteristics of the muffins and on in vitro bile acid binding and fermentation with human fecal flora. Wheat flour muffins were prepared with the addition of β-glucan extracts with high-, medium-, or low-MW. For oat flour muffins, the native oat flour contained high-MW β-glucan; the oat flours were treated to create medium- and low-MW β-glucan within the prepared muffin treatments. For each 60-g muffin, the amounts of β-glucan were 0.52, 0.57, and 0.59 g for high-, medium-, and low-MW β-glucan wheat flour muffins, and 2.38, 2.18, and 2.23 g for high-, medium-, and low-MW β-glucan oat flour muffins, respectively. The lower the MW of the β-glucan in muffins, the lower the height and volume of the muffins. The oat flour muffins were less firm and springy than the wheat flour muffins as measured on a texture analyzer; however, MW had no effect on muffin texture. The oat flour muffins bound more bile acid than did the wheat flour muffins. The muffins with high-MW β-glucan bound more bile acid than did those with low- and medium-MW β-glucan. Muffin treatment affected the formation of gas and total short-chain fatty acids (SCFA) compared with the blank without substrate during in vitro fermentation. There were no differences in pH changes and total gas production among muffin treatments. The high-MW β-glucan wheat flour muffins produced greater amounts of SCFA than did the wheat flour muffin without β-glucan and the oat flour muffins; however, there were no differences in SCFA production among muffins with different MW. In general, the β-glucan MW affected the physical qualities of muffins and some potential biological functions in humans.

Consumption of dietary fiber has many health benefits such as reducing the incidence of obesity, type-II diabetes, gastrointestinal disease, hypercholesterolemia, and coronary heart diseases (Anderson et al 2004b). Specifically, increased consumption of dietary fiber improves serum lipid concentrations, lowers blood pressure, improves blood glucose control in diabetes, and aids in weight loss by increasing satiety (Brown et al 1999; Keenan et al 2002; Anderson et al 2004b; Birketvedt et al 2005). Current recommendations for dietary fiber intake are 20–35 g/day for healthy adults, and an amount equal to the age of a child plus 5 g/day for children >2 years (USDA 2005). However, most people in the United States consume less than half the recommended level of dietary fiber daily despite the widely acknowledged nutritional health benefits of dietary fiber consumption (Park et al 2005). The growing interest of consumers and food scientists in dietary fiber has encouraged the development of high-fiber food products that provide the recommended level of dietary fiber.

Oats are an excellent source of dietary fiber. The (1→3)/(1→4)-β-D-glucan (referred to as β-glucan) is the essential soluble dietary fiber in oats. β-Glucan has two major physiological effects: it lowers cholesterol, especially in people with high cholesterol levels, and it attenuates postprandial blood glucose levels, especially in diabetics (Lazaridou and Biliaderis 2007; Wood 2007). The cholesterol-lowering effect is related to the ability of β-glucan to bind bile acids, lowering the reabsorption of bile acids by increasing fecal excretion of bile acids (Drzikova et al 2005). The cholesterol lowering also occurs as a secondary reaction of microbial fermentation of β-glucan in the large intestine (Malkki and Virtanen 2001; Queenan et al 2007). In 1997, the U.S. Food and Drug Administration (FDA) approved a health claim stating that oat β-glucan at a level of 0.75 g/serving in a product may reduce cholesterol and lower the risk of coronary heart disease (FDA 1997).

Numerous factors such as concentration, structure, and molecular weight (MW) affect the β-glucan health benefits in foods. Oat-bran muffins with 8 g of β-glucan/serving reduced the glycemic response in healthy human subjects in greater amounts than consumption of the same amount of muffins with 4 g of β-glucan/serving; however, the efficacy decreased as the MW of β-glucan decreased (Tosh et al 2008). Alternatively, in another study, oat breads with high- and low-MW had similar cholesterol-lowering effects in human subjects (Frank et al 2004). Understanding the physiological effects of β-glucan with different structural characteristics is vital to developing oat-based food products with desirable health benefits.

The objectives of this study were to develop muffins containing different amounts and MW of β-glucan and to evaluate the effect on physical characteristics of the muffins and on in vitro bile acid binding and fermentation of the muffins with human fecal flora.

MATERIALS AND METHODS

Materials

An experimental oat line, N979-5-4 (unpublished data), developed at Iowa State University was used to produce high-, medium-, and low-MW β-glucan and as flour in the preparation of muffins. 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 ultra-centrifugal mill (ZM-1, Retch GmbH&Co., Haan, Germany) with a 0.5-mm sieve to create oat flour. Oat flours were then stored in plastic bags at 4°C until used.

Other muffin ingredients (all-purpose wheat flour, light brown sugar, baking powder, soybean oil, egg, and salt) were purchased from a local grocery (Hy-Vee, Ames, IA). Each muffin pan was lined with 4.1-cm baking cups (Reynolds, Henrietta, NY) and held 12 muffins with a cup size of 5-cm diameter top × 2-cm height × 3-cm diameter bottom.

Extraction and Hydrolysis of β-Glucan from Oat Flour

Oat flours were refluxed with 82% ethanol (v/v) for 2 hr at 85°C to inactivate endogenous enzymes and to remove fat. Water-soluble β-glucans were extracted from the treated oat flours using water with heat-stable α-amylase and pancreatin (Sigma-Aldrich, St. Louis, MO) according to the procedure of Yao et al (2008) and Kim and White (2010). Extracted β-glucan suspension (defined as high-MW β-glucan) was hydrolyzed using lichenase (1,3- 1,4-β-D-glucan-4-glucanohydrolase; 330 U of lichenase/mg of protein, <0.0001 U of β-glucosidase, cellulase, and endo-1,3-β-glucanase,

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and <0.0004 U of α-amylase and amyloglucosidase; Megazyme International, Wicklow, Ireland) 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 concentrated using a rotary evaporator (Rotavapor R-205, Buchi Labortechnik AG, Postfach, Switzerland) to 1.3% β-glucan (w/v), the maximum amount that could be physically incorporated into muffin batter before the batter became too viscous to handle. Moisture content of the β-glucan extract suspension was measured using a moisture analyzer (MB45, Ohaus, Pine Brook, NJ) at 105°C.

Preparation of Muffins

Two types of muffins, wheat flour muffins and oat flour muffins, were prepared (Table I). The wet and dry ingredients were mixed separately. The wet ingredients were added to the dry ingredients and the batter was stirred (25 strokes) until all of the dry ingredients were wetted. For wheat flour muffins, the extracted β-glucan fractions with different MW (1.3% β-glucan in water) were added to all-purpose wheat flour to create high-, medium-, and low-MW β-glucan muffins. The oat flour muffins were prepared by mixing water with oat flour for the high-MW oat flour muffin treatment. A mixture of oat flour and water was incubated at 50°C for 10 min to produce a medium-MW β-glucan oat flour muffin and incubated with lichenase (0.48 U/g of oat flour) at 50°C for 10 min to produce a low-MW oat flour muffin. A wheat flour muffin without added β-glucan also was prepared to serve as the control muffin. Batter (14 g/muffin) was poured into the paper-lined muffin pans. Muffins were baked in a conventional oven at 205°C for 12 min. After cooling, muffins were removed from the pans and packaged in plastic bags and stored at 4°C until needed for the analyses.

Proximate Composition

All muffin treatments were analyzed for moisture content according to Approved Method 44-15A.02 (AACC International 2010). The concentration of available carbohydrates in muffins was determined by measuring reducing sugars that were converted from acid hydrolysis of total carbohydrates (James 1995). Ground muffins (0.1 g) were mixed with 10 mL of 1.5*M* sulfuric acid and heated in a boiling water bath for 20 min. After cooling, 12 mL of 10% sodium hydroxide was added, mixed, and centrifuged at 3,000 × *g* for 10 min. The supernatants were collected and diluted depending on sugar content. An aliquot (1 mL) of diluted supernatant was transferred to a test tube and mixed with 1 mL of DNS reagent (mixture of 2 g of 3,5-dinitrosalicylic acid [Sigma-Aldrich], 40 mL of 2*M* sodium hydroxide, and 60 g of sodium potassium tartrate tetrahydrate [Sigma-Aldrich] in 100 mL of deionized water) and 2 mL of deionized water. All tubes were heated in a boiling water bath for 5 min to allow the reducing sugar and the DNS reagent to react. After cooling, the absorbance of each solution was monitored spectrophotometrically at 540 nm. A calibration curve was prepared by plotting absorbance against the known concentration of glucose and the % of available carbohydrates in muffins was determined as % of available carbohydrate = glucose concentration/weight of muffin × dilution factor × 100.

Starch content of the muffin treatments was analyzed using Approved Method 76-13.01 using the Total Starch Kit (Megazyme International Ireland) (AACC International 2010). Total dietary fiber (TDF) was determined using a kit (Megazyme) (AACC Approved Method 32-07.01; AOAC Official Method 991.43). The β-glucan concentrations in muffins were analyzed enzymatically using Approved Method 32-23.01 with a mixed β-glucan linkage kit (Megazyme) (AACC International 2010). Proteins were determined by using an automatic nitrogen analyzer (Elementar Analysen System GmbH, Germany) with a nitrogen conversion factor of 6.25. Fat was analyzed by the gravimetric method after extraction with a mixture of petroleum ether and 2-propanol (3:2) in a Goldfisch system (AACC Approved Method 30-25.01). All analyses were run in triplicate and the average reported on a dry weight basis.

TABLE I
Muffin Formulations

Ingredients (g)	Wheat Flour Muffins	Oat Flour Muffins
All purpose wheat flour	31.5	–
Oat flour	–	31.5
Baking powder	1.8	1.8
Salt	0.1	0.1
Sugar	15.0	15.0
Dry milk, skim	6.0	6.0
Vegetable oil	5.0	5.0
Egg	15.0	15.0
β-Glucan solution (1.3% w/v)	35.0	–
Water	–	35.0
Total	109.4	109.4

Physical Characteristics of Muffins

Muffin height was measured from the highest point of the muffin to the paper muffin cup bottom with a micrometer (Mitutoyou, Tokyo, Japan). The volume of muffins was measured using seed displacement according to Approved Method 10-05.01 (AACC International 2010). The moisture loss (%) upon baking was determined by measuring the weight of muffins before and after baking and calculated as Moisture loss (%) = (weight of muffin batter – weight of muffin after baking)/weight of muffin batter × 100. Texture analyses, including firmness and springiness, were performed at room temperature using a texture analyzer (Stable Micro Systems TA-XT2, Texture Technologies, Scarsdale, NY) with Texture Expert v.1.11 software (Stable Micro Systems). A method based on Approved Method 74-09.01 (AACC International 2010) for bread firmness was developed for muffins. The upper rounded side of the muffin was cut off to leave a height of 20 mm. The muffin without the round top was compressed using an 11-mm diameter cylinder probe (TA-212) with test speed at 1.0 mm/sec to a depth of 5.0 mm. The up and down motion was repeated three times at the same location on the muffin, recording the force required for each descent into the muffin. Four muffins were tested for each different treatment.

Molecular Weight Determination of β-Glucan

Water-soluble β-glucans were extracted from the treated muffins using the method described above in extraction of β-glucan from oat flour. Relative MW distributions of extracted β-glucan from muffins were analyzed by using size-exclusion high-performance liquid chromatography (SE-HPLC) (Sayar et al 2007). The SE-HPLC included a solvent delivery module (model 210, ProStar, Varian, 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). The temperatures of the column and detector were 40°C. The flow rate of the mobile phase, MilliQ water (Milipore, Bedford, MA) containing 0.02% sodium azide, was 0.5 mL/min. Samples were filtered through a 0.45-µm nylon syringe filter (25 mm, i.d., Whatman, NY) before the injection. β-Glucan MW standards (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 β-glucans. 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 as M_n =

$\sum w_i/\sum(w_i/MW_i)$, 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 (Yao et al 2007).

Structural Features of β-Glucan

Structural features of oat β-glucans extracted before and after baking of wheat flour muffins were determined using fluorophore-assisted capillary electrophoresis (FACE) after complete hydrolysis using lichenase (Colleoni-Sirghie et al 2003). For complete enzymatic hydrolysis, β-glucans at 2 mg/mL of 20 m*M* sodium phosphate buffer (pH 6.5) were incubated with lichenase at 1 U/mg of β-glucan at 50°C for 24 hr. The mixtures were centrifuged at 10,000 × *g* for 5 min. Supernatants were transferred and evaporated to dryness in a Speed Vac. Dried samples were suspended in 2 µL of 1*M* sodium cyanoborohydride in tetrahydrofuran (Sigma-Aldrich) and 2 µL of 8-amino-1,3,6-pyrenetrisulfonic acid (APTS); 0.1 mg/µL in 15% acetic acid. The reaction was incubated at 42°C overnight and then diluted with 46 µL of MilliQ water (Milipore), vortexed, and centrifuged at 10,000 × *g* for 2 min. A 5-µL aliquot was added to 195 µL of MilliQ water and this was applied to a Beckman P/ACE capillary electrophoresis instrument with the cathode on the injection side and monitored with a laser-induced fluorescence detector (Beckman Instrument, Fullerton, CA) fitted with an argon-ion laser as the excitation source. The oligosaccharides (degree of polymerization; DP 3≈10) were detected according to migration time. The ratio of DP3 to DP4 and the ratio of β-(1→4)/β-(1→3) linkages are reported.

In Vitro Bile Acid Binding of Muffins

In vitro bile acid bindings of wheat flour muffins and oat flour muffins with high-, medium-, and low-MW β-glucan were measured according to previous procedures with modifications (Kahlon and Woodruff 2003; Yao et al 2008). The bile acid mixture was prepared with sodium cholate, sodium deoxycholate, sodium glycocholate, and sodium taurocholate (Sigma-Aldrich) with proportions as 35, 35, 15, and 15% (w/w), respectively, in a 50 m*M* phosphate buffer at pH 6.9. Cholestyramine (a bile acid binding anionic resin) and cellulose (a nonbile acid binding fiber) were used as a positive and a negative control, respectively (Kahlon and Woodruff 2003; Drzikova et al 2005). Muffins, cholestyramine, and cellulose were weighed at 50 mg into centrifugal tubes and digested with 1 mL of 0.01*N* hydrochloric acid in a shaking water bath at 37°C for 1 hr, which simulated gastric digestion.

The solution was adjusted to pH 6.9 with 0.1*N* sodium hydroxide. 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 m*M* phosphate buffer, pH 6.9; to provide amylase, protease, and lipase for digestion) were added and incubated at 37°C for 1 hr in a shaking water bath. Sample mixtures were centrifuged at 3,100 × *g* for 10 min and 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 using a bile acid diagnostic kit (Trinity Biotech, Wicklow, Ireland). Samples were diluted to fall within the range of the test kit. The concentration of bile acid was calculated based on a standard curve developed from the bile acid at different concentrations. The bile acid binding was determined as the difference between the amounts of bile acid added and the amounts of bile acid recovered after in vitro digestion. Bile acid binding was presented as bile acid bound/100 mg of muffin or TDF.

In Vitro Digestion and Fermentation of Muffins

An in vitro digestion process, simulating the human digestion system, was accomplished using human salivary α-amylase (EC 3.2.1.1) and porcine pepsin (EC 3.4.23.1) with pancreatin (from porcine pancreas, Sigma-Aldrich) enzymes before in vitro fermentation according to the method of Sayar et al (2007). The digested residue was collected and freeze-dried for the in vitro fermentation study.

In vitro fermentation was conducted using a batch fermentation system under strict anaerobic conditions for 24 hr with human fecal flora. The anaerobic fermentation medium was prepared with brain heart infusion (Difco Laboratories, Detroit, MI) according to the method of Zheng et al (2000). Digested residues of muffins 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, Bellefonte, PA) and digested residues of muffins were hydrated overnight at 4°C. Blanks without any substrate and lactulose (Sigma-Aldrich) as a completely fermentable substrate were prepared as controls. The inoculums were prepared from fresh feces collected from the volunteers who had not received antibiotics for at least three months and had not suffered from indigestion problems within the previous week. Feces from two volunteers were immediately pooled, mixed with three parts of the fermentation medium, and filtered through four layers of cheesecloth in an Erlenmeyer flask under continuous CO_2 flow. The 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 hr. Total gas production was measured by overpressure in the headspace of the bottle using a digital manometer (Fisher Scientific, Pittsburgh, PA). Fermentation was terminated by adding 0.1 mL of saturated mercury chloride solution. The fermented slurry was transferred to a centrifuge tube and the pH was measured. After centrifugation at 3,100 × *g* for 10 min, a 1-mL of aliquot from the supernatant was taken for the SCFA analysis.

Short-Chain Fatty Acids Analysis

Short-chain fatty acids (SCFA) such as acetate, propionate, isobutyrate, butyrate, iso-valerate, and valerate were analyzed as silyl derivatives by gas chromatography (Sayar et al 2007). An aliquot (1 mL) of fermentation solution 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 using a vortex. The ether layer (1 mL) was transferred and derivatized by 100 µL of *N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoroacetamide (MTBSTFA, Sigma-Aldrich) at 80°C for 20 min. After standing at room temperature in the dark for 24 hr for complete derivatization, 1 µL of material was injected into a gas chromatograph (5890 GC, Hewlett-Packard, Palo Alto, CA). The column was an SPB-5 (30 m × 0.25 mm; Supelco); helium was used as the carrier 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. Injector and detector temperatures were 220 and 250°C, respectively. The SCFA were identified and quantified by comparison with known fatty acid standards.

Statistical Analyses

Data were analyzed using the analysis of variance (ANOVA), followed by least significant difference (LSD) to compare the differences among muffin treatments using GLM procedure (v.9.1, SAS Institute, Cary, NC) at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Characteristics of Muffins with High-, Medium-, and Low-MW β-Glucan

Table II shows the concentrations of total available carbohydrates including starch, total dietary fiber (TDF), β-glucan, and remaining available carbohydrates, protein, and fat in the wheat flour muffins and oat flour muffins with high-, medium-, and low-

MW β-glucan. Wheat flour muffins contained greater concentrations (69.3–71.1%) of total available carbohydrates, including starch, TDF, and remaining available carbohydrates, than did oat flour muffins (59.6–62.6%), along with lower protein and fat concentrations. The starch concentrations of wheat flour muffins were greater than those of oat flour muffins ($P < 0.05$), but there were no differences in starch concentrations between the high-, medium-, and low-MW muffins. Total dietary fiber (TDF), including β-glucan of wheat flour muffins and oat flour muffins, were 4.8–5.9% and 11.4–12.0%, respectively. Oat flour muffins contained greater amounts of TDF than did wheat flour muffins. The remaining available carbohydrates were calculated as total available carbohydrates minus starch and TDF were mainly from sugar added during muffin preparation. The total chemical composition of wheat flour muffins and oat flour muffins accounted for 94–97% of the total weight. Likely, the remaining composition of the muffins was ash (3–4%) and other complex carbohydrates not determined by measuring reducing sugars converted from acid hydrolysis of carbohydrates (Grigelmo-Miguel et al 1999; Frank et al 2004).

A mixture of oat flour and water was incubated at 50°C for 10 min to produce a medium-MW β-glucan oat flour muffin, and incubated with lichenase at 50°C for 10 min to produce a low-MW oat flour muffin. Very little starch and protein hydrolysis would be expected during incubation at this temperature (Belitz and Grosch 1999). This observation is further confirmed in Table II, which reveals no differences in starch and protein composition among oat flour muffin treatments.

The wheat flour muffins and oat flour muffins contained ≈1% and 4% of β-glucan, respectively. The high-, medium-, and low-MW β-glucan fractions extracted from oat flour were concentrated to 1.3% β-glucan in water before being added to the wheat flour muffin batter. The moisture concentration of the β-glucan extract suspension added to the muffin batter was 98%. Previous laboratory work showed the composition of the freeze-dried β-glucan extracts with different MW as 64–67% β-glucan, 5–6% starch, and 4–5% protein (Kim and White 2010). Thus, 0.7% of the β-glucan extract suspension was composed of starch, protein, and remaining carbohydrates. The amounts of β-glucan/serving size in 60-g muffins were 0.52, 0.57, and 0.59 g for high-, medium-, and low-MW β-glucan wheat flour muffins, and 2.38, 2.18, and 2.23 g for high-, medium-, and low-MW oat flour muffins, respectively. Current U.S. FDA guidelines require >0.75 g of β-glucan/serving for a health claim (FDA 1997). Thus, the oat flour muffins provided more than enough β-glucan to meet this claim.

Molecular features (M_n and peak MW) and structural features (DP3/DP4 ratio and β-(1→4)/β-(1→3) linkages ratio) of high-, medium-, and low-MW β-glucan extracted from wheat flour muffins before and after baking are shown in Table III. The M_n and peak MW were determined from the peak retention time of the SE-HPLC chromatograms. The M_n of the high-, medium-, and low-MW β-glucan fractions extracted before muffin baking was 4.58×10^5, 2.08×10^5, and 0.92×10^5 g/mol, respectively. After baking, the M_n of high-, medium-, and low-MW β-glucan in the wheat flour muffins was measured at 2.39×10^5, 1.20×10^5, and 0.62×10^5 g/mol, respectively. The baking process for muffins decreased MW of β-glucan (Beer et al 1997; Anderson et al 2004a).

The capillary gel electrophoretic method using laser-induced fluorescence detection (FACE) enabled full quantification of the cello-oligosaccharides released from β-glucan during hydrolysis with lichenase. High-, medium-, and low-MW β-glucan extracted from wheat flour muffins before and after baking contained mainly DP3 and DP4 (91≈93%) with small amounts of >DP5 (data not shown) after complete hydrolysis of β-glucan. In the β-glucans

TABLE II
Chemical Composition (%, dwb)[a] of Wheat-Flour Muffins and Oat-Flour Muffins with High-, Medium-, and Low-MW β-Glucan

	Carbohydrates Available[b]					
Muffin[a]	Starch	TDF[c]	β-Glucan	Remaining[d]	Protein	Fat
Control	41.7 ± 0.7b	4.8 ± 0.2c	–	23.2 ± 1.3b	13.4 ± 0.9c	13.4 ± 0.3d
W-H	43.1 ± 0.9a	5.5 ± 0.4b	0.95 ± 0.05b	22.0 ± 1.5b	13.3 ± 0.2c	13.1 ± 0.8c
W-M	41.4 ± 0.2b	5.9 ± 0.3b	0.86 ± 0.15b	22.0 ± 1.5b	13.2 ± 0.0c	12.8 ± 0.3e
W-L	40.1 ± 0.3c	5.8 ± 0.4b	0.98 ± 0.08b	25.2 ± 1.7a	13.8 ± 0.1c	12.6 ± 0.1e
O-H	33.8 ± 0.4d	12.0 ± 0.4a	3.96 ± 0.22a	16.6 ± 1.5cd	15.3 ± 0.1b	17.3 ± 0.5b
O-M	33.7 ± 0.9d	11.4 ± 0.8a	3.64 ± 0.18a	17.5 ± 0.8c	16.1 ± 0.4b	15.5 ± 0.3c
O-L	33.0 ± 0.9d	11.6 ± 0.7a	3.71 ± 0.15a	15.0 ± 1.1d	17.3 ± 0.6a	18.4 ± 0.3a

[a] Control, wheat flour muffin without β-glucan; W, wheat flour muffin; O, oat flour muffin; H, M, and L, muffins containing high-, medium-, and low-MW β-glucan.
[b] Mean values ± SD. Values followed by different letters within a column are significantly different ($P < 0.05$).
[c] Total dietary fiber included β-glucan.
[d] Calculated as total available carbohydrates minus starch and total dietary fiber concentration.

TABLE III
Molecular and Structural Features of β-Glucans in Wheat Flour Muffins Before and After Baking, and Molecular Weight of Oat Flour Muffins After Baking

	Molecular Features[b]				Structural Features[b]			
	M_n[c] (× 10^5 g/mol)		Peak MW (× 10^5 g/mol)		DP3/DP4 Ratio		β-(1→4)/β-(1→3) Linkage Ratio	
Muffin[a]	Before	After	Before	After	Before	After	Before	After
W-H	4.58a*	2.39a	6.27a*	3.61a	1.86b*	2.14a	2.54*	2.51
W-M	2.08b*	1.20b	3.21b*	1.58b	2.02a	2.06b	2.53	2.53
W-L	0.92c*	0.62c	1.88c*	0.66c	1.92ab*	2.01c	2.53	2.54
O-H	–	2.46a	–	3.19a	–	–	–	–
O-M	–	1.18b	–	1.14b	–	–	–	–
O-L	–	0.48c	–	0.40c	–	–	–	–

[a] W, wheat flour muffin; O, oat flour muffin; H, M, and L, muffins containing high-, medium-, and low-MW β-glucan.
[b] Values followed by different letters within a column and a muffin type (wheat or oat flour) are significantly different ($P < 0.05$). Values with an asterisk, within a muffin treatment, indicate a significant difference before and after baking ($P < 0.05$).
[c] M_n, number-average MW.

from oat flours, Colleoni-Sirghie et al (2003) reported DP3 and DP4 accounted for 89≈90% of the total oligosaccharides measured. In the current study, the ratio of DP3/DP4 of β-glucan extracted from wheat flour muffins increased after baking. This result may be related to the decrease of MW after baking, as just noted, in that higher MW β-glucans are broken into smaller cello-oligosaccharides with lower MW and more chains of DP3 length. The ratios between β-(1→4) and β-(1→3) linkages were similar for before and after baking.

After baking, the M_n and peak MW of the high-, medium-, and low-MW β-glucan in the oat flour muffins were similar to the corresponding values per high-, medium-, and low-MW β-glucan in the wheat flour muffins. Also, the M_n and peak MW of high-, medium-, and low-MW β-glucan all differed within the same muffin type ($P < 0.05$). During preparation of the oat flour muffins, the β-glucan MW was allowed to decrease during mixing and incubating of the oat flour in the water at 50°C for 10 min. The reduction of MW was caused by endogenous enzymes such as β-glucanase in the oat flour. All the β-glucan MW in this study were in the MW range reported for oat or wheat breads: 1.0–9.0 × 10^5 g/mol (Aman et al 2004; Frank et al 2004). Similar to findings in the current study, the MW of β-glucans decreased during baking of oat bran muffins and breads made with hull-less barley and wheat flour during studies of Beer et al (1997) and Anderson et al (2004a). Endogenous enzymes in oat flours might be used to create oat-flour-based food products with optimum MW for desirable health effects.

Physical Characteristics of Muffins with High-, Medium-, and Low-MW β-Glucan

The height, volume, moisture loss upon baking, and texture analyses of the wheat flour muffins and oat flour muffins with high-, medium-, and low-MW β-glucan are given in Table IV. The height and volume of wheat flour muffins were not different among different MW treatments. In oat flour muffins, the MW of β-glucan affected the height and volume of muffins. The lower the MW of the β-glucan in oat flour muffins, the lower the height and volume of muffins. Oat flour muffins with low-MW β-glucan had the least viscous batter and the lowest height and volume among muffin treatments after baking. A decrease in viscosity of batter has been associated with an increase in bubble buoyancy and subsequent low height and volume (Lee et al 2005; Lakshminarayan et al 2006). In the current study, the greater quantity of TDF, including β-glucan, in the oat flour muffins (11% TDF and 4% β-glucan) likely reduced the height and volume when compared with the wheat flour muffins (5% TDF and 1% β-glucan). In other work, the addition of dietary fiber to baked products decreased height and volume (Pomeranz et al 1977).

The percent of moisture loss upon baking varied from 17.0% to 20.2%. The high-MW muffin lost less moisture than the low-MW muffin. Likely, the high water-holding capacity of high-MW β-glucan (Tosh et al 2008) reduced water loss during baking. The degraded β-glucan molecule in low-MW oat flour muffin treatment likely had less water-holding capacity.

The oat flour muffins were less firm and springy than wheat flour muffins as measured on a texture analyzer; however, the MW had no effect on muffin texture (Table IV). Likely, the lack of gluten in the oat flour muffins decreased the viscoelastic nature of the batter and the density, thereby decreasing the force needed for compression (Grigelmo-Miguel et al 1999). Thus, the oat flour muffins had less protein structure along with more gummy fiber, which resulted in a less firm product. Further study investigating the sensory qualities of wheat flour muffins with high-, medium-, and low-MW β-glucan and oat flour muffins with high-, medium-, and low-MW β-glucan will help determine the relationship of these mechanical texture parameters and sensory attributes.

In Vitro Bile Acid Binding of Muffins

In vitro bile acid binding of cholestyramine, cellulose, wheat flour muffins, and oat flour muffins with high-, medium-, and low-MW β-glucan (dwb) are shown in Table V. The total bile acid added was 11.2 μmol/100 mg of treatment.

TABLE IV
Physical Characteristics[a] of Wheat Flour Muffins and Oat Flour Muffins with High-, Medium-, and Low-MW β-Glucan

Muffin[b]	Height (mm)	Volume (mL)	Moisture Loss (%)	Firmness (g)	Springiness (g)
Control	34.5 ± 0.1a	43.5 ± 2.0a	20.2 ± 0.5a	73.4 ± 5.7a	69.4 ± 5.4a
W-H	35.4 ± 0.9a	38.7 ± 1.0b	18.8 ± 0.4c	62.1 ± 1.8b	58.4 ± 1.4b
W-M	34.6 ± 0.3a	37.9 ± 0.3b	19.1 ± 0.1bc	77.2 ± 7.7a	71.0 ± 6.8a
W-L	34.6 ± 0.4a	38.3 ± 0.6b	19.5 ± 0.3ab	70.5 ± 4.7a	66.2 ± 4.9a
O-H	35.6 ± 0.2a	39.0 ± 0.6b	17.0 ± 0.5d	38.0 ± 1.9d	34.6 ± 1.7d
O-M	32.2 ± 0.2b	35.8 ± 1.2c	17.4 ± 0.3d	46.2 ± 1.1c	41.8 ± 3.5c
O-L	29.2 ± 0.5c	33.7 ± 1.4c	19.8 ± 0.5a	30.7 ± 2.1e	28.2 ± 2.2e

[a] Mean values ± SD. Values followed by different letters within a column are significantly different ($P < 0.05$).
[b] Control, wheat flour muffin without β-glucan; W, wheat flour muffin; O, oat flour muffin; H, M, and L, high-, medium-, and low-MW β-glucan.

TABLE V
In Vitro Bile Acid Binding by Wheat Flour Muffins and Oat Flour Muffins with High-, Medium-, and Low-MW β-Glucan

	Bile Acid Bound[b]		
Muffin Treatments[a]	Treatment (μmol/100 mg, dwb)	Relative % to Cholestyramine[c]	TDF (μmol/100 mg, dwb)[d]
Cholestyramine	9.811 ± 0.071	100.0	–
Cellulose	0.019 ± 0.001	0.2	–
Control	1.957 ± 0.003d	20.0	–
W-H	2.027 ± 0.003c	20.7	0.369 ± 0.005a
W-M	1.948 ± 0.033d	19.9	0.330 ± 0.003b
W-L	1.940 ± 0.036d	19.8	0.334 ± 0.003b
O-H	2.217 ± 0.049a	22.6	0.185 ± 0.004c
O-M	2.086 ± 0.019b	21.3	0.183 ± 0.002c
O-L	2.095 ± 0.024b	21.4	0.181 ± 0.003c

[a] Control, wheat flour muffin without β-glucan; W, wheat flour muffin; O, oat flour muffin; H, M, and L, high-, medium-, and low-MW β-glucan.
[b] Mean values ± SD. Values followed by different letters within a column are significantly different ($P < 0.05$).
[c] Relative bile acid bound percentage (%) when cholestyramine is considered to bind 100% bile acid.
[d] Calculated on the basis of total dietary fiber (TDF), including β-glucan, composition of the muffins.

Cholestyramine, a component of a drug frequently used to reduce cholesterol, was used as a positive control for bile acid binding. It bound 9.811 μmol of bile acid/100 mg of cholestyramine (dwb). Cellulose, a negative control, bound only 0.019 μmol of bile acid/100 mg of cellulose (dwb). If the amount bound by cholestyramine were considered to bind bile acid at 100%, then the amount bound by cellulose would be calculated as 0.2% (0.019 μmol/9.811 μmol × 100%).

The relative bile acid binding values of wheat flour muffins without β-glucan and with high-, medium-, and low-MW β-glucan were calculated as 20.0, 20.7, 19.9, and 19.8%, respectively. Bile acid binding values of oat flour muffins with high-, medium-, and low-MW β-glucan were 22.6, 21.3, and 21.4%, respectively. The oat flour muffins bound more bile acid than did the wheat flour muffins. Muffins with high-MW β-glucan bound more bile acid than did the low- and medium-MW β-glucan.

For further examination, bile acid binding effects were evaluated by calculating binding values based on TDF (including β-glucan) concentration in muffins, which was previously determined to correlate with bile acid binding (Kahlon and Woodruff 2003; Drzikova et al 2005; Sayar et al 2005). Unlike the values of bile acid binding/100 mg of muffin, wheat flour muffins exhibited greater bile acid binding values expressed as μmol of bile acid/100 mg of TDF than did oat flour muffins (Table V). These data suggested that components other than TDF and β-glucan or interactions of TDF with other components may have affected bile acid binding.

Two mechanisms have been proposed for the effect of β-glucan on bile acids. One mechanism suggested that β-glucan may inhibit the reabsorption of bile acid merely by increasing the viscosity of ileum fluids (Bowles et al 1996; Wood et al 2002). The other suggested that binding may occur between β-glucan and micelles formed from bile acid and fatty acids rather than from isolated bile acid alone (Bowles et al 1996). Further study is needed to explore these hypotheses.

In Vitro Fermentation of Muffins

Muffins were digested to 58–68% of the original weight before in vitro fermentation. The in vitro fermentation progress of the blank, lactulose, and the digested residues of wheat flour muffins and oat flour muffins was monitored by pH changes, total gas production, and total SCFA formation over 0, 2, 4, 8, 12, and 24 hr of fermentation. The pH of all treatments decreased up to 4–8 hr of fermentation and then slightly increased until the end of fermentation (Fig. 1). Total gas production of muffins with high-, medium- and low-MW β-glucans increased as fermentation proceeded. Muffin treatments containing β-glucan affected pH changes and gas production compared to the blank without substrate during in vitro fermentation. Lactulose, a standard for complete fermentation in the colon, resulted in the lowest pH and the greatest gas production during fermentation ($P < 0.05$). However, there were no differences in pH and total gas production among wheat flour muffins without β-glucan and with different MW β-glucans and any of the oat flour muffins. These trends are similar to the results from previous work with raw oat flours and extracted β-glucan fractions (Kim and White 2009, 2010); however, in the current study, higher pH and lower gas production were observed.

The SCFA production for all treatments increased at fermentation time of 0–24 hr (Table VI). Lactulose tended to produce greater amounts of total SCFA than did muffins at 8 hr of fermentation. Total SCFA amounts did not differ among all muffin treatments at 8 hr of fermentation. At 12 and 24 hr of fermentation, the wheat flour muffin treatments produced greater amounts of SCFA than did lactulose and the oat flour muffin treatments. At 24 hr of fermentation, the high-MW β-glucan wheat flour muffin treatment (W-H) produced greater amounts of SCFA than did the wheat flour muffin without β-glucan (control), or the oat flour muffin treatments (O-H, O-M, and O-L). Likely, components other than the β-glucan of the digested residues of wheat flour muffins also contribute to the formation of SCFA during in vitro fermentation. The components in muffins may interact with β-

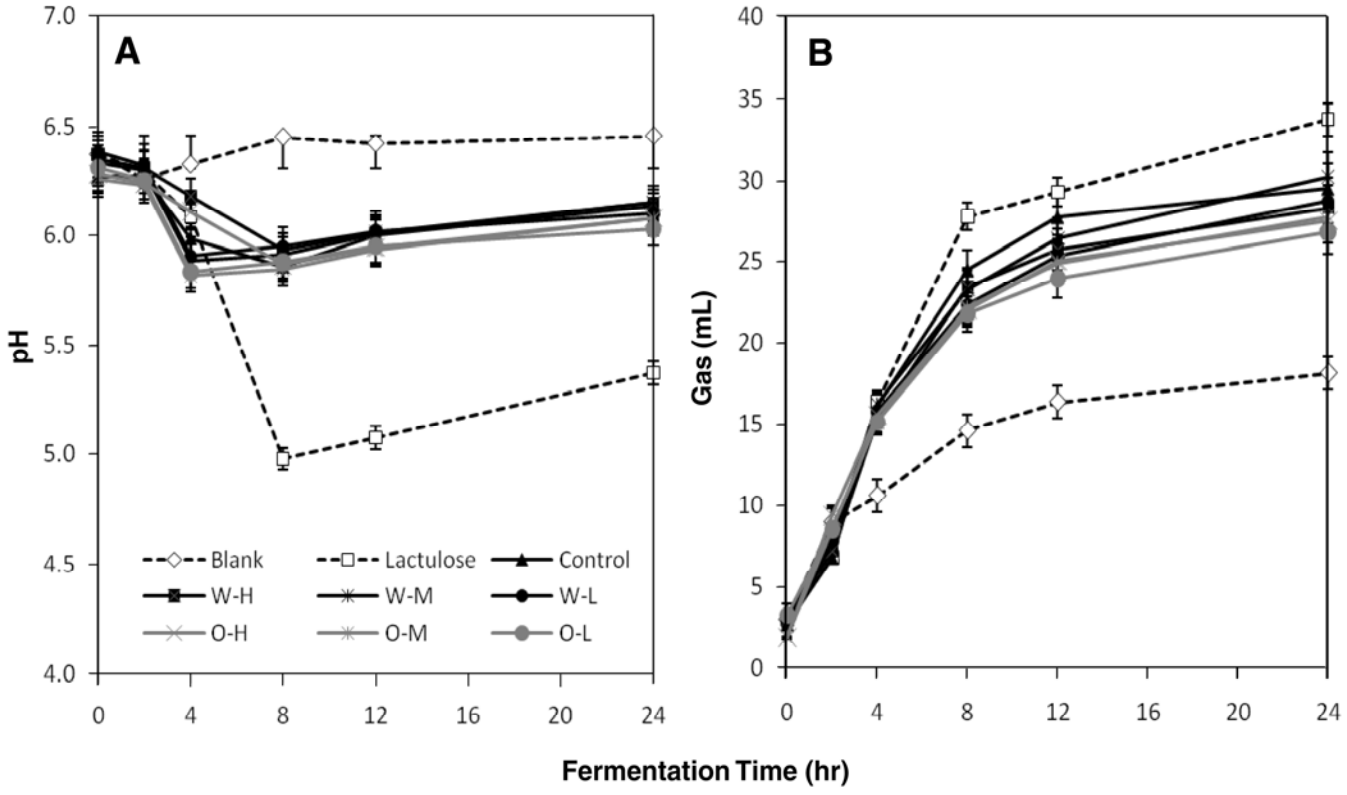

Fig. 1. pH Changes (**A**) and total gas production (**B**) during in vitro fermentation of wheat flour muffins and oat flour muffins with high-, medium-, and low-MW β-glucan.

TABLE VI
Total Short-Chain Fatty Acid (SCFA, m*M*) Production During In Vitro Fermentation of Wheat Flour Muffins and Oat Flour Muffins with High-, Medium-, and Low-MW β-Glucan

Muffin Treatments[a]	Fermentation Time[b]					
	0 hr	2 hr	4 hr	8 hr	12 hr	24 hr
Blank	2.66 ± 0.04	5.76 ± 0.33	11.12 ± 0.86	25.94 ± 2.29	30.44 ± 0.50	39.93 ± 1.89
Lactulose	2.91 ± 0.36	5.32 ± 0.18	16.16 ± 0.45	36.92 ± 1.09	37.81 ± 1.46	47.37 ± 1.10
Control	2.44 ± 0.21a	5.60 ± 0.47a	13.60 ± 0.57a	30.53 ± 2.38a	41.16 ± 1.44a	46.54 ± 0.85b
W-H	2.95 ± 0.43a	5.73 ± 0.45a	13.36 ± 0.78ab	27.68 ± 1.86ab	40.29 ± 2.24a	49.14 ± 1.38a
W-M	2.67 ± 0.03a	5.63 ± 0.13a	13.28 ± 1.61ab	29.17 ± 3.17a	42.49 ± 1.55a	49.01 ± 1.34a
W-L	2.44 ± 0.01a	4.95 ± 0.34b	12.95 ± 0.75b	26.10 ± 1.63b	42.15 ± 0.53a	47.90 ± 0.76a
O-H	2.78 ± 0.11a	5.62 ± 0.23a	11.83 ± 1.55b	30.57 ± 2.84a	38.40 ± 2.37b	44.98 ± 0.57c
O-M	2.51 ± 0.09a	4.25 ± 0.47b	13.84 ± 0.47a	28.57 ± 0.46a	39.15 ± 1.53b	43.83 ± 0.92c
O-L	2.75 ± 0.14a	4.52 ± 0.20b	14.58 ± 0.47a	29.03 ± 1.69a	36.83 ± 2.55b	43.48 ± 1.37c

[a] Control, wheat flour muffin without β-glucan; W, wheat flour muffin; O, oat flour muffin; H, M, and L, high-, medium-, and low-MW β-glucan.
[b] Mean values ± SD. Values followed by different letters within a column are significantly different ($P < 0.05$).

TABLE VII
Molar Proportions of Acetate, Propionate, Butyrate, and Valerate After 24 hr of In Vitro Fermentation of Wheat Flour Muffins and Oat Flour Muffins with High-, Medium-, and Low-MW β-Glucan

Muffin Treatments[a]	SCFA[b]			
	Acetate	Propionate	Butyrate[c]	Valerate[d]
Lactulose	57a	13c	28d	2
Control	50b	17ab	30c	3
W-H	51b	17ab	29cd	3
W-M	50b	16b	31c	3
W-L	48c	18a	31c	3
O-H	42e	18a	38a	2
O-M	44d	19a	34b	3
O-L	43d	18a	35b	3

[a] Control, wheat-flour muffin without β-glucan; W, wheat flour muffin; O, oat flour muffin; H, M, and L, high-, medium-, and low-MW β-glucan.
[b] Values followed by different letters within a column are significantly different ($P < 0.05$).
[c] Included iso-butyrate.
[d] Included iso-valerate.

glucan or contribute on their own (Liu et al 2010). The wheat flour muffins had greater amounts of starch than did the oat flour muffins (Table II). Thus, after in vitro digestion of muffins, the undigested starch polysaccharides may have undergone fermentation with the fecal microorganisms, which could produce enzymes catalyzing hydrolysis of starch polysaccharide molecules (BeMiller and Huber 2008). Starch polysaccharides not cleaved during in vitro digestion may be broken down and utilized by the fecal bacteria during in vitro fermentation, producing SCFA.

Acetate, propionate, and butyrate were the main SCFA formed from in vitro fermentation and small amounts of iso-butyrate, valerate, and iso-valerate were produced from all treatments. In all treatments (Table VII), acetate tended to be produced in the greatest proportions, followed by butyrate and propionate. Oat flour muffin treatments tended to produce more butyrate than did the wheat flour muffin treatments. The production of propionate and butyrate at high concentrations is physiologically important because propionate reduces serum cholesterol levels by converting to glucose in the liver and butyrate helps to stimulate cell proliferation and protect against colon cancer (Chen et al 1984; Topping and Clifton 2001). Casterline et al (1997) reported that fermentation of β-glucan produced propionate and butyrate in greater amounts than did starch, pectin, and resistant starch. Similar results occurred in the current study, with the oat flour muffins containing the most β-glucan, producing more propionate and butyrate than the wheat flour muffins. Thus, despite the greater amounts of total SCFA formed during microbial fermentation from wheat flour muffins, the oat flour muffins likely would provide better health benefits.

CONCLUSIONS

The amounts of β-glucan/60 g of muffin were 0.52, 0.57, and 0.59 g for high-, medium-, and low-MW β-glucan wheat flour muffins, and 2.38, 2.18, and 2.23 g for high-, medium-, and low-MW β-glucan oat flour muffins, respectively. The low-MW of β-glucan in the oat flour muffins reduced the height and volume of muffins. The oat flour muffin with high-MW β-glucan bound more bile acid than did the wheat flour muffins, and low- and medium-MW β-glucan oat flour muffins. The high-MW β-glucan wheat flour muffins produced greater amounts of SCFA than did the wheat flour muffin without β-glucan or the oat flour muffins; however, the oat flour muffins produced more butyrate, which is more physiologically important than acetate. These results demonstrate the importance of β-glucan MW to the sensory qualities of food products containing oats, and to the potential biological functions in humans when consumed as a dietary fiber.

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