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Processing Affects the Physicochemical Properties of β-Glucan in Oat Bran Cereal

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The tendency of mixed linkage oat β-glucan to form viscous solutions is generally assumed to be related to its ability to lower serum cholesterol levels in humans. However, the association has not been clearly demonstrated. To conduct a clinical trial showing the relationship between LDL-cholesterol levels and viscosity, a series of extruded oat bran cereals were prepared in which the β-glucan had a range of molecular weights and modified solubility. An extraction protocol using physiological enzymes at 37 °C was used to estimate the effect that the cereals would have on gut viscosity. By reducing the molecular weight from 1,930,000 to 251,000 g/mol, the apparent viscosity in the physiological extract dropped from 2900 to 131 mPa·s (at 30 s^{-1}). Microscopic examination showed that as the extrusion conditions were made more severe, to cause depolymerization, the integrity of the cell walls was lost and β-glucan dispersed throughout the cereal. Differences in the hardness and density of the extruded cereals were also evident as the molecular weight was reduced.

KEYWORDS: Oats; β-glucan; breakfast cereal; solubility; molecular weight

INTRODUCTION

The (1→3)(1→4)-β-D-glucan (β-glucan) found in oats has been widely studied because consumption results in reduced serum cholesterol levels in human subjects (*1*, *2*) and also attenuated serum glucose and insulin levels after a meal (*3*, *4*). Newer research suggests that it also affects gut hormones associated with satiety (*5*) and modulates immune function (*6*). Lower levels of serum cholesterol, specifically the low-density lipoprotein (LDL) cholesterol fraction, are associated with a reduced risk of developing coronary heart disease (*7*). Accordingly, various jurisdictions have approved health claims for oats, the first of which was in the United States (*7*). More recently, the European Food Safety Authority has indicated that it will allow a claim that consumption of oat β-glucan can help maintain normal blood cholesterol (*8*).

Although the overall trend shows a reduction in serum cholesterol during the consumption of oat products (*1*, *2*), the magnitude of this effect has been variable, and in some cases no statistically significant effect was reported (*9*). In allowing a health claim, the U.S. FDA (*7*) suggested a number of possible explanations for this variability, including poor solubility or reduced molecular weight of the β-glucan in the food consumed, which would decrease the ability of the β-glucan to form viscous solutions in the gut.

Ready-to-eat (RTE) cereals are commonly used in clinical trials to study the effect of β-glucan on serum cholesterol (*10*−*15*). In recent studies, subjects were fed between 3.0 and 7.3 g of β-glucan per day. Five of the studies showed a significant reduction in LDL-cholesterol (*10*−*14*), whereas a study specifically designed to test the effect of oat β-glucan on normocholesterolemic subjects showed no significant effect on LDL-cholesterol (*15*). For these six trials (*10*−*15*), which all included RTE cereals, the average LDL-cholesterol reduction was 0.32 mmol/L or 0.064 mmol/L per gram of β-glucan/day, showing that, despite the high temperatures and shear used for extrusion, the β-glucan in RTE cereals remains effective in lowering serum cholesterol levels. By comparison, a meta-analysis comparing studies that had used a variety of oat foods (*2*) reported a lesser reduction of 0.037 mmol/L per gram of soluble fiber per day.

Unlike LDL-cholesterol reduction, a relationship between β-glucan's ability to form viscous solutions and reduction in postprandial glucose levels has been demonstrated. The viscosity of drinks made from oat β-glucan isolate was varied both by changing the concentration and by reducing the molecular weight (*3*), and peak blood glucose rise in human subjects was shown to increase with decreasing drink viscosity. A similar response of peak blood glucose rise to concentration and molecular weight of oat β-glucan has been demonstrated using solid foods (*16*−*18*). The influence that the β-glucan in solid foods would have on the viscosity of the gut contents was estimated using a 37 °C extraction method, with pH changes and enzymes similar to those found in the upper gastrointestinal (GI) tract (*19*). There was an exponential relationship between the peak blood glucose rise and the product of molecular weight and concentration of soluble β-glucan (PBGR ∝ log(MW × c_e)).

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Table 1. Extrusion Parameters Used in Production and Physical Characteristics of Extruded Cereals[a]

name	3H	4M	3M	4L	W
extrusion temperature[b] (°C)	181	220	228	237	160
water (%)	18.7	14.5	10	7	16
standard mechanical energy (Wh/kg)	135	125	145	148	84
bulk density (g/L), $n = 5$	270 (11)	170 (10)	160 (9)	160 (10)	325 (25)
color, $n = 3$					
L^*	75.2 (0.1)	71.6 (0.1)	69.3 (0.1)	62.2 (0.2)	57.0 (0.2)
a^*	4.6 (0.1)	7.6 (0.1)	8.2 (0.1)	9.8 (0.1)	8.7 (0.1)
b^*	24.9 (0.2)	26.3 (0.1)	25.3 (0.1)	26.2 (0.2)	23.6 (0.1)
hardness (N), $n = 3$	974 (6)	531 (8)	610 (13)	567 (15)	464 (18)
work of shearing (N·s), $n = 3$	5090 (140)	3600 (150)	3900 (200)	3900 (310)	1800 (80)

[a] Standard deviation is shown in parentheses. The number of replicates (n) is indicated. [b] At die head.

Although viscosity is also thought to play a major role in the cholesterol-lowering ability of oat β-glucan (*7*, *8*), it has not been demonstrated in clinical trials, as it has for glycemic response. The development of viscosity caused by β-glucan is thought to result in decreased mixing of gut contents, which then inhibits absorption of fat and cholesterol (*20*, *21*). Viscosity is also thought to decrease reabsorption of bile acids from the gut, which results in compensatory synthesis of bile acids from hepatic cholesterol (*20*, *21*). These viscosity-dependent mechanisms would be affected by the physicochemical properties of the β-glucan in the foods. There are exponential relationships between viscosity and either concentration or molecular weight (*22*). Thus, if the β-glucan is partially depolymerized or has low solubility in a food, the expected gut viscosity may be greatly reduced.

To test the hypothesis that development of viscosity in the gut is necessary for serum LDL-cholesterol reduction, a set of foods was required that would contain different quantities of soluble β-glucan of different molecular weights. Despite the high temperatures and shear rates used in extruders, β-glucan molecular weight in commercial extruded breakfast cereals remains high (*5*, *23*). However, preliminary experiments showed that it was possible to manipulate β-glucan molecular weight by increasing the temperature and shear beyond what is commonly used and decreasing the water added during extrusion. The objective of this study was to measure the effects of changing the extrusion parameters on the physical as well as nutritional characteristics of oat bran cereal and compare these with a wheat bran cereal. Changes in color, texture, and microstructure were measured. The proximate analyses of the oat bran cereals were compared with a wheat bran cereal. To determine whether the cereals were likely to change the viscosity of the gut contents, an in vitro digestion protocol was used to extract the β-glucan from the cereal (*19*) and the viscosity of the extract was measured.

MATERIALS AND METHODS

Oat bran cereals were produced from a premix containing OatWell oat bran, corn flour, fructose, and salt. The control cereal was made from a premix of wheat bran, sucrose, barley malt flour, and salt. The cereals were processed in a Bühler BCTG 62/200 industrial twin-screw extruder. Water was injected into the first stage of the extruder. The temperature and standard mechanical energy (SME, a measure of the energy input during the extrusion process) were increased from the typical conditions, as indicated in **Table 1**, to cause depolymerization of the β-glucan during extrusion.

One kilogram samples were removed from the production line at five points during the production run of each product. These samples were mixed to produce a representative sample. The representative sample was divided into subsamples for the different analyses. The samples are named 3H, 4M, 3M, and 4L in order of decreasing molecular weight and increasing solubility for consistency with a companion clinical trial paper (*24*). The control wheat bran cereal is designated W. The physical characteristics and nutrient composition were measured before the beginning of the clinical trial. The total β-glucan content was measured before the beginning of the trial and three more times during the trial. The characteristics of the soluble β-glucan were measured before the clinical trial and twice more during the clinical trial to ensure that the cereal was not changing over time.

Physical Properties. Cereal density was determined by weighing a tared 1 L container of cereal. The average and standard deviation of five replicates are reported.

Color measurements were performed on ground cereal samples using a HunterLab LabScan XE Spectrocolorimeter (Hunter Associates Laboratory Inc., Reston, VA). Each sample was measured using a 2.5 in. glass sample cup that was placed over a sample port with a 2 in. aperture and covered with a sample cup cover. Readings were taken using the 10° observer and the D65 illuminant. CIE $L^*a^*b^*$ values were recorded, where L^* measures black (0) to white (100), a^* measures green to red (negative is green, positive is red), and $3b^*$ measures blue to yellow (negative is blue, positive is yellow). The average and standard deviation of three replicates are reported.

Shear tests were performed on cereal samples using the TA.HDPlus Texture Analyzer (Stable Micro Systems, Godalming, Surrey, U.K.). Intact cereal (1.5 g) was weighed into a five-blade miniature Kramer Shear Press, and a constant cross-head speed of 1.0 mm/s was applied to a depth of 20 mm. The force required to shear and extrude the sample through the slots in the base of the cell was recorded. The maximum force and work of shearing (total area under the shearing curve) were used as indices of hardness and total shearing force. The average and standard deviation of three replicates are reported.

Nutrient Composition. The nutritional values of the cereals were determined to evaluate whether the different extrusion conditions had an effect on the macronutrient profile. The nitrogen in the cereals was determined by combustion and converted to protein using a factor of 5.7 (AOAC 990.03). Fat was determined gravimetrically (AOAC 933.05), moisture by oven-drying (NMKL 23), and ash by combustion at 550 °C overnight (NMKL 173). Sugars were extracted with water at 85 °C for 15 min and analyzed by anion exchange HPLC (Dionex Corp., Sunnyvale, CA) with an electrochemical detector. Starch was determined by enzymatic digestion to glucose (AOAC 996.11) and dietary fiber by the standard gravimetric method (AOAC 985.29). Total β-glucan content in the cereals was determined by the enzymatic assay (AOAC 995.18) using a commercial assay kit (Megazyme International, Bray, County Wicklow, Ireland). Total β-glucan was measured at four different time points during the clinical trial on duplicate samples. To determine the molecular weight distribution of total β-glucan, ground cereal was extracted by stirring in 0.1 N NaOH at room temperature for 2 h. The dispersion was filtered through a 0.45 μm filter, and the peak molecular weight (M_p) was measured by size exclusion HPLC. The sample was injected into a Shodex (Showa Denko K.K., Tokyo, Japan) OHpak SB-806 M column (with OHpak guard) followed by a Waters Ultrahydrogel linear column (40 °C) using a Waters 717plus autosampler and eluted at 1 mL/min in 0.1 M Tris buffer (pH 8.0) with a Shimadzu model LC-20AT pump. A Shimadzu LC-10ATVP pump was used for post-column addition of Calcofluor (20 mg/L in 0.1 M Tris buffer, pH 8.0, at 1 mL/min) (Calcofluor White M2R New, C.I. 40622, fluorescent brightener 28, American Cyanamid Co., Bound

Table 2. Nutrient Composition of Oat Bran and Control Wheat Bran Cereals[a]

	premix	3H	4M	3M	4L	W
moisture (%), $n = 2$	7.2 (0.4)	4.5 (0.2)	3.6 (0.2)	3.5 (0.2)	3.3 (0.2)	5.6 (0.3)
protein (N × 5.7) (%), $n = 2$	14.9 (1.6)	15.4 (1.7)	15.6 (1.7)	16.1 (1.7)	15.0 (1.6)	13.9 (1.5)
crude fat (%), $n = 2$	3.5 (0.3)	3.4 (0.3)	3.8 (0.3)	3.5 (0.3)	3.5 (0.3)	5.6 (0.5)
ash (%), $n = 2$	3.1 (0.3)	3.3 (0.3)	3.1 (0.3)	3.2 (0.3)	3.2 (0.3)	5.6 (0.5)
starch (%), $n = 2$	36.0 (3.6)	35.1 (3.5)	36.3 (3.5)	37.0 (3.8)	37.6 (3.8)	11.0 (1.5)
sugars[b] (%), $n = 2$	8.37 (0.6)	7.07 (0.5)	7.17 (0.5)	6.05 (0.5)	5.60 (0.4)	15.01 (1.5)
total dietary fiber (%), $n = 2$	25.4 (2.5)	27.8 (2.7)	27.7 (2.8)	28.5 (2.8)	28.3 (2.8)	38.3 (3.8)
total β-glucan (%), $n = 24$	14.53 (0.2)	14.95 (0.52)	14.20 (0.58)	14.22 (0.52)	14.08 (0.40)	2.21 (0.34)
peak molecular weight (g/mol), $n = 4$	2,484,000 (180,000)	1,930,000 (200,000)	950,000 (150,000)	527,000 (42,000)	251,000 (20,000)	422,000 (22,000)

[a] The data are presented on a wet weight basis. Standard deviation is shown in parentheses. The number of replicates (n) is indicated. [b] Includes glucose, fructose, sucrose, and maltose.

Brook, NJ), enabling fluorescence detection in a Shimadzu RF-10Axl fluorescence detector (excitation, 360 nm; emission, 450 nm). Fluorescence intensity was collected by a Viscotek DM 400 data manager. Data integration was performed using TriSEC 3.0 (Viscotek, Houston, TX) software. Five β-glucan molecular weight standards (20,000−1,200,000 g/mol), both prepared in-house (*25*) and obtained commercially (Megazyme International), were used to construct a calibration curve for β-glucan by plotting retention time versus log M_p.

Microscopy. Two layers of Whatman no. 1 filter paper were placed in Petri dishes and moistened with deionized water; samples of each formulation were placed on the filter paper to hydrate with minimal solubilization or leaching of components. The cereal samples were allowed to hydrate for 4 h at 4 °C before sectioning. For sectioning, samples were mounted and frozen onto stubs using Tissue-Tek OCT Compound (Miles Laboratories, Elkhart, IN), and 6 μm sections were cut at −20 °C and placed on Fisherbrand Superfrost/Plus glass slides (Fisher Scientific). Sections were allowed to dry and bond to the slides overnight before staining and microscopy. For autofluorescence, sections on slides were mounted in CFM-1 Mountant solution (Electron Microscopy Sciences, Hatfield, PA) before application of the coverslip. All fluorescence microscopy was performed on a Zeiss AxioPlan2 Imaging microscope equipped with epi-illumination, using a UV filter cube (dichromatic beam splitter and exciter/barrier filter set with maximum transmission at 365 and 420 nm). For analysis of β-glucan distribution, sections were stained for 1 min in 0.01% Calcofluor in 25 mM Tris buffer (pH 8.0), gently rinsed twice with deionized water, mounted in Acid Fuchsin (C.I. 42685, Fisher Scientific; 0.01% in 1% acetic acid), and imaged immediately. Images were captured using a Zeiss Axiocam digital camera. For the unprocessed cereal premix, approximately 10 mg was measured into a 2 mL microcentrifuge tube, 1.0 mL of Calcofluor (as above) was added, the solution vortexed and allowed to stand for 1 min, then centrifuged briefly at 10,000 rpm to pellet. The supernatant was drawn off and discarded, and the pellet was washed and pelleted briefly twice with deionized water. The final pellet was suspended in 0.5 mL of Acid Fuchsin, and droplets were placed on slides and covered with a coverslip before microscopic analysis.

Physiological Extraction. An in vitro extraction method essentially as developed by Beer and co-workers (*19*) was used. The cereal was ground to pass a 30 mesh screen, and 10 g was dispersed in 95 mL of phosphate buffer (pH 6.9). The suspension was treated with 58.5 U of salivary α-amylase (EC 3.2.1.1, from human saliva) for 15 min with mild agitation at 37 °C. The pH was then adjusted to 2.0 with HCl, 700 U of pepsin (EC 3.4.23.1, from porcine stomach) was added, and agitation was continued for another 30 min. The pH was then adjusted to 6.9 with NaOH, 0.625 mg of pancreatin (EC 232.468.9, from porcine pancreas) was added, and mild agitation at 37 °C was continued for an additional 90 min. Enzymes were purchased from Sigma-Aldrich (Oakville, ON, Canada). The final volume of the digest was calculated (100−105 mL). The mixture was centrifuged (10 min at 9000*g*), and the characteristics of the β-glucan in the supernatant were determined. Samples were taken at three time points before and during the clinical trial to ensure that the solubility and molecular weight were stable. Each sample was analyzed in duplicate.

Characterization of β-Glucan Solubilized at 37 °C. The viscosity of the supernatant was measured using a controlled strain rheometer (ARES, TA Instruments, New Castle, DE) fitted with a cone-and-plate geometry (angle = 0.04 radians, diameter = 50 mm). Apparent viscosity was measured at 37 °C in a shear rate range of 400−0.1 s^{-1}. Viscosities measured at the shear rate of 30 s^{-1} were used for calculations, primarily for consistency with earlier publications. The molecular weight distribution of the extracted β-glucan was measured using size exclusion HPLC with post-column Calcofluor addition and a fluorescence detector as described above. Duplicate measurements were made on each of the supernatants.

To determine the solubility of the β-glucan at 37 °C, the concentration in the extract (c_e) was determined using flow injection analysis (FIALab Instruments, Bellevue, WA) essentially as described by Jørgensen (*26*). A standard curve was prepared by solubilizing pure β-glucan (Megazyme International) in water (1 mg/mL) at 90 °C for 2 h and diluting to concentrations from 10 to 100 μg/mL. The extracts were diluted with water to give β-glucan concentrations in the same range. The standards and extracts were mixed 1:1 with 50 mg/L Calcofluor solution (in 0.1 M Tris-HCl, pH 8.0) in the FIA, and the fluorescence intensity was measured (excitation, 360 nm; emission, 450 nm). It should be noted that the Calcofluor binding and, therefore, the increase in fluorescence response decline linearly as the molecular weight decreases below 20000 g/mol. The percent extractable (solubilized) β-glucan was calculated from the two values (% soluble β-glucan = soluble β-glucan/total β-glucan × 100). Duplicate measurements were made on each of the supernatants.

Statistical Analysis. Statistical analysis was performed in Analysis ToolPak for Microsoft Excel. One-way ANOVAs were performed on all data sets. Linear regression was performed to demonstrate significant trends. Student t tests were used for individual comparisons.

RESULTS

Physical Properties. The OatWell cereal, 3H, had dense pieces that were consistent in size and shape. With increasing temperature and SME, the pieces expanded more as they exited the extruder. There was a decrease in bulk density from 270 to 160 g/L (**Table 1**), although all had lower density than the control wheat bran cereal ($p < 0.002$). As the temperature and pressure were increased, CIE $L^*a^*b^*$ values indicated that the cereal became darker ($p < 0.0001$) and redder ($p < 0.0001$), but all oat cereals were lighter than the control wheat cereal ($p < 0.001$).

Nutrient Composition. Increasing the temperature and shear of extrusion resulted in progressively lower moisture content ($p = 0.002$) (**Table 2**). As the extrusion conditions became more harsh, the sugars were degraded through nonenzymatic browning, resulting in a decrease in sugar content ($p = 0.02$) and a darkening of the cereal. Other macronutrients were not affected by the changes in extrusion parameters ($p > 0.05$). The average β-glucan content in the finished cereals was 14.4 ± 0.6% (wwb) compared to 2.21 ± 0.34% in the wheat bran cereal. In the cereal premix, the peak molecular weight of the β-glucan was outside the range of the standard curve and was estimated to be approximately 2,450,000 g/mol. There was minimal depolymerization of the β-glucan during typical commercial processing (3H), but each increase in temperature and SME had the desired effect of reducing the molecular weight (**Table 2**). An exponential relationship ($p < 0.0001$) was observed between the product of the standard mechanical energy and temperature and the resulting molecular weight of the oat bran cereals (**Figure 1**).

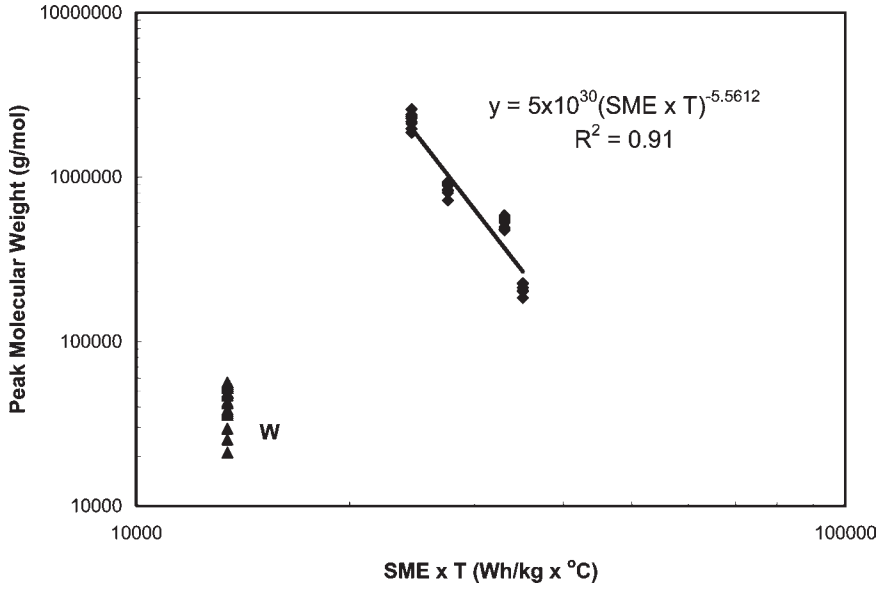

Figure 1. Relationship between standard mechanical energy and temperature at the extruder head and the molecular weight of the β-glucan in the cereals. 3H, 4M, 3M, and 4L are oat cereals; W is control wheat cereal.

Microscopy. The influence of increasing the temperature and shear on the distribution of β-glucan in the cereals is evident in **Figure 2**. In the premix (**Figure 2f**), β-glucan in the oat bran cell walls is labeled by Calcofluor. Large pieces of subaleurone with thick cell walls are visible. Because the bran was not sectioned, cross sections of the subaleurone layer are not seen; rather, the honeycomb-like transverse view is displayed. Protein in the cell contents is stained red. During extrusion, the β-glucan was partially solubilized and started to spread through the mix. In 3H (**Figure 2a**), the cell walls appeared swollen and dominated the field of view. As the β-glucan was further solubilized in 4M (**Figure 2b**) and 3M (**Figure 2c**), β-glucan staining was diminished. The number of small protein particles visible increased with increasing severity of the extrusion conditions. For the cereal with the lowest molecular weight β-glucan (4L), the polysaccharide is less visible (**Figure 2d**), despite the fact that all of the oat bran cereals have the same β-glucan content (**Table 2**). This may be because the β-glucan was more dispersed. In addition, a portion of the β-glucan was small enough that it did not bind Calcofluor well. Intact cell walls and protein bodies are visible in the wheat cereal (**Figure 2e**).

Autofluorescence of the cereals is shown in **Figure 3**. Phenolic compounds in cereal cell walls autofluoresce blue in UV light, whereas proteins and lignins appear yellow-green. Fragments of intact endosperm (from the subaleurone region) and aleurone are visible in cereal 3H (**Figure 3a**). In cereal 4M (**Figure 3b**) large segments of aleurone cells remain, both intact and with cells broken open. As the extrusion conditions became harsher, the extent of cell disruption increased, so that pieces of endosperm, which has thinner cell walls that are more easily broken, are smaller and less frequent. In 3M, there is evidence that the phenolics have leached out of the cell walls, leaving a brown skeleton. In 4L, little cellular structure remains, but disrupted protein particles and a diffuse phenolic haze are evident. In the wheat cereal (**Figure 3e**) the aleurone cell walls, which are rich in phenolic compounds, autofluoresce bright blue. Some of the aleurone cell walls appear brown, which may indicate leaching of phenolics.

Characterization of Physiological Extract. The in vitro digestion process extracted 38.7 ± 1.5% of the β-glucan from the unprocessed oat bran, less than in any of the extruded cereals ($p < 0.0001$). Increasing the temperature and SME markedly ($p < 0.0001$) increased the solubility of the β-glucan from 66.8 to 100%. The percentage of soluble β-glucan in each physiological extract was used to calculate the dose of soluble β-glucan for the clinical trial (C = % solubility/100 × % β-glucan/100 × daily dose in g) (*24*). The concentration of β-glucan in the extract increased with increasing extrusion temperature and SME (**Table 3**), but the viscosity of the extracts decreased ($p < 0.0001$) because of the concurrent decrease in β-glucan molecular weight. The oat bran cereal extracts exhibited shear thinning behavior typical of polymer solutions (**Figure 4**). The viscosity observed at all shear rates declined in the order 3H > 4M > 3M > 4L. The extract from the wheat bran cereal had a very low viscosity and near-Newtonian behavior.

Increasing the temperature and SME, and decreasing the water added to the extruder, resulted in depolymerization of the β-glucan (**Figure 5**). With the exception of 3H, which had a tail at the low molecular weight end, the peaks were fairly symmetrical. A comparison of the peak molecular weights (M_p) of the total β-glucan as extracted by NaOH (**Table 2**) with the β-glucan soluble at 37 °C (**Table 3**) shows that the soluble portion had a molecular weight similar to that of the total β-glucan ($p > 0.05$), except for the wheat cereal. Thus, in these oat cereals neither the high nor the low molecular weight β-glucans are preferentially extracted from the cereal at 37 °C with the pH and enzyme treatments. In contrast, the β-glucan in the wheat cereal shows preferential extraction of the low molecular weight fractions using the in vitro extraction protocol. Although the peak molecular weight of the total β-glucan in the wheat cereal was 422,000 g/mol, the portion that was solubilized at 37 °C had a peak molecular weight of 36,000 g/mol.

The cereals were shelf stable at room temperature, facilitating distribution and storage during the clinical trial. The β-glucan molecular weight and solubility were measured before the clinical trial began and again at the middle and end of the trial, over a period of 18 months. No significant changes in the physicochemical parameters were observed ($p > 0.05$).

Figure 6 shows the relationship between viscosity and $M_p \times c_e$ for the extract viscosities. The viscosity of the wheat cereal extract did not show shear thinning behavior and was only slightly higher than the viscosity of water at 25 °C (1.0 mPa·s). Therefore, the

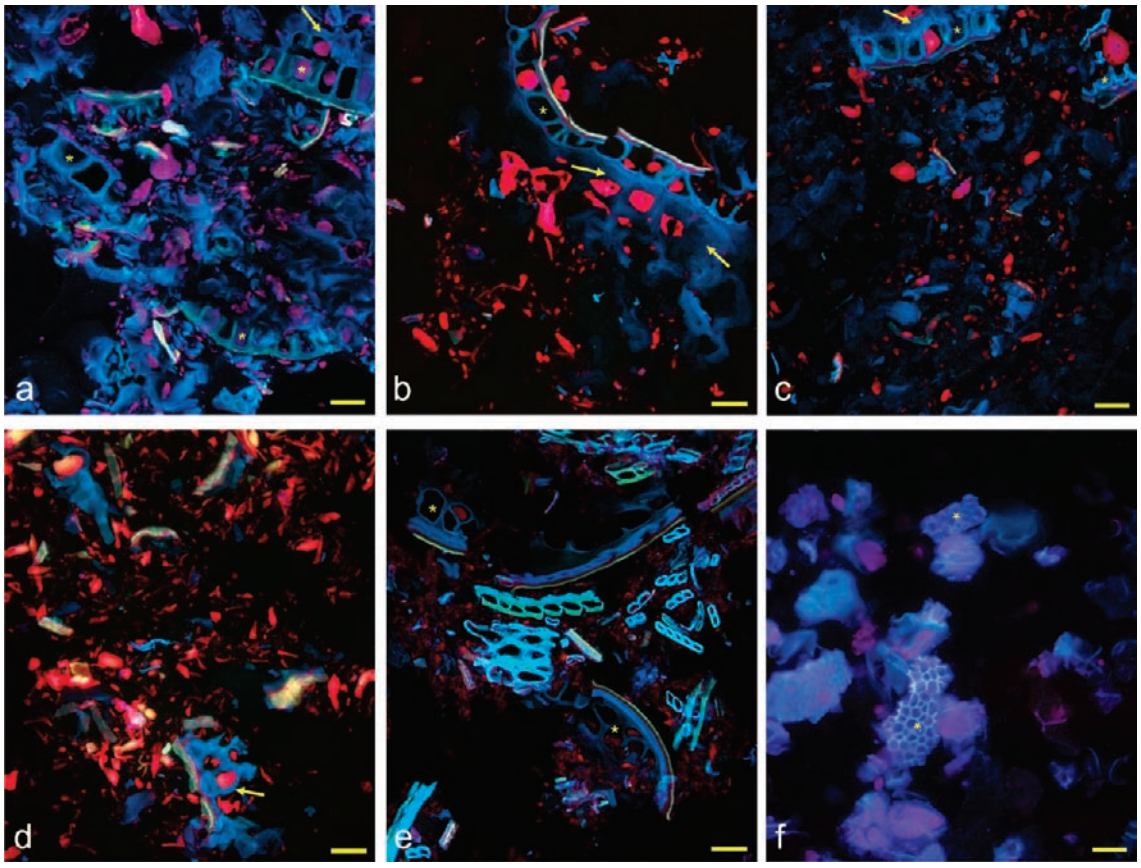

Figure 2. Cryosections of test cereals, stained with Calcofluor (0.01% in 25 mM Tris buffer, pH 8) and mounted in Acid Fuchsin (0.01% in 1% acetic acid): (**a**) 3H; (**b**) 4M; (**c**) 3M; (**d**) 4L; (**e**) W; (**f**) P. ∗, aleurone cells; →, subaleurone cell walls. For **a**−**e**, bars represent 50 μm; for **f**, bar represents 100 μm.

Table 3. Characteristics of Soluble β-Glucan in in Vitro Extract[a]

	oat bran	3H	4M	3M	4L	W
extract viscosity (mPa·s), n = 8	280 (14)	2900 (800)	1700 (160)	800 (96)	131 (12)	2.7 (3.2)
β-glucan concentration[b] (mg/mL), n = 16	3.13 (0.1)	9.9 (1.1)	11.2 (0.9)	12.8 (0.8)	14.5 (1.5)	0.16 (0.08)
soluble β-glucan (% of total), n = 16	38.7 (1.5)	66.8 (7.3)	80.2 (6.9)	92.2 (4.0)	101.7 (5.0)	8.7 (0.6)
peak molecular weight (g/mol), n = 10	2,537,000 (30,000)	2,213,000 (235,000)	847,000 (80,000)	528,000 (42,000)	211,000 (13,000)	36,000 (19,000)

[a] Standard deviation is shown in parentheses. The number of replicates (n) is indicated. [b] Concentration in in vitro extract, c_e.

wheat cereal extract falls in the dilute viscosity regime. The oat cereal extracts were in the semidilute region, and the viscosity increased exponentially with increasing concentration and molecular weight ($p < 0.0001$).

DISCUSSION

Despite widespread acceptance of the role of viscosity in the lipid-lowering activity of β-glucan (*7*, *8*), few studies have attempted to demonstrate or quantify this. Test foods developing different viscosities in the GI tract are required to demonstrate a correlation of effect with viscosity. To achieve this, viscosity must be varied by varying the amount solubilized and the molecular weight distribution of the β-glucan, rather than simply varying the dose, where dose response and viscosity response may be indistinguishable.

There has been much speculation and insufficient investigation over the years concerning the effects of processing on the physicochemical characteristics of oat β-glucan incorporated into food products. This speculation has frequently supposed that processing would be detrimental to bioactivity. However, our results show that, at least where extrusion is concerned, processing is more likely to increase bioactivity. The β-glucan in the commercially produced OatWell cereal, 3H, maintained its physicochemical properties and was expected to maintain high bioactivity. The molecular weight remained high, similar to the original ingredient, whereas the solubility increased from 38.7 to 66.8%. This resulted in high viscosity in the extract, which is associated with physiological effectiveness. Thus, the commercial extruded cereal would be expected to increase viscosity in the upper gut and reduce serum cholesterol levels to a greater extent than the original bran.

Depolymerization of β-glucan during processing of some foods has been reported. A survey of molecular weights in processed foods (*23*) showed a range of β-glucan molecular weights in common oat foods. Average molecular weights were > 1,800,000 g/mol in extruded flakes, macaroni, and muffins, but < 600,000 g/mol in apple juice, fresh pasta, and teacakes. In the foods where partial depolymerization occurred, the molecular weight distributions were frequently wide and bimodal. Rye and barley breads that include wheat flour are especially prone to β-glucan depolymerization (*27*, *28*), due to the presence of β-glucanases in wheat flour, which degrade the β-glucan during mixing and proofing. Depolymerization may also result from oxidative−reductive depolymerization due to ingredients such as ascorbate, which is present, for example, in fruit juices (*29*).

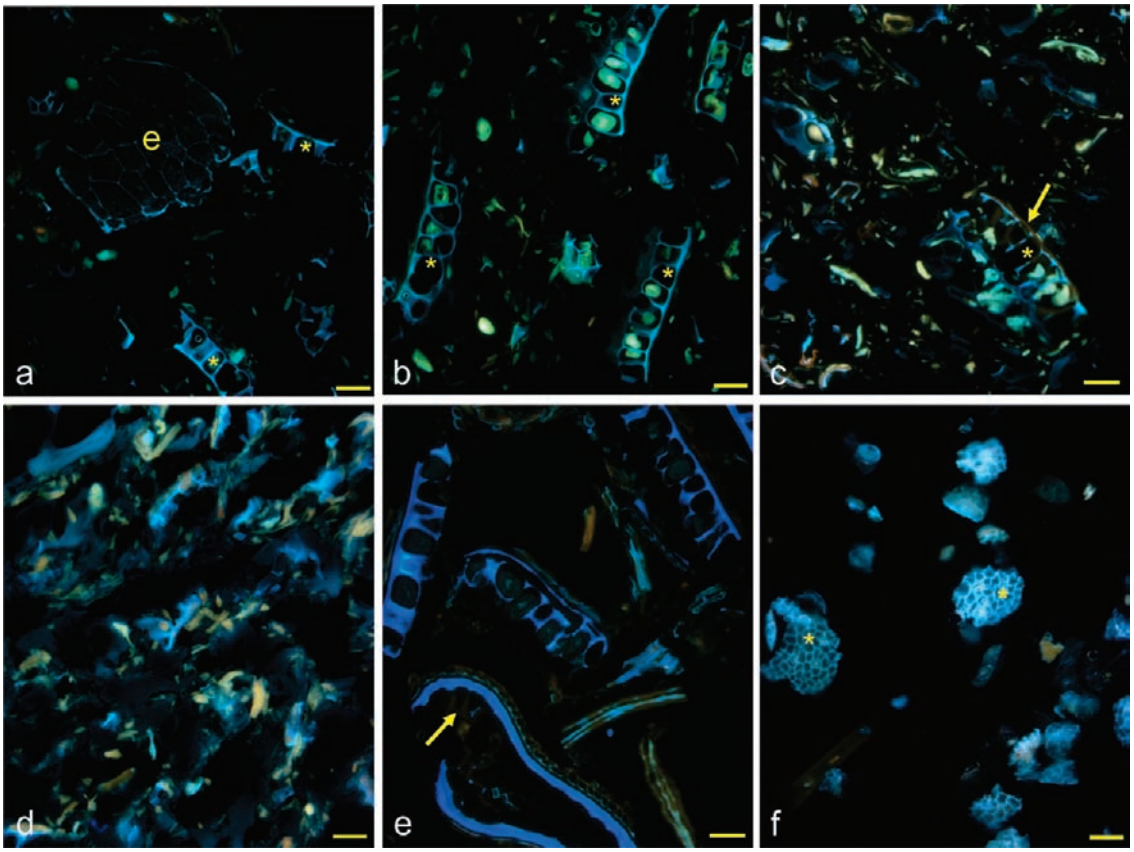

Figure 3. Cryosections of test cereals, autofluorescence: (**a**) 3H; (**b**) 4M; (**c**) 3M; (**d**) 4L; (**e**) W; (**f**) P. e, endosperm fragment; ∗, aleurone cells; →, aleurone cells showing loss of autofluorescence. Note amorphous blue matrix of solubilized phenolics in **d**. For **a**−**e**, bars represent 50 μm; for **f**, bar represents 100 μm.

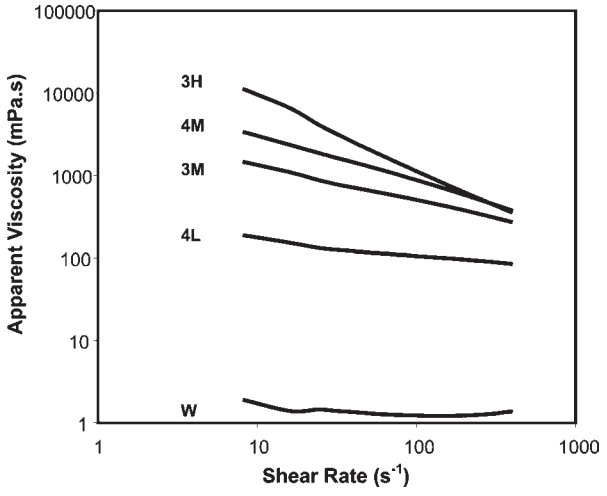

Figure 4. Apparent viscosity of in vitro extracts (37 °C) for oat cereals 3H, 4M, 3M, and 4L and for wheat cereal W. Concentrations and molecular weights shown are in **Table 3**.

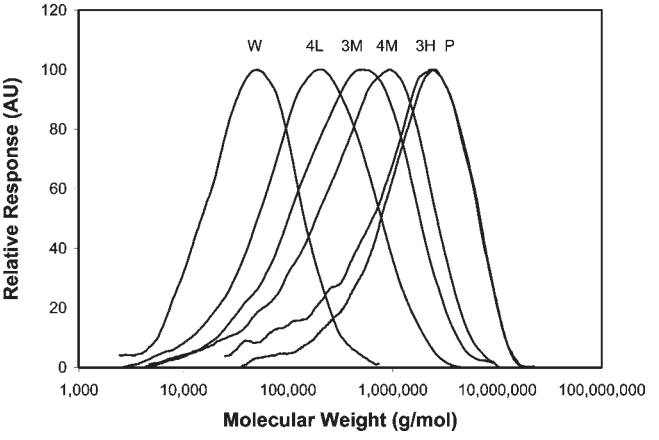

Figure 5. Molecular weight distributions of β-glucan in in vitro extracts (37 °C) for oat cereals 3H, 4M, 3M, and 4L and for control wheat cereal W. The molecular weight distribution in the original premix (P) is shown for comparison.

Solubility of β-glucan in foods is also a concern because β-glucan not solubilized at 37 °C under physiological conditions would not produce viscosity in the aqueous lumen of the gut. Oat β-glucan is more soluble in hot water than at room temperature, so processing steps that involve moisture and heat are likely to increase β-glucan solubility. Amundsen and co-workers (*14*) reported solubilities ranging from 22% for pasta to 70% in an apple drink, although the method used to measure solubility was not described. The solubility of the β-glucan in a breakfast cereal was 48%. A decline in solubility has been observed in foods stored frozen for extended periods of time (*19*) or subjected to freeze−thaw cycling (*17*). During breadmaking, β-glucan solubility increases (*28*). However, in muffins treated with β-glucanase, solubility initially increased as molecular weight decreased and then decreased with further depolymerization (*16*). In the work reported here, the solubility of the β-glucan in the extruded oat bran cereals was much higher than in the original uncooked oat bran. Indeed, following the highest temperature and SME treatment (4L) the β-glucan appeared to be completely soluble at 37 °C. These high solubilities are consistent with previous work (*5*). During extrusion, the β-glucan was dispersed throughout the

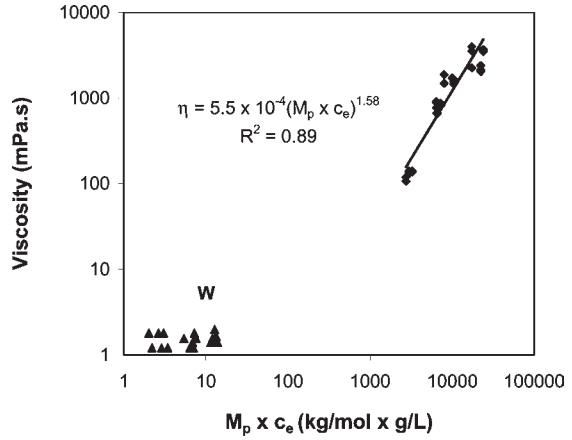

Figure 6. Relationship between viscosity and the product of M_p and c_e, where viscosity is the apparent viscosity of the in vitro extract measured in mPa · s at 30 s^{-1}, M_p is the peak molecular weight (kg/mol) of the extracted β-glucan, and c_e is the concentration of β-glucan in the extract (g/L). A regression is fitted to the semidilute region of the graph. W, wheat control.

cereal pieces so that it was more bioaccessible (**Figure 2**). The cell walls appear to swell under typical commercial extrusion conditions, but under harsher conditions they begin to lose their integrity.

The physiological extraction demonstrated that the oat bran cereals would be expected to produce a range of viscosities in the gut. There was a 20-fold difference in extract viscosities at 30 s^{-1} between the highest and lowest molecular weight oat bran cereals. The viscosity of wheat bran cereal extract was very low because of the low solubility (8.65%) and low molecular weight (36,000 g/mol) of the extracted β-glucan. However, extrusion had also increased the solubility of the wheat β-glucan, which was essentially zero in the original wheat bran. Kahlon and co-workers (*30*) reported that the soluble dietary fiber (100 °C extraction) in wheat bran extruded under a range of conditions was 1.97−2.95%.

The relationship between the viscosity of polymer solutions and the characteristics of the polymers in the dilute and semidilute regimes can be described by the equation

$$\eta_{sp,0} = C[\eta] + b'(C[\eta])^{n'}$$

where $\eta_{sp,0}$ is the zero shear viscosity, c is the polymer concentration, $[\eta]$ is the intrinsic viscosity, and b' and n' are fitting parameters (*22*). Intrinsic viscosity, $[\eta]$, is a measure of the volume of individual molecular domains, and therefore the dimensionless product $c[\eta]$ (overlap parameter, reduced concentration) represents the volume occupancy of the polymer in the solvent. A plot of zero shear viscosity against $c[\eta]$ shows an area of discontinuity at the point where the polymers become entangled and the viscosity changes from increasing linearly with increasing polymer concentration (dilute regime) to increasing exponentially (semidilute regime). Because $[\eta]$ is dependent on molecular weight (Mark−Houwink−Sakurada, $[\eta] = KM^{\alpha}$), a plot of apparent viscosity against $M_p \times c_e$ should have a similar shape modified by shear rate and different fitting parameters (*31*). The data of **Figure 6** are consistent with this. These fundamentals of polymer behavior in solutions mean that to know the potential for a polymer to increase the viscosity of water, as in the GI tract, knowledge of its molecular weight distribution (or intrinsic viscosity) and its solubility in water is required. Typically there is also particulate matter in the GI tract, which will also modify the rheological behavior (*32*).

The test cereals were used in a human clinical trial using a randomized, controlled, double-blind, parallel design (*24*), where subjects consumed the wheat control (W), 1.5 g of high molecular weight (3H), 2 g of medium molecular weight (4M), 1.5 g of medium molecular weight (3M), or 2 g of low molecular weight (4L) oat β-glucan in cereal twice daily for 4 weeks. The clinical trial confirmed that a dose of 3 g of β-glucan/day from 3H and 3M cereals significantly reduced serum LDL-cholesterol in mildly hypercholesterolemic subjects by 0.21 ($p = 0.002$) and 0.18 mmol/L ($p = 0.012$), respectively, compared to W (*24*). A dose of 4 g of β-glucan/day from 4M significantly reduced serum LDL-cholesterol by 0.25 mmol/L ($p = 0.007$), but 4 g of β-glucan/day from 4L did not significantly reduce serum LDL-cholesterol ($p = 0.21$). Significant correlations were found between the cholesterol reduction of the subjects and both the extract viscosity, η ($p = 0.001$), and ($M_p \times C$) ($p = 0.003$), where M_p is the peak molecular weight of the soluble β-glucan and C is the soluble β-glucan in the daily dose.

In summary, the objectives of making and characterizing oat bran cereals with a range of molecular weights and solubilities of β-glucan were achieved. The molecular weights of the β-glucans in the oat bran cereals ranged from 1,930,000 g/mol for the cereal made under typical conditions to 251,000 g/mol when the extrusion temperature and shear were increased and the water was decreased. A control cereal made from wheat bran was also produced for the clinical trial. When a physiological extraction process was conducted at 37 °C with gut enzymes, a range of viscosities, which were expected to correspond to the bioactivity of the β-glucan, was evident. Microscopic examination of the cereals revealed progressive disruption of the cell walls and dispersal of the β-glucan as the extrusion conditions became more severe.

ABBREVIATIONS USED

RTE, ready to eat; SME, standard mechanical energy; 3H, 4M, 3M, and 4 L, oat bran cereals in descending order of molecular weight; W, control wheat cereal; P, oat bran premix; M_p, peak molecular weight; c, concentration; c_e, β-glucan concentration in extract; wwb, wet weight basis; C, daily dose of soluble β-glucan; η, viscosity; $[\eta]$, intrinsic viscosity.

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