Impact of the Molecular Weight, Viscosity, and Solubility of β-Glucan on in Vitro Oat Starch Digestibility

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ABSTRACT: The impacts of the molecular weight (MW), viscosity, and solubility of β-glucan on the rate of in vitro starch digestion and estimated glycemic index (GI) were evaluated. Extracted oat starch and β-glucan suspensions with high, medium, and low MW were heated to gelatinize the starch. The viscosity increased and the solubility decreased with an increase in the MW of β-glucan. The in vitro starch hydrolysis of the mixtures and a control, white bread, increased as the digestion time increased. As the MW of β-glucan increased, the starch hydrolysis decreased during in vitro digestion. The in vitro estimated GI of the mixture without β-glucan, determined from the starch hydrolysis rate, was 88.3 for Jim and 80.0 for N979, which decreased to 68.4 and 66.8, respectively, with the inclusion of high-MW β-glucan. The estimated GI values were negatively correlated with the β-glucan peak and final viscosities ($r = -0.81$ and -0.82). These results illustrated the importance of viscosity attributed to the β-glucan MW on starch hydrolysis during in vitro digestion. These findings will help to develop new food products with a low GI by using oat β-glucan.

KEYWORDS: *oat β-glucan, viscosity, oat starch digestion, estimated glycemic index*

■ INTRODUCTION

Oats are highly recommended for consumption, partly because they are an excellent source of dietary fiber (DF), especially of the water-soluble mixed linkage (1→3)- and (1→4)-β-D-glucan, referred to as β-glucan.[1] β-Glucans, primarily located in the subaleurone and endosperm cell walls, are linear homopolysaccharides composed of D-glucopyranosyl units linked via a mixture of β-(1→3) and β-(1→4) linkages. Because of the consecutive (1→4)-linked β-glucan in blocks separated by a single (1→3) linkage, β-glucan forms viscous solutions.[2] The consumption of β-glucan has been shown to reduce insulin sensitivity of glucose intolerance, lower cholesterol in the blood, and induce and prolong satiety.[2,3] The U.S. Food and Drug Administration has allowed a health claim stating that oat β-glucan at a level of 0.75 g per serving in a product, leading to consumption of 3 g per day, may reduce cholesterol and lower the risk of coronary heart disease.[4] These physiological effects of β-glucan have been mostly attributed to its contribution to viscosity in the gastrointestinal tract.[5] In addition, the increase in gut viscosity retards the rate of starch digestion and absorption of glucose by reducing the activity of pancreatic amylase and the movement of released sugars to the gut wall.[5] Symons and Brennan[6] reported that 5% inclusion of a β-glucan-rich fraction in bread resulted in a significant decrease in the release rate of reducing sugars as well as the in vitro digestion rate of bread by α-amylase and pepsin. Moreover, the increased viscosity caused by β-glucan reduced the plasma glucose and insulin response of human subjects consuming liquid meals containing β-glucan.[7]

The glycemic index (GI) characterizes the carbohydrates consumed in different types of foods on the basis of the postprandial level of blood glucose and allows the foods to be ranked on the basis of a rate of release and absorption of carbohydrates.[8,9] In general, low-GI foods are defined as having a GI of less than 55, medium-GI foods a GI of 56−69, and high-GI foods a GI of over 70.[10] Low-GI diets improved glucose control and insulin sensitivity.[11,12] The rate of blood glucose rise is determined by the rate and extent of starch digestion.[13] Several studies showed a high correlation between the rate of starch digestion and the glycemic response by in vitro digestion methods that mimic in vivo digestion.[9,14,15] The structural properties of starch and the presence of DF influence the rate of starch digestion in foods by decreasing susceptibility of starch-degrading enzymes, limiting water availability, and restricting starch gelatinization.[16,17]

β-Glucan, as a soluble DF, influences the starch digestibility, which may help to lower the GI in foods. In our previous study, starch digestion in oat-flour slurries was negatively correlated with β-glucan concentration.[18] The physicochemical properties of β-glucan, influenced by its structural and molecular features, impact the viscosity of foods,[19−21] thus reducing starch digestibility in foods. If the attenuation of glucose release is a function of the increased viscosity in the digestive tract, the physicochemical properties of β-glucan should be critical in their capacity to reduce glucose release from foods. Therefore, the objective of this study was to evaluate the impact of the physicochemical properties of β-glucan, including MW,

viscosity, and solubility, on in vitro oat starch digestion and estimated GI.

■ MATERIALS AND METHODS

Oat Flour. A publically available oat line, 'Jim', with a typical concentration of β-glucan and an experimental oat line developed at Iowa State University, N979-5-4 (N979), with a high level of β-glucan were selected for this study to investigate the impact of different oat lines. These oat types were grown at the Agronomy and Agricultural Engineering Field Research Center in Ames, IA, and harvested in August 2011. The harvested oat kernels were dried and dehulled with an air-pressure dehuller (Codema, Eden Prairie, MN). The resulting oat 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 use.

Extraction of Oat Starch. Starch was extracted from Jim and N979 oat flour according to Sayar et al.[22] Oat flour (25 g) was mixed with 200 mL of 0.02 M sodium hydroxide solution at room temperature for 30 min. The mixture passed through a sieve (no. 140) with a 106 μm pore diameter, and the filtrate was centrifuged at 3000g for 20 min. The supernatant was discarded; the brown portion at the top of the solids was removed by using a medium-size spatula. The remaining white portion of starch was washed twice with distilled water. After centrifugation at 3000g for 10 min, the pellet was mixed with distilled water and neutralized with 1 M hydrochloric acid. The mixture was centrifuged at 3000g for 10 min, and the decanted starch was dried at 40 °C overnight. After drying, the moisture and starch concentration were determined by following AACC method 44-15A and AACC method 76-13, respectively, by using a total starch kit (Megazyme International Ireland Ltd., Bray, Ireland).[23]

Extraction and Preparation of Oat β-Glucan with High, Medium, and Low MW. Water-soluble β-glucans were extracted from Jim and N979 oat flour according to Sayar et al.[24] with modifications as described. Oat flour (20 g) was refluxed with 200 mL of 82% ethanol (v/v) for 2 h at 85 °C to inactivate endogenous enzymes and to remove fat. After centrifugation at 3000g for 10 min, the pellets were washed with 50 mL of 95% ethanol twice and centrifuged. The residue was then dried at 40 °C overnight. The dried flour was transferred to a 250 mL centrifuge bottle and 150 mL of distilled water added. The water-soluble β-glucan was extracted by placing the bottles in a shaking water bath at 47 °C for 3 h. The solution was centrifuged at 3000g for 10 min, and the supernatant was collected. The extracted β-glucan solution was then concentrated in a rotary evaporator at 75 °C and 200 mbar until the solution reached one-third its starting volume. The concentration of β-glucan in the extracted β-glucan suspension was measured by using AACC method 32-23 with the application of a mixed β-glucan linkage kit (Megazyme).[23]

The extracted β-glucan suspension (defined as high-MW β-glucan) was hydrolyzed by using lichenase (330 U of lichenase/mg of protein, catalog no. E-BLAAM100, Megazyme), which cleaves the 1,4-linkage of the 3-O-substituted glucose residues in β-glucan, to yield medium- and low-MW β-glucans. Lichenase (0.15 U/g of oat flour for medium and 0.25 U/g for low MW) was added to the extracted β-glucan suspension and incubated at 50 °C for 20 min. The hydrolyzed β-glucan suspensions for medium and low MW were heated in a boiling water bath for 10 min to inactivate the lichenase. After the hydrolyzed β-glucan suspensions were cooled to room temperature, their MW and viscosity of were determined.

Determination of β-Glucan MW. The relative MW distribution of extracted β-glucan suspensions with high, medium, and low MW was determined by using size-exclusion high-performance liquid chromatography (SE-HPLC) according to Sayar et al.[24] The SE-HPLC consisted of a solvent delivery module (model 210, ProStar, Varian Inc., Reodyne, CA), a 100 μL loop injection valve, a guard column (Ohpak SB-G, Shodex Showa Denko K. K., Tokyo, Japan), three serially connected columns (Ohpak SB-806 HQ, Ohpak SB-805 HQ, and Ohpak SB-804 HQ, Shodex Showa Denko K. K.), and a refractive index detector (model 350, ProStar, Varian Inc.). 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. An aliquot was 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 were used to estimate the actual MW ranges of the extracted β-glucan. The number-average MW and peak MW were obtained by a first-order polynomial curve of log MW versus retention time of the HPLC chromatogram.[25,26]

Preparation of the Mixtures of Oat Starch and β-Glucan with High, Medium, and Low MW. To study the impact of β-glucan on in vitro starch digestibility, extracted dried oat starch (~125 mg, wet weight basis) and extracted β-glucan suspension (2.5 g, wet weight basis, ~1% β-glucan solution in water) with high, medium, and low MW were mixed to have five different treatments: (1) oat starch and water (5% starch solution) without heating treatment (S, no heat), (2) oat starch and water (S), (3) oat starch and high-MW β-glucan suspension (S + HBG), (4) oat starch and medium-MW β-glucan suspension (S + MBG), and (5) oat starch and low-MW β-glucan (S + LBG). Total starch amounts in all mixtures were adjusted to have the same concentration. The ratio of oat starch to β-glucan was 5:1, by weight, which was established after preliminary studies in which ratios of 20:1, 15:1, 10:1, and 5:1 for oat starch and β-glucan were evaluated. Among those conditions, the ratio of 5:1 was the most effective in demonstrating starch hydrolysis among all ratios (data not shown). The mixtures of oat starch and water or β-glucan suspensions with different MWs were heated at 90 °C for 10 min to gelatinize starch[24] and cooled to room temperature before undergoing in vitro digestion.

In Vitro Starch Digestibility. In vitro starch digestibility of the mixtures of oat starch and β-glucan with high, medium, and low MW was determined by using the method of Englyst et al.[27] and Regand et al.[28] with modifications. The enzyme solution for digestion was prepared: A 0.9 g portion of porcine pancreatin (EC 232.468.9, from porcine pancreas, activity 8 × USP/g, Sigma-Aldrich, St. Louis, MO) was dispersed in 8 mL of distilled water and centrifuged at 1500g for 10 min. The supernatant (5.4 mL) was mixed with 0.8 mL of diluted amyloglucosidase (0.64 mL of amyloglucosidase, EC 3.2.1.3., 3300 U/mL, Megazyme, diluted to 0.8 mL with distilled water), and 0.5 mL of distilled water was added. This enzyme solution was freshly prepared for each digestion.

The mixtures of S (no heat), S, S + HBG, S + MBG, S + LBG, and a control (white bread made from wheat flour) were prepared from heat treatment in 50 mL tubes, in triplicate, with 10 glass beads (5 mm diameter) added to each tube. The mixtures were heated immediately before the in vitro digestion procedure to avoid starch retrogradation. A 2 mL volume of 0.05 M hydrochloric acid and 10 mg of pepsin were added to the tubes and the tubes incubated at 37 °C in a shaking water bath for 30 min. A 4 mL volume of sodium acetate buffer (0.5 M, pH 5.2) was added to each tube; the freshly prepared enzyme solution (1 mL) was added after 1 min intervals. The mixtures were then incubated at 37 °C in a shaking water bath. Aliquots (100 μL) were taken at 0, 10, 20, 30, 60, 90, 120, and 180 min intervals and mixed with 50% ethanol (1 mL). These solutions were centrifuged at 800g for 5 min, and the released glucose content of the supernatant was measured by using the glucose oxidase–peroxidase assay (Megazyme). The time and temperature during in vitro digestion were strictly controlled.

Estimated Glycemic Index. The kinetics of in vitro starch digestion was followed by a nonlinear model established by Goni et al.[29] The first-order equation is $C = C_\infty(1 - e^{-kt})$, where C is the percentage of starch hydrolyzed at time t (min), C_∞ is the equilibrium percentage of starch hydrolyzed after 180 min, and k is the kinetic constant. The parameters C_∞ and k were estimated for each treatment on the basis of the data obtained from the in vitro starch digestion. The area under the hydrolysis curve (AUC) was calculated by the following equation: $AUC = C_\infty(t_f - t_0) - (C_\infty/k)[1 - \exp^{\{-k(t_f - t_0)\}}]$, where C_∞ is the equilibrium percentage of starch hydrolyzed after 180 min, t_f is the final time (180 min), t_0 is the initial time (0 min), and k is the kinetic constant.

Table 1. Characteristics of β-Glucan Extracted from the Jim and N979 Oat Lines

β-glucan extract		extract suspension composition[a] (%)		MW[a] (×10⁵)		viscosity[a] (cP)		β-glucan solubility (%) after in vitro digestion[a]
oat line	MW	β-glucan	starch	average	peak	peak	final	
Jim	H	0.95 ± 0.02	0.4 ± 0.01 b	9.04 ± 0.10 a	12.13 ± 0.27 a	3308 ± 79 b	2820 ± 153 b	68.2 ± 0.9 b
	M	0.95 ± 0.02	0.4 ± 0.01 b	6.98 ± 0.03 b	9.25 ± 0.54 c	1569 ± 47 c	1234 ± 106 c	71.2 ± 0.7 ab
	L	0.95 ± 0.02	0.4 ± 0.01 b	1.13 ± 0.01 c	1.93 ± 0.07 e	223 ± 4 e	114 ± 7 e	72.8 ± 1.3 a
N979	H	0.92 ± 0.01	0.7 ± 0.01 a	8.51 ± 0.54 a	14.28 ± 0.71 a	3877 ± 124 a	3477 ± 161 a	67.2 ± 1.6 b
	M	0.92 ± 0.01	0.7 ± 0.01 a	7.05 ± 0.02 b	8.22 ± 0.26 d	1096 ± 66 d	972 ± 72 d	69.3 ± 1.8 ab
	L	0.92 ± 0.01	0.7 ± 0.01 a	1.65 ± 0.05 c	3.01 ± 0.02 f	218 ± 15 e	100 ± 3 e	72.3 ± 2.4 a

[a]Values are means ± standard deviation. Values followed by different letters within a column are significantly different ($p < 0.05$).

A hydrolysis index (HI) represents the rate of starch digestion, and the estimated glycemic index (GI) indicates the digestibility of oat starch in relation to the digestibility of starch in a reference material, white bread. The HI, suggesting a predictor of glycemic response, was calculated by dividing the AUC of each treatment by the AUC of a reference (control, white bread). The GI was then estimated by using the following equation of Goni et al.:[29] GI = 39.71 + 0.549(HI).

Solubility of β-Glucan after in Vitro Digestion. To determine the amount of β-glucan remaining after in vitro digestion, the β-glucan concentration in the supernatant from the in vitro digestion extraction was measured by using AACC method 32-23 with a mixed β-glucan linkage kit (Megazyme).[23] The solubility of β-glucan after in vitro digestion was calculated as solubility (%) = (β-glucan concentration in the mixture after digestion)/(β-glucan concentration in the mixture before digestion) × 100.

Viscosity Measurement. A Rapid Visco Analyzer (RVA; Newport Scientific, Warriewood, Australia) was used to determine the viscosity of extracted β-glucan suspensions with high, medium, and low MW and the pasting profiles of the mixtures of oat starch and β-glucan during heating and in vitro digestion. The conditions to measure the apparent viscosity of high-, medium-, and low-MW β-glucan suspensions involved a stirring speed of 960 rpm for 10 s, followed by stirring at 115 rpm for 4 min at 25 °C. The viscosity was recorded every 4 s, and the peak viscosity and final viscosity were measured.

To measure the pasting profile of the mixtures of oat starch and β-glucan suspensions with different MWs during heating, the mixtures were prepared in the RVA canister with a total mass of 20 g and 5:1 ratio of starch to β-glucan by weight. The test profile of the RVA to simulate heating prior to in vitro digestion included a stirring speed of 960 rpm for 10 s and 115 rpm for the remainder of the test and a temperature program increasing from 25 to 90 °C over 1 min, holding at 90 °C for 9 min, and decreasing to 37 °C over 1 min. After heating, the enzyme solution (8 mL containing pancreatin and amyloglucosidase) was added to the canister containing the mixture and the viscosity measurement made at a speed of 115 rpm at 37 °C for 180 min.

Statistical Analysis. Oat starch and β-glucan from each oat line were prepared in triplicate. All analyses were done in duplicate, and the values were averaged. Data were analyzed by using the analysis of variance (ANOVA), followed by least significant differences (LSDs) for the comparison among treatments using the GLM procedure found in SAS version 9.1 (SAS Institute, Cary, NC) at $\alpha = 0.05$. Linear regression analyses were used to establish a relationship between estimated GI and MW, viscosity, or solubility of β-glucan with $p < 0.05$.

■ **RESULTS AND DISCUSSION**

Characterization of Extracted β-Glucan with High, Medium, and Low MW. The Jim and N979 oat flours contained 4.1% and 7.5% β-glucan and 62.5% and 53.0% starch (dry weight basis), respectively (data not shown). The extracted oat starch from Jim and N979 oat flour contained 95−97% starch (dry weight basis). After extraction of β-glucan with water from oat flour and concentration of the β-glucan

solutions, the extracted β-glucan suspensions of Jim and N979 oat contained 0.95% and 0.92% β-glucan, respectively (Table 1). This concentration of β-glucan in water was able to be mixed well with oat starch for the in vitro starch digestion test. The majority of extracted β-glucan suspensions were water (∼98%), with 0.4% and 0.7% starch present in the suspensions of Jim and N979, respectively.

The average MW and peak MW of high-, medium-, and low-MW β-glucan determined from the peak retention time of the SE-HPLC chromatograms after the hydrolysis of β-glucan from Jim and N979 are shown (Table 1). When compared with the SE-HPLC chromatogram of β-glucan reported in our previous study,[26] the SE-HPLC chromatogram of β-glucan in the extract suspension was similar. In our previous study, the β-glucan was extracted from oat flour to have ∼90% purity. Therefore, the calculated MW from the SE-HPLC chromatogram in the current study was the MW of β-glucan. The average MW of high-MW β-glucan was greatly decreased to that of medium- or low-MW β-glucan by the hydrolysis procedure depending upon the lichenase amount. The peak MW values of high-, medium-, and low-MW β-glucan significantly differed within the oat line ($p < 0.05$). The average MW of high-MW β-glucan extracted from N979 oat was similar to that of the Jim oat, but the peak MW of N979 was greater than that of the Jim oat line, likely because of differences in heritable traits of oat lines.[30]

The peak and final viscosities of the high-, medium-, and low-MW β-glucan suspensions from Jim and N979 oats were determined by using the RVA at 25 °C (Table 1). The viscosity of the suspensions decreased greatly with a decrease in MW of β-glucan ($p < 0.05$). The final viscosity values of β-glucan suspensions were lower than the peak viscosity. The peak MW of β-glucan was more closely correlated with the peak and final viscosity values than with the average MW for both oat lines ($R^2 = 0.88$). In agreement with these findings, in previous work, a greater peak MW of β-glucan in some oat types caused greater slurry viscosities than a lower MW in another oat type.[25] The enzymatic degradation by lichenase to create medium- and low-MW β-glucans selectively degraded β-glucan and led to modifications in the viscosity of the solutions.

The solubility of β-glucan with high, medium, and low MW after in vitro digestion was calculated on the basis of the amount of β-glucan before and after digestion (Table 1). The low-MW β-glucan was soluble in water at a greater concentration than the high-MW β-glucan suspension after digestion. A lower diffusion rate in solution caused by high MW might lower the solubility of β-glucan.[31]

In Vitro Starch Digestibility. In vitro starch digestibility of the mixtures of oat starch and β-glucan with high, medium, and low MW extracted from the Jim and N979 oat lines are shown
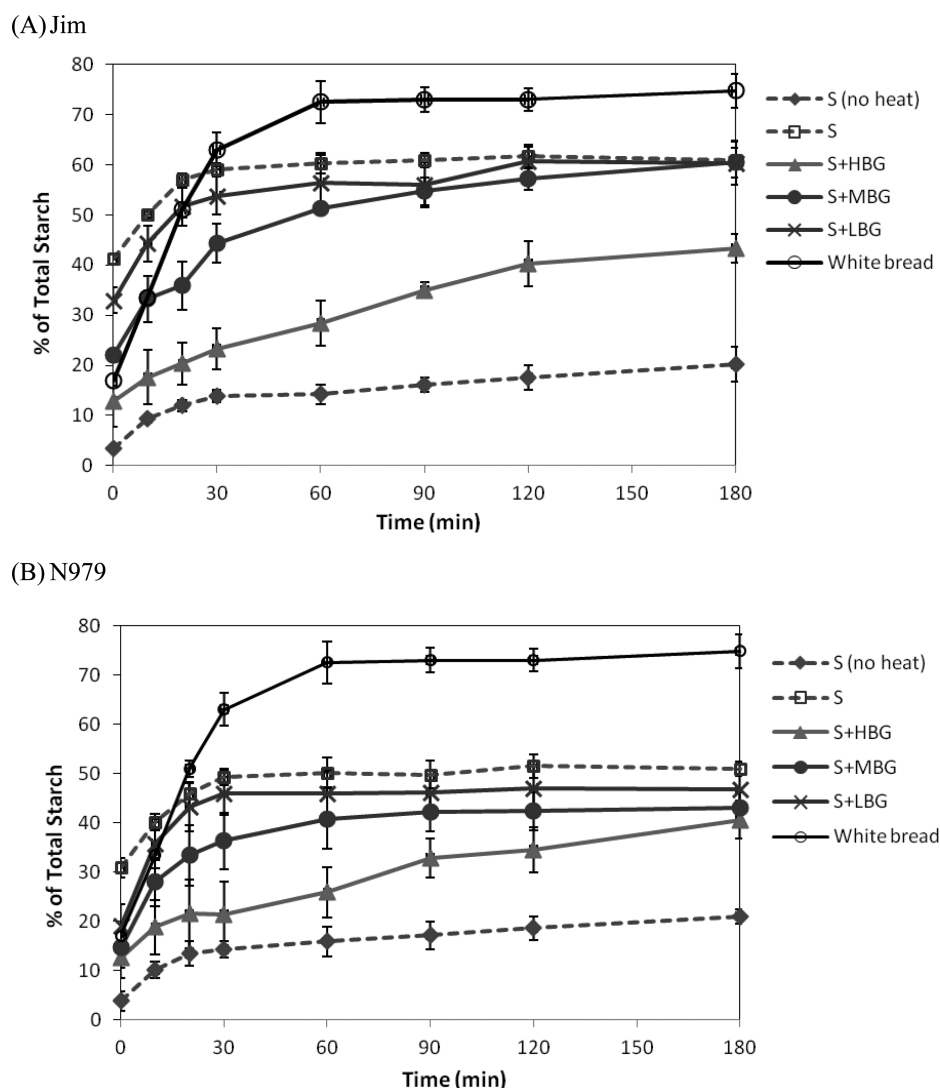
(A) Jim

(B) N979

Figure 1. In vitro starch hydrolysis of the mixture of oat starch and β-glucan with high, medium, and low MW extracted from the Jim (A) and N979 (B) oat lines. S = starch, HBG = high-MW β-glucan, MBG = medium-MW β-glucan, and LBG = low-MW β-glucan.

(Figure 1). The starch hydrolysis of all mixtures and the control, white bread, increased as the digestion time increased. The control showed a digestion value of ~70% after 180 min of digestion, which agreed with the value reported in the original protocol by Goni et al.[29] The starch digestion rate of raw oat starch without heating (S, no heat) was much lower than that of the starch with heating (S) ($p < 0.05$). Heating of oat starch extracted from Jim and N979 oats increased starch digestion from ~20% to 51% and 61%, respectively, measured after 180 min of in vitro digestion. Heating changes the crystallites of starch.[32] When starch granules were gelatinized by heating, a part of the amylose diffused out of the granules and went into solution, resulting in breakage of the granule. The starch crystallites were disrupted, thus increasing the susceptibility of enzymatic digestion.[32,33]

The mixture containing high-MW β-glucan (S + HBG) retarded starch digestion; however, as the MW of β-glucan decreased to medium and low MW, the starch digestion increased (Figure 1). The starch digestion rate of the mixture with low-MW β-glucan (S + LBG) was not different from that of the mixture without β-glucan (S) during in vitro digestion. The high-MW β-glucan increased the viscosity of the mixture

and likely decreased the water availability for starch gelatinization and the susceptibility of digestion enzymes. Indeed, previous work showed the presence of dietary fiber slowed starch digestion by formation of a fiber network, thus decreasing the susceptibility of enzyme attack.[16] The soluble dietary fiber, oat β-glucan in the current study, limited the susceptibility of starch-degrading enzymes in the mixtures.[16,17] Others showed that, as the MW or chain length of the fiber increased, the viscosity of fiber in solution increased and the water migration was inhibited.[34]

The starch digestibility of the mixtures containing oat starch extracted from Jim was greater than that from N979, regardless of the β-glucan MW (Figure 1), which may have resulted from different structures or conformations of starch, such as the amylose and amylopectin ratio, granule size and type, and degree of crystallinity.[35] For example, the unit chain length of amylopectin was negatively correlated to the digestibility of starch; longer chains formed longer and more stable helices and decreased digestibility.[36] High amounts of resistant starch were found in high-amylose corn starch.[37] In addition, a smaller granule size and a large and smooth surface in corn and cassava starch displayed greater enzymatic susceptibility, which could

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Table 2. Calculated Hydrolysis Index (HI) and Estimated Glycemic Index (GI) for the Mixtures of Oat Starch and β-Glucan with High, Medium, and Low MW Extracted from the Jim and N979 Oat Lines and Correlations with Estimated GI and β-Glucan Characteristics

treatment[a]	Jim[b]		N979[b]		correlations with GI		
	HI (%)	GI	HI (%)	GI	β-glucan characteristics	r	p value
S (no heat)	25.2 ± 1.4 d	53.5 ± 1.8 d	26.6 ± 0.7 e	54.3 ± 1.7 d	average MW	−0.70	0.12
S	88.6 ± 2.6 a	88.3 ± 1.4 a	73.4 ± 1.3 a	80.0 ± 2.2 a	peak MW	−0.78	0.07
S + HBG	52.3 ± 2.8 c	68.4 ± 2.2 c	49.3 ± 1.4 c	66.8 ± 2.1 c	peak viscosity	−0.81	0.05
S + MBG	78.7 ± 2.4 b	82.8 ± 2.4 b	60.1 ± 2.8 c	72.7 ± 1.6 b	final viscosity	−0.82	0.04
S + LBG	83.5 ± 2.1 ab	85.6 ± 2.8 ab	67.0 ± 2.5 b	76.5 ± 1.4 a	solubility	0.88	0.02
white bread	100		100				

[a]S =starch, HBG = high-MW β-glucan, MBG = medium-MW β-glucan, and LBG = low-MW β-glucan [b]Values are means ± standard deviation. Values followed by different letters within a column are significantly different (p < 0.05).
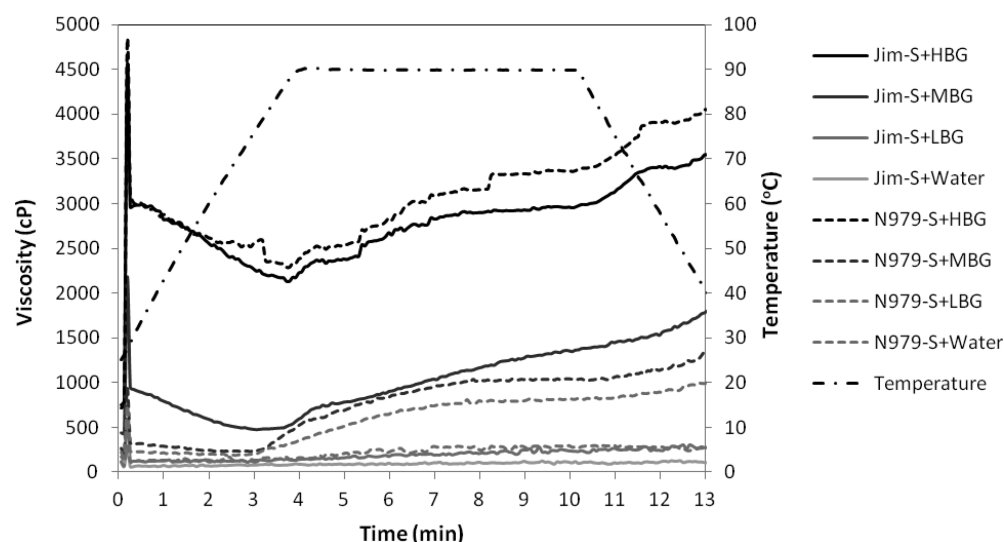

Figure 2. Change of viscosity during heating of the mixture of oat starch and β-glucan with high, medium, and low MW extracted from the Jim and N979 oat lines. S = starch, HBG = high-MW β-glucan, MBG = medium-MW β-glucan, and LBG = low-MW β-glucan.

have impacted digestibility.[38,39] Finally, shorter double helices and interior crystallites in corn, potato, and rice starch are more readily digestible.[40] Further study is needed to explore the structure of oat starch extracted from different oat lines and its impact on starch digestibility.

Hydrolysis Index and Estimated Glycemic Index. The estimated GIs of raw oat starch for Jim and N979 oat were 53.5 and 54.3, which increased to 88.3 and 80.0, respectively, after heating (Table 2). The addition of high-MW β-glucan to Jim and N979 oat starch greatly reduced the estimated GI to 68.4 and 66.8, respectively; however, the low-MW β-glucan did not change the estimated GI values, which were similar to the estimated GI value of the mixture without β-glucan. The decrease in estimated GI values, which indicated low starch digestibility, was attributed to the high MW of β-glucan. The high-MW β-glucan formed highly viscous solutions. The viscous property of the mixture retarded the susceptibility of enzymes to digest starch and reduced the rate of enzymatic hydrolysis of starch during in vitro digestion.[6] Furthermore, the presence of β-glucan influenced the rate of starch digestion by decreasing the susceptibility of starch-degrading enzymes, limiting water availability, and restricting starch gelatinization.[16,17] The result of this in vitro test is in agreement with several in vivo studies.[7,20,41,42] An in vivo study showed that the viscosity of β-glucan ranging from 9.4 to 1548 cP reduced by 79−96% the plasma glucose and insulin response of human

subjects who consumed drink meals containing oat β-glucan and 50 g of available carbohydrates.[7] Similar results have been reported supporting findings of an exponential relationship between the peak blood glucose concentration and the MW, concentration, and solubility of β-glucan, which were directly related to the viscosity.[20,41,42] It is established that β-glucan slows gastric emptying and limits the diffusion of glucose toward the walls of the small intestine as a result of increased viscosity.[20,42] It is likely that the viscosity contributed by β-glucan would impact not only in vitro but also in vivo studies.

To better understand the impact of β-glucan characteristics on in vitro starch digestion, the correlation between estimated GI and the average and peak MWs, peak and final viscosities, and solubility of β-glucan are shown (Table 2). The estimated GI was negatively correlated with the peak and final viscosities of β-glucan suspensions (r = −0.81 and −0.82, p = 0.05 and 0.04). These findings suggest that the greater the viscosity of a β-glucan solution, the lower the estimated GI values, a finding in agreement with the studies of Wood et al.[7] and Tosh et al.[20] The estimated GI values and the solubility of β-glucan after in vitro digestion were highly correlated (r = 0.88, p = 0.02). Although the solubility of high-MW β-glucan after digestion was low, high-MW β-glucan greatly reduced the estimated GI values. The high-MW β-glucan had a lower diffusion rate in solution, allowing for easy aggregation.[31] This property of high-
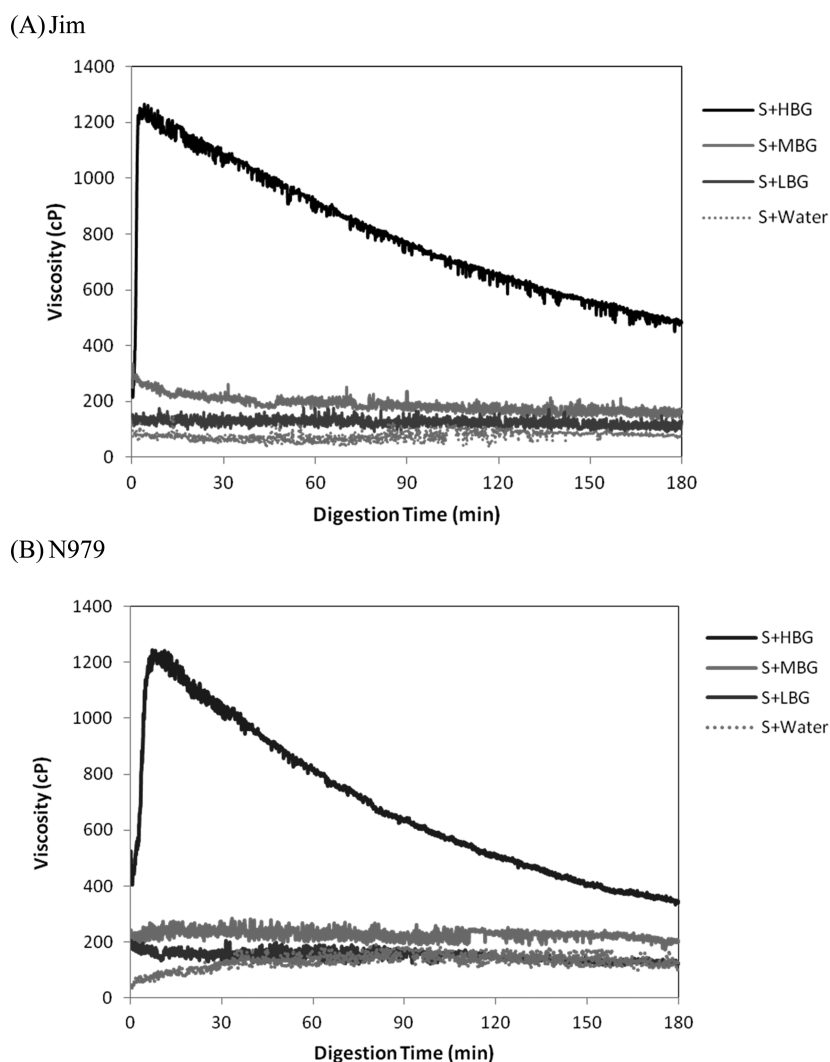
(A) Jim

(B) N979

Figure 3. Change of viscosity during in vitro digestion of the mixture of oat starch and β-glucan with high, medium, and low MW extracted from the Jim (A) and N979 (B) oat lines. S = starch, HBG = high-MW β-glucan, MBG = medium-MW β-glucan, and LBG = low-MW β-glucan.

MW β-glucan might inhibit the mobility of starch-degrading enzymes, thus lowering the rate of starch digestion.

Viscosity Changes during Heating and in Vitro Digestion. The changes of viscosity during heating of the mixture of oat starch and β-glucan with high, medium, and low MW are shown (Figure 2). The mixture without β-glucan for Jim and N979 did not demonstrate any pasting properties. Oat starch was swollen and gelatinized, but this did not change the viscosity of the solutions (5% starch, w/w) during heating. As the MW of β-glucan increased, the viscosity increased. The inclusion of high-MW β-glucan, even at low concentrations, significantly changed the pasting behavior.

To understand the change of viscosity during in vitro digestion, the enzyme solution was added to the mixture and the incubation simulated at 37 °C for 180 min. The viscosity profiles of all mixtures except the one with high-MW β-glucan (S + HBG) were not changed during digestion (Figure 3). The high-MW β-glucan increased the viscosity of the mixture. At the end of digestion of 180 min, the viscosity of the mixture with high-MW β-glucan was reduced greatly but still remained greater than those of the other mixtures. These results indicated that the viscosity of β-glucan played a key role in reducing starch digestibility. The reduction of starch digestibility was a

consequence of altered rheological properties of starch dispersions in the solution by high-MW β-glucan, which increased viscosity and inhibited enzyme accessibility to gelatinized starch granules.[6] Therefore, it is important to understand the physicochemical properties of β-glucan when attempting to achieve a particular purpose for adding β-glucan in the development of health-promoting food products.

This study demonstrated that the inhibition of starch digestibility by β-glucan was greatly influenced by the physicochemical properties of β-glucan, such as MW, viscosity, and solubility. The inclusion of high-MW β-glucan, providing greater viscosity and lower solubility than medium- and low-MW β-glucan, retarded the rate of starch digestion and thus lowered the estimated GI values after in vitro digestion. There were significant correlations between the estimated GI values and the peak and final viscosities of β-glucan. Moreover, the viscosity of β-glucan was highly correlated with the peak MW of β-glucan. These results illustrated that the inclusion of oat soluble dietary fiber, β-glucan, retarded starch digestibility, resulting in a reduced estimated GI value; however, the molecular and physicochemical properties of β-glucan should be considered to optimize the impact. This finding will help in

developing food products containing oat β-glucan with the benefit of providing a low GI.

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Notes

The authors declare no competing financial interest.

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