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Hyun Jung Kim
Iowa State University, hjkim@iastate.edu

Pamela J. White
Iowa State University, pjwhite@iastate.edu

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Abstract
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Keywords
oat β-glucan, starch, protein, viscosity, in vitro bile acid binding

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Interactional Effects of β-Glucan, Starch, and Protein in Heated Oat Slurries on Viscosity and In Vitro Bile Acid Binding

Hyun Jung Kim and Pamela J. White*

Department of Food Science and Human Nutrition, Iowa State University, Ames, Iowa 50011, United States

ABSTRACT: Three major oat components, β-glucan, starch, and protein, and their interactions were evaluated for the impact on viscosity of heated oat slurries and in vitro bile acid binding. Oat flour from the experimental oat line "N979" (7.45% β-glucan) was mixed with water and heated to make oat slurry. Heated oat slurries were treated with α-amylase, lichenase, and/or proteinase to remove starch, β-glucan, and/or protein. Oat slurries treated with lichenase or lichenase combined with α-amylase and/or proteinase reduced the molecular weight of β-glucan. Heat and enzymatic treatment of oat slurries reduced the peak and final viscosities compared with the control. The control bound the least amount of bile acids ($p < 0.05$); heating of oat flour improved the binding. Heated oat slurries treated with lichenase or lichenase combined with α-amylase and/or proteinase bound the least amount of bile acid, indicating the contribution of β-glucan to binding. Oat slurries treated with proteinase or proteinase and α-amylase together improved the bile acid binding, indicating the possible contribution of protein to binding. These results illustrate that β-glucan was the major contributor to viscosity and in vitro bile acid binding in heated oat slurries; however, interactions with other components, such as protein and starch, indicate the importance of evaluating oat components as whole system.

KEYWORDS: *oat β-glucan, starch, protein, viscosity, in vitro bile acid binding*

INTRODUCTION

Oats are well-recognized as a whole-grain cereal, highly recommended as an important part of the daily diet. Health benefits of oat-based food products are attributed to the dietary fiber in oats, (1→3), (1→4)-β-D-glucan, referred to as β-glucan. The consumption of β-glucan decreases glucose uptake and insulin responses, lowers cholesterol in the blood, and induces and prolongs satiety.[1,2] In particular, 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.[3,4] 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, equal to a level of 3 g per day, may reduce cholesterol and lower the risk of coronary heart disease.[5] These health benefits are caused by the increase in viscosity formed by β-glucan, which is related to its concentration and molecular weight.[6] Processing and cooking of foods can influence the molecular, structural, and functional properties of β-glucan, depending on the methods and conditions of processing. Cooking and extrusion methods increased the physiological activity of β-glucan by increasing solubility and extractability[7,8] and possibly reducing the molecular size of the polymer.[9]

Along with β-glucan, other major components in oats are starch and protein. The interactions of all components affect the physical properties and consequently impact the health benefits of oat-based food products. Starch is the most abundant component in oats and impacts the viscosity, as related to the ratio of amylose to amylopectin and the branch-chain length distribution of amylopectin.[10] Protein concentration in oats ranges from 12 to 24%, which is the highest among cereals. The hydrolysis of protein by protease in oatmeal slurries caused minor changes of some pasting parameters, demonstrating minimal impact of protein on viscosity of oatmeal slurries compared with the oat slurries treated with β-glucanase.[11]

For the evaluation of the contribution of starch, β-glucan, protein, and their interactions, α-amylase, lichenase, and proteinase K are used to break down those components.[12] α-Amylase randomly hydrolyzes α-(1→4)-glucosidic linkages along the starch chain, yielding a mixture of linear and branched oligosaccharides, maltotriose, and maltose from amylose and maltose, glucose, and α-limit dextrin from amylopectin.[13] Lichenase specifically cleaves β-(1→4)-glucosidic linkages of the 3-*O*-substituted glucose residues in β-glucan, resulting in oligosaccharides with different degrees of polymerization.[14] Proteinase K is a stable and highly reactive serine protease, catalyzing the hydrolysis of a wide variety of peptide bonds.

Complex interactions of oat components during food processing and cooking, and with other food ingredients, altered the physical functions of the macronutrients in oat.[11,12] Clearly, more study is needed to understand the interaction and impact of oat components on the physical and physiological functions. In this study, the contribution of oat components β-glucan, starch, and protein and their interactions to the viscosity and to in vitro bile acid binding of heated oat slurries was evaluated.

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Table 1. Composition of Oat Slurry Treatments

treatments[a]	composition[b] (%, dry weight basis)			
	β-glucan	starch	glucose	protein
control	7.5 ± 0.1 a	51.3 ± 1.3 a	0.9 ± 0.1 c	13.0 ± 0.6
no-enzyme	7.4 ± 0.1 a	49.9 ± 1.2 ab	2.6 ± 0.6 c	14.5 ± 1.3
amylase (A)	6.8 ± 0.2 bcd	16.7 ± 0.7 d	33.3 ± 3.5 ab	12.9 ± 1.2
lichenase (L)	6.6 ± 0.1 cde	45.4 ± 4.9 b	2.5 ± 0.1 c	12.0 ± 0.8
proteinase (P)	7.2 ± 0.4 b	49.8 ± 3.3 ab	2.6 ± 0.2 c	12.5 ± 1.4
A + L	6.3 ± 0.1 e	23.6 ± 3.3 c	31.7 ± 3.6 b	14.5 ± 1.5
L + P	6.6 ± 0.1 cd	48.8 ± 1.5 ab	2.9 ± 0.1 c	13.4 ± 1.9
P + A	6.9 ± 0.1 bc	22.1 ± 4.2 c	33.3 ± 1.9 ab	12.0 ± 0.8
A + L + P	6.2 ± 0.1 e	11.0 ± 1.4 e	35.6 ± 0.7 a	14.0 ± 0.6

[a]Control is raw whole oat flour. No-enzyme is oat slurry without enzyme treatment. Oat slurry A + L was treated with amylase and lichenase, L + P with lichenase and proteinase, P + A with proteinase and amylase, and A + L + P with amylase, lichenase, and proteinase. [b]Values are means ± standard deviation. Values followed by different letters within a column are significantly different ($P < 0.05$).

MATERIALS AND METHODS

Preparation of Heated Oat Slurries. An experimental oat line, N979-5-4, developed at Iowa State University and grown in 2010 at the Agronomy and Agricultural Engineering Field Research Center in Ames, IA, was chosen for this study because this line has a greater β-glucan concentration (7−8%) than publicly available cultivars (4−5%). The harvested oat kernels were dried and dehulled with an air-pressure dehuller (Codema, Eden Prairie, MN). The resulting oat groats were ground in an ultracentrifugal mill (ZM-1, Retch GmbH, Hann, Germany) with a 0.5 mm sieve. Oat flour was mixed with water (13% w/v) and heated at 90 °C for 10 min with continuous stirring to prepare oat slurries. Heated oat slurries were cooled down to 40 °C for enzyme treatments. Oat flour (raw) was used as a control.

Eight different enzyme treatments of heated oat slurries were used: (1) no-enzyme treatment, (2) hydrolysis of starch by adding α-amylase (125 U/g of flour; EC 3.2.1.1.; contained <0.08% of amyloglucosidase, 0.0001% of xylanase, and 0.02% of cellulase; Megazyme, Wicklow, Ireland),[12] (3) hydrolysis of β-glucan with added lichenase (100 U/g of flour; EC 3.2.1.73; 1,3−1,4-β-D-glucan-4-glucanohydrolase; 330 U lichenase/mg protein, <0.0001 U of β-glucosidase, cellulase, and endo-1,3-β-glucanase, and <0.0004 U of α-amylase and amyloglucosidase; Megazyme),[12,15] (4) hydrolysis of protein with added proteinase K (20 U/g of flour; EC 3.4.21.64, maximum activity at 37 °C and pH range of 7.5−12.0; Sigma-Aldrich Co., St. Louis, MO),[11] (5) hydrolysis of both starch and β-glucan by adding α-amylase and lichenase, (6) hydrolysis of β-glucan and protein by adding lichenase and proteinase K, (7) hydrolysis of starch and protein by adding α-amylase and proteinase K, and (8) hydrolysis of all three components, starch, β-glucan, and protein, by adding α-amylase, lichenase, and proteinase K. An aliquot of heated oat slurry (28 g), after cooling down to 40 °C, was mixed and incubated with specific enzymes by using a rapid Visco analyzer (RVA, Newport Scientific, Warriewood, Australia) at 115 rpm at 40 °C for 1 h to evaluate the contribution of starch, β-glucan, and protein and their interactions. All measurements were prepared in triplicate and the results averaged.

Viscosity Measurement. The apparent viscosity of oat slurry treatments was measured by using the RVA as a function of temperature, time, and stirring speed. The test profile of the RVA 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 40 to 90 °C over 3 min, holding at 90 °C for 6.5 min, decreasing to 40 °C over 4.5 min, and holding at 40 °C for 5 min.[12] To measure the viscosity of the control, oat flour (13% w/v, total mass 28 g) was dispersed in silver nitrate solution (16.7 mM) to inactivate natural β-glucan-degrading enzymes.[12] The peak and final viscosities were measured in triplicate and the results averaged for all oat slurries.

Proximate Composition. Moisture concentration of oat slurries was analyzed by using AACC Method 44-15A.[16] The concentrations of β-glucan in oat slurries were measured enzymatically by AACC Method 32-23, with the application of a Mixed β-Glucan Linkage Kit (Megazyme). Starch concentration was determined by following AACC Method 76-13 by using a Total Starch Kit (Megazyme). Proteins were analyzed by using an automatic nitrogen analyzer (Elementar Analzen System GmbH) with a nitrogen conversion factor of 5.7. Free glucose concentration in oat slurries was determined by using the D-Glucose Assay Procedure (Megazyme). All analyses were run in triplicate and the averages are reported on a dry-weight basis.

Determination of β-Glucan Molecular Weight. Water-soluble β-glucans were extracted from oat slurries according to the procedure of Yao et al.[17] with modification as described. Starch and protein in oat slurries were removed by heat-stable α-amylase (200 U/g of oat flour, Megazyme) and pancreatin (1.3 mg/g of oat flour, Sigma-Aldrich), respectively. After ethanol precipitation of polymers, the precipitate was isolated by centrifugation at 3100*g* for 20 min and redissolved in deionized water at 80 °C under magnetic stirring. The relative molecular weight (MW) of the extracted β-glucan suspension (5 mg of β-glucan/mL) was determined by using size-exclusion high-performance liquid chromatography (SE-HPLC) according to the method of Sayar et al.[18] 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, 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, NY) before injection. β-Glucan MW standards (Cat No. P-MWBG, Megazyme) with MW values of 3.59×10^5, 2.45×10^5, 1.83×10^5, 1.23×10^5, and 0.40×10^5 g/mol were used to estimate the actual MW ranges of the extracted β-glucan fractions. The number-average MW (M_n) and peak MW were obtained by a first-order polynomial curve of log MW versus retention time of the HPLC chromatogram.[17]

In Vitro Bile Acid Binding. In vitro bile acid binding of oat slurry treatments was determined by a previously published procedure.[19] The bile acid mixture was freshly prepared with sodium cholate, sodium deoxycholate, sodium glycocholate, and sodium taurocholate (Sigma-Aldrich) with proportions as 35, 35, 15, and 15% (w/w) in 50 mM phosphate buffer at pH 6.9, respectively. The total amount of bile acid initially added was 11.2 μmol/100 mg of treatment. Cholestyramine (Sigma-Aldrich) as a positive control and cellulose (Sigma-Aldrich) as a negative control were used.[20] Fifty milligram portions of cholestyramine, cellulose, and oat slurries were digested with 1 mL of 0.01 N hydrochloric acid and incubated in a shaking water bath at 37 °C for 1 h, which simulated gastric digestion. The pH of the materials was then adjusted to 6.9 with 0.1 N sodium hydroxide. Four milliliters of bile acid mixture (1.4 μmol/mL) and 5 mL of porcine pancreatin (Sigma-Aldrich; activity at least equivalent to 8× USP specifications; 6.25 mg/mL in a 50 mM phosphate buffer, pH 6.9) were added, and the

mixture was incubated in a shaking water bath at 37 °C for 1 h to digest the starch and protein. After centrifugation at 3100*g* for 10 min, the supernatant was removed. An additional 5 mL of phosphate buffer was used to rinse out the residue, and the mixtures were centrifuged again. The supernatant was removed and combined with the previous supernatant. Unbound bile acid in the supernatant was analyzed by using a Bile Acid Diagnostic Kit (Trinity Biotech, Bray Co., Wicklow, Ireland). The mixtures were diluted to fall within the range of the test kit. The concentration of bile acid was calculated on the basis of a standard curve developed from the bile acid at different concentrations.

Statistical Analysis. Data were analyzed by using the analysis of variance (ANOVA), followed by least significant differences (LSD) for comparison among oat slurry treatments using the GLM procedure in SAS version 9.1 (SAS Inst., Cary, NC) at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Characterization of Oat Slurry Treatments. Oat flour from the experimental oat line N979 contained 7.45% β-glucan. β-Glucan concentrations of oat slurries treated with amylase, lichenase, and/or proteinase were lower than that of the oat slurry without enzyme treatment (Table 1). This reduction might be a result of the minor contaminants in α-amylase acting during incubation at 40 °C for 1 h and breaking down parts of β-glucan molecules. α-Amylase purchased from Megazyme contains 0.02% cellulase. This particular enzyme might cleave parts of β-glucan linked by β-1,4-linkage. Starch concentrations in oat slurries treated with amylase (A) or amylase combined with lichenase (A + L) and/or proteinase (A + L + P) decreased from 51.3% (control, oat flour) to 11.0 to 23.6% and glucose concentrations increased from 0.9% to 31.7 to 35.6%. Protein concentrations in oat slurries ranged from 12.0 to 14.5%. Heating of oat flour to prepare oat slurries did not change the chemical composition of the oat flour (control vs no-enzyme treatment). Enzymatic treatments with amylase and/or lichenase hydrolyzed starch and β-glucan molecules to glucose. Oat slurries treated with proteinase did not change the concentration of protein in oat slurries. Likely, proteins in oat slurries were broken down by proteinase to low molecular proteins; however, both low and high molecular weight proteins were detected by a nitrogen analyzer,[21] thus few differences in protein concentrations were noted among oat-slurry treatments.

Molecular Weight of β-Glucan Extracted from Oat Slurry Treatments. The number-average molecular weight (MW) and peak MW of the control oat flour were 5.78×10^5 and 6.47×10^5 g/mol, respectively (Table 2). These values are lower than the values of N979-5-4 grown in 2009 (7.09×10^5 and 8.98×10^5 g/mol) reported in our previous study.[15] The molecular weights of β-glucan from both the 2009 and 2010 crops were analyzed by the same researcher, in the same lab, using the same equipment. These MW differences might be attributed to environmental and growth factors associated with the growing years of 2009 and 2010.[2] The weather conditions during 2010 were wetter than those during 2009 with average temperatures being similar each year.[22] Wetter years result in lower β-glucan MW. For example, the MW of oat lines grown in 2004 was lower than those grown in 2003 with less rainfall.[17] Further, Ajithkumar et al.[23] reported that the MW of β-glucan seems to be controlled more by environmental factors, especially rainfall distribution over the years, rather than by genetic factors.

Heating of oat flour to make slurries did not change the number-average MW and peak MW (5.39×10^5 and 7.31×10^5 g/mol); however, the enzyme treatments reduced the MW of β-glucan (Figure 1). The study of Regand et al.[7] showed that cooking of porridge made with oat bran and oat flakes did not affect the MW and solubility of β-glucan, because of the inactivation by heat treatment of endogenous enzymes, such as β-glucanase, in the oat flour, which prevents further destruction of β-glucan molecules.

Oat slurries treated with amylase, proteinase, and both amylase and proteinase together had lower values of the number-average MW and peak MW than did the treatment with no enzyme. This finding might be a result of the minor contaminants in α-amylase and proteinase K products being able to act during incubation at 40 °C for 1 h. Oat slurries treated with lichenase or lichenase combined with amylase and/or proteinase reduced the size of molecules, creating particles too small for detection by size-exclusion HPLC, thus causing the loss of viscosity in oat slurries. Lichenase is the enzyme that specifically breaks the 1,4-bonds adjacent to a 1,3-bond from β-glucan molecules to produce 1,4-linked glucose oligosaccharides with one 1,3-link at the reducing end.[14] Likely, most β-

Table 2. Molecular Weight of β-Glucan Extracted from N979 Oat Flour and Oat Slurry Treatments

	molecular weight (MW) of β-glucan[b] ($\times 10^5$ g/mol)	
treatments[a]	number-average MW	peak MW
control	5.78 ± 0.4 a	6.47 ± 0.7 a
no-enzyme	5.39 ± 0.8 a	7.31 ± 0.7 a
amylase	3.28 ± 0.8 c	5.90 ± 0.7 b
lichenase	nd[c]	nd
proteinase	4.93 ± 0.6 b	7.52 ± 0.4 a
A + L	nd	nd
L + P	nd	nd
P + A	2.65 ± 0.3 d	5.00 ± 0.8 c
A + L + P	nd	nd

[a]Control is raw whole oat flour. No-enzyme is oat slurry without enzyme treatment. Oat slurry A + L was treated with amylase and lichenase, L + P with lichenase and proteinase, P + A with proteinase and amylase, and A + L + P with amylase, lichenase, and proteinase. [b]Values are means ± standard deviation. Values followed by different letters within a column are significantly different ($P < 0.05$). [c]Molecular weight was not detected with SE-HPLC.

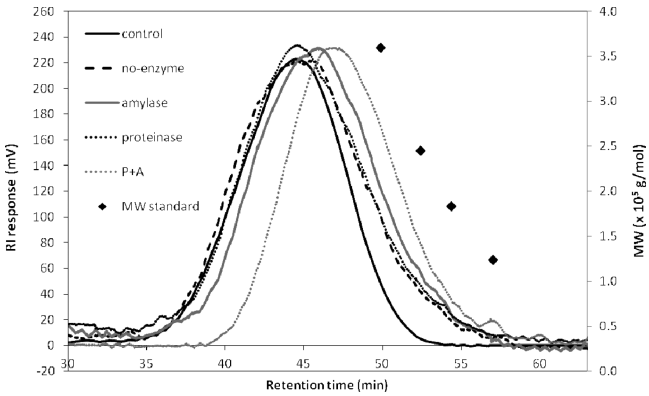

Figure 1. Distribution of β-glucan molecular weight (MW) from oat-slurry treatments and MW standards (MW = 0.4×10^5, 1.23×10^5, 1.83×10^5, 2.45×10^5, and 3.59×10^5 g/mol). The MW of β-glucan from lichenase-treated oat slurries (L, A + L, L + P, and A + L + P) was not detectable by SE-HPLC.

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glucan molecules in oat slurries were broken down by lichenase to β-1,4-linked glucose oligosaccharides.

Viscosity of Oat Slurry Treatments with the Rapid Visco Analyzer. Heating of oat flour in water to make oat slurries, such as in the control and in the no-enzyme oat slurry treatment, reduced peak viscosities from 7476 to 5371 cP and final viscosities from 10 901 to 6267 cP, respectively (Figure 2).

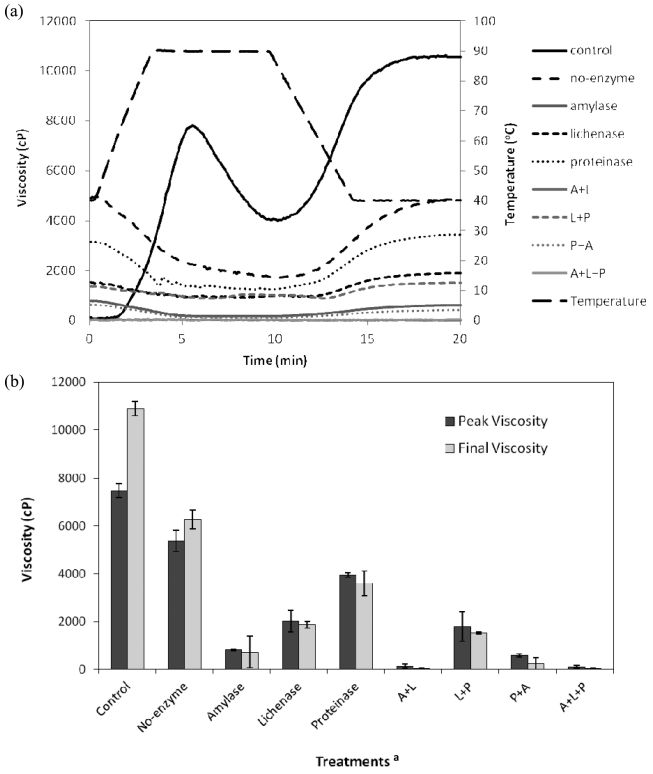

Figure 2. Viscosity profiles (a) and peak and final viscosity (b) of oat-slurry treatments. The control is raw whole oat flour. No-enzyme is oat slurry without enzyme treatment. Oat slurry A + L was treated with amylase and lichenase, L + P with lichenase and proteinase, P + A with proteinase and amylase, and A + L + P with amylase, lichenase, and proteinase.

Although the chemical compositions of the control and no-enzyme oat slurry treatment were comparable, during heating, starch and other soluble carbohydrates, the primary contributors to viscosity in cereal-based slurries, were degraded and their swelling ability was reduced.[24] Likely, these degradations led to lower peak and final viscosities when compared to the control without heat treatment.[24]

Enzymatic hydrolysis of starch, β-glucan, and/or protein, in all oat slurry treatments, reduced peak and final viscosities compared to the control (Figure 2). The oat slurry treatment with amylase had the least peak and final viscosities among oat slurries treated with one enzyme added. Also, the combination treatments of amylase with lichenase and/or proteinase had substantial loss of viscosity compared with the no-enzyme treatment. These results indicated that the hydrolysis of starch caused the loss of viscosity in oat slurries. The addition of α-amylase to oat slurries reduced the starch concentration by hydrolyzing the amylose and amylopectin, also interfering with starch interactions with other components, substantially lowering the viscosity of oat slurries.[12]

The lichenase specially broke down β-glucan molecules in oat slurries and eliminated the contribution of β-glucan to viscosity. Under this condition, starch became the major component responsible for swelling and pasting. The peak and final viscosities obtained with lichenase treatment were greater than those of the amylase treatment. These results indicated that, overall, starch-related factors contributed more to the viscosity than did β-glucan. However, given that the starch concentration was considerably greater than the β-glucan concentration in oat flour (Table 1), the β-glucan likely contributed more per unit weight to viscosity than did starch.

The hydrolysis of protein by proteinase K resulted in oat slurries with the least viscosity reduction among all enzyme-treated oat slurries (Figure 2). The relative percentage of viscosity difference was calculated on the basis of the peak and final viscosities of the no-enzyme treatment. The relative decreases of peak and final viscosities with the addition of proteinase were 26.5 and 42.6%, respectively, values much less than those obtained with the amylase treatment (86.5 and 89.0%, respectively) and lichenase treatments (62.5 and 70%, respectively). These results demonstrate a lesser contribution of protein to viscosity than of starch and β-glucan, findings in agreement with those of other studies.[11,12,21]

The addition of α-amylase and lichenase (A + L) greatly reduced starch and β-glucan, lichenase and proteinase K (L + P) greatly reduced β-glucan and protein, and proteinase K and α-amylase (P + A) greatly reduced protein and starch-related effects to peak and final viscosity. These treatments presented almost the sole contribution of protein, starch, and β-glucan, respectively, to viscosity. With these conditions, the peak and final viscosities decreased in the order of L + P, P + A, and A + L treatments, indicating that the greatest contributors to viscosity were in descending order of starch, β-glucan, and protein. These results reconfirmed the minimal effect of protein on viscosity.

In Vitro Bile Acid Binding of Oat Slurry Treatments. Cholestyramine, the positive control for bile acid binding, bound 9.54 μmol of the 11.2 μmol of bile acid/100 mg of cholestyramine (Table 3). Cellulose, the negative control, bound 0.03 μmol of bile acid/100 mg of cellulose. If the amount bound by cholestyramine was considered to bind bile acid at 100%, then the amount bound by cellulose would be calculated as 0.31% (0.03 μmol/9.54 μmol $\times$ 100%).

The relative bile acid binding values of oat-slurry treatments were calculated on the basis of 100% bile acid bound to cholestyramine to eliminate methodological effects. The control, oat flour, bound the least amount of bile acid (22.1%, Table 3). Heating of oat flour improved bile acid binding to 25.5% (no-enzyme treatment, Table 3). The high temperature during heating might cause optimal exposure of components in oat flour, leading to increased bile acid binding.[25,26]

The bile acid binding of oat-slurry treatments was altered by the type of enzyme treatment (Table 3). Oat slurries treated with lichenase (L) or lichenase combined with amylase and/or proteinase (A + L, L + P, and A + L + P treatments) had the lowest bile acid binding among heat-treated oat slurries (23.7, 23.7, 22.1, and 23.4%). The hydrolysis of β-glucan in oat slurries treated with lichenase to create very small molecular oligosaccharides likely reduced the bile acid binding.[15] Kim and White[15] reported an optimum MW range of β-glucan [(1.61–2.42) $\times$ 10^5 g/mol] for in vitro bile acid binding. β-Glucan in the oat-slurry treatment with lichenase was broken down to

Table 3. In Vitro Bile Acid Binding of Oat Slurry Treatments

	bile acid bound[b]	
treatments[a]	μmol/100 mg total weight, dwb	relative % to cholestyramine[c]
control	2.109 ± 0.052 c	22.1 ± 0.5 d
no-enzyme	2.435 ± 0.082 b	25.5 ± 0.9 c
amylase	2.472 ± 0.120 b	25.9 ± 1.2 bc
lichenase	2.259 ± 0.008 c	23.7 ± 0.1 d
proteinase	2.595 ± 0.137 ab	27.2 ± 1.3 ab
A + L	2.262 ± 0.007 c	23.7 ± 0.1 d
L + P	2.108 ± 0.098 c	22.1 ± 1.0 d
P + A	2.700 ± 0.081 a	28.3 ± 0.8 a
A + L + P	2.236 ± 0.015 c	23.4 ± 0.2 d
cholestyramine	9.540 ± 0.010	100
cellulose	0.03 ± 0.008	0.31

[a]Control is raw whole oat flour. No-enzyme is oat slurry without enzyme treatment. Oat slurry A + L was treated with amylase and lichenase, L + P with lichenase and proteinase, P + A with proteinase and amylase, and A + L + P with amylase, lichenase, and proteinase. [b]Values are means ± standard deviation. Values followed by different letters within a column are significantly different ($P < 0.05$). [c]Relative bile acid binding percentage (%) when cholestyramine is considered to bind 100% bile acid.

very small MW β-glucans with MW of 0.46×10^5 g/mol, which were highly self-aggregated,[27] resulting in reduced ability to bind bile. The peak viscosity of oat slurries was highly correlated with β-glucan number-average MW ($R^2 = 0.94$); however, there was no clear relationship between viscosity or MW and in vitro bile acid binding (Figure 2). The low mobility of the high MW β-glucan and the high self-aggregation of the lowest MW β-glucan reduced the bile acid binding to β-glucan.[15] The oat slurry treated with proteinase and amylase (P + A treatment) bound 28.3% of the bile acid. Under this condition, β-glucan was almost the sole contributor to bile acid binding. These results indicated that β-glucan greatly contributed to bile acid binding of oat slurries. Further study will be needed to explore the mechanism of bile acid binding by β-glucan.

Alternatively, oat slurries treated with proteinase (P) or proteinase combined with amylase (P + A) bound bile acid at values of 27.2 and 28.3%, respectively. Likely, the hydrolysis of protein by proteinase K to peptides influenced bile acid binding. Previous studies showed involvement of postdigestion hydrophobic peptides in plasma cholesterol-lowering by bile acid binding of dietary plant proteins, particularly the bile acid binding activity of buckwheat protein and corn protein hydrolysate.[28,29] Sayar et al.[30] fractionated oat flour into bran, protein concentrate, and starch to examine the bile acid binding of each component. Among three types of fractions, bran and protein bound more bile acid than did starch.[30] Further study is needed to explore the impact of protein or peptides in oat flour on bile acid binding.

In conclusion, heating of oat flour in water to prepare oat slurries reduced the peak and final viscosities but improved in vitro bile acid binding of oat slurries. Oat slurries treated with lichenase or lichenase combined with amylase and/or proteinase reduced the β-glucan MW, resulting in reduction of in vitro bile acid binding. Treatment of the oat slurries with proteinase and/or amylase improved the bile acid binding. These results indicated that β-glucan contributed greatly to viscosity and to in vitro bile acid binding in heated oat slurries. Interactions of β-glucan with protein and starch also contributed to in vitro bile acid binding. These findings indicate the importance of evaluating oat components as a whole system when considering potential health impacts.

AUTHOR INFORMATION

Corresponding Author

*Phone: (515) 294-5380. Fax: (515) 294-7802. E-mail: pjwhite@iastate.edu.

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