8-25-2010

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Abstract
Seven experimental oat lines with high (6.2–7.2%), medium (5.5–5.9%), and low (4.4–5.3%) β-glucan concentrations were evaluated for contributions of β-glucan, starch, protein, and their interactions, to pasting properties of oat flours by using a Rapid Visco Analyser (RVA, Newport Scientific, Warriewood, NSW, Australia). Significant correlations ($P < 0.05$) between β-glucan concentration and pasting parameters of oat slurries were obtained under autolysis without 1 h of incubation, inhibition, and amylolysis. The relative decrease of viscosity after enzymatic hydrolysis of β-glucan correlated with β-glucan concentration ($P < 0.05$). These data demonstrated the important contribution of β-glucan to pasting. The relative decrease of viscosity after either amylolysis or enzymatic removal of protein correlated with β-glucan concentration ($P < 0.1$), which might be explained by the considerable contribution of the interaction of β-glucan with starch and protein, to pasting. The viscosity decrease by hydrolysis of protein was much greater than the actual viscosity remaining after hydrolysis of both β-glucan and starch, reconfirming the importance of interactions between protein and other oat components to pasting. Optimal multiple linear regression (MLR) models were generated to predict key pasting parameters in both buffer without 1 h of incubation and silver nitrate solution by using a stepwise procedure. The β-glucan concentration alone or together with the concentration of starch, rather than protein, was selected as the predictor under certain conditions. These results illustrated the major unit contribution of β-glucan, secondary unit contribution of starch, and minimal unit contribution of protein to pasting.

Keywords
Statistics, β-Glucan, starch, protein, oat, RVA; pasting, multiple linear regression

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DOI:10.1021/jf1007852

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Individual and Interactional Effects of β-Glucan, Starch, and Protein on Pasting Properties of Oat Flours

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Seven experimental oat lines with high (6.2−7.2%), medium (5.5−5.9%), and low (4.4−5.3%) β-glucan concentrations were evaluated for contributions of β-glucan, starch, protein, and their interactions, to pasting properties of oat flours by using a Rapid Visco Analyser (RVA, Newport Scientific, Warriewood, NSW, Australia). Significant correlations ($P < 0.05$) between β-glucan concentration and pasting parameters of oat slurries were obtained under autolysis without 1 h of incubation, inhibition, and amylolysis. The relative decrease of viscosity after enzymatic hydrolysis of β-glucan correlated with β-glucan concentration ($P < 0.05$). These data demonstrated the important contribution of β-glucan to pasting. The relative decrease of viscosity after either amylolysis or enzymatic removal of protein correlated with β-glucan concentration ($P<0.1$), which might be explained by the considerable contribution of the interaction of β-glucan with starch and protein, to pasting. The viscosity decrease by hydrolysis of protein was much greater than the actual viscosity remaining after hydrolysis of both β-glucan and starch, reconfirming the importance of interactions between protein and other oat components to pasting. Optimal multiple linear regression (MLR) models were generated to predict key pasting parameters in both buffer without 1 h of incubation and silver nitrate solution by using a stepwise procedure. The β-glucan concentration alone or together with the concentration of starch, rather than protein, was selected as the predictor under certain conditions. These results illustrated the major unit contribution of β-glucan, secondary unit contribution of starch, and minimal unit contribution of protein to pasting.

KEYWORDS: β-Glucan; starch; protein; oat; RVA; pasting; multiple linear regression

INTRODUCTION

Oat (*Avena sativa*), a multifunctional food, is nutritionally superior to many other unfortified cereals. Consumption of oat-based products can lower serum cholesterol levels, reduce glucose uptake, decrease plasma insulin response, and control weight through prolonged satiety (*1*−*3*). These physiological effects of oats are primarily attributable to the elevation of viscosity in the gastrointestinal tract (*4*), caused mainly by β-glucans. The increased luminal viscosity may lower the reabsorption of bile acid (BA) in the ileum, thus increasing BA excretion in the feces (*5*). Physical elimination of BA from enterohepatic circulation necessitates increased synthesis of BA, consequently increasing cholesterol conversion into BA in the liver, and eventually decreasing serum cholesterol (*6*). The elevation of viscosity also slows intestinal transit and delays gastric emptying and intestinal absorption of nutrients, such as digestible carbohydrates, thereby reducing postprandial hyperglycemia and insulin secretion. These actions, in turn, increase satiety and promote weight loss (*7*, *8*).

The pasting properties of oat flours also impact textural attributes and consumer acceptance of food products. Oat slurries having short time to peak viscosity and high relative mean values of peak viscosity and final viscosity were related to greater acceptability by Australian consumers (*9*). Similarly, rolled oats with a relatively low peak viscosity and a delay in time to peak viscosity were unacceptable to consumers, although none of the standard quality control tests identified a problem (*10*).

Three oat components may primarily impact the pasting properties of oat slurries: β-glucan, starch, and protein. β-Glucan, a major component of starchy endosperm and aleurone cell walls of oat seeds, is an unbranched homopolysaccharide composed of (1 → 4)-linked (∼70%) and (1 → 3)-linked (∼30%) β-D-glucopyranosyl units. β-Glucans with a high water-binding capacity exhibit high viscosity at relatively low concentrations. Correlations between β-glucan concentration in oats and the viscosity of oat slurries were previously reported (*11*−*13*). The contribution of β-glucan to pasting was demonstrated by the significant decrease of apparent viscosity after enzymatic degradation of β-glucan with lichenase (EC 3.2.1.73) (*14*). Starch, the most abundant component in oats, constitutes nearly 60% of the dry matter of oat grain. Most studies on oats have focused on functions of isolated starch because of the difficulties of separating the effects of starch, β-glucans, and other oat components on pasting (*15*). In general, the pasting properties of starch are impacted by the ratio of amylose to amylopectin and by the branch chain-length distribution of amylopectin. Amylopectin contributes to swelling of starch granules and pasting, whereas

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 Published on Web 07/21/2010

amylose inhibits swelling (*16*). Pasting properties of starch can also be influenced by its interactions with other components, such as sugars, lipids, proteins, emulsifiers, gums, salts, and pH modifiers (*17*−*19*). The protein content of oat groats ranges from 12 to 24%, the highest among cereals. The action of protease had no effect on the viscosity of barley slurries, indicating that proteins contributed little to the overall viscosity of barley-flour slurries (*20*). The addition of proteinase into oatmeal slurries caused significant but minor changes to some pasting parameters compared with the addition of β-glucanase (*12*), demonstrating minimal impact of protein on pasting properties of oatmeal slurries compared with that of β-glucan.

The objective of this study was to investigate the contributions of β-glucan, starch, protein, and their interactions to the pasting properties of oat flours as measured with a Rapid Visco Analyser (RVA, Newport Scientific, Warriewood, NSW, Australia). Understanding how the individual and interactional effects of these three components in oats impact the pasting properties of oat flours will help in the development of oat-based food products with desirable health benefits and sensory qualities.

MATERIALS AND METHODS

Plant Materials. Seven experimental oat lines, including IA03144-7 (IA44), IA03146-6 (IA46), IA03147-1 (IA47), IA03150-3 (IA50), IA03176-2 (IA76), IA03187-6 (IA87), and IA03192-4 (IA92), developed at Iowa State University, were grown in 2007 at the Agronomy and Agricultural Engineering Field Research Center near Ames, Iowa. These oat lines were previously shown to vary in β-glucan concentration and apparent viscosity (*21*). The oats were dehulled with an air-pressure dehuller (Codema, Eden Prairie, MN). The groats were ground in an ultracentrifugal mill (ZM-1, Retch GmbH & Co, Haan, Germany) fitted with a 0.5 mm sieve.

Oat Composition. The moisture concentration of oat flours was determined by AACC Method 44-15A (*22*). The β-glucan concentration in flours was enzymatically determined by using AACC Method 32-23 (*22*), with the application of a mixed β-glucan linkage kit (Megazyme Int. Ireland Ltd., Wicklow, Ireland). The starch concentration in flours was analyzed by using AACC Method 76-13 (*22*). Oat flour proteins were determined by an automatic nitrogen analyzer (Elementar Analyzensysteme GmbH, Hanau, Germany) with a protein conversion factor of 6.25. Lipids were analyzed by the gravimetric method after extraction with petroleum ether on a Goldfisch system (*22*). All analyses were done at least in triplicate, averaged, and reported on a dry matter basis (db).

Rapid Visco Analyser Measurement. An aliquot of oat flours (3.68 g, db) was dispersed either in sodium phosphate buffer (50 mM, pH 6.9) for all enzymatic treatments and autolysis or in silver nitrate solution (16.7 mM) to promote inhibition conditions. The total mass of each slurry was 28 g. The Rapid Visco Analyser was used to measure the apparent viscosity of oat-flour slurries as a function of temperature, time, and stirring speed. The Rapid Visco Analyser test profile developed for oats included (1) a stirring speed of 960 rpm for 10 s and 115 rpm for the remainder of the test; (2) 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*). The measured viscosity was read directly from the Rapid Visco Analyser and reported in centipoise (cP). The Rapid Visco Analyser pasting parameters were peak viscosity (PV, maximum viscosity developed during or soon after sample heating), trough (T, minimum viscosity after PV), breakdown (BD, PV minus T), final viscosity (FV, viscosity at the end of test), setback (SB, FV minus T), and time to peak time (TTPV, time to reach PV).

To evaluate the contribution of β-glucan, starch, protein, and their interactions, to pasting, specific enzymes were used to systematically eliminate one or two components in oat slurries at a time. The impact of each enzymatic treatment was determined by comparison with the corresponding control. Seven different conditions (four enzymatic treatments and three controls) were used to measure the viscosities: (1) removal of starch from the system by adding thermostable α-amylase (125 U/g of flour, db; EC 3.2.1.1, Sigma-Aldrich Co., St Louis, MO) immediately before pasting measurements; (2) removal of β-glucan with added lichenase (100 U/g flour, db, EC 3.2.1.73, Megazyme Int. Ireland Ltd., Wicklow, Ireland) at 40 °C for 1 h before pasting; (3) removal of protein with added proteinase K (16.47 U/g of flour, db; EC 3.4.21.64, Sigma-Aldrich Co., St Louis, MO) at 40 °C for 1 h prior to pasting; (4) removal of both β-glucan and starch with added lichenase at 40 °C for 1 h, followed by the addition of α-amylase immediately before pasting; (5) autolysis (sodium phosphate buffer for dispersion) during pasting (control for α-amylase treatment); (6) autolysis at 40 °C for 1 h prior to pasting (control for both proteinase K treatment and lichenase plus α-amylase treatment); and (7) inhibition (dispersion in silver nitrate solution) during pasting (control for lichenase treatment).

The α-amylase randomly hydrolyzes α-(1 → 4)-glycosidic linkages along the starch chain, resulting in a mixture of linear and branched oligosaccharides and eventually yielding maltotriose and maltose from amylose or maltose, glucose, and α-limit dextrin from amylopectin (*23*). The lichenase specifically cleaves β-(1 → 4)-glycosidic linkages of the 3-O-substituted glucose residues in β-glucan, yielding oligosaccharides with different degrees of polymerization (*24*). Proteinase K is a stable and highly reactive serine protease, catalyzing the hydrolysis of a wide variety of peptide bonds. Unlike α-amylase, lichenase and proteinase K are not thermostable during pasting. The hydrolysis of β-glucan or protein was conducted by mixing the slurries at 115 rpm for 1 h in the Rapid Visco Analyser right before applying the Rapid Visco Analyser test profile just described. The sodium phosphate buffer was used to study the autolytic effect (hydrolysis by endogenous β-glucanases) of oat-flour slurries on viscosity. Silver nitrate solution was employed to study the intrinsic viscosity of oat slurries by inhibiting endogenous β-glucanases (*25*). The viscosity profiles developed with enzymatic treatments were compared with profiles in sodium phosphate buffer or silver nitrate solution obtained with the same program. The Rapid Visco Analyser measurements were performed at least in duplicate for all oat flours.

Statistical Analysis. Results were analyzed by using statistical analysis software (SAS 9.1, SAS Institute Inc., Cary, NC). The paired *t* test was conducted to determine whether the means of each Rapid Visco Analyser pasting parameter in enzymatic treatments differ from those in the controls with $P < 0.05$. Multiple comparisons among the means of each Rapid Visco Analyser pasting parameter for different oat lines were performed by using the least significant difference (LSD) test at $\alpha = 0.05$. Linear regression analyses were used to establish a relationship among variables with $P < 0.05$. Prediction models for the key pasting parameters under certain conditions were developed by multiple linear regression (MLR) using a stepwise procedure at a 0.1 significance level.

RESULTS AND DISCUSSION

Chemical Composition of Oat Groats. The seven experimental oat lines chosen for this study had a broad range of β-glucan concentrations (**Table 1**). The IA47 line contained a typical level of β-glucan (4.4%). The β-glucan concentrations in the other six oat lines were between 5.3% and 7.2%, which are greater than the typical values reported for domestic *A. sativa* cultivars (3.7%−5.0%) (*26*). Starch concentrations were between 51.1% and 59.6%, with the greatest concentration in the IA50 line. Protein percentages were between 17.5% and 21.3%, which is within the range of reported values for common oat cultivars (16.7%−22.1%) (*26*). Lipid concentrations were between 5.3% and 7.8%,

Table 1. Chemical Composition (%, db) of Flours from Different Oat Lines[a]

oat lines	β-glucan (*n* = 4)	starch (*n* = 4)	protein (*n* = 3)[b]	lipid (*n* = 3)
IA46	7.2 ± 0.29 a	55.5 ± 1.00 abc	20.1 ± 0.18 b	7.8 ± 0.10 a
IA92	6.2 ± 0.37 b	56.9 ± 1.22 ab	17.8 ± 0.02 f	5.6 ± 0.22 bc
IA44	5.9 ± 0.26 b	52.1 ± 0.99 bc	18.3 ± 0.14 e	7.1 ± 0.27 abc
IA87	5.9 ± 0.27 b	51.1 ± 0.46 c	21.3 ± 0.14 a	6.5 ± 0.20 abc
IA50	5.5 ± 0.29 c	59.6 ± 0.99 a	19.7 ± 0.08 c	6.5 ± 0.30 abc
IA76	5.3 ± 0.19 c	56.5 ± 0.55 ab	19.2 ± 0.08 d	7.2 ± 0.29 ab
IA47	4.4 ± 0.30 d	58.1 ± 0.60 a	17.5 ± 0.03 g	5.3 ± 0.21 c

[a] Values are the means of *n* measurements ± standard deviation. Values within a column followed by a common letter (a−g) are not significantly different ($P > 0.05$). [b] N × 6.25.

Table 2. Pasting Properties of Oat Flours under Autolytic Conditions[a,b]

	autolysis (0 h)				autolysis (1 h)			
	viscosity (cP)				viscosity (cP)			
oat lines	PV	T	FV	TTPV (min)	PV	T	FV	TTPV (min)
IA46	10513 ± 54.8 a	6322 ± 31.9 a	16383 ± 75.5 a	9.0 ± 0.00 a	2867 ± 26.6 b	2159 ± 15.6 b	2857 ± 25.3 bc	10.6 ± 0.05 a
IA92	8838 ± 51.3 b	6682 ± 29.4 a	14280 ± 75.6 ab	8.3 ± 0.11 bc	5017 ± 44.6 a	4223 ± 20.6 a	5318 ± 46.2 a	10.6 ± 0.05 ab
IA44	8781 ± 49.5 b	6389 ± 30.1 a	12438 ± 36.4 b	8.0 ± 0.09 c	2991 ± 26.6 b	2453 ± 19.6 b	2665 ± 21.8 bc	10.5 ± 0.04 ab
IA87	7916 ± 47.9 cd	5497 ± 20.5 b	11938 ± 73.8 b	8.4 ± 0.08 bc	3079 ± 17.1 b	2496 ± 8.1 b	2753 ± 21.5 bc	10.6 ± 0.05 a
IA50	8454 ± 52.3 bc	6397 ± 30.3 a	13095 ± 74.8 b	8.1 ± 0.12 bc	4166 ± 38.0 ab	3282 ± 18.4 ab	4171 ± 32.8 ab	10.5 ± 0.09 ab
IA76	7395 ± 42.8 d	5257 ± 25.7 b	12752 ± 67.1 b	8.6 ± 0.09 ab	3194 ± 24.3 b	2434 ± 12.3 b	2975 ± 26.0 bc	10.6 ± 0.14 ab
IA47	8159 ± 46.0 bcd	6688 ± 32.6 a	12296 ± 69.6 b	7.8 ± 0.10 c	2818 ± 29.2 b	2256 ± 16.9 b	1925 ± 15.7 c	10.4 ± 0.05 b

[a] Values are the means of $n = 3$ measurements ± standard deviation. Values within a column followed by a common letter (a-d) are not significantly different ($P > 0.05$). [b] PV, peak viscosity; T, trough; FV, final viscosity; TTPV, time to peak viscosity.

Table 3. Pasting Properties of Oat Flours under Inhibition Conditions[a,b]

	viscosity (cP)			
oat lines	PV	T	FV	TTPV (min)
IA46	12034 ± 56.5 a	8499 ± 42.6 a	18187 ± 81.3 a	7.5 ± 0.14 a
IA92	10644 ± 60.1 b	6848 ± 33.0 b	14919 ± 72.4 b	6.9 ± 0.12 c
IA44	10445 ± 63.5 bc	6293 ± 31.5 c	14213 ± 78.4 b	6.9 ± 0.10 c
IA87	9794 ± 53.0 cd	6632 ± 33.6 bc	13860 ± 75.9 b	7.1 ± 0.07 b
IA50	10083 ± 68.7 bcd	6837 ± 32.9 b	13628 ± 72.5 b	6.9 ± 0.14 c
IA76	9592 ± 52.7d	6742 ± 33.9 b	14390 ± 79.2 b	7.3 ± 0.07 b
IA47	10011 ± 57.7 bcd	6804 ± 33.8 b	13248 ± 71.6 b	6.9 ± 0.08 c

[a] Values are the means of $n = 3$ measurements ± standard deviation. Values within a column followed by a common letter (a-d) are not significantly different ($P > 0.05$). [b] PV, peak viscosity; T, trough; FV, final viscosity; TTPV, time to peak viscosity.

in accord with the normal range of groat oil concentration reported in the literature (5%−9%) (*27*).

Pasting Properties of Oat Flours under Autolytic and Inhibition Conditions. Buffer (as required for enzymatic treatments), instead of water, was used to prepare oat slurries for Rapid Visco Analyser measurements under autolytic conditions. The use of buffer or water did not affect the pasting properties of freshly prepared oat flours but did impact slurries held for 1 h (to allow enzymatic hydrolysis) (*12*). Therefore, buffer was used as the solution to study the autolysis of oat flours and also to serve as the control for certain enzymatic treatments. Moreover, holding of oat slurries in buffer for 1 h caused significant changes in pasting properties (**Table 2**). After holding, the PV decreased by 43.2% to 72.7%, and the T decreased by 36.8% to 66.3% ($P < 0.001$). The greatest changes occurred with the FV, which decreased by 62.8% to 84.3% ($P < 0.001$). The TTPV was 17.8% to 33.3% longer in held slurries than in fresh slurries ($P < 0.001$). The decrease of PV, T, and FV and the increase of TTPV might be explained by physical changes during flour hydration, action of endogenous enzymes in flours, or interactions between them (*11*, *12*).

The key pasting parameters corresponding to each oat line under inhibition conditions are reported in **Table 3**. Use of silver nitrate solution significantly increased PV ($P < 0.001$), T ($P = 0.051$), and FV ($P = 0.001$), and decreased TTPV ($P < 0.001$) compared with the buffer alone without 1 h of incubation (**Tables 2** and **3**). These changes could be partly explained by the inactivation of natural β-glucan-degrading enzymes in silver nitrate solution. The increase of PV under inhibition conditions has also been reported in oats, barley, and malts by previous researchers (*14*, *25*).

The β-glucan concentration correlated with PV and FV under conditions of autolysis without 1 h of incubation ($r = 0.789$, $P = 0.035$ for PV and $r = 0.777$, $P = 0.040$ for FV) and inhibition conditions ($r = 0.812$, $P = 0.027$ for PV and $r = 0.869$, $P = 0.011$ for FV), confirming the important contribution of β-glucan to the pasting properties.

Effect of Thermostable α-Amylase on Pasting Properties. With amylolysis, all starch-related viscosity-contributive factors, such as starch concentration, amylose content, amylopectin structure, and interaction between starch and other oat components were concomitantly eliminated. Clearly, β-glucan, a component with a high water-binding capacity, was the major pasting contributor under this condition. β-Glucanase, a possible trace contaminant in α-amylase, should not interfere with β-glucan contribution to pasting under amylolysis because of its thermolabile property (*14*).

The β-glucan concentration positively correlated with PV ($r = 0.829$, $P = 0.021$), T ($r = 0.951$, $P = 0.001$), and FV ($r = 0.994$, $P < 0.001$) (**Tables 1** and **4**). The increasing correlation from PV, T, to FV could be explained by the decreasing starch contribution to pasting. However, the TTPV depended on oat lines, not on β-glucan concentration ($P > 0.05$). The correlations between β-glucan concentration and actual viscosities after amylolysis demonstrated the importance of β-glucan contribution to pasting. The results also indicated that Rapid Visco Analyser was sensitive enough to detect viscosity differences, which were mainly attributable to β-glucan concentration variations among oat lines (*14*).

The starch hydrolysis caused by added α-amylase decreased PV, T, FV, and TTPV compared with the profile obtained in buffer without 1 h of incubation (**Figure 1**). The effect of α-amylase on pasting parameters was evaluated by comparing viscosity profiles obtained during amylolysis with those during autolysis without 1 h of incubation. The data were calculated as the relative percentage of viscosity or time difference: $(PV_{buffer\ 0\ h} - PV_{\alpha\text{-amylase}})/PV_{buffer\ 0\ h}$, $(T_{buffer\ 0\ h} - T_{\alpha\text{-amylase}})/T_{buffer\ 0\ h}$, $(FV_{buffer\ 0\ h} - FV_{\alpha\text{-amylase}})/FV_{buffer\ 0\ h}$, and $(TTPV_{buffer\ 0\ h} - TTPV_{\alpha\text{-amylase}})/TTPV_{buffer\ 0\ h}$. The most affected parameter was T followed by PV and FV; and the least was TTPV (**Tables 2** and **4**). The relative viscosity decreases for T and FV after amylolysis were correlated with β-glucan concentration ($r = -0.945$, $P = 0.001$ for T and $r = 0.839$, $P = 0.018$ for FV). Since the relative viscosity decreases after amylolysis reflected the contribution of starch-related factors, including starch alone and its interactions with other oat components to pasting, these correlations might be explained by the considerable contribution of the interaction between β-glucan and starch to pasting.

Effect of Lichenase on Pasting Properties. The addition of lichenase specifically removed β-glucan in oat slurries and eliminated the contribution of β-glucan to pasting. Under this situation, starch became the major component responsible for swelling and pasting. To verify the absence of α-amylase contamination in lichenase, preliminary experiments were conducted by using Sigma corn starch (3.68 g, db) instead of oat flours. The pasting

Table 4. Pasting Properties of Oat Flours after Treatment with Added α-Amylase and Lichenase[a,b]

	α-amylase				lichenase			
	viscosity (cP)				viscosity (cP)			
oat lines	PV	T	FV	TTPV (min)	PV	T	FV	TTPV (min)
IA46	638 ± 4.5 a	404 ± 3.5 a	1687 ± 15.5 a	3.2 ± 0.07 a	715 ± 6.8 b	404 ± 4.5 c	233 ± 2.5 d	10.2 ± 0.08 ab
IA92	447 ± 4.3 b	187 ± 2.2 bc	1144 ± 10.2 b	2.7 ± 0.07 d	1612 ± 12.9 a	1080 ± 10.1 a	488 ± 4.4 ab	10.2 ± 0.13 ab
IA44	574 ± 3.0 a	231 ± 1.5 b	1134 ± 10.5 b	2.9 ± 0.07 c	1461 ± 11.6 a	1140 ± 9.2 a	447 ± 4.2 bc	10.2 ± 0.08 ab
IA87	411 ± 4.9 b	167 ± 1.7 bc	995 ± 10.0 bc	2.8 ± 0.04 c	1566 ± 11.9 a	1029 ± 8.3 ab	590 ± 5.2 a	10.4 ± 0.04 a
IA50	460 ± 4.4 b	148 ± 1.6 c	806 ± 8.4 cd	3.0 ± 0.04 b	1412 ± 10.8 a	1004 ± 7.8 ab	366 ± 3.4 bcd	9.8 ± 0.13 b
IA76	465 ± 3.4 b	118 ± 1.1 cd	693 ± 6.8 d	2.9 ± 0.07 c	805 ± 7.2 b	588 ± 4.4 bc	245 ± 2.4 d	10.1 ± 0.12 ab
IA47	328 ± 3.5 c	53 ± 0.6 d	313 ± 3.1 e	2.8 ± 0.04 c	1235 ± 8.9 ab	940 ± 8.6 ab	337 ± 3.2 cd	10.1 ± 0.12 ab

[a] Values are the means of $n = 3$ measurements ± standard deviation. Values within a column followed by a common letter (a-e) are not significantly different ($P > 0.05$). [b] PV, peak viscosity; T, trough; FV, final viscosity; TTPV, time to peak viscosity.

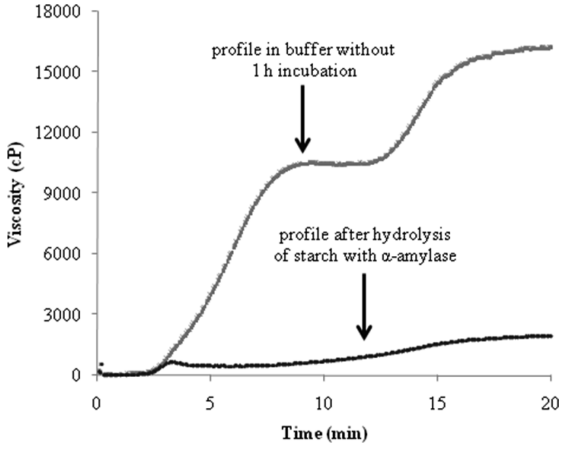

Figure 1. Viscosity profiles of IA46 slurries in buffer without 1 h of incubation and after hydrolysis of starch with thermostable α-amylase.

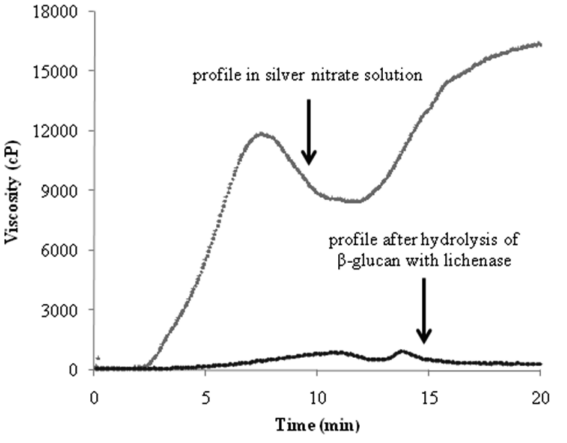

Figure 2. Viscosity profiles of IA46 slurries in silver nitrate solution and after the hydrolysis of β-glucan with lichenase for 1 h.

parameters (PV, T, FV, and TTPV) were not different among treatments with and without the addition of lichenase ($P > 0.05$), indicating no existence of α-amylase in lichenase.

Compared with amylolysis, the PV and T obtained under lichenase treatment were greater, whereas FV was less ($P < 0.05$, **Table 4**). These results indicated that, overall, the starch-related factors contributed more to PV and T, whereas β-glucan played a more important role for FV. The relationship between starch concentration and pasting parameters under lichenase treatments was investigated (**Tables 1** and **4**). According to the proximate analyses of all samples summarized in **Table 1**, the IA46, IA92, IA50, IA76, and IA47 oat flours were similar in starch content ($P > 0.05$). However, IA92 and IA50 slurries had greater PV than IA46 and IA76 ($P < 0.05$). IA92, IA50, and IA47 slurries had greater T values than IA46 ($P < 0.05$). IA92 slurry had a greater FV than IA46, IA76, and IA47 ($P < 0.05$). Similarly, IA44 and IA87 oat flours were similar in starch content ($P > 0.05$) but different in FV ($P < 0.05$). These results might be explained by underlying structural differences in the amylose and amylopectin components of the different oat lines, and their interactions with other oat components under test conditions.

β-Glucan hydrolysis by added lichenase dramatically decreased PV, T, and FV while increasing TTPV compared with the profile obtained in silver nitrate solution (**Figure 2**), confirming the important contribution of β-glucan to pasting properties. The effect of lichenase on pasting parameters was evaluated by comparing viscosity profiles obtained under enzymatic hydrolysis of β-glucan with added lichenase with those in silver nitrate solution, and calculated as the relative percentage of viscosity or time difference: $(PV_{AgNO3} - PV_{lichenase})/PV_{AgNO_3}$, $(T_{AgNO_3} - T_{lichenase})/T_{AgNO_3}$, $(FV_{AgNO_3} - FV_{lichenase})/FV_{AgNO3}$, and $(TTPV_{lichenase} - TTPV_{AgNO_3})/TTPV_{AgNO_3}$. Different from amylolysis, the most affected parameter was FV, followed by PV and T; while the least affected was TTPV (**Tables 3** and **4**). The relative decrease of FV was significantly correlated with β-glucan concentration ($r = 0.987$, $P < 0.001$). In other words, when β-glucan concentration in oats was greater, the relative viscosity decrease for FV was greater, which reconfirmed the critical role of β-glucan in pasting parameters, especially for FV. Other researchers also noted that enzymatic removal of β-glucans led to modifications of pasting properties, confirming the important contribution of β-glucan to pasting properties (*12*, *14*, *20*, *25*).

Effect of Proteinase K on Pasting Properties. The removal of protein by proteinase K resulted in slurries with the highest PV, T, and FV among all of the enzymatic treatments (**Tables 4** and **5**). The effect of proteinase K on pasting parameters was evaluated by comparing viscosity profiles obtained after enzymatic removal of protein with those under autolysis with 1 h of incubation (**Figure 3**) and calculated as the relative percentage of viscosity or time difference: $(PV_{buffer\ 1\ h} - PV_{proteinase\ K})/PV_{buffer\ 1\ h}$, $(T_{buffer\ 1\ h} - T_{proteinase\ K})/T_{buffer\ 1\ h}$, $(FV_{buffer\ 1\ h} - FV_{proteinase\ K})/FV_{buffer\ 1\ h}$, and $(TTPV_{buffer\ 1\ h} - TTPV_{proteinase\ K})/TTPV_{buffer\ 1\ h}$. The PV decreased by 25.4% to 51.9% ($P < 0.001$); the T decreased by 24.9% to 53.4% ($P = 0.003$); and the TTPV decreased by 10.3% to 10.6% ($P = 0.020$) (**Tables 2** and **5**). The greatest decreases occurred for the FV, with declines ranging from 36.4% to 62.1% ($P = 0.002$). The relative decrease of PV, T, and FV by adding proteinase K was much smaller than that obtained by adding α-amylase or lichenase. These results demonstrated that proteins contribute to pasting much less than do β-glucans and starches. Moreover, the relative decrease

Table 5. Pasting Properties of Oat Flours after Treatment with Added Proteinase K and Lichenase Plus α-Amylase[a,b]

	proteinase K				lichenase + α-amylase			
	viscosity (cP)				viscosity (cP)			
oat lines	PV	T	FV	TTPV (min)	PV	T	FV	TTPV (min)
IA46	1378 ± 11.5 c	1115 ± 10.3 c	1083 ± 6.4 b	10.4 ± 0.05 ab	99 ± 1.1 b	32 ± 0.3 a	41 ± 0.4 ab	2.8 ± 0.05 ab
IA92	2517 ± 22.3 ab	1970 ± 17.7 ab	2078 ± 19.4 ab	10.4 ± 0.10 ab	110 ± 1.4 ab	25 ± 0.4 a	37 ± 0.3 ab	2.6 ± 0.06 b
IA44	2029 ± 19.7 bc	1684 ± 16.0 bc	1579 ± 12.7 ab	10.3 ± 0.04 b	101 ± 1.3 b	15 ± 0.4 a	23 ± 0.1 b	2.6 ± 0.05 b
IA87	2015 ± 19.3 bc	1613 ± 15.2 bc	1296 ± 8.0 b	10.3 ± 0.14 b	106 ± 1.3 ab	27 ± 0.5 a	46 ± 0.6 ab	2.8 ± 0.04 ab
IA50	3106 ± 30.1 a	2465 ± 19.8 a	2651 ± 20.3 a	10.5 ± 0.09 ab	118 ± 0.8 ab	31 ± 0.3 a	44 ± 0.2 ab	3.0 ± 0.06 a
IA76	2142 ± 20.4 bc	1719 ± 15.0 abc	1695 ± 15.7 ab	10.6 ± 0.14 a	116 ± 0.8 ab	29 ± 0.1 a	49 ± 0.1 a	2.8 ± 0.05 ab
IA47	1846 ± 14.6 bc	1503 ± 13.4 bc	1143 ± 12.9 b	10.3 ± 0.09 ab	139 ± 0.6 a	39 ± 0.5 a	41 ± 0.4 ab	2.8 ± 0.00 ab

[a] Values are the means of $n = 2$ measurements ± standard deviation. Values within a column followed by a common letter (a-c) are not significantly different ($P > 0.05$). [b] PV, peak viscosity; T, trough; FV, final viscosity; TTPV, time to peak viscosity.

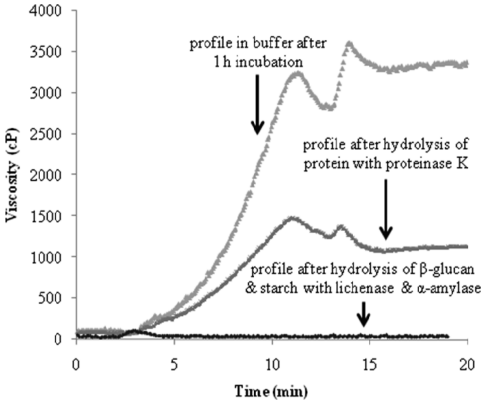

Figure 3. Viscosity profiles of IA46 slurries in (1) buffer, (2) proteinase K, and (3) α-amylase plus lichenase solution, all after 1 h of incubation at 40 °C with mixing.

of FV, T, and FV was weakly correlated with β-glucan concentration ($r = 0.705$, $P = 0.077$ for PV; $r = 0.675$, $P = 0.096$ for T; and $r = 0.728$, $P = 0.064$ for FV), which might be explained by the contribution of interactions between β-glucan and protein to pasting. Other researchers also reported that enzymatic removal of protein from oat-flour slurries decreased PV and T by about 15% (*12*).

Effect of Lichenase Plus Thermostable α-Amylase on Pasting Properties. The addition of lichenase and α-amylase eliminated all β-glucan- and starch-related effects (including their interaction with protein) to pasting and presented almost the sole contribution of protein to pasting. The PV under this condition was negatively correlated with β-glucan concentration ($r = -0.881$, $P = 0.009$), which indicated that the higher the β-glucan concentration, the less the sole contribution of protein to pasting in the selected oat lines. The actual viscosities for PV, T, and FV obtained under this condition were much smaller than the viscosity decrease after enzymatic removal of protein, demonstrating the important contribution of interactions between protein and other oat components to pasting (**Table 5**). The effect of lichenase plus α-amylase on pasting parameters was evaluated by comparing viscosity profiles obtained after enzymatic removal of β-glucan and starch with profiles under autolysis with 1 h of incubation (**Figure 3**) and calculated as the relative percentage of viscosity or time difference: $(PV_{buffer\ 1\ h} - PV_{lichenase+\alpha\text{-}amylase})/PV_{buffer\ 1\ h}$, $(T_{buffer\ 1\ h} - T_{lichenase+\alpha\text{-}amylase})/T_{buffer\ 1\ h}$, $(FV_{buffer\ 1\ h} - FV_{lichenase+\alpha\text{-}amylase})/FV_{buffer\ 1\ h}$, and $(TTPV_{buffer\ 1\ h} - TTPV_{lichenase+\alpha\text{-}amylase})/TTPV_{buffer\ 1\ h}$. The PV decreased by 95.1% to 97.8% ($P < 0.001$); the T decreased by 98.3% to 99.4% ($P < 0.001$); the FV decreased by 97.9% to 99.3% ($P < 0.001$); and the TTPV decreased by 10.6% to 11.3% ($P < 0.001$) (**Tables 2** and **5**). In other words, less than 5% of the values for PV, T, and FV were attributable to the sole contribution of protein, reconfirming the minimal effect of protein on pasting.

Table 6. Optimal Multiple Linear Regression Models to Predict the Key Pasting Parameters by a Stepwise Procedure[a]

model		standard estimate	*P* value	R square
PV in $AgNO_3$				0.657
	intercept	5956	0.009	
	β-glucan	764	0.027	
PV in buffer				0.621
	intercept	3372	0.126	
	β-glucan	901	0.035	
FV in $AgNO_3$				0.754
	intercept	5035	0.098	
	β-glucan	1661	0.011	
FV in buffer				0.904
	intercept	−12905	0.078	
	β-glucan	1770	0.004	
	starch	287	0.026	

[a] PV, peak viscosity; FV, final viscosity.

MLR Models to Predict the Key Pasting Parameters. To better understand the unit effects of β-glucan, starch, and protein to pasting as a whole system, we attempted to construct optimal MLR models to predict the key pasting parameters (PV, T, and FV) in buffer without 1 h of incubation and in silver nitration solution. Three specific predictors (concentration of β-glucan, starch, and protein) were chosen for MLR model selection, primarily for two reasons. First, previous literature and our current findings showed these variables as the most potentially influential factors to pasting. Second, adding more predictors would threaten model accuracy. Four prediction models were generated for PV and FV on the basis of a sequence of F-tests (**Table 6**). As to T, no prediction model was obtained at the 0.1 significance level.

For PV prediction in both buffer without 1 h of incubation and silver nitrate solution, only β-glucan concentration was selected as the predictor (explanatory variable), indicating that the unit contribution of β-glucan to PV was much greater than that of starch or protein under both conditions. The model R square represents a measure of the global fit of the model. The R square equal to 0.657 for the silver nitrate solution model may be interpreted as follows: approximately 65.7% of the variation in PV can be explained by β-glucan concentration. Therefore, the model in silver nitrate solution accounted for more proportion of variability in PV (65.7%) than did the buffer model (62.1%).

Similarly, for FV prediction in silver nitrate solution, the β-glucan concentration selected as the only predictor indicated

that unit β-glucan contributed more to FV than did unit starch or protein under this condition. Nevertheless, the concentrations of both β-glucan and starch were selected in the FV prediction model in buffer without 1 h of incubation, suggesting that both were more important for FV than was protein under this condition. The predictor difference of FV models in silver nitrate solution and in buffer without 1 h of incubation might be explained by the partial hydrolysis of β-glucan caused by endogenous β-glucanases during pasting. Moreover, the model in buffer accounted for more proportion of variability in FV (90.4%) than did the model in silver nitrate solution (75.4%).

These findings demonstrated the important unit contribution of β-glucan to PV and FV in both silver nitrate solution and buffer without 1 h of incubation, and of starch to FV in buffer without 1 h of incubation. Similar results were reported by using a partial least-squares (PLS) model, specifically showing that the β-glucan concentration in oats could be better predicted by measuring the pasting profile in silver nitrate solution than in deionized water or in alkali solution (*13*).

Overall, the individual effects of β-glucan, starch, and protein to pasting properties of oat flours were demonstrated. Their unit contributions to pasting were β-glucan > starch > protein. The interaction of β-glucan with starch or protein also contributed considerably to pasting. From a practical view, these findings showed β-glucan to be the major contributor to viscosity in oat-flour slurries; however, its interactions with other ingredients, especially starch and protein, illustrated the importance of evaluating oat components as a whole system and not just in parts.

LITERATURE CITED

(1) Welch, R. W. Oats in Human Nutrition and Health. In *The Oat Crop: Production and Utilization*; Welch, R. W., Ed.; Chapman and Hall: London, U.K., 1995; pp 433−471.

(2) Food and Drug Administration. Food labeling: Health claims; oats and coronary heart disease. *Fed. Regist*. **1997**, *62*, 3584–3601.

(3) Hallfrisch, J.; Facn; Behall, K. M. Mechanisms of the effects of grains on insulin and glucose responses. *J. Am. Coll. Nutr*. **2000**, *19*, 320S–325S.

(4) Wood, P. J. Cereal β-glucans in diet and health. *J. Cereal Sci*. **2007**, *46*, 230–238.

(5) Drzikova, B.; Dongowski, G.; Gebhardt, E.; Habel, A. The composition of dietary fibre-rich extrudates from oat affects bile acid binding and fermentation in vitro. *Food Chem*. **2005**, *90*, 181–192.

(6) LaRusso, N. The Role of Bile Acids in Intestinal Absorption of Cholesterol. In *Bile Acids in Gastroenterology*; Barbara, L., Dowling, R. H., Hofmann, A. F., Roda, E., Eds.; MTP Press Ltd.: Boston, MA, 1983; pp 183−191.

(7) Mälkki, Y.; Virtanen, E. Gastrointestinal effects of oat bran and oat gum. *Lebensm.-Wiss. Technol*. **2001**, *34*, 337–347.

(8) Lazaridou, A.; Biliaderis, C. G. Molecular aspects of cereal β-glucan functionality: Physical properties, technological applications and physiological effects. *J. Cereal Sci*. **2007**, *46*, 101–118.

(9) Hall, M. B.; Tarr, A. W. Oat Starch Quality and Relationships to Other Quality Traits. In *6th International Oat Conference Proceedings*; Cross, R. J., Ed.; New Zealand Institute for Crop & Food Research Ltd.: Christchurch, New Zealand, 2000; pp 11.

(10) Glennie-Holmes, M. Rapid visco applications: Rolled oats. *Newport Scientific-RVA World* **1996**, *8*, 4.

(11) Doehlert, D. C.; Zhang, D.; McMullen, M. S.; Moore, W. R. Estimation of mixed linkage beta-glucan concentration in oat and barley from viscosity of whole grain flour slurry. *Crop Sci*. **1997**, *37*, 235–238.

(12) Zhou, M.; Robards, K.; Glennie-Holmes, M.; Helliwell, S. Effects of enzyme treatment and processing on pasting and thermal properties of oats. *J. Sci. Food Agric*. **2000**, *80*, 1486–1494.

(13) Colleoni-Sirghie, M.; Jannink, J. L.; Kovalenko, I. V.; Briggs, J. L.; White, P. J. Prediction of β-glucan concentration based on viscosity evaluations of raw oat flours from high β-glucan and traditional oat lines. *Cereal Chem*. **2004**, *81*, 434–443.

(14) Colleoni-Sirghie, M.; Jannink, J. L.; White, P. J. Pasting and thermal properties of flours from oat lines with high and typical amounts of β-glucan. *Cereal Chem*. **2004**, *81*, 686–692.

(15) Zhou, M.; Robards, K.; Glennie-Holmes, M.; Helliwell, S. Structure and pasting properties of oat starch. *Cereal Chem*. **1998**, *75*, 273–281.

(16) Tester, R.; Morrison, W. R. Swelling and gelatinization of cereal starches. I. Effect of amylopectin, amylose, and lipids. *Cereal Chem*. **1990**, *67*, 551–557.

(17) Kim, C. S.; Walker, C. E. Changes in starch pasting properties due to sugars and emulsifiers as determined by viscosity measurement. *J. Food Sci*. **1992**, *57*, 1009–1013.

(18) Morrison, W. R.; Tester, R. F. Chemical and Physical Factors That Affect Cereal Starches. In *Cereals International*; Martin, D. J., Wrigley, C. W., Eds; Royal Australian Chemistry Institute: Melbourne, Australia, 1991; pp 135−138.

(19) Sopade, P. A.; Hardin, M.; Fitzpatrick, P.; Desmee, H.; Halley, P. Macromolecular interactions during gelatinisation and retrogradation in starch-whey systems as studied by Rapid Visco-Analyser. *Int. J. Food Eng*. **2006**, *2*, 1–17.

(20) Izydorczyk, M. S.; Storsley, J.; Labossiere, D.; MacGregor, A. W.; Rossnagel, B. G. Variation in total and soluble β-glucan content in hulless barley effects of thermal, physical, and enzymic treatments. *J. Agric. Food Chem*. **2000**, *48*, 982–989.

(21) Chernyshova, A. A. Selection for High β-Glucan Content and Good Agronomic Performance in Oat Grain. Master's Thesis, Iowa State University, Ames, IA, 2006.

(22) AACC International. *Approved Methods of the AACC*, 10th ed.; Methods 44-15A, 32-23, 76-13, 30-25; The Association: St. Paul, MN, 2000.

(23) Xie, S. X.; Liu, Q.; Cui, S. W. Starch Modifications and Applications. In *Food Carbohydrates: Chemistry, Physical Properties, and Applications*; Cui, S. W., Ed.; CRC Press: Boca Raton, FL; 2005; pp 357−406.

(24) Cui, S. W. Structural Analysis of Polysaccharides. In *Food Carbohydrates: Chemistry, Physical Properties, and Applications*; Cui, S. W., Ed.; CRC Press: Boca Raton, FL; 2005; pp 105−160.

(25) Glennie-Holmes, M. Studies on barley and malt with the Rapid Viscoanalyser: [I] The effect of variations in physical and chemical parameters. *J. Inst. Brew*. **1995**, *101*, 11–18.

(26) Miller, S. S.; Wood, P. J.; Pietrzak, L. N.; Fulcher, R. G. Mixed linkage β-glucan, protein content, and kernel weight in *Avena* species. *Cereal Chem*. **1993**, *70*, 231–233.

(27) Morrison, W. R. Cereal Lipids. In *Advances in Cereal Science and Technology*; Pomeranz, Y., Ed.; American Association of Cereal Chemists: St. Paul, MN; 1978; Vol. 2, pp 221−348.

Received for review February 25, 2010. Revised manuscript received May 13, 2010. Accepted May 17, 2010. This project was supported by the USDA-NRI Competitive Grants Program, award number 2007-02701.