

Prediction of β -Glucan Concentration Based on Viscosity Evaluations of Raw Oat Flours from High β -Glucan and Traditional Oat Lines

Mirela Colleoni-Sirghie,¹ Jean-Luc Jannink,² Igor V. Kovalenko,³ Jenni L. Briggs,^{1,3,4} and Pamela J. White^{1,5}

ABSTRACT

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Rheological properties of raw oat flour slurries were determined in experimental high β -glucan ($\leq 7.8\%$) and traditional oat lines (4–5% β -glucan) grown in two consecutive years. Three different media were used to disperse oat flours: deionized water, silver nitrate solution (to inactivate endogenous enzymes), and alkali solution (to solubilize both water-soluble and water-insoluble β -glucans). Significant correlations ($P < 0.05$) between viscosity of slurries and β -glucan concentration obtained in either deionized water ($r = 0.833$), silver nitrate ($r = 0.940$), or alkali ($r = 0.896$) solutions showed that β -glucans were the main contributor to oat extract viscosity. The highest correlation was obtained in silver nitrate

solution, suggesting that inactivating endogenous enzymes is important to obtain high correlations. Predictive models of oat β -glucan concentration based on the viscosity profile were developed using partial least squares (PLS) regression. Prediction of β -glucan concentration based on viscosity was most effective in the silver nitrate solution ($r = 0.949$, correlation coefficient of predicted vs. analyzed β -glucans) and least effective in the alkali solution ($r = 0.870$). These findings demonstrate that the β -glucan in oat could be predicted by measuring the viscosity of raw flours in silver nitrate solution, and this method could be used as a screening tool for selective breeding.

A low-fiber diet is thought to partly contribute to the development of heart disease, obesity, cancer, and type-2 diabetes. Consequently, the American Cancer and American Heart Associations and the National Institutes of Health recommend substantial increases in fiber intake (Krauss et al 2000; Anonymous 2001). The component responsible for the hypocholesterolemic and hypoglycemic effects in oat is (1,3)(1,4)- β -D-glucan, a cell-wall polysaccharide found in the endosperm and subaleurone layers of cereal seeds (Behall et al 1997). In 1997, the U.S. Food and Drug Administration (FDA) registered oat bran, and specifically β -glucan, at a level of 3 g/day, as the first cholesterol-reducing food and, therefore, a food that reduces the risk of heart disease (Anonymous 1997).

Soluble dietary fibers, such as β -glucans, lower blood cholesterol, glucose, and insulin concentrations partly because of their capacity to increase the viscosity of intestinal chyme (Welch 1995). The viscosity of polymer solutions depends on molecular weight (MW), concentration, and polymer solubility. Thus, to develop physiologically effective food products from oat, it is important to have oat with a high concentration of β -glucan and good extractability, and to minimize factors reducing the MW of β -glucans (Mälkki 2001). Processing procedures can produce substantial fragmentation of β -glucan with subsequent effects on the physiological response, though a loss in MW can to a certain extent be compensated for by an increase in concentration. Consequently, plant breeders seek to increase levels of β -glucans in oat lines, which may be processed to create new effective nutritional and functional foods (Cervantes-Martinez et al 2001).

To select oat lines with high nutritional potential, reflected by both quantity (β -glucan content) and quality (extractability and viscosity), rapid and inexpensive methods are necessary in oat breeding programs. Several methods have been reported in the literature for the measurement of β -glucan concentration in cereals, including enzymatic, colorimetric, and viscosimetric methods. The

enzymatic method (McCleary and Glennie-Holmes 1985) is costly and laborious. The colorimetric method (Jørgensen 1988), based on the use of the fluorophore compound Calcofluor to specifically bind the β -glucan molecule, requires expensive equipment. Measurement of extract viscosity in cereals indirectly estimated soluble nonstarch polysaccharide content, represented mainly by β -glucans in oats and barley (Doehlert et al 1997a). Proteins and starch had only minimal influence on viscosity of oat extracts (Doehlert et al 1997b). Most of the apparent viscosity in oat slurries resulted from the β -glucans, as demonstrated by a significant decrease in apparent viscosity when a β -glucan-degrading enzyme, lichenase (EC 3.2.1.6), was added (Doehlert et al 1997b). Similarly, the viscosity of barley extracts was caused largely by β -glucan and to a lesser extent by pentosans (Bhatty et al 1991). Treatments of barley extracts with either α -amylase or pronase did not significantly reduce the viscosity; xylanase led to a small drop in viscosity and β -glucanase produced an almost complete loss in viscosity (Bhatty et al 1991). Recently, Izydorczyk et al (2000) showed that the addition of lichenase caused an immediate decline in the viscosity of water-barley slurries, whereas the action of α -amylase was somewhat hindered by the relatively slow enzymatic hydrolysis of starch granules. Protease and xylanase had no effect on the viscosity of barley slurries, demonstrating that proteins and arabinoxylans contributed little to viscosity (Izydorczyk et al 2000).

Correlations between β -glucan concentration in oat (Doehlert et al 1997a, Luhalo et al 1998) or barley (Bhatty et al 1991, Izydorczyk et al 2000) and the viscosity of their extracts have been reported in the literature. Because the viscosity of polymer solutions depends not only on the polymer concentration, but also on the MW, factors potentially affecting the MW should be eliminated to establish successful correlations. For example, inactivation of oat endogenous enzymes by steaming improved the regression between β -glucan concentration and flour slurry viscosity from $r^2 = 0.626$ for raw groats to $r^2 = 0.717$ for steamed groats (Doehlert et al 1997a).

The objectives of this study were to 1) better understand the role of β -glucans and the contribution of other factors related to oat genotype and environment to the rheological behavior of oat flour slurries by analyzing oat lines with a wide range of β -glucan concentrations (3.9–7.8%) grown in two years (2001 and 2002); 2) investigate new procedures other than heat treatment to inactivate the endogenous enzymes improving the correlations between oat β -glucan concentration and viscosity of extracts; and 3) evaluate new methods to screen oat lines for potential functional quality as determined by β -glucan viscosity.

¹ Department of Food Science and Human Nutrition, Iowa State University, Ames, IA 50011.

² Department of Agronomy, Iowa State University, Ames, IA 50011.

³ Department of Agricultural & Biosystems Engineering, Iowa State University, Ames, IA 50011.

⁴ Present address: Thermo Electron Corporation, Madison, WI 53711.

⁵ Corresponding author. Phone: 515-294-9688. Fax: 515-294-8181. E-mail: pjwhite@iastate.edu

MATERIALS AND METHODS

Plant Material

We grew six experimental oat lines (IA95111, IA95205, and IA95258, described in Cervantes-Martinez et al [2001] and N979-5-2-4, IA91463, IA91524-1-5-1[*unpublished data*]) and three publicly available cultivars (Paul, a naked cultivar described in McMullen et al [1997]; JIM and IN09201 developed at the University of Minnesota and Purdue University, respectively). The total of nine lines was chosen to span a broad range of β -glucan concentrations (3.9–7.8% β -glucans). All nine lines were grown in two years (2001 and 2002), resulting in 18 samples with distinct chemical compositions. Field plots were located at the Agronomy and Agricultural Engineering Field Research Center near Ames, IA, on a Nicollet loam soil (fine-loamy, mixed, mesic Aquic Hapludoll). The planting dates were April 25, 2001, and April 4, 2002, and the harvest dates were July 25, 2001, and July 23, 2002, respectively. After harvest, oat samples were stored at room temperature. Weather data including temperature, rainfall, and sunshine records at the growing site were gathered by Iowa Environmental Mesonet of Iowa State University and Department of Agronomy (<http://mesonet.agron.iastate.edu/agclimate>).

Oat Composition

Oat samples, except the naked cultivar Paul, were dehulled with an air-pressure dehuller (Codema, Eden Prairie, MN), and the kernels were ground with a 0.5-mm sieve on an ultracentrifugal mill (ZM-1, Retch GmbH&Co, Haan, Germany). All analyses of oat groats were done in triplicate and reported on a dry matter basis. The moisture of oat flours was determined by Approved Method 44-15A (AACC 2000). The β -glucan concentration in flours and in extracts was determined enzymatically by Approved Method 32-23 (AACC 2000), by using a mixed β -glucan linkage kit (Megazyme Int., Wicklow, Ireland). Pentosan in flours was analyzed by the phloroglucinol colorimetric method (Douglas 1981). Oat groat flour proteins were determined by the Kjeldhal procedure (Approved Method 46-12, AACC 2000) with a protein conversion factor of 6.25. Starch content in flour was analyzed by Approved Method 76.13 (AACC 2000) using a total starch kit from Megazyme. Lipids were analyzed by the gravimetric method after extraction with petroleum ether over 6 hr on a Soxhlet system. Ash was determined by Approved Method 08-01 (AACC 2000). β -Glucanase activity in flours was determined by the Azo-barley β -glucan method using the malt beta-glucanase assay kit from Megazyme. Because of the low level of the β -glucanase in oat compared with malt, the incubation time was increased from 10 min (incubation time indicated for malt) to 240 min, with the activity determined as recommended in the Megazyme kit and divided by a time factor of 24.

Rheological Measurements

Oat groats were ground in an ultracentrifugal mill (ZM-1, Retch GmbH&Co, Haan, Germany) fitted with a ring sieve with 0.5-mm diameter conidur holes, and with the speed selection set at switch 1. Each sample was ground immediately before the experiments. To avoid differences in fines of grinding, which can have an important impact on rheological properties (Yoon et al 1995; Grosjean et al 1999a), highly controlled conditions were applied, including strict control of seed quantity (milled in 20-g batches), time of milling (30 sec), and position of the sieve in the mill. All samples were ground in duplicate for rheological measurements. Particle distribution of flours obtained from replicate millings was determined by using U.S. standard sieves 35, 50, 100, and 120. Very low variations in particle size distribution were observed between replicate millings after sieving with $8.48 \pm 0.06\%$ of particles over U.S. 35; $22.3 \pm 0.64\%$ between U.S. 35 and U.S. 50; $57.3 \pm 0.08\%$ between U.S. 50 and U.S. 100; $7.52 \pm 0.31\%$ between U.S. 100 and U.S. 120; and $4.37 \pm 0.82\%$ through U.S. 120.

The viscosity of raw oat flour slurries at 25°C was determined by using a Haake VT550 viscometer equipped with an MV1 rotor (Thermo Electron Corp., Madison, WI). Fresh ground oat (15 g at 8% moisture) was suspended in 60 g of either deionized water, silver nitrate solution (AgNO_3 0.1 mmol/g of flour) according to Glennie-Holmes (1995), or an alkali solution (Na_2CO_3 6.36 g/L, NaHCO_3 3.36 g/L, pH 10), according to Ullrich et al (1986).

Before the rheological measurements, samples were stirred manually with a spatula for 5 min to ensure that all flour was incorporated into the solution. The changes in viscosity were followed for 4 hr during mixing of oat flour slurries at a shear rate of 50 sec^{-1} and were automatically monitored with the Rheowin software to obtain viscosity readings every 24 sec. The effect of storage on the rheological properties was checked by comparing viscosity profiles of both a high- and low-viscosity oat at the beginning and at the end of the rheological experiments. Whole-oat seeds were stored during this period at room temperature and dehulled and ground just before the rheological measurements. Identical viscosity profiles were obtained in deionized water, silver nitrate solution, or alkali solution at the two times of analysis, suggesting that during this period, no enzymatic or physico-chemical modifications affecting rheological parameters occurred (data not shown).

Oat-Soluble Extracts

Soluble extracts from the oats were obtained in two replicate batches by extraction of 1 g of oat flour with 9 mL of deionized water for 1 hr at 25°C, in a shaker (New Brunswick Scientific, Edison, NJ) at 200 strokes/min, followed by centrifugation of the suspension at $10,000 \times g$ for 20 min. The supernatants were collected and incubated at 100°C for 15 min to inactivate the enzymes, then frozen and stored at -20°C until further analysis. The composition of the soluble extracts (β -glucan, pentosan, and starch) was determined by the methods described for oat flour analysis. Proteins in soluble extracts were analyzed by the Bradford dye-binding method (Bradford 1976) with bovine serum albumin as a standard. Free glucose was analyzed by using the glucose oxidase/peroxidase system from Megazyme. Results were expressed as % (w/w) in the dry flour. Extractability of each component was determined by the ratio between the % of soluble component and the % of the total component in the dry flour. Soluble β -glucans were correlated with the viscosity of flour slurries developed in deionized water.

Statistical Analysis

Results were analyzed with a statistical analysis computer program (SAS Institute, Cary, NC). Analysis of variance (ANOVA) was performed by using SAS proc GLM. Comparison of means was performed by least significant difference (LSD) at $\alpha = 0.05$. Predictive models of oat β -glucan concentration based on the whole viscosity profile were developed by using partial least squares (PLS) regression as reported by SAS proc PLS. Separate models were developed for each of the slurry solutions: deionized water, silver nitrate, and alkali. For each oat and slurry solution combination, viscosity data consisted of readings every 24 sec over 4 hr for a total of 600 data points. For each model, therefore, we had 18 observations (β -glucan concentrations of nine genotypes for two years) and 600 predictor variables. From these data, the PLS procedure extracts linear combinations of the predictor variables (called factors) that represent a maximum amount of variation in the predictor variables and that explain a maximum amount of variation in the response variables.

To avoid model overfitting and consequent poor prediction of β -glucan levels in future samples, cross-validation of the PLS procedure was performed by using the option CV=SPLIT. This option deletes one out of seven observations from the data set, performs PLS on the remaining observations, and uses the PLS results to predict the β -glucan concentration of the deleted observations.

A predicted residual sum of squares (PRESS) statistic is calculated from this procedure; R_j is the residual from the cross-validation prediction for observation j . That is, if Y_j is the β -glucan concentration for deleted observation j and \hat{Y}_j is the prediction obtained from PLS without using data from observation j , $R_j = Y_j - \hat{Y}_j$. The sum of R_j^2 across all observations is the PRESS, which should be minimized. Models from PLS with different numbers of factors will have different PRESS statistics: models with too few factors do not take full advantage of the information in the predictor variables, whereas models with too many factors overfit the data, producing spurious predictions. The option CVTEST retains the PLS model with a minimum number of factors that has a PRESS not significantly greater than the smallest PRESS obtained among all models. Relationships among functional parameters (maximum attainable viscosity and time-to-peak viscosity) and β -glucan concentration in flours were investigated by using Pearson's correlations with SAS proc CORR.

RESULTS AND DISCUSSION

Oat Composition

The oat lines chosen for this study displayed a large range of β -glucan concentration (3.9–7.8%) with significant differences between oat lines (Table I). Starch content was 50–66%, with the highest concentrations in JIM and IN090201. Protein concentrations were 18.3–21.6% (dwb) except JIM and IN090201, which had lower protein contents (15.5–17.8%, dwb). A wide range of values was observed for lipids (4.86–9.54%, dwb), but this amount of vari-

ation is typical within common oat cultivars (5–9%) (Morrison 1978). Small variations were observed in pentosan and ash content among the oat lines. β -Glucanase activity originated from grain, and possible microbial contaminants developed after harvest and during storage. β -Glucanase levels were very low, ranging from 1.51 U/kg (IN09201, grown in 2001) to 4.02 U/kg (IA95205, grown in 2002).

ANOVA of the nine oat lines grown in two years showed a significant effect of line, year, and line-by-year interaction ($P < 0.0001$) on the starch, β -glucan, protein, pentosan, and lipid concentrations, and a significant effect of line and line-by-year interaction on the endoglucanase level ($P < 0.0001$) (values not shown). For most of the oat lines, lower concentrations of β -glucans and higher starch were observed for the same line grown in 2002 compared with 2001. The year effect might be a consequence of weather, harvest date, or storage conditions. Weather conditions for 2001 and 2002 are presented in Table II. More rainfall and lower high temperature and low temperature means were recorded in 2002 than in 2001. However, the influence of climate on β -glucan concentration is currently poorly understood. As in our study, Brunner and Freed (1994) found lower mean β -glucan concentrations in years with high precipitation during the grain filling periods (June and July). A rainy climate also induced lower mixed-linkage β -glucan concentration and extract viscosity in barley, whereas high temperature during growth induced high mixed-linkage β -glucan concentration (Pérez-Vendrell et al 1996). The recent study by Doehlert et al (2001), however, showed opposite findings, with a positive correlation between total precipitation in the last two months before

TABLE I
Chemical Composition (% w/w, dmb) and β -Glucanase Activity in Oats^a

Oat Line	Year	Starch	Protein ^b	Lipid	β -Glucan	Ash	Pentosan	β -Glucanase (U/kg) ^c
IA95111	2001	54.0h	20.9ab	6.85hi	7.84a	2.31c-e	2.26h	3.74ab
	2002	56.0f	20.6bc	6.18i	6.19d	2.68b	2.63d	2.97c-e
N979-5-2-4	2001	51.3i	21.1ab	5.09j	7.45b	2.34cd	2.20j	2.93c-e
	2002	55.2fg	19.4de	7.93de	6.94c	2.22c-f	2.83a	2.10g-i
IA 95258	2001	50.2i	21.6a	4.86k	7.28b	2.30c-e	2.16j	nd ^d
	2002	55.0fg	20.4fg	6.66i	6.70c	2.97a	2.46f	2.86c-f
IA91524-1-5-1	2001	56.2f	18.8ef	8.07cd	6.15de	2.24c-e	1.90n	2.68d-f
	2002	58.9e	18.3fg	7.29e-g	5.46g	2.23c-e	2.36g	2.49e-h
IA 95205	2001	54.8gh	20.9ab	6.42i	6.00ed	2.37c	2.20j	3.21b-d
	2002	59.1e	19.9cd	8.03cd	5.90ef	2.16d-g	2.68c	4.02a
IA91463	2001	59.0e	18.8ef	6.70hi	5.32g	2.26c-e	2.07k	2.97c-e
	2002	60.4cd	18.9ef	6.85hi	5.72f	2.18c-g	2.56e	1.96h-j
Paul	2001	55.5fg	19.7d	7.41f-h	4.41i	2.20c-e	2.22i	3.86a
	2002	59.5de	19.2de	8.94b	4.98h	2.03fg	2.82b	3.30bc
JIM	2001	66.6a	15.5i	7.50ef	4.85h	2.02g	1.82l	1.75ij
	2002	64.0b	16.9h	6.82hi	4.32i	2.00fg	2.26h	2.36f-h
IN090201	2001	61.5c	16.8h	9.54a	4.97h	2.10fg	2.00m	1.51j
	2002	60.8c	17.8g	8.24c	3.94j	2.15g	2.28k	2.63e-g

^a Values are means of replicate measurements ($n = 3$). Values within a column followed by a common letter are not significantly different ($P > 0.05$).

^b $N \times 6.25$.

^c Units of activity with the Azo-Barley Glucan method (Megazyme).

^d Not determined (limited quantity of seeds).

TABLE II
Weather Data for Environment Evaluated in this Study (Ames, IA) in the 2001 and 2002 Growing Period^a

Date	Mean High Temperature (°C)	Mean Low Temperature (°C)	Total Seasonal Precipitation (mm)	Mean Daily Solar Radiation (Langleys)
25–30 April 2001	26	11	7	516
1–31 May 2001	21	11	159	400
1–30 June 2001	27	15	42	549
1–7 July 2001	29	19	40	489
25 April –7 July 2001	26	14	248	489
4–30 April 2002	15	3	86	374
1–31 May 2002	21	8	112	519
1–30 June 2002	28	17	71	560
1–23 July 2002	30	18	122	548
4 April –23 July 2002	24	11	391	500

^a Source: Iowa Environmental Mesonet and Department of Agronomy of Iowa State University.

harvesting and the β -glucan concentration of 10 oat cultivars. Lim et al (1992) did not find differences in the β -glucan mean content of two years when one year was drier and hotter than the other. All these studies, however, involved traditional oat lines with normal ranges in β -glucan. Doehlert (1992) reported that β -glucan accumulation occurs late in grain development, suggesting that environmental stresses leading to an early end to grain development also reduce the β -glucan concentration. More work is needed to understand the intricate role of the environmental factors on oat β -glucan concentration, and particularly involving high β -glucan oat lines.

Oat-Soluble Extracts

The β -glucan extractability of the nine oat lines grown in two years, measured after 1 hr of mixing at 25°C in deionized water, ranged from 63% (IN090201, grown in 2001) to 100% (Paul, 2001) with high variations between oat samples (Table III). A significant increase of β -glucan extractability was observed with an increase in the level of the β -glucanase activity (Table I) ($r = 0.586$, $P < 0.0001$), which might be explained by an increase in solubility for more degraded polymers. The endogenous enzymes might have acted slowly during the storage of oat grains and intensively during the extraction of flours. The low correlation coefficient, however, suggests that other factors in addition to the β -glucanase activity determined the β -glucan extractability. A positive correlation also was observed for the free glucose content in soluble extracts with the increase of β -glucanase level ($r = 0.728$, $P < 0.0001$). This molecule may have been generated by the endogenous β -glucanase and amylases during the extraction. Extractability and viscosity of β -glucans are important in determining oat functionality. Only the highly viscous soluble fibers lowered serum cholesterol concentrations in humans. In fact, sources containing little or no soluble fiber had limited effect on serum cholesterol levels (Shinnick et al 1991).

A significant effect of year ($P < 0.0001$) was observed on the soluble protein content and protein extractability, with greater levels for oat grown in 2002 than in 2001. One explanation for these differences might be related to the postharvest aging of oat during the storage period, which was different for the two series of oat (18 months of storage for oat grown in 2001 and six months of storage for oat grown in 2002). Although no reports are available

in the literature on the effect of aging on oat, several authors found major changes in rice proteins during storage, such as a decrease of solubility or changes in their properties (Dhaliwal et al 1991; Zhou et al 2003). The aging of oat might also produce a decrease in protein solubility. Another explanation for differences observed between oat samples grown in the two years may be related to a greater level of proteases from grains and microbial contamination in oat grown in 2002, with a subsequently higher proteolysis, generating an increased soluble protein content.

Viscosity Profile of Oat Flours in Deionized Water

The viscosity measured in deionized water increased, reaching a peak at 600–1,380 sec of extraction, depending on oat sample, and decreased shortly after (Fig. 1). The solubilization of β -glucan during mixing of the slurries increased the soluble polymer concentration, causing an increase in viscosity. At the same time, the fragmentation of polymers in solution by enzymatic hydrolysis with endogenous enzymes decreased MW, thus causing a decrease in viscosity. The increase in viscosity observed at 600–1,380 sec of extraction may have been because there was a greater solubilization than fragmentation of β -glucans during that period. The sharp decrease following the viscosity peak may be explained by further molecular fragmentation.

An interesting profile was observed for the relationship (expressed as correlation coefficients over time of mixing) between total β -glucans and viscosity development and between soluble β -glucans and viscosity, (Fig. 2A and B, respectively). The correlation between β -glucans and viscosity increased during the first 900 sec of dispersion, along with the increase of viscosity, due to an increase of the concentration of solubilized β -glucans. In this short interval, endogenous enzymes possibly did not have enough time to act or the substrate was not yet available in solution. Between 900 sec and 4,620 sec, the correlation decreased, which could be explained by hydrolysis of β -glucans, which occurred at different rates for different samples, depending on the level of endogenous enzymes in each oat sample. During this interval, we also observed the maximum rate in viscosity decrease (Fig. 1). After 900 sec, viscosity declined more slowly.

The change in rate of viscosity decrease during the experiment may be explained in two ways. First, the viscosity of polymers in solution is related to MW by the Mark-Houwink equation $k(MW)^a$.

TABLE III
Water-Solubility (S) and Extractability (E) of Raw Oat Flour Compounds^a

Oat Line	Year	Starch + Dextrin		Protein		β -Glucan		Pentosan		Glucose
		S ^b	E ^c	S	E	S	E	S	E	S
IA95111	2001	25.8b	47.6cd	0.99g	4.7g	6.32b	81.1hi	0.29a	13.0ab	0.22b
	2002	25.5b	45.6de	1.52bc	7.5c-e	5.48e	88.5e-g	0.26a-d	9.9c-f	0.17de
N979-5-2-4	2001	25.6b	49.8ab	1.36c-e	6.4ef	6.97a	92.9c-e	0.26a-d	11.9a-c	0.18d
	2002	25.5b	46.2d	1.60ab	8.3bc	5.85cd	84.8gh	0.27a-d	9.4d-f	0.16e-g
IA95258	2001	nd ^d	nd	nd	nd	nd	nd	nd	nd	nd
	2002	25.5b	50.7a	1.26ef	6.2ef	4.95g	73.9jk	0.25a-d	10.3c-e	0.17d-f
IA91524-1-5-1	2001	26.4ab	47.1cd	1.52bc	8.1b-d	5.35ef	86.2f-h	0.26a-d	13.5a	0.16fg
	2002	25.9b	43.9ef	1.76a	9.6a	4.31hi	78.25ij	0.26a-d	10.9b-d	0.15g
IA 95205	2001	26.6ab	48.6bc	1.15e-g	5.5fg	5.98c	99.4ab	0.28a-c	12.7ab	0.21c
	2002	25.7b	43.5fg	1.76a	8.8a-c	5.59de	94.7b-d	0.27a-d	9.8c-f	0.22b
IA91463	2001	27.3a	46.3d	1.55a-c	8.3a-c	5.11fg	97.6a-c	0.28ab	12.8ab	0.17ef
	2002	25.6b	42.4fg	1.66ab	8.8a-c	4.00i	70.2kl	0.22d	8.6ef	0.13h
Paul	2001	25.4b	45.8de	1.04gf	6.7d-f	4.44h	100.0a	0.22d	9.8c-f	0.28a
	2002	25.5b	42.8fg	1.44b-d	8.5a-c	4.43h	90.3d-f	0.23cd	8.1f	0.27a
JIM	2001	25.7b	38.6i	1.25ef	6.3ef	3.36j	68.6lm	0.24b-d	11.2b-d	0.12h
	2002	25.6b	40.0ih	1.25ef	6.5ef	3.03k	70.4kl	0.23cd	9.8c-f	0.13h
IN090201	2001	25.5b	41.4gh	1.08gf	6.5ef	3.11jk	63.4m	0.23cd	11.3b-d	0.12h
	2002	25.6b	42.1f-h	1.66ab	9.3ab	3.20jk	81.9hi	0.22d	9.7d-f	0.13h

^a Values are means of two replicate analyses of soluble extracts obtained in two replicate extractions. Values within a column followed by a common letter are not significantly different ($P > 0.05$).

^b Soluble compounds (% w/w, dmb).

^c Extractability (E%) calculated as ratio between soluble compound and total compound in oat flour.

^d Not determined (limited quantity of seeds).

The fragmentation of high MW polymers in the early stages of hydrolysis can drastically affect the viscosity. The resultant low MW fragments had less impact than on viscosity as the experiment continued. Second, high MW polymers are a better substrate for endoglucanases than are the low MW fragments (Chen et al 1995). Thus, a decrease in rate of hydrolysis might be explained by a decrease in specificity of enzymes toward the substrate as hydrolysis occurs. Viscosity profiles for raw barley slurries in water, with an initial rise in viscosity followed by a decrease, were similarly explained by the presence of the β -glucan-degrading enzymes in barley (Izydorczyk et al 2000).

In some studies reported in the literature, evaluation of rheological properties of flour slurries involved viscosity measurement of the supernatant obtained after slurry centrifugation, which was previously mixed (Bhatty et al 1991; Grosjean et al 1999b) or held undisturbed (Doehlert et al 1997a) for a defined time to allow the extraction of soluble components. In other studies (Luhalo et al 1998; Izydorczyk et al 2000), as in the present work, viscosity was monitored continuously during the time of extraction with mixing, giving full information about the rheological behavior of oat slurries during extraction. The advantage of recording viscosity during mixing is that the correlations with β -glucan concentration could be made by using the whole profile rather than by using viscosity values at a single time.

Viscosity Profile of Oat Flours in Silver Nitrate Solution

The dispersion of slurries in silver nitrate solution used to inhibit endogenous enzymes resulted in higher and more stable viscosity

values during mixing than those obtained in deionized water (Fig. 3). Most of the flours reached a maximum viscosity value followed by a plateau. However, for certain flours, the maximum viscosity was followed by a steady decrease of viscosity. The profiles differed in time-to-peak and the length-of-plateau region, depending on oat sample. The decrease of viscosity during mixing that occurred for certain oat samples could not be explained by an enzymatic hydrolysis of polymers in solution because, in the presence of silver nitrate at 0.1 mmol/g meal, the amylases, proteases, and glucanases were inactivated (Glennie-Holmes 1995). One explanation for the viscosity decrease might be that the molecular features of components or interactions between the molecules contributing to the viscosity in solution, such as β -glucans, proteins, and arabinoxylans, changed during rheological measurements. Wood et al (1978) showed that changes in molecular organization can occur in β -glucan solutions and might lead to a loss of viscosity or precipitation of some material. Similar observations previously reported for wheat flour (Moore and Hosney 1990) were suggested by the authors to result from an aggregation of the polymers. They ruled out enzymatic degradation because no pH dependence was observed (pH 2–8). Grosjean et al (1999a) also observed a linear decrease with time of relative viscosity for a wheat-water extract after inactivation of enzymes by heating.

The evolution of correlation coefficients between total β -glucan concentration and viscosity values showed an increase over time, with a maximum value at 7,000–14,400 sec of dispersion ($r = 0.940$, $P < 0.05$), when it is likely that the maximum amount of β -glucans was extracted (Fig. 4). Higher correlations were obtained in silver nitrate solution than in deionized water.

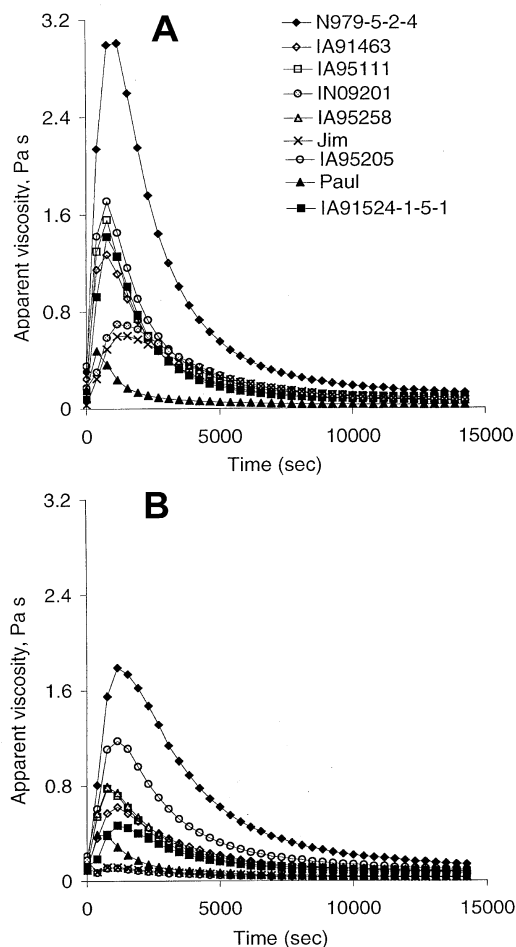


Fig. 1. Viscosity development of raw oat flours with dispersion in deionized water at 25°C and mixing at a shear rate of 50 sec⁻¹. **A**, Oats grown in 2001. **B**, Oats grown in 2002. Results presented are averages of replicate measurements with flours obtained in two different millings.

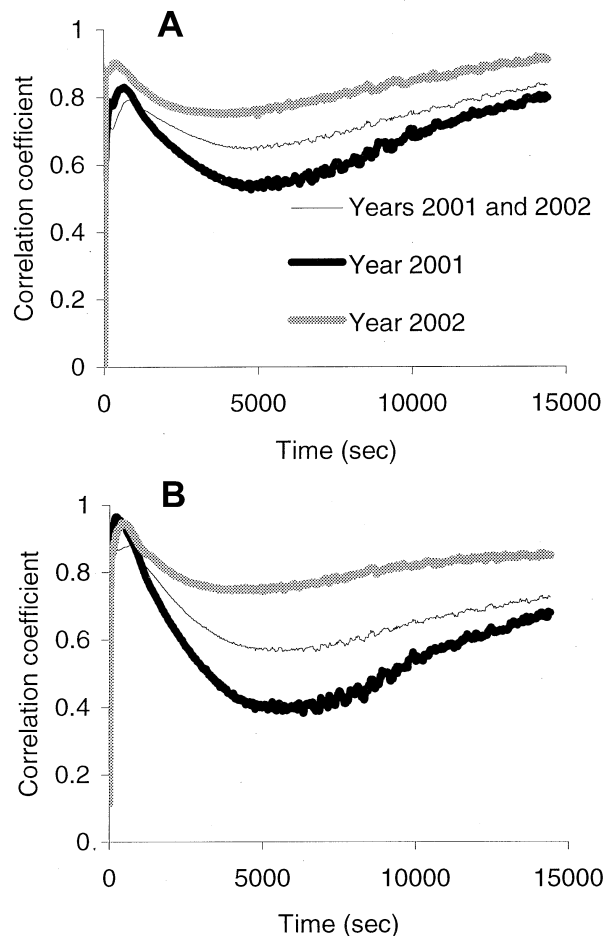


Fig. 2. Correlation between total β -glucan concentration and viscosity (**A**) and between soluble β -glucan and viscosity of slurries (**B**) developed in deionized water over time of mixing.

TABLE IV
Maximum Attainable Viscosity of Raw Oat Flour Slurries During Dispersion^a

Oat Line	Year	Maximum Viscosity (Pa·sec)			Ratio Maximum Viscosity	
		Water	AgNO ₃	Alkali	AgNO ₃ /Water	Alkali/Water
IA95111	2001	1.58 ± 0.04c	5.03 ± 0.17b	3.36 ± 0.45b	3.19	2.13
	2002	0.78 ± 0.03f	3.35 ± 0.04fg	2.29 ± 0.08de	4.29	2.94
N979-5-2-4	2001	3.10 ± 0.19a	6.32 ± 0.04a	4.32 ± 0.02a	2.04	1.39
	2002	1.81 ± 0.02b	4.44 ± 0.27cd	2.36 ± 0.11cd	2.46	1.30
IA 95258	2001	nd ^b	4.11 ± 0.59de	3.07 ± 0.02b	nd	nd
	2002	0.79 ± 0.06f	3.72 ± 0.08ef	1.56 ± 0.01g	4.71	1.97
IA91524-1-5-1	2001	1.42 ± 0.00d	3.73 ± 0.15ef	2.18 ± 0.08de	2.62	1.54
	2002	0.47 ± 0.02i	2.03 ± 0.13h	1.10 ± 0.04h	4.35	2.35
IA95205	2001	1.75 ± 0.13b	4.50 ± 0.04c	2.64 ± 0.29c	2.57	1.51
	2002	1.18 ± 0.03e	3.07 ± 0.01g	1.98 ± 0.08ef	2.6	1.67
IA91463	2001	1.29 ± 0.05de	3.22 ± 0.08g	1.83 ± 0.00fg	2.5	1.42
	2002	0.62 ± 0.06gh	2.26 ± 0.19h	1.15 ± 0.04h	3.64	1.85
Paul	2001	0.49 ± 0.02hi	1.89 ± 0.13hi	1.15 ± 0.17h	3.89	2.37
	2002	0.42 ± 0.01i	1.94 ± 0.19h	1.09 ± 0.03h	4.61	2.6
JIM	2001	0.61 ± 0.04gh	1.54 ± 0.06i	0.60 ± 0.01i	2.52	0.98
	2002	0.12 ± 0.01j	0.65 ± 0.04j	0.50 ± 0.05i	5.61	4.30
IN090201	2001	0.71 ± 0.03fg	1.89 ± 0.11hi	0.55 ± 0.04i	2.65	0.77
	2002	0.11 ± 0.07j	0.83 ± 0.06j	0.55 ± 0.01i	7.47	5.00

^a Values are means of rheological measurements ($n = 2$) with flours obtained in two different millings ± standard deviation. Values within a column followed by a common letter are not significantly different ($P > 0.05$).

^b Not determined (limited quantity of seeds).

TABLE V
Correlation Coefficients (r) of Maximum Attainable Viscosity^a and Total β -Glucan Content

	β -Glucan 2001	MV _w 2001	MV _s 2001	MV _{alk} 2001	β -Glucan 2002	MV _w 2002	MV _s 2002	MV _{alk} 2002
β -Glucan 2001	1.000							
MV _w 2001	0.810 ^{ab}	1.000						
MV _s 2001	0.918 ^{***}	0.950 ^{***}	1.000					
MV _{alk} 2001	0.905 ^{***}	0.933 ^{***}	0.981 ^{***}	1.000				
β -Glucan 2002	0.781 [*]	0.884 ^{**}	0.900 ^{***}	0.954 ^{**}	1.000			
MV _w 2002	nd ^c	nd	nd	nd	0.846 ^{**}	1.000		
MV _s 2002	nd	nd	nd	nd	0.971 ^{***}	0.904 ^{***}	1.000	
MV _{alk} 2002	nd	nd	nd	nd	0.871 ^{**}	0.883 ^{**}	0.928 ^{***}	1.000

^a MV_w, MV_s, MV_{alk}, maximum attainable viscosity in water, silver nitrate solution, and alkali solution, respectively, for oats grown in 2001 and 2002 ($n = 9$).

^b *, **, *** = significant at $P < 0.001$, $P < 0.01$, and $P < 0.05$, respectively.

^c Not determined.

TABLE VI
Correlation Coefficients (r) of Time-to-Peak Viscosity^a and Total β -Glucan Content

	β -Glucan 2001	TPV _w 2001	TPV _s 2001	TPV _{alk} 2001	β -Glucan 2002	TPV _w 2002	TP _s 2002	TPV _{alk} 2002
β -Glucan 2001	1.000							
TPV _w 2001	-0.110	1.000						
TPV _s 2001	0.138	0.959 ^{***b}	1.000					
TPV _{alk} 2001	0.359	-0.170	0.076	1.000				
β -Glucan 2002	0.781	nd	nd	0.311	1.000			
TPV _w 2002	nd ^c	nd	nd	0.647	0.311	1.000		
TPV _s 2002	nd	nd	nd	0.801 ^{**}	0.647	0.903	1.000	
TPV _{alk} 2002	nd	1.000	1.000	1.000	0.801 ^{**}	0.703 [*]	0.871 ^{**}	1.000

^a TPV_w, TPV_s, TPV_{alk}, time-to-peak viscosity in water, silver nitrate solution, and alkali solution, respectively, for oats grown in 2001 and 2002 ($n = 9$).

^b *, **, *** = significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

^c Not determined.

TABLE VII
Partial Least Squares (PLS) Analysis of Viscosity Profiles with Cross-Validation

Slurry Solution	Retained PLS Factors	% Variation				Cross-Validation Root Mean PRESS ^a
		Predictor Variables		β -Glucan Content		
		Current Factor	Total	Current Factor	Total	
Deionized water	3	93.1	93.1	57.8	57.8	0.80
		4.1	97.2	22.1	79.9	0.63
		2.4	99.6	10.1	90	0.46
Silver nitrate	2	94.7	94.7	82.2	82.2	0.53
		4.5	99.2	7.9	90.1	0.40
Alkali	1	96.7	96.7	75.7	75.7	0.53

^a PRESS, predicted residual sum of squares.

Viscosity Profile of Oat Flours in Alkali Solution

To ensure complete solubility of the oat β -glucan, an alkali solution at pH 10 was used for the dispersion of flours. In alkali solution, β -glucans were solubilized along with the other components (starch and proteins) that also contributed to viscosity development. At pH 10, endoglucanases also were inactivated, preventing changes in viscosity caused by enzymatic degradation. Viscosity values in alkali solution were lower than those obtained in silver nitrate solution. In alkali conditions, the system was more homogenous, with most of components being soluble. In contrast, in heterogenous water systems, unsolubilized flour particles contributed to viscosity. As in silver nitrate solutions, the oat samples differed in their rheological behavior during mixing, having either a continuous increase in viscosity or an increase followed by a decrease after reaching a maximum (Fig. 5). A slight settling of particles or mechanical shear forces associated with alkaline conditions might explain the decrease in viscosity that occurred for some oat samples.

Correlation coefficients between β -glucan content and viscosity increased over time with a maximum ($r = 0.896$, $P < 0.05$) at 10,440–14,400 sec of mixing (Fig. 6). The correlation coefficient values calculated early in the experiment (after 300 sec of dispersion) were higher ($r = 0.800$) than those obtained by dispersion in silver nitrate solution at the same time ($r = 0.600$), which may be explained by the rapid solubilization of β -glucans in alkali solution.

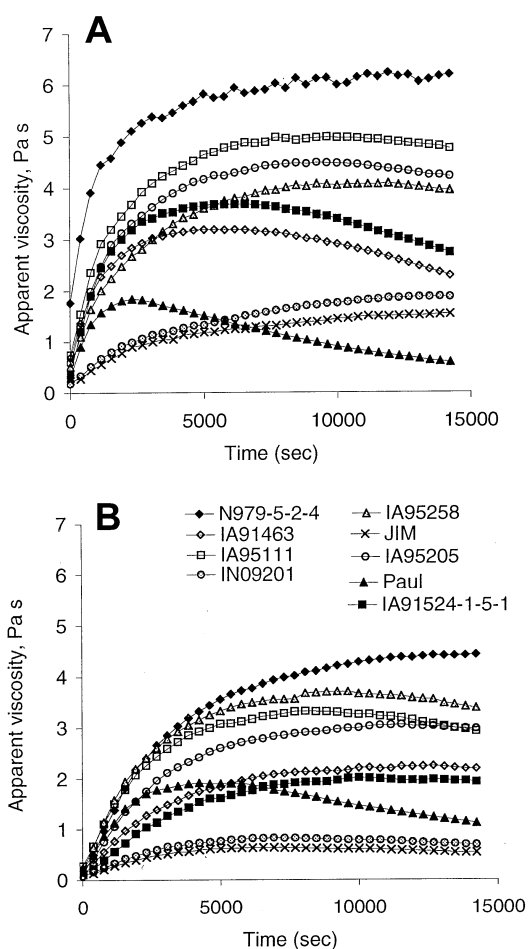


Fig. 3. Viscosity development of raw oat flours with dispersion in silver nitrate solution (0.1 mmol/g of oat flour) at 25°C and mixing at a shear rate of 50 sec⁻¹. **A**, Oats grown in 2001. **B**, Oats grown in 2002. Results presented are averages of replicate measurements with flours obtained in two different millings.

Maximum Attainable Viscosity

Maximum attainable viscosity values of oat flours dispersed in deionized water, silver nitrate solution, and alkali solution are presented in Table IV. Highly significant Pearson's correlations were observed between total β -glucan and maximum attainable viscosity (Table V), suggesting that the β -glucan concentration made an important contribution to viscosity. The highest correlation between maximum attainable viscosity and β -glucan concentration was obtained for silver nitrate solution, followed by alkali solution, and then deionized water, results which are consistent with those obtained by analyzing the entire profile. High correlation coefficients obtained between the maximum attainable viscosity in the three different solutions from two years showed that this parameter is a characteristic of the oat line, independent of the extraction conditions and the year of growing.

The extraction time needed to reach the maximum viscosity reflected the β -glucan extractability, which was an important factor determining β -glucan viscosity and oat functionality and quality. The time-to-peak viscosity was significantly affected by oat line. Analysis of variance showed a significant effect of line and line-by-year interaction ($P < 0.0001$) and a nonsignificant effect of year on time-to-peak viscosity. The time-to-peak viscosity in deionized water significantly decreased with the increase in β -glucanase level ($r = -0.704$, $P < 0.005$), suggesting that more degraded polymers were extracted more easily. High Pearson's correlation coefficients were obtained between time-to-peak-viscosity in deionized water, silver nitrate, and alkali solutions (Table VI), suggesting that the extractability of β -glucans was related to the genotype and growth environment year.

Generally, higher values of maximum attainable viscosity were reached in 2001 (an average of 1.37 Pa-sec in deionized water, 3.58 Pa-sec in silver nitrate solution, and 2.19 Pa-sec in alkali solution) than in 2002 (an average of 0.70 Pa-sec in deionized water, 2.47 Pa-sec in silver nitrate solution, and 1.40 Pa-sec in alkali solution). Moreover, the inactivation of endogenous enzymes using the silver nitrate or alkali solution produced a greater effect on viscosity from oat grown in 2001 than from oat grown in 2002, reflected by greater viscosity ratios from silver nitrate/water and alkali/water for oat grown in 2001 than in 2002. Analysis of variance showed a significant effect of line ($P < 0.0001$), year ($P < 0.0001$) and line-by-year interaction ($P < 0.0001$) on maximum viscosity reached in deionized water, silver nitrate, and alkali solution. Higher viscosity values observed for oat grown in 2001 than for oat grown in 2002 are only partly explained by greater concentrations in β -glucans in the oat grown in 2001. For example, for IA95205, the β -glucan concentration was comparable for the two years of growing (6%); however, viscosity values in

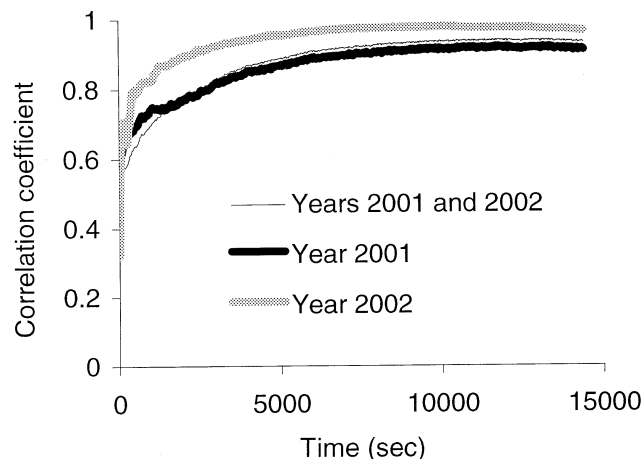


Fig. 4. Correlation between total β -glucan concentration and viscosity developed in silver nitrate solution over time of mixing.

deionized water, silver nitrate, and alkali solution were lower in oat grown in 2002 than in oat grown in 2001. Similarly, Paul grown in 2001 and JIM grown in 2002 had equal β -glucan concentrations ($\approx 4.4\%$); however, viscosity values of oat grown in 2001 were greater than those of oat grown in 2002.

Several other factors in addition to that of the total β -glucan concentration could explain the viscosity differences between the two years, such as levels of endoglucanase, solubility of β -glucans, and other components, changes in molecular properties of protein, starch, and interactions due to aging, and MW of β -glucan polymers.

Differences in magnitude of the effects of silver nitrate and alkali solution on the viscosity between the two growing years might suggest different levels in endogenous enzymes (β -glucanase, protease, amylase) with higher values in oat from 2002 than from 2001. However, a nonsignificant year effect was observed for the endoglucanase level.

The significant year effect observed for soluble protein content may have contributed to differences in viscosity between the two years. A decrease in maximum viscosity was observed with the decrease in protein extractability, which might be explained by a decrease in protein solubility during aging or the higher level of proteases in oat grown in 2002 than in 2001. The aging of oat might have affected enzyme levels originating from grain or from

microbial contamination, or physicochemical properties such as hydration, swelling, and solubility.

In general, the differences in viscosity observed between the genotypes from the two years are due to complex factors not yet elucidated. These factors will be studied in future research.

PLS Models to Predict β -Glucan Content

Three different models were constructed by using data from viscosity profiles obtained by dispersing flours from all nine oat lines grown in two consecutive years in 1) deionized water, 2) silver nitrate, and 3) alkali solution (Table VII). The iterative nature of the PLS algorithm does not allow the results to be presented as simple regression models. The information in Table VII indicates that the PLS prediction of β -glucan will be more successful when viscosity in a silver nitrate solution is used for prediction than when deionized water or alkali solutions are used. First, the two-factor silver nitrate PLS model had the lowest root mean PRESS statistic among all models. The PRESS statistic provides an assessment of the effectiveness of the model at predicting the β -glucan content of samples that have not been used for model development, as would be the case for future oat samples. The root mean PRESS of 0.40 for the silver nitrate model indicates that the PLS model should be able to predict $1 - \sqrt{0.40} = 84\%$ of the variation in β -glucan content of future oat samples based on their viscosity in silver nitrate solution. Second, the two-factor silver nitrate PLS model accounts for more variation in the predictor variables (99.2%) than does the single-factor alkali model (96.7%) and almost as much variation as the three-factor deionized water model (99.6%). The assumption behind PLS is that factors that account for much of the variation among current predictors should provide better prediction for future observations. Consequently, PLS models that account for more variation in the predictor variables are favored over models that account for less variation in the predictor variables and models that account for the same amount of variation with fewer factors are favored over models that require more factors. The silver nitrate model accounts for much variation with just two factors, which makes it desirable.

Figure 7 shows the predicted versus actual β -glucan content of the oat samples by experiment year. The figure shows that the same model could consistently predict β -glucan content in both years: predictions were not biased up or down for samples from one year versus the other year, indicating that environmental effects did not perceptibly affect the relationship between viscosity measurements and β -glucan concentration. These results suggest that the PLS models obtained thus far should provide robust predictions on future samples from different oat genotypes. Nevertheless, further validation experiments should be conducted.

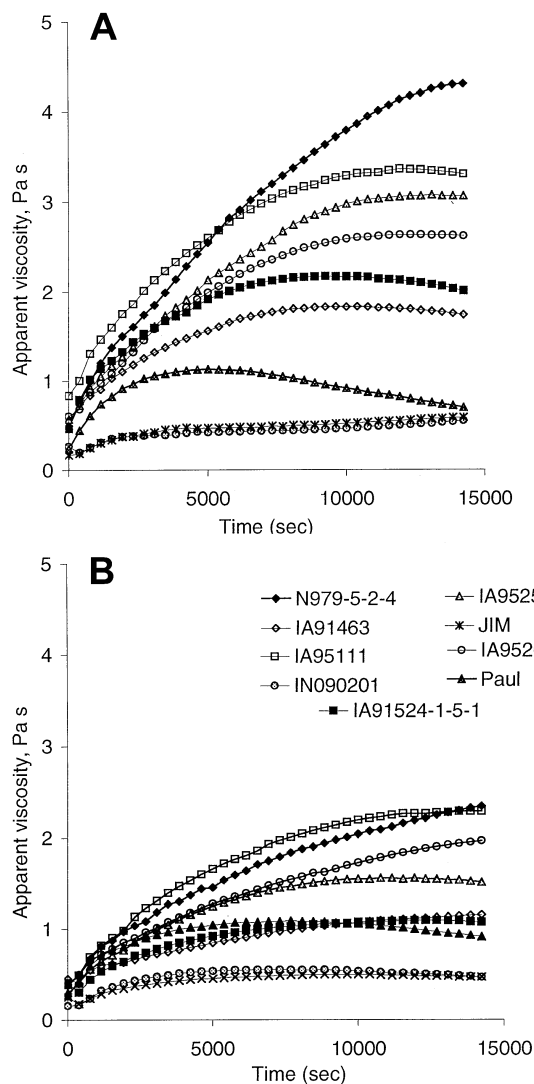


Fig. 5. Viscosity development of raw oat flours with dispersion in alkali solution (pH 10) at 25°C and mixing at a shear rate of 50 sec⁻¹. **A,** Oats grown in 2001. **B,** Oats grown in 2002. Results presented are averages of replicate measurements with flours obtained in two different millings.

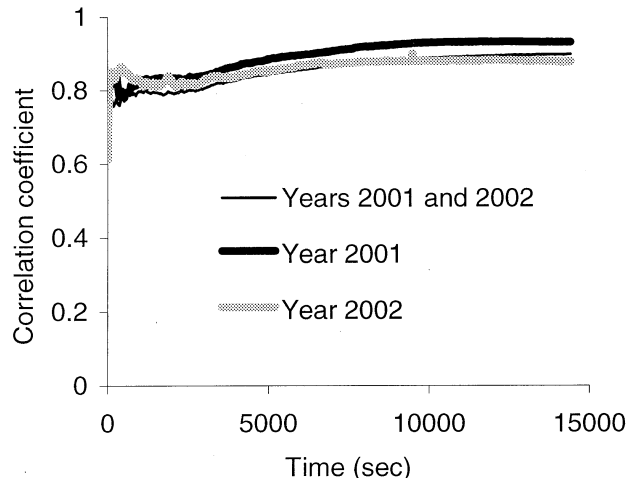


Fig. 6. Correlation between total β -glucan concentration and viscosity developed in alkali solution over time of mixing.

CONCLUSIONS

This study, made with oat lines containing a wide range in β -glucan concentrations grown during two consecutive years, confirmed the contribution of β -glucans to the viscosity of oat flour slurries and emphasized the potential role of other factors such as endoglucanase levels and environmental factors on rheological properties. In addition, high correlations were obtained between the β -glucan concentrations of nine oat lines grown in two consecutive years and the viscosity of their slurries when dispersed in deionized water, silver nitrate solution, or alkali solution. The overall findings demonstrated that the β -glucan provided the main contribution to viscosity and that the measure

of viscosity of raw oat flour slurries could be a useful screening method for selecting breeding lines. Viscosity profiles obtained in silver nitrate, which created higher viscosity values, versus those obtained in deionized water, showed the importance of inactivating β -glucanases to maintain the functional properties of β -glucans. By using PLS, a quantitative relationship between the viscosity profile and total β -glucan concentration was established. The PLS models were developed using oat samples spanning a broad range of β -glucan concentration and grown in two years. This broad range and the use of cultivars from two different environments should make the models applicable to a wide variety of future oat samples.

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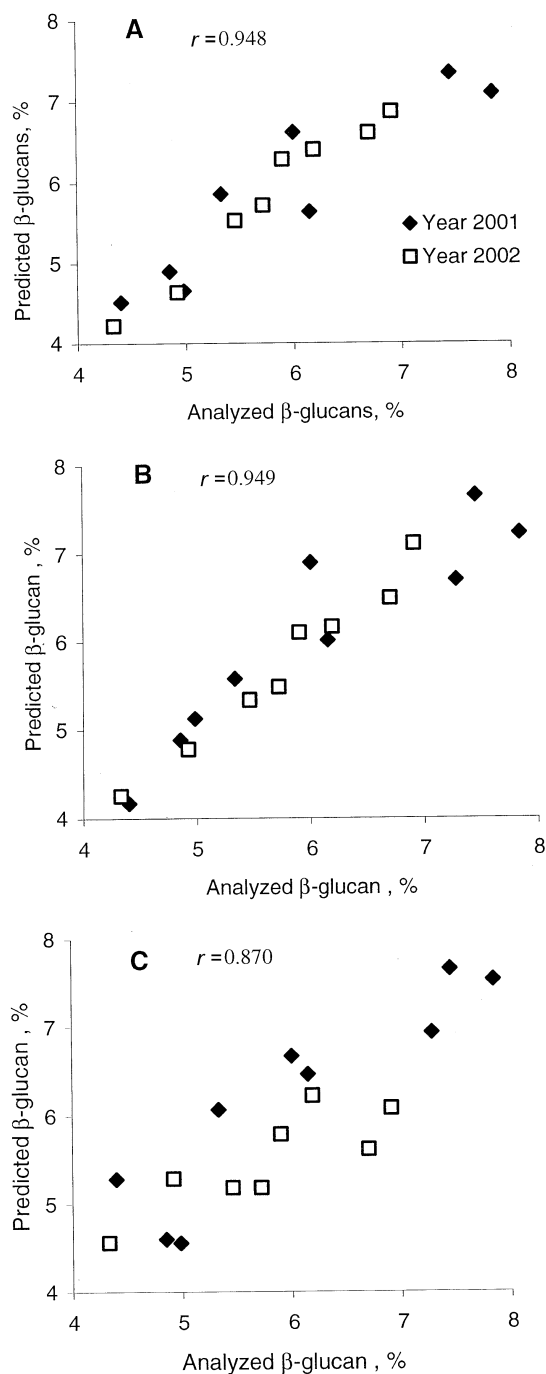


Fig. 7. Predicted vs. actual β -glucan content calculated with the PLS calibration method. Models were constructed with data points from viscosity development profiles created by dispersing oat flours in deionized water (A), silver nitrate (B), and alkali solution (C).

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