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Rheological and molecular properties of water soluble (1,3) (1,4)-β-D-glucans from high-β-glucan and traditional oat lines

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Abstract

Water-soluble β-glucans were extracted, with a recovery yield of ~70%, from five oat lines with increased β-glucan concentrations (up to 7.8% β-glucan) and one traditional oat line (4.4% β-glucan). The rheological properties of the extracted β-glucan gums were studied at concentrations of 0.25, 0.5 and 1% (w/w) over shear rates of 0–300 s^{-1}. Statistically significant differences in the rheological properties of β-glucans were found among the oat lines when measured at 1% (w/w) β-glucan. β-glucan from the enhanced oat lines were more viscous (IA95258 had the highest and IA95205 the lowest viscosity) than those from the traditional line (Paul). The effect of concentration on apparent viscosity was accurately described by an exponential model for the six oat lines; however, the rate of change was significantly different among IA95258, IA95205 and Paul lines. Differences in viscosity between oat lines measured at the same β-glucan concentration and in rate of viscosity change with concentration might be explained by differences in peak molecular weight and polymer distribution.
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Keywords: β-glucan; Oats; Rheological behavior; Viscosity; Molecular weight

1. Introduction

Soluble fibers from oats lower the blood cholesterol level when consumed in the daily diet, particularly for subjects with high blood cholesterol levels (Welch, 1995). The component responsible for lowering cholesterol is (1,3)(1,4)-β-D-glucan, a cell-wall polysaccharide found in the endosperm and subaleurone layers of cereal seeds (Behall, Scholfield, & Hallfrisch, 1997). The mechanism by which β-glucans lower the cholesterol level is not well established, but one factor believed to play a significant role is the increase of luminal viscosity in the gastrointestinal tract, related to the high molecular weight of β-glucans (Anderson, 1995).

Rheological properties of β-glucans extracted from cereal sources have been studied by many researchers (Bohm & Kulicke, 1999; Doublier & Wood, 1995; Gomez, Navarro, Garnier, Horta, & Carbonell, 1997a,b) with information reported on the effect of different factors on the rheological properties, such as the extraction procedure (Beer, Arrigoni, & Amado, 1996) or cereal processing (Doehlert, Zhang, & Moore, 1997). Rheology also was used to characterize oat-based beta-glucan/amylodextrin blends, called Oatrim, obtained by hydrolysis of oat flours (Carriere & Inglett, 1999), and to characterize mechanically solubilized β-glucan products called NutrimX (Carriere & Inglett, 2000).

Only a limited number of studies have been reported, however, regarding variations in β-glucan rheological properties among cereal cultivars. Autio, Mllymäki, Suortti, Saatamoinen and Poutanen (1992) found differences in rheological properties of β-glucans extracted from different oat varieties, when measured at the same β-glucan concentration, which was reflected in the wide variation of molecular weights (MW). More studies have been carried out for barley. Yoon, Berglund and Fastnaught (1995) noticed that waxy hull-less barley cultivars had greater β-glucan content and extract viscosities than did non-waxy cultivars and the basis for the viscosity increase was only

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partly explained by greater content in β-glucan. Ullrich, Clancy, Eslick and Lance (1986) emphasized that both the quantity and quality of β-glucans influence barley extract viscosity, with larger polymers having greater influence than smaller polymers. Xue, Newman, Newman and McGuire (1991) suggested that the measurement of viscosity, only, is not enough to estimate β-glucan content across barley genotypes, and that other factors affect this parameter. Indeed, Saulnier, Gévaudan and Thibault (1994) observed differences in rheological behavior and polymer distribution of β-glucans extracted from two barley cultivars with similar content in β-glucan. Furthermore, Izydorczyk, Storsley, Labossiere, MacGregor and Rossnagel (2000) observed substantial differences in molecular weight and viscosity of β-glucans isolated from several barley genotypes.

The aim of this study was to identify oat types with a high nutritional potential reflected by both an increased level of β-glucans and a high viscosity of their extracts. As suggested above, the viscosity of a polysaccharide may control its physiological behavior. In addition, understanding the rheological properties could lead to the development of new food applications of β-glucans, such as stabilizers, thickeners textural agents, or fat replacers in food products, or even in biomedical applications. Five high-β-glucan oat lines, developed at Iowa State University, and one traditional variety were investigated in this study for the rheological behavior and molecular features of their β-glucans.

2. Experimental

2.1. Oat grain

Oat (*Avena sativa*) grain types were grown in 2001 at the Agronomy and Agricultural Engineering Field Research Center near Ames, IA, on a Nicollet loam soil (fine-loamy, mixed, mesic Aquic Hapludoll). Experimental lines, IA95111, IA95205, and IA95258, had high β-glucan concentrations, as previously described (Cervantes-Martinez, Frey, White, Wesenberg, & Holland, 2001). Experimental lines, N979-5-2-4 and IA91524-1-5-1 were derived from unpublished selection experiments for high β-glucan content at Iowa State University. Paul, a traditional oat cultivar used as control in this study, is a naked oat variety (McMullen, Doehlert, & Miller, 1997).

2.2. Chemical analysis

The moisture of oat flours was determined by Approved Method 44-15 A (AACC, 1995). The β-glucan content in flours and in extracts was determined enzymatically by the method of McCleary and Glennie-Holmes (1985). Pentosan in flours and in extracts was analyzed by the phloroglucinol colorimetric method (Douglas, 1981). Proteins in extracts were analyzed by the Bradford dye-binding method (Bradford, 1976) with bovine serum albumin as a standard. Oat-groat flour proteins were determined by the Kjeldhal procedure (AACC method 46-12, 1995) with a protein conversion factor of 6.25. Starch content in flour, residual starch and dextrin in extracts were analyzed by the enzymatic procedure of McCleary, Gibson and Meyford (1997). Lipids were analyzed by the gravimetric method after extraction with hexane on a Soxhlet system (method 60-50A, AACC, 1995). Ash was determined by Approved Method 08-01 (AACC, 1995). Results were reported on a dry weight basis (dwb).

2.3. Extraction of β-glucans

Samples were dehulled with an air-pressure dehuller (Codema, Eden Prairie, MN) and the kernels then ground in an ultracentrifugal mill (ZM-1, Retch GmbH&Co, Haan, Germany). Water-soluble β-glucans were extracted in replicate by the procedure of Westerlund, Andersson and Aman (1993) with a few modifications. The flour (10 g), defatted in a Soxhlet system by 2-propanol: petroleum ether (2:3) during 6 h, was extracted with 40 ml of 90% ethanol for 1 h, under mixing. The mixture was centrifuged (20 min. at 3500 g), the supernatant discarded, and the ethanol extraction repeated. The pellets were suspended in 150 ml deionized water containing 2 mg thermostable amylase (Cat no. A-4551, Sigma-Aldrich, St.Louis, MO) and 28 mg calcium chloride, and the mixture incubated at 80 °C for 3 h, with occasionally shaking. Next, the mixture was centrifuged (3500, 20 min), the supernatant decanted and pellets washed twice with 25 ml water. Pancreatin (10 mg, Cat no. P-8096, Sigma-Aldrich) and 200 μl of 10% sodium azide were added to the three pooled supernatants and the mixture incubated at 40 °C for 4 h. Polymers were precipitated by 60% ethanol overnight at 4 °C. The precipitate was isolated by centrifugation (3500 g, 20 min., 4 °C) and re-dissolved in deionized water at 70° C to 80°C under magnetic stirring. The β-glucans were precipitated by 20% ammonia sulfate for 72 h at 4°C. Finally, the mixture was centrifuged (3500 g, 20 min., 4 °C), the supernatant decanted and the pellets placed in a dialysis tube (Spectrapor, molecular cut-off 12,000 Da), dissolved in deionized water at 80 °C and dialyzed for 72 h. A solution of 0.02% sodium azide was added to the final suspension to inhibit growth of microorganisms. One part of the suspension was frozen at −80 °C for immediate analysis and another part freeze-dried for long-term storage.

2.4. Rheological measurements

The β-glucan solutions were prepared at three levels of concentration (0.25, 0.5 and 1% w/w) and tested by using a Haake RS 150 rotational rheometer (Haake, Karlsruhe, Germany) equipped with a cone attachment C60/2°. A shear rate ($\dot{\gamma}$, dimensionless) ramp from 0 to 300 s^{-1} was applied over 60 s. The temperature during testing was maintained at

20 °C by a TC-81 Peltier temperature control system (Haake, Karlsruhe, Germany). All samples were tested in triplicate and all data analyzed.

Rheological behavior of the samples was evaluated by fitting the data to a power law model

$$\sigma = K\dot{\gamma}^n \quad (1)$$

where σ is the shear stress (Pa), K is the consistency coefficient (Pa-s^n), and n is the flow behavior index (dimensionless). The values of apparent viscosity (η, Pa-s) were calculated as

$$\eta = \frac{\sigma}{\dot{\gamma}} = K\dot{\gamma}^{n-1} \quad (2)$$

2.5. Molecular weight distribution

The relative molecular weight of β-glucans was estimated by size-exclusion chromatography (SEC). The SEC was performed in a glass column (75 × 1.5 cm i.d.) packed with TSK HW 75 gel (Toso Haas, Montgomeryville, PA) (fractionation range: 10^4–10^7 Da) with 10 mM NaOH as the mobile phase. The flow rate was 0.3 ml/min, the concentration of the applied sample was 3 mg/ml in the mobile phase, and the injected sample volume was 1 ml. The elution volume was determined with glucose. Dextrans of known molecular weight, 2×10^6 Da (Cat no. D-5376, Sigma), 0.519×10^6 Da (D5251, Sigma) and 0.207×10^6 Da (D-4876, Sigma), were used as standards. The concentration of the polymer in the effluent (2-ml fractions collected, collector Biorad, Hercules, CA) was determined by the phenol-sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956).

2.6. Statistical analysis

Experiments and individual analyses were conducted in replicate. Results were analyzed by using the Statistical analysis computer Program (SAS Institute, Cary, NC). Analysis of variance (ANOVA) was performed by using the linear model (GLM) procedure of SAS. Multiple comparison of means was performed by least significant difference (LSD) and Tukey–Kramer tests at $\alpha = 0.05$.

3. Results and discussion

3.1. Chemical composition of oat groats

The oat lines chosen for this study had relatively high β-glucan concentrations (Table 1). The Paul line, used as a control, is a traditional variety with a normal β-glucan level (4.4%, dwb). The five high-β-glucan oat lines contained between 6 and 7.8% (dwb) β-glucan, which is greater than typical values for domestic cultivars of *A. sativa* reported in the literature (3.7–5.0%, dwb) (Miller, Wood, Pietrzak, & Fulcher, 1993). Small variations were observed in pentosan, starch, and protein percentages, and no variation was observed in ash content among the oat lines. Protein concentration was between 18.8 and 21.6% (dwb) in accord with results reported by Miller et al. (1993), where the mean protein level for five domestic cultivars of *A. sativa* was 20.7%). A wide range of values was observed for lipids (4.8–8.5%, dwb), but the variation was typical within common oat varieties (5–9%). Two oat lines with high-β-glucan percentages (IA95258 and N979-5-2-4) had low lipid concentrations. Such oat types might be used in foods contributing to a high-fiber, low-fat diet.

3.2. Extraction and purification of β-glucans

β-glucans were extracted from six oat lines with a total recovery yield of ~70%. Solutions were adjusted at 1% (w/w) β-glucans for the rheological study and main contaminants were analyzed. Starch, dextrin and free glucose were not detected. The 1% (w/w) β-glucan solutions had less than 0.015% (w/w) proteins and less than 0.10% (w/w) pentosan. A low amount of pentosan could have a small influence on viscosity, whereas the contribution of a small amount of proteins is expected to be negligible (Bhatty, MacGregor, & Rossnagel, 1991).

Table 1
Chemical composition (%db) of flours ground from groats of control and high-β-glucan oat lines

Oat line	Starch	Protein[a]	Lipid	β-glucan	Ash	Pentosan
IA 95111	46.88 ± 0.82a	20.91 ± 0.41a	6.85 ± 0.06	7.84 ± 0.19	2.31 ± 0.09a	2.26 ± 0.03a
N979-5-2-4	49.75 ± 0.35bc	21.10 ± 0.62a	5.09 ± 0.04	7.45 ± 0.04a	2.34 ± 0.06a	2.20 ± 0.15a
IA 95258	49.17 ± 0.70cd	21.59 ± 0.29a	4.86 ± 0.07	7.28 ± 0.19a	2.30 ± 0.08a	2.16 ± 0.06ab
IA 91524-1-5-1	49.44 ± 0.78bcd	18.82 ± 0.73	8.50 ± 0.09	6.15 ± 0.08b	2.24 ± 0.08a	1.90 ± 0.20b
IA 95205	47.94 ± 0.94ad	20.98 ± 0.18a	6.42 ± 0.01	6.01 ± 0.12b	2.37 ± 0.07a	2.20 ± 0.17a
Paul	51.50 ± 0.71	19.69 ± 0.20	7.41 ± 0.08	4.41 ± 0.19	2.20 ± 0.01a	2.22 ± 0.14a

Values are means of $n = 3$ measurements, ± standard deviation. Values within a column followed by a common letter (a–d) are not significantly different ($p > 0.05$).

[a] $N \times 6.25$

Table 2
Mass balance of oat groat components during extraction

Extraction step	Oat line	β-glucan (mg)	Starch/Dextrin (mg)	Glucose (mg)	Protein (mg)	Pentosan (mg)
Starting material	IA 95205	486.8	3883.1a	0	1699.9a	177.4a
	IA 95258	589.7	3982.8a	0	1748.8a	162.0a
	Paul	365.1	4264.2a	0	1629.9a	182.7a
Water extraction	IA 95205	409.7ab	2676.9	246.9	42.1	33.8
	IA 95258	508.7	3557.7b	311.9a	60.4	45.0
	Paul	310.6c	3228.1b	288.4a	66.6	24.1b
Ethanol precipitation	IA 95205	399.4a	117.5c	9.7b	10.1bc	14.3c
	IA 95258	460	151.6c	13.3b	8.7bc	15.8c
	Paul	308.8c	131.6c	13.5b	7.0c	20.2bc
Ammonia precipitation	IA 95205	358.3	ND[a]	ND	4.3d	3.7d
	IA 95258	424.7b	ND	ND	4.0d	4.2d
	Paul	265.7d	ND	ND	4.0d	3.1d
Dialysis	IA 95205	349.6a	ND	ND	3.2d	2.2d
	IA 95258	414.4ab	ND	ND	4.9d	4.0d
	Paul	251.7d	ND	ND	4.1d	2.3d

All values are means of three extraction trials with 10 g (wet basis) starting material. Means within a column, followed by a common letter (a–d) are not significantly different ($p > 0.05$).
[a] ND: non detected.

Two oat lines (IA95205 and IA95258) were selected (based on rheological results), along with the Paul line as a control, for further in depth studies. The mass balance of the main oat groat components during extraction was established by conducting three separate extractions (Table 2). At each step the purity of β-glucans was enhanced but the recovery yield, based on the expected β-glucan measured on the whole groat flour, decreased. Total recovery yield of β-glucans, and composition of 1% (w/w) β-glucan solutions, prepared for rheological study are presented in Table 3. No significant differences in yield and purity were observed among the three oat lines.

The treatment with hot petroleum ether/isopropanol was carried out to inactivate endogenous β-glucanase activity originating from the grain. Stopping the β-glucanase activity is important to prevent degradation of molecular characteristics and functional properties of β-glucans. If β-glucanases are present, β-glucans could be depolymerized and, even a slight breakdown, could have a drastic effect on viscosity. Several procedures, employing different solvents and conditions, were detailed in the literature to extract β-glucans. We focused on evaluating the water-soluble β-glucans in the current study, because these soluble fibers are involved in decreasing total and LDL cholesterol in human blood. The water-insoluble fibers have no effect, unless they displace foods supplying saturated fats and cholesterol (Anderson, 1995). The extract obtained by water extraction, simultaneous with amylolysis, was rich in dextrin and glucose from starch degradation. A complete extraction of β-glucans was not possible. With ethanol precipitation, high molecular weight (MW) polymers and dextrins with a degree of polymerization (DP) of >20 were recovered after centrifugation. Glucose, malto-oligosaccharides, and small peptides were removed by discarding the supernatant. Because pentosans could enhance viscometric properties of β-glucans, it was desirable to reduce their level in the β-glucan extract. Precipitation with 20% ammonium sulfate (method of Preece &

Table 3
Total recovery yield of β-glucans and composition of final extracts from groats of control and two selected high-β-glucan oat lines

Oat line	Total recovery yield of β-glucans[a] (%)	Composition of β-glucan solutions[b] (%, w/w)				
		β-glucan	Starch and/or maltodextrins	Free glucose	Protein	Pentosan
IA95205	71.8 ± 3.9a	1.005 ± 0.01a	ND[c]	ND	0.009 ± 0.001a	0.006 ± 0.001a
IA95258	70.3 ± 0.9a	1.008 ± 0.01a	ND	ND	0.012 ± 0.001a	0.010 ± 0.002b
Paul	68.9 ± 1.9a	1.007 ± 0.01a	ND	ND	0.016 ± 0.002b	0.009 ± 0.001b

Values are means from $n = 3$ extraction trials ± standard deviation; means within a column followed by same letter (a, b) are not significantly different ($p > 0.05$).
[a] Recovery yield is reported as a percentage of the starting material (oat groats).
[b] Solutions were prepared from freeze-dried extracts at 1% (w/w) β-glucan.
[c] ND, non-detected.

Mackenzie, 1952), followed by dialysis, allowed reduction of the amount of pentosans and proteins, and complete elimination of the dextrin and glucose. The β-glucans from IA 95258 and IA 95205, precipitated by ammonium sulfate, were difficult to dissolve, whereas the purified β-glucan from the Paul line dissolved more easily. The behavior difference between the Paul and high-β-glucan lines was likely related to the differences in molecular weight and polymer conformation, because the extracts have the same composition in the main contaminants, proteins and pentosans.

3.3. Rheological measurements

To avoid changes in molecular organization, which can occur during storage and might lead to a loss of viscosity or precipitation of some material (Wood, Siddiqui, & Paton, 1978) rheological properties were measured on fresh β-glucan solutions, immediately after preparation. Typical rheograms collected in this experiment are shown in Fig.1. Rheological behavior of all extracts was adequately described by a power law model ($R^2 > 0.99$). As expected, β-glucan solutions were shear-thinning fluids. Flow behavior index 'n' was dependent on both oat line and β-glucan concentration (Table 4), and ranged from 0.504 (IA95258, 1%) to 0.895 (Paul, 1%). In general, a more pronounced non-Newtonian character of the samples was observed for solutions with higher β-glucan concentrations (Fig.1(b)).

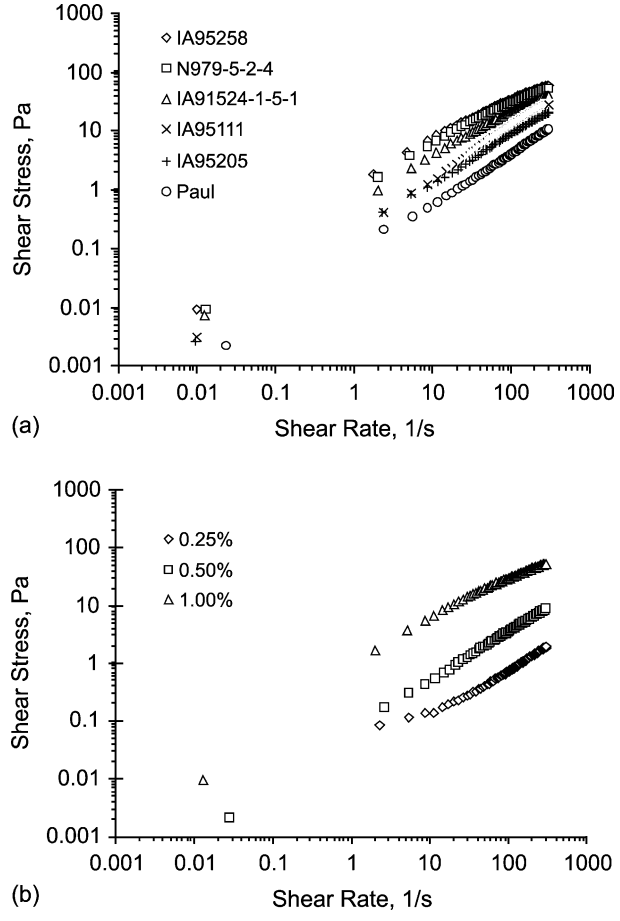

Fig. 1. Typical rheograms collected in rheological measurements: (a) 1% (w/w) β-glucan solutions from all six oat lines; (b) N979-5-2-4 β-glucan solutions at 0.25, 0.5, and 1% concentrations.

Table 4
Power law model parameters in rheological measurements of β-glucan solutions prepared at 0.25, 0.5, 1% (w/w) β-glucan

Oat sample	β-glucan concentration (%, w/w)					
	0.25	0.50	1.00	0.25	0.50	1.00
	Consistency coefficient, K[a]			Flow behavior index, n[b]		
IA95258	0.019	0.181	3.816	0.855[c]	0.737	0.504
IA91524-1-5-1	0.014[c]	0.109[c]	2.570[c]	0.867[ca]	0.795[c]	0.562[c]
N979-5-2-4	0.013[ca]	0.085[ca]	2.261[ca]	0.877[a]	0.815[c]	0.565[c]
IA95111	0.011[ab]	0.044[ab]	1.360[ab]	0.869[ca]	0.859	0.623
IA95205	0.009[db]	0.029[b]	0.555[db]	0.879	0.891[a]	0.733
Paul	0.007[d]	0.012[b]	0.064[d]	0.820	0.891[a]	0.895

[a] Consistency coefficient, K, is the power law value of viscosity (Pa s) at a shear rate of 1 s^{-1}.
[b] Flow behavior index, n, is a measure of deviation from Newtonian ($n = 1$) behavior.
[c] Values are means of three rheological measurements of β-glucans solutions prepared in two replicate extractions. The means in the same column followed by a common letter are not significatntly different ($p > 0.05$).

For further comparison of the samples, apparent viscosity at a shear rate of 50 s^{-1} was calculated (Table 5) and plotted versus β-glucan concentration (Fig. 2). Both factors, oat line and β-glucan concentration, affected the value of apparent viscosity. For all oat lines, apparent viscosity increased with an increase in concentration; although, the rate of change of the response was not the same for all types. The most dramatic effect of β-glucan concentration was observed for N979-5-2-4, IA95258, IA91524-1-5-1, and IA95111. The increase in concentration from 0.25 to 1% caused approximately a 50-fold increase in apparent viscosity for

Table 5
Mean values of apparent viscosity evaluated at $\dot{\gamma} = 50\ s^{-1}$ for all tested oat lines at 0.25, 0.5, and 1% (w/w) β-glucan concentration

Sample	Mean values of apparent viscosity at $\dot{\gamma} = 50\ s^{-1}$, Pa s		
	0.25% β-glucan	0.5% β-glucan	1% β-glucan
IA95258	0.010	0.062	0.528
IA91524-1-5-1	0.008[a]	0.045[a]	0.420[a]
N979-5-2-4	0.007[a]	0.040[a]	0.412[a]
IA95111	0.006[b]	0.025[b]	0.307
IA95205	0.005[b]	0.018[b]	0.177
Paul	0.003	0.007	0.042

[a] Values were calculated from three rheological measurements of β-glucan solutions prepared in two replicate extractions. The means within a column followed by a common superscript are not significantly different ($p > 0.05$).

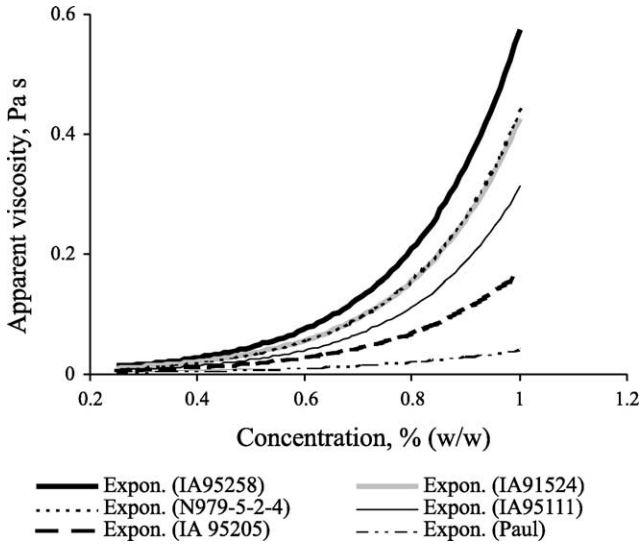

Fig. 2. Exponential fit of apparent viscosity (at 50 s^{-1}) as a function of β-glucan concentration. Values are means of three rheological measurements of β-glucan solutions, prepared in two replicate extractions.

the above oat lines, whereas IA 95205 responded with a 30-fold increase and Paul with only a 12-fold increase. Apparent viscosity values of 1% (w/w) solutions were significantly different among all oat lines ($p < 0.05$), except for IA91524-1-5-1 and N979-5-2-4. The samples prepared from all high-β-glucan oat lines were more viscous than those from the traditional variety, Paul. Among the oat lines with increased β-glucan content, IA95258 showed the greatest apparent viscosity and IA 95205 the lowest.

A wide variation was reported in the literature in the orders of magnitude among viscosities of oat β-glucan solutions. These discordances could be attributed to differences in sample sources, extraction conditions and measurement techniques. For this reason, comparison of reported data remains difficult. Our preliminary studies indicated that variations in viscosity values, between replicate extractions, could result during the large number of steps, including stirring and heating, in a fastidious extraction procedure. Therefore, to further study the effect of extraction, β-glucans from three oat lines were extracted in four replicate, and carefully controlled, extractions. Apparent viscosity of 1% solutions at a shear rate of 50 s^{-1} was used as a comparative measure. Two representatives from oat lines with increased β-glucan concentration, IA95205 and IA95258 (min and max viscosity, Fig. 2), and the traditional variety, Paul, were chosen. One-way ANOVA showed that both factors, oat line and extraction, affected the value of apparent viscosity ($p < 0.05$). Extraction effect was responsible for 92% of the total variance of apparent viscosity. In spite of higher variation of the response, as compared to two-extraction data of Table 5, comparison of all pairs by Tukey-Kramer HSD test ($\alpha = 0.05$) showed significant differences between viscosity means of the three β-glucan solutions (Fig. 3). In both two- and four-extraction experiments apparent viscosity decreased in order IA95258, IA95205, Paul.

3.4. Molecular weight and polymer distribution by size-exclusion chromatography (SEC)

The β-glucan preparations extracted from IA95258, IA95205, and Paul lines were fractionated by SEC to compare their apparent MW and polymer distributions (Fig. 4). Insertion of log Mp (molecular weight peak) obtained by injecting dextran standards gave information of the MW distribution over the elution profile. The exact molecular weight of β-glucans was, however, overestimated by using dextran for calibration (Wood, Weisz, & Mahn, 1991).

The extrapolated calibration curves indicated that β-glucans from IA95258 and IA95205 sources had a MW peak slightly higher than or equal to 2×10^6 Da, with the β-glucans from the Paul extract eluting shortly thereafter. More significant differences, however, were observed in the elution patterns of the β-glucans among oat lines, in

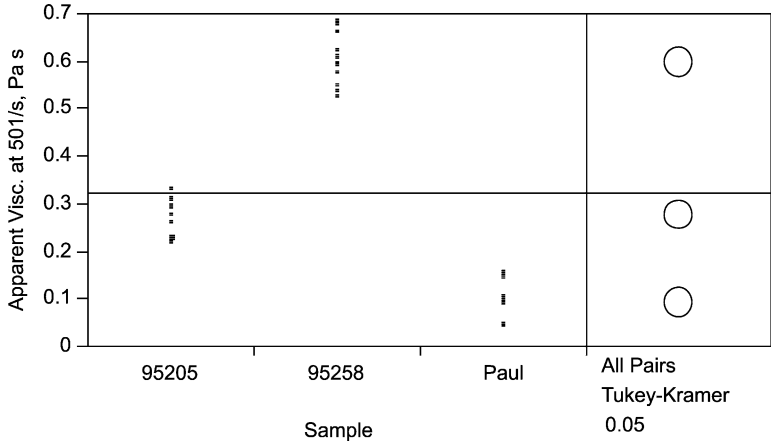

Fig. 3. Effect of extraction on apparent viscosity (at 50 s^{-1}) analyzed by one-way ANOVA for 1% (w/w) β-glucan solutions, by oat sample. Results are means from four replicate extractions with three replicate rheological measurements for each sample.

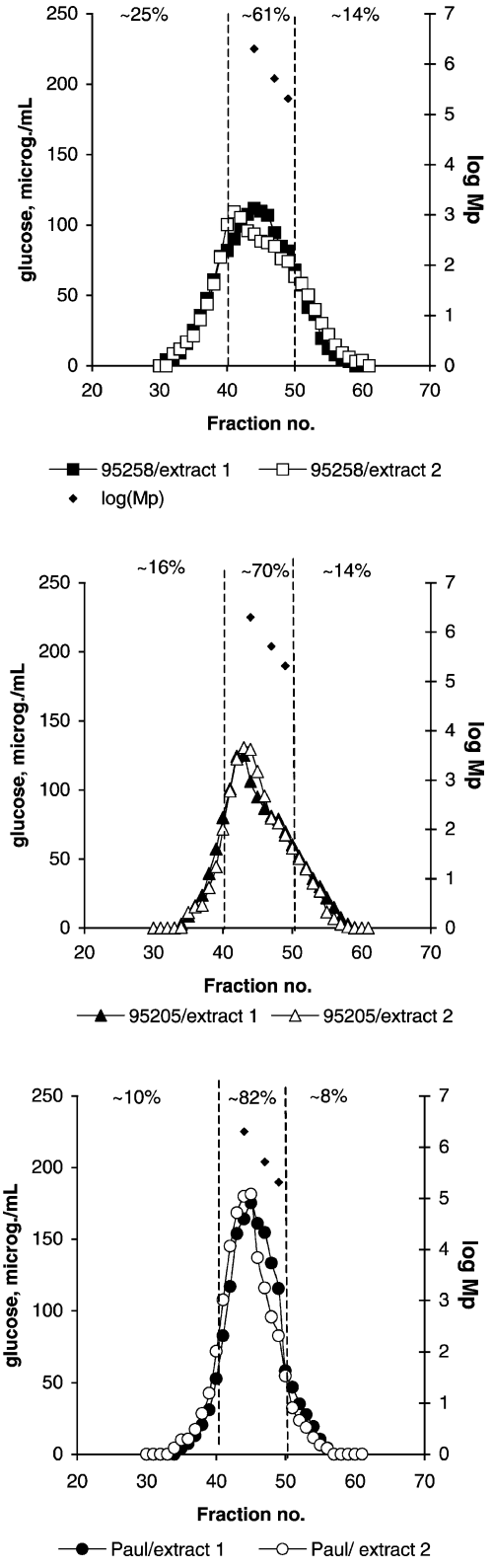

Fig. 4. Size exclusion chromatography showing the polymer distribution of β-glucans prepared in two separate extractions from IA95258, IA95205 and Paul oat lines. β-glucans were analyzed by total sugar (Dubois et al., 1956). The peak fractions of dextran standards with known molecular mass (2×10^6, 0.514×10^6 and 0.207×10^6) are marked (◆). Relative percentage of β-glucans eluted in different fractionation ranges (fractions 30–40, 40–50, or 50–60) are indicated on the top of each chromatogram.

relative peak width and peak asymmetry, probably due to differences in polydispersity. Relative percentages of polymers eluted within different MW regions (fractions F30–40, F40–50, and F50–60) were calculated for each sample and compared. The IA95258 β-glucans were eluted as a broad peak, suggesting a wide range of polymer sizes, with the greatest proportion of high-MW polymers (~25%, eluted between F30 and 40), compared to the other two samples. The lowest dispersity, suggesting the highest homogeneity in polymer distribution, was observed for the Paul β-glucans. The polysaccharides from the Paul extract were eluted in a symmetric peak and had the lowest proportion of high-MW polymers (~10%, eluted between F30 and 40) among all samples analyzed. The IA95205 β-glucans had an intermediate proportion of high-MW polymers (~16%, eluted between F30 and 40). A linear increase was observed between viscosity and the proportion of high-MW polymers eluted between F30 and 40 ($r = 0.95, p = 0.001$), a linear decrease for polymers eluted between F50 and 60 ($r = 0.935, p = 0.0016$), and a non significant correlation was noted between viscosity and proportion of polymers eluted between F50 and 60 ($r = 0.549, p = 0.0916$). Variations in the viscosity among samples seem to be related to the differences in the proportion of high-MW polymers present in β-glucans solutions, since the low-MW polymers do not have a significant effect.

A wide variation of MW ($1.5–3 \times 10^6$) ranges for oat β-glucans is reported in the literature (Autio et al. 1992; Beer, Wood, & Weisz, 1997; Wood, Weisz, & Mahn, 1991). This variation may be generated by differences in extraction methodology (water or sodium carbonate as solvents, temperatures of between 60 and 90 °C), method of MW determination (detection, standards used for calibration) and source (oat flour or bran). The Mp of β-glucans from the three oat lines reported in this paper are consistent, however, with those reported by Wood et al. (1991) (2.5×10^6 Da), Doehlert et al. (1997) (slightly less than 2×10^6 Da) and Beer et al. (1997) ($2–2.5 \times 10^6$ Da). Consistent with our studies, Beer et al. (1997) also reported only a small variation in peak molecular weight of β-glucans extracted from seven oat cultivars.

4. General conclusions

This study revealed that β-glucans purified from certain high-β-glucan oat lines were significantly more viscous than β-glucans purified from a traditional oat variety, when measured at the same concentration. The most significant differences in viscosity among oat varieties could be explained in terms of differences in molecular weight, because viscosity is highly sensitive to MW. This relationship previously was demonstrated by the value of the exponent a in the Mark-Houwink viscosity equation $[\eta] \propto \mathrm{MW}^{\alpha}$, as 0.75 for oat β-glucans (Vårum and

Smidsrød, 1988). Significant differences among certain high-β-glucan oat lines and a traditional variety also were observed in the rate of viscosity increase with concentration. The β-glucans from the traditional oat line were the least sensitive to changes in concentration, and this behavior could be explained by its low MW, which might lead to less entanglement with an increase in concentration, compared to the samples prepared from high-β-glucan oat lines.

There is great likelihood that these new high β-glucan oat lines will have enhanced physiological effects. Because cereal-based foods with high β-glucan concentrations are desirable for human consumption, plant breeders are therefore interested in creating oat cultivars with high levels of β-glucan (Cervantes-Martinez et al. 2001). This study shows that the analysis of β-glucan content, alone, is not enough to make selections in the breeding of new oat cultivars for great viscosity and, therefore, enhanced hypocholesterolemic effects. Measurement of rheological properties might be an important criterion for selection of new oat cultivars. The evaluation of additional oat cultivars for their MW and rheological properties, would assist with these conclusions. The effect of other factors in the rheological behavior of β-glucans from different oat lines, such as structure, conformation, and intermolecular interactions with arabinoxylans, are under investigation.

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