Molecular Characterization of Cereal β-Glucans. II. Size-Exclusion Chromatography for Comparison of Molecular Weight[1]

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ABSTRACT

Cereal (1→3),(1→4)-β-D-glucan (β-glucan) and pullulan standards were fractionated by size-exclusion chromatography (SEC) using a high-performance liquid chromatography gel column (TSK-60). The β-glucans were used to calibrate the column after their molecular weight was determined by SEC with low-angle laser-light-scattering detection. Using pH 10 sodium carbonate buffer at 60°C, β-glucan was extracted from the groats and brans of four cultivars of oats, four barley cultivars, four malts, four wheats, and one rye sample. Some commercial products were also analyzed. The increase in fluorescence intensity of Calcofluor in the presence of β-glucan was used for peak detection, allowing direct chromatography of the crude extracts. β-Glucans from the oat cultivars and brans had the highest molecular weights (3.00×10^6), and commercially milled samples were similar (2.70×10^6), followed by barley (2.14×10^6), malts (1.22×10^6), and rye (1.13×10^6). Wheat β-glucan was not sufficiently solubilized for detection. β-Glucan in ready-to-eat breakfast cereals ranged in molecular weight from 0.60 to 2.93×10^6. The method was used to monitor changes in molecular weight of β-glucan through the digestive tract of the rat.

Recent years have seen a considerable increase in interest in oat products. This can be attributed to health-conscious consumers aware of clinical and animal studies indicating that consumption of oat products may lower serum cholesterol (Anderson 1986). High-serum cholesterol is a risk factor for coronary heart disease (Anderson and Tietyen-Clark 1986), and reduction of cholesterol levels decreases the risk. In addition, recommendations have been made that the general (North American) population should increase dietary fiber intake from a variety of sources (Jenkins et al 1985). There is good evidence that dietary fiber, particularly the soluble dietary fiber component, may assist in glucoregulation, be of benefit to diabetics, and lower serum cholesterol (Anderson 1980, Jenkins 1980). Oat bran, unlike wheat bran, is a good source of soluble dietary fiber. Recent studies have demonstrated that oat bran and a partially purified soluble fiber extract, oat gum, reduce postprandial glucose and insulin rise (Wood et al 1990). It has been reported that a positive correlation exists between viscosity and effectiveness of soluble fibers in reducing postprandial glucose (Jenkins et al 1978). The situation in oats is therefore contrary to that of barley, where viscosity of β-glucan may reduce the nutritional value of poultry feed and, in the brewing industry, may interfere with process efficiency (Woodward and Fincher 1983).

The major component of the soluble dietary fiber of oat groats and barley is (1→3),(1→4)-β-D-glucan (Wood 1986). Cultivar variation in β-glucan content, extensively studied in barley, is less well documented in oats. This information is needed, but since the main nutritional emphasis at present is on oat bran, it is equally important to know the capacity of any cultivar to produce an adequate enrichment of β-glucan in the bran. Furthermore, total β-glucan may not be the most important factor in determining viscosity characteristics, since solubility, molecular weight, and structure will have an effect. There is a need for rapid methods of assessment of products as a whole rather than of isolated fractions whose properties may be different from intact or unfractionated material (Wilkie 1979). In this study, dye-binding of Calcofluor to (1→3),(1→4)-β-D-glucan (Wood 1980a, Jørgensen 1988) was used for peak detection in size-exclusion chromatography, allowing comparison of molecular weights of β-glucans from different sources without prior purification. The principle of using Calcofluor for peak detection has previously been applied to analysis of wort β-glucans by gel chromatography (Foldager and Jørgensen 1984).

MATERIALS AND METHODS

Oats were obtained from V. Burrows, and barley and wheat from D. Flynn, of the Plant Research Centre, Agriculture Canada. Rye (cv. Musketeer) was from J. G. McCleod, Swift Current Research Station, Agriculture Canada, and malt was provided by W. Pitz, Canada Malting, Toronto. Breakfast cereals were purchased in a supermarket. Samples were normally milled with a coffee grinder to pass 30-mesh (U.S. Standard) (600-μm) screen. Oat flour and bran, however, were prepared by P. Fedec (POS Pilot Plant, Saskatoon, SK) as follows. Dehulled or hulless (Tibor and no. 03669) oats were milled at setting "0" in an AB Falling Number mill (model KT-30, AB Falling Number, Stockholm, Sweden). A portion of the flours obtained (10 g) was fractionated into coarse (bran) and fine fractions in an ATM sonic sifter (model L-3P, ATM Corp., Sonic Sifter Div., Milwaukee, WI) fitted with a 40-mesh (U.S. Standard) (425-μm) screen. Samples were sifted for 5 min at setting 5. Raw (i.e., not heat-treated) commercial

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oats, heat-treated groats, and rolled oats were provided by the Quaker Oats Co. Heat-treated groats receive a drying heat (104–116°C) prior to cutting and flaking, whereas rolled oats receive further moist (steam) heat treatment (Deane and Commers 1986). Other oat samples were obtained commercially. Pilot-plant oat gum was obtained as described by Wood et al (1989) and bench oat gum as described by Wood et al (1978). Purified oat β-glucan was obtained from oat gum by two precipitations with 20%, w/v, ammonium sulfate followed by two precipitations with 50%, v/v, 2-propanol (Wood et al 1991). Oat gums contain ≈80% β-glucan (McCleary and Glennie-Holmes 1985), and purified oat β-glucan contained ≈95% anhydroglucose as determined by acid hydrolysis and <0.5% starch as determined by an automated version (Wood et al 1991) of the method of Batey (1982). Barley β-glucan was obtained from Biocon USA (Lexington, KY) and lichenan was from Sigma Chemical Co. (St. Louis, MO; catalogue no. L 8378, Lot 27C-0047). Pullulans, (1→4),(1→6)-α-D-glucan (Showa Denko K.K., Japan), were obtained from Mandel Scientific, Rockwood, ON, and dextrans were from Pharmacia Canada Ltd., Dorval, PQ. Calcofluor (Calcofluor White M2R New, C.I. 40622, fluorescent brightener 28) was provided by Cyanamid Co. Bound Brook, NJ. Other chemicals were purchased commercially and were of analytical grade. Samples were dried in vacuo at 80°C for >3 hr. Statistical comparisons were made using a general linear model analysis of variance (SAS Institute).

Extraction of Flours

Aqueous ethanol (5 ml, 50%, w/v) was added to ground sample (100 mg), and the mixture was heated in a boiling water bath for 5 min. After heating, a further 5 ml of the aqueous ethanol was added, and the suspension was mixed with a vortex mixer and centrifuged (14,500 × *g*) for 10 min at room temperature. The supernatant was discarded, and a further 10 ml of the aqueous ethanol was added. The sample was recentrifuged and the supernatant again discarded. Ethanol-treated sample was then extracted with 20 ml of water or sodium carbonate buffer (pH 10 and ionic strength 0.2) at 45, 60, or 80°C for 2 hr with constant stirring. Evaporative losses were compensated for by determining weight after extraction. Samples were then centrifuged (33,000 × *g*) for 20 min, and the supernatant was decanted and stored frozen or immediately analyzed, following dilution as appropriate.

Size-Exclusion Chromatography

A Bio-Rad (Bio-Rad, Mississauga, ON) TSK-60 column (7.5 × 300 mm) and a Waters (Milford, MA) model 590 pump were used for size-exclusion chromatography (SEC). Samples were filtered (1.2 μm) before analysis. The column, fitted with a Bio-Rad TSK guard column of 75 × 7.5 mm, was maintained at 40°C and eluted with 0.05*M* sodium 2-(*N*-morpholino) ethane sulfonate (MES) buffer, pH 6.5, containing 5 m*M* sodium azide, at 0.5 or 0.6 ml/min. A Perkin-Elmer ISS 100 autosampler and injector was used with an injection volume of 50 μl, with detection by Calcofluor (0.01%, w/v) in 0.1*M* tris(hydroxymethyl)aminomethane (Tris) adjusted to pH 8.0 with HCl. The Calcofluor solution, protected from light, was introduced into the postcolumn stream through a T-junction at 0.5 ml/min, pumped by a Waters model 510 pump fitted with a 50-ft × 0.007-in. pulse-damping coil. Mixing was effected in a 10 ft × 0.01 in coil prior to measurement of fluorescence intensity in a Perkin-Elmer 6505 Spectrofluorimeter, set at sensitivity range 3 and normal gain. Excitation and emission were at 360 and 425 nm, respectively. Tubing containing Calcofluor was opaque. For refractive index (RI) detection, a Waters R401 detector was used with the same postcolumn tubing as required for fluorescence detection. The system was controlled and the data processed by a Perkin-Elmer 7500 computer. However, for retention time of peaks where instrument noise or peak asymmetry was clearly a problem, peak retention time was obtained from the midpoint of the peak at half height rather than as determined by the computer. Quantitation was by determination of the area under the peak using purified oat β-glucan in the range 0.25 to 1.0 mg/ml as a standard.

SEC Low-Angle Laser-Light-Scattering Chromatography

SEC with low-angle laser-light-scattering (LALLS) chromatography of duplicate solutions of β-glucan standards was on a TSK 5000pw column with a TSK 6pw guard. A flow rate of 1.0 ml/min of 0.05*M* MES/5m*M* azide buffer (pH 6.5) was used to elute samples through an in-line 0.45-μm filter. An LDC/Analytical KMX-6 LALLS Photometer and RI-IV differential refractometer were used. The dn/dc of barley β-glucan was determined to be 0.132 ml/g with an LDC/Analytical KMX-16 differential refractometer.

Comparison of Viscosity Changes with SEC Retention Volume

Duplicate samples (1 g) of oat bran were extracted with 20 ml of sodium carbonate buffer (pH 10) at 22.5°C for 1 hr, centrifuged (33,000 × *g*) 1 hr, and the viscosity of the supernatant, maintained at 37°C, was monitored at intervals in a Cannon-Manning semi-micro capillary viscometer, viscosity constant ≈0.1. At the same time as viscosity was measured, aliquots were removed, diluted with sodium carbonate buffer, and stored frozen until SEC analysis.

Analysis of Rat Digestive Tract Contents

Rats were fed ad libitum a diet containing 7% β-glucan for 15 days as described by Bégin et al (1989), then killed by decapitation; digestive tract contents were washed out with water (stomach) or 0.9% NaCl and freeze-dried. The intestinal section from the duodenum to the ileum was divided into three equal parts and labeled a, b, and c. The freeze-dried material was extracted with refluxing 75% ethanol, rinsed with 75% ethanol, 95% ethanol, and then air-dried. Samples were extracted as usual with pH 10 sodium carbonate buffer at 60°C and the supernatants were analyzed by SEC.

RESULTS

Chromatography System

SEC of β-glucan standards and an oat extract are shown in Figure 1. Signal noise sometimes caused error in peak sensing and recording of retention time by the computer, but this was easily detected and corrected.

Standards for Chromatography

The retention time (16.64 ± 0.09 min) of oat β-glucan dissolved at 80°C in 0.05*M* MES buffer (pH 6.5) was the same as that (16.65 ± 0.09 min) for a sample dissolved at 80°C in water to which four volumes of carbonate buffer (pH 10, ionic strength

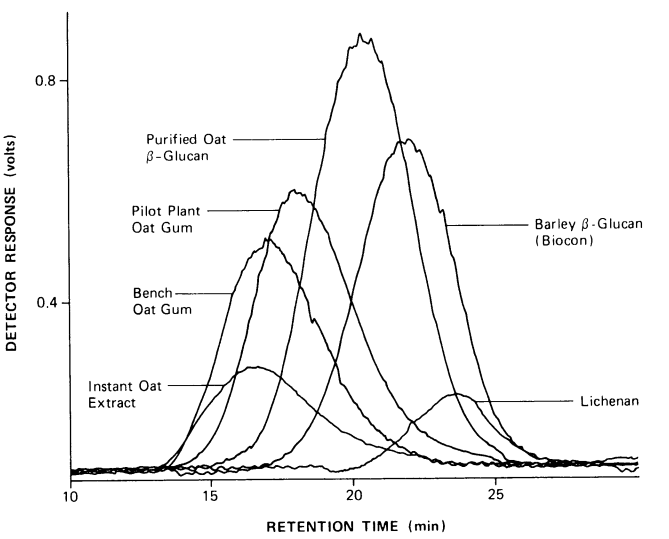

Fig. 1. Size-exclusion chromatography using a TSK-60 column of lichenan, barley β-glucan, oat β-glucan, pilot-plant oat gum, bench oat gum, and carbonate buffer (pH 10, 60°C) extract from instant oatmeal: flow rate 0.5 ml/min.

0.2) were added prior to chromatography (0.6 ml/min). When the sample was dissolved directly in the carbonate buffer at 80°C, retention time increased to 17.15 ± 0.10 min. At lower temperatures (45 and 60°C), solution in the carbonate buffer had no effect on the retention time. Routinely, standards were dissolved by heating (70–80°C) and stirring in 0.05*M* MES buffer, pH 6.5, containing 5 m*M* sodium azide. Pullulan standards, however, were dissolved according to manufacturers' instructions.

Retention volumes (RV) and molecular weights (MW) of β-glucan and pullulan standards are shown in Table I. The β-glucan standards detected by Calcofluor showed highly reproducible retention volumes, which were the same as those observed with RI detection. RI peak detection was somewhat less reproducible, and this was reflected in the standard deviation of the retention volumes of the pullulan standards. The data in Table I were obtained with the system in continuous operation. Greater changes in retention volume may occur when restarting the system after a shutdown, making the use of a standard essential for valid comparisons between such runs. Retention volumes were unchanged on varying the flow rate from 0.5 to 0.6 ml/min.

A plot of log [MW] against retention volume is shown in Figure 2 for the β-glucans and for pullulan and dextran standards. For pullulan and dextran, the manufacturers' MW values (M_w, weight average MW) were used. For the β-glucans, the MW determined by LALLS at the refractive index peak was used. Constants for the regression equation

$$\log [MW] = a + b(RV)$$

are shown in Table II. Data from the LALLS calculated M_w and M_n (number average MW) for the cereal β-glucans are included.

The slope difference and log nature of the plot would mean significant overestimates of the molecular weight of β-glucan extracts if the pullulan or dextran calibration were used. Dextrans were similar to the pullulans. Lichenan, of much lower MW than the cereal β-glucan standards, was not included in calibration since it showed departure from the straight line.

A linear relationship existed between the area under the curve and β-glucan concentration for purified oat β-glucan in the range 0.25–1.0 mg/ml. This is an imprecise quantitation but allows an approximate estimate of β-glucan. As β-glucan concentration was increased, saturation dye-binding was approached, and the incremental increase in fluorescence intensity approached zero, leading to a flattening of peak and nonlinear response. This, and broad peaks and signal noise at low concentrations, may lead to difficulties in estimation of retention volumes, which were 10.24, 10.14, 10.11, and 10.05 ml at 1.0, 0.75, 0.5, and 0.25 mg/ml, respectively. Measurements were normally done in this concentration range.

β-Glucan Extraction from Oat Bran by TSK-60 Chromatography with Fluorescence Detection

The effect of conditions of extraction on the yield of solubilized β-glucan is shown in Table III. The amount of β-glucan remaining in the residue and the total β-glucan content of the bran were

TABLE I
Retention Volumes (RV) and Molecular Weights[a] (MW) of β-Glucan and Pullulan Standards

Standard	RV (ml) RI[b]	RV (ml) Calcofluor	MW × 10^{-3}
Pullulans[c]			
P-5	12.99 ± 0.16[d]	NA[e]	5.8
P-10	12.74 ± 0.16	NA	12.2
P-20	12.45 ± 0.16	NA	23.7
P-50	12.05 ± 0.16	NA	48.0
P-100	11.58 ± 0.16	NA	100.0
P-200	11.12 ± 0.15	NA	186.0
P-400	10.63 ± 0.17	NA	380.0
P-800	10.26 ± 0.23	NA	853.0
β-Glucans			
Bench oat gum[f]	ND[h]	8.50 ± 0.05	2,188.0
Pilot-plant oat gum[g]	9.18 ± 0.10	9.09 ± 0.02	1,175.0
Purified oat β-glucan[f]	10.20 ± 0.18	10.13 ± 0.03	363.0
Barley β-glucan[f]	10.98 ± 0.13	11.00 ± 0.02	195.0
Lichenan[g]	ND	11.84 ± 0.02	30.9

[a] Molecular weights of pullulans, as quoted by supplier; molecular weights of β-glucans, as determined for size-exclusion chromatographic peak (RI detection) using low-angle laser-light-scattering chromatography.
[b] Refractive index.
[c] $n = 5$.
[d] ± Standard deviation.
[e] Not applicable.
[f] $n = 14$.
[g] $n = 7$.
[h] Not done.

TABLE II
Linear Regression Constants from Plots of Log (MW) Against Retention Volume for Pullulan (M_w) and β-Glucans (M_w, M_n, and RI peak)[a]

Molecular Weight Type and Standard	Regression Constants Correlation (*r*)	Intercept (*a*)	Slope (*b*)
Pullulan M_w	−0.997[b]	12.83	−0.68
β-Glucan M_w	−0.983	9.54	−0.38
β-Glucan M_n	−0.993	10.66	−0.52
β-Glucan RI peak	−0.995	10.21	−0.45

[a] M_w = Weight average molecular weight; M_n = number average molecular weight; RI = refractive index.
[b] P-50 to P-800 only.

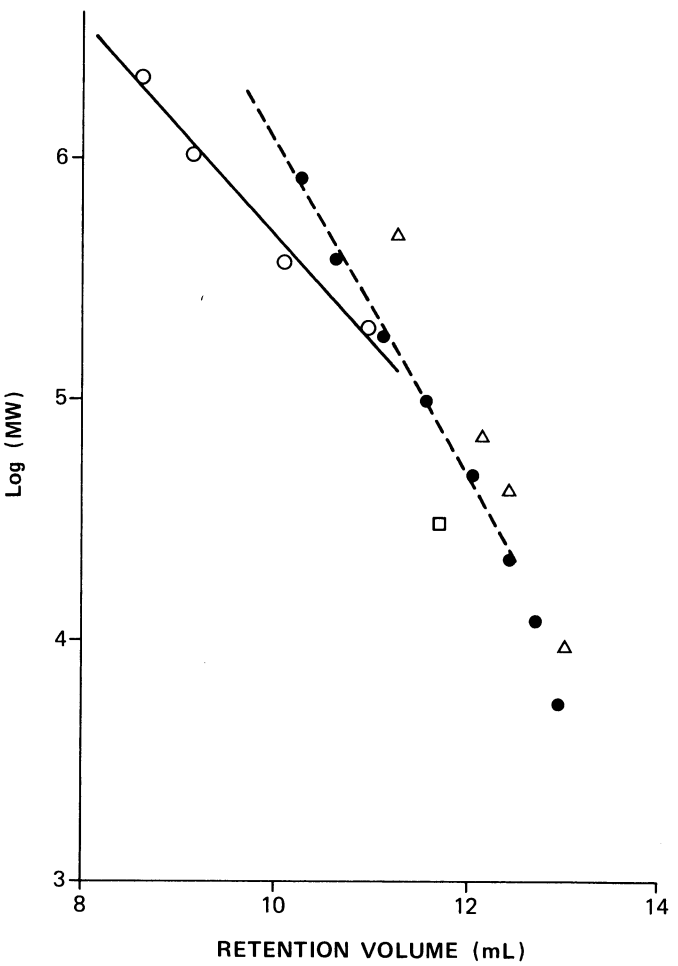

Fig. 2. Plot of log molecular weight (MW) against retention volume for cereal β-glucans (○), lichenan (□), pullulans (●) and dextrans (△).

determined by the method of McCleary and Glennie-Holmes (1985) as a check on the validity of the chromatographic measurement. Totals of extract plus residue (14.4 ± 0.7 %) were in good agreement with the total found in the original bran (15.5 ± 0.4%).

Effect of Extraction Conditions on SEC

The effects of conditions of extraction on peak retention volume, and on retention volume relative to purified oat β-glucan, of extracts from oats, oat bran, and barley are shown in Table IV. For oats, somewhat lower retention volumes were observed in water extracts compared with carbonate at the same temperature. However, the peak of water extracts was skewed, making peak retention volume more difficult to estimate and less reproducible than for the carbonate extracts. For barley, the difference between water and carbonate was reversed or not observed. Retention volumes of fractions extracted into 80°C carbonate buffer increased relative to 45 and 60°C. In general, the optimum condition for best reproducibility, maximum extraction yield, and highest MW was 60°C in carbonate buffer, and this condition was chosen for further study.

TABLE III
Effect of Temperature and Extractant on Solubilization of β-Glucan of Oat Bran,[a] as Determined by Fluorescence Peak Area

Extraction Condition	β-Glucan Extracted[b] Percent of Total	β-Glucan Extracted[b] Percent of Bran	β-Glucan[c] in Residue (% of bran)	Total, Extract + Residue[d] (% of bran)
Water				
45°C	33	4.54	9.43	13.98
60°C	37	5.03	8.85	13.89
80°C	46	6.63	7.95	14.57
Carbonate buffer				
45°C	44	6.46	8.21	14.67
60°C	73	11.02	4.16	15.18
80°C	88	12.11	1.90	14.01

[a] Bran was prepared in the POS Pilot Plant by a combination of dry sieving and sieving in aqueous ethanol (Wood et al 1989). Data are the average of duplicates, dry weight basis.
[b] Determined from the area under the curve of sample eluted from TSK-60 and detected by Calcofluor fluorescence.
[c] Determined by the method of McCleary and Glennie-Holmes (1985).
[d] Total β-glucan, determined by method of McCleary and Glennie-Holmes (1985), was 15.5 ± 0.4%.

Effect of Heat Treatments on Oat Extracts

The effect of commercial heat treatments and processing on the molecular weight of extracts was studied (Table V). Dehulled raw oats as received for processing, heat-treated groats, and rolled oats were extracted without the usual pretreatment with hot aqueous ethanol. More β-glucan was released from oats that had not been heat-treated, but the product was of lower MW than heat-treated samples. Furthermore, the peak MW of the untreated sample decreased further with storage at room temperature. Decrease in MW with room temperature storage was also apparent, but was less in the heat-treated samples. Frozen storage protected these samples against depolymerization. Ethanol pretreatment of the samples stabilized the carbonate extracts, allowing room temperature storage in the carbonate buffer during one day or overnight, but over four days a small increase (≈0.2 ml) in retention volume was noted. Thus samples not analyzed within 24 hr should be stored frozen until ready for analysis.

Comparison of Alternate Pretreatment or Extraction

Increase in the time of heat treatment with 50% ethanol to a total of 4 hr under reflux did not increase the MW of extract (Table VI). Extraction with carbonate containing sodium borohydride did not protect against possible depolymerization by alkaline degradation, but rather resulted in a decrease in the peak MW. Extracts were stable over 24 hr with each treatment.

Determination of Peak Molecular Weight of Extracts from Varied Sources

The milling of four oat cultivars gave bran yields of ~50% and an average β-glucan enrichment between groat and bran of 1.6, determined by the method of McCleary and Glennie-Holmes (1985). These brans are not therefore highly enriched in the outer layers of the kernel but are similar to commercial brans. The

TABLE IV
Effect of Solvent and Temperature of Extraction on Extract Peak Retention Volume for Oat and Barley Cultivars and Oat Bran (average of duplicate extractions)

	Water						Carbonate (pH 10)					
	45°C		60°C		80°C		45°C		60°C		80°C	
Sample	RV[a]	RRV[b]	RV	RRV	RV	RRV	RV	RRV	RV	RRV	RV	RRV
Oats												
Donald	8.24	0.79	7.97	0.76	8.09	0.77	8.49	0.81	8.50	0.81	8.94	0.86
Marion	8.24	0.79	8.08	0.77	8.16	0.78	8.41	0.81	8.42	0.81	8.87	0.85
Bran	8.22	0.81	8.22	0.81	8.34	0.82	8.10	0.80	8.28	0.82	8.76	0.86
Barley												
Bruce	8.81	0.84	8.72	0.83	8.84	0.85	8.64	0.83	8.64	0.83	9.18	0.88
Birka	9.32	0.89	9.29	0.89	9.33	0.89	8.71	0.83	8.73	0.84	9.49	0.91

[a] Retention volume (ml).
[b] Retention volume relative to oat β-glucan standard.

TABLE V
Peak Retention Volumes of β-Glucan Extracted by Carbonate Buffer at pH 10 and 60°C from Commercial Oats and Effect of Pretreatment with Hot Aqueous Ethanol (average of triplicate extractions ± SD)

	β-Glucan Extracted (% of groat)		Retention Volume (ml)				
				No EtOH Treatment			
Sample	EtOH Treated[a]	No EtOH Treatment	EtOH Treated[a]	Immediate	4.5 hr[b]	24 hr[b]	Frozen[c]
Raw oats	2.38 ± 0.08	4.09 ± 0.34	8.48 ± 0.02	10.73 ± 0	11.03 ± 0.04	11.57 ± 0.04	10.99 ± 0.07
Heat-treated oats[d]	2.61 ± 0.27	2.66 ± 0.61	8.56 ± 0.03	8.41 ± 0.09	8.48 ± 0.07	8.75 ± 0.02	8.43 ± 0.48
Rolled oats	2.41 ± 0.13	3.01 ± 0.42	8.56 ± 0.02	8.47 ± 0.03	8.52 ± 0.03	8.75 ± 0.23	8.48 ± 0.23

[a] 50% EtOH, 5 min in boiling water bath.
[b] Held at room temperature 4.5 or 24 hr.
[c] Frozen until analysis.
[d] Groats were heated (104–116°C) to reduce moisture content.

β-glucan contents and the peak MW of extracts obtained at 60°C in carbonate buffer are shown in Table VII. There was no statistically significant difference in the MW of extracts from whole groat or bran or between cultivars. The average peak MW of the groat extracts was 2.95 ± 0.06 × 10^6, and that of the brans was 3.05 ± 11 × 10^6. On average, 72 ± 4% of the groat β-glucan and 73 ± 6% of the bran β-glucan were extracted by the buffer.

On average, 47 ± 8% of the total barley β-glucan was extracted (Table VIII). Insufficient β-glucan was solubilized from wheat to assess peak MW. The average peak MW of the four barley cultivars, 2.14 ± 0.43 × 10^6, was significantly lower than that of the four oat cultivars ($P < 0.01$) and showed a greater variation between cultivars. The average molecular weight of the malts, 1.22 ± 0.23 × 10^6, was in turn significantly lower than that of barley ($P < 0.01$). Malts 1 and 2 had higher MWs (mean, 1.41 × 106) and more solubilized β-glucan than the reportedly (W. Pitz, *personal communication*) more modified malts 3 and 4 (mean MW, 1.03 × 10^6). Extracts from commercially milled oats had MW values (2.70 ± 0.35 × 10^6) similar to oat cultivars, and the one rye sample examined showed the lowest MW for an unmodified cereal of 1.13 × 10^6.

Analysis of breakfast cereals was done using a Bio-Rad TSK-60XL column instead of a TSK-60 column but otherwise under identical conditions. Peak MW of extracts ranged from 0.60 × 10^6, which is lower than malt, to 2.93 × 10^6, which is more typical of oats (Table VIII).

Relationship of Differences in Peak Molecular Weights to Viscosity

Changes with time in retention volumes of extracts from oat bran are compared with viscosity changes in the same extract in Table IX. Functionally significant changes in viscosity resulted from relatively small changes in peak MW. Whereas viscosity in these extracts (β-glucan concentration 0.15%, w/v) dropped by 70%, the loss in MW was only 23%.

Analysis of Rat Digestive Tract Contents

β-Glucan that was insoluble in ethanol (75%, w/v) was detected throughout the digestive tract of the rat, and some remained detectable in the feces. The identity of this material was confirmed by enzyme assay (McCleary and Glennie-Holmes 1985). The retention volume of extracted β-glucan increased significantly upon consumption, remained similar through the small intestine, then increased somewhat again on reaching the cecum (Table X).

TABLE VI
Effect of Aqueous Alcohol Pretreatments and Extraction Treatment on Retention Volumes of β-Glucan Extracted from Oat Bran[a] (average of triplicate extractions ± SD)

Day of Measurement	Retention Volume (ml)			
	1 × 2 hr Reflux in 50% EtOH	2 × 2 hr Reflux in 50% EtOH	5 min Heat in 50% EtOH[b]	$NaBH_4$[c] Extraction
1	8.46 ± 0.11	8.36 ± 0.13	8.38 ± 0.07	9.05 ± 0.08
2	8.46 ± 0.11	8.38 ± 0.05	8.41 ± 0.10	9.11 ± 0.12

[a] All samples were extracted with sodium carbonate buffer at pH 10, ionic strength 0.2.
[b] Neutralized with HCl following extraction.
[c] 0.1 *M* $NaBH_4$ incorporated into sodium carbonate buffer.

DISCUSSION

SEC of cereal β-glucans on TSK-60 columns was reported previously (Wood et al 1989). Postcolumn automated chemistry (orcinol/sulfuric acid reaction) was used for peak detection. Although more sensitive and selective than refractive index detection, other carbohydrates interfere in this reaction, particularly pentosan, which has a 7-fold higher response than glucan. Use of 70% sulfuric acid in this chemistry makes it cumbersome and somewhat hazardous.

It has been shown that the binding of the fluorescent dye Calcofluor, disodium 4,4′-bis{4-anilino-6-[bis(2-hydroxyethyl)-amino]-1,3,5-triazin-2-yl}amino-2,2′-stilbenedisulfonate, is specific for (1→3),(1→4)-β-D-glucans in cereal extracts (Wood et al 1983) and results in large increases in the fluorescent intensity of the dye. The dye was designed for cellulose, but this presents no problem of interference in solution studies. The reactions of substituted celluloses (e.g., O-[hydroxyethyl] celluloses and xyloglucan) (Wood 1980a, 1980b, 1982) could be studied by these techniques and in real food mixtures might interfere. Proteins with hydrophobic regions might also interfere (Takenaka and Shibata 1969), but in our experience (with casein, *unpublished*) the response to such protein is weak.

The difference in molecular weight between pilot-plant oat gum and bench oat gum was documented (Wood et al 1989), and possible explanations were presented. Viscosity determinations (Wood et al 1990) showed that at 1%, w/v β-glucan basis), the bench oat gum had an apparent viscosity, at a shear rate of 30 sec^{-1}, of 2.4 times the pilot-plant gum and a calculated low shear (1 sec^{-1}) apparent viscosity of 10 times the pilot-plant oat gum. This order of magnitude of difference in low shear viscosity between bench and pilot-plant oat gum arises from a MW loss in the pilot plant of 50%. Although viscosity is clearly a more sensitive indicator of small changes in molecular size than SEC, reliable interpretation of viscosity differences between extracts requires knowledge of concentration and MW. SEC with Calcofluor detection allows approximate estimation of both of these. Relatively minor changes in concentration or MW can profoundly affect viscosity. The considerable effect on viscosity of a relatively small amount of depolymerization is demonstrated by the data in Table IX.

There is no explanation at present for the large decrease in peak MW that occurs during purification of oat β-glucan. A similar phenomenon occurs with barley β-glucan. Material isolated (from cv. Minerva; provided by K. Jørgensen, Carlsberg Research Laboratories) by alkaline (pH 10) extraction and 2-propanol precipitation had a retention volume of 8.63 ml, compared with 11.67 ml for material extracted by water at 45°C and precipitated by ammonium sulfate. This difference in RV

TABLE VII
Peak Molecular Weight and Amount of β-Glucan Extracted from Groats and Brans from Four Oat Cultivars[a] (average of triplicates ± SD)

Cultivar	Yield of Bran[b] (% of groat)	β-Glucan Extracted[c] (%)		β-Glucan[d] (% of total)		Molecular Weight of Extract × 10^{-6}	
		Groat	Bran	Groat	Bran	Groat	Bran
Donald	52	3.4[e]	5.48 ± 0.56	72	71	2.89 ± 0.15	2.89 ± 0.36
Marion	56	4.32 ± 0.01	5.74 ± 0.15	69	67	2.95 ± 0.19	3.14 ± 0.23
Tibor	39	3.23 ± 0.15	5.58 ± 0.06	78	82	2.95 ± 0.39	3.11 ± 0.33
No. 03669	50	3.26 ± 0.30	5.55 ± 0.18	70	73	3.03 ± 0.14	3.06 ± 0.35

[a] All samples were extracted with sodium carbonate buffer, pH 10, 60°C, 2 hr.
[b] As-is basis.
[c] Amount extracted, determined from area under curve, as a percentage of groat or bran (dry weight basis).
[d] β-Glucan extracted expressed as percentage of total determined by method of McCleary and Glennie-Holmes (1985).
[e] Single determination.

TABLE VIII
Peak Molecular Weight and Amount of β-Glucan Extracted[a] from Various Sources (average of triplicates ± SD)

Sample	β-Glucan Extracted[b] (%)	β-Glucan[c] (% of total)	Molecular Weight of Extract ($\times 10^{-6}$)
Barley			
Bruce	2.81 ± 0.14	54	2.66 ± 0.14
Rodeo	2.44 ± 0.29	53	1.90 ± 0.06
Birka	1.79 ± 0.18	38	2.32 ± 0.28
Mingo	2.01 ± 0.14	43	1.70 ± 0
Malt			
1	1.71 ± 0.06	ND[d]	1.34 ± 0.07
2	0.63 ± 0.07	ND	1.48 ± 0.12
3	0.56 ± 0.09	ND	1.09 ± 0.03
4	0.32 ± 0.04	ND	0.97 ± 0.05
Rye	1.50 ± 0.49	86	1.13 ± 0.03
Commercial oats			
Instant oats	2.59 ± 0.70	58	2.52 ± 0.22
Regular rolled oats	2.82 ± 0.93	67	2.54 ± 0.19
Oat bran 1	7.36 ± 0.30	85	2.96 ± 0.04
Oat bran 2	6.30 ± 0.10	72	2.80 ± 0.04
Ready-to-eat cereals			
Balance Oat Bran (Nabisco)	2.48 ± 0.01	78	1.05 ± 0
Common Sense Oat Bran (Kellogg)	2.38 ± 0.13	83	1.44 ± 0.08
Crackling Oat Bran (Kellogg)	1.74 ± 0.07	62	2.93 ± 0
Oat Bran (Quaker)	4.20 ± 0.07	73	2.93 ± 0
Oh!s (Quaker)	0.32 ± 0.03	44	0.60 ± 0.05
Multigrain Flakes (Nature Path)	0.87 ± 0.04	71	2.05 ± 0.10

[a] All samples were extracted with sodium carbonate buffer, pH 10, 60°C, 2 hr.
[b] Amount extracted, determined from area under the curve, as a percentage of flour (dry weight basis).
[c] β-Glucan extracted expressed as percentage of total determined by method of McCleary and Glennie-Holmes (1985).
[d] Not determined.

TABLE IX
Viscosity and Retention Volume Changes in Oat Bran Extracts (average of duplicates)

Time (hr)	Viscosity (cS)[a]	Retention Volume (ml)
0	32.2	8.51
1	28.5	8.57
4	18.4	8.62
22	9.9	8.77

[a] Centistokes.

TABLE X
Retention Volume of β-Glucan in Digestive Tract of Rats Fed a Diet Containing 7% Oat β-Glucan (average ± SD, $n = 4$)

Sample	Retention Volume (ml)
Diet	9.28 ± 0.06
Stomach	11.44 ± 0.03
Intestine[a]	11.51 ± 0.23
Cecum	12.31 ± 0.03
Feces	12.38 ± 0.07

[a] Data shown for ileum, section c; sections a and b showed similar retention volumes.

represents a 20-fold difference in MW. Forrest and Wainwright (1977) reported that ammonium sulfate appears to preferentially precipitate lower-MW barley β-glucan, and this has since been reported for oat (Vårum and Smidsrød 1988). Whatever the basis, the availability of this material, purified barley β-glucan, and bench and pilot-plant oat gums provided four standards with a suitable MW range for column calibration. Using pullulan calibration, the MWs of the cereal β-glucans appear much larger. The pullulan standards have a low polydispersity (M_w/M_n 1.1; manufacturer's data obtained by sedimentation analysis), whereas the cereal β-glucan standards were more polydisperse (M_w/M_n: oat gums, 1.1 and 1.2; oat β-glucan, 1.6; barley β-glucan, 2.1). The β-glucans and lichenan precipitated with ammonium sulfate showed evidence of small amounts of high-MW aggregates. This does not, however, appear to be the basis for the calibration difference between pullulan (or dextran) and β-glucans (Table II). These data indicate that defined β-glucan standards should be used for MW determinations. Comparisons, however, can be based on retention volumes, but a standard should be included, and data reported as relative retention volume (Table IV) if significant changes in the standard are observed. It should be recognized that small changes in retention volume, because of the logarithmic relationship used, can represent large apparent differences in MW. For example, a difference of 0.1 ml in retention volume, which may occur between replicate extracts, could represent a difference in MW of 200,000.

Although the difference in MW between barley and malt presumably reflects the process of malting, the difference between oats and barley, although statistically significant, may reflect differences in extract yield and requires further survey for confirmation.

The MW differences in ready-to-eat breakfast cereals may arise in part from differences in starting material, but some effect of process (e.g., extrusion cooking) and ingredient use (e.g., malt in Oh!s) seems likely. Without access to starting material or a fuller knowledge of the effects of cultivar and environment, the basis for these marked differences cannot be assessed. The relative amounts of β-glucan in each cereal are in general agreement with ingredient labeling, but it is not our intention to make brand name comparisons that would require considerably more sampling.

Although wheat contains less β-glucan than oats or barley, amounts are similar to those in malts. The lack of peaks adequate for analysis therefore suggests a low solubility for wheat β-glucan. A negligible solubility of wheat β-glucan in water (65°C) was reported previously (Beresford and Stone 1983). In general, the order of solubility appears to be oats > barley > wheat. Caution is required in these comparisons, because the determination of β-glucan from the area under the curve is subject to large errors (e.g., instant and regular rolled oats).

The data on MW changes in the rat digestive tract illustrate the utility of the specific peak detection system. The MW loss between diet and stomach contents would not be expected in humans unless digestive system proteases were responsible, acting on possible peptide linkages (Forrest and Wainwright 1977, Vårum and Smidsrød 1988). Pepsin and chymotrypsin, however, were without effect on oat gum viscosity, and incubation of the diet in dilute acid 0.1 *N* HCl, 37°C, 5 hr) did not affect the retention volume of the β-glucan (*unpublished*).

In conclusion, suitable extraction conditions are described that allow a simple and rapid comparison by SEC of the MW of β-glucan in cereals, cereal-based food products, and contents of the gastrointestinal tract. Although considerably less sensitive than viscosity for detecting MW differences in high-MW β-glucans, the method is independent of concentration within the range 0.25–1.0 mg/ml. This and the selective detection of β-glucans make rapid assessment of crude extracts possible. The MW of the cereal β-glucans studied ranged from 1.1 to 3.1 × 10^6, based on calibration with β-glucans of known MW. Use of pullulan as a MW standard would lead to overestimation of the MW of cereal β-glucans. Future studies will be directed towards improving the chromatography system, increasing extraction yield without depolymerization, and more extensive comparisons of oats, barley, and food products.

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