

# Molecular Weight Distribution and (1→3)(1→4)-β-D-Glucan Content of Consecutive Extracts of Various Oat and Barley Cultivars<sup>1</sup>

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## ABSTRACT

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The content and molecular weight (MW) of β-glucan in extracts from a selection of oat and barley cultivars were compared using flow-injection analysis and high-performance size-exclusion chromatography. From 60 to 75% of the β-glucan was extracted from oat and waxy barley by hot water (90°C) containing heat-stable α-amylase, whereas just 50–55% was extracted from nonwaxy barley. Consecutive extractions with hot water and dimethylsulfoxide (DMSO) extracted 65% (nonwaxy barley)

or 75–80% (oat and waxy barley) of the total β-glucan. An extraction with sodium hydroxide and sodium borohydride (NaOH/NaBH<sub>4</sub>) increased the percentage of β-glucan extracted to 86–100% but decreased the MW. The MW of β-glucan in the oat cultivars selected was significantly higher than those in the barley cultivars. The β-glucan extracted from the nonwaxy barley cultivars showed significantly higher peak MW than that from the waxy barley cultivars.

(1→3)(1→4)-β-D-Glucan (β-glucan) is a cell-wall polysaccharide of cereal grains which is present in greatest amounts in oats and barley. There is good evidence that consumption of oat and barley products may lead to lower cholesterol levels (Anderson and Chen 1986, Gold and Davidson 1988, Newman et al 1989, Anderson et al 1991, Braaten et al 1994), and β-glucan has been proposed as an active factor. β-Glucan has been shown to attenuate glycemic response (Wood et al 1990, Braaten et al 1991, Wood et al 1994). Mechanisms of action in the human gastrointestinal tract remain uncertain, although attenuation of blood glucose and insulin levels is viscosity-related (Wood et al 1994) and increased luminal viscosity is believed to be of importance in lowering serum cholesterol levels (Jenkins et al 1987). Increased viscosity in the gut will be dependent, among other things, on molecular weight (MW), structure, and concentration of the β-glucan in the intestinal fluid. Solubility, or extractability, as well as total amount of β-glucan, determines the concentration and, therefore, the viscosity in the gastrointestinal tract. Several techniques to determine extractability and MW using different solvents and temperatures have been described (Anderson et al 1978, Åman et al 1989, Wood et al 1991, Beer et al 1995). However, only 30–70% of total β-glucan was extracted by mild reagents and conditions. More vigorous reagents and conditions (McCleary 1988, Bhaty 1993) have achieved apparent total extraction. In this study, the amount and MW of β-glucan extracted from selected cultivars of oats and barley by consecutive treatments with hot water followed by dimethylsulfoxide (DMSO) or sodium hydroxide (NaOH), were compared using flow-injection analysis and high performance size exclusion chromatography (HPSEC).

## MATERIALS AND METHODS

The oat cultivars were dehulled and provided by V. Burrows (Agriculture and Agri-Food Canada, Ottawa, Canada). The barley samples provided by W. Newman (Montana State University, Bozeman) were isolines of Compana developed by R.F. Eslick (Montana State University, Bozeman). Genetic patterns were: Compana (covered, long awn, nonwaxy); Nupana (hull-less, long

awn, nonwaxy); Shopana (covered, short awn, nonwaxy); Shonupana (hull-less, short awn, nonwaxy); Wapana (covered, long awn, waxy); Wanupana (hull-less, long awn, waxy); Washopana (covered, short awn, waxy); Washonupana (hull-less, short awn, waxy). Both oat (grown in 1994) and barley (grown in 1992) samples were stored frozen. Termamyl (120 L), a heat-stable α-amylase, was obtained from Novo BioLabs (Danbury, CT). Calcofluor White M2R New (fluorescent brightener 28) was provided by American Cyanamid (Bound Brook, NJ). β-Glucans of different molecular weights used as standards were provided by Megazyme Australia Pty. Ltd. (Warriewood, Australia, now Megazyme International Ireland Ltd., Bray, Ireland) or produced as described by Wood et al (1989).

## Extraction

Samples were ground in a cyclone sample mill (Udy Corp., Fort Collins, CO) to pass a 1-mm screen. Before extractions, ≈5 g of flour was blended with 100 mL of aqueous ethanol (70%) and stirred under reflux for 2 hr at 85°C to inactivate β-glucanases. The supernatant was removed after centrifugation (8,000 × g, 15 min), and the residue was washed with 20 mL of aqueous ethanol (95%) and dried on a hot plate with gentle warming. The drying step was completed by drying for 3 hr in a vacuum oven at 80°C. A schematic outline of the extraction procedure is presented in Fig. 1.

## Analytical Methods

β-Glucan content in extracts and in flour from the oat and barley samples was determined by flow-injection analysis (FIA) (Jørgensen 1988) and by the method of McCleary and Glennie-Holmes (1985) using a β-glucan assay kit (Megazyme), respectively. The final assay of glucose used an automated glucose oxidase procedure (Wood et al 1991)

HPSEC was performed using three columns (300 × 7.5 mm) in series (Polymer Laboratories PL-aquagel-OH 60, Shodex OHPak KB806, Bio-Rad TSK-20) and a Waters (Milford, MA) model 590 pump. Samples were filtered (0.45 μm) before analysis. The columns were maintained at 40°C and eluted with 0.1M NaNO<sub>3</sub> buffer at 1 mL/min. A Perkin-Elmer ISS 100 autosampler and injector was used with an injection volume of 150 μL, with detection by refractive index, viscosity (model 250, Viscotek, Houston, TX), and right-angle laser light-scattering (RALLS, Viscotek) for β-glucan standards. The system was controlled and the data processed by TRISEC V2.7 software (Viscotek) to obtain number average ( $M_n$ ), weight average ( $M_w$ ) and chromatographic peak MW ( $M_p$ ). β-Glucan standards were used to calibrate the SEC columns. Chromatographic peak MW of extracts was measured

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using Calcofluor postcolumn detection (Perkin-Elmer LS-5 Spectrofluorimeter, Waters model 510 pump) as described by Wood et al (1991).

### Statistics

Differences between means of oat, waxy, and nonwaxy barley samples were evaluated for significance by analysis of variance (ANOVA) calculated using the statistical program Statgraphics Plus 5.01. Values of  $P < 0.05$  were considered statistically significant.

## RESULTS

### MW Standards

MW of the  $\beta$ -glucan standards used to calibrate SEC columns are shown in Table I. The regression equation ( $r = 0.995$ ) used to calculate peak MW ( $M_p$ ) of  $\beta$ -glucan in extracts was:

$$\log[M_p] = 11.15 - 0.37(RV)$$

where RV is retention volume (mL). The refractive index and Calcofluor fluorescence chromatographic peaks were equivalent, as determined from off-set values of standards (Fig. 2).

### Extraction

The  $\beta$ -glucan content of oats and barley ranged from 4.7 to 8.1% (dwb) and was higher in waxy barley than nonwaxy barley and oats (Table II). In oats and waxy barley, 60–65% of the total  $\beta$ -glucan was extracted using a single extraction with water and heat-stable  $\alpha$ -amylase. Significantly lower amounts (50–55%) were extracted from nonwaxy barley (Table II). No significant effects of the hull-less or the short awn gene on extractability of  $\beta$ -glucan was seen, except a significant difference of short awn and long awn samples in amount of  $\beta$ -glucan in extract E2. Two additional consecutive hot-water treatments (E2 and E3) extracted

a further 10–15% of the total  $\beta$ -glucan from both oats and barley. DMSO treatment extracted a further 6–8% of the  $\beta$ -glucan, to give a total extraction of 76–83% in oats, 71–78% in waxy barley, and 60–65% in nonwaxy barley. Quantitative extraction was only achieved, following hot water extraction, by a final NaOH/NaBH<sub>4</sub> treatment, which increased the total amount extracted to 86–105%, but decreased the peak MW of the  $\beta$ -glucan by 20–60% (Table III). Direct treatment of ethanol-deactivated flours with NaOH/NaBH<sub>4</sub> (16 hr at room temperature) led to nearly total extraction (AC Lotta 95.78%, Compana 89.14%), but also decreased peak MW (AC Lotta  $1.272 \times 10^6$ , Compana  $1,258 \times 10^6$ ).

The peak MW's of  $\beta$ -glucan in the first two hot-water extracts (E1 and E2) were significantly higher in oats than in barley. The peak MW of  $\beta$ -glucan in the first extract (E1) was significantly lower in waxy barley as compared to nonwaxy barley. In both oat and barley, the peak MW of  $\beta$ -glucan in E1 and E2 were similar, but the peak MW of  $\beta$ -glucan in E3 was lower. The  $\beta$ -glucan extracted by DMSO was similar in peak MW to E1 and E2 in oats, whereas in barley it was higher. No significant differences between oats and barley were detected in peak MW of  $\beta$ -glucan in DMSO and NaOH extracts (Table III). The hull-less gene and the short awn gene in the barley isotypes had little effect on MW of extracted  $\beta$ -glucan. A significant difference was noted in extract E2 of long awn and short awn samples, but the small amounts extracted limit their functional significance.

## DISCUSSION

### MW Determination

Dextran and pullulan are often used as standards in SEC of polysaccharides, and MW values are reported as dextran or pullulan equivalents (Mälkki et al 1992). This can lead to significant overestimation of the MW of the  $\beta$ -glucan due to differences in hydrodynamic volumes (Vårum et al 1991). To calculate the MW

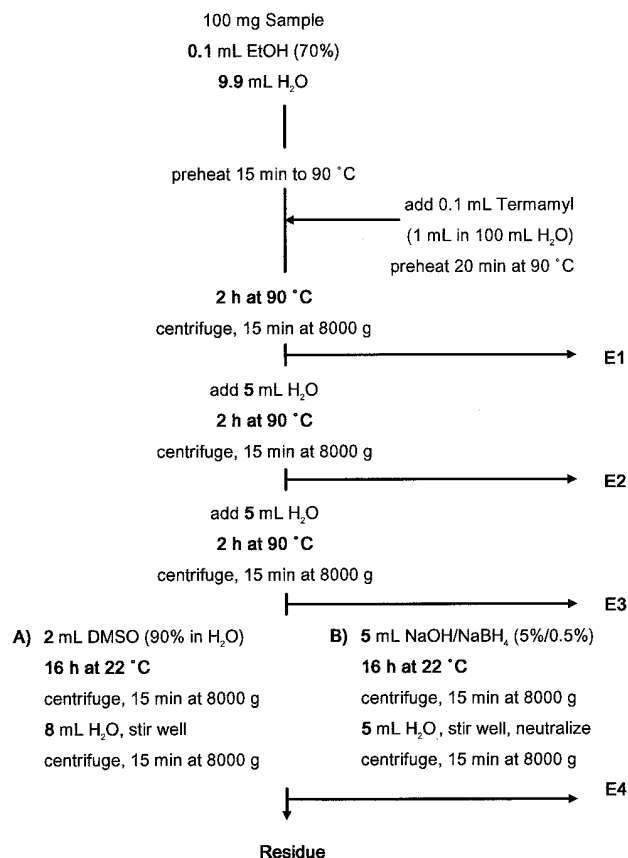


Fig. 1. Procedure for  $\beta$ -glucan extraction.

TABLE I  
Molecular Weight<sup>a</sup> of  $\beta$ -Glucan Standards

Sample <sup>b</sup>	$M_n$	$M_w$	$M_p$
Bench	665,000	1,634,000	1,420,000
POS	376,000	932,000	941,000
J271	361,000	700,000	857,000
A	68,000	106,000	72,000
B	286,000	376,000	356,000
C	155,000	215,000	191,000
D	144,000	220,000	226,000
E	137,000	229,000	261,000

<sup>a</sup> Number average ( $M_n$ ), weight average ( $M_w$ ), and chromatographic peak molecular weight ( $M_p$ ).

<sup>b</sup> Bench, POS, and J271 are oat  $\beta$ -glucan standards produced by Wood et al (1989). A, B, C, D, and E are barley  $\beta$ -glucan standards produced by Megazyme (Aust.) Pty. Ltd., Warriewood, Australia.

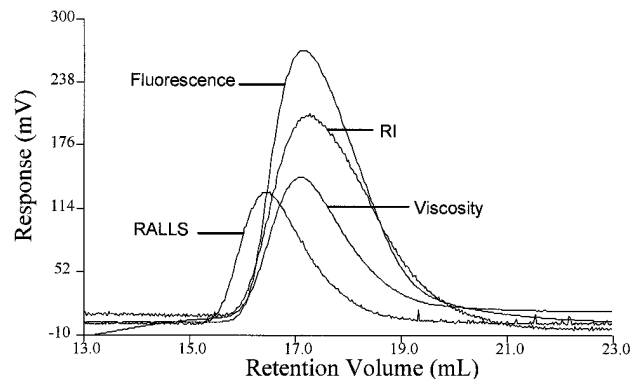


Fig. 2. High-performance size-exclusion chromatogram of an oat  $\beta$ -glucan standard (J271) using refractive index (RI), viscosity, right-angle laser light-scattering (RALLS), and fluorescence detection.

of  $\beta$ -glucan samples,  $\beta$ -glucan standards of known MW, or detectors sensitive to molecular size (MALLS or RALLS with Viscometer), are needed. However, in crude extracts of oats, the use of these nonspecific detectors to evaluate  $\beta$ -glucan is not possible because of unknown contributions from coextracted substances (starch, proteins). This problem may be overcome by addition of a fluorescence detector exploiting the specific dye-binding of Calcofluor by  $\beta$ -glucan (Wood et al 1991). Signal is proportional to  $\beta$ -glucan concentration in the concentration range used and MW range of interest in this study. In our system, problems with decreasing response at lower MW were not encountered, but clearly there is a MW at which declining response will occur (Jørgensen 1988, Manzanares et al 1991)

The combination of HPSEC with an in-line viscometer and a right-angle light-scattering detector allows measurement of MW, intrinsic viscosity, and radius of gyration, and was used to calcu-

late the MW of six  $\beta$ -glucan standards. Values found in this study are somewhat lower than reported by Wood et al (1991), reflecting a difference in calibration standards. The system has several advantages: it is rapid (a single chromatographic analysis takes 40 min) and automated, allowing analysis of a large number of samples; the values obtained are comparable with different columns and should enable interlaboratory comparisons; the system is especially useful in detecting changes during processing, cooking, or digestion (Beer et al 1996). There are some disadvantages: the equipment is expensive; the viscometric detector does not perform well with highly viscous samples (radius of gyration > 60 nm); standards presently available are of lower  $M_p$  than  $\beta$ -glucan of some crude extracts, which are therefore outside the established calibration range; at higher MW, the system is not very sensitive to MW differences, and small differences in retention volume may translate into large MW differences.

**TABLE II**  
 **$\beta$ -Glucan Content (% Total  $\beta$ -Glucan) of Consecutive Oat and Barley Extracts (E1-E4)**

Sample	$\beta$ -Glucan (g/100g)	Consecutive Extracts (% of Total Amount)						
		H <sub>2</sub> O			DMSO <sup>a</sup>	NaOH	E4 DMSO	E4 NaOH
		E1	E2	E3	E4	E4	Total	Total
<b>Oat</b>								
AC Lotta	5.89 ± 0.03	58.50 ± 2.37	10.04 ± 1.35	3.03 ± 0.07	7.17 ± 0.56	21.70 ± 1.80	78.83 ± 0.83	91.90 ± 2.90
Capitol	6.23 ± 0.13	61.66 ± 1.01	8.93 ± 0.37	2.16 ± 0.39	6.07 ± 0.35	13.85 ± 1.25	80.15 ± 0.94	85.70 ± 0.50
Rigodon	5.02 ± 0.14	66.20 ± 2.63	9.90 ± 1.81	2.75 ± 0.24	6.57 ± 0.27	14.25 ± 0.45	83.00 ± 2.75	93.65 ± 0.85
AC Steward	6.06 ± 0.16	57.54 ± 2.01	11.09 ± 1.23	3.58 ± 0.56	6.91 ± 0.09	...	76.05 ± 1.99	...
Newman	5.38 ± 0.12	63.43 ± 1.41	9.31 ± 0.74	2.96 ± 0.15	7.88 ± 0.55	...	78.68 ± 2.83	...
Marion	6.08 ± 0.04	65.81 ± 0.87	9.06 ± 0.51	2.85 ± 0.23	7.22 ± 0.13	...	81.30 ± 1.79	...
Mean	5.77 ± 0.09a <sup>b</sup>	62.18 ± 0.79a	9.72 ± 0.28a	2.89 ± 0.11a	6.96 ± 0.14a	16.60 ± 1.71a	79.67 ± 0.86c	90.42 ± 1.71a
<b>Barley (nonwaxy)</b>								
Compana	5.79 ± 0.17	50.03 ± 3.77	7.37 ± 0.27	2.97 ± 0.25	7.44 ± 0.68	33.67 ± 1.78	62.35 ± 3.95	92.05 ± 0.45
Nupana	5.38 ± 0.08	53.77 ± 2.02	8.19 ± 0.24	3.19 ± 0.52	8.29 ± 0.99	33.32 ± 2.59	65.70 ± 2.60	97.90 ± 2.90
Shopana	4.71 ± 0.22	49.13 ± 3.14	7.24 ± 0.52	2.70 ± 0.20	7.71 ± 0.11	33.75 ± 0.51	59.30 ± 2.70	92.60 ± 4.80
Shonupana	5.71 ± 0.04	50.36 ± 0.89	7.36 ± 0.31	3.04 ± 0.09	6.00 ± 0.37	36.84 ± 1.68	61.25 ± 0.05	97.10 ± 0.40
Mean	5.40 ± 0.14a	50.83 ± 0.83b	7.53 ± 0.13b	2.98 ± 0.09ab	7.36 ± 0.39a	34.39 ± 0.97b	62.15 ± 1.35a	94.91 ± 1.45ab
<b>Barley (waxy)</b>								
Wapana	6.15 ± 0.07	64.90 ± 3.66	11.16 ± 2.12	3.40 ± 0.27	6.66 ± 0.33	21.41 ± 0.34	78.65 ± 1.65	101.75 ± 1.25
Wanupana	6.69 ± 0.06	65.34 ± 4.99	8.44 ± 0.77	2.82 ± 0.26	5.27 ± 0.73	23.80 ± 0.98	71.10 ± 2.00	105.85 ± 0.55
Washopana	6.23 ± 0.06	59.96 ± 5.90	6.98 ± 1.21	3.06 ± 0.26	6.03 ± 0.12	22.71 ± 1.90	72.60 ± 6.40	90.10 ± 1.50
Washonupana	8.07 ± 0.06	59.65 ± 4.11	7.59 ± 0.31	4.07 ± 0.53	6.92 ± 0.11	23.86 ± 0.38	72.80 ± 1.20	93.65 ± 5.85
Mean	6.79 ± 0.23b	62.46 ± 1.41a	8.54 ± 0.53a	3.34 ± 0.15b	6.21 ± 0.29b	22.95 ± 0.56c	73.79 ± 1.71b	97.84 ± 2.64b

<sup>a</sup> Dimethylsulfoxide.

<sup>b</sup> Values followed by the same letter in the same column are not significantly different ( $P < 0.05$ ).

**TABLE III**  
**Peak Molecular Weight ( $M_p \times 10^{-3}$ ) of Consecutive Oat and Barley Extracts**

Sample	H <sub>2</sub> O				E4 DMSO <sup>a</sup>	E4 NaOH
	E1	E2	E3	E4		
<b>Oat</b>						
AC Lotta	2,485 ± 76	2,846 ± 91	1,029 ± 244		2,694 ± 155	1,010 ± 21
Capitol	2,510 ± 95	2,123 ± 73	647 ± 75		2,672 ± 132	1,067 ± 36
Rigodon	2,287 ± 79	2,226 ± 179	333 ± 83		1,886 ± 31	1,227 ± 20
AC Steward	2,469 ± 132	2,581 ± 267	1,398 ± 294		1,908 ± 18	...
Newman	2,269 ± 127	1,947 ± 81	645 ± 165		1,671 ± 89	...
Marion	2,094 ± 92	2,032 ± 214	528 ± 49		1,428 ± 161	...
Mean	2,346 ± 39a <sup>b</sup>	2,329 ± 82a	788 ± 87ab		1,918 ± 111a	1,102 ± 43a
<b>Barley nonwaxy</b>						
Compana	1,685 ± 42	1,425 ± 109	723 ± 248		1,919 ± 36	1,244 ± 4
Nupana	1,647 ± 32	1,465 ± 129	671 ± 203		1,919 ± 36	1,157 ± 8
Shopana	1,686 ± 56	1,673 ± 86	494 ± 91		1,937 ± 148	1,097 ± 17
Shonupana	1,643 ± 36	1,722 ± 51	428 ± 189		1,931 ± 0	1,107 ± 10
Mean	1,665 ± 12b	1,571 ± 42b	579 ± 59a		1,926 ± 30a	1,151 ± 22a
<b>Barley waxy</b>						
Wapana	1,315 ± 80	1,422 ± 106	829 ± 158		1,918 ± 36	1,151 ± 5
Wanupana	1,317 ± 40	1,680 ± 92	601 ± 159		1,968 ± 38	1,034 ± 3
Washopana	1,512 ± 85	1,794 ± 119	911 ± 66		2,310 ± 90	1,088 ± 43
Washonupana	1,398 ± 86	2,115 ± 46	1,230 ± 118		2,496 ± 0	1,124 ± 31
Mean	1,386 ± 28c	1,754 ± 69b	893 ± 67b		2,181 ± 91a	1,100 ± 20a

<sup>a</sup> Dimethylsulfoxide.

<sup>b</sup> Values followed by the same letter in the same column are not significantly different ( $P < 0.05$ ).

## Extraction

There have been several studies dealing with the extraction of  $\beta$ -glucan from oat and barley (Anderson et al 1978, Åman et al 1989, Wood et al 1991), and much variability in the results is evident, due to differences in both starting material and extraction technique. However, all studies show that total extraction of cereal  $\beta$ -glucan with mild reagents and conditions is difficult. There are as yet no fully satisfactory molecular or microstructural explanations for differences in extractability (Wood 1993). McCleary (1988) reported that  $\approx 90\%$  of the total barley  $\beta$ -glucan was extracted in successive treatments with water at 40, 65, and 95°C, whereas Wood et al (1991) found  $\approx 45\%$  soluble  $\beta$ -glucan in barley and 70% in oats using a carbonate extraction (pH 10) at 60°C. In this study,  $\approx 65\%$  of the total  $\beta$ -glucan in oats and waxy barley, and  $\approx 50\%$  in nonwaxy barley, was soluble using a single hot-water Termamyl extraction. Termamyl, like many sources of  $\alpha$ -amylase, contains  $\beta$ -glucanases, but these are less heat-stable than the  $\alpha$ -amylase. Nevertheless, inactivation of the  $\beta$ -glucanase is a kinetic process. Under the conditions used here (Fig. 1), no residual  $\beta$ -glucanase was detectable in the extraction media. Bengtsson et al (1990), using a similar hot-water Termamyl methodology, extracted 25–42% of total  $\beta$ -glucans in waxy and 29% in nonwaxy barley samples. However, extracted  $\beta$ -glucan was not directly measured in the supernatant but after ammonium sulfate and ethanol precipitation of soluble  $\beta$ -glucans. Because the total recovery of  $\beta$ -glucan (soluble and insoluble) was 70–80%, it is likely that some of the soluble  $\beta$ -glucan was lost during isolation.

Additional hot-water extracts and a final DMSO extraction increased the total soluble  $\beta$ -glucan to  $\approx 80\%$ , 20% remaining insoluble. Sodium hydroxide extraction led to nearly total extraction in both oat and barley samples, but also decreased MW. Bhatti (1993) reported that 84% of  $\beta$ -glucan in Azhul barley bran was extracted using 4% sodium hydroxide at room temperature. No difference in the MW of  $\beta$ -glucan extracted by water and sodium hydroxide extracts was reported, whereas in this study, using sodium hydroxide for total extraction of  $\beta$ -glucan appeared to be accompanied by degradation of the  $\beta$ -glucan macromolecule. The MW of the  $\beta$ -glucans extracted by Bhatti (1993) were apparently lower than those reported here, although methodologies used were different. Lack of inactivation of endogenous  $\beta$ -glucanase under relatively mild conditions led to reduced MW but increased total amount extracted (Wood et al 1991); without enzyme deactivation, evaluation of the significance of MW differences between samples is difficult. Harsher extraction conditions, such as high or low pH, can also result in depolymerization.

The peak MW of  $\beta$ -glucan extracted by hot water from oats was significantly higher than from barley, and was lowest in the waxy barley cultivars. The difference between waxy and nonwaxy barley in peak MW of  $\beta$ -glucan was only evident in the first hot water extract (E1). Waxy barleys are noted for higher extract viscosity and  $\beta$ -glucan solubility (Xue et al 1991, Yoon et al 1995). Clearly, the increased amount extracted must predominate over the reduced MW to produce the higher viscosity extracts. Previously, Wood et al (1991) found lower peak retention volumes (i.e., higher MW) in oats than in barley samples in both water and carbonate (pH 10) at different temperatures.

In the present study, no change in peak MW in consecutive extracts E1, E2, and DMSO extract was detected. The significantly lower peak MW in E3 could be due either to degradation during extraction process or to a low MW fraction of the  $\beta$ -glucan that is less readily extracted. However, the very small  $\beta$ -glucan concentrations make determination of the MW difficult. It has been suggested that increasing temperature of extraction leads to increase in MW of the extracted barley  $\beta$ -glucan (McCleary 1988). This possibility was not examined in this study. The peak MW of  $\beta$ -glucan in sodium hydroxide extracts,  $\approx 1 \times 10^6$ , was lower than  $\beta$ -glucan from water extracts. This was observed whether extraction in sodium hydroxide was direct or following hot water treatment,

so total extraction of the  $\beta$ -glucan by NaOH appears to be accompanied by degradation of the molecule. A similar relationship between extractability and MW of cereal  $\beta$ -glucan was reported by Wood et al (1991). A carbonate extraction of untreated oat samples at 60°C yielded higher amounts of extractable  $\beta$ -glucan but lower MW than a similar extraction of the ethanol-pretreated sample. This may be due to  $\beta$ -glucanases, possibly from contaminating microorganisms in the kernel, but the true basis for this, and for the lower MW of  $\beta$ -glucans from sodium hydroxide extraction, needs further investigation.

With the techniques used here, no evidence that ease of extractability was related to MW was found. The significantly different MW of  $\beta$ -glucan from oats, waxy, and nonwaxy barley did not parallel differences in ease of extraction. The true or native MW of the  $\beta$ -glucan that resists extraction in water and DMSO remains unclear and needs further investigation.

Both the lower MW and lower extractability might result in lower effectiveness of nonwaxy barley in applications where viscosity is important. Whether the differences between samples detected in this study might translate into advantages or disadvantages as food or feed will require further study. The methodologies described will be of value in such investigations.

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