

Structure of (1→3)(1→4)-β-D-Glucan in Waxy and Nonwaxy Barley

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ABSTRACT

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The endosperm cell walls of barley are composed largely of a (1→3)(1→4)-β-D-glucan commonly known simply as β-D-glucan (Wood 2001). There has been much research into the characteristics of barley β-glucan because of the influence of this polysaccharide on performance of barley in malting and subsequent brewing of beer, and in feed value, especially for young chicks (MacGregor and Fincher 1993). The potential for β-glucan to develop high viscosity is a problem in these uses, but

from the perspective of human nutrition, this characteristic may be an advantage. The glycemic response to oat β-glucan is inversely related to (log)viscosity (Wood et al 1994a) and there is evidence to suggest that the lowering of serum cholesterol levels associated with oat and barley products (Lupton et al 1994; Wood and Beer 1998) is at least in part due to the β-glucan (Braaten et al 1994) and probably also its capacity to develop viscosity in the gastrointestinal tract (Haskell et al 1992).

Factors contributing to viscosity of β-glucan have been extensively studied over the years. Waxy cultivars of barley generally are reported to have a higher total β-glucan than nonwaxy. For viscosity to develop, the β-glucan must be in solution and, consequently, extractability from intact cell walls is a key first step. Waxy barley has a higher content of β-glucan than nonwaxy and, additionally, the percentage readily extracted from waxy barley cultivars is greater (Beer et al 1997; Xue et al 1997). Assuming there is no counteracting difference in molecular size, this would lead to a higher extract viscosity for waxy cultivars as generally observed. The reason for this is not known, and may be based on either chemical or morphological structure or both. Molecular weight (MW) may also play a role in extractability (McCleary 1988) and certainly molecular size, a function of MW and structure, and concentration, control the viscosity of the resultant solution.

The major structural feature of cereal β-glucan is β-(1→3)-linked cellotriosyl and cellotetraosyl units (Parrish et al 1960), apparently randomly distributed (Staudte et al 1983). In addition there are more cellulose-like regions in the molecule with 4 to >10 successive β-(1→4)-linked D-glucopyranosyl units (Woodward et al 1983; Wood et al 1994b). These may increase the extension of the molecule in space (Buliga and Brant 1982). Increased radius of gyration, leading to increased volume occupancy of the molecule will increase viscosity (Robinson et al 1982).

In this study we compare the composition, extract viscosity, and β-glucan structure of covered and naked, long awn and short awn, and waxy and nonwaxy barley lines.

MATERIALS AND METHODS

The barleys were grown in small plots (1.2 × 30.5 m) and harvested using a small plot combine and cleaned to remove chaff and foreign material. Samples were stored in a controlled seed storage room before chemical analyses. Samples were milled (Cyclotek 1093 mill, Tecator, Sweden; or Tekmar analytical mill Tekmar, Cincinnati, OH); 1-mm screen size was used for proximate analysis and 0.5-mm screen size was used for viscosity and lichenase digestion.

Analyses were made for protein and fat (AOAC 1984) and soluble and insoluble dietary fiber (Prosky et al 1988).

Alkaline extract viscosity of the kernels was measured by the falling ball technique (Bendelow 1975) as modified by Coon et al (1978). Milled grain (4.0 g) was added to 100 mL of 4.0% (w/v)

sodium carbonate buffered to pH 10 with sodium bicarbonate. Samples were shaken for 45 min at 45°C and 50-mL portions of the slurry were centrifuged at 23,500 × g for 15 min at room temperature. Viscosity was then determined at room temperature using a viscometer (model B/H 7210-2 Haake).

β-Glucan in milled barley flours was determined using a β-glucan assay kit (Megazyme Intl. Ireland) in which the enzyme lichenase, a (1→3)(1→4)-β-D-glucan-4-glucanohydrolase, specifically hydrolyses the mixed linkage β-glucan to oligosaccharides which are then converted to glucose using β-glucosidase. The final assay of glucose used an automated glucose oxidase procedure (Wood et al 1991).

Barley samples were prepared for structural analysis of β-glucan. Milled grain was treated with refluxing 70% (v/v) aqueous ethanol (1:20 solid to liquid) for 2 hr and the mixture was centrifuged (8,000 × g, 15 min); the supernatant was discarded and the residue then washed with the aqueous ethanol followed by 95% ethanol before drying by gentle warming in a stream of air. Final drying was in vacuo at 80°C to yield ethanol-treated flour.

The procedure for production of oligosaccharides released by lichenase (Megazyme) was essentially as that used for determination of β-glucan, but with the β-glucosidase step omitted. Ethanol-treated samples (≈100 mg, accurately weighed, triplicate) were suspended in 4.8 mL of 20 mM sodium phosphate buffer, pH 6.5, and lichenase (200 μL, 10 units) added and the mixture incubated at 50°C for 1.5 hr with constant stirring. The samples were centrifuged and the supernatant diluted with water (5.0 mL) and analyzed by high performance chromatography, both as is and after dilution (10–25 fold) with water. As controls, barley and oat β-glucan standards (Megazyme, 5 mg) were dissolved by heating (70°C for 2.0 hr) in phosphate buffer (5.0 mL), cooled to room temperature, and lichenase added (200 μL, 10 units). After digestion, samples were diluted with water (4.8 mL) and analyzed as is or further diluted 15-fold. For one set of samples, the ethanol treatment was done without isolation of the dried flour. The samples (100 mg) were suspended in 5 mL of aqueous ethanol (50%, v/v) and heated at 100°C for 10 min, cooled, and 5 mL of aqueous ethanol (50%, v/v) added, and the sample was centrifuged. The treatment with aqueous ethanol was repeated, the residue was washed with 10 mL of ethanol, and the lichenase treatment then continued on the ethanol wet residue. This procedure was performed in a single tube.

A Dionex system (Sunnyvale, CA) using a CarboPac PA1 column (4 × 250 mm) and guard (3 × 25 mm) was used for oligosaccharide analysis by high-performance anion-exchange chromatography (HPAEC). Detection was by pulsed amperometry with a gold electrode. Samples were filtered (0.45 μm) before analysis. Eluent A was 150 mM sodium acetate in 150 mM sodium hydroxide and eluent B was 150 mM sodium hydroxide. Elution was with 70% eluent A and 30% eluent B for 1 min, then with a gradient to 100% eluent A by 9 min, which was continued for 11

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min. The initial conditions were maintained for 10 min between each injection of sample. The flow rate was 1.0 mL/min at ambient temperature. Pulse potentials (E , volts) and durations (t , ms) initially were $E_1 = 0.1$, $t_1 = 300$; $E_2 = 0.6$, $t_2 = 120$; $E_3 = -0.8$, $t_3 = 300$. Response time of the detector was 3.0 sec. After an electrode change, pulse potentials and durations were changed to $E_1 = 0.05$, $t_1 = 480$; $E_2 = 0.6$, $t_2 = 180$; $E_3 = -0.6$, $t_3 = 60$, with a detector response time of 1.0 sec. Instrument conditions were controlled and data were processed using Dionex AI 450 software. The weight ratio of tri-to-tetrasaccharide was calculated as the peak area ratio and converted to molar ratio by the factor 1.321. The proportion of DP 5–9 material, obtained from the more concentrated sample was calculated as a proportion of all peak areas from DP 3 to DP 9 normalized at 100%. Statistical evaluations used least squares procedures (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

The yield of ethanol-extracted barley flour overall was $86.0 \pm 1.7\%$ (dwb); from waxy cultivars, the yield was $84.9 \pm 1.3\%$ and from nonwaxy cultivars, the yield was $87.1 \pm 1.2\%$. The ethanol treatment deactivates enzymes and removes low molecular weight oligosaccharides that would otherwise interfere in the analysis of oligosaccharides from β -glucan. The difference in yields probably reflects the higher free sugar content of waxy cultivars (Xue et al 1997).

Analytical data (Table I) shows that awn type was without significant influence on any parameters measured. Protein and viscosity were the only variables significantly affected by location. There were two significant interactions: for β -glucan and viscosity with hull versus starch type.

Hull obviously greatly increased the fiber content, mainly as insoluble fiber (from 7.8 to 14.7%). Soluble fiber was not

significantly different but did not show the decrease observed for all other components diluted by the presence of the hull, which is mostly fiber. In protein, this reduction (14.9–14.2%) was nonsignificant. There is no β -glucan in hull, and the increase on going from hulled to hullless (5.0–5.6%) would be expected from a change based solely on the basis of measurement being with or without 11% hull. The increased β -glucan was reflected in an increased extract viscosity in hullless types. In general, there is an association between β -glucan content and extract viscosity. Above $\approx 0.3\%$ (w/v), the zero shear rate viscosity of high molecular weight β -glucan increases very rapidly (approximately to the 4th power) with β -glucan concentration (Doublrier and Wood 1994; Böhm and Kulicke 1999a).

Waxy barleys had significantly higher β -glucan content ($6.10 \pm 0.47\%$) than nonwaxy ($4.53 \pm 0.37\%$), and greater viscosity of extract (4.45 ± 1.17 and 2.38 ± 0.53 cP, respectively) as was previously reported (Xue et al 1997). The structure of the waxy barley β -glucan was also different with a significantly higher ratio of β -(1 \rightarrow 3) linked cellotriosyl units to (1 \rightarrow 3) linked cellotetraosyl units (3.02 ± 0.05 and 2.74 ± 0.05 , respectively). The detailed analysis of the oligosaccharides released by lichenase (Table II) shows an overall pattern similar to previous reports (Wood et al 1991, 1994b; Izydorczyk et al 1998; Jiang and Vasanthan 2000) in which the amount detected declines to $<1\%$ at DP 7, but then increases again at DP 9. The oligosaccharide reaction products become mostly insoluble at this DP, like cellodextrins but with a single β -(1 \rightarrow 3) linked unit at the reducing end. The level of oligosaccharides of DP 5–9, representing more cellulose like regions of the β -glucan, was somewhat lower in waxy cultivars but this difference was not significant. The main significant difference (the ratio of trisaccharide to tetrasaccharide) was previously reported by Jiang and Vasanthan (2000), but these authors found lower ratios overall (waxy 2.64, nonwaxy 2.38). Others

TABLE I
Effect of Barley Genotype on Selected Components and β -Glucan Properties

	Hull Type ^a		Awn Type		Starch Type ^b	
	Hulled	Hullless	Long	Short	Nonwaxy	Waxy
Protein %	14.16	14.91	14.62	14.45	14.19	14.88
Fat %	2.32	2.47	2.36	2.44	2.13	2.67
TDF %	17.39	10.24	13.37	14.26	13.38	14.25
SDF %	2.59	2.42	2.46	2.54	2.25	2.75
IDF %	14.71	7.82	10.91	11.63	11.03	11.49
β -Glucan %	5.01	5.61	5.21	5.42	4.53	6.10
Viscosity cP	2.87	3.96	3.33	3.50	2.38	4.45
DP ^c 5–9	8.18	8.13	8.20	8.10	8.37	7.94
T:T ^d ratio	2.87	2.89	2.87	2.90	2.74	3.02

^a Hull effect P values: TDF ≤ 0.005 ; IDF ≤ 0.001 ; β -glucan ≤ 0.05 ; viscosity ≤ 0.025 .

^b Starch effect P values: β -glucan ≤ 0.007 ; viscosity ≤ 0.006 ; trisaccharide-to-tetrasaccharide ratio ≤ 0.002 .

^c Degree of polymerization of oligosaccharide.

^d Trisaccharide-to-tetrasaccharide ratio.

TABLE II
Analysis of Oligosaccharides (%) Released by Lichenase from β -Glucan in Barley Genotypes^a

Sample	tri	tetra	penta	hexa	hepta	octa	nona	tri+tetra	penta-nona
NWL ^b	61.5	30.1	3.7	2.2	0.4	0.5	1.5	91.7	8.4
NWl	61.6	29.8	4.0	2.2	0.4	0.4	1.5	91.4	8.6
nWL	61.9	29.7	3.9	2.2	0.4	0.5	1.5	91.6	8.4
nWl	62.3	29.6	3.6	2.2	0.4	0.5	1.5	91.8	8.2
W Mean ^c	61.8	29.8	3.8	2.2	0.4	0.5	1.5	91.6	8.4
NwL	64.0	28.0	3.4	2.1	0.4	0.5	1.3	92.0	8.0
Nwl	64.3	27.9	3.7	2.1	0.4	0.5	1.2	92.2	7.8
nwL	63.8	28.2	3.7	2.1	0.4	0.5	1.3	92.0	8.1
nwl	64.2	27.9	3.6	2.2	0.4	0.5	1.3	92.1	7.9
w Mean ^d	64.1	28.0	3.6	2.1	0.4	0.5	1.3	92.1	7.9

^a Mean percentages from three locations, based on peak areas and normalized to 100% for peaks of DP 3–9.

^b N, covered; n, hullless. W, nonwaxy (normal); w, waxy. L, long awn (normal), l, short awn.

^c Mean of all nonwaxy types from three locations.

^d Mean of all waxy types from three locations.

have also reported lower ratios than we have observed (Woodward et al 1983; Izydorczyk et al 1998). These discrepancies relate, inter alia, to uncertainties in the response factors for the oligosaccharides in the analytical system used. We have not corrected for the likely different response factors for tri- and tetrasaccharide, previously estimated as 0.49 and 0.42 relative to glucose, respectively (Wood et al 1994b) because lack of appropriate pure standards make these corrections uncertain. However, the true ratios of tri- to tetrasaccharide are probably somewhat lower, and the amounts of higher oligosaccharides, representing cellulose-like regions in the intact polymer, higher.

In the present analysis, the lichenase is used on flour, without isolation of the β -glucan. A comparison of the patterns of oligosaccharides released from flour and a barley β -glucan standard is shown in Fig. 1A–D. The hexasaccharide peak region sometimes indicates the presence of a major and minor component, either as shoulder or partially separated peak. Methylation analysis of an isolated hexasaccharide fraction indicated more than one component (Wood et al 1991). It is possible that the hexasaccharide released from two adjacent β -(1 \rightarrow 3) linked cellotriosyl units is resistant to further action of the lichenase, giving G1 \rightarrow 4G1 \rightarrow 3G1 \rightarrow 4G1 \rightarrow 4G1 \rightarrow 3G in addition to G1 \rightarrow 4G1 \rightarrow 4G1 \rightarrow 4G1 \rightarrow 3G (where G is a glucopyranosyl residue). Analysis of whole flour ensures that all the β -glucan is analyzed, but if there is structural heterogeneity (Izydorczyk et al 1998) an average picture is obtained. Thus, patterns from purified material and whole flour might be slightly different; the standard barley of Fig. 1A showed a somewhat higher molar tri- to tetrasaccharide ratio (3.2) relative to the similarly analyzed flours.

There was a nonsignificant trend to less DP 9 oligosaccharide in the lichenase digest of waxy cultivars. This analysis represents only the soluble portion of DP 9. Lichenase digestion of β -glucan normally produces an insoluble material in \approx 3–5% by weight, consisting mainly of DP 9 and higher oligosaccharides (Wood et al 1994b, and unpublished data). This material was not analyzed in the present study. It might be expected that more cellulose-like regions would decrease solubility in the intact polysaccharide through cellulose like interactions, but this has not been demonstrated experimentally.

Although there seems to be an association between the ratio of trisaccharide to tetrasaccharide and the β -glucan extractability and extract viscosity of waxy cultivars, there may be no causal relationship. Indeed, current understanding might suggest the opposite

because it would appear that an increase in proportion of β -(1 \rightarrow 3) linked cellotriosyl units leads, statistically, to a greater structural regularity. This increases the capacity for intermolecular associations, which leads to gel formation in solution and decreased solubility (Böhm and Kulicke 1999b; Cui and Wood 2000). It would be anticipated that the waxy barley β -glucan, particularly if of lower MW as tentatively indicated by Beer et al (1997), would be more susceptible to gelation. This possibility was not examined.

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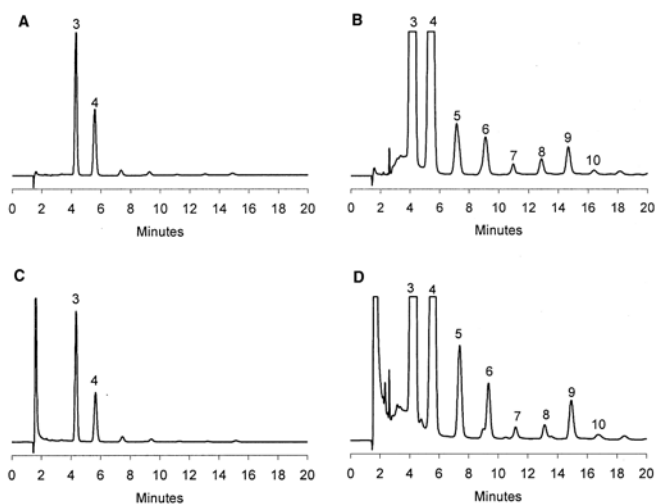


Fig. 1. High-performance anion exchange chromatography of oligosaccharides released by lichenase from barley β -glucan (A,B) and barley (naked, waxy, long awn) flour (C,D). Concentration in (B) and (D) 15 \times that in (A) and (C). Numerals represent oligosaccharide DP.

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