Total and Acid-Soluble β-Glucan Content of Hulless Barley and Its Relationship to Acid-Extract Viscosity[1]

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ABSTRACT

The relationship between total and acid-soluble β-glucan and acid extract viscosity (AEV) was investigated in 13 genotypes of barley (12 hulless and one hulled low β-glucan mutant for comparison). The range in AEV was 3.0–145.5 cS; in total β-glucan, 3.9–5.4%. AEV was correlated (+0.83, $P < 0.01$) with total β-glucan percentage but with none of the other components of barley measured, such as starch, protein, pentosans, or their constituent sugars (arabinose and xylose). The acid-soluble fraction of barley contained 1.0–2.7% β-glucan, 0.8–2.7% starch, 1.6–2.2% nitrogen, and 0.2–0.6% pentosans (determined by gas chromatography), representing an average of 44.7, 2.4, 81.8, and 14.8%, respectively, of their concentrations in the grain. Thus, besides β-glucan, the major components of the acid-soluble fraction were nitrogen and pentosans. The correlations between AEV and soluble β-glucan, starch, and pentosans were +0.71, $P<0.01$), +0.90, $P<0.01$), and +0.61, $P<0.05$), respectively. The positive correlation between AEV and soluble starch seemed coincidental, as the latter contributed very little to AEV; viscosities of acid extracts of three genotypes of barley containing 0.9–2.7% starch were completely abolished in 15 min on addition of β-glucanase to the extracts. Earlier addition of protease and α-amylase to an acid extract of one genotype of barley did not lower viscosity significantly, but the viscosity was lowered by the addition of xylanase, a pentosan-hydrolyzing enzyme. The present data confirm that soluble β-glucan is largely, and pentosans are to a minor extent, responsible for AEV in spite of AEV's large variability in genotypes of hulless barley.

Barley and oats are unique among cereals, containing relatively high concentrations of the mixed-linked, nonstarchy polysaccharides, β-D-glucans (β-glucans). Hulled barley may typically contain 3–7% β-glucan (Åman and Graham 1987) and hulless barley as much as 16% (Newman et al 1989a). Soluble β-glucan is a major component of the soluble dietary fiber (Frølich and Nyman 1988) implicated in hypocholesterolemia in chicks (Fadel et al 1987), humans (Newman et al 1989b), and rats (Bhatty et al 1990).

The β-glucan content of barley and its products may be determined by measuring the specific binding of the fluorochrome Calcofluor to β-glucan or enzymatically, using specific β-glucanase (and β-glucosidase) preparations. Several modifications of these basic procedures have been reported. Jørgensen and Aastrup (1988) reviewed methods available for β-glucan determination; Aastrup and Jørgensen (1988) obtained almost identical β-glucan values for barley, malt, wort, and beer using fluorometric flow injection analysis (FIA) and the enzymatic procedure of McCleary and Glennie-Holmes (1985). Sendra and Carbonell (1989) modified and improved the FIA-fluorometric method for determining β-glucan in wort and beer. Bhatty (1987) reported the proportions of soluble, insoluble, and total β-glucans and their relationship to acid extract viscosity (AEV) in hulled and hulless cultivars of barley grown at different locations under Canadian prairie conditions. The β-glucan content of three barley cultivars grown at 15 locations ranged from 2.3 to 3.5% and in AEV from 6 to 30 cS. These ranges were limited. A higher range of β-glucan values was reported for Australian (Henry 1986), Scandinavian (Lehtonen and Aikasalo 1987b), and U.S. barleys (Åman and Graham 1987).

Hulless barleys, like hulled barleys, vary widely in AEV (Aastrup 1979, Bhatty 1987). This variation may be due to differences in soluble β-glucan, starch, protein, pentosans, or possibly other hemicelluloses in the extract. Pentosan and β-glucan contents of a large number of two- and six-rowed hulled barley cultivars were reported (Henry 1986, Lehtonen and Aikasalo 1987a), but these two components were not correlated. Aastrup (1979) found that AEV was highly correlated with soluble β-glucan but not with protein, carbohydrate, or fat content. Smith et al (1980 a,b) reported that analysis of biochemical constituents of acid extracts of barley revealed contrasting malting quality. Both studies indicated that soluble β-glucan was largely responsible for AEV, and that in poor malting barley, the soluble proportion (up to 67%) of β-glucan was greater than that of good malting barley (about 25%).

We routinely use AEV in our laboratory in the preliminary selection of hulless lines of barley for low and high β-glucan content. A positive correlation of 0.83 ($P < 0.01$; n = 15) was obtained between AEV and total β-glucan in barley, although soluble β-glucan formed only about one third of the total β-glucan content (Bhatty 1987). The present study is a continuation of our previous work on hulless barley. A great deal of interest has developed in such a barley as a source of soluble dietary fiber in foods for human consumption. The objective of this study was to further investigate the relationship between soluble and total β-glucan in hulless barley lines that have a large range in AEV.

MATERIALS AND METHODS

Grain Samples

Thirteen lines of barley grown on experimental plots at the University of Saskatchewan in Saskatoon in 1986 were selected from material routinely screened for AEV in our laboratory. The barley lines included the hulled Danish two-rowed, low β-glucan Minerva mutant 737 (Aastrup 1983) for comparison, and Scout—a registered, two-rowed Canadian hulless barley. The hulless barleys were cleaned of residual hull and ground in a Udy cyclone mill to pass through a 0.5-mm screen. The ground grain (meal) was stored at 5°C.

The assay kit for β-glucan determination, which included low and high β-glucan barley standards, was obtained from Biocon (Lexington, KY).

Analytical Methods

Meal and the acid-soluble fraction. Moisture and total nitrogen were determined by AACC procedures (AACC 1983), starch by the method of Fleming and Reichert (1980), and AEV as follows. Barley meal (1.5 g) was extracted with 15.0 ml of acid buffer (82.8 ml of 1*N* HCl and 7.46 g of KCl) for 1 hr at room temperature in a Udy shaker. The extract was centrifuged at 1,000 × *g* for 20 min. The viscosity of the extract was measured at 25°C using an Ubbelohde viscometer (No. A 629) and was expressed in centiStokes, calculated by multiplying efflux time (*t*) in seconds with the calibration constant (0.097) provided with the instrument. After measuring viscosity, the acid extracts were neutralized by adding solid sodium bicarbonate and were freeze-dried to yield the acid-soluble fraction.

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In some experiments, viscosity was determined and expressed differently. For extraction of the meal with water, any endogenous β-glucanase present was denatured by boiling with ethanol for 10 min, followed by centrifugation at 1,000 × *g* for 10 min. The supernatant was poured off. Such denaturation of β-glucanase was not necessary for extracting the meal with the acid buffer that denatures the enzyme. Barley meal (0.5 g) was vortexed with 10 ml of water or acid buffer. The mixture stood in a centrifuge tube at room temperature (40°C for water extraction) for 1 hr and then was centrifuged at 1,000 × g for 10 min. An aliquot of the supernatant (4.5 ml) was poured into the viscometer (Fisher Scientific 200 C907), and flow viscosity was measured at 25°C. Results were expressed as specific viscosity (η_{sp}), calculated as $t\text{-}-t_0/t_0$, where t and t_0 were running times, in seconds, of the sample and the extraction buffer, respectively.

Total and soluble β-glucan content was determined as follows. A 250-mg subsample of the meal or acid-soluble fraction and 5.0 ml of 50 m*M* perchloric acid were added to a polyallomer centrifuge tube and heated for 10 min in a water bath at 90°C. After centrifugation at 3,000 × g for 5 min, the pellet was washed with 1.0 ml of cold perchloric acid, recentrifuged, and the supernatants combined (Ahluwalia and Ellis 1984). To a 0.5-ml aliquot was added 0.4 ml of 100 m*M* sodium acetate buffer, pH 5.4. This mixture was incubated with 100 μl (100 μg/ml) of β-glucanase (Bhatty 1987) at 37°C for 1 hr. A 0.25-ml aliquot was withdrawn, and reducing sugars were measured using 3,5-dinitrosalicylic acid (Bernfeld 1955). The β-glucan concentration was obtained from a standard curve prepared with β-glucan from commercial barley (Biocon), extracted, and hydrolyzed under identical conditions. This procedure and two others (McCleary and Glennie-Holmes 1985, Henry and Blakeney 1988) were used to determine β-glucan content of standards of barley with low and high β-glucan.

Pentosan content was determined by a colorimetric procedure (Hashimoto et al 1987) and gas-liquid chromatography after acid hydrolysis of the meal and the acid-soluble fraction; this procedure was followed by reduction and acetylation (Blakeney et al 1983). The alditol acetates were separated under the following conditions: fused silica column SP 2330 (Supelco, Oakville, ON); injection port and detection temperatures of 250 and 300°C, respectively; temperature program, 170–230°C at 8°C/min; carrier gas (nitrogen) flow rate adjusted to complete the run in about 13 min; internal standard, *myo*-inositol; integration system, HP 3385A. Response factors were calculated for each sugar with commercial samples (Sigma Chemical Co., St. Louis, MO). Pentosan concentration was calculated as the sum of arabinose and xylose × 0.88.

Soluble and Insoluble β-Glucan Content of Water and Acid Extracts

Sodium phosphate buffer, pH 6.5 (1.0 ml), was added to 1.0 ml of water extract. Sufficient sodium hydroxide was added to the acid extract to bring the pH to 6.5, followed by the addition of 2 .0 ml of the phosphate buffer. β-Glucan content and the insoluble β-glucan content of water and acid extracts were determined using the procedure of McCleary and Glennie-Holmes (1985).

TABLE I
β-Glucan Content of Barley Standards Determined by Three Enzymatic Procedures

Procedure	Low β-Glucan (%)	High β-Glucan (%)	Difference (%)
Assay kit (as-is moisture basis)	3.2	4.4	38
McCleary and Glennie-Holmes (1985)	3.07 ± 0.03[a]	4.15 ± 0.10	37
Bhatty (1987)	3.77 ± 0.08	4.99 ± 0.06	32
Henry and Blakeney (1988)[b]	3.06 ± 0.10	3.95 ± 0.11	29

[a]Mean ± SD.
[b]Used β-glucanase preparation described by Bhatty (1987).

Effect of α-Amylase, Protease, Xylanase, and β-Glucanase on Viscosity

Barley line R86153 was extracted with the acid buffer as described above; the extract was neutralized to pH 6.5 and the viscosity of an aliquot (4.5 ml) determined in a viscometer as described above. A pronase solution (2 mg/ml) (45,000 PUK/g from *Streptomyces griseus* obtained from Calbiochem Corp. (San Diego, CA) was prepared in acid extraction buffer and neutralized to pH 6.5. An aliquot (50 μl) of this solution was added to the barley extract in the viscometer. The viscosity of the extract was measured after 8, 20, 30, and 40 min. A 50-μl portion of malt barley α-amylase 2 (415 IDC units/μl), purified as described by Westlake et al (1983), was added to the extract, and the viscosity was measured for another 35 min. Finally, a portion (50 μl, 2.5 μm) of lichenase (β-glucanase) from the β-glucan assay kit was added to the extract and the viscosity again measured after 1, 5, and 10 min. Another barley extract was treated in the viscometer with 50 μl of a xylanase solution prepared as follows. Xylanase (200 μl) (4,000 μ/ml, obtained from Biocon [Australia] Ltd., Melbourne) was centrifuged at 10,000 × g for 1 min, the supernatant was discarded, and the pellet was dissolved in 900 μl of phosphate buffer (0.1*M*, pH 6.5). The viscosity of the extract was measured before xylanase was added and at 2, 15, 20, and 25 min after it was added. Finally, a portion (50 μl, 2.5 μm) of lichenase from the β-glucan assay kit was added to the extract-xylanase mixture; viscosity was measured at 1 and 5 min after this addition.

Data Analyses

All analytical data are means of duplicate and, in some cases, quadruplicate determinations. Simple and multiple correlations between AEV and β-glucan, starch, pentosans, and nitrogen content of barley and the acid-soluble fraction were calculated, the latter by multiple linear regression as described by Snedecor and Cochran (1967).

Fluorescence Microscopy

Barley kernels were fixed in 5% glutaraldehyde, dehydrated, embedded in glycol methacrylate (Eastman Kodak), sectioned as described previously (Bhatty and MacGregor 1988), and stained with Calcofluor White M2R (Polysciences). Stained sections were examined under a Wild Leitz Orthoplan fluorescence microscope using Filter Block A (exciting filter BP 340–380 nm, barrier filter LP 430 nm). Illumination was provided by a super pressure mercury lamp (HBO, 5O W). Micrographs were photographed on Kodacolor VR 400 film.

RESULTS AND DISCUSSION

The enzymatic procedure routinely used in our laboratory to determine the β-glucan content of barley is based on perchloric acid extraction (Ahluwalia and Ellis 1984). This procedure is followed by hydrolysis of the extract by β-glucanase prepared from BAN 1000, a crude α-amylase preparation (Bhatty 1987). The oligosaccharide content of the extract is compared, without further hydrolysis, with that of commercial β-glucan extracted and hydrolyzed under identical conditions. Such an approach was first suggested by Henry (1984) and again by Henry and Blakeney (1988). The procedure is simple and economical. Barley standards available with the assay kit and containing low (3.2%) and high (4.4%) levels of β-glucan were analyzed by three enzymatic procedures. The results obtained with the Bhatty (1987) procedure showed that the relative difference between the low and high values was within the range obtained with the procedures of Henry and Blakeney (1988) and McCleary and Glennie-Holmes (1985) (Table I). The higher β-glucan values obtained with this procedure were due to endogenous reducing sugars. These may be removed, if necessary, by ethanol extraction before enzyme hydrolysis or, as in the procedure of Henry and Blakeney (1988), by reducing them with sodium borohydride.

Table II shows statistical parameters of AEV, of other barley components, and of the acid-soluble fraction obtained from the

barleys. Barley samples were selected on the basis of AEV, which varied from 3.0 to 145.5 cS, a range of almost 50-fold (Table II). In contrast, the ranges in β-glucan, pentosans, starch, nitrogen, arabinose, and xylose were smaller and even negligible in some cases. The ranges for the same components were somewhat larger in the acid-soluble fraction because of the different solubilities of these compounds in the acid buffer. The coefficient of variability (CV) for the components in barley and the acid-soluble fraction reflected their ranges. For example, the CV was 96% for AEV but only 9% for total β-glucan. In the acid soluble fraction, higher CVs were obtained for xylose, arabinose, starch, and soluble β-glucan than for the same components in whole barley.

In the present study, the barley contained, on average, only slightly more xylose than arabinose (1.7 vs. 1.4%, Table II). The range and mean for both the sugars were lower than those reported for Australian barleys (Henry 1986), where the mean content for 17 cultivars of barley grown at three different locations was 1.9% for arabinose and 4.7% for xylose. The ratio of arabinose to xylose was 0.40. The mean pentosan content of Australian barleys was 5.4%, whereas in our study it was only 2.7%. Lehtonen and Aikasalo (1987a) reported a mean pentosan content of 8.3.% in two-rowed (n = 68) and 9.9% in six-rowed (n = 50) Finnish barleys determined by a colorimetric procedure. Obviously, pentosan content is influenced by hull, cultivar, environment, and method of determination.

Table II compares pentosan data determined by gas-liquid chromatography and by colorimetric determination. Colorimetric determination of pentosan content of the barley samples by the reaction of ferric chloride and orcinol (Hashimoto et al 1987) gave a mean value of 5.5%, which was twice that of the mean obtained with gas-liquid chromatography (2.7%). The correlation between the two methods was +0.82 ($P < 0.01$) for barley and +0.74 ($P < 0.01$) for the acid-soluble fraction. The higher value obtained with the colorimetric procedure was most likely due to interference by hydrolytic products of the carbohydrates (most likely dextrins) not fermented by yeast (Hashimoto et al 1987).

AEV was positively correlated (0.83, $P < 0.01$) with total β-glucan but with none of the other components of barley (Table II), suggesting that barley composition, except for β-glucan, is unlikely to affect AEV. This correlation was identical to one obtained previously from fewer barley samples having a narrow range in AEV (Bhatty 1987). Figure 1A shows the distribution of the correlation data, where the natural log of AEV is plotted against total β-glucan content of the barley. Conversion of AEV to natural log increased the correlation value from +0.83 to +0.94, indicating sensitivity of AEV to increases in total β-glucan. Aastrup (1979) concluded from a similar study, conducted with 18 genotypes of barley, that a good correlation does not exist between AEV and total β-glucan. In that study, the correlation obtained was +0.78, which increased to +0.94 on dividing the barley samples into low and high AEV and excluding the four high lysine genotypes of barley. We did not think it necessary to divide the data in the same way, as the number of observations would become too small to be of significance.

In the present study, soluble β-glucan formed, on average, 44.7% of the total β-glucan, exceeding the value of 32.7% previously obtained for hulled and hulless barleys (Bhatty 1987). The average solubilities for starch, nitrogen, and pentosans were 2.4, 81.8, and 14.8% (determined by gas-chromatography), respectively (Table II). The range for soluble β-glucan content was 1.0–2.7%, compared with 0.8–1.5% obtained previously (Bhatty 1987). Aastrup (1979) reported genotypic variations in soluble β-glucan

TABLE II
Statistical Data for Various Components of 13 Barleys Investigated and the Acid-Soluble Fraction Prepared from the Barleys

Composition	Range	Mean	SD[a]	CV	R[b]
Barley, dry basis					
Acid extract viscosity, cS	3.0–145.5	45.9	43.9	95.6	+1.00
Total β-glucan, %	3.9–5.4	4.7	0.4	8.5	+0.83**
Starch, %	49.5–64.7	57.6	3.9	6.8	+0.33
Nitrogen, %	2.1–2.4	2.2	0.1	4.5	+0.63*
Pentosans					
Gas chromatography, %	1.2–4.7	2.7	0.8	29.6	−0.36
Colorimetry, %	4.4–7.3	5.5	0.9	16.4	−0.51
Arabinose, %	0.7–2.0	1.4	0.3	21.4	+0.10
Xylose, %	0.7–3.3	1.7	0.7	41.2	−0.49
Acid-soluble fraction, % by weight					
Soluble β-glucan	1.0–2.7	2.1	0.5	23.8	+0.71**
Starch	0.8–2.7	1.4	0.6	42.9	+0.90**
Nitrogen	1.6–2.2	1.8	0.2	11.1	+0.13
Pentosans					
Gas chromatography	0.2–0.6	0.4	0.1	25.0	+0.61*
Colorimetry	1.1–2.3	1.7	0.4	23.5	+0.39
Arabinose	0.1–0.4	0.3	0.1	33.3	+0.60*
Xylose	0.1–0.3	0.2	0.1	50.0	+0.63*

[a]SD = standard deviation, CV = coefficient of variation, R = correlation with acid extract viscosity.
[b]**, * Significant at $P < 0.01$ and $P < 0.05$, respectively.

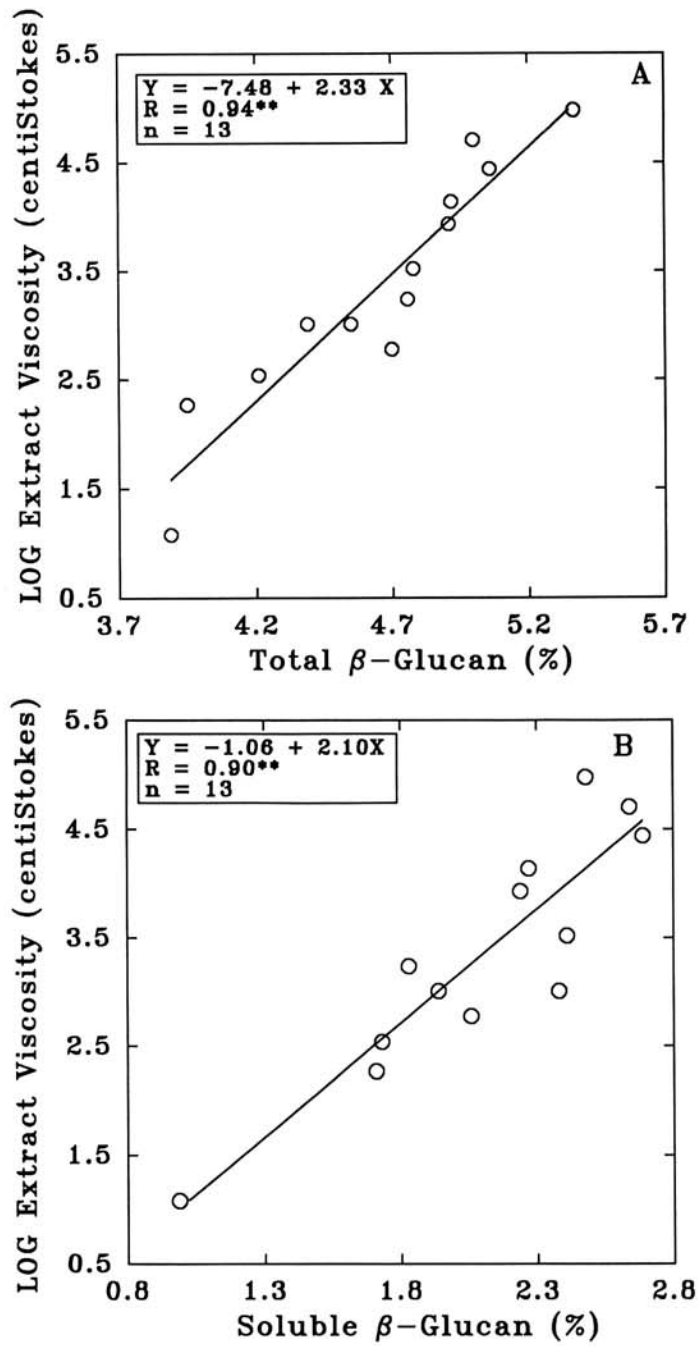

Fig. 1. Correlation between log acid extract viscosity of barley and total β-glucan (**A**) and acid-soluble β-glucan (**B**). The correlations without log conversion of acid extract viscosity were 0.83 (**A**) and 0.71 (**B**).

content extracted by acid buffer. Smith et al (1980a) reported that up to 67% of total β-glucan was soluble in poor malting barley compared with only about 25% in good malting barley. However, the correlation between AEV and soluble β-glucan was lower (+0.71, $P < 0.01$) in the present study than in the previous case, but it reached the same value (+0.90, $P < 0.01$) on logarithmic expression of the data (Fig. 1B).

Nevertheless, the present data, like those of previous studies (Aastrup 1979; Smith et al 1980a,b; Bhatty 1987) confirm the suitability of AEV as an indicator of total β-glucan content of barley, particularly at medium and high concentrations of β-glucans. Multiple linear regression calculated between AEV (dependent variable) and β-glucan, starch, pentosans, arabinose, xylose, and nitrogen contents of barley and the acid-soluble fraction are given in Table III. AEV (expressed on logarithmic scale) could be predicted as linear functions of β-glucan in both barley and the acid-soluble fraction. Multiple linear regression indicated that pentosans, arabinose, xylose, starch, and nitrogen failed to contribute any additional information concerning variation in AEV (Table III). However, Ullrich et al (1986) found a low correlation (+0 64, $P < 0.01$) between AEV and β-glucan in 21 genotypes of barley. The correlations were highest within Compana barleys (+0.94–0.97, $P < 0.01$; n = 6–9), intermediate for normal barleys (+0.71, $P < 0.01$; n = 9), and lowest for waxy barleys (+0.52, $P < 0.05$; n = 12). They suggested that these differences were due to different sizes of β-glucan in the extract (no evidence presented) and that AEV can estimate total β-glucan only in some genotypes of barley.

Biochemical analysis showed that acid extracts of barley contained carbohydrate fractions of both high and low molecular weight; the former consisted principally of β-glucan and, to a minor extent, starch and pentosans (Smith et al 1980b). The high-molecular-weight fraction was almost exclusively responsible for AEV. The low-molecular-weight fraction, which consisted principally of fructose and sucrose, with small concentrations of glucose, xylose, arabinose, maltose, and raffinose, made little difference to AEV. The acid extract contained some protein derived almost exclusively from the salt-soluble fraction of barley proteins, but the protein had no effect on extract viscosity (Smith et al 1980a). In the present study, nitrogen solubility in the acid buffer was, on average, 82% (Table II). Thus it seems that the acid buffer extracted more than the salt-soluble fraction of barley proteins.

AEV was positively correlated with pentosan (+0.61, $P < 0.05$) and starch (+0.90, $P < 0.01$) contents of the acid-soluble fraction

TABLE III
Regression of Acid Extract Viscosity (Logarithmic Scale) on β-Glucan, Starch, Arabinose, Xylose, Pentosans, and Nitrogen in Barley and the Acid-Soluble Fraction

Source	Degrees of Freedom	Sum of Squares	
		Barley	Acid-Soluble Fraction
Regression on β-glucan	1	12.52**[a]	11.34**
Regression on starch, arabinose, xylose, pentosans and nitrogen (after β-glucans)	5	0.22[ns]	1.55[ns]
Deviations	4[b]	0.99	1.30

[a] ** = Significant at $P < 0.01$; [ns] = nonsignificant.
[b] Six degrees of freedom for the acid-soluble fraction.

TABLE IV
Components of Three Genotypes of Barley and the Acid-Soluble Fraction Prepared from the Barleys

Component	Genotype		
	M737 (Hulled)	R86137 (Hulless)	R86153 (Hulless)
Barley, dry basis			
Acid extract viscosity, cS	3.0	51.1	145.5
Total β-glucan, %	3.9	4.9	5.4
Starch, %	49.5	55.4	59.1
Nitrogen, %	2.3	···	2.3
Pentosans, %[a]	3.2	2.4	2.3
Arabinose, %	1.4	1.5	1.3
Xylose, %	2.3	1.2	1.3
Acid-soluble fraction, % by weight			
Soluble β-glucan	1.0	2.2	2.5
Starch	0.9	1.4	2.7
Nitrogen	2.1	2.2	1.8
Pentosans[a]	0.3	0.5	0.5
Arabinose	0.2	0.3	0.3
Xylose	0.1	0.3	0.3

[a] Determined by gas chromatography.

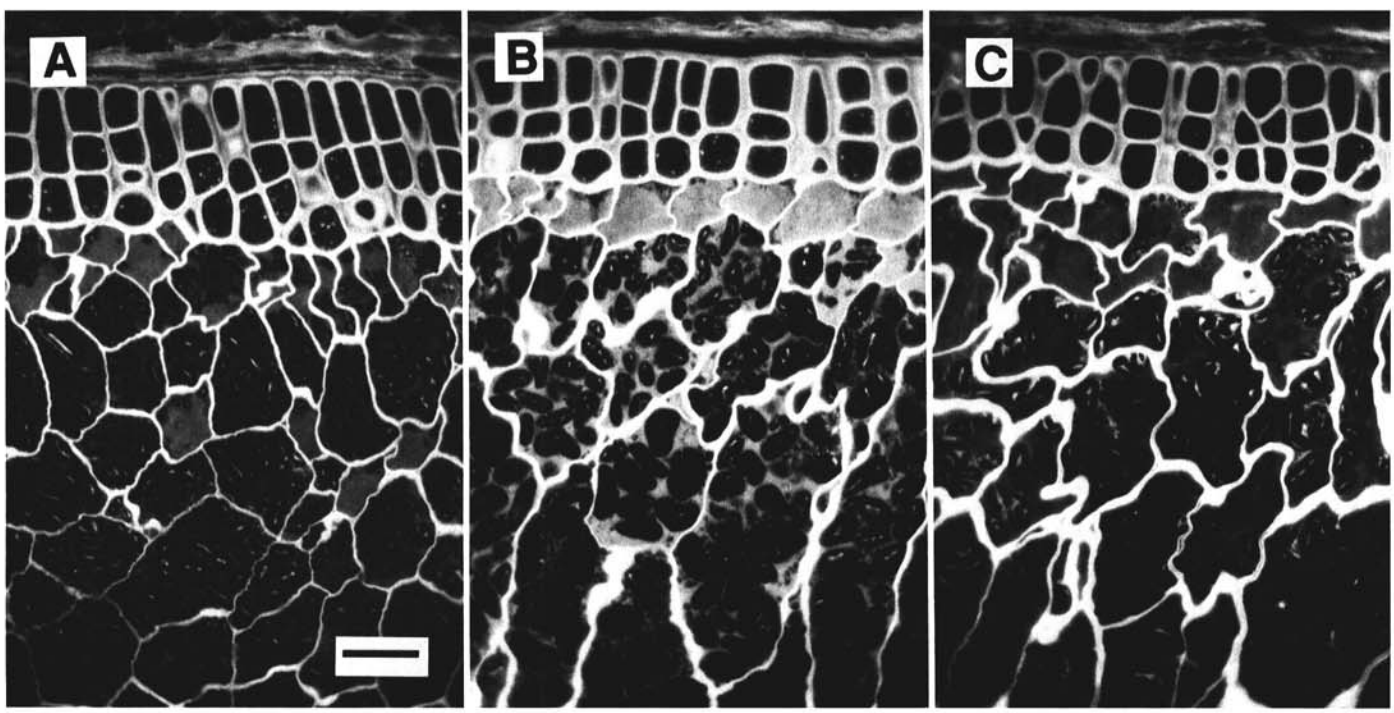

Fig. 2. Transverse sections of barley endosperms stained with Calcofluor to visualize β-glucan in barley with low (**A**, 3.9%, M737), medium (**B**, 4.9%, R86137), and high (**C**, 5.4%, R86153).

(Table IV). Therefore, the data indicate that pentosans and starch contributed to AEV. To determine the extent of this contribution, three genotypes of barley with low, medium, and high AEV were selected for further investigation. The AEV and analytical composition of meals from the three genotypes and of the acid- soluble fraction are given in Table IV. The major differences among the genotypes were in AEV, total β-glucan and starch contents of the meal, and soluble β-glucan, starch, and pentosan contents of the acid-soluble fraction. The AEV of genotype R86153 was almost three times higher, but its soluble β-glucan was only 14% higher than that of genotype R86137. The AEV of the latter genotype was 17 times higher and had 26% more β-glucan than did the mutant M737. The acid buffer extracted 1.8–4.6% of barley starch, 78.3–91.3% of nitrogen, 9.4–21.7% of pentosans, and 25.6–50.3% of β-glucan in the three samples. Soluble protein was thus the major component of the acid-soluble fraction from the three genotypes of barley.

The three genotypes had 3.9, 4.9, and 5.4% total β-glucan (Table IV). This material was present largely in the endosperm cell walls and was visualized readily with Calcofluor (Fig. 2). The cell wall thickness in the three genotypes corresponded to their β-glucan content; M737 had the thinnest walls (Fig. 2A), R86137 had walls of intermediate thickness (Fig. 2B), and R86153 had the thickest walls (Fig. 2C). Some heavier Calcofluor staining was evident in the subaleurone cells, particularly in genotypes M737 and R86137.

TABLE V
β-Glucan Content and Viscosity of Water and Acid Buffer Extracts of Three Genotypes of Barley

Component	Genotype		
	M737 (Hulled)	R86137 (Hulless)	R86153 (Hulless)
Total β-glucan, %[a]	3.4	4.8	5.1
Water-soluble β-glucan, %	0.7	1.7	2.0
Water-insoluble β-glucan, %	2.5	2.5	3.0
Viscosity of water extract, η_{sp}	0.2	3.0	4.9
Acid-soluble β-glucan, %	0.9	2.2	2.4
Acid-insoluble β-glucan, %	2.4	1.9	2.7
Viscosity of acid buffer extract, η_{sp}	0.6	4.8	7.0

[a] Determined by the procedure of McCleary and Glennie-Holmes (1985). Values slightly different than reported in Table II for the same samples.

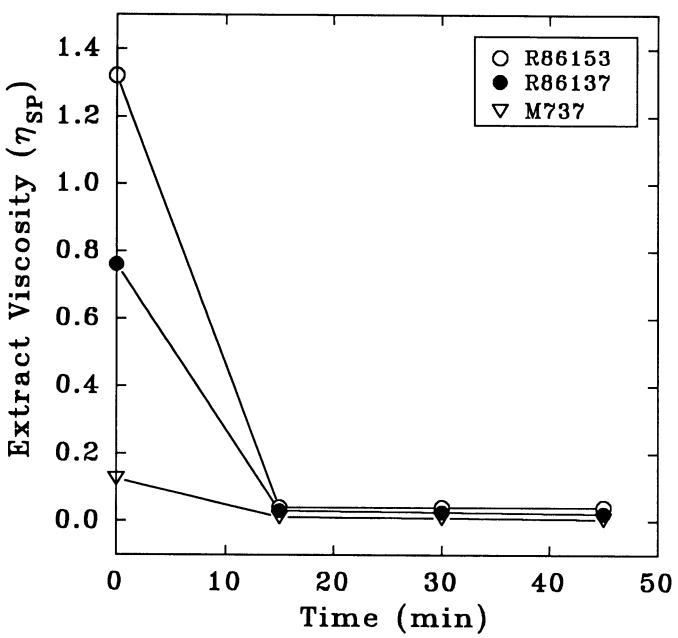

Fig. 3. Effect of β-glucanase addition on the viscosity of acid extracts of barleys with low (3.9%, M737), medium (4.9%, R86137), and high (5.4%, R86153) β-glucan content.

Table V shows a comparative distribution of soluble and insoluble β-glucan in water (40°C) and acid extracts (25°C) of the three genotypes of barley. The water-soluble β-glucan formed 21–39% and the acid-soluble formed 27–47% of the total β-glucan. Because of the slightly higher solubilities of β-glucan in the acid buffer, its specific viscosity was also higher. Acid buffer was thus slightly more suitable than water for extracting β-glucan. However, soluble β-glucan has been extracted from barley with water at different temperatures (Woodward et al 1988). The 40°C water-soluble β-glucan accounted for about 20% and 65°C water-soluble β-glucan accounted for about 50–70% of the endosperm cell wall β-glucan of barley. The two fractions showed only minor differences in their fine structure. The higher extraction temperature (65°C) is traditionally used for extraction of malt, and the soluble β-glucan may be a useful indicator of malting quality of barley. Such an extraction has little relevance for nonmalting barleys. In feed barley, extraction in acid buffer at pH 1.5 more closely resembles physiological conditions in the gastrointestinal tract and may be a better indication of the feed quality of barley, particularly for broiler chickens.

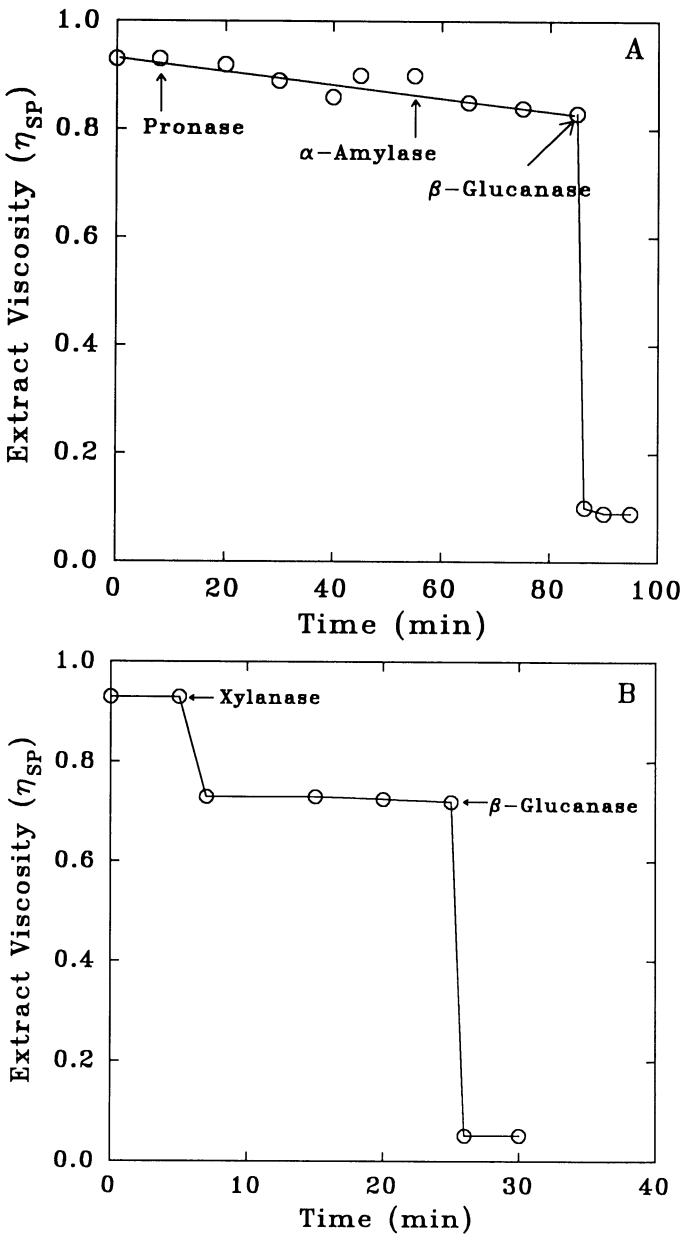

Fig. 4. Effect of sequential addition of various enzymes on the acid extract viscosity of hulless barley with high (5.4%, R86153) β-glucan content. **A,** effect of pronase, α-amylase, and β-glucanase; **B,** effect of xylanase and β-glucanase.

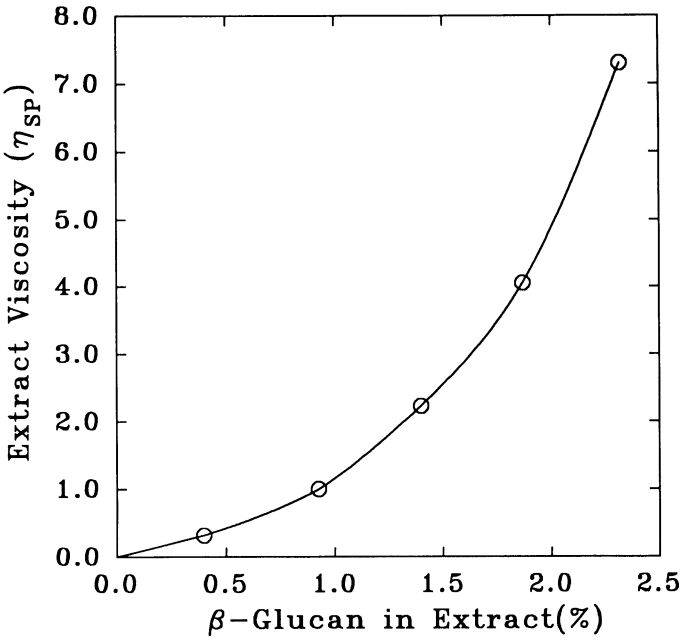

Fig. 5. Relationship between β-glucan concentration and viscosity of acid buffer extracts. Viscosity determinations were made on a series of dilutions of an acid extract of barley genotype R86153.

In another experiment, effects of enzyme addition to acid extracts were investigated. Specific viscosity of acid extracts from the three genotypes of barley was measured, the extracts were hydrolyzed with β-glucanase, and viscosity was measured at 15-min intervals. The results (Fig. 3) show that in all three samples, viscosity was completely abolished after 15 min of hydrolysis with the enzyme, indicating that soluble β-glucan was a major contributor to AEV.

Treatment of a neutralized acid extract of genotype R86153 with either pronase or α-amylase did not significantly reduce the viscosity of the extract (Fig. 4A). Addition of xylanase, however, led to a rapid, small drop in viscosity (Fig. 4B), indicating that pentosans contributed to AEV. When β-glucanase was added to the extracts, an immediate, almost complete loss of viscosity was observed (Fig. 4). These results confirm those shown in Figure 3 and indicate clearly that high viscosities of barley extracts were caused largely by β-glucan and, to a lesser extent, by pentosans. Acid-buffer soluble starch and protein made a negligible contribution to the viscosity of such extracts.

A nonlinear relationship was observed between β-glucan content and viscosity of an extract (Fig. 5). At higher β-glucan concentrations, large increases in viscosity were obtained for relatively small increases in β-glucan concentration. Other studies have reported similar results for oat (Wood et al 1978) and barley β-glucan (Bourne et al 1976; Aastrup 1979). In the present study, we used diverse lines of hulless barley and the low β-glucan hulled barley mutant 737, which varied widely in AEV (50-fold). The results were in general agreement with those reported by Aastrup (1979) and Smith et al (1980a,b), indicating that soluble β-glucan is largely responsible for AEV. The contribution of pentosans to AEV is clear, and this effect should be taken into account in the measurement of barley viscosity.

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