Variation in Total and Soluble β-Glucan Content in Hulless Barley: Effects of Thermal, Physical, and Enzymic Treatments

M. S. Izydorczyk,*,† J. Storsley,† D. Labossiere,† A. W. MacGregor,‡ and B. G. Rossnagel§

Department of Food Science, University of Manitoba, Winnipeg, MB, Canada R3T 2N2, Grain Research Laboratory, 1404-303 Main Street, Winnipeg, MB, Canada R3C 3G8, and Crop Development Centre, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK, Canada S7N 5A8

Total and soluble β-glucan content and effects of various treatments of barley grain on extractability and molecular characteristics of soluble β-glucan were studied. Four types of hulless barley (normal, high amylose, waxy, and zero amylose waxy) from 29 registered and experimental genotypes were analyzed. For each, moisture, protein, amylose, 100 kernel weight, starch, β-glucan (total and soluble), β-glucanase activity, and slurry viscosity were determined. Significant differences in total β-glucan were observed among the groups, with average values of 7.49%, 6.86%, 6.30%, and 4.38% for high amylose, waxy, zero amylose waxy, and normal barley, respectively. The extractability of β-glucan in high amylose barley was relatively low (20.6−29.7%) compared to that in normal (29.8−44.3%), zero amylose waxy (34.0−52.5%), and waxy (36.7−52.7%) barley genotypes. Viscosity of barley flour slurries was affected by the content of soluble β-glucans, β-glucanase activity, and molecular weight of β-glucans. Hydrothermal treatments (autoclaving and steaming) of barley had no effect on extractability of β-glucans, but prevented enzymic hydrolysis of β-glucans, and thereby substantially improved their molecular weight. The addition of enzymes (protease and esterase) during extraction and/or physical treatments (sonication) increased extractability of β-glucans from barley.

Keywords: *Barley; β-glucans; extractability; viscosity*

INTRODUCTION

With the increase in knowledge about the beneficial role of barley β-glucans in the human diet, there is a growing demand for incorporation of barley into various food systems. Although cultivation and utilization of barley have long traditions, barley has been used mainly for malting and feed purposes, and only a small portion has been utilized by the food industry (Jadhav et al., 1998). In contrast to well-developed guidelines for desirable quality characteristics of malting barley, there are no clear or well-defined quality parameters which would determine the suitability of any particular type of barley for food uses. Generally, hulless barley with an easily detachable hull is considered to be well suited as a food grain (McIntosh et al., 1995). Some recently developed hulless barley genotypes not only exhibit unusual characteristics, such as waxy endosperm, high-amylose starch, or high lysine level, but also contain higher levels of β-glucans than is usual in malting varieties (Jadhav et al., 1998). While certain unique characteristics of barley starches and proteins may predispose the grain for specific uses, it is likely that the content and properties of β-glucans will play a key role in determining the extent and breadth of barley utilization for food purposes because of the many health attributes ascribed to these polymers. Barley β-glucans bear the advantage over some other sources of dietary fiber of being partially soluble in water. Therefore, in addition to physiological effects associated with insoluble fiber, such as an increase in fecal bulk or ability to relieve constipation, barley β-glucans also possess the biological benefits of soluble fiber. Soluble β-glucans have been reported to reduce plasma cholesterol and postprandial serum glucose levels in humans and animals (Newman and Newman, 1991; McIntosh et al., 1991; Wood et al., 1994; Jenkins et al., 1995; Kahlon and Chow, 1997; Yokoyama et al., 1997). Because of the very different physiological effects of soluble and insoluble dietary fiber, it is important to distinguish the two types of β-glucans and examine their ratios in various barley varieties. Moreover, because soluble β-glucans have the capacity to form highly viscous solutions which seems to be responsible for the biological activity of these polymers, factors affecting viscosity properties of β-glucans should also be investigated.

In this study we have examined the content and solubility of β-glucans in several genotypes of hulless barley with substantially different starch characteristics. We have also assessed various thermal, physical, and enzymic treatments of barley as potential strategies for increasing the content and viscosity of soluble β-glucans from barley.

MATERIALS AND METHODS

Material. Twenty-nine samples of registered varieties and experimental genotypes of hulless barley were assessed. Varieties Falcon, Condor, CDC Gainer, Bear, AC Hawkeye, and AC Bacon, obtained from James Farms Ltd. (Winnipeg, MB), were grown in 1998 in Manitoba, Canada. The remaining genotypes were grown in 1997 in Saskatchewan, Canada, at the Crop Development Centre, University of Saskatchewan, Saskatoon, Canada. All four lines of zero amylose samples are full sisters from the same cross SB85750 × Azhull. Waxy, SR93139 and SR93135, are full sisters from the cross SB85738 × SB88490 and SB93965 is a half-sister to both being from the cross SB88579/SB85738. High amylose, 92-55-06-04 and -54, are full sisters from the cross SB86106/Glacier. SB94897

† University of Manitoba.
‡ Grain Research Laboratory.
§ University of Saskatchewan.

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and SB94893 are full sisters from the cross SB88488/Glacier and thus all are half-sisters. Before analysis, grain samples were ground in a cyclone mill (Udy Corp., Fort Collins, CO) to pass a 0.5 mm screen.

Chemical Analyses. All results of chemical analyses are reported on a dry matter basis as an average of at least two measurements. Moisture and protein contents were determined according to AACC Methods 44.15A and 46.13 ($N \times 5.7$), respectively. Amylose content in defatted samples was determined by potentiometric titration (Schoch, 1964). Starch content was determined enzymatically using the Megazyme kit for total starch assay (Megazyme). The mixed-linkage β-glucan assay kit (Megazyme International Ireland, Ltd.) was used for total and soluble β-glucan determinations. Soluble β-glucan was determined after extraction (30 min) of ground barley with water at 25 °C using a Burrell wrist action shaker (Burrell Corp., Pittsburgh, PA). The activity of β-glucanase in barley samples was assessed by measuring the rate of decrease in relative viscosity of control β-glucan solutions after addition of barley extracts. Barley extracts were obtained after extraction of 1 g barley samples with water (9 mL) at 25 °C for 30 min in the Burrell shaker and subsequent centrifugation of the suspension for 20 min at 9000*g*. The assay involved addition of 2.0 mL of barley extracts to a control solution of purified β-glucans (0.1% w/v) in a Ubbelohde viscometer (International Research Glassware, Kenilworth, NJ) and monitoring the changes of relative viscosity over a 4 h period at 25 °C. The activity of β-glucanase in barley samples is reported as the change of the relative viscosity of control β-glucan solutions per minute upon addition of barley extracts.

Viscosity Measurements. Viscosity of barley slurries was determined in a Bohlin VOR rheometer (Bohlin Reologi, Edison, NJ) equipped with a concentric cylinder geometry. Ground barley samples were suspended in deionized water (1:6 barley-to-water ratio) and the development of viscosity was followed for 90 min at 25 °C at a shear rate of $147 s^{-1}$. To evaluate the effects of various enzymes on viscosity of barley slurries, the following enzymes were added to the suspensions prior to viscosity measurements: lichenase, xylanase, α-amylase (Megazyme), protease, proteinase K, amyloglucosidase, and esterase (Boehringer Mannheim, Canada). To evaluate the effects of ultrasonic disruption on extractability of β-glucans and viscosity of barley slurries, the barley water suspensions were sonicated for 3 min using a high-intensity ultrasonic processor equipped with a 3 mm probe (Sonics and Materials Inc., Danbury, CO).

Heat Treatments. Heat treatments were applied to barley grain prior to grinding. Grain was steamed for 20 min in a vegetable steamer over boiling water. Roasting consisted of placing 100 g of grain in a baking pan (25 × 25 cm) and roasting in a convection oven at 105 °C for 30 min. Autoclaving was performed at 121 °C and 15 psi for 30 min in open containers. The layers of grain were not thicker than 1 cm. After heat treatments grain was left exposed to the atmosphere, at room temperature to allow moisture equilibration, and subsequently ground in the Udy mill.

Extraction and Purification of β-Glucans. Ground barley samples, control and heat treated, were extracted (30 min) at 25 °C using deionized water (1:10 ratio). Esterase (65 U/g barley) and proteinase K (30 U/g barley) were added to some; other samples were sonicated prior to extraction. After centrifugation (20 min, 9000*g*), supernatants were heated for 5 min at 95 °C, filtered, and subsequently treated with α-amylase (40 U/mL extract) and proteinase K (10 U/mL extract) for 24 h. After extensive dialysis, the samples were lyophilized and used for the determination of β-glucan molecular weight.

Molecular Weight Determination. Molecular weights of purified β-glucan preparations were estimated by high-performance size-exclusion chromatography. A Jordi Gel DVB sulfonated mixed bed column (10 × 250 mm, 5 μm particle size, Jordi associates, Bellingham, MA) and Waters 510 pump, 712 WISP sample injector, and a 410 differential refractometer were used. Samples were dissolved in 20% (v/v) DMSO (3 mg/mL) by heating and stirring, and filtered through a Whatmann GF/A glass fiber filter. Samples were eluted isocratically for 35 min with 0.7 mL/min 20% DMSO at 35 °C. A Shodex standard P-82 kit containing pullulan standards with molecular weights from 5.8×10^3 to 1660.0×10^3 was used to calibrate the column.

Statistical Analysis. Analysis of variance was determined by the Duncan's multiple range test using SAS statistical software (release 6.12; SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Chemical Composition. Several genotypes of hulless barley, with four distinct compositions of starch polysaccharides, amylose and amylopectin, were investigated in the present study. The amylose content of barley with normal starch composition varied between 23.8 and 27.1%, whereas in high amylose varieties amylose content ranged between 37.3 and 41.8%. Waxy barleys contained only 3.8−6.0% amylose. Amylose was entirely absent in the zero amylose waxy type barley.

The starch content of barley with normal starch polymers distribution was significantly greater than that for barley with anomalous amylose-to-amylopectin ratios. When taken as a group, normal barley genotypes had significantly greater starch content (60.7% w/w) than high amylose (52.9% w/w), waxy (54.0% w/w), and zero amylose waxy (53.9% w/w).

The 100 kernel weight of various barley samples is given in Table 1. On average, the 100 kernel weight of the zero amylose waxy barleys was largest (4.35 g) and significantly different from that of waxy (3.78 g) and normal barley (3.86 g). The 100 kernel weight of high amylose samples was not significantly different from any other group. There were also significant differences in the 100 kernel weight within each type of barley (Table 1). The greatest differences were among normal varieties with values ranging from 3.19 to 5.05 g. Waxy barleys, despite the relatively large number of samples, exhibited narrower variation from 3.31 to 4.23 g.

Significant differences were observed in the total β-glucan content among samples. High amylose barley samples ranked first, with average β-glucan content of 7.49% (w/w), followed by waxy barley (6.86%), zero amylose waxy barley (6.30%), and normal barley (4.38%). The greatest variations in β-glucan content were found within the normal barleys, with values ranging from 3.30% to 6.28%. These results indicate that it is possible to identify normal barley varieties with relatively high β-glucan content. The waxy barleys also displayed a relatively large range of β-glucan, from 5.52% to 8.07%. Narrower ranges of total β-glucan in high amylose and zero amylose waxy barleys might be due to a lower number of samples within these two groups as compared to the larger number of samples of normal and waxy barley. The concentrations of total β-glucan for the 29 varieties of hulless barley, obtained in this study, represent a slightly wider range of values (3.30−8.23% w/w) than normally reported (4−7% w/w; MacGregor and Fincher, 1993). The values did not, however, exceed the most extreme case ever reported: 2% and up to 13.2% for wild barley (Henry and Brown, 1987).

When the total β-glucan content was expressed in milligrams per kernel, the ranking of the samples remained the same. No clear relationship was found between the β-glucan content and 100 kernel weight. When taken as a group, the high amylose barleys, with their significantly higher total β-glucan content than the normal barleys, did not have significantly different weight of 100 kernels. Moreover, there were no signifi-

Table 1. Chemical Composition of Barley Samples

sample	amylose (% w/w)	starch (% w/w)	protein (% w/w)	weight/100 kernels (g)	total β-glucans (% w/w)	total β-glucans (mg/kernel)	soluble β-glucans (% w/w)
				Normal Starch Genotypes			
Falcon	23.8	61.3 ± 0.3a[a]	11.6± 0.1	4.07 ± 0.06b	3.64 ± 0.03e	1.48	1.65 ± 0.07bc
SB90354	24.3	58.9 ± 2.1a	12.5 ± 0.1	5.05 ± 0.06a	5.38 ± 0.03b	2.72	1.68 ± 0.04bc
CDC Dawn	25.8	61.9 ± 1.5a	11.6 ± 0.1	3.93 ± 0.07c	3.88 ± 0.02d	1.52	1.16 ± 0.08e
SR93102	27.1	59.6 ± 0.4a	11.9 ± 0.1	3.79 ± 0.02d	6.28 ± 0.11a	2.38	1.28 ± 0.11de
CDC Silky	24.6	61.1 ± 1.6a	13.0 ± 0.3	3.19 ± 0.04e	5.17 ± 0.10bc	1.65	1.30 ± 0.08de
Condor	24.6	61.5 ± 0.7a	12.2 ± 0.4	3.22 ± 0.01e	5.14 ± 0.03c	1.65	1.78 ± 0.11ab
CDC Gainer	25.8	60.2 ± 0.4a	12.5 ± 0.3	3.75 ± 0.06d	3.45 ± 0.21ef	1.29	1.48 ± 0.11cd
Bear	26.0	61.4 ± 0.6a	11.9 ± 0.4	4.05 ± 0.06b	3.30 ± 0.14f	1.34	1.82 ± 0.18ab
AC Hawkeye	25.5	61.0 ± 1.4a	12.3 ± 0.3	3.81 ± 0.06d	3.58 ± 0.10e	1.36	1.90 ± 0.13ab
AC Bacon	24.5	60.7 ± 1.1a	12.0 ± 0.5	3.78 ± 0.02d	3.96 ± 0.08d	1.50	1.96 ± 0.08a
average	25.2 ± 1.0	60.7 ± 1.0a	12.2 ± 0.4	3.86 ± 0.51b	4.38 ± 1.02c	1.69 ± 0.48	1.60 ± 0.28b
				High Amylose Genotypes			
92-55-06-54	41.4	53.3 ± 0.4ab	11.7 ± 0.6	4.24 ± 0.05c	7.04 ± 0.03c	2.98	2.02 ± 0.03a
92-55-06-04	39.8	54.7 ± 0.1a	12.5 ± 0.1	3.47 ± 0.01d	8.23 ± 0.32a	2.86	1.78 ± 0.11a
92-55-06-48	41.8	54.0 ± 1.0ab	11.8 ± 0.3	3.26 ± 0.02e	7.96 ± 0.27ab	2.60	2.00 ± 0.14a
SB94893	39.1	49.6 ± 3.2b	14.4 ± 0.1	4.55 ± 0.06b	7.36 ± 0.27bc	3.35	1.52 ± 0.03b
SB94897	37.3	52.8 ± 1.6ab	12.7 ± 0.1	4.75 ± 0.08a	6.86 ± 0.28c	3.26	1.82 ± 0.10a
average	39.9 ± 1.8	52.9 ± 2.0b	12.6 ± 1.1	4.05 ± 0.66ab	7.49 ± 0.59a	3.01 ± 0.30	1.83 ± 0.20b
				Waxy Genotypes			
CDC Candle	4.3	56.9 ± 0.8a	11.3 ± 0.5	3.99 ± 0.07b	6.89 ± 0.27b	2.75	2.53 ± 0.04f
SB94917	4.2	53.0 ± 3.0abc	13.1 ± 0.1	3.62 ± 0.07ef	8.07 ± 0.10a	2.92	2.22 ± 0.06g
SB93965	3.9	55.6 ± 0.8a	13.1 ± 0.1	4.23 ± 0.02a	6.76 ± 0.23bc	2.86	3.32 ± 0.09ab
SR93135	4.8	54.8 ± 0.5ab	12.8 ± 0.2	3.58 ± 0.04ef	7.01 ± 0.16b	2.51	3.55 ± 0.14a
SR93139	5.9	55.2 ± 0.8a	12.8 ± 0.2	3.31 ± 0.15g	7.20 ± 0.28b	2.38	3.21 ± 0.08bc
SH96054	3.8	50.8 ± 2.6bc	13.7 ± 0.1	3.45 ± 0.06gf	6.96 ± 0.03b	2.40	3.06 ± 0.08bcd
SH96076	4.0	53.3 ± 0.8abc	12.5 ± 0.1	3.79 ± 0.09cd	6.95 ± 0.11b	2.63	2.64 ± 0.06ef
SH96090	4.2	50.0 ± 2.0c	12.9 ± 0.1	3.65 ± 0.03ed	5.52 ± 0.54d	2.02	2.91 ± 0.16cde
SH96093	5.5	54.2 ± 0.4ab	12.7 ± 0.1	3.85 ± 0.03bc	6.250.35c	2.41	2.80 ± 0.28def
SB95134	3.8	56.1 ± 2.3a	13.4 ± 0.1	4.31 ± 0.01a	6.99 ± 0.06b	3.01	3.07 ± 0.07bcd
average	4.4 ± 0.7	54.0 ± 2.2b	12.8 ± 0.6	3.78 ± 0.32b	6.86 ± 0.65b	25.9 ± 0.30	2.93 ± 0.40a
				Zero Amylose Waxy Genotypes			
SB94785	0	56.6 ± 0.3a	12.2 ± 0.6	4.78 ± 0.03a	5.83 ± 0.14c	2.79	3.07 ± 0.10a
SB94783	0	52.4 ± 0.3b	13.0 ± 0.1	4.64 ± 0.04a	6.32 ± 0.03b	2.93	2.16 ± 0.21b
SB94792	0	53.7 ± 0.6b	13.6 ± 0.1	3.34 ± 0.09b	6.95 ± 0.07a	2.32	2.84 ± 0.01a
SB94794	0	52.8 ± 1.7b	13.8 ± 0.5	4.64 ± 0.04a	6.11 ± 0.01b	2.84	2.77 ± 0.04a
average	0	53.9 ± 1.9b	13.2 ± 0.7	4.35 ± 0.68a	6.30 ± 0.48b	2.72 ± 0.27	2.71 ± 0.39a

[a] For each group of barley samples, values followed by the same letter (column) are not significantly different ($p \leq 0.05$).

cant differences in the β-glucan content between the waxy and zero amylose waxy samples, but the 100 kernel weights were significantly different between these two groups. These results indicate, therefore, that the differences in total β-glucan content among the four groups of hulless barley are likely due to genetic differences rather than differences in size and/or amount of endosperm in barley kernels.

An inverse relationship between total β-glucan content and starch content was observed. Generally, barley varieties with the anomalous amylose-to-amylopectin ratio had lower starch but a higher β-glucan content than the varieties with normal starch. These results agree with findings from previous studies (Andersson et al., 1999; Bhatty, 1999). Interestingly, despite the significantly higher starch content in barley with normal starch polymer distribution, the 100 kernel weight of this group was not significantly higher than that of groups with anomalous starch.

Although high total β-glucan content is indicative of high dietary fiber content in barley, it is important to recognize that it is actually the soluble component of β-glucan that is responsible for the beneficial ability of β-glucans to lower serum cholesterol and blood glucose levels (McIntosh et al., 1991; Wood et al., 1994; Jenkins et al., 1995; Kahlon and Chow, 1997; Yokoyama et al., 1997). Therefore, it may not be enough to determine the content of total β-glucan in the grain when searching for the most suitable barley for human consumption. The content of soluble β-glucans found in the present investigation ranged from 1.16% to 3.55% (w/w), indicating 20.6−52.5% extractability. Marked differences in β-glucan extractability were observed among the four types of barley analyzed. Clearly, the β-glucans in waxy and zero amylose waxy barleys exhibited the highest extractability, ranging from 36.7% to 52.7% and from 34.0% to 52.5%, respectively. The extractability of β-glucans in normal barleys varied between 29.8% and 44.3% and in high amylose barleys between 20.6% and 29.7%. Because of the unexpectedly low solubility of these polymers in the high amylose barley, the correlation between the soluble and total β-glucans was poor ($r = 0.45$) (Figure 1). However, when the high amylose samples were excluded from consideration, the correlation improved ($r = 0.73$). Our results indicate that high amylose barley cultivars, despite their high content of total β-glucans, might not be the best source of soluble polysaccharides. Conclusions must, however, be drawn with caution. Because the solubility of β-glucans is dependent on extraction parameters, such as solvent type, temperature, and duration of extraction, substantial differences in solubility might be expected with changes of any of these parameters. It is prudent to expect, therefore, that if the extraction conditions in our studies were changed, the relative ranking of extractability of β-glucans among the four groups of barley might have been different. Solubility of β-glucans in oat and barley has been investigated in the past by many researchers, but the methodologies used were quite different and direct comparison of results is difficult. Also, most previous investigations dealt with hulled, malting barley rather than hulless genotypes. Recently Andersson et al. (1999) and Oscarsson et al. (1996), who extracted barley for 2 h at 38 °C, also reported relatively

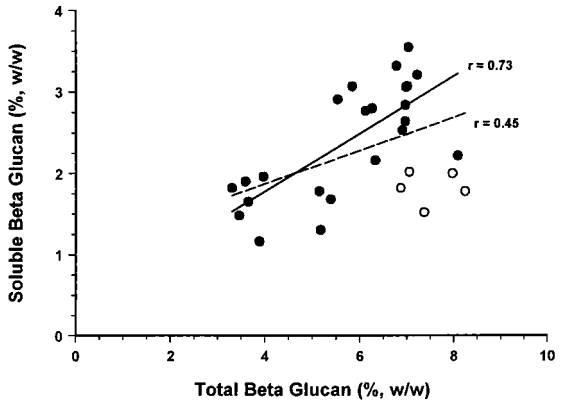

Figure 1. Relationship between the content of water-soluble and total β-glucans in hulless barleys. Open circles indicate the high amylose varieties, filled circles indicate the normal, waxy, and zero amylose waxy varieties. Dashed line indicates the linear regression line obtained for all barley varieties; solid line indicates the linear regression line obtained when high amylose varieties are excluded from calculations.

low extractability of β-glucans in two Swedish high amylose naked barleys (35.1% for cultivar Hashonucier and 36.7% for cultivar Glacier). On the other hand, Xue et al. (1991), who examined the solubility of β-glucans in waxy and normal barley isogenic lines, reported that waxy varieties, in contrast to the present study, had significantly lower amounts of soluble β-glucans. Bhatty et al. (1991) used acidic buffers and 1 h extraction at 40 °C, and reported an average of 44.7% extractability of β-glucans from 13 Canadian hulless barley genotypes.

Viscosity of Barley Slurries. It has recently been postulated that some biological benefits of β-glucans associated with reduction of plasma cholesterol and of postprandial serum glucose levels in humans and animals stem from their solubility in water and capacity to form highly viscous solutions (Schneeman, 1998). Viscosity is also one of the most important physical characteristics of food components affecting their functionality in the food system. Although viscosity properties of barley extracts containing β-glucans have been previously studied (Aastrup, 1979; Bhatty et al., 1991; Perez-Vendrell et al., 1996), those studies were prompted by problems caused by barley β-glucans in brewing and animal nutrition rather than by the potential benefits of β-glucans in the human diet. Traditionally, the determination of extract viscosity of barley was performed in acidic solutions to avoid interferences associated with endogenous β-glucanases. Good relationships between the viscosity of acid extracts and the amount of soluble and total β-glucans in barley have been generally obtained, and therefore, the viscosity of barley extracts is often routinely used as a predictor of β-glucan content. Aastrup (1979) reported that the logarithm of viscosity of acid extracts of malting barley was highly correlated with the extractable β-glucan content (r = 0.99) and with the total β-glucan content (r = 0.94). Bhatty et al. (1991) examined 13 varieties of hulless barley and also found a significant logarithmic relationship between acid extract viscosity of barley flour and its total β-glucan content (r = 0.94).

In our studies, we have monitored the changes in viscosity of barley flour slurries during a 2 h period of mixing at a constant shear rate (147 s^{-1}). The samples varied not only in the maximum attainable viscosity but also in the stability of the viscosity during measurements (Figure 2). Some samples exhibited a steady evolution of viscosity within the initial 20−30 min period, followed by a pseudo plateau region with very little change in viscosity (e.g., SR93139, SB94792, 92-55-06-48). These samples also attained relatively high final viscosity values. Other samples showed a similar initial rise in viscosity, but a decrease of viscosity thereafter. Preliminary studies have shown that starch granules and β-glucans were the two main contributors to overall barley flour slurry viscosity as the addition of both starch and β-glucan degrading enzymes drastically reduced viscosity (results not shown). The addition of the β-glucan degrading enzyme lichenase can cause an immediate decline in viscosity, whereas the action of α-amylase is somewhat hindered by the relatively slow enzymic hydrolysis of starch granules. Protease and xylanase had no effect on the viscosity of barley slurries, and it was concluded that proteins and arabinoxylans contribute little to the overall viscosity of barley flour slurries. The initial increase in viscosity during continuous mixing of the slurry was, therefore, ascribed mainly to the solubilization of β-glucans, with the subsequent viscosity decline attributed to the degradation of β-glucans by endogenous β-glucanases. Because α-amylase could also contribute to the decline of slurry viscosity, its effect was eliminated by the addition of silver nitrate ($AgNO_3$). Only samples which showed a rapid decrease in viscosity were somewhat affected by the addition of $AgNO_3$. Despite the slight elevation of viscosity values in the presence of $AgNO_3$, the general profile of viscosity development and decline remained the same for the samples with (results not shown) and without $AgNO_3$.

In an attempt to explain the differences in the maximum attainable viscosity (Table 2), the content of total and soluble β-glucans in the samples was examined. A better correlation was obtained between slurry viscosity and the amount of soluble β-glucans (r = 70) than between viscosity and total β-glucans (r = 0.49) (Figure 3). These results suggest, however, that factors other than β-glucan content might also affect the barley flour slurry viscosity. This was especially apparent for the high amylose barley samples, which, despite the relatively low content of extractable β-glucans, on average, attained much higher viscosities than the normal barley samples with only slightly lower β-glucan content. Also, the highest viscosity exhibited by one of the waxy samples, SR93139 a cultivar specifically selected for this trait (Rossnagel, University of Saskatchewan, personal communication), cannot be explained exclusively by the highest content of soluble β-glucan. Other waxy samples contained comparable or even higher amounts of this polymer but attained much lower viscosity values.

The presence of β-glucan-degrading enzymes could substantially affect the viscosity and stability of barley slurries. Two isoenzymes of (1→3)(1→4)-β-D-glucan 4-glucanohydrolase (referred to here as β-glucanase) have been found responsible for the degradation of barley β-glucan as judged by their ability to rapidly depolymerize the polysaccharide (Woodward et al., 1982). The enzymes are abundant in the endosperm of germinating barley. However, very low β-glucanase activity is expected in barley before germination, and little scientific data on the levels of β-glucanase in mature barley grains are available. Also, most tests suitable for detection of β-glucanase activity in malt might not be appropriate for detection of the very low

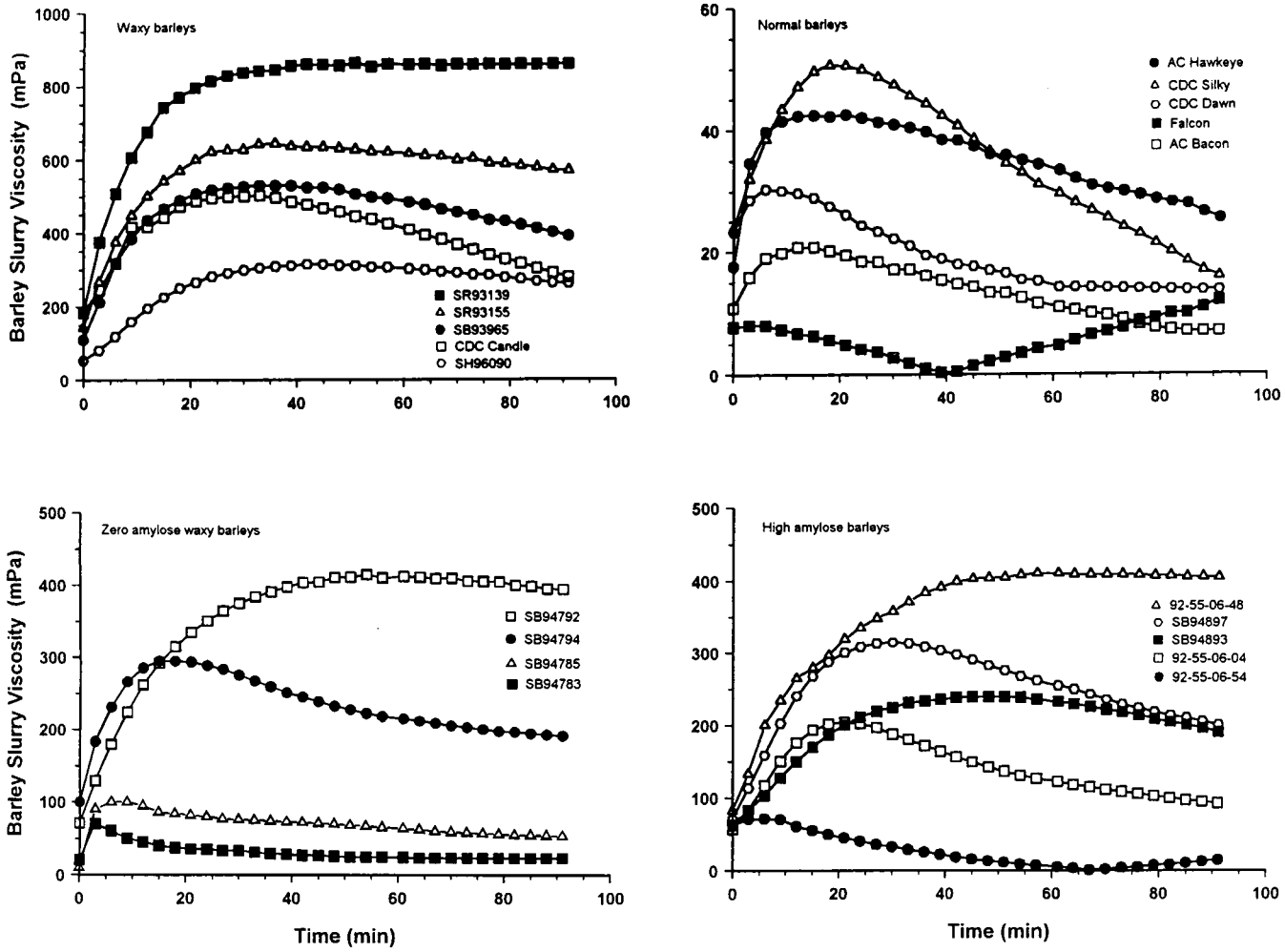

Figure 2. Development of viscosity of barley flour slurries (1:6 barley-to-water ratio) at 25 °C at a constant shear rate (147 s^{-1}).

levels of these enzymes in barley. Ellis et al. (1997) examined two malting varieties of barley grown in Scotland and Spain, and reported some genetic and environmental differences in the levels of β-glucanase activity, although the values were obviously very low compared to those reported for malt samples. Knuckles and Chiu (1999) reported insignificant differences in β-glucanase activity among 10 barley varieties grown in the United States. In both studies, β-glucanase was estimated by the method of McCleary and Shameer (1987), recommended for determination of the enzyme in malt. In our studies, β-glucanase activity in barley samples was estimated by determining the viscosity decline rate of β-glucan solutions after addition of aqueous barley extracts. This test allowed for a clear distinction between levels of activity of β-glucanase in the various samples (Table 2). The relatively high β-glucanase activity found in normal barley could probably explain the low viscosity values of the slurries and fast decline of viscosity upon mixing of barley flour with water. On this basis Falcon had almost twice as much β-glucanase activity as the other normal samples, and this was reflected in the extremely low viscosity of this sample. On the other hand, the very high and stable viscosities of the waxy SR93139, zero amylose waxy SB94792, and high amylose 92-55-06-48 samples corroborated well with the findings that these samples also contained the lowest levels of β-glucanase. The amount of β-glucanase activity in barley samples seems to be a very important factor affecting the overall viscosity of barley slurries. Therefore, if barley is to be incorporated into food systems and we are to take full advantage of the biological action of β-glucans, we should ensure that β-glucanase activity in barley is arrested and β-glucans are not degraded by enzymic activity during food preparation, processing, or storage.

It is well-known that the molecular weight of polymers is one of the principal properties determining their viscosity. It is, therefore, possible that intrinsic differences in the molecular weight of β-glucans contributed to the differences in the viscosity of barley flour slurries. In fact, when the molecular weight of β-glucans isolated and purified from several different barleys was assessed by the size exclusion chromatography, substantial differences were observed. However, prior to the evaluation of molecular weight of isolated β-glucans, inactivation of endogenous β-glucanases must be ensured.

Effect of Thermal, Enzymic, and Physical Treatments of Barley on Extractability and Viscosity of β-Glucans. Since it is postulated that solubility and viscosity of β-glucans are responsible for the biological activity of these polymers, several thermal, enzymic, and physical treatments of barley grain were assessed as potential strategies for increasing the level and viscosity of β-glucans in barley extracts. The two hydrothermal treatments, autoclaving and steaming, had substantial effects on the viscosity developments of barley slurries (Figure 4). The samples obtained much higher maximum viscosity values than the control samples, and no detectable decline of viscosity was

Table 2. Maximum Attainable Viscosity of Barley Slurries and β-Glucanase Activity in Barley Grains

sample	maximum viscosity of barley slurry[a] (mPas)	β-glucanase activity[b]
Normal Starch Genotypes		
Falcon	8 ± 1	0.0150
SB90354	140 ± 5	0.0073
CDC Dawn	30 ± 2	0.0077
SR93102	50 ± 3	0.0077
CDC Silky	50 ± 2	0.0074
Condor	20 ± 3	0.0073
CDC Gainer	89 ± 5	0.0073
Bear	12 ± 2	0.0077
AC Hawkeye	42 ± 3	0.0077
AC Bacon	20 ± 3	0.0067
High Amylose Genotypes		
92-55-06-54	71 ± 5	0.0065
92-55-06-04	203 ± 8	0.0063
92-55-06-48	410 ± 5	0.0036
SB94893	240 ± 8	0.0056
SB94897	313 ± 9	0.0055
Waxy Genotypes		
CDC Candle	403 ± 5	0.0042
SB94917	120 ± 6	0.0039
SB93965	529 ± 8	0.0046
SR93135	644 ± 9	0.0053
SR93139	850 ± 9	0.0024
SH96054	630 ± 10	0.0045
SH96076	320 ± 8	0.0043
SH96090	313 ± 9	0.0043
SH96093	530 ± 8	0.0045
SB95134	518 ± 10	0.0045
Zero Amylose Waxy Genotypes		
SB94785	75 ± 5	0.0065
SB94783	60 ± 6	0.0101
SB94792	416 ± 9	0.0041
SB94794	295 ± 10	0.0057

[a] Maximum attainable viscosity of barley slurries (1:6 ratio of barley to water) obtained at 25 °C at shear rate of 147 s^{-1}. [b] Reported as a change of relative viscosity of control β-glucan solutions per minute upon addition of barley extracts.

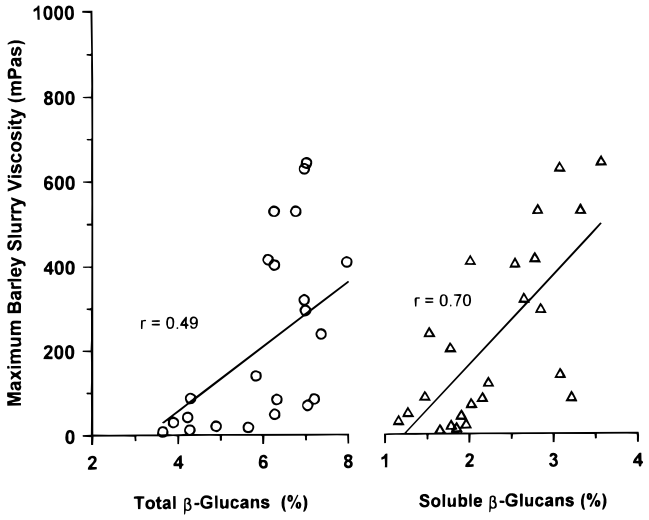

Figure 3. Relationship between the maximum attainable viscosity of barley slurries and the content of water-soluble and total β-glucans in hulless barleys.

observed thereafter. Roasting grain at 100 °C, on the other hand, did not result in any increase of viscosity and only slightly slowed its decline. Autoclaving in conjunction with enzyme addition, esterase (3 U/mL barley slurry) and proteinase K (2 U/mL barley slurry) during mixing experiments resulted in slurries exhibiting slight but steady increases of viscosity throughout the measurement time (100 min). This observation most likely indicates a continuous solubilization of β-glucans in the presence of enzymes. Autoclaving in conjunction with a physical disruption (ultrasonication) resulted in slurries with very high initial viscosities, indicating the solubilization of β-glucans prior to measurements. It should be noted that starch was not gelatinized during the heat treatments as assessed by differential scanning calorimetry and rapid viscoanalysis tests (results not shown). The changes in the slurry viscosity must be, therefore, attributed to changes in the amount and/or characteristics of β-glucans.

Interestingly, when the amount of soluble β-glucan was assessed in the treated samples, it became evident that the two hydrothermal treatments did not increase the amount of extractable polymers (Figure 5). The increase of the viscosity resulted rather from the much higher molecular weights of β-glucans extracted from the treated versus untreated samples, as evidenced by the profiles obtained from size-exclusion chromatography (Figure 6). These results indicate, therefore, that although hydrothermal treatments did not significantly affect the solubility of β-glucans, they had a positive effect on the molecular weight of β-glucans. This improvement was likely due to inhibition of β-glucanase activity and subsequent prevention of polymer degradation. The elution pattern of β-glucans from the untreated Falcon barley, with very high activity of β-glucanase, indicated the presence of a polysaccharide population with relatively small molecular weights. The autoclaved samples, on the other hand, which exhibited a definite shift toward higher molecular weights, had no β-glucanase activity. In fact, no detectable β-glucanase activity was found in any of the autoclaved or steamed samples as determined by the viscosity tests utilized in our studies. These results do not agree with the findings of Knuckles and Chiu (1999), who reported some residual β-glucanase activity in autoclaved barley samples. Discrepancies might have arisen from the use of different tests used to assess the activity of the enzyme in both studies as well as from different heat treatment conditions. It appears that the presence of moisture during the heat treatments was crucial for effective inactivation of β-glucanase, as application of dry heat (roasting) did not have the same effect (Figure 4). It is known that heat penetration of moist air is much faster and occurs to a greater extent than that of dry air and might, therefore, more effectively infuse into the kernel, accomplishing inactivation of β-glucanase not only on the surface but also inside the kernel. Furthermore, enzymes are less heat stable in moist than in dry environments. The hydrothermal treatments of barley grains might, therefore, constitute an effective means of preventing the depolymerization of β-glucans, and should be considered as a potential pretreatment of barley before incorporation of the grain into food systems. Various aspects and conditions of hydrothermal treatments should, however, be investigated more thoroughly to optimize desirable effects. Hydrothermal treatments of barley for animal feed, on the other hand, should be avoided as the degradation of β-glucans in this case is desirable and might improve the feed utilization by animals. According to the recent study of Vukic Vranjes and Wenk (1995), a significant depression in feed efficiency, feed apparent metabolizable energy, and fat and protein utilization resulted from feeding chickens extruded barley. The results were explained by higher amounts of soluble fiber and higher extract viscosities in heat treated versus untreated barleys. These results agree with our observations.

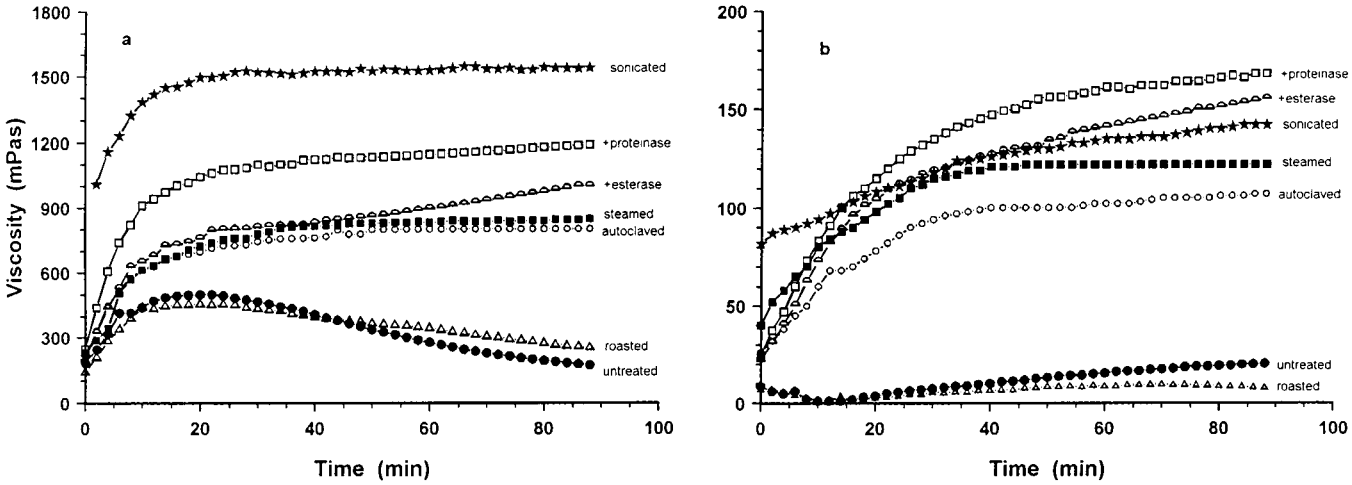

Figure 4. Effects of various treatments on the development of viscosity of barley slurries: (a) CDC Candle, (b) Falcon.

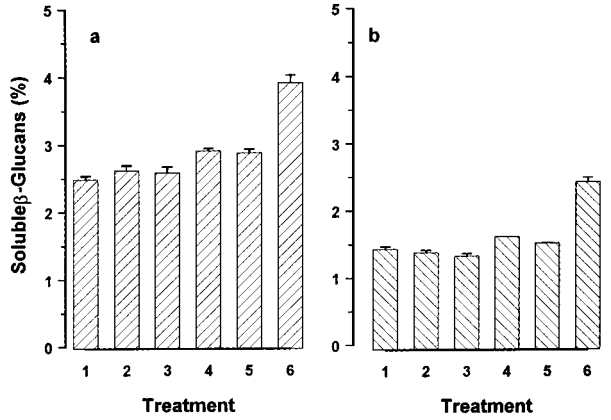

Figure 5. Effects of various treatments on the solubility of β-glucans in CDC Candle (a) and Falcon (b) barleys. Treatments: (1) control; (2) autoclaved, 30 min; (3) steamed, 15 min; (4) autoclaved followed by addition of esterase; (5) autoclaved followed by addition of proteinase K; and (6) autoclaved followed by sonication.

Hydrothermal treatments, aided by the presence of enzymes during extraction or by the additional physical disruption (sonication), resulted not only in improved molecular weight of β-glucans but also in increased β-glucan solubilization. The soluble β-glucan content of in the sonicated samples or in samples treated with esterase or proteinase K was greater than that in the untreated, autoclaved, or steamed samples (Figure 5). The elution profiles of these samples also indicated the presence of an additional population of polymers, as evidenced by broader and more numerous peaks (Figure 6). This study has demonstrated that with the aid of appropriate enzymes and/or physical disruption of the cell wall material, it is possible to increase the pool of soluble β-glucans in barley grain. So far, the reasons for water insolubility of β-glucans have not been fully understood. Although it has been speculated that ester linkages might be responsible for rendering a portion of β-glucans insoluble, the nature of such linkages has not yet been revealed (Bamforth et al., 1996). Also, the partial insolubility of these polymers has been attributed to the presence of non covalent interactions between β-glucans and other cell wall components (Izydorczyk et al., 1998). It seems, therefore, that the disruption of either covalent or noncovalent bonds within barley cell wall material might bring about the release and solubilization of the initially insoluble

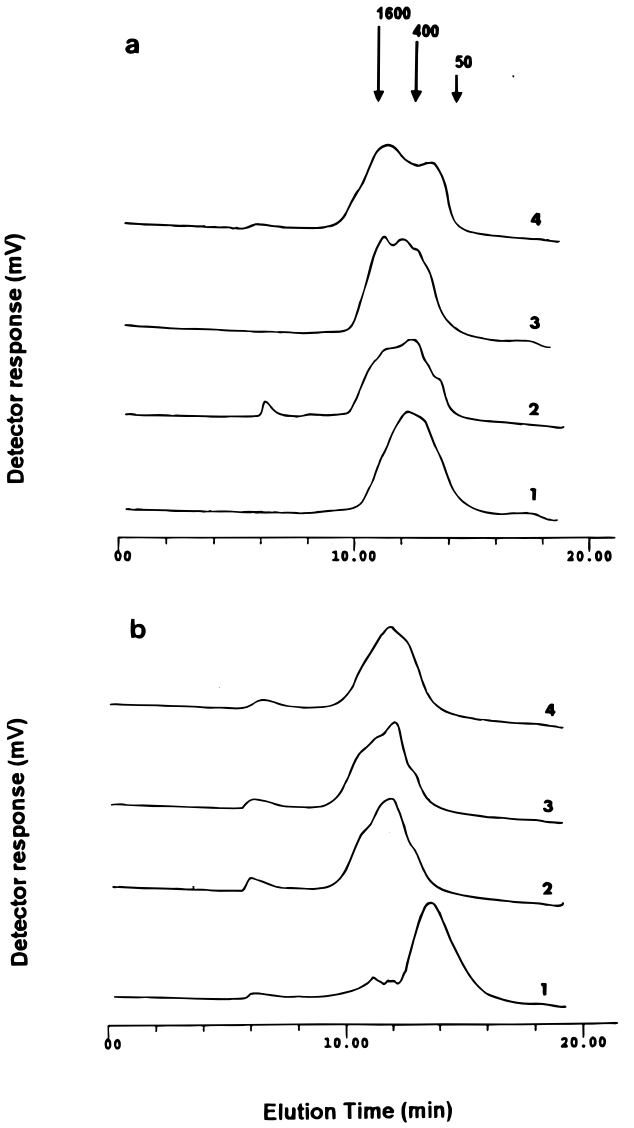

Figure 6. Effects of various treatments on the molecular weight of β-glucans isolated from CDC Candle (a) and Falcon (b) barley samples. Treatments: (1) control; (2) autoclaved, 30 min; (3) autoclaved followed by addition of proteinase K; (4) autoclaved followed by sonication. Arrows indicate elution time of pullulan standards with various molecular weight (MW): 1600, MW 1660 $\times$ 10^3; 400, MW 380 $\times$ 10^3; and 50, MW 48 $\times$ 10^3.

β-glucans. The improved extractability of β-glucans from barley, through proteolytic actions, also implies an association of a portion of β-glucans with proteins in the cell wall materials of barley.

CONCLUSION

Large variations in the chemical and physical characteristics of a range of barley samples were found in this study. Significant differences in the content of starch, total and soluble β-glucans, β-glucanase activity, 100 kernel weight, and slurry viscosity were observed among the samples. Samples with normal starch composition had significantly higher starch but lower total β-glucan content than barley containing starches with anomalous amylose-to-amylopectin ratios. A relatively poor correlation between the soluble and total β-glucans ($r = 0.41$) was attributed mainly to the high amylose barley samples, which, despite the highest content of total β-glucans, exhibited very low extractability of these polymers in aqueous media. Viscosity development profiles, as measured in this study, cannot be explained by the extractability and β-glucan content only. The presence of β-glucanase in barley samples had a significant effect on the molecular weight of β-glucans and thus on viscosity development profiles.

The extractability and physical characteristics of barley β-glucans can be modified by thermal, enzymic, and physical treatments of barley grain. Hydrothermal treatments of barley had no effect on the extractability of β-glucans from barley but prevented fragmentation of β-glucans due to enzymic hydrolysis and thereby substantially improved their molecular weight and, consequently, their viscosity. These treatments, therefore, might have a potential to positively affect the physiological responses to barley β-glucans in human diets. The enzymic and physical treatments were, on the other hand, more important in accounting for the increased extractability of β-glucans. Considering that it is soluble dietary fiber that can induce the desirable physiological effects in humans, it is essential to investigate, in greater detail, potential strategies for improving the ratio of soluble to insoluble dietary fiber in barley.

LITERATURE CITED

Aastrup, S. The relationship between the viscosity of an acid flour extract of barley and its β-glucan content. *Carlsberg Res. Commun.* **1979**, *44*, 289−304.

Andersson, A. A. M.; Elfverson, C.; Andersson, R.; Regner, S.; Aman, P. Chemical and physical characteristics of different barley samples. *J. Sci. Food Agric.* **1999**, *79*, 979−986.

Bamforth, C. W.; Moore, J.; Proudlove, M. O.; Bartholome, B.; Williamson, G. The dissolution of β-glucans. New dimensions. Abstract. *J. Inst. Brew.* **1996**, *102*, 313.

Bhatty, R. S. β-Glucan and flour yield of hull-less barley. *Cereal Chem.* **1999**, *76*, 314−315.

Bhatty, R. S.; MacGregor, A. W.; Rossnagel, B. G. Total and acid-soluble β-glucan content of hulless barley and its relationship to acid-extract viscosity. *Cereal Chem.* **1991**, *68*, 221−227.

Ellis, R. P.; Swanston, J. S.; Rubio, A.; Perez-Vendrell, A. M.; Romagosa, I.; Molina-Cano, J. L. The development of β-glucanase and degradation of β-glucan in barley grown in Scotland and Spain. *J. Cereal Sci.* **1997**, *26*, 75−82.

Henry, R. J.; Brown, A. H. D. Variation in the carbohydrate composition of wild barley (*Hordeum spontaneum*). *Plant Breeding.* **1987**, *98*, 97−103.

Izydorczyk, M. S.; Macri, L. J.; MacGregor, A. W. Structure and physicochemical properties of barley non-starch polysaccharides − II. Alkali-extractable β-glucans and arabinoxylans. *Carbohydr. Res.* **1998**, *35*, 259−269.

Jadhav, S. J.; Lutz, S. E.; Ghorpade, V. M.; Salunkhe, D. K. Barley: Chemistry and value-added processing. *Crit. Rev. Food Sci.* **1998**, *2*, 123−171.

Jenkins, D. J. A.; Jenkins, A. L.; Wolever, T. M. S.; Vuksan, V.; Venket Rao, A.; Thompson, L. U.; Josse R. G. Effect of reduced rate of carbohydrate absorption on carbohydrate and lipid metabolism. *Eur. J. Clin. Nutr.* **1995**, *49*, S68−S73.

Kahlon, T. S.; Chow, F. I. Hypocholesterolemic effects of oat, rice, and barley dietary fibers and fractions. *Cereal Food World* **1997**, *42*, 86−92.

Knuckles, B. E.; Chiu, M.-C. M. Beta-glucanase activity and molecular weight of beta-glucans in barley after various treatments. *Cereal Chem.* **1999**, *76*, 92−95.

MacGregor, A. W.; Fincher G. B. Carbohydrates of the barley grain. In *Barley Chemistry and Technology*; MacGregor, A. W., Bathy, R. S., Eds.; American Association of Cereal Chemists: St Paul, MN, 1993; pp 73−130.

McCleary, B. V.; Shameer, I. Assay of malt β-glucanase using azo barley glucan: an improved precipitant. *J. Inst. Brew.* **1987**, *93*, 87−90.

McIntosh, G. H.; Newman, R. K.; Newman, C. W. Barley foods and their influence on cholesterol metabolism. *Plants Hum. Nutr.* **1995**, *77*, 89−108.

Newman, R. K.; Newman, C. W. Barley as a food grain. *Cereal Food World* **1991**, *36*, 800−805.

Oscarsson, M.; Andersson, R.; Salomonsson, A.-C.; Aman, P. Chemical composition of barley samples focusing on dietary fibre components. *J. Cereal Sci.* **1996**, *24*, 161−170.

Perez-Vendrell, A. M.; Brufau, J.; Molina-Cano, J. L.; Francesch, M.; Guasch, J. Effects of cultivar and environment on β-(1,3)−(1,4)-D-glucan content and acid extract viscosity of Spanish barleys. *J. Cereal Sci.* **1996**, *23*, 285−292.

Schoch, T. J. Iodometric determination of amylose. In *Methods in Carbohydrate Chemistry*; Whistlet, R. L., Ed.; Academic Press: New York, NY, 1967; Vol. 4, pp 157−160.

Schneeman, B. *Properties of nondigestible polysaccharides associated with health* (Abstract); Plant Polysaccharide Symposium; University of California: Davis, CA, 1998.

Vukic Vranjes, M.; Wenk, C. The influence of extruded vs. untreated barley in the feed, with and without dietary enzyme supplement on broiler performance. *Animal Feed Sci. Technol.* **1995**, *54*, 21−32.

Wood, P. J.; Braaten, J. T.; Scott, F. W.; Riedel, K. D.; Wolynetz, M. S.; Collins, M. W. Effect of dose and modification of viscous properties of oat gum on plasma glucose and insulin following an oral glucose load. *Brit. J. Nutr.* **1994**, *72*, 731−743.

Woodward, J. R.; Fincher, G. B. Substrate specificities and kinetic properties of two (1,3),(1,4)-β-D-glucan endo-hydrolases from germinating barley (*Hordeum vulgare*). *Carbohydr. Res.* **1982**, *106*, 11−122.

Yokoyama, W. H.; Hudson, C. A.; Knuckles, B. E.; Chiu, M.-C. M.; Sayre, R. N.; Turnlund, J. R.; Schneemam, B. O. Effect of barley β-glucan in durum wheat pasta on human glycemic response. *Cereal Chem.* **1997**, *74*, 293−296.

Xue, Q.; Newman, R. K.; Newman, C. W.; McGuire, C. F. Waxy gene effects on β-glucan, dietary fiber content and viscosity of barleys. *Cereal Res. Commun.* **1991**, *19*, 399−404.

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