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Analysis of Total and Insoluble Mixed-Linked (1→3),(1→4)-β-D-Glucans in Barley and Oats

Per Åman* and Hadden Graham

An enzymatic method for analysis of total mixed-linked (1→3),(1→4)-β-D-glucans (β-glucans), and insoluble β-glucans after extraction of soluble β-glucans with water for 2 h at 38 °C, has been developed. The method involves complete removal of starch, hydrolysis of β-glucans to glucose with a technical β-glucanase preparation, and analysis of formed glucose by the glucose oxidase method. Soluble β-glucans are calculated as the difference between total and insoluble β-glucans. Total and insoluble β-glucans were analyzed in barley samples from Montana and Scandinavia and in Swedish oat samples. The average content of total β-glucans in barley was 4.5%, with a range from 3.0 to 6.9%, and that in oats was 3.2%, with a range from 2.2 to 4.2%. In barley, on average, 54% of the β-glucans was soluble and in oats 80%.

Mixed-linked (1→3),(1→4)-β-D-glucans, referred to hereafter as β-glucans, are frequently present in endosperm cell walls of cereals (Anderson et al., 1978; Bacic and Stone, 1981). Compared to other cereals, barley and oats have relatively high contents of total β-glucans and figures between 2 and 10% for barley (Bamforth, 1982) and 2 and 4% for oats (Wood and Weisz, 1984) have been reported. The total content of β-glucans varies with both genetic and environmental factors (Hesselman, 1983; Henry, 1986). Both soluble and insoluble β-glucans are present in cereals with factors such as particle size, β-glucanase activity of the flour, and temperature, pH, and ionic strength of the extraction media affecting solubility (Wood et al., 1978; Ahluwalia and Ellis, 1985).

In the brewing industry a high content of β-glucans in the barley may lead to problems such as diminished rate of wort filtration, haze formation in the beer, and possibly reduced extraction efficiency (Bamforth, 1985; McCleary and Glennie-Holmes, 1985). β-Glucans can also have antinutritional properties, particularly in chicken diets where they may give sticky droppings and affect food intake, growth rate, and feed-conversion efficiency (Hesselman, 1983; Hesselman and Åman, 1986). Oat bran diets, rich in soluble fibers (β-glucans), may have distinct hypocholesterolemic effects in humans, with decreased serum and low-density lipoprotein cholesterol and increased fecal bile acid excretion (Kirkby et al., 1981; Anderson et al., 1984). Porridge oats have been shown to have a lowering effect on the glycemic index, indicating that β-glucan-rich products may be useful food ingredients for people with low blood glucose tolerance (Jenkins et al., 1981).

Insoluble β-glucans in grain cell walls encapsulate easily available nutrients such as starch, intracellular protein, and fat and act as a physical hindrance to nutrient hydrolysis and utilization, while soluble or solubilized β-glucans give rise to viscous solutions, which also may interfere with nutrient availability (Hesselman and Åman, 1986). In order to further evaluate the technical and nutritional effects of β-glucans in cereals, reliable methods for the

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quantitative estimation of soluble and insoluble β-glucans are necessary. Recently an enzymatic method for analysis of total β-glucans was published (Åman and Hesselman, 1985). In the present paper a more rapid modification of this method, which also allows quantification of insoluble β-glucans, and studies on the content and solubility of β-glucans in barley and oats are presented.

EXPERIMENTAL SECTION

Materials. Samples of covered spring-sown barley (n = 64) were grown in Scandinavia between 55 and 61° N and in Montana around 45° N and represent 21 cultivars and lines and 5 different growing seasons (1981–1985). Samples of covered spring-sown oats (n = 121) were grown in Sweden and represent 42 cultivars and lines, 15 localities from Skellefteå (65° N) in the north to Malmö (56° N) in the south and 3 different growing seasons (1981–1983). The samples were grown under commercial conditions and stored as whole kernels until analyzed.

Three purified β-glucan preparations were obtained from Biocon (Ireland), Grindstedt (Denmark), and the Technical Research Centre of Finland (VTT), respectively. Cellulose powder (CF 11) was purchased from Whatman (Sweden), curdlan [(1→3)-β-glucan] from Wasco (Japan), and soluble starch (M 1252) from Merck. Cotton lint cellulose and xyloglucan from tamarind seeds were a gift from Professor O. Theander (Uppsala) and wheat starch granules from Svalöv AB (Sweden). Arabinoxylan from barley straw had previously been isolated in this laboratory (Theander and Åman, 1978).

Analysis of Total and Insoluble β-Glucans. *Step 1a.* Cereal grain (about 50 g, moisture content around 10%) was ground in a Tecator Cyclotec sample mill to pass a 0.5-mm screen. Duplicate samples (200 mg) were weighed into 35-mL thick-walled Pyrex glass tubes with screw caps containing Teflon liners. Racks of 26 test tubes containing 24 test and two standard samples were used.

Step 1b. Soluble β-glucans were extracted with water (15 mL) for 2 h at 38 °C in a shaking water bath (75 rpm). The tubes were then centrifuged (1500g, 5 min), the supernatant was decanted, and the pellets were washed twice with water (15 mL) and recovered by centrifugation (1500g, 5 min).

Step 2. For analysis of total β-glucans the sample from step 1 and for insoluble β-glucans the pellet from step 1b was used. Sodium acetate buffer (0.1 M, pH 5.0, 5 mL) and thermostable α-amylase (EC 3.2.1.1.; Termamyl 120L, Novo A/S, Demark; 100 μL) were added, the tubes capped tightly, and the contents mixed. Immediately after mixing, the samples were incubated for 1 h in a boiling water bath, and during this time the tubes were shaken by hand on three occasions. After the mixtures were cooled to 40 °C, a suspension of amyloglucosidase from *Aspergillus niger* (EC 3.2.1.3.; Boehringer Mannheim; 14 U/mg, 100 μL) was added, and the tubes were capped, shaken, and incubated for 4 h at 60 °C in a shaking water bath (75 rpm).

Step 3. Soluble polymers were precipitated with absolute ethanol (21 mL) and the samples placed in a refrigerator overnight. The samples were then centrifuged (1500g, 5 min), the supernatant was decanted, and the pellets were washed twice with 80% ethanol (25 mL) and recovered by centrifugation (1500g, 5 min). After the last washing the test tubes were inverted on a filter paper for about 10 min.

Step 4. Sodium acetate buffer (0.05 M, pH 4.8, 25.0 mL) and a commercial β-glucanase preparation from *A. niger* (GV-L, Grindstedt, Denmark; 2 U/mL, 200 μL) were added to the insoluble residues. The capped tubes were mixed vigorously on a vortex mixer, incubated for 3 h at 50 °C in a water bath with shaker, and centrifuged (1500g, 5 min).

Step 5. The glucose concentrations in the supernatants were determined by the glucose oxidase method according to the suppliers manual (Merkotest 3395, Merck). The total or insoluble β-glucans were calculated from the amount of free glucose in the supernatants.

Analysis of Degraded β-Glucans by HPLC. Barley (cv. Tellus, 200 mg), oats (cv. Selma, 200 mg), or β-glucan (Biocon, 10 mg) was treated as described in the procedures for enzymatic analysis of total β-glucans by Åman and Hesselman (1985) using purified β-glucanase (Hydrozyme, 6.4 EU/mL; Norsk Hydro, Norway) or as described here (steps 1–4) using GV-L from Grindstedt. Supernatants containing degraded β-glucans were isolated by centrifugation and evaporated (<40 °C) to dryness. The samples were dissolved in water (0.5 mL) and filtered (0.45 μm, Millipore), and qualitative analyses were performed on a Waters liquid chromatograph as described by Cheetham et al. (1981). The Dextropak plastic cartridge column (Waters, 10 × 1 cm) was pressurized in a Z-radial compression module and eluted with water.

Effect of Extraction Time on Solubility. Barley (cv. Tellus) or oat (cv. Selma) samples (200 mg) were weighed into test tubes as described in step 1 above. Soluble β-glucans were extracted with distilled water (15 mL) or HCl–KCl buffer (0.1 M, pH 1.5, 15 mL) according to Greenberg and Whitmore (1974) in a shaking water bath at 38 °C or ultrasonic bath for five different periods of time. The water temperatures in the ultrasonic bath were 23, 27, 30, 36, 41, and 45 °C at zero time and the five sampling times, respectively. Soluble β-glucans were immediately removed by centrifugation and the contents of insoluble β-glucans in the residue analyzed as described above.

Effect of Extraction Temperature on Solubility. Barley samples (200 mg) were weighed into test tubes as described in step 1 above. Soluble β-glucans were extracted with distilled water (15 mL) or HCl–KCl buffer (0.1 M, pH 1.5, 15 mL) for 2 h at temperatures between 20 and 100 °C. During the incubation time samples were shaken after 15, 60, and 90 min on a vortex mixer. Soluble β-glucans were removed by centrifugation and the contents of insoluble β-glucans in the residues analyzed as described above.

Statistical Analysis. The statistical analysis were performed with use of the Statistical Analysis System (SAS, 1982).

RESULTS AND DISCUSSION

Analysis of Total β-Glucans. A major problem with enzymatic methods for the determination of β-glucans is to obtain enzyme preparations free from amylase and, to a lesser extent, cellulase activity. This problem may be overcome by the prior removal of starch, followed by β-glucan degradation with a cellulase-free enzyme preparation as described in Åman and Hesselman (1985). However, the enzyme preparation utilized in that method degraded β-glucans to a mixture of oligosaccharides of which the two major had retention times similar to maltodextrins with DP 6 and 7, respectively (Figure 1A). Thus, a hydrolysis step was necessary to complete the degradation to glucose. As shown in Figure 1B, however, another β-glucanase preparation (GV-L, Grindstedt) has been found that completely degraded isolated β-glucans and β-glucans in the cell walls of barley or oats to glucose.

Steps 1–3 in the described method for total β-glucan analysis have previously been studied by Åman and Hesselman (1985). These studies established complete

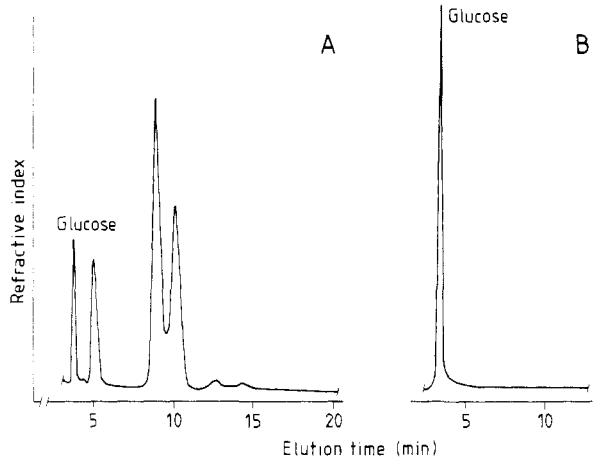

Figure 1. *Fractionation of hydrolyzed β-glucan by HPLC on* Dextropak. The column was eluted with water. Key: A, β-glucan hydrolyzed with β-glucanase from Norsk Hydro; B, β-glucan hydrolyzed with β-glucanase from Grindstedt.

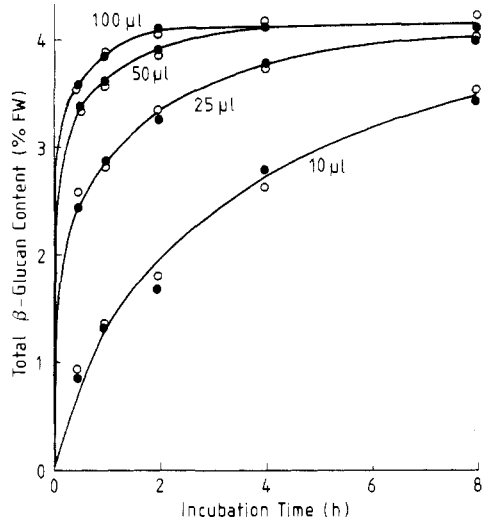

Figure 2. Analysis of total β-glucans in barley (cv. Tellus) using hydrolysis with different amounts (10, 25, 50, 100 µL) of β-glucanase from Grindstedt (GV-L, 2 U/mL) for different periods of time and by two different analytical methods for the determination of released glucose: ●, glucose oxidase method; ○, *hexokinase/glucose 6-phosphate dehydrogenase method.*

degradation of starch and complete recovery of soluble and insoluble β-glucans. During step 4 it is important that the β-glucanase used is capable of completely degrading the total β-glucans in the sample. In previous studies the β-glucanase from Grindstedt has been shown to degrade both soluble and insoluble β-glucans by chemical (De Silva et al., 1983) and microscopic methods (Hesselman and Åman, 1985).

Variable amounts of β-glucanase, different incubation times, and two different methods for analyzing released glucose were investigated when a barley sample was analyzed for total β-glucans (Figure 2). It was shown that incubation with 100 µL of β-glucanase for 2 h ensured maximal yield of total β-glucan in the analysis and that incubation for 30 h did not significantly change the yield. The same results were also obtained when released glucose was measured with the Merck glucose oxidase or the Boehringer Mannheim hexokinase/glucose 6-phosphate dehydrogenase methods according to the suppliers manuals.

Different polysaccharide preparations were analyzed with the described method for total β-glucans in order to evaluate the specificity of the method. Three different

Table I. Analyzed Content of Total β-Glucan in Different Polysaccharide Preparations (% of Sample ± SEM)

polysaccharide	content[a]
β-glucan (Biocon)	84.6 ± 0.8
β-glucan (Grindstedt)	94.0 ± 0.3
β-glucan (VTT)	97.0 ± 0.3

[a] $n = 4$.

Table II. Precision of the Described Method for Analysis of Total and Insoluble β-Glucans in Barley (cv. Tellus) and Oats (cv. Selma)

	barley			oats	
	total	total[a]	insol	total	insol
no. of replicates	24	19	24	24	24
mean value (% dry grain)	4.2	3.8	1.4	3.0	0.65
range of values	4.1–4.3	3.5–3.9	1.3–1.4	2.9–3.2	0.60–0.69
coeff of variation, %	1.9	3.2	2.8	2.6	3.7

[a] For comparison the barley sample was also analyzed for total β-glucans by the method of Åman and Hesselman (1985).

Table III. Analysis of Total β-Glucans (% Dry Grain) in a Barley Sample (cv. Tellus) Using the Described Method (A) and Modifications Thereof (B and C)

procedure	content[c]
A	4.17 ± 0.01
B[a]	4.10 ± 0.02
C[b]	3.82 ± 0.02

[a] B = precipitation of the centrifuged hydrolysate from step 4 (5.2 mL) with absolute ethanol (21 mL) and determination of free glucose in the supernatant. [b] C = part of supernatant from B hydrolyzed with trifluoroacetic acid (2.0 M, for 2 h at 125 °C) and free glucose in the hydrolysate determined [see Åman and Hesselman (1985)]. [c] $n = 8$; ±SEM.

β-glucan preparations were found to contain 85–97% of total β-glucans (Table I). Analysis of these preparations by the dietary fiber method of Theander and Westerlund (1986) indicated complete recovery of glucose residues as β-glucans. Arabinoxylans, cellulose, and starch are major carbohydrates in barley and oats and do not interfere with the method since only very small amounts of these polysaccharide preparations were analyzed as β-glucan (<0.1%). It is, however, important to ensure complete degradation of starch and complete resistance of cellulose, as these polysaccharides, like β-glucans, contain glucose units that could easily interfere in the analysis. Curdlan and xyloglucan, which also contain glucose units, both contained about 1% of material analyzed as β-glucan. During the analysis these polysaccharides formed strong insoluble gels that probably diminished accessibility for the enzymes involved in the analysis. However, these polysaccharides are known not to be present in significant amounts in barley or oats. Blank determinations, without addition of the β-glucanase or the grain sample during the analysis, revealed no detectable color in the glucose oxidase assay.

The precision of the described method was estimated on one barley and one oat sample that contained 4.2 and 3.0%, respectively, of total β-glucans (Table II). The coefficient of variation was significantly higher for oats (2.6%) compared to barley (1.9%). For comparison, the precision of the previously published method (Åman and Hesselman, 1985) was also investigated for the barley sample. This method gave a somewhat lower mean value for total β-glucans and a significantly higher coefficient of variation. Purification of the hydrolysate in step 4 by ethanol precipitation did not significantly change the yield of total β-glucans, while acid hydrolysis of the hydrolysate

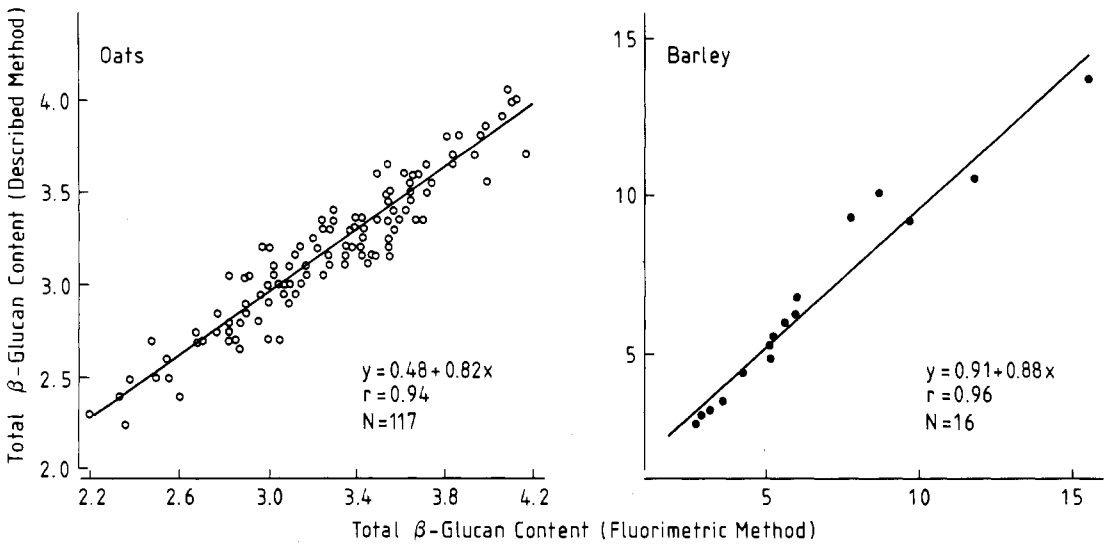

Figure 3. Plot of total β-glucans in barley and oats (% DM) determined by the described enzymatic method vs. a fluorimetric method using Calcofluor.

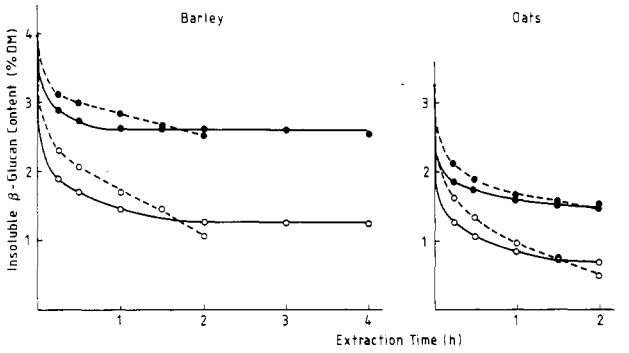

Figure 4. Content of insoluble β-glucans in barley and oats (% DM) after extraction of soluble β-glucans with water (○) or acidic buffer, pH 1.5 (●). The extractions were carried out in a water bath at 38 °C (—) or in an ultrasonic bath (- - -) for different periods of time.

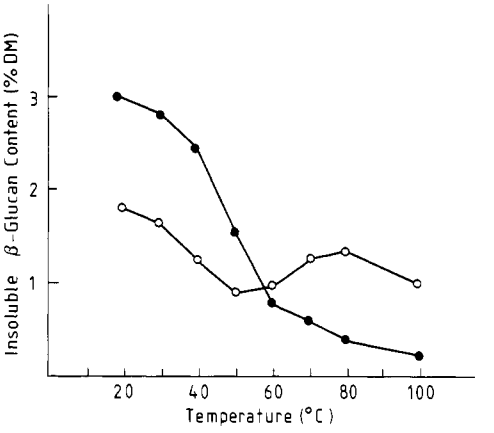

Figure 5. Content of insoluble β-glucans in barley (% DM) after extraction of soluble β-glucans with water (○) or acidic buffer, pH 1.5 (●), at different temperatures.

reduced the yield by about 8% (Table III). These results indicate that reversion or other losses during the acid hydrolysis step employed by Åman and Hesselman (1985) may, at least partly, account for the difference between the two methods.

The described method for total β-glucan analysis was compared with a very rapid fluorometric method using the specific binding of Calcofluor to β-glucans and flow injection analysis. Close correlations between the two methods were obtained both for 16 barleys with very different β-glucans content and for 117 oats (Figure 3).

Analysis of Soluble and Insoluble β-Glucans. Soluble β-glucans can be extracted with a range of different methods. It is evident, however, that pretreatment and extraction conditions will have a great influence on the yield of soluble β-glucans (Ahluwalia and Ellis, 1985). When a barley or oat sample was extracted with water at 38 °C, considerably more β-glucans were solubilized compared to when extracted with an acidic buffer (pH 1.5) formulated to inactivate endogenous enzymes (Figure 4). In a water bath at 38 °C, soluble β-glucans were completely extracted after about 1 h in acidic buffer but not until after 2 h with water, indicating the importance of endogenous enzymatic activity. Extraction with ultrasonic treatment showed a steadily decreasing yield of insoluble β-glucans with time for both water and acidic buffer. This is probably mainly an effect of temperature since ultrasonic treatment generates heat, which increased the bath temperature from 23 to 45 °C over the 2-h experimental period.

The effect of temperature on the solubility of β-glucans was further studied in an experiment where a barley sample was incubated with water or acidic buffer (pH 1.5) (Figure 5). When extracted with water, the yield of insoluble β-glucans decreased from 1.8 to 0.9% as the temperature increased from 20 to 50 °C. Thereafter, the yield increased to 1.3% at 80 °C and decreased again to 1.0% at 100 °C. This curve is probably a combination of the increased solubility at elevated temperature and the deactivation of endogenous β-glucanases in the barley at temperatures above 50 °C. When extracted with acidic buffer the yield of insoluble β-glucans decreased rapidly from 3.0 to 0.2% when the temperature increased from 20 to 100 °C. This increased solubility of β-glucans is probably partly due to acid hydrolysis at elevated temperature. The effect of endogenous enzymes would only be of limited importance at this pH.

From a physiological point of view extraction at body temperature, 38 °C, would seem appropriate, and as seen in Figure 4, maximum yield of soluble β-glucans is obtained after 2 h. Thus, for routine determinations of soluble and insoluble β-glucans in barley and oats, extraction with water for 2 h at 38 °C is used.

Content of β-Glucans in Barley and Oats. Covered barleys from Scandinavia contained, on average, 4.4%

Table IV. Variation in Content of Total, Insoluble, and Soluble β-Glucans (% Dry Grain) and in Soluble β-Glucans as Percent of Total β-Glucans of Scandinavian (n = 51) and Montana (n = 13) Barleys and Swedish Oats (n = 121)

β-glucan constituent	mean value	range of values	coeff of variation, %
	Scandinavian Barley		
total	4.4	3.0–5.6	11
insoluble	2.0	1.2–2.7	18
soluble	2.4	1.3–3.4	20
soluble, % of total	55	38–69	14
	Montana Barley		
total	5.2	4.0–6.9	15
insoluble	2.6	1.9–3.3	16
soluble	2.6	1.6–4.0	28
soluble, % of total	49	38–61	16
	Oats		
total	3.2	2.2–4.2	12
insoluble	0.64	0.30–1.1	22
soluble	2.5	1.5–3.6	16
soluble, % of total	80	65–90	6.5

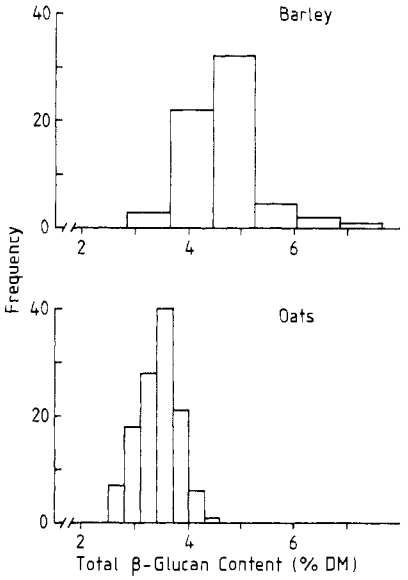

Figure 6. Histograms of the frequency of classes of barley (n = 64) or oats (n = 121) with different content of total β-glucans (% DM).

total, 2.0% insoluble, and 2.4% soluble β-glucans. Between 38 and 69% of the β-glucans were soluble in water at 38 °C (Table IV). The contents of total and insoluble β-glucans and the solubility of the β-glucans were somewhat higher in the covered Montana barleys compared to the barleys from Scandinavia, while the content of soluble β-glucans was more similar. The coefficients of variation were higher for insoluble and soluble β-glucans than for total β-glucans.

The average content of total β-glucans in this investigation (4.4%) is higher than the previously published average content (3.6%) in Swedish barley (Åman, 1986). As discussed in the present paper, however, the method used in that investigation will give a somewhat lower result. The short, dry growing season in Montana, resulting in a rapid maturation of the barley crop, may explain the differences in β-glucan contents between barleys from Scandinavia and Montana, although differences in genotype and agricultural practice also may be important. Barleys from Sweden and Montana have a different gross chemical composition, with a higher crude protein, lower starch, and similar total fiber contents in the Montana barleys (Åman and Newman, 1985).

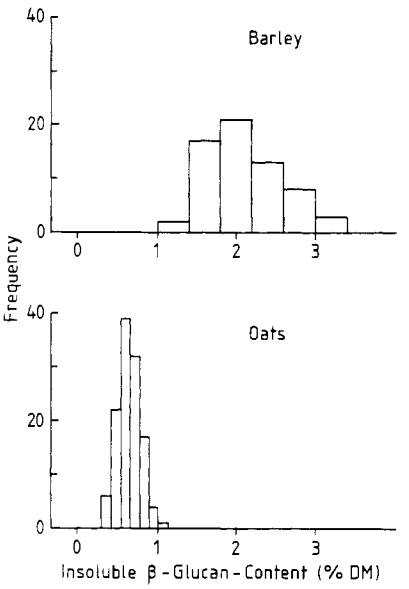

Figure 7. Histograms of the frequency of classes of barley (n = 64) or oats (n = 121) with different content of insoluble β-glucans (% DM).

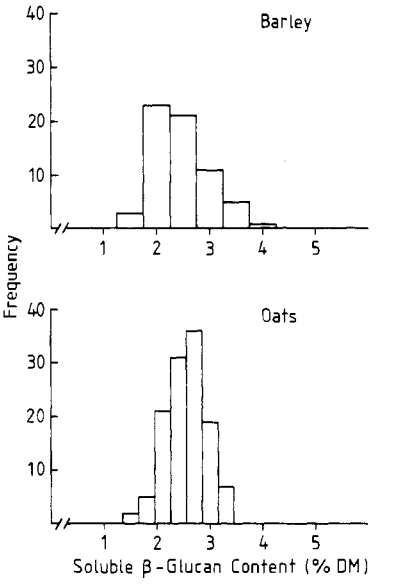

Figure 8. Histograms of the frequency of classes of barley (n = 64) or oats (n = 121) with different content of soluble β-glucans (% DM).

Compared to barley, the oat samples contained less total and insoluble β-glucans but similar amounts of soluble β-glucans (Table IV). The solubility of the β-glucans in oats was very high, on average 80%. The coefficients of variation for the different types of β-glucans were similar to those of barley.

Histograms of the frequency of classes of barley with different amounts of total β-glucans in oats and barley, including both the barley from Scandinavia and Montana, are presented in Figure 6. A higher average content and a wider variation in barley were evident. This difference between barley and oats was mainly due to the higher content and wider variation of insoluble β-glucans in the former (Figures 7 and 8). Evident differences in the solubility in water at 38 °C between barley and oat β-glucans were also notable, with a range between 38 and 69% for barley β-glucans and between 65 and 90% for oat β-glucan (Figure 9).

General Discussion. An improved enzymatic method for analysis of total β-glucans in cereals has been devel-

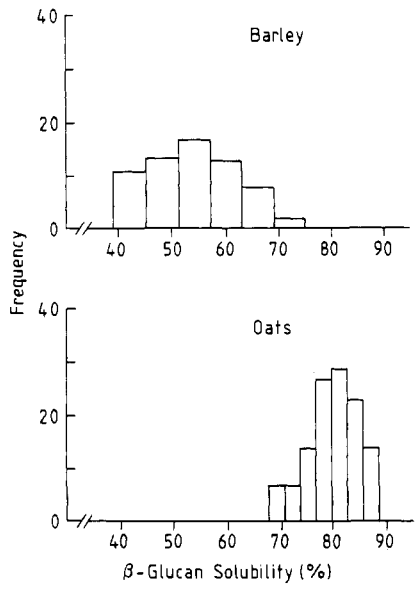

Figure 9. Histograms of the frequency of classes of barley (n = 64) or oats (n = 121) with different solubility of the β-glucans (soluble β-glucans as percent of total β-glucans).

oped. With our facilities 25 samples can be analyzed in duplicate per day. No sophisticated equipment is needed, and the method is accurate and has high precision.

Different extraction procedures for β-glucans gave very variable yields, and factors such as temperature, pH, and endogenous enzyme activity were found to be of great importance. Extraction of soluble β-glucans in barley and oats for 2 h at 38 °C produced a stable value, and extraction for a longer period of time did not significantly increase the yield. When the effects of soluble β-glucans in the upper gut are considered, these extraction conditions may be a reasonable physiological compromise and have been used in this investigation. After the extraction of soluble β-glucans, insoluble β-glucans were analyzed in the residue by the same procedure as for total β-glucans and soluble β-glucans calculated as the difference between the total and insoluble components. A wide variation was found in both content and solubility of β-glucans in covered barleys. However, barleys with an even greater divergence in β-glucan contents are found, for example, a nude, high-protein line from Montana (Prowashonupana) that contained 13.5% total and 10.4% insoluble β-glucans.

The wide variation that exists in both content and solubility of β-glucans has a large influence on the nutritional and technical properties of the grains. With accurate analytical procedures it should be possible in the future to better utilize these differences when evaluating or selecting grain or grain fractions for feed, food, or industrial uses.

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Registry No. β-Glucan, 9041-22-9; glucose oxidase, 9001-37-0; glucanase, 9074-98-0.

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