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Hydrolysis of β-glucan

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Abstract

The acid and enzymatic hydrolyses of oat β-glucan were compared for commercial oat β-glucan and β-glucan isolated from oat grain and oat bran. The resulting mono- and oligosaccharides were analysed by high-performance anion-exchange chromatography, combined with pulsed amperometric detection. The acid hydrolysis was studied with HCl, TFA and H_2SO_4 at two concentrations, with three durations of hydrolysis and at three temperatures.

Under the mildest acid conditions (37 °C and pH 1, corresponding to those in the human stomach) no degradation of β-glucan was observed with HCl over a 12 h period. At 120 °C, total hydrolysis to glucose occurred with high-concentration acids. Total recovery was also achieved with lichenase hydrolysis. Hydrolysis, with lichenase and β-glucosidase together, produced glucose, which was analysed spectrophotometrically according to the AOAC 995.16 method. Since the results with the original method were not satisfactory, the method was further modified to determine the best conditions for samples with high β-glucan content and poor solubility.

Keywords: Acid hydrolysis; Enzymatic hydrolysis; β-Glucan; HPAEC-PAD

1. Introduction

Mixed-linkage (1 → 3),(1 → 4)-β-D-glucan, usually referred to as β-glucan, is the main component of the soluble dietary fibre of oats and barley. It is a linear polymer, consisting mostly of cellotriosyl and cellotetraosyl blocks linked by single (1 → 3)-linkages (Wood, Weisz, & Blackwell, 1994). Longer sequences of (1 → 4)-linkages, mainly cellopentaosyl, cellohexaosyl etc., have also been identified in cereals (Izydorczyk, Macri, & MacGregor, 1998a, 1998b,Wood et al., 1994). Oat β-glucan positively affects human health by lowering serum cholesterol and glucose levels (Braaten et al., 1994; Ripsin et al., 1992; Wood, 2004).

The composition analysis of polysaccharides and their fractions is commonly based on hydrolysis with hydrochloric acid (HCl), trifluoroacetic acid (TFA) or sulphuric acid (H_2SO_4) at elevated temperatures (Lebet, Arrigoni, & Amado, 1997; Manthey, Hareland, & Huseby, 1999; Olson, Gray, Chiu, Betschart, & Turnlund, 1988; Salvador, Suganuma, Kitahara, Tanoue, & Ichiki, 2000; Theander, Åman, Westerlund, Andersson, & Pettersson, 1995). HCl at different concentrations has been used for determination of the total pentosan content of wheat products (Houben, de Ruijter, & Brunt, 1997) and also for partial hydrolysis of β-glucan (Doublier & Wood, 1995; Tosh, Wood, & Wang, 2003). Under the conditions encountered in the human stomach at pH 1–1.5 and temperature of 37 °C, HCl is not believed to

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hydrolyse carbohydrates (Kutchai, 1998). TFA is effective in hydrolysing glycosidic bonds in soluble polysaccharides but is not efficient enough to hydrolyse the β-(1 → 4) glycosidic bonds of cellulose (Lebet et al., 1997; Puls, 1993; Yu Ip, Manam, Hepler, & Hennessey, 1992). Sulphuric acid hydrolysis with a presolubilization step with 72% H_2SO_4 (Saeman, Moore, Mitchell, & Millett, 1954) is commonly used for total hydrolysis of polysaccharide samples containing cellulose and insoluble hemicelluloses (Miron, Yosef, & Ben-Ghedalia, 2001; Puls, 1993). It was suggested by Puls (1993) that acid hydrolysis is often a compromise between complete solubilization and possible sugar destruction and incomplete hydrolysis. After TFA and especially after HCl hydrolysis, some amounts of the sample may be unhydrolysed or structurally changed while, with H_2SO_4, the hydrolysis to neutral sugars is complete but some of the monomer units may degrade.

Enzymatic hydrolysis is a specific and gentle method for fragmentation of many polysaccharides. A commonly used method for analysis of the structure of β-glucan is hydrolysis with a specific β-D-glucanase enzyme, i.e., lichenase that cleaves the β-(1 → 4)-linkages adjacent to a (1 → 3)-linkage. The oligosaccharides thus obtained are the (1 → 4)-linked building blocks of β-glucan with (1 → 3)-linked end group (Johansson et al., 2000; Wood, Weisz, & Blackwell, 1991, 1994). Other enzymes used for depolymerization of β-glucan samples are cellulase, which cleaves only β-(1 → 4)-glycosidic linkages (Roubroeks, Andersson, Mastromauro, Christensen, & Åman, 2001) and β-glucosidase, which together with lichenase is used for quantitative measurement of β-glucan in cereal products (McCleary & Codd, 1991; Boyac, Seker, & Mutlu, 2002).

The mono-and oligosaccharides resulting from acid and enzyme hydrolysis are further analysed by gas chromatography (GC) or high-performance liquid chromatography methods (HPLC), of which the high-performance anion-exchange chromatography equipped with pulse amperometric detection (HPAEC-PAD) method is currently the most used (Cheng & Kaplan, 2001; Lee, 1990; Panagiotopoulos, Sempere, Lafont, & Kerherve, 2001; Talaga, Vialle, & Moreau, 2002).

The aim of the present study was to examine the acid and enzymatic hydrolysis of β-glucan to find a reliable method for determining the amount of β-glucan in different types of sample and at high β-glucan concentrations. We further modified the AOAC method 995.15 to make it suitable for samples with high β-glucan content. Acid hydrolysis was studied under different conditions to determine the most effective procedure for total hydrolysis of β-glucan. The results of acid hydrolyses at 120 °C were compared with those obtained by enzymatic hydrolysis, with lichenase, or with both lichenase and β-glucosidase.

2. Materials and methods

2.1. Materials

The materials were high-viscosity commercial β-glucan of oats (CB) (Megazyme International, Wicklow, Ireland), with molecular mass (M_w) 327,000 g/mol and purity >97% according to the supplier and wholegrain oats (YTY) grown and harvested in 1997 and obtained from Boreal Plant Breeding Ltd., Jokioinen, Finland. The grains were dehulled manually and milled (Cyclotec 1093, Tecator AB, Höganäs, Sweden) to a particle size of <0.5 mm. The β-glucan of whole grain oats (WB) was isolated from the grains using the method of Westerlund, Andersson, and Åman (1993) with minor changes (Johansson et al., 2000). The β-glucan from oat bran (BB) was extracted in the same way was used as reference (Johansson et al., 2000). The molar masses (M_w) of the β-glucans were 1.1×10^6 for BB (Johansson et al., 2000), and 5.1×10^5 for WB (Johansson, Tuomainen, Ylinen, Ekholm, & Virkki, 2004).

Water was purified with a Milli-Q-Plus system (Millipore Corporation, Bedford, MA, USA). The maltooligosaccharides with degree of polymerization (DP) 3–6 were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Glucose was obtained from J.T. Baker (Devender, Holland), cellobiose from Merck (Darmstadt, Germany) and laminaribiose from Sigma. NaOH solution was purchased from Fluka (Buchs, Switzerland). Lichenase (E.C. 3.2.1.73) was purchased from Megazyme (Wicklow, Ireland). Termamyl 300 L DX was from Novo Nordisk A/S (Bagsvaers, Denmark) and pancreatin 8 × U.S.P. from Sigma Chemical Co. (St. Louis, MO, USA). The β-glucan content in the samples was determined by the Association of Analytical Chemists (AOAC) 995.15 method, using a β-glucan assay kit (Megazyme International, Wicklow, Ireland) (McCleary & Codd, 1991). All other chemicals used were the purest obtainable.

2.2. Extraction of β-glucan from oats

The ground grains were defatted, using Soxhlet extraction with hot isopropanol (IPA) and petroleum ether. The polysaccharides were solubilized in water at 96 °C and starch was hydrolysed using Termamyl. The insoluble fraction was separated by centrifugation. The proteins in the supernatant were degraded with pancreatin. The polysaccharides were precipitated, using 60% ethanol at 4 °C. The ethanol precipitate was removed with centrifugation and dissolved in water at 70–80 °C for 2–3 h. The β-glucan was precipitated with 30% $(NH_4)_2SO_4$ and separated by centrifugation. This precipitate was dissolved in water at 80 °C in the dialysis tube for 1–2 h (CelluSep T1, MW cutoff 3500, Membrane Filtration Products, Inc., San Antonio, TX,

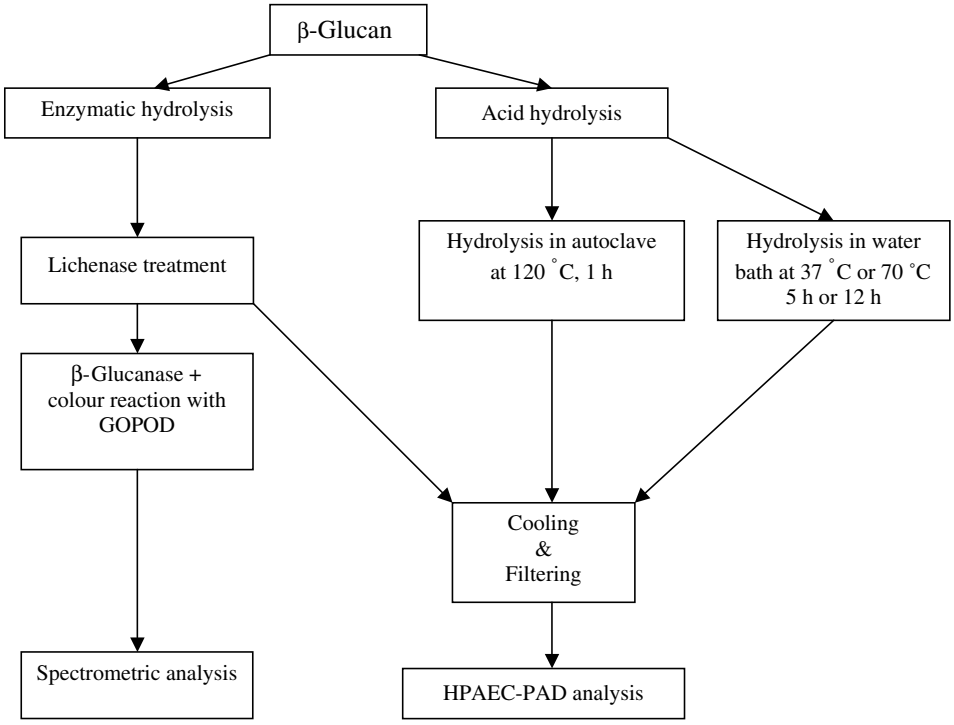

Fig. 1. Hydrolysis and analysis procedures for β-glucan samples (CB,WB and BB).

USA) and dialysed against purified water at room temperature for 3×24 h. The resulting β-glucan solution was freeze-dried. Prior to analyses with the AOAC 995.16 method, the WB and BB samples were treated with IPA overnight with magnetic stirring and then dried in a vacuum oven at 80 °C for 4 h. The purpose of this final step was to improve their solubility (Doublier & Wood, 1995; Tosh et al., 2003). The samples were hydrolysed with acids and enzymes and further analysed according to Fig. 1.

2.3. Acid hydrolysis

The acid hydrolysis (Fig. 1) of CB (20 mg) was performed with three acids: HCl, TFA and H_2SO_4. The concentrations were 0.1 and 3 M for HCl and TFA and 0.05 and 1.5 M for H_2SO_4. The temperatures of hydrolysis were 37, 70 and 120 °C. For 120 °C, an autoclave (Steris Finn-Aqua 46-E, Wilmington, DE, USA) and, for temperatures 37 and 70 °C, a water bath (Certomat WR) were used. The hydrolysis times were 1 h at 120 °C and 5 and 12 h at temperatures of 37 and 70 °C. The amount of glucose produced in the hydrolyses was analysed using HPAEC-PAD. In the hydrolysis with high-concentration acids, both unhydrolysed (PAD1) and hydrolysed (PAD2) glucose were used as external standards, while with low-concentration acids only PAD1 glucose standards were used. In the hydrolysis of WB, 1.5 M H_2SO_4 was used for 1 h at 120 °C in an autoclave. The glucose thus produced was analysed with HPAEC-PAD, using PAD2 glucose as standard. The BB was hydrolysed with 3 M TFA at 120 °C for 1 h. The glucose thus produced was analysed by GC (Johansson et al., 2000). The free sugar residues were corrected by a factor of 0.9 to anhydro sugar, as present in polysaccharides (McCleary & Codd, 1991).

2.4. Enzymatic hydrolysis

2.4.1. Hydrolysis to oligosaccharides

The β-glucans (CB, WB and BB) were hydrolysed to oligosaccharides with lichenase, as previously described (Johansson et al., 2000). The method was modified from the McCleary method (AOAC 995.16 method). The β-glucans were dried at 70 °C for 3 h. The samples (60–110 mg) were weighed accurately and pretreated with 0.2 ml of aqueous ethanol (50%), dissolved in phosphate buffer (4.0 ml, 20 mM, pH 6.5) in a boiling water bath and stirred on a vortex mixer. The samples were then incubated with lichenase at 60 °C for 2 h. The resulting solution was again incubated in a boiling water bath for 10 min to inactivate the lichenase and then diluted with water. The oligosaccharides thus produced were analysed with HPAEC-PAD.

2.4.2. Hydrolysis to glucose

The β-glucan contents of the CB and WB samples were determined with the AOAC 995.16 method (McCleary & Codd, 1991; McCleary & Mugford, 1997). In this method, the lichenase and β-glucosidase

enzymes were used for hydrolysis of β-glucan and the amount of β-D-glucose was spectrophotometrically measured with glucose oxidase-peroxidase reagent. With the approved AOAC 995.16 method, the obtained recovery of pure CB β-glucan sample was too low and therefore the method was optimized for analysing samples with high β-glucan content. The CB was used throughout the optimization tests. The amount of sample material, reaction time and amount of buffer in the lichenase treatment, dilution with buffer in the β-glucosidase treatment and the concentration of lichenase were tested. The solubility of β-glucan was improved by treatment with IPA and vacuum-drying (Doublier & Wood, 1995; Tosh et al., 2003). The amount of sample material recommended for use in the AOAC method for β-glucan in oat and barley flours and fibre samples is 80–120 mg. However, with our high β-glucan samples, the smallest amount, that was possible to weigh accurately, was 30 mg. The lichenase additions used were 10 U as also used in the AOAC method, 30 and 60 U. The incubation times for lichenase treatment were 60 min (used in the AOAC method) and 90, 180, 540 and 1440 min. Dilutions (1-, 3- and 4-fold) with 50 mM acetate buffer (pH 4.0) for β-glucosidase treatment were used as McCleary and Mugford (1997) recommended for samples containing more than 10% β-glucan. All measurements were made at least in triplicate.

2.5. HPAEC-PAD-analysis

The mono- and oligosaccharides produced by acid and enzymatic hydrolyses were analysed using two different HPLC systems equipped with two Waters 515 HPLC pumps (Waters Corporation, Milford, MA, USA). One system consisted of a Waters Automated Gradient Controller, a Merck Hitachi D2500 Chromato-Integrator (Hitachi Ltd., Japan) and a Rheodyne 7725 injector (Rohnert Park, CA, USA). The second system included a Waters 717 autosampler using Millenium[32] software for instrument control and data handling. The analytical column was a CarboPac PA1 type (250 × 4 mm, i.d.) and the guard column was PA1 (25 × 3 mm, i.d., Dionex, Sunnyvale, CA, USA) maintained at 30 °C. All samples were filtered before analysis (0.2 μm Acrodisc 13 GHP filter; Pall Corporation, Ann Arbor, MI, USA) and the injection volume was 20 μl in all measurements.

The monosaccharide analyses were performed after acid hydrolysis by isocratic elution with 8 mM NaOH at a flow rate of 1 ml/min. The filtered mobile phase was degassed before use. A Decade detector with gold electrode (Antec Layden, Zoeterwoude, The Netherlands) was used in pulse mode at 30 °C. The pulse potentials and durations were: $E_1 = 0.15$ V, $t_1 = 400$ ms, $E_2 = 0.75$ V, $t_2 = 120$ ms, $E_3 = -0.8$ V, $t_3 = 300$ ms, $t_s = 20$ ms. To regenerate the column, a 100 mM NaOH solution was used at a flow rate of 0.2 ml/min overnight after each analysis day. Quantification was performed with PAD1 and glucose hydrolysed by the same process as the samples (PAD2). The calibration curve for analysing the acid hydrolysates was obtained in the concentration range 5–50 μM. Three parallel samples were analysed with duplicate injections.

The oligosaccharide analysis was performed as follows: the eluents were: A, 150 mM NaOH and B, 500 mM sodium acetate in 150 mM NaOH. The flow rate was 1 ml/min. The linear gradient was from 90% A/10% B to 100% B in 15 min, and thereafter, back to the initial state (90% A/10% B) during 2 min. The column was stabilized for 10 min before the following injection. The Decade detector with gold electrode was used at the same pulse potentials as for monosaccharides. Quantitation was performed with glucose, cellobiose and malto-oligosaccharides with DP 3–6. We established calibration curves for each of the analysed compounds, since the molar response of the solute, measured with PAD, is different for each compound and the concentration ranges of the analysed oligosaccharides differ in the samples. For malto-oligosaccharides, the concentration range varied from 200 to 1200 μM for DP 3–4 and from 5 to 60 μM for DP 5–6. For glucose and cellobiose, the concentration range was 5–50 μM and the correlation coefficients were 0.9989 and 0.9997, respectively. All analyses were done in triplicate. Millennium[32] software was used for instrument control and data handling.

2.6. Viscosity

For viscosity measurements, a 2.5% stock solution of CB was prepared in water by incubating at 70 °C. A dilution series (series A) was formed thereof, with water, to give concentrations between 0.3% and 1.8%. Another set of samples (series B) was also prepared from the stock solution. These samples were acidified to pH 1 with 0.1 M HCl and diluted with water to give the same concentrations as for the water solutions (series A). The samples were then incubated at 37 °C for 5 h. A third set of samples (series C), with the same concentrations as the two other sets, was prepared by incubating β-glucan in 0.1 M HCl at 70 °C. The viscosities were measured with a ThermoHaake RheoStress 600 (Thermo Electron GmbH, Dreieich, Germany) using a cone-and-plate geometry with 35 mm diameter and an angle of 2°. Three parallel samples were analysed in triplicate at 37 °C.

2.7. Statistics

The statistical calculations were performed using one-way analysis of variance (ANOVA) at the 95% confidence level using Statgraphics Plus 3.0.

3. Results

3.1. Acid hydrolysis

The results of hydrolysis for CB with high-concentration acids (3 M for HCl and TFA and 1.5 M for H_2SO_4) at 120 °C are shown in Fig. 2. The results marked with PAD1 were calculated with unhydrolysed glucose standards in water solutions. The recoveries were about 75% for H_2SO_4, 63% for TFA and 27% for HCl. Under these conditions, a part of the glucose is converted, with all three acids, to products such as 5-hydroxymethylfurfural (Johnson, Alford, & Kinzer, 1969; Kaar, Cool, Merriman, & Brink, 1991; Sjöström, 1993). Various coloured substances, through the Maillard reaction (Lerche, Pischetsrieder, & Severin, 2002; Whistler & BeMiller, 1997) have been reported for saccharides. The results show that much less glucose was degraded in the hydrolysis with H_2SO_4 and TFA than with HCl. The loss of glucose was about 73% with 3 M HCl. The recoveries of analysis with hydrolysed glucose standards marked with PAD2 varied from 88% to 89%, indicating similar ability of all three acids to hydrolyse β-glucan. In the hydrolysis of WB with 1.5 M H_2SO_4 at 120 °C for 1 h, the glucose recovery was of the same order of magnitude (89.4%) as with CB. For BB, hydrolysed with TFA and analysed with GC, the recovery was 86% (Johansson et al., 2000). In these experiments the standard curves had correlation coefficients of at least 0.9943, thus showing the reliability of the analyses.

Hydrolysis with low-concentration acids (0.1 M for HCl and TFA and 0.05 M for H_2SO_4) at 120 °C, in an autoclave for 1 h, produced a series of products (Fig. 3). The main saccharide found in HPAEC-PAD analysis was glucose but cellobiose was also found in all samples (Table 1). The amount of glucose and also the total amount of glucose and cellobiose decreased,

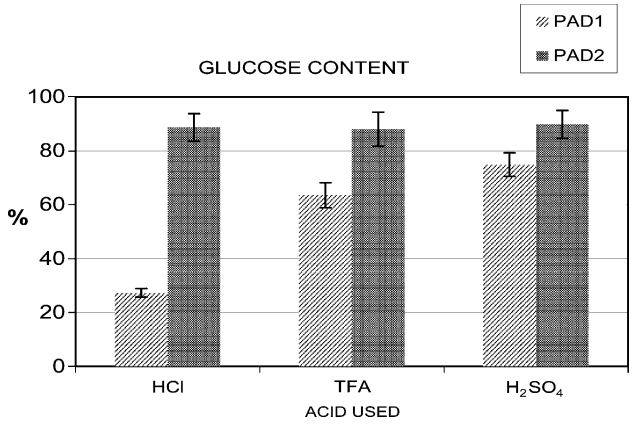

Fig. 2. Percentage recoveries of glucose (% dry matter) in acid hydrolysis of CB with HCl and TFA (3 M) and H_2SO_4 (1.5 M) at 120 °C for 1 h analysed by HPAEC-PAD. The standard deviations are shown as vertical bars in each column.

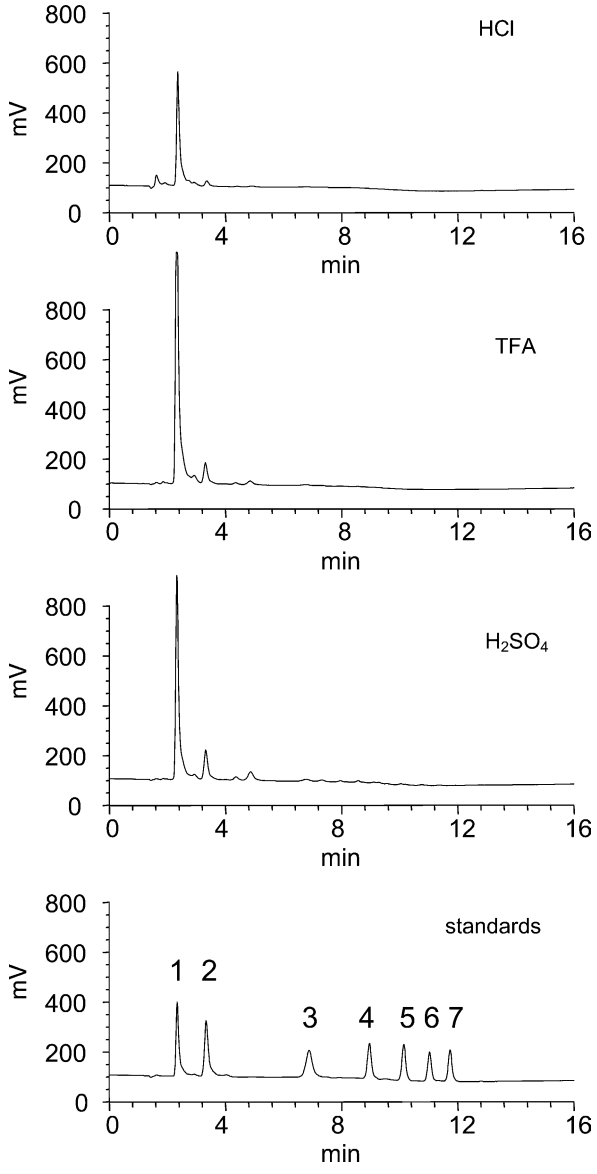

Fig. 3. Chromatograms of the CB hydrolysis products produced with 0.1 M HCl and TFA and 0.05 M H_2SO_4 at 120 °C and chromatogram of a standard mixture of glucose, cellobiose and malto-oligosaccharides, DP 3–7, analysed by HPAEC-PAD. The dilutions of the sample solutions varied.

Table 1
The relative amounts (wt% ± S.D.) of glucose and cellobiose produced in the acid hydrolysis of CB during 1 h at 120 °C analysed by HPAEC-PAD

	Glucose	Cellobiose	Total
HCl	101 ± 12.5	6.6 ± 0.6	108
TFA	69.6 ± 10.7	17.8 ± 5.4	87.4
H_2SO_4	44.8 ± 2.7	19.9 ± 2.3	64.6

The concentrations of the acids were 0.1 M for HCl and TFA and 0.05 M for H_2SO_4.

but the amount of cellobiose increased from HCl to TFA and further to H_2SO_4. For the sample hydrolysed with HCl, no oligosaccharides with DP of 3 or over were

detected. The hydrolysis with TFA and H_2SO_4 produced a series of oligosaccharides with DP 3 or more which were not quantified. Trace amounts of laminaribiose were detected but not determined.

At 70 °C with low-concentration acids for 5 and 12 h, a complex series of products was found. The main component was glucose, the amounts of which, for a 5 h hydrolysis time in HCl, TFA and H_2SO_4, were 0.13%, 0.12% and 0.09%, respectively. The other products found in the chromatograms were partially overlapped peaks and too complicated for analysis. When the hydrolysis time was increased to 12 h, the amounts of glucose obtained with HCl, TFA and H_2SO_4 increased to 0.27%, 0.20% and 0.15%, respectively. When CB was hydrolysed under stomach-mimicking conditions at pH 1 (0.1 M) and at 37 °C, no hydrolysis products were detected for any acid during the 5 or 12 h incubation times.

3.2. Enzymatic hydrolysis

3.2.1. Hydrolysis to oligomers

Fig. 4 shows the chromatograms of the hydrolysis products of β-glucan with lichenase, for both CB and WB and oligosaccharide standards with DP 3–6. The oligosaccharides produced were (1 → 4)-β-D-gluco-oligosaccharides with a (1 → 3)-linkage at the reducing end. Oligosaccharides with DP 3–6 were quantified (Table 2). The correlation coefficients were 0.9988 for DP3, 0.9981 for DP4, 0.9987 for DP5 and 0.9994 for DP6. Total recovery was achieved for both CB and WB (Johansson et al., 2004) and also for BB (Johansson et al., 2000). The main products were oligomers with DP 3 and 4 which constitute about 94% of the total recovery; oligomers longer than DP 4 constitute the remaining products.

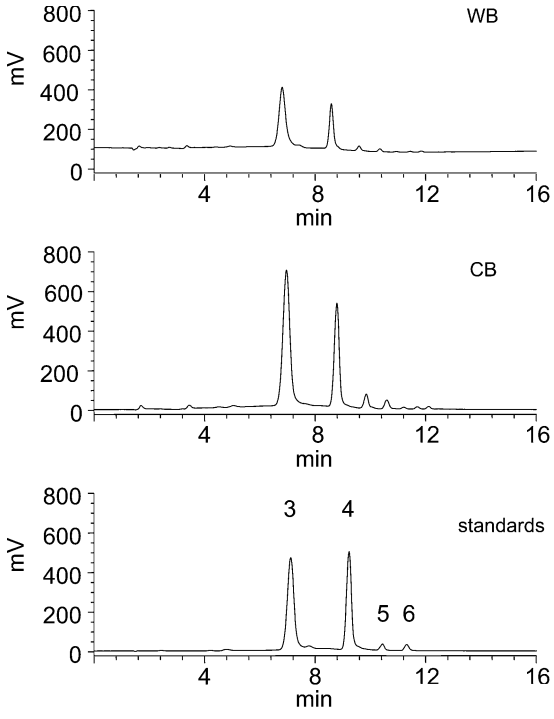

Fig. 4. Chromatograms of oligosaccharides produced by lichenase treatment of WB and CB and malto-oligosaccharide standards, with DP 3–6, analysed by HPAEC-PAD.

Table 2
The relative amounts (wt% ± S.D) of oligosaccharides, with degrees of polymerization (DP) 3–6, produced, using lichenase digestion of CB and WB analysed by HPAEC-PAD

	DP 3	DP 4	DP 5	DP 6	Total	DP3:DP4
WB[a]	54.2 ± 1.9	39.8 ± 1.2	4.7 ± 0.4	3.5 ± 0.4	102	1.4
CB	62.7 ± 0.5	43.1 ± 0.4	4.1 ± 0.01	2.7 ± 0.1	113	1.4
BB[b]					99.1	1.3

[a] From Johansson et al. (2004).
[b] From Johansson et al. (2000).

3.2.2. Hydrolysis to glucose

The results of enzymatic hydrolysis to glucose for CB are shown in Table 3. The β-glucan content was about 34% with the AOAC 995.16 method. The results show that increased incubation time alone did not improve the recovery of β-glucan. A 180 min incubation and double the amount of buffer in lichenase treatment was essential for the solubility of β-glucan. Three-fold dilution with β-glucosidase treatment increased the content to 80% and 4-fold dilution to 91.4%. The recovery was not significantly enhanced with increased lichenase concentration (30 and 60 U). The optimized conditions for the pure CB sample were: 10 U lichenase enzyme, double the amount of buffer, 180 min incubation time with lichenase and 4-fold dilution with buffer in the β-glucosidase treatment. The WB content with 10 U lichenase, 4-fold dilution and 90 min incubation with lichenase was about 67% while the 180 min incubation with lichenase gave 73%. IPA treatment increased the solubility of β-glucan and resulted in the highest β-glucan content of 78% for WB and 85% for BB.

Table 3
The β-glucan contents (% ± S.D.) of CB sample obtained from all optimizing steps for AOAC 995.16 method

Incubation time (min)	Dilution for β-glucosidase	Lichenase concentration (U)	β-Glucan content
90	1	10	33.6 ± 0.2
180	1	10	34.8 ± 0.8
540	1	10	33.4 ± 2.3
1440	1	10	32.7 ± 1.2
90	3	10	80.0 ± 4.5
1440	3	10	83.5 ± 2.0
180	4	10	91.4 ± 2.1
180	4	30	92.7 ± 1.8
180	4	60	91.0 ± 1.1

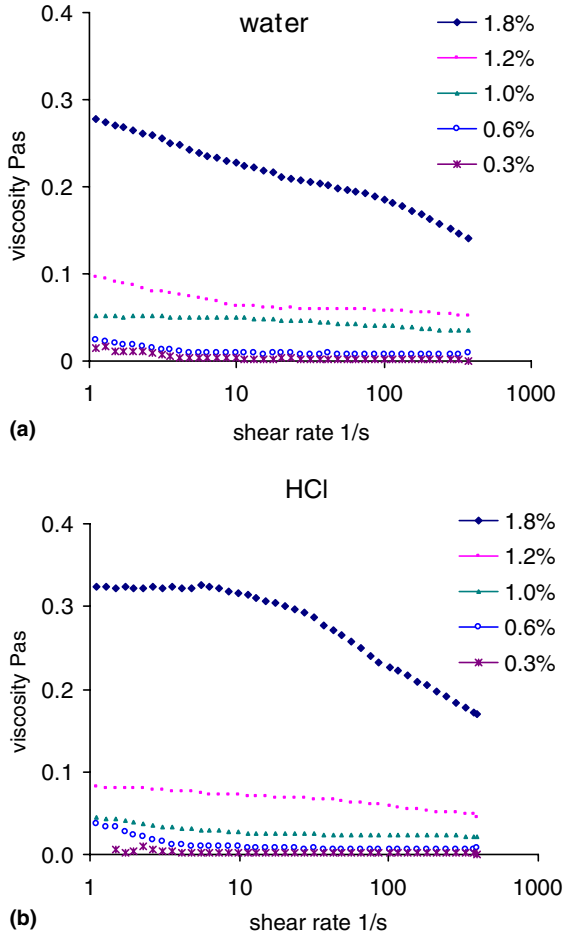

Fig. 5. Viscosities of CB in an aqueous solution and after treatment with 0.1 M HCl.

3.3. Viscosity

The viscosities of the samples incubated at 37 °C showed the shear-thinning behaviour typical of polysaccharides. The viscosity dropped rapidly at low shear rates and levelled off to a plateau, the value of which varied with the concentration. The viscosity drop was very rapid with low-concentration samples and became less so as the concentrations increased. Fig. 5 shows the viscosities of the water and HCl solutions as a function of the logarithm of the shear rate. Small differences can be seen at low shear rates, especially at a concentration of 1.8% where the viscosity of the HCl solution begins to drop later than that of the water solution. For series C, incubated at 70 °C with HCl, the viscosity dropped to the lowest level of concentration.

4. Discussion

The aim of the work was to compare various hydrolysis methods for determining the amount of β-glucan in samples of high β-glucan content. The AOAC 995.16 enzymatic hydrolysis with lichenase and acid hydrolysis methods were used. Acid hydrolysis of CB was tested under several conditions and with both hydrolysed (PAD2) and unhydrolysed (PAD1) standards. All three acids used (HCl, TFA and H_2SO_4) gave essentially the same results with high acid concentrations at 120 °C for 1 h quantified with hydrolysed glucose. The hydrolysed standards were also used by Theander et al. (1995) in a collaborative study of dietary fibre determination and by Puls (1993) in a study of forest and agricultural waste. With the unhydrolysed standard, the results for the three acids differed remarkably, due to different amounts of glucose being converted during the hydrolysis (Puls, 1993). Lebet et al. (1997) compared gas chromatography (GC) and HPAEC-PAD methods for the analysis of neutral monosaccharides. According to the authors, the advantage of HPAEC-PAD over GC is that derivatization can be omitted but the disadvantages are that the resolution and reproducibility of the retention times are lower than with GC. The method we used here included a regeneration step after each analysis that allowed reproducible retention times. The correlation coefficients of the standard curves and the low standard deviations in this work indicate the reliability and repeatability of the method.

Hydrolysis of CB with low-concentration acids at 120 °C produced glucose as the main product, as well as cellobiose and a series of oligomers. At 70 °C, the main product was also glucose but the amounts of degradation products were very low. Hydrolysis under stomach-mimicking conditions did not result in degradation of CB or changes in its viscosity. Despite small differences observed at low shear rates, no statistically significant differences were observed at shear rates of 100, 10 and 1 1/s between samples in water and in HCl incubated at 37 °C and analysed with one-way ANOVA ($p > 0.05$) (series A and B). This is consistent with Wood, Braaten, Scott, Riedel, and Poste (1990), who reported no difference in kinematic viscosity between samples of oat gum in water and in 0.1 N HCl at 37 °C. In the present paper, the viscosity of samples incubated at 70 °C (series C) dropped to the lowest level of concentration, which is consistent with the partial degradation observed at 70 °C in the acid hydrolysis experiments.

Hydrolysis with lichenase alone gave total recovery for both CB and WB (Johansson et al., 2004) as well as BB (Johansson et al., 2000) using HPAEC-PAD. Separate standard curves for each component are required for quantification. The correlation coefficients of the standard curves and the low standard deviations observed between parallel samples indicate the reliability of the method. Analyses of oligosaccharides with DP > 7 were not possible since the necessary standards are not commercially available. This could have resulted in minor errors in the results. Also, Jiang and Vasanthan

(2000) analysed the oligosaccharides from the β-glucans of barley with HPAEC-PAD using malto-oligosaccharides to produce standard curves for quantification. The recoveries were between 90% and 97% and the standard deviations were low. Wood et al. (1994) have analysed oligosaccharides up to DP 15 and Izydorczyk et al. (1998a, 1998b) up to DP 20 and gained total recovery. In these reports the calculations were based on relative response factors.

The AOAC 995.16 method is widely used for determining β-glucan content (Gruppen, Hamer, & Voragen, 1992; Jiang & Vasanthan, 2000; Manthey et al., 1999). Wood et al. (1994) used the AOAC method to determine the amount of β-glucan in an oat gum preparation and a purified β-glucan and values of ≈80% and 96%, respectively, were reported. The samples in their work were precipitated from IPA and air-dried. Boyac et al. (2002) developed a method using barley and wheat flours as sample materials. The glucose produced by enzymatic hydrolysis was analysed with an amperometric glucose electrode. The results were the same as those obtained using the original AOAC 995.16 method.

In the present paper, the method as such gave poor results for CB and WB and was therefore further modified to determine the optimal conditions for analysing high-β-glucan samples. A longer incubation time of 180 min, instead of the 60 min used in the AOAC method, was required. The samples were also diluted 4-fold for treatment with β-glucosidase. The optimized AOAC 995.15 method, gave a β-glucan content of 91% for CB, whereas the manufacturer assures at least 97% purity. For WB, the optimized AOAC method gave a content of 75%, acid hydrolysis gave 89% and lichenase hydrolysis gave total recovery. For BB, the values obtained with the optimized AOAC method and acid hydrolysis were the same, 85% and 86%, respectively. These values are lower than those obtained with lichenase hydrolysis, which gave total recovery. The samples differed in their solubilities and molar masses. The CB was readily soluble in water, but the solubilities of WB and BB were lower than that of CB. Treatment with IPA and vacuum-drying improved the solubilities of WB and BB but the results still deviated from the β-glucan content obtained with lichenase hydrolysis. The results obtained in this work suggest that acid hydrolysis and lichenase hydrolysis, combined with HPAEC-PAD, are reliable alternatives for the determination of the amount of β-glucan in samples with high β-glucan content and low solubility.

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