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Structural characterization of water-insoluble nonstarchy polysaccharides of oats and barley

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Abstract

Water-insoluble dietary fibre of dehulled oat and barley grain was isolated with enzymatic hydrolysis of starch and protein and further extracted sequentially. The extractants used were saturated barium hydroxide, water, 1 M potassium hydroxide and 4 M sodium hydroxide. The fractions were dialysed against pure water, freeze-dried and weighed. Repeatable recoveries of extracts were obtained. The ground grains and extracted fractions were characterized for their fat and protein content. The fractions were hydrolysed and characterized by measuring their neutral sugar composition using gas chromatography. Comparison of the amounts of cellulosic and noncellulosic polysaccharides was made. Further characterization of fractions was performed with solid-state ^{13}C CP/MAS NMR spectroscopy and IR spectroscopy. The differences in composition of insoluble material between oats and barley were small. The distribution of masses and monosaccharides of the five fractions obtained were similar and the measured spectra show the same characteristic signals as saccharide structures.

Keywords: β-Glucan; Oats; Barley; Insoluble fibre

1. Introduction

Dietary fibre (DF) is defined as the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine (American Association of Cereal Chemistry, AACC Report, 2001). The main nonstarchy polysaccharides (NSPs) of oat and barley dietary fibre are the mixed-linkage (1→3),(1→4)-β-D-glucan, referred to as β-glucan, arabinoxylans and cellulose. In addition to other polysaccharides, glycoproteins and lignin they are important components of the endosperm cell wall of cereals. For analytical and nutritional reasons dietary fibre is often divided into water-soluble and water-insoluble fractions. There is generally no sharp distinction between soluble and insoluble fractions and the ratio is highly dependent on the extraction conditions of soluble fibre. β-Glucan and arabinoxylans are found both as soluble and insoluble components in DF. Cellulose consists only of (1→4)-β-D linkages and is therefore stiff, highly crystalline and nonsoluble (Fincher & Stone, 1986). Other nonsoluble parts included in dietary fibre are lignin, resistant starch, unhydrolysed protein, tannins and cutins (AACC Report, 2001; Theander, Westerlund, & Åman, 1993).

Cereal products of oats and barley have positive effects on human health. Oat and barley β-glucan were reported to lower serum cholesterol and glucose levels (Anderson, Jones, & Riddell-Manson, 1994; Braaten et al., 1994; Kahlon, Chow, Knuckles, & Chiu, 1993; Kalra & Jood, 2000; Wood, Beer, & Butler, 2000). These effects are related to soluble DF and its ability to raise the viscosity of intestinal contents. On the other hand, the viscous nature of barley β-glucan may cause problems in the brewing process (Bamforth, 1982; Gomez, Navarro, Manzanares, Horta, & Carbonell, 1997). However, a large part of the NSP in oats and barley is water insoluble. Insoluble DF was shown to have protective effects against cancer (Slavin, Marquart,

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& Jacobs, 2000). The structure and solubility of DF components are important since they influence the physical properties of the polysaccharides and therefore the nutritional properties of food products.

DF was isolated and fractionated from cereal materials mainly by the use of enzymatic gravimetric methods (Asp, Johansson, Hallmer, & Siljeström, 1983; Claye, Idourane, & Weber, 1996; Prosky, Asp, Schweitzer, DeVries, & Furda, 1988) whereby soluble and insoluble fibres are isolated separately and weighed. Water-insoluble dietary fibre (WIS) is further fractionated with different extractants depending on the purpose of treatment. The extractants commonly used for cereal NSPs are alkali solutions, which dissolve most of the hemicelluloses and leave cellulose as an unextracted residue (Gruppen, Hamer, & Voragen, 1992). Mild alkaline extraction was used by Carr, Glatter, Jeraci, and Lewis (1990), Wood, Weisz, and Mahn (1991) and Claye et al. (1996). $Ba(OH)_2$, used as the primary extractant, extracts arabinoxylans from WIS material (Gruppen, Hamer, & Voragen, 1991). Subsequent extraction with water releases β-glucan, and further extraction with concentrated alkali solutions releases other nonstarch carbohydrates (Gruppen et al., 1992; Izydorczyk, Marci, & MacGregor, 1998; Nilsson, Saulnier, Andersson, & Åman, 1996; Vinkx, Stevens, Gruppen, Grobet, & Delcour, 1995). Isolated fractions of water-insoluble DF were analysed for their sugar content, molecular weight, viscosity and structure (Gruppen et al., 1991, 1992; Izydorczyk et al., 1998; Manthey, Hareland, & Haseby, 1999; Nilsson et al., 1996; Roubroeks, Andersson, & Åman, 2000).

Nuclear magnetic resonance (NMR) spectroscopy was used to obtain structural information on high-molecular weight materials and their building blocks (Bock, Duus, Norman, & Pedersen, 1991). This nondestructive method provides direct information on the chemical structure of polysaccharides. One-dimensional ^{1}H and ^{13}C NMR spectra were used for investigation of anomeric protons and carbons of β-glucan and arabinoxylans and for comparison of β-glucans of different origins (Dais & Perlin, 1982; Westerlund, Andersson, & Åman, 1993; Wood, Weisz, & Blackwell, 1994). Solid-state cross polarization-magic angle spinning (CP/MAS) ^{13}C NMR was used to examine barley β-glucan and its interaction with bile acids (Bowles, Morgan, Furneaux, & Coles, 1996). Dudley et al. (1983) used CP/MAS NMR for analyses of cellulose oligomers and the structure of cellulose II. Gidley (1992) reviewed the use of solid-state NMR of food material. Morgan, Roberts, Tendler, Davies and Williams (1999) analysed the structure of Glucagel™, the β-glucan extract from barley, with CP/MAS NMR. Davies, Harris and Newman (2002) used solid-state ^{13}C NMR for structural analyses of cellulose and various polysaccharide extracts from thale cress *Arabidopsis thaliana* leaves.

Fourier transform-infrared (FT-IR) spectroscopy is also a tool used for the structural analysis of polysaccharide material and was used to investigate glycosidic linkages of oligosaccharides (Sekkal, Dincq, Legrand, & Huvenne 1995) and structural features of arabinoxylans (Kačuráková, Ebringerová, Hirsch, & Hromádlová, 1994). Kačuráková and Wilson (2001) reviewed the use of FT-IR for selected carbohydrates. Kačuráková, Capek, Sasinková, Wellner, and Ebringerová (2000) also studied model compounds with FT-IR to identify plant cell wall polysaccharides as pectin and hemicelluloses.

The aim here was to study the structure of water-insoluble dietary fibre in oats and barley. In our earlier studies we investigated the structure of soluble β-glucan of oat bran (Johansson et al., 2000) and mineral solubility in oat bran and flakes (Ekholm, Virkki, Ylinen, & Johansson 2003; Ekholm, Virkki, Ylinen, Johansson, & Varo, 2000). Structural study of insoluble dietary fibre is needed to reveal the structure–properties relationships of the polymer, which can aid in understanding the behaviour of oat and barley dietary fibre in binding minerals, in the digestive track, and in baking and other processes of industrial importance.

2. Materials and methods

2.1. Materials

The starting materials used were whole-grain cultivars of oats (Yty) and barley (Saana). The samples were grown and harvested in 1997 and were obtained from Boreal Plant Breeding Ltd, Jokioinen, Finland. The oats and barley were dehulled manually and the grain samples milled (Cyclotec 1093, Tecator AB, Höganäs, Sweden) to a particle size of less than 0.5 mm.

For standard monosaccharides, glucose was obtained from J.T. Baker (Devender, Holland), arabinose, xylose and galactose from Merck (Darmstadt, Germany) and mannose and myo-inositol from Sigma Chemicals Co. (St. Louis, MO, USA). All other chemicals used were the purest obtainable.

2.2. Isolation of water-insoluble dietary fibre

The oat and barley samples were treated according to the method of Asp et al. (1983) for DF with some modifications. In this method the starch and proteins of the sample were digested enzymatically. Each milled sample (7.5 g) was weighed, suspended in water and kept in a boiling water bath with horizontal agitation for 15 min to gelatinize the starch. The starch was hydrolysed with Termamyl 300 L (Novo Nordisk A/S, Bagsvaerd, Denmark) in a boiling water bath for 1 h 45 min. The presence of any unhydrolysed starch was checked with an I_2/KI solution, and additional enzyme was added if necessary. The pH of the reaction mixture was adjusted to 1.5 and the proteins were digested with pepsin (0.7 FIP-U/mg, EC 3.4.23.1, Merck, Darmstadt, Germany) at 40 °C for 60 min. The pH was adjusted to 6.8 and degradation was continued with pancreatin (8× U.S.P. Sigma Chemical Co., St. Louis,

MO) by incubation for an additional 60 min at 40 °C. The samples were centrifuged (16,000 rcf, 20 min) and the precipitate suspended in 50 ml water. The suspension was filtered with a sintered funnel, washed with water and dried by solvent exchange (95% ethanol and acetone). The sintered funnels with the precipitate were further dried at 70 °C overnight and weighed. This method was used to obtain the insoluble fraction of DF. Four parallel isolations were performed for both oats and barley.

2.3. Fractionation of water-insoluble dietary fibre

Samples of water-insoluble dietary fibre (WIS) were extracted with sequential alkaline extraction, based on a method described by Gruppen et al. (1992) with some modifications. The fractionation procedure is shown in Fig. 1. A 1.5 g amount of insoluble fibre was suspended in a saturated $Ba(OH)_2$ solution (250 ml) containing 0.26 M $NaBH_4$ to prevent alkaline peeling. Extraction was carried out with magnetic stirring overnight at room temperature and the suspension centrifuged (16,000 rcf, 20 min). The residue was re-extracted with $Ba(OH)_2$ (125 ml) for an hour and again centrifuged. The extracts were combined, neutralized with acetic acid (pH 5) and dialysed (Cellu Sep, molecular-weight cut-off 12,000–14,000), first against sodium acetate buffer (0.2 M, pH 5, 5 l) for 3 h and then against tap water for 3 days and finally against Milli-Q water (Millipore Corporation, Bedford, MA, USA) for 3 h. The extract was then frozen (−18 °C), freeze-dried and weighed (Fr. 1).

The insoluble material from the $Ba(OH)_2$ extraction was suspended in 150 ml Milli-Q water, acidified to pH 5 with acetic acid, stirred for 1 h and centrifuged as previously described. Extraction with Milli-Q water was repeated four times, supernatants were combined, acidified, dialysed against tap water and Milli-Q water as described above, freeze-dried and weighed (Fr. 2). The residue from the water extraction was extracted overnight at room temperature with 1 M KOH (100 ml) containing 0.26 M $NaBH_4$, centrifuged and re-extracted with 50 ml KOH for 1 h. The combined extracts were acidified and dialysed as described above (Fr. 3). The insoluble residue remaining after these extractions was suspended in 4 M NaOH (100 ml) containing 0.26 M $NaBH_4$ and extracted with magnetic stirring overnight at room temperature. After centrifugation the residue was re-extracted (50 ml, 1 h) and centrifuged again. The residue was washed with 4×50 ml Milli-Q water and the extracts were combined, acidified (pH 5, acetic acid) dialysed and freeze-dried (Fr. 4). The final residue was freeze-dried (Fr. 5). Four parallel fractionations were performed for the WIS material of oats and barley.

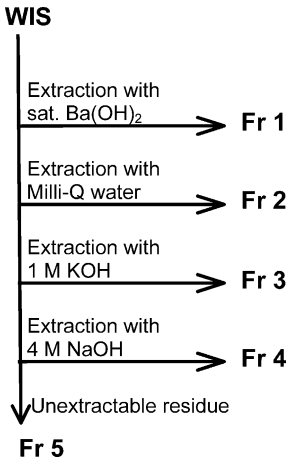

Fig. 1. Extraction procedure for fractionation of WIS material.

2.4. General analysis

The dry material content of the grain was determined by drying at 105 °C overnight. The ash content was determined as the residue after ashing at 550 °C in a temperature-programmed muffle oven (Nabertherm, Germany). The crude grain protein and isolated insoluble DF samples were analysed using the Kjeldahl method (6.25×*N*). The fat content of the grains and WIS samples was determined using the Association of Official Analytical Chemists (AOAC) method 922.06 (AOAC, 1995). The β-glucan content was determined with the method of McCleary (McCleary & Codd, 1991, AOAC method 995.16), using the mixed-linkage β-glucan assay kit (Megazyme Ltd, Wicklow, Ireland).

In the WIS fractionation procedure (Gruppen et al., 1992) concentrated alkaline solutions of barium, potassium and sodium were used as well as acetic acid, sodium acetate buffer and tap water. For this reason the fractions were analysed for their Na, K, Mg, Al, Ca, Fe, Cu, Zn and Ba contents with a Perkin Elmer ELAN 6000 inductively coupled plasma/mass spectrometer (ICP/MS) equipped with a Perkin Elmer auto sampler AS-91. The dried samples and blanks were wet-digested in 10 ml of conc. HNO_3 with a Tecator Digestion System 41 with an auto step 1012 controller. Rhodium was used as an internal standard at a 0.01 μg dm^{-3} concentration. NBS (National Bureau of Standards, Gaithersburg, MD) 1567a Wheat Flour was used as the reference sample. A TotalQuant analysis was performed which gives semi-quantitative results. An ICP Multielement Standard VI (Merck) was used.

2.5. Analysis of monosaccharides

The monosaccharide compositions of the alkali-extracted fractions of WIS were determined after acid hydrolyses of the fraction. The hydrolyses were performed with both H_2SO_4 and trifluoroacetic acid (TFA) to study the difference between the noncellulosic and cellulosic origins of the monosaccharides. Neutral sugars were released by pre-treatment with 72% (w/w) H_2SO_4 for 1 h at room

temperature followed by hydrolysis with 1 M H_2SO_4 for 3 h at 120 °C (Pettersen & Schwandt, 1991). Hydrolyses with 2.0 M TFA were performed at 120 °C for 1 h (Olson, Gray, Chiu, Betschart, & Turnlund, 1988). The sugars obtained from the hydrolysis steps and the monosaccharide standards (glucose, arabinose, xylose, galactose, mannose) were acetylated with acetic anhydride as described by Blakeney, Harris, Henry, and Stone (1983). The acetylated monosaccharides were analysed with gas chromatography (GC) using a Micromat HRGC 412 (Orion Analytica, Finland) gas chromatograph equipped with a flame ionization detector. The column was a NB-17 fused silica capillary column (25 m × 0.32 mm i.d., film thickness 0.25 μm, Nordion, Finland). Helium was used as carrier gas (pressure 0.7 bar). Split injection (split ratio 1:20) was performed at 225 °C, the detector operated at 280 °C. The column oven was programmed from 190 °C (4 min hold) to 230 °C (6 min hold) at a rate of 4 °C/min. The monosaccharides were identified according to their retention times and quantitated using an internal standard method involving myo-inositol. Free sugar residues were corrected by a factor of 0.9 to anhydro sugars, as present in polysaccharides (McCleary & Codd, 1991). All analyses were made in duplicate.

2.6. Spectrometric analyses

^{13}C CP/MAS measurements were done with a Varian ^{UNITY}*INOVA* 300 NMR spectrometer operating at 75 MHz for carbons. A 5 kHz spinning speed and 7 mm rotor were used. The contact time was 0.5 ms, acquisition time 20 ms and delay between pulses 3 s. The chemical shifts were adjusted using external secondary referencing hexamethylbenzene (HMB, methyl line set to 17.3 ppm). The same spectral window was used during the sample measurements.

The FT-IR spectra were recorded on a Perkin Elmer Spectrum One spectrometer (Perkin Elmer Ldt, Bucos, England) equipped with a Universal ATR sampling accessory and MIR TGS detector. The spectral range was 4000–600 cm^{-1}, resolution 4 cm^{-1} and 8 cans were collected for each sample.

3. Results and discussion

3.1. Characterization of ground grain and WIS

The compositions of oat and barley grains are shown in Table 1. The values for ash, crude protein, fat and β-glucan are in good agreement with the results reported earlier for oat and barley grains (Åman & Newman, 1986; McCleary & Codd, 1991; Wilhelmson et al., 2001). The amount of WIS material isolated by the method of Asp et al. (1983) in oats was 6.1 ± 0.9% and in barley 13.7 ± 1.7%. These values (%, dry matter) are averages of four parallel isolations. Manthey et al. (1999) determined that the content of WIS for six genotypes of oats was 6.0–7.1%. Aalto, Lehtonen and Varo (1988) found a content of 11.1–19.2% for water-insoluble fibre of barley grown in Finland. The total sugar residue was obtained from hydrolysis with H_2SO_4 with which all polysaccharides are hydrolysed to monomeric sugars. The sugars were calculated as the sum of all detected monosaccharides (glucose, arabinose and xylose) and corrected to anhydro sugars as present in polysaccharides (conversion factor 0.9; Table 1). Under the conditions of hydrolysis with TFA, only noncellulosic polysaccharides are hydrolysed and the monosaccharides released originate from starch, β-glucan and arabinoxylan.

Table 1
Chemical composition (% dry weight) of ground grains

Components	Oat grain	Barley grain
Moisture	8.6	8.7
Ash	2.2	2.1
Protein	13.4	11.8
Fat	8.5	4.4
Water insoluble fibre (WIS)	6.1	13.7
Total sugar residue[a]	57.9	60.1
Noncellulosic sugars[b]	48.0	55.1
β-Glucan	4.0	3.7

[a] From hydrolysis with H_2SO_4; includes starch.
[b] From hydrolysis with TFA; includes starch.

Isolated WIS of oats and barley was characterized for its crude protein, crude fat, total sugar and β-glucan contents (Table 2). The high values of protein and fat in the WIS show that a large part of these components are undigested in the procedure used (Asp et al., 1983). The undigested protein is partly of cell wall origin, as was also found in WIS material by Asp et al. (1983) and Vinkx et al. (1995), and partly originates from residual enzymes used in the procedure. The amount of fat in the WIS fraction of oats is two times higher than in barley, as also occurred in ground grain. The total contents of NSP in WIS are 41.7% for oats and 48.6% for barley. The β-glucan content in the WIS of oats (11.5%) is much higher compared with that of barley (6.7%). Since the contents were similar in ground grain, the lower barley β-glucan content in WIS may be an indication of its better extractability in water.

3.2. Analysis of WIS fractions

The extraction procedure (Gruppen et al., 1992) was chosen to fractionate the WIS and thus enable its analysis,

Table 2
Chemical composition (% dry weight) of WIS material

Components	Oat WIS	Barley WIS
Fat	9.2	4.2
Protein	17.3	21.8
Total sugars[a]	41.7	48.6
β-Glucan	11.5	6.7

[a] From hydrolysis with H_2SO_4.

Table 3
Percentages yields (% dry matter) of WIS fractions

Fraction	Oats	Barley
Fr. 1	27.0±4.7	23.4±5.0
Fr. 2	15.1±1.6	18.2±1.0
Fr. 3	12.4±2.5	17.9±3.1
Fr. 4	7.7±2.1	6.8±1.7
Fr. 5	7.1±1.6	11.1±1.2
Total	69.3	77.4

characterization and comparison in oats and barley. The DF of oats and barley contains β-glucan as the major and arabinoxylans as the minor NSP components. The water unextractability of arabinoxylans and β-glucan is believed to be caused by their noncovalent interactions between themselves and each other and also with other cell wall material such as cellulose and lignin (Izydorczyk et al., 1998; Maes & Delcour, 2001; Morgan et al., 1999). Another reason for this insolubility may be diferulic acid cross-links occurring between the arabinoxylan chains (Fincher & Stone, 1986). Arabinoxylans are first selectively extracted with saturated $Ba(OH)_2$, while most of the β-glucan remains insoluble (Gruppen et al., 1991). After this strong alkali extraction most of the β-glucan is released from the WIS material using water as an extractant. The remaining polysaccharide components can be extracted with alkali solutions. The unextracted residue contains mostly cellulose (Gruppen et al., 1991). The lignin content was not separately determined.

The results shown in Table 3 are averages of four parallel extractions. The total recovery of extractions of WIS was 69.3% for oats and 77.4% for barley. These relatively low recoveries show that losses occurred during the extraction procedure. Distributions of the five fractions (Fr. 1–Fr. 5) obtained show that the differences between oats and barley are small. The amount of polysaccharides was largest in Fr. 1 for both cereals, 27% for oats and 23% for barley. According to Gruppen et al. (1991) arabinoxylans and part of the β-glucan are extracted in this fraction. In subsequent water extraction (Fr. 2) the proportions for oats and barley were 15 and 18%, respectively. The amounts extracted with 1 M KOH are similar to those of fraction 2 (12 and 18%). When the alkali concentration was increased (4 M NaOH, Fr. 4), a small amount of WIS material (8 and 7%) was extracted. Residual alkali insoluble-material (Fr. 5) was 7% for oats and 11% for barley.

The protein content in fractions 1–5 was determined by the Kjeldahl method. $Ba(OH)_2$ (Fr. 1) and 1 M KOH (Fr. 3) released most of the WIS protein (data not shown). In the other fractions the amounts of protein were small. ^{13}C CP/MAS NMR spectra of the fractions confirm this distribution of protein (Figs. 2 and 3). The ICP-MS analyses of WIS material were performed to study the residual mineral elements remaining from alkali extractants, enzymes and solvents used in dialyses. The amount of Ba, probably originating from the extractant, was 2–3% in Fr. 1 and Fr. 2 and 8% in Fr. 5. Fractions 1–4 contained small amounts of Ca (1–4%). The amounts of other minerals measured in the fractions were negligible (< 1%). In our previous studies of oat grain (Ekholm et al., 2000) we showed that DF has a strong mineral binding capacity, especially for Ca, which may explain this element's contents in Fr. 1–Fr. 4.

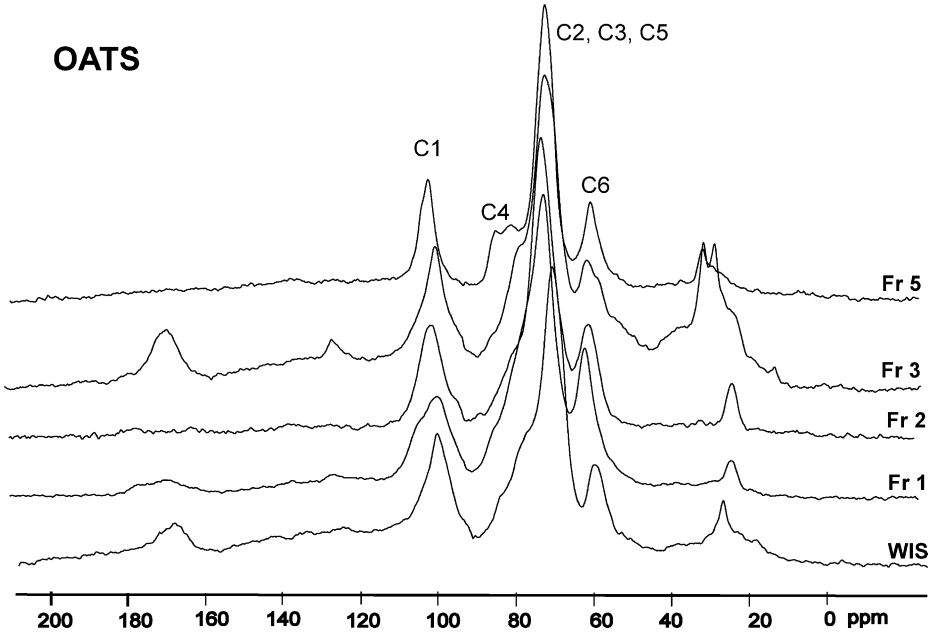

Fig. 2. ^{13}C CP/MAS NMR spectra of oat WIS and fractions (Fr. 1–Fr. 3 and Fr. 5) extracted from oat WIS material. The numbers refer to carbon atoms of glycosyl residue.

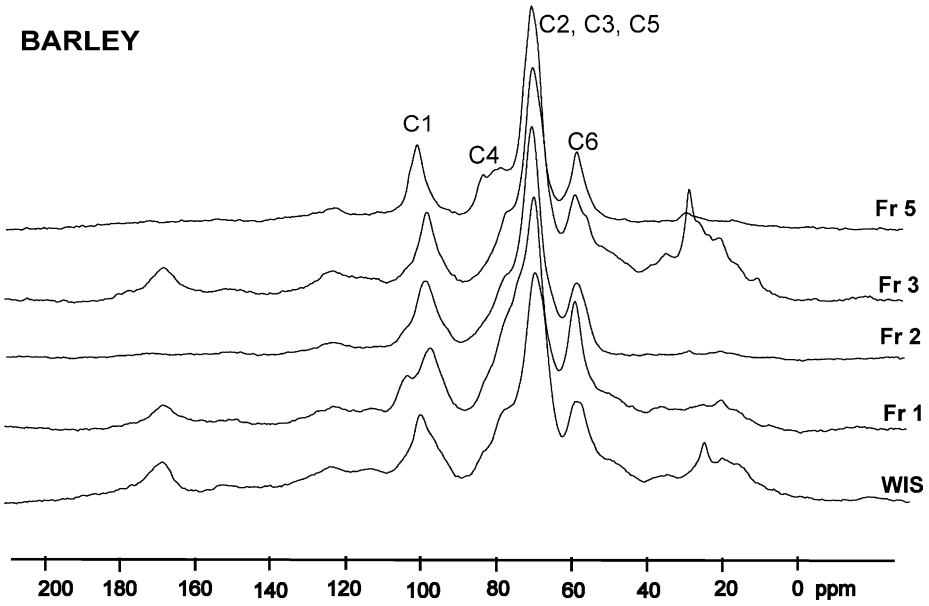

Fig. 3. ^{13}C CP/MAS NMR spectra of barley WIS and fractions (Fr. 1–Fr. 3 and Fr. 5) extracted from barley WIS material. The numbers refer to carbon atoms of glycosyl residue.

3.2.1. Monosaccharide analysis

Table 4 shows the contents of sugar residues for the extracted fractions (Fr. 1–Fr. 5) of oat and barley grain obtained using the two acid hydrolysis methods described above. The monosaccharide contents are presented as weight percentages of dry matter and are means of four parallel fractionations. Standard error of the mean (SEM) varied between 0.0 and 5.1% (Table 4). The results show that the fractions of WIS were composed of arabinose, xylose and glucose, mannose and galactose were not found in the samples analysed.

The total recoveries of sugar residues in the fractions were relatively small, varying between 9.8 and 66.6% for oats and 17.6 and 70.5% for barley. Analyses of the fractions described above show that they contained residual protein and also small amounts of mineral elements, lignin or other insoluble fibre components were not determined in this work. Possible losses of polysaccharides may have occurred due to incomplete hydrolysis by TFA and acid degradation during the hydrolysis by H_2SO_4.

As expected, arabinose and xylose were the main components in Fr. 1 extracted with $Ba(OH)_2$ (Gruppen et al., 1991), accounting for 80–95% of total monosaccharide content. Most of the glucose was extracted in Fr. 2, as was also found for rye grain by Nilsson et al. (1996). In Fr. 1–Fr. 3 the amount of arabinose, xylose and glucose differed only slightly in hydrolysis with TFA and H_2SO_4, showing that both acids used hydrolysed polysaccharides in a similar way. The results in Table 4 show clear differences in Fr. 4 and Fr. 5, in which the amount of glucose is increased significantly when H_2SO_4 was used in hydrolysis. The major monosaccharide component in Fr. 5 is glucose, indicating the presence of cellulose which is hydrolysed totally with H_2SO_4 but only slightly with TFA.

The ratio of arabinose and xylose (ara/xyl) is an indication of branching in arabinoxylan polysaccharide. A low ara/xyl value shows a low-branched structure, while

Table 4
Monosaccharide content (mean ± SEM % dry matter) and ara/xyl ratios of WIS fractions

	Fr. 1	Fr. 2	Fr. 3	Fr. 4	Fr. 5
Oats					
TFA					
Total	54.4	49.8	31.6	15.1	9.8
Ara	18.2 ± 1.6	4.8 ± 0.6	5.8 ± 1.0	2.0 ± 0.6	2.3 ± 0.5
Xyl	28.4 ± 1.9	7.3 ± 1.1	12.3 ± 2.1	4.0 ± 0.8	2.2 ± 0.3
Glc	7.8 ± 0.8	37.7 ± 5.1	13.5 ± 1.1	9.1 ± 1.9	5.3 ± 0.3
Ara/xyl	0.64	0.66	0.47	0.50	1.0
H_2SO_4					
Total	44.4	47.1	28.8	28.8	66.6
Ara	13.6 ± 1.3	4.0 ± 0.6	4.5 ± 0.9	1.8 ± 0.0	1.8 ± 0.2
Xyl	22.5 ± 1.7	6.7 ± 1.4	10.1 ± 1.9	3.3 ± 0.6	2.7 ± 0.2
Glc	8.3 ± 0.8	36.4 ± 2.7	14.2 ± 1.1	23.7 ± 5.1	62.1 ± 3.6
Ara/xyl	0.60	0.60	0.45	0.55	0.66
Barley					
TFA					
Total	46.4	49.3	32.5	33.4	17.6
Ara	16.9 ± 1.2	10.6 ± 1.2	7.0 ± 0.7	6.5 ± 0.8	7.1 ± 1.0
Xyl	27.3 ± 1.8	15.1 ± 1.5	15.0 ± 0.9	13.6 ± 1.4	6.6 ± 0.7
Glc	2.2 ± 0.4	23.6 ± 3.0	10.5 ± 1.2	13.3 ± 1.6	3.9 ± 0.4
Ara/xyl	0.62	0.70	0.47	0.48	1.10
H_2SO_4					
Total	39.4	52.6	32.8	34.6	70.5
Ara	13.6 ± 1.4	9.8 ± 1.5	6.3 ± 0.5	4.9 ± 0.7	6.9 ± 1.0
Xyl	22.9 ± 1.9	13.7 ± 1.7	13.6 ± 0.8	10.7 ± 1.1	6.4 ± 0.7
Glc	2.9 ± 0.9	29.1 ± 2.0	12.9 ± 0.9	19.0 ± 2.4	57.2 ± 2.4
Ara/xyl	0.59	0.72	0.46	0.46	1.10

high values indicate a high degree of branching. The ara/xyl values (Table 4) varied between 0.60 and 0.66 for oats and 0.59 and 0.72 for barley in Fr. 1 and Fr. 2. The values decreased in Fr. 3 and Fr. 4, with the average being approximately 0.50 for both oats and barley and indicating a more branched structure of arabinoxylane in these fractions. A highly branched state for arabinoxylan is indicated in Fr. 5 with an ara/xyl value close to 1. These results of ara/xyl are in good agreement with those of Nilsson et al. (1996) for rye grain.

3.3. Spectrometric analysis

The solid-state ^{13}C CP-MAS technique was used to obtain direct information on the materials extracted from the WIS fractions of oats and barley. The solubility of the extracted fractions in water or any dilute solvent was poor due to the nature of the polymeric material itself and also to intermolecular associations occurring after freeze-drying. The amounts of the fractions were also small. Although the resonances in the spectra were broad, it was possible to monitor the chemical composition of samples without interfering effects caused by strong solvents. Detailed NMR analysis of the fractions was not possible without further purifications, but solid-state measurements made comparison of the fractions possible.

The spectra of the oat and barley fractions are shown in Figs. 2 and 3. The amounts in Fr. 4 were too small for reliable measurements and the spectra are not shown in the figures. The fractions of WIS were mixtures of NSPs and other constituents. All the spectra of WIS and its fractions in both oats and barley show broad resonances in the region 50–110 ppm that are partially overlapping signals of polysaccharide carbons (Figs. 2 and 3). Resonances in the region 10–40 ppm are of aliphatic origin, probably polyethylene chains originating mainly from fat residues and partly from aliphatic carbons of proteins (Gidley, 1992; Pizzoferrato et al., 2000). These resonances are present in all oat fractions but missing from Fr. 2 and Fr. 5 of barley. In the WIS material of oats the amount of residual fat is two times higher than in barley (Table 2) and thus fat appears in all extracted fractions. Signals at 173 ppm in WIS and Fr. 1 and Fr. 3 were of carbonyl carbons from cell wall proteins (Davies et al., 2002) and partly also from enzymes used in isolation. These signals were present only in alkali-extracted fractions 1 and 3, as also observed in Kjeldahl analyses.

The results of the sugar analysis (Table 4) discussed above suggest that the fractions of WIS (Fr. 1–Fr. 5) are

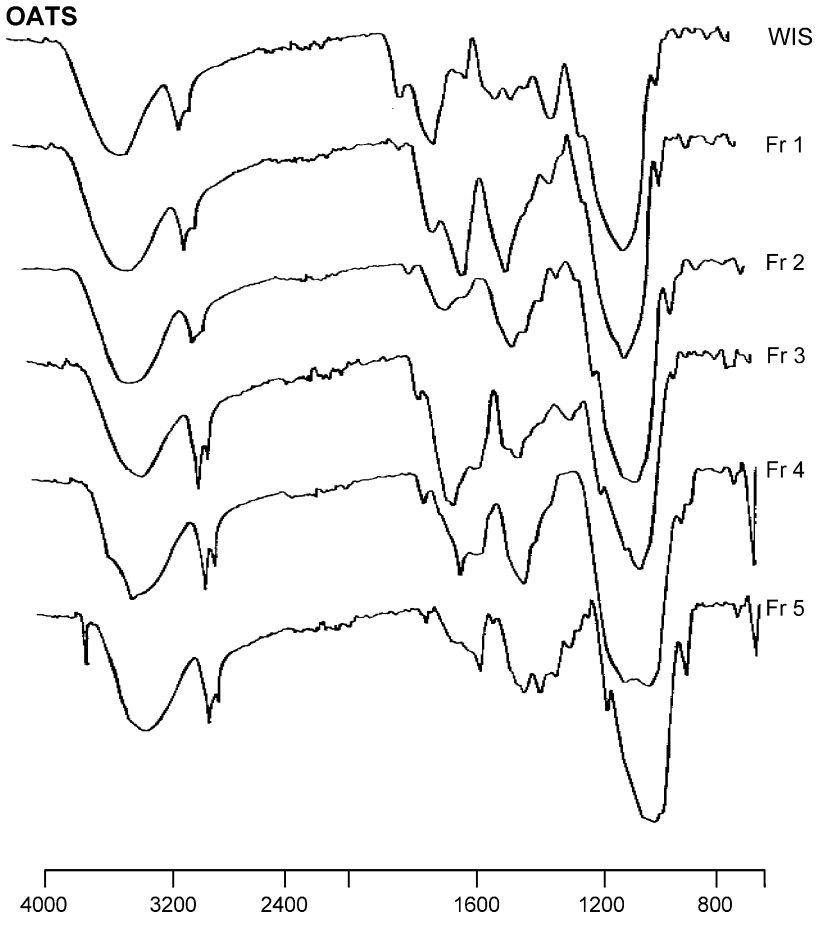

Fig. 4. FT-IR spectra of oat WIS and fractions (Fr. 1–Fr. 5) extracted from oat WIS material.

mixtures of NSPs, arabinoxylans, β-glucan and cellulose. Fraction 5 (the residue) contains mostly cellulose. The CP/MAS spectra of polysaccharides differ from each other only slightly. Signal assignment was based on data obtained from the literature for the solid- and solution-state spectra of arabinoxylans, β-glucan and cellulose (Bengtsson & Åman, 1990; Bock et al., 1991; Dais & Perlin, 1982; Davies et al., 2002; Dudley et al., 1983; Johansson et al., 2000) and reference spectra run from model compounds of β-glucan and arabinoxylan. The resonance at 103–105 ppm was assigned to anomeric carbon C-1 and signal at about 60 ppm to C-6. The polysaccharide carbons C-2, C-3 and C-5 appear at 74–75 ppm. The broad shoulder in this intense signal at 80 ppm is from C-4. Another shoulder in the spectra of Fr.1 at 108 ppm is due to C-1 in the arabinose side chain of the arabinoxylan structure (Bengtsson and Åman, 1990). Broad signals are present in the spectra of Fr. 5 in the spectral region 80–90 ppm originating from the C-4 carbon of cellulose (Davies et al., 2002; Dudley et al., 1983).

The FT-IR spectra of WIS and the fractions (Fr. 1–Fr. 5) of oats and barley are shown in Figs. 4 and 5. The spectra were assigned according to data presented in the literature (Kačuráková et al., 2000, 1994; Proniewicz et al., 2001; Sekkal et al., 1995). The oat and barley spectra show similar characteristic broad absorption bands typical of polysaccharide structures. All the spectra are mixtures of NSPs as discussed above. The spectra have bands at 3000–3600 cm^{-1} of O–H-stretching and at 2800–3000 cm^{-1} of CH_2-stretching. The strongest bands in the spectra at 1000–1200 cm^{-1} are overlapped ring vibrations, stretching bands of side groups (C–OH) and glycosidic bond vibrations (Kačuráková et al., 2000). Some differences between the fractions arise in the region 1200–1700 cm^{-1} where the specific assignments of bands are difficult. The bands in this region are due to mixed and complex vibrations related to OCH deformation and CH2, CCH and COH bending rather than to characteristic group frequencies (Proniewicz et al., 2001). This region also shows the bands of residual proteins (Hromádková et al., 2003) and bending vibrations of water molecules (1600–1650 cm^{-1}). The sharp absorption occurring at about 895 cm^{-1} shows the β configuration of the glucan linkages (Gutiérrez, Prieto, & Martínez, 1996). Comparison of the spectra of different oat fractions with those of barley show that the absorptions are similar but the band intensities differ slightly.

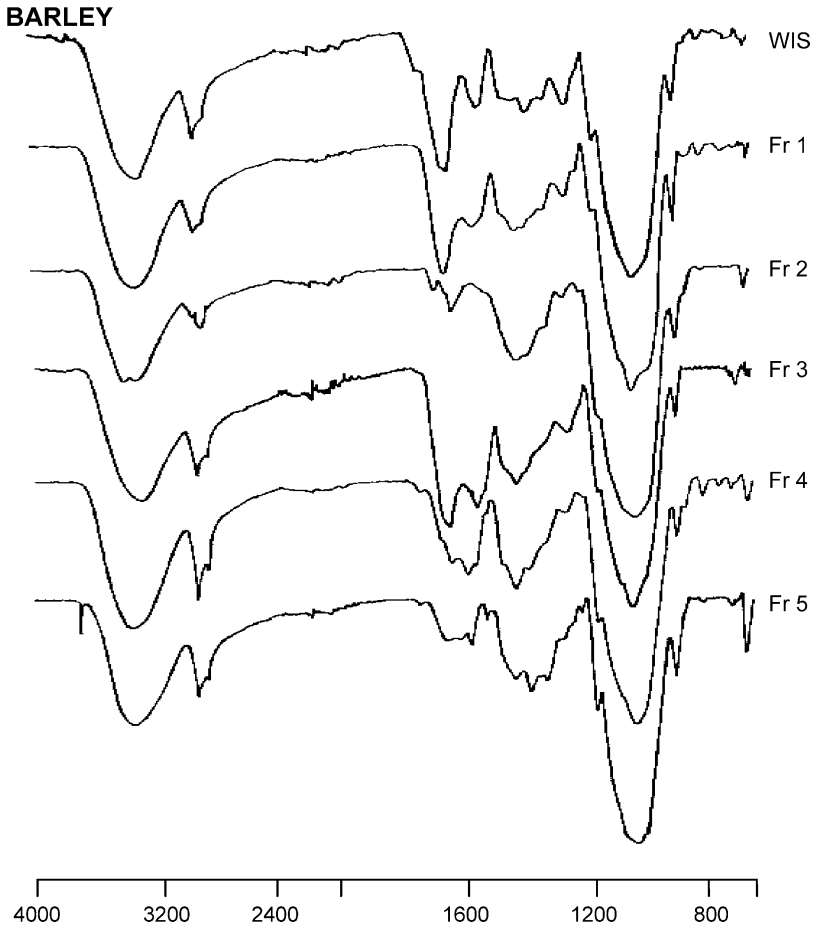

Fig. 5. FT-IR spectra of barley WIS and fractions (Fr. 1–Fr. 5) extracted from barley WIS material.

4. Conclusions

The results of this study show that the amount of water-insoluble material in barley is much higher than in oats. The extraction procedure used here separated the insoluble material of oats and barley into five fractions with different types of polysaccharides. Repeatable recoveries of extracts were obtained and the total yields of the fractions were about 70–80%. The distribution of the five fractions obtained show only small differences between oats and barley.

TFA and H_2SO_4 were used to hydrolyse the fractions in order to separate the cellulosic and noncellulosic sugar residues. The monosaccharides found in WIS material were glucose, arabinose and xylose. The results of both oats and barley show that in Fr. 1–Fr. 3 both acids hydrolysed the material similarly, thus demonstrating that the polysaccharides were mostly noncellulosic. In fraction 4 and especially fraction 5, most of the material originated from cellulose. The ara/xyl ratio indicates the degree of branching in arabinoxylan polysaccharide. Branching appears to strongly affect the solubility of arabinoxylans. Arabinoxylans showing an intermediate ara/xyl ratio are soluble in $Ba(OH)_2$ and water after this alkali treatment, lower or higher ara/xyl ratios of arabinoxylans indicate weaker solubility.

The results of spectrometric measurements with solid-state ^{13}C CP/MAS NMR and with FT-IR showed that the spectra of oats and barley were very similar and demonstrated the characteristic signals of saccharide structures. Only small differences were found between the structures of the extracted fractions of the water-insoluble material.

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