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Isolation and characterization of physicochemical and material properties of arabinoxylans from barley husks

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Abstract

Different methods of isolating arabinoxylans from barley husks were compared and the effect of isolation method on yield, composition and physicochemical as well as material properties was investigated. The isolation involved HCl or enzyme pretreatment, chlorite or organosolv delignification and an extraction with aqueous alkali. Four fractions of isolated arabinoxylan were obtained. The arabinoxylan content in the isolated fractions varied between 50 and 83%, with the highest content in the chlorite-delignified sample. In addition to the sugar composition, the amount of lignin and protein was determined. The molecular weights of these samples varied between 35,000 and 45,000 g/mol, determined by SEC in water and DMSO. Films were made by water casting, and the delignified samples formed clear and flexible films. The mechanical properties of the chlorite-delignified films were evaluated by tensile testing. The stress at break was 50 MPa and strain at break 2.5%. Measurement of the equilibrium moisture content at 50% RH and 100% RH showed that the films were highly hygroscopic.

Keywords: Arabinoxylan; Isolation; Barley husk

1. Introduction

1.1. Renewable polymers

Today, plastic materials are almost exclusively made from petroleum. Intensive research and development during the 20th century generated a wide variety of polymers from this fossilic, non-renewable material. As oil wells begin to run dry it will become more and more expensive to produce petroleum-based polymers. This development will certainly pave the way for new materials created from renewable resources. However, although we still have an abundant supply of inexpensive oil, it has become commercially interesting to find replacements for traditional plastics because of the requirements of authorities in different countries, a surplus of waste materials from agriculture and forestry and growing environmental awareness among a great number of populations.

Packaging accounts for 41% of the total plastics consumption and is the largest user sector (APME, 2004). There is interest in replacing synthetic polymers with materials that can be made from biomass, providing these alternative materials have equivalent properties and can be produced easily enough to make them economically competitive with traditional plastics.

Hemicelluloses are the second most abundant plant biopolymers on earth (Gatenholm & Tenkanen, 2004). They are hetero-polysaccharides present in large quantities in wood and annual plants, where they are interconnected together with cellulose and lignin in the cell wall. The most abundant hemicellulose in annual plants is arabinoxylan. Arabinoxylan contains a backbone of D-xylopyranosyl residues, linked together by β-(1→4)-glycosidic bonds. To these are attached (through positions C-2 or C-3 or both) a number of L-arabinofuranose and D-glucuronic acid (or its 4-*O*-methyl derivative) residues as single-unit side chains (MacGregor & Fincher, 1993). Naturally occurring xylan contains *O*-acetyl groups located at some of the hydroxyl groups in the xylan backbone. These groups are removed when alkali is used during the isolation process (Gabrielii, Gatenholm, Glasser, Jain, & Kenne, 2000).

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1.2. Isolation of hemicelluloses

Many attempts have been made to isolate hemicelluloses from various biomass sources (see the review by Ebringerová & Heinze, 2000). Hemicelluloses can be isolated by extraction and consist of two different types, watersoluble and waterinsoluble. A general method for water extraction of hemicelluloses was described by Bengtsson & Åman (1990). Only a smaller part of the hemicelluloses are watersoluble and can be extracted with hot water. A somewhat more effective neutral solvent is dimethyl sulfoxide (DMSO), and a mixture of 90% DMSO and 10% water has been found to be most effective (Saake, Kruse, & Puls, 2001). Although only a part of the arabinoxylan can be extracted with DMSO, the advantage is that no chemical changes take place.

Larger amounts of arabinoxylan can be extracted with alkali, which, however, results in deacetylation (Gabrielii et al., 2000). The release of arabinoxylans from biomass during alkali extraction may be assisted by pretreatment procedures, such as mild hydrolysis (Gabrielii et al., 2000; Glasser, Kaar, Jain, & Sealey, 2000; Gustavsson Bengtsson, Gatenholm, Glasser, Teleman, & Dahlman, 2001). A delignification step using chlorite or ethanol can also be included to remove lignin and further facilitate the extraction. A variety of multi-step extraction and purification procedures has been proposed to prepare arabinoxylans of desired purity from cereal grains (Doner & Hicks, 1997; Fang, Sun, & Tomkinson, 2000; Fang, Sun, Salisbury, Fowler, & Tomkinson, 1999; Hromádková & Ebringerová, 1995; Izydorczyk & Biliaderis, 1995; Nilsson, Saulnier, Andersson, & Åman, 1996; Oscarsson, Andersson, Salomonsson, & Åman, 1996; Puls, Poutanen, Körner, & Viikari, 1985; Sun, Lawther, & Banks, 1996; Viëtor, Kormelink, Angelino, & Voragen, 1994; Zhuang & Vidal, 1997), even on a pilot scale (Annison, Choct, & Cheetham, 1992; Bataillon, Mathaly, Nunes Cardinali, & Duchiron, 1998; Faurot et al., 1995; Glasser, Jain, & Sjöstedt, 1995). Some experiments have used different enzymes such as amylase and protease in the pretreatment step to remove protein and starch (Buchanan et al., 2002; Izydorczyk, Macri, & MacGregor, 1998).

The yield from alkali extraction of annual plant biomass has been reported to be around 50% of the available hemicelluloses (Doner et al., 1997; Fang et al., 2000). Sun and Sun (2002) were able to extract 91% of the available hemicelluloses from barley straw using eight sequential extraction steps with sodium hydroxide, hydrogen peroxide and potassium hydroxide. The influence of different extraction conditions on the yield and physicochemical properties of the isolated hemicelluloses has also been investigated (Methacanon, Chaikumpollert, Thavorniti, & Suchiva, 2003).

This study compared different methods of isolating arabinoxylans. The aim was to find a suitable method for isolating arabinoxylans from barley husks in order to achieve a product with high molecular weight that could later be used for the preparation of oxygen barrier films or coatings. Further aims were to determine the structure of the isolated arabinoxylan in order to understand the effect of the isolation method on the structure and to make an initial evaluation of the material properties.

2. Experimental

2.1. Material

Barley husks (*Hordeum Vulgare*) were obtained from Lyckeby Stärkelsen, Kristianstad, Sweden. The barley used is a special kind called Cindy or waxy barley, which is a high-amylopectin barley, containing 96% amylopectin. The husk is very strongly attached to the grain and must be removed by abrasion or peeling. Thus, the husks contain some starch and protein from the kernel. The raw material was first ground in a mill (Urschel Laboratories Inc. 3600) to a particle size of less than 1 mm.

2.2. Isolation

Four different isolation methods were compared in order to find the simplest means of extraction and to identify how the isolation process affects the structure. Hence, four fractions of isolated arabinoxylan were obtained, AX1–AX4. The barley husks were extracted sequentially with different liquids. The stepwise separation of components from barley husk biomass is illustrated in Fig. 1.

Samples AX1–AX3 were prepared according to the methods described by Glasser et al. (2000). One-hundred grams of ground, oven-dried barley husks were mixed with 1 L of 0.05 M hydrochloric acid (HCl) and the suspension was stirred at room temperature for 16 h. Thirty milliliters of 25% ammonium hydroxide (NH_4OH) was added to sample AX2 to remove pectins, and the slurry with a pH of 10.3 was stirred at room temperature for 16 h. The fiber was then recovered by centrifugation and washed with water.

The fiber from sample AX2 was mixed with 0.5 L of 1% sodium hydroxide (NaOH) in 50% ethanol and the suspension was heated to 75 °C. The fiber was extracted for 2 h for delignification. The mixture was allowed to cool to room temperature and the holocellulose (solid residue) was recovered by centrifugation and thoroughly washed with water. The fiber from sample AX3 was mixed with 1 L of water and the pH was adjusted to 4.0 with glacial acetic acid. Twenty-two grams of sodium chlorite (80% purity) was added and the temperature was raised to 75 °C. After 2 h of extraction, the mixture was allowed to cool to room temperature and the fiber was recovered by centrifugation and washed thoroughly with water.

Sample AX4 was prepared by mixing 100 g of barley husks with 1 L of water in a bottle, and the pH was adjusted to six using HCl. One-hundred microliters of thermostable α-amylase (Termamyl Ultra 300 L, Novozymes) were

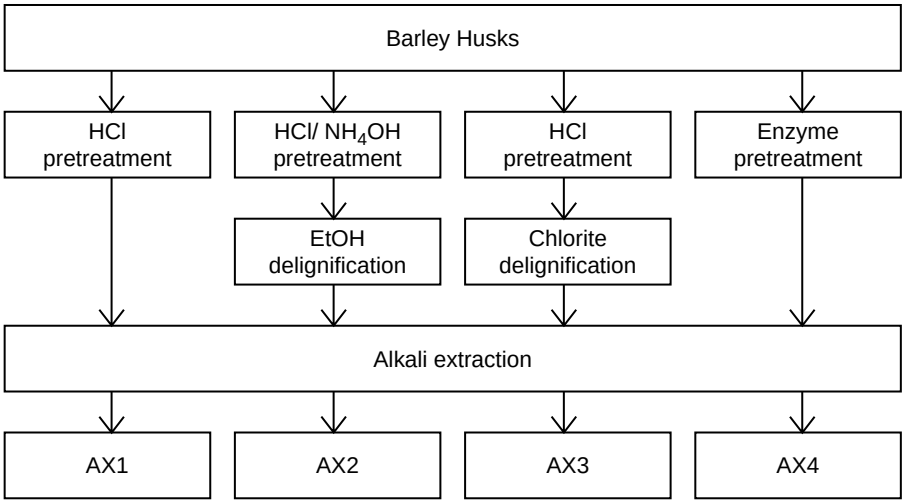

Fig. 1. Schematic presentation of the isolation methods used.

added and the bottle was placed in a 80 °C water bath. The bottle was shaken every 15 min. After 45 min the temperature was slowly reduced to 60 °C. One-hundred microliters each of protease (Subtilisin A, Megazymes) and amyloglucosidase (Roche Diagnostics) were added. After a further 45 min, the temperature was raised to 100 °C for 15 min in order to inactivate the enzymes. The suspension was allowed to cool to room temperature and the fiber was recovered by centrifugation and washed with water.

All samples were then treated similarly. The fiber was mixed with 1 L of 1 M NaOH solution containing 0.5% of sodium borohydride ($NaBH_4$) in order to prevent degradation of the hemicelluloses by peeling reactions. The fiber was extracted at room temperature for 16 h. The extract was recovered and the pH was adjusted to seven using HCl to stop any further hydrolysis. The extract was concentrated by rotor evaporation and dialyzed for 2 days against deionized water (Spectra/Por 2 membrane, MWCO: 12-14,000, Spectrum laboratories Inc.) The products were collected by freezedrying (Labconco Freezone 4.5).

2.3. Composition analysis

The starch content was determined by enzymatic degradation followed by glucose quantification with High Performance Liquid Chromatography (HPLC). The equipment used was an Aminex HPX-87H column and guard columns from BioRad, an HPLC pump, an auto-sampler, an RI detector, a column oven, a degasser and a computer. The mobile phase was 0.005 M sulfuric acid (H_2SO_4) and the flow rate was 0.6 mL/min The column temperature was 35 °C and the detector temperature 30 °C. One gram of dry sample was mixed with 50 mL of water and 5 mL of 5 M potassium hydroxide (KOH) and was placed in a shaking water bath at 95 °C for 5 min. The pH was adjusted to 5.0 with 5 M acetic acid and five drops of 50% calcium chloride ($CaCl_2$). One-hundred microliters of α-amylase (Termamyl 300 L DX, Novo Nordisk) was added. The solution was again placed in a shaking water bath at 95 °C for 1 h. One-hundred microliters of amyloglucosidase (Boehringer–Mannheim Scandinavia AB) was added and the solution was placed in a shaking water bath at 60 °C for 1 h. The solution was finally filtered, washed and diluted to 200 mL before injection of 27 μL onto the HPLC.

The protein content was calculated from the nitrogen content, determined by the Kjeldahl method using the Kjeltec System 1 analysis system. The system consists of three units, Digestion system 6, Kjeltec Auto Distillation 2200 and Metrohm Dosimat 665. Brome-cresol green and methyl red were used as indicators. The samples were dried to 100% dryness in a moisture content meter (Mettler LP 15) at 135 °C. One gram of sample was placed in the boiler tube, and two catalyst tablets (Special Kjeltabs Cu 3.5) and 12 mL of concentrated H_2SO_4 were added. The sample was refluxed for 30 min. Twenty-five milliliters of 4% boric acid was placed in a receiving flask. The boiler tube and the receiving flask were placed in the distillation unit. After analysis, the receiving solution was green, indicating the presence of a base: ammonium. The receiving solution was titrated with 0.1 M HCl until a change in color was observed. A blank sample was also analyzed according to the same procedure. The percentage of nitrogen was calculated as follows:

$$\%N = \frac{(\text{mL HCl sample} - \text{mL HCl blank}) \times (\text{M of acid}) \times 14.007 \times 100}{\text{weight of sample (mg)}}$$

The percentage of protein was then calculated as follows:

$$\%\ \text{protein} = \%N \times 6.25.$$

The lignin content and carbohydrate composition of the samples was analyzed by the Uppsala method for determination of total dietary fiber (Theander, Åman, Westerlund,

Andersson, & Pettersson, 1995) with minor adjustments. The hydrolyses were done in test tubes with pressure-safe lids with a total volume of 10.4 mL instead of a total volume of 100 mL. A Reactiterm was used instead of an autoclave. Furthermore, the amount of standard solution (*myo*-inositol) added before hydrolyses was 1 mL instead of 10 mL. Similarly, a smaller amount of sample was hydrolyzed, 50 mg instead of the recommended amount of 250–500 mg. The basic outline of the analysis is as follows: the sample material is hydrolyzed to its monosaccharide components and the solution is filtered. The undissolved material is defined as Klason lignin and determined by gravimetry. The monosaccharide solution is then divided into two parts. One part of the solution is used to determine the amount of uronic acids by a colorimetric method (Scott, 1979). The other part of the solution is first reduced with $NaBH_4$ to sugar alditols and subsequently acetylated with acetic acid anhydride. These acetylated sugar alcohols are volatile and can be separated, identified and quantified by Gas–Liquid Chromatography (GLC).

2.4. Nuclear magnetic resonance

For proton Nuclear Magnetic Resonance (^{1}H NMR) analysis, a portion of the dried samples (10 mg) was dissolved in D_2O and freezedried to dryness again. This procedure was repeated five times, and the samples were finally dissolved in 0.75 mL D_2O. One drop of tetramethyl silane (TMS) was added as an internal standard. ^{1}H NMR spectra were recorded at 85 °C on a Varian VRX 300 instrument. The pulse repetition time was 4.2 s and the radio frequency pulse angle 30°.

2.5. Size exclusion chromatography

The molecular weights of the arabinoxylan fractions, AX1–AX4, were examined using an HPSEC-MALLS-RI-UV system consisting of a Waters 2690 with on-line degasser, an auto-sampler and a column oven (Waters, Milford, MA, USA) and two serially connected columns controlled at 50 °C (TSK gel G6000 WXL and TSK gel GMPWXL, TosoHaas, Sturtgart, Germany). The detectors were multi-angle laser light scattering (Dawn DSP equipped with an He–Ne laser at 632.8 nm, Wyatt Technology Corp., Santa Barbara CA, USA), refractive index (RI) controlled at 35 °C (Optilab DSP, Wyatt Technology Corp.) and UV monitor set to record at 280 nm (Shimadzu SPD-10A, Shimadzu Corp., Kyoto, Japan). The eluent was 0.1 M sodium nitrate ($NaNO_3$) containing 0.02% sodium azide (NaN_3), and the flow rate was 0.4 mL/min. Different pullulan standards (Pullulan 5, 10, 20, 100 and 200) were used to give approximate values of the molecular weights. The samples were prepared by dissolving 20 mg of arabinoxylan in 10 mL of 0.1 M $NaNO_3$. The solution was then filtered with a 0.22 μm filter before injection of 100 μL in the Size Exclusion Chromatography (SEC) system.

SEC was also run with DMSO as solvent to prevent agglomeration of the arabinoxylans. This was done according to a procedure described elsewhere (Saake et al., 2001).

2.6. Solubility

The solubility of the arabinoxylans was tested by mixing 20 mg of sample and 10 mL of solvent at room temperature by magnetic stirring for 16 h. The solvents tested were water, 0.5 M NaOH, DMSO, acetone, chloroform and hexane.

The water solubility of the isolated fractions was further evaluated by mixing 1 g of sample with 100 mL of deionized water during magnetic stirring for 72 h. The samples were centrifuged at 3000 rpm for 15 min and the solution was separated from the insoluble material. The amount of insoluble material was determined gravimetrically after freezedrying.

2.7. Preparation of films

Films were prepared by mixing 1 g of the isolated fractions with 35 mL of deionized water during magnetic stirring at 95 °C for 15 min. The solutions were poured onto polystyrene Petri dishes with a diameter of 14 cm, and films were allowed to form upon drying at a temperature of 23 °C and a relative humidity (RH) of 50%. The films were stored in these conditions until analysis.

2.8. Tensile testing

The mechanical properties of the conditioned films of fraction AX3 were measured with a tensile testing machine (Lloyd L2000R) with a load cell with a capacity of 100 N. The samples were cut into dogbone-shaped strips with a width of 7 mm. The thickness of the samples, measured with a micrometer, was 49 ± 7 μm. The initial distance between the grips was 20 mm, and the separation rate of the grips was kept constant at 5 mm per min. Fifteen replicates were tested. The stress–strain curve was recorded for each sample, and Young's modulus, strength at break and strain at break were calculated. The measurements were made at 50% RH and 23 °C after conditioning for at least 1 week.

2.9. Wide angle X-ray scattering

The crystallinity of the AX3 fraction was examined with Wide Angle X-ray Scattering (WAXS). The film was milled in liquid nitrogen and investigated with a Siemens D5000 goniometric diffractometer. Cu K_α radiation with a wavelength of 1.54 Å was used, and 2θ was varied between 5 and 30° at a rate of 1° (2θ) per min and a step size of 0.1° (2θ).

2.10. Water content

Three pieces of the AX3 films were conditioned in relative humidities of 50 and 100%, respectively, at ambient temperature. The samples were weighed and, after equilibration of the water content, dried at 130 °C overnight.

The equilibrium water content was calculated as the weight of water in the sample at equilibrium compared to the total weight.

3. Results and discussion

3.1. Chemical composition

The chemical composition of the barley husks is shown in Fig. 2. The products from the isolation did not consist of pure arabinoxylan. Analysis of products showed great differences in composition. The arabinoxylan content varied between 50 and 83%, with the highest content in the chlorite-delignified sample. Fig. 3a–d show the chemical composition of the isolated fractions, AX1–AX4. The origin of glucose is both starch and β-glucan.

The purpose of the pre-hydrolysis step was to remove starch, proteins and fat. Starch granules are swollen when a water mixture is heated and the granules are finally dissolved. Hence, heat is required to remove the starch from the sample. A hot acid solution causes severe degradation of hemicelluloses by acid hydrolysis reactions, however, and must be avoided or the isolated product will be of low molecular weight and not suitable for film applications. Thus, the pre-hydrolysis was carried out at room temperature. As a result, the amount of starch left after pretreatment is still high, which can be seen in sample AX1.

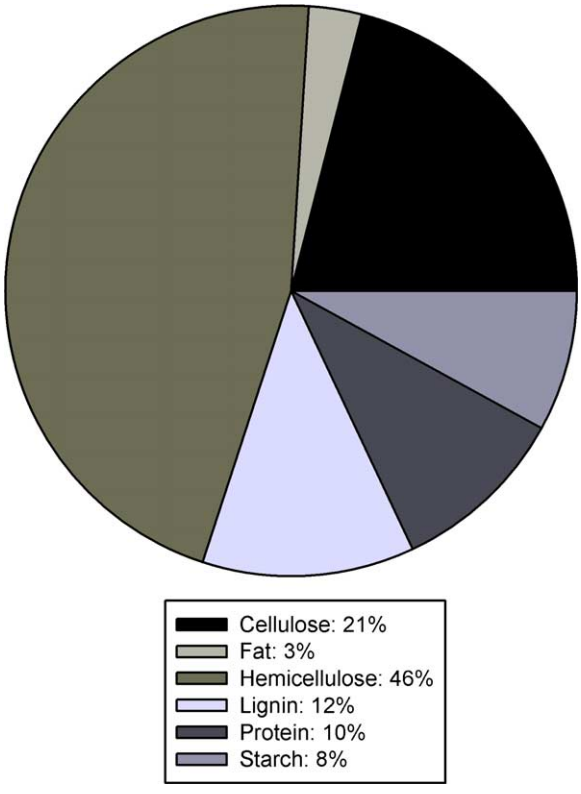

Fig. 2. Chemical composition of the barley husks.

It can be discussed whether the pre-hydrolysis step at room temperature could be eliminated as starch and proteins are not sufficiently removed even with pre-hydrolysis. However, between 15 and 20% by weight of the starting material is actually removed during the step. Further, the pre-hydrolysis makes the fiber swell and become more susceptible to subsequent treatments. NH_4OH was added to the solution in order to remove pectin. However, the amount of pectin in barley husks is negligible and the step could be eliminated.

The enzyme treatment done in this study did not meet our expectations as regards protein and starch removal. On the contrary, sample AX4 had the highest protein content of all samples. Even if the protein was degraded by the enzymes, it was not removed sufficiently during washing. Another method that may be more effective is treating the arabinoxylan product with enzymes after extraction.

Both delignification methods worked well. Most of the lignin was removed without any noticeable degradation of the arabinoxylan. The chlorite treatment was somewhat more effective than the organosolv method, as 2.5% of lignin was left in AX3 as compared to 3.9% in AX2. The chlorite treatment is also the most selective.

Another interesting aspect is that the protein content is considerably lower in delignified samples, AX2 and AX3, than in AX1. The higher temperature could be the reason for the proteins being removed during delignification and not in the pretreatment step. During the chlorite delignification step, starch is also removed very effectively because it is dissolved in hot water. However, starch is not soluble in ethanol, which explains the high starch content in organosolv-delignified sample AX2 despite the fact that the same temperature was used. The pretreatment step may be unnecessary when using chlorite delignification, since the chlorite step also removes starch and protein. This would be an economic advantage in an industrial scale, but the effect of leaving out the pretreatment must be evaluated.

Alkali extraction was done at room temperature. The cold step can be followed by a hot alkali extraction step, which will dissolve some more arabinoxylan, but the yield is normally much lower in the hot step. Furthermore, hot alkali causes degradation of arabinoxylan by end-wise peeling reactions, depending on the temperature (Glasser et al., 2000). Since the aim was to reach as high molecular weights as possible, only cold extraction was used.

3.2. Monosaccharide composition

The sugar composition was monitored with GLC. The results are shown in Table 1. The samples consisted mainly of the monosaccharides xylose, arabinose and glucose. The origin of glucose is both starch and β-glucan.

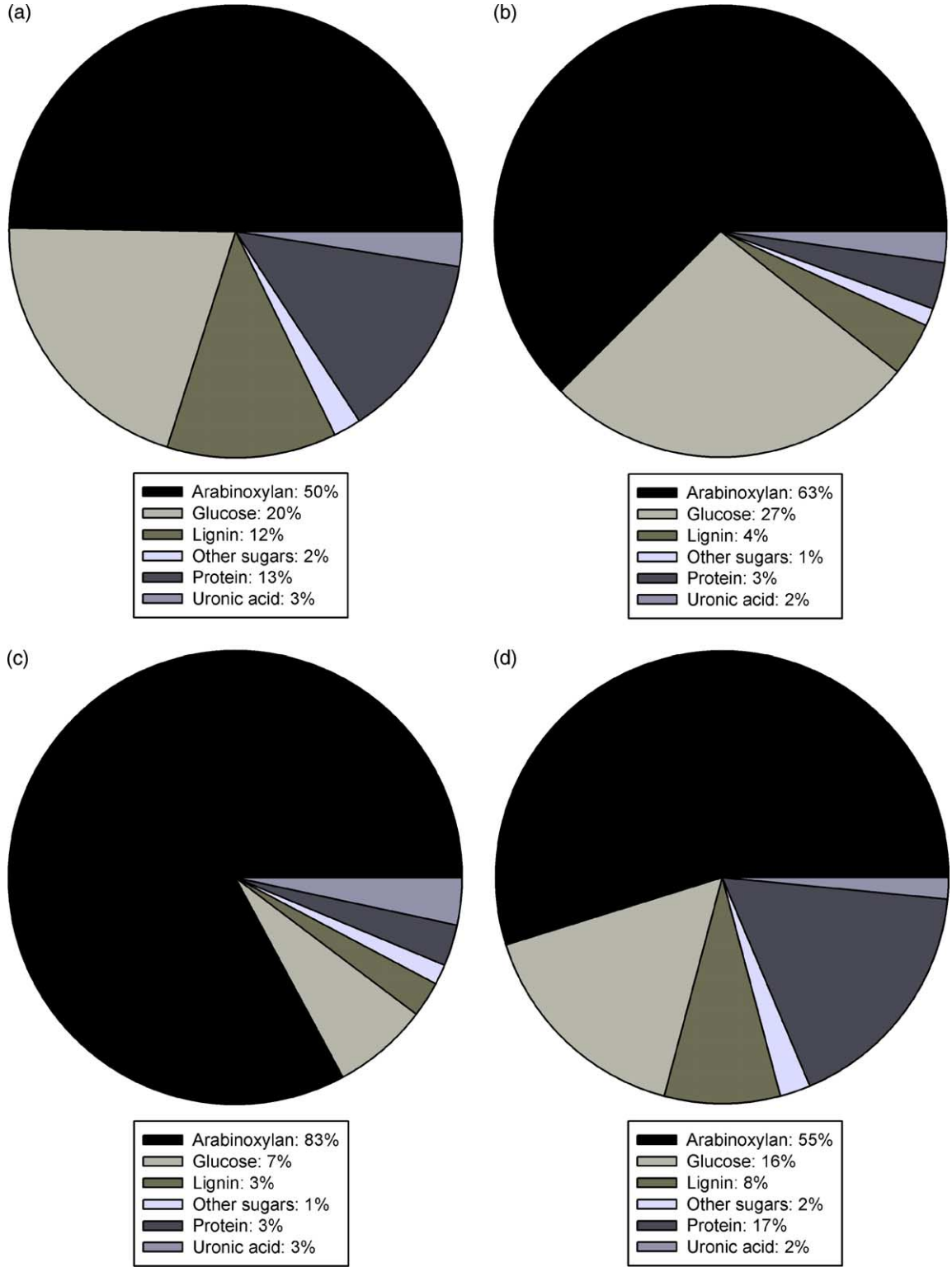

Fig. 3. (a) Chemical composition of fraction AX1. (b) Chemical composition of fraction AX2. (c) Chemical composition of fraction AX3. (d) Chemical composition of fraction AX4.

3.3. Yield

The yield of arabinoxylan from the different methods, as percentage of total available arabinoxylan, was 26.1% in AX1, 31.1% in AX2, 57.0% in AX3 and 25.1% in AX4. The yield is much higher in the chlorite method (AX3), probably because chlorite is the most selective delignification agent. The two samples that are

Table 1
% of total carbohydrate, determined by GLC after total hydrolysis, and xylose to arabinose ratio

Sample	Ara	Xyl	Glc	Xyl/Ara
AX1	13.1	55.9	28.3	4.28
AX2	10.7	58.3	29.6	5.44
AX3	16.5	74.4	7.5	4.52
AX4	20.4	54.6	22.0	2.68

not delignified (AX1 and AX4) have very similar yields.

3.4. NMR characterization

The four isolated fractions were analyzed by ^{1}H NMR spectroscopy to characterize the structural features of the isolated arabinoxylans. The spectrum of fraction AX1 is shown in Fig. 4. There are several strong signals at 1–3 ppm in samples AX1 and AX4 that are absent in the delignified samples, AX2 and AX3. These signals are most likely from proteins. This is in accordance with the results of the composition analysis, indicating that the proteins were not effectively removed during pretreatment with mild acid and NH_4OH, nor with protease. On the other hand, the proteins seem to have been sufficiently removed during delignification.

The broad peak around 7 ppm is from aromatic compounds, which originate mainly from lignin. It is not possible to calculate the lignin content by integration of the lignin signal because lignin is not completely dissolved. It can be seen, however that the signal is larger in AX1 and AX4 than in the delignified samples, AX2 and AX3, which means that there is more lignin in these samples, as already determined (Klason lignin).

The signals at 3.2–5.6 ppm are caused by the protons of the arabinose and xylose residues (Bengtsson et al., 1990) except for the strong signal at 4.23 ppm, which is residual

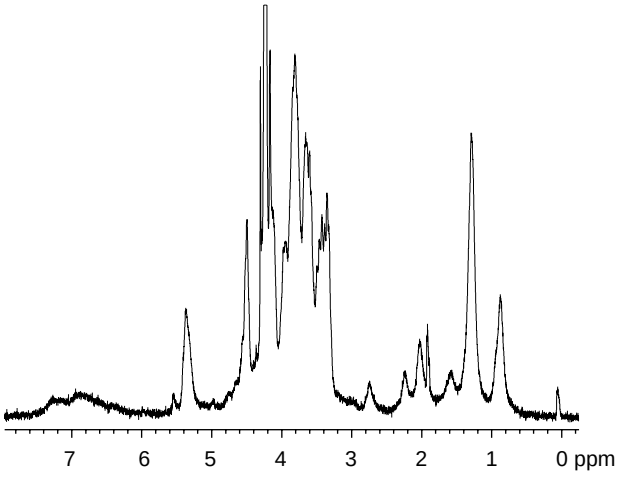

Fig. 4. The full ^{1}H NMR spectrum of AX1 showing protein signals (1–3 ppm), arabinoxylan (3–6 ppm) and lignin (~7 ppm).

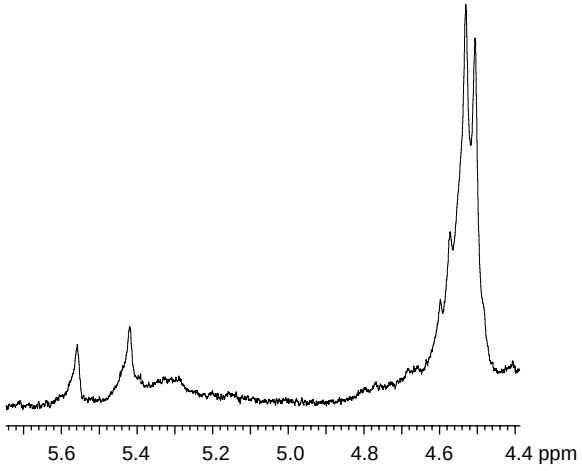

Fig. 5. The anomeric region of the ^{1}H NMR spectrum of AX3.

water. Especially interesting is the anomeric region from 4.4–5.6 ppm, see Fig. 5. Signals at 5.2–5.6 ppm in the ^{1}H NMR spectra have been assigned to anomeric protons of the α-L-Ara*f* residues of arabinoxylan. The anomeric signals from the xylose residues occur at 4.4–4.7 ppm (Bengtsson et al., 1990).

The distribution pattern of side groups in arabinoxylan is important for the solubility, interactions with other polymeric cell wall substances, degradability by enzymes and other functional properties. The substituted xylose residues may be randomly distributed along the chain, but they are more likely gathered in highly substituted regions, leaving longer sections with no arabinose residues. This has to do with the polymer's function in the cell wall. The presence of larger unsubstituted regions in the xylan chains can give rise to strong hydrogen bonds, causing interchain aggregation, and can also make the isolated material partly crystalline (Dervilly-Pinel, Tran, & Saulnier, 2004).

3.5. SEC characterization

The molecular weights (MW) of the four arabinoxylan fractions were determined by SEC. All isolation methods yielded polymeric arabinoxylan. However, the isolation method significantly affected the molecular weight distributions. All samples had one fraction with a very high MW, one with a MW ranging from 35,000 to 45,000, and a tailing of low MW components. According to the work of Bikova and Treimanis (2002), the medium MW components would be expected to be hemicelluloses and the low MW components are lignin (aromatic substances). SEC was done using water as the solvent. However, studies show that in some cases xylan in water is present as aggregates (Gabrielii et al., 2000). To avoid possible aggregation, SEC was also done in a 90:10 DMSO:water solution. There was also a peak at very high MW in DMSO, indicating that this is a high molecular weight polymer, most probably starch,

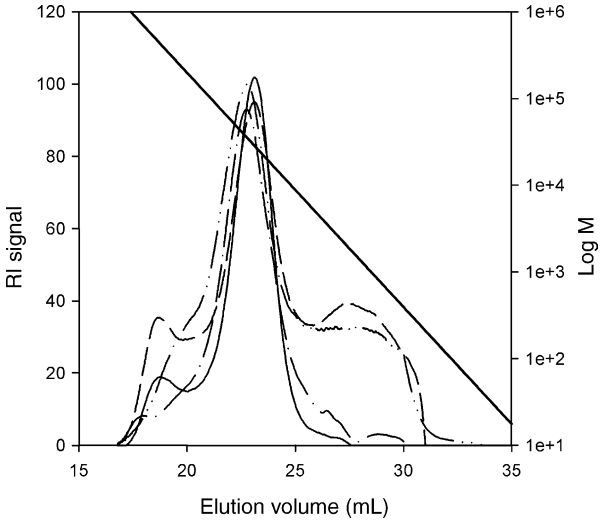

Fig. 6. Molecular weight distributions of fractions AX1–AX4 as RI signals from water SEC. Thick solid line: log *M*, thin solid line: AX1, dashed line: AX2, dash–dot: AX3, dash–dot–dot: AX4.

rather than aggregates. The RI signals from the water SEC are combined in Fig. 6, showing the molecular weight distributions. The calculated molecular weights from both the aqueous and the DMSO systems and viscosities in DMSO are shown in Table 2.

3.6. Solubility

The solubility of arabinoxylans is affected by the patterns of intra- and inter-molecular hydrogen bonds. The isolated arabinoxylans were poorly soluble in cold water but soluble in hot water. They were completely soluble in a 0.5 M NaOH solution and in a 90:10 DMSO:water mixture. All fractions were insoluble in acetone, chloroform and hexane.

The water-insoluble fraction was calculated in % of the total amount of arabinoxylan and was 7.3% in AX1, 6.4% in AX2, 6.1% in AX3 and 7.8% in AX4.

3.7. Material properties

All four arabinoxylan fractions formed films when a water solution was left to dry on Petri dishes. Films of AX1 were quite dark, with a few visible particles. There were some cracks along the edges, and the films were relatively brittle. Films of AX2 were lighter; a few insoluble particles could bee seen, but the films were clear near the edges. The AX3 films were light, homogeneous and transparent. The films of AX4 were dark and smooth but very brittle.

The mechanical properties of the films prepared from the AX3 fraction were evaluated using tensile testing at 50% RH. Fig. 7 shows a typical stress–strain curve for the AX3 films. The films are strong, with a stress at break above 50 MPa, and have an elongation at break of around 2.5%. The Young's modulus was 2930 ± 300 MPa. The mechanical properties of these films can be compared with those of glucuronoxylan films. Aspen glucuronoxylan films plasticized with 20% by weight of sorbitol have a stress at break of about 40 MPa and a strain at break of about 2% (Gröndahl, Eriksson, & Gatenholm, 2004), and hydroxypropyl xylan shows similar properties (Jain, Sjöstedt, & Glasser, 2000). The water content plays an important role in xylan films, since water acts as a plasticizer and softens the material (Gröndahl et al., 2004; Salmén & Olsson, 1998). The water content at 50% RH, at which the measurements were carried out, was 35.50 ± 4.44%, which is quite high as compared to glucuronoxylan films that have a water content of between 14 and 20% at 54% RH (Gröndahl et al., 2004; Gröndahl, Teleman, & Gatenholm, 2003). At 100% RH, the water content was as high as 82.27 ± 0.79%, which indicates that arabinoxylans are very hygroscopic.

The morphology of the AX3 films was investigated using WAXS. The diffractograms showed that the AX3 fraction is mainly amorphous but has small crystalline peaks in the region of $2\theta = 17 - 21°$, which is where semicrystalline glucuronoxylan has its crystalline peaks (Horio & Imamura, 1964). This indicates that some of the xylan is present in its crystalline form. It is probable that unsubstituted regions crystallize and that substituted regions are amorphous. The hindrance of crystallization by substituents has also been observed in the case of acetyl groups (Gröndahl & Gatenholm, 2004).

4. Summary of results

Hemicellulose-rich fractions were isolated from barley husks by alkali extraction. The lignin content was reduced from 11.5% in the starting material to 2.5% by chlorite delignification and to 3.9% by organosolv treatment. The starch content was low in the chlorite-treated sample, and the protein content was low in both delignified samples. The hemicelluloses proved to be composed mainly of xylose

Table 2
Molecular weights determined by SEC in DMSO and water and viscosities determined by a viscosity detector in the DMSO system

Sample	(η) (cm^3/g)	M_w (g/mol) DMSO system	M_w/M_n DMSO system	M_w (g/mol) aqueous system	M_w/M_n aqueous system
AX1	63	43,000	2.0	35,100	1.3
AX2	48	44,700	1.9	34,100	1.3
AX3	48	35,900	2.4	39,100	1.4
AX4	47	34,300	1.8	40,400	1.3

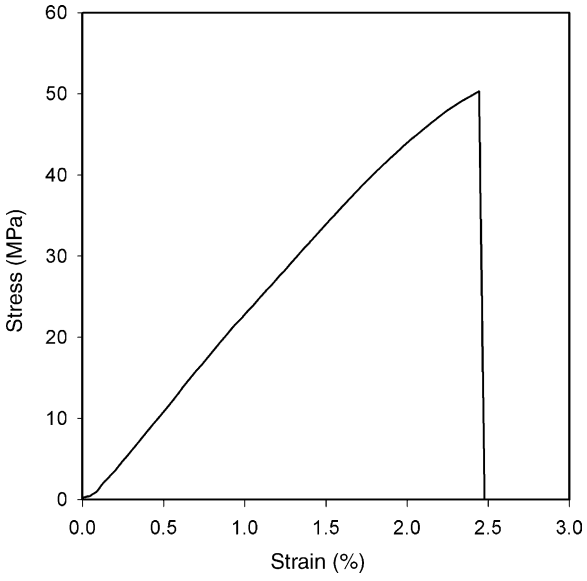

Fig. 7. Stress–strain behavior of AX3 films at 23 °C and 50% RH.

and arabinose. The process recommended for achieving high yield and a pure product is chlorite delignification followed by alkali extraction. The yield in this extraction process is approximately 57% of the available arabinoxylan in dry barley husks.

The weight average molecular weights determined by SEC were between 35,000 and 45,000 g/mol. Films were prepared by water casting and the properties of the chlorite-delignified sample were further evaluated. The films were strong, stiff and rather brittle, had high water content and were mainly amorphous.

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