Extraction, Fractionation, and Characterization of Structural Polysaccharides from Wheat Straw

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Wheat straw polysaccharides were fractionated into water soluble, pectic, 80% ethanol soluble, sodium chlorite soluble, hemicellulosic, and cellulose fractions using essentially gravimetric methods. The hemicellulosic material was further separated into a dimethyl sulfoxide soluble fraction and hemicellulose types A–C. The sugar and uronic acid compositions of each fraction were determined by gas chromatography after hydrolysis and conversion to trimethylsilyl ether derivatives and by a spectrophotometric method. The average molecular weights (M_w) of hemicellulosic fractions were estimated using gel permeation chromatography. The major polysaccharides in wheat straw were found to be cellulose (37.19–38.55%), followed by hemicellulosic fractions (30.28–35.01%) and small amounts of water soluble, pectic, 80% ethanol soluble, and sodium chlorite soluble materials. Xylose was found to be the major sugar in all of the hemicellulosic fractions with arabinose, glucose, and galactose as minor constituents. From hemicellulosic fractions A to C, the relative amount of xylose decreased, while the contents of arabinose, glucose, and galactose increased. The contents of phenolic acids and aldehydes remaining in wheat straw hemicellulosic fractions A and B and in cellulose were 0.72, 0.85, and 0.19%, respectively.

Keywords: *Wheat straw; pectin; holocellulose; hemicellulosic fraction; lignin; cellulose; extraction; sugars; uronic acids; protein; molecular weight; phenolic acids and aldehydes*

INTRODUCTION

Agricultural waste residues such as wheat straw, corn stover, and oat hulls contain significant cellulose and hemicellulosic fractions (Reddy et al., 1983). These materials are potential sources of energy for ruminant animals and of raw material for paper and board production (Morrison, 1980).

Hemicellulosic fractions are a large group of well-characterized polysaccharides found in the primary and secondary cell walls of all land and fresh water plants and in some seaweeds. Hemicellulosic fractions are made up of a relatively limited number of sugar residues, principally D-xylose, D-mannose, D-glucose, D-galactose, L-arabinose, D-glucuronic acid, 4-*O*-methyl-D-glucuronic acid, D-galacturonic acid, and, to a lesser extent, L-rhamnose, L-fucose, and various O-methylated neutral sugars. Wood and annual plants contain about 20–35% of hemicellulosic polymers (dry weight basis). The hemicellulosic fraction of wheat straw is mainly thought to be composed of β-1–4 linked D-xylopyranose units with side chains of various lengths containing L-arabinose, D-glucuronic acid or its 4-*O*-methyl ether, D-galactose, and possibly D-glucose (Bailey, 1973).

As part of a continuing study of wheat straw polysaccharides, we have extensively investigated the pectic substances and evaluated several methods of extraction of hemicellulosic fractions from wheat straw. This is described in other papers (Sun et al., 1994; Lawther et al., 1994). The overall aim of the present research into wheat straw in our laboratories is to establish the effects of mechanical pulping, refining, and alkaline pretreatments on the polymeric components of the straw. To achieve this aim, a thorough analysis and characterization of our starting material is required. The work described in this paper therefore had two primary objectives. The first one was to fractionate wheat straw polysaccharides using gravimetric methods. The second objective was to characterize structural polysaccharides by determination of their neutral sugar composition, uronic acid content, average molecular weight (M_w), and yield of phenolic acids and aldehydes after alkaline nitrobenzene oxidation of residual lignin in polysaccharides. The water soluble, 80% ethanol soluble, and sodium chlorite soluble polysaccharides, pectic substances, and α-cellulose were also studied.

EXPERIMENTAL PROCEDURES

Wheat straw was obtained from Silsoe Research Institute (Silsoe, Bedfordshire) and was ground using a Christie Laboratory mill to pass a 1 mm screen. The ground straw was then dried in a cabinet oven with air circulation at 60 °C for 16 h before use. All chemicals used were of analytical or reagent grade.

The component polysaccharides in wheat straw were determined by sequential extraction followed by weighing, using methods modified from those described by Harper et al. (1981), Summerell et al. (1989), and Carré et al. (1985). The methods for extraction of wheat straw pectins have been described elsewhere. We have carried out extensive extractions of the hemicellulosic cell wall components using various amounts of potassium hydroxide (at 20 °C and 0.05% sodium borate) and fractionated these into a dimethyl sulfoxide (DMSO) soluble fraction and fractions A–C. In addition, the composition of the hemicellulosic fractions have also been thoroughly studied. The scheme for fractional extraction and isolation of hemicellulosic fractions from wheat straw is shown in Figures 1 and 2. All experiments in this study were conducted in duplicate, and all weights and yields are given on a moisture-free basis. In general, concentrations were performed under reduced pressure at bath temperatures below 40 °C .

Moisture was determined by drying ground wheat straw in an air-circulated oven at 105 °C for 16 h. Ash content was

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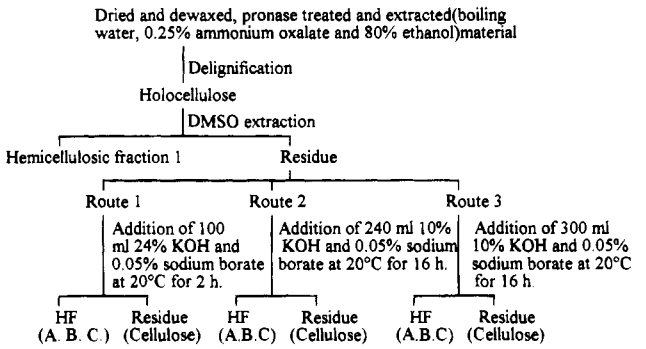

Figure 1. Scheme for extraction and isolation of hemicellulosic fractions from wheat straw (routes 1–3; HF, hemicellulosic fractions).

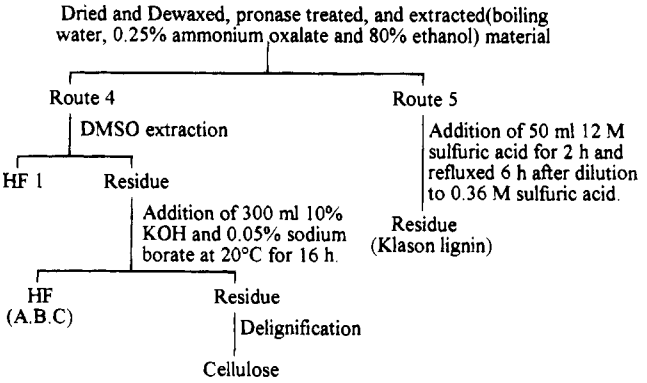

Figure 2. Scheme for extraction and isolation of hemicellulosic fractions from wheat straw (routes 4 and 5; HF, hemicellulosic fractions).

measured by incinerating the sample for 5 h in a muffle furnace at 550 °C. Lipids were extracted using chloroform (5 h) in a Soxhlet apparatus.

The extractive-free wheat straw was suspended in 0.1 M phosphate buffer, pH 7.5, containing 0.02% sodium azide as bactericide. Proteolysis was then started by the addition of Pronase (trypsin, 2.5 mg/g wheat straw) and was continued for 6 h at 40 °C with magnetic stirring. The medium was filtered under vacuum and the residue was exhaustively washed with water, ethanol, and ether. Finally, nearly protein-free material was first dried in an air-circulated oven for 16 h at 60 °C, then cooled in a desiccator, and weighed to 0.1-mg accuracy. Proteins (N × 6.25) in wheat straw were measured by a Kjeldahl procedure (AOAC, 1984). Supernatant from the Pronase treatment was extensively concentrated on a rotary evaporator under reduced pressure at 40 °C. The dried supernate contained polysaccharides solubilized during proteolysis and was stored at 5 °C until commencement of analysis.

The residue from defatted and nearly protein-free wheat straw was gently boiled in two successive batches of distilled water (30 g/L, 2 × 2 h). The straw was filtered and the residue was washed twice with boiling water, dried for 16 h at 60 °C, and weighed. The weight lost was defined as the hot water soluble component. The supernatants from boiling water treatments were pooled and then evaporated on a rotary evaporator under reduced pressure at bath temperatures not exceeding 40 °C. The dried supernate was kept at 5 °C until analyzed.

Pectic polysaccharides were isolated from the boiling water extracted straw using 0.25% ammonium oxalate (AO) for 4 h at 85 °C (Phatak et al., 1988). The supernatants were concentrated on a rotary evaporator under reduced pressure at 40 °C and polymer precipitated using 5 volumes of 96% ethanol. After filtration and drying in an air-circulated oven for 16 h at 60 °C, the resultant white powder was labeled "pectic polysaccharides" or "pectin fraction" and kept in a refrigerator at 0 °C until analysis. The residue was rinsed once with water, twice with 96% ethanol, and once with ether and then dried in an oven for 16 h at 60 °C.

Eighty percent ethanol (1.5 g of straw/100 mL of extractant) was added to the straw remaining after the previous extraction and the mixture boiled gently for 3 h. The residue was recovered by filtration and washed twice with 80% ethanol and water. The weight of residue was obtained by heating at 60 °C for 16 h followed by equilibration to constant weight in a desiccator over anhydrous silica gel. The weight lost was defined as "hot ethanol soluble materials". The supernatant from 80% ethanol treatment was concentrated as described above.

Lignin content in wheat straw was determined according to the methods described by Bagby et al. (1971), Collings et al. (1978), and Asensio and Seoane (1987). The residue, extracted from the above procedures (6.70 g), was stirred with water (300 mL) and 10% acetic acid (20 mL) and delignified using sodium chlorite ($NaClO_2$, 10.0 g) in a Pyrex flask. The mixture was heated for 1 h at 75 °C, more acid (10 mL) and sodium chlorite (5.0 g) were then added, and the mixture was heated for a further hour. After 2 h of oxidation, the residue was filtered out on a nylon cloth and washed with water (three times), 96% ethanol (twice), and ether (once) and then dried at 60 °C for 16 h and reweighed. The difference in weight was defined as "sodium chlorite lignin". The supernatant was concentrated on a rotary evaporator under reduced pressure at 40 °C. The dried supernate was kept in a refrigerator at 5 °C until the commencement of analysis of polysaccharides solubilized during delignification.

Klason lignin (route 5 in Figure 2) was measured according to the methods of Theander and Åmon (1978) and Brillouet and Riochet (1983). Fifty milliliters of 12.0 M sulfuric acid was added to the straw remaining (2.0 g) after the 80% ethanol extraction. The polysaccharide hydrolysis was performed at room temperature for 2 h using concentrated acid. The acid was then diluted to 0.36 M, and the subsequent mixture was refluxed for a further 6 h. After filtration, the residue was thoroughly washed with water, dried at 60 °C for 16 h, weighed, and defined as "Klason lignin".

Hemicellulosic fractions were first extracted from the remaining straw with dimethyl sulfoxide (2.2 g of straw residue/100 mL of DMSO for routes 1–3) for 2 h at 80 °C. After filtration and drying at 60 °C for 16 h, the residue was extracted with 24% potassium hydroxide (1.8 g of straw residue/100 mL of extractant) and 0.05% sodium borate (route 1) in air for 2 h at 20 °C or with 10% potassium hydroxide (1.8 g of straw residue/240 mL of extractant for route 2 and 1.6 g of straw residue/300 mL of extractant for route 3) and 0.05% sodium borate in air for 16 h at 20 °C. The residue was recovered, washed three times with water, once with 5% aqueous acetic acid, once with distilled water, twice with 96% ethanol, and once with ether, then dried, and reweighed. The weights lost both in DMSO and in KOH solution were defined as DMSO soluble and alkali soluble hemicellulosic fractions, respectively. The fraction in DMSO was concentrated to dryness by evaporation at diminished pressure at about 80–90 °C. The dried sample was kept in a refrigerator at 0 °C until analysis. Hemicellulosic fraction A was precipitated from 24 or 10% potassium hydroxide supernatant by acidifying to pH 5.0 with acetic acid, recovered by centrifugation, rinsed with 96% ethanol, and dried for 16 h at 60 °C. Hemicellulosic fraction B was obtained from the mother liquor by precipitation with 5 volumes of 96% ethanol (24 h at 20 °C), then filtered and washed by solvent exchange, and finally dried for analysis. The fraction that remained soluble in aqueous ethanol was named hemicellulosic fraction C and isolated by evaporation on a rotary evaporator under reduced pressure at 40 °C against water and ethanol. The residue was finally dried in an oven for 16 h at 60 °C.

In route 4 (see Figure 2) the residue of the 80% ethanol treated straw was first extracted with DMSO (1.0 g of straw residue/70 mL of DMSO) for 2 h at 80 °C and then with 10% KOH and 0.05% Na_3BO_3 (3.0 g of straw residue/300 mL of extractant) at 20 °C for 16 h. Finally, the residue was delignified as described above; i.e. delignification was carried out after hemicellulose removal.

The weight of the residue remaining after the alkaline or sodium chlorite extraction (route 4 only), corrected for ash content, was taken as cellulose.

The contents of the neutral sugars—arabinose, xylose, mannose, galactose and glucose—in water soluble materials (both free and polymer bound), pectic polysaccharides, hemicellulosic fractions, and cellulose were measured by gas chromatography after conversion to trimethylsilyl (TMS) ether derivatives. For the measurement of free sugars in water soluble materials including those obtained during proteolysis, hot water extraction, and delignification, the neutral sugars were determined directly by gas chromatography after conversion to trimethylsilyl ether derivatives. For the measurement of polysaccharide sugars, the water soluble materials, pectic polysaccharides, and hemicellulosic fractions were first hydrolyzed with 2 N trifluoroacetic acid for 2 h at 121 °C in sealed pressure tubes (Baig et al., 1982). Trifluoroacetic acid was removed by vacuum evaporation at 40 °C. The mixtures of sugars produced were then converted to corresponding mixtures of trimethylsilyl derivatives.

All of the trimethylsilyl sugar derivatives were analyzed by gas chromatography, using flame ionization detection, on a glass column (1.70 m × 2 mm i.d.) packed with 3% OV-17 on 80–100 Supelcoport. The injector and detector temperatures were 200 and 250 °C, respectively, and the oven temperature was programmed to rise from 100 to 190 °C at 2 °C/min with a 10-min hold at 190 °C. Nitrogen was used as a carrier gas, and its flow was maintained at 36 mL/min. In general, *myo*-inositol was used as an internal standard during the analyses.

For the measurement of neutral sugars in "cellulose", the material was first hydrolyzed with 72% sulfuric acid for 2 h at 20 °C. Distilled water was then added to decrease the acid concentration to 3% (w/v), and the hydrolysate was boiled for 6 h and filtered immediately. The filtrate was neutralized with ammonia to pH 7.0, water was removed under low pressure on a rotary evaporator, and the sugars were extracted from the residue into dry pyridine. The pyridine was then removed on the rotary evaporator. Finally, the sugars were determined by gas chromatography after conversion to trimethylsilyl ether derivatives as described above.

The sugar alcohols arabitol and mannitol in the 80% ethanol extract were determined as their alditol acetates by gas chromatography according to the method described by Blakeney et al. (1983).

Finally, identification of sugars in trifluoroacetic acid hydrolyzed and water soluble nonhydrolyzed polysaccharide fractions was achieved by comparison of the relative retention times of the TMS derivatives of wheat straw sugars with TMS derivatives of authentic sugars or comparison of the relative retention times of alditol acetates of arabitol and mannitol in 80% ethanol extract with their acetates of the standard in cochromatography (Sabir et al., 1975). After identification, the TMS derivatives of sugars in pectin, hemicellulosic fractions, cellulose, and water soluble polysaccharides were quantitated from the peak areas and weight ratio of TMS derivatives of authentic sugars and the internal standard, *myo*-inositol (Mason and Slover, 1971).

Total uronic acids were assayed colorimetrically as anhydrogalacturonic acid in pectin or as glucuronic acid in hemicellulosic fractions using 3-phenylphenol color reagent according to the procedure outlined by Blumenkrantz and Asboe-Hanson (1973) with a modification by Wedig et al. (1987). A Hewlett-Packard 8452A diode array spectrophotometer was used to measure anhydrogalacturonic acid or glucuronic acid at a wavelength of 520 nm. The relative amount of rhamnose in pectin was determined by the quantitative colorimetric procedure of Gibbons (1955) and Dische and Shettles (1948) after hydrolysis for 4 h in 2 N trifluoroacetic acid. Methyl ester content was determined according to the method described by Wood and Siddiqui (1971). Acetic acid content was estimated using the transesterification method outlined by Browning (1967).

The average molecular weights (M_w) of pectin and hemicellulosic fractions were determined by gel permeation chromatography (Will and Dietrich, 1992) on a PL Aquagel-OH 50 column (300 × 7.7 mm, Polymer Laboratories Ltd.), calibrated with PL pullulan polysaccharide standards (peak average molecular weights 667, 5 800, 12 200, 23 700, 48 000, 100 000, 186 000, and 386 000; Polymer Laboratories). The pump used was a Knauer HPLC Model 64 with a flow rate of 0.1 mL/min. The eluent was 0.02 M NaCl in 0.005 M sodium phosphate buffer, pH 7.5. Detection was achieved using a Knauer differential refractometer. The column oven was maintained at 30 °C. The samples were dissolved in 0.005 M sodium phosphate buffer with 0.02 M NaCl to a concentration of 0.1%.

The method for alkaline nitrobenzene oxidation of lignin remaining attached to/associated with hemicellulosic fractions and cellulose was based on the procedure published by Scalbert and Monties (1986) with some modifications. Sodium hydroxide (2 M, 7 mL) and nitrobenzene (0.4 mL) were added to hemicellulosic fractions or cellulose (0.15 g) in steel autoclaves. The autoclaves were then heated in an oil bath for 2.5 h at 170 °C. The solutions were filtered, and the filtrates were extracted with chloroform (3 × 30 mL), acidified to pH 1 with 20% chlorhydric acid, and extracted again with chloroform (3 × 30 mL). These last chloroform extracts were evaporated at 40 °C to dryness and resolubilised in methanol (4 mL) containing 3′-dimethoxy-4′-hydroxyacetophenone as an internal standard. The lignin oxidation products were then analyzed by high-performance liquid chromatography (HPLC) on a Hichrom H50DS column of dimensions 250 × 4.6 mm. Separations were obtained using a linear gradient of two solvent systems: solvent A (water–methanol–acetic acid 89:10:1) and solvent B (methanol–water–acetic acid 90:9:1). A linear gradient was run over 31 min from 0 to 40% B at a flow rate of 1 mL/min. Products were detected at 280 and 320 nm. Peak areas (280 nm) were calculated relative to the internal standard using Kontron MT 450 software. Calibration curves were established with appropriate mixtures of authentic phenolic acids and aldehydes.

RESULTS AND DISCUSSION

Plant cell walls contain three main types of structural polysaccharides, namely cellulose, hemicellulosic fractions, and pectic substances. In wheat straw cellulose and hemicellulosic fractions are the predominant components, comprising about 70% of dry straw. The third major cell wall component in straw is lignin, which makes up around 14% (sodium chlorite lignin) of the dry weight. The results of the fractionation and the determination of the chemical composition of wheat straw, treated under different conditions (routes 1–5) are shown in Table 1. Besides the main constituents of cellulose, xylan, and lignin, small amounts of pectic substances, crude lipids, protein, and ash are also indicated in the table. Characterization of the chloroform extractives is currently being done in our laboratory. More details of the composition of pectic polysaccharides are given elsewhere (Sun et al., 1994).

During time course preliminary studies, it became evident that Pronase treatments were partially successful but prolonged use could lead to the release of a small portion of the cell wall polysaccharides, mostly pectic substances. This was mainly due to the action of the buffer (Stevens and Selvendran, 1980). The total yield of water soluble materials from proteolysis at 40 °C for 6 h was 4.44%, which contained 65.84% water soluble polysaccharides. The yield of released protein was 1.52%. The content of protein (N × 6.25) in the residue of proteolysis was low, 0.21%.

The neutral sugar composition, both free and polymer bound, of the water soluble polysaccharides released during proteolysis is shown in Table 2. Arabinose and galactose were the prominent constituent sugars, while minor sugars were found to be xylose, mannose, and glucose. Mannose was not detected as free monosaccharide. Xylose was present in small amounts in free

Table 1. Fractionation and Chemical Composition of Wheat Straw (Percent Dry Weight)

		proteolysis						hemicellulose			
route	extractives	removed protein (N × 6.25)	WS-P[a]	hot water soluble part WS-P[a]	pectin	80% ethanol soluble part ES-P[b]	lignin	DMSO[c] soluble part	alkali soluble part	cellulose	ash
1	1.65	1.52	2.92	4.67	1.52	1.27	14.13	4.78	28.78	38.55	7.93
2	1.65	1.52	2.92	4.67	1.52	1.27	14.13	4.78	29.25	38.09	7.93
3	1.65	1.52	2.92	4.67	1.52	1.27	14.13	4.78	30.23	37.17	7.93
4	1.65	1.52	2.92	4.67	1.52	1.27	7.42	0.64	36.24 (HC,[d] 29.64 LG,[d] 6.60)	38.45	7.93
5	1.65	1.52	2.92	4.67	1.52	1.27	25.83 (Klason lignin)		61.24		7.93

[a] Abbreviation for water soluble polysaccharides. [b] Abbreviation for ethanol soluble polysaccharides. [c] Abbreviation for dimethyl sulfoxide. [d] HC and LG stand for hemicellulose and lignin, respectively.

Table 2. Content (Percent Dry Weight) of Free and Polymer Bound Neutral Sugars Solubilized during Proteolysis, Boiling Water Extraction, and Delignification

	proteolysis			boiling water extraction			delignification (route 1)		
neutral sugar	free[a]	bound[b]	total	free	bound	total	free	bound	total
arabinose	1.74	1.75	3.49	ND	ND	ND	1.15	1.47	2.62
xylose	0.14	0.72	0.86	0.48	ND	0.48	0.12	3.47	3.59
mannose	ND[c]	0.31	0.31	ND	ND	ND	ND	0.57	0.57
galactose	1.12	1.53	2.65	1.25	5.80	7.05	1.26	1.26	2.52
glucose	0.21	0.02	0.23	0.60	1.78	2.38	ND	0.26	0.26

[a] Determined directly in duplicate on hydrolysate fraction by gas chromatography after conversion to trimethylsilyl (TMS) ether derivatives. [b] Determined by hydrolysis with 2 N trifluoroacetic acid at 121 °C for 2 h in sealed pressure tubes and then by gas chromatography after conversion to trimethylsilyl (TMS) ether derivatives (mean of duplicate analyses). [c] Not detectable.

form but in noticeable amounts as a polymer bound sugar. Glucose was present only in trace amounts in the bound form. The arabinose/galactose molar ratios in free, polymer bound, and total sugars were 1.87, 1.37, and 1.58, respectively.

The yield of hot water soluble polysaccharides extracted with boiling water for 4 h, from defatted and nearly protein-free straw, was 4.67%. The neutral sugar composition of the extract is shown in Table 2. As can be seen, galactose was the major sugar in both free and polymeric form. Low concentrations of glucose and xylose were noted in these hydrolysates. In total, the polymer bound sugar content in the extract was significantly higher than that of the free. A lower xylose concentration indicated proportionately less hemicellulosic fraction in the hot water soluble polysaccharides, while a higher galactose content in the extract was taken to indicate correspondingly more galactans. Rhamnose, arabinose, and mannose were not detected. The presence of hot water soluble polysaccharides in agricultural residues has been widely demonstrated. For example, Kavitha and Chandrashekar (1992) showed that soft sorghum grains contain more water soluble polysaccharides than hard grains. Daveby and Åman (1993) have demonstrated the presence of water soluble polysaccharides in dehulled legume seeds and their hulls.

A total of 1.27% of the dry straw matter was dissolved during 80% ethanol extraction, which removes low molecular weight polysaccharides and other water–ethanol soluble components. The contents of free fructose, glucose, arabitol, and mannitol in the 80% ethanol soluble fraction were 2.0, 4.1, 1.7, and 1.7%, respectively. Traces of galactose were also detected in the extract. The total free sugars and sugar alcohols comprised 9.5% of the dry extracts, an amount which was lower than that obtained in Theander and Åman's (1978) study of Swedish wheat straw (0.3–1.3% of the straw). This was mainly due to the previous extensive extractions of water soluble polysaccharides and pectin in our experiment.

The yields of sodium chlorite lignin were 14.13% (obtained before extraction of hemicellulosic fractions, routes 1–3) and 7.42% (obtained after extraction of hemicellulosic fractions, route 4), respectively. The major reason for this difference was the dissolution of a proportion of the lignin during the alkali extraction of hemicellulosic fractions prior to isolation of lignin in route 4. Such an effect might originate either from alkali cleavage of ferulic acid cross-link between lignin and hemicellulosic fractions or from modification of lignin polyelectrolyte properties induced by free carboxyl groups of phenolic acid ethers (Scalbert et al., 1985). Jackson (1977) has observed that alkali treatment of straw greatly increased the solubility of lignin, and the effects of alkali treatments are currently used to increase the biodegradability of straws.

The Klason lignin represents not only the lignin but also polyphenols, cutins, some carbohydrate degradation products, and nitrogenous matter (Theander and Åman, 1977). Hence, the method generally produces an overestimate of lignin. As expected, the Klason lignin content was 25.83% (route 5) and considerably exceeded the sodium chlorite lignin (about 14%). Theander and Åman (1978) also found that the Klason lignin in Swedish wheat straw was between 19 and 24%. Harper and Lynch (1981) have concluded that Klason lignin always accounted for more than 25% of the weight of wheat straw.

The monosaccharides and polysaccharides associated with the lignin during delignification were determined either without or after trifluoroacetic acid hydrolysis. The total recovered monosaccharides (route 1) were measured by gas chromatography and accounted for 1.35% of dry wheat straw. The contents of free and polymer bound sugars in the hydrolysates of delignification are shown in Table 2. They are rich in xylose, arabinose, and galactose and low in mannose and glucose. Furthermore, the content of polymer bound sugars was much higher than that of free. The molar ratio of xylose/arabinose/galactose/mannose/glucose was 2.4:1.7:1.4:0.3:0.14, respectively, in the total sugar. This

Table 3. Composition of Wheat Straw Hemicellulosic Fractions (Percent Dry Weight)

route	total hemicellulose	DMSO[a] soluble part	alkali soluble part			
			total amt	A[b]	B[b]	C[b]
1	33.56	4.78	28.78	11.29	15.37	2.12
2	34.03	4.78	29.25	11.63	14.93	2.69
3	35.01	4.78	30.23	13.51	13.69	3.03
4	30.28	0.64	29.64	13.30	13.90	2.44

[a] Abbreviation for dimethyl sulfoxide. [b] Abbreviation for hemicellulosic fractions A–C.

is in contrast to the results of the Ahlgren and Goring (1971) investigation of wood samples. They established that no polysaccharides were released from wood by sodium chlorite until at least 60% delignification was achieved. At later stages of delignification with chlorite, small quantities of glucomannan from the hemicellulosic fraction were removed. However, Bouveng and Meier (1959) reported that during the delignification of wood meal from spruce compression wood, more than 10% of the wood polysaccharides were dissolved and could be recovered from the delignification liquors. Most of this material was a galactan, typical for compression wood. Xylans and glucomannans were also dissolved. The authors illustrated that there was an important difference between wood meal and intact wood fibers. From studies on nonwood samples, such as grasses, jute fiber, and sunflower palm-kernel meal, Das et al. (1981), Ford (1986), and Düsterhöft et al. (1992) have shown that (arabino)xylans are involved in the linkage between lignin and polysaccharides. Fidalgo et al. (1993) also suggested that wheat straw hemicellulosic fractions appeared as an arabinoxylan, associated with lignin. Nordkvist et al. (1987) obtained 1.9% (dry matter) soluble lignin–polysaccharide complexes from wheat straw using an in vitro method. Further study of wheat straw lignin–polysaccharide complexes is needed and is presently underway in our laboratory.

Much of the hemicellulosic fraction is of the xylan type, with a backbone of (1→4)-β-D-xylose residues, having mostly single arabinofuranosyl units attached to some C-3 positions of the main xylan chain and glucuronic acid and/or its 4-*O*-methyl ether linked to some xylose units. The latter are probably mainly linked to the C-2 position (Theander, 1985). Table 3 clearly shows that hemicellulosic fractions, including DMSO soluble and alkali soluble portions, represent over 30% of the dry weight of the straw in routes 1–3 extractions. In route 4 the treatment of straw with 10% KOH for 16 h at 20 °C resulted in the dissolution of nearly half of the wheat straw lignin. The yield of alkali soluble hemicellulosic fractions in route 4 was 29.64% of dry weight of the straw after precipitation of the extract with 5 volumes of 96% ethanol (24 h at 20 °C). The total yield of hemicellulosic fractions, including the DMSO soluble part obtained using route 4, was still over 30% of the initial dry weight. In addition, from route 1 to route 3, the yields of hemicellulosic fractions increased with increase in extractant volume, extracting time, and content of alkali in the extracting solutions. The yields of hemicellulosic fractions obtained via routes 1–4, in descending order, are hemicellulosic fractions B, A, and C, which represents less than 10% of the total hemicellulosic fractions.

In xylans it is thought that most of the *O*-acetyl groups present are attached to C-3 and the remainder to C-2 of the D-xylose residues. These acetylated xylans are soluble in water and in solvents such as dimethyl sulfoxide, formamide, and *N,N*-dimethylformamide.

Table 4. Composition of Neutral Sugars (Relative Percent) in Hemicellulosic Fractions Extracted from Wheat Straw

route	hemicellulose fractions	Ara	Xyl	Man	Gal	Glu
1	DMSO soluble	8.4	80.6	tr[a]	4.3	6.8
1	A	4.7	90.2	tr	1.2	4.0
1	B	5.1	89.2		1.3	4.3
1	C	14.2	73.8		4.2	7.7
2	DMSO soluble	8.4	80.6	tr	4.3	6.8
2	A	5.0	89.7	tr	1.3	4.0
2	B	11.3	82.4		2.6	4.0
2	C	13.8	71.2		3.8	11.4
3	DMSO soluble	8.4	80.6	tr	4.3	6.8
3	A	5.0	86.6	tr	2.5	5.8
3	B	6.8	84.2		2.6	6.4
3	C	16.1	65.7		10.4	7.8
4	DMSO soluble	9.9	71.1		7.0	12.1
4	A	5.9	85.4	tr	2.8	5.9
4	B	7.2	83.4		3.3	6.3
4	C	14.4	74.9		3.3	7.5

[a] Traces.

When xylans are isolated by alkaline extraction, any ester groups present are simultaneously saponified. Therefore, to extract xylans in their native, acetylated form, we isolated the acetylated xylans using dimethyl sulfoxide as a solvent before application of alkaline solutions. The yields of the DMSO extractions from straw holocellulose and cell wall materials (prior to lignin extraction, route 4) were 4.78 and 0.64%, respectively. It is postulated that the low yield of DMSO soluble material obtained in the latter case is mainly due to the presence of lignin–polysaccharide complexes. The acetyl contents in DMSO extracts were 12.38% from holocellulose and 7.03% from cell wall materials, respectively. However, extraction with KOH rapidly hydrolyzes ester linkages. No acetyl groups could hence be detected in hemicellulosic fractions A–C.

The relative sugar compositions of hemicellulosic fractions after hydrolysis are presented in Table 4. The sugar analyses of the hydrolysates show that xylose is an extremely predominant component sugar in wheat straw hemicellulosic fractions and that it comprises 65–90% of the total sugars in the hemicellulosic fractions. It is apparent that (Table 4) glucose, galactose, and arabinose are all present as minor constituents in hemicellulosic fractions. Mannose is present in trace amounts. This is in accordance with the study of Hatfield (1989), in which he states that most of the xylose and arabinose in grasses is present in hemicellulosic fractions rather than the less abundant pectic polysaccharides.

The molar ratio of arabinose/xylose/galactose/glucose in DMSO extracts isolated from straw holocellulose was 5.6:54:2.4:3.8. These data illustrated that DMSO extracted hemicellulosic fraction probably contained some glucoarabinoxylans. By extraction of a softwood holocellulose with hot water after a dimethyl sulfoxide treatment, Bouveng and Meier (1959) found that the mixture contained, besides the galactoglucomannan, some 15% glucuronoarabinoxylan and 4.5% *O*-acetyl groups.

From Table 3, one can see that most of the hemicellulosic polymers were accumulated in the A and B fractions, accounting for about 80% of the material. As stated above (Table 4), xylose was the major sugar in all of the hemicellulosic fractions. Furthermore, hemicellulosic fractions A–C also contained appreciable amounts of arabinose and glucose as well as small

Table 5. Content (Percent) of Uronic Acids in Wheat Straw Hemicellulosic Fractions

route	DMSO[a] soluble part	hemicellulose A	hemicellulose B	hemicellulose C
1	5.2	2.4	7.5	0.6
2	5.2	3.4	7.6	0.5
3	5.2	3.8	5.7	0.5
4	2.2	3.3	6.3	0.6

[a] Abbreviation for dimethyl sulfoxide.

Table 6. Average Molecular Weights of Wheat Straw Hemicellulosic Fractions

route	DMSO[a] soluble part	hemicellulose A	hemicellulose B	hemicellulose C
1	8000	10000	14000	9000
2	8000	11000	29000	14000
3	8000	11000	21000	12000
4	6000	26000	30000	16000

[a] Abbreviation for dimethyl sulfoxide.

amounts of galactose. The only significant difference among the hemicellulosic fractions A–C was that the proportion of xylose decreased from A to C, whereas the proportions of glucose, galactose, and arabinose increased accordingly. These data are in agreement with the results of Theander and Åman (1977). Aspinall and Meek (1956) also stated that the same sugars were present in wheat straw hemicellulosic fractions A and B. However, some authors have indicated that hemicellulosic fraction A of wheat straw contained only three neutral sugar residues (xylose, arabinose, and glucose) and glucuronic acids, whereas hemicellulosic fraction B possessed four neutral sugar residues (xylose, arabinose, glucose, and galactose) and glucuronic acids (Summerell and Burgess, 1989; Bishop, 1953; Aspinall and Meek, 1956; Dekker, 1979). This variation in findings may reflect the influence of factors such as soil, climate, and method of analysis on the sugar content of wheat straw hemicellulosic fractions.

The molar ratios of xylose/arabinose/galactose/glucose in hemicellulosic fractions A–C (route 1) were found to be 60:3.1:0.7:2.2, 59:3.4:0.7:2.4, and 49:9.5:2.3:4.3, respectively. Xylose to arabinose ratios are indicative of the degree of linearity or branching of hemicellulosic fraction (Wedig et al., 1987). A high xylose to arabinose ratio would indicate a high degree of polymerization with little bonding to other monosaccharide constituents. On the other hand, a low xylose to arabinose ratio suggests a short-chain polymer with a large amount of branching with other monosaccharides. Our results, showing a relatively large amount of xylose with relatively low levels of other neutral sugars, points to the presence of mainly xylan or arabinoxylan. Fidalgo et al. (1993) concluded that the wheat straw hemicellulosic fractions are largely arabinoxylans and that a percentage of the arabinose units are linked to lignin. In addition, the above authors also stated that, in the alkali lignin fraction, the percentage of arabinose units linked to lignin (as a percentage of total arabinose) was higher than the percentage of linked xylose, in accordance with the findings of An et al. (1989).

The content of uronic acids in hemicellulosic fractions showed only minor changes between the analysis routes, but significant differences appeared between hemicellulosic fractions (Table 5). The corresponding values for uronic acids were 5.2 for the DMSO extracted hemicellulosic fraction and 2.4, 7.5, and 0.6 for hemicellulosic fractions A–C obtained via route 1, respectively. Hemicellulosic fraction B was maximal for uronic acid content, whereas hemicellulosic fraction C was found to be minimal. Reddy et al. (1983) have also indicated that wheat straw hemicellulosic fractions average about 3% of uronic acids, with hemicellulosic fraction B possessing nearly twice this amount.

The average GPC estimated molecular weights of hemicellulosic fractions are given in Table 6, and the molecular weight ranges of hemicellulosic fractions A–C obtained using route 2 are shown in Figure 4. As expected, the average molecular weights of DMSO soluble

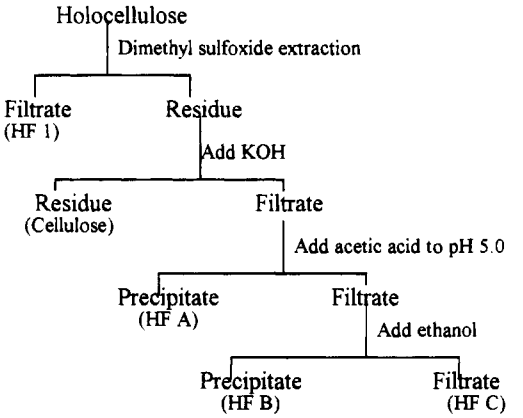

Figure 3. Scheme for hemicellulosic subfractionation from wheat straw holocellulose (HF, hemicellulosic fraction).

hemicellulosic fraction and hemicellulosic fractions A and C were lower than that of hemicellulosic fraction B. The data generally agreed with the findings of Aspinall and Mahomed (1954). Their study involved molecular weight determination using the isothermal distillation method, producing values of 8 000–11 800 ± 400 (degree of polymerization 47–76) for methylated wheat straw xylan. In their study of the isolation and characterization of hemicellulosic fractions from sugar beet pulp, Wen et al. (1988) indicated that the molecular weight distributions of hemicellulosic fractions A, the more linear and less acidic hemicellulosic fraction, and B, the more acidic or branched fraction, exhibited two major carbohydrate peaks. The first appeared at a molecular weight greater than or equal to 150 000 and the second at a molecular weight of around 40 000. This trend is not observed with the wheat straw hemicellulosics.

Because of the degradation of hemicellulosic fractions in a high concentration of alkaline solution, the average molecular weights of hemicellulosic fractions extracted with 24% potassium hydroxide and 0.05% sodium borate at 20 °C for 2 h in route 1 were lower than those determined for the materials extracted with 10% potassium hydroxide and 0.05% sodium hydroxide at 20 °C for 16 h in routes 2–4. This is mainly due to hydrolytic degradation of hemicellulosic fractions in high concentration of alkaline solution. In addition, the average molecular weights of hemicellulosic fractions obtained before delignification (route 4) were observed to be higher than those obtained from holocelluloses isolated after lignin removal (routes 2 and 3) using an equivalent concentration of potassium hydroxide. The reason for this difference can probably be ascribed to the presence of soluble lignin–polysaccharide complexes the material extracted prior to delignification.

The FTIR spectra of hemicellulosic fractions A and B (Figure 5) appeared to be rather similar. However, on closer examination of the spectrum of the hemicellulosic fraction B, it can be seen that there are two definite peaks at 1626 and 1562 cm^{-1}, whereas that of fraction

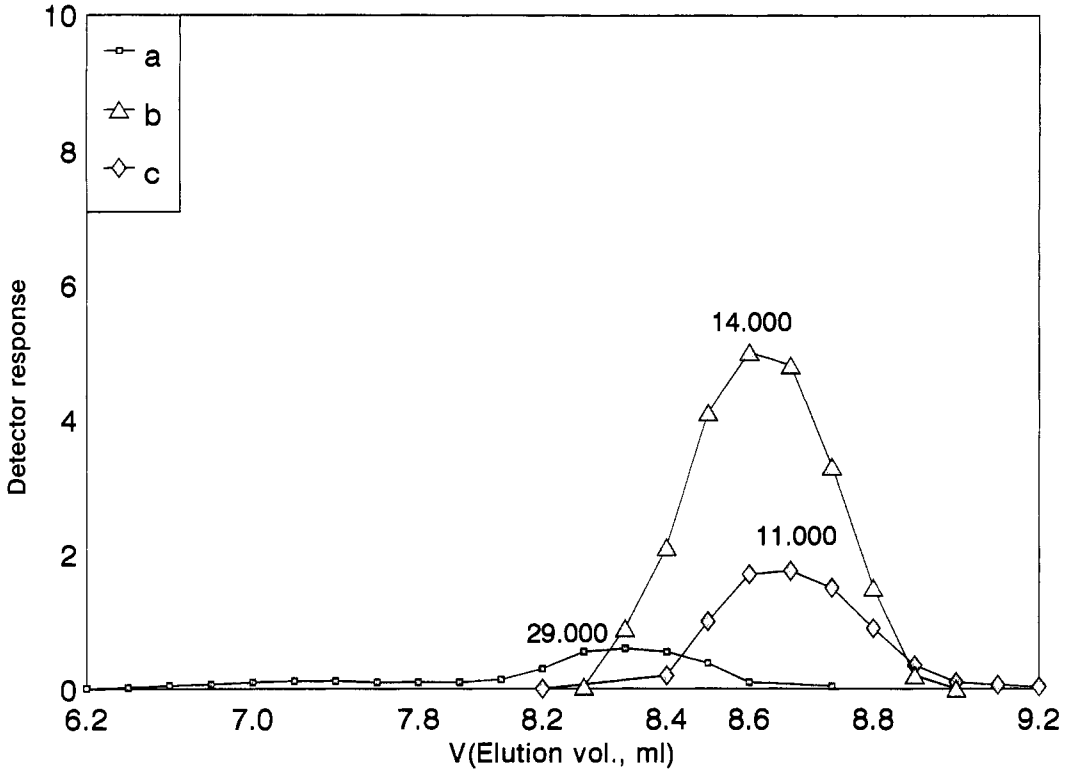

Figure 4. GPC range of the molecular weight of wheat straw hemicellulosic fractions A(c), B(a), and C(b) for route 2.

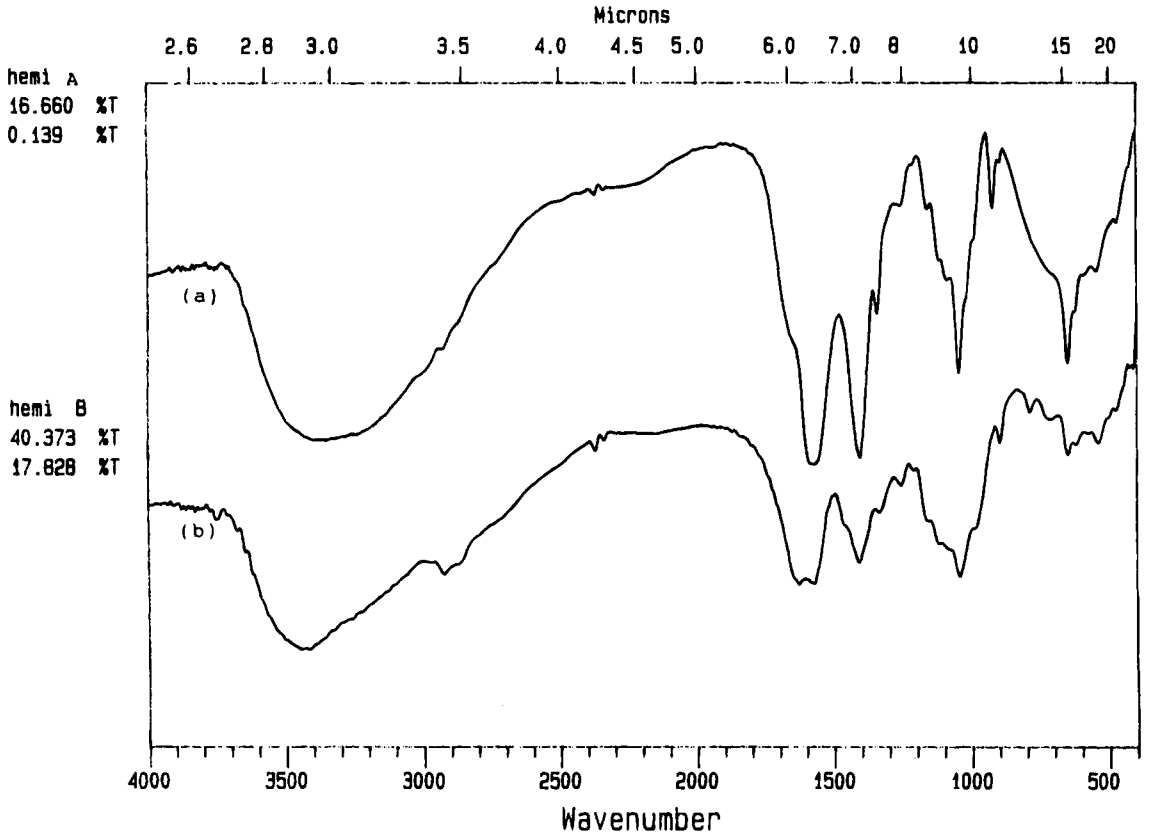

Figure 5. FTIR spectra of wheat straw hemicelluloses A(a) and B(b) extracted with 10% KOH and 0.05% Na_3BO_3 at 20 °C for 16 h in route 2.

A has a single flattened peak at 1562 cm^{-1}. The absorbance around 1562 cm^{-1} was due to aromatic groups in associated lignin. This figure indicates that the extracted hemicellulosic fractions A and B still contain some residual lignin.

The phenolic composition of the hemicellulosic fractions A and B is summarized in Table 7. The total phenolic contents in hemicellulosic fractions A and B were 0.72 and 0.85%, respectively. The major components were found to be *p*-hydroxybenzoic acid and vanillin in both fractions. Hemicellulosic fraction A showed higher contents of protocatechuic acid, *p*-hydroxybenzoic acid, and ferulic acid. The contents of vanillin, *p*-hydroxybenzaldehyde, gallic acid, and vanillic

Table 7. Content (Percent) of Phenolic Acids and Aldehydes of Alkaline Nitrobenzene Oxidation Lignin in Wheat Straw Hemicellulosic Fractions and Cellulose Extracted with 10% Potassium Hydroxide and 0.05% Sodium Borate at 20 °C for 16 h in Route 2

phenolic acids and aldehydes[a]	hemicellulose A (%)	hemicellulose B (%)	cellulose (%)
gallic acid	0.0077	0.063	0.0038
protocatechuic acid	0.022	0.013	ND[b]
p-hydroxybenzoic acid	0.52	0.31	0.048
p-hydroxybenzaldehyde	0.0030	0.052	0.0089
vanillic acid	0.019	0.031	0.023
vanillin	0.056	0.19	0.028
syringic acid	0.0035	0.015	ND
p-coumaric acid	0.023	0.035	ND
syringaldehyde	ND	0.0026	ND
ferulic acid	0.036	0.025	0.036
cinnamic acid	0.0071	0.066	0.011
unknown	0.020	0.050	0.028
total	0.72	0.85	0.19

[a] Determined by HPLC after alkaline nitrobenzene oxidation at 170 °C for 2.5 h in steel autoclaves. [b] Not detectable

acid were higher in fraction B. Syringaldehyde was not detected in hemicellulosic fraction A, whereas it appeared at a level of about 0.003% in fraction B. The characterization of wheat straw lignin and lignin–polysaccharide complexes is currently the subject of detailed further study in our laboratory.

It is evident that α-cellulose is contaminated with hemicellulosic fractions and pectic substances that have not been extracted during the previous fractionation procedures. Treatment with 72% H_2SO_4 (2 h, 20 °C) and 3% H_2SO_4 (6 h, 100 °C) hydrolyzed the cellulose, producing a neutral sugar composition (relative percent) of arabinose 3.4, xylose 6.2, galactose 2.8, and glucose 87.6, with a trace amount of mannose. The resistance to extraction by 24% KOH suggests that hemicellulosic fractions and pectic substances are very strongly associated with the cellulose.

According to the model proposed by Preston (1974), the hemicellulosic fraction and cellulose are closely associated and the cellulose mircofibrils are coated with hemicellulosic polymers. Studies of sycamore cell wall suspension by Darvill et al. (1980) illustrated that the neutral and acidic pectic polysaccharides were covalently attached to the hemicellulosic fraction. In a study of the enzyme hydrolysis of hemicelluloses and cellulose, using pectinase and cellulase, Ben-Shalom (1986) concluded that the cellulose and hemicellulosic fraction in the cell wall are sterically masked by the pectic substances. In effect, the pectic substances in the cell wall appear to surround the hemicellulosic fraction and cellulose, sterically hindering enzyme binding and concomitant hydrolysis.

In addition to the presence of small amounts of heteropolysaccharides, the final α-cellulose material also contained some residual lignin. The degradation products of lignin in α-cellulose, obtained by alkaline nitrobenzene oxidation, are shown in Table 7. The total yield of phenolic acids and aldehydes was 0.19%. The major components were found to be *p*-hydroxybenzoic acid, ferulic acid, vanillin, and vanillic acid.

In conclusion, this study has shown that the major polysaccharide in wheat straw is cellulose (37.19–38.55%), followed by hemicellulosic fractions (30.28–35.01%). Other minor components are small amounts of water soluble, pectic, 80% ethanol soluble, and sodium chlorite soluble materials. Extraction of the holocellulose (routes 1–3) and cell wall material (route 4) by DMSO and using different concentrations of potassium hydroxide with 0.05% sodium borate leads to four hemicellulosic fractions (1, A–C). Hemicellulosic fraction 1 extracted from straw holocellulose corresponds to 4.78% of the dry straw mass and has an acetyl content of 12.38%. The yield of hemicellulosic material from the extraction regimes (fixed temperature: 20 °C and 0.05% sodium borate) is dependent on concentration of potassium hydroxide and extraction time. For example, the use of 10% potassium hydroxide for extraction for 16 h (route 2) produces only a slightly higher yield than treatment with 24% potassium hydroxide for 2 h (route 1). However, GPC molecular weight determinations have shown significant polymer degradation at higher alkaline concentrations, even at room temperature. In addition, as the volume of 10% potassium hydroxide increased from route 2 to route 3, the yield of the hemicellulosic fractions was observed to reproducibly increase from 34.0 to 35.0%. It should also be noted that extraction of hemicellulosic fractions A–C using 10% potassium hydroxide and 0.05% sodium borate at 20 °C for 16 h (route 4), from the residue of the DMSO extract, prior to delignification, resulted in the dissolution of 47% of the lignin.

The above demonstrates the importance of correct selection of separation techniques for characterization of heterogeneous materials such as wheat straw and illustrates the necessity of obtaining as much data as possible in the initial phases of the analysis.

Xylose is an extremely predominant component sugar in all of the hemicellulosic fractions while glucose, galactose, and arabinose are present as minor constituents. The content of uronic acids in hemicellulosic fraction B was higher than in hemicellulosic fractions A and C. The average molecular weights of hemicellulosic fractions 1, A, and C were much lower than that of hemicellulosic fraction B. In addition, the extracted hemicellulosic fractions A and B contained a small amount of lignin. The final α-cellulose residue still contained an amount of hemicellulosic polymer and a very small amount of lignin.

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