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Fractional and structural characterization of wheat straw hemicelluloses

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Six hemicellulosic fractions were extracted successively from dewaxed wheat straw with sodium hydroxide at increasing strength from 0.25 to 2.00M, and the chemical composition are reported. The structure of the hemicellulosic fraction 2 was investigated using acid hydrolysis, methylation analysis and ^{13}C-NMR experiments. The hemicelluloses were confirmed to be a (1→4)-linked β-D-xylan with D-glucopyranosyluronic acid (or 4-*O*-methyl-α-D-glucopyranosyluronic acid) group attached at position 2, and L-arabinofuranosyl and D-xylopyranosyl groups attached at position 3. For every 26 D-xylopyranosyl residues in the main chain, there was one uronic acid unit. For 13 such D-xylopyranosyl residues, there was one L-arabinofuranosyl group, and for 18 such D-xylopyranosyl residues, there was one D-xylopyranosyl group. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Hemicelluloses rank second to cellulose in abundance in agricultural waste residues such as wheat straw (Reddy *et al.*, 1984). Wheat straw contains 35–40% cellulose and 30–35% hemicelluloses composed mainly of L-arabino-(4-*O*-methyl-D-glucurono)-D-xylan (Wilkie, 1979). Earlier studies in our laboratories (Sun *et al.*, 1995b) showed that it was possible to fractionate the wheat straw hemicelluloses into hemicellulosic fractions A, B and C. The hemicelluloses B, isolated from pressure refined wheat straw sample −300μm, appeared to be essentially a (1→4) linked β-D-xylan with 4-*O*-methyl-α-D-glucopyranosyluronic acid attached at position 2, while L-arabinofuranosyl and D-xylopyranosyl groups attached at position 3. These investigations were carried out on hemicelluloses isolated by alkaline extraction from straw which had previously been treated with acidified sodium chlorite solution for delignification. In view of the possibility of degradation of polysaccharides during acidic chlorite delignification, we have now examined the hemicelluloses extracted directly with alkali from dewaxed wheat straw rather than from the holocelluloses.

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MATERIAL AND METHODS

Fractional extraction of hemicelluloses from dewaxed wheat straw

The wheat straw (winter) was obtained from Silsoe Research Institute (Silsoe, Bedfordshire). It was ground in a Christie Laboratory mill to pass a 1mm screen and extracted (Soxhlet) with chloroform: methanol (2:1, v/v) to remove waxes. The air-dried dewaxed straw (50g) was successively extracted in a thermostated reactor at 30°C under nitrogen atmosphere and stirred with the following extraction conditions (Table 1): (1) 0.25M NaOH, 2h, 50g/2000ml; (2) 0.50M NaOH, 2h, 40g/2000ml; (3) 0.75M NaOH, 2h, 34g/2000ml; (4) 1.00M NaOH, 2h, 32g/2000ml; (5) 1.50M NaOH, 4h, 30g/2000ml; (6) 2.00M NaOH, 24h, 27g/2000ml.

The extracts in each of the fractions were acidified to pH 5 with glacial acetic acid, concentrated under reduced pressure to about 800ml, and then mixed with 5 volumes of 95% ethanol (24h, 20°C). The crude hemicelluloses were filtered, washed with 75% ethanol, purified 3 times by dissolving in water and re-precipitated with 4 volumes of 95% ethanol for 12h at 20°C, respectively, and dried in an oven at 40°C.

Table 1. Yield and extraction conditions of hemicelluloses

Fraction no.	NaOH concentration (M)	Temperature (°C)/ times (h)	Dry material (g)/ extractant (ml)	Yield (% dry straw)
1	0.25	30°C/2h	50/2000	12.3
2	0.50	30°C/2h	40/2000	6.3
3	0.75	30°C/2h	34/2000	3.9
4	1.00	30°C/2h	32/2000	2.4
5	1.50	30°C/4h	30/2000	3.9
6	2.00	30°C/24h	27/2000	5.1

Methylation and analysis

Sample of hemicellulosic fraction 2 was methylated by a modified procedure (Sandford & Conrad, 1966; Asensio, 1987). At room temperature, a stirred solution of sodium methlsulfinylmethanide (prepared from 1.70g (60%) of sodium hydride and 25ml of methylated sulfoxide under nitrogen) was added to the solution of hemicellulosic fraction 2 (0.21g) in methyl sulfoxide (5ml). After stirring for 4h, the solution of hemicelluosic alkoxide was cooled to 20°C in an ice-water bath and methyl iodide (4ml) was added. Stirring was continued for 12h, water (60ml) was then added, and the mixture was extracted 3 times with 80ml of chloroform. The combined extracts were washed 3 times with 30ml of water, and the extracts in chloroform were concentrated to a yellow solid under reduced pressure at 40°C. Benzene (5ml) was added to dissolve the yellow solid, and the solution was diluted with light petroleum to precipitate the methylated hemicellulose (0.17g), $[\alpha]_D^{17}$ −87° (c 0.1, chloroform). A portion (20mg) of the material was hydrolysed with 3M trifluoroacetic acid (15ml) at 120°C for 3h and the resulting sugars were converted into alditol acetates and analysed by GC (Blakeney *et al.*, 1983).

IR spectra were obtained on an IR spectrophotometer (Mattson cygnus 100), using a KBr disc containing 1% finely ground samples. The solution ^{13}C NMR spectra (62.9MHz) were measured at 25°C on the Bruker 250 AC spectrometer. The number of Scans was 40000. The dried hemicellulosic fraction 2 (200mg/ml) were dissolved in 1ml D_2O with 1M sodium deuteroxide.

The methods of neutral sugar and uronic acid analysis, molecular weight measurement and alkaline nitrobenzene oxidation of lignin and determination of phenolic acids and aldehydes with HPLC in extracted hemicellulosic fractions have been described in previous papers (Lawther *et al.*, 1995; Sun *et al.*, 1995a). All nitrobenzene oxidation results represent the mean of at least triplicate analyses and each oxidation mixture was chromatographed twice. Other experiments were performed in duplicate. The standard errors or deviations were always observed to be lower than 5% except the variations among triplicate nitrobenzene oxidation (9–16%).

RESULTS AND DISCUSSION

Yield and composition of hemicellulosic fractions

Due to partial depolymerization during the delignification of straw with acid-chlorite (Wilkie, 1979), each of the hemicellulosic fractions was directly and sequentially extracted with alkali at increasing strength (0.25–2.00M) from the dewaxed straw under low temperature. The yield and the extraction conditions are shown in Table 1. As can be seen, with the successive increase of sodium hydroxide concentrations from 0.25 to 0.50, 0.75, 1.00, 1.50 and 2.00M, the yields of each fraction were 12.3, 6.3, 3.9, 2.4, 3.9 and 5.1%, respectively, and the total yield of extracted hemicelluloses was 33.9%, which indicated that most of the hemicelluloses were dissolved during these sequential extraction conditions. The foregoing data also showed that more than 30% of hemicelluloses were released during the first step extraction with 0.25M NaOH. This result was in agreement with our previous study (Sun *et al.*, 1995a). Treatment of extractive-free wheat straw with 1.5% NaOH at 20°C for 0.5h released 37.4% of hemicelluloses and for 144h dissolved more than 80% of hemicelluloses. This high solubility of hemicelluloses at low alkaline concentrations was due to cleavage of the ester-bond between ferulic acid and hemicelluloses by dilute alkali. It is of interest to note that lignin impedes dissolution of hemicelluloses in the other alkaline extraction steps. Extractions with 0.50, 0.75 and 1.00M NaOH at 30°C for 2h yielded, in descending order, 6.3, 3.9 and 2.4% of hemicelluloses, respectively. These data were in good agreement with our assumption reported earlier (Lawther & Sun, 1995). In addition to the ester bond between ferulic acid and hemicelluloses, or *p*-coumaric acid and lignin, and the ether bond between ferulic acid and lignin, the majority of lignin in atmospheric refined and alkali pre-treated wheat straw is directly ether-linked to arabinose in hemicelluloses.

The data on sugar and uronic acid composition are summarized in Table 2. Xylose was the major sugar in all the hemicellulosic fractions, comprising about 70–80% of the total sugars in these fractions. Galactose, glucose and mannose were observed as minor sugar constituents. Arabinose appeared in noticeable

Table 2. Monosaccharide constituent (relative %) and uronic acid content (%) of hemicelluloses

Fraction no.	Ara	Xyl	Man	Gal	Glc	Uronic acids
1	16.5	71.1	2.0	6.7	3.6	4.1
2	14.0	77.0	2.0	4.2	2.8	3.8
3	13.0	79.5	2.0	3.7	1.8	4.4
4	13.0	76.8	1.8	5.6	2.8	4.5
5	12.5	69.5	1.7	12.3	4.0	4.8
6	9.2	68.5	1.7	14.4	6.2	5.4

amounts. The maximum relative amount of xylose was detected in the hemicellulosic fraction 3 obtained from 0.75M NaOH treatment. With the increase of sodium hydroxide concentration from 0.25 to 2.00M, the content of arabinose decreased from 16.5 to less than 10%, while the content of glucose increased to 6.2%. Uronic acid values averaged about 4–5% for the fractions, which was higher than that in the hemicelluloses extracted from straw holocelluloses (3.0%). Compared to high content of xylose and low content of arabinose in hemicelluloses extracted from straw holocelluloses (Sun *et al.*, 1995a), the hemicellulosic fractions extracted directly from dewaxed straw contained a relatively high amount of arabinose and low amount of xylose. This phenomenon provides evidence that in wheat straw cell walls arabinose, probably as a side chain in hemicelluloses, is bound to ferulic acid or directly to lignin and is easily released, whereas the xylose in the main chain of hemicellulose is prevented from extraction prior to delignification.

Structural characterization of hemicellulosic fraction 2

Methylation of the hemicellulosic fraction 2 gave a product with an $[\alpha]_D^{17}$ value of $-87°$ indicative of β-linkages, which was confirmed by the ^{13}C NMR spectrum (δ 104.8 for C-1). Reduction and hydrolysis of methylated fraction 2 yielded the following methylated sugars: 2,3,5-tri-*O*-methyl-L-arabinose (6.8%), 2,3,4-tri-*O*-methyl-D-xylose (4.9%), 2,3-di-*O*-metyl-D-xylose (77.4%), 2-*O*-methyl-D-xylose (7.2%) and 3-*O*-methyl-D-xylose (3.7%). The formation of 2,3,5-tri-*O*-methyl-L-arabinose indicated the existence of 1 terminal arabinofuranosyl group per 13 xylose residues. The formation of a small proportion of 2,3,4-tri-*O*-methyl-D-xylose showed that xylopyranosyl group was also present as a terminal unit, and for every 18 xylopyranosyl residues in the main chain, there was one xylopyranosyl group. That the backbone consisted of (1→4) linked β-D-xylose residues was indicated by the formation of a large proportion of 2,3-di-*O*-metyl-D-xylose. The side-chains were attached to positions 2 and 3 of the xylose residues, as indicated by the formation of 3 or 2 -*O*-methyl-D-xylose.

In agreement with previous studies (Aspinall & Meek, 1956; Toman & Chimidcogzol, 1988) there was nearly double the content of 2-*O*-methyl-D-xylose compared with 3-*O*-methyl-D-xylose in xylan fraction 2. We are of the opinion that the L-arabinose and 4.9% D-xylose residues occurred as side-chains linked to the backbone of (1→4) β-D-xylopyranose residues through position 3 of xylose. Bishop (1956) isolated the trisaccharide, *O*-L-arabinofuranosyl-(1→3)-*O*-D-xylopyranosyl-(1→4)-D-xylopyranose, from the enzymic hydrolysis of wheat straw hemicelluloses, and proved that L-arabinose residue was attached to position 3 in the main chain of the xylosyl residues. The methylated aldobiouronic acid isolated from the hydrolysis of the methylated xylan showed that the D-glucuronic acid residues (partially present as the 4-methyl ether) are linked directly to the main chain through position 2 of xylose (Aspinall & Meek, 1956).

Identification of the 4-*O*-methyl-α-D-glucopyranosyluronic acid was not possible by GC on OV-17, but it was identified by the fragments obtained by GC–MS of the partially methylated alditol acetates (Asensio, 1987). It is also possible that uronide-like substances were eliminated during the methylation step (Ehrenthal *et al.*, 1954; Wilkie, 1979). Therefore, this loss of D-glucopyranosyluronic acid, or its 4-methyl-ether, or both is not surprising and indeed would lend support to the theory advanced above, that the D-glucuronic acid or its 4-methyl ether are attached to the main chain of xylose residues. As can be seen from Fig. 1 and Table 2, the chemical shifts for D-glucuronic acid residues appeared at 100.8 (C-1), 75.4 (C-2, C-3), 83.2 (C-4), 74.4 (C-5) and 179.8 (C-6). The molar ratio of uronic acid:-xylose in the hemicellulosic fraction 2 was 1.9:50. Thus, for every 26 D-xylopyranosyl residues in the main chain, there was one uronic acid unit.

The structural features of hemicellulosic fraction 2 evaluated by methylation analysis are reflected by the ^{13}C NMR spectrum (Fig. 1). The spectrum was interpreted (Table 3) on the basis of reported data for structurally-defined arabinoxylan-type, glucuronoxylan-type and L-arabino-(4-*O*-methyl-D-glucurono)-D-xylan, as well as those of wheat straw hemicelluloses isolated after delignification (Ebringerová *et al.*, 1992; Simkovic *et al.*, 1986; Sun *et al.*, 1995b). As expected, most signals of the spectrum (Fig. 1) are attributed to the corresponding carbon atoms of the L-arabino-(4-*O*-methyl-D-glucurono)-D-xylan.

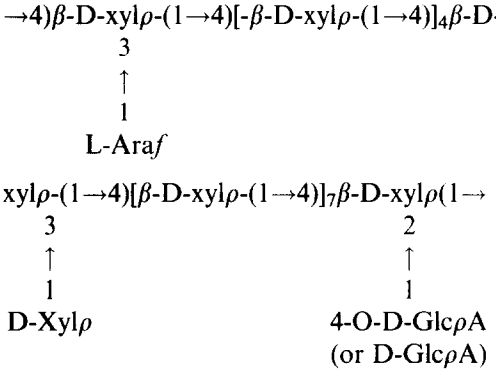
Thus, it is concluded that the hemicellulosic fraction 2 had a structure composed of a main chain of (1→4)-linked β-D-xylopyranosyl residues, with (1→2)-linked D-glucopyranosyluronic acid (or 4-*O*-methyl-α-D-

Table 3. Methyl derivatives from the hydrolysate of methylated hemicellulosic fraction 2 and the corresponding chemical shifts in ^{13}C NMR data

Methyl derivative	Mole %	Chemical shift (ppm)					
		C-1	C-2	C-3	C-4	C-5	MeO
2,3,5-Me_3-Ara[a]	6.8	112.1	83.2	78.9	89.4	64.6	—
2,3,4-Me_3-Xyl	4.9	104.8	75.9	81.4	78.9	66.1	—
2,3-Me_2-Xyl	77.4	104.8	75.9	77.2	78.9	66.1	—
2-Me-Xyl	7.2	104.2	80.4	77.2	78.9	66.1	—
3-Me-Xyl	3.7	100.8	74.4	75.4	83.2	73.0	60.5

[a] 2,3,5-Me_3-Ara = 2,3,5-tri-O-methyl-L-arabinose, etc.

glucopyranosyluronic acid), and (1→3)-linked L-arabinofuranosyl or D-xylopyranosyl residues at the branch points. For every 13 D-xylopyranosyl residues in the main chain, there was one L-arabinofuranosyl group branch; for 18 such D-xylopyranosyl residues, there was one D-xylopyransyl group branch and for 26 such D-xylopyranosyl residues, there was one uronic acid unit as the side chain. These results are consistent with the general type of structure shown:



Physico–chemical characterization of hemicellulosic fractions

The hemicellulosic fractions extracted from dewaxed straw before delignification had a high degree of polymerization with molecular-average weights between 28 000 and 40 900 Da (Table 4), which were much higher than those of hemicelluloses (9000–13 100 Da) extracted from straw holocelluloses after delignification. In the fractions between 1 and 4, with the increase of sodium hydroxide concentration from 0.25 to 1.00 M, the mole-

Table 4. Molecular-average weight of hemicelluloses

Fraction no.	$\overline{M}_w$	$\overline{M}_n$	$\overline{M}_w/\overline{M}_n$
1	34 800	25 700	1.35
2	32 200	21 100	1.53
3	30 800	21 100	1.46
4	28 000	20 900	1.34
5	33 900	20 200	1.67
6	40 900	21 400	1.91

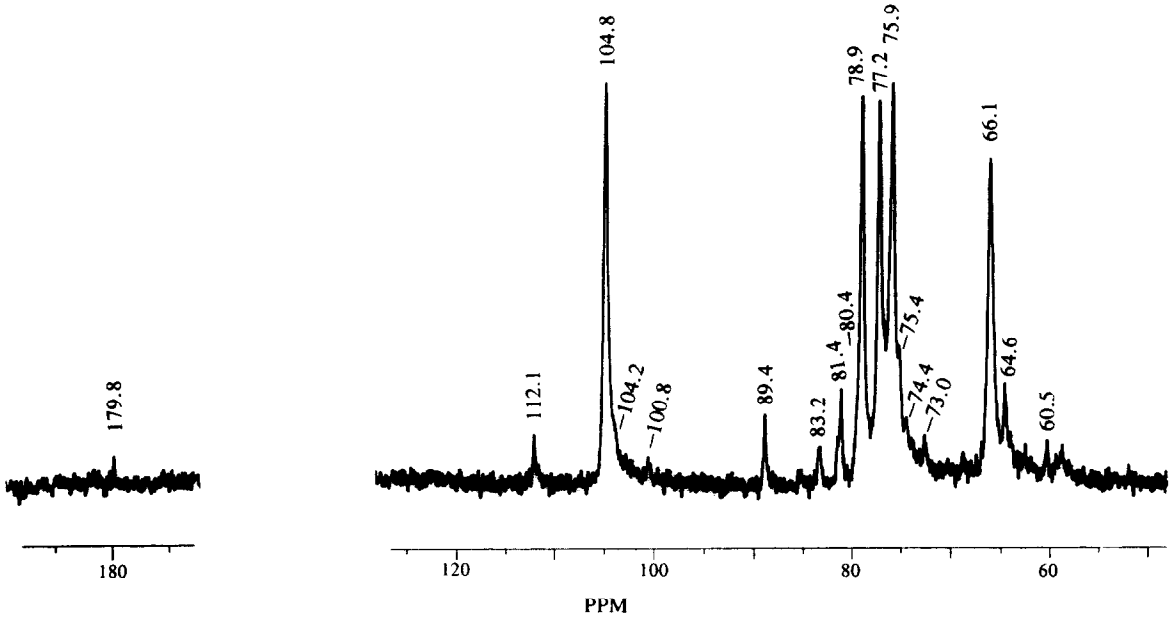


Fig. 1. ^{13}C-NMR spectrum of hemicellulosic fraction 2 in D_2O.

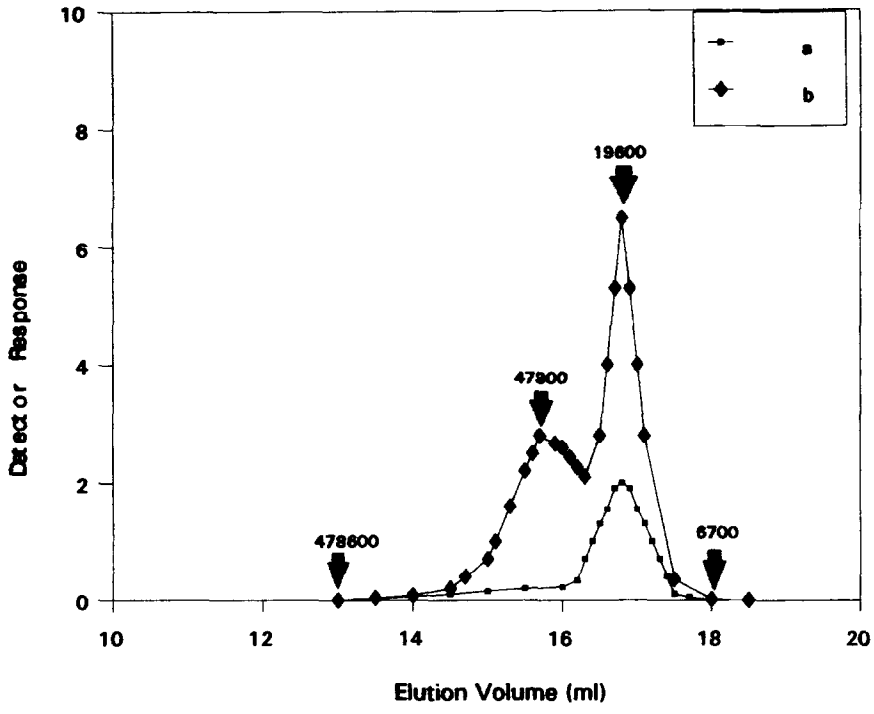


Fig. 2. The molecular weight range of hemicelluloses (a) extracted with 1.00M NaOH at 30°C for 2h and (b) extracted with 0.25M NaOH at 30°C for 2h.

cular-average weights decreased from 34800 to 28000Da. This result was in agreement with our previous study (Sun *et al.*, 1995a). We indicated that weak alkaline solutions generally solubilized straw hemicelluloses B, the more acidic and branched fraction, to a greater extent than hemicelluloses A, the more linear and less acidic hemicelluloses. Therefore, hemicelluloses B (high molecular weight) can be more or less selectively extracted from straw with very weak alkaline solution, such as 0.25M NaOH, while hemicelluloses A (low molecular weight) can be more or less selectively extracted with relatively high concentration of alkali, such as 1.00M NaOH. Increasing the sodium hydroxide concentration from 1.00 to 2.00M, raised the molecular-average weights from 28000 to 40900Da. The reason for this increasing tendency was probably because more lignin–hemicellulosic complex was co-extracted with hemicelluloses.

The elution profiles of hemicellulosic fraction 1, extracted with 0.25M NaOH at 30°C for 2h, contained two major peaks (Fig. 2) Peak I eluted in the void volume (15.7ml) and a molecular weight equal to 479000Da. Peak II had a molecular weight around 19600Da. The molecular weight distribution ranged between 478600 and 6700Da. However, the fraction 4, extracted with 1.00M NaOH at 30°C for 2h, produced

Table 5. The content of phenolic acids and aldehydes in the products of alkaline nitrobenzene oxidation of lignin in hemicellulosic fractions

Phenolic acids and aldehydes	Fraction no.					
	1	2	3	4	5	6
Gallic acid	0.26	0.21	0.21	0.30	0.34	0-31
Protocatechuic acid	0.020	0.0066	0.0068	0.013	0.027	0.018
p-Hydroxybenzoic acid	0.096	0.024	0.012	0.024	0.097	0.030
p-Hydroxybenzaldehyde	0.23	0.093	0.075	0.099	0.19	0.13
Vanillic acid	0.15	0.074	0.056	0.093	0.16	0.14
Syringic acid	0.35	0.20	0.20	0.095	0.41	0.16
Vanillin	1.67	0.99	0.89	1.13	1.27	1.20
Syringaldehyde	1.67	0.99	0.93	1.26	1.80	1.70
p-Coumaric acid	0.065	0.23	0.023	0.023	0.073	0.041
Acetovanillone	0.032	0.012	0.011	0.021	0.043	0.024
Ferulic acid	0.21	0.11	0.054	0.11	0.14	0.18
Total	4.75	2.94	2.47	3.17	4.55	3.93

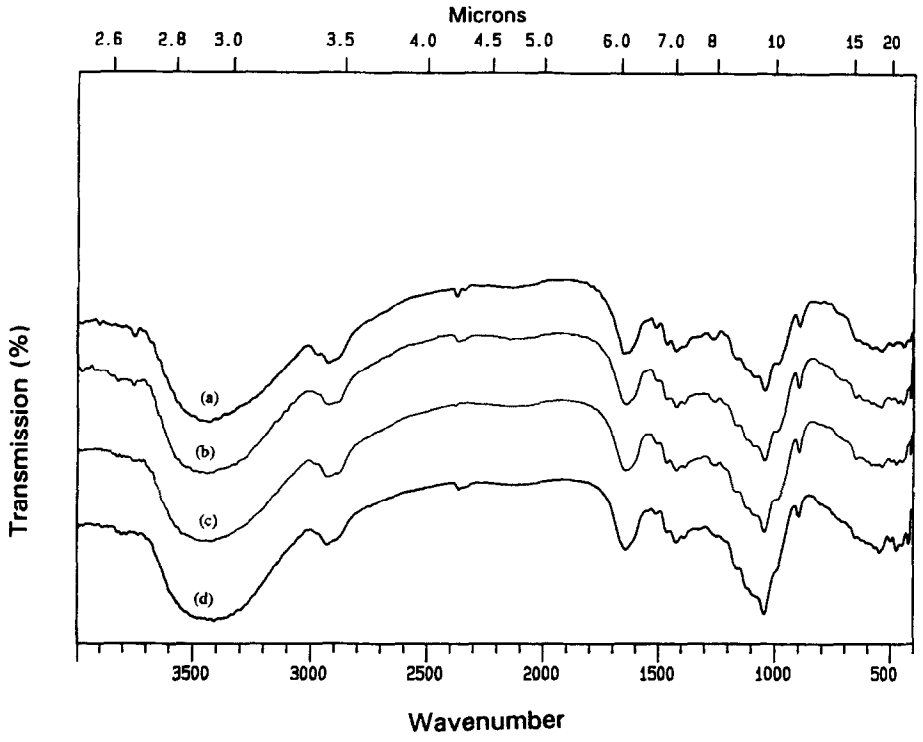


Fig. 3. FT–IR spectra of hemicellulosic fractions, 1 (a), 2 (b), 4 (c) and 6 (d).

an apparent lower molecular weight distribution with a major peak corresponding to 19 600 Da. Hence higher concentrations of alkali could cause fragmentation of hemicelluloses.

The FT–IR spectra of hemicellulosic fractions (1, 2, 4, 6) are shown in Fig. 3. As can be seen from the figure, four spectra of hemicellulosic fractions appeared to be rather similar. All of the spectra had a sharp band at 890cm^{-1}, which is characteristic of beta-glucosidic linkages between the sugar units (Gupta *et al.*, 1987). This suggested that the xylose residues forming the backbone of the macromolecule are linked by β form bonds. The other prominent bands corresponding to hemicelluloses also appeared at 1370 and 1030cm^{-1}. The broad band at 1635cm^{-1} was probably due to linked water (Fidalgo *et al.*, 1993), and the small bands at 1510, 1460, 1420, 1245, and 1150cm^{-1} showed low lignin content in hemicellulosic fractions. Interestingly, in the spectra of fractions 1 and 6 the intensities of 1510, 1460, 1420 and 1150cm^{-1} were stronger than those in fractions 2 and 4, indicating higher content of lignin in fractions 1 and 6.

It is well known that low amounts of phenolic substances were found in each of the hemicellulosic fractions. After alkaline nitrobenzene oxidation of lignin in hemicellulosic fractions at 170°C for 2.5h, the content of phenolic acids and aldehydes in the products is given in Table 5. As mentioned earlier, due to the lignin–hemicellulosic complex in the cell walls of wheat straw, the content of phenolics in the hemicellulosic fractions extracted directly from dewaxed straw was 5–10 times higher than that in the fractions extracted from straw holocelluloses. The content of phenolic monomers in the fractions 1 (4.75%) and 6 (3.93%) was higher than in the fractions 2 (2.94%) and 4 (3.17%), which was supported by the infrared spectra. In all the extracted hemicellulosic fractions, the major components of phenolics in the alkaline nitrobenzene oxidation of residual lignin were found to be syringaldehyde and vanillin.

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