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Comparative study of hemicelluloses from rice straw by alkali and hydrogen peroxide treatments

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Abstract

First extraction of the dewaxed rice straw with 1% NaOH at 55°C for 2 h and then following treatment with 0.0, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0% hydrogen peroxide at 45°C for 12 h at pH 11.5 solubilized 67.2, 77.2, 78.7, 83.7, 85.5, 87.3 and 88.5% of the original hemicelluloses, and 2.5, 9.8, 11.8, 12.1, 15.6, 16.4 and 17.8% of the original cellulose, respectively. Meanwhile, the two-stage treatment together resulted in a dissolution of 68.3, 85.4, 89.4, 92.7, 92.7, 94.3 and 95.1% of the original lignin under the conditions given, respectively. Comparison of these hemicellulosic preparations indicated that alkali treatment in the absence of H_2O_2 favoured solubilization of the small molecular size of hemicelluloses, which are rich in glucose, probably originating from α-glucan, while the second stage treatment by alkaline peroxide enhanced dissolution of larger molecular size hemicelluloses, which are rich in xylose, principally resulting from L-arabino-(4-*O*-methyl-D-glucurono)-D-xylan. The treatment by alkaline peroxide under the conditions used did not affect the overall structure of macromolecular hemicelluloses except for a noticeable degradation of cellulose and hemicelluloses during the treatment with more than 1.0% H_2O_2. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Rice straw; Hemicelluloses; Hydrogen peroxide; Sugars; Lignin

1. Introduction

Rice straw is one of the main cereal straws and is produced in large quantities world-wide every year. In developing countries, these large quantities of fibrous crop residues are currently under-utilized either as raw material for paper making, or as potential animal feed sources. However, such straw is considered to be agricultural waste in developed countries since it cannot be converted into valuable products (Pan, Sano & Ito, 1999). As we attempt to reduce the adverse impact on the environment and to use this renewable biomass to produce various chemicals, the development of effective technologies for utilization of straw is considered to be both important and significant.

Hemicelluloses are one of the most abundant natural polysaccharides and comprise over 30% of the dry mater of rice straw. They, unlike cellulose, which is a unique molecule differing only in degree of polymerization and crystallinity, are inhomogeneous fractions and classically defined as the alkali soluble material after removal of the pectic substances (Sun, 1996). Most of the hemicellulosic preparations are soluble in water after alkaline extraction. Their isolation actually involves alkaline hydrolysis of ester linkages to liberate them from the lignocellulosic matrix followed by extraction into aqueous media. Hemicelluloses are branched polymers of low molecular weight with a degree of polymerization of 80–200. Their general formulae are $(C_5H_8O_4)_n$ and $(C_6H_{10}O_5)_n$ and called pentosans and hexosans, respectively (Cai & Paszner, 1988). Hemicelluloses are made of a relatively limited number sugar residues. The principle ones are D-xylose, L-arabinose, D-glucose, D-galactose, D-mannose, 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. The sugar composition of the hemicelluloses from wheat straw found that xylose was the predominant sugar component, with arabinose appearing in noticeable amounts. Glucose, galactose, mannose, and rhamnose were the minor sugar constituents. Further structural studies of the hemicelluloses from wheat straw showed that the β-(1 → 4)-D-xylopyranose backbone is substituted by α-L-arabinofuranose and α-D-glucuronic acid, mainly 4-*O*-methyl-D-glucuronic acid (MeGlcA) at C-3 and C-2 of xylose residues in the main chain, respectively. Galactose and xylose residues are potentially linked to arabinofuranosyl branches (Sun, Lawther & Banks, 1996).

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The hemicelluloses are potentially very useful. Properties of wheat straw hemicelluloses worth exploiting are their ability to serve as adhesives, thickeners, and stabilisers, and as film formers and emulsifiers (Doner & Hicks, 1997). More recently, with the extensive study of the hemicelluloses from wheat straw, we found that the native hemicelluloses lattices showed good properties for making decorative paints, indicating a possibility of using hemicelluloses for commercial decorative paint systems (Sun, Fang, Rolands & Bolton, 1998). However, the hemicelluloses prepared by aqueous alkali extraction from lignified wheat straw, were, in general brown and this impedes their industrial utilization. The aim in our laboratory is to develop a commercial process for fractionation of straw components using an environmental friendly procedure for the extraction of hemicelluloses in a large scale with a light colour. In an earlier paper (Sun & Tomkinson, 1999), we reported that hydrogen peroxide in alkaline media could serve as a mild agent for solubilizing the macromolecular hemicelluloses besides its dual role in delignifying and bleaching. It is generally accepted that the hydroperoxide anion (HOO^-), formed in alkaline media, is the principal active species in hydrogen peroxide bleaching systems. On the contrary, hydrogen peroxide is unstable in alkaline conditions and readily decomposes particularly in the presence of certain transition metals such as manganese, iron, and copper. The decomposition products such as hydroxyl radicals ($HO\cdot$) and superoxide anion radicals ($O_2^-\cdot$) are thought to cause the oxidation of lignin structures which leads to the introduction of hydrophilic (carboxyl) groups, cleavage of some interunit bonds and eventually, the dissolution of lignin and hemicelluloses even though it has been claimed recently that these radicals may participate in the bleaching mechanism, at least to some small extent (Dence, 1996; Pan, Bolton & Leary, 1998). By using this method, the nonfibrous constituents, both hemicelluloses and lignin, could be recovered simply from the hydrolysates in large quantities by fractional precipitation. The lignin and hemicelluloses could be converted to valuable products by chemical modifications.

The aim of this study was to develop a fractional treatment procedure for rice straw hemicelluloses with maximal yield but minimal degradation and light colour. The dewaxed straw was first treated with 1% sodium hydroxide at 55°C for 2 h and then with 0.0–5.0% hydrogen peroxide (H_2O_2) at 45°C for 12 h at pH 11.5, respectively. The chemical composition and physico-chemical properties of alkali-soluble and alkaline peroxide-soluble hemicelluloses were comparatively studied and the results are reported.

2. Experimental

2.1. Materials

Rice straw was obtained from the experimental farm of the North-Western University of Agricultural and Forest Sciences and Technology (Yangling, P.R. China). It was dried in sunlight and then cut into small pieces. The cut straw was ground to pass a 0.8 mm size screen. All weights and calculations were made on an oven-dried (60°C, 16 h) basis. The composition (w/w) of the rye straw used was cellulose 36.5%, hemicelluloses 33.8%, chlorite lignin 12.3%, wax 3.8%, and ash 13.3%, which contains 70.8% silica.

2.2. Alkaline peroxide treatment

The dried powder was first extracted with toluene–ethanol (2:1,v/v) in a Soxhlet for 8 h. The dewaxed straw was then soaked in a 1% NaOH solution with a 1:25 straw to liquor ratio at 55°C for 2 h. The alkali extracts were neutralized with 6 M HCl to pH 8.5 to precipitate the release of sodium silicate. After filtration of the silicate, the supernatants were neutralized with 6 M HCl to pH 5.5 and then concentrated. The alkali-soluble hemicelluloses were obtained by precipitation of the concentrated supernatants in 3 volumes of ethanol. After filtration, the isolated hemicelluloses were washed with 70% ethanol and then air-dried. Samples free of wax and alkali-solubles (5.0 g) were added to 250 ml of distilled water in the absence of H_2O_2 and containing 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0% H_2O_2 (w/v) in a jacketed reaction vessel heated with water from a thermostat-controlled circulating bath. The suspension was adjusted to pH 11.5 with 4 M NaOH and allowed to stir gently for 12 h at 45°C, respectively. During initial stages of stirring, oxygen evolution was active, and substantial frothing occurred, requiring that extractions were conducted in vessels with volumes two to three times those of extraction mixtures. No further adjustments in pH were made during the course of the treatment. Under these conditions, the reaction pH remained nearly constant for two hours before slowly rising to a final value of ca 13.1. After the indicated period of time, the insoluble residue was collected by filtration, washed repeatedly with distilled water until the filtrate was neutral, and then oven-dried at 60°C for 16 h. The supernatant fluid was neutralized to pH 8.5 and 5.5 with 6 M HCl for isolation of sodium silicate and residual hemicelluloses as in the method mentioned above. The isolated residual hemicelluloses were thoroughly washed with 70% ethanol and anhydrous ethyl alcohol, then air-dried. The preparation procedure is illustrated in Fig. 1.

2.3. Determination of ash and silica in the straw and residues

The contents of ash and silica were determined according to the methods described by Pan et al. (1999). The sample was transferred to a crucible and carbonised gently in a muffle furnace at 550 ± 10°C for 6 h. The ash obtained was treated with concentrated HCl. The acid-insoluble residue was filtered, washed with hot water until no

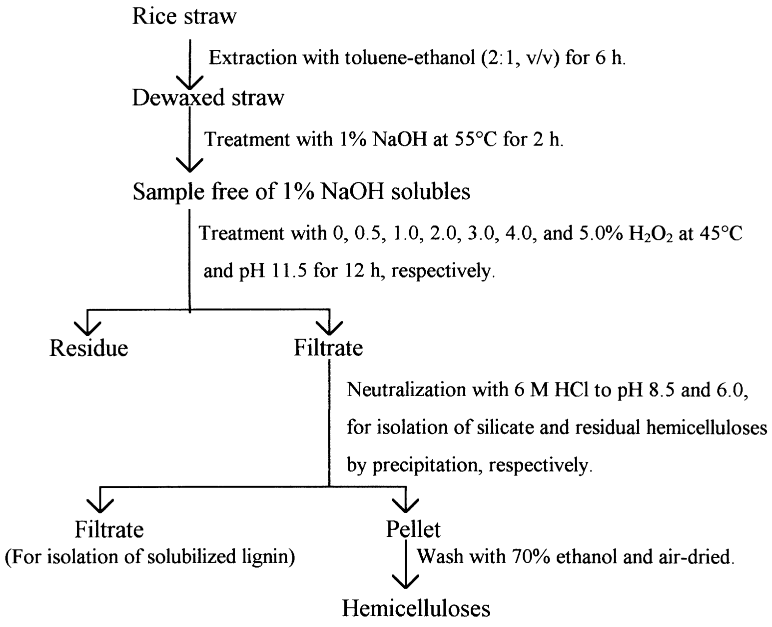

Fig. 1. Scheme for extraction of hemicelluloses from rice straw.

chlorides were detectable, ignited, and finally weighed as silicon dioxide.

2.4. Characterization of the solubilized hemicelluloses

The neutral sugar composition of the isolated hemicelluloses was determined by gas chromatography (GC) analysis of their alditol acetates (Blakeney, Harris, Henry & Stone, 1983). Alkaline nitrobenzene oxidation of associated lignin from the solubilized hemicellulosic preparations was performed at 170°C for 3 h. The lignin content in hemicellulosic preparations was calculated multiplying by 4.0, the yield of phenolics obtained by nitrobenzene oxidation (Sun et al., 1998). Methods of uronic acid analysis, determination of phenolic acids and aldehydes in nitrobenzene oxidation mixtures with high performance liquid chromatography (HPLC), and measurement of the molecular weights have been described in previous papers (Lawther, Sun & Banks, 1995; Sun, Lawther & Banks, 1995). The FT-IR spectra were recorded from KBr pellets containing 1% finely ground samples on a Nicolet-750 FT-IR spectrophotometer. The solution-state ^{13}C NMR spectrum was obtained on a Bruker 250 AC spectrometer operating in the FT mode at 62.4 MHz under total proton decoupled conditions. They are recorded at 25°C from 140 mg of sample dissolved in 1.0 ml D_2O after 12,000 scans. A 60° pulse flipping angle, a 3.9 μs pulse width and 0.85 s acquisition time were used.

3. Results and discussion

3.1. Yield of hemicelluloses

The yields of hemicelluloses, cellulose, and lignin, solubilized during the alkali extraction and the following alkaline peroxide treatment processes, and the residues are listed in Table 1. All the values are calculated based on the mass of untreated starting material. As can be seen from the table, extraction of the dewaxed rice straw with 1% NaOH at 55°C for 2 h and the following treatment in the absence of H_2O_2 and with 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0% hydrogen peroxide at 45°C for 12 h at pH 11.5 solubilized 67.2, 77.2, 78.7, 83.7, 85.5, 87.3 and 88.5% of the original hemicelluloses, and 2.5, 9.8, 11.8, 12.1, 15.6, 16.4 and 17.8% of the original cellulose, respectively. Meanwhile, the two-stage treatment together also resulted in a dissolution of 68.3, 85.4, 89.4, 92.7, 92.7, 94.3 and 95.1% of the original lignin under the conditions given, respectively. This result indicated that alkaline peroxide is a strong agent both for

Table 1
The yield of residue, hemicelloluses, cellulose, and lignin (%dry matter) solubilized during the treatment of rice straw with 1% NaOH and the following treatments using various concentrations of hydrogen peroxide

	AS[a]	H_2O_2 concentration (%)						
		0[b]	0.5[b]	1.0[b]	2.0[b]	3.0[b]	4.0[b]	5.0[b]
Hemicelluloses[c]	18.6	4.1	7.5	8.0	9.7	10.3	10.9	11.3
Cellulose	ND[d]	0.9	3.5	4.3	4.4	5.7	6.0	6.5
Lignin	7.2	1.2	3.3	3.8	4.2	4.2	4.4	4.5
Residue	59.7	50.4	41.9	41.0	39.0	37.4	36.4	35.4

[a] Abbreviation for the alkali-soluble hemicelluloses and lignin obtained by extraction with 1% NaOH at 55°C for 2 h from the dewaxed rice straw.

[b] The preparations obtained by extraction with different concentrations of H_2O_2 at 45°C for 12 h at pH 11.5 from the alkali-treated straw.

[c] Obtained by precipitation of the neutralized and desilicated extracts with 3 vols ethanol.

[d] ND: not detectable.

delignifying and for solubilizing hemicelluloses, and the alkaline peroxide treatment under the conditions used significantly cleaved the ether bonds between lignin and hemicelluloses from the cell walls of rice straw. The hemicellulosic preparations, obtained from the second stage of alkaline peroxide treatment, were slight brown in colour, while the two hemicellulosic fractions, obtained by dilute alkali solution in the absence of H_2O_2 were dark brown in colour, that is, the hemicelluloses prepared by alkaline peroxide extraction were much lighter in colour than those obtained under similar conditions but in the absence of peroxide.

Interestingly, the data in Table 1 showed that treatment of the dewaxed rice straw with 1% NaOH at 55°C for 2 h released more than 50% of the original hemicelluloses and lignin. This high solubility of hemicelluloses and lignin was presumed to be due to the cleavage of the ester bonds between hydroxycinnamic acids such as *p*-coumaric and ferulic acids and hemicelluloses or lignin, and the α-benzyl ether linkages between lignin and hemicelluloses from the cell walls of rice straw by alkali. It was also found that prior to alkaline peroxide treatment, extraction with 1% NaOH at 55°C for 2 h removed 62.2% of the original silica from rice straw. A similar phenomenon was also observed by Eroglu and Deniz (1993) from wheat straw. The authors stated that a three-stage treatment with 1% NaOH was the most convenient condition for desilication. In this case, more than 73% silica could be removed under the relatively mild alkaline conditions. As the silica is concentrated in the outer parts of cereal straw, it is very likely that pre-treatment with selected chemicals such as 1% NaOH solution may be very effective for desilication from this straw. Further treatment of the alkali-extracted straw with 0.5–5.0% H_2O_2 under the alkaline media given led to a further ca 26.6% desilication. Hence, less than ca 2.7% silica was found in the end residues. It is thought that this two-stage treatment with dilute alkali solution followed by alkaline peroxide may propose a convenient and effective method for the desilication of rice straw.

The extent of the delignification and hemicellulose solubilization catalyzed by H_2O_2 was a strong function of the reaction pH, with a sharp optimum at pH 11.5 (Gould, 1985). When properly adjusted, the total alkalinity of the solution should be high enough to ensure an adequate concentration of hydroperoxide anion (HOO^-), the active bleaching species, throughout the course of reaction as dictated by the equilibrium (Gould, 1984):

$$H_2O_2 + HO^- \leftrightarrow HOO^- + H_2O$$

On the contrary, when the alkalinity is high enough to decompose the peroxide, the reactive hydroxyl radicals ($HO\cdot$) and superoxide anion radicals ($O_2^-\cdot$) are formed, which are responsible for delignification and hemicellulose solubilization (Doner & Hicks, 1997):

$$H_2O_2 + HOO^- \rightarrow HO\cdot + O_2^-\cdot + H_2O$$

These radicals may react further with each other and give rise to oxygen and hydroxyl anions as the final products, leading to an increase in the reaction pH (Dence, 1996):

$$HO\cdot + O_2^-\cdot \rightarrow O_2 + HO^-$$

To obtain maximum rate of delignification and yield of hemicellulose solubilization, it is, therefore, not necessary to continuously regulate the reaction pH, even though over the course of the treatment the reaction pH increased from 11.5 at the beginning to ca 13.1 in the end, since similar levels of delignification were also attained using H_2O_2 at $pH > 12.5$. Interestingly, as the reaction pH becomes more alkaline, the hemicelluloses begin to substantially solubilize. Nearly 64% of the residual hemicelluloses present in the alkali-treated rice straw was solubilized after 12 h of 2% H_2O_2 treatment at 45°C in the absence of continuous pH control, while only about 39% of the residual hemicelluloses were released in 12 h when the reaction pH was maintained at pH 11.5 ± 0.2 (Sun & Tomkinson, 1999).

Table 1 also shows the effect of various H_2O_2 concentrations from 0.5 to 5.0% on the yield of hemicelluloses released. In the absence of H_2O_2, only about 27% of the residual hemicelluloses originally present in alkali-treated rice straw was solubilized during the treatment with dilute alkali solution at pH 11.5 for 12 h at 45°C, whereas as much as 50% of the hemicelluloses was solubilized during the treatment with 1.0% H_2O_2 at 45°C for 12 h at pH 11.5. In the presence of 5.0% H_2O_2, more than 74% of the residual hemicelluloses originally present in the alkali-treated rice straw were solubilized under the condition given. The current results were consistent with the study on the optimisation of the alkaline peroxide pretreatment for delignification of rice straw by Patel and Bhatt (1992). The authors showed that the substantial hemicelluloses were solubilized beyond 1% H_2O_2 and 1.5% NaOH treatment at room temperature for 18 h from rice straw.

As observed by Gould and Freer (1984), it was also noted here that substantial solubilization of lignin and hemicelluloses was also associated with a severe disruption of the physical integrity of the straw. Subsequently, the straw particles disintegrated into small, highly dispersed fibres, and the suspension acquired a more homogeneous pulp-like consistency. Further, in contrast to other treatments, which only reduce cellulose crystallinity, the alkaline peroxide treatments under the conditions used resulted in a noticeable degradation of cellulose. As can be seen from the table, treatment of the alkali-extracted rice straw under the similar alkaline conditions but in the absence of peroxide led to only 2.5% of the original cellulose dissolution. While the treatment with alkaline peroxide under concentrations of 0.5–5.0% resulted in a solubilization of 9.8–17.8% of the original cellulose. This is rather surprising, since alkaline peroxide treatment did not cause detectable changes in the structure of highly polymerized cellulose but attributed to a possible decrease in cellulose crystallinity (Martel & Gould, 1990). However, a similar observation

Table 2
The content of neutral sugars (relative% hemicellulosic sample, w/w) and uronic acids (%hemicellulosic sample, w/w) in isolated hemicellulosic preparations

Neutral sugars/Uronic acids	ASH[a]	H_2O_2 concentraion (%)						
		0[b]	0.5[b]	1.0[b]	2.0[b]	3.0[b]	4.0[b]	5.0[b]
Rhamnose	0.66	0.36	0.85	0.80	0.75	0.53	0.66	0.75
Fucose	0.09	0.10	0.31	0.34	0.34	0.32	0.40	0.67
Arabinose	11.15	11.75	15.10	15.80	16.53	16.43	16.93	15.95
Xylose	27.27	26.87	37.82	41.55	42.08	43.89	50.21	53.42
Glucose	53.06	54.37	39.76	35.57	35.00	33.35	27.01	24.58
Galactose	7.77	6.55	6.16	5.94	5.30	5.50	4.79	4.66
Uronic acids	1.75	5.62	4.63	2.63	2.50	2.63	4.00	5.63

[a] Abbreviation for the alkali-soluble hemicellulosic preparation obtained by extraction with 1% NaOH at 55°C for 2 h from the dewaxed rice straw.

[b] The hemicellulosic preparations obtained by extraction with different concentrations of H_2O_2 at 45°C and pH 11.5 for 12 h from the alkali-treated rice straw.

has been found by Patel and Bhatt (1992) from the alkaline peroxide treatment of rice straw. The authors mentioned that over 30% of the original cellulose from rice straw was solubilized when the treatment was performed with 4.5% H_2O_2 and 1.5% NaOH at room temperature for 18 h. This suggested that the concentration of H_2O_2 has to be used as low as possible in order to prevent any degradation of cellulose from rice straw. Further studies found that the depolymerization of cellulose resulted mainly from the hydroxyl radical, generated from the decomposition of H_2O_2 under the alkaline solution, particularly in the presence of transition of metals (Dence, 1996).

3.2. Sugar composition and content of uronic acids

The data on sugar composition and content of uronic acids in eight recovered hemicellulosic preparations are given in Table 2. Obviously, 1% NaOH-soluble hemicellulosic fraction (AS) and the dilute alkali-soluble hemicelluloses, obtained under similar conditions but in the absence of peroxide, mainly comprised neutral chains, including glucose (53.1–54.4%), xylose (26.9–27.3%), galactose (6.6–7.8%), and arabinose (11.2–11.8%). These data showed that the two dilute alkali-soluble hemicellulosic fractions were probably similar in structure and the high percentage of glucose and xylose was taken to indicate correspondingly more glucans and xylans. In contrast, xylose was the major sugar in other six hemicellulosic preparations, extracted with 0.5–5.0% H_2O_2 at pH 11.5 for 12 h at 45°C from the alkali-treated rice straw, comprising 37.8–53.4% of the total sugars. Glucose and arabinose appeared as the second and third major sugars, comprising 24.6–39.8% and 15.1–16.9% of the total sugars, respectively. Galactose was identified as a noticeable amount, and fucose and rhamnose were observed as minor constituents. An increase in concentration of H_2O_2 from 0.5 to 5.0% resulted in an increase of xylose from 37.8 to 53.4% and arabinose from 15.1 to 16.0%, respectively, but a decrease of glucose from 39.8 to 24.6% and galactose from 6.2 to 4.7% in the hemicellulosic preparations. This significant increase in xylose content and a substantial decrease in glucose content with the increase of H_2O_2 from 0.5–5.0% provided evidence that in the rice straw cell walls, glucose is probably present in side chains of hemicelluloses and easily released at low concentration of H_2O_2, while xylose in the main chain of hemicelluloses is favourably extracted at a relatively high concentration of H_2O_2. This observation implied that the hemicelluloses, extracted by alkaline peroxide from rice straw, are mainly composed of a xylan structure similar to that found in wheat straw or grass (Sun,

Table 3
The content of neutral sugars (relative% residual sample, w/w) in alkali and alkaline peroxide treated residues

Neutral sugars/Uronic acids	ATR[a]	H_2O_2 concentraion (%)						
		0[b]	0.5[b]	1.0[b]	2.0[b]	3.0[b]	4.0[b]	5.0[b]
Rhamnose	0.15	0.32	0.11	0.10	0.10	0.14	0.10	0.11
Fucose	0.10	0.08	ND[c]	ND	ND	ND	ND	ND
Arabinose	4.80	4.33	3.17	2.89	2.84	2.89	2.40	2.66
Xylose	16.27	16.15	15.31	14.66	11.84	11.50	11.18	10.08
Glucose	77.86	78.33	80.76	81.67	84.71	84.79	85.58	86.35
Galactose	0.82	0.79	0.69	0.67	0.60	0.70	0.82	0.81

[a] Abbreviation for the alkali-treated residue obtained by treatment with 1% NaOH at 55°C for 2h from the dewaxed rice straw.

[b] The residual fractions obtained by treatment with different concentrations of H_2O_2 at 45°C and pH 11.5 for 12 h from the alkali-treated rice straw.

[c] ND: not detectable.

Table 4
The content (%hemicellulosic sample, w/w) of phenolic acids and aldehydes from nitrobenzene oxidation of the associated lignin in various isolated hemicellulosic preparations

Phenolic acids and aldehydes	ASH[a]	H_2O_2 concentraion (%)						
		0[b]	0.5[b]	1.0[b]	2.0[b]	3.0[b]	4.0[b]	5.0[b]
p-Hydroxybenzoic acid	0.04	0.05	0.051	0.041	0.036	0.032	0.029	0.028
p-Hydroxybenzaldehyde	0.14	0.16	0.18	0.14	0.11	0.098	0.090	0.084
Vanillic acid	0.023	0.034	0.035	0.033	0.032	0.032	0.031	0.031
Syringic acid	0.067	0.087	0.14	0.14	0.12	0.12	0.10	0.088
Vanillin	0.33	0.28	0.40	0.34	0.30	0.28	0.25	0.24
Syringaldehyde	0.33	0.37	0.49	0.40	0.35	0.33	0.31	0.27
Acetovanillone	0.021	0.022	0.030	0.029	0.029	0.030	0.018	0.012
p-Coumaric acid	0.017	0.021	0.026	0.020	0.016	0.010	0.088	0.0072
Acetosyringone	0.15	0.21	0.27	0.23	0.18	0.16	0.18	0.20
Ferulic acid	0.022	0.039	0.040	0.030	0.026	0.024	0.026	0.032
Total	1.14	1.27	1.66	1.40	1.20	1.12	1.12	0.99
Lignin content (%)	4.56	5.08	6.64	5.60	4.80	4.48	4.48	3.96

[a] Abbreviation for the alkali-soluble hemicellulosic preparation obtained by treatment with 1% NaOH at 55°C for 2 h from the dewaxed rice straw.

[b] The hemicellulosic preparations obtained by treatment with different concentrations of H_2O_2 at 45°C and pH 11.5 for 12 h from the alkali-treated rice straw.

1996), that is, the hemicelluloses, obtained by alkaline peroxide treatment, seemed more linear than those extracted by alkali in the absence of H_2O_2. However, this observation needs to be confirmed by methylation analysis of the hemicelluloses solubilized. In addition, as can be seen in Table 2, all the hemicellulosic fractions contained small proportions of uronic acids, mainly glucuronic acid or MeGlcA, ranging between 1.8 and 5.6%, corresponding to the hemicellulosic preparations extracted with alkali from wood or straw samples.

Table 3 shows the sugar composition in the alkali and the following alkaline peroxide treated residues. Clearly, glucose was the predominant sugar component (77.9–86.4%) and increased with raised H_2O_2 concentration. This increasing content of glucose corresponded to the higher in cellulose content and the lower in residual hemicelluloses in the residues, and the yield of hemicelluloses solubilized during the various treatment processes. The residual fraction, extracted with 5.0% H_2O_2 at 45°C for 12 h from the alkali-treated straw, still contained a noticeable amount of xylose (10.1%), together with small amounts of arabinose (2.7%), minimal quantities of galactose (0.8%) and rhamnose (0.1%). The resistance to extraction with a relatively high concentration of H_2O_2 at pH 11.5 for 12 h at 45°C suggested that the hemicelluloses in rice straw cell walls are very strongly associated to the surface of cellulose.

3.3. Content of associated lignin in the isolated hemicellulosic preparations and its phenolic composition

Results concerning the characterization of lignin bound to hemicelluloses in the cell walls of rice straw are summarized in Table 4. The data showed that all the hemicellulosic preparations contained relatively low amounts of associated lignins, ranging between 4.0 and 6.6%. This low content of bound lignin in hemicelluloses indicated that the α-benzyl ether linkages between lignin and hemicelluloses were significantly cleaved during the treatment by both alkali and alkaline peroxide under the conditions given. This cleavage is particularly true at the relatively high concentration of H_2O_2, since an increase in concentration of H_2O_2 from 0.5 to 5.0% led to a decrease in lignin content from 6.6% to less than 4.0%. The major products, obtained from alkaline nitrobenzene oxidation, were identified to be syringaldehyde and vanillin, which together represented 50.0–57.9% of the total phenolic monomers. This suggested that the associated lignin in the hemicelluloses contained almost equal amounts of non-condensed syringyl and guaiacyl units. A noticeable amount of *p*-hydroxybenzaldehyde, and traces of acetosyringone, syringic acid, vanillic acid, *p*-hydroxybenzoic acid, acetovanillone, *p*-coumaric acid, and ferulic acid were also found to be present in the nitrobenzene oxidation mixtures.

Hydroxycinnamic acids such as *p*-coumaric and ferulic acids occur widely in the cell wall of monocotyledonous plants, and studies (He & Terashima, 1991; Sharma, Brillouet, Scalbert & Monties, 1986) have been on the composition of these hydroxycinnamic acids and their association with lignin and polysaccharides. It has been suggested that *p*-coumaric acid was mostly esterified to lignin or polysaccharides, while ferulic acid appeared almost equally in esterified bonds to arabinose in hemicelluloses and in etherified linkages with lignin (Kato, Azuma & Koshijima, 1987; Sun & Tomkinson, 1999). Further studies indicated that these phenolic acids occur in every kind of cell wall and increase in amount with the progress of lignification, further the deposition of ferulic acid is predominant over *p*-coumaric acid in the early stage of cell wall formation (He & Terashima, 1989). As can be seen from the Table 4, the occurrence of traces of esterified or etherified ferulic and *p*-coumaric acids in the hemicellulosic fractions, obtained by both alkali and alkaline peroxide treatment, indicated

Table 5
The content (%residual sample, w/w) of phenolic acids and aldehydes from nitrobenzene oxidation of the lignin in various isolated residual preparations

Phenolic acids and aldehydes	ATR[a]	H_2O_2 concentraion (%)						
		0[b]	0.5[b]	1.0[b]	2.0[b]	3.0[b]	4.0[b]	5.0[b]
p-Hydroxybenzoic acid	0.025	0.039	0.039	0.039	0.059	0.060	0.010	0.010
p-Hydroxybenzaldehyde	0.26	0.21	0.093	0.067	0.049	0.049	0.049	0.060
Vanillic acid	0.042	0.041	0.016	0.011	0.009	0.007	0.007	0.004
Syringic acid	0.096	0.095	0.047	0.033	0.022	0.021	0.018	0.013
Vanillin	0.57	0.50	0.23	0.16	0.093	0.071	0.062	0.060
Syringaldehyde	0.54	0.51	0.26	0.19	0.12	0.12	0.11	0.11
Acetovanillone	0.018	0.019	0.006	0.006	0.004	0.003	ND[c]	ND
p-Coumaric acid	0.024	0.026	0.009	0.005	0.002	0.002	ND	ND
Acetosyringone	0.013	0.011	0.14	0.11	0.11	0.12	0.13	0.098
Ferulic acid	0.027	0.027	0.026	0.024	0.021	0.022	0.015	0.015
Total	1.73	1.58	0.87	0.65	0.49	0.48	0.40	0.37
Lignin content (%)	8.48	7.74	4.26	3.19	2.40	2.35	1.96	1.81

[a] Abbreviation for the alkali-treated residue obtained by treatment with 1% NaOH at 55°C for 2 h from the dewaxed rice straw.

[b] The residual preparations obtained by treatment of the alkali-treated rice straw with different concentrations of H_2O_2 at 45°C and pH 11.5 for 12 h.

[c] ND: not detected.

that these two phenolic acids are strongly associated with hemicelluloses or lignin in the cell walls of rice straw. This observation suggested that treatment of rice straw with alkali or further extraction of the alkali-treated straw with alkaline peroxide can only result in a partial cleavage of these esterified or etherified linkages, since a large proportion of the ferulic acid was quantitatively oxidized to vanillin, and most of the *p*-coumaric acids were quantitatively oxidized to *p*-hydroxybenzaldehyde by nitrobenzene under the reaction conditions given (170°C, 3 h). However, the alkaline peroxide treatment was particularly significant in the cleavage of the direct ether bonds between hemicelluloses and lignin, as evidenced by the release of 63.8% of residual hemicelluloses and 86.3% of the residual lignin present in the alkali-treated rice straw during the treatment with 2% H_2O_2 at 45°C for 12 h at pH 11.5.

The pigmentation associated with the hemicellulosic preparations is probably due to the presence of strongly associated lignin having various coloured carbonyl groups. During the treatment with alkaline peroxide, the hydroperoxide anion, formed in the alkaline media, is the principle active species in hydrogen peroxide bleaching systems. This anion is a strong nucleophile that, during bleaching, preferentially attacks ethylenic and carbonyl groups present in lignin. Consequently, chromophores such as quinones, cinnamaldehyde, and ring-conjugated ketones are converted to nonchloromophoric species (Dence, 1996; Pan et al., 1999). In this study, the hemicelluloses, prepared by alkaline peroxide extraction, were much lighter in colour than those obtained by 1% NaOH extraction or under similar conditions but in the absence of peroxide, indicating a significant bleaching efficiency of the alkaline peroxide on the associated lignins in solubilized hemicelluloses. It should be noted that some of the hemicellulosic preparations, obtained by alkaline treatment, still appeared brown. This is probably that at the elevated pH, chromophores are generated by alkali-catalyzed modification of reducing end groups on the hemicelluloses. Similar phenomenon was observed by Doner and Hicks (1997) in the isolation of hemicelluloses from corn fibre by alkaline hydrogen peroxide extraction at high pH, especially at 60°C.

All the residues were almost white. The content of residual lignin and yield of phenolic acids and aldehydes obtained from alkaline nitrobenzene oxidation of residual

Table 6
Weight-average ($\bar{M}_w$) and number-average ($\bar{M}_n$) molecular weights and polydispersity ($\bar{M}_w/\bar{M}_n$) of alkali and hydrogen peroxide-soluble hemicellulosic preparations isolated from rice straw

	ASH[a]	Hemicellulosic preparation[b]						
		0	0.5	1.0	2.0	3.0	4.0	5.0
M_w	36,000	31,280	46,950	52,950	41,280	38,000	36,450	34,740
M_n	17,060	13,030	11,010	10,500	10,640	10,040	14,240	15,830
(M_w/M_n)	2.11	2.40	4.26	5.04	3.88	3.78	2.56	2.19

[a] Abbreviation for the alkali-soluble hemicellulosic preparation obtained by extraction with 1% NaOH at 55°C for 2 h from the dewaxed rice straw.

[b] The hemicellulosic preparations obtained by extraction with different concentrations of H_2O_2 at 45°C and pH 11.5 for 12 h from the alkali-treated rice straw.

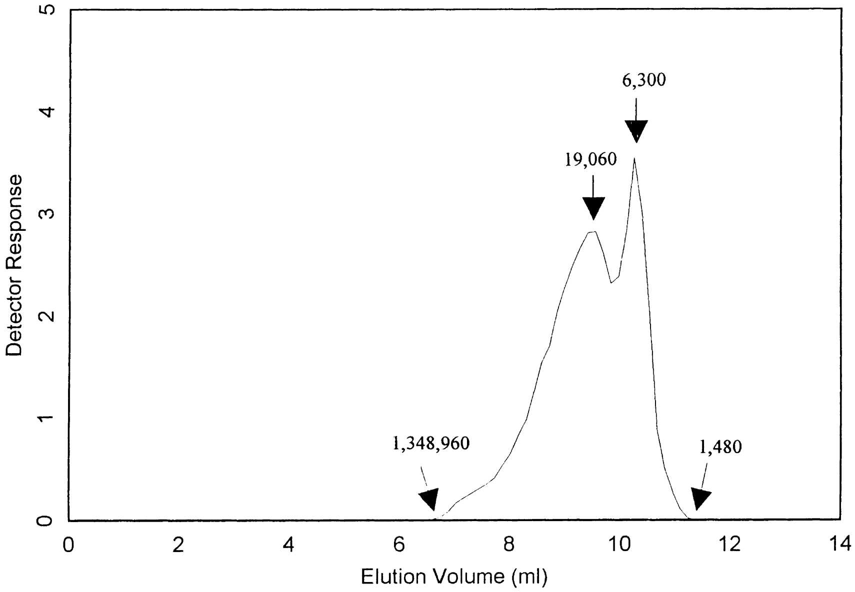

Fig. 2. GPC molecular weight distribution of hemicelluloses extracted with 2.0% hydrogen peroxide at 45°C and pH 11.5 for 12 h from the alkali-treated rice straw.

lignin in the corresponding residues are summarized in Table 5. As discussed earlier, due to the significant delignifying effect of H_2O_2, the content of lignin in the residues, obtained by alkaline peroxide treatment, was much lower than that in the residues, treated by 1% aqueous NaOH and followed by dilute alkali solution under similar conditions but in the absence of H_2O_2. Syringaldehyde, vanillin, and *p*-hydroxybenaldehyde were found to be the remaining major constituents of phenolics in the nitrobenzene oxidation of bound lignin from the residues. In these cases, the results presented here also showed that the increase of alkaline peroxide concentration favoured the reduction of bound lignin in the residues, as indicated by a decrease in lignin content from 4.3 to 1.8% with an increase in H_2O_2 concentration from 0.5 to 5.0%.

3.4. Molecular weight distribution

The weight-average ($\bar{M}_w$) and number-average ($\bar{M}_n$) molecular weights and the polydispersity ($\bar{M}_w/\bar{M}_n$) of the hemicellulosic preparations are presented in Table 6. The hemicellulosic fractions, solubilized during the second stage of alkaline peroxide treatment, showed a relatively higher degree of polymerization with $\bar{M}_w$ between 34,740 and 52,950 g mol^{-1} than those of the two alkali-soluble hemicellulosic preparations ($\bar{M}_w = 31,280–36,000$ g mol^{-1}), extracted by 1% aqueous NaOH and followed by dilute alkali solution under similar conditions but in the absence of H_2O_2, respectively. This suggested that the extractions with alkali in the absence of H_2O_2 favoured solubilization of the small molecular size of hemicelluloses, and the alkaline peroxide treatment under the conditions used did not result in any significant degradation of the macromolecular structure of hemicelluloses. On the contrary, Table 6 showed that the alkaline peroxide treatment with an increase in H_2O_2 concentration from 0.5 to 1.0% under the conditions used resulted in an increase in $\bar{M}_w$ from 46,950 to 52,950 g mol^{-1}, indicating that increasing concentration of H_2O_2 between 0.5 and 1.0% at least in part, enhanced dissolution of large molecular size hemicelluloses during the alkaline peroxide treatment of the straw. In contrast, as the H_2O_2 concentration was further increased from 1.0 to 2.0, to 3.0, to 4.0, and to 5.0%, the $\bar{M}_w$ decreased from 52,950 to 41,280, to 38,000, to 36,450, and to 34,740 g mol^{-1}, respectively, implying that a noticeable degradation occurred during the treatment with over 1.0% H_2O_2. This degradation was particularly obvious at high H_2O_2 concentration of 5.0%, in which the $\bar{M}_w$ obtained was lower than the value measured at 1.0% H_2O_2 by 34%.

The elution profiles of the hemicelluloses, extracted with 2.0% H_2O_2 at 45°C for 12 h and pH 11.5 showed two major peaks having $\bar{M}_w$ of 19,060 and 6,300 g mol^{-1}, respectively (Fig. 2). The molecular weight distribution ranged between 1,348,960 and 1,480 g mol^{-1}. This elution profile of the hemicelluloses showed wide polymolecularity as shown from oligosaccharides up to polysaccharides of $\bar{M}_w$ over one million. Occurrence of a second small peak was largely due to the fragmentation of hemicelluloses obtained by degradation of the macromolecular structure of hemicelluloses during the alkaline peroxide treatment process.

3.5. FT-IR spectra

The FT-IR spectra of 1% NaOH-soluble hemicelluloses (spectrum a), extracted with 1% NaOH at 55°C for 2 h from dewaxed rice straw; dilute alkali-soluble hemicelluloses (spectrum b), extracted with dilute alkali solution (pH 11.5) under similar conditions but in the absence of peroxide

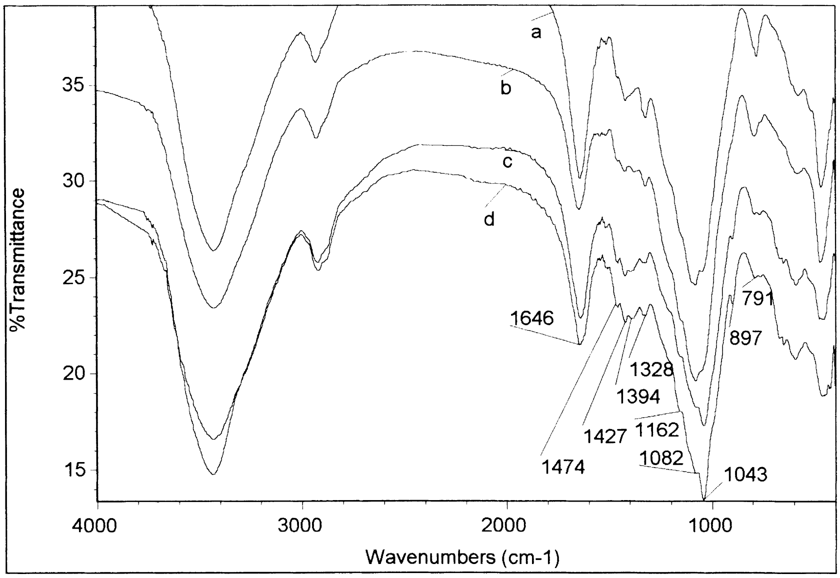

Fig. 3. FT-IR spectra of 1% NaOH-soluble hemicelluloses (spectrum a), extracted with 1% NaOH at 55°C for 2 h from dewaxed rice straw; dilute alkali-soluble hemicelluloses (spectrum b), extracted with dilute alkali solution (pH 11.5) under similar conditions but in the absence of peroxide from 1% NaOH-treated straw; and alkaline peroxide-soluble hemicelluloses, extracted with 2.0% H_2O_2 (spectrum c) and 5.0% H_2O_2 (spectrum d) at 45°C for 12 h at pH 11.5, respectively from the 1% NaOH treated straw.

from the 1% NaOH-treated straw; and alkaline peroxide soluble hemicelluloses, extracted with 2.0% H_2O_2 (spectrum c) and 5.0% H_2O_2 (spectrum d) at 45°C for 12 h at pH 11.5, respectively, from the 1% NaOH treated straw are illustrated in Fig. 3. As can be seen, the first two spectral profiles and relative intensities of the bands were rather similar, indicating similar structures between the two alkali-soluble hemicelluloses. Similar features appeared in the spectra c and d, indicating analogous structures between the alkaline peroxide soluble hemicellulosic preparations. The absorption at 1646 cm^{-1} is principally associated with absorbed water (Kacurakova, Belton, Wilson, Hirsch & Ebringerova, 1998). Bands between 1125 and 1000 cm^{-1} are typical of xylans. The prominent band at 1043 cm^{-1} is attributed to the C–O, C–C stretching or C–OH bending in hemicelluloses (Kacurakova, Ebringerova, Hirsch & Hromadkova, 1994). The band at 1082 cm^{-1} corresponds to the C–OH bending, which is strongly influenced by degree of branching. The sharp band at 897 cm^{-1}, which corresponds to the C_1 group frequency or ring frequency,

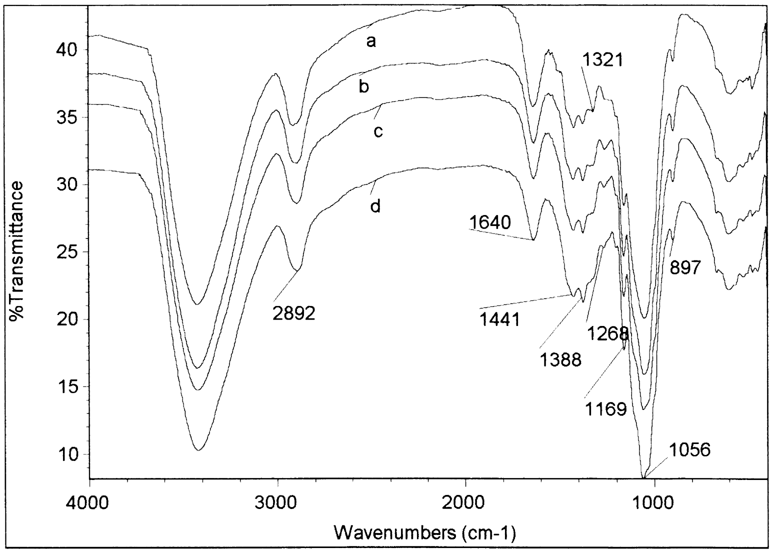

Fig. 4. FT-IR spectra of the alkali-treated straw residue (spectrum a), treated with 1% NaOH at 55°C for 2 h, and the alkaline peroxide treated residues, obtained by treatment of the alkali-treated rice straw with the 1.0% H_2O_2 (spectrum b), 3.0% H_2O_2 (spectrum b), 3.0% H_2O_2 (spectrum c), and 5.0% H_2O_2 (spectrum d) at 45°C and pH 11.5 for 12 h.

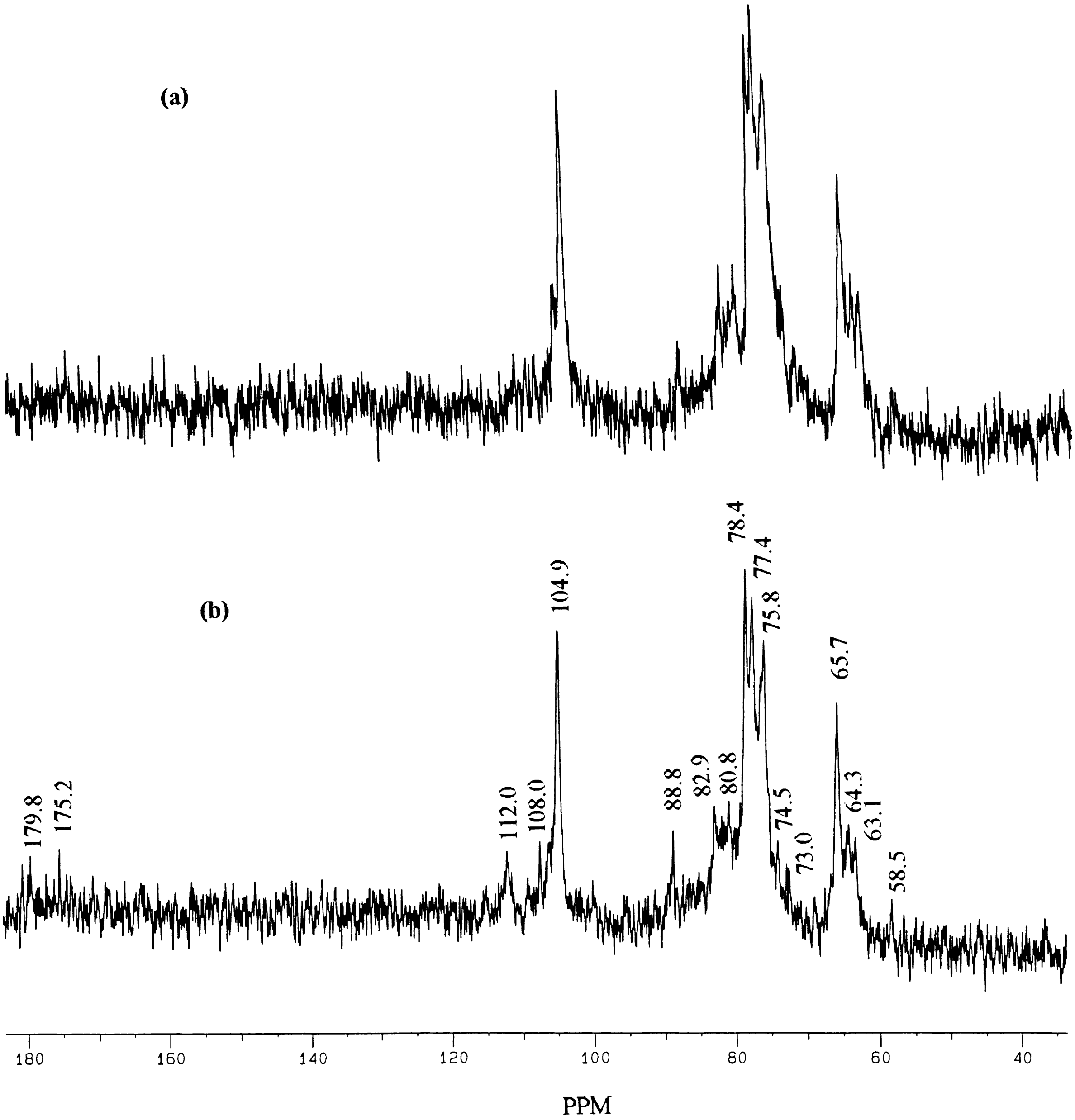

Fig. 5. ^{13}C NMR spectra (in D_2O) of the hemicellulosic preparations extracted with 2.0% H_2O_2 (spectrum a) and 3.0% H_2O_2 (spectrum b) at 45°C and pH 11.5 for 12 h from the alkali-treated rice straw.

is characteristic of β-glycosidic linkages between the sugar units (Gupta, Madan & Bansal, 1987). The absence of this band in the two alkali-soluble hemicellulosic preparations (spectra a and b) implied that the alkali-soluble hemicelluloses are mainly composed of α-glucan. This observation, however, needs further examination by methylation analysis. The bands at 1474, 1427, 1394, and 1328 cm^{-1} represent C–H, OH or CH_2 bendings. The presence of the arabinosyl side-chains is identified by the low intensity shoulder at 1162 cm^{-1}, corresponding to the C–O–C vibration in hemicelluloses. The typical values of xylan in the spectra of c and d indicated that alkaline peroxide treatment under the conditions used did not result in any significant change in the macromolecular structure of hemicelluloses. The occurrence of a very small band at 1512 cm^{-1} in all the spectra is presumed due to the presence of small amounts of associated lignin in the hemicelluloses, which corresponded to the results obtained by alkaline nitrobenzene oxidation.

Fig. 4 shows the FT-IR spectra of the alkali-treated straw residue (spectrum (a) treated with 1% NaOH at 55°C for 2 h, and the alkaline peroxide treated residues obtained by treatment of the alkali-treated rice straw with the 1.0% H_2O_2 (spectrum b), 3.0% H_2O_2 (spectrum c), and 5.0% H_2O_2 (spectrum d) at 45°C and pH 11.5 for 12 h. The most obvious feature is the similarity of these spectra. The absorbances at 1441, 1388, 1321, 1169, 1056, 1049, and

897 cm^{-1} in the four spectra are associated with the typical values of cellulose. All the spectra have an intense absorbed water-related absorbance at 1640 cm^{-1}. The lignin-related absorbance at 1512 cm^{-1} is rather weak in the spectrum a and poorly resolved in the other three spectra, reflecting the low level of associated lignin in the residues.

3.6. ^{13}C NMR spectra

The ^{13}C NMR spectra of the two hemicellulosic preparations, obtained by treatment of the alkali-extracted rice straw with 2.0% H_2O_2 (a) and 3.0% H_2O_2 (b) at 45°C for 12 h at pH 11.5 are shown in Fig. 5. The spectra were interpreted on the basis of reported data for structurally defined arabinoxylan-type, glucoronoxylan-type and L-arabino-(4-*O*-methyl-D-glucurono)-D-xylan, as well as those of wheat straw hemicelluloses extracted before delignification (Ebringerova, Hromadkova, Aldoldi & Berth, 1992; Imamura, Watanabe, Kuwahara & Koshijima, 1994; Kato et al., 1987; Sun et al., 1996). The spectra were complicated by the relatively large molecular weights of the hemicelluloses and by the high pH of sample. Three drops of 40% sodium deuteroxide (which was necessary to dissolve the polymer samples) caused line broadening and baseline problems. As can be seen from this figure, the two spectra were qualitatively very similar, indicating a similar structure of the hemicelluloses. The main 1,4-linked β-D-Xylp units are obviously characterized by the signals at 104.9, 78.4, 77.4, 75.8, and 65.7 ppm, which attributes to C-1, C-4, C-3, C-2, and C-5 of the β-D-Xylp units, respectively. The signals at 112.0, 88.9, 82.9, 80.8, and 64.3 ppm correspond to C-1, C-4, C-2, C-3, and C-5 of α-L-Araf residues, respectively. Two signals at 175.2 and 58.5 ppm originate from C-6 and 4-*O*-methoxyl group of glucuronic acid residue in the xylan which are very weak and correspond to the low uronic acid content. The signal at 108.0 and 63.1 ppm relate to C-1 and C-6 of the glucose residue in the xylan, respectively. The presence of minimal quantities of associated lignin was identified by a very weak signal at 179.8 ppm, which originates from the carbonyl group in associated lignin. These typical signals for L-arabino-(4-*O*-methyl-D-glucurono)-D-xylan revealed that the alkaline peroxide treatment under the conditions used did not affect the overall structure of the macromolecular hemicelluloses.

In conclusion, this study has shown that extraction of dewaxed rice straw with 1.0% NaOH at 55°C for 2 h and the following treatment of the alkali-extracted straw with dilute alkali solution under similar conditions but in the absence of peroxide, solubilized 55.0% of the original hemicelluloses and 27.0% of the residual hemicelluloses, respectively. While the treatment of the alkali-extracted straw with 0.5–5.0% H_2O_2 released 49.3–74.3% of the residual hemicelluloses originally present in the alkali-extracted straw. Alkaline peroxide is capable of significant release of both hemicelluloses and lignin from rice straw under the conditions used, but the treatment did not affect the overall structure of macromolecular hemicelluloses although a noticeable degradation of both hemicelluloses and cellulose occurred during the treatment with over 1.0% H_2O_2. The initial study showed that a major difference between the alkali-soluble and alkaline peroxide soluble hemicellulosic preparations is that the hemicelluloses in the former are mainly composed of α-glucan, whereas the hemicelluloses in the latter comprise L-arabino-(4-*O*-methyl-D-glucurono)-D-xylan as the main constituent. However, all the hemicellulosic preparations were contaminated with small amounts of associated lignin (4.0–6.6%).

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