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Fractional and structural characterization of hemicelluloses isolated by alkali and alkaline peroxide from barley straw

R.C. Sun[a,*], X.F. Sun[b]

[a]*State Key Laboratory of Pulp and Paper Engineering, College of Paper and Environment Engineering, South China University of Technology, Guangzhou, People's Republic of China*

[b]*Centre for Straw and Wood Utilization, The North-Western Sciences and Technology University of Agriculture and Forestry, Yangling, People's Republic of China*

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Abstract

Eight hemicellulosic fractions were obtained by sequential treatment of dewaxed barley straw with 0.1 M NaOH at 45 °C for 3 h, 0.25, 0.5, 1.0, 1.5, 2.0, and 3.0% H_2O_2 at 45 °C for 3 h at pH 11.5, and 10% KOH–1% $Na_2B_4O_7{\cdot}10H_2O$ at 28 °C for 15 h under continuous agitation. The yields of the fractions were 8.0, 3.1, 3.3, 3.3, 2.2, 2.0, 2.0, and 9.9%, respectively, of the initial amount of barley straw, corresponding to the dissolution of 21.6, 8.4, 8.9, 8.9, 5.9, 5.4, 5.4, and 26.7% of the original hemicelluloses. Meanwhile, the successive treatment also solubilized 29.1, 15.8, 14.6, 10.8, 4.5, 3.2, 2.7, and 3.7% of the original lignin, respectively. This sequential extraction together resulted in dissolution of 91.1% of the original hemicelluloses and 84.8% of the original lignin. The 0.1 M NaOH-soluble hemicellulosic fraction contained mainly xylose, glucose, and arabinose, 44.2, 15.7, and 15.2%, respectively, while the 10% KOH–1% $Na_2B_4O_7{\cdot}10H_2O$-soluble fraction predominated in xylose, 75.0%. The six alkaline peroxide-soluble fractions were composed of 50.3–54.4% xylose, 14.7–16.9% arabinose, 6.8–10.7% glucose, 6.8–8.5% glucuronic acid or 4-*O*-methyl-D-glucuronic acid, 0.4–1.5% mannose, and 0.3–1.2% rhamnose. All the hemicellulosic fractions contained substantial amounts of glucuronoarabinoxylans and noticeable quantities of β-glucans. In comparison, the six hemicellulosic fractions, isolated with alkaline peroxide, had much higher molecular weights (56,890–63,810 g mol^{-1}) than those of the two hemicellulosic preparations (28,000–29,080 g mol^{-1}), isolated with alkali in the absence of hydrogen peroxide. The thermal stability of the hemicelluloses increased with an increment of their molar mass.

Keywords: Barley straw; Hemicelluloses; Alkali; Alkaline peroxide; Sugars; Lignin

1. Introduction

Hemicelluloses, the second most abundant natural polysaccharides after cellulose, comprise roughly one-fourth to one-third of most plant materials, and this amount will vary according to the particular plant species, such as wheat straw 32–38%, barley straw 33–36% (Sun, Lawther, & Banks, 1995). The hemicelluloses are usually defined as that polysaccharide part of plant tissue, which is accessible to the action of dilute acids and alkalis. They are chemically complex and comprise a mixture of heteropolymers, including arabinans, galactans, mannans, and xylans. Since the latter are most abundant of the hemicelluloses, the bulk of relevant research to date has been concentrated on xylans (Cai & Paszner, 1988). Xylans from straw and grass have the same backbone as the hardwood xylans, which consist of about 200 β-xylopyranose residues, linked together by 1,4-glycosidic bonds. However, they contain smaller proportions of uronic acids, but are more highly branched and contain large proportion of L-arabinofuranosyl units. The former, consisting of 4-*O*-methylglucuronic acid, attaches directly to the C-2 position of xylose, while the latter are linked mainly to the C-3 position of xylose (Puls & Schuseil, 1993).

Nevertheless, when compared with cellulose and starch, hemicelluloses have been somewhat neglected topics, even though their alkaline isolation and structural characterization have been investigated as early as in 1950s (Aspinall, 1959). However, since the 1980s depletion of the world's forests has steadily forced up the price of wood and wood-based materials. At the same time, the burning of straw and other agricultural by-products is also a serious environmental hazard. In some countries, straw-burning has already

* Corresponding author. Present address: The BioComposites Centre, University of Wales, Bangor Gwynedd LL57 2UW, UK. Tel.: +44-1248-370588; fax: +44-1248-370594.

E-mail address: bcs00a@bangor.ac.uk (R.C. Sun).

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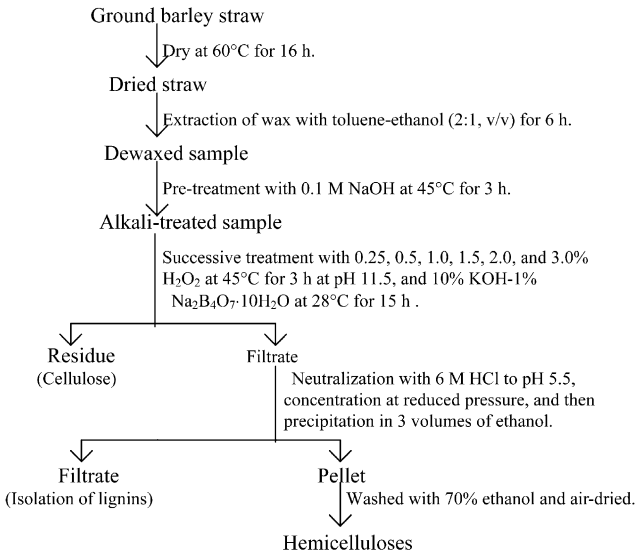

Fig. 1. Scheme for isolation of hemicellulosic fractions from the hydrolysates of alkaline peroxide extraction of the 0.1 M NaOH-treated barley straw.

been banned. During the past 10 years, therefore, the situation has changed markedly and such studies have received fresh impetus. In particular, suitable extraction procedures for a potential commercial production of polymeric hemicelluloses have been developed (Glasser, Kaar, Jain, & Sealey, 2000; Kosikova & Ebringerova, 1991). However, in general, the hemicelluloses, isolated directly from the biomass contained noticeable amounts of bound lignins, which limits their industrial utilizations. In recent years, we have carried out the isolation and structural characterization of hemicelluloses, extracted from cereal straws and other agricultural residues (Lawther, Sun, & Banks, 1995; Sun, Lawther, & Banks, 1996; Sun, Fang, Rolands, & Bolton, 1998). A simple and environmental friendly procedure for a large-scale production of hemicelluloses from cereal straws has been proposed in our laboratory and it has been successfully used in the pilot plant. We found that alkaline peroxide, which is widely used in the pulp and paper industry to bleach lignin-rich pulps, is an effective agent for both delignification and solubilization of hemicelluloses from straw and grass. It is generally accepted that the hydroperoxide anion (HOO^-), formed in alkaline media, is the principal active species in hydrogen peroxide bleaching systems. In contrast, 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 (Dence, 1996; Pan, Bolton, & Leary, 1998).

In this comparative study, we investigated the hemicellulosic substances, isolated sequentially with alkali and alkaline peroxide with an increase of concentration from barley straw, and characterized their structural and physico-chemical properties. The emphasis is on the lignin components associated with the hemicellulosic fractions.

2. Experimental

2.1. Materials

Barley straw was obtained from the experimental farm of the North-Western Science and Technology University of Agriculture and Forestry (Yangling, P.R. China). It was dried in sunlight and ground to pass a 0.8 mm size screen. The composition (w/w) of the straw used was cellulose 37.5%, hemicelluloses 37.1%, lignin 15.8%, protein 2.6%, extractives 2.6%, and ash 4.2%, which contain 68.6% silica. All weights and calculations were made on an oven-dried (60 °C, 16 h) basis.

2.2. Alkali and alkaline peroxide extraction

The procedure used to isolate alkali- and alkaline peroxide-extractable polysaccharides from barley straw was based partially on a method for isolation of alkali-extractable cell wall materials from wheat straw, as described by Lawther et al. (1995). The dried powder of barley straw was first extracted with toluene–ethanol (2:1, v/v) in a Soxhlet for 6 h. The dewaxed straw (50 g) was sequentially extracted with 0.1 M NaOH at 45 °C for 3 h, 0.25, 0.5, 1.0, 1.5, 2.0, and 3.0% H_2O_2 for 3 h at 45 °C at pH 11.5, and 10% KOH–1% $Na_2B_4O_7{\cdot}10H_2O$ for 15 h at 28 °C under continuous agitation. The extractant to straw ratio was fixed to 20 ml g^{-1} in all the extracting steps. After filtration on a nylon cloth, the residues were washed with water and ethanol, then dried at 60 °C for 16 h. The hemicelluloses solubilized were isolated from each of the hydrolysates by precipitation of the neutralized hydrolysates (pH 5.5 adjusted with 6 M HCl) with three volumes of 95% ethanol. After filtration, the hemicellulosic pellets were washed with 70% aqueous ethanol and air-dried. The scheme for fractional extraction of hemicelluloses from barley straw is shown in Fig. 1.

2.3. Physico-chemical and thermal analysis

The neutral sugar composition of the isolated hemicelluloses was determined by gas chromatography (GC) analysis of their alditol acetates (Blakeney, Harris, Henry, & Stone, 1983). The hemicelluloses were hydrolyzed with 2 M trifluoroacetic acid at 120 °C for 2 h and the resulting monosaccharides were reduced and acetylated. The content of uronic acid was determined colorometrically by the method of Blumenkrantz and Asboe-Hanson (1973)). Alkaline nitrobenzene oxidation of associated lignin from the solubilized hemicellulosic preparations was performed at 170 °C for 2.5 h. The lignin content in hemicellulosic preparations was calculated multiplying by 1.9, the yield of phenolics obtained by nitrobenzene oxidation.

Table 1
The yield of hemicelloluses and lignin (% dry matter) solubilized during the successive treatments of barley straw with 0.1 M NaOH, various concentrations of alkaline hydrogen peroxide, and 10% KOH–1% $Na_2B_4O_7{\cdot}10H_2O$ at 28 °C for 15 h

	AS1[a]	H_2O_2 concentration (%)[b]						AS2[c]	Total
		0.25	0.5	1.0	1.5	2.0	3.0		
Hemicelluloses[d]	8.0	3.1	3.3	3.3	2.2	2.0	2.0	9.9	33.8
Lignin	4.6	2.5	2.3	1.7	0.73	0.51	0.42	0.59	13.4
Residue	77.5	72.0	66.9	61.9	58.8	56.0	55.0	42.7	

[a] Abbreviation for the alkali-soluble hemicelluloses and lignin obtained by extraction with 0.1 M NaOH at 45 °C for 3 h from the dewaxed barley straw.
[b] The preparations obtained by successive extractions of the 0.1 M NaOH treated straw with different concentrations of H_2O_2 at 45 °C for 3 h at pH 11.5.
[c] The fraction obtained by extraction with 10% KOH–1% $Na_2B_4O_7{\cdot}10H_2O$ at 28 °C for 15 h from the 3.0% H_2O_2 treated straw.
[d] The hemicellulosic fractions obtained by precipitation of the neutralized extracts with three volumes of ethanol.

The average molecular weights of the hemicellulosic preparations were determined by gel permeation chromatography on a PL Aquagel-OH 50 column (300×7.7 mm^2, Polymer Laboratories), calibrated with PL pullulan polysaccharide standards. The eluent was 0.02 M NaCl in 0.005 M sodium phosphate buffer, pH 7.5 with a flow rate of 0.1 ml min^{-1}. Detection was achieved using a Knauer differential refractometer. The column oven was maintained at 40 °C. The samples were dissolved in 0.005 M sodium phosphate buffer with 0.02 M NaCl to a concentration of 0.1%.

The FT-IR spectra were recorded from KBr pellets containing 1% finely ground samples on a Nicolet-510 FT-IR spectrophotometer. The solution-state ^{13}C-NMR spectrum was obtained on a Bruker MSI-300 spectrometer operating in the FT mode at 74.5 MHz under total proton decoupled conditions. It was recorded at 25 °C from 150 mg of sample dissolved in 1.0 ml D_2O after 16,000 scans. A 60° pulse flipping angle, a 3.9 μs pulse width and 0.85 s acquisition time were used.

Thermal analysis of the hemicellulosic preparations was performed using thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) on a simultaneous thermal analyzer (NETZSCH STA-409). The apparatus was continually flushed with nitrogen. The sample weighed between 8 and 12 mg. Each sample was heated from room temperature to 600 °C at a rate of 10 °C min^{-1}.

3. Results and discussion

3.1. Fractional yield of hemicelluloses

Eight distinct fractions of hemicelluloses were obtained by sequential extraction of barley straw with 0.1 M NaOH at 45 °C for 3 h, 0.25, 0.5, 1.0, 1.5, 2.0, and 3.0% H_2O_2 at 45 °C for 3 h at pH 11.5, and 10% KOH–1% $Na_2B_4O_7{\cdot}10H_2O$ at 28 °C for 15 h. The yields of the fractions were 8.0, 3.1, 3.3, 3.3, 2.2, 2.0, 2.0, and 9.9%, respectively, of the initial amount of barley straw, corresponding to dissolution of 21.6, 8.4, 8.9, 8.9, 5.9, 5.4, 5.4, and 26.7% of the original hemicelluloses (Table 1). Meanwhile, as might be expected, the successive treatments also solubilized 29.1, 15.8, 14.6, 10.8, 4.5, 3.2, 2.7, and 3.7% of the original lignin, respectively. This result indicated that the sequential extractions together resulted in dissolution of 91.1% of the original hemicelluloses and 84.8% of the original lignin. It should be noted that the first treatment of the dewaxed barley straw with 0.1 M NaOH at 45 °C for 3 h also removed 81.0% of the original protein and 78.9% of the original ash except for

Table 2
The content of neutral sugars and uronic acids (% dry weight, w/w) in isolated hemicellulosic fractions and cellulose

	AS1[a]	H_2O_2 concentration (%)[b]						AS2[c]	Cellulose
Sugars (%)		0.25	0.5	1.0	1.5	2.0	3.0		
Rhamnose	0.3	0.3	0.8	1.2	1.5	0.7	0.6	Tr[d]	ND
Arabinose	15.2	16.2	16.0	16.6	16.9	16.0	14.7	7.8	0.9
Xylose	44.2	52.6	54.4	51.9	50.3	50.7	53.9	75.0	6.8
Mannose	3.6	1.5	0.8	0.8	0.8	0.5	0.4	ND[e]	ND
Glucose	15.7	9.3	7.1	6.8	7.8	10.2	10.7	4.9	86.0
Galactose	8.0	6.0	4.9	4.6	4.6	4.4	3.5	1.1	ND
Uronic acids	6.3	6.8	7.1	7.5	7.7	8.1	8.5	5.6	Tr

[a] Abbreviation for the alkali-soluble hemicelluloses and lignin obtained by extraction with 0.1 M NaOH at 45 °C for 3 h from the dewaxed barley straw.
[b] The preparations obtained by successive extractions of the 0.1 M NaOH treated straw with different concentrations of H_2O_2 at 45 °C for 3 h at pH 11.5.
[c] The fraction obtained by extraction with 10% KOH–1% $Na_2B_4O_7{\cdot}10H_2O$ at 28 °C for 15 h from the 3.0% H_2O_2 treated straw.
[d] Trace.
[e] Not detectable.

Table 3
The yield (% hemicellulosic sample, w/w) of phenolic acids and aldehydes from alkaline nitrobenzene oxidation of the associated lignin in isolated hemicellulosic fractions and cellulose

Phenolic acids and aldehydes	AS1[a]	H_2O_2 concentration (%)[b]						AS2[c]	Cellulose
		0.25	0.5	1.0	1.5	2.0	3.0		
p-Hydroxybenzoic acid	0.60	0.72	0.68	0.57	0.58	0.62	0.48	0.58	0.74
p-Hydroxybenzaldehyde	0.22	0.23	0.33	0.36	0.33	0.31	0.17	0.14	0.10
Vanillic acid	0.08	0.08	0.08	0.13	0.22	0.25	0.17	0.10	0.06
Syringic acid	0.26	0.29	0.41	0.53	0.51	0.56	0.52	0.25	0.14
Vanillin	0.94	0.98	1.26	1.29	1.22	1.10	0.98	0.66	0.75
Syringaldehyde	0.57	0.55	0.73	1.00	0.90	0.90	0.84	0.46	0.77
Acetovanillone	0.16	0.18	0.26	0.26	0.29	0.28	0.22	0.25	0.24
p-Coumaric acid	0.22	0.16	0.28	0.23	0.28	0.26	0.18	0.08	0.06
Acetosyringone	0.14	0.15	0.18	0.21	0.23	0.20	0.10	0.12	0.10
Ferulic acid	0.21	0.19	0.20	0.22	0.21	0.20	0.20	0.10	0.05
Total	3.38	3.53	4.41	4.80	4.77	4.68	3.86	2.74	3.01
Content of lignin	6.42	6.70	8.38	9.12	9.06	8.89	7.33	5.21	5.72

[a] Abbreviation for the alkali-soluble hemicelluloses and lignin obtained by extraction with 0.1 M NaOH at 45 °C for 3 h from the dewaxed barley straw.
[b] The preparations obtained by successive extractions of the 0.1 M NaOH treated straw with different concentrations of H_2O_2 at 45 °C for 3 h at pH 11.5.
[c] The fraction obtained by extraction with 10% KOH–1% $Na_2B_4O_7{\cdot}10H_2O$ at 28 °C for 15 h from the 3.0% H_2O_2 treated straw.

solubilization of 8.0% hemicelluloses and 4.6% lignin. It is believed that hydroxyl ions cause swelling of cellulose, disruption of hydrogen bonds between cellulose and hemicelluloses, and hydrolysis of ester bonds most likely connecting cell wall polysaccharides, resulting in the solubilization of substantial amounts of hemicelluloses and lignin (Izdorczyk, Macri, & MacGregor, 1998). In the case of alkaline media, lignin removal involves a different set of reactions (Sultanov & Wallis, 1991). These are generally initiated by the removal of a proton from a phenolic group under alkaline conditions. An electronic rearrangement follows resulting in the cleavage of an aryl–ether bond to give a small but soluble lignin fragment and a deprotonated phenolic group on the residual lignin (Stewart & Morrison, 1992).

3.2. Content of neutral sugars and uronic acids

Table 2 gives the neutral sugar composition and content of uronic acid in isolated eight hemicellulosic fractions. Evidently, the 0.1% NaOH-soluble hemicellulosic fraction mainly comprised of xylose (44.2%), glucose (15.7%), arabinose (15.2%), galactose (8.0%), and glucuronic acid or 4-*O*-methyl-D-glucuronic acid (MeGlcA) (6.3%). Minor quantities of mannose (3.6%) and trace amounts of rhamnose (0.3%) were also identified in this fraction. This monosaccharide analysis revealed that the hemicellulosic extract contained a mixture of primarily two polysaccharides, glucuronoarabinoxylans and β-glucans. A relatively higher content of xylose, arabinose, and glucuronic acid or MeGlcA, but lower quantities of glucose in six alkaline peroxide-soluble hemicellulosic fraction than in 0.1% NaOH-soluble hemicellulosic fraction indicated that the six alkaline peroxide-soluble hemicellulosic fractions contained progressively more glucuronoarabinoxylans and less β-glucans. Alkaline peroxide-soluble arabinoxylans in six fractions differed slightly in the relative proportions of xylose and arabinose residues; the Xyl/Ara ratios ranged between 2.98 and 3.67. In general, arabinoxylans obtained at the initial extraction step with low concentration of alkali were more highly substituted, as indicated by the lower Xyl/Ara ratio of 2.91 in 0.1% NaOH-soluble hemicellulosic fraction. This phenomenon provided evidence that in barley straw cell walls arabinose, probably as a side chain in hemicelluloses, is easily solubilized during the initial and dilute alkaline extraction process, whereas this side chain was partially cleaved or degraded in the strong alkaline solution such as in 10% KOH–1% $Na_2B_4O_7{\cdot}10H_2O$ extraction process for 15 h even though it was performed at room temperature, as shown a higher Xyl/Ara ratio of 9.62. These observations were in agreement with similar trends reported from maize stems and wheat straw alkali-soluble arabinoxylans fractionated with various concentrations of alkaline peroxide (Fang, Sun, Salibury, Fowler, & Tomkinson, 1999; Sun, Tomkinson, Zhu, & Wang, 2000). Similar results have been reported by Kato, Iki, and Matsuda (1981a,b, 1988) in the studies of hemicelluloses from cell walls of immature barley plant. They indicated that the major non-cellulosic polysaccharides in barley straw are arabinoxylans together with small portions of β-D-glucan. The acidic arabinoxylan had a linear backbone chain of β-1,4-D-xylose residues, about 50% of which were substituted at the 2 and/or 3 position, mainly with arabinofuranose and glucuronic acid residues. They also revealed the β-D-glucan had (1 → 3)- and (1 → 4)-linked D-glucopyranosyl residues in an approximate molar ratio of 1.0:2.3, as shown by methylation analysis and enzyme degradation studies.

Table 4
Weight-average ($\bar{M}_w$) and number-average ($\bar{M}_n$) molecular weights and polydispersity ($\bar{M}_w/\bar{M}_n$) of the hemicellulosic fractions isolated successively with 0.1 M NaOH at 45 °C for 3 h, 0.25, 0.5, 1.0, 1.5, 2.0, and 3.0% H_2O_2 at pH 11.5 for 3 h at 45 °C, and 10% KOH–1% $Na_2B_4O_7{\cdot}10H_2O$ at 28 °C for 15 h from barley straw

	AS1[a]	H_2O_2 concentration (%)[b]						AS2[c]
		0.25	0.5	1.0	1.5	2.0	3.0	
$\bar{M}_w$	28000	57910	60890	63130	63810	59080	56890	29080
$\bar{M}_n$	8070	13280	14740	12450	11160	10820	11150	7240
$\bar{M}_w/\bar{M}_n$	3.47	4.36	4.13	5.07	5.72	5.46	5.10	4.02

[a] Abbreviation for the alkali-soluble hemicelluloses and lignin obtained by extraction with 0.1 M NaOH at 45 °C for 3 h from the dewaxed barley straw.
[b] The preparations obtained by successive extractions of the 0.1 M NaOH treated straw with different concentrations of H_2O_2 at 45 °C for 3 h at pH 11.5.
[c] The fraction obtained by extraction with 10% KOH–1% $Na_2B_4O_7{\cdot}10H_2O$ at 28 °C for 15 h from the 3.0% H_2O_2 treated straw.

3.3. Content of associated lignin and its phenolic composition

It is well known that lignin is tightly linked to polysaccharides in the cell walls of plants by various linkage types, and the most commonly covalent linkage is the ether linkage of the hydroxyl group at the α-position of the lignin side chain with alcoholic hydroxyl of sugar residue (Freudenberg, 1965; Xie, Yasuda, Wu, & Liu, 2000). To verify the associated lignin in eight hemicellulosic fractions, all the hemicellulosic preparations were performed by alkaline nitrobenzene, and the lignin oxidation products, phenolic acids and aldehydes are given in Table 3. As expected, we found that a substantial cleavage of α-ether linkages between lignin and hemicelluloses occurred during the sequential extractions as shown by a low amount of bound lignins between 5.21 and 9.12% in the isolated hemicellulosic fractions. The content of bound lignin was maximized in the hemicellulosic fraction isolated with 1.0% H_2O_2 (9.12%) and minimized in the fraction AS2 (5.21%) isolated with 10% KOH–1% $Na_2B_4O_7{\cdot}10H_2O$ at 28 °C for 15 h from the 3.0% H_2O_2 treated straw. A decrease of lignin content from 9.12 to 9.06, to 8.89, and to 7.33 with an increase in alkaline peroxide concentration from 1.0 to 1.5, to 2.0 and to 3.0% indicated that a higher concentration of alkali favoured cleavage the α-ether bonds between lignin and hemicelluloses. After successive extractions with various concentrations of alkali and alkaline peroxide, the residue (crude cellulose) contained relatively low amounts of associated lignin, 5.72%. The major products obtained from the alkaline nitrobenzene oxidation, were identified to be vanillin (0.66–1.29%), *p*-hydroxybenzoic acid (0.48–0.74%), and syringaldehyde (0.46–1.00%). A small amount of syringic acid, *p*-hydroxybenaldehyde, vanillic acid, acetovanillin, acetosyringone, *p*-coumaric acid, and ferulic acid was also found to be present in the nitrobenzene oxidation products.

In the plant cell wall, *p*-coumaric and ferulic acids are widely distributed along with polylignol moiety. Their composition and content are dependent on the morphological location and the differentiation stage. Ferulic acid rapidly deposits in the cell walls at the early stage of lignification, subsequently *p*-coumaric acid residue deposits continuously throughout the lignification and become a predominant constituent of hydroxycinnamic acids (He & Terashima, 1991). In other words, ferulic acid plays an important role in lignification (possibly as nucleation sites for attachment of lignin precursors) and influences the physical and textural attributes of plants, while the esters of *p*-coumaric acid cannot serve as initiation sites for lignification because though some *p*-coumaric acid is esterified to arabinoxylan in the same manner as ferulic acid, most is esterified to the lignin fraction (Morrison, Jung, Buxton, & Hatfield, 1998). On the other hand, ferulic acid is bound through ester linkages to the arabinoxylan of gramineae or to the pectins of dicotyledons in addition to ether-linked to lignin, while *p*-coumaric acid is not involved in this cross-linkage bridge (Ralph, Grabber, & Hatfield, 1995). Our previous study (Sun, Xiao, & Sun, 2001) on barley straw hydroxycinnamic acids found that the straw contained 0.66% ferulic acid and 0.36% *p*-coumaric acid, in which the bulk of cell wall *p*-coumaric acid (66.7%) was ester-linked to cell wall components, mainly to lignin, and over 40% of cell wall ferulic acid is etherified through its phenolic oxygen to the cell wall lignin component, whereas more than 50% of ferulic acid is identified to be esterified to the cell wall hemicelluloses and/or lignin in barley straw. Similar result has been reported by Harvey, Hartley, Harris, and Curzon (1986) on the basis of the study on the linkage of *p*-coumaroyl and feruloyl groups to cell wall polysaccharides of barley straw. They showed that one in every 121 pentose residues was feruloylated and one in every 243 pentose residues was *p*-coumaroylated. One in every 31 arabinose residues was esterified with *p*-coumaric acid, and one in every 15 with ferulic acid.

3.4. Molecular mass

Molecular weights of eight hemicellulosic fractions were estimated in this study by gel permeation chromatography, and their weight-average ($\bar{M}_w$) and number-average ($\bar{M}_n$) molecular weights and polydispersity ($\bar{M}_w/\bar{M}_n$) are given in Table 4. Obviously, the two alkali-soluble hemicellulosic fractions AS1 and AS2 showed a much lower degree of

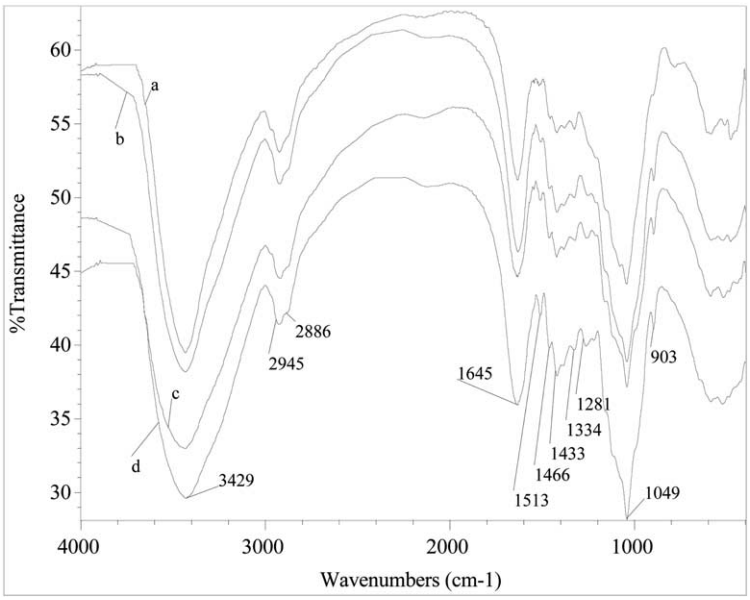

Fig. 2. FT-IR Spectra of hemicellulosic fractions extracted successively with 0.1 M NaOH at 45 °C for 3 h (spectrum a), and 0.25% (spectrum b), 0.5% (spectrum c), and 1.0% H_2O_2 (spectrum d) at pH 11.5 for 3 h at 45 °C from dewaxed barley straw.

polymerization with $\bar{M}_w$ values between 28,000 and 29,080 g mol^{-1} than those of the six alkaline peroxide-soluble hemicellulosic preparations, ranging from 56,890 to 63,810 g mol^{-1}. This implied that the first extraction with dilute alkali such as 0.1 M NaOH in the absence of H_2O_2 favoured solubilization of the small molecular size of hemicelluloses, and the following alkaline peroxide treatments under the concentrations used did not result in any significant degradation of the macromolecular structure of hemicelluloses. However, the data in Table 4 showed that the values of $\bar{M}_w$ increased from 57,910 to 63,810 g mol^{-1} as the H_2O_2 concentration rose from 0.25 to 1.5%, indicating that an increment of H_2O_2 concentration from 0.25 to 1.5% at least in part, increased dissolution of large molecular size hemicelluloses from barley straw. In contrast, as the H_2O_2 concentration was further increased from 1.5 to 2.0, and to 3.0%, the $\bar{M}_w$ decreased from 63,810 to 58,080, and to 56,890 g mol^{-1}, respectively, implying that a slight degradation occurred during the treatment with over 1.5% H_2O_2. Similarly, a final treatment with a much higher concentration of alkali (10% KOH–1% $Na_2B_4O_7{\cdot}10H_2O$) led to a substantial degradation of the polysaccharide polymers as

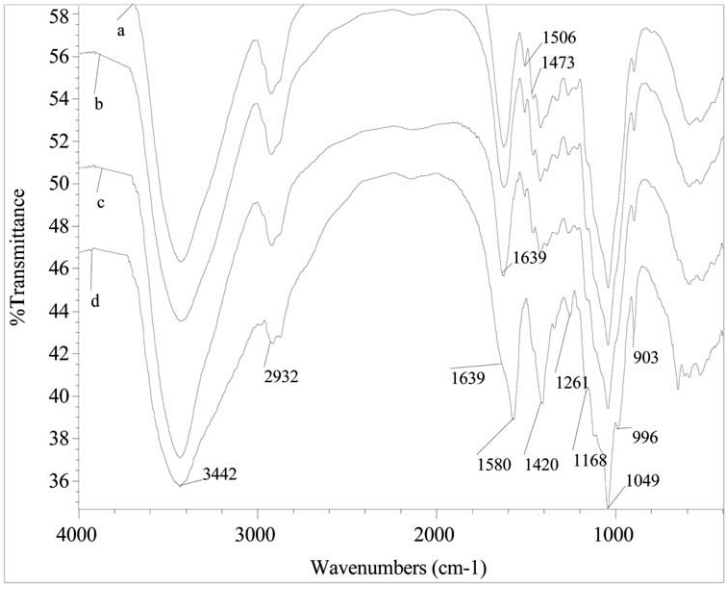

Fig. 3. FT-IR Spectra of hemicellulosic fractions extracted successively with 1.5% (spectrum a), 2.0% (spectrum b), and 3.0% H_2O_2 (spectrum c) at pH 11.5 for 3 h at 45 °C, and 10% KOH–1% $Na_2B_4O_7{\cdot}10H_2O$ at 28 °C for 15 h (spectrum d) from the 1.0% H_2O_2 treated barley straw.

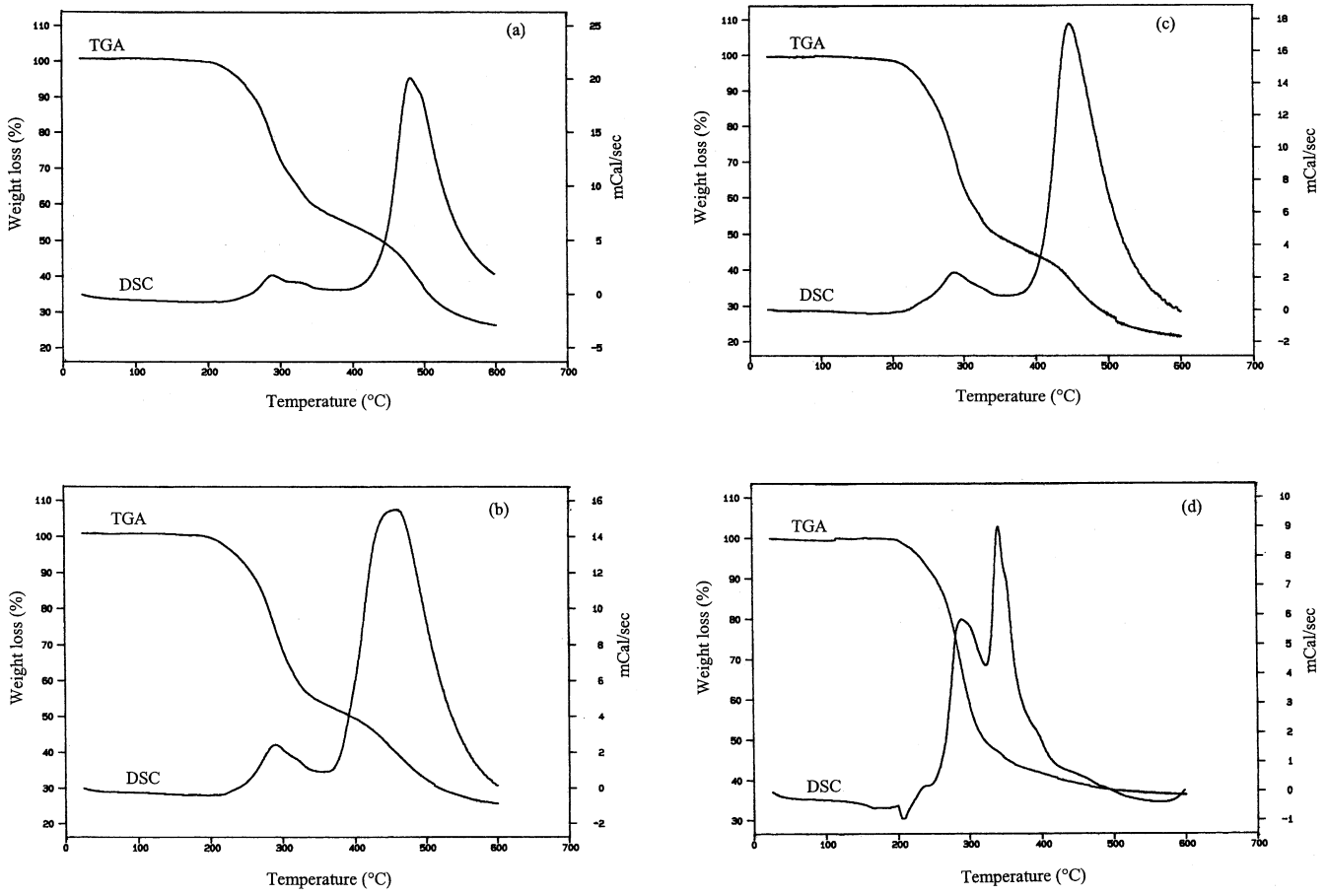

Fig. 4. Thermograms of the hemicellulosic fractions extracted with: (a) 0.5% H_2O_2, (b) 2.0% H_2O_2, (c) 3.0% H_2O_2, and (d) 10% KOH–1% $Na_2B_4O_7{\cdot}10H_2O$.

shown a much lower $\bar{M}_w$ value of 29,080 g mol^{-1}. Additionally, the analysis showed that the two alkali-soluble polymeric hemicelluloses, gave a more narrow molecular weight distribution, corresponding to polydispersity indexes of 3.47 for AS1 and 4.02 for AS2 as compared to those of the alkaline peroxide soluble hemicellulosic products having a more broad molar mass distribution with polydispersity indexes between 4.13 and 5.72. These broad elution profiles might indicate either a heterogeneous nature of the hemicelluloses or possibly some intermolecular interactions (Izdorczyk et al., 1998).

3.5. FT-IR spectra

The FT-IR spectra of the eight hemicellulosic fractions, illustrated in Figs. 2 and 3, clearly showed the typical signal pattern expected for a hemicellulosic moiety. In particular, all the spectra are dominant by signals at 3429 or 3442 and 2945 or 2932 cm^{-1} due to stretching vibrations of OH and CH and by signals in the C–O stretching region between 1200 and 950 cm^{-1} (Kacurakova, Ebringerova, Hirsch, & Hromadkova, 1994). An intensive and sharp band at 1049 cm^{-1} in all the spectra is attributed to the typical of xylans, indicating a dominant xylan of the alkali-soluble and alkaline peroxide-soluble hemicelluloses, which corresponded to the results obtained by sugar analysis. In the anomeric region (950–700 cm^{-1}), a small sharp band at 903 cm^{-1} is indicated typical for β-anomers in hemicelluloses. This indicated the presence of dominant β-glycosidic linkages between the sugar units in all the hemicellulosic fractions. In the carbonyl stretching region, a signal at 1645 cm^{-1} arises from the absorbed water. The absence of a signal at 1745 cm^{-1} for the acetyl and uronic ester groups of the hemicelluloses or from the ester linkage of carboxylic group of the ferulic acid in all the spectra implied that both the alkali and the alkaline peroxide treatments under the conditions given completely saponified these ester bonds from the hemicelluloses. More importantly, the absence of a signal at 1720 cm^{-1} for carbonyl stretching in all the eight spectra of hemicelluloses revealed that the sequential extraction with alkali and alkaline peroxide under the conditions used did not significantly attack or oxidise the glycosidic linkages and hydroxyl groups of hemicelluloses. The fact that the glycosidic linkages and hydroxyl groups of hemicelluloses remained unattached during the successive treatments indicated lignin protects hemicelluloses from being attracted by alkaline peroxide since the degradation of phenolic structure in the lignin was very rapid as shown by a noticeable band at 1720 cm^{-1} for carboxylic groups (unpublished data).

In addition, as can be seen from the two figures, the associated lignin absorbance at 1513 or 1506 cm^{-1} is clearly observed in the hemicellulosic spectra except for the fraction of AS2, although it is rather weak, while the corresponding absorbance at 1600 cm^{-1} is virtually absent since the latter is masked by the stretch of absorbed water at 1645 or 1639 cm^{-1}. Treatment with increasing alkali between 0.1 M NaOH and 10% KOH and alkaline peroxide concentration from 1.0 to 3.0% resulted in a progressive reduction in the lignin absorbance (1513 or 1506 cm^{-1}), indicating a decrease in lignin content. The occurrence of this very small band for principal lignin absorbance indicated that the hemicelluloses isolated contained only small amounts of bound lignin, which corresponded to the results obtained by alkaline nitrobenzene oxidation.

3.6. ^{13}C-NMR spectra

In order to characterize the structural features of the isolated hemicelluloses, the hemicellulosic fraction AS1 and 1.0% H_2O_2 soluble hemicelluloses were analyzed by ^{13}C-NMR spectroscopy (spectra not shown). No significant differences in the spectra of the two hemicellulsic fractions were observed. The spectra were interpreted on the basis of previously reported data for structurally-defined arabinoxylan-type, glucuronoxylan-type, and L-arabino-(4-*O*-methyl-D-glucurono)-D-xylan (Ebringerova, Hromadkova, Alfoldi, & Berth, 1992; Imamura, Watanabe, Kuwahara, & Koshijima, 1994; Sun et al., 1996). The main 1,4-linked β-D-Xyl*p* units are characterized by the strong signals at 104.7, 78.5, 77.5, 75.6, and 65.6 ppm, which respectively are assigned to C-1, C-4, C-3, C-2, and C-5 of the β-D-Xyl*p* units. Another group of signals at 111.8, 88.9, 82.8, 80.8, and 64.0 ppm are originated from C-1, C-4, C-2, C-3, and C-5 of α-L-Ara*f* residues, respectively. The signal at 59.4 ppm arises from 4-*O*-methoxyl group of glucuronic acid residue in the xylan, which is very weak and in accord with the low uronic acid content. These observations stated that both alkali and alkaline peroxide treatments under the condition given did not affect the main structural feature of the hemicelluloses from barley straw.

3.7. Thermal stability

The thermal properties of the hemicellulosic fractions were analyzed by TGA and DSC. Fig. 4 gives the thermograms of the hemicellulosic fractions extracted with 0.5% H_2O_2 (Fig. 4a), 2.0% H_2O_2 (Fig. 4b), 3.0% H_2O_2 (Fig. 4c), and 10% KOH–1% $Na_2B_4O_7{\cdot}10H_2O$ (Fig. 4d). As illustrated, all the four hemicellulosic fractions began to decompose at an approximate temperature of 200 °C. However, at 10% weight loss the degradation temperature was observed at 265 °C in Fig. 4a, 261 °C in Fig. 4b, 258 °C in Fig. 4c, and 255 °C in Fig. 4d. When weight loss arrived at 50%, the temperature raised to 441, 384, 341, and 319 °C for the hemicellulosic fractions extracted by 0.5, 2.0, 3.0% H_2O_2, and 10% KOH–1% $Na_2B_4O_7{\cdot}10H_2O$, respectively. This decreasing trend of temperature was paralleled to the decreasing data of molecular weight from 60,890, to 59,080, to 56,890, and to 29,080 g mol^{-1}, respectively, in Table 4, indicating that the thermal stability of the hemicelluloses declined with the decreasing molecular weight.

As can be seen from Fig. 4, the three thermograms of hemicelluloses extracted with 0.5, 2.0, 3.0% H_2O_2 showed two regions with variation of mass loss between 200–320 and 320–500 °C, while the hemicellulosic fraction, extracted with 10% KOH–1% $Na_2B_4O_7{\cdot}10H_2O$, gave only one main decomposition stage ranging between 200 and 320 °C. In addition, the hemicellulosic fraction, extracted with 10% KOH–1% $Na_2B_4O_7{\cdot}10H_2O$, showed a small endothermic peak at 205 °C. Then there appeared two big exothermic peaks between 250 and 400 °C. At higher temperatures over 500 °C, all the hemicellulosic degradations proceeded to a lesser extent.

In short, the present study revealed that the sequential treatment of barley straw with 0.1 M NaOH, 0.25, 0.5, 1.0, 1.5, 2.0, and 3.0% H_2O_2, and 10% KOH–1% $Na_2B_4O_7{\cdot}10H_2O$ was an effective technique for isolation of large proportions of hemicelluloses and lignin, which together solubilized more than 90% of the original hemicelluloses and approximately 85% of the original lignin. It was identified that the hemicelluloses from barley straw were composed mainly of L-arabino-(4-*O*-methyl-D-glucurono)-D-xylan, and contaminated with small amounts of associated lignin (5.21–9.12%). Treatments with 0.25–3.0% alkaline peroxide solubilized the hemicellulosic fractions having higher $\bar{M}_w$ values between 56,890 and 63,810 g mol^{-1} than those of the two alkali-soluble hemicellulosic fractions isolated with 0.1 M NaOH and 10% KOH–1% $Na_2B_4O_7{\cdot}10H_2O$ with $\bar{M}_w$ values of 28,000 and 29,080 g mol^{-1}. The FT-IR and liquid-state ^{13}C-NMR analyses showed that the alkaline peroxide treatment of the straw under the conditions used did not result in any significant degradation in the macromolecular structure of the polymeric hemicelluloses. The thermal stability of the hemicelluloses decreased with a decrement of molecular weight.

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