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Physico-chemical and structural characterization of hemicelluloses from wheat straw by alkaline peroxide extraction

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Abstract

The treatment of wheat straw with 2% H_2O_2 at 50°C and pH 11.5 for 4–30 h or with 2% H_2O_2–0.05% anthraquinone at 50°C and pH 11.5 for 4.5 h resulted in the release of 79–86% of the original lignin and 77–91% of the original hemicelluloses, which contained 3.8–6.5% of associated lignins. Xylose was the major sugar constituent in all the solubilized hemicellulosic fractions, and arabinose, glucose, and galactose were present in small amounts. The isolated seven hemicellulosic samples were further characterized by Fourier transform infrared, and carbon-13 magnetic resonance spectroscopy as well as gel permeation chromatography. Comparison of these hemicellulosic fractions with those obtained by alkali extraction from wheat straw in the absence of hydrogen peroxide provided evidence of similar chemical composition and structure. The treatment by alkaline peroxide under the conditions used did not result in any significant change in the macromolecular structure of hemicelluloses.

Keywords: Wheat straw; Hemicelluloses; Hydrogen peroxide

1. Introduction

For the last 10 years, there has been an important resurgence of interest in the development of non-feed applications of straws from agricultural residues, e.g. paper making, especially in the developing countries such as China and India. Among these, wheat straw has been widely studied in this area as a raw material for paper making [1]. However, effluents from the chemical pulps have become the focus of environmental concern. Recently, a new pulping method for environmental friendly processing has been developed in the division of pulp and paper at the BioComposites Centre (University of Wales, Bangor, UK), which was firstly proposed by Gould [2] in the treatments of agricultural residues for increasing fermentation. In brief, the straw was treated with 2% H_2O_2 at 50°C and pH 11.5 for 20 h. The pulp was filtered and washed thoroughly to remove solubilized components. This one-step treatment resulted in approximately 84% of the original lignin degradation and 90% of the original hemicellulose solubilization [3].

The hydrogen peroxide delignification of agricultural residues is strongly pH-dependent, with an optimum pH of 11.5–11.6, pK_a for the dissociation reaction of H_2O_2:

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

Alkaline solutions of H_2O_2 react rapidly with lignin and lignin model compounds to form low molecular weight, water-soluble oxidation products. The lignin oxidizing species in these reactions is apparently the highly reactive hydroxyl radical ($HO^{\cdot}$) that is formed during the degradation of H_2O_2 in a reaction with the hydroperoxy anion (HOO^-) [4]:

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

Hydroperoxide anion (HOO^-) is the active species and is responsible for the bleaching action of hydrogen peroxide under alkaline conditions. On the other hand, hydroperoxyl and hydroxyl radicals generated by the decomposition of hydrogen peroxide are responsible for delignification and solubilization of hemicelluloses. Nowadays, hydrogen peroxide is mainly used in chemical pulping and bleaching. It functions as a replacement for hypochlorite, either partially or totally. It can be used in the first extraction state with or without oxygen to help reduce oxidant levels in the chlorination state. Hydrogen peroxide continues to reduce demands for chlorine dioxide, where it effectively adds an extra bleaching stage [5].

When used in both the delignification and bleaching of

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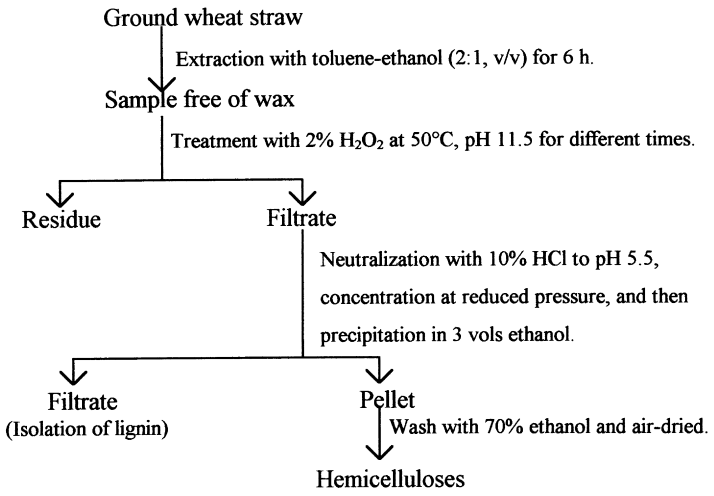

Fig. 1. Scheme for isolation of hemicelluloses from the hydrolysates of 2% H_2O_2 treatment of wheat straw.

chemical pulps, hydrogen peroxide produces a less-colored effluent, reduces pollution load on the effluent treatment system, and improves product quality as is apparent in strength, brightness, and brightness stability [5]. Other advantages of hydrogen peroxide include environmental compatibility and mild oxidation during the delignification process. In the chemical pulping, hemicelluloses were significantly degraded or oxidized into monocarboxylic acids such as formic, acetic, lactic, and glycolic acids, whereas during the delignifying process using alkaline peroxide the macromolecular hemicelluloses were only solubilized or partially degraded. The solubilized hemicelluloses were then recovered by precipitation in ethanol and modified as a novel material for industries [6]. To achieve this aim, a thorough study and characterization of the starting hemicelluloses solubilized during the alkaline peroxide treatment is necessary.

The main constituents of wheat straw cell walls are cellulose (40.1%), hemicelluloses (32.8%), and lignin (14.1%). The cell walls also contain small amounts of *p*-hydroxycinnamic acids such as ferulic and *p*-coumaric acids, glycoproteins, pectins, wax, and ash [1]. All the cell wall constituents are closely bound either by weak bonds such as hydrogen and Van der Waals bonds or by covalent bonds. β-D-glucans (β 1-3, 1-4) are hemicelluloses characteristic for Gramineae but xylans are quantitatively the dominant hemicelluloses [7], which contain a main chain formed by D-xylopyranose residues linked by β-1,4 linkages. They form hydrogen bonds with cellulose, covalent bonds with lignins, and ester linkages with acetyl units and *p*-hydroxycinnamic acids. Xylans can be substituted by arabinose, galactose, glucuronic acid or 4-*O*-methyl-glucuronic acid (MeGlcA). In the cell walls of Gramineae, arabinoxylans generally predominate. The substitution on C-2 or C-3 may occur by MeGlA and α-arabinofuranose, respectively, or they may be esterified by acetyl groups. Cellulose is a polydispersed linear homopolymer of poly-β-(1,4)-D-glucose with a degree of polymerization greater than 15 000. This is found in the microfibrils forming a para-crystalline structure, stabilized by intra- and intermolecular hydrogen bonds oriented in a parallel or antiparallel manner [8]. X-ray diffraction studies suggest that this crystalline structure is surrounded by xylans, having a structure similar to those of the amorphous phase. This finding is supported by fact that the bonds between cellulose and xylan are extremely resistant and cannot be broken even by treatment with acids [8].

This present work concentrated on the minimizing degradation of the hemicellulose fraction and maximizing hemicellulose extraction during the alkaline peroxide treatment at different periods. The isolated hemicelluloses were purified, chemically defined, and physico-chemically characterized.

2. Experimental

2.1. Material

Wheat straw was collected from the farm fields at the University of North-Western Forestry (Yangling, People's Republic of China) and was ground to pass a 1.0 mm size screen. The dried powder was extracted with toluene–ethanol (2:1, v/v) in a Soxhlet for 6 h. The dewaxed straw was then dried in an oven for 16 h at 50°C before use. All chemicals used were of analytical or reagent grade.

2.2. Alkaline peroxide treatment

Samples of dewaxed wheat straw (5.0 g) were added to 150 ml of distilled water containing 2% 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 at 50°C for 4, 8.5, 12, 16, 20, and 30 h, respectively. For comparison, one sample was treated with 2% H_2O_2–0.05% anthraquinone (AQ) (w/v) at pH 11.5 and 50°C for 4.5 h. During initial stages of stirring, oxygen evolution was

Table 1
The yields of hemicelluloses and lignin (% dry matter) solubilized during the 2% H_2O_2 treatment of wheat straw at 50°C and pH 11.5 for various periods and the yield of residues

Yield (% dry matter)	2% H_2O_2 (50°C, pH 11.5) treatment time (h)						2% H_2O_2–0.05% AQ
	4[a]	8.5[a]	12[a]	16[a]	20[a]	30[a]	4.5 h[b]
Hemicelluloses[c]	25.2	27.3	29.5	30.0	29.5	29.3	28.0
Other solubilized materials[d]	9.6	9.9	7.9	8.1	8.6	11.2	6.9
Lignin	11.1	11.4	11.5	11.5	11.8	12.1	11.3
Residue	54.1	51.4	51.1	50.4	50.1	47.4	53.8

[a] The hemicellulosic and lignin fractions obtained by treatment of the dewaxed wheat straw with 2% H_2O_2 at 50°C and pH 11.5 for various periods.
[b] The hemicellulosic and lignin fractions obtained by treatment of the dewaxed wheat straw with 2% H_2O_2–0.05% AQ at 50°C and pH 11.5 for 4.5 h.
[c] Obtained by precipitation of the neutralized extracts with 3 vol ethanol.
[d] Represent for the 2% H_2O_2 solubilized non-polysaccharide materials such as ash and proteins.

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 2 h before slowly rising to a final value of ca. 12.9. The insoluble residue was collected by filtration, washed with distilled water until the pH of the filtrate was neutral, and then dried at 60°C. The supernatant fluid was adjusted to pH 5.5 with 10% HCl and then concentrated. The solubilized hemicelluloses were precipitated by pouring the concentrated supernatant fluid into three volumes of ethanol, from which they were settled out as a white flocculent precipitate. The hemicelluloses were collected after carefully decanting off the supernatant fluid and washed with 70% ethanol. Next the hemicelluloses were air-dried in a fume hood overnight, finely fragmented with a conventional chopper–grinder, and dried to constant weight in an oven at 50°C (Fig. 1).

2.3. Characterization of the solubilized hemicelluloses

The neutral sugar composition of the isolated hemicelluloses and residues, was determined by gas chromatography (GC) analysis of their alditol acetates [9]. Alkaline nitrobenzene oxidation of associated lignin from solubilized hemicelluloses and residues was performed at 170°C for 3 h. The lignin content in hemicellulosic and residual fractions was calculated multiplying by 2.41 the yield of phenolics, obtained by nitrobenzene oxidation [10]. 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 [11,12].

FT-IR spectra were obtained on an FT-IR spectrophotometer (Nicolet, 750) using a KBr disc containing 1% finely ground samples. 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 150 mg of sample dissolved in 1.0 ml D_2O after 15 000 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 solubilized hemicelluloses

The yields of alkaline peroxide soluble hemicelluloses resulting from the fractionation procedures were expressed as a percentage of the dried wheat straw. As expected, treatment with 2% H_2O_2 at 50°C and pH 11.5 for 4, 8.5, 12, 16, 20, and 30 h resulted in 79, 81, 82, 82, 84, and 86% of the original lignin and 77, 83, 90, 91, 90, and 89% of the original hemicelluloses removal, respectively (Table 1). An extraction in which the duration was increased from 4 to 16 h resulted in an increase in the yield of solubilized hemicelluloses from 25.2 to 30.0%. The hemicelluloses were nearly white, and the recovered residue was off-white. Prolonging extraction duration from 16 to 20, and to 30 h resulted in a slightly decreasing yield from 30.0 to 29.5, and to 29.3%, respectively. A maximum yield of hemicelluloses (30.0%) was obtained by treatment of dewaxed wheat straw with 2% H_2O_2 at 50°C and pH 11.5 for 16 h.

Previous studies on the mechanism of H_2O_2-dependent lignin and hemicellulose solubilizations from wheat straw indicated that the reaction rate was strongly dependent on pH, with a sharp optimum at pH 11.5 [2,13]. It was generally not necessary to continuously regulate the reaction pH, even though over the course of the treatment (4–30 h) the reaction pH increased from 11.5 to 12.8–13.0. As can be seen in Table 1, most of the lignin and hemicelluloses were solubilized in the first 4 h, when the reaction pH remained $<$12.8. Interestingly, as the reaction pH became more alkaline, more hemicelluloses were solubilized, and the yield of insoluble residues decreased. About 77% of the hemicelluloses originally present in wheat straw was solubilized after 4 h of 2% H_2O_2 treatment in the absence of continuous pH control, and 91% was solubilized after 16 h. In comparison, only about 52% of the original

Table 2
The content of neutral sugars (relative % dry weight) and uronic acid (% dry weight) in extracted hemicellulosic fractions

2% H_2O_2 treatment time (h)	Neutral sugars						Uronic acids
	Rha	Ara	Xyl	Man	Glc	Gal	
4[a]	0.65	15.11	63.18	0.50	16.19	4.36	5.46
8.5[a]	0.46	15.81	65.37	0.42	14.01	3.92	5.25
12[a]	0.66	15.41	66.13	0.35	13.86	3.60	5.04
16[a]	0.96	15.44	65.02	0.41	13.81	4.35	4.90
20[a]	0.96	15.49	64.20	0.50	13.85	5.00	4.83
30[a]	1.20	15.38	63.90	0.71	13.84	4.97	4.90
4.5[b]	1.12	14.82	62.30	0.39	15.73	5.66	4.97

[a] The hemicellulosic fractions obtained by treatment of the dewaxed wheat straw with 2% H_2O_2 at 50°C and pH 11.5 for various periods.
[b] The hemicellulosic fraction obtained by treatment of the dewaxed wheat straw with 2% H_2O_2–0.05% AQ at 50°C and pH 11.5 for 4.5 h.

hemicelluloses were solubilized in 4 h when the reaction pH was maintained at pH 11.5 (data not shown). These data indicated that the hemicelluloses in wheat straw can be significantly solubilized by treatment with 2% H_2O_2 at pH 11.5 in the absence of continuous pH control.

As compared to the yield of solubilized hemicelluloses (50% originally present in wheat straw) obtained by treatment with 1% H_2O_2 at room temperature and pH 11.5 for 6 h, a much higher yield (77% originally present in wheat straw) of the dissolved hemicelluloses was observed during the treatment with 2% H_2O_2 at 50°C and pH 11.5 for 4 h in our studies, indicating that increases of alkaline peroxide concentration or reaction temperature favored solubilization of hemicelluloses. In the absence of H_2O_2, approximately 30% hemicelluloses originally present in wheat straw were solubilized at 25°C and pH 11.5–11.7 for 4 h treatment (data not shown), while the yield was enhanced to 77% after addition of 2% H_2O_2 at 50°C, and it continued to increase to 91% after 16 h treatment. However, the results obtained by Gould [2] indicated that in the absence of H_2O_2, all of the wheat straw hemicelluloses remained insoluble after treatment as high as pH 12, and essentially complete solubilization of hemicelluloses occurred during the treatments at pH 13 whether H_2O_2 was present or not. This dispersion of values may reflect the influence of factors such as soil, climate, method of analysis, etc. on the degradation of wheat straw hemicelluloses.

One explanation for the improved solubility efficiency of hemicelluloses observed at a relatively high concentration of H_2O_2 (2%) is that the delignifying reactions complete more favorably with increase of peroxide concentration, which subsequently results in an enhancement of the hemicelluloses released. The effect of increasing temperature on solubilizing rate of hemicelluloses may result from increasing the concentration of hydroxyl and perhydroxyl radicals ($HO^{\cdot}$; $O_2^{-\cdot}$) that are formed during the degradation of H_2O_2 in a reaction with the hydroperoxy anion (HOO^-). That is, increasing the reaction temperature not only enhances the rate of delignification but enhances the peroxide decomposition as well [14]. The optimum treatment temperature, therefore, was used at 50°C with retention times up to 30 h.

As can be seen in Table 1, alkaline peroxide is capable of reducing the contents of both lignin and hemicelluloses from wheat straw significantly under the conditions applied. It is noteworthy that the introduction of hydrogen peroxide stabilizer such as sodium silicate has no effect (or an adverse effect, data not shown) on straw delignification and hemicellulose solubilization. This observation is consistent with the finding that improvement in H_2O_2 stabilization is not a prerequisite for good delignification even under particularly severe conditions (120°C) because, contrary to classical peroxide bleaching, the active species are the decomposition products of hydrogen peroxide, i.e., hydroxyl and perhydroxyl radicals ($HO^{\cdot}$, $O_2^{-\cdot}$) and oxygen [15].

It has been reported that addition of small amounts of AQ in alkaline pulping of hardwood and nonwood materials enhances removal of lignin by promoting cleavage of some interunit bond in the lignin which is not cleaved in the absence of AQ, minimizing recondensation reactions, and reacting with the carbohydrates to improve lignin removal [16,17]. In order to gain a more efficient delignification and significant dissolution of hemicelluloses by alkaline peroxide, 0.05% AQ (w/v) was added to the treatment solution, and the reaction was performed at 50°C and pH 11.5 for 4.5 h. The data in Table 1 showed that addition of 0.05% AQ had a minimal effect on the delignification as shown by the nearly equal yield of solubilized lignin (11.1–11.3%) obtained by 2% H_2O_2 treatment at 50°C and pH 11.5 for 4 h in the absence of AQ. While the treatment with 2% H_2O_2–0.05% AQ led to a slight increase of solubilization of hemicelluloses by 2.8% as compared to the yield of hemicelluloses solubilized during the treatment with 2% H_2O_2 for 4 h in the absence of 0.05% AQ.

3.2. Composition of neutral sugars and uronic acids

The composition of neutral sugars and content of uronic acid in the solubilized hemicellulosic fractions are given in Table 2. Obviously, xylose was the predominant sugar component in all of the hemicellulosic fractions, comprising 63.2–66.1% of the total sugars, with arabinose and glucose present in smaller amounts. Galactose, rhamnose, and

Table 3
The composition of neutral sugars (relative % dry weight) in 2% H_2O_2 treated wheat straw residues

2% H_2O_2 treatment time (h)	Neutral sugars					
	Rha	Ara	Xyl	Man	Glc	Gal
4[a]	Tr[b]	2.65	15.12	1.81	78.49	1.93
8.5[a]	Tr	2.46	13.03	2.19	80.72	1.60
12[a]	Tr	2.05	12.05	1.11	84.00	0.79
16[a]	Tr	1.89	10.05	1.09	86.16	0.85
20[a]	Tr	1.85	10.72	1.54	85.04	0.84
30[a]	Tr	2.06	9.70	1.43	85.73	1.08
4.5[c]	Tr	2.82	15.04	1.58	79.77	0.79

[a] The residue preparations obtained by treatment of the dewaxed wheat straw with 2% H_2O_2 at 50°C and pH 11.5 for various periods.
[b] Tr = trace.
[c] The residue preparation obtained by treatment of the dewaxed wheat straw with 2% H_2O_2–0.05% AQ at 50°C and pH 11.5 for 4.5 h.

mannose were observed as minor sugar constituents. The content of uronic acids, mainly glucuronic acid or MeGlcA, ranged between 4.8 and 5.5%. These results were in good agreement with those of hemicellulosic preparations obtained by alkali extraction of lignified wheat straw in the absence of hydrogen peroxide, indicating that alkaline peroxide treatment did not affect the whole chemical composition of the hemicelluloses. Previous studies showed that the hemicelluloses extracted with 0.5 M NaOH, were 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 group attached at position 3 in the main chain [12].

The residues after alkaline treatment were mainly formed of crude cellulose, as evidenced by sugar analysis. The data, shown in Table 3, indicated that glucose was the extremely predominant sugar component, comprising 78–86% of the total sugar constituents. An increase of treating time from 4 to 20 h led to growth of cellulose in the residues as shown by an increase of glucose from 78 to 86% and a decrease of xylose from 15.1 to 10.1%, which corresponded to the increasing yield of hemicelluloses solubilized. These measurable amounts of sugars corresponding to non-cellulose polysaccharides undoubtedly resulted from the associated hemicelluloses. This finding was supported by the fact that the bonds between cellulose and xylan are extremely resistant and cannot be broken by treatment with alkaline peroxide even with acids [8]. Further studies found that alkaline peroxide treatment did not cause detectable changes in the structure of highly polymerized cellulose [18]. Interestingly, the alkaline peroxide treatment of straw apparently loosened the lignocellulosic matrix, causing, at the molecular level, a more open three-dimensional relationship between the cellulose, lignin, and possibly hemicellulose polymers, and subsequently resulted in significant solubilization of lignin and hemicelluloses.

3.3. Associated lignin

The hemicelluloses prepared by alkaline peroxide extraction was much lighter in color than those obtained under similar conditions but in the absence of peroxide. The effects were focused on extractions of hemicelluloses using H_2O_2 in NaOH rather than other alkalis in order to compare with alkali-soluble hemicelluloses. The pigmentation associated with the hemicellulosic preparations was found to be likely due to the presence of co-extracted lignin, which was associated with hemicelluloses through ether linkages [19]. To verify whether lignin fragments that may be contaminating wheat straw hemicellulose preparations could be removed by including H_2O_2 in the extraction medium, all the hemicellulosic fractions were oxidized by alkaline nitrobenzene at 170°C for 3 h. The content of lignin and the composition of phenolic acids and aldehydes from nitrobenzene oxidation of the associated lignin in the isolated hemicellulosic fractions are given in Table 4. As can be seen, lignin removal in the solubilized hemicelluloses was maximized in 4 h, corresponding to the

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

Phenolic acids and aldehydes	2% H_2O_2 (50°C, pH 11.5) treatment time (h)						2% H_2O_2–0.05% AQ
	4[a]	8.5[a]	12[a]	16[a]	20[b]	30[a]	4.5 h[b]
p-Hydroxybenzoic acid	0.052	0.060	0.064	0.068	0.060	0.048	0.072
p-Hydroxybenzaldehyde	0.084	0.088	0.13	0.13	0.11	0.072	0.096
Vanillic acid	0.096	0.076	0.056	0.060	0.096	0.10	0.10
Syringic acid	0.13	0.17	0.22	0.26	0.21	0.18	0.16
Vanillin	0.55	0.68	1.06	1.04	0.86	0.71	0.71
Syringaldehyde	0.61	0.77	1.04	1.02	0.79	0.74	0.73
p-Coumaric acid	0.036	0.048	0.048	0.068	0.052	0.056	0.042
Ferulic acid	0.031	0.044	0.044	0.048	0.044	0.048	0.042
Total	1.59	1.94	2.66	2.69	2.22	1.95	1.95
Lignin content	3.83	4.68	6.41	6.48	5.35	4.70	4.70

[a] The hemicellulosic fractions obtained by treatment of the dewaxed wheat straw with 2% H_2O_2 at 50°C and pH 11.5 for various periods.
[b] The hemicellulosic fraction obtained by treatment of the dewaxed wheat straw with 2% H_2O_2–0.05% AQ at 50°C and pH 11.5 for 4.5 h.

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

Phenolic acids and aldehydes	2% H_2O_2 (50°C, pH 11.5) treatment time (h)						2% H_2O_2–0.05% AQ
	4[a]	8.5[a]	12[a]	16[a]	20[a]	30[a]	4.5 h[b]
p-Hydroxybenzoic acid	0.044	0.040	0.038	0.039	0.037	0.038	0.044
p-Hydroxybenzaldehyde	0.095	0.077	0.070	0.064	0.060	0.064	0.061
Vanillic acid	0.060	0.055	0.051	0.043	0.042	0.041	0.092
Syringic acid	0.14	0.12	0.12	0.13	0.12	0.11	0.13
Vanillin	0.80	0.68	0.65	0.64	0.54	0.46	0.67
Syringaldehyde	0.97	1.03	1.04	1.07	0.94	0.88	0.97
p-Coumaric acid	0.060	0.060	0.062	0.045	0.038	0.040	0.041
Ferulic acid	0.050	0.045	0.041	0.041	0.035	0.034	0.035
Total	2.22	2.11	2.07	2.07	1.81	1.67	2.04
Lignin content	5.35	5.09	5.00	5.00	4.36	4.02	4.92

[a] The residual preparations obtained by treatment of the dewaxed wheat straw with 2% H_2O_2 at 50°C and pH 11.5 for various periods.
[b] The residual preparation obtained by treatment of the dewaxed wheat straw with 2% H_2O_2–0.05% AQ at 50°C and pH 11.5 for 4.5 h.

lowest associated lignin (3.8%). The extension of treatment period or addition of 0.05% AQ in H_2O_2 solution, in general, resulted in an increase of associated lignin up to 6.5%. This was presumed due to the lignin condensation during the prolonging alkaline peroxide extraction processes.

With the studies of delignifying agricultural residues using alkaline peroxide, Gould [13] demonstrated that delignification was most effective at pH 11.5, the pK_a for the dissociation of H_2O_2, and that the concentration of the species active in delignification, $HO^{\cdot}$ and $O_2^{-\cdot}$ were optimal at pH 11.6. In a milk alkaline peroxide process, only minimal amounts of hemicelluloses were solubilized. When the pH increased to >11.5, increased solubilization of hemicellulose was noted. Similar results of high yield of hemicelluloses were observed in our experiments in the absence of continuously regulating the reaction pH. In this case, the extraction medium can convert the significant amounts of both lignin and hemicelluloses into water-soluble fractions. Further studies showed that the alkaline peroxide process was most effective when conducted at pH 11.5 in the beginning of the reaction as the hemicellulose color and yield is considered. Possibly at the elevated pH, chromophores were generated by alkali-catalyzed modification of reducing end groups on the polysaccharides. The hemicelluloses produced at pH 11.5 were off-white, while those exposed to pH value at 13.0 were tannish. This phenomenon was in good accordance with the findings by Doner and Hicks [19].

As compared to the associated lignin content in the hemicelluloses extracted with 7.5% NaOH at 25°C for 16 h on a large scale in the absence of H_2O_2 from lignified wheat straw, only one-half amount of lignin was found to be associated in the hemicellulosic fractions extracted by alkaline peroxide under the conditions given (data not shown). These data suggested that 2% H_2O_2 treatment at a relatively high temperature (50°C) and pH 11.5 can peel off the hemicelluloses from most of their neighboring lignins, resulting in an solubilized hemicelluloses low in etherified lignins.

The results obtained by alkaline nitrobenzene oxidation of associated lignin in isolated hemicelluloses showed that the oxidation produced approximately equal amounts of vanillin and syringaldehydes, resulting from the degradation of guaiacyl and syringyl noncondensed units, respectively. The presence of small quantities of *p*-hydroxybenzaldehyde is generally considered to be indicative of *p*-hydroxyphenyl units within the lignin 'core' [20]. Small amounts of syringic, vanillic, *p*-hydroxybenzoic, *p*-coumaric, and ferulic acids were also identified in the nitrobenzene oxidation products. Among theses, syringic, vanillic and *p*-hydroxybenzoic acids originated from the further oxidation of syringaldehyde, vanillin, and *p*-hydroxybenzaldehyde, respectively. Ferulic and *p*-coumaric acids are two major phenolic acids in a wide variety of graminaceous plants. Particularly, ferulic acid was found to be implicated in cross-linking cell wall polymers. With detailed studies we demonstrated that 60–64% ferulic acid is linked to lignin side chains by ether bonds, whereas 77% *p*-coumaric acid is esterified to lignin at the side chains [21].

Table 5 summarizes the content of residual lignin and its phenolic composition from residual fractions obtained by alkaline nitrobenzene. Clearly, a much lower lignin content (4.0–5.4%) was observed in the alkaline peroxide treated residues as compared to the residues obtained by alkaline treatment in the absence of H_2O_2 [1], indicating that alkaline peroxide was an effective agent for delignification of agricultural residues. Furthermore, as can be seen from the table, extension of treatment time from 4 to 30 h led to a decrease of residual lignin from 5.4 to 4.0% in the residues. The major phenolic components were found to be syringaldehyde and vanillin in the nitrobenzene oxidation products. A measurable higher yield of syringaldehyde than vanillin implied that the polysaccharides such as cellulose and hemicelluloses in wheat straw cell walls are tightly associated with lignin by syringyl units.

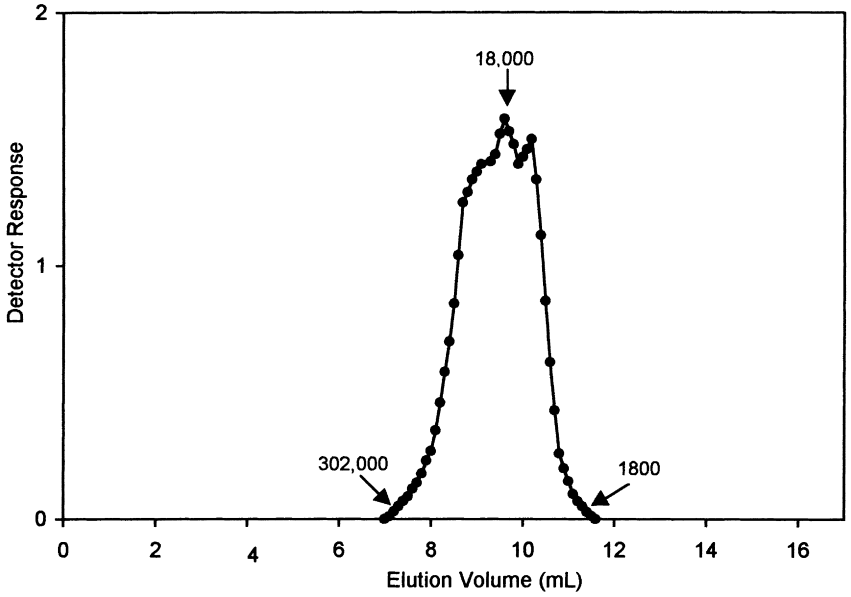

Fig. 2. GPC molecular weight distribution of hemicelluloses extracted with 2% H_2O_2 at 50°C and pH 11.5 and 12 h from wheat straw.

3.4. Molecular weight distribution

The molar mass distribution of the hemicelluloses extracted with 2% H_2O_2 at 50°C and pH 11.5 for 12 h, is shown in Fig. 2. In this plot, the area under distribution curve is proportional to the yield of hemicelluloses recovered in the process, and the molar mass scale indicates polysaccharide-equivalent molar mass. As can be seen from the figure, the molar mass distribution ranged between 302 000 and 1800 g mol^{-1} with a main peak having a molecular weight of 18 000 g mol^{-1}. The weight ($\bar{M}_w$) and number ($\bar{M}_n$) molar mass averages of the solubilized hemicellulosic fractions, calculated from the GPC chromatograms, are listed in Table 6 as a function of alkaline peroxide treatment time. $\bar{M}_w$ increased from 28 400 in 4 h treatment to a maximum of 30 700 in 12 h treatment, from where it decreased to 24 400 in a maximum treatment time (30 h) used. $\bar{M}_n$ presented an equivalent evolution, growing from 5700 for 4 h treatment to a maximum of 6300 and decreasing to 5100 in 30 h treatment. The data in Table 6 indicated that prolonging treatment time between 4 and 12 h resulted in an increasing molecular weight of solubilized hemicelluloses, while it decreased with a further extension of treatment time from 12 to 30 h, suggesting that a prolonging treatment period of 12–30 h might produce a further degradation of the hemicelluloses. Interestingly, as compared to the molar mass of hemicelluloses extracted with 3% NaOH from delignified wheat straw in the absence of H_2O_2 (data not shown), an approximately equal molecular size was obtained, implying that 2% H_2O_2 treatment at 50°C and pH 11.5 did not significantly degrade the macromolecule of hemicelluloses. Our research confirmed earlier studies, which found that alkaline peroxide was a mild agent both for delignification and for solubilization of hemicelluloses from agricultural residues such as straw and grass [3,13].

3.5. FT-IR spectra

FT-IR spectroscopy has been shown to be a powerful tool for the study of the physicochemical and conformational properties of polysaccharides [22]. The spectra of the four hemicellulosic fractions extracted with 2% H_2O_2 at 50°C and pH 11.5 for 4 h (spectrum 1), 12 h (spectrum 2), 16 h

Table 6
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 with 2% H_2O_2 at 50°C and pH 11.5 for various periods from wheat straw

	2% H_2O_2 (50°C, pH 11.5) treatment time (h)						2% H_2O_2–0.05% AQ
	4[a]	8.5[a]	12[a]	16[a]	20[a]	30[a]	4.5 h[b]
($\bar{M}_w$)	28 400	29 100	30 700	28 800	24 600	24 400	24 500
($\bar{M}_n$)	5700	5700	5700	6300	5200	5100	4900
($\bar{M}_w/\bar{M}_n$)	5.0	5.1	5.4	4.6	4.7	4.8	5.0

[a] The hemicellulosic fractions obtained by treatment of the dewaxed wheat straw with 2% H_2O_2 at 50°C and pH 11.5 for various periods.
[b] The hemicellulosic fraction obtained by treatment of the dewaxed wheat straw with 2% H_2O_2–0.05% AQ at 50°C and pH 11.5 for 4.5 h.

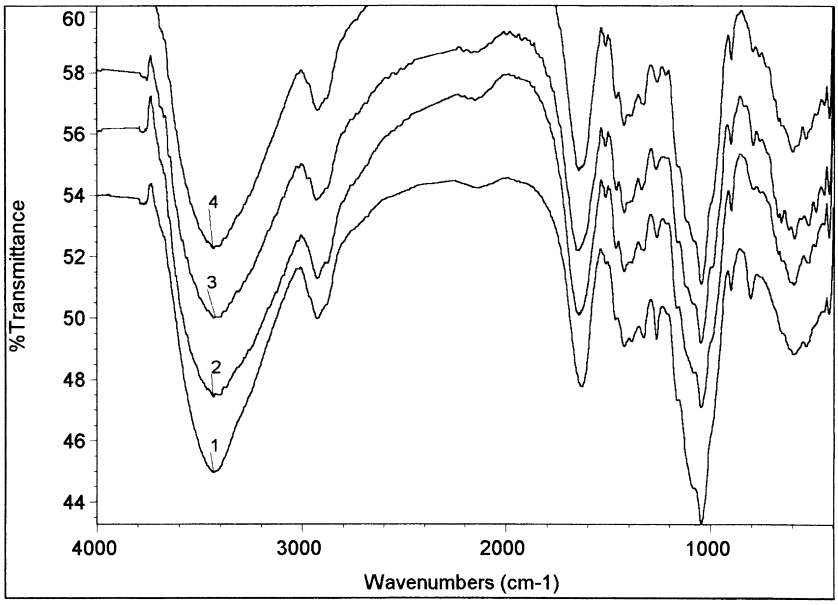

Fig. 3. FT-IR spectra of wheat straw hemicelluloses extracted with 2% H_2O_2 at 50°C and pH 11.5 for (1) 4 h; (2) 12 h; (3) 16 ; and (4) 20 h.

(spectrum 3), and 20 h (spectrum 4) are shown in Fig. 3. All the spectra showed a typical feature of xylan, indicating once again that alkaline peroxide treatment did not result in any significant change in the macromolecular structure of hemicelluloses. The spectral profiles and relative intensities of the bands were rather similar, indicating similar structures of the hemicelluloses isolated in various conditions used. The absorption at 1638 cm^{-1} is principally associated with absorbed water, since the hemicelluloses usually have a strong affinity for water, and in the solid state these macromolecules may have disordered structures which can easily be hydrated [22]. The prominent band at 1044 cm^{-1} is attributed to the C–O, C–C stretching or C–OH bending in hemicelluloses [23]. The sharp band at 895 cm^{-1}, which corresponds to the C_1 group frequency or ring frequency, is characteristic of β-glycosidic linkages between the sugar units [24]. The bands at 1460, 1376, 1336, 1260, and 1220 cm^{-1} represent C–H, OH or CH_2 bendings. An

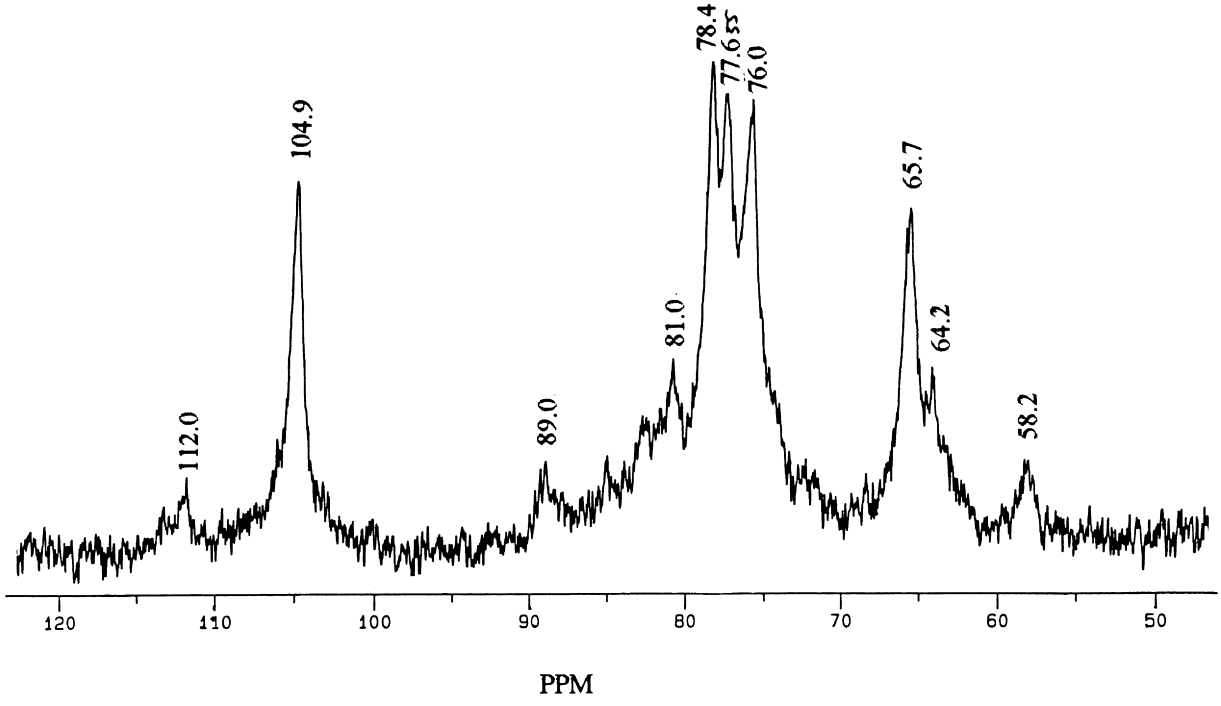

Fig. 4. ^{13}C-NMR spectrum of hemicellulosic fraction (in D_2O) extracted with 2% H_2O_2 at 50°C and pH 11.5 for 12 h from dewaxed wheat straw.

intensive band at 1420 cm^{-1} corresponds to the C=O stretch of carboxylic anions (salt) for uronic acids in hemicelluloses [23]. The occurrence of a small band at 1512 cm^{-1} is undoubtedly due to the presence of small amounts of associated lignin in the hemicelluloses, and the band intensity increased from spectrum 1 to 2, and to 3, and then decreased to spectrum 4, which corresponded to the content of associated lignin obtained by alkaline nitrobenzene oxidation.

3.6. ^{13}C-NMR spectrum

To further confirm the structural changes of the hemicelluloses solubilized during the alkaline peroxide treatment process, the isolated hemicelluloses (2% H_2O_2, 50°C, pH 11.5, 12 h) were analyzed by ^{13}C-NMR spectroscopy in D_2O (Fig. 4). This allows elucidation of the polymer backbone and can also be employed to evaluate the type of side-chains branching along the backbone [25,26]. The spectrum was 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 [12,27–29]. The spectrum was complicated by the relatively large molecular weights of the hemicelluloses and by the high pH of sample. The three drops of 40% sodium deuteroxide, which was necessary to dissolve the compounds, caused line broadening and baseline problems. The one-dimensional ^{13}C-NMR spectra of both, extracted by 3% NaOH in the absence of H_2O_2 (spectrum not shown) or by alkaline peroxide, were qualitatively very similar, indicating a similar structural feature. The main 1,4-linked β-D-Xylp units are obviously characterized by the signals at 104.9, 78.4, 77.6, 76.0, and 65.7 ppm, which respectively attributes to C-1, C-4, C-3, C-2, and C-5 of the β-D-Xylp units. The signals at 112.0, 89.0, 83.0 (data not shown), 81.0, and 64.2 ppm correspond to C-1, C-4, C-2, C-3, and C-5 of α-L-Araf residues, respectively. The signal at 58.2 ppm originates from the 4-*O*-methoxyl group of glucuronic acid residue in the xylan. This relatively broad weak signal is in accord with the low uronic acid content. These signals stated that the alkaline peroxide treatment did not affect the macromolecular structure of hemicelluloses to any noticeable extent. The proportional glycosidic linkages in the main chain and side chains were comparable for the hemicelluloses isolated from lignified wheat straw by 3% NaOH in the absence of H_2O_2, which indicated that no substantial changes occurred during the alkaline peroxide treatment process under the conditions used. Similar results have been reported by Schonebaum [30] in as early as 1992 in the studies of mono- and disaccharide behaviors in hydrogen peroxide solution. He demonstrated that D-glucose, D-fructose, D-xylose, D-arabinose, lactose, and maltose, at low levels of hydrogen peroxide, even at 70°C, are not significantly changed.

4. Conclusions

The treatments of wheat straw with 2% H_2O_2 at 50°C and pH 11.5 for 4–30 h or with 2% H_2O_2–0.05% AQ at 50°C and pH 11.5 for 4.5 h solubilized 77–91% of the original hemicelluloses, which contained 3.8–6.5% associated lignin, and had a degree of polymerization giving weight-average molecular weights between 24 400 and 30 700 g mol^{-1}. Xylose was a predominant component sugar in all the hemicellulosic fractions. Arabinose, glucose, and galactose were present in small amounts, and rhamnose and mannose were identified as minimal quantities. Vanillin and syringaldehyde were found to be the major phenolics in the nitrobenzene oxidation products from the associated lignin in isolated hemicelluloses. Under an optimum condition (2% H_2O_2, 50°C, pH 11.5, 16 h), the treatment solubilized 91% of the original hemicelluloses from wheat straw. All of the hemicellulosic fractions had similarly physicochemical properties as compared to those isolated by alkali in the absence of H_2O_2. Supporting the evidence was provided by ^{13}C-NMR analysis which indicated that the alkaline peroxide treatment under the conditions used did not affect the overall structure of macromolecular hemicelluloses.

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References

[1] Sun RC, Lawther JM, Banks WB. Ind Crops Prod 1995;4:127.
[2] Gould JM. Biotechnol Bioengng 1984;26:46.
[3] Sun RC, Tomkinson J. Characterization of lignins from wheat straw by alkaline peroxide treatment, 1999. Submitted for publication.
[4] Lewis SM, Holzgraefe DP, Berger LL, Fahey GC, Gould JM, Fanta GF. Animal Feed Sci Technol 1987;17:179.
[5] Walsh PB. Tappi J 1991;January:81.
[6] Fang JM, Sun RC, Fowler F, Tomkinson J, Hill CAS, Bolton J. Esterification of wheat straw hemicelluloses in *N,N*-dimethylacetamine/lithium chloride homogenous system. Submitted for publication.
[7] Migne C, Prensier G, Grenet E. Biol Cell 1994;81:267.
[8] Heredia A, Jimenez A, Guillen RZ. Lebensm Unters Forsch 1995;200:24.
[9] Blakeney AB, Harris PJ, Henry RJ, Stone BA. Carbohydr Res 1983;113:291.
[10] Sun RC, Fang JM, Goodwin A, Lawther JM, Bolton J. J Agric Food Chem 1998;46:2817.
[11] Lawther JM, Sun RC, Banks WB. J Agric Food Chem 1995;43:667.
[12] Sun RC, Lawther JM, Banks WB. Carbohydr Polym 1996;29:325.
[13] Gould JM. Biotechnol Bioengng 1985;27:893.
[14] Dence CW, Omori S. Tappi J 1986;10:120.

[15] Lachenal D, Choudens CD, Monzie P. Tappi 1980;63:119.
[16] Venica A, Chen CL, Gratzl JS. Tappi Proc 1989;263.
[17] Suckling ID. Tappi Proc 1989;503.
[18] Martel P, Gould JM. J Appl Polym Sci 1990;39:707.
[19] Doner LW, Hicks K. Cereal Chem 1997;74:176.
[20] Scalbert A, Monties B, Guittet E, Lallemand JY. Holzforschung 1986;40:119.
[21] Sun RC, Xiao B, Lawther JM. J Appl Polym Sci 1998;68:1633.
[22] Kacurakova M, Belton PS, Wilson RH, Hirsch J, Ebringerova A. J Sci Food Agric 1998;77:38.
[23] Marchessault RH, Liang CY. J Polym Sci 1962;59:367.
[24] Gupta S, Madan RN, Bansal MC. Tappi J 1987;70:113.
[25] Focher B, Marzetti A, Naggi A, Torri G. Makromol Chem 1989;190:129.
[26] Williams DL, Pretus HA, McNamee RB, Jones EL, Ensley HE, Browder IW. Carbohydr Res 1992;235:247.
[27] Kato A, Azuma J, Koshijima T. Agric Biol Chem 1987;51:1691.
[28] Ebringerova A, Hromadkova Z, Alfoldi J, Berth G. Carbohydr Polym 1992;19:99.
[29] Imamura T, Watanabe T, Kuwahara M, Koshijima T. Phytochemistry 1994;37:1165.
[30] Schonebaum CW. Rec Trav Chim 1922;41:503.