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FRACTIONAL SEPARATION AND STRUCTURAL CHARACTERIZATION OF LIGNINS AND HEMICELLULOSES BY A TWO-STAGE TREATMENT FROM RICE STRAW

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ABSTRACT

Rice straw was separated into two lignin fractions and two hemicellulosic preparations through a two-stage treatment. Pretreatment with ethanol–H_2O (60/40, v/v) under acid catalyst (0.2 *N* HCl) at 70°C for 4 hr degraded 48.8, 24.9, and 23.3% of the original lignin, hemicelluloses, and cellulose, respectively. Following treatment with 2% H_2O_2 at pH 11.5 for 16 hr at 45°C resulted in a dissolution or degradation of 44.7, 55.2, and 12.1% of the original lignin, hemicelluloses, and cellulose, respectively. By this two-stage process, 93.5% of the original lignin and 88.2% of the original hemicelluloses were separated from rice straw. In comparison, the isolated two lignin fractions and two hemi-

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cellulosic preparations were characterized for their chemical composition, physico-chemical properties, structural features, and thermal stability. Both of the lignin fractions contained higher amounts of noncondensed guaiacyl units than those of noncondensed syringyl units, together with small amounts of *p*-hydroxyphenyl units. Pre-treatment with acidic organosolv, under the condition used, favored solubilization of the small molecular size of hemicelluloses, which were mainly composed of α-glucan, while the post-treatment with alkaline peroxide, under the condition given, enhanced dissolution of large molecular size of hemicelluloses, which comprised L-arabino-(D-glucurono)-D-xylan as the major constituent. The thermal analysis showed that the two hemicellulosic fractions were substantially decomposed between 200 and 330°C, while the lignin decomposition process covered a large temperature range, between 200 and 600°C, indicating more thermal stability of the lignin polymers.

Key Words: Rice straw; Hemicelluloses; Lignin; Separation; Characterization

INTRODUCTION

Nowadays, the demand for nonwood plant fibers for papermaking is expected to increase due to depleting forest resources and the environmental problems with the disposal of agricultural residues. At present, China produces more than two-thirds of the nonwood pulp produced worldwide. This is not only due to a shortage of wood fiber but also because there are abundant agricultural residues available in China. Among these, straw materials are by far the largest source of nonwood fibers, followed by bagasse and bamboo. In particular, rice straw and wheat straw are the top two in terms of availability and amount in pulping and papermaking (1). However, conventional pulping processes for straws, such as soda–anthraquinone and neutral or alkaline sulfite processes, face many problems, one of which is that the recovery of the spent liquors is very difficult because of the high viscosity, low calorific value, and high silica content of the liquors. As a result, most of the spent liquors are discharged without any treatment and cause serious water pollution (2). Furthermore, environmental concern has led to the development of bleaching processes without chlorine or chlorine dioxide, and, nowadays, great interest is focused on organosolvent pulping and hydrogen peroxide bleaching (3). By this process, straw fiber can be separated simply into pulp, lignin, and hemicelluloses, which makes it easy to

utilize them for more valuable products without impact on the environment. The pulp can be used for either paper or cellulose derivative. The lignin can be converted to valuable products, e.g., carbon fiber (4,5) and adhesives (6). From the hemicelluloses, chemicals, sweetening materials, and food additives can be produced (7).

A number of organic solvents have been proposed for use in organosolv delignification, either as solvents as such or combined with water, but so far only aqueous methanol and ethanol have shown potential for industrial application. In principle, organosolv pulping can be divided into uncatalyzed, base-catalyzed, and acid-catalyzed processes. In the first category, pulping is actually promoted by acetic acid released from wood. During the base-catalyzed process, such as delignification using NaOH in aqueous methanol, the reaction probably follows a course similar to that of soda pulping, but with methanol promoting lignin dissolution and reducing condensation processes. The rates of acid-catalyzed processes appear to be governed by the hydrolysis of α-ether bonds between lignin and polysaccharides. Other reactions include partial hydrolysis of β-*O*-4 ether linkages between lignin units and lignin condensation. Of these, the α-ether hydrolysis is the faster reaction than the cleavage of β-*O*-4 ether linkages (8–10).

Hydrogen peroxide is a weak acid and dissociates in water to the perhydroxyl ion, HO_2^-, with $pK_a = 11.6$ at 25°C. It has been used widely for many years to bleach high-lignin wood pulps. The bleaching effect of hydrogen peroxide generally has been attributed to the action of the perhydroxyl anion, which is a strong nucleophilic and reacts with various colored carbonyl-containing structures in lignin (11–13). In general, the peroxide bleaching is performed in an alkaline solution because the concentration of perhydroxyl anions increases with increasing alkalinity. On the other hand, hydrogen peroxide is unstable in alkaline conditions and readily decomposes, particularly in the presence of certain transition metals such as manganese, iron, and copper. This decomposition proceeds predominantly through the formation of radical intermediates, such as hydroxyl radicals ($HO^\bullet$) and superoxide anion radicals ($O_2^{\bullet -}$). These radicals oxidize the lignin, leading to depolymerization and the introduction of hydrophilic groups, e.g., carboxylic acid groups (3).

Model compound work has indicated that at least two mechanisms are involved in the lignin-degrading reactions occurring during the bleaching of pulps, viz. a side-chain elimination via the "Dakin-like" reaction (14–16) and an oxidation of aromatic rings through the involvement of radicals. The Dakin-like reaction is ionic and requires the presence of a phenolic benzylalcohol structure, whereas, the other takes place only when hydrogen peroxide is allowed to decompose (17,18). Interestingly, these radicals have a somewhat higher reactivity toward the aromatic structure in lignin than polysaccharides, such as cellulose and hemicelluloses, and this promotes the oxidation and degradation of the lignins (19). Therefore, it is very important to note that a slight decomposition

of hydrogen peroxide may be, thus, beneficial since unreactive aromatic structures in the straw lignin can be degraded and oxidized while the hemicelluloses, at the same time, are solubilized or degraded, and cellulose is left fairly intact (20).

Although many different organosolv delignifications and various model compounds of lignin oxidation by hydrogen peroxide have been investigated during the last 50 years, the chemistry of delignification behind acidic organosolv pulping and alkaline peroxide delignification is still not fully understood (21). This is particularly true for the structural changes of lignin and hemicelluloses, and no suitable range of their physico-chemical properties have been identified for acid-catalyzed solvent and alkaline-catalyzed peroxide. In this study, lignin and hemicelluloses from rice straw were separated by sequential treatments with ethanol–H_2O (60/40, v/v) under acid catalyst (0.2 *N* HCl) at 70°C for 4 hr and 2% H_2O_2 at pH 11.5 for 16 hr. The isolated lignin and hemicellulosic fractions were analyzed to characterize their chemical compositions, physico-chemical properties, and structural features by using the degradation method, such as alkaline nitrobenzene oxidation, acid hydrolysis, and nondestructive techniques, e.g., gel permeation chromatography (GPC), ultraviolet (UV), Fourier transform infrared (FT-IR), and ^{13}C nuclear magnetic resonance (^{13}C-NMR) spectroscopies.

EXPERIMENTAL METHODS

Materials

Ricestraw was obtained from the experimental farm of the North-Western Sciences and Technology University of Agriculture and Forestry (Yangling, People's Republic of China). It was dried in sunlight and then cut into small pieces. The cut straw was ground to pass through a 1.0 mm size screen. All weights and calculations were made on an oven-dried (60°C, 16 hr) basis. The chemical composition (w/w) of the rice 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. Crude wax was removed by extraction with toluene–ethanol (2:1, v/v) in a Soxhlet for 6 hr. The extractives free sample was dried in a cabinet oven with air circulation at 60°C for 16 hr and then kept at 5°C before treatment.

A Two-Stage Method for Separation of Lignin and Hemicelluloses

The wax-free powder of rice straw (9.75 g) was pretreated with ethanol–H_2O (195 mL, 60/40, v/v) under acid catalyst (0.2 *N* HCl) at 70°C for 4 hr. After filtration on a nylon cloth, the residue was washed sequentially with ethanol and

distilled water, and then oven dried at 60°C for 16 hr. Ethanol in the combined supernatant was removed with a rotary vacuum evaporator at a temperature not exceeding 40°C. The supernatant was then adjusted to pH 5.5 with 1 *M* NaOH, concentrated on a rotary evaporator under reduced pressure to about 120 mL, and then mixed with 360 mL of 95% ethanol (12 hr, 25°C) for separation of hemicelluloses. The precipitated hemicelluloses were filtered, washed with 70% ethanol, and air-dried. After filtration and evaporation of ethanol, the organosolv lignin was obtained by precipitation at pH 1.5, adjusted with 6 *M* HCl, from the corresponding supernatants. The separated lignin preparation was then washed with acidified water (pH 2.0) and freeze-dried. To further isolate alkaline peroxide soluble hemicelluloses and lignin fractions, the above residue was post-treated with 2% H_2O_2 with a solid to extractant ratio of 1:25 at pH 11.5 for 16 hr at 45°C. The dissolved hemicelluloses and lignin fractions were separated according to the method mentioned earlier. During the course of the post-treatment, no further adjustments in pH were made. It was found that the reaction pH remained nearly constant for 2 hr before slowly rising to a final value of ca. 12.9. Note that the fractions 1 (F_1) and 2 (F_2) represent the two acid-insoluble lignin fractions extracted successively with ethanol–H_2O (60/40, v/v) under acid catalyst (0.2 *N* HCl) at 70°C for 4 hr and 2% H_2O_2 at pH 11.5 for 16 hr at 45°C from dewaxed rice straw, while the fractions 3 (F_3) and 4 (F_4) represent the two hemicellulosic preparations, solubilized during the acidic organosolv pre-treatment and alkaline peroxide post-treatment, respectively (Fig. 1).

Characterization of Separated Lignin and Hemicellulosic Fractions

The neutral sugar constituents in isolated hemicelluloses and lignin fractions were determined as their alditol-acetate derivatives by gas chromatography (GC) after hydrolysis with 2 *M* trifluoroacetic acid for 2 hr at 120°C (22). The uronic acid content was assayed colorimetrically using the 3-phenylphenol reagent according to the procedure outlined by Blumenkrantz and Asboe-Hanson (23). The composition of phenolic acids and aldehydes, liberated from alkaline nitrobenzene oxidation of the two acid-insoluble lignin preparations and two hemicellulosic fractions at 175°C for 2.5 hr, were determined on a Hichrom H5ODS HPLC column of dimensions 250 mm × 4.6 mm (purchased from Phenomenex Co., Beijing, People's Republic of China). The identification of the individual compounds were detected at 280 nm by computer comparison of the retention times and peak areas with the authentic phenolics (24). Methods for recording UV spectra, determination of chemical composition, including ash and silica of the straw and residue, and measurement of molecular-average weights of two lignin fractions and two hemicellulosic preparations are described in previous papers (25–27).

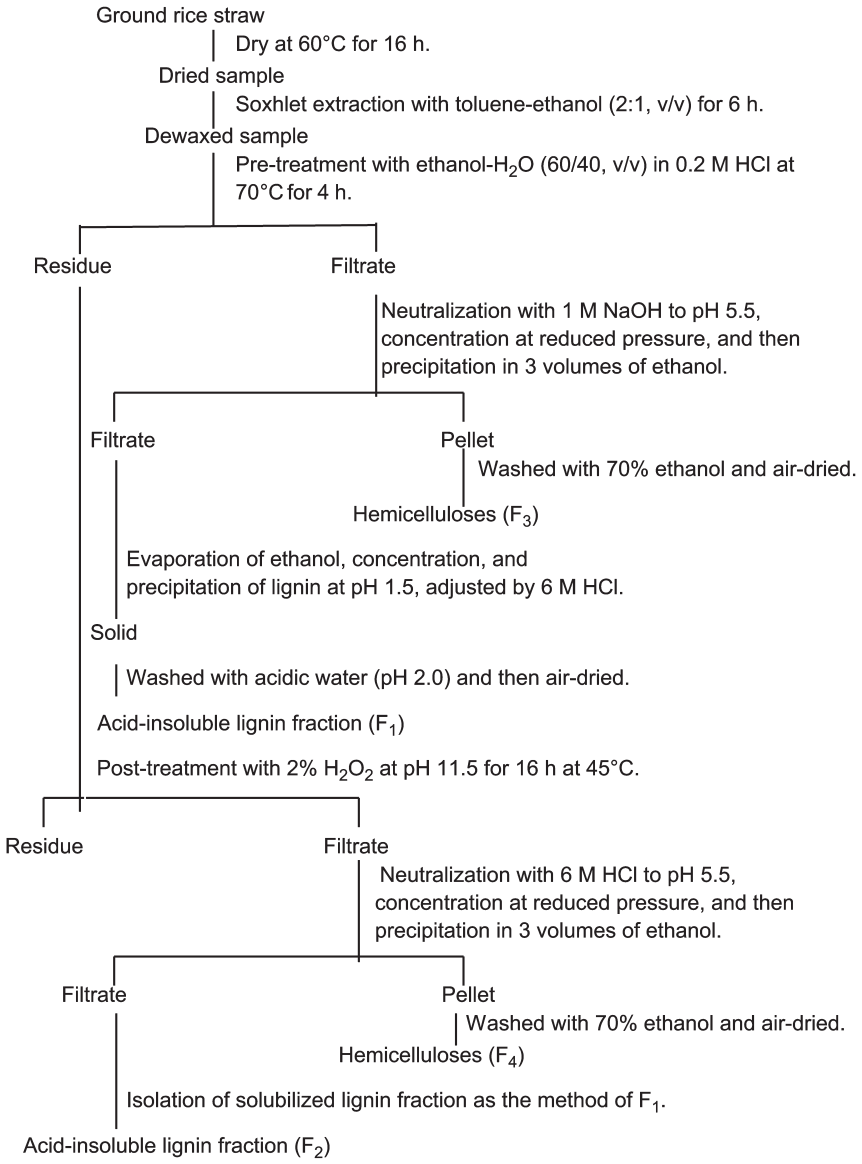

Figure 1. Scheme for separation of hemicelluloses and lignin from dewaxed rice straw.

FT-IR spectra were obtained on a Nicolet 510 spectrophotometer (Warwick, England) using a KBr disc containing 1% finely ground samples. The solution ^{13}C-NMR spectra of lignin fraction F_1 and hemicellulosic preparation F_4 were recorded on a Bruker MSI-300 spectrometer (Oxford, England) at 74.5 MHz from 180 mg of sample dissolved in 1.0 mL DMSO-d_6 and 1.0 mL D_2O after 15,000 scans, respectively. Thermal stability of the lignin and hemicellulosic fractions was performed using thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) on a simultaneous thermal analyzer (NETZSCH STA-409, Netzsch-Geratebau GmbH, Hamburg, Germany). 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}.

RESULTS AND DISCUSSION

Yield and Chemical Composition

Table 1 gives the yield of solubilized lignin, hemicelluloses, and degraded cellulose during the pre- and post-treatments. As can be seen from the table, pre-treatment of dewaxed rice straw with ethanol–H_2O (60/40, v/v) under acid catalyst (0.2 *N* HCl) at 70°C for 4 hr resulted in a dissolution or degradation of 48.8, 24.9, and 23.3% of the original lignin, hemicelluloses, and cellulose, respectively. Obviously, cellulose degradation to soluble products became significant under the acidic condition given (0.2 *M* HCl). This suggested that the concentration of the mineral acid has to be as low as possible in order to prevent any degradation of cellulose from rice straw, because the catalytic effect of mineral acid is dictated by the hydrogen ion concentration it provided to the medium (8). It has been reported previously that furanosides are more acid labile than pyranosides (28) and that the β(1 → 4) xylopyranose chains and arabinofuranosyl side chains in Graminaea are acetylated (29). These arabinofuranosyl residues are acid labile and are removed during treatment with mineral acid of low molarity. As the molarity increases, the more resistant xylopyranoside backbone is hydrolyzed (30). In addition, the pre-treatment removed 67.7% of the original silica from rice straw. As the silica is concentrated in the outer parts of rice straw, it is very likely that pre-treatment with selected chemicals such as 60% aqueous ethanol may be very effective for desilication from this straw.

As shown in Table 1, the following treatment with 2% H_2O_2 at pH 11.5 for 16 hr at 45°C led to a dissolution or degradation of 44.7, 55.2, and 12.1% of the original lignin, hemicelluloses, and cellulose, respectively. Clearly, pre-treatment with acidic organosolv favored degradation of lignin macromolecules, while post-treatment with alkaline peroxide favored solubility of hemicellulosic polymers. In this case, the hydroxide ion also disrupted the internal hydrogen

Table 1. The Yield of Lignin and Hemicelluloses (% Dry Matter) Solubilized in Pre-treatment of Dewaxed Rice Straw with Ethanol–H_2O (60/40, v/v) Under Acid Catalyst (0.2 *N* HCl) at 70°C for 4 hr and Post-treatment with 2% H_2O_2 at pH 11.5 for 16 hr

Lignin, Hemicelluloses, or Cellulose	Yield (%)
Total solubilized lignin in pre- and post-treatments	11.5
Total solubilized hemicelluloses in pre- and post-treatments	29.8
Total degraded cellulose in pre- and post-treatments	12.9
Residue	28.5
Total solubilized lignin in pre-treatment[a]	6.0
Acid-insoluble lignin[b]	4.2
Lignin associated in the isolated hemicelluloses	0.8
Acid-soluble lignin[c]	1.0
Hemicelluloses solubilized in pre-treatment[a]	8.4
Cellulose degraded in pre-treatment[a]	8.5
Total solubilized lignin in post-treatment[d]	5.5
Acid-insoluble lignin[b]	3.5
Lignin associated in the isolated hemicelluloses	1.0
Acid-soluble lignin[c]	1.0
Hemicelluloses solubilized in post-treatment[d]	21.4
Cellulose degraded in post-treatment[d]	4.4
Lignin in the post-treated residue (crude cellulose)[e]	0.9
Hemicelluloses in the post-treated residue (crude cellulose)[e]	4.0

[a] Represent for the lignin, hemicellulosic, or cellulose fraction degraded during the pre-treatment of dewaxed rice straw with ethanol–H_2O (60/40, v/v) under acid catalyst (0.2 *N* HCl) at 70°C for 4 hr.

[b] Represent for acid-insoluble lignin fraction obtained by precipitation of the supernatant solution at pH 1.5 after isolation of the solubilized hemicelluloses and degraded cellulose.

[c] Represent for acid-soluble lignin fraction that is still solubilized in the supernatant (pH 1.5) after precipitation of the acid-insoluble lignins.

[d] Represent for lignin, hemicellulosic, or cellulosic fractions degraded during the post-treatment.

[e] Represent for the content of lignin or hemicelluloses in the two-stage treated residues.

bonds of the crystalline cellulose microfibril, thereby resulting in further degradation by peroxide. In other words, the acidic organosolv pre-treatment promoted cleavage of α-aryl ether bonds in lignin. However, when approximately half of the original lignin was degraded previously in the first stage of treatment, the action of the alkaline peroxide, during the second stage of

treatment, appeared to concentrate on the hemicellulosic breakdown, thus limiting further degradation of cellulose. Similarly, the post-treatment also removed 30.8% of the original silica. In short, the two-stage treatment together resulted in a dissolution or degradation of 93.5, 88.2, and 35.3% of the original lignin, hemicelluloses, and cellulose under the given conditions, respectively.

Interestingly, as expected, the acid-insoluble lignin fraction was the major lignin fraction, comprising 70.0 and 63.6% of the total solubilized lignins in pre- and post-treatment, respectively, while the lignin fraction, associated in the solubilized hemicellulosic preparation, accounted for only 13.3 and 18.2% of the total released lignins. These data revealed that both acidic organosolv pre-treatment and alkaline peroxide post-treatment, under the conditions used, significantly cleaved the ether linkages between lignin and hemicelluloses from the cell walls of rice straw. Furthermore, the two-stage treatment yielded 28.5% of the residue, which was rich in cellulose (82.8%), and contained 14.0% hemicelluloses and 3.2% lignin. The former is significantly affected by the quality of the cellulosic fibers and is considered responsible for several important properties of the fibers, such as tearing resistance and tensile strength (31).

Table 2. The Content of Neutral Sugars and Uronic Acids (Relatively % Sample, w/w) in Isolated Acid-Insoluble Lignin and Hemicellulosic Fractions Obtained from Dewaxed Rice Straw

	Lignin Fractions		Hemicellulosic Fractions	
Sugars (%)	F_1[a]	F_2[a]	F_3[b]	F_4[b]
Rhamnose	Tr[c]	Tr	0.66	0.67
Arabinose	0.36	0.10	2.28	7.29
Xylose	0.62	0.58	15.22	51.46
Mannose	ND[d]	ND	0.53	0.25
Glucose	0.23	0.15	73.86	32.12
Galactose	0.10	Tr	2.60	2.27
Uronic acids	Tr	Tr	4.6	5.3
Total	1.31	0.83	99.75	99.36

[a] Fractions 1 (F_1) and 2 (F_2) represent the acid-insoluble lignin fractions extracted successively with ethanol–H_2O (60/40, v/v) under acid catalyst (0.2 *N* HCl) at 70°C for 4 hr and 2% H_2O_2 at pH 11.5 for 16 hr at 45°C from dewaxed rice straw.

[b] F_3 and F_4 represent the solubilized hemicellulosic preparations extracted successively with ethanol–H_2O (60/40, v/v) under acid catalyst (0.2 *N* HCl) at 70°C for 4 hr and 2% H_2O_2 at pH 11.5 for 16 hr at 45°C from dewaxed rice straw.

[c] Tr, trace.

[d] ND, not detected.

Contents of neutral sugars and uronic acids in two acid-insoluble lignin fractions and two hemicellulosic preparations are given in Table 2. Evidently, the two lignin fractions contained rather low amounts of chemically linked polysaccharides as shown by 1.3 and 0.8% neutral sugar content. Xylose, arabinose, and glucose were identified as the main sugar components. A slightly higher content of polysaccharides in lignin fraction F_1 indicated that there were more chemical linkages between lignin and hemicelluloses in F_1 than in F_2, which was stable to acid hydrolysis. Hydroxycinnamic acids, such as *p*-coumaric acid and ferulic acid, present in rice straw might be favorable to this kind of linkages (32,33), while the post-treatment with alkaline peroxide, under the condition used, substantially saponified these ester linkages between hydroxycinnamic acids and lignin or polysaccharides.

The composition of neutral sugars in two hemicellulosic fractions showed significant differences. The acidic organosolv soluble hemicellulosic fraction F_1 consisted mainly of glucose (73.9%) together with a small amount of xylose (15.2%) and minor quantities of galactose (2.6%) and arabinose (2.3%). These data implied that the hemicellulosic fraction F_1, solubilized during the acidic organosolv pre-treatment, comprised significant amounts of α-glucans together with small amounts of xylans. In contrast, xylose (51.5%) was the major sugar in the alkaline peroxide soluble hemicellulosic fraction F_4 with glucose (32.1%) and arabinose (7.3%) as the second and third sugars in addition to less amounts of galactose, rhamnose, and mannose. This observation suggested that the hemicellulosic fraction F_4, isolated by post-treatment with alkaline peroxide from rice straw, contained higher amounts of xylans than those of α-glucans. In addition, as can be seen in Table 2, the hemicellulosic fraction F_4 also contained a slightly higher proportion of uronic acids (5.3%), mainly glucuronic acid or 4-*O*-methyl-D-glucuronic acid (MeGlcA) than that of the fraction F_3 (4.6%), solubilized during the pre-treatment with acidic organosolv. These differences in neutral sugars and uronic acids between the two hemicellulosic fractions revealed that the treatment conditions of rice straw had a significant influence on the solubilized hemicellulosic compositions.

The yield and composition of phenolic acids and aldehydes from alkaline nitrobenzene oxidation of the isolated two lignin fractions and two hemicellulosic preparations are listed in Table 3. A total yield of the phenolic products from the acidic organosolv soluble lignin fraction F_1 was higher than that of alkaline peroxide soluble lignin fraction F_2, indicating a higher degree of condensation of the latter lignin fraction F_2 than that of the former lignin preparation F_1. In addition, the lower yield of F_2 could also be explained either as the removal of hydroxycinnamic acids, e.g., *p*-coumaric and ferulic acids, ester linked to the protolignin, which exist in a remarkable amount in rice straw lignin, or as the occurrence of condensation between the lignin units during the post-treatment with alkaline peroxide. Nevertheless, protolignin of rice straw gave vanillin and

Table 3. The Yield (% Sample, w/w) of Phenolic Acids and Aldehydes from Alkaline Nitrobenzene Oxidation of the Isolated Four Fractions Obtained from Dewaxed Rice Straw

	Lignin Fractions		Hemicellulosic Fractions	
Phenolic Acids and Aldehydes	F_1[a]	F_2[a]	F_3[b]	F_4[b]
p-Hydroxybenzoic acid	0.38	1.62	0.41	0.29
p-Hydroxybenzaldehyde	3.48	2.65	0.16	0.10
Vanillic acid	1.35	0.84	0.018	0.013
Syringic acid	3.81	3.04	0.084	0.12
Vanillin	11.70	9.89	0.51	0.44
Syringaldehyde	10.01	7.12	0.42	0.38
Acetovanillin	1.30	0.29	0.19	0.10
p-Coumaric acid	4.01	1.67	0.054	0.022
Acetosyringone	0.62	0.12	0.042	0.020
Ferulic acid	1.81	1.64	0.078	0.031
Total	38.47	28.88	1.97	1.52
Klason lignin (%)	86.4	80.1	4.9	3.8

[a] Fractions 1 (F_1) and 2 (F_2) represent the acid-insoluble lignin fractions extracted successively with ethanol–H_2O (60/40, v/v) under acid catalyst (0.2 *N* HCl) at 70°C for 4 hr and 2% H_2O_2 at pH 11.5 for 16 hr at 45°C from dewaxed rice straw.

[b] F_3 and F_4 represent the solubilized hemicellulosic preparations extracted successively with ethanol–H_2O (60/40, v/v) under acid catalyst (0.2 *N* HCl) at 70°C for 4 hr and 2% H_2O_2 at pH 11.5 for 16 hr at 45°C from dewaxed rice straw.

syringaldehyde as dominant products accompanied with fewer *p*-hydroxybenzaldehyde and their corresponding aromatic acids in minor quantities. In comparison, the two lignin fractions yielded a higher amount of vanillin than that of syringaldehyde by 1.7 and 2.8%. These results implied that lignin in rice straw was rich in guaiacyl (G) units, and G units constituted an important portion in high-reactive lignin, and could be easily removed during the treatments, while the syringyl (S) units had a great stability and were difficult to degrade under the given treatment conditions. Similar observations have been reported by Chen et al. (34) in the study of rice straw lignins with NaOH–oxygen pulping.

Table 3 also gives the composition of phenolic monomers obtained by alkaline nitrobenzene oxidation of the chemically linked lignin in solubilized two hemicellulosic preparations. The major products were identified to be vanillin (0.44–0.51%), syringaldehyde (0.38–0.42%), and *p*-hydroxybenzoic acid (0.29–0.41%), which together represented 68.0–73.0% of the total phenolic acids and aldehydes. Small amounts of *p*-hydroxybenzaldehyde (0.10–0.16%)

and acetovanillone (0.10–0.19%), and traces of vanillic acid, syringic acid, acetosyringone, *p*-coumaric acid, and ferulic acid were also detected in the mixtures of alkaline nitrobenzene oxidation. A relatively lower amount of associated lignin in alkaline peroxide soluble hemicellulosic preparation F_4 (3.8%) than that of the acidic organosolv soluble hemicellulosic fraction F_3 (4.9%) implied that the α-benzyl ether linkages between lignin and polysaccharides were significantly cleaved during the sequential treatments, and this cleavage was particularly high during the post-treatment with alkaline peroxide under the condition used.

It is well known that hydroxycinnamic acids, such as *p*-coumaric and ferulic acids, occur widely in the cell wall of monocotyledonous plants, and many studies (34,35) have been made with respect to the composition of these phenolic acids and their association with lignin and polysaccharides. It was suggested that the concentration of *p*-coumaric and ferulic acids in rice internodes was similar to those of the wheat samples. The content of ester-linked *p*-coumaric acid, however, was significantly higher than that in wheat internodes, but considerably lower than in the internode cell walls of maize and sorghum stems (36). It was found that the ferulic acids are deposited in the developing primary cell walls as feruloyl-arabinoxylans by ester bonds. These FA esters are subsequently linked to lignin monomers via a peroxidase-catalyzed reaction to form ether and other free radical derived linkages (37). In other words, 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 becomes a predominant constituent of hydroxycinnamic acids, mainly ester linked to lignin (38). The rice straw, investigated in this study, contained 0.86% *p*-coumaric acid and 0.87% ferulic acid, in which about 56% *p*-coumaric acid was identified to be esterified to lignin and/or polysaccharides, and approximately 60% ferulic acid was detected to be ester linked to hemicelluloses or lignin (39).

Molecular Weight

With GPC analyses, the values of 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 two acid-insoluble lignin preparations and two hemicellulosic fractions are given in Table 4. Between the two lignin fractions, the acidic organosolv soluble lignin fraction F_1 had a much lower $\bar{M}_w$ of 2580 g mol^{-1} than that of the alkaline peroxide soluble lignin fraction F_2 ($\bar{M}_w$, 3880 g mol^{-1}). A similar phenomenon was observed between the two hemicellulosic fractions. The alkaline peroxide soluble hemicellulosic fraction F_4 gave a much higher degree of polymerization with a $\bar{M}_w$ value of 36,270 g mol^{-1} than that of the hemicellulosic fraction F_3 ($\bar{M}_w$, 15,900 g mol^{-1}), solubilized during the acidic organosolv pre-treatment.

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 Lignin and Hemicellulosic Fractions Isolated Sequentially with Ethanol–H_2O (60/40, v/v) at 70°C for 4 hr and 2% H_2O_2 at pH 11.6 for 16 hr at 45°C from Dewaxed Rice Straw

	Lignin Fractions		Hemicellulosic Fractions	
	F_1[a]	F_2[a]	F_3[b]	F_4[b]
$\bar{M}_w$	2580	3880	15,900	36,270
$\bar{M}_n$	2230	2780	14,550	10,610
$\bar{M}_w/\bar{M}_n$	1.16	1.40	1.09	3.42

[a] Fractions 1 (F_1) and 2 (F_2) represent the acid-insoluble lignin fractions extracted successively with ethanol–H_2O (60/40, v/v) under acid catalyst (0.2 *N* HCl) at 70°C for 4 hr and 2% H_2O_2 at pH 11.5 for 16 hr at 45°C from dewaxed rice straw.

[b] F_3 and F_4 represent the solubilized hemicellulosic preparations extracted successively with ethanol–H_2O (60/40, v/v) under acid catalyst (0.2 *N* HCl) at 70°C for 4 hr and 2% H_2O_2 at pH 11.5 for 16 hr at 45°C from dewaxed rice straw.

This relatively low molecular weight of the former lignin fraction F_1 and the hemicellulosic preparation F_3 indicated that the organosolv pre-treatment under the acidic condition used resulted in a noticeable degradation of the macromolecular structures of lignin and hemicelluloses, while the post-treatment with alkaline peroxide, under the given condition, did not lead to any significant degradation of the macromolecular structures of both lignin and hemicelluloses. In addition, a similar lower value of the polydispersity of the former lignin fraction F_1 (1.16) and the hemicellulosic preparation F_3 (1.09) indicated that the lignin and hemicelluloses, solubilized during the pre-treatment with acidic organosolv, had a more narrow molecular weight distribution, whereas the latter fractions of lignin and hemicelluloses with a polydispersity of 1.40 and 3.42, solubilized during the post-treatment with alkaline peroxide, had a more broad molecular weight distribution.

Spectroscopic Characterization

The two lignin and two hemicellulosic samples were also characterized by spectroscopic methods, which constitute important tools in connection with the characterization of the polymers. In this study, we focused on UV, FT-IR, and ^{13}C-NMR spectroscopic methods. Figure 2 shows UV absorption spectra of the two acid-insoluble lignin fractions F_1 and F_2, degraded during the acidic organosolv pre-treatment and alkaline peroxide post-treatment, respectively.

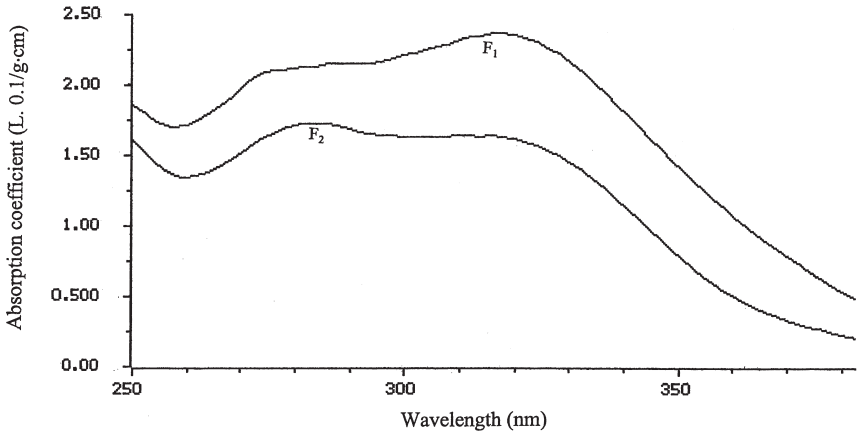

Figure 2. Ultraviolet spectra of the acid-insoluble lignin fractions obtained by pre-treatment with ethanol–H_2O (60/40, v/v) at 70°C for 4 hr (spectrum F_1) and post-treatment with 2% H_2O_2 at pH 11.6 for 16 hr at 45°C (spectrum F_2) from dewaxed rice straw.

Clearly, the two lignin fractions exhibited the basic UV spectrum typical of lignins with two maxima at 280 nm, originating from nonconjugated phenolic groups in the lignin, and 318 nm, resulting from the *p*-coumaric and ferulic acids in the lignins (32). A much higher extinction coefficient at 318 nm in the spectrum F_1 as compared to that in the spectrum F_2 indicated that the pre-treatment of the dewaxed rice straw with 60% aqueous ethanol, under the acidic condition used, only partially cleaved the linkages between lignin and hydroxycinnamic acids, whereas the much lower absorption coefficients at 280 and 318 nm in spectrum F_2 implied that the post-treatment with 2% H_2O_2, under the alkaline condition given, significantly cleaved the chemical bonds between lignin and *p*-coumaric and ferulic acids. Furthermore, this lower absorption coefficient at 280 nm in spectrum F_2 was also probably due to the higher amounts of co-precipitated nonlignin materials, such as ash and salts.

Figure 3 shows the FT-IR spectra of the acidic organosolv soluble lignin (spectrum a) and alkaline peroxide soluble lignin (spectrum b). The relative intensities of the bands for aromatic skeleton vibrations, assigned at 1613, 1520, 1467, and 1427 cm^{-1}, were similar in the two spectra of lignin fractions, indicating a similar lignin structure. However, the changes of the carbonyl absorption region and particularly in the C—O stretching and aromatic C—H in-plane deformation regions might enable the evaluation of the structural differences in the two lignin fractions. A shoulder at 1726 cm^{-1} was assigned to unconjugated carbonyls, and the absorbance of this shoulder was relatively intensive for the lignin fraction F_1, solubilized during the acidic organosolv pre-

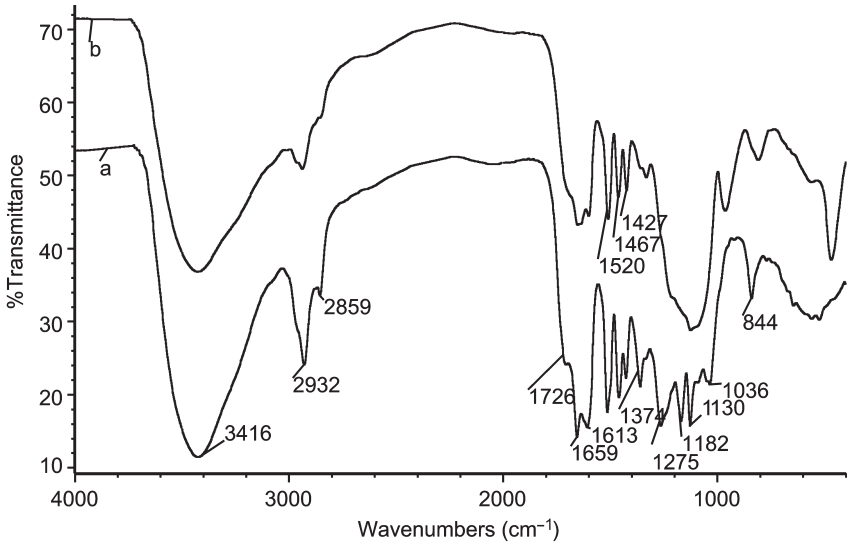

Figure 3. The FT-IR spectra of lignin fractions extracted sequentially with ethanol–H_2O (60/40, v/v) at 70°C for 4 hr (spectrum a) and 2% H_2O_2 at pH 11.6 for 16 hr at 45°C (spectrum b) from dewaxed rice straw.

treatment. Similarly, the sharp band at 1659 cm^{-1}, due to the conjugated carbonyl groups, was stronger for F_1 fraction than for F_2 fraction, degraded during the alkaline peroxide post-treatment. Further evidence for more carbonyl moieties in F_1 fraction was the absorbance at 1182 cm^{-1} due to the —C—O in ester groups (conj.), which is also typical for annual cereal straw and grass lignins (40,41). Thus, the acidic organosolv soluble lignin fraction F_1 had more carbonyl groups than the alkaline peroxide soluble lignin preparation F_2. On the other hand, a much stronger absorbance between 1200 and 1050 cm^{-1} in spectrum b than in spectrum a suggested that the lignin fraction F_2, degraded during the post-treatment with alkaline peroxide, contained noticeable amounts of nonlignin materials, e.g., silica, which corresponded to a relatively lower Klason lignin content in this lignin fraction given in Table 3.

In agreement with the chemical analysis, the FT-IR spectrum of the two hemicellulosic fractions F_3 and F_4, shown in Fig. 4, clearly shows the typical signal pattern expected for a hemicellulosic moiety. In particular, the spectra are dominated by signals in the region 3600–2800 cm^{-1}, corresponding to stretching vibrations of OH and CH, and by signals in the C—O stretching region (1200–950 cm^{-1}). In the carbonyl stretching region, a band at 1646 cm^{-1} is due to the absorbed water. In the anomeric region (950–700 cm^{-1}), two bands for

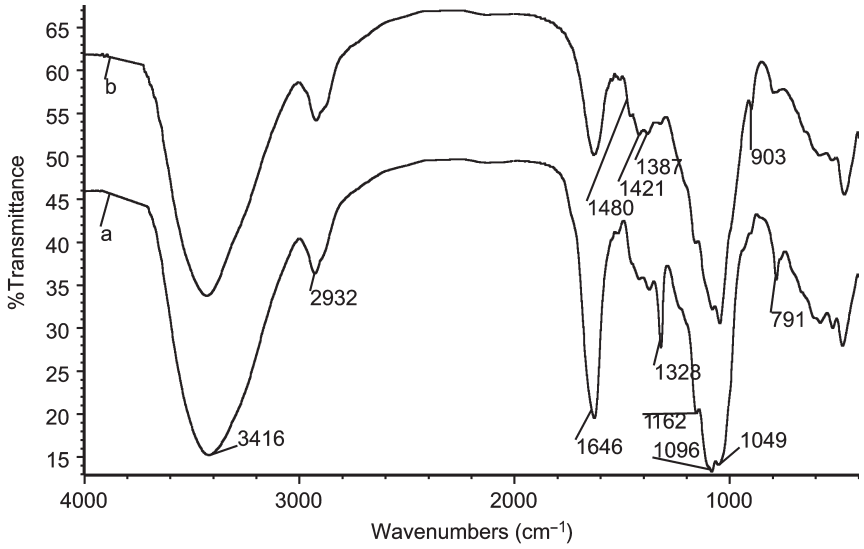

Figure 4. FT-IR spectra of hemicellulosic fractions extracted sequentially with ethanol–H_2O (60/40, v/v) at 70°C for 4 hr (spectrum a) and 2% H_2O_2 at pH 11.6 for 16 hr at 45°C (spectrum b) from dewaxed rice straw.

α-linkage (791 cm^{-1}) obviously in spectrum a and β-linkage (903 cm^{-1}) in spectrum b distinguish well between the two glycosidic linkage types of the two hemicellulosic fractions (42,43). The absorbances at 1096, 1049, and 791 cm^{-1}, seen in the spectrum a, are associated with α-glucans, while bands between 1125 and 1000 cm^{-1} are typical of β-xylans. The prominent band, at 1049 cm^{-1}, is attributed to the C—O and C—C stretching, or C—OH bending in hemicelluloses. The presence of arabinosyl side chains is identified by a low intensity band at 1162 cm^{-1}. This observation revealed again that the acidic organosolv soluble hemicellulosic fraction dominated in α-glucans, whereas the alkaline peroxide soluble hemicellulosic preparation contained substantial amounts of β-xylans, which were consistent with the data of sugar analysis.

To further investigate the structural features of the lignins, the acidic organosolv soluble lignin fraction F_1 was studied by ^{13}C-NMR spectroscopy (Fig. 5). Most of the observed signals have been assigned previously in straw and wood lignin spectra by Scalbert et al. (32), Chen et al. (34), Himmelsbach and Barton (44), and Nimz et al. (45). The signals (ppm) at 181.9, 174.6 (data not shown), 172.8 (data not shown), and 171.3 (data not shown) belong to aliphatic COOH. The signals (ppm) at 166.1 (C-γ, PC ester, data not shown), 159.8 (C-4, PC ester), 130.3 (C-2/C-6, PC ester), 125.1 (C-1, PC ester), and 115.9 (C-3/C-5,

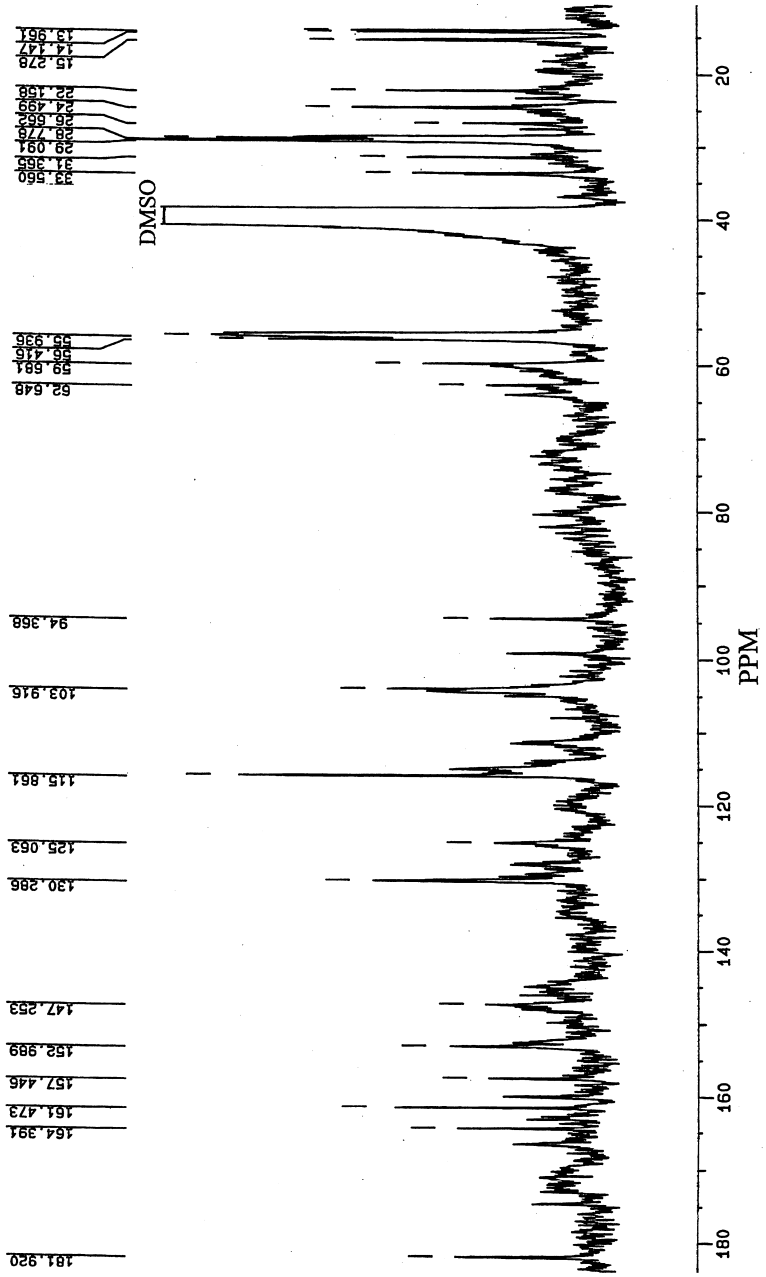

Figure 5. ^{13}C-NMR spectrum of lignin fraction F_1 solubilized during pre-treatment with ethanol–H_2O (60/40, v/v) at 70°C for 4 hr from dewaxed rice straw.

PC ester) are indicative of the esterified *p*-coumaric acid. The absence of etherified ferulic acid signals could be the result of the removal of substantial amounts of etherified ferulic acid by 60% aqueous ethanol pre-treatment under the acidic conditions. It is possible that the acidic organosolv pre-treatment cleaved almost all of the ether bonds between ferulic acid and lignin.

The aromatic carbon atoms in lignin appeared in the region between 103.9 and 160.0 ppm. Guaiacyl residues were identified by signals (ppm) at 149.0 (C-3, G etherified, data not shown), 147.3 (C-4, G etherified), 145.2 (C-4, G nonetherified, data not shown), 114.8 (C-5, G, data not shown), and 111.5 (C-2, G). The S residues were detected by signals (ppm) at 153.0 (C-3/C-5, S etherified), 147.3 (C-3/C-5, S nonetherified), and 104.3 (C-2/C-6, S). The *p*-hydroxyphenyl (H) residues were verified by a signal at 128.0 ppm (C-2/C-6, H).

In the aliphatic region of the spectrum from 50 to 90 ppm, methoxyl groups give a strong signal at 55.9 ppm. Carbon atoms β, α, and γ in β-*O*-4 structures absorb at 82.0, 72.3, and 59.7 ppm, respectively. Weaker signals at 82.0 and 72.3 ppm implied that the acidic organosolv pre-treatment, under the condition given, removed significant amounts of the moieties with structure C-β and C-α in β-*O*-4 linkages. The signals between 14.0 and 33.6 ppm are originated from the γ-methyl, α- and β-methylene groups in *n*-propyl side chains of the lignin. The signals at 99.0 (C-1, α-D-glucopyranosiduronate, data not shown) and 62.6 ppm (C-5, xylose internal unit) arise from the associated polysaccharides in the lignin fraction.

The ^{13}C-NMR spectrum of the alkaline peroxide soluble hemicellulosic fraction F_4 is shown in Fig. 6. The spectrum was interpreted on the basis of previously reported data for structurally defined arabinoxylan type, glucuronox-

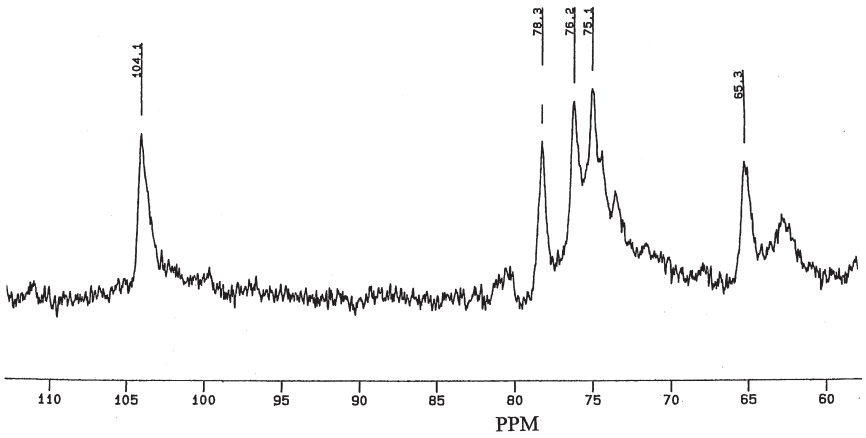

Figure 6. ^{13}C-NMR spectrum of hemicellulosic fraction F_4 solubilized during post-treatment with 2% H_2O_2 at pH 11.6 for 16 hr at 45°C.

ylan type, and L-arabino-(4-*O*-methyl-D-glucurono)-D-xylan (46–49). The main 1,4-linked β-D-Xyl*p* units are characterized by the strong signals at 104.1, 78.3, 76.2, 75.1, and 65.3 ppm, which are, respectively, assigned to C-1, C-4, C-3, C-2, and C-5 of the β-D-Xyl*p* units. The signals at 81.0 and 63.2 ppm (data not shown) represent C-4 and C-6 of the α-glucan. A small signal at 112.0 ppm (data not shown) is indicative of C-1 of α-L-Ara*f* residues. An additional chemical shift at 73.5 ppm (data not shown) arises from C-5 of glucuronic acid residues. These observations revealed that the hemicellulosic fraction F_4, solubilized during the alkaline peroxide post-treatment, consisted of substantial amounts of L-arabino-(4-*O*-methyl-D-glucurono)-D-xylan and noticeable quantities of α-glucan. However, this observation needs to be further confirmed by methylation analysis.

Thermal Analysis

The thermal properties of the two lignin fractions and two hemicellulosic preparations were investigated by TGA and DSC. Figure 7 shows the thermograms of the alkaline peroxide soluble lignin fraction F_2. As can be seen from Fig. 7, the lignin fraction decomposed at about 200°C. At 10% weight loss, the decomposition temperature of the lignins was observed at 295°C. The

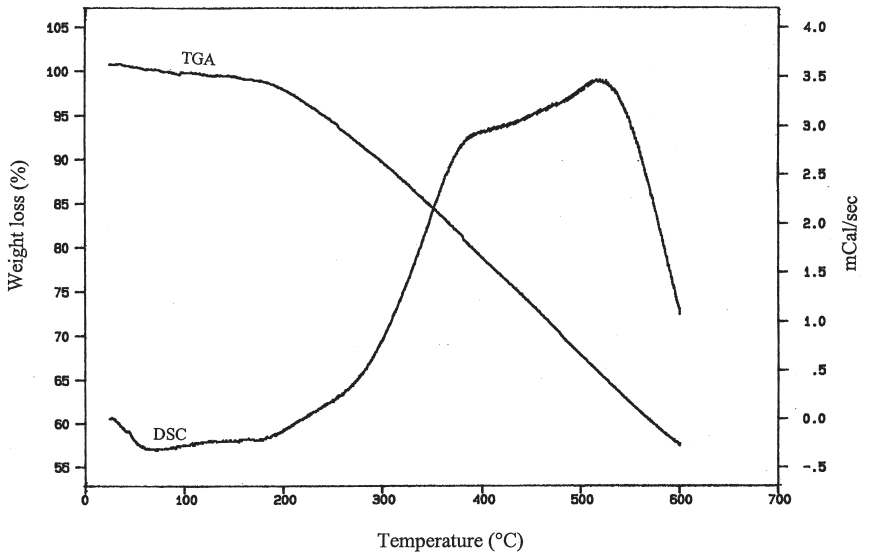

Figure 7. Thermogram of the acid-insoluble lignin fraction F_2 solubilized during the post-treatment with 2% H_2O_2 at pH 11.6 for 16 hr at 45°C.

decomposition process of the lignin fraction covered a large temperature range, between 200 and 600°C, although at temperatures lower than 570°C, only 40% was decomposed rendering charcoal as the residue product. The DSC curve showed that the exothermic reaction of the lignin occurred mainly between 300 and 600°C.

The two hemicellulosic fractions, solubilized during the acidic organosolv pre-treatment (Fig. 8a) and the alkaline peroxide post-treatment (Fig. 8b), started to decompose at a temperature of 243 and 205°C, respectively. However, at 50% weight loss, the decomposition temperature of the two hemicellulosic fractions was observed at 324 and 446°C in Fig. 8a and b, respectively. This latter weight loss correlated well with the hemicellulosic molecular weights given in Table 4 showing an increase in the thermal stability with an increment in molecular weight. In addition, the two DSC curves showed that the acidic organosolv soluble hemicellulosic fraction F_3 gave an exothermic peak centered at 302°C, while the alkaline peroxide soluble hemicellulosic fraction F_4 exhibited an exothermic peak maximized at 503°C due to the exothermic reaction of the polymers (50) and corresponding to the increasing trend of the molecular weights.

CONCLUSIONS

The two-stage treatment has been shown to be convenient and useful in the fractional separation of lignins and hemicelluloses from rice straw. Pre-treatment with 60% aqueous ethanol, under the acidic condition used, degraded more lignins than the hemicelluloses, while the post-treatment with 2% H_2O_2, under the alkaline condition given, solubilized more hemicelluloses than the lignin polymers. Both acidic organosolv pre-treatment and alkaline peroxide post-treatment, under the conditions used, significantly cleaved the ether linkages between lignin and hemicelluloses from the cell walls of rice straw. The two lignin fractions contained higher amounts of noncondensed G units than those of noncondensed S units, together with small amounts of *p*-hydroxyphenyl units. They are relatively free of chemically linked polysaccharides, while the alkaline peroxide soluble lignin fraction contained relatively higher amounts of co-precipitated nonlignin materials, such as ash and salts, as compared to the acidic organosolv soluble lignin fraction. In addition, the acidic organosolv pre-treatment, under the condition given, removed significant amounts of the moieties with structure C-β and C-α in β-*O*-4 linkages, resulting in the lignin fraction having a lower molecular weight. *p*-Coumaric acid was identified to be ester linked to lignin molecules in the cell wall of rice straw.

The results also showed that the hemicellulosic fraction, solubilized during the 60% aqueous ethanol, was mainly composed of α-glucan and had a lower molecular weight ($\bar{M}_w$, 15,900 g mol^{-1}), while the hemicellulosic preparation,

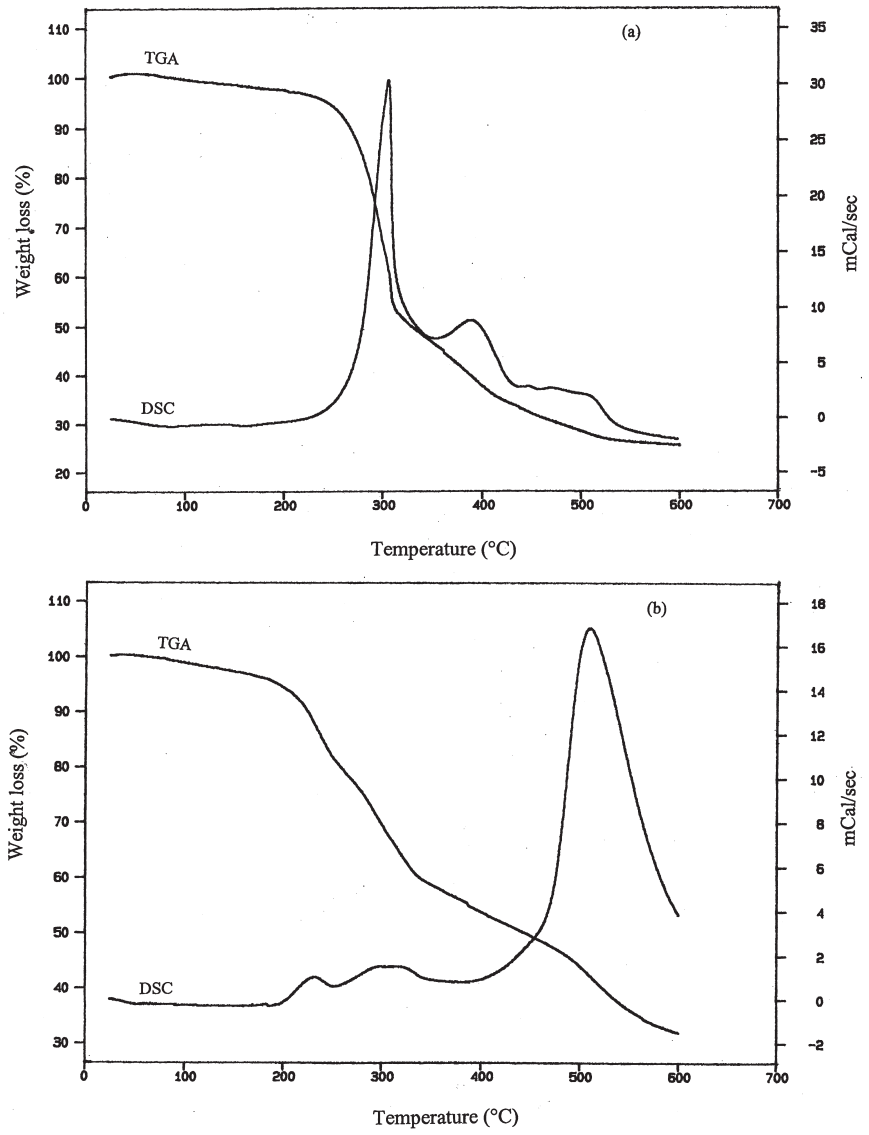

Figure 8. Thermograms of hemicellulosic fractions solubilized during pre-treatment with ethanol–H_2O (60/40, v/v) at 70°C for 4 hr (a) and post-treatment with 2% H_2O_2 at pH 11.6 for 16 hr at 45°C (b) from dewaxed rice straw.

obtained during the 2% H_2O_2 post-treatment under the alkaline condition given, comprised L-arabino-(D-glucurono)-D-xylan as the major constituent and had a higher molecular weight ($\bar{M}_w$, 36,270 g mol^{-1}). The thermal analysis showed that the two hemicellulosic preparations decomposed significantly between 200 and 330°C. The decomposition process of the lignins covered a larger temperature range between 200 and 600°C.

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