Physicochemical Characterization of Lignins from Rice Straw by Hydrogen Peroxide Treatment

RUNCANG SUN,[1] J. TOMKINSON,[1] F. C. MAO,[2] X. F. SUN[2]

[1] The BioComposites Centre, University of Wales, Bangor, Gwynedd, LL57 2UW, United Kingdom

[2] The North-western University of Agricultural and Forest Sciences, Yangling, People's Republic of China

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ABSTRACT: Extraction of the dewaxed rice straw with 1% NaOH at 55°C for 2 h and following treatment without and with 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0% hydrogen peroxide (H_2O_2) at 45°C for 12 h at pH 11.5 resulted in a dissolution of 68.3, 85.4, 89.4, 92.3, 92.3, 94.3, and 95.1% of the original lignin, respectively. Meanwhile, the two-stage treatment together solubilized 67.2, 77.2, 78.7, 83.7, 85.5, 87.3, and 88.5% of the original hemicelluloses and degraded 2.5, 9.8, 11.8, 12.1, 15.6, 16.4, and 17.8% of the original cellulose under the conditions given, respectively. Analyses of these lignins revealed that alkali-soluble lignin fractions did not suffer sever oxidation, but nearly 60% of the original lignin was dissolved out during the first stage of alkali treatment. In the second stage of alkaline peroxide treatment, the residual lignins were substantially released and enriched in oxidized carbonyl and carboxyl groups. In comparison, the isolated eight pure lignin samples were further characterized by both destructive methods such as alkaline nitrobenzene oxidation and nondestructive techniques such as ultraviolet (UV), Fourier transform infrared (FTIR), and carbon-13 magnetic resonance spectroscopy (^{13}C-NMR) as well as gel permeation chromatography (GPC), and the results are reported. © 2000 John Wiley & Sons, Inc. J Appl Polym Sci 79: 719–732, 2001

Key words: rice straw; hydrogen peroxide; lignin; oxidation; phenolic acids and aldehydes

INTRODUCTION

Rice, *Oryza sativa L.,* is the third gramineous species after maize, *Zea mays L.,* and sorghum, *Sorghum bicolor L.,* and its corresponding straw production averaged about 629 $\times$ 10^6 Mg per year.[1,2] These large quantities of fibrous crop residues are currently under-utilized as the raw material for paper making and as the potential animal feed sources in developing countries. The digestibility in pulping and in animal stomachs is affected greatly by the content and properties of the lignin.[3]

Research has sought to improve the nutritive value and pulp quality by chemical, physical, and enzymatic treatments to remove lignin and decrease cellulose crystallinity. Moreover, processes such as autohydrolysis, alkaline cooking, and steam explosion require substantial energy input in the form of heat and tend to generate toxic side products. Another drawback typical of conventional treatments includes loss of the hemicelluloses with the oxidized products such as aliphatic carboxylic acids. Among the chemical treatments, oxidative treatments are directed to principally affect the degradation of lignin, whereas hydro-

Correspondence to: R. Sun.

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lytic agents are expected to cleave the lignin–carbohydrate linkages. Coupling of the hydrolysis and the oxidative treatment was shown to achieve a better delignification efficiency.[4]

As an alternative, alkaline peroxide treatment may offer a more practical and environmentally benign approach toward the improvement of these shortages by selective delignification. Hydrogen peroxide is known to react with lignin under certain conditions and has been widely used for many years to bleach high-lignin wood pulps. More recently, some Swedish, Spanish, and Canadian mills started the commercial production of bleached kraft pulps using sequences containing at least one extensive peroxide delignification stage.[5] Lachenal et al.[6] found that at 80–120°C under alkaline conditions hydrogen peroxide will delignify kraft pulps with partial degradation of the cellulose. However, the requisite for high energy and reagent inputs diminish the appeal of applying this process to agricultural residues. Gould[7] reported that approximately one-half of the lignin and most of the hemicelluloses present in agricultural residues such as wheat straw and corn stover are solubilized when the residue is treated at 25°C in an alkaline solution of hydrogen peroxide. The delignification reaction is most efficient when the ratio of hydrogen peroxide to substrate is at least 0.25 (w/w) and the pH is 11.5. Further studies showed that hydroperoxide anion (HOO^-), formed in the alkaline media, is the principal active species in hydrogen peroxide bleaching processes. This anion is a strong nucleophile that, during bleaching, preferentially attacks ethylenic and carbonyl groups present in lignin. As a consequence, such chromophores as quinones, cinnamaldehyde, and ring-conjugated ketones are converted to nonchromophoric species under the alkaline conditions. On the other hand, hydrogen peroxide is unstable in alkaline conditions and readily decomposed to more active radicals such as hydroxyl and superoxide anion radicals ($HO^\bullet$, $O_2^{-\bullet}$), which participate in the delignifying mechanism.[8]

The advantages of delignification with hydrogen peroxide are low investment cost and the accompanying strong bleaching effect. In addition, the degraded or solubilized lignins during the alkaline peroxide treatment under mild conditions are both theoretically interesting for lignin structural studies and commercially interesting for the cosmetics, adhesions, and pharmaceutical industries. In other words, delignification using alkaline peroxide might enable the utilization of by-products and therefore a more complete use of the raw material.

As an extensive investigation of using rice straw lignin as a novel polymer material for industrial utilizations, a thorough study of its isolation and structural characterization of the solubilized lignin are necessary. As the first part of this program, in this article, the alkaline peroxide treatment was characterized with respect to the H_2O_2 concentration affecting delignification efficiency and the solubilization of hemicelluloses from rice straw during the treatment. Structural characterization of the released hemicellulosic fractions will be published elsewhere. The solubilized lignin preparations were analyzed to characterize their physicochemical properties, composition of monomer units, linkages between units, and association with hydroxycinnamic acids or hemicelluloses by using a destructive method such as alkaline nitrobenzene oxidation and nondestructive techniques such as ultraviolet (UV), Fourier transform infrared (FTIR), and carbon-13 magnetic resonance (^{13}C-NMR) spectroscopies.

EXPERIMENTAL

Materials

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

Alkaline Peroxide Treatment

The dried powder was first extracted with toluene–ethanol (2:1, v/v) in a Soxhlet for 8 h. The dewaxed straw was then soaked in a 1% NaOH solution with a 1:25 straw-to-liquor ratio at 55°C for 2 h. After isolation of the alkali-soluble hemicelluloses by precipitation of alkali extracts in 3 vol ethanol, an alkali-soluble lignin preparation was obtained by reprecipitation at pH 1.5, adjusted by 10% HCl, from the supernatant solution. Samples free of wax and alkali solubles (5.0

g) were added to 250 mL of distilled water containing 0.0 (absence of H_2O_2), 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0% H_2O_2 (w/v) in a jacketed reaction vessel heated with water from a thermostat-controlled circulating bath. The suspension was adjusted to pH 11.5 with 4*M* NaOH and allowed to stir gently for 12 h at 45°C. During the initial stages of stirring, oxygen evolution was active and substantial frothing occurred, requiring that extractions were conducted in vessels with volumes two to three times those of the 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 increasing to a final value of about 13.1. After the indicated period of time, the insoluble residue was collected by filtration, washed repeatedly with distilled water until the filtrate was neutral, and then oven-dried at 60°C for 16 h. The supernatant fluid was subjected to neutralization to pH 5.5 with 10% HCl and subsequently concentrated. The released hemicelluloses were precipitated by pouring the concentrated supernatant fluid into 3 vol of ethanol. The solubilized lignins were obtained from the corresponding supernatants by precipitation at pH 1.5. The lignin preparations were washed with acidified water (pH 2.0), freeze-dried, and named pure lignin (PL) fractions. The preparation procedure is illustrated in Figure 1. Treatment was repeated twice, giving very reproducible yields.

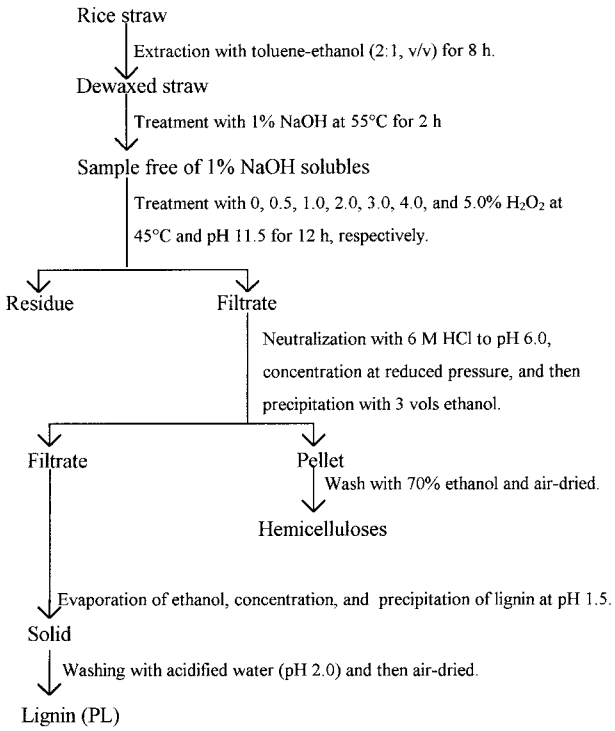

Figure 1 Scheme for extraction of hemicelluloses and lignin from rice straw.

Determination of Ash and Silica in the Straw and Residues

The contents of ash and silica were determined according to the methods described by Pan et al.[9] The sample was transferred to a crucible and carbonized gently in a muffle furnace at 550 ± 10°C for 6 h. The ash obtained was treated with concentrated HCl. The acid-insoluble residue was filtered, washed with hot water until no chloride was detectable, ignited, and finally weighed as silicon dioxide.

Lignin (PL) Analysis

The monomeric composition of the noncondensed monomeric units of these lignin preparations was characterized by nitrobenzene oxidation and analysis of the resulting aromatic aldehydes and acids by high-performance liquid chromatography (HPLC) as previously reported.[10] All nitrobenzene oxidation results represent the mean of at least triplicate samples and each oxidation mixture was chromatographed twice. The hemicellulosic moieties associated with lignin preparations were hydrolyzed with 2*M* trifluoroacetic acid for 2 h at 120°C. Liberated neutral sugars were analyzed as their alditol–acetate derivatives by gas chromatography (GC).[11,12]

UV spectra were recorded on a Hewlett–Packard 8452A diode array spectrophotometer. A lignin sample (5 mg) was dissolved in 95% (v/v) dioxane–water (10 mL). A 2-mL aliquot was diluted to 10 mL with 50% (v/v) dioxane–water, and the absorbances between 250 and 380 nm were measured.

The molecular-average weights of the lignin fractions were determined by gel permeation chromatography on a PLgel 5μ Mixed-D column. The samples were dissolved in tetrahydrofuran at a concentration of 0.2%, and a 200-μL sample in solution was injected. The column was operated at 40°C and eluted with tetrahydrofuran at a flow rate of 1 mL min^{-1}. Monodisperse polystyrene was used as the standard for the molecular weight ($\bar{M}_w$) of lignin.

FTIR spectra were recorded from KBr pellets containing 1% finely ground samples on a Nicolet-

Table I Yield of Lignin (% Dry Matter) Solubilized During Treatment of Rice Straw with 1% NaOH and During Following Treatments Using Various Concentrations of Hydrogen Peroxide

		H_2O_2 Concentrations (%)						
	ASL[a]	0[b]	0.5[b]	1.0[b]	2.0[b]	3.0[b]	4.0[b]	5.0[b]
PL[c]	5.2	0.8	2.1	2.6	2.8	2.7	2.7	2.3
Lignin solubilized in the supernatant (pH 1.5)[d]	1.1	0.2	0.5	0.6	0.8	0.9	1.1	1.6
Lignin associated in the isolated hemicelluloses	0.9	0.2	0.7	0.6	0.6	0.6	0.6	0.6
Total solubilized lignin	7.2	1.2	3.3	3.8	4.2	4.2	4.4	4.5

[a] Abbreviation for the alkali-soluble lignin obtained by treatment of the dewaxed rice straw with 1% NaOH at 55°C for 2 h.
[b] Lignin preparations obtained by treatment of the alkali-treated rice straw with different concentrations of H_2O_2 at 45°C for 12 h at pH 11.5.
[c] Lignin fractions obtained by precipitation of the supernatant solution at pH 1.5 after isolation of the solubilized hemicelluloses.
[d] Lignin fractions which are still solubilized in the pH 1.5 supernatant after precipitation of the PL fractions and obtained by difference.

750 FTIR spectrophotometer. The solution-state ^{13}C-NMR spectrum was obtained on a Bruker 250 AC spectrometer operating in the FT mode at 62.4 MHz under total proton decoupled conditions. They were recorded at 25°C from 250 mg of the sample dissolved in 1.0 mL DMSO-d_6 after 28,000 scans. A 60° pulse flipping angle, a 3.9-μs pulse width, and 0.85-s acquisition time were used.

RESULTS AND DISCUSSION

Lignin Yield

It is impossible to isolate pure lignin quantitatively from cell walls in an intact state and to depolymerize the lignin quantitatively into its building stones. The lignin isolated by known methods is a mixture of lignin from various unidentified morphological regions.[13] Furthermore, the content of lignin appeared different depending on the isolating method used. The yield of Klason lignin, in general, was significantly higher than that of the acid-insoluble lignin. This overestimation of the Klason lignin is probably due to the lignin association with other cell-wall components, while the content of acid-insoluble lignin is undoubtedly underestimated because of the loss of acid-soluble monomeric products and oligomeric fractions in the acidified solution.[1] Hence, the yield of solubilized lignin, given in Table I, includes the acid-insoluble lignin, named pure lignin (PL), acid-soluble lignin (solubilized in the pH 1.5 supernatant), and the lignin associated in the released hemicelluloses. As can be seen in Table I, extraction of the dewaxed rice straw with 1% NaOH at 55°C for 2 h and following the treatment without and with 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0% hydrogen peroxide (H_2O_2) at 45°C for 12 h at pH 11.5 solubilized 8.4, 10.5, 11.0, 11.4, 11.4, 11.6, and 11.7% lignin (percent dry starting material), corresponding to a dissolution of 68.3, 85.4, 89.4, 92.3, 92.3, 94.3, and 95.1% of the original lignin, respectively. Meanwhile, the two-stage treatment together solubilized 67.2, 77.2, 78.7, 83.7, 85.5, 87.3, and 88.5% of the original hemicelluloses and degraded 2.5, 9.8, 11.8, 12.1, 15.6, 16.4, and 17.8% of the original cellulose under the conditions given, respectively. As expected, the isolated PL was the major fraction, composing 51.1–72.2% of the total solubilized lignins, while the lignin fraction, associated in the solubilized hemicelluloses, accounted for only 12.5–21.2% of the total released lignins. This result indicated that the alkaline peroxide treatment under the conditions used significantly cleaved the ether linkages between the lignin and hemicelluloses from the cell walls of rice straw.

Interestingly, the data in Table I shows that prior to alkaline peroxide treatment saponification of the dewaxed rice straw with 1% NaOH at 55°C for 2 h led to the release of 58.5% of the original lignin and 55.0% of the original hemicelluloses. During the alkaline-extracting process, some alkali-labile linkages between lignin monomers, or between lignin and polysaccharides, might be broken by alkali treatment. Acidic moieties such as carboxylic or phenolic groups, ionized in alkaline solution, might also promote the

solubilization of the lignin, either by increasing the solubility of individual fragments or by inducing the swelling of the cell wall.[14] In addition, the participation of ester-linked hydroxycinnamic acids, particularly ferulic acids, as a linkage bridge between lignin and hemicelluloses in straw and grass, is considered to play a important role in the significant dissolution of lignin during the alkali extraction. It has been identified that *p*-coumaric acid is linked mainly to lignin via ester bonds while ferulic acid constitutes a bridge binding to polysaccharides via ester bonds and to lignin via ether bonds.[14] Obviously, such cross-linking has been regarded to have a significant influence on the physicochemical properties of straw and grass lignins. One of the most striking properties is that straw lignin has a high solubility in an alkaline solution. Our earlier studies showed that pretreatment of wheat straw with 1.5% NaOH at 60°C for 6 h resulted in dissolution of 53.8% of the original lignin and 74.7% of the original hemicelluloses.[11] Similar results have reported that 60–70% of the original lignin from wheat straw is extracted with dilute alkali at temperature below 100°C, even at room temperature. In woody materials such as pine and birch, however, these percentages reach only about 20 or 30%, respectively.[15]

It is of interest to note that rice straw has a higher solubility of lignin in a dilute alkaline solution than that of wheat straw. Based on the structural study of rice straw lignin and wood lignin, Chen et al.[16] indicated that lignin condensation also plays a very important factor affecting its solubility in alkaline media. For the wood lignins, the obvious condensations of the lignins result in many macromolecular products, whereas the degradations of the lignins are depressed greatly. For the straw lignin, however, the degradation is not so depressed. The condensation is not as important as in the wood lignins, which, therefore, leads to a significant dissolution of the macromolecular lignin from straw in alkali media.

Another feature of rice straw is its high silica content (9.4%, w/w). It was found that silica has the function of increasing the degree of resistance against pathogens and against lodging of the plants, but may inhibit the degradability, cause rather serious difficulties during the recovery process, and slow drainage of the straw pulp during papermaking. This side effect, however, can be altered by chemical treatment.[17] In the condition of our experiment, treatment with 1% NaOH at 55°C for 2 h removed 62.2% of the original silica from the rice straw. This observation was consistent with the studies on predesilication of wheat straw with 1% NaOH solution by Eroglu and Deniz.[18] The authors revealed that a three-stage treatment with 1% NaOH was the most convenient condition for desilication. In this case, more than 73% silica could be removed under relatively mild alkaline conditions. A photograph shows that silica is concentrated on the surface and only a little is present in the inner part of the stalk. In other words, the silica is located mainly in epidermal cells that are on the surface of the rice straw stalk.[9]

During the pretreatment with dilute alkaline solution, silica was separated from the fibers and appeared in the hydrolysate in the form of sodium silicate or in some other complex compound. Although the handling and cleaning of the raw material prior to cooking can remove a high percentage of silica from the outer surface, the remaining silica content can still be high on entering digester. As the silica is concentrated in the outer parts of cereal straw, it is presumed that pretreatment with selected chemicals such as 1% NaOH solution may be very effective for the desilication of this raw material.[18] Further treatment of the alkali-extracted rice straw with 0.5–5.0% H_2O_2 at 45°C for 12 h at pH 11.5 resulted in about another 26.6% desilication. Hence, the silica content in the two-stage treated rice straw was lower than about 2.7%. It is, therefore, likely that this two-stage treatment with a dilute alkaline solution followed by alkaline peroxide may propose a convenient and effective method for desilication from cereal straws.

As mentioned earlier, hydrogen peroxide is a mild oxidant which is largely used in the bleaching of high-yield pulps and also in chemical-bleaching sequences. Its high efficiency in bleaching and delignification is observed when the reaction is conducted in an alkaline medium.[19] Under these conditions, the active species responsible for the elimination of chromophores in lignin structures, particularly conjugated carbonyl structures, are the hydroperoxide anion, formed in hydrogen peroxide dissociation via an ionic pathway ($H_2O_2 + HO^- \leftrightarrow HOO^- + H_2O$, p$Ka$ = 11.5–11.6 at 25°C). On the other hand, radical species, generated from the decomposition of hydrogen peroxide in alkaline media, are responsible for delignification processes by cleavage of some interunit bonds and, eventually, dissolution of the lignin. In this case, alkaline peroxide decomposes

to hydroxyl radicals ($HO^{\bullet}$) and superoxide anion radicals ($O_2^{-\bullet}$) in the presence as well as in the absence of transition metals ($H_2O_2 + HOO^- \rightarrow HO^{\bullet} + O_2^{-\bullet} + H_2O$). The radicals formed may react further with each other and give rise to oxygen and hydroxyl anions as the final products ($HO^{\bullet} + O_2^{-\bullet} \rightarrow O_2 + HO^-$), which lead to an increase in the reaction pH.[5]

Although the delignification reaction is strongly dependent on pH, with a sharp optimum at pH 11.5, it is not necessary to continuously regulate the reaction pH; even in the case of the alkaline peroxide treatment, the reaction pH increased from 11.5 in the beginning to about 13.1 at the end, since similar levels of delignification were also attained using H_2O_2 at pH > 12.5.[20] Interestingly, as the reaction pH became more alkaline, increasing amounts of hemicelluloses were solubilized and the yield of residue decreased. About 64% of the residual hemicelluloses originally present in the alkali-treated rice straw were solubilized after 12 h of 2% H_2O_2 treatment in the absence of continuous pH control, while only 39% of the originally residual hemicelluloses were released in 12 h when the reaction pH was maintained at pH 11.5 ± 0.2 (data not shown).

Besides the reaction pH, the extent of the delignification reaction was also a function of the H_2O_2 concentration. As can be seen in Table I, in the absence of H_2O_2, only about 24% of the residual lignin originally present in the alkali-treated rice straw was dissolved at pH 11.5 for 12 h at 45°C in the absence of H_2O_2, whereas more than 80% of the residual lignin was solubilized when the alkali-treated straw was further treated with 2.0% H_2O_2 at pH 11.5 for 12 h at 45°C. In the presence of 5.0% H_2O_2, substantially more lignin (nearly 90% originally residual lignin) was solubilized during the conditions given. In contrast, with the study of the effect of the initial H_2O_2 concentration on the degree of delignification from untreated rice straw, Patel and Bhatt[4] indicated that delignification increased to a maximum of 52% at 1% H_2O_2 for 18 h at room temperature. However, further increasing the concentration of H_2O_2 had a marginally adverse effect on the delignification of the substrate, with the delignification efficiency falling to 45% at 4.5% H_2O_2. The reason for this decrease in lignin yield was presumed to be that lignin condensation occurred during the alkaline peroxide treatment at a low ratio of liquid/solid (25/1, v/w) in the Patal and Bhatt's study[4] as compared to our experiments performed at a high ratio of liquid/solid (50/1, v/w). In addition, as can be seen in Table I, although increasing the concentration of H_2O_2 from 0.5 to 5.0% produced a growth of the total solubilized lignin from 1.2 to 4.5%, an increment in H_2O_2 concentration from 0.5 to 2.0% led to a noticeable increase of the PL fraction from 2.1 to 2.8%; however, as the concentration was further increased to 5.0%, a decreasing yield of PL of 17.8% was found. On the other hand, on increasing the concentration of H_2O_2 from 0.5 to 5.0%, the yield of the lignin solubilized in the supernatant (pH 1.5) was enhanced by more than threefold (from 0.5% to 1.6%), indicating that treatment with the relatively high concentration of H_2O_2 over 2.0% under the conditions used could lead to a substantial degradation of the solubilized lignin into small and dilute acid-soluble fragments. This increasing yield of lignin, solubilized in the pH 1.5 supernatant, therefore, offset the negative effect of the H_2O_2 concentration on the yield of PL.

The data in Table I reveal that alkaline peroxide was capable of reducing the lignin content from alkali-treated rice straw significantly at pH 11.5 for 12 h at 45°C. The addition of sodium silicate had no effect on the straw delignification, since, during the alkaline peroxide treatment, the residual silica was separated from the fibers and appeared in the hydrolysates in the form of sodium silicate. This observation was consistent with the finding that improvement in H_2O_2 stabilization was not a prerequired condition for good delignification of soft wood even under particularly severe conditions (120°C).[6] Similar results were reported by Dence and Omori.[21] The authors demonstrated that the use of silicate was deemed superfluous when sufficient sodium hydroxide was available.

UV Absorption

UV spectroscopy has been proved to be useful in the study of lignin distribution among various tissues of gymnosperm and dicotyledonous angiosperm in respect to the concentration. Figure 2 shows UV absorption spectra of alkali lignins (PL fraction) isolated with 1% NaOH at 55°C for 2 h from dewaxed rice straw (spectrum a) and with a dilute alkaline solution (pH 11.5) at 45°C for 12 h in the absence of H_2O_2 from the 1% NaOH-treated rice straw (spectrum b) and alkaline peroxide soluble lignins (PL fraction) extracted with 0.5% H_2O_2 (spectrum c) and 5% H_2O_2 (spectrum d) at 45°C for 12 h at pH 11.5 from the 1% NaOH

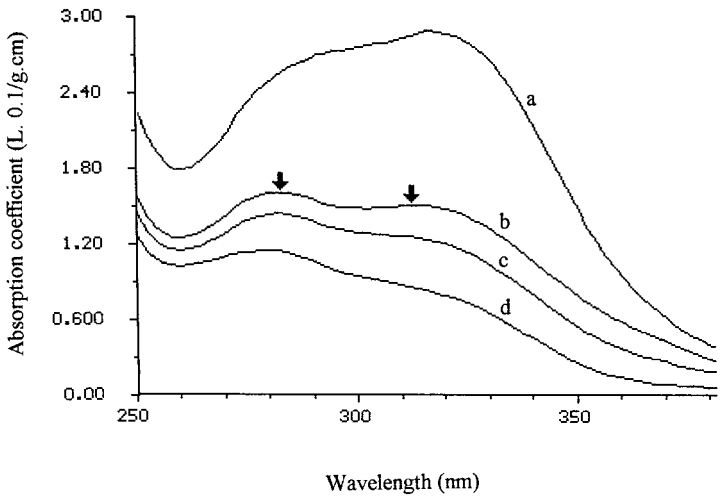

Figure 2 UV spectra of alkali lignins isolated (spectrum a) with 1% NaOH at 55°C for 2 h from dewaxed rice straw and (spectrum b) at pH 11.5, 45°C for 12 h from the 1% NaOH-treated rice straw and aqueous hydrogen peroxide soluble lignins extracted with (spectrum c) 0.5% H_2O_2 and (spectrum d) 5% H_2O_2 at 45°C, pH 11.5 for 12 h from 1% NaOH treated rice straw.

treated rice straw. The spectra exhibited two absorption maxima around 280 and 310–320 nm. The absorption at 280 nm is assigned mainly to the polylignol, adehydrogenative copolymer of sinapyl alcohol, coniferyl alcohol, and a small amount of *p*-coumaryl alcohol, and the absorption in the 310–320 nm region, mainly to the esters of *p*-coumaric and ferulic acids.[22] In the lignin spectra, the absorption at 280 nm also partially results from the associated hydroxycinnamic acid esters.

All these structural moieties give different absorption maxima and extinction coefficients. The extinction coefficient of the G unit at 280 nm is 3.5 times of that of the S unit, and the extinction coefficient of the H unit is lower than that of G unit, but higher than that of the S unit.[23] Moreover, the extinction coefficient of the *p*-coumaric acid ester is about 1.5 times of that of the ferulic acid ester at 320 nm. Therefore, a maximum absorption at 316 nm in spectrum a shown in Figure 2 is undoubtedly due to the association of *p*-coumaric and ferulic acids. This indicated that treatment of the dewaxed rice straw with 1% NaOH under the condition used only partially cleaved the linkages between lignin and hydroxycinnamic acids such as the ester bond between lignin and *p*-coumaric acid and the ether bond between lignin and ferulic acid. Similarly, the much lower absorption coefficients at 280 nm with a shoulder around 310–320 nm in spectra b, c, and d implied that the treatment of the alkali-extracted rice straw with a dilute alkaline solution in the absence of H_2O_2 or with 0.5–5.0% H_2O_2 at 45°C for 12 h at pH 11.5 significantly cleaved the linkages between lignin and hydroxycinnamic acids, and the treatment resulted in a noticeable degradation of the lignin. The lowest absorption coefficient of the PL fraction, obtained by 5.0% H_2O_2 treatment, was also probably due to the highest amount of coprecipitated nonlignin materials, such as ash and salts.

Composition of the Associated Polysaccharides

The composition of monosaccharide constituents of the PL fractions is given in Table II. Obviously, all the PL fractions contained rather low amounts of bound polysaccharides as shown by a 0.57–1.12% neutral sugar content, indicating that treatment of the straw with alkali in the absence of H_2O_2 or with alkaline peroxide under the conditions used significantly cleaved the ether bonds between lignin and hemicelluloses in the cell walls of rice straw in addition to partial saponification of hydroxycinnamic esters such as between *p*-coumaric acid and lignin/polysaccharides or between ferulic acid and hemicelluloses. Glucose, galactose, arabinose, and xylose were identified as the only four sugar components. As expected, an increase in the concentration of H_2O_2 from 0.5 to 5.0% led to a decrement in the level of associ-

Table II Content of Neutral Sugars (% Lignin Sample, w/w) in the Isolated PL Fractions

		H_2O_2 Concentrations (%)						
Neutral Sugars	ASL[a]	0[b]	0.5[b]	1.0[b]	2.0[b]	3.0[b]	4.0[b]	5.0
Arabinose	0.23	0.20	0.18	0.19	0.17	0.16	0.16	0.12
Xylose	0.12	0.10	0.08	0.08	0.07	0.07	0.06	0.05
Glucose	0.51	0.42	0.38	0.28	0.30	0.30	0.31	0.18
Galactose	0.26	0.40	0.38	0.30	0.29	0.31	0.23	0.22
Total	1.12	1.12	1.02	0.85	0.83	0.84	0.76	0.57

[a] Abbreviation for the alkali-soluble lignin obtained by treatment of the dewaxed rice straw with 1% NaOH at 55°C for 2 h.
[b] Lignin preparations obtained by treatment of the alkali-treated rice straw with different concentrations of H_2O_2 at 45°C for 12 h at pH 11.5.

ated polysaccharides from 1.0% to less 0.6% in the PL fractions. These differences largely depended on the alkaline peroxide treatment and cannot be used directly for discussion on the structure of lignin–polysaccharide associations.

Content of Phenolic Acids and Aldehydes

Alkaline nitrobenzene oxidation has been widely used for assaying and identifying the structural units of lignin. In the case of oxidation, the three constitutive monomeric lignin units *p*-hydroxyphenyl, guaiacyl, and syringyl produce the corresponding *p*-hydroxybenzaldehyde, vanillin, and syringaldehyde. Generally, lignins can be classified into three types based on the constituent of the basic structural units, that is, G, GS, and GSH types. In other words, they consist of G units, G and S units, and G, S, and H units. According to the order of increment of S unit portions, the GS type also can be subdivided into GS1, GS2, GS3, and GS4 lignins.[16] Results concerning the characterization of phenolic acids and aldehydes in each of the PL preparations, obtained by alkaline nitrobenzene oxidation at 170°C for 3 h, are shown in Table III. The predominant oxidation products were found to be vanillin and syringaldehyde. The presence of fewer *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid was considered most probably to be indicative of noncondensed *p*-hydroxyphenyl units, indicating the incorporation of *p*-hydroxycinnamoyl alcohol in rice straw lignin.

The occurrence of large amounts of non-condensed guaiacyl and syringyl units with relatively fewer *p*-hydroxyphenyl units implied that the eight lignin preparations can be justified as SGH lignin such as cereal straw and grass type lignin. In comparison, in the case of alkali (1% NaOH) soluble lignin (ASL), the content of vanillin was higher than that of syringaldehyde, while the reverse trend appeared in the PL fractions, solubilized during the second stage of alkali treatment at pH 11.5 and the alkaline peroxide treatment under the conditions given. This suggested that noncondensed guaiacyl units were more easily degradable than were the noncondensed syringyl units in the rice straw cell walls during the first alkaline treatment. As shown in Table III, the relative molar ratios of S (the relatively total moles of syringaldehyde, acetosyringone, and syringic acid) to V (the relatively total moles of vanillin, acetovanillone, and vanillic acid), and to H (the relatively total moles of *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid) increased from 2:2:1 in the first and second dilute alkali-soluble lignin fractions to 4:3:1 in the 5.0% H_2O_2-soluble lignin fractions, indicating that the PL fractions, obtained during the processes of alkaline treatment in the absence of H_2O_2, contained relatively more noncondensed *p*-hydroxyphenylpropane units, while the PL lignin fractions, solubilized during the courses of alkaline peroxide treatments at relatively higher concentrations of H_2O_2 such as at 4.0–5.0% hydrogen peroxide, contained more noncondensed syringyl and guaiacyl units. These results indicated that treatment by alkali in the absence of H_2O_2 seemed to degrade or solubilize the lignins mainly from vessels of the protoxylem and metaxylem, whereas the treatments by alkaline peroxide at relatively higher concentrations such as 4.0–5.0% H_2O_2 favored degradation or release the lignins mainly from middle lamella of parenchyma in the rice straw cell walls.[13]

Table III Content (% Lignin Sample, w/w) of Phenolic Acids and Aldehydes from Nitrobenzene Oxidation of the Isolated PL Fractions

Phenolic Acids and Aldehydes	ASL[a]	H_2O_2 Concentrations (%)						
		0[b]	0.5[b]	1.0[b]	2.0[b]	3.0[b]	4.0[b]	5.0[b]
p-Hydroxybenzoic acid	1.26	1.45	0.97	0.82	0.80	0.68	0.32	0.25
p-Hydroxybenzaldehyde	2.58	2.09	1.44	1.40	1.51	1.30	1.23	1.07
Vanillic acid	0.35	0.35	0.34	0.25	0.24	0.28	0.35	0.48
Syringic acid	1.03	1.36	1.44	1.58	2.13	1.92	1.87	1.75
Vanillin	9.82	7.48	5.45	5.33	6.21	5.40	4.57	4.21
Syringaldehyde	7.07	7.61	7.15	6.99	7.76	7.01	5.84	5.42
Acetovanillone	0.50	0.20	0.25	0.32	0.28	0.24	0.22	0.23
p-Coumaric acid	0.42	0.27	0.9	0.24	0.23	0.21	0.22	0.18
Acetosyringone	0.92	0.81	0.80	0.61	0.52	0.40	0.42	0.39
Ferulic acid	0.54	0.38	0.45	0.52	0.54	0.40	0.35	0.20
Total	24.49	22.00	18.58	18.06	20.22	17.84	15.39	15.18
Molar ratio (*S*:*V*:*H*)[c]	2:2:1	2:2:1	3:2:1	3:2:1	3:2:1	3:2:1	4:3:1	4:3:1

[a] Abbreviation for the alkali-soluble lignin obtained by treatment of the dewaxed rice straw with 1% NaOH at 55°C for 2 h.
[b] The lignin preparations obtained by treatment of the alkali-treated rice straw with different concentrations of H_2O_2 at 45°C for 12 h at pH 11.5.
[c] *S* represents the relatively total moles of syringaldehyde, syringic acid, and acetosyringone; *V*, the relatively total moles of vanillin, vanillic acid, and acetovanillone; and *H*, the relatively total moles of *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid.

Table III also shows that the relatively higher yields of nitrobenzene oxidation appeared in the PL fractions obtained by alkali in the absence of H_2O_2 than those in the PL fractions solubilized during the alkaline peroxide treatment. This observation suggested that alkaline peroxide-soluble PL fractions had a relatively higher degree of condensation than did those of the alkali-soluble lignins obtained in the absence of H_2O_2. In addition, the occurrence of hydroxycinnamic acids such as *p*-coumaric and ferulic acids in the cell walls of rice straw were also identified as minimal amounts in the mixture of nitrobenzene oxidation. It was found that *p*-coumaric acids are mainly esterified to lignin, while ferulic acids are linked by their phenolic groups via ether bonds to lignin and by their carboxyl groups via ester bonds to hemicelluloses.[1,24]

Molecular Weight Distribution

With GPC analysis, the distribution of the PL fraction, isolated with 3% H_2O_2 at 45°C for 12 h at pH 11.5 from the alkali-treated rice straw, is shown in Figure 3, and 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 eight PL fractions are given in Table IV. As can be seen from the diagram, the molecular weight distribution showed two peaks: a strong peak at the side of a high molecular weight and a shoulderlike peak at the side of a low molecular weight, which had its maximum corresponding to $\bar{M}_w$ 6650 and 1090 g mol^{-1}, respectively. The elution profile showed a wide polymolecularity, ranging from the oligomer up to polystyrene of molecular weight over 20,000 g mol^{-1}. In Table IV, the eight lignin fractions show no significant difference in their molecular-average weights, which ranged from $\bar{M}_w$ 4540 to 6270 g mol^{-1}. An increase in concentration of H_2O_2 from 0.5 to 2.0% during the alkaline peroxide treatment at pH 11.5 for 12 h at 45°C led to a growth of $\bar{M}_w$ from 5240 to 6270 g mol^{-1}, indicating that by increasing the H_2O_2 concentration between 0.5 and 2.0% at least, in part, enhanced the solubilization of large molecular-size lignins. In contrast, as the concentration was further increased to 3.0, 4.0, and 5.0%, the $\bar{M}_w$ slightly decreased to 6170, 5880, and 5700 g mol^{-1}, respectively. This phenomenon revealed that a minimal degradation of the lignins occurred at a relatively higher concentration of H_2O_2 over 2.0%. Similarly, a relatively lower $\bar{M}_w$ (4540–4890 g mol^{-1}) in the alkali-soluble PL fractions obtained in the absence of H_2O_2 than

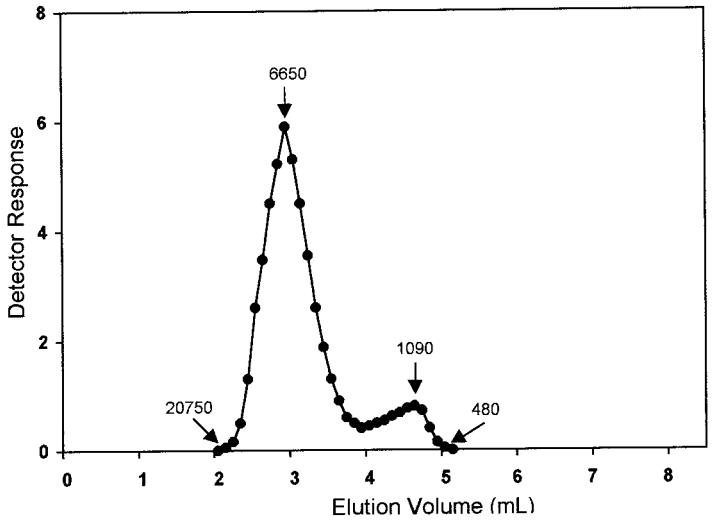

Figure 3 GPC molecular weight distribution of pure lignin (PL) fraction isolated with 3% H_2O_2 at 45°C for 12 h at pH 11.5 from the alkali-treated rice straw.

those obtained by alkaline peroxide treatment implied that alkali treatment favored dissolution of small molecular-size lignins, and the alkaline peroxide treatment under the conditions used did not degrade the macromolecular structure of lignin to any noticeable extent. Furthermore, based on an $\bar{M}_w$ between 4540 and 6270 g mol^{-1}, it is therefore very likely that both alkali and hydrogen peroxide treatment did not apparently decompose the lignin except its important dissolution.

In the cell walls of rice straw, lignification always is accompanied by the deposition of hydroxycinnamic acids that make alkali-labile ester linkages among the lignin building stones and between lignin and polysaccharides. Further studies found that these ester linkages are distributed widely and rather evenly regardless of the morphological region, which results in high solubility of the lignin under alkaline media.[24] Conversely, in the case of wood, owing to the large degree of condensation of lignin in the middle lamellae, delignification is much slower than those from straw and grass.

FTIR Spectra

Figure 4 shows FTIR spectra of rice straw alkali lignin (spectrum a) extracted with 1% NaOH at 55°C for 2 h in the absence of H_2O_2 and alkaline peroxide soluble lignins extracted with 0.5% H_2O_2 (spectrum b), 2.0% H_2O_2 (spectrum c), and 5.0% H_2O_2 (spectrum d) at 45°C for 12 h at pH 11.5 from the alkali-treated rice straw. As can be seen from the diagram, the relative intensities of the bands for aromatic skeleton vibrations, assigned at 1601, 1510, 1464, and 1421 cm^{-1}, were rather similar in the four PL spectra, which confirmed that the "core" of the lignin structure did not

Table IV Weight-average ($\bar{M}_w$) and Number-average ($\bar{M}_n$) Molecular Weights and Polydispersity ($\bar{M}_w/\bar{M}_n$) of Alkali and Hydrogen Peroxide Soluble PL Fractions

		Lignin Fractions[b] Extracted with Different Concentrations of H_2O_2 (%)						
	ASL[a]	0[b]	0.5[b]	1.0[b]	2.0[b]	3.0[b]	4.0[b]	5.0[b]
$\bar{M}_w$	4540	4890	5240	6250	6270	6170	5880	5700
$\bar{M}_n$	1860	2200	2460	3370	3220	2940	2770	2650
$\bar{M}_w/\bar{M}_n$	2.44	2.22	2.13	1.85	1.96	2.09	2.12	2.15

[a] Abbreviation for the alkali-soluble lignin obtained by treatment of the dewaxed rice straw with 1% NaOH at 55°C for 2 h.
[b] The lignin preparations obtained by treatment of the alkali-treated rice straw with different concentrations of H_2O_2 at 45°C for 12 h at pH 11.5.

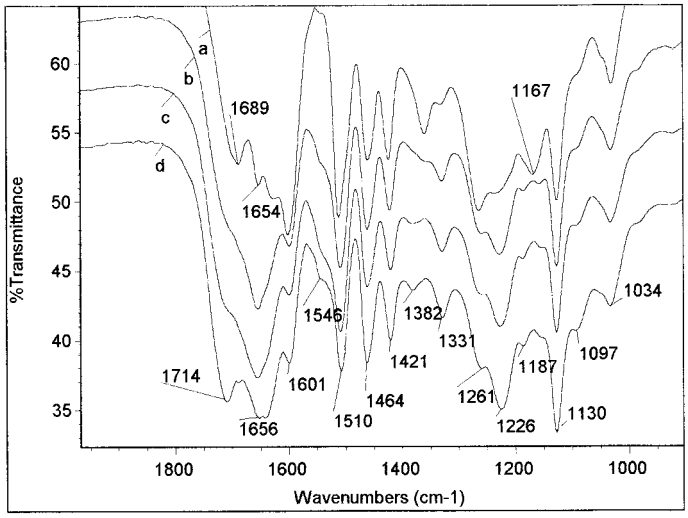

Figure 4 FTIR spectra of (spectrum a) rice straw alkali lignin extracted with 1% NaOH at 55°C for 2 h in the absence of H_2O_2, and alkaline peroxide soluble lignins extracted with (spectrum b) 0.5% H_2O_2, (spectrum c) 2.0% H_2O_2, and (spectrum d) 5.0% H_2O_2 at 45°C for 12 h at pH 11.5 from the alkali-treated rice straw.

change significantly during the alkaline peroxide treatment under various concentrations given. However, the changes of the carbonyl absorption region might enable the evaluation of the effects of the alkaline peroxide treatment. The band at 1714 cm^{-1} in spectrum d corresponds to the unconjugated ketone and carboxyl group stretching, while the bands at 1656 cm^{-1} in spectra b, c, and d and at 1689 and 1654 cm^{-1} in spectrum a are attributed to conjugated carbonyl stretching in the lignin, respectively. The absorption at 1628 cm^{-1} in spectrum a might originate from an enol structure in the lignin.[25] Clearly, remarkable increases of carbonyl absorption were observed in the PL fractions, obtained from the alkaline peroxide treatments. Particularly, in spectrum d, an apparent carboxyl absorption at 1714 cm^{-1}, which was not identified in spectrum a, but appeared as a small shoulder in spectra b and c, indicated a noticeable oxidation of the lignin structure during the 5.0% H_2O_2 treatment process. A similar phenomenon was observed by Backman and Gellerstedt[26] in studies on the behavior of lignin in kraft pulp. The authors demonstrated that delignification with alkaline peroxide at 90°C led to some depolymerization of lignin and the creation of carboxyl groups. The intensive bands at 1382 and at 1261, and 1167 cm^{-1} in spectrum a indicate the aliphatic C—H stretch in CH_3 (not OMe) and ester linkages in the lignin molecule such as esterified *p*-coumaric acid, respectively, while they became rather weak in the spectra b, c, and d. This once again implied that the significant oxidation and saponification occurred during the treatment by alkaline peroxide under the conditions used.

Analogously, the intensive bands at 1331 and 1226 cm^{-1} in spectra b, c, and d were assigned to syringyl and guaiacyl ring breathing with C═O stretching, respectively, and the almost disappearance of these two bands in spectrum a indicated a noticeable increase in carbonyl groups in the lignin fractions, obtained by alkaline treatment. This increase in carbonyl groups is undoubtedly due to the oxidation of lignin by hydrogen peroxide under the conditions given. On the other hand, the similar intensity bands at 1130, 1097, and 1034 cm^{-1} indicate the aromatic C—H in-plain deformation, suggesting that a great similarity in the aromatic ring skeleton existed among the spectra of alkaline peroxide-soluble lignins and the alkali-soluble lignin preparations, solubilized under alkaline conditions but in the absence of peroxide. This supported the previous finding that alkaline peroxide treatment did not affect the overall structure of lignin from rice straw except for the remarkable increases of carboxyl and carbonyl groups in the lignin.

^{13}C-NMR Spectrum

The ^{13}C-NMR spectrum of the PL fraction, obtained by treatment of the alkali-extracted straw

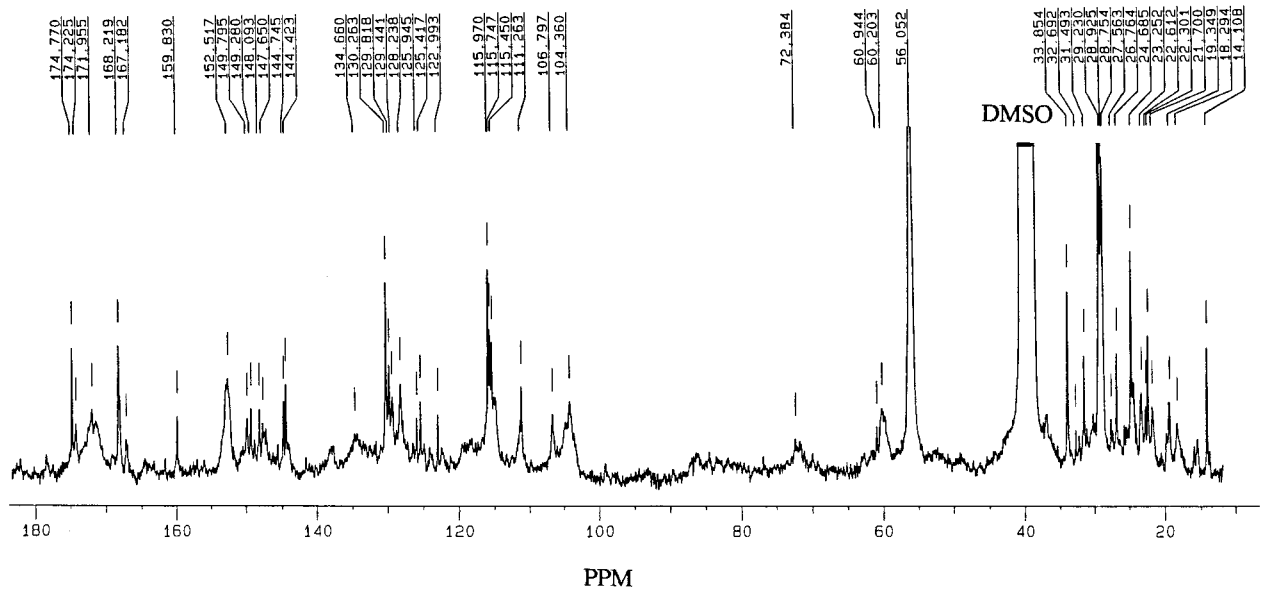

Figure 5 ^{13}C-NMR spectrum of PL fraction extracted with 2% H_2O_2 at 45°C for 12 h at pH 11.5 from alkali-treated rice straw.

sample with 2% H_2O_2 at 45°C for 12 h at pH 11.5, is shown in Figure 5. Most of the observed signals were previously assigned in straw and wood lignin spectra.[27–30] As can be seen from Figure 5, one of the most striking characteristics of the ^{13}C-NMR spectrum is the almost absence of typical polysaccharide signals between 57 and 103 ppm. The spectrum does show a signal at 63.1 ppm (C-5, Xyl internal unit) for the associated hemicelluloses, and the signal at 83.8 ppm can be assigned to the C-α of lignin moieties with an α-benzyl ether linkage to polysaccharides. However, the peak intensity is rather weak. This implied that D-xylose is probably associated with lignin through an α-benzyl ether bond. The presence of an α-benzyl ether linkage between the lignin and polysaccharides was also observed by Xie and Terashima[3] in the study of rice straw lignin traced by ^{13}C NMR. The carbonyl resonances from uronic acids and esters may contribute to the signals at 174.2 and 174.8 ppm and at 60.9 ppm, which indicate C-6 in methyl uronates and the 4-*O*-methoxyl group of the glucuronic acid residue, respectively.[31]

Another of the most striking characteristics of the ^{13}C-NMR spectrum is the increase in the carboxylic groups in the prominent intensities at 170.9 and 172.0 ppm (aliphatic COOH), resulting from the alkaline peroxide oxidation effect.[32] It is presumed that the cutting linkages in lignin macromolecules occurred in the side chains of the structural units, that is, the alkaline peroxide oxidation may act on almost all carbons in the side chains of the lignin structure and convert them to carboxylic acids to a varying extent. It is also very likely that the alkaline peroxide favored oxidation of the carbons linked by hydroxyl, aldehyde, and ketone groups and aryl ethers in the lignin side chains. As these carbons were oxidized to carboxyl, aldehyde groups, or ketone groups, the contiguous aryl ethers could be easily cleaved simultaneously and be subjected to further degradation. Such an oxidation may be the main reason for the substantial degradation of lignins from rice straw by alkaline peroxide treatment.[32]

The signals for aromatic part of the lignin appear in the region between 104.4 and 160.0 ppm. The syringyl (S) residues were indicated by signals at 152.5 (C-3/C-5, S), 138.4 (C-4, S etherified, data not shown in the spectrum), 134.7 (C-1, S etherified), 106.8 (C-2/C-6, S with α-CO), and 104.4 ppm (C-2/C-6, S). Guaiacyl (G) residues gave signals at 149.8 and 149.3 ppm (C-3, G etherified), 148.0 and 147.7 (C-4, G etherified), 145.8 (C-4, G nonetherified), 134.7 (C-1, G etherified), 114.9 (C-5, G, data not shown in the spectrum), and 111.3 ppm (C-2, G). The *p*-hydroxyphenyl (H) residues appeared as three signals at 129.8, 129.4, and 128.2 ppm (C-2/C-6, H). These signals confirmed that the lignin preparation could be justified as SGH lignin. Clearly, the signals for syringyl units had strong relative inten-

sities, while the signals for guaiacyl units exhibited obvious weak ones. This suggested that more S units remained in the macromolecular portions of the lignin, whereas G units were subjected to more degradation upon the alkaline peroxide oxidation, that is, S units had smaller reactivities with the oxidation by alkaline peroxide than did G and H unit structures, which corresponded to the results obtained by alkaline nitrobenzene oxidation and was well consistent with the results obtained by Chen and Hayashi[32] from the study of rice straw lignin with NaOH–oxygen pulping.

The signals at 168.2 (C-γ, PC ester), 159.8 (C-4, PC ester), 144.7 (C-α, PC ester), 130.3 (C-2/C-6, PC ester), 125.9 and 125.4 (C-1, PC ester), and 116.0, 115.7, and 115.5 ppm (C-3/C-5, PC ester) represented the esterified *p*-coumaric acid. Etherified ferulic acid was observed with signals at 167.2 (C-γ, FE ether), 144.4 (C-α, FE ether), and 122.4 ppm (C-6, FE ether). Esterified ferulic acid was identified with a signal at 123.0 ppm (C-6, FE ester). It seems clear that *p*-coumaric is linked to lignin by ester bonds, while ferulic acid is linked to lignin by ether and ester bonds. Based on the studies of associations between lignin and polysaccharides from rice straw, Sharma et al.[1] reported that *p*-coumaric acid was mostly esterified to lignin, while ferulic acid appeared almost equally in etherified linkages with lignin and in esterified bonds to polysaccharides. Similarly, in the cell walls of rice straw, in addition to the etherified linkages between ferulic acid and lignin, ferulic acid, at least in part, also esterified to hemicelluloses. Further studies on the deposition and distribution of hydroxycinnamic acids in the cell wall of a rice plant indicated that *p*-coumaric and ferulic acids occur in every kind of cell wall and increase in amount with the progress of lignification. In the seconary cell walls, less ferulic acid is involved, while in the middle lamellae of vessels and parenchyma, varying amounts of *p*-coumaric and ferulic acids are present, and the molar ratio of *p*-coumaric acid to ferulic acid varies with the degree of lignification. In addition, it was also found that ferulic acid is deposited in the early stage of cell wall formation prior to the deposition of *p*-coumaric acid and is linked mainly to polysaccharides by ester bond, while *p*-coumaric acid is almost esterified to lignin.[24]

The signals below 104 ppm are the resonance of aliphatic carbons. Of them, signals at 86.2 (data not shown in the spectrum), 72.4, and 60.2 ppm belong to the resonances of C-β, C-α, and C-γ in β-*O*-4, respectively. The relatively weak signal at 86.2 ppm implied that removal of the moieties with structure C-β in β-*O*-4 or cleavage of the β-*O*-4 ether linkages occurred during the alkaline peroxide treatment. Besides, the intensity of the β-*O*-4 linkage at 72.4 ppm for C-α in β-*O*-4 also appeared rather weak. These decreases in the signal intensities indicated that the peroxide treatment may cut the β-*O*-4 ether bonds to some extent. However, as shown from the diagram, they were still the major linkages between the lignin structural units. The common carbon–carbon linkages such as β–β (C-γ in β–β units, 71.8 ppm, data not shown in the spectrum) and β-5 (C-4 in β-5 units, 144.7 ppm, overlapped with C-α, PC ester; C-1 in β-5 units, 129.8 and 129.4 ppm, overlapped with C-2/C-6, H) were also present. These signals indicated that the linkages in this rice straw lignin is composed mainly of β-*O*-4 ether bonds together with small amounts of β–β and β-5 carbon–carbon linkages. These results suggested that alkaline peroxide under the conditions used here may not attack the β-aryl ether structure to a significant extent, but to some extent. Similar results were reported by Dence[5] and Lachenal et al.[33] in studies on the behavior of lignin in kraft pulp during hydrogen peroxide delignification. The authors revealed that hydrogen peroxide was unable to attack phenols of the type present in lignin under alkaline conditions, that is, no degradation of the phenolic ring was observed during the alkaline peroxide treatment. However, at a relatively higher temperature such as 90°C, some depolymerization of lignin may occur and carboxyl groups are created.[5] The signals representing the γ-methyl and α- and β-methylene groups in *n*-propyl side chains appeared in the spectrum between 14.1 and 33.9 ppm. A very strong signal at 56.1 ppm corresponded to the OCH_3 in syringyl and guaiacyl units.

SUMMARY

In short, the results presented in this study clearly showed that the alkaline peroxide treatment of the alkali-extracted rice straw under the conditions used resulted in some oxidative degradation of lignin by producing some amounts of carbonyl and carboxyl groups and cleavage of β-*O*-4 ether bonds as compared to the lignins, isolated under similar alkaline conditions but in the absence of peroxide. It is very likely that the peroxide selectively oxidized the carbons linked by hydroxyl, aldehyde, and ketone groups and

aryl ethers in the side chains of the lignin structures, and this subsequently led to a substantial degradation or solubilization of the macromolecular lignins. However, it was found that β-*O*-4 ether bonds remain the major linkages between the lignin units. The treatment by alkaline peroxide under the conditions given did not degrade the macromolecular lignin structure from rice straw to a significant extent. Meanwhile, the lignin in the rice straw cell walls appeared to be very closely associated to glucuronic acid or 4-*O*-methylglucuronic acid by ester bonds. *p*-Coumaric acid was found to be linked to lignin mainly by ester bonds, while ferulic acid was linked to lignin via ether bonds and also linked to hemicelluloses via ester bonds.

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