Delignification of Maize Stems by Peroxymonosulfuric Acid, Peroxyformic Acid, Peracetic Acid, and Hydrogen Peroxide. 1. Physicochemical and Structural Characterization of the Solubilized Lignins

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Water-treated maize stems were subjected to delignification with peroxymonosulfuric acid at 20 °C for 144 h, with peroxyformic acid at 80 °C for 6 h, with peracetic acid at 50 °C for 6 h, and with 2% hydrogen peroxide at 45 °C for 12 h at pH 1.5, 4.4, 9.5, 11.5, 12.0, and 12.6, respectively, which solubilized 47.1, 91.3, 33.3, 16.6, 15.9, 17.4, 86.2, 87.7, and 91.3% of the original lignin, respectively. Substantial lignins were released during the treatment with peroxyformic acid and hydrogen peroxide at pH ≥11.5, whereas an insignificant effect on delignification was observed by using peroxymonosulfuric acid, peracetic acid, and hydrogen peroxide under acidic, natural, and weakly alkaline media conditions. The structures of the isolated lignin preparations were investigated by chemical analysis, gel permeation chromatography, and UV, FT-IR, and ^{13}C NMR spectroscopy.

Keywords: *Maize stem; delignification; peroxymonosulfuric acid; peroxyformic acid; peracetic acid; hydrogen peroxide; lignin; phenolic acids and aldehydes; FT-IR and ^{13}C NMR spectroscopy*

INTRODUCTION

Hydrogen peroxide (H_2O_2) and peracids, such as peroxymonosulfuric acid (Ps; H_2SO_5), peroxyformic acid (Pf; HCOOH plus H_2O_2), and peracetic acid (Pa; CH_3-CO_3H), have been identified as promising alternatives to chlorine-containing chemicals for delignification of wood and agricultural residues and bleaching of chemical pulps (Hortling et al., 1991; Perez et al., 1998; Ruggiero et al., 1998; Yuan et al., 1998a; Zhang et al., 1998). In the alkaline peroxide treatment process, hydroperoxide ion (HOO^-), formed in alkaline media, is the principal active species in hydrogen peroxide bleaching systems. This anion is a strong nucleophile that 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. 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 metal-catalyzed decomposition of hydrogen peroxide is undesirable in the bleaching operation. However, this decomposition generates more active radicals, such as hydroxyl radicals ($HO^•$) and superoxide anion radicals ($O_2^{•-}$), participating in the degradation reaction of lignin and hemicelluloses which, therefore, results in a significant solubility of lignin and hemicelluloses (Dence, 1996; Pan et al., 1998).

Organic solvent-based delignification has been widely investigated by the Finnish Pulp and Paper Research Institute. It consists of the treatment of the lignocellulosic raw material with peroxyformic acid, generated in situ by mixing formic acid and hydrogen peroxide at 80 °C for 3 h, followed by a reflux formic acid stage, and finally another peroxyformic acid stage identical to the first. Delignification of softwoods (pine and spruce), hardwood (birch), and agricultural plants has been reported to yield pulps that exhibit good mechanical properties and excellent peroxide bleachability (Perez et al., 1998). During the Pf stage electrophilic HO^+ ions are formed ($HCOOOH + H^+ \rightarrow HCOOH + HO^+$). According to model compound studies the main reactions for the HO^+ ions with lignin are expected to be ring hydroxylation, oxidative ring opening, substitution of side chains, cleavage of β-aryl ether bonds, and expoxidation (Gierer et al., 1982; Hortling et al., 1991).

Another study of organic solvent-based delignification uses Pa as a solvent, and the results have shown that Pa is a good delignifier as well as a good brightening agent when optimum conditions are employed. It is generally accepted that its reaction pathways include hydroxylation of the lignin aromatic ring by an electrophilic substitution, resulting in the formation of hydroquiones. Quinones may be further oxidized to form water-soluble carboxylic acids via Baeyer−Villiger oxidation (Strumila and Rapson, 1975). Furthermore, lignin structures containing α-carbonyl groups are oxidized via Baeyer−Villiger oxidation (Yuan et al., 1998b).

As mentioned above, wood and other lignocelluloses can be readily delignified using organic peroxides such as Pf and Pa. With the exception of studies using alkaline hydrogen peroxide, research on delignification with an inorganic peroxide such as Ps has also been performed (Springer, 1990). It has been found that low pH solutions of the peroxymonosulfate anion are much more effective in delignifying aspen wood than are

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alkaline solutions of hydrogen peroxide. This is presumed to be the case because, under these conditions, peroxymonosulfate is a much stronger oxidizing agent than is hydrogen peroxide. The very low pH of the peroxymonosulfate solutions, produced by mixing hydrogen peroxide with concentrated sulfuric acid, results in marked attack on the polysaccharide constituents of the wood, resulting in low residue yields and low viscosities (Springer, 1990).

Lignin can be divided into core lignin, polymerized and ether-bonded phenolics, and noncore lignin, esterified hydroxycinnamic acids (Jung, 1989). Of the hydroxycinnamic acids, ferulic acid predominates in highly digestible primary (parenchymal) tissues, whereas *p*-coumaric acid is closely related to secondary cell wall formation in less degradable (sclerenchymal) tissues (Engels and Schuurmans, 1992). Ferulic acid in grasses and straw has received considerable attention because of its intimate association with the plant cell wall and ability to function as a cross-link between wall polysaccharides (notably arabinoxylans) and the phenylpropanoid lignin polymers. Small amounts of *p*-coumaric acid are esterified to arabinoxylans early in primary wall development in much the same way as ferulic acid, but, later in wall development, *p*-coumaric acid is found to be more extensively esterified to lignin (Ralph et al., 1994). Dissolution and oxidation of lignin by peroxide under mild conditions have been used to produce low molecular weight aromatic products, which are both theoretically interesting for lignin structural studies and commercially interesting for the cosmetics, adhesions, and pharmaceutical industries (Quesada et al., 1997). In other words, delignification using various peroxides might enable the utilization of byproducts and therefore a more complete use of the raw material.

We were interested in a mild delignification method that can be run on agricultural residues such as straw and sugar cane bagasse, at atmospheric pressure and lower temperature, and recovery of the byproducts such as lignin and hemicelluloses for industrial utilizations. As the first part of the study, the present work investigates the structures and physicochemical properties of the lignins that dissolve during the different peroxide treatment procedures. The results obtained will be used in explaining the reactions that occur during the various peroxide treatments.

MATERIALS AND METHODS

Materials. The maize (*Zea mays* L.) stalk samples were obtained from the experimental farm of The North-Western University of Agricultural and Forest Sciences and Technology (Yangling, People's Republic of China). After being dried in sunlight, the maize stems were manually isolated from leaf and sheath fractions and then cut into small pieces. The cut stems were ground to pass a 1-mm size screen. All weights and calculations were made on an oven-dried (60 °C, 16 h) basis. The chemical compositions (w/w) of the maize stems used in this work are 38.5% cellulose, 28.0% hemicelluloses, 15.0% chlorite lignin, 5.2% protein (N × 6.25), 3.6% wax, 4.2% ash, and 5.8% others, such as hydroxycinnamic acids, starch, and pectins. Hydrogen peroxide (27.5% H_2O_2) and peracetic acid, which consists of ~32% Pa (w/w), 6% H_2O_2 (w/w), and 40% acetic acid (w/w) with the remainder being water, were purchased from Aldrich (Dorset, U.K.). Peroxyformic acid (2%) is a mixture of formic acid (300 mL) and hydrogen peroxide (39.2 g of 27.5% H_2O_2) with an H_2O_2 concentration of 2%. Peroxymonosulfuric acid (8%) was produced by the addition of concentrated sulfuric acid (38.1 g) to cold 27.5% hydrogen peroxide (49.5 g, 2 °C), and the two were thoroughly mixed and diluted with 315 g of distilled water. Using 27.5% H_2O_2 and a mole-to-mole ratio of acid to peroxide of 1:1, the yield of peroxymonosulfate was 8% (% H_2O_2 basis) (Springer, 1990).

Peroxide Treatments. The dried powder was first extracted in a Soxhlet apparatus with toluene/ethanol (2:1, v/v) for 6 h. The dewaxed maize stems were then soaked in distilled water with a 1:40 straw-to-liquor ratio at 55 °C for 2 h. After isolation of the water-soluble hemicelluloses by precipitation of water extracts in 3 volumes of ethanol, water-soluble lignin preparation was obtained by reprecipitation at pH 1.5, adjusted with 6 M HCl, from the supernatant solution. Samples free of wax and water-solubles (8.0 g) were treated with the concentrated Pa solution (320 mL) at 50 °C for 6 h, with Pf solution (320 mL) at 80 °C for 6 h, and with Ps solution (320 mL) at 20 °C for 144 h in a round-bottom flask immersed in a constant-temperature bath under stirring, respectively. The spent liquor was removed, and the residue was pressed to recover the maximum possible amounts of liquor. The residue was then suspended in hot water (45 °C), and 0.1 M NaOH was carefully added to raise the pH to 7. The residue was filtered and washed thoroughly with water and ethanol and then dried in an oven at 60 °C for 16 h. The degraded hemicelluloses were recovered by precipitation of the concentrated filtrates with 3 volumes of ethanol, respectively. After filtration of the hemicelluloses and evaporation of the ethanol, the solubilized phenolic polymers were precipitated from the spent liquors and separated by centrifugation and washed with acidified water (pH 2.0). Finally, the lignins were freeze-dried and kept at 5 °C before analysis.

In the treatment with hydrogen peroxide, samples (8.0 g) free of wax and water-solubles were added to 320 mL of distilled water containing 2.0% 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 1.5, 4.4 (natural H_2O_2), 9.5, 11.5, 12.0, and 12.6 with 4 M H_2SO_4 or 4 M NaOH and allowed to stir gently for 12 h at 45 °C. During the initial stages of stirring under alkaline conditions, particularly at initial pH values of 11.5, 12.0, and 12.6, oxygen evolution was active and substantial frothing occurred, requiring that extractions be conducted in vessels with volumes 2−3 times those of extraction mixtures. No further adjustments in pH were made during the course of the treatment. Under alkaline conditions, the reaction pH remained nearly constant for 2 h before slowly rising from 11.5, 12.0, and 12.6 to final values of 12.6, 12.9, and 13.2, respectively. 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 6.0 with 6 M NaOH or 6 M HCl and subsequently concentrated. The released hemicelluloses were precipitated by pouring the concentrated supernatant fluid into 3 volumes 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 as precipitated lignin (PL) preparation. The preparation procedure is illustrated in Figure 1. All of the treatments were repeated twice, giving very reproducible yields.

Lignin (PL) Analysis. The monomeric composition of the noncondensed monomeric units of precipitated 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 (Lawther et al., 1995). All nitrobenzene oxidation results represent the mean of triplicate samples, and each oxidation mixture was chromatographed twice. The amounts of the hemicellulosic moieties associated with PL preparations were determined by acid hydrolysis of the polysaccharides to monosaccharides with 2 M trifluoroacetic acid for 2 h at 120 °C. Liberated neutral sugars were analyzed as their alditol−acetate derivatives by gas chromatography (Blakeney et al., 1983; Sun et al., 1995).

UV spectra were obtained using a Hewlett-Packard 8452A diode array spectrophotometer. FT-IR spectra of the PL preparations were recorded from KBr pellets containing 1% finely

Table 1. Yield of Lignin (Percent Dry Matter) Solubilized during the Various Treatments of Maize Stems

	lignin preparations[a]									
	1	2	3	4	5	6	7	8	9	10
precipitated lignin (PL)[b]	0.8	2.5	4.6	1.9	1.0	1.2	1.8	9.1	9.5	10.2
lignin solubilized in the supernatant (pH 1.5)[c]	0.1	4.0	8.0	2.7	1.3	1.0	0.6	2.3	2.1	1.8
lignin associated in the isolated hemicelluloses	0.3	0.03	0.01	0.03	0.01	0.01	0.03	0.55	0.53	0.55
total solubilized lignin	1.2	6.5	12.6	4.6	2.3	2.2	2.4	11.9	12.1	12.6

[a] Lignin preparation 1 was extracted with water at 55 °C for 2 h from dewaxed maize stems, preparation 2 was extracted with peroxymonosulfuric acid at 20 °C for 144 h from water-treated maize stems, preparation 3 was extracted with peroxyformic acid at 80 °C for 6 h from water-treated maize stems, preparation 4 was extracted with peracetic acid at 50 °C for 6 h from water-treated maize stems, and preparations 5−10 were extracted with 2% H_2O_2 (45 °C) for 12 h at pH 1.5, 4.4, 9.5, 11.5, 12.0, and 12.6, respectively, from water-treated maize stems. [b] Represents the lignin fraction obtained by precipitation of the supernatant solution at pH 1.0−1.5 after isolation of the hemicelluloses. [c] Represents the lignin fraction that is still solubilized in the pH 1.0−1.5 supernatant after precipitation of the solubilized lignin fraction (PL) and obtained by difference.

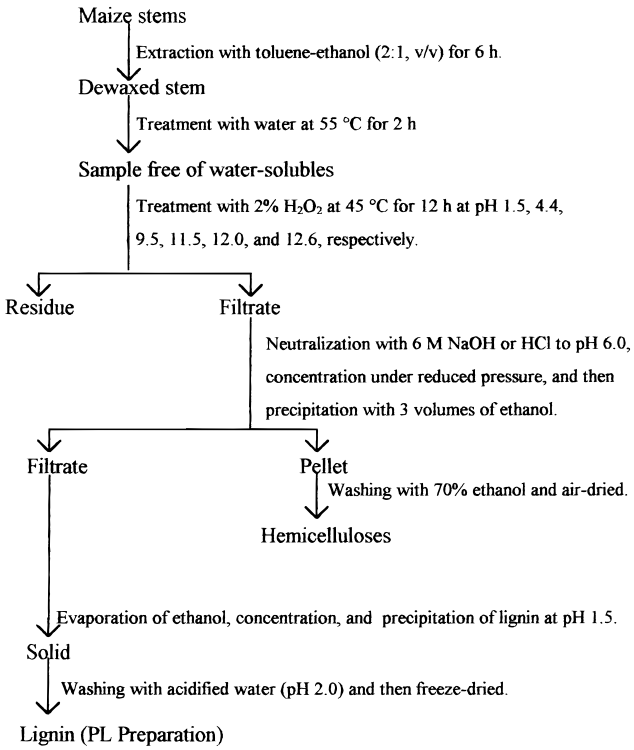

Figure 1. Scheme for extraction of hemicelluloses and lignin from maize stems by hydrogen peroxide.

ground samples on a Nicolet-750 FT-IR spectrophotometer. The weight-average molecular weights of PL fractions were determined by gel permeation chromatography (GPC) on a PLgel 5μ Mixed-D column. The samples were dissolved in tetrahydrofuran at a concentration of 0.2%, and a 200 FL 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.

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. It is recorded at 25 °C from 250 mg of sample dissolved in 1.0 mL of DMSO-d_6 after 25000 scans. A 60° pulse flipping angle, a 3.9 μs pulse width, and 0.85 s acquisition time were used.

RESULTS AND DISCUSSION

Yield of Lignin. Table 1 gives the yield of solubilized lignin including acid-insoluble lignin (precipitated lignin, named PL), acid-soluble lignin (solubilized in the pH 1.5 supernatant), and the lignin associated with the released hemicelluloses. Obviously, the PL and acid-soluble lignin fractions were the predominant totally solubilized lignins, whereas the lignin associated in the released hemicelluloses appeared in a minimal amount, indicating that the linkages between lignin and hemicelluloses were significantly cleaved during the treating conditions used. Treatment of the dewaxed maize stems with water at 55 °C for 2 h, in general, mainly solubilized the low molecular weight of hemicelluloses as shown by the release of 20.0% of the original hemicelluloses and 8.0% of the original lignin together with 42.9% of the original ash from dewaxed maize stems.

As can be seen from Table 1, delignification with Pf in one stage was very efficient, in which 91.3% of original lignin was solubilized or degraded during the treatment at 80 °C for 6 h, whereas the treatment of the dewaxed maize stems with Ps or Pa was ineffective because only 47.1 and 33.3% of the originally present lignin were released or degraded during the treatment processes at 20 °C for 114 h or at 50 °C for 6 h, respectively. Similar results for delignification with Pf have been reported by Perez et al. (1998) during the peroxyformic acid treatment of *Eucalyptus grandis* wood chips and sugar cane bagasse in one stage. The authors found that the concentration of water in the formic acid is an important parameter for the pulping. The pulping conditions included the application of moderate quantities of hydrogen peroxide (5% for eucalyptus and 3% for bagasse based on lignocellulosic material) and recovered formic acid (94% concentration or less) for the bagasse. In contrast, the eucalyptus wood required high-quality formic acid (98%) for good pulping. Further studies found that the HO^+ ions formed from Pf caused demethylation and formation of additional phenolic hydroxyl groups, which makes the bleaching reactions easier (Hortling et al., 1991). On the other hand, the current results obtained from Ps and Pa were not consistent with the observations obtained by Springer (1990) and Yuan et al. (1998a) from the studies of delignification of aspen wood using Ps and bleaching using Pa. Springer (1990) revealed that low-pH solutions of the peroxymonosulfate anions are much more effective in delignifying aspen wood than are alkaline solutions of hydrogen peroxide. During the delignification of aspen wood with Ps, the very low pH of the peroxymonosulfate solutions, produced by mixing hydrogen peroxide with sulfuric acid, resulted in marked attack on the carbohydrate constituents of the wood, resulting in low residue yields and low viscosities, whereas in our experiment, treatment of the water-extracted maize stems using Ps and Pa degraded only 12.1 and 4.5% of the original hemicelluloses, respectively. It is therefore very likely that at this higher liquor-to-stem ratio, increasing Ps and Pa concentration or rising treatment temperature is needed to increase lignin and hemicellulose removal greatly.

Due to its dual role of delignifying and bleaching, alkaline hydrogen peroxide is widely used in the pulp industry to delignify lignocellulosic materials such as wood and agricultural residues or to bleach lignin-rich pulps to brightness levels of 80−83% ISO (Dence, 1996). Gould (1985) found that approximately half the lignin present in agricultural residues, such as wheat straw, could be solubilized when the residue was treated at 25 °C with an alkaline solution of hydrogen peroxide. The lignification was most effective at pH 11.5. McDonough et al. (1989) studied the delignification of southern pine kraft pulp with alkaline hydrogen peroxide. They also found that approximately half of the lignin present in the pulp could be removed. On the basis of delignification of aspen wood using hydrogen peroxide, Springer (1990) showed that treatment of the wood with H_2O_2 at optimum pH (pH 11) removed at most 36% of the original lignin. More recently, the results obtained from our experiment showed that treatment of rice straw with 1% NaOH at 55 °C for 2 h and following treatment with 2.0% H_2O_2 at 45 °C for 12 at pH 11.5 resulted in releases of >90% of the original lignin and 84% of the original hemicelluloses (Sun and Tomkinson, 2000). In these cases, the most efficient delignification occurred under reaction conditions where no stabilizers (to inhibit peroxide decomposition) were present and the rate of peroxide decomposition was maximum. As mentioned earlier, the action of alkaline peroxide as a bleaching agent has been explained through the reaction of the hydroperoxide anion (HOO^-), formed in an alkaline medium. This anion is believed to be the principal active species involved in the elimination of chromophores in lignin structures, particularly conjugated carbonyl structures that are prone to react with the hydroperoxide anion. On the other hand, hydroxyl radicals ($HO^\bullet$) and superoxide anion radicals ($O_2^{\bullet-}$), produced by decomposition of H_2O_2 in alkaline media, are thought to cause the oxidation of lignin structures, which leads to the introduction of hydrophilic (carboxyl) groups, the cleavage of some interunit bonds, and, eventually, the dissolution of lignin (Dence, 1996).

On the basis of extensive studies of the mechanism of H_2O_2 delignification from wheat straw, Gould (1984, 1985) stated that the delignification reaction is strongly dependent on pH, with a sharp optimum at pH 11.5. However, it was generally not necessary to continuously regulate the reaction pH, even though over the course of the treatment in alkaline media, the reaction pH rose from 11.5, 12.0, and 12.6 to final values of 12.6, 12.9, and 13.2, respectively. As the reaction pH became more alkaline, substantially more lignin was solubilized over the pH range of 11.5 and increasing amounts of hemicelluloses were solubilized. Delignification of the water-treated maize stems with 2% H_2O_2 at 45 °C for 12 h at pH 11.5, 12.0, and 12.6 released 86.2, 87.7, and 91.3% of the original lignin together with 63.3, 64.7, and 83.0% of the original hemicelluloses, respectively. However, there was no significant lignin dissolution or degradation below pH 10 as the data show in Table 1. Treatment of the stems with 2% H_2O_2 at pH 1.5, 4.4, and 9.5 solubilized only 16.6, 15.9, and 17.4% of the original lignin, respectively. This phenomenon implied that acidic or almost neutral hydrogen peroxide was ineffective in the delignification of maize stems, and substantial lignin was removed under strong alkaline conditions.

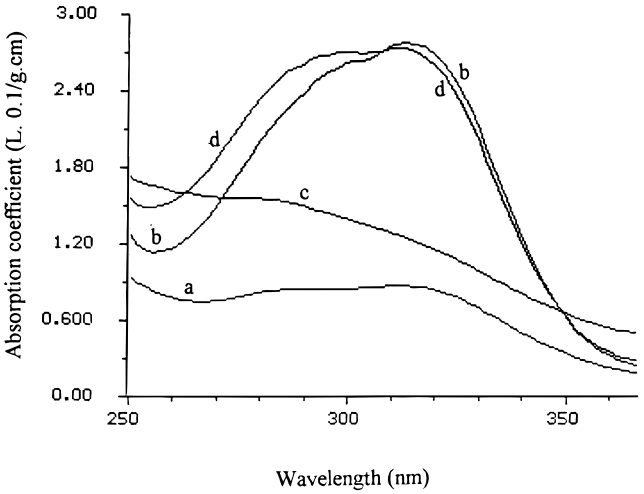

Figure 2. UV spectra of water-soluble lignin preparation extracted with water at 55 °C for 2 h from dewaxed maize stems (a), aqueous peroxymonosulfuric acid-soluble lignin preparation extracted with 8% H_2SO_5 (% H_2O_2 basis) at 20 °C for 144 h from water-treated maize stems (b), peroxyformic acid-soluble lignin preparation extracted with peroxyformic acid at 80 °C for 6 h from water-treated maize stems (c), and peracetic acid-soluble lignin preparation extracted with peracetic acid at 50 °C for 6 h from water-treated maize stems (d).

According to the results obtained by studies on the reactions of lignin model compounds with Pa or H_2O_2 under acidic conditions, protonation of the hydrogen peroxide and formation of a hydroxonium ion is the first reaction step.

$$H_2O_2 + H^+ \leftrightarrow HO^+ + H_2O$$

The hydroxonium ion is a strong electrophilic agent. It attacks the lignin's aromatic nuclei. Ring hydroxylation, oxidative demethylation, displacement of side chains, and oxidative ring opening are the main reaction types (Suss and Helmling, 1986). Therefore, the main reason for this insignificant delignification by H_2O_2 under acidic, neutral, and weakly alkaline conditions is probably due to the homolytic cleavage of H_2O_2 by the reactive phenolics, because these phenols are apparently converted into carboxylic acids under the conditions given (Kubelka et al., 1992).

UV Spectra. Although UV spectroscopy of lignins is not well-suited for structure elucidation because of the overlapping of absorption bands from the different chromophores found in the macromolecule, it is used for a preliminary characterization at qualitative and quantitative levels with respect to the concentration (Perez et al., 1998). Figure 2 shows UV absorption spectra of water-soluble lignin extracted with water at 55 °C for 12 h from dewaxed maize stems (spectrum a), of aqueous peroxymonosulfuric acid-soluble lignin extracted with 8% H_2SO_5 (H_2O_2 basis) at 20 °C for 6 144 h (spectrum b), of peroxyformic acid-soluble lignin extracted with 2% peroxyformic acid at 80 °C for 6 h (spectrum c), and of peracetic acid-soluble lignin extracted with 32% peracetic acid at 50 °C for 6 h from the water-treated maize stems (spectrum d). All of the spectra exhibit an absorption maximum at 210 nm (spectra not shown), resulting from $\pi-\pi^*$ transitions of the lignin aromatic skeleton (Perez et al., 1998). From the spectra recorded, it is possible to identify clearly the maxima at 280 and 318 nm for the Ps- and Pa-soluble

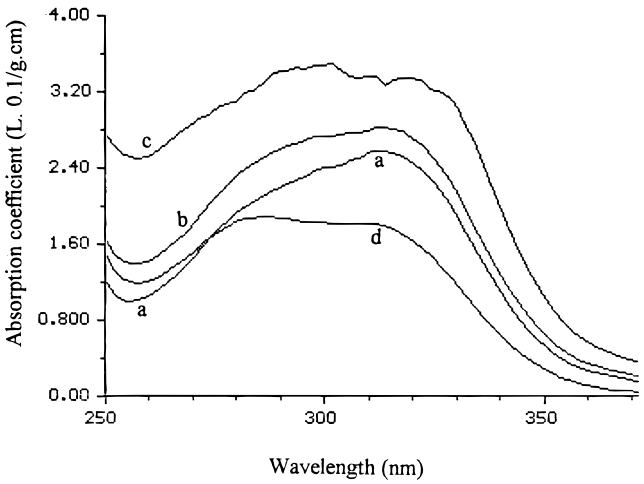

Figure 3. UV spectra of aqueous hydrogen peroxide-soluble lignin preparations extracted with 2% H_2O_2 at 45 °C for 12 h at pH 1.5 (a), 4.4 (s b), 9.5 (c), and 12.6 (d) from water-treated maize stems.

lignins and shoulders for the water-soluble lignin in the same region as well as a shoulder around 280 nm for Pf-soluble lignin. In the UV spectra of lignins in gramineous monocotyledons, the absorption in the 280 nm region is mainly assigned to the polylignol, a dehydrogenative copolymer of sinapyl alcohol, coniferyl alcohol, and a small amount of *p*-coumaryl alcohol, and the absorption at 318 nm mainly to the esters of *p*-coumaric and ferulic acids (He and Tetrashima, 1991). It is very likely that the significant absorption of Ps- and Pa-soluble lignins indicated the higher lignin concentration of the two samples, whereas the low absorption coefficient of the Pf- and water-soluble lignin fractions implied the lower lignin content of the samples. This lower content of lignin in the samples is largely due to the coprecipitation of more non-lignin materials such as ash and salts.

The UV spectra of aqueous hydrogen peroxide-soluble lignin fractions extracted with 2% H_2O_2 at 45 °C for 12 h at pH 1.5 (spectrum a), 4.4 (spectrum b), 9.5 (spectrum c), and 12.6 (spectrum d) from water-treated maize stems are illustrated in Figure 3. Similarly, the four PL preparations exhibited the basic UV spectrum typical of gramineous lignins with maxima in the region of 280−320 nm. The continuing appearance of a maximum absorption at 316 nm revealed that treatment of the water-extracted maize stems with 2% H_2O_2 under the acidic or basic conditions used only partially cleaved the linkages between lignin and hydroxycinnamic acids, such as the ester bond between *p*-coumaric acid and lignin or hemicelluloses and the ether bond between lignin and ferulic acid.

Content of Associated Hemicelluloses. To elucidate the effect of various peroxide treatments on the content of polysaccharides in the isolated PL preparations, the associated hemicellulose compositions of the PL fractions were determined. The results are given in Table 2. A slightly high percentage of associated hemicelluloses in water-soluble, Ps, Pf, and Pa lignin samples may be explained by the assumption that some of the soluble hemicelluloses are chemically bonded to and/or absorbed onto the lignin and not washed off. Results here are also supported by evidence from Seisto and Poppius-Levlin (1997) showing that the lignins, solubilized during Pf pulping of reed canary grass and tall fescue, did not contain more than 3 and 7.5% of carbohydrates, respectively. On the other hand, a much higher content of bound polysaccharides (~23.2%) was obtained in the lignins isolated from spent liquors of Pf pulping (Hortling et al., 1991), which was probably due to the different lignin isolation methods used. As can be seen in Table 2, xylose, glucose, and arabinose were the most abundant of the six major neutral sugars in all of the PL preparations.

Table 2. Content of Neutral Sugars (Percent Lignin Sample, w/w) in Isolated PL Preparations

neutral sugar	lignin preparations[a]									
	1	2	3	4	5	6	7	8	9	10
rhamnose	0.51	0.36	0.34	0.12	0.18	0.11	ND[b]	ND	ND	ND
arabinose	0.18	0.62	1.10	0.31	0.58	0.60	0.51	0.48	0.29	0.09
xylose	1.63	1.96	3.56	2.06	1.18	0.40	0.25	0.23	0.23	0.05
mannose	0.28	0.28	0.29	0.13	0.14	0.08	ND	ND	ND	ND
glucose	1.26	0.74	1.28	0.80	0.88	0.66	0.46	0.39	0.24	0.16
galactose	0.33	0.31	0.37	0.43	0.28	0.39	0.38	0.29	0.27	0.16
total	4.19	4.27	6.94	3.85	3.24	2.24	1.60	1.39	1.03	0.46

[a] Corresponding to the lignin preparations in Table 1. [b] ND, not detectable.

In general, the carbohydrate content of milled grass lignins, even after purification, is 5−10% (Seisto and Poppius-Levlin, 1997). Interestingly, the PL preparations, obtained by treatment with 2% H_2O_2 at pH 1.5, 4.4, 9.5, 11.5, 12.0, and 12.6, contained only 3.2, 2.2, 1.6, 1.4, 1.0, and 0.5% bound neutral sugars, respectively. This minimal amount of associated hemicelluloses in these PL fractions implied that hydrogen peroxide under the conditions given, particularly in the alkaline solution, seems to have a significant effect on the cleavage of the α-ether bonds between lignin and hemicelluloses. Furthermore, as can be seen in Table 2, the amount of arabinose is significantly higher in these PL preparations except for the fraction obtained with 2% H_2O_2 at pH 12.6, whereas the xylose contents are much lower than in the water-, Ps-, Pf-, and Pa-soluble lignin fractions. The current results indicated once again that some of the arabinose side chains of xylan connect to lignin in the cell walls of maize stems. Chemical studies on linkages between hemicelluloses, especially arabinoxylans, and lignin have emphasized the important role of arabinose residues in the formation of these linkages. Ralph et al. (1994) stated that small amounts of *p*-coumaric acid are esterified to arabinoxylans early in primary wall development in much the same way as ferulic acid, but, later in wall development, *p*-coumaric acid is found to be more extensively esterified to lignin. Further strong evidence suggested that ferulic acid esters act as lignin initiation sites and direct cell-wall cross-linking during plant growth and development. The ferulic acid esters are deposited in the developing primary cell wall as feruloyl-arabinoxylans, and they are subsequently linked to lignin monomers via a peroxidase-catalyzed reaction to form ether- and other free-radical-derived linkages (Morrison et al., 1998). Iiyama et al. (1994) have reached similar conclusions by quite different methods and showed that *p*-coumaric acid is mostly esterified to lignin or hemicelluloses, whereas ferulic acid occurs almost equally in esterified and etherified forms in the cell walls of wheat internodes.

Monomeric Composition of PL Preparations. For the purposes of comparing compositions, all of the PL fractions have been characterized by the method of common alkaline nitrobenzene oxidation, which has a broader action on lignin structures as well as causes a

Table 3. Content (Percent Lignin Sample, w/w) of Phenolic Acids and Aldehydes from Nitrobenzene Oxidation of the Isolated PL Preparations

phenolic acid or aldehyde	lignin preparations[a]									
	1	2	3	4	5	6	7	8	9	10
p-hydroxybenzoic acid	0.69	1.62	0.72	1.06	2.54	2.88	3.65	1.67	1.47	0.84
p-hydroxybenzaldehyde	1.83	11.77	3.81	11.09	9.39	8.48	7.21	5.44	5.28	4.34
vanillic acid	0.17	0.21	0.18	0.29	0.14	0.12	0.10	0.11	0.11	0.12
syringic acid	0.63	1.60	1.65	1.80	1.29	1.26	1.24	1.97	2.50	2.41
vanillin	2.34	4.22	3.45	5.71	4.93	4.60	3.74	5.95	6.91	7.82
syringaldehyde	4.79	13.47	2.97	6.28	13.47	11.20	8.57	17.04	19.07	15.64
acetovanillone	0.11	0.25	0.24	0.41	0.11	0.12	0.14	0.14	0.14	0.17
p-coumaric acid	0.32	0.36	0.24	0.90	0.90	0.68	0.56	0.56	0.61	0.61
acetosyringone	0.78	4.31	1.92	1.39	1.64	1.80	2.44	0.53	0.52	0.29
ferulic acid	0.43	0.75	0.29	0.34	0.36	0.34	0.42	0.20	0.18	0.13
total	12.09	38.56	15.47	29.27	34.77	31.48	28.07	34.02	36.79	32.37
molar ratio (*S*:*V*:*H*)[b]	2:1:1	3:1:4	1:1:1	1:1:3	3:1:3	2:1:3	3:1:3	3:1:1	3:1:2	2:1:1

[a] Corresponding to the lignin preparations in Table 1. [b] *S* represents the relative total moles of syringaldehyde, syringic acid, and acetosyringone; *V* represents the relative total moles of vanillin, vanillic acid, and acetovanillone; and *H* represents the relative total moles of *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid.

cleavage of the side chains of the lignin phenylpropane units involved not only in *β-O*-4 noncondensed structures but also in other bonding patterns such as diarylpropane (Chabbert et al., 1994). Table 3 shows the composition of PL as obtained by alkaline nitrobenzene oxidation at 170 °C for 3 h, including the monomeric products originating from guaiacyl (G), syringyl (S), and *p*-hydroxyphenyl (H) units involved in the noncondensed structures of lignin. The total recovery yields of monomeric products from water-soluble (12.1%) and Pf-soluble (15.5%) lignins were much lower than those from Ps-, Pa-, and 2% hydrogen peroxide-soluble lignins (28.1−38.6%), indicating that fewer lignin monomers are involved in alkyl aryl ether linkages in water- and Pf-soluble lignins (Iiyama and Lam, 1990). The water- and Pf-soluble lignins therefore apparently contain a more condensed lignin polymer than other PL preparations and have a higher degree of condensation. Another reason for these lower yields of oxidation products from water- and Pf-soluble lignins is also presumed to be due to the higher amounts of coprecipitated ash or salts.

The monomeric composition of lignin varied according to the different peroxide treating procedures as shown in Table 3. The presence of a significantly higher yield of *p*-hydroxybenzaldehyde and relatively lower content of syringaldehyde in Ps-, Pf-, and Pa-soluble lignins revealed that the *p*-hydroxyphenyl units present in maize stem lignins are easily solubilized and more reactive than syringyl structures toward Ps, Pf, and Pa except that it is also considered most probably to result partly from *p*-coumaric acid oxidation (Seisto and Poppius-Levlin, 1997; Billa et al., 1996). A significantly higher yield of syringaldehyde was identified in the oxidation products of 2% H_2O_2-soluble PL preparations, indicating that a large amount of the noncondensed syringyl units appeared in these lignin preparations and hydrogen peroxide treatment had a more significant effect on the release of syringyl units from the cell walls of maize stems. Such a result has been reported by Chabbert et al. (1994) in the studies of biological variability in lignification of maize by alkaline nitrobenzene oxidation.

Table 3 also gives the effect of 2% H_2O_2 treatment pH on the monomeric composition of PL fractions. The relative molar ratios of *S* (the relative total moles of syringaldehyde, syringic acid, and acetosyringone) to *V* (the relative total moles of vanillin, vanillic acid, and acetovanillone) and to *H* (the relative total moles of *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid), corresponding to ratios of syringyl to guaiacyl and to *p*-hydroxyphenyl units, engaged in noncondensed structures were not significantly different among the PL lignin preparations (between 3:1:3 and 2:1:1), indicating the almost same original lignins. The occurrence of a large proportion of noncondensed syringyl units and noticeable amounts of guaiacyl and *p*-hydroxyphenyl units indicated that the six PL preparations can be considered as SGH lignins such as wheat straw and grass type lignin. However, the appearance of a relatively higher content of *p*-hydroxybenzaldehyde in the oxidation mixtures of PL preparations 5−7 indicated that *p*-hydroxyphenyl units present in the stem lignins had a significant reactivity toward the hydrogen peroxide treatment under acidic, natural, and weakly alkaline media. In addition, the appearance of a minimal amount of ferulic acid in all of the oxidation products implied that a considerable proportion of this compound is oxidized into vanillin or vanillic acid under the nitrobenzene oxidation conditions given because a significant yield of ferulic acid can be observed only at temperatures <170 °C (Billa et al., 1998).

Molar Mass Distribution. The molar mass distribution curves of PL preparations obtained by GPC relative to polystyrene standards indicate similar elution patterns and molecular mass distribution of the lignin fraction isolated with 2% H_2O_2 at 45 °C for 12 h at pH 11.5 from the water-treated maize stems, as illustrated in Figure 4. Peak I eluted in the volume of 3.0 mL and had a higher molecular mass value of 7160 g mol^{-1}, whereas peak II had a much lower molecular mass value of 1230 g mol^{-1}, which is presumed to be due to degradation of the solubilized lignins during the 2% alkaline peroxide treatment process. The elution profile showed a wide polydispersity, ranging from oligomer up to polystyrene of molecular mass >20000 g mol^{-1}. The average molar masses of all PL preparations are presented in Table 4. In comparison, it is easily seen that the phenolic lignin solubilized during the water treatment belongs to a lower molecular mass fraction, and a large proportion of the higher mass fragments is extracted from the water-treated maize stems by Ps, Pf, Pa, and 2% H_2O_2, respectively. A slight increase in the molar mass of the PL fraction released during the 2% H_2O_2 treatment at pH 12.6 is apparently due to the fact that the high molar mass lignin is the most difficult to solubilize from the stems, whereas at

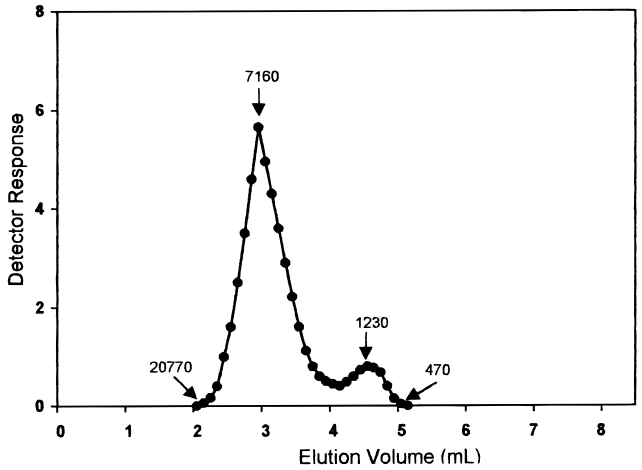

Figure 4. GPC molecular mass distribution of PL preparation isolated with 2% H_2O_2 at 45 °C for 12 h at pH 11.5 from the water-treated maize stems.

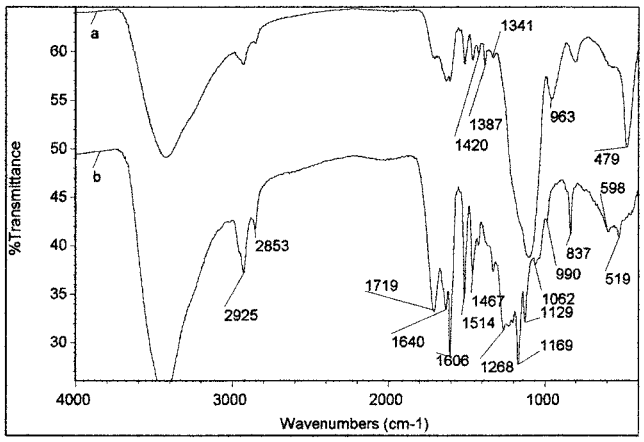

Figure 5. FT-IR spectra of water-soluble lignin preparation (a) extracted by treatment of the dewaxed maize stems with water at 55 °C for 2 h and aqueous peroxymonosulfuric acid-soluble lignin preparation and (b) extracted with 8% H_2SO_5 (% H_2O_2 basis) at 20 °C for 144 h from water-treated maize stems.

Table 4. Weight-Average ($\bar{M}_w$) and Number-Average ($\bar{M}_n$) Molecular Masses and Polydispersity ($\bar{M}_w/\bar{M}_n$) of the PL Preparations Extracted from Maize Stems

	lignin preparations[a]									
	1	2	3	4	5	6	7	8	9	10
$\bar{M}_w$	3890	5340	5170	6190	5910	5820	5780	5880	5850	6140
$\bar{M}_n$	1920	1920	1820	3080	2880	2870	2810	2840	2850	3140
$\bar{M}_w/\bar{M}_n$	2.03	2.78	2.84	2.01	2.05	2.03	2.06	2.07	2.05	1.96

[a] Corresponding to the lignin preparations in Table 1.

the same time intermolecular condensation reactions are probable.

FT-IR Spectra. FT-IR spectroscopy is an important tool to study lignin polymer structure (Faix, 1991). The FT-IR absorptions of water-soluble (spectrum a) and 8% Ps-soluble (spectrum b) lignins dispersed in KBr matrix were measured, and the spectra are reported in Figure 5. The two lignins display an important ester absorption band at 1719 cm^{-1}, indicating the presence of carboxylic acids and/or ester groups such as *p*-coumarate esters (Ruggiero et al., 1998). The band at 1640 cm^{-1} indicates the carbonyl groups in conjugated para-substituted aryl ketones (Seisto and Poppius-Levlin, 1997). The three bands at 1606, 1514, and 1420 cm^{-1} are characteristic of aromatic compounds and are due to vibrations of the

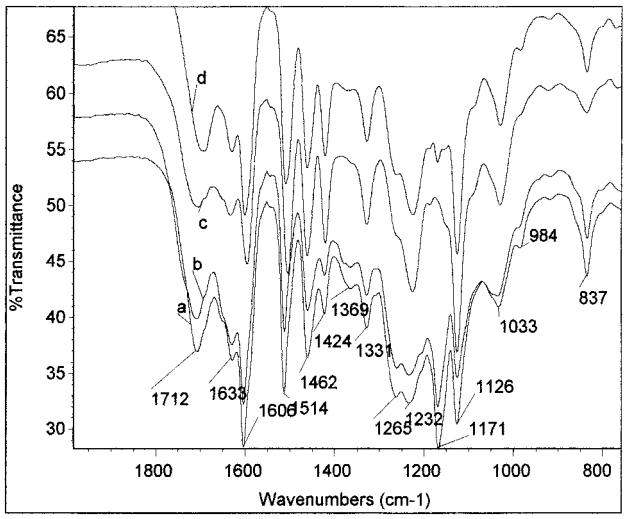

Figure 6. FT-IR spectra of aqueous hydrogen peroxide-soluble lignin preparations extracted with 2% H_2O_2 at 45 °C for 12 h at pH 1.5 (a), 4.4 (b), 11.5 (c), and 12.6 (d) from water-treated maize stems.

aromatic skeletal (Scalbert et al., 1986). The intensity of the 1606 cm^{-1} band is much stronger than that of the 1514 cm^{-1} band, indicating a typical feature of hardwood and grass lignins. A band at 1467 cm^{-1} corresponds to the C−H deformations and aromatic ring vibrations. Aliphatic C−H stretching in CH_3 (not in OMe) is clearly seen at 1387 cm^{-1} in the spectrum of water-soluble lignin, whereas it appears only as a shoulder in the spectrum of Ps-soluble lignin, implying that a significant oxidation of the solubilized lignin occurred during the treatment with Ps. Moreover, the syringyl and guaiacyl ring breathings are obviously seen at 1341 and 1268 cm^{-1}, respectively, in the Ps-soluble lignin fraction, whereas they appear only as shoulders in the water-soluble lignin preparation. Unambiguous carbonyl stretching of conjugated ester groups can be observed in the spectrum of Ps lignin at 1169 cm^{-1} and is characteristic of straw lignins. Another important spectral feature of the straw lignins is the appearance of a band at 837 cm^{-1}, which is due to the aromatic C−H out of plane vibrations in *p*-hydroxyphenylpropane units (Perez et al., 1998). The presence of a huge band at 3400 cm^{-1} is largely due to the hydroxyl functions (alcohol and phenols), and this band is more intense for the Ps lignin fraction.

FT-IR spectra of the lignins solubilized during the treatment with 2% H_2O_2 at 45 °C for 12 h at pH 1.5 (spectrum a), 4.4 (spectrum b), 11.5 (spectrum c), and 12.6 (spectrum d) from water-treated maize stems (Figure 6) differ only slightly from those of the lignin fractions obtained at pH 9.5 and 12.0, indicating that the core of the lignins did not change dramatically during the 2% H_2O_2 treatment under acidic, natural, and alkaline conditions. Similarly, the bands at 1712 and 1633 cm^{-1} can be assigned to be the esters from carboxylic groups and conjugated carbonyl groups, respectively. Aromatic skeleton vibrations in the four PL fractions are assigned at 1606, 1514, and 1424 cm^{-1}. The relative decrease in intensity of the bands at 1369 cm^{-1} for aliphatic C−H stretch in CH_3 (not OMe), at 1265 cm^{-1} for guaiacyl ring breathing with CO stretching, and at 1171 cm^{-1} for ester linkages in lignin molecule such as esterified *p*-coumaric acid in spectra c and d compared to the lignins in spectra a and b indicates that noticeable oxidation and saponification

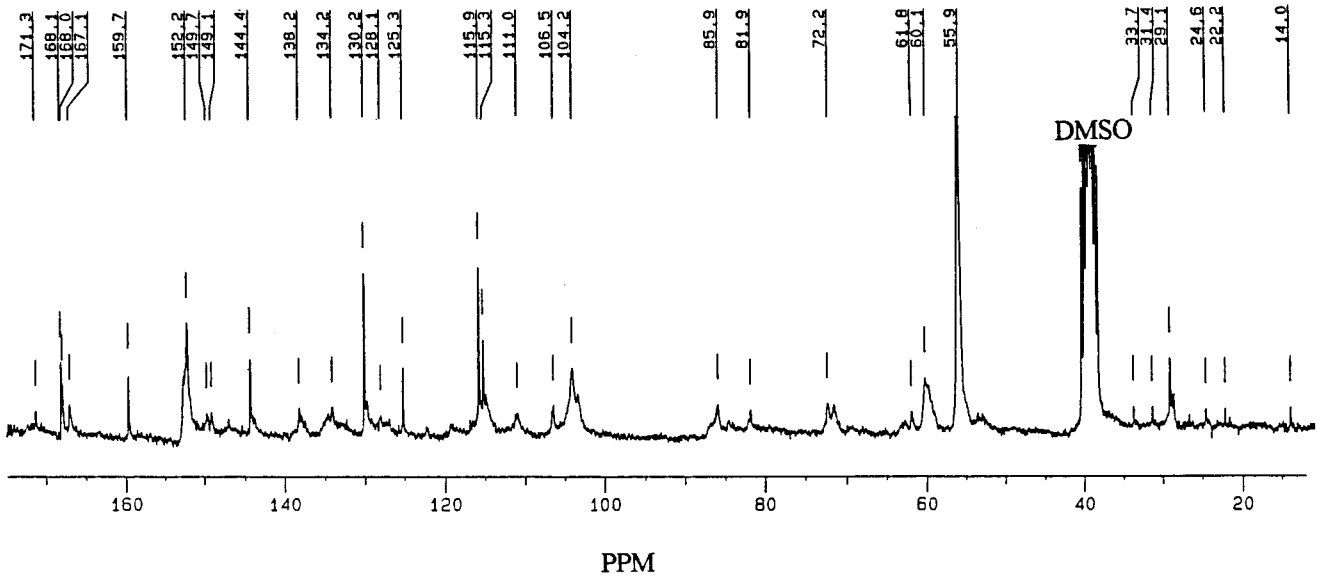

Figure 7. ^{13}C NMR spectrum of PL preparation extracted with 2% H_2O_2 at 45 °C for 12 h at pH 11.5 from dewaxed and water-treated maize stems.

occurred during the treatment by alkaline peroxide under the conditions given. Analogously, the intense bands at 1331 and 1232 cm^{-1} in spectra c and d are assigned to syringyl and guaiacyl ring breathing with C═O stretching, whereas a decrement in these two bands in spectra a and b indicates an increase in carbonyl groups in the PL preparations obtained by 2% H_2O_2 treatment under alkaline conditions. This increase in carbonyl groups is undoubtedly due to the oxidation of lignin by alkaline peroxide. On the other hand, the similar intensities of bands for the aromatic ring skeleton in all of the lignin spectra suggest that hydrogen peroxide treatment did not affect the overall structure of lignin from maize stems except for the remarkable increase of carbonyl groups and saponification of lignins obtained under alkaline conditions.

^{13}C NMR Spectrum. To gain a more complete understanding of the structure in isolated lignins, the ^{13}C NMR spectrum of the PL preparation, obtained by treatment of the water-extracted maize stems with 2% H_2O_2 at 45 °C for 12 h at pH 11.5, was qualitatively obtained (Figure 7). Most of the observed signals have been previously assigned in straw and wood lignin spectra (Himmelsbach and Barton, 1980; Nimz et al., 1981; Lapierre et al., 1984; Scalbert et al., 1986; Jung and Himmelsbach, 1989; Ralph et al., 1994; Pan et al., 1994; Kondo et al., 1995). As can be seen from Figure 7, one of the most striking characteristics of the ^{13}C NMR spectrum is the near absence of typical polysaccharide signals between 57 and 103 ppm. The spectrum does show a signal at 63.2 ppm (C-5, Xyl internal unit, data not shown in the spectrum) for the associated hemicelluloses, and the small signal at 84.2 ppm (data not shown in the spectrum) can be assigned to C-α of lignin moieties with an α-benzyl ether linkage to hemicelluloses, suggesting that D-xylose is probably associated with lignin through an α-benzyl ether bond. The carbonyl resonances from uronic acids and esters may contribute to the signals at 171.3 and 81.9 ppm, which indicate C-6 and C-4 in 4-*O*-methyl-D-glucuronic acid residue, respectively (Himmelsbach and Barton, 1980; Imamura et al., 1994).

Another very important feature of the lignin observed from the ^{13}C NMR spectrum is the occurrence of noticeable amounts of bound hydroxycinnamic acids, particularly *p*-coumarate ester. As can be seen from the spectrum, the signals at 168.1 and 168.0 ppm (C-*γ*, PC ester), 159.7 ppm (C-4, PC ester), 144.4 ppm (C-α, PC ester), 130.2 ppm (C-2/C-6, PC ester), 125.3 ppm (C-1, PC ester), 115.9 ppm (C-3/C-5, PC ester), and 115.3 ppm (C-*β*, PC ester) represented the esterified *p*-coumaric acid. Etherified ferulic acid was observed with signals at 167.1 ppm (C-*γ*, FE ether), 144.0 ppm (C-α, FE ether, data not shown in the spectrum), and 122.3 ppm (C-2/C-6, FE ether, data not shown in the spectrum). It seems clear that the *p*-coumaric is linked to lignin by ester bonds, whereas the ferulic acid is linked to lignin by ether bonds. On the basis of extensive studies on the pathway of *p*-coumaric acid incorporation into maize lignin by NMR, Ralph et al. (1994) unambiguously revealed that *p*-coumaric acid is attached exclusively at the *γ*-position of lignin side chains by ester bond and not at the α-position. The chemical shifts observed in the lignin are in agreement with those of *p*-coumarate esters in which the phenolic hydroxyl is unetherified. In other words, the relative sharpness of the peaks also indicates that the *p*-coumarate unit has not been incorporated into the lignin structure and remains as a pendant, terminal group on the polymer (Ralph et al., 1994). With a quantitative study on cell wall composition of maize stem internodes, Jung and Buxton (1994) reported that the cell walls contained 2.37% esterified and 0.45% etherified *p*-coumaric acid together with 0.42% esterified and 0.34% etherified ferulic acid except for the main components of polysaccharides and lignin. Obviously, etherified *p*-coumaric acid concentrations observed were greater than the concentration found for etherified ferulic acid. However, it was found that *p*-coumaric acid was mostly esterified to lignin or polysaccharides, whereas ferulic acid occurred almost equally in esterified and etherified forms (Migne et al., 1998; Pan et al., 1998). It should be, therefore, noted that all etherified ferulic acid concentrations reported by Jung and Buxton (1994) were probably underestimates of the involvement of ferulic acid in lignin−polysaccharide complex cross-linkages because ferulic acid attached to the solubilized complex fraction is substantial and cannot be ignored.

The region between 104.4 and 160.0 ppm can be assigned to the aromatic part of the lignin. The syringyl (S) units were identified by signals at 152.2 ppm (C-3/C-5, S), 138.2 ppm (C-4, S etherified), 134.2 ppm (C-1, S etherified), 106.5 ppm (C-2/C-6, S with α-CO), and 104.2 ppm (C-2/ C-6, S). Guaiacyl (G) units gave signals at 149.7 and 149.1 ppm (C-3, G etherified), 147.2 ppm (C-4, G etherified, data not shown in the spectrum), 145.4 ppm (C-4, G nonetherified, data not shown in the spectrum), 134. 2 ppm (C-1, G etherified), and 111.0 ppm (C-2, G). The *p*-hydroxyphenyl (H) units appeared as one signal at 128.1 ppm (C-2/C-6, H). These signals confirmed that the lignin preparation could be justified as SGH lignin such as straw or grass lignins (Sun and Lawther, 1998). Signals at 85.9, 72.2, and 60.1 ppm belong to the resonances of C-*β*, C-α, and C-*γ* in *β*-*O*-4, respectively. Interestingly, the proportion of *threo*-*β*-*O*-4 ethers is significantly higher than that of *erythro*-*β*-*O*-4 in the stem lignin preparation as shown by the noticeable signals at 72.2 and 60.1 ppm for C-α and C-*γ* in *threo*-*β*-*O*-4 and the slightly weak signals at 71.8 and 59.7 ppm (data not shown in the spectrum) for C-α and C-*γ* in *erythro*-*β*-*O*-4, respectively. This implied that the presence of sinapyl alcohol was predominant during the maize lignification, corresponding to the results obtained by alkaline nitrobenzene oxidation. The common carbon−carbon linkages such as *β*-*β* (C-*γ* in *β*-*β* units, 71.8 ppm, overlapped with the C-α in *erythro*-*β*-*O*-4; C-*β* in *β*-*β* units, 53.8 ppm, data not shown in the spectrum) and *β*-5 (C-*β* in *β*-5 units, 52.9 ppm, data not shown in the spectrum) were also present. The signals representing the *γ*-methyl and α- and *β*-methylene groups in *n*-propyl side chains appeared in the spectrum between 14.0 and 33.7 ppm. A very strong signal at 55.9 ppm corresponds to the OCH_3 in syringyl and guaiacyl units. These signals indicated that the linkages in this maize stem lignin are still mainly composed of *β*-*O*-4 ether bonds together with small amounts of *β*-*β* and *β*-5 carbon−carbon linkages. These results suggested that the proportions of the main lignin interunit linkages are comparable for the organosolv lignins from rind tissue of maize stem internodes studied by Ralph et al. (1994), and alkaline peroxide under the conditions used here may not attack the *β*-aryl ether structure to a significant extent. Similar results have been reported by Dence (1996) and Lachenal et al. (1992) 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. In other words, 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 (Dence, 1996).

In conclusion, the results obtained in this study showed that delignification with Pf and alkaline peroxide (under pH ≥11.50) resulted in a substantial release of the lignins from water-treated maize stem, whereas treatment with Ps and Pa under the conditions given showed an insignificant effect on the delignification. The lignin preparations obtained contained minimal amounts of bound hemicelluloses due to the selective cleavage of the ether bonds such as α-ether linkages between lignin and hemicelluloses and had $\bar{M}_w$ between 5170 and 6140 g mol^{-1}. The water- and Pf-soluble lignin preparations apparently contained a more condensed lignin polymer than other PL preparations and had a higher degree of condensation. The *p*-hydroxyphenyl units present in maize stem lignins are more reactive (more *p*-hydroxyphenyl units go into solution) than syringyl and guaiacyl structures toward peroxymonosulfuric acid, peroxyformic acid, and peracetic acid, whereas guaiacy units are less reactive (fewer guaiacyl units go into solution) than *p*-hydroxyphenyl and syringyl structures toward hydrogen peroxide in acidic, natural, and weakly alkaline media. A significant oxidation of the solubilized lignin occurred during the treatment with Ps under the condition given. ^{13}C NMR revealed that maize stem lignin is a syringyl/guaiacyl/*p*-hydroxyphenyl copolymer with substantial amounts of *p*-coumarate esters and small amounts of ferulic ethers. The treatment by alkaline peroxide under the conditions given did not degrade the macromolecular lignin structure from maize stem to a significant extent except for the remarkable increase of carbonyl groups and saponification of lignins because it was found that *β*-*O*-4 ether bonds were the major linkages between the lignin units. The delignification occurs mainly by cleavage of α-ether linkages between lignin and polysaccharides. Thorough characterization of the lignins is important for the understanding of the mechanism of the delignification process as well as the possible utilization of such byproducts.

LITERATURE CITED

Billa, E.; Tollier, M. T.; Monties, B. Characterisation of the monomeric composition of *in situ* wheat straw lignins by alkaline nitrobenzene oxidation: Effect of temperature and reaction time. *J. Sci. Food Agric.* **1996**, *72*, 250−256.

Billa, E.; Koukios, E. G.; Monties, B. Investigation of lignins structure in cereal crops by chemical degradation methods. *Polym. Degrad. Stability* **1998**, *59*, 71−75.

Blakeney, A. B.; Harris, P. J.; Henry, R. J.; Stone, B. A. A simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohydr. Res.* **1983**, *113*, 291−299.

Chabbert, B.; Tollier, M. T.; Monties, B. Biological variability in lignification of maize: expression of the brown midrib bm2 mutation. *J. Sci. Food Agric.* **1994**, *64*, 455−560.

Dence, C. W. Chemistry of mechanical pulp bleaching. In *Pulp Bleaching—Principle and Practice*; Dence, C. W., Reeve, D. W., Eds.; TAPPI Press: Atlanta, GA, 1996; pp 349−361.

Engels, F. M.; Schuurmans, J. L. L. Relationship between structural development of cell walls and degradation of tissues in maize stems. *J. Sci. Food Agric.* **1992**, *59*, 45−51.

Faix, O. Classification of lignins from different botanical origins by FT-IR spectroscopy. *Holzforschung* **1991**, *45* (Suppl.), 21−27.

Gierer, J. The chemistry of delignification. A general concept. Part II. *Holzforschung* **1982**, *36*, 55−64.

Gould, J. M. Alkaline peroxide delignification of agricultural residues to enhance enzymatic saccharification. *Biotechnol. Bioeng.* **1984**, *26*, 46−52.

Gould, J. M. Studies on the mechanism of alkaline peroxide delignification of agricultural residues. *Biotechnol. Bioeng.* **1985**, *27*, 225−231.

He, L.; Terashima, N. Formation and structure of lignin in monocotyledons. IV. Deposition process and structural diversity of the lignin in the cell wall of sugarcane and rice plant studied by ultraviolet microscopic spectroscopy. *Holzforschung* **1991**, *45*, 191−198.

Himmelsbach, D. S.; Barton, F. E. ^{13}C Nuclear Magnetic Resonance of Grass Lignins. *J. Agric. Food Chem.* **1980**, *28*, 1203−1208.

Hortling, B.; Poppius, K.; Sundquist, J. Formic acid/peroxyformic acid pulping IV. Lignins isolated from spent liquors of three-stage peroxyformic acid pulping. *Holzforschung* **1991**, *45*, 109−120.

Iiyama, K.; Lam, T. B. T. Lignin in wheat internodes. Part 1: The reactivities of lignin units during alkaline nitrobenzene oxidation. *J. Sci. Food Agric.* **1990**, *51*, 481−491.

Iiyama, K.; Lam, T. B. T.; Stone, B. A. Covalent cross-links in the cell wall. *Plant Physiol.* **1994**, *104*, 315−320.

Imamura, T.; Watanable, T.; Kuwahara, M.; Koshijima, T. Ester linkages between lignin and glucuronic acid in lignin-carbohydrate complexes from *Fagus crenata*. *Phytochemistry* **1994**, *37*, 1165−1173.

Jung, H. G. Forage lignins and their effects on fiber digestibility. *Agron. J.* **1989**, *81*, 33−38.

Jung, H. G.; Buxton, D. R. Forage quality variation among maize inbreds: Relationships of cell-wall composition and in-vitro degradation for stem internodes. *J. Sci. Food Agric.* **1994**, *66*, 313−322.

Jung, H. G.; Himmelsbach, D. S. Isolation and characterization of wheat straw lignin. *J. Agric. Food Chem.* **1989**, *37*, 81−87.

Kondo, T.; Watanabe, T.; Ohshita, T.; Kyuma, T. Comparative characterisation of dioxane-soluble lignins released by ball milling and by sheep digestion from forage grasses. *J. Sci. Food Agric.* **1995**, *68*, 383−388.

Kubelka, V.; Francis, R. C.; Dence, C. W. Delignification with acidic hydrogen peroxide activated by molybdate. *J. Pulp Paper Sci.* **1992**, *18*, J108−J114.

Lachenal, D.; Wable, F.; Damiens, P.; Ledon, H. The potential of H_2O_2 as delignifying and bleaching agent—Application to new bleaching sequences. *Pan Pacific Pulp and Paper Technology Conference Proceedings*; JTAPPI: Tokyo, Japan, 1992; pp 33−37.

Lapierre, C.; Monties, B.; Guittet, E.; Lallemand, J. Y. Photosynthetically ^{13}C-labelled polar lignins: ^{13}C NMR experiments. *Holzforschung* **1984**, *38*, 333−342.

Lawther, J. M.; Sun, R. C.; Banks, W. B. Extraction, fractionation, and characterization of structural polysaccharides from wheat straw. *J. Agric. Food Chem.* **1995**, *43*, 667−675.

McDonough, T. J.; Kirk, R. C.; Backlund, B.; Winter, L. *Oxygen Delignification Conference Proceedings*; TAPPI Press: Atlanta, GA, 1989; pp 165−168.

Migne, C.; Prensier, G.; Utille, J. P.; Angibeaud, P.; Cornu, A.; Grenet, E. Immunocytochemical location of *p*-coumaric acid and feruloyl-arabinose in the cell walls of maize stem. *J. Sci. Food Agric.* **1998**, *78*, 373−381.

Morrison, T. A.; Jung, H. G.; Buxton, D. R.; Hatfield, R. D. Cell-wall composition of maize internodes of varying maturity. *Crop Sci.* **1998**, *38*, 455−460.

Nimz, H. H.; Robert, D.; Faix, O.; Nemr, M. Carbon-13 NMR Spectra of lignins, 8: Structural differences between lignins of hardwoods, softwoods, grasses and compression wood. *Holzforschung* **1981**, *35*, 16−26.

Pan, G. X.; Bolton, J. L.; Leary, G. J. Determination of ferulic and *p*-coumaric acids in wheat straw and the amounts released by mild acid and alkaline peroxide treatment. *J. Agric. Food Chem.* **1998**, *46*, 5283−5288.

Pan, X. Q.; Lachenal, D.; Neirinck, V.; Robert, D. Structure and reactivity of spruce mechanical pulp lignins: ^{13}C-NMR Spectral studies of isolated lignins. *J. Wood Chem. Technol.* **1994**, *14*, 483−506.

Perez, D. da S.; Terrones, M. G. H.; Grelier, S.; Nourmamode, A.; Castellan, A. Peroxyformic acid pulping of *Eucalyptus grandis* wood chips and sugar cane bagasse in one stage and characterization of the isolated lignins. *J. Wood Chem. Technol.* **1998**, *18*, 333−365.

Quesada, J.; Rubio, M.; Gomez, D. Chemical characterization of ozonated lignin solutions from corn (*Zea mays*) stalk and poplar (*Populus deltoides*) wood by capillary gas chromatography. *J. High Resolut. Chromatogr.* **1997**, *20*, 565−568.

Ralph, J.; Hatfield, R. D.; Quideau, S.; Helm, R. F.; Grabber, J. H.; Gung, H. G. Pathway of *p*-coumaric acid incorporation into maize lignin as revealed by NMR. *J. Am. Chem. Soc.* **1994**, *116*, 9448−9456.

Ruggiero, R.; Machado, A. E. H.; Perez, D. da S.; Grelier, S.; Nourmamode, A.; Castellan, A. Bleached chemical pulp from *Eucalyptus grandis* wood produced by peroxyformic acid pulping and photochemical bleaching. *Holzforschung* **1998**, *52*, 325−332.

Scalbert, A.; Monties, B.; Guittet, E.; Lallemand, J. Y. Comparison of wheat straw lignin preparations I. Chemical and spectroscopic characterizations. *Holzforschung* **1986**, *40*, 119−129.

Seisto, A.; Poppius-Levlin, K. Peroxyformic acid pulping of nonwood plants by the MILOX method—Part 1: Pulping and bleaching. *Tappi J.* **1997**, *80*, 215−221.

Springer, E. L. Delignification of aspen wood using hydrogen peroxide and peroxymonosulfate. *Tappi J.* **1990**, Jan., 175−178.

Strumila, G.; Rapson, H. Reaction products of neural peracetic acid oxidation of model lignin phenols. *Pulp Paper Can.* **1975**, *76*, 72−76.

Sun, R. C.; Lawther, J. M. Fractional and structural characterization of ball-milled and enzyme lignins from wheat straw. *J. Appl. Polym. Sci.* **1998**, *68*, 1633−1641.

Sun, R. C.; Tomkinson, J. Comparative study of hemicelluloses from rice straw by alkali extraction and alkaline peroxide treatment. *Carbohydr. Polym.* **2000**, in press.

Sun, R. C.; Lawther, J. M.; Banks, W. B. Influence of alkaline pre- treatments on the cell wall components of wheat straw. *Ind. Crops Prod.* **1995**, *4*, 127−145.

Suss, H. U.; Helmling, O. Delignification using hydrogen peroxide and oxygen under acidic conditions-the Po_s-treatment. *Jahrgang. Heft* **1986**, *40*, 258−262.

Yuan, Z.; Entremont, M.; Ni, Y.; Heiningen, A. V. The formation of gaseous products and its relation to pulp bleaching during the peracetic acid treatment. *J. Wood Chem. Technol.* **1998a**, *18*, 267−288.

Yuan, Z.; Ni, Y.; Heiningen, A. V. An improved peracetic acid bleaching process. *Appita J.* **1998b**, *51*, 377−380.

Zhang, X. Z.; Francis, R. C.; Dutton, D. B.; Hill, R. T. The role of transition metal species in delignification with distilled peracetic acid. *J. Wood Chem. Technol.* **1998**, *18*, 253−266.

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