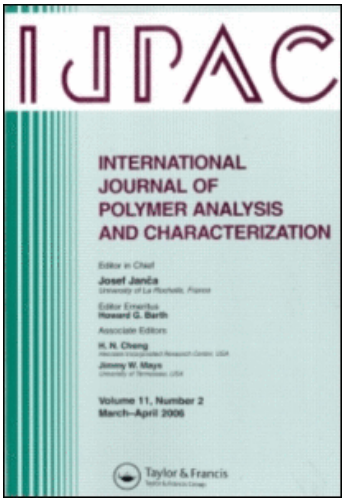

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Structural Characterization of Residual Lignins Isolated with Tetraacetylethylenediamine-Activated Peroxide from Ultrasonically Irradiated Organosolv Pretreated Wheat Straw

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Abstract: Residual lignin (14.5–30.8% of the original wheat straw lignin) was obtained by post-treatment with 2% H_2O_2-0.2% tetraacetylethylenediamine (TAED) at pH 11.8 for 12 h at 48°C from ultrasonic irradiation (0–35 min) following by organosolv treatment of wheat straw. Analyses of the released residual lignins revealed that TAED-activated peroxide post-treatment led to side-chain oxidation of the lignin as shown by a slight increase of aliphatic carboxyl groups. The eight residual lignin preparations were relatively free of bound polysaccharides, indicating substantial cleavage of the α-ether linkages between lignin and polysaccharides. The lignin preparation solubilized during the TAED-activated peroxide post-treatment had a higher molecular weight and thermal stability than that of the corresponding lignin fraction released during the alkaline organosolv pretreatment with or without ultrasonic assistance. Detailed structural characterization of the residual lignin was performed by ^{13}C NMR.

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Lignin is a polymeric material composed of phenylpropanoid units derived from three cinnamyl alcohol (monolignols): *p*-coumaryl, coniferyl, and sinapyl alcohols.[1] Lignin is randomly cross-linked phenylpropane type subunits, consisting of *p*-hydroxyphenyl, guaiacyl, and syringyl units.[2] Hardwood, softwoods, and grass lignins differ considerably in the relative amounts of these subunits.[3] The most frequent linkage between subunits is a β-aryl ether linkage (48 and 60% in spruce and birch lignins, respectively),[4] whereas for lignins formed in vitro by mixing coniferyl alcohol, hydrogen peroxide, and peroxidase, higher percentages of β-β and β-5 carbon-carbon linkages are found.[5,6] In addition, lignin is chemically linked to polysaccharides in the cell wall to form large macromolecules. It has been reported that lignin is linked to arabinoxylans in grasses via ferulic acid molecules,[7] although chemical extraction data do point toward α-ether linkages of lignin directly to polysaccharides.[8] However, unlike ferulic acid, *p*-coumarate in grasses does not involve a lignin-hemicellulose cross-linking function and most *p*-coumarate is esterified to lignin rather than carbohydrate.[9] From a functional point of view, lignins impart strength to cell walls, facilitate water transport, and impede the degradation of wall polysaccharides, thus acting as a major line of defence against pathogens, insects, and other herbivores.[1]

Although lignin has been recognized as a distinct chemical entity of plant cell walls for over 100 years,[10] there is still considerable scientific argument concerning its structure, biosynthesis, and measurement. These uncertainties arise mainly from the complexity of lignin synthesis and the resultant complexity of molecular lignin structure. Up to now, there is no standard lignin structure for reference, and measurement of lignin concentration is empirical and very dependent on methodology.[11] In addition, due to the heterogeneous nature of lignin, the extent of the different reactions taking place during the isolation process is difficult to assess. That is, precise knowledge of the composition of lignins cannot be obtained directly, but must be gained indirectly by nuclear magnetic resonance (NMR) of soluble lignin fractions or by identification of lignin degradation products.[12] Degradation methods used include pyrolysis-GC-MS, hydrolysis, and oxidation,[13,14] in which the first two methods can provide information about the interunit linkages in the parent lignin, since a portion of the products from these methods retain their C-3 side chains.[15] In contrast, information from oxidation methods, such as nitrobenzene oxidation, on the nature of the side chains is lost because oxidation methods degrade lignins into benzaldehyde or benzoic acid

analogues of their parent lignols.[16] However, through careful analyses of these compounds a detailed picture of the original lignin can emerge. Moreover, the properties of lignin can be obtained from the study of the development of functional groups and the changes in molecular weight in isolated lignin.[17]

The aim in our laboratory is to develop a commercial process for fractionation of straw components using an environmentally friendly procedure for the extraction of lignin and hemicelluloses in a large scale with a light color. In an earlier article[18] we reported that hydrogen peroxide in alkaline media could serve as a mild agent for delignifying and bleaching besides its dual role in solubilizing macromolecular hemicelluloses. It is generally accepted that the hydroperoxide anion (HOO^-), formed in alkaline media, is the principal active species in hydrogen peroxide bleaching systems. In contrast, hydrogen peroxide is unstable in alkaline conditions and readily decomposes, particularly in the presence of certain transition metals, such as manganese, iron, and copper. These decomposition products, such as hydroxyl radicals ($HO^{\bullet}$) and superoxide anion radicals ($O_2^{-\bullet}$), are thought to cause oxidation of lignin structures, cleavage of some interunit bonds, and eventually dissolution of lignin and hemicelluloses.[19–21] More recently, it has been reported that using activators, such as tetraacetylethylenediamine (TAED), enhances the performance of peroxide in bleach under milder bleaching conditions. This enhancement is achieved because the reaction of TAED with peroxide produces a strong oxidizing agent that is a highly effective bleaching agent at low temperature.[22]

This article describes the feasibility of using TAED-activated peroxide treatment as an environmentally friendly procedure for removal of residual lignins from ultrasound irradiated and organosolv pretreated wheat straw. Eight isolated lignin preparations were characterized using alkaline nitrobenzene oxidation and UV, FT-IR, ^{13}C-NMR, and GPC. The results obtained are discussed in relation to those data obtained from lignin isolated during organosolv pretreatment with ultrasonic irradiation, reported in a previous article.[23]

EXPERIMENTAL SECTION

Materials

Wheat straw was obtained from the experimental farm of the North-Western University of Agricultural and Forest Sciences and Technology (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.

The composition (%, w/w) of the straw was cellulose 38.9%, hemicelluloses 38.2%, lignin 17.2%, ash 2.1%, and wax 2.3% on a dry weight basis.

Isolation of Residual Lignins with TAED-Activated Peroxide

The procedure used to isolate residual lignins with TAED-activated peroxide from ultrasound-assisted and organosolv pretreated wheat straw was based partially on a method for extraction of alkaline peroxide-soluble lignins from cereal straws;[24,25] the isolation scheme is shown in Figure 1. The pretreated wheat straw was soaked in 2% H_2O_2-0.2% TAED aqueous solution with a 1:25 straw to liquor ratio (g/mL). The dispersions were stirred at pH 11.8 for 12 h at 48°C. Upon completion, the residue was filtered off, washed thoroughly with water and ethanol until the filtrate was neutral, and then dried in an oven at 60°C for 16 h. Each of the supernatant fluids was neutralized to pH 5.5 with 6 M HCl, and the solubilized hemicelluloses were isolated by precipitation of the concentrated filtrates with three volumes of 95% ethanol. The TAED-activated peroxide-soluble residual lignins were obtained by reprecipitation at pH 1.5 adjusted with 6 M HCl from the corresponding supernatants after evaporation of ethanol. The acid-insoluble lignin preparations isolated were washed with acidified water (pH 1.5–2.0), freeze-dried overnight, and kept at 5°C until analysis. Triplicate runs were done for each lignin preparation. The relative standard deviation, determined by dividing the standard deviation by the mean value, was less than 3.8%.

Characterization of Acid-Insoluble Lignin Preparations

Nitrobenzene oxidation was used to compare monolignol composition among isolated residual lignin samples. A mixture of the sample (corresponding to ~10 mg of lignin), 7 mL of 1 N NaOH, and nitrobenzene (0.4 mL) in a 20 mL stainless-steel pressure vessel was heated at 170°C for 2.5 h. The oxidation products were separated on a Hichrom H5ODS 250 × 4.6 mm high-performance liquid chromatography (HPLC) column (Phenomenex Co., Beijing). The identification of the individual compounds was made at 280 nm by computer comparison of the retention times and peak areas with the authentic phenolics. The neutral sugar composition of the associated hemicelluloses in isolated acid-insoluble lignin preparations was determined as its alditol acetate derivatives by gas chromatography (GC).[26] The molecular-average weights of the acid-insoluble lignin preparations were determined by gel permeation chromatography (GPC) on a PL gel 4 μ Mixed-D column. The samples

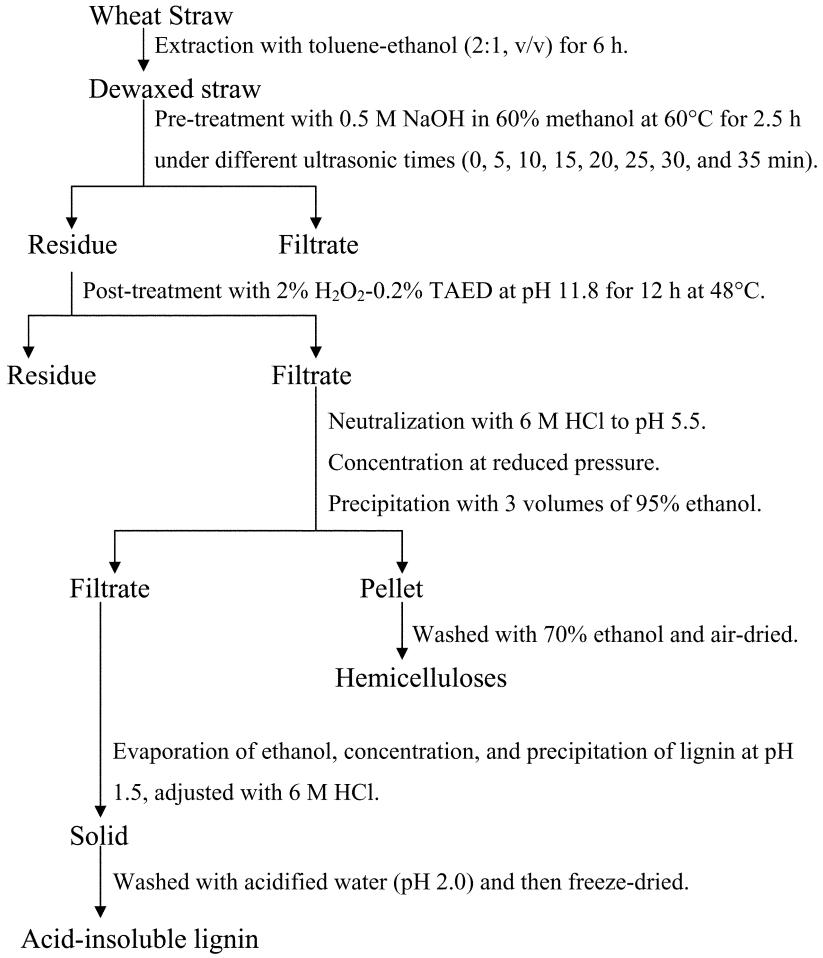


Figure 1. Scheme for isolation of acid-insoluble lignins with 2% H_2O_2-0.2% TAED at pH 11.8 for 12 h at 48°C from ultrasonically irradiated and organosolv pretreated wheat straw.

were dissolved with tetrahydrofuran with a concentration of 0.2%, and a 200 µL sample in solution was injected. The column was operated at 45°C and eluted with tetrahydrofuran at a flow rate of 1 mL in^{-1}. The column was calibrated using polystyrene standards.[25] The results of nitrobenzene oxidation and sugar analysis represent the mean of at least triplicate samples and each sample was chromatographed twice. The standard errors or deviations were observed to be $<4.2\%$, except for the variation among the triplicate nitrobenzene oxidation analyses (6.6–12.9%).

To carry out lignin purity determination, the samples were measured by UV spectroscopy. About 5 mg of the residual lignin sample was dissolved in 10 mL of 90% dioxane. Of this solution, 1 mL was further diluted with 50% dioxane to 10 mL. The resulting solution was transferred to a quartz cell, and the UV absorption was determined in the 250–400 nm range (Varian double-beam spectrophotometer) with 50% dioxane in the reference cell. Fourier transform-infrared (FT-IR) spectroscopy was performed on a Nicolet 510 spectrophotometer by using KBr (approximately 0.3 mg lignin sample with 300 mg KBr; resolution 4 cm^{-1}, 32 scans). The solution ^{13}C NMR spectrum was recorded on a Bruker MSI-300 spectrometer at 74.5 MHz from 250 mg of sample dissolved in 1.0 mL DMSO-d_6 after 28,000 scans. A 70° pulse flipping angle, a 10° pulse width, and a 15 s delay time between scans were used.

Thermal analysis of the acid-insoluble lignin preparations was performed using thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) on a simultaneous thermal analyzer (Netzsch STA-409). The apparatus was continually flushed with nitrogen. The sample weighed approximately 10 mg. Each sample was heated from room temperature to 600°C at a rate of 10°C per min.

RESULTS AND DISCUSSION

Fractional Yield and Purity of Lignin

Due to the complex lignin structure and linkage to other cell-wall polymers, isolation of lignin samples relatively free of polysaccharides is difficult. As shown in Table I, post-treatment with 2% H_2O_2-0.2% TAED

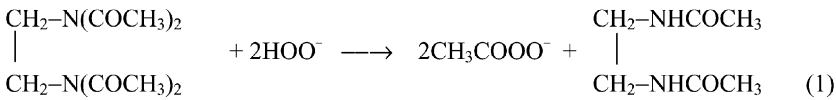
Table I. Yield of lignin fractions (% dry matter) obtained by post-treatment with 2% H_2O_2-0.2% TAED at pH 11.8 for 12 h at 48°C from alkaline organosolv pretreated wheat straw under different ultrasonic times

	Ultrasonic time (min)							
Lignin fractions	0	5	10	15	20	25	30	35
Total solubilized lignins	5.3	4.3	4.2	3.1	2.7	2.7	2.6	2.5
Acid-insoluble lignins[a]	3.4	2.6	2.6	1.8	1.5	1.5	1.5	1.5
Acid-soluble lignins[b]	0.9	0.8	0.7	0.5	0.5	0.5	0.6	0.6
Lignin associated in isolated hemicelluloses	1.0	0.9	0.9	0.8	0.7	0.7	0.5	0.4

[a]The lignin fractions obtained by precipitation of the supernatant solution at pH 1.5 after isolation of the solubilized hemicelluloses.
[b]The lignin fractions still solubilized in the pH 1.5 supernatant after precipitation of the acid-insoluble lignin fractions and obtained by difference.

at pH 11.8 for 12 h at 48°C ultrasonically irradiated for 0, 5, 10, 15, 20, 25, 30, and 35 min with subsequent organosolv treatment solubilized 5.3, 4.3, 4.2, 3.1, 2.7, 2.7, 2.6, and 2.5% lignin (percent dry starting material), corresponding to a dissolution of 30.8, 25.0, 24.4, 18.0, 15.7, 15.7, 15.1, and 14.5% of the original lignin, respectively. Post-treatment released 53.4, 50.8, 49.7, 46.9, 45.0, 45.0, 44.5, and 44.5% of the original hemicelluloses, respectively. The reason for this decreasing trend of lignin and hemicelluloses during post-treatment is probably the extensive solubilization of the lignin and hemicelluloses during pretreatment with 60% aqueous methanol containing 0.5 M NaOH at prolonged ultrasonic treatment. Taken together, the pretreatment of dewaxed wheat straw with 60% aqueous methanol in alkaline media at 60°C for 2.5 h under ultrasonic times of 0–35 min and sequential post-treatment with 2% H_2O_2-0.2% TAED at pH 11.8 for 12 h at 48°C resulted in a dissolution of 85.8–86.1% of the original hemicelluloses and 91.7–93.2% of the original lignin, respectively. The result implied that alkaline organosolv pretreatment combined with ultrasonic irradiation favoured the release of lignin, whereas the following TAED-activated peroxide post-treatment preferably dissolved hemicelluloses. Interestingly, the data in Table I showed that the acid-insoluble lignin fraction isolated was the major fraction, comprising 53.8–64.2% of the total solubilized lignins, whereas the lignin fraction, associated in the released hemicelluloses, accounted for only 16.0–25.9% of the total solubilized lignins. The results suggest that the TAED-activated peroxide post-treatment substantially cleaved ether linkages between lignin and hemicelluloses from the cell walls of ultrasonically irradiated and organosolv pretreated wheat straw.

Bleaching activators, such as TAED, are compounds with O– or N–bounded acetyl groups, that are able to react with the strong nucleophilic hydroperoxy anion to yield peroxyacetic acid for which peroxide alone would be ineffective.[27] During the post-treatment with TAED-activated peroxide, peroxide (perhydroxyl anion) reacts with TAED to form the low-temperature bleaching agent peracetic acid, as illustrated in reaction (1) below. The rate of peracetic acid formation increased with increasing alkalinity of the medium, probably due to the dissolution of peroxide and formation of the perhydroxyl anions (reaction (2)). In alkaline medium, theoretically 1 mol of TAED reacts with 2 mol of perhydroxyl ion (hydrogen peroxide) to produce 2 mol of peracetic anion, which is considered to be a better and more active bleaching agent than peroxide.[27] However, about 1.5 mol of peracetic anion is formed from 1 mol of the bleaching activator. The lower yield is a consequence of the competition between peracetic acid formation and simple saponification of TAED with hydroxide anions.[28] Both TAED and the reaction product DAED are nontoxic and non-sensitive and biodegrade to give carbon dioxide, water, nitrate, and ammonia as end products.



Under alkaline conditions, the rate of peracid release is rapid and increases with increasing temperature and pH. Increasing the concentration of peroxide in the system also increases the rate of formation of peracid anion in solution.[22] Therefore, during the post-treatment an excess of peroxide (2%) over TAED (0.2%) used to ensure sufficient peroxide was available to force the reaction to completion. More importantly, the results also showed that the brightness of the pulps achieved using a low-temperature (48°C) TAED-activated peroxide bleaching process was clearly improved as compared to a peroxide process without TAED.[29]

The content of neutral sugars in eight acid-insoluble lignin preparations is given in Table II. Evidently, all the lignin fractions contained rather low amounts of contaminated hemicelluloses as shown by a 0.81–0.87% neutral sugar content, indicating again that post-treatment with TAED-activated peroxide significantly cleaved the ether bonds between lignin and hemicelluloses in the cell walls of ultrasonically irradiated and organosolv pretreated wheat straw in addition to partial saponification of hydroxycinnamic esters, such as between ferulic acid and hemicelluloses or between *p*-coumaric acid and lignin. These linkages may be one of the main obstacles for the removal of residual lignin from pretreated wheat straw together with unreactive structures in residual

Table II. Content of neutral sugars (% lignin sample, w/w) in isolated acid-insoluble lignin preparations obtained by post-treatment with 2% H_2O_2-0.2% TAED at pH 11.8 for 12 h at 48°C from alkaline organosolv pretreated wheat straw under different ultrasonic times

	Ultrasonic time (min)							
Neutral sugars	0	5	10	15	20	25	30	35
Arabinose	0.22	0.21	0.21	0.20	0.20	0.21	0.21	0.20
Xylose	0.35	0.33	0.32	0.34	0.32	0.33	0.34	0.33
Galactose	0.10	0.11	0.11	0.11	0.11	0.10	0.11	0.10
Glucose	0.20	0.19	0.18	0.19	0.19	0.19	0.19	0.18
Total	0.87	0.84	0.82	0.84	0.82	0.83	0.85	0.81

lignins and the inaccessibility in the fiber wall. The results showed that the content of xylose (0.32–0.35%) and arabinose (0.20–0.22%) was slightly higher than that of glucose (0.18–0.20%), suggesting that the associated polysaccharides originated from hemicelluloses.

UV absorbance behavior between 270 and 280 nm is of special interest because of its dependence upon the delocalized π-electron system in aromatic ring structures.[3] Clearly, all the UV spectra of the lignin preparations (figure not shown) give a guaiacyl peak at 280 nm and a broad shoulder between 300 and 320 nm, since the pronounced maximum absorbance at 280 nm usually indicates the presence of strongly absorbing guaiacyl lignin.[30] Grass lignin, like wheat straw lignin, is composed of guaiacyl-, syringyl-, and *p*-hydroxyphenolpropanes. As a unique feature, it also contains 5–10% of *p*-coumaric acid esters,[31] which are at the γ-positions of grass lignins, predominantly on syringyl units.[32] The shoulder in the lignin spectra, therefore, can be linked to the presence of *p*-coumaroylation as demonstrated by Higuchi.[31] No obvious chromophore absorption maximum beyond that of the aromatic ring without (around 280 nm) and with simple (around 330 nm) conjugation can be seen, and a monotonously declining curve extends into the visible region (>400 nm).

Lignin Composition

To compare monolignol composition among isolated acid-insoluble lignin samples, nitrobenzene oxidation of the lignin preparations was performed at 170°C for 2.5 h. Nitrobenzene analysis does not result in the complete disassociation of lignin molecules into their monolignol subunits. Owing to possible effects of heterogeneity of lignin structures upon the yields of nitrobenzene reaction products, no attempt was made to apply correction factors to account for total lignin composition.[33] The values given in Table III are the yield of phenolic acids and aldehydes and the molar proportions of S (relative total moles of syringaldehyde and syringic acid):V (relative total moles of vanillin and vanillic acid):H (relative total moles of *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid) from nitrobenzene oxidation of each acid-insoluble lignin preparation. As can be seen, all the lignins composed of both guaiacyl and syringyl units resulted in a mixture of vanillin and syringaldehyde as the most abundant oxidative products, which together represented 83.7, 86.9, 88.5, 87.5, 87.0, 86.4, 84.8, and 86.7% of the total phenolic compounds in the lignin fractions isolated by TAED-activated peroxide from the organosolv pretreated without ultrasonic irradiation and with ultrasound assistance for 0, 5, 10, 15, 20, 25, 30, and 35 min, respectively. The occurrence of minor quantities of *p*-hydroxybenzoic acid and

Table III. Content (% lignin sample, w/w) of phenolic acids and aldehydes from nitrobenzene oxidation of the acid-insoluble lignin preparations obtained by post-treatment with 2% H_2O_2-0.2% TAED at pH 11.8 for 12 h at 48°C from alkaline organosolv pretreated wheat straw under different ultrasonic times

	Ultrasonic time (min)							
Phenolic acids and aldehydes	0	5	10	15	20	25	30	35
p-hydroxybenzoic acid	0.77	1.26	1.18	1.08	1.20	1.02	1.05	0.86
p-hydroxybenzaldehyde	0.82	1.06	1.22	1.26	1.24	1.21	1.05	0.76
Vanillic acid	0.32	0.52	0.55	0.51	0.58	0.51	0.41	0.38
Syringic acid	1.73	2.08	2.15	2.38	2.36	2.38	2.01	1.46
Vanillin	13.68	22.55	27.59	24.69	24.33	21.84	17.36	15.54
Syringaldehyde	10.46	19.56	23.02	21.87	20.04	17.52	13.81	11.90
p-coumaric acid	0.46	0.56	0.58	0.53	0.45	0.38	0.36	0.32
Ferulic acid	0.61	0.84	0.92	0.90	0.80	0.72	0.66	0.42
Total	28.85	48.43	57.21	53.22	51.00	45.58	36.76	31.64
Molar ratio (S:V:H)[a]	5:7:1	6:8:1	7:9:1	7:9:1	6:8:1	6:8:1	5:7:1	6:8:1

[a]S represents the relatively total moles of syringaldehyde and syringic acid; V represents the relatively total moles of vanillin and vanillic acid; and H represents the relatively total moles of *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid.

p-hydroxybenaldehyde resulted from the oxidation of fewer non-condensed *p*-hydroxyphenyl units in the lignin preparations. A similar higher molar ratio of V:S (7:5, 8:6, 9:7) in the acid-insoluble lignins indicated that guaiacyl lignin units slightly predominate the syringyl ones in the TAED-activated peroxide-soluble lignin preparations, indicating that guaiacyl lignin units engaged in β-O-4 lignin structures were more easily degraded than syringyl lignin units. The reason for this higher degradation of guaiacyl units is that they have more free bare hydroxyls than do syringyl units.[34] A relatively lower yield of nitrobenzene oxidation for the lignin preparation obtained with TAED-activated peroxide from the organosolv pretreated wheat straw in the absence of ultrasonic irradiation implied that this lignin fraction had a relatively higher degree of condensation than the lignin preparations isolated with TAED-activated peroxide from the ultrasonically irradiated and organosolv pretreated wheat straw.

It is well known that *p*-coumaric and ferulic acids are the prominent components of hydroxycinnamic acids in grass walls.[35] During the nitrobenzene oxidation process, part of *p*-coumaric acid was converted to *p*-hydroxybenzaldehyde and ferulic acid to vanillin, respectively, and was counted as part of the lignin. Recovery of these hydroxycinnamic acids was determined to be a function of both temperature and time.[24] As shown in Table III, the remaining small amounts of ferulic acid (0.42–0.92%) and *p*-coumaric acid (0.32–0.58%) in all the lignin oxidation products obtained at 170°C for 2.5 h indicated that these two hydroxycinnamic acids are closely linked with lignins in the cell walls of wheat straw.

Molecular Weights

To investigate the extent of degradation occurring during the TAED-activated peroxide post-treatment, the eight acid-insoluble lignin preparations were subjected to GPC in nonaqueous medium, and their weight-average (M_w) and number-average (M_n) molecular weights and polydispersity (M_w/M_n) are listed in Table IV. As can be seen in Table IV, the eight lignin preparations isolated by TAED-activated peroxide from the organosolv pretreated straw without or with ultrasound assistance for 5–35 min showed no significant difference in their molecular-average weights, which M_w ranged from 4,090 to 4,680 g mol^{-1}. In comparison, the lignin preparations isolated with TAED-activated peroxide during the post-treatment had much higher M_w than the corresponding lignin samples (M_w, 2,140–2,600 g mol^{-1})[23] solubilized during the organosolv pretreatment combined with ultrasonic irradiation, indicating that the post-treatment with TAED-activated peroxide did not lead to

Table IV. Weight-average (M_w) and number-average (M_n) molecular weights and polydispersity (M_w/M_n) of the isolated acid-insoluble lignin preparations obtained by post-treatment with 2% H_2O_2-0.2% TAED at pH 11.8 for 12 h at 48°C from alkaline organosolv pretreated wheat straw under different ultrasonic times

	Ultrasonic time (min)							
	0	5	10	15	20	25	30	35
M_w	4090	4560	4500	4510	4450	4440	4610	4680
M_n	1350	1550	1560	1590	1580	1570	1480	1460
M_w/M_n	3.02	2.95	2.88	2.85	2.81	2.83	3.11	3.21

substantial degradation of the lignin polymers. In addition, the eight lignin samples also showed a fairly analogous elution pattern and were clearly polydisperse.

Spectroscopic Characterization

The FT-IR spectra (Figure 2) of the acid-insoluble lignin preparations isolated with TAED-activated peroxide from the ultrasonically irradiated and alkaline organosolv pretreated wheat straw appear to be rather similar, indicating a similar structure of the polymers, and give the bands of Graminous lignins, which are made up of three types of phenylpropane units: *p*-hydroxyphenylpropane, guaiacylpropane, and syringylpropane. The broad band at 834 cm^{-1} originated from the *p*-hydroxyphenyl units and the one at 1126 cm^{-1} is attributed to guaiacyl and syringyl units.[36] A shoulder at 1162 cm^{-1} arises from carbonyl groups in a conjugated ester structure, indicating minor quantities of ester linkages in lignin molecules such as the ester linkage between *p*-coumaric acid and lignin. Absorption at 1037 cm^{-1} indicates aromatic CH in-plane deformation from non condensed guaiacyl units.[23] Aromatic skeleton vibrations (around 1500 cm^{-1} for syringyl and 1515 cm^{-1} for guaiacyl units) in the lignin preparations give bands at 1598, 1510, and 1423 cm^{-1}. Another intense band at 1465 cm^{-1} is indicative of C−H deformations and aromatic methyl group vibrations. The 1330 cm^{-1} band is due to the syringyl ring breathing with CO stretching, while the 1224 cm^{-1} relates to guaiacyl ring breathing with CO stretching. More importantly, two shoulders at about 1706 and 1636 cm^{-1} represent the unconjugated and conjugated carboxyl-carbonyl stretching, respectively, and are of particular interest since oxidative post-treatment with TAED-activated peroxide should result in an increase in this band. However, occurrence of the two rather

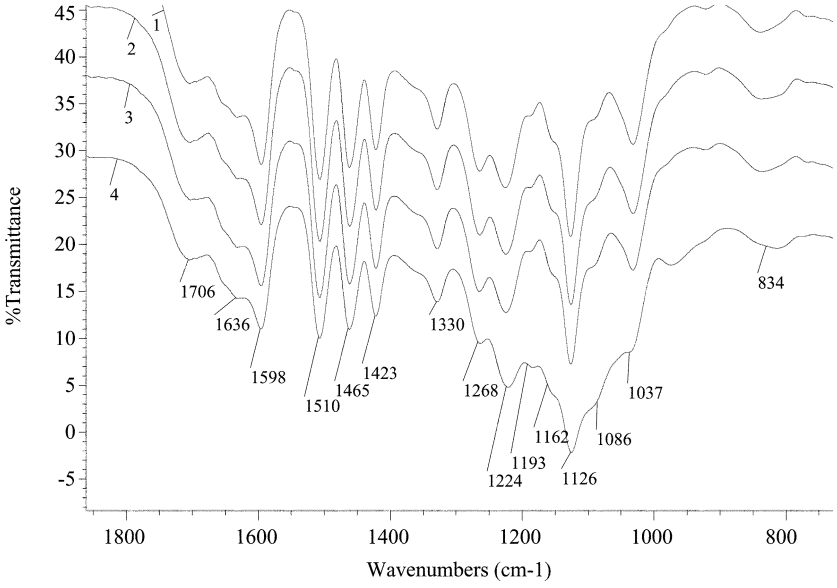


Figure 2. FT-IR spectra of acid-insoluble lignin preparations obtained by post-treatment with 2% H_2O_2-0.2% TAED at pH 11.8 for 12 h at 48°C from alkaline-organosolv pretreated wheat straw under ultrasonic assistance for 5 (spectrum 1), 15, (spectrum 2), 25 (spectrum 3), and 35 min (spectrum 4).

weak shoulders indicated that post-treatment with TAED-activated peroxide did not result in significant oxidation of the lignin polymer.

^{13}C-NMR Spectrum

The lignin preparation obtained by post-treatment of the ultrasonic-assisted (20 min) and organosolv pretreated wheat straw with TAED-activated peroxide was studied by ^{13}C NMR spectroscopy (figure not shown). Most of the observed signals have been previously assigned in straw and wood lignin spectra.[37–39] Evidently, the most striking characteristic of the ^{13}C NMR spectrum is the almost complete absence of typical polysaccharide signals between 57 and 103 ppm. The spectrum does show a very small signal at 63.1 ppm (C-5, Xyl internal unit)[39] for the associated hemicelluloses, however, the peak intensity is rather weak, indicating trace amounts of contaminated polysaccharides. Another pronounced structural difference between the native lignins (ball-milled lignins)[40] is the amount of carboxylic acid groups. A slight increase in the amount of carboxylic acid groups was noticed from ball-milled lignin

as compared to TAED-activated peroxide-soluble lignin as shown by a intense signal at 174.6 ppm, which originated from aliphatic carboxyl groups. Such groups can be formed directly as a result of oxidation reactions in the lignin side chains, since this signal in ball-milled lignin is very small. This indicated that some carboxylic acid groups were formed during the TAED-activated peroxide post-treatment process. That is, the TAED-activated peroxide post-treatment did lead to the formation of carboxyl groups. These results demonstrated that side-chain oxidation in lignin is pronounced in TAED-activated peroxide post-treatment. This agrees with recent results from TAED-activated peroxide bleaching of mechanical pulp,[41] peroxide bleaching alone,[42] and model compound studies.[43] In addition, the presence of carboxyl groups from C-6 in methyl uronates, which were esterified to lignin side chains, would appear in the same spectral region as other aliphatic acids.[44]

The region between 104.2 and 167.9 ppm represents the aromatic part of the lignin. The syringyl (S) residues were identified by signals at 152.2 (C-3/C-5, S etherified), 147.0 (C-3/C-5, S nonetherified), 138.1 (C-4, S etherified), 134.3 (C-1, S etherified), 106.7 (C-2/C-6, S with α-CO), and 104.2 ppm (C-2/ C-6, S). Guaiacyl (G) residues were verified by 149.1 (C-3, G etherified), 147.0 (C-4, G etherified), 145.4 (C-4, G nonetherified), 134. 3 (C-1, G etherified), 119.4 (C-6, G), 114.8 (C-5, G), and 111.1 ppm (C-2, G). The *p*-hydroxyphenyl (H) residues were detected at 128.1 ppm (C-2/C-6, H). The signals at 167.0 (C-γ, PC ester), 159.8 (C-4, PC ester), 130.1 (C-2/C-6, PC ester), and 115.8 ppm (C-3/ C-5, PC ester) are indicative of esterified *p*-coumaric acid. Etherified ferulic acids give signals at 167.9 (C-γ, FE ether), 144.2 (C-α, FE ether), and 122.2 ppm (C-6, FE ether, data not shown). These observations demonstrated that *p*-coumaric is linked to lignin by ester bonds, while ferulic acid is linked to lignin by ether bonds.

In native wood lignin, the β-O-4 linkage is the predominant interunit linkage with smaller amounts of carbon-carbon and (aromatic) carbon-oxygen linkages being present. The latter types are referred to as condensed lignin structures and may be present in structures of the β-5, 5-5, and 4-O-5 types.[42,45] During the TAED-activated peroxide post-treatment process, these structures may increase due to the partial cleavage of β-O-4 structures. This side-chain displacement reaction (the Dakin-like reaction) has been suggested to be responsible for lignin degradation during hydrogen peroxide bleaching.[46] The mechanism involves cleavage of the lignin polymer between the aromatic ring and the C-α atom in phenolic benzyl alcohol structures.[42] As shown in Figure 4, side-chain carbons carrying an oxygen substituent are centered at 86.0, 72.3, and 60.1 ppm, representing for oxygenated C-β (C-β in β-O-4), C-α (C-α in β-O-4), and C-γ (C-γ in β-O-4), respectively. This magnitude of β-O-aryl ether structures revealed that post-treatment with

TAED-activated peroxide did not attack the β-aryl ether structure to a significant extent. In other words, the lignin that went into solution regained most of the lignin-like structure, an appreciable amount of β-O-4 linkage.

In the NMR spectrum, certain signals belonging to condensed structures can easily be distinguished. In the aromatic region, a signal at 125.2 ppm (data not shown) is due to C-5/C-5′ in 5-5′ structures. Other common carbon-carbon linkages, such as β-β (C-γ in β-β units, 71.8 ppm), were also present. A very strong signal at 55.9 ppm is attributed to the OCH_3 in syringyl and guaiacyl units, indicating that the TAED-activated peroxide post-treatment did not lead to a substantial degree of demethylation.

In the spectral region below the methoxyl signal at 55.9 ppm, a variety of methine, methylene, and methyl groups, bound only to other carbon atoms, can be found. As illustrated in Figure 4, the largest group of signals in the aliphatic region consists of the methylene carbons located in the area of 22–37 ppm. Among the latter, the signal at 29.1 ppm is the largest and originated from a symmetric 5-5′ coupled diguaiacylmethane.[42,47] An intense signal at 14.0 ppm is assigned to methyl groups in lignin side chains. However, due to interference from the solvent signal (DMSO), it is not possible to make any quantitative conclusions about the broad methine carbon signal centered around 40–50 ppm.[42]

Thermal Stability

Thermograms of the acid-insoluble lignin preparation isolated with 2% H_2O_2-0.2% TAED at pH 11.8 for 12 h at 48°C from the alkali-organosolv treated wheat straw under ultrasonic assistance for 25 min showed a decomposition temperature ranging between 200 and 600° C (thermograms not shown). At 50% weight loss the decomposition temperature of the lignin preparation occurred at 500°C. In comparison with the corresponding lignin fraction solubilized during the alkaline organosolv pretreatment combined with ultrasonic irradiation, the lignin preparation obtained by post-treatment with TAED-activated peroxide had a higher thermal stability, which corresponded to an increased molecular weight. The DSC curve of the lignin preparation gave a large exothermic peak centered at 510°C, due to exothermic reactions of the lignin. This exothermic peak shifted to a higher temperature of over 450°C, as compared to that of the corresponding lignin sample solubilized during the alkaline organosolv pretreatment combined with ultrasonic irradiation, indicative again that the TAED-activated peroxide-soluble lignin preparation had a higher thermal stability.

CONCLUSIONS

The results presented in this study clearly showed that alkali-organosolv pretreatment combined with ultrasonic irradiation for 5–35 min favored the release of lignin (67.4–78.5% of the original lignin), whereas subsequent TAED-activated peroxide post-treatment preferably dissolved hemicelluloses (44.5–53.4% of the original hemicelluloses) except for dissolution of 14.5–30.8% of the original lignin. In TAED-activated peroxide post-treatment, aliphatic carboxyl groups were formed in the dissolved lignin, indicating oxidation of the residual lignin side chain caused by TAED-activated peroxide. However, the occurrence of strong peaks in aromatic region and three intensive signals for β-O-4 linkages between lignin units in the ^{13}C NMR spectrum indicated that the TAED-activated peroxide post-treatment did not degrade the macromolecular lignin structure to a significant extent, except for substantial cleavage of α-ether bonds between lignin and hemicelluloses.

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