Structural Characterization of Residual Lignins Isolated with Cyanamide-activated Hydrogen Peroxide from Various Organosolvs Pretreated Wheat Straw

F. Xu,[1,2] Z. C. Geng,[3] C. F. Liu,[4] J. L. Ren,[4] J. X. Sun,[3] R. C. Sun[2,3,4]*

[1]*College of Light and Textile Industry, Qiqihar University, Wenhua Street, Qiqihar, China*
[2]*College of Material Science and Technology, Beijing Forestry University, Beijing, 100083, China*
[3]*College of Forestry, the North-Western University of Agricultural and Forest Sciences and Technology, Yangling 712100, China*
[4]*State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou 510641, China*

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ABSTRACT: The posttreatment of various organosolvs pretreated wheat straw with cyanamide-activated hydrogen peroxide was studied. About 44–80% of the total residual lignin and 38–85% of the total residual hemicelluloses were released or degraded during the posttreatment with 1.8% H_2O_2–0.18% cyanamide at 50°C under pH 10.0 for 4 h from different aqueous organic acids or alcohols pretreated straw. The seven degraded residual lignin preparations were subjected to a comprehensive physicochemical and structural characterization by UV, FTIR, and ^{1}H and ^{13}NMR spectroscopy, and GPC. The nitrobenzene oxidation method was also applied to the *in situ* lignins. It was found that the seven residual lignin preparations contained large amounts of noncondensed syringyl and guaiacyl units, together with fewer noncondensed *p*-hydroxyphenyl units, esterified *p*-coumaric acid, and mainly etherified ferulic acid. All of the lignin fractions are free of associated polysaccharides and had molecular-average weights ranging between 2980 and 3820 g mol^{-1}. Analysis of these low molecular weight degradation products revealed an oxidation of residual lignin had occurred. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 109: 555–564, 2008

Key words: wheat straw; residual lignins; cyanamide-activated hydrogen peroxide; degradation

INTRODUCTION

Lignin is an extremely complex three-dimensional polymer formed by dehydrogenative polymerization of *p*-hydroxycinnamyl, coniferyl, and sinapyl alcohols. These three lignin precursors (''monolignols'') give rise to the so-called H (*p*-hydroxyphenyl), G (guaiacyl), and S (syringyl) phenylpropanoid units, which show different abundances in lignins from different groups of vascular plants, as well as in different plant tissues and cell-wall layers. During polymerization of the above *p*-hydroxycinnamyl alcohols involved, the formation of aryl ether (involving C_4) interunit linkages is strongly favored. In addition, a small proportion of lignin units remains as phenolic, being linked only by carbon–carbon bonds, such as β-5, β-1, β-5, β-β, and α-β linkages.[1] In woody tissues lignin acts as an adhesive that binds together the cellulose fiber structure and imparts stiffness to the wood matrix. In this case, the various covalent linkages and physicochemical interactions between lignin, cellulose, hemicelluloses, phenolic acids, and other polyphenolic or proteinaceous or mineral extractives all contribute structural integrity to the plant cell wall composite.[2] Lignin is a natural polymer of plants that is conventionally obtained as a by-product of pulping process. Delignification in pulping processes consists of the degradation and dissolution of lignin macromolecule in smaller fragments.[3] In other words, the release of lignin from the cell wall is mainly related to the hydrolysis by alkali of ester bonds between lignins and phenolic acids and/or acidic sugars from pectins or hemicelluloses associated with lignin.[4–8] These lignins from different processes have a wide range of potential applications, such as carbon fibers, adhesives, ion-exchange resins, and metal ion-chelating resinds.[9–11]

This is a growing concern, from an environmental perspective, about chlorinated organic compounds

Correspondence to: R. C. Sun (bcs00a@bangor.ac.uk).
**Present address:* College of Forestry, the North-Western University of Agricultural and Forest Sciences and Technology, Yangling, China.

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present in pulp mill effluents. Oxygen-containing chemicals such as oxygen, hydrogen peroxide, and ozone have been used as nonchlorine bleaching agents for commercial pulp.[12] In particular, hydrogen peroxide has become increasingly important in the today's bleaching technology as the pulp and paper industry is moving toward minimization of environmental impact. For many years it was extensively accepted that perhydroxyl anion HOO^- was the most important active species involved in the suppression of chromophores in lignin macromolecules.[13] Moreover, it is also believed that this system is much more complex, and various radicals species including $OH^{\cdot}$ and O_2^{-} can particulate in chromophore elimination.[14] However, because of the limited reactivity towards possible structure in residual lignin and effectiveness in removing lignin, they are unable to replace chlorine-containing bleaching reagents.[15] To increase their reactivity, strong reaction conditions are being employed. Although high temperature and chemical charge facilitates delignification it also promotes polysaccharide degradation, particularly in the presence of transition-metal ions.[16] For this reason the activation of oxygen-based reagents is being extensively studied. One particular area of interest has been in the activation of hydrogen peroxide by cyanamide.[17,18] In this article residual lignins were isolated or degraded with hydrogen peroxide in the presence of cyanamide from various organosolvs pretreated wheat straw. The lignins obtained were physicochemically and structurally characterized by both degradation methods such as alkaline nitrobenzene oxidation and acid hydrolysis, and nondestructive techniques, e.g., ultraviolet (UV), Fourier transform infrared (FTIR) and hydrogen-1 and carbon-13 nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectroscopy, and gel permeation chromatography (GPC).

EXPERIMENTAL

Material

Wheat straw (*Variety Riband*) was kindly supplied by B Lloyd, Llangefni. The chemical composition (%, w/w) of the straw used in this work is cellulose 39.0%, hemicelluloses 38.7%, lignin 17.0%, ash 1.8%, and wax 1.9% on a dry weight basis. After being dried at 60°C in an oven for 16 h, the straw was ground to pass through a 0.7-mm screen. Prior to treatment, the dried powder of the straw was first extracted with toluene–ethanol (2 : 1, v/v) in a Soxhlet extractor for 6 h so as to dewax, and then air-dried.

Organosolv pretreatment and cyanamide-activated hydrogen peroxide posttreatment

Organosolv pretreatment was carried out in a 500-mL glass reactor equipped with mechanical stirring at atmospheric pressure. The extractive free powder (10.0 g) was pretreated with acetic acid-H_2O (65/35, v/v), acetic acid-H_2O (80/20, v/v), acetic acid-H_2O (90/10, v/v), formic acid-acetic acid-H_2O (20/60/20, v/v/v), formic acid-acetic acid-H_2O (30/60/10, v/v/v), methanol-H_2O (60/40, v/v), and ethanol-H_2O (60/40, v/v) using 0.1% HCl as a catalyst at 85°C for 4 h with a liquor to solid ratio of 20 : 1 (mL/g), respectively. The residue was subsequently washed with distilled water and ethanol, and then oven dried at 60°C for 16 h. The cyanamide-activated hydrogen peroxide posttreatment of the above residues was performed at 50°C under pH 10.0 for 4 h with 1.8% H_2O_2-0.18% cyannmide (residue : extractant, 1 : 30, g/mL), respectively. The solubilized residual hemicelluloses were separated from the insoluble residue (cellulose) by filtration with a nylon cloth. The residue was subsequently washed with distilled water and ethanol, and then oven dried at 60°C for 16 h. Then the supernatant was neutralized to pH 5.5 with 6*M* HCl, concentrated on a rotary evaporator under reduced pressure to about 100 mL, which consisted of a mixture of residual hemicelluloses and lignins, and then mixed with three volumes of 95% ethanol (5 h, 20°C) for isolation of residual hemicelluloses released. The residual lignins degraded were then recovered by re-precipitation at pH 1.5 from the supernatant solution after evaporation of ethanol and purified by washing with acidified water (pH 2.0). Finally, the acid-insoluble residual lignin fractions were freeze-dried before analysis. The residual lignins obtained from the corresponding organosolv pretreated straw residue with acetic acid-H_2O (65/35, v/v), acetic acid-H_2O (80/20, v/v), acetic acid-H_2O (90/10, v/v), formic acid-acetic acid-H_2O (20/60/20, v/v/v), formic acid-acetic acid-H_2O (30/60/10, v/v/v), methanol-H_2O (60/40, v/v), and ethanol-H_2O (60/40, v/v) were designated as residual lignin preparation L_1, L_2, L_3, L_4, L_5, L_6, and L_7, respectively. Scheme for isolation of residual lignin preparations with 1.8% H_2O_2-0.18% NH_2CN at 50°C under pH 10.0 for 4 h from the corresponding organosolv pretreated wheat straw is shown in Figure 1. All experiments were performed at least in duplicate. Yields of the residual lignins are given on a dry weight basis related to the wheat straw.

Characterization of the residual lignins

The neutral sugar composition in isolated residual lignins was determined as sugar alditol-acetate derivatives by gas chromatography (GC) after hydrolysis with 2*M* trifluoroacetic acid for 2 h at 120°C.[19] Alkaline nitrobenzene oxidation of lignin preparations was performed at 170°C for 3 h. Methods for determination of phenolic acids and aldehydes via HPLC of nitrobenzene oxidation mixtures and measurement of lignin molecular weights have been described in a previous paper.[20]

UV-visible spectra were obtained using a Hewlett–Packard 8452A diode array spectrophotometer. FTIR

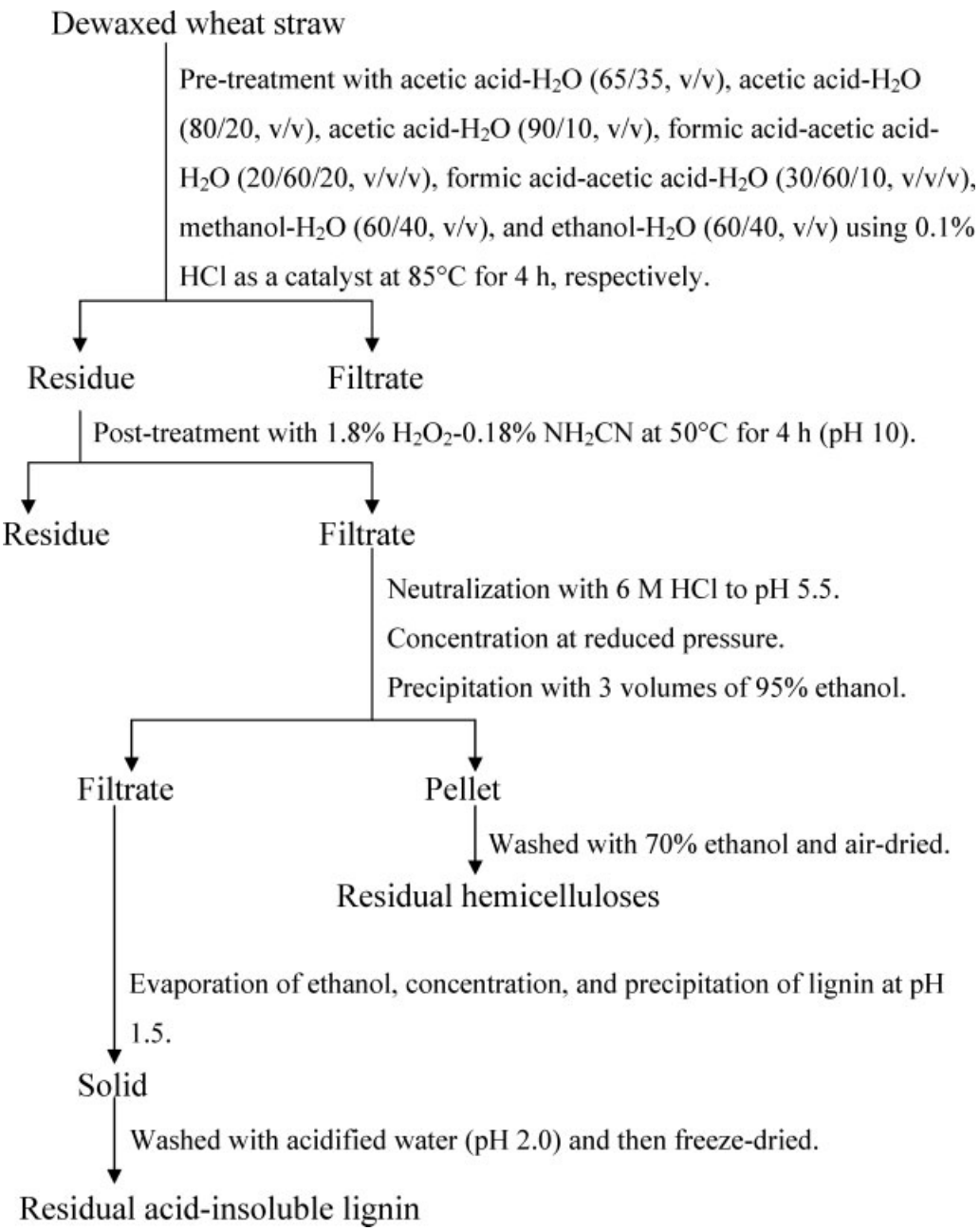

Figure 1 Scheme for isolation of acid-insoluble residual lignins with 1.8% H_2O_2–0.18% NH_2CN at 50°C under pH 10.0 for 4 h from the corresponding organosolv pretreated wheat straw.

spectra were obtained on an FTIR spectrophotometer (Nicolet 750) in the range 4000–400 cm^{-1} using a KBr disc containing 1% finely ground samples. The solution-state ^1H NMR spectrum of the lignin was recorded on a Bruker MSL300 spectrometer using 25 mg of lignin in 1.0 mL of DMSO-d_6. For each sample, 1000 scans were collected. ^{13}C NMR spectrum was obtained on a Bruker MSL300 spectrometer at 300 and 74.5 MHz. The spectrum was recorded at 25°C from 250 mg of sample dissolved in 1.0 mL DMSO-d_6 after 30,000 scans. A 70° pulse flipping angle, a 10 μs pulse width and a 15 s delay time between scans were used.

RESULTS AND DISCUSSION

Yield and purity of residual lignins

On the basis of studies on selective oxidative decomposition of kraft pulp lignin and lignin model compounds by cyanamide-activated hydrogen peroxide, Kadla et al.[21] reported that over 70% of the kraft lignin was degraded and solubilized in the presence of cyanamide as compared with less than 20% by hydrogen peroxide alone. In this case, a large decrease in the phenolic hydroxyl and methoxyl content, as well as an increase in the carboxylic acid content was observed

TABLE I
The Yield of the Residual Lignin Preparations Obtained by Posttreatment with 1.8% H_2O_2–0.18% NH_2CN at 50°C under pH 10.0 for 4 h from the Corresponding Organosolvs Pretreated Wheat Straw

Lignin fractions	Lignin preparation						
	L_1	L_2	L_3	L_4	L_5	L_6	L_7
Total solubilized lignins	1.7	1.5	1.0	1.3	0.8	6.8	5.3
Acid-insoluble lignins[a]	0.7	0.8	0.6	0.9	0.4	5.3	4.3
Acid-soluble lignins[b]	0.1	0.1	0.1	Tr[c]	Tr	1.0	0.5
Lignin associated in solubilized hemicelluloses	0.9	0.6	0.3	0.4	0.4	0.5	0.5

L_1, L_2, L_3, L_4, L_5, L_6, and L_7 represent the residual lignin preparations obtained by posttreatment with 1.8% H_2O_2–0.18% cyanamide at 50°C under pH 10.0 for 4 h from the corresponding pretreated wheat straw with acetic acid-H_2O (65/35, v/v), acetic acid-H_2O (80/20, v/v), acetic acid-H_2O (90/10, v/v), formic acid-acetic acid-H_2O (20/60/20, v/v/v), formic acid-acetic acid-H_2O (30/60/10, v/v/v), methanol-H_2O (60/40, v/v), and ethanol-H_2O (60/40, v/v) using 0.1% HCl as a catalyst at 85°C for 4 h, respectively.

[a] Represent the lignin fractions obtained by precipitation of the supernatant solution at pH 1.5 after isolation of the solubilized hemicelluloses.

[b] Represent the lignin fractions which are still solubilized in the pH 1.5 supernatant after precipitation of the acid-insoluble lignin fractions and obtained by difference (total solubilized lignin - acid-insoluble lignin - lignin associated in the solubilized hemicelluloses).

[c] Tr = Trace.

in the insoluble residue. In the case of lignin model compounds, reaction optimization studies revealed that only the free-phenolic compounds underwent oxidative degradation. The reactions are strongly dependent on pH and cyanamide-to-peroxide ratio, with optimal reactivity at a pH of 9–10 with equimolar amounts of cyanamide and hydrogen peroxide. In addition, based on the investigation of the nitrilamine reinforced hydrogen peroxide bleaching of kraft pulps, Sturm[22] proposed that the reactions proceed ionically via a peroxyimidic acid intermediate I, which is isoelectronic with peroxyacids. In the absence of added substrate, intermediate I reacts with hydrogen peroxide or another intermediate I to give the corresponding amide and oxygen.[23] It is obvious that the reactivity of the cyanamide-hydrogen peroxide system is quite unique in relation to other traditional peroxide regents, and in the reaction with lignin, the lignin macromolecule will be rendered more hydrophilic and a substantial degree of degradation of the lignin polymer can be expected to take place.

Table I shows the yield of residual lignins obtained by posttreatment with 1.8% H_2O_2–0.18% NH_2CN at 50°C under pH 10.0 for 4 h from the corresponding organosolv pretreated wheat straw. As can be seen, posttreatment of acetic acid-H_2O (65/35, v/v), acetic acid-H_2O (80/20, v/v), acetic acid-H_2O (90/10, v/v), formic acid-acetic acid-H_2O (20/60/20, v/v/v), formic acid-acetic acid-H_2O (30/60/10, v/v/v), methanol-H_2O (60/40, v/v), and ethanol-H_2O (60/40, v/v) using 0.1% HCl as a catalyst (85°C, 4 h) pretreated straw with cyanamide-activated hydrogen peroxide under the condition used solubilized 1.7, 1.5, 1.0, 1.3, 0.8, 6.8, and 5.3% lignin (% dry starting material), corresponding to the release of 46.0, 44.1, 50.0, 72.2, 80.0, 52.3, and 50.0% of the total residual lignin, respectively. Meanwhile, the posttreatment also released 78.0, 78.1, 84.5, 81.5, 83.5, 37.7, and 48.2% of the total residual hemicelluloses, respectively. It should be noted that a much lower yield of lignins between L_1 and L_5 released during the posttreatment with activated peroxide was undoubtedly because of the substantial dissolution or degradation of lignins during the pretreatment with various organic acids. Taken together, the two-step treatment released 88.2, 88.8, 94.1, 97.1, 98.8, 63.5, and 68.8% of the original lignin from wheat straw, respectively, by first treatment with acetic acid-H_2O (65/35, v/v), acetic acid-H_2O (80/20, v/v), acetic acid-H_2O (90/10, v/v), formic acid-acetic acid-H_2O (20/60/20, v/v/v), formic acid-acetic acid-H_2O (30/60/10, v/v/v), methanol-H_2O (60/40, v/v), and ethanol-H_2O (60/40, v/v) using 0.1% HCl as a catalyst (85°C, 4 h) and sequential treatment with 1.8% H_2O_2-0.18% NH_2CN. This indicated that significant amounts of lignin were released sequentially with organic acids and cyanamide-activated hydrogen peroxide under the conditions used. In addition, as the data shown in Table I, the recovered acid-insoluble lignin fraction was the major lignin fraction, comprising 41.2%–81.1% of the total solubilized residual lignins. This is particularly true for the lignin preparations isolated from the aqueous methanol or ethanol pretreated straw, in which the acid-insoluble residual lignin fraction accounted for 77.9% and 81.1% of the total degraded residual lignins.

Figure 2 illustrates the UV absorption parameters and spectra of acid-insoluble residual lignin fractions L_2 (spectrum 2), L_5 (spectrum 5), L_6 (spectrum 6), and L_7 (spectrum 7). Obviously, all the spectra exhibited well known lignin characteristics such as the two maxima at 278–282 and 314–318 nm. The first absorption maximum is originated from nonconjugated phenolic

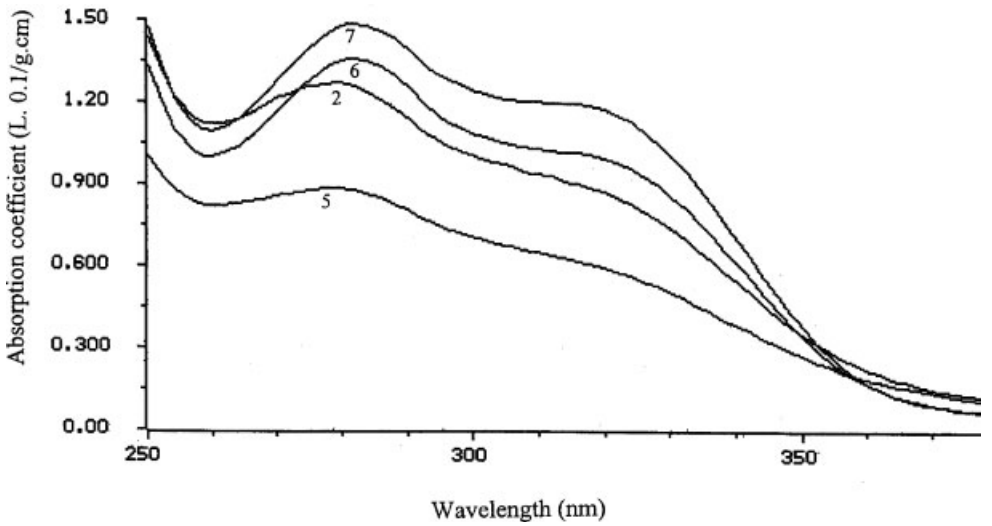

Figure 2 UV spectra of the acid-insoluble residual lignin fractions isolated with 1.8% H_2O_2–0.18% NH_2CN at 50°C under pH 10.0 for 4 h from the formic acid-acetic acid-H_2O (30/60/10, v/v/v, sample L_5, spectrum 5), acetic acid-H_2O (80/20, v/v, sample L_2, spectrum 2), methanol-H_2O (60/40, v/v, sample L_6, spectrum 6), and ethanol-H_2O (60/40, v/v, sample L_7, spectrum 7) using 0.1% HCl as a catalyst pretreated (85°C, 4 h) wheat straw.

groups (aromatic ring) in lignin. The second maximum at 314–318 nm is due to conjugated phenolic groups in *p*-coumaric and ferulic acids. In comparison, the lower absorption coefficient of the lignin fraction L_5 isolated with cyanamide-activated hydrogen peroxide from formic acid-acetic acid-H_2O (30/60/10, v/v/v) pretreated straw, was probably because of the association of noticeable amounts of nonlignin materials such as coprecipitated ash or salts.

The polysaccharide content of the seven residual lignin samples was remarkably low as shown by 0.8%–1.1% neutral sugar content. This may be explained by the substantial alkaline cleavage of linkages between lignin and hemicelluloses in addition to saponification of hydroxycinnamic esters, such as between *p*-coumaric acid and lignin or between ferulic acid and hemicelluloses, during the posttreatment with cyanamide-activated hydrogen peroxide under the condition given. Moreover, as the data shown in Table II, a relatively high amount of xylose (0.4%–0.5%) together with noticeable quantities of arabinose (0.2%–0.3%), glucose (0.1%–0.2%), and galactose (0.1%) indicated that these bound polysaccharides mainly originated from the hemicelluloses such as arabinoxylans in the secondary cell walls of wheat straw.

Composition of phenolic acids and aldehydes of the nitrobenzene oxidation

Alkaline nitrobenzene oxidation is one of the most frequently used methods for destructive analyzing lignins by chemical degradative methods, which derives information about the composition of the original polymer.[24] Results obtained by alkaline nitrobenzene oxidation at 170°C for 3 h are given in Table III. The predominant products were detected to be syringaldehyde and vanillin, which result from the degradation of noncondensed syringyl and guaiacyl units, respectively. The presence of small quantities of *p*-hydroxybenzoic acid and *p*-hydroxybenzaldehyde is considered most probably to be indicative of noncondensed *p*-hydroxyphenyl units with the lignin ''core,'' since they also results from the degradative oxidation of *p*-coumaric acid. The occurrence of large amounts of noncondensed syringyl and guaiacyl units together with relatively fewer *p*-hydroxyphenyl units in each of the oxidation mixtures of acid-insoluble residual lignin fractions revealed that these residual lignins could be justified as grass type lignin. The relative molar ratios of G (the relative total moles of vanillin, vanillic acid, and acetovanillin) to S (the relative total

TABLE II
The Content of Neutral Sugars (% Acid-Insoluble Lignin Sample, w/w) in the Residual Lignin Preparations Obtained by Posttreatment with 1.8% H_2O_2–0.18% NH_2CN at 50°C under pH 10.0 for 4 h from the Corresponding Pretreated Wheat Straw

Neutral sugars/ uronic acids	Lignin preparation						
	L_1	L_2	L_3	L_4	L_5	L_6	L_7
Arabinose	0.2	0.2	0.2	0.2	0.2	0.3	0.3
Xylose	0.5	0.4	0.4	0.4	0.4	0.5	0.5
Mannose	Tr[a]	Tr	Tr	Tr	Tr	Tr	Tr
Galactose	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Glucose	0.2	0.2	0.2	0.1	0.1	0.2	0.1
Total	*1.0*	*0.9*	*0.9*	*0.8*	*0.8*	*1.1*	*1.0*

Corresponding to the lignin fractions in Table I.
[a] Tr = trace

TABLE III
The Composition (% Acid-Insoluble Lignin Sample, w/w) of Phenolic Acids and Aldehydes from Nitrobenzene Oxidation of the Residual Lignin Preparations Obtained by Posttreatment with 1.8% H_2O_2-0.18% NH_2CN at 50°C under pH 10.0 for 4 h from the Corresponding Organosolv Pretreated Wheat Straw

	Lignin preparation						
Phenolic acids and aldehydes	L_1	L_2	L_3	L_4	L_5	L_6	L_7
p-Hydroxybenzoic acid	0.40	0.89	0.66	0.99	0.86	0.31	0.55
p-Hydroxybenzaldehyde	0.74	0.62	0.73	1.69	1.44	0.70	1.12
Vanillic acid	0.25	0.35	0.39	1.77	1.64	0.37	0.49
Vanillin	7.27	5.33	5.80	14.18	8.04	8.05	11.16
Syringic acid	0.23	0.36	0.36	0.99	1.04	0.13	0.17
Syringaldehyde	10.45	7.94	6.90	16.76	12.96	6.56	10.94
Acetovanillin	ND	ND	ND	ND	ND	0.17	0.32
Acetosyringone	1.38	0.47	0.80	3.24	0.96	0.37	0.96
p-Coumaric acid	0.10	0.082	0.058	0.21	0.18	0.11	0.11
Ferulic acid	0.18	0.19	0.30	0.54	0.56	0.13	0.18
Cinnamic acid	0.14	0.13	0.10	0.081	0.084	0.12	0.10
Total	*21.14*	*16.36*	*16.10*	*40.45*	*27.76*	*17.02*	*26.10*
Molar ratio (G : S : H)[a]	6 : 7 : 1	3 : 4 : 1	4 : 4 : 1	5 : 5 : 1	4 : 5 : 1	7 : 5 : 1	6 : 5 : 1

Corresponding to the lignin fractions in Table I.
ND = not detected.
[a] G represents the sum of total moles of vanillin, vanillic acid, and acetovanillin; S represents the sum of total moles of syringaldehyde, syringic acid, and acetosyringone; and H represents the sum of total moles of *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid.

moles of syringaldehyde, syringic acid, and acetosyringone) and to H (the relative total moles of *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid) in residual lignin fractions L_1, L_2, L_3, L_4, and L_5 were found to be 6 : 7 : 1, 3 : 4 : 1, 4 : 4 : 1, 5 : 5 : 1, and 4 : 5 : 1, whereas these ratios increased to 7 : 5 : 1 in L_6 and 6 : 5 : 1 in L_7, respectively. These higher amounts of noncondensed syringyl units between lignin fraction L_1 and L_5 suggested that the acid-insoluble residual lignin fractions isolated with cyanamide-activated peroxide from aqueous organic acids pretreated wheat straw, arose from the secondary wall lignin. While the L_6 and L_7 residual lignin fractions extracted with cyanamide-activated hydrogen peroxide from aqueous organic alcohols pretreated straw, were originated from the middle lamella lignin, since the secondary wall lignin contains many more syringly units than the middle lamella lignin.[25]

TABLE IV
Weight–Average (M_w) and Number–Average (M_n) Molecular Weights and Polydispersity (M_w/M_n) of the Residual Acid-Insoluble Lignin Preparations Obtained by Posttreatment with 1.8% H_2O_2-0.18% NH_2CN at 50°C under pH 10.0 for 4 h from the Corresponding Organosolv Pretreated Wheat Straw

Lignin preparation	M_w	M_n	M_w/M_n
L_1	3730	1870	2
L_2	3690	1530	2.41
L_3	3820	2070	1.85
L_4	2980	1740	1.71
L_5	3410	1660	2.05
L_6	3480	1830	1.9
L_7	3250	1490	2.18

Corresponding to the lignin fractions in Table I.

In comparison, the lower yields (16.1–17.0%) of alkaline nitrobenzene oxidation of residual lignin fractions L_2, L_3, and L_6 demonstrated that a higher degree of condensation of these residual lignins. In contrast, the residual lignin fraction L_4 isolated with cyanamide-activated hydrogen peroxide from formic acid-acetic acid-H_2O (20/60/20, v/v/v) pretreated straw, gave the highest yield of phenolics (40.5%), indicating a minimally condensed residual lignin fraction. In addition, although considerable amounts of *p*-coumaric and ferulic acids were converted into *p*-hydroxybenzaldehyde or *p*-hydroxybenzoic acid and vanillin or vanillic acid, respectively, during the alkaline nitrobenzene oxidation at 170°C, the remaining occurrence of small amounts of *p*-coumaric acid (0.06%–0.21%) and ferulic acid (0.13%–0.56%) suggested that these two hydroxycinnamic acids are strongly linked to lignin and/or hemicelluloses in the cell walls of wheat straw. Similar results have been already found in our previous studies on ball-milled and enzyme lignin fractions from wheat straw.[26]

Molecular weight

Weight–average (M_w), number–average (M_n) molecular weights, and polydispersity (M_w/M_n) of the seven acid-insoluble residual lignin preparations obtained by posttreatment with 1.8% H_2O_2–0.18% NH_2CN at 50°C under pH 10.0 for 4 h from the corresponding organosolv pretreated wheat straw are given in Table IV. It can be seen that the differences in average molecular weights of the seven residual lignin preparations were not significant (M_w 2980–3820 g mol^{-1}).

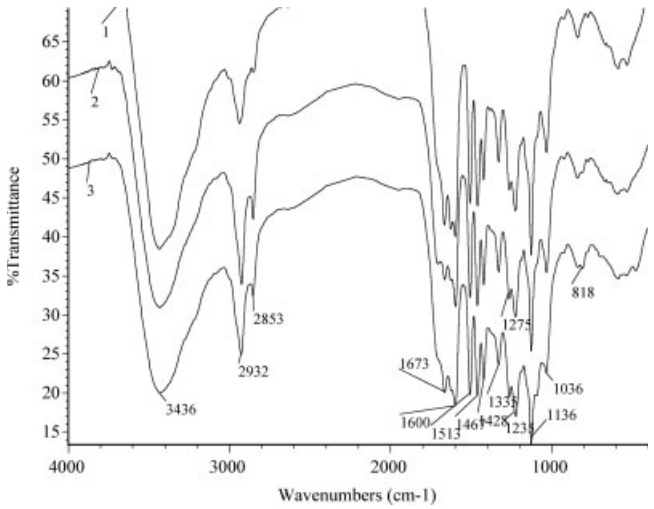

Figure 3 FTIR spectra of acid-insoluble residual lignin preparations isolated with 1.8% H_2O_2–0.18% NH_2CN at 50°C under pH 10.0 for 4 h from the acetic acid-H_2O (65/35, v/v, sample L_1, spectrum 1), acetic acid-H_2O (80/20, v/v, sample L_2, spectrum 2), and formic acid-acetic acid-H_2O (20/60/20, v/v/v, sample L_4, spectrum 3) using 0.1% HCl as a catalyst pretreated (85°C, 4 h) wheat straw.

The reason for this low molecular weight was probably because of the extensive cleavage of the interunit linkages in lignin molecules during the posttreatment with cyanamide-activated hydrogen peroxide. In comparison, the residual lignins isolated from aqueous acetic acid pretreated straw, appeared to have relatively higher molecular weights as shown by their M_w ranging between 3690 and 3820 g mol^{-1}, whereas the residual lignin fraction extracted from mixture of formic acid-acetic acid-H_2O (20/60/20, v/v/v) pretreated straw, had a lowest value of M_w (2980 g mol^{-1}), strongly suggesting that the pretreatment with various organsolvs had a remarkable effect on the level of degradation of the residual lignin released during the posttreatment with cyanamide-activated hydrogen peroxide.

FTIR spectra

Among the analysis techniques described in the literature, FTIR spectroscopy shows interesting characteristics, such as high sensivity and selectivity, high signal-to-noise ratio, accuracy, data handling facility, mechanical simplicity and short time and small amount of sample required for the analysis.[27] In addition, FTIR spectroscopy serves as a fast method for characterization of lignins, which can be applied to whole solid samples, thus avoiding the formation of artifacts that may occur during wet chemical degradation methods. Furthermore, the spectrum of a lignin sample gives an overall view of its chemical structure.[28] Figure 3 shows FTIR spectra of acid-insoluble residual lignin preparations isolated with 1.8% H_2O_2–0.18% NH_2CN at 50°C under pH 10.0 for 4 h from the acetic acid-H_2O (65/35, v/v, sample L_1, spectrum 1), acetic acid-H_2O (80/20, v/v, sample L_2, spectrum 2), and formic acid-acetic acid-H_2O (20/60/20, v/v/v, sample L_4, spectrum 3) using 0.1% HCl as a catalyst pretreated (85°C, 4 h) wheat straw. Clearly, the spectral profiles and the relative intensities of the bands were rather similar, indicating a similar structure of the residual lignins.

Aromatic skeleton vibrations in the three residual lignin fractions are assigned at 1600, 1513, and 1428 cm^{-1}, in which the aromatic semicircle vibration (a vibration involving both carbon–carbon stretching and a change of the H-C-C bond angle) is attributed at 1513 cm^{-1}.[29] Absorption at 1467 cm^{-1} is due to methoxyl C—H deformations and bendings of lignin. A shoulder peak at 1725 cm^{-1} (data not shown) is indicative of the carbonyl and unconjugated ketone and carboxyl group stretching, while the intensive bands at 1673 and 1640 cm^{-1} arise from conjugated carbonyl stretching in residual lignins. Syringyl ring breathing with C—O stretching gives an intensive band at 1335 cm^{-1}. In the wavenumber region from 1300 to 1200 cm^{-1}, aromatic carbon–oxygen (in methoxyl and phenol groups) stretching vibrations from guaiacyl units exhibit two bands at 1275 and 1235 cm^{-1}.[30] The bands at 1136 and 1036 cm^{-1} are originated from C—H in-plan bending in guaiacyl lignin. Also, at about 1036 cm^{-1}, C—O deformation in combination with aromatic C—H deformation has been reported in lignins.[31] Aromatic C—H out of bending exhibits at 818 cm^{-1}.

Figure 4 shows FTIR spectra of acid-insoluble residual lignin preparations isolated with 1.8% H_2O_2-0.18%

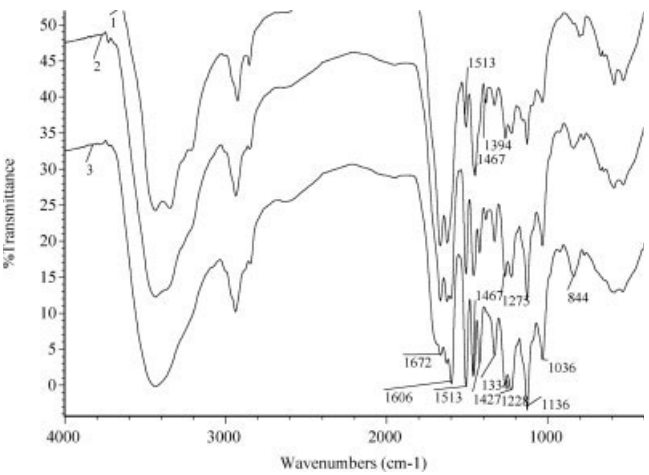

Figure 4 FTIR spectra of acid-insoluble residual lignin preparations isolated with 1.8% H_2O_2–0.18% NH_2CN at 50°C under pH 10.0 for 4 h from the formic acid-acetic acid-H_2O (30/60/10, v/v/v, sample L_5, spectrum 1), methanol-H_2O (60/40, v/v, sample L_6, spectrum 2), and ethanol-H_2O (60/40, v/v, sample L_7, spectrum 3) using 0.1% HCl as a catalyst pretreated (85°C, 4 h) wheat straw.

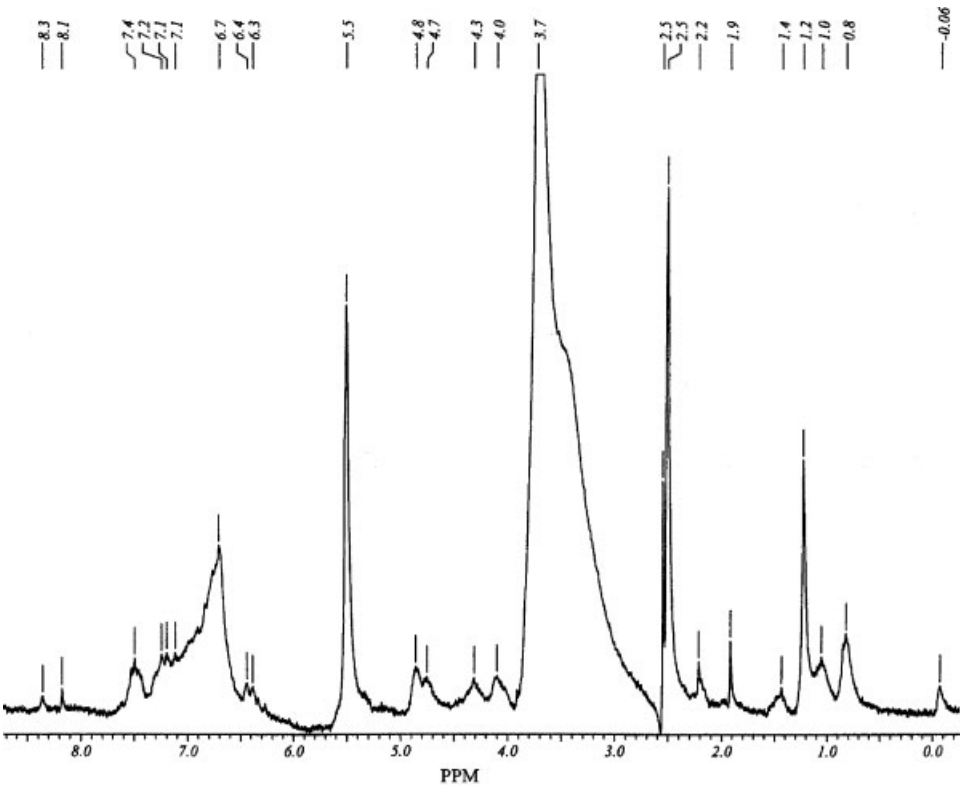

Figure 5 ^{1}H NMR spectrum of acid-insoluble residual lignin preparation L_7 extracted from aqueous ethanol pretreated straw with cyanamide-activated hydrogen peroxide.

NH_2CN from the formic acid-acetic acid-H_2O (30/60/10, v/v/v, sample L_5, spectrum 1), methanol-H_2O (60/40, v/v, sample L_6, spectrum 2), and ethanol-H_2O (60/40, v/v, sample L_7, spectrum 3) pretreated wheat straw. In comparison, a remarkable decrease of aromatic skeletal vibrations was observed in spectrum 1 at 1513 and 1394 cm^{-1} (1427 cm^{-1} in spectra 2 and 3). In addition, a more intensive peak at 1672 cm^{-1} in spectra 1 and 2 implied that the residual lignin preparations L_5 and L_6 contained higher amounts of conjugated carbonyl groups than those of the residual lignin fraction L_7.

^{1}H and ^{13}C NMR spectra

The ^{1}H NMR spectrum of acid-insoluble residual lignin preparation L_7 extracted from aqueous ethanol pretreated straw with cyanamide-activated hydrogen peroxide is illustrated in Figure 5. The integrals of signals centered at 6.7 ppm are assignated to aromatic protons in syringylpropane and guaiacylpropane structures,[32] indicating the presence of syringyl and guaiacyl units in the residual lignin. The small signals around 7.4 ppm are attributed to the aromatic protons in positions 2 and 6, in structures containing a C_α=O group, to aromatic protons in positions 2 and 6 of *p*-hydroxyphenyl units conjugated with a double bond, to the proton in C_α=C_β structures, and to aromatic protons in *p*-coumaric and ferulic acids, confirming the presence of *p*-hydroxyphenyl units, C_α=O groups, and *p*-coumaric and ferulic acids in the lignin fraction.[33] The H_α and H_β in β-*O*-4 structures give signals at 5.5 and 4.8 ppm. Methoxyl protons (–OCH_3) produce a strong signal at 3.7 ppm. The signal at 3.3 ppm arises from the protons in water in DMSO. Two intense signals around 2.5 ppm are characterized by protons in DMSO. Protons in aliphatic groups exhibit signals between 2.2 and 0.8 ppm.

The acid-insoluble residual lignin preparation L_7, isolated with cyanamide-activated peroxide from the aqueous ethanol pretreated straw, was also investigated by ^{13}C NMR spectroscopy and its spectrum is shown in Figure 6. Most of the observed signals have been previously assigned in straw and wood lignin spectra.[34,35] The most striking characteristic of the spectrum is the almost complete absence of typical hemicellulosic signals between 57 and 103 ppm. The spectrum does show three very small signals at 63.6 (C-5 in xylose internal unit, data not shown), 81.7 (C-4 in 4-*O*-methylglucuronic acid (4-*O*-MeGlcA)) and 174.6 ppm (C-6 in 4-*O*-MeGlcA) for the contaminated hemicelluloses[36]; however, the peak intensities are rather weak, implying trace amounts of associated hemicelluloses. This phenomenon observed was consistent with the results obtained by sugar analysis. Another remarkable feature of the spectrum is the

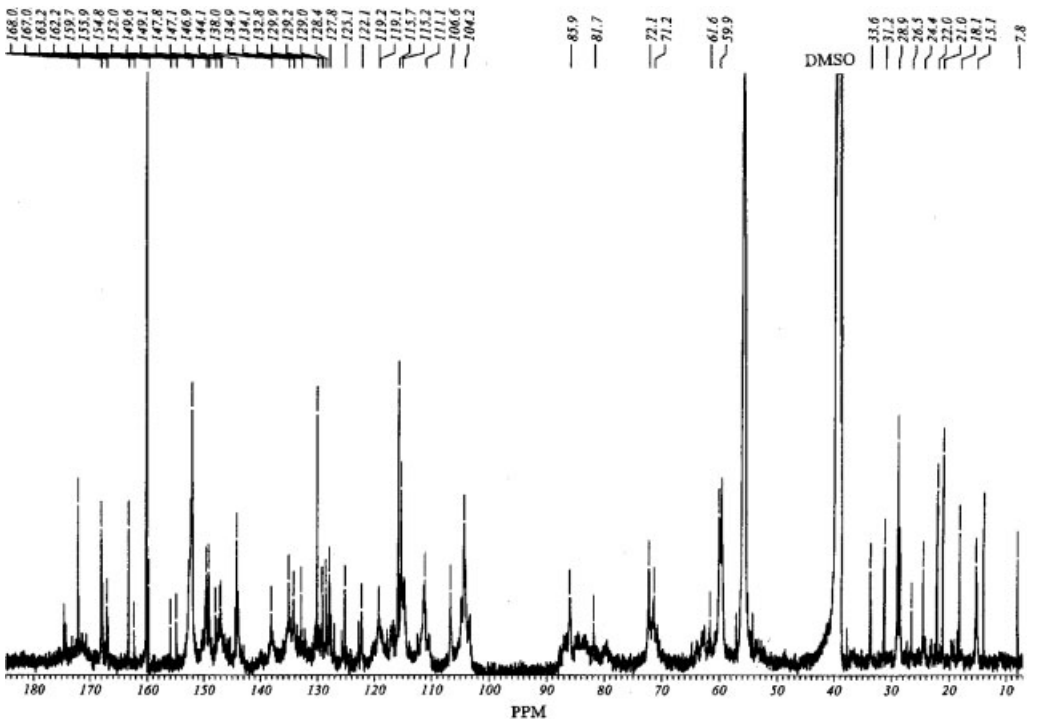

Figure 6 ^{13}C NMR spectrum of acid-insoluble residual lignin preparation L_7 extracted from aqueous ethanol pretreated straw with cyanamide-activated hydrogen peroxide.

occurrence of a signal at 171.9 ppm, which represents –COOH groups in aliphatic acids, indicating that the oxidation reactions do occur at the lignin side chains during the posttreatment with cyanamide-activated peroxide under the condition given.

One of most important features of lignin can be seen in the aromatic region (104.2-168.0 ppm). The syringyl (S) residues were detected by signals at 152.0 (C-3/C-5, S etherified), 147.8, 147.1, and 146.9 (C-3/C-5, S nonetherified), 138.0 (C-4, S etherified), 134.9 and 134.1 (C-1, S etherified), 132.8 (C-1, S nonetherified), and 104.2 ppm (C-2/C-6, S). Guaiacyl (G) residues were identified by signals at 149.6 and 149.1 (C-3, G etherified), 147.8 147.1, and 146.9 (C-4, G etherified), 134.9 and 134.1 (C-1, G etherified), 132.8 (C-1, G nonetherified), 119.2 and 119.1 (C-6, G), 114.8 (C-5, G, data not shown), and 111.1 ppm (C-2, G). The *p*-hydroxyphenyl (H) residues give a small signal at 127.8 ppm (C-2/C-6, H). These signals indicated that the lignin preparation could be justified as GSH grass lignin, which corresponded to the results obtained by alkaline nitrobenzene oxidation. The signals at 167.0 (C-γ, *p*-coumaric acid (PC) ester), 159.7 (C-4, PC ester), 129.9, 129.2, and 129.0 (C-2/C-6, PC ester), 125.1 (C-1 PC ester), and 115.7 and 115.2 ppm (C-3/C-5, PC ester) are assigned to esterified *p*-coumaric acid. Etherified ferulic acids give signals at 168.0 (C-γ, ferulic acid (FE) ether) and 122.1 ppm (C-6, Fe ether). Esterified ferulic acid was verified with a small signal at 122.6 ppm (C-6, FE ester, data not shown). These observations stated that *p*-coumaric acid is bonded to lignin by ester bonds, while the ferulic acid is linked to lignin by both ether and ester bonds.

The most abundant substructure in lignin is an β-*O*-4 structure. Side chain carbons: C-α, C-β, and C-γ in β-*O*-4 can be seen at 72.1 and 71.2, 85.9, and 61.6 and 59.9 ppm, respectively. In addition, certain signals belonging to condensed structures such as carbon–carbon linkages, can be easily distinguished in the spectrum. The presence of β-β substructures can be seen from the C-γ signal at 70.4 ppm (data not shown), although C-α and C-β signals are overlapped with other signals. Side chain carbons of C-γ in β-5 substructures can be observed at 63.0 ppm. Signal at 125.1 ppm could also arise from 5 to 5′ substructures. These observations demonstrated that the residual lignin preparation is mainly composed of β-*O*-4 ether bonds together with small amounts of β-β, β-5, and 5-5′ carbon–carbon linkages. The strong signal at 56.0 ppm is due to the OCH_3 group in syringyl and guaiacyl units. The signals representing the γ-methyl, α and β-methylene groups in *n*-propyl side chains occur at 15.1 and 18.1–33.6 ppm, respectively.

CONCLUSIONS

The activation of hydrogen peroxide with cyanamide released 44–80% of the total residual lignin and 38–85% of the total residual hemicelluloses from the various organosolvs pretreated wheat straw. The results showed that the cyanamide-activated peroxide system under the condition given do react with lignin mole-

cules by oxidation as shown by a noticeable amount of carboxylic groups generated, further resulting in a high residual lignin degradation. GPC and ^{1}H and ^{13}C NMR analyses revealed that the low molecular weights of residual lignin is preferentially degraded by cyanamide-activated peroxide system, however, the system did not cause a significant change of the overall structure of the lignin, and no differences in interunit linkages were observed. β-*O*-4 ether bonds are the major linkages between the residual lignin substructural units together with small amounts of carbon–carbon linkages such as β-β, β-5, and 5-5′.

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