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Mild Acetosolv Process To Fractionate Bamboo for the Biorefinery: Structural and Antioxidant Properties of the Dissolved Lignin

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Supporting Information

ABSTRACT: Fractionation of lignocellulosic material into its constitutive components is of vital importance for the production of biofuels as well as other value-added chemicals. The conventional acetosolv processes are mainly focused on the production of pulp from woody lignocelluloses. In this study, a mild acetosolv process was developed to fractionate bamboo under atmospheric pressure to obtain cellulosic pulp, water-soluble fraction, and acetic acid lignin. The structural features of the lignins obtained under various conditions were characterized with elemental analysis, sugar analysis, alkaline nitrobenzene oxidation, gel permeation chromatography (GPC), ^{1}H nuclear magnetic resonance (^{1}H NMR), and heteronuclear single-quantum coherence (HSQC) spectroscopy. As compared to milled wood lignin (MWL) of bamboo, acetic acid lignins had low impurities (carbohydrates 2.48−4.56%) mainly due to the cleavage of linkages between lignin and carbohydrates. In addition, acetic acid lignins showed a low proportion of syringyl (S) units. Due to the cleavage of linkages between lignin units, acetic acid lignins had weight-average molecular weights ranging from 4870 to 5210 g/mol, less than half that of MWL (13000 g/mol). In addition, acetic acid lignins showed stronger antioxidant activity mainly due to the significant increase of free phenolic hydroxyls. The lignins obtained with such low impurities, high free phenolic hydroxyls, and medium molecular weights are promising feedstocks to replace petroleum chemicals.

KEYWORDS: *acetosolv, fractionation, lignin, HSQC, antioxidant activity*

INTRODUCTION

The major chemical industry for utilization of lignocellulosic materials is mainly conducted with a destructive strategy to prepare a single cellulosic fraction; other accompanying fractions are either disposed of as wastes or used in low value for energy.[1] In fact, the separation of lignocellulosic materials has been conducted in the chemical pulping industry all over the world for a long time. However, the main objective of the process is to a produce cellulose-rich fraction (accounting for half of the starting material). Especially, problematic issues in the process are reasonable recovery and application of the components dissolved in the spent liquor (i.e., the spent cooking liquor from the pulping process when lignocellulose is digested into pulp).[2] On the other hand, with the rapidly growing demand for energy and the dwindling and unstable supply of petroleum, conversion of lignocellulosic biomass into biofuels is attracting increasing attention.[3,4] Due to the protective characteristic of lignin (i.e., lignin serves as the "glue" that binds cellulose and hemicelluloses together, imparting rigidity, together with moisture and microbial resistance to lignocellulose) as well as its inability to undergo rapid biotransformation into useful chemicals, pretreatment of lignocellulose is needed to achieve a reduction of lignin content in the raw material.[5,6] If the pretreatment approach is achieved in a fractionation manner, that is, separation of the constitutive components of lignocelluloses (cellulose, hemicelluloses, and lignin) with good selectivity and high yield, all lignocellulosic components can be fully exploited for the subsequent conversion into biofuels, commodities (i.e., pulp board, dissolving pulp, xylose, commercial sulfur-free lignin including alkali lignin and organosolv lignin, etc.), and other high value-added products.[7,8]

In the past decades, a variety of approaches have been developed to pretreat lignocellulosic materials, which include physical, chemical, physiochemical, and biological methods.[9] Among them, ionic liquids, as a new class of designer solvents, are attracting increasing attention in lignocellulose processing due to their good dissolving capacity on a variety of lignocelluloses. They have been used to pretreat lignocelluloses for enzymatic hydrolysis as well as to fractionate lignin and cellulose from the starting material.[10−13] Although extensive investigations have been reported in the literature, these processes are currently mainly performed on an experimental scale due largely to the high cost of ionic liquids. Besides, several organic solvents, including alcohols, organic acids, phenols, and cresols, etc., have attracted increasing attention as fractionation agents as compared to the conventional alkalis and inorganic acids.[14] The process with organic compounds can delignify the raw material in a gentler and more effective way than the conventional process does, resulting in separation of lignocellulose in a less degraded way. Among them, acetic acid shows good features for lignocellulose fractionation, mainly due to its ability to achieve an extensive delignification and simultaneous hemicelluloses hydrolysis. Actually, acetic acid was proposed as an agent for delignification of wood for

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structural studies as early as the 1920s, but no systematic investigation was conducted with regard to the component separation until the 1980s. Acetic acid based processes have been proposed to fractionate lignocellulosic material during the past decades, for pulping and, more recently, for multiple products. Acetic acid can be easily recovered by evaporation and reused in the whole process. It is worth noting that on an industrial scale, some of the equipment should be manufactured with corrosion-resistant materials because acetic acid aqueous solution shows corrosion propriety.[15] As a pretreatment agent, acetic acid has been used to pretreat biomass for enhancing the enzymatic hydrolysis of cellulose, and minimizing the formation of inhibitors.[16] For instance, after pretreatment with a 25% acetic acid solution at 160 °C, 67.6% of the glucan in wheat straw was hydrolyzed into glucose via cellulase.[17] In the acetic acid pulping process, the cellulosic pulp obtained is a good feedstock for biofuels as well as cellulose derivatives. For example, carboxymethyl cellulose with a degree of substitution of 1.16 was successfully synthesized with bleached acetic acid pulp.[18] In addition, lignin is also degraded and dissolved in the spent liquor during the acetic acid fractionation process.

The approaches applying acetic acid for fractionation can be summarized as aqueous acetic acid,[19] acid-catalyzed aqueous acetic acid (acetosolv process),[20] and salt-catalyzed aqueous acetic acid processes.[21] In the acetosolv process, acetic acid and HCl were recovered by evaporation and distillation and recycled as cooking agents due to their volatile properties. As the hemicelluloses and lignin were extensively removed, cellulose remained in the pulp. The dissolved lignin in the spent liquor can be easily recovered by the addition of water as compared to neutralization in the alkaline pulping (such as soda and kraft) process. The conventional acetosolv processes are mainly focused on the production of pulp and the properties of the pulp, and they were usually applied to woody lignocelluloses and conducted at elevated temperatures (130−160 °C).[15,21,22] To fractionate the major components from nonwoody lignocelluloses under mild conditions, the process can be designed in a modified way, that is, a mild acetosolv process. In the mild acetosolv process, the cost of the equipment can be largely reduced because the process was performed under atmospheric pressure. Obviously, the recovery of the chemicals can be achieved by a shorter sequence as compared to a multistep operation in the alkali recovery system of the conventional process. The dissolved sugars are also readily convertible into chemicals and fuels. The recovery and application of the dissolved lignin and degraded carbohydrate products achieve a more effective utilization of the lignocelluloses; thus, the whole process is more profitable than the conventional processes.

Among the various available lignocelluloses, nonwoody materials (such as residues from agricultural crops, annual and perennial grasses, etc.), have attracted increasing attention because they represent inexpensive and abundant feedstocks, particularly in countries with a scarcity of wood. Bamboo, a perennial grass widely distributed in many Asian countries, is a promising feedstock for chemical utilization in the context of biorefinery due to its fast growth, short renewal cycle, and easy propagation.[23] In the present study, an acetosolv process was developed to fractionate bamboo in a mild way. To make the whole process feasible, it is of vital importance that the dissolved lignin in the spent liquor can be efficiently recovered and its structural modifications be well characterized. Therefore, the dissolved lignin was recovered and their structural features were characterized as compared to the native lignin of bamboo with a set of chemistry and spectroscopy methods. The combination of wet chemistry (e.g., elemental analysis, sugar analysis, and alkaline nitrobenzene oxidation) and multiple modern spectroscopy techniques (e.g., 1D and 2D nuclear magnetic resonance spectroscopy (NMR)) was examined, aiming at providing complementary information on the reactive groups as well as the types of lignin units. Additionally, antioxidant activity was also investigated because it is closely related to the chemical structure of lignin. The protocol for lignin extraction and structural characterization will help us maximize the industrial exploitation of nonwoody lignocellulose.

MATERIALS AND METHODS

Materials. Bamboo (*Phyllostachys sulphurea*) was harvested from Yunnan province, in southwestern China. Once harvested, the stem was air-dried, and then leaves and branch were manually removed. The obtained stem was chopped into small pieces and then ground to obtain a fraction sized in 40−60 mesh. This fraction was extracted with toluene/ethanol (2:1, v/v) in a Soxhlet apparatus, air-dried, and stored in hermetic polypropylene containers before use. The chemical composition of the dewaxed bamboo was carbohydrates, 77.03% (arabinose, 2.18%; galactose, 0.35%; glucose, 50.02%; xylose, 22.98%; glucuronic acid, 1.50%); lignin, 21.24%; and ash, 1.27%, determined according to the literature.[24−26]

Fractionation of Bamboo and Recovery of Acetic Acid Lignin. Fractionation of bamboo was conducted under stirring in aqueous acetic acid with the addition of various amounts of HCl (0, 0.5, 1.0, 1.5, 2.0, 4.0, and 6.0% (w/w) based on the weight of the raw material, respectively). In a typical run, 2 g of bamboo sample was delignified in 30 mL of solution of 90% (w/w) acetic acid aqueous solution containing HCl at boiling point (114 °C) under atmospheric pressure for 2 h. After that, the mixtures were filtered to obtain cellulosic pulp and spent liquor rich in lignin and degraded carbohydrates. The cellulosic pulp was washed with 90% acetic acid and then with water to a neutral pH. The spent liquor and the washing liquor (acetic acid-soluble fraction) were combined and concentrated with a rotatory evaporator under reduced pressure at 60 °C to 10 mL. Then the concentrated liquor was added into 100 mL of water. Subsequently, the lignin was precipitated by centrifugation (at 3000*g* for 5 min). The precipitated preparation was washed with acidic water (pH 2, adjusted with HCl) and dried to obtain acetic acid lignin. The supernatant was evaporated under reduced pressure to dryness to obtain the water-soluble fraction.

Preparation of Milled Wood Lignin. To compare the structural modifications of lignin after the fractionation process, milled wood lignin was isolated after milling of the dewaxed bamboo in a ball mill prior to extraction with dioxane/water according to the classical procedure.[27] Briefly, the ball milled bamboo (20 g) was extracted twice with dioxane/water (200 mL; 96:4, v/v) for 24 h. After the suspension was removed by centrifugation, the solution obtained was concentrated under reduced pressure to obtain crude milled wood lignin. Subsequently, the crude lignin was dissolved in acetic acid/water (20 mL; 9:1, v/v) and precipitated into water (400 mL). The precipitated lignin was filtered, dissolved in 1,2-dichloroethane/ethanol (10 mL; 2:1, v/v), and precipitated into ether (200 mL). The lignin sample obtained was referred as MWL.

Component Analysis and Structural Characterization. The yields of cellulosic pulp, acetic acid lignin, and water-soluble fraction were measured gravimetrically. Lignin content in the cellulosic pulp was determined according to the acetyl bromide method,[25] and delignification degree was calculated with the formula

$$\text{delignification degree (\%)} = \frac{1 - \text{wt of lignin in pulp}}{\text{wt of lignin in original raw material}} \times 100$$

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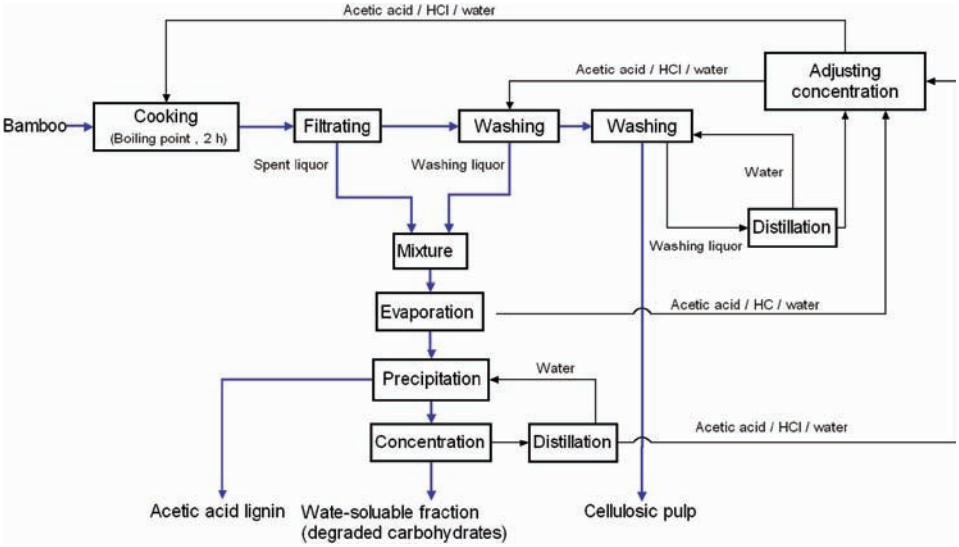

Figure 1. Acetosolv process for fractionation of bamboo (operated in 90% (w/w) acetic acid aqueous solution containing HCl at boiling point under atmospheric pressure for 2 h).

Results of the yield and lignin content in the samples are presented as mean values of three parallels, and the relative standard deviation was below 2.0%. The ash content of the lignin specimen was determined according to TAPPI standard T 211 om-85 (relative standard deviation was below 2.0%). Elemental analysis (C, H, and N contents) of lignin samples was performed using a Vario EL III elemental analyzer instrument (Elementar, Germany). Oxygen content in lignin sample was calculated from the difference between the sample weight and the C, H, and N contents. Results of the elemental contents are presented as mean values of two parallels, and the relative standard deviation was below 5%. Methoxyl content of lignin sample was determined as described by Mousavioun and Doherty.[28]

The chemical composition of phenolic acids and aldehydes liberated from alkaline nitrobenzene oxidation of the lignin in the lignin preparation was determined by high-performance liquid chromatography (HPLC, Agilent).[26] The measurements were conducted with three parallels, and the relative standard deviation was found to be below 15%. Sugar analysis (neutral sugars and uronic acids) on the lignin sample was conducted by using high-performance anion exchange chromatography (HPAEC).[26] The neutral sugars and uronic acids in the lignin fractions were liberated by hydrolysis with 72% H_2SO_4 for 45 min at 25 °C followed by a high-temperature hydrolysis at 105 °C for 2.5 h after dilution to 3% H_2SO_4. After hydrolysis, the samples were diluted and injected into the HPAEC system (Dionex ISC 3000) with an amperometric detector, a CarbopacTMPA-20 column (4 × 250 mm, Dionex), and a guard PA-20 column (3 × 30 mm, Dionex). Neutral sugars and uronic acids were separated in isocratic 5 mM NaOH (carbonate-free and purged with nitrogen) for 20 min, followed by a 0.75 mM NaAc gradient in 5 mM NaOH for 15 min with a flow rate of 0.4 mL/min. Calibration was performed with standard solutions of sugars, and the relative standard deviation of the results was below 6%.

Molecular weights of the lignin fractions were determined by gel permeation chromatography (GPC) on a PL-gel 10 mm Mixed-B 7.5 mm i.d. column. The calibration of GPC was conducted against a polystyrene calibration curve. The calibration curve was created by fitting a polynomial equation to the retention volumes obtained from a series of narrow molecular weight distribution polystyrene standards. The samples were acetylated with acetic anhydride before determination according to the method described by Gellerstedt with some modifications.[29] Briefly, lignin samples (~0.2 g) were added to mixtures of pyridine/acetic anhydride (2:1, v/v) for 72 h under nitrogen atmosphere in darkness. At the end of this time, the acetylated lignin samples were recovered by precipitation with diethyl ether. Then they were washed thoroughly with diethyl ether and dried before use. Two milligrams of acetylated sample was dissolved in 2 mL of tetrahydrofuran, and 20 μL of solution was injected. The column was operated at ambient temperature and eluted with tetrahydrofuran at a flow rate of 1 mL/min. The measurements were conducted in triplicate, and the relative standard deviation was below 5%.

The ^{1}H NMR spectra of lignin samples were recorded at 100 MHz using 15 mg of acetylated lignin fractions in 1 mL of $CDCl_3$. For heteronuclear single-quantum correlation (HSQC) spectra of the lignin samples, the data were acquired by HSQC GE experiment mode using 20 mg of sample in 1 mL of deuterated dimethyl sulfoxide (DMSO-d_6). The spectral widths for the HSQC spectra were 2200 and 15400 Hz for the ^{1}H and ^{13}C dimensions, respectively. The number of collected complex points was 1024 for the ^{1}H dimension with a recycle delay of 1.5 s. The number of scans was 128, and 256 time increments were recorded in the ^{13}C dimension. The $^1J_{CH}$ was set to 146 Hz. Prior to Fourier transform the data matrices were zero filled up to 1024 points in the ^{13}C dimension. Lignin antioxidant capacity was studied by evaluating the free radical scavenging effect. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activities of lignin samples were measured using the spectrophotometric procedure according to a previous paper.[30]

RESULTS AND DISCUSSION

Fractionation with Acetosolv Process. The overall concept of the fractionation process is illustrated in Figure 1. Bamboo was subjected to acetic acid fractionation under mild condition (114 °C, atmospheric pressure). The addition of a small amount of HCl as catalyst resulted in enhancement of the degradation and dissolution of lignin as well as carbohydrates (especially hemicelluloses and amorphous cellulose) in the raw material.

To validate the process scheme and the potential applications of the extracted lignin from bamboo, a set of experiments was carried out with various amounts of HCl (0–6%). The cellulosic pulp yield and delignification degree in the fractionation process are illustrated in Figure 2. Obviously, the process without the addition of HCl as catalyst showed a high yield of cellulosic residue (90.48%) and a low delignification degree (only 10.12%). This result indicated that the process with acetic acid under atmospheric pressure only slightly defibrated the lignocellulosic material due to its relatively weaker acidity as compared to other strong organic acids. For instance, it has been reported that fractionation with

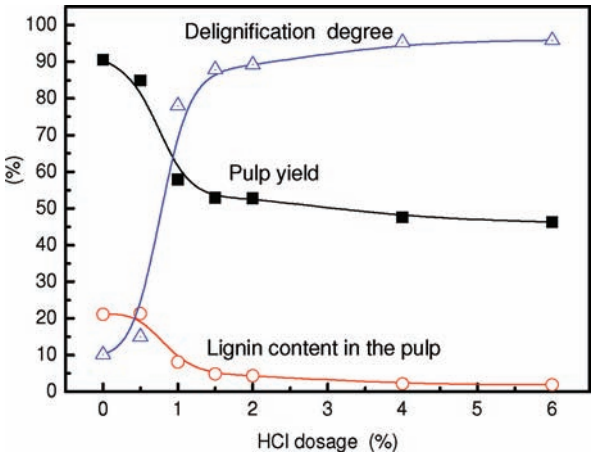

Figure 2. Pulp yield, residual lignin content in the pulp, and delignification degree in acetosolv fractionation of bamboo with various HCl dosages.

formic acid (90%) resulted in a cellulosic pulp yield of 48.3% and a delignification degree of 71.9%.[31] To achieve an effective delignification, more drastic conditions, such as high temperature and the addition of catalyst, are needed. In the present study, HCl was selected to increase the acidity, and the effect of the dosage was investigated. With an increase of HCl dosage from 0.5 to 1.0%, there was a sharp decrease of cellulosic pulp yield, together with a marked increase of delignification degree from 14.98 to 77.99%. With a further increase of HCl dosage, the cellulosic pulp yield decreased slightly and the delignification degree showed a slowly increasing trend. It should be noted that the addition of 4.0% HCl achieved a high delignification degree up to 95.28%, producing a cellulosic pulp with a yield of 47.55% and a residual lignin content of 2.11%. In addition, a further increase of the dosage to 6% significantly increased the cost but showed a negligible increase of the delignification degree. It has been reported that a high amount of HCl in organic acid resulted in a relatively low viscosity (a parameter indicating the degree of polymerization of cellulosic macromolecule) of cellulosic pulp and some structural modifications due to serious degradation of cellulose.[20]

The evolution of lignin and carbohydrates in the solid residue together with the selectivity of delignification is summarized in a Ross diagram (Figure 3). The Ross diagram is a graphical method representing the solubilization behavior of different extraction processes. In this figure, the ideal extraction process must follow the lines A→B, hemicelluloses removal from lignocellulose, and A→D, delignification of lignocellulose. In the acetosolv fractionation process, the simultaneous solubilization of lignin and carbohydrates (mainly hemicelluloses and amorphous cellulose) resulted in the L/C value (the ratio of the yield of lignin to that of carbohydrates) varying in the region of both below the line AB and left of the line AD. As expected, an increase of HCl dosage resulted in a decreased yield of lignin (L) and carbohydrates (C) in solid residue (pulp), indicating that delignification and carbohydrate degradation (mainly hemicelluloses and amorphous cellulose) occurred simultaneously. When the HCl dosage was increased from 0 to 0.5 and 4.0%, L/C kept a steady value at 0.27 as compared to the raw material, followed by a sharp decrease to 0.09 and a steady decrease to 0.02, whereas a further increase of HCl dosage to 6.0% resulted in only a negligible decrease of L/C. Therefore, the addition of 4.0% HCl was reasonable for the fractionation of bamboo in the present study.

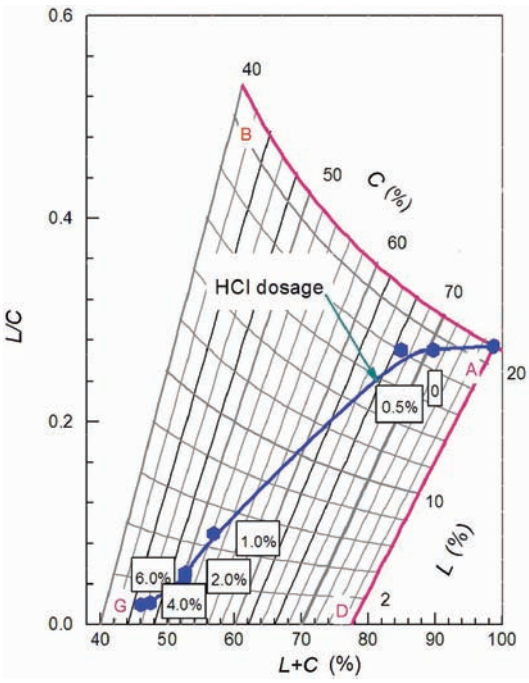

Figure 3. Ross diagram of acetosolv fractionation of bamboo with various HCl dosages. (L represents grams of lignin in the pulp per 100 g of raw material, and C represents gram of carbohydrates (celluloses and hemicelluloses) in the pulp per 100 g of raw material.).

Yield and Chemical Compositions of Acetic Acid Lignins. The dissolved fractions of bamboo were further investigated. With an increase of HCl dosages from 1.0 to 2.0 and 4.0%, the water-soluble fractionation yield increased from 23.36 to 24.88 and 23.57% (note: characterization of these fractionations is under investigation in our laboratory), and the acetic acid lignin yield increased from 12.10 to 17.38 and 20.21%, respectively. Obviously, an increase of HCl usage led to more acetic acid lignins recovered. To obtain detailed information on the compositional and structural features, the three acetic acid lignins (AL1, AL2, and AL3 correspond to samples prepared with HCl dosages of 1.0, 2.0, and 4.0%, respectively) were further characterized as compared to MWL of bamboo. MWL is considered to be a representative of the native lignin in lignocelluloses, despite its low yield and some modifications during the milling. Analysis of the sugar and ash contents of the lignin fractions is illustrated in Table 1. The bound sugars in acetic acid lignin decreased with increasing HCl dosage. The total bound sugars decreased from 4.56 to 2.48% with increasing HCl dosage from 1.0 to 4.0%, indicating that the increase of the severity of fractionation resulted in more intense cleavage of the linkages between lignin and carbohydrates. On the contrary, bamboo MWL isolated in the present study showed a high proportion of carbohydrates (15.47%) containing mainly xylose (up to 13.30%). This was attributed to the mild cleavage of the linkages between lignin and carbohydrates during the extraction process, similar to the case in the preparation of MWL from other materials, such as *Miscanthus* × *giganteus*.[32] In addition, there was a slight increase of ash content from AL1 to AL3, but it was below 0.5% for these preparations. Overall, acetic acid lignins had lower impurities as compared to MWL.

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Table 1. Ash Content and Bound Carbohydrate Composition of Bamboo Acetic Acid Lignins As Compared to MWL

			carbohydrate composition (%)				
lignin sample[a]	ash (%)	total	arabinose	galactose	glucose	xylose	glucuronic acid
MWL	0.19	15.47	0.75	0.06	0.28	13.30	1.08
AL1	0.07	4.56	1.24	0.03	0.24	1.35	1.69
AL2	0.15	3.33	0.96	0.01	0.31	1.16	0.88
AL3	0.39	2.48	0.50	0.01	1.13	0.69	0.15

[a]MWL, milled wood lignin; AL1, AL2, and AL3, lignin samples prepared with HCl dosages of 1, 2, and 4%, respectively.

Table 2. Elemental Analysis, Methoxyl Content, and C_9 Formula of Bamboo Acetic Acid Lignins As Compared to MWL

	elemental and methoxyl content (%)						
lignin sample[a]	C	H	O	N	OCH_3	C_9 formula	C_9 wt (g/mol)
MWL	59.10	6.24	34.54	0.13	19.42	$C_9H_{8.38}O_{2.71}(OCH_3)_{1.48}$	205.70
AL1	61.95	5.88	31.87	0.31	18.88	$C_9H_{7.81}O_{2.64}(OCH_3)_{1.23}$	196.25
AL2	62.63	5.80	31.27	0.31	18.80	$C_9H_{7.58}O_{2.55}(OCH_3)_{1.20}$	193.65
AL3	63.43	5.71	30.68	0.18	18.67	$C_9H_{7.32}O_{2.45}(OCH_3)_{1.18}$	191.17

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

Table 2 shows the elemental composition, methoxyl group, and C_9 formula of the lignin samples. With increasing HCl dosage from 1.0 to 4.0%, the carbon content of acetic acid lignin increased from 61.95 to 63.43%, higher than that of MWL (59.10%), whereas the hydrogen and oxygen content showed a decreasing trend, from 5.88 to 5.71% and from 18.88 to 18.67%, respectively. The small amount of nitrogen in the lignin fractions was derived from protein bound to lignin. The methoxyl numbers in C_9 formulas of acetic acid lignins were lower than that of MWL and decreased with increasing HCl dosage. This meant that more demethoxylation reaction occurred with increasing delignification. In addition, the decrease of the number of oxygen (apart from that in OCH_3) suggested that the lignins were slightly condensed. The condensation reaction mainly resulted from the formation of the carbon cation at Cα of the side chain, which was blinded with an electron-rich carbon atom in the aromatic ring of another lignin unit through the free C5- or C6-position.[33]

Alkaline nitrobenzene oxidation analysis allows identification and quantification of oxidation products derived from lignin, providing the molar proportions of different substructures of lignin. The results of alkaline nitrobenzene oxidation of acetic acid lignins and MWL are given in Table S1 of the Supporting Information. Protolignin of all lignins gave syringaldehyde, vanillin, and *p*-hydroxybenzaldehyde as main products accompanying their corresponding aromatic acids in minor quantity, indicating that all lignins showed a guaiacyl/syringyl/*p*-hydroxyphenyl (GSH) type. In addition, *p*-coumaric and ferulic acids, which exist in a remarkable amount in Gramineae lignin, were also detected. Compared with 30.31% of MWL, the total yields of the phenolic acids and aldehydes decreased from 21.46% in AL1 to 15.01% in AL3. The low yields of products from AL3 can be explained by the occurrence of condensation between the units of lignin in a high-acidity medium. Compared with the molar ratio 47:41:12 of guaiacyl unit (G)/syringyl unit (S)/*p*-hydroxyphenyl unit (H) from MWL, acetic acid lignins showed a low proportion of S units, which decreased from 36% in AL1 to 32% in AL3. This suggested that S units were condensed to some extent in the fractionation process. In addition, demethylation of S units was probably another cause.[34]

Molecular Weight Distributions. GPC chromatograms of acetic acid lignins (AL1, AL2, and AL3) and MWL are shown in Figure S1 of the Supporting Information. The chromatogram of MWL presented a bimodal curve in the high M_w part, with a shoulder peak at 20440 g/mol and a main peak at 9350 g/mol, whereas the peaks shifted to the low M_w region for curves of acetic acid lignins. From AL1 to AL2 and AL3, the peak in the high M_w part decreased from 6130 to 5780 and 5650 g/mol, and the peak in the low M_w part decreased from 2580 to 2370 and 2320 g/mol. The values of the weight-average ($\bar{M}_w$) and number-average ($\bar{M}_n$) molecular weights, estimated from the GPC curves (relative values to polystyrene), and the polydispersity ($\bar{M}_w/\bar{M}_n$) of the lignin samples, are summarized in Table 3. Acetic acid lignins had $\bar{M}_w$ ranging from 4870 to

Table 3. Weight-Average ($\bar{M}_w$) and Number-Average ($\bar{M}_n$) Molecular Weights and Polydispersity ($\bar{M}_w/\bar{M}_n$) of Bamboo Acetic Acid Lignins As Compared to MWL[a]

sample[b]	$\bar{M}_w$ (g/mol)	$\bar{M}_n$ (g/mol)	$\bar{M}_w/\bar{M}_n$
MWL	13000	6450	2.02
AL1	5210	2850	1.83
AL2	5060	2940	1.72
AL3	4870	2710	1.80

[a]Molecular weights were determined by GPC after acetylation of the lignin samples. [b]Corresponding to the lignin samples in Table 1.

5210 g/mol, less than half that of MWL (13000 g/mol), because the treatment in the present study degraded the macromolecule to a noticeable extent. A similar low value of polydispersity for acetic acid lignins ($\bar{M}_w/\bar{M}_n$ ranging from 1.72 to 1.83) indicated a narrower molecular weight distribution. In addition, the decrease of $\bar{M}_w$ with increasing HCl dosage resulted from the significant cleavage of linkages under stronger acidity condition, in good agreement with the NMR results afterward. This phenomenon has also been observed in other organosolv fraction processes, such as ethanol extraction of lignin from lignocelluloses.[35]

NMR Spectroscopy. ^{1}H NMR spectra of the acetylated lignin samples are shown in Figure S2 of the Supporting Information. Assignments of the main signals were made according to the guidelines of Islam et al.,[36] and subdivisions of

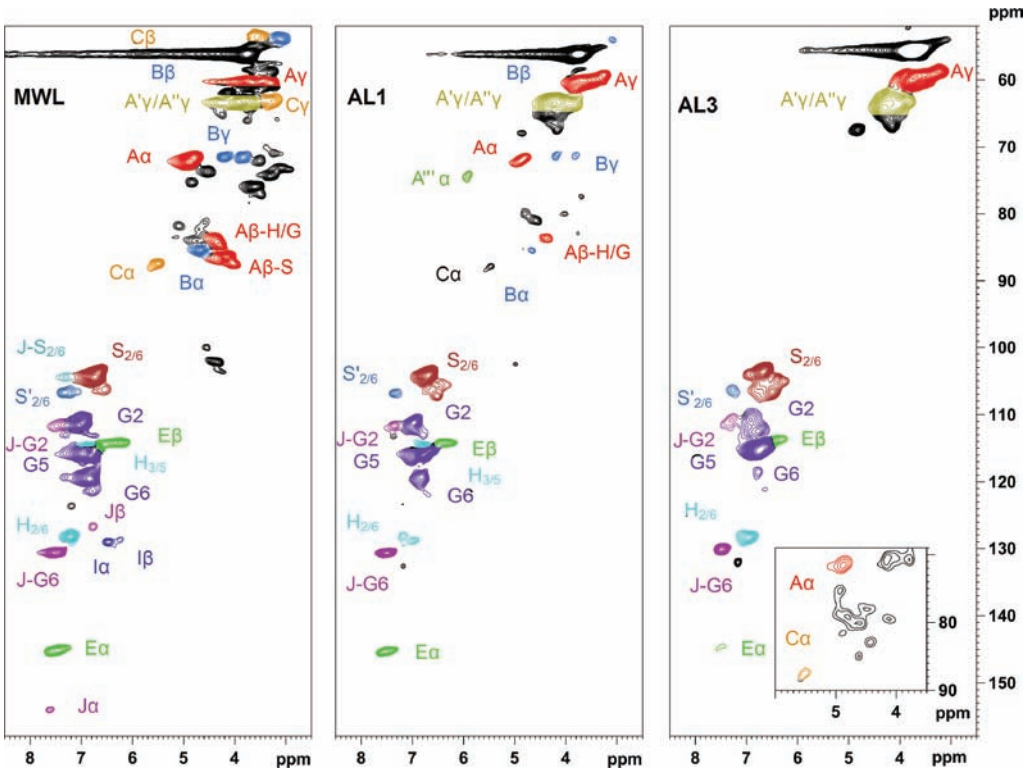

Figure 4. HSQC spectra of bamboo acetic acid lignins (AL1 and AL3) as compared to MWL. The label definitions correspond to the assigned signals of lignin substructures in Table S4 of the Supporting Information. See Figure 5 for the main lignin substructures.

the integration curve were conducted using the resonance of methoxyl groups as internal standard (Table S2 of the Supporting Information). The functional groups in the original lignins are listed in Table S3 of the Supporting Information based on C_9. Obviously, the acetic acid lignins had relatively higher amounts of phenolic protons (0.37−0.53 per C_9) and lower amounts of alcoholic hydroxyl protons (0.84−1.09 per C_9) as compared to MWL. The total hydroxyl protons of acetic acid lignins (1.37−1.46 per C_9) were lower than those in MWL (1.80 per C_9). An increase of the HCl dosage resulted in an increase of phenolic hydroxyl groups but a loss of alcohol hydroxyl groups as well as total hydroxyl groups. The increase of the phenolic protons in acetic acid lignins was due to the enhancement of aryl ether cleavage, whereas the reduction of the alcoholic hydroxyl protons may be partially due to the acidity of the medium promoting the intermolecular condensation reactions and the loss of formaldehyde.[37]

There were averages of 2.21 (AL1), 2.15 (AL2), and 2.03(AL3) aromatic protons per C_9 in acetic acid lignins, as compared to 2.39 aromatic protons per C_9 in MWL. This suggested that condensation of lignin occurred during the acetic acid fractionation, and higher amounts of HCl resulted in more condensation. In addition, the numbers of protons in carbon−carbon (β-β' and β-1′) and (β-O-4′) ether linkages in acetic acid lignins were less than those in MWL and decreased with increasing HCl dosage. However, the numbers of protons in β-5′ linkages in acetic acid lignins (0.29−0.31 per C_9) were slightly higher than MWL (0.27 per C_9), indicating the stability of this type of linkage in acetic acid medium.

The HSQC spectra of acetic acid lignin fractions (AL1 and AL3) and MWL are presented in Figure 4. The main lignin cross-signals observed in the HSQC spectra are listed in Table S4 of the Supporting Information by comparison with literature data,[38,39] and the main indentified substructures are depicted in Figure 5. Because the spectrum of MWL shows strong and relatively complete signals, the signal interpretation was conducted mainly on MWL as shown below. The side-chain region of the spectrum (δ_C/δ_H50−90/2.60−5.80) gives useful information on the interunit linkages. The spectrum shows predominant signals corresponding to β-O-4′ alkyl-aryl ether linkages (A). The C_α−H_α and C_γ−H_γ correlations in β-O-4′ substructures (A) were observed at δ_C/δ_H 72.4/4.86 and 56.0/3.17−3.68, respectively, whereas the C_β−H_β correlations were observed at δ_C/δ_H 84.4/4.35 for the substructures linked to G and H units and at δ_C/δ_H 86.5/4.09 for substructures linked to H units, respectively. In addition, γ-acylated β-O-4′ alkyl−aryl ether linkages (A′/A″/A‴) were observed with their correlations at δ_C/δ_H 63.5/3.63−4.40 for the γ-C-position, whereas α-acylated β-O-4′ alkyl−aryl ether linkages were observed with their correlations at δ_C/δ_H 74.9/5.93 for the α-C-position. Strong signals for resinol (β-β', B) substructures were observed with their correlations at δ_C/δ_H 85.5/4.66, at δ 54.0/3.09 for α- and β-C-positions, and at δ 71.6/3.82 and 71.5/4.18 for the γ-C-position. Phenyl coumaran substructures (C) were also found, the signals for C_α−H_α, C_β−H_β, and C_γ−H_γ being observed at δ_C/δ_H 87.5/5.52, 54.2/3.51, and 63.2/3.16−3.48, respectively.

The main cross-signals in the aromatic region of the HSQC spectrum (δ_C/δ_H 100−160/6.0−8.0) correspond mainly to the substituted benzenic rings of the lignin units. Clearly, typical signals of GSH units were observed. Strong signals corresponding to S units were observed at δ_C/δ_H 104.5/6.73, whereas G units showed different correlations for C_2−H_2 (δ_C/δ_H 111.5/6.98), C_6−H_6 (δ_C/δ_H 119.6/6.82), and C_5−H_5 (δ_C/δ_H 116.1/6.79). In addition, the oxidized (α-ketone) structure of syringyl lignin (S′) was identified at δ_C/δ_H 106.8/7.30 along with

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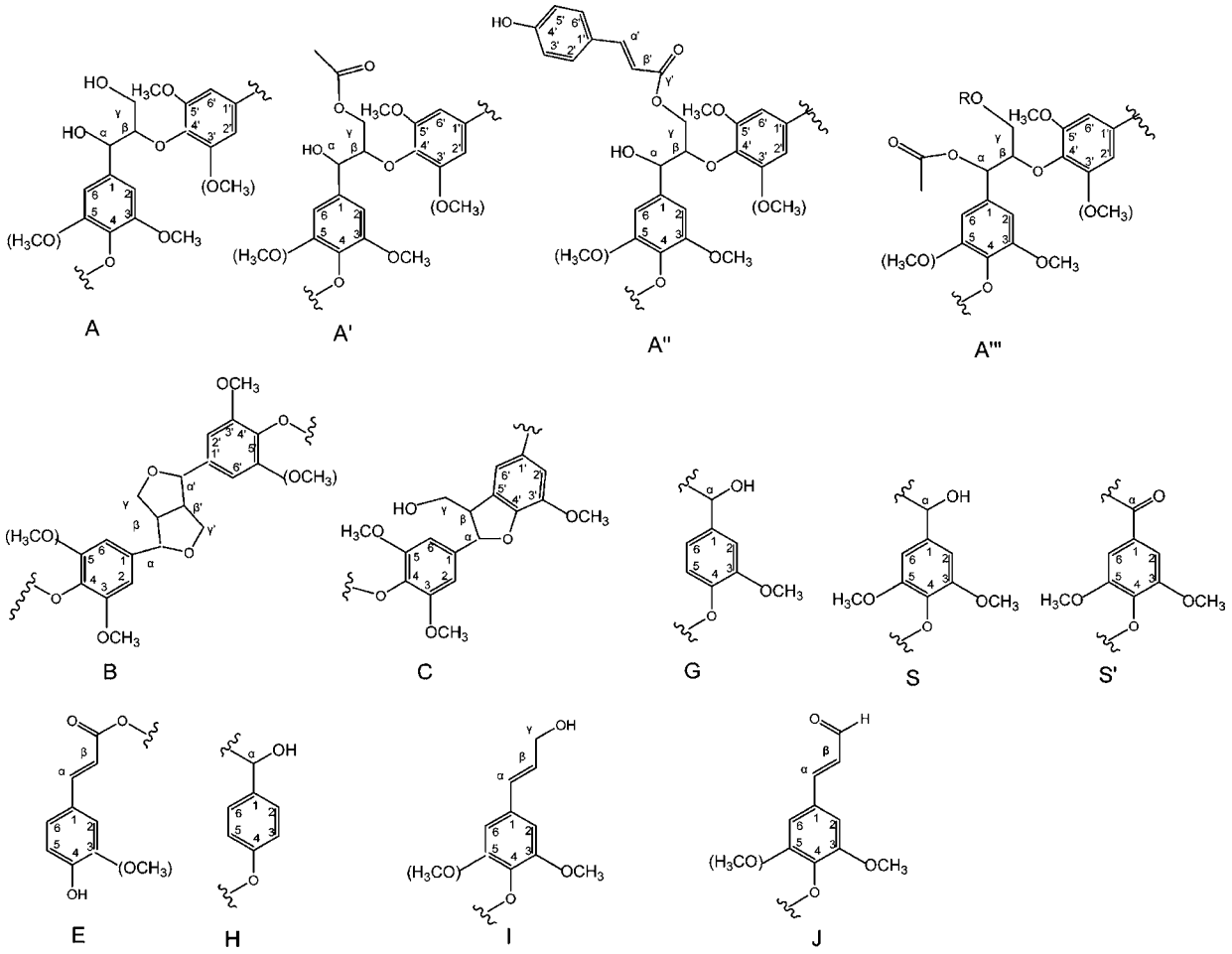

Figure 5. Main substructures of bamboo lignins involving different side-chain linkages and aromatic units identified by HSQC: (A) *β-O-4′* linkages; (A′) *β-O-4′* linkages with a carbonyl group at C*γ*; (A″) *γ-p*-coumaroylated *β-O-4′* linkages; (A‴) *β-O-4′* linkages with a carbonyl group at C*α*; (B) resinol structures formed by *β-β′/α-O-γ′/γ-O-α′* linkages; (C) phenylcoumaran structures formed by *β-5′/α-O-4′* linkages; (E) cinnamate unit; (G) guaiacyl unit; (S) syringyl unit; (S′) oxidized syringyl unit with a carbonyl group at C_α (phenolic); (H) *p*-hydroxyphenyl unit; (I) cinnamyl alcohol end-groups; (J) cinnamaldehyde end-groups.

normal $S_{2,6}$ correlations.[39,40] Signals of H units were detected at δ_C/δ_H 115.0/6.71 and 128.3/7.21 for $C_{3,5}$–$H_{3,5}$ and $C_{2,6}$–$H_{2,6}$, respectively. The signals at δ_C/δ_H 145.3/7.45 and 114.3/6.26 are attributed to C_α–H_α and C_β–H_β of the cinnamate unit. In addition, signals from cinamyl alcohol end-groups (I) were observed with their C_α–H_α and C_β–H_β correlations at δ 129.1/6.45 and 128.5/6.24, respectively, and cinnanmaldehyde end-groups (J) were observed with their C_α–H_α and C_β–H_β correlations at δ 154.1/7.60 and 126.7/6.75, respectively. The aromatic cross-signals of the cinnamyl alchol end-groups overlapped with the same signals in G and S units, whereas the presence of coniferaldehyde end-groups (JG) is characterized by C_2–H_2 and C_6–H_6 correlation signals at δ_C/δ_H 117.0/7.35 and 130.8.2/7.45, respectively, and the sinapaldehyde end-groups (JS) are characterized by a unique $C_{2,6}$–$H_{2,6}$ correlation (δ_C/δ_H 104.5/7.28).

The relative abundance of the main interunit linkages (referred to the total side chains) in lignin samples was calculated by integration from the HSQC spectra, and the results are shown in Table 4. The main substructures in MWL of bamboo were *β-O-4′* ones, accounting for 75% of all side chains, followed by *β-5′* phenylcoumaram substructures with 11% and *β-β′* resinol substructures involving 9% of all side chains. Other lignin substructures were present in lower proportions. In the case of acetic acid lignins, the significant reduction of signal intensity of the side chains (based on the same intensity of aromatic region) indicated the degradation of lignin during acetic acid extraction. The proportion of *β-O-4′* substructures in AL1 increased to 81%, mainly owing to the cleavage of end-groups (I and J), whereas it decreased to 54% in AL3 due to extensive degradation of this type of linkages. In addition, the proportion of phenylcoumaram structures in AL3 increased to 32% due to its relatively high stability in acid medium.

Table 4. Relative Abundance of Main Interunit Linkages (as Percentages of Side Chains Involved) of Bamboo Acetic Acid Lignins As Compared to MWL[a]

	abundance (%)		
	MWL	AL1	AL3
β-O-4′ aryl ether (A, A′, A″, A‴)	75	81	54
β-β′ resinols (B)	10	8	13
β-5′ phenylcoumarans (C)	10	11	32
cinnamyl alcohol end-groups (I)	1	0	0
cinnamaldehyde end-groups (J)	3	0	0

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

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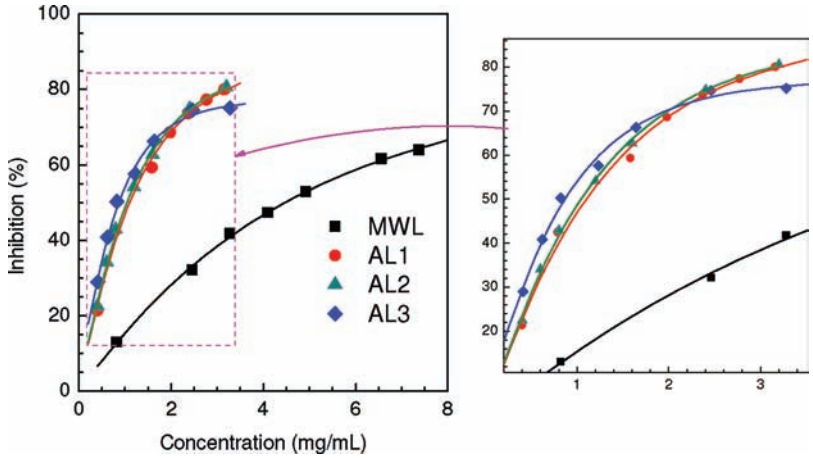

Figure 6. Scavenging activity of bamboo acetic acid lignins (AL1, AL2, and AL3) as compared to MWL.

Antioxidant Activity Analysis. The DPPH inhibitory effects of the lignin solutions are shown in Figure 6. Obviously, the DPPH inhibitory effect increased with increasing solution concentration. The curves of the DPPH free radical-scavenging activity for the tested lignins indicated that the acetic acid lignins had significantly higher antioxidant activity than MWL. The radical-scavenging index (RSI) values of MWL, AL1, AL2, and AL3 were 0.22, 0.91, 0.96, and 1.15, respectively. Obviously, the antioxidant activity of lignin samples decreased in the order AL3 > AL2 > AL1 > MWL. The determined values for acetic acid lignins were higher than those of *Miscanthus* × *giganteus* lignins (0.25−0.40) extracted with formic/acetic acid solution.[41] It has reported that the RSI was positively correlated to phenolic hydroxyl groups but has no effect with aliphatic hydroxyl groups in lignins.[42] The stronger DPPH-scavenging capacity of the acetic acid lignins was mainly due to the fact that acetic acid fractionation induced a large reduction of aliphatic hydroxyl groups together with a significant increase of free phenolic hydroxyls, in good agreement with the results of ^{1}H NMR analysis aforementioned. Additionally, the lower radical-scavenging activity of MWL was partly attributed to the presence of high amounts of coexisting carbohydrates because hydroxyl groups in hemicelluloses may interact with lignin phenolic groups via hydrogen bonds.[43]

In summary, an integrated fractionation process for the separation of bamboo into cellulosic pulp, degraded carbohydrates, and lignin has been investigated. The degraded lignin can be recovered by a simple precipitation operation. With an increase of HCl dosage from 1.0 to 2.0 and 4.0%, the yield of acetic acid lignin increased from 12.10% (AL1) to 17.38% (AL2) and 20.21% (AL3). Under the optimal conditions, that is, 4.0% HCl dosage in 90% aqueous acetic acid at 114 °C, 95.28% of the lignin in the raw material was released, accompanying a cellulosic pulp yield of 47.55% and a residual lignin content in the pulp of 2.11%. As compared to MWL, acetic acid lignins had low impurities (carbohydrates, 2.48−4.56%) mainly due to the cleavage of linkages between lignin and carbohydrates. Acetic acid lignins showed a low proportion of S units, which decreased from 36 to 32% from ALl to AL3. Acetic acid lignins had $\bar{M}_w$ ranging from 4870 to 5210 g/mol, less than half that of MWL (13000 g/mol), mainly due to the cleavage of linkages between lignin units as evidenced by ^{1}H NMR and HSQC spectroscopy. As compared to MWL, the stronger antioxidant activity of acetic acid lignins was mainly due to a large reduction of aliphatic hydroxyl groups together with a significant increase of free phenolic hydroxyls. The lignins were obtained with low impurities, high free phenolic hydroxyls, and medium molecular weights, suggesting that they are promising feedstocks to replace petroleum chemicals (such as phenol) in various applications.[44] These lignins can be used as dispersants and binders or in many thermosetting applications, for instance, in conjunction with phenolic or epoxy resins.[45] The lignin obtained may be readily modified with alkylene oxides as potential polyols for the manufacture of polyurethane foams.[46]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Tables containing results on nitrobenzene oxidation (Table S1), assignments of signals and protons per C_9 structural unit in ^{1}H NMR spectra of acetylated lignin samples (Table S2), analysis of functional groups of lignin samples (abundance based on C_9) (Table S3), and assignments of main ^{13}C−^{1}H correlation signals in the HSQC spectra of bamboo lignin (Table S4); figures containing GPC chromatograms (Figure S1) and ^{1}H NMR spectra (Figure S2) of bamboo acetic acid lignins and MWL. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

HPLC, high-performance liquid chromatography; AL, acetic acid lignin; DMSO-d_6, deuterated dimethyl sulfoxide; DPPH, 2,2-diphenyl-1-picrylhydrazyl; G, guaiacyl; GPC, gel permeation chromatography; H, *p*-hydroxyphenyl; HPAEC, high-performance anion exchange chromatography; HSQC, hetero-

nuclear single-quantum correlation; MWL, milled wood lignin; RSI, radical scavenging index; S, syringyl.

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