ISOLATION AND PHYSICO-CHEMICAL CHARACTERIZATION OF LIGNIN FROM HYBRID POPLAR IN DMSO/LiCl SYSTEM INDUCED BY MICROWAVE-ASSISTED IRRADIATION

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In this paper, the effect of microwave-assisted heating (60, 80, 100 and 120 °C) on lignin separation from ball-milled hybrid poplar wood in dimethyl sulfoxide and lithium chloride (DMSO/LiCl) system was examined. The isolated lignin fractions were characterized by HPAEC (high-performance anion exchange chromatography), FT-IR, ^{13}C NMR and 2D-HSQC NMR techniques. The results showed that extraction of lignin assisted by microwave irradiation at 60, 80, 100 and 120 °C resulted in an increase of lignin yield by 2.4, 8.8, 13.5 and 24.6% (% Klason lignin), respectively. The content of neutral sugars in these lignin fractions was relatively lower as compared with the milled wood lignin (MWL) obtained by the classical method. Structural characterization by 1D and 2D NMR demonstrated that these lignin fractions were classified as syringyl-guaiacyl lignin as they were mainly composed of syringyl units with noticeable amounts of guaiacyl units. In addition, the results of semi-quantitative NMR spectroscopy showed that these lignin fractions mainly consisted of *β*-aryl ether linkage (*β*-*O*-4', 53.3-74.1%) and resinol substructure (*β*-*β*', 15.1-28.5%) combined with small quantities of phenylcoumaran substructure (*β*-5', 3.1-8.2%) and spirodienone substructure (*β*-1', 3.2-8.4%).

Keywords: Hybrid poplar, lignin, microwave-assisted heating, DMSO/LiCl, 2D-HSQC NMR

INTRODUCTION

Lignin is an irregular aromatic biopolymer consisting of phenylpropane units linked by ether and carbon-carbon bonds. Lignin forms an amorphous matrix, binding individual wood cells together, and thus providing mechanical strength properties to wood.[1] However, it has long been recognized for its negative impact on forage quality, papermaking and cellulosic biofuel production. Therefore, elucidating the lignin structure can play an important guiding role on efforts to remove lignin fractions from lignocellulosic materials. Moreover, effectively overcoming the recalcitrance is an important and urgent research priority for the development and implementation of the lignocellulosic biorefinery concept.[2]

One of the most important problems in elucidating the lignin structure has been the isolation of the total lignin from wood in a chemically unaltered form. Early lignin preparation techniques used strong mineral acids to reach high lignin yield,[3] and also used strong alkalis to cleave the ester bonds between hemicelluloses and lignin macromolecules.[4] Such drastic conditions, however, were found to cause irreversible reactions that severely alter the structure of the isolated material. Currently, the most used techniques aimed at isolating lignin from wood in a virgin form are based on the extraction of ball-milled wood by neutral solvents.[5] The classical approach was established by Björkman,[5] who extracted lignin from

ball-milled wood with aqueous dioxane-water (96:4, v/v) mixture. The milled wood lignin (MWL) is usually considered to be more or less representative of native lignin. The yields, relative to the total lignin in the plant sample, range from 10% to 50%, depending on the nature of the plant material and ground severity. However, a steady decrease in *β-O*-4 linkages with increasing ball-milling intensity has been observed,[6-8] showing that substantial lignin depolymerization via the cleavage of uncondensed *β*-aryl ether linkages may take place under severe mechanical action. In addition, MWL is not entirely free of polysaccharide contaminants. In general, the carbohydrate content in isolated lignin fractions obtained by the classical method was more than 10%. An expanded approach subjected milled wood to enzymatic treatment to remove most of the carbohydrate components, which was followed by a liquid-solid extraction.[9] Many modifications based on both methods described above have been proposed, and all of them involved a tedious liquid-solid extraction as the crucial step.

Recently, lignin separation from ball-milled wood based on a complete dissolution in dimethyl sulfoxide/N-methylimidazole (DMSO/ NMI) followed by precipitation in dioxane/water was established.[10] The result showed that the structure of the separated lignin fraction was quite similar to that of MWL obtained by the classical Björkman method. Furthermore, a novel complete dissolution solvent system dimethyl sulfoxide and lithium chloride (DMSO/LiCl) for ball-milled wood powder was developed by Wang *et al*.[11] It was reported that the wood sample milled for 2 h using a planetary ball mill could be completely dissolved in this system at room temperature. In our previously published work, the separation of lignin with a total dissolution of ball-milled wood in DMSO/LiCl system at room temperature was investigated.[12] It was found that the yields of the isolated lignin fractions were relatively lower, as compared to the average yield of milled wood lignin. Therefore, in an effort to further improve the yields of the isolated lignin fractions, a heating procedure induced by microwave irradiation was applied in this study.

Microwave irradiation, as an alternative to conventional heating technology, has been successfully applied in synthesis, catalysis and analytical chemistry. It has the advantages of rapid volumetric heating, high reaction rate, short reaction time, enhanced reaction selectivity and energy conservation.[13] Microwave radiation lies in the range of the electromagnetic spectrum between infrared and radio frequencies and corresponds to wavelengths of 1 cm to 1 m (frequencies of 30 GHz to 300 MHz). When a molecule is irradiated with microwaves, it rotates to align itself with the applied field. The frequency of molecular rotation is similar to the frequency of microwave radiation, and consequently the molecule continually attempts to realign itself with the changing field and energy is absorbed.[14] The use of microwave as an energy source for chemical reactions and processes has been extensively investigated during the last decades. More recently, considerable efforts have been devoted to examine the effect of microwave-assisted reaction on the biomass pretreatment and dissolution.[15,16] It has been shown that the environmentally friendly microwave heating caused the decrease in the dissolution time and energy consumption when this heating process was applied to the dissolution of cellulose in N-methylmorpholine-N-oxide (NMMO). Furthermore, the conversion of 5-hydroxymethylfurfural (HMF) and furfural from lignocellulosic biomass has been studied in an ionic liquid in the presence of $CrCl_3$ as a catalyst under microwave irradiation. The results demonstrated that this method was valuable in facilitating conversion of biomass into biofuels and platform chemicals.

In order to establish a cost-effective separation method for lignin from biomass, microwave irradiation heating has been performed in this study. The objective of this work was to investigate the effect of microwave irradiation heating on the lignin separation efficiency based on complete dissolution in a DMSO/LiCl system. The structural features of the isolated lignin fractions were characterized by FT-IR, ^{13}C-NMR and 2D HSQC NMR techniques.

EXPERIMENTAL

Materials

Hybrid poplar (*Populus bolleana*×*Populus tomentosa Carr*), 7 years old, was obtained from the experimental farm of Beijing Forestry University. Wood samples were dried and milled with a cutting mill to pass through a 40-mesh sieve. The main composition (%, w/w) of dried hybrid poplar wood was 44.5% cellulose, 32.3% hemicelluloses, 21%

lignin and 0.49% ash, respectively. The milled wood powder was extracted with 90% (v/v) acetone/water in a Soxhlet apparatus for 24 h. The extractive-free wood powder was dried under vacuum for several days. The dry wood was ground for 48 h in a 1-gallon porcelain jar containing 9 porcelain balls. All chemicals used were of analytical grade, and standard sugar materials were purchased from Sigma-Aldrich Company (Beijing). The microwave irradiation oven (XH100B) used for sample preparation was purchased from Beijing Xiang-Hu Science and Technology Development Reagent Corporation, and was equipped with a magnetic stirring system and a water-cooling condenser outside the microwave cavity.

Separation and purification of lignin fraction

The separation of lignin fractions in hybrid poplar wood was carried out by the following procedures. 3 g ball-milled wood powder and 1.2 g lithium chloride (LiCl) were placed in a round-bottomed flask. Then, 30 mL of DMSO was added and the mixture was stirred at room temperature for 1 h. After that, the mixture was sequentially stirred at room temperature for 1 h, and heated to 60, 80, 100 and 120 °C using a laboratory microwave irradiation oven and held for 1 h under continuous stirring, respectively. Then, the brown solution was poured into 300 mL 95% ethanol to precipitate and regenerate the carbohydrates under vigorous stirring. The precipitated bulky material was then filtered and washed with an additional 50 mL of 95% ethanol. The filtrate dissolving lignin fraction was concentrated using a rotary evaporator under a reduced pressure to get rid of ethanol and DMSO, and the crude lignin fraction was obtained. Then, 5 mL of deionized water was added, and the lignin was obtained by precipitation at pH 1.5-2.0 and centrifugation. After that, the lignin fraction was thoroughly washed twice with acidified water (pH 2.0) and centrifuged again, and then freeze-dried. Figure 1 shows the fractional separation procedure of lignin fractions by applying microwave-assisted extraction in DMSO/LiCl complete dissolution system. The obtained lignin fractions, which were dissolved in DMSO/LiCl system at different extraction temperatures (room temperature, 60, 80, 100 and 120 °C), were labelled as L_1, L_2, L_3, L_4 and L_5, respectively.

Analytical methods

The monosaccharide in the lignin fractions was determined by hydrolyzing 5 mg samples using 1.475 mL of 10% H_2SO_4 for 2.5 h at 105 °C. At the end of hydrolysis, the mixture was filtered over a 0.22 μm PTFE filter to remove unhydrolyzed residues. The hydrolyzate was diluted 50-fold and injected into the high-performance anion-exchange chromatography (HPAEC) system (Dionex ICS3000, USA) with pulsed amperometric detector and an ion exchange Carbopac PA-1 column (4×250 mm). The neutral sugars were separated in 18 mM NaOH (carbonate-free and purged with nitrogen) with post-column addition of 0.3 M NaOH at a rate of 0.5 mL/min. Run time was 45 min, followed by a 10 min elution with 0.2 M NaOH to wash the column and then a 15 min elution with 18 mM NaOH to reequilibrate the column. The acidic sugars were separated using 0.4 M NaOH for 20 min at a rate of 1 mL/min, and a post-column addition of 0.3 M NaOH was used. Calibration was performed with a standard solution of L-rhamnose, L-arabinose, L-glucose, L-galactose, D-mannose and D-xylose.

Fourier transform infrared (FT-IR) spectra of the lignin fractions were obtained on a FT-IR spectrophotometer (Bruker Tensor 27) using a KBr disk containing 1% finely ground samples. 32 scans were taken of each sample recorded from 4000 to 400 cm^{-1} at a resolution of 2 cm^{-1} in the transmission mode. The ^{13}C-NMR spectra were performed on a Bruker AV III NMR spectrometer at 400 M Hz. The sample (80 mg) was dissolved in 0.5 mL of DMSO-d_6, and the spectra were recorded at 25 °C after 30000 scans. A 30° pulse flipping angle, a 9.2 μs pulse width, 1.89 s delay time and 1.36 s acquisition time between scans were used. The spectral widths for the HSQC (Heteronuclear Single Quantum Correlation) (semi-quantitative mode) were 5000 and 20000 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 (d_1) of 2 s. The number of transients for the HSQC spectra was 128, and 256 time increments were always recorded in the ^{13}C dimension. The $^1J_{C\text{-}H}$ used was 146 Hz. Prior to Fourier transformation, the data matrixes were zero filled up to 1024 points in the ^{13}C-dimension. Data processing was performed using standard Bruker Topspin-NMR software.

RESULTS AND DISCUSSION

Yields of lignin fractions

The use of microwaves as an energy source for chemical reactions and processes can shorten the reaction time and give a uniform heating. Therefore, the effects of various energy inputs induced by microwave irradiation on the lignin yields were determined, and the results are listed in Table 1. It was noted that the yields of isolated lignin fractions increased with the increment of treatment temperature. Compared with the yield of lignin fraction L_1 obtained at room temperature, the results showed that applying microwave-assisted extraction at 60, 80, 100 and 120 °C resulted in an increase of lignin yield by 2.4, 8.8, 13.5 and 24.6% (% Klason lignin),

respectively. Obviously, the use of microwave can liberate higher amounts of lignin from lignin-carbohydrate complexes. The principle of microwave irradiation is called dielectric heating. The rapid change of electric field in the microwave radiation leads to the rotation of the molecules. Based on this process, "internal friction" takes place in the polar medium, which leads to a direct and almost even heating of the reaction mixture. Although some data for the bond energy pointed out that microwave irradiation does not give an energy high enough to break typical chemical bonds commonly found in organic molecules (C-C, C-O, or C-H),[13] there was no doubt that microwave irradiation made a significant contribution on lignin separation.

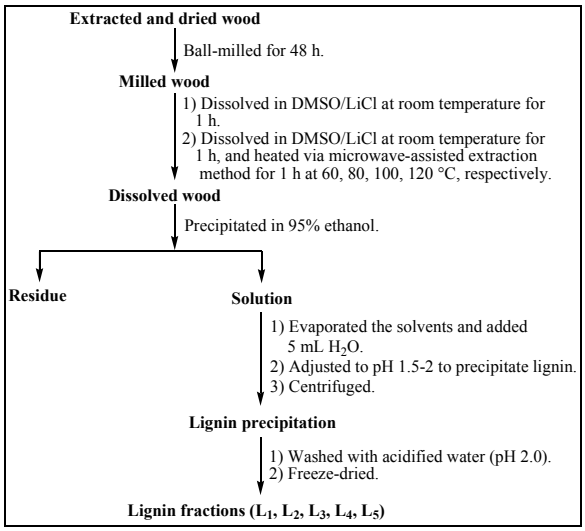

Figure 1: Scheme for lignin separation induced by microwave-assisted irradiation in DMSO/LiCl dissolution system

Table 1
Yields of lignin fractions (% Klason lignin) extracted in DMSO/LiCl dissolution system induced by microwave-assisted irradiation

Yield	Lignin fractions[a]				
(%)	L_1	L_2	L_3	L_4	L_5
Total	8.7	11.1	17.5	22.2	33.3

[a]L_1, L_2, L_3, L_4 and L_5 represent the lignin fractions separated at room temperature, 60, 80, 100 and 120 °C, respectively

Table 2
Content of neutral sugars and uronic acids (% dry weight, w/w) in lignin fractions extracted in DMSO/LiCl system induced by microwave-assisted irradiation

Sugars	Lignin fractions[a]				
(%)	L_1	L_2	L_3	L_4	L_5
Rhamnose	0.01	0.04	0.06	0.08	0.14
Arabinose	0.02	0.10	0.15	0.44	0.34
Galactose	0.04	0.05	0.06	0.10	0.19
Glucose	0.02	0.23	0.40	0.67	1.38
Xylose	0.24	0.86	1.15	1.52	3.35
Mannose	0.04	0.10	0.17	0.05	0.18
Uronic acids	0.05	0.10	0.05	0.21	0.51
Total	0.42	1.47	2.05	3.07	6.09

[a]Corresponding to the lignin fractions in Table 1

Sugar analysis

It is well known that lignin is tightly linked to polysaccharides in the cell walls of plants by various linkage types. In order to analyze the purity of these isolated lignin fractions, the composition of bound neutral sugars in these five lignin fractions was determined, and the results are given in Table 2. The data showed that the sugar contents of these lignin fractions increased from 1.47 to 6.09% with the increase of irradiation temperature from 60 °C to 120 °C. Noticeably, although the yield of lignin fraction L_5 almost reached 33.3%, the sugar content of this lignin sample was relatively lower, as compared to MWL.[7] Obviously, xylose (0.24 to 3.35%) was found to be the major sugar component. In addition, minor amount of glucose was also identified in these lignin fractions. Accordingly, high lignin yield and the low amounts of sugars could be achieved based on the method for isolation of lignin assisted by microwave irradiation.

FT-IR spectra

Figure 2 shows the FT-IR spectra of the lignin fractions L_1 (spectrum L_1), L_4 (spectrum L_4) and L_5 (spectrum L_5). The spectra of these lignin fractions show major O-H stretching absorption at 3445 cm^{-1}, which mainly comes from alcohol hydroxyl and phenolic hydroxyl group in lignin fraction and residual sugars. The bands at 2919 and 2846 cm^{-1} are originated from the C-H asymmetric and symmetrical vibrations in the aromatic methyl group and methylene group. The aromatic skeletal vibrations give four relatively strong peaks at 1599, 1508, 1460 and 1423 cm^{-1}, respectively. The band at 1711 cm^{-1} assigned to unconjugated carbonyl groups was observed in spectrum L_4, while this band occurred as a shoulder in spectrum L_5. Meanwhile, the strong band at 1649 cm^{-1}, originated from the carbonyl stretching in conjugated *p*-substituted aryl ketone, gradually increased from spectra L_4 to L_5 as the heating temperature increased. This indicated that a higher amount of unsaturated bonding was generated due to the heating treatment by microwave irradiation. In addition, the band at 1032 cm^{-1} is indicative of the C-O-C stretching of glycosidic linkages among carbohydrate polymers. The stronger intensity of this band in lignin fraction L_5 indicated that lignin fraction L_5 contained a larger amount of sugar components, which was in accordance with the data obtained by sugar analysis. Furthermore, the band at 1328 cm^{-1} is attributed to syringyl units and the band at 1266 cm^{-1} is associated with guaiacyl units. It was observed that the ratio of band intensity in 1328/1266 cm^{-1} increased as the irradiation temperature increased, which indicated that a higher amount of syringyl units was released with the increase of treatment temperature.[17] It has been reported that lignin with more guaiacyl units is deposited in the early stages of xylem differentiation in poplar wood, and that syringyl units in lignin appear in the late stages of the secondary cell wall.[18] Therefore, the use of microwave irradiation made a contribution to the release of syringyl unit lignin from the secondary cell wall.

^{13}C and 2D-HSQC NMR spectra

To further characterize the structural change, the non-acetylated lignin preparations L_1, L_2 and L_5 were investigated by ^{13}C-NMR and HSQC NMR spectra. Figure 3 shows the ^{13}C-NMR spectrum of the isolated lignin fractions. As shown in this figure, information about the aromatic region was presented in the range from 103 to 160 ppm. It was noted that the intensities of peaks became weaker and fewer signals appeared as the microwave-assisted heating temperature increased, which demonstrated that higher heating temperature resulted in the decrease of purity of isolated lignin contaminated by degraded sugars. The syringyl units are identified by signals at 152.1 ($S_{3/5}$, etherified units), 147.5 ($S_{3/5}$, non-etherified units), 138.5 (S_4, etherified units), 138.1 (S_4, non-etherified units), 135.0 (S_1, non-etherified units), 106.8 ($S_{2/6}$, etherified units with α-CO), and 104.3 ppm ($S_{2/6}$, etherified units). The guaiacyl units give signals at 148.0 (G_3, non-etherified units), 134.3 (G_1, etherified units), 119.3 (G_6, etherified units), 115.3 (G_5, non-etherified units), and 111.1 (G_2, etherified units).[19] The relative intensities of both syringyl and guaiacyl signals revealed that the isolated lignin fractions were mainly composed of syringyl units in combination with considerable amounts of guaiacyl units. In addition, the small signal at 194.3 ppm is indicative of the occurrence of C=O in the cinnamaldehyde structure. Moreover, carboxylic acid or acetyl

groups in xylans give very weak signals at 174.5, 169.7 and 22.1 ppm, which suggested that minor amounts of lignin-carbohydrate complexes (LCC) were still preserved in these lignin fractions. The signal at 55.9 is assigned to -OCH_3 groups in syringyl and guaiacyl units. In addition, the quantification of *β*-*O*-aryl ether structures in lignin preparations could also be successfully identified in ^{13}C-NMR spectra. In the region of aliphatic carbons, signals at 86.1 (C-*β* in S *β*-*O*-4' erythro), 85.1 (C-*β* in G *β*-*O*-4' threo), 72.2 (C-*α* in *β*-*O*-4' G and S erythro), 71.3 (data not shown, C-*α* in *β*-*O*-4' G and S threo), and 59.6 (C-*γ* in *β*-*O*-4' G and S threo and erythro) ppm belong to the resonances of C-*β*, C-*α*, and C-*γ* in *β*-*O*-4' linkages, respectively. Obviously, the intensity of signals of *β*-*O*-4' linkage indicated that larger amounts of *β*-aryl ether structures were preserved in lignin fraction L_1 than those in L_2 and L_5 fractions. The common carbon-carbon linkages, such as *β*-*β*' (C-*β* in *β*-*β*' units, 53.7 ppm) and *β*-5' (C-*γ* in *β*-5' units, 63.1 ppm) were also detected in this spectrum. These signals indicated that the isolated lignin fractions were mainly composed of *β*-*O*-4' ether bonds together with small amounts of *β*-*β*' and *β*-5' carbon-carbon linkages.

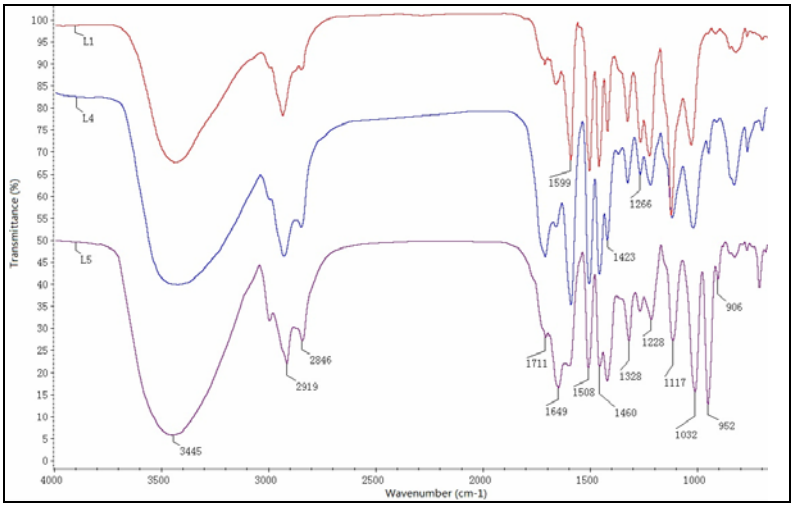

Figure 2: FT-IR spectra of the lignin fractions L_1, L_4 and L_5 extracted in DMSO/LiCl dissolution system from ball-milled poplar wood

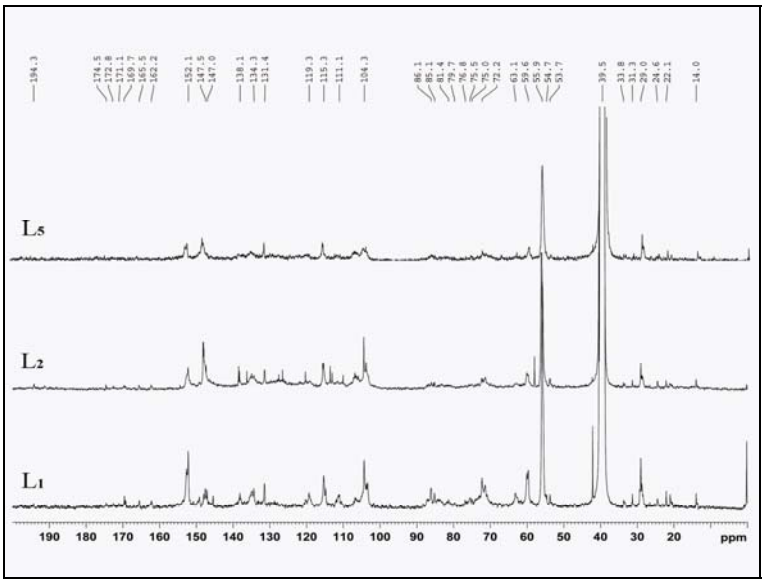

Figure 3: ^{13}C-NMR spectrum of lignin fractions L_1, L_2 and L_5 from ball-milled poplar wood

The 1D NMR spectra were unable to assign minor signals due to overlapping. 2D NMR spectra provide more diagnostic information about the structure of a macromolecular polymer than 1D NMR, due to its well-resolved signals from various structural environments in a complex molecule. Recently, 2D NMR techniques have been increasingly applied to natural polymers, such as lignin.[20,21] The HSQC NMR spectra of L_1, L_2 and L_5 fractions are shown in Figures 4 and 5. Moreover, the relative abundances of the main side chains involved in the primary substructures A-F, as well as the percentage of *γ*-acetylation and the molar S/G ratios, were measured by integrating the anomeric carbon contours area, and the results are listed in Table 3.

In the side chain region, useful information for various linkages between structural units is clearly observed in Figure 4. The main correlating signals observed in the side chain region are the *β*-*O*-4' ether linkages (substructure A). The correlations of C_α-H_α, C_β-H_β and C_γ-H_γ in the *β*-*O*-4' ether linkages of fraction L_1 are obviously characterized by signals at δ_C/δ_H 71.0-73.5/4.5-5.2, 83.5-88.1/3.9-4.5 and 60.0/3.1-4.2, respectively. The intensity of substructure A correlations in L_2 and L_5 fractions extracted by microwave-assisted heating at 60 °C and 120 °C were weaker, as compared to that in L_1 fraction, which implies that higher amounts of *β*-*O*-4' ether bonds were broken down due to the microwave-assisted heating treatment. This result can be demonstrated by Table 3, which shows that the percentage of substructure A in L_1 to L_5 decreased from 74.1% to 53.3%. Interestingly, a small but clear signal corresponding to the C_γ-H_γ correlations in *γ*-acetylated lignin units (A') was also identified at δ_C/δ_H 63.8-64.5/4.2-4.5 in L_1 and L_2 fractions. The result indicates that the separated lignin fractions were partially acetylated at the *γ*-carbon in the side chain. In addition, the signals for C_α-H_α, C_β-H_β and C_γ-H_γ from the phenylcoumaran (*β*-5', C) unit were also determined by major signals at δ_C/δ_H 87.3/5.49, 52.8-54.1/3.46-3.72 and 62.5/3.75, respectively.[22] A strong signal standing for the resinol (*β*-*β*', B) linkage was also observed, as shown by the C_α-H_α, C_β-H_β and C_γ-H_γ cross-peaks at δ_C/δ_H 85.1/4.67, 53.7/3.07 and 71.2/3.72-4.19, respectively. Another C_γ-H_γ correlation (δ_C/δ_H 61.9/4.15) is assigned to *p*-hydroxycinnamyl (F) terminal structures. A spirodienone structure (D) was also detected in these poplar lignin samples, including signals corresponding to C_α-H_α and $C_{\alpha'}$-$H_{\alpha'}$ correlations.[23] In the aromatic region of the HSQC spectrum (Figure 5), C-H correlation contours corresponding to syringyl (S) and guaiacyl (G) units could be qualitatively observed. The primary cross-peak at δ_C/δ_H 104.7/6.69 is attributed to the C-2/6 of S units, and the correlation contour at δ_C/δ_H 106.8/7.09-7.43 is assigned to C-2/6 of S units with *α*-ketone (S') and *α*-carboxyl (S'') structures. Moreover, G units give correlations for C_2-H_2 (δ_C/δ_H 111.6/6.99), C_5-H_5 (δ_C/δ_H 115.4/6.72 and 6.94), and C_6-H_6 (δ_C/δ_H 119.5/6.77), respectively. The main lignin cross-signals present in the aromatic region of the HSQC spectra showed that S units occupied the predominant portion combined with noticeable amounts of G units in lignin substructures.

The molar S/G ratios of all these lignin fractions were estimated by the integration of contours in HSQC plots and the result is listed in Table 3. It was shown that the S/G ratios of lignin fractions L_2 and L_5 were of 2.4 and 3.1, which was higher than those of L_1 fraction and other acetylated samples.[24,25] This result implies that a higher amount of S units from lignin fractions L_1 to L_5 were released as the microwave irradiation temperature increased, and these lignin fractions were mainly composed of S units combined with considerable amounts of G units. It was also noted that the *p*-hydroxybenzoate groups (PB) were also present in the lignin preparations, with the C-2/6 correlation contours at δ_C/δ_H 132.1/7.58-7.83.[26] As shown in Table 3, the main substructure was the *β*-*O*-4' ether linkage (A), which accounted for 53.3-74.1% of all detectable side chains. The second abundant linkage in these lignin fractions was the resinol substructure (B), which involved 15.1-28.5% of all the side chains. In addition, it was noted that small amounts of the phenylcoumaran unit C (3.1-8.2%) were detected in these lignin fractions. Since both the 3- and 5-positions of syringyl units are substituted with methoxyl groups, there are relatively limited probabilities of generating C units, which can be only made when a monolignol couples to a guaiacyl (G) or to *p*-hydroxyphenyl (H) units.[27,28] Accordingly, the main linkages of lignin substructures were the *β*-*O*-4' linkage, followed

by resinol and spirodienone substructures in the isolated lignin fractions.

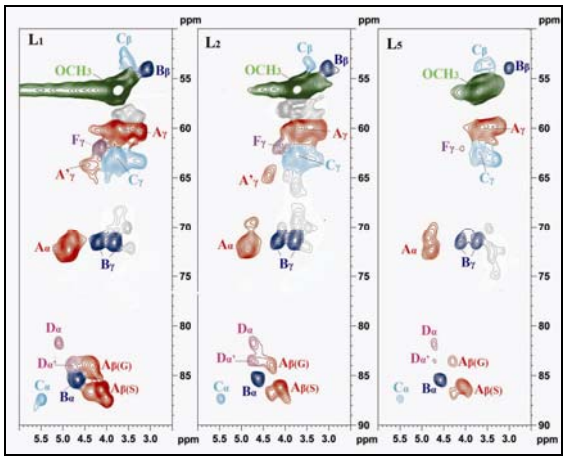

Figure 4: The side chain region of the HSQC spectrum of lignin fractions L_1, L_2 and L_5 extracted from ball-milled poplar wood

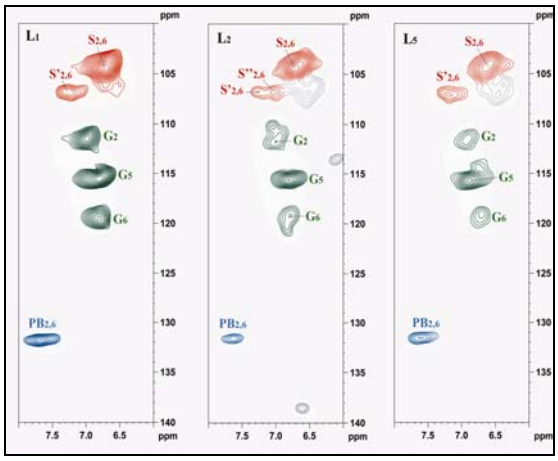

Figure 5: The aromatic region of the HSQC spectrum of lignin fractions L_1, L_2 and L_5 extracted from ball-milled poplar wood

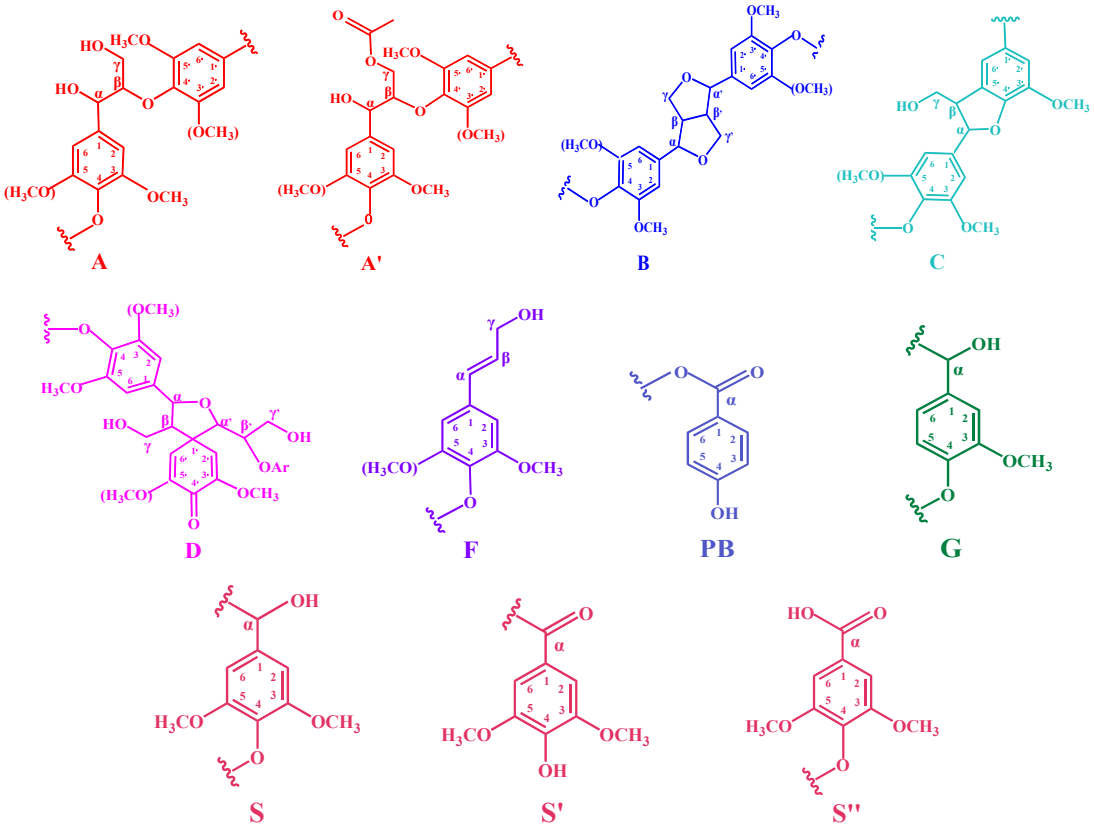

Figure 6: Main structures of isolated lignin fractions: (A) *β-O-*4’ linkages; (A’) *β-O-*4’ linkages with acetylation at *γ*-carbon; (B) resinol formed by *β-β*’, *α-O-γ*’ and *γ-O-α*’ linkages; (C) phenylcoumarane structures formed by *β*-5’ and *α-O-*4’ linkages; (D) spirodienone structures formed by *β*-1’ linkages; (F) *p*-hydroxycinnamyl alcohol terminal units; (PB) *p*-hydroxybenzoate substructures; (G) guaiacyl units; (S) syringyl units; (S’) oxidized syringyl units with a C_α ketone; and (S’’) oxidized syringyl units with a C_α carboxyl

Table 3
Structural characteristics (relative abundance of the main interunit linkages, percentages of *γ*-acetylation and S/G ratio) from integration of ^{13}C-^{1}H correlation signals in the HSQC spectra of lignin fractions from poplar wood

Linkage relative abundance (% of side chains involved)	L_1	L_2	L_5
β-Aryl-ether units (*β*-*O*-4', A/A')	74.1	60.6	53.3
Resinol substructures (*β*-*β*', B)	15.1	18.6	28.5
Phenylcoumaran substructures (*β*-5', C)	3.1	6.9	8.2
Spirodienone structures (*β*-1', D)	3.2	8.4	6.0
p-Hydroxycinnamyl alcohol end groups (F)	4.5	5.5	4.0
Percentage of *γ*-acetylation	8.5	5.7	ND [a]
S/G ratio	2.1	2.4	3.1

[a] ND = not detected

CONCLUSIONS

This study provides a facile method for lignin isolation, based on complete dissolution in DMSO/LiCl system, assisted by microwave irradiation heating. It was found that the yields of these lignin fractions significantly increased with the increase of heating temperature. Moreover, the contents of neutral sugars in these lignin fractions were relatively lower, as compared with the MWL obtained by the classical method. Further studies by ^{13}C and 2D HSQC NMR revealed that the lignin fractions were mainly composed of syringyl units with noticeable amounts of guaiacyl units. The results also showed that the lignin preparations consisted mainly of *β*-aryl ether (*β*-*O*-4') linkages and resinol substructures (*β*-*β*'), combined with small quantities of phenylcoumaran (*β*-5') and spirodienone (*β*-1') substructures.

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