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In this paper, the physicochemical properties and structural features of six lignin preparations separated with a total dissolution of ball-milled wood in dimethyl sulfoxide and lithium chloride (DMSO/LiCl) followed by extraction with ethanol/water were investigated. These isolated lignin fractions were characterized using wet chemical analysis, FT-IR, and ^{1}H and^{13}C-NMR techniques. Experimental results showed that separated lignin preparations were relatively free of associated polysaccharides. These lignin fractions were classified as guaiacyl-syringyl lignin type: mainly composed of guaiacyl units with noticeable amounts of syringyl units and fewer *p*-hydroxyphenyl units. The molar ratio of non-condensed guaiacyl units to syringyl units (G/S) decreased as the ratio of LiCl to poplar weight increased. The results also showed that these lignin preparations consisted mainly of *β-O*-4 ether bonds combined with small quantities of *β-β′* and *β*-5 carbon-carbon linkages. Furthermore, considerable amounts of esterified *p*-hydroxybenzoic acids and minor amounts of esterified *p*-coumaric acid were also detected in these lignin fractions.

Keywords DMSO/LiCL; lignin separation; poplar; wood dissolution

INTRODUCTION

Lignin is the second most abundant renewable biomaterial on the earth (1). It exists in all woody plants and plays a vital role in plant growth and development by improving water conduction through xylem tracheary elements and enhancing the strength of fibrous tissues (2,3). This complex polymer is mainly composed of substituted phenylpropane units, which are linked together to form a polymer lacking regularity, crystallinity, or optical activity. Therefore, it is a significant factor in the manufacture of products derived from plants, for example pulp and paper (4).

For decades, a great deal of interest has been focused on lignin separation and its structure characterization. In order to study its structure and properties, lignin must first be separated from wood or pulp with a minimum change in structure. However, lignin is difficult to separate intact due to the chemical and physical associations between carbohydrates and lignins. This difficulty is further compounded by our inability to fully characterize lignin and lignin matrix structures in cell walls (5). Traditionally, a mild separation method was established by Björkman who extracted lignin from ball-milled wood with aqueous dioxane (6). The milled wood lignin (MWL) was separated after milling disrupted the crystallinity of cellulose in cell wall and depolymerized lignin polymer to some unknown extent (7). So far, the milled wood lignin (MWL) is considered to be representative of native lignin structure and has been extensively used in the elucidation of native lignin structure. However, concerns exist over the similarity between the milled wood lignin and native lignin based on the low yields (25–50% of protolignin) and structural alterations due to ball milling. An expanded approach was subjected to milled wood to enzymatic treatment to remove most of the carbohydrate components and followed by a liquid-solid extraction (8,9). Many modifications based on both methods described above were proposed and all of them involved a liquid-solid extraction as the crucial step (10).

The prospects for lignin characterization have improved considerably with the recent discovery of solvent systems capable of fully dissolving ball-milled cell walls for analysis by NMR (11). Lu and Ralph (11) found that ball-milled wood could be completely dissolved in a mixture of dimethyl sulfoxide and N-methylimidazole (DMSO/NMI) by stirring the wood-solvent suspension at room temperature for 1–3 h. The entire wood material was then acetylated and a cryoprobe device with the help of which low lignin concentration in the solvent could be reliably detected (12). Complete dissolution of wood using an appropriate solvent gives a broad insight for lignin separation. Recently, a new and facile method for lignin separation from wood based on complete dissolution was

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established by Fasching et al. (13). This method involved three steps:

1. mechanical degradation of wood (by ball milling),
2. complete dissolution using dimethyl sulfoxide and N-methylimidazole (DMSO/NMI),
3. precipitation of carbohydrates in dioxane/water and various purification stages.

In evidence, this new approach avoided the tedious liquid-solid extraction step. The results indicated that the structure of separated lignin from the described method was quite similar with the milled wood lignin obtained by the classical Björkman method. Up to date, dimethyl sulfoxide and lithium chloride (DMSO/LiCl) dissolution system, which provided much stronger dissolution ability to wood, was performed by Wang et al. (14). It was proposed that wood samples milled for 2 h using a planetary ball mill could be completely dissolved in this system in which the concentration of ball-milled woods could reach as high as 10%. Furthermore, lignin structure did not change significantly due to the short milling time.

So far, a new type of non-volatile solvent, named ionic liquids, has been widely applied as solvents in synthesis, catalyst, and separation processes (15–17). Ionic liquids are often fluid at room temperature, and consist entirely of ionic species and represent a new class of solvents with high polarity. Since no toxic or explosive gases are formed due to their low vapor pressure, ionic liquids are considered as "green solvents". The complete or partial dissolution behaviors for lignocellulose using ionic liquid were investigated (18–20). The results showed that a variety of ionic liquids can only partially dissolve wood chips, whereas it had good solvating power for sawdust and thermomechanical pulp (TMP) fibers. The dissolution of wood in ionic liquids could offer a variety of new possibilities for its structural and macromolecular characterization, without the prior isolation of its individual components. Moreover, the dissolution and separation of cellulose and lignin in lignocellulose using ionic liquid were also studied (21–23). It was shown that the cellulose regenerated from ionic liquids was found essentially amorphous and porous, and was much more prone to degradation by cellulase. It was also found that up to 20 wt% lignin could be dissolved in certain ionic liquids and the dissolved lignin could be performed for ^{13}C nuclear magnetic resonance (NMR) analyses. In addition, extraction of lignin from sugarcane bagasse waste using ionic liquids was investigated by Tan et al. (24). The results showed that over 93% lignin yield was attained using an ionic liquid mixture containing the 1-ethyl-3-methylimidazolium cation and a mixture of alkylbenzenesulfonates with xylenesulfonate as the main anion, and the regenerated ionic liquid showed good retention of structure and properties.

For the production of biofuels and chemicals, it is necessary to decompose fiber structure and utilize all components of lignocellulose (25). One approach, besides gasification and pyrolysis, is to separate lignocellulose into its components by fractionation. The aim of our study was to develop a fractional separation method for lignocellulosic components using DMSO/LiCl dissolution system, avoiding the liquid-solid extraction step of the classical milled wood lignin (MWL) separation. As the first part of this program, milled poplar wood was totally dissolved in DMSO/LiCl dissolution system and then the lignin component was first separated according to the method described previously (26). Due to the very complex constitution of lignin, the separated lignins were characterized by their chemical composition and physicochemical properties and the results were reported.

EXPERIMENTAL

Materials

A hybrid poplar tree (*Populus bolleana* × *Populus tomentosa Carr*), 7 years old, was obtained from the experimental farm of Beijing Forest 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 as follows: cellulose 44.5%, hemicellulose 32.3%, lignin 21%, and ash 0.49%. 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 with 22.39 g in weight and 2.7 cm in diameter, 16 porcelain balls with 9.48 g in weight and 1.9 cm in diameter, and 41 porcelain balls with 2.86 g in weight and 1.3 cm in diameter, respectively. All chemicals used were analytical grade and sugar reference materials were purchased from Sigma-Aldrich Company (Beijing).

Separation of Lignin Fractions

DMSO (30 ml) was placed in a round bottomed flask. Then, 3 g of the ball-milled wood and different weight of lithium chloride (LiCl), according to LiCl to poplar weight ratios of 0.3:1, 0.4:1, 0.5:1, 0.6:1, 0.7:1, and 0.8:1, were added and the mixture was stirred at room temperature for 2 h. The brown solution was poured into 300 ml 95% ethanol to precipitate the carbohydrates under continuous agitation. The suspension was filtrated and the carbohydrates pad was washed with additional 50 ml 95% ethanol. The lignin-containing solution combined with the washing solution was transferred to a flask and the solvents were recovered with a rotary evaporator under a reduced pressure. Then, 5 ml deionized water was added. The lignin was precipitated at pH 1.5–2.0 by adding 1 M HCl dropwise and centrifuged at the maximum achievable velocity,

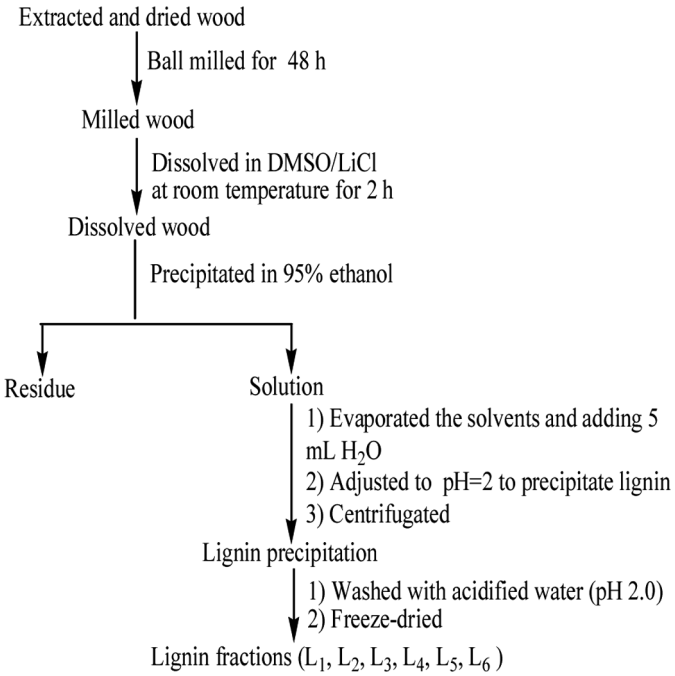

FIG. 1. Scheme for separation of lignin fractions from poplar wood based on a complete dissolution.

usually 4000 rpm. The lignin precipitation was washed twice with acidified water (pH 2.0) and centrifuged under the conditions describe above. After that, the lignin preparations were freeze-dried for 24 h. Figure 1 shows the fractional separation sequence for lignin preparations based on complete dissolution. These lignin fractions obtained from the hybrid poplar dissolved with DMSO and various ratios of LiCl to poplar weight at 0.3:1, 0.4:1, 0.5:1, 0.6:1, 0.7:1, and 0.8:1 were named as L_1, L_2, L_3, L_4, L_5, and L_6, respectively.

Physicochemical Characterization of Lignin Fractions

The monosaccharide in the lignin fractions was determined by hydrolyzing ~5 mg samples using 1.475 ml of 6.1% H_2SO_4 for 2.5 h at 105°C with occasional vibration. At the end of hydrolysis, the mixture was filtered over a 0.22 μm PTFE filter to remove unhydrolyzed residues. The hydrolyzate was diluted 30-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^{-1}. 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^{-1}, 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. UV spectra were recorded on an ultraviolet/visible spectrophotometer (Techcomp, UV2300). The lignin sample (5 mg) was first dissolved in 10 ml of 90% (v/v) dioxane/water, and then a 1 ml aliquot was diluted to 10 ml with 50% (v/v) dioxane/water. The absorbance of the dissolved samples was measured between 250 and 380 nm. The phenolic aldehydes and acids from alkaline nitrobenzene oxidation were analyzed by high performance liquid chromatography (HPLC, Agilent, USA) as previously reported (27). The weight-average (*M*w) and number-average (*M*n) molecular weights of the lignin fractions were determined by gel permeation chromatography on a PL-gel 10 mm Mixed-B 7.5 mm ID column. A 4 mg lignin sample was dissolved in 2 ml tetrahydrofuran and 20 μl solutions were injected. The column was operated at ambient temperature and eluted with tetrahydrofuran at a flow rate of 1 ml min^{-1}. Monodisperse polystyrene was used as the reference materials for determining the molecular weight of lignin.

Fourier transform infrared (FT-IR) spectra of the lignin preparations were recorded from an FT-IR spectrophotometer (Tensor 27, Bruker, Germany) using a KBr disc containing 1% finely ground samples. The solution-state ^1H NMR and ^{13}C NMR spectra were obtained on a Bruker MSL300 spectrometer operating in the FT mode at 74.5 MHz. The lignin sample (25 mg for ^1H, 250 mg for ^{13}C) was dissolved in 1 ml DMSO-d_6 (99.8% D). The ^{13}C NMR spectrum was recorded at 25°C after 30000 scans.

RESULTS AND DISCUSSION

Yield of Totally Dissolved Lignin

A ball-milling time of 48 h was chosen in order to ensure a complete dissolution of the wood in DMSO/LiCl at room temperature and minimize damage to the lignin structure. Therefore, ball-milled wood samples followed by complete dissolution in DMSO/LiCl system was carried out in this study. The effects of various ratios of LiCl to poplar weight on the lignin yield are present in Table 1. The results showed that lignin yields represented 7.7, 8.7, 10.8, 7.6, 6.2, and 8.5% (Klason lignin) among these lignin preparations separated using 30 ml DMSO (25°C, 2 h) with LiCl to poplar weight ratios of 0.3:1, 0.4:1, 0.5:1, 0.6:1, 0.7:1, and 0.8:1, respectively. The relatively low yield of separated lignin was in accordance with milled wood lignin obtained from Alfa grass (28), which revealed that chemical linkages such as *β-O*-aryl ether and carbon-carbon bonding between lignin units were quantitatively preserved. It was demonstrated that ball-milling treatment degraded *β-O*-4 structures in lignins while less than 25% of the

TABLE 1
Yield of lignin fractions (%, Klason lignin) separated using DMSO/LiCl dissolution system from dewaxed poplar at various ratios of LiCl to poplar weight (g/g)

	Lignin fractions[a]					
Yield (%)	L_1	L_2	L_3	L_4	L_5	L_6
Total	7.7	8.7	10.8	7.6	6.2	8.5

[a]Represents the lignin fractions separated based on a complete dissolution in DMSO/LiCl system with LiCl to poplar weight ratios of 0.3:1, 0.4:1, 0.5:1, 0.6:1, 0.7:1, and 0.8:1, respectively.

β-O-4 structures were degraded even after extensive ball milling (9). Moreover, only hydrogen bonding in lignocellulose components was broken during the total dissolution process with DMSO/LiCl system since chloride anions could compete with the lignocellulose components for hydrogen bonding, thus disrupting inter-chain hydrogen bonds of cellulose at a sufficient LiCl concentration (29). Accordingly, the relatively low lignin yield was mainly caused by the milder rotary ball-milling treatment conditions. In addition, the incomplete lignin separation was also ascribed to unbroken ether or ester linkages between lignin and hemicellulose as the absence of acid and base in this dissolution system.

Purity of Lignin Fractions

To verify the purity of separated lignin fractions, the composition of bound neutral sugars in these six lignin fractions was determined and the results were given in Table 2. It was noted that all lignin fractions contained extremely lower amounts of bound polysaccharides as shown by 0.14–0.33% neutral sugars. Compared with dissolved wood, lignin obtained from DMSO/NMI dissolution system (13), the lower contents of carbohydrate contamination in these lignin fractions was an important advantage. As shown in Table 2, the sugar contents of the separated lignins decreased from 0.33% to 0.14% as the ratio of LiCl to poplar weight increased from 0.4:1 to 0.7:1 (lignin fractions L_2 to L_5). It was previously suggested that numerous attachments of LiCl molecules onto the lithium-oligosaccharide adduct ion were observed using electrospray ionization (ESI) mass spectra. Furthermore, the number of attachments increased with the rising value of sugar numbers, corresponding to a higher degree of polymerization of the oligosaccharides (30). Accordingly, the decrease of sugar contents with an increase of LiCl to poplar weight ratio was probably caused by the formation of lithium-oligosaccharide adduct ion during total dissolution. Xylose (0.1–0.24%) was found to be the dominant sugar component, which was consistent with Gabrielii's research results that xylan was the main pentose in hardwood (31). Trace amounts of rhamnose, arabinose, galactose, and glucose were also identified in these lignin fractions.

TABLE 2
The content of neutral sugars (% dry weight, w/w) in lignin fractions separated using DMSO/LiCl dissolution system from dewaxed poplar at various ratios of LiCl to poplar weight (g/g)

	Lignin fractions[a]					
Sugars (%)	L_1	L_2	L_3	L_4	L_5	L_6
Rhamnose	ND[b]	0.01	ND	ND	ND	ND
Arabinose	0.02	0.02	0.02	0.01	0.01	0.02
Galactose	0.04	0.04	0.01	0.02	0.02	0.04
Glucose	0.02	0.02	0.02	0.02	0.02	0.01
Xylose	0.24	0.24	0.24	0.18	0.10	0.21
Total	0.31	0.33	0.29	0.23	0.14	0.28

[a]Corresponding to the lignin fractions in Table 1.
[b]ND = not detected.

UV Spectra of Lignin Fractions

In this study, UV spectroscopy was used to semi-quantitatively determine the purity of lignin in respect of the concentration. Figure 2 shows the UV absorption spectra of lignin fractions L_1, L_2, and L_3 dissolved using 30 ml DMSO in combination with LiCl to poplar weight ratios of 0.3:1, 0.4:1, and 0.5:1, respectively. It was evident that all three lignin fractions exhibited the typical UV spectrum with a maximum around at 274 nm, originating from non-conjugated phenolic groups (aromatic ring) in

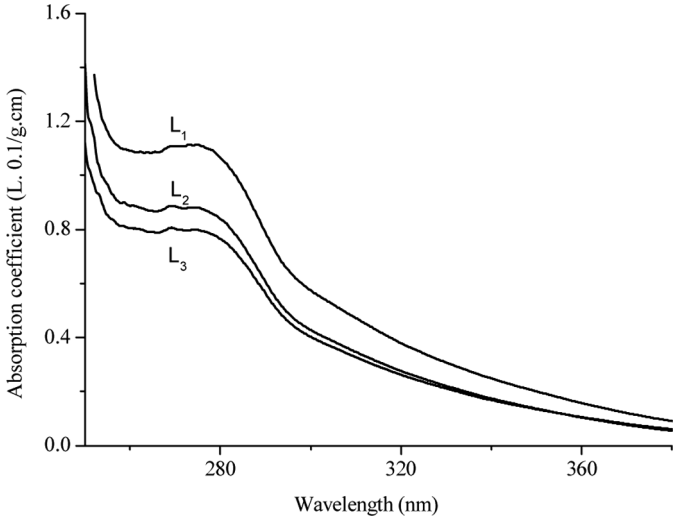

FIG. 2. UV spectra of lignin fractions separated from dewaxed poplar wood.

lignin, which was known to be characteristic of guaiacyl-syringyl lignin (26,32). Furthermore, lignin fractions L_1, L_2, and L_3 showed maximum absorbance at 273.2 nm, 269.2 nm, and 269 nm, respectively. This implied that the syringyl unit content gradually increased in lignin fractions from L_1 to L_3 since syringyl units exhibited the bands at somewhat shorter wavelengths. Moreover, the sole absorption peak that existed in spectra demonstrated that all lignin fractions had a quantitatively high purity. In comparison, a much lower adsorption coefficient of the lignin fraction L_3, dissolved using 30 ml DMSO and LiCl to poplar weight ratio of 0.5:1 at room temperature for 2 h, revealed a low content of lignin in this sample. This was probably due to more co-precipitated non-lignin materials, such as ash and salts in L_3 fraction.

Content of Phenolic Acids and Aldehydes

Traditionally, alkaline nitrobenzene oxidation has been used to estimate the extent of non-condensed units in lignin based on the yield of vanillin, syringaldehyde, and *p*-hydroxybenzaldehyde. Table 3 gives the yields of monomeric products obtained from alkaline nitrobenzene oxidation of separated lignin preparations. Clearly, the predominant oxidation products were identified to be vanillin, syringic, acid and syringaldehyde, which together represented 86.5, 86.9, 89.2, 88.2, 86.4, and 89.9% of the total phenolic compounds in these lignin preparations. Furthermore, it was found that vanillin yield was higher than that of the syringic acid in all the lignin preparations. The presence of fewer *p*-hydroxybenzoic acid and *p*-hydroxybenzaldehyde was most probably considered to be indicative of non-condensed *p*-hydroxyphenyl units, indicating the incorporation of *p*-hydroxycinnamoyl alcohol in poplar lignin. In light of the results discussed above, all the six lignin fractions were composed of large amounts of guaiacyl units with noticeable amounts of syringyl units and fewer *p*-hydroxyphenyl units. It was reported previously that lignin with more guaiacyl units deposited in the early stages of xylem differentiation in poplar wood, and that syringyl units in lignin appeared in late stages of the secondary cell wall (33). Therefore, the higher molar ratio of *G* (relatively total moles of vanillin, vanillic acid, and acetovanillone) to *S* (relatively total moles of syringaldehyde, syringic acid, and acetosyringone) ranging from 1.4 to 1.7 in lignin preparations indicated that these separated lignin fractions were mainly released from the guaiacyl units' richer region such as the middle lamella or the primary cell wall. In addition, the ratio of G/S in all lignin fractions decreased as LiCl to poplar weight ratio increased, which implied that more content of syringyl lignin was liberated from the secondary cell wall under higher LiCl concentration in the dissolution system. In comparison, the lower yields of nitrobenzene oxidation of the lignin fraction L_3 (25.50%) and L_6 (29.95%) suggested the higher content of condensed lignin fractions or non-lignin materials including ash and salts in these two lignin preparations.

TABLE 3
The content (% lignin sample, w/w) of phenolic acids and aldehydes by nitrobenzene oxidation in six lignin fractions separated using DMSO/LiCl complete dissolution system

	Lignin fractions[a]					
Phenolic acids and aldehydes	L_1	L_2	L_3	L_4	L_5	L_6
p-Hydroxybenzoic acid	0.52	0.70	ND[b]	0.47	0.65	ND
p-Hydroxybenzaldehyde	0.45	0.54	0.39	0.57	0.60	0.46
Syringaldehyde	5.94	6.27	5.05	7.39	7.52	6.32
Vanillic acid	3.93	3.19	2.16	3.50	3.95	2.56
Syringic acid	9.75	8.48	6.30	9.43	8.99	7.39
Vanillin	17.88	16.14	11.41	17.03	16.66	13.21
Acetosyringone	0.33	0.23	ND	ND	ND	ND
Acetovanillone	ND	ND	0.19	ND	ND	ND
Total	38.82	35.54	25.50	38.40	38.37	29.95
Molar ratio (*G*:*S*:*H*)[c]	19/11/1	13/8/1	28/19/1	16/11/1	14/9/1	27/19/1
Molar ratio (*G*:*S*)	1.7:1	1.6:1	1.5:1	1.5:1	1.5:1	1.4:1

[a]Corresponding to the lignin fractions in Table 1.
[b]ND = Not detected.
[c]*G* represents the relatively total moles of vanillin, vanillic acid and acetovanillone; *S* represents the relatively total moles of syringaldehyde, syringic acid and acetosyringone; *H* represents the relatively total moles of *p*-Hydroxybenzaldehyde and *p*-Hydroxybenzoic acid.

TABLE 4
Weight-average (M_w) and number-average (M_n) molecular weights and polydispersity (M_w/M_n) of six lignin fractions separated using DMSO/LiCl complete dissolution system

	Lignin fractions[a]					
	L_1	L_2	L_3	L_4	L_5	L_6
M_w	3040	3090	2570	2270	3350	1920
M_n	2350	2440	2140	1910	2330	1740
M_w/M_n	1.29	1.27	1.20	1.17	1.44	1.10

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

Molecular Weight Distribution

The question as to whether the DMSO/LiCl treatment caused lignin depolymerization was addressed by investigating the gel permeation chromatographic elution curves for all six lignin fractions. The values of the weight-average (M_w) and number-average (M_n) molecular weight and the polydispersity (M_w/M_n) of all lignin preparations are listed in Table 4. The results indicated that all six lignin preparations showed no significant difference in their molecular-average weights (M_w 1920–3350 g mol^{-1}). In comparison, the molecular weights of the separated lignin fractions were more than that of poplar exploded wood lignin (34). With the exception of lignin fraction L_5, the molecular weight distribution decreased at higher salt concentration in this dissolution system, which demonstrated that more sugars were removed under a higher LiCl concentration due to the formation of lithium-oligosaccharide adduct ions (30).

FT-IR Spectra

In order to study and compare the structural changes taking place during separation procedures, FT-IR spectra of six lignin fractions were recorded. Figure 3 illustrates the FT-IR spectra of lignin fractions extracted using 30 ml DMSO in combination with LiCl to sample weight ratios of 0.4:1 (spectrum L_2), 0.6:1 (spectrum L_4) and 0.8:1 (spectrum L_6) from the dewaxed poplar. As shown in Fig. 3, the relative intensities of the bands for aromatic skeleton vibrations, assigned at 1597, 1506, 1460, and 1421 cm^{-1}, were rather similar, indicating a similar structure of lignin fractions. No intense polysaccharide bands were observed in the spectra, which implied that these lignin fractions were relatively free of these compounds. It should be noted that the intensity of band at 1726 cm^{-1} assigned to unconjugated carbonyl groups was observed in spectrum L_2 while this band occurred as a shoulder in spectra of L_4 and L_6, which indicated that lignin fraction L_2 contained a greater amount of carbonyl groups than lignin fractions L_4 and L_6. Compared with the spectra of exploded wood lignin from poplar, the band at 1658 cm^{-1} due to conjugated carbonyl groups were observed in all three lignin spectra, whereas only background absorption was detected in this region of the contrasted spectrum (34). Absorption at 1460 cm^{-1} was indicative of C-H deformations and aromatic ring vibrations (35). The bands at 1328, 1267, and 1227 cm^{-1} related to ring breathing with

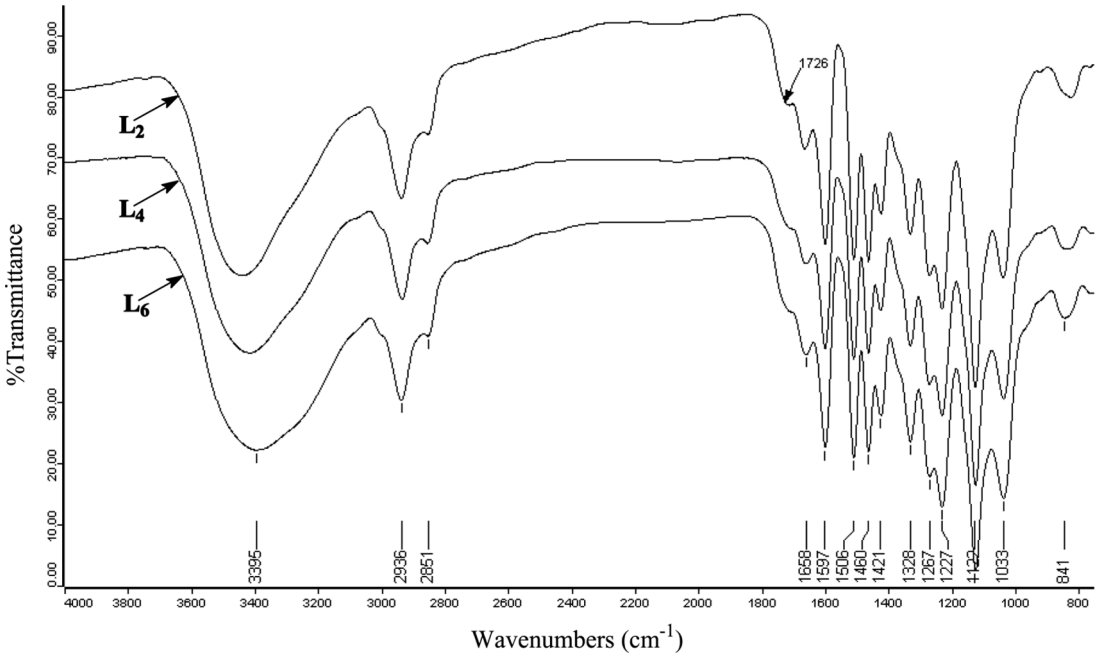

FIG. 3. FT-IR spectra of lignin preparations of L_2, L_4, and L_6 from dewaxed poplar wood.

C-O stretching, and the 1328 cm^{-1} band was associated with syringyl units and 1267 cm^{-1} band with guaiacyl units. The strong intensities of the bands at 1122 and 1033 cm^{-1} corresponded to the aromatic C-H in-plain deformation. Aromatic C-H out of bending exhibited at 841 cm^{-1} (36).

In addition, the spectra of lignin exhibited clearly the characteristic of G-S lignins type according to the lignin classification system by Faix (37): the intensity of the band at 1506 cm^{-1} was higher than that of the band at 1460 cm^{-1} as well as with a maximum intensity around at 1125 cm^{-1}. In general, G-lignins type showed a maximum band at 1140 cm^{-1} while a few per cent S units in lignin was enough to change the maximum peak from 1140 cm^{-1} to a wave number below 1128 cm^{-1} (38). In light of the above discussion, the isolated lignin fractions were mainly composed of guaiacyl units combined with noticeable amounts of syringyl units, which corresponded to the results obtained from nitrobenzene oxidation in Table 3.

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

To further characterize the structural changes of the lignins separated with DMSO/LiCl total dissolution system, the non-acetylated lignin preparation L_1 was investigated by ^{1}H-NMR and ^{13}C-NMR spectroscopy, shown as Figs. 4 and 5. Most of the observed signals were previously assigned in wood lignin spectra (39–41). In the proton NMR spectrum of lignin fraction L_1, signals at 3.7 and 6.4–7.0 ppm were assigned to protons of methoxy and aromatic groups. The strong signal at 3.4 ppm was assigned to hydroxyl protons, which indicated that large amounts of phenolic or acyclic hydroxyl groups existed in this lignin fraction. The free phenolic hydroxyl groups indicated the cleavage of etherified β-*O*-4 linkages could not be avoided to ensure the total dissolution of the wood sample during the milling procedure. It was noteworthy that a weak but distinct peak at 5.3 ppm was observable in the spectrum, which revealed that phenyl coumaran structure was still present in this lignin fraction. An intense signal at 2.4 ppm was indicative of protons in DMSO. Signals of methyl and methylene protons in saturated aliphatic side chains between 0.8 and 1.4 ppm were more intense than the signals of methyl or methylene protons adjacent to double bond or carbonyl group between 1.9 and 2.1 ppm, suggesting that the side chains in C_9 units were saturated or reduced in the lignin fraction. In particular, the signal at 6.9 ppm was attributed to the aromatic protons in G unit while the signal at 6.7 ppm was originated from the S unit. The shoulder at 5.0 ppm arised from the protons of xylan residue (42), corresponding to the sugar analysis results in Table 2. The signals at 4.6 and 4.1 ppm corresponding to H_β and H_γ attached to carbons involved in aryl ether bonds, suggesting that quantitative amounts of β-*O*-4 linkages had been preserved and a comparison with the ^{13}C spectrum (see below) strongly supported this interpretation.

The ^{13}C-NMR spectroscopy of lignin preparation L_1 was shown in Fig. 5. The weak signal at 174.9 ppm (data not shown) was attributed to carbon in carbonyl groups from aliphatic carboxyl carbon (43), which was confirmed by the presence of a signal at 167.2 ppm corresponding to α carboxylic carbons. As to be expected, the lignin fraction was almost the absence of typical polysaccharide signals between 100 and 60 ppm. The spectrum showed signals at 66.6 (C-5 in xylose non-reducing end unit) for the chemically linked polysaccharides. However, this peak intensity

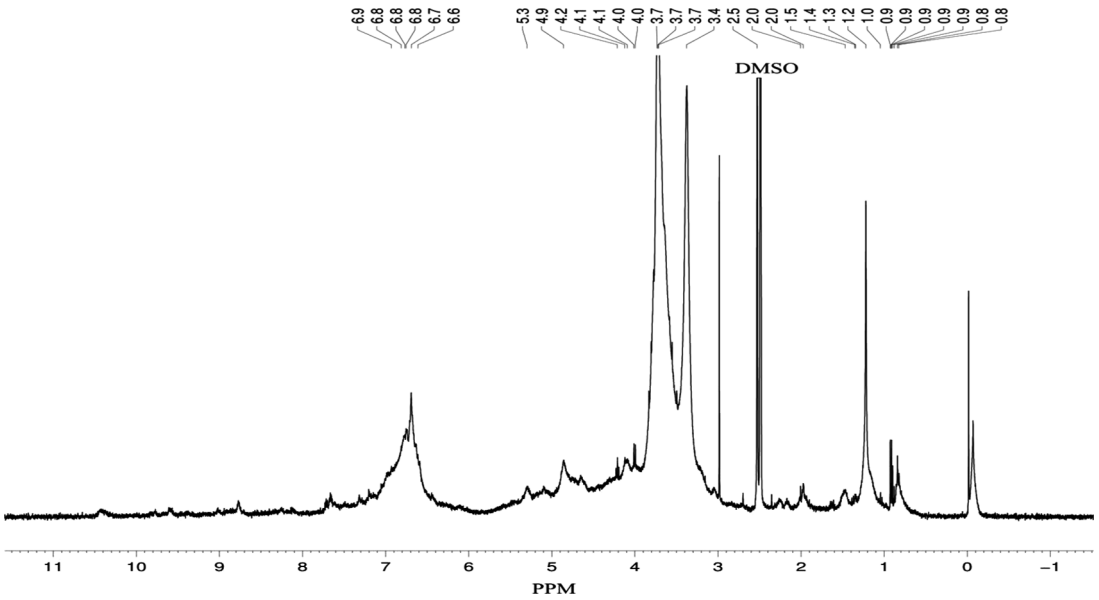

FIG. 4. ^{1}H NMR spectrum of non-acetylated lignin fraction L_1.

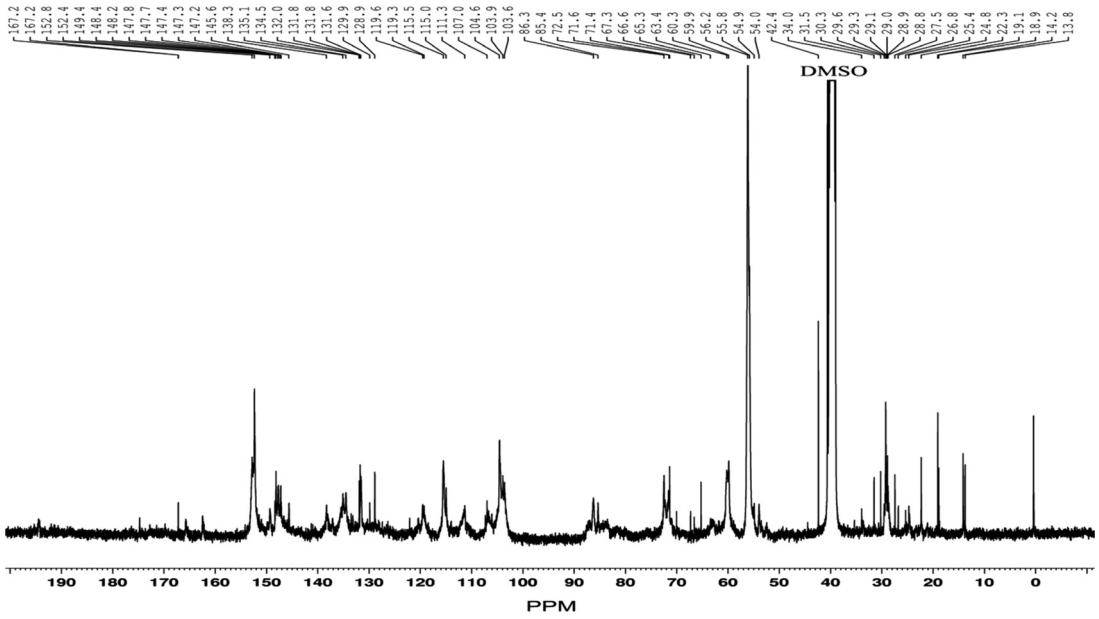

FIG. 5. ^{13}C NMR spectrum of non-acetylated lignin fraction L_1.

was rather weak, indicating that a trace amount of associated polysaccharides existed in this lignin fraction. This qualitative observation was confirmed by data obtained from sugar analysis (Table 2).

In the aromatic part of the lignin from 104.4 to 167.4 ppm, the syringyl, guaiacyl, and *p*-hydroxyphenyl aromatic carbons were detected qualitatively. The guaiacyl units gave signals at 149.4 (C-3 in etherified guaiacyl units), 147.8 and 147.2 (C-4 in etherified guaiacyl units), 145.6 (C-4 in non-etherified guaiacyl units), 134.5 (C-1 in etherified guaiacyl units), 132.0 (C-1 in non-etherified guaiacyl units), 119.3 (C-6 in etherified guaiacyl units), 115.0 (C-5 in non-etherified guaiacyl units), and 111.3 ppm (C-2 in etherified guaiacyl units). The syringyl units were identified by signals at 152.4 (C-3/C-5 in etherified syringyl units), 147.3 (C-3/C-5 in non-etherified syringyl units), 138.3 (C-4 in etherified syringyl units), 107.0 (C-2/C-6 in syringyl units with α-CO), and 104.6 ppm (C-2/C-6 in syringyl units). The *p*-hydroxyphenyl units appeared as a signal at 128.9 ppm (C-2/C-6). The relative intensities of these guaiacyl, syringyl, and *p*-hydroxyphenyl signals clearly revealed that the separated lignin contained a much higher content of guaiacyl units in combination with a considerable amount of syringyl units and a fewer amount of *p*-hydroxyphenyl units. This qualitative observation was confirmed by similar results obtained from alkaline nitrobenzene oxidation (Table 3).

Qualitative ^{13}C-NMR analysis of lignin preparation also allowed for the quantification of *β-O*-aryl ether structures. In the region of aliphatic carbons, compared to alkali-soluble and exploded lignin from poplar (32,34), signals at 86.3 (C-*β* in S *β-O*-4 erythro) and 85.4 (C-*β* in G *β-O*-4 threo), 72.5 (C-α in *β-O*-4 G and S erythro) and 71.4 (C-α in *β-O*-4 G and S threo), 60.3 (C-*γ* in *β-O*-4 G and S threo and erythro) ppm belonged to the resonances of C-*β*, C-α, and C-*γ* in *β-O*-4 linkages, respectively. These observations revealed that large amounts of *β*-aryl ether structures were preserved in lignin fraction L_1 after a milder ball-milling treatment. It was also shown that C-α signal intensity of erythro at 72.5 ppm was lower than that of threo at 71.4 ppm although the poplar contained a high erythro/threo ratio, which was mainly caused by the erythro isomer degraded preferentially during the ball-milling treatment (9). The common carbon-carbon linkages, such as *β-β′* (C-*β* in *β-β* units, 54.0 ppm) and *β*-5 (C-*β* in *β*-5 units, 52.3 ppm, data not shown; C-*γ* in *β*-5 units, 63.4 ppm) were also present. The signals for the *γ*-methyl, α, and *β*-methylene groups in n-propyl side chains of the lignin fraction appeared in the spectrum between 14.1 and 33.7 ppm. In addition, the resolution of the spectra allowed the observation of two distinct signals at 56.2 and 55.8 ppm, which were assigned respectively to the $-OCH_3$ syringyl and $-OCH_3$ guaiacyl groups. These signals indicated that the separated lignins of poplar wood were mainly composed of *β-O*-4 ether bonds together with small amounts of *β-β′* and *β*-5 carbon-carbon linkages. Furthermore, the signals at 167.2 (C=O), 131.8 (C-2/C-6) and 115.5 (C-3/C-5) ppm represented the esterified *p*-hydroxybenzoic acid were higher than the signal at 129.9 (C-2/C-6) ppm attributed to the esterified *p*-coumaric acid, which indicated that considerable amounts of esterified *p*-hydroxybenzoic acids and minor amounts of esterified

p-coumaric acid linked to lignin were qualitatively detected. These findings were consistent with previous work reported by Lapierre and co-workers on the lignins obtained from poplar wood (44).

CONCLUSION

The results of this investigation showed that the yield of all six lignin fractions obtained from fully dissolved ball-milled poplar cell walls using DMSO/LiCl was relatively lower, which was mainly caused by the milder ball milling treatment. In addition, these lignin fractions were mainly extracted from the middle lamella or the primary cell wall since guaiacyl units occupied the main component in these lignin fractions. Moreover, it was noteworthy that the separated lignins contained trace amounts of associated polysaccharides. Further studies by ^{13}C-NMR revealed that these lignin fractions contained considerable amounts of esterified *p*-hydroxybenzoic acids and minor amounts of esterified *p*-coumaric acid. The results also showed that the lignin preparations were mainly composed of β-*O*-4 ether bonds combined with small quantities of β-β' and β-5 carbon-carbon linkages between the lignin structural units. Further work should be performed to improve the yield of lignin combined with a minimum change in its virgin structure.

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