Yields Analysis and Structural Characterizations of Lignin Extracted from *Eucalyptus globulus* with Catalyzed Ethanol Systems

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A mild and successive method was proposed and evaluated for fractionating lignin samples from *Eucalyptus globulus*. The extraction efficiency and structural features of the extracted lignin samples were comprehensively investigated by a yields analysis, ^{1}H-nuclear magnetic resonance (NMR), ^{13}C-NMR, and two-dimensional heteronuclear single quantum coherence (2D-HSQC) NMR. The catalyzed ethanol systems consisting of ethanol, 4-methyl-2-pentanone, dimethyl sulfoxide (DMSO), formic acid, and distilled water were effective for extracting lignin from *Eucalyptus globulus*, and the yield of lignin was 75.2% when the extraction process was held at 145 °C for 180 min. Compared with that of the milled wood lignin (MWL), the NMR spectra of the extracted lignin fractions supported the destruction of β-O-4 units for the disappearance of H_α signals. Moreover, the striking characteristics of the extracted lignin were the destruction of ether bonds of S3,5 and the condensation of syringyl (S) and guaiacyl (G) units. In short, lignin fractions with relatively complete structures were effectively extracted with the catalyzed ethanol systems.

Keywords: Lignin; Eucalyptus globulus; Extraction; Yield; NMR

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INTRODUCTION

Eucalyptus species are widely distributed in many regions of the world, and they are regarded as fast-growing feedstock for pulp and paper production (Luo *et al.* 2012). In addition to the pulping industry, more attention has been paid to biorefining of *Eucalyptus* feedstock (Xu *et al.* 2017). As a potential feedstock for biorefinery, improving the extraction effect of *Eucalypt* lignin is necessary to achieve an efficient delignification for further value-added applications (Rico *et al.* 2014).

Different extraction processes result in different chemical composition and structural features of the extracted lignin samples (Sequeiros *et al.* 2014). The complex structures of lignin samples as well as the fundamental chemistry of lignin has not been completely elucidated, although the primary structure has been well depicted. To improve the fundamental understanding of lignin chemistry, it is necessary to broaden the knowledge of its structure features. Researchers in this area have made many advances, such as understanding the effects of NaOH:lignin ratio and CH_2O:lignin ratio on the hydroxymethylation of alkali lignin in alkaline solution (Malutan *et al.* 2008). The structural characteristics of lignin fractions dissolved by acetic acid were analyzed, and the hydroxycinnamic acids were found in the lignin macromolecules (Ibarra *et al.* 2005; Rencoret *et al.* 2008; Abdelkafi *et al.* 2011). The lignin fractions isolated from cotton stalks by catalytic methods consist mainly of β-O-4 aryl ether linkages, and the decrease of β-O-4 linkages is consistent with the acidity of the catalysts (Wu *et al.* 2015). The lignin separated from birch wood sawdust in the moderately alkaline water solution at 90 °C is rich in p-oxyphenylpropane fragments and carbonyl groups, and contains a relatively small amount of aliphatic hydroxyl and methoxyl groups (Shulga *et al.* 2012). Moreover, some combined technologies based on ethanol organosolv delignification were proposed (Huijgen *et al.* 2012). However, the delignification in that process generally requires a higher temperature (> 170 °C) and an acid catalyst (H_2SO_4), which undoubtedly increases the cost of the whole process (Garcia *et al.* 2010).

In this study, the yields of the *Eucalyptus globulus* lignin samples obtained from the extraction with the catalyzed ethanol systems are analyzed. The catalyzed ethanol systems are composed of ethanol, 4-methyl-2-pentanone, DMSO, formic acid, and distilled water, in which formic acid can be regarded as the primary catalyst. The structural features of the extracted lignin samples are investigated by several NMR techniques, including ^{1}H-NMR, ^{13}C-NMR, and 2D-HSQC NMR. The results obtained can contribute to the understanding of the structure of *Eucalypt globulus* lignin and potentially allow an efficient delignification of *Eucalypt globulus* biomass to be achieved.

EXPERIMENTAL

Materials

Eucalyptus globulus wood chips were obtained from Anhui province, China. The chips were sectioned using a mechanical chipper, and the dimensions of the chips were 20 mm × 10 mm × 3 mm. Ethanol (Beijing Chemical Works, Beijing, China), 4-methyl-2-pentanone (Xilong Chemical Works, Shantou, China), dimethyl sulfoxide (DMSO) (Zhiyuan Chemical Reagent Company, Tianjin, China), and formic acid (Xilong Chemical Works, Shantou, China) were analytical grade and were used as received without any further purification.

Methods

Isolation of milled wood lignin (MWL)

The MWL was extracted from *Eucalyptus globulus* chips with 96% aqueous dioxane according to the classic method as follows. Specifically, the ball-milled *Eucalyptus globulus* chips were extracted with 96% aqueous dioxane for 24 h (twice) at 25 °C in the dark. After centrifugation, the extracting solution was concentrated to 50 mL and slowly dripped into 150 mL of 95% ethanol to accelerate the precipitation of hemicellulose. After removing hemicellulose by centrifugation, the lignin solution was concentrated to 50 mL under stirring and then dried at 45 °C (Bjorkman 1956).

Extraction of lignin with catalyzed ethanol systems

The oven-dried *Eucalyptus globulus* chips were placed in an oil bath pan with eight 1.2-L stainless steel tubes. The mass of chips in every stainless steel tube was 100 g. The catalyzed ethanol systems were mixed with the *Eucalyptus globulus* chips. The liquid-to-solid ratio during the extraction process was 10:1. The samples in the stainless steel tubes were heated from 25 °C to 145 °C in 90 min. The holding periods at 145 °C were 90 min, 120 min, 150 min, and 180 min, separately. The black liquor in the stainless steel tubes was acquired after the extraction process. The lignin was precipitated from the black liquor at pH 2 (adjusted with sulfuric acid). The precipitated lignin was filtered through a sintered glass Büchner funnel and washed with water at 75 °C to remove residual sugars. The purification was performed by dissolving 5.0 g of lignin in 25.0 mL of acetone (dried over molecular sieve) and filtered to remove impurities using a membrane filter. Acetone was evaporated under reduced pressure and pure lignin was dried over P_2O_5 in a vacuum desiccator. The formulas of the catalyzed ethanol systems are indicated in Table 1. All of the experiments were conducted under air atmosphere.

Table 1. Formulas of the Catalyzed Ethanol Systems

Serial number	Ethanol (%)	4-methyl-2-pentanone (%)	DMSO (%)	Formic acid (%)	Distilled water (%)
1#	40	5	20	10	25
2#	40	10	15	10	25
3#	40	15	10	10	25
4#	40	20	5	10	25

Yields of lignin samples

The yields of MWL and the lignin samples extracted by the catalyzed ethanol systems were calculated as follows,

$$X\% = \frac{m_2}{m_1 \times n} \times 100\% \qquad (1)$$

where m_1 is the weight of oven-dried untreated *Eucalyptus globulus* chips, n is the Klason lignin content of untreated wood, and m_2 is the weight of oven-dried extracted lignin samples.

Tests of extracted lignin samples

Traditional MWL isolated from *Eucalyptus globulus* chips was used as a standard sample to analyze the structural features of the lignin samples extracted with the catalyzed ethanol systems. The ^{1}H-NMR spectra of the extracted lignin samples were recorded on a Bruker AV III NMR spectrometer (Bruker, Germany) at 400 MHz using 10 mg of lignin in 0.5 mL of DMSO-d_6.

The chemical shifts were calibrated relative to the signals from DMSO-d_6, used as an internal standard at 2.49 ppm for the ^{1}H-NMR spectra. The acquiring time was 3.98 s, and relaxation time was 1.0 s. In addition, the quantitative ^{13}C-NMR spectra of the lignin extracted with the catalyzed ethanol systems were obtained on a Bruker spectrometer at 100 MHz.

The lignin sample (80 mg) was dissolved in 0.5 mL of DMSO-d_6, and the spectrum was recorded at 25 °C after 3000 scans. A 30° pulse flipping angle, a 9.2 μs pulse width, a 1.89 s delay time, and a 1.36 s acquired time between scans were used. A semi-quantitative analysis of the 2D-HSQC cross-signal intensities was also performed in this study. The integration of the cross-signals was performed separately for the different regions of the 2D-HSQC NMR spectra, which contained signals that correspond to chemically analogous carbon-proton pairs (in similar samples).

RESULTS AND DISCUSSION

Components of *Eucalyptus globulus*

The *Eucalyptus globulus* chips were oven-dried at 105 °C for 24 h and then the chemical compositions of chips were analyzed. The components of *Eucalyptus globulus* are shown in Table 2, which is consistent with the findings of Wen *et al*. (2013a).

Table 2. Chemical Compositions of *Eucalyptus globulus* Wood Chips

Glucan^a (%)	Xylan (%)	Arbinan (%)	Galactan (%)	Klason lignin (%)
36.5	16.8	0.29	0.93	29.8

[a] The data in this table are average values of duplicate experiments.

Characterizations of Lignin Extracted with Catalyzed Ethanol Systems

Yields of extracted lignin samples

MWL was used as a standard sample to analyze the structural features of lignin extracted from *Eucalyptus globulus*. However, the low yield of MWL (23.5%±2.9%) indicated that it only represented a part of lignin fragments in the plant. The extraction efficiency of lignin samples extracted with catalyzed ethanol systems was affected by the formulas of the catalyzed ethanol systems and holding times. The variations of the yields of extracted lignin are shown in Fig. 1.

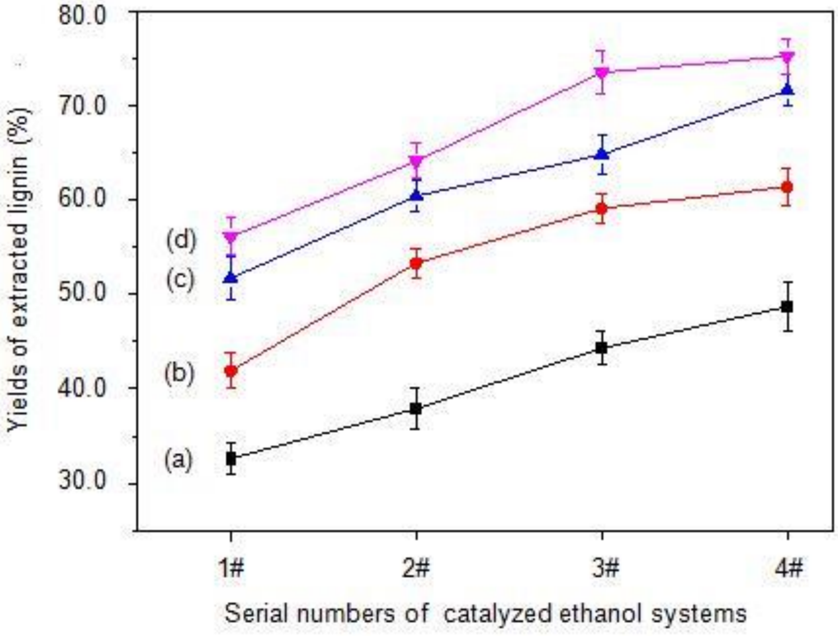

Fig. 1. Yields of the lignin samples extracted with 4 kinds of catalyzed ethanol systems under different holding times: (a) 90 min; (b) 120 min; (c) 150 min; and (d) 180 min

When the holding time was 90 min, the treatment with 1# catalyzed ethanol system led to the extraction of 32.6% lignin. With the increase of the content of 4-methyl-2-pentanone and the decrease of DMSO content, the yield of extracted lignin was enhanced 49.3% (from 32.6% to 48.7%) (Fig. 1a). The increase in holding time resulted in the addition of lignin dissolved in the catalyzed ethanol systems. As indicated in Fig.

1b, after a 120-min holding time, 41.9% lignin was extracted with 1# catalyzed ethanol system. And the 4# catalyzed ethanol system resulted in a yield of 61.4% of the extracted lignin. With further extension of the holding time, the increase of yields of the extracted lignin samples slowed down. When the extraction process was held at 145 °C for 180 min, the yield of the lignin extracted with the 4# catalyzed ethanol system was 75.2%, which was 5.1% more than that extracted with a holding time of 150 min (Figs. 1c and d).

^{1}H-NMR Analysis

The spectroscopy method of ^{1}H-NMR was used to assess the structural features of lignin samples extracted by 4# catalyzed ethanol system with a 180-min holding period. The ^{1}H-NMR spectra of the extracted lignin are indicated in Fig. 2. The characteristic signals of extracted lignin were assigned by comparing with the previous literature (Vazquez *et al.* 1997).

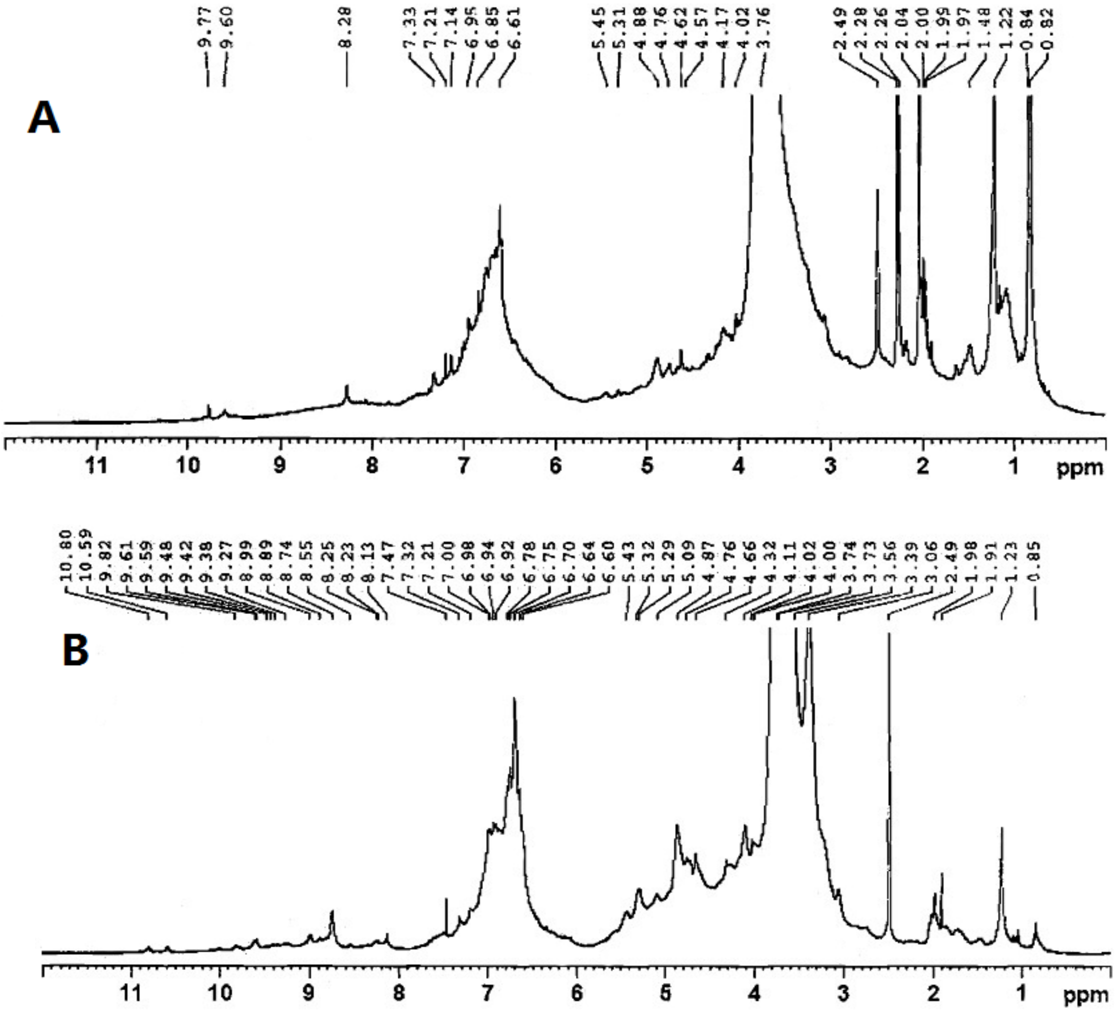

Fig. 2. ^{1}H-NMR spectra of lignin samples: A: lignin isolated by the catalyzed ethanol system; B: MWL

According to the ^{1}H-NMR spectra of the extracted lignin samples (Fig. 2A), the signals between 8.00 ppm and 6.00 ppm mainly indicated the assignments of aromatic protons in syringyl (S), guaiacyl (G), and p-hydroxyphenyl (H) units, as well as p-coumaric acid (Kuhnel *et al.* 2015). The signal at 7.33 ppm was assigned to aromatic protons of ester-linked p-coumaric and ether-linked ferulic acid. The obvious signals at 6.85 ppm and 6.61 ppm were attributed to the 3, 5-position, and the β-position of esterified p-coumaric acid and etherified ferulic acid, respectively. The weak signal at 5.31 ppm was attributed to the H_α of β-5 structure and the signal at 4.88 ppm that originated from the H_α of β-O-4 structure. The signals between 4.88 ppm and 4.17 ppm stood for H_β in the β-5 and β-β structures. The methoxyl protons ($-OCH_3$) gave a sharp signal at 3.76 ppm. An intense signal at 2.49 ppm was indicative of residual protons in DMSO-d_6. The aromatic acetyl and aliphatic acetyl appeared at the region between 2.49 ppm and 0.82 ppm.

Compared with the ^{1}H-NMR spectra of MWL in Fig. 2B, the striking characteristic of lignin samples extracted with the catalyzed ethanol system was the disappearance of H_α signals at 5.31 ppm and 4.88 ppm. That phenomenon indicated the destruction of β-O-4 units (Fig. 2A), in agreement with the results already reported for the lignin extracted with the mixture of acid and water (Wen *et al.* 2013b). Moreover, the enhancements of aromatic acetyl signals at 2.28 ppm and 2.04 ppm (indicated in Fig. 2A) showed that the hydrolysis of acetyl was limited at 145 °C during this separation process of lignin samples (Santos *et al.* 2014).

^{13}C-NMR Analysis

Further information on the chemical structures of lignin extracted with the catalyzed ethanol systems was obtained by ^{13}C-NMR. The ^{13}C-NMR spectra (Fig. 3) revealed the carbon chain structures and inter-unit linkages in the lignin fractions. According to the previous publications, the signal assignments for ^{13}C-NMR of the extracted lignin samples are shown in Table 3 (Balakshin *et al.* 2011; Idstrom *et al.* 2016).

Table 3. Chemical Shift Values (Δ, ppm) and Signal Assignments of the Lignin Extracted with the Catalyzed Ethanol Systems

δ/ppm	Assignment
160-140	Aromatic C-O
140-123	Aromatic C-C
123-103	Aromatic C-H
60.2-58	β-O-4
58-54	OCH_3
54-51.8	β-β, β-5

Compared with the ^{13}C-NMR spectrum of MWL in Fig. 3B, the absence of the S 3,5 signal at 152.2 ppm demonstrated the breaking of ether bonds, and the enhancement of the signal at 147.9 ppm indicated the synthesis of β-β structures in G units (Fig. 3A). A similar effects on the *Miscanthus* lignin extracted by the ethanol solvent was reported (Hage *et al.* 2009). Further, the peaks at 119.5 ppm, 115.5 ppm, and 109.6 ppm were observed in the spectrum of MWL (Fig. 3B), while the peaks at the same chemical shift values were inconspicuous in Fig. 3A. The attenuation of those characteristic peaks was evidence of the condensation of G units in the acidic environment (Shimada *et al.* 1997). According to Figs. 3A and B, the signals at 85.4 ppm, 72.4 ppm, and 60.0 ppm of the lignin extracted with the catalyzed ethanol systems were noticeably weaker than the same signals of MWL. This phenomenon confirmed the decomposition of β-O-4 units of the extracted lignin samples, confirmed the result obtained by ^{1}H-NMR.

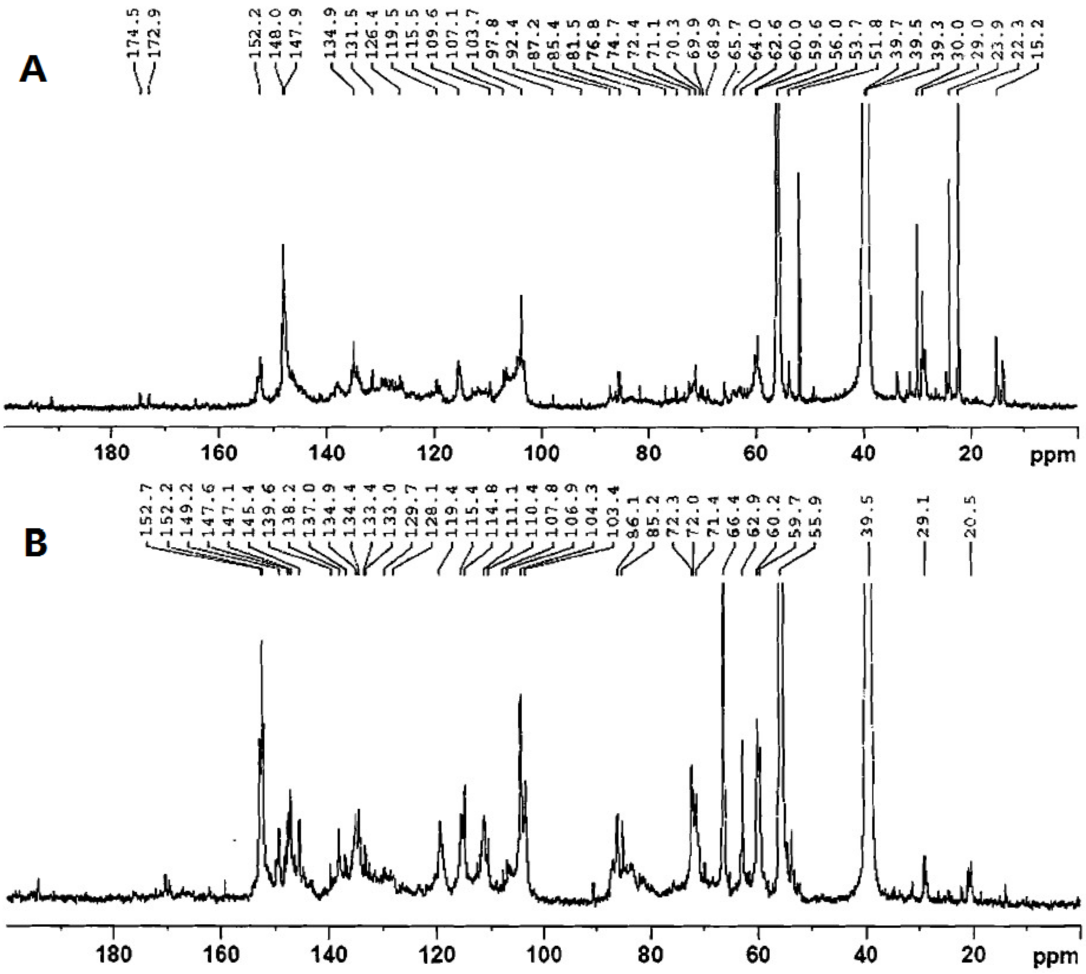

Fig. 3. ^{13}C-NMR spectra of lignin samples: A: lignin extracted with the catalyzed ethanol system; B: MWL

2D-HSQC Analysis

To investigate the comprehensive structural characterizations, the lignin samples extracted with the catalyzed ethanol systems were subjected to 2D-HSQC analysis (Rio *et al.* 2012). Compared with the MWL (Fig. 4B), the typical structural features of the extracted lignin samples were identified in the region of the side chain (δ_C/δ_H 50.0 to 95.0/2.5 ppm to 5.5 ppm) and the aromatic region (δ_C/δ_H 95.0 to 140.0/5.5ppm to 8.0 ppm) (Fig. 4A) (Rio *et al.* 2009).

The side-chain region of the spectra yielded useful information about the inter-unit linkages of the extracted lignin, such as β-O-4 aryl ether (A), phenylcoumaran (β-5, α-O-4) (B), and resinol (β-β, α-O-γ) (C) (Fig. 5). As indicated in Fig. 4, the signals of C_α-H_α, C_β-H_β, and C_γ-H_γ in β-O-4 aryl ether (A) substructures were respectively indicated at δ_C/δ_H 71.8/4.86 ppm, δ_C/δ_H 83.4/4.38 ppm (G), δ_C/δ_H 85.8/4.12 ppm (S), and δ_C/δ_H 59.9/3.35 ppm to 3.80 ppm. The signals of C_α-H_α, C_β-H_β, and C_γ-H_γ in phenylcoumaran (B) were shown at δ_C/δ_H 86.8/5.45 ppm, 53.1/3.46 ppm, and 62.2/3.76 ppm. The signals of C_α-H_α, C_β-H_β, and C_γ-H_γ in resinol (C) were indicated at δ_C/δ_H 84.8/4.66 ppm, δ_C/δ_H

53.5/3.07 ppm, and δ_C/δ_H 71.2/3.82 ppm to 4.18 ppm, respectively. In addition, signals corresponding to the S and G units of the extracted lignin samples were observed in the aromatic region. The strong absorption signals for $C_{2,6}$-$H_{2,6}$ in the S units were shown at δ_C/δ_H 103.9/6.70 ppm. The absorption signal at δ_C/δ_H 106.3/7.32 ppm was the characteristic spectrum of the C_α-CO substructures in the S' units. The chemical shifts of C_2-H_2, C_5-H_5, and C_6-G_6 in the G units of lignin were δ_C/δ_H 110.8/6.97 ppm, δ_C/δ_H 114.5/6.70 ppm, and δ_C/δ_H 119.0/6.78 ppm, respectively.

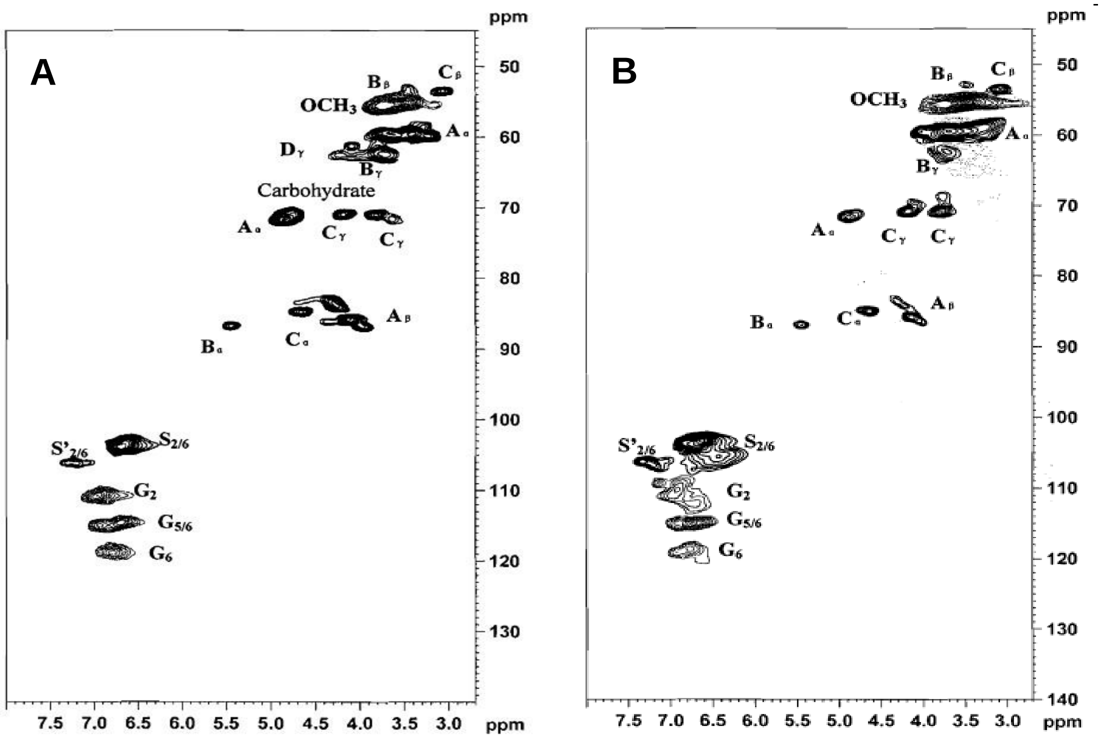

Fig. 4. 2D-HSQC NMR spectra of lignin samples: A: lignin extracted with the catalyzed ethanol system; B: MWL

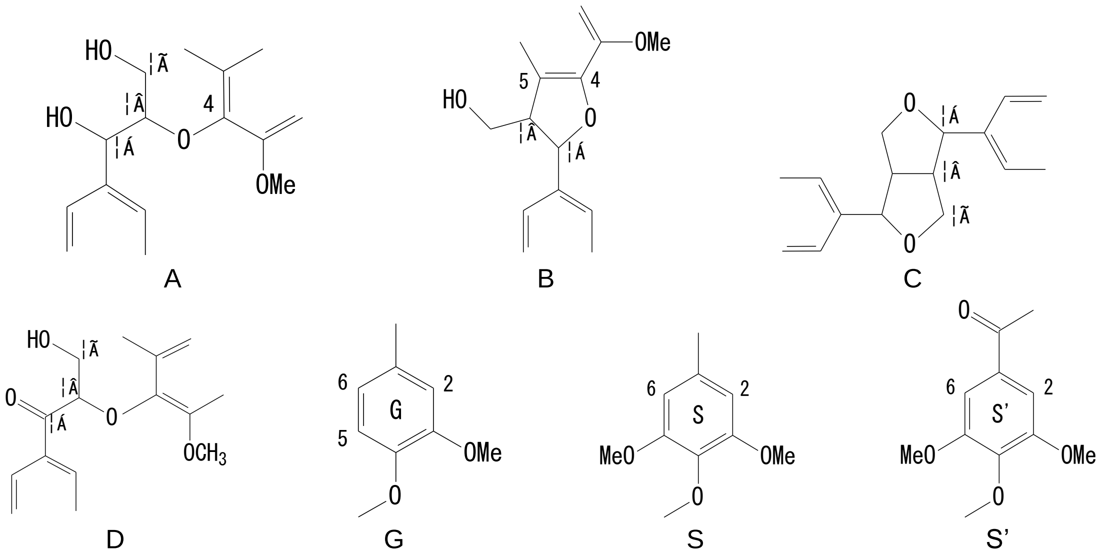

Fig. 5. Main structures present in the lignin fractions: A: β-O-4 ether; B: phenylcoumaran (β-5); C: resinol (β-β); D: α-CO/β-O-4 ether; G: guaiacyl units; S: syringyl units; S': syringyl units with α-CO

Compared with the 2D-HSQC NMR spectrum of the MWL (Fig. 4B), the spectrum of the extracted lignin samples exhibited variations of absorption signals (Fig. 4A). This phenomenon mainly resulted from the condensation reactions of the S and G units during the extraction process, and the condensation of phenolic structures was similar to that reported by Yanez-S *et al.* (2014). Structural information of the lignin extracted with the catalyzed ethanol systems was analyzed, and the detailed assignments of the lignin fractions in the 2D-HSQC spectra are listed in Table 4 by comparison with previous literature (Balakshin *et al.* 2003; Ibarra *et al.* 2007).

Table 4. Assignments of ^{13}C-^{1}H Cross Signals in the 2D-HSQC Spectra of the Lignin Extracted with the Catalyzed Ethanol Systems

Label	δ_C/δ_H/ppm	Assignment
B_β	53.1/3.46	C_β-H_β in phenylcoumaran substructures
C_β	53.5/3.07	C_β-H_β in β-β’ resinol substructures
$-OCH_3$	56.4/3.70	C-H in methoxyls
A_γ	59.9/3.35-3.80	C_γ-H_γ in β-O-4 aryl ether substructures
B_γ	62.2/3.76	C_γ-H_γ in phenylcoumaran substructures
A_α	71.8/4.86	C_α-H_α in β-O-4 aryl ether substructures
C_γ	71.2/3.82-4.18	C_γ-H_γ in β-β’ resinol substructures
C_α	84.8/4.66	C_α-H_α in β-β’ resinol substructures
A_β	83.4/4.38, G units; 85.8/4.12, S units	C_β-H_β in β-O-4 aryl ether substructures
B_α	86.8/5.45	C_α-H_α in phenylcoumaran substructures
$S_{2/6}$	103.9/6.70	$C_{2,6}$-$H_{2,6}$ in S units
$S'_{2/6}$	106.3/7.32	$C_{2,6}$-$H_{2,6}$ in S' units with α-CO substructures
G_2	110.8/6.97	C_2-H_2 in G units
G_5	114.5/6.70	C_5-H_5 in G units
G_6	119.0/6.78	C_6-G_6 in G units

CONCLUSIONS

1. The catalyzed ethanol systems consisting of ethanol, 4-methyl-2-pentanone, DMSO, formic acid, and distilled water were used to extract the lignin samples at 145 °C. The yields of the extracted lignin were greatly affected by the formulas of the catalyzed ethanol systems and the holding times.
2. Compared with the ^{1}H-NMR spectrum of MWL, the spectrum of the extracted lignin fractions supported the destruction of β-O-4 units, which accounted for the disappearance of the H_α signal. In addition, the acetyl substructures were introduced into the lignin fractions during the extraction process.
3. According to the ^{13}C-NMR spectra, the striking characteristics of the extracted lignin samples, such as the ether bonds of S3,5 broken by the formic acid and the condensation of G units accelerated by the acidic environment, were markedly different from that of MWL. In addition, the decomposition of β-O-4 units was observed by the ^{13}C-NMR spectrum of the lignin extracted with the catalyzed ethanol systems.

4. The 2D-HSQC NMR spectra indicated that the linkages of the lignin fractions were mainly formed by β-O-4 aryl ether, phenylcoumaran, and resinol. The main differences between the extracted lignin samples and the MWL were the condensations of S and G units.

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REFERENCES CITED

Abdelkafi, F., Ammar, H., Rousseau, B., Tessier, M., El, G. R., and Fradet, A. (2011). "Structural analysis of alfa grass (*Stipa tenacissima* L.) lignin obtained by acetic acid/formic acid delignification," *Biomacromolecules* 12(11), 3895-3902. DOI: 10.1021/bm2008179

Balakshin, M. Y., Capanema, E. A., Chen, C. L., and Gracz, H. S. (2003). "Elucidation of the structures of residual and dissolved pine kraft lignins using an HMQC NMR technique," *J. Agr. Food Chem.* 51(21), 6116-6127. DOI: 10.1021/jf034372d

Balakshin, M. Y., Capanema, E. A., Gracz, H., Chang, H. M., and Jameel, H. (2011). "Quantification of lignin-carbohydrate linkages with high-resolution NMR spectroscopy," *Planta* 233(6), 1097-1110. DOI: 10.1007/s00425-011-1359-2

Bjorkman, A. (1956). "Studies of finely divided wood. Part 1. Extraction of lignin with neutral solvents," *Sven. Papperstidn.* 59(13), 477-485. DOI: 10.1177/004057368303900411

Garcia, A., Toledano, A., Andres, M. A., and Labidi, J. (2010). "Study of the antioxidant capacity of *Miscanthus sinensis* lignins," *Process Biochem.* 45(6), 935-940. DOI: 10.1016/j.procbio.2010.02.015

Hage, R. E., Brosse, N., Chrusciel, L., Sanchez, C., Sannigrahi, P., and Ragauskas, A. (2009). "Characterization of milled wood lignin and ethanol organosolv lignin from *miscanthus*," *Polym. Degrad. Stabil.* 94(10), 1632-1638. DOI: 10.1016/j.polymdegradstab.2009.07.007

Huijgen, W. J., Smit, A. T., Wild, P. J. D., and Den, U. H. (2012). "Fractionation of wheat straw by prehydrolysis, organosolv delignification and enzymatic hydrolysis for production of sugars and lignin," *Bioresource Technol.* 114(3), 389-398. DOI: 10.1016/j.biortech.2012.02.143

Ibarra, D., Chavez, M. I., Rencoret, J., del Rio, J. C., Gutierrez, A., Romero, J., Camarero, S., Martinez, M. J., Jimenez-Barbero, J., and Martinez, A. T. (2007). "Structural modification of eucalyptpulp lignin in a totally chlorine free bleaching sequence including a laccase-mediator stage," *Holzforschung* 61(6), 634-646. DOI: 10.1515/HF.2007.096

Ibarra, D., Rio, J. C. D., Gutierrez, A., Rodriguez, I. M., Romero, J., Martinez, M. J., and Martinez, A. T. (2005). "Chemical characterization of residual lignins from eucalypt paper pulps," *J. Anal. Appl. Pyrol.* 74(1-2), 116-122. DOI: 10.1016/j.jaap.2004.12.009

Idstrom, A., Schantz, S., Sundberg, J., Chmelka, B. F., Gatenholm, P., and Nordstierna, L. (2016). "^{13}C NMR assignments of regenerated cellulose from solid-state 2D NMR spectroscopy," *Carbohyd. Polym.* 151, 480-487. DOI: 10.1016/j.carbpol.2016.05.107

Kuhnel, I., Podschun, J., Saake, B., and Lehnen, R. (2015). "Synthesis of lignin polyols *via* oxyalkylation with propylene carbonate," *Holzforschung* 69(5), 531-538. DOI: 10.1515/hf-2014-0068

Luo, J. Z., Arnold, R. J., Cao, J. G., Lu, W. H., Ren, S. Q., and Xie, Y. J. (2012). "Variation in pulp wood traits between eucalypt clones across sites and implications for deployment strategies," *J. Trop. For. Sci.* 24(24), 70-82.

Malutan, T., Nicu, R., and Popa, V. I. (2008). "Contribution to the study of hydroxymethylation reaction of alkali lignin," *BioResources* 3(1), 13-20. DOI: 10.15376/biores.3.1.13-20

Rencoret, J., Marques, G., Gutierrez, A., Ibarra, D., Li, J. B., Gellerstedt, G., Santos, J. I., Jimenez-Barbero, J., Martinez, A. T., and Rio, J. C. D. (2008). "Structural characterization of milled wood lignins from different eucalypt species," *Holzforschung* 62(5), 514-526. DOI: 10.1515/HF.2008.096

Rico, A., Rencoret, J., Rio, J. C. D., Martinez, A. T., and Gutierrez, A. (2014). "Pretreatment with laccase and a phenolic mediator degrades lignin and enhances saccharification of eucalyptus feedstock," *Biotechnol. Biofuels* 7(1), 6-19. DOI: 10.1186/1754-6834-7-6

Rio, J. C. D., Rencoret, J., Marques, G., Li, J. B., Gellerstedt, G., Jimenez-Barbero, J., Martinez, A. T., and Gutiérrez, A. (2009). "Structural characterization of the lignin from Jute (*Corchorus capsularis*) fibers," *J. Agr. Food Chem.* 57(21), 10271-10281. DOI: 10.1021/jf900815x

Rio, J. C. D., Rencoret, J., Prinsen, P., Martínez, A. T., Ralph, J., and Gutiérrez, A. (2012). "Structural characterization of wheat straw lignin as revealed by analytical pyrolysis, 2D-NMR, and reductive cleavage methods," *J. Agr. Food Chem.* 60(23), 5922-5935. DOI: 10.1021/jf301002n

Santos, P. S. B. D., Cademartori, P. H. G. D., Prado, R., Gatto, D. A., and Labidi, J. (2014). "Composition and structure of organosolv lignins from four eucalypt species," *Wood Sci. Technol.* 48(4), 873-885. DOI: 10.1007/s00226-014-0646-z

Sequeiros, A., Gatto, D. A., Labidi, J., and Serrano, L. (2014). "Different extraction methods to obtain lignin from almond shell," *J. Biobase. Mater. Bioenerg.* 8(3), 370-376. DOI: 10.1166/jbmb.2014.1443

Shimada, K., Hosoya, S., and Ikeda, T. (1997). "Condensation reactions of softwood and hardwood lignin model compounds under organic acid cooking conditions," *J. Wood Chem. Technol.* 17(1-2), 57-72. DOI: 10.1080/02773819708003118

Shulga, G., Vitolina, S., Shakels, V., Belkova, L., Cazacu, G., Vasile, C., and Nita, L. (2012). "Lignin separated from the hydrolyzate of hydrothermal treatment of birch wood and its surface properties," *Cell. Chem. Technol.* 46(5), 307-318.

Vazquez, G., Antorrena, G., Gonzalez, J., and Freire, S. (1997). "FTIR, ^{1}H and ^{13}C NMR characterization of acetosolv-solubilized *Pine* and *Eucalyptus* lignins," *Holzforschung* 51(2), 158-166. DOI: 10.1515/hfsg.1997.51.2.158

Wen, J. L., Sun, S. L., Yuan, T. Q., Xu, F., and Sun, R. C. (2013a). "Structural elucidation of lignin polymers of *Eucalyptus* chips during organosolv pretreatment and extended delignification," *J. Agric. Food Chem.* 61(46), 11067-11075. DOI: 10.1021/jf403717q

Wen, J. L., Sun, S. N., Yuan, T. Q., Xu, F., and Sun, R. C. (2013b). “Fractionation of bamboo culms by autohydrolysis, organosolv delignification and extended delignification: Understanding the fundamental chemistry of the lignin during the integrated process,” *Bioresource Technol.* 150(1), 278-286. DOI: 10.1016/j.biortech.2013.10.015

Wu, M., Zhao, D. H., Pang, J. H., Zhang, X. M., Li, M. F., Xu, F., and Sun, R. C. (2015). “Separation and characterization of lignin obtained by catalytic hydrothermal pretreatment of cotton stalk,” *Ind. Crop. Prod.* 66, 123-130. DOI: 10.1016/j.indcrop.2014.12.056

Xu, J., Liu, B., Hou, H., and Hu. J. (2017). “Pretreatment of eucalyptus with recycled ionic liquids for low-cost biorefinery,” *Bioresource Technol.* 234, 406-414. DOI: 10.1016/j.biortech.2017.03.081

Yanez-S., M., Matsuhiro, B., Nunez, C., Pan, S. B., Hubbell, C. A., Sannigrahi, P., and Ragauskas, A. J. (2014). “Physicochemical characterization of ethanol organosolv lignin (EOL) from *Eucalyptus globulus*: Effect of extraction conditions on the molecular structure,” *Polym. Degrad. Stabil.* 110(15), 184-194. DOI: 10.1016/j.polymdegradstab.2014.08.026

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