Organic Solvent Isolation and Structural Characterization of Willow Lignin

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Autocatalyzed isopropanol-water extraction is a potentially high-value lignin isolation methodology. A solution of isopropanol-water (2-PrOH/ H_2O, 7:3, v/v) was used on willow powder (*Salix matsudana cv. Zhuliu*), at varying temperatures (160 °C to 210 °C) and reaction times (6 h to 12 h). The highest yield (61 wt%) and purity (93.7%) with low weight/number average molecular weight (1681/1061 g/mol) was observed at a treatment temperature and time of 190 °C and 10 h, respectively. Fourier-transform infrared (FTIR) and two-dimensional nuclear magnetic resonance (2D-NMR) spectroscopy indicated that the lignin was composed of syringyl (S) and guaiacyl (G) units that were not severely damaged. More specifically, ^{31}P-NMR showed that S units were dominant. Finally, a majority of glucan was preserved.

Keywords: Lignin; Characterization; Willow; Isopropanol; ^{31}P-NMR

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INTRODUCTION

With the continued depletion of fossil resources, renewable resources such as biomass have attracted increasing attention. Biomass has the potential to become a highly productive, low-polluting, renewable energy source that is easy to store and can be directly converted into transportation fuels (Dwivedi *et al*. 2009). Willow (*Salix matsudana cv. Zhuliu*) is a new plant variety in the family Salicaceae, with high survival rate, fast growth, and strong resistance, that is widely applied in wind prevention, sand fixation, and pulp and paper (Guo *et al*. 2016). Lignin is biosynthesized from three phenylpropanoid monomers (coniferyl alcohol, coumaryl alcohol, and sinapyl alcohol); it is a non-crystalline, aromatic compound that is widely present as structural units in plants (Boerjan *et al*. 2003).

Lignin is a widely abundant natural resource that can act as a substitute for petroleum-based counterparts (Gabov *et al*. 2014). It had been viewed as a low-value waste product of pulp and paper (Stewart 2008), but it now has potential to be economically converted into valuable materials because of its molecular structure and wide availability (Nsimba *et al.* 2013). It can be applied in many fields, such as biomaterials, chemicals, fuels, biomedicals, etc. (Lora and Glasser 2002). However, utilization of lignin requires, in many typical applications, high yield and high purity. The isolation process for alkali lignin is usually inefficient. In addition, the structure extracted from pulping liquors is seriously

damaged, which limits valorization (Yang *et al*. 2017). Ionic liquid treatment is also an alternative for lignin separation; however, its toxicity has attracted increasing attention (Strehmel *et al*. 2017). Deep eutectic solvents (DESs) have recently been reported to be a better way to separate lignin, but their economic cost is still relatively high (Li *et al.* 2017). Milled wood separation has high purity, but it requires too much time to be considered feasible, with low yields (Zhang *et al*. 2017). Another similar method of high purity lignin separation is the so-called enzymatic mild/acidolysis lignin (EMAL); however the complex process, time-consuming steps, and high energy consumption become the main drawbacks. (Guerra *et al*. 2008). Organic acids such as formic, acetic, or aqueous organic acids may be used to overcome these latter deficiencies, but the acidic conditions cause corrosion (Shui *et al*. 2016). Ferrini and Rinaldi (2014) demonstrated that lower-molecular-weight (M_w) lignin could be solvolytically extracted from the plant cell wall in an aqueous solution of isopropanol without catalysts. However, the separation and structural characterization was not thoroughly studied or discussed.

EXPERIMENTAL

Materials

The willow was generously provided by Shandong Shengshi Forestry Development Group (Linyi, Shandong Province, P.R. China). It was cut into 3- to 5-cm sticks and ground in a star mill (FW-102, Everbright, Beijing, China). The 40- to 60-mesh fractions were used as the raw material for compositional analysis and subsequent fractionation. The chemical composition of the willow, analyzed according to the laboratory analytical procedure for biomass provided by NREL (Sluiter *et al.* 2008), is listed in Table 1.

Table 1. Composition Analysis of Willow (*Salix matsudana cv. Zhuliu*)

Component	Content (wt-%)
Acid Insoluble Lignin (AIL)	17.3 ± 0.4
Acid Soluble Lignin (ASL)	3.8 ± 0.1
Glucan	39.2 ± 0.4
Xylan	17.5 ± 0.2
Arabinan	2.2 ± 0.1
Glucan	6.7 ± 0.2
Mannan	1.5 ± 0.1
Extractives (Benzene/Ethanol, 2:1, v/v)	0.8 ± 0.1
Ash	1.0 ± 0.1

Separation of Organosolv Lignin

Lignin isolation was performed in a 100-mL stainless steel micro-reactor (SLM100, Beijing Century Senlong Experimental Apparatus Co., Ltd., Beijing, China). In each run, 4 g of extractive-free willow and 40 mL of isopropanol-water solution (2-PrOH/H_2O, 7:3, v/v) were loaded into the reactor. The fractionation reactions were conducted under stirring at 150 rpm for 6 h to 16 h between 160 °C and 210 °C. After reaction, the mixture was cooled to room temperature and vacuum filtered. The solid residue was washed 3 times with 2-PrOH/H_2O (7:3, v/v) and dried *in vacuo*. The collected filtrate was concentrated and added to acidic deionized (DI) water (2000 mL, pH 1 to 2 adjusted with sulfuric acid) to precipitate lignin-rich fractions. The precipitate was thoroughly washed with DI water until

neutral pH and freeze-dried. Each run was repeated at least twice to ensure good reproducibility. Solid residue yield and organosolv lignin yield were calculated by Eqs. 1 and 2, respectively.

$$\frac{\text{Mass of the solid residue}}{\text{Mass of the initial dry sample}} \times 100\% \quad (1)$$

$$\frac{\text{Mass of the separated lignin}}{\text{Mass of the lignin in the initial sample}} \times 100\% \quad (2)$$

Composition Analysis of Solid Residue, Organosolv Lignin, and Dissolved Carbohydrates

The chemical composition, including carbohydrates and acid-insoluble lignin, of the resulting solid residue (pulp fibers) and organosolv lignin were determined using two-step acid saccharification. The dissolved carbohydrates in the fractionated mixture solution were quantitatively assessed following NREL protocols (Sluiter *et al*. 2008). The determination of the sugar content was performed on a Thermo Fisher ion chromatography system ICS-5000 (Sunnyvale, CA, USA) with a Dionex CarboPac PA20 column (3 mm × 150 mm, Thermo Fisher, Shanghai, China). The analyses were carried out at 30 °C using a 2-M NaOH solution as the eluent at a flow rate of 0.4 mL/min.

Molecular Weight Determination using GPC

The apparent molecular weight distribution of organosolv lignin was estimated using a gel-permeation chromatography (GPC) system (GPC-50, Agilent Technologies Inc., Santa Clara, CA, USA). Approximately 10 mg of organosolv lignin was dissolved in 10 mL of tetrahydrofuran (THF). Then, 20 μL of the THF solution was injected to the GPC column with an eluent (THF) flow rate of 1.0 mL/min at 40 °C. Six different polystyrene standards with M_p ~374, ~1620, ~3420, ~9130, ~18100, ~32500 g/mol (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) were used for calibration.

FT-IR

Fourier-transform infrared (FT-IR) spectra of the isolated organosolv lignin and initial samples were recorded on a Bruker ALPHA FT-IR (Karlsruhe, Germany). The dried samples were embedded in KBr pellets at a concentration of 1 mg/100 mg KBr. The spectra were recorded in the range of 500 cm^{-1} to 4000 cm^{-1} at 4 cm^{-1} resolution, and 64 scans per sample were collected.

2D-NMR

Approximately 80 mg of oven-dried organosolv lignin was dissolved in 0.5 mL of DMSO-d_6. The solution was analyzed using an Advance II 400 MHz spectrometer (Bruker, Germany) at 25 °C for 12 h using a Bruker standard pulse library to provide a pulse sequence "hsqcetgpsisp.2" with a spectral width of 5000 Hz (from 10 ppm to 0 ppm) and 20843 Hz (from 165 ppm to 0 ppm) for the ^{1}H and ^{13}C dimensions, respectively, for a total of 75,000 scans. The number of transients was 32; 256 time increments were recorded in the ^{13}C dimension, and the number of collected complex points was 1024 for the ^{1}H dimension, with a recycle delay of 1.5 s. Data processing was recorded using standard Bruker Topspin-NMR software.

^{31}P-NMR

The ^{31}P-nuclear magnetic resonance (NMR) spectrum of lignin was acquired at 298 K on a 400-MHz Bruker AVANCE III 400 spectrometer (Bruker, Germany). Lignin (40 mg) was dissolved in 400 µL of anhydrous pyridine and deuterated chloroform (1.6:1, v/v). In the next step 150 µL of cyclohexanol/chromium (III) acetylacetonate solution (4 mg·mL^{-1}/3.6mg·mL^{-1} in anhydrous pyridine and deuterated chloroform 1.6:1, v/v) was added as internal standard and relaxation reagent, respectively. The mixture was reacted with 75 µL of phosphating reagent (2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane-tetramethyl-1,3,2-dioxaphospholane, TMDP) for approximately 5 min prior to analysis.

RESULTS AND DISCUSSION

Effect of Reaction Time on Extraction of Lignin from Willow

To investigate the effect of reaction time on extraction, six different reaction times from 6 h to 16 h were carried out in 2-PrOH/H_2O (7:3, v/v) at a fixed temperature of 180 °C. The yields of organosolv lignin and solid residue are shown in Fig. 1. Reaction time had an important effect on the extraction of lignin from willow in isopropanol-water. The yield of lignin increased from 37% to 54% from 6 h to 10 h. With further increases in time, the lignin yield reduced gradually. For example, at 16 h, the yield was 46%. As the reaction time was extended, lignin may have degraded into small molecular compounds, as confirmed previously (Buranov *et al.* 2010). The reaction time had little effect on the yield of solid residues. For example, from 6 h to 16 h, the yield decreased by 4%, *i.e.*, from 60% to 56%, indicating that prolonged reaction time did not cause excessive loss of carbohydrates.

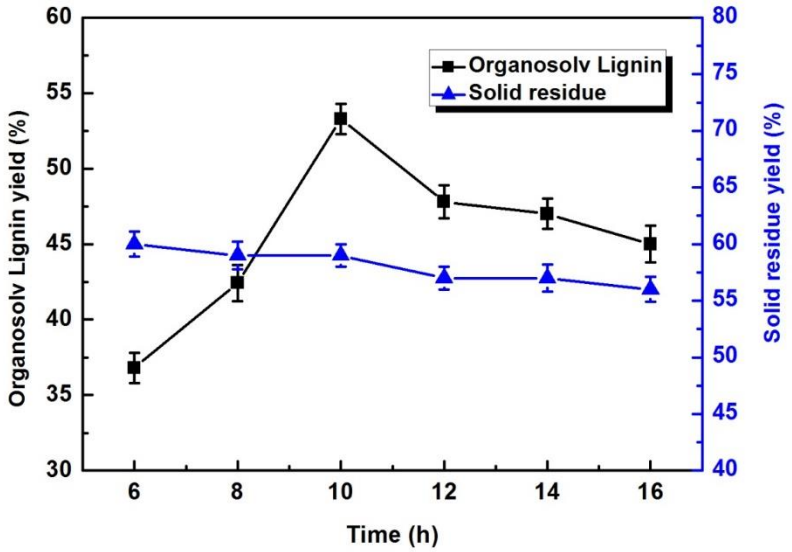

Fig. 1. The yield of organosolv lignin and solid residue at various reaction times (at 180 °C in 2-PrOH/H_2O (7:3, v/v)).

Effect of Temperature

Extraction treatments of willow in 2-PrOH/H_2O (7:3, v/v) were conducted at temperatures ranging from 160 °C to 210 °C at a constant reaction time of 10 h. The yields

of lignin and solid residue are shown in Fig. 2. The yields changed substantially with temperature. Increasing temperature resulted in yield increases from 36% to 72% from 160 °C to 210 °C, but it had a negative impact on the yield of solid residue, which decreased from 67% to 35% with increasing temperature. It is worth noting that although the higher temperature improved lignin yield, it also caused excessive carbohydrates degradation.

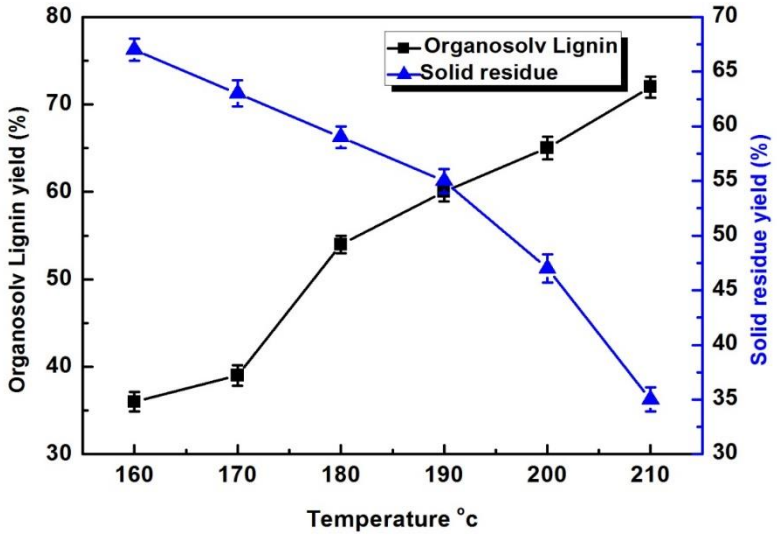

Fig. 2. The yield of organosolv lignin and solid residue at various temperatures (in 2-PrOH/H_2O (7:3, v/v) for 10 h).

Chemical Composition Analysis of Solid Residue and Dissolved Carbohydrates

The sugars and lignin in the solid residue and the dissolved carbohydrates in the mixtures after treatment with 2-PrOH/H_2O (7:3, v/v) at 190 °C for 10 h were characterized (Table 2, basis of the initial component). The results indicated that 82.3% of the initial glucan was preserved, while only 36.1% of the initial lignin was retained in the solid residue. A considerable amount of lignin (61.5%) was extracted and then isolated after 2-PrOH/H_2O treatment. Additionally, 19.3% of xylan and 41.8% of mannan were retained in the solid residue. In contrast, 63.5% of xylan, 75.5% of arabinan, 70.3% of galactan, and 35.6% of mannan were degraded into oligosaccharides and monosaccharides during processing and dissolved in the reaction mixtures. The mass losses of the carbohydrate occurring along the way was thought to be due to excessive degradation of carbohydrates and then formation of furfural, hydroxymethylfurfural *etc*. (Gao *et al*. 2016).

Table 2. The Chemical Composition of Solid Residue and Dissolved Carbohydrates after 2-PrOH/H_2O (7:3, v/v) Treatment at 190 °C for 10 h (Percentage of Initial Component)

Component	Arabinan (%)	Galactan (%)	Glucan (%)	Xylan (%)	Mannan (%)	Lignin (%)
Solid Residue	-	-	82.3	19.3	41.8	36.1
Dissolved in the Mixtures	75.5	70.3	10.2	63.5	35.6	61.5*

* The amount of lignin extracted by 2-PrOH/H_2O at 190 °C for 10 h.

Lignin Purity Analysis

The purity of the isolated lignin was as high as 93.7% and contained only 1.15% of carbohydrate impurities (Table 3).

Molecular Weight Analysis of Lignin

The weight-averaged molecular weight (M_w) and the number-averaged molecular weight (M_n) of the isolated organosolv lignin were 1681 g/mol and 1061 g/mol, respectively (Table 4), much lower than the values reported for enzymatic mild/acidolysis lignin (EMAL) (M_w 4127.0 and M_n 3324.9) and pulping black liquor lignin (AL) (M_w 3522.5 and M_n 1871.2) isolated from the same biomass material (Guo *et al.* 2016). In addition, the polydispersity (PD, M_w/M_n) of the isolated lignin was 1.58, which is lower than the values reported for milled wood lignin (MWL) from pine stem wood, residue, and bark (PD of 1.92, 2.19, and 3.02, respectively) (Huang *et al*. 2011). The present isolation was effective for high purity, low molecular weight, and homogeneous lignin.

Table 3. Purity Analysis of Lignin Extracted from Willow using 2-PrOH/H_2O Treatment at 190 °C for 10 h

Component	Amount (%)
Acid Insoluble Lignin (AIL)	91.2 ± 0.1
Acid Soluble Lignin (ASL)	2.5 ± 0.1
Glucan	0.76 ± 0.06
Xylan	0.39 ±0.04
Lignin Purity*	93.7 ± 0.2

* Lignin purity is calculated from the sum up of AIL and ASL of willow.

Table 4. Average Molecular Weight and Polydispersity of Organosolv Lignin

M_w (g/mol)	M_n (g/mol)	M_z (g/mol)	PD (M_w/M_n)
1681	1061	2770	1.58

FT-IR

Compared to the initial willow sample, the intensities of the absorption bands at 1605, 1515, 1270, and 1120 cm^{-1} were enhanced in the organosolv lignin (Fig. 3). The bands at 1605 cm^{-1} and 1515 cm^{-1} were assigned to aromatic skeletal vibrations, while the band at 1461 cm^{-1} was assigned to the C–H deformation vibration in $–CH_2–$ to reveal the primary structure of lignin (Xiao *et al*. 2012). The typical absorptions of syringyl and guaiacyl units were observed at 1327 and 1270 cm^{-1}, respectively (Gong *et al*. 2016). The absorption band at 1373 cm^{-1} was associated with C–H bending vibration of aliphatics, and the band at 1175 cm^{-1} was assigned to C–O–C symmetrical stretching vibration in carbohydrate (Li *et al*. 2017). In addition, high intensity of band at 1710 cm^{-1}, which related to stretching vibrations of C=O bonds, indicate the greater amount of carboxyl groups in hemicellulose (Dominguez-Robles *et al*. 2017). The bands at 1710 cm^{-1}, 1373 cm^{-1}, and 1175 cm^{-1} were absent in organosolv lignin compared to the initial willow sample, indicating negligible carbohydrate residual consistent with the purity analysis (Table 3).

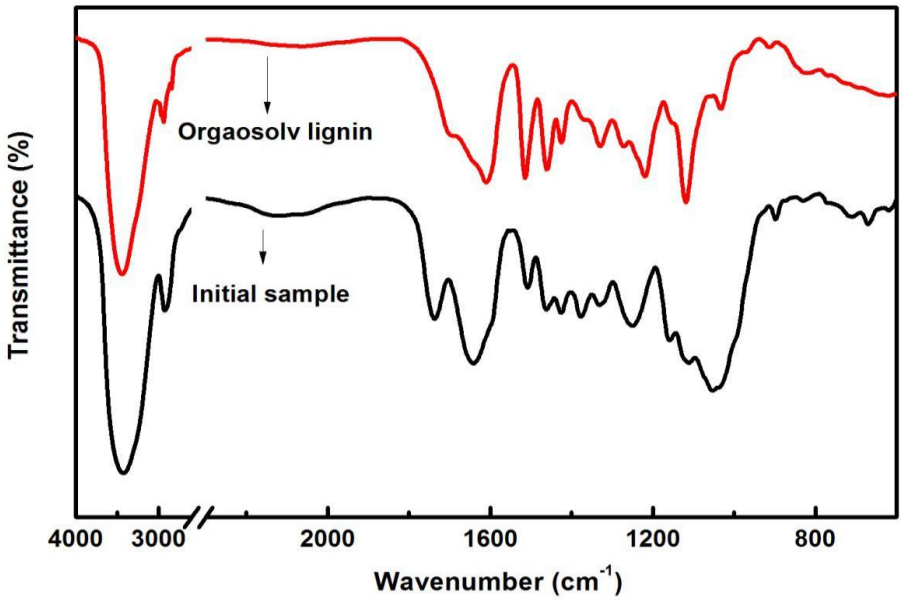

Fig. 3. FT-IR spectra of the initial sample and organosolv lignin.

2D-NMR

Two-dimensional heteronuclear single-quantum coherence (2D-HSQC) NMR can reveal both the aromatic units and various inter-unit linkages in lignin (Zhang *et al*. 2013; Huang *et al*. 2018). The ^{13}C-^{1}H HSQC spectra are shown in Fig. 4, with peaks assigned based on past work (Samuel *et al*. 2010; Naron *et al*. 2017), as listed in Table 5.

Table 5. Assignments of ^{13}C-^{1}H Correlation Signals in the HSQC Spectra of Organosolv Lignin Isolated from Willow *via* 2-PrOH/H_2O (7:3, v/v) Treatment at 190 °C for 10 h

Labels	δ_C/δ_H (ppm)	Assignment
C_β	53.5/3.11	C_β-H_β in resinol (β–β) substructure
–OMe	55.6/3.73	C-H in methoxyl group
A_γ	59.4/3.75	C_γ-H_γ in β-O-4 substructure
A_α	71.5/4.86	C_α-H_α in β-O-4 linkage
A_β-G	83.6/4.15	C_β-H_β in β-O-4 substructures linked to a G unit
A_β-S	86.0/4.23	C_β–H_β in β-O-4 substructures linked to a S unit
C_α	85.0/4.77	C_α–H_α in β-β (resinol) substructures
$S_{2,6}$	104.5/6.55	$C_{2,6}$-$H_{2,6}$ in etherified syringyl units
G_2	110.9/6.88	C_2-H_2 in guaiacyl units
G_5	115.6/6.73	C_5-H_5 in guaiacyl units
G_6	119.0/6.79	C_6-H_6 in guaiacyl units
H	132.5/7.66	$C_{2,6}$–$H_{2,6}$ in p-coumaroylated substructures

The C_β–H_β correlations in β-β substructures were observed at δC/δH 53.5/3.11 ppm, and the C_γ–H_γ correlations were at δC/δH 59.4/3.75 ppm, respectively, for the β-O-4 substructures in organosolv lignin. The C_β-H_β correlations were observed at δC/δH 86.0/4.23 ppm for β-O-4 structures linked to S units, and at δC/δH 83.6/4.15 ppm for β-O-4 structures linked to G units. The C_α-H_α correlations in β-O-4 substructures were observed at δC/δH 71.5/4.86 ppm, and the signals at δC/δH 85.0/4.77 ppm corresponded to C_α–H_α

in β-β substructures (Samuel *et al*. 2010). The main resonances of the S units for $C_{2,6}$–$H_{2,6}$ correlations were observed at δC/δH 104.5/6.55 ppm. The G lignin units displayed three different correlation signals for C_2–H_2, C_5–H_5, C_6–H_6, and the correlations were observed at δC/δH 110.9/6.88, δC/δH 115.6/6.73, and δC/δH 119.0/6.79 ppm, respectively. Compared to EMAL isolated from willow and/or other well-prepared lignin, organosolv lignin extracted by 2-PrOH/H_2O had nearly all the same functional groups and exhibited similar structural features with that of original lignin (Guerra *et al*. 2008; Buranov *et al*. 2010; Guo *et al*. 2016).

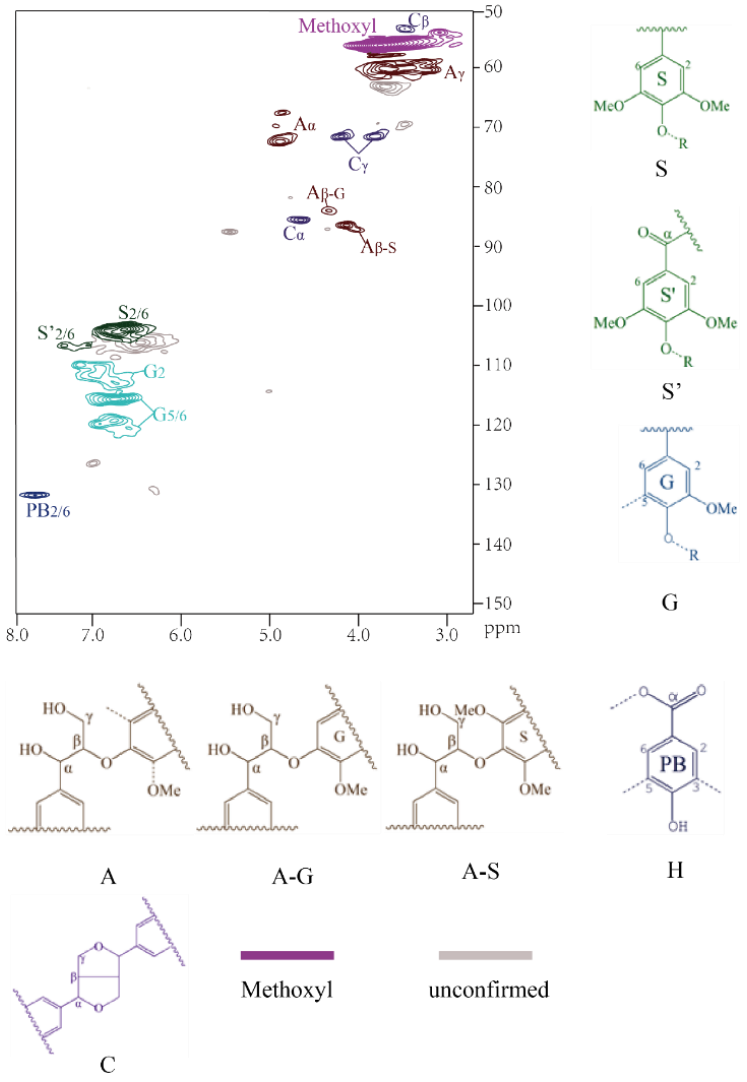

Fig. 4. The 2D HSQC NMR of the organosolv lignin. Main structures observed in organosolv lignin: (S) Syringyl units; (S') Syringyl (α-ketone); (G) guaiacyl units; (A) β-aryl ether (β-O-4); (C) Resinol (β-β); (A-G) β-aryl ether (β-O-4-G); (A-S) β-aryl ether (β-O-4-S); (H) p-Hydroxybenzoate.

^{31}P-NMR

The ^{31}P-NMR spectra are shown in Fig. 5, and functional group identification and content analysis are listed in Table 6. The signals in the range of 144.2 ppm to 134.5 ppm represent lignin phenolic moieties (Pu *et al*. 2011). The syringyl (S) type resonated at 143.7 ppm to 142.1 ppm, guaiacyl (G) derived adducts showed signals at 140.5 ppm to 139.2

ppm, and signals in the 138.4 ppm to 137.5 ppm region were attributed to p-hydroxyphenyl units (H) (Tohmura and Argyropoulos 2001; Savy *et al*. 2015). The phenolic hydroxyl group derived from S, G, and H moieties were 0.96, 0.51, and 0.11 mmol/g, respectively, which were higher than that of the reported acetic acid lignin isolated from shoot shell of bamboo (Gong *et al*. 2016). The S:G:H ratio was 1.88:1:0.22, indicating that the S unit was dominant in willow lignin. The results of Table 6 illustrate that the content of total phenol hydroxyl group was 1.87 mmol/g, higher than that of ball-milled lignin isolated from switchgrass and deep eutectic solvents lignin isolated from willow (Samuel *et al*. 2010; Li *et al*. 2017).

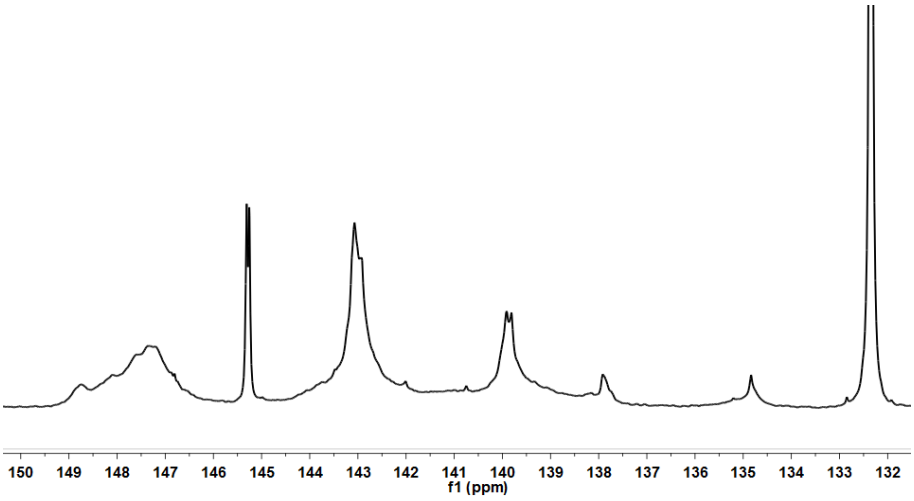

Fig. 5. Quantitative ^{31}P-NMR spectra of organosolv lignin.

Table 6. ^{31}P-NMR Analysis of Organosolv Lignin

Chemical Shift (ppm)	Assignment	Content (mmol/g)
134.5-135.3	Carboxyl	0.12
137.5-138.4	*p*-phenol hydroxyl (H)	0.11
139.2-140.5	Guaiacyl phenol hydroxyl (G)	0.51
142.1-143.7	Syringyl phenol hydroxyl (S)	0.96
140.3-142.4 /144.2-143.6	Condensed phenol hydroxyl	0.29
145.6-150.1	Aliphatic hydroxyl	0.75
-	Total phenol hydroxyl	1.87
-	Total hydroxyl	2.62

CONCLUSIONS

1. Isopropanol-water (2-PrOH/H_2O) extraction was an effective method for isolating lignin from willow (*Salix matsudana cv. Zhuliu*). A lignin yield of 61% (wt-% based on the initial lignin in willow) with a high purity (93.7%) was obtained using 2-PrOH/H_2O (7:3, v/v) at 190 °C for 10 h. The solid residue contained 82.3% of the initial glucan, which is conducive to further utilization of the processable carbohydrates.

2. The weight-averaged molecular weight (M_w) and the number-averaged molecular weight (M_n) were 1681 g/mol and 1061 g/mol, respectively, with a polydispersity (PD, M_w/M_n) of 1.58, suggesting low molecular weight and homogeneous lignin fragments. The FTIR and 2D-NMR spectra indicated that the lignin extracted by isopropanol-water was composed of S and G units and was not severely damaged.
3. The ^{31}P-NMR spectra indicated that S units were dominant, with a S:G:H ratio of 1.88:1:0.22, and total phenolic hydroxyl content of 1.87 mmol/g.

ACKNOWLEDGMENTS

The authors are grateful for the support of the National Key Research and Development Program of China (Grant No. 2017YFB0307900), the National Natural Science Foundation of China (Grant No. 31770630; 31770626), the Taishan Scholars Program of Shandong Province, and the Open Fund of the Guangxi Key Laboratory of Clean Pulp & Papermaking and Pollution Control (KF201704).

REFERENCES CITED

Boerjan, W., Ralph, J., and Baucher, M. (2003). "Lignin biosynthesis," *Annu. Rev. Plant. Biol*. 54, 519-546. DOI: 10.1146/annurev.arplant.54.031902.134938

Buranov, A. U., Ross, K. A., and Mazza, G. (2010). "Isolation and characterization of lignins extracted from flax shives using pressurized aqueous ethanol," *Bioresource Technol.* 101(19), 7446-7455. DOI: 10.1016/j.biortech.2010.04.086

Dominguez-Robles, J., Sanchez, R., Espinosa, E., Savy, D., Mazzei, P., Piccolo, A., and Rodriguez, A. (2017). "Isolation and characterization of gramineae and fabaceae soda lignins," *Int. J. Mol. Sci.* 18(2), 327. DOI: 10.3390/ijms18020327

Dwivedi, P., Alavalapati, J. R. R., and Lal, P. (2009). "Cellulosic ethanol production in the United States: Conversion technologies, current production status, economics, and emerging developments," *Energ. Sustain. Dev.* 13(3), 174-182 DOI: 10.1016/j.esd.2009.06.003

Ferrini, P., and Rinaldi, R. (2014). "Catalytic biorefining of plant biomass to non-pyrolytic lignin bio-oil and carbohydrates through hydrogen transfer reactions," *Angew. Chem. Int. Edit.* 53(33), 8634-8639. DOI:10.1002/anie.201403747

Gabov, K., Gosselink, R. J., Smeds, A. I., and Fardim, P. (2014). "Characterization of lignin extracted from birch wood by a modified hydrotropic process," *J. Agr. Food Chem.* 62(44), 10759-10767. DOI: 10.1021/jf5037728

Gao, Y., Wang, H., Guo, J., Peng, P., Zhai, M., and She, D. (2016). "Hydrothermal degradation of hemicelluloses from triploid poplar in hot compressed water at 180–340 °C," *Polym. Degrad. Stabil*. 126, 179-187. DOI: 10.1016/j.polymdegradstab.2016.02.003

Gong, W., Xiang, Z., Ye, F., and Zhao, G. (2016). "Composition and structure of an antioxidant acetic acid lignin isolated from shoot shell of bamboo (*Dendrocalamus latiforus*)," *Ind. Crop. Prod.* 91, 340-349. DOI: 10.1016/j.indcrop.2016.07.023

Guerra, A., Lucia, L. A., and Argyropoulos, D. S. (2008). "Isolation and characterization of lignins from *Eucalyptus grandis* Hill ex Maiden and *Eucalyptus globulus* Labill.

by enzymatic mild acidolysis (EMAL)," *Holzforschung* 62(1), 24-30. DOI: 10.1515/hf.2008.004

Guo, T., Liu, Y., Yang, G., Chen, J., and Lucia, L. A. (2016). "Chemical elucidation of structurally diverse willow lignins," *BioResources* 11(1), 2043-2054 DOI: 10.15376/biores.11.1.2043-2054

Huang, F., Singh, P. M., and Ragauskas, A. J. (2011). "Characterization of milled wood lignin (MWL) in Loblolly pine stem wood, residue, and bark," *J. Agr. Food Chem.* 59(24), 12910-12916. DOI:10.1021/jf202701b

Huang, Y., Liu, H., Yuan, H., Zhan, H., Zhuang, X., Yuan, S., Yin, X., and Wu, C. (2018). "Relevance between chemical structure and pyrolysis behavior of palm kernel shell lignin," *Sci. Total Environ.* 633, 785-795. DOI: 10.1016/j.scitotenv.2018.03.238

Li, T., Lyu, G., Liu, Y., Lou, R., Lucia, L. A., Yang, G., Chen, J., and Saeed, H. A. M. (2017). "Deep eutectic solvents (DESs) for the isolation of willow lignin (*Salix matsudana cv. Zhuliu*)," *Int. J. Mol. Sci.* 18(11). DOI: 10.3390/ijms18112266

Lora, J. H., and Glasser, W. G. (2002). "Recent industrial applications of lignin: A sustainable alternative to nonrenewable materials," *J. Polym. Environ.* 10, 39-48. DOI: 10.1023/A:1021070006895

Naron, D. R., Collard, F. X., Tyhoda, L., and Görgens, J. F. (2017). "Characterisation of lignins from different sources by appropriate analytical methods: Introducing thermogravimetric analysis-thermal desorption-gas chromatography-mass spectroscopy," *Ind. Crop. Prod.* 101, 61-74. DOI: 10.1016/j.indcrop.2017.02.041

Nsimba, R. Y., Mullen, C. A., West, N. M., and Boateng, A. A. (2013). "Structure-property characteristics of pyrolytic lignins derived from fast pyrolysis of a lignin rich biomass extract," *ACS Sustain. Chem. Eng.* 1(2), 260-267. DOI: 10.1021/sc300119s

Pu, Y., Cao, S., and Ragauskas, A. J. (2011). "Application of quantitative ^{31}P NMR in biomass lignin and biofuel precursors characterization," *Energ. Environ. Sci.* 4(9), 3154-3166. DOI: 10.1039/c1ee01201k

Samuel, R., Pu, Y., Raman, B., and Ragauskas, A. J. (2010). "Structural characterization and comparison of switchgrass ball-milled lignin before and after dilute acid pretreatment," *Appl. Biochem. Biotechnol.* 162(1), 62-74. DOI: 10.1007/s12010-009-8749-y

Savy, D., Nebbioso, A., Mazzei, P., Drosos, M., and Piccolo, A. (2015). "Molecular composition of water-soluble lignins separated from different non-food biomasses," *Fuel Process. Technol.* 131, 175-181. DOI: 10.1016/j.fuproc.2014.11.011

Shui, T., Feng, S., Yuan, Z., Kuboki, T., and Xu, C. C. (2016). "Highly efficient organosolv fractionation of cornstalk into cellulose and lignin in organic acids," *Bioresource Technol*. 218, 953-61. DOI: 10.1016/j.biortech.2016.07.054

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., and Crocker, D. (2008). *Determination of Structural Carbohydrates and Lignin in Biomass* (TP-510–42618), National Renewable Energy Laboratory, Golden, CO.

Stewart, D. (2008). "Lignin as a base material for materials applications: Chemistry, application and economics," *Ind. Crop. Prod.* 27(2), 202-207. DOI: 10.1016/j.indcrop.2007.07.008

Strehmel, V., Strunk, D., Wetzel, H., and Strehmel, N. (2017). "Investigation of lignin obtained by processing of Betula pendula with ionic liquids," *Sustainable Chemistry and Pharmacy*. 6, 107-113. DOI: 10.1016/j.scp.2017.10.006

Tohmura, S-I., and Argyropoulos, D. S. (2001). "Determination of arylglycerol-β-aryl ethers and other linkages in lignins using DFRC/^{31}P NMR," *J. Agr. Food Chem.* 49(2), 536-542. DOI: 10.1021/jf001026c

Xiao, L-P., Shi, Z-J., Xu, F., and Sun, R-C. (2012). "Characterization of lignins isolated with alkaline ethanol from the hydrothermal pretreated *Tamarix ramosissima*," *Bioenerg. Res.* 6(2), 519-532. DOI:10.1007/s12155-012-9266-3

Yang, L., Wang, D., Zhou, D., Zhang, Y., and Yang, T. (2017). "Isolation and further structural characterization of lignins from the valonea of *Quercus variabilis*," *Int. J. Biol. Macromol*. 97, 164-172. DOI: 10.1016/j.ijbiomac.2017.01.008

Zhang, A., Liu, C., Sun, R., and Xie, J. (2013). "Extraction, purification, and characterization of lignin fractions from sugarcane bagasse," *BioResources* 8(2), 1604-1614. DOI: 10.15376/biores.8.2.1604-1614

Zhang, J., Kim, K. H., Choi, Y. S., Motagamwala, A. H., Dumesic, J. A., Brown, R. C., and Shanks, B. H. (2017). "Comparison of fast pyrolysis behavior of cornstover lignins isolated by different methods," *ACS Sustain. Chem. Eng.* 5(7), 5657-5661. DOI:10.1021/acssuschemeng.7b01393

Article submitted: July 10, 2018; Peer review completed: August 18, 2018; Revised version received and accepted: August 31, 2018; Published: September 4, 2018.
DOI: 10.15376/biores.13.4.7957-7968