Isolation of Lignin from Masson Pine by Liquid-Liquid Extraction Based on Complete Dissolution in NaOH Aqueous Solution

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A method for lignin isolation from softwood based on complete dissolution in NaOH aqueous solution and liquid-liquid extraction was introduced. The structural features of milled alkali-soluble lignin (MAL) were comparatively analyzed with those of classical milled wood lignin (MWL) by means of alkaline nitrobenzene oxidation (NBO) and molecular weight, as well as Fourier transform infrared (FTIR) and nuclear magnetic resonance (NMR) spectra analyses. The results showed that the yield of crude MAL (34.2%) was about twice as much as that of MWL (16.4%). The NBO product yields of MWL and MAL were quite similar. The weight-average molecular weight of MAL (10,400 g mol^{-1}) was much higher than for MWL (6,970 g mol^{-1}). Both MWL and MAL displayed similar FTIR, UV, ^{1}H NMR, and ^{1}H-^{13}C HSQC NMR spectra. The total OH content of MAL (4.48 mmol g^{-1}) was higher than that of MWL (3.89 mmol g^{-1}). Compared with MWL, MAL showed similar structural characteristics but better isolation yield and higher molecular weight.

Keywords: Ball-milled alkali-dissolved lignin (MAL); Liquid-liquid extraction; Masson pine; NaOH aqueous solution; Structural characteristics

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INTRODUCTION

Higher plant cell walls are primarily composed of cellulose, hemicelluloses, and lignin. Lignin consists of an extensive three-dimensional network resulting from radical-coupling polymerization reactions occurring after polysaccharides have been accumulated (Lu and Ralph 2003; Fasching *et al.* 2008). Consequently, molecular associations and covalent bonds can be produced between lignin and carbohydrates (Yamamoto *et al.* 1989; Wang *et al.* 2009). As the most abundant aromatic polymer in nature, lignin can be utilized as a potential clean and sustainable feedstock for liquid fuel and chemical production, which has attracted increasing interest in the utilization of lignocellulose. Developing efficient methods for isolating lignin from plant cell walls is beneficial for increasing the understanding of the structural characterization of lignin, thus facilitating better utilization of lignin resources (Zhang *et al.* 2010). However, the complexity of lignin and its chemical and physical associations with carbohydrates make isolation impossible in high yield without serious degradation. Being able to isolate as much lignin as possible while minimizing the structural modification is the key problem in elucidating the structural characterization of native lignin (Xu *et al.* 2008).

A good lignin isolation strategy usually contains three main steps: (1) degrading

the wood mechanically (typically by ball-milling), (2) a solid-liquid extraction step for dissolving lignin in an appropriate solvent, and (3) various purification steps (Fasching *et al.* 2008). At present, milled wood lignin (MWL) established by Björkman (1956), who extracted lignin from ball-milled wood by aqueous 1,4-dioxane, is the main material used to investigate the chemical structure of lignin. However, MWL does not represent the total lignin. Another lignin isolation approach involves removing most of the carbohydrate by subjecting milled wood to enzymatic treatment before aqueous 1,4-dioxane extraction, resulting in cellulolytic enzyme lignin (CEL) (Pew and Weyna 1962; Chang *et al.* 1975). In order to improve the efficiency of solid-liquid extraction, various modifications of both methods have been proposed to reduce the amount of undissolved residual lignin in the wood (Wu and Argyropoulos 2003; Hu *et al.* 2006).

Currently, efficient isolation technologies and solvents are still required to get a better utilization of the lignin resources. The topic of complete dissolution of lignocelluloses with appropriate solvents has attracted more attention and become a research point for lignin isolation. Lu and Ralph (2003) established that wood can dissolve in dimethyl sulfoxide and tetrabutylammonium fluoride solvent systems after extensive ball-milling. Fasching *et al.* (2008) established a facile method with high lignin yield and a low number of separation steps for lignin isolation from wood based on complete dissolution in dimethylsulfoxide and N-methylimidazole (DMSO/NMI). Recently, using ionic liquids as a dissolution system has attracted extensive attention as a result of the environmental and process benefits (Zavrel *et al.* 2009; Yu *et al.* 2012). The development of a solvent system for dissolving lignocellulosic materials provides new approaches to isolate lignin from cell walls. For example, lithium chloride/dimethyl sulfoxide (LiCl/DMSO) was developed as a solvent system for dissolving wood and grass with short ball-milling time (Wang *et al.* 2009; Wu *et al.* 2014a). Based on this solvent system, a novel lignin isolation method featuring the dissolution of ball-milled wood in LiCl/DMSO solvent and then regeneration in water prior to enzymatic hydrolysis was developed. Regeneration cellulolytic enzyme lignin (RCEL) preparations, with a higher yield than classical CELs, have been successfully isolated from wood (Capanema *et al.* 2015) and straw (Wu *et al.* 2014b).

Liquid-liquid extraction is more efficient than the classical solid-liquid extraction for lignin isolation. For this reason, complete wood dissolution systems have great potential to isolate a much higher yield of lignin from wood. Almost all of the current wood dissolution systems are organic solvents or ionic liquids, and most of the related dissolution processes are water-free. Previous work by the authors reported that ball-milled Masson pine could be dissolved in NaOH aqueous solution at room temperature under vigorous stirring (Zhu *et al.* 2016). The inorganic NaOH aqueous solution is more environmentally friendly and easier to access than organic solvents and ionic liquids. Additionally, it is not necessary to control the dissolution conditions in the absence of water. In this work, the isolation and characterization of lignin from Masson pine based on complete dissolution in NaOH aqueous solution is described and comparisons are made with the classical MWL to get a better evaluation of this method.

EXPERIMENTAL

Materials

Masson pine (*Pinus massoniana* Lamb.) wood was provided by a paper plant in

Fujian, China. The air-dried chips were ground using a Wiley mill, and then the particles between the sizes of 40-mesh and 80-mesh were collected and extracted with benzene-ethanol (2:1, v/v) in a Soxhlet apparatus for 12 h. The extractive-free samples were further vacuum-dried at 40 °C for several days prior to ball-milling. Klason lignin in the extractive-free Masson pine was determined to be 28.3% according to the method described by Sluiter *et al*. (2008). The ball milling was carried out with a planetary ball mill (Pulverisette 7 premium line, Fritsch GMBH, Idar-Oberstein, Germany) at 600 rpm for 4 h at room temperature. Two grinding bowls made of silicon nitride (80 mL) with 25 zirconium dioxide grinding balls (1-cm diameter) in each bowl were prepared. In each milling run, 4 g of oven-dried, extractive-free Masson pine was charged for each bowl, and a 10-min break, followed by 5 min of milling, was conducted to avoid over-heating. All weights and calculations were made on an oven-dried basis. All of the chemicals, such as sodium hydroxide and 1,4-dioxane, were analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd of China (Beijing, China) and used as received.

Dissolution of Milled Wood in Sodium Hydroxide Aqueous Solution

The 4-h ball-milled Masson pine powder was put into 6% (w/w) NaOH aqueous solution (wood concentration in the solution was 5%), and the milled wood was completely dissolved in the alkaline solution in approximately 10 min under vigorous magnetic stirring at room temperature (Zhu *et al.* 2016). A dark, but clear, wood-NaOH solution was obtained.

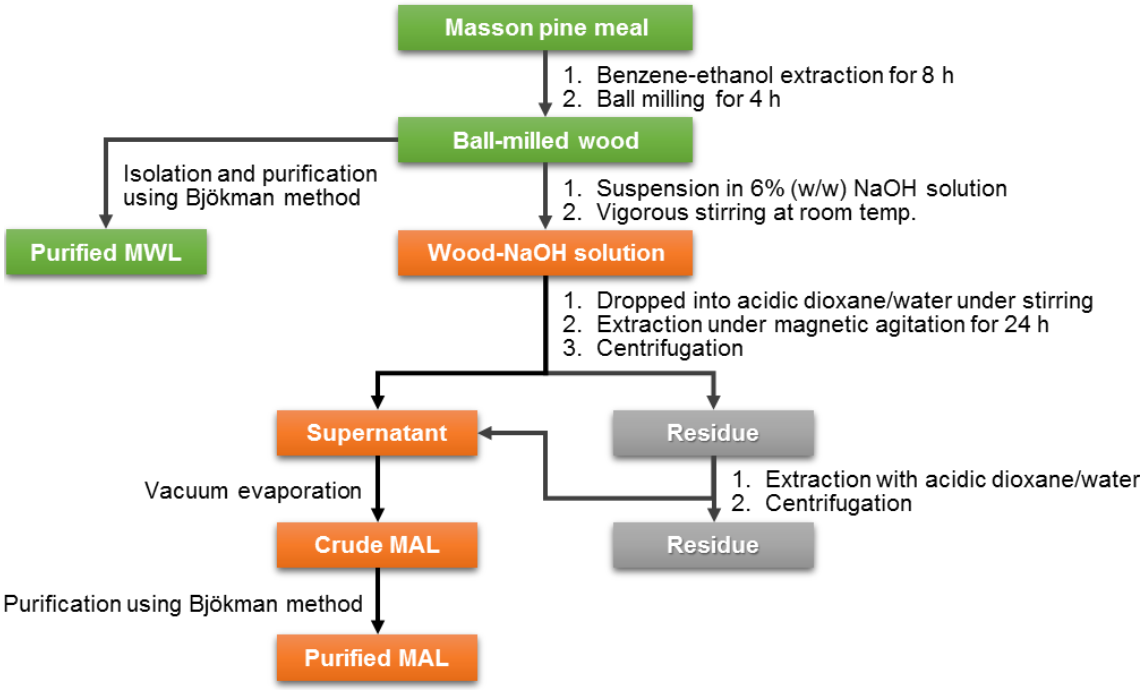

Fig. 1. Scheme of the isolation and purification of lignin fractions from ball-milled Masson pine based on complete dissolution in NaOH aqueous solution

Isolation and Purification of Lignins

The scheme for the isolation and purification of lignin fractions is illustrated in Fig. 1. Milled wood lignin was isolated from 4-h ball-milled Masson pine according to the classical procedure established by Björkman (1956). Liquid-liquid extraction of lignin

fractions was conducted based on the complete wood dissolution in NaOH aqueous solution. The wood-NaOH solution was added dropwise into acidic 1,4-dioxane/water (96:4, v/v) solution under magnetic stirring and continued agitation for 24 h for sufficient lignin extraction. A certain amount of HCl was added in advance to ensure that the final pH of the mixture was 2. The suspension was centrifuged off, and the residue was again extracted with the acidic 1,4-dioxane/water solution for 24 h. The combined lignin-contained supernatant was rotary evaporated under reduced pressure to remove the 1,4-dioxane solution and give a crude lignin fraction. The lignin fraction isolated by liquid-liquid extraction based on the complete wood dissolution in NaOH aqueous solution was named ball-milled alkali-dissolved lignin (MAL).

The purification of isolated MWL and MAL was completed following the classical procedure of Björkman (1956).

Acetylation of Isolated Lignins

The acetylation of the isolated MWL and MAL was conducted according to the published method (Lundquist 1992). In brief, the lignin sample (approximately 100 mg) was acetylated with 1 mL to 2 mL of acetic anhydride/pyridine (1:1, v/v) at room temperature overnight. Repeated addition and removal of ethanol (five to ten times, by film evaporation) was conducted to remove the acetic acid and pyridine from the sample. The acetate was dissolved in 1 mL to 2 mL of chloroform, and the solution was dripped into 100 mL of magnetically stirred ether. The precipitated acetate was centrifuged off, washed with ether, and then dried in a vacuum oven at 45 °C with KOH and P_2O_5.

Structure Characterization of Isolated Lignins

Alkaline nitrobenzene oxidation (NBO) analyses were applied to the original milled wood, MWL, and MAL according to the common procedure (Chen 1992). The NBO products were silylated with N,O-bis(trimethylsilyl)acetamide at 100 °C for 10 min and then analyzed by gas chromatography under the following conditions: gas chromatograph, GC-14B with flame ionization detector (Shimadzu Co., Kyoto, Japan); column, InertCap 1 (fused-silica capillary column, 30 m, 0.25-mm i.d.) (GL Science Inc., Tokyo, Japan); column program, kept for 5 min at 150 °C, raised by 5 °C min^{-1} to 210 °C, and then by 20 °C min^{-1} to 280 °C for 6 min; injection temperature, 280 °C; detector temperature, 280 °C. The main monomer of softwood lignin is guaiacyl propane (G unit). Therefore, the yield of vanillin and vanillic acid in the oxidation products, which were mainly generated from the nitrobenzene oxidation of G-unit lignin, was equivalent to the whole yield of nitrobenzene oxidation products.

The average molecular weight of isolated lignin fractions was determined on a gel permeation chromatography system (Waters 600-GPC, Waters Corp., Milford, MA, USA) instrument equipped with a Waters 2410 refractive index detector and 1515 isocratic HPLC pump. Mono GPC-100, -300, and -500 (Sepax Technologies Inc., Newark, DE, USA) were used as analytical column. The acetylated samples were dissolved in tetrahydrofuran (THF) with a concentration of 0.2%, and the injection sample size was 200 µL. The column was operated at 35 °C and eluted with THF at a flow rate of 1 mL min^{-1}, calibrated using polystyrene standards (Xu *et al.* 2008).

The Fourier transform infrared (FTIR) spectra of MAL and MWL were recorded from a KBr disc containing 0.5% finely ground samples on an IR spectrophotometer (FTIR-650, Tianjin Gangdong Technology Development Inc., China) in the range of 4,000 cm^{-1} to 400 cm^{-1}. Ultraviolet (UV) spectra were recorded on a UV-vis spectrophotometer

(Agilent, Cary 60 Conc, Mulgrave, Victoria, Australia) in the range of 260 nm to 400 nm. The lignin sample (5 mg) was dissolved in 10 mL of 95% 1,4-dioxane solution (1,4-dioxane/water = 95:5, v/v), and 1 mL of the solution was diluted to 10 mL with 50% 1,4-dioxane solution (1,4-dioxane/water = 50:50, v/v) (Wen *et al.* 2010).

Nuclear magnetic resonance (NMR) spectra were obtained on a Bruker Avance 500 spectrometer (Bruker, Boston, MA, US) at 500 MHz (^{1}H NMR), 125 MHz (^{13}C NMR), or 202 MHz (^{31}P NMR) using a 5-mm inverse probe. 2D ^{1}H-^{13}C correlation spectra were recorded through a phase-sensitive gradient-enhanced HSQC (heteronuclear single quantum correlation) using an echo-antiecho experiment (HSQCETGP sequence). Samples for ^{1}H NMR and 2D analysis were prepared by dissolving approximately 50 mg lignin samples with and without acetylation into 0.5 mL deuterated dimethylsulfoxide (DMSO-d_6), respectively. Chemical shifts were referenced to DMSO-d_6 (2.50 ppm) for ^{1}H NMR and to DMSO-d_6 (39.52 ppm) for ^{13}C NMR. ^{31}P NMR experiments were carried out using a slightly modified procedure, as reported by Granata and Argyropoulos (1995). The lignin (20 mg) was dissolved in a deuterated chloroform/pyridine ($CDCl_3/C_5D_5N$) mixture (1:1.6, v/v; 0.5 mL) at 60 °C for 12 h. The phosphitylation reagent, 2-chloro-4,4,5,5-tetramethyl-1,2,3-dioxaphospholane (TMDP, 100 μL), and the internal standard, N-hydroxy-6-norbornene-2,3-dicarboximide (NHND, 100 μL of 0.1212 M solution in $CDCl_3/C_5D_5N$, 1:1.6, v/v), were added successively. In order to homogenize and accelerate phosphorus relaxation, 25 μL of 0.0312 M solution of chromium acetylacetonate in the same $CDCl_3/C_5D_5N$ mixture was added. The spectra were recorded with a 5-s relaxation time and an average number of 200 scans. Chemical shifts were relative to the signal of the phospholane hydrolysis product at 132.2 ppm. The integral value of the internal standard was used for the calculations of the absolute amount of each functional group.

RESULTS AND DISCUSSION

Isolation Yield and Klason Lignin Content of Crude MAL and MWL

According to our previous result, 4-h ball-milled Masson pine powder is able to be dissolved in 6% (w/w) NaOH aqueous solution (Zhu *et al.* 2016). After that, MAL was isolated from the alkali-wood solution by liquid-liquid extraction based on the complete dissolution, as described in the Experimental section. The MWL was also isolated from the 4-h ball-milled Masson pine using the traditional solid-liquid extraction (Björkman 1956). Before purification, the yields and Klason lignin contents of crude MAL and classical MWL were compared. The results showed that although the Klason lignin content of crude MAL (84.6%) was slightly lower than that of MWL (86.0%), the yield of crude MAL (34.2%) was about twice as much as that of MWL (16.4%). These results indicated that the liquid-liquid extraction was much more effective than the traditional solid-liquid extraction in lignin isolation. Fasching *et al.* (2008) also found that a higher yield of lignin could be extracted by liquid-liquid extraction after ball-milled wood was totally dissolved in DMSO/NMI. The alkali-dissolution and liquid-liquid extraction process is favorable for separating lignin in higher yields from wood with the same ball-milling conditions, or a shorter ball-milling time can be conducted on wood to separate lignin with the same yield.

Alkaline Nitrobenzene Oxidation Analysis of MAL and MWL

Nitrobenzene oxidation of lignin is important in terms of the characterization of the lignin by providing the information on the minimal quantities and the relative amounts of

the uncondensed *p*-hydroxyphenyl-, guaiacyl-, and syringylpropane units present in lignin (Chen 1992). Masson pine, a kind of softwood, was used in this study as raw material. The lignin of softwood is primarily composed of G units, which give rise to vanillin and vanillic acid as the major products in nitrobenzene oxidation. Therefore, the yield of vanillin and vanillic acid in oxidation products is equivalent to the total yield of NBO products. The NBO products yield of purified MWL and MAL is given in Table 1. The NBO products yield of MWL (1.9 mmol g^{-1}) and MAL (1.8 mmol g^{-1}) was quite similar, with both yields being slightly lower than that of raw Masson pine. This result demonstrates that the alkali-dissolution and liquid-liquid extraction process caused little damage to the aromatic ring of the lignin unit, which was similar to that seen with the traditional solid-liquid extraction method. Neither method caused much degradation of the aromatic part of the lignin when compared with the lignin in raw Masson pine.

Table 1. Nitrobenzene Oxidation Products Yield of Purified MWL and MAL

	MWL	MAL	Masson Pine [b]
NBO Products Yield [a] (mmol g^{-1})	1.9 ± 0.2	1.8 ± 0.1	2.0 ± 0.1
Klason Lignin Content (%)	94.0 ± 0.7	93.6 ± 0.2	28.3 ± 0.1
[a] Based on Klason lignin; [b] Data cited from Zhu *et al.* (2016)			

Molecular Weights of MAL and MWL

Gel permeation chromatographic elution curves for the isolated lignin samples MWL and MAL were investigated to check whether the alkali-dissolution and liquid-liquid extraction process caused any chemical depolymerization to the lignin. Table 2 shows the weight-average (M_w) and number-average (M_n) molecular weights and polydispersity (M_w/M_n) of the purified MWL and MAL. The M_w of MAL (10,400 g mol^{-1}) was much higher than that of MWL (6,970 g mol^{-1}). This result indicates that the alkali-dissolution and liquid-liquid extraction process did not significantly degrade the macromolecular structure of lignin, and a lignin fraction with higher molecular weight could be extracted in MAL. The more effective liquid-liquid extraction contributes to the higher yield and molecular weight of MAL. Xu *et al.* (2008) also found the combination of mild ball-milling and alkaline organic solvent extraction or alkali treatment did not apparently degrade the lignin structure.

Table 2. The Weight-Average (M_w) Molecular Weight, Number-Average (M_n) Molecular Weight, and Polydispersity (M_w/M_n) of Purified MWL and MAL

Sample	M_w (g mol^{-1})	M_n (g mol^{-1})	M_w/M_n
MWL	6970	4170	1.67
MAL	10400	4350	2.39

Spectral Analysis of MAL and MWL

The spectra of FTIR (Fig. S1), UV, and ^{1}H NMR (Fig. S2) were applied to the structural analysis of the isolated lignins. The results indicated that MWL and MAL have very similar spectral characteristics. The details of the spectral analysis are given in the supplementary material.

The resolution of the signals overlapping in the ^{1}H and ^{13}C NMR spectra, along with the main structural characteristics of the lignin, including various units linked by ether

and C-C bonds, can be observed in the HSQC spectra. The side chain (δ_C/δ_H 90–50/6.0–2.5 ppm) and the aromatic region (δ_C/δ_H 125–100/8.0–6.0 ppm) of the HSQC spectra of the purified MWL and MAL, as well as the substructures detected in the spectra, are presented in Fig. 2. From these well-resolved NMR spectra, C-H correlations from all major units, representing the various linkages between lignin units, can be readily assigned.

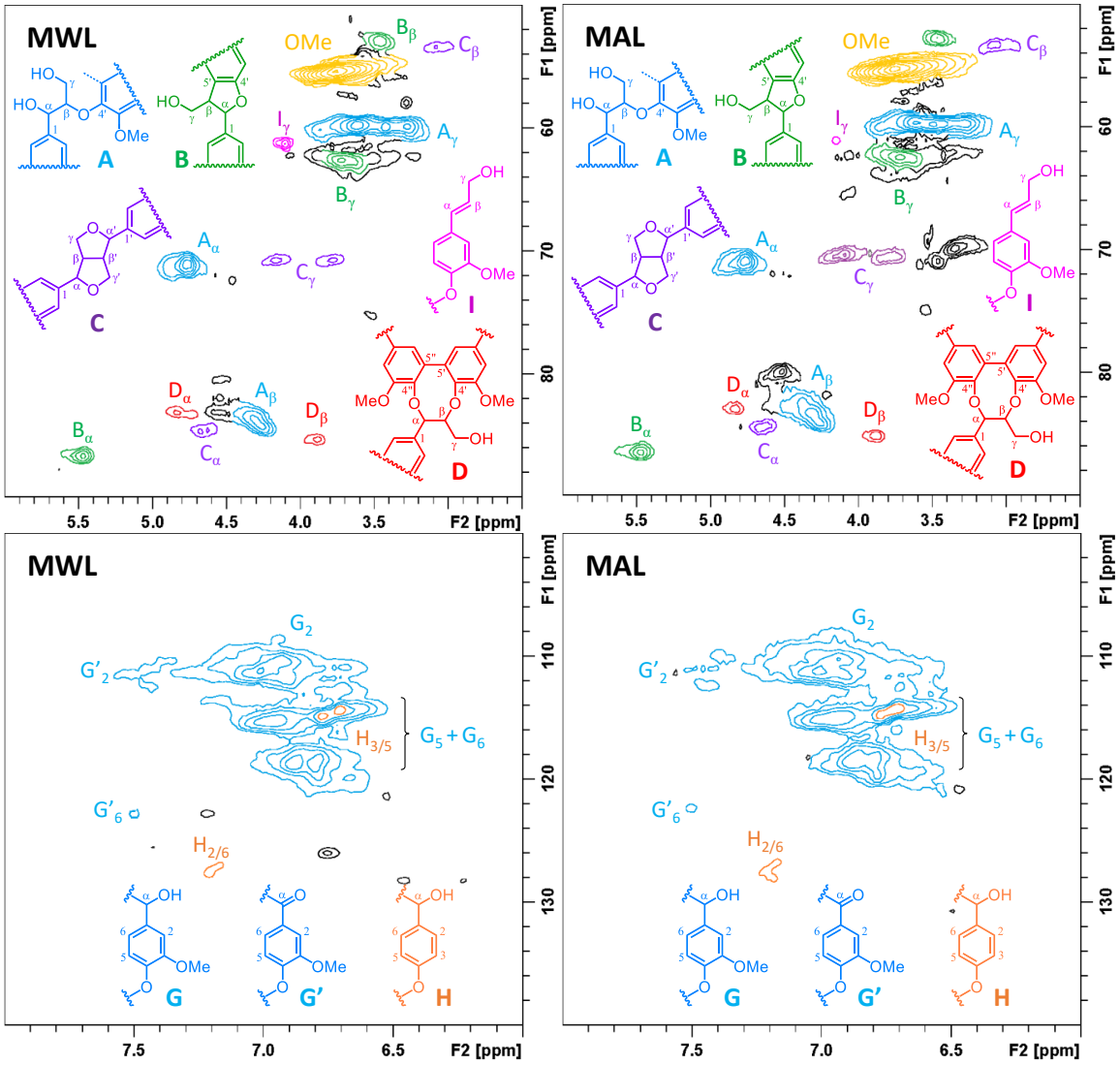

Fig. 2. 2D HSQC NMR spectra of the purified MWL and MAL. A: β-aryl ether (β–O–4'); B: phenylcoumaran (β–5'); C: resinol (β–β'); D: dibenzodioxocin (5'–5"/β–O–4'); I: cinnamyl alcohol end-group; G: guaiacyl unit; H: p-hydroxyphenyl unit

In the side chain region, C-H correlation signals at δ_C/δ_H 70–72/4.7–5.0, 81–85/4.2–4.7, and 58–61/3.1–4.0 belong to the C_α-H_α, C_β-H_β, and C_γ-H_γ correlations of the β–O–4' ether substructures A. The correlations at δ_C/δ_H 85–87/5.4–5.7, 52–54/3.4–3.6, and 61–64/3.3–4.0 are from the C_α-H_α, C_β-H_β, and C_γ-H_γ correlations of the phenylcoumaran B (β–5') structures. The C_α-H_α, C_β-H_β, and C_γ-H_γ correlations of the resinol C (β–β') structures appear at δ_C/δ_H 83–85/4.6–4.8, 53–54/3.0–3.2, and 70–72/3.7–4.3. The C_α-H_α and C_β-H_β correlations from the dibenzodioxocin D structures are also distinguishable at δ_C/δ_H 83.0/4.9 and 85.0/3.95, although the corresponding C_γ-H_γ correlations are obscured by

those from the β–O–4' ether structures. In the aromatic region, the typical C-H correlations in the aromatic ring of guaiacyl lignin (G) were shown at δ_C/δ_H 109–113/6.9–7.4 (C_2-H_2), 113–116/6.4–7.2 (C_5-H_5), and 117–120/6.7–7.1 (C_6-H_6). The double C_5-H_5 signal revealed some heterogeneity among the G-units, especially affecting the C_5-H_5 correlation, probably because of the different substituents at C_4 (*e.g.*, phenolic or etherified in different substructures) (Rencoret *et al.* 2009a,b; Wen *et al.* 2013). Weak C-H correlations of δ_C/δ_H 114.4/6.70 ($C_{3,5}$-$H_{3,5}$) and δ_C/δ_H 127.5/7.19 ($C_{2,6}$-$H_{2,6}$) were also detected, which indicates the existence of a very small amount of *p*-hydroxyphenyl units in Masson pine lignin. The 2D ^{1}H-^{13}C HSQC NMR spectra of the MWL and MAL also revealed the similarity of the two isolated lignin fractions, and the alkali-dissolution and liquid-liquid extraction caused no obvious damage to the lignin structure.

The two isolated lignin samples, MWL and MAL, were phosphitylated with TMDP in the presence of NHND as an internal standard, and analyzed with quantitative ^{31}P NMR. The concentration of each hydroxyl functional group (in mmol g^{-1}) was calculated on the basis of the hydroxyl content of the internal standard and its integrated peak area. The obtained spectra showed well-resolved signals for the different hydroxyl groups presented within the two lignin preparations. The signal at 145.6 ppm to 149.0 ppm was attributed to aliphatic OH, the signal at 137.6 ppm to 140.4 ppm was from guaiacyl phenolic OH, and carboxylic OH gave a signal at 133.8 ppm to 136.0 ppm. Table 3 displays the quantitative data on the distribution of the various OH groups for the lignin samples. The OH contents of aliphatic, guaiacyl phenolic, and carboxylic groups of MAL were all higher than those of MWL, and the total OH contents of MAL and MWL were 4.48 mmol g^{-1} and 3.89 mmol g^{-1}, respectively. The increase of phenolic OH content in MAL may have been caused by the cleavage of the β–O–4' ether structure during the alkaline dissolution process. The signal for condensed phenolic OH was not detected in the ^{31}P NMR spectra of either MWL and MAL, demonstrating that the alkali-dissolution and liquid-liquid extraction did not cause condensation of the lignin units (Zhang *et al.* 2010).

Table 3. The OH Content Based on Klason Lignin of MWL and MAL (mmol g^{-1})

	Aliphatic OH	Guaiacyl Phenolic OH	Carboxylic OH	Total OH
MWL	3.27	0.53	0.09	3.89
MAL	3.72	0.61	0.15	4.48

CONCLUSIONS

1. The introduced isolation method (alkali-dissolution and liquid-liquid extraction) can isolate lignin from wood in much higher yield. The liquid-liquid extraction was much more effective than the traditional solid-liquid extraction in lignin isolation.
2. The NBO products yield of the MWL and MAL showed that the two isolation methods did not cause much degradation of the aromatic part of the lignin. The weight-average molecular weight of MAL was much higher than that of MWL.
3. MWL and MAL displayed quite similar FTIR, ^{1}H, and 2D ^{1}H-^{13}C HSQC NMR. The total OH content of MAL from ^{31}P NMR analysis was higher than that of MWL. However, the relative content of phenolic OH in MWL and MAL was very similar at approximately 13.6%.

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REFERENCES CITED

Björkman, A. (1956). "Studies on the finely divided wood. Part I. Extraction of lignin with neutral solvents," *Svensk Paperstid.* 59, 477-485.

Capanema, E., Balakshin, M., Katahira, R., Chang, H.-M., and Jameel, H. (2015). "How well do MWL and CEL preparations represent the whole hardwood lignin?" *J. Wood Chem. Technol.* 35(1), 17-26. DOI: 10.1080/02773813.2014.892993

Chang, H.-M., Cowling, E. B., Brown, W., Adler, E., and Miksche, G. (1975). "Comparative studies on cellulolytic enzyme lignin and milled wood lignin of sweetgum and spruce," *Holzforschung* 29(5), 153-159. DOI: 10.1515/hfsg.1975.29.5.153

Chen, C-L. (1992). "Nitrobenzene and cupic oxide oxidations," in: *Method of Lignin Chemistry*, S. Y. Lin and C. W. Dence (eds.), Springer-Verlag, Berlin, Germany, pp. 301-321.

Fasching, M., Schroder, P., Wollboldt, R. P., Weber, H. K., and Sixta, H. (2008). "A new and facile method for isolation of lignin from wood based on complete wood dissolution," *Holzforschung* 62(1), 15-23. DOI: 10.1515/HF.2008.003

Granata, A., and Argyropoulos, D. S. (1995). "2-Chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane, a reagent for the accurate determination of the uncondensed and condensed phenolic moieties in lignins," *J. Agric. Food Chem.* 43(6), 1538-1544. DOI: 10.1021/jf00054a023

Hu, Z., Yeh, T.-F., Chang, H.-M., Matsumoto, Y., and Kadla, J. F. (2006). "Elucidation of the structure of cellulolytic enzyme lignin," *Holzforschung* 60(4), 389-397. DOI: 10.1515/HF.2006.061

Lu, F., and Ralph, J. (2003). "Non-degradative dissolution and acetylation of ball-milled plant cell walls: High-resolution solution-state NMR," *Plant J.* 35, 535-544. DOI: 10.1046/j.1365-313X.2003.01817.x

Lundquist, K. (1992). "Proton (^{1}H) NMR spectroscopy," in: *Methods in Lignin Chemistry*, S. Y. Lin and C. W. Dence (eds.), Springer-Verlag, Berlin, Germany, pp. 242-249.

Pew, J. C., and Weyna, P. (1962). "Fine grinding, enzyme digestion, and the lignin-cellulose bond in wood," *Tappi J.* 45(3), 247-256.

Rencoret, J., Marques, G., Gutiérrez, A., Nieto, L., Jiménez-Barbero, J., Martínez, Á. T., and del Río, J. C. (2009a). "Isolation and structural characterization of the milled-wood lignin from *Paulownia fortunei* wood," *Ind. Crop. Prod.* 30(1), 137-143. DOI: 10.1016/j.indcrop.2009.03.004

Rencoret, J., Marques, G., Gutiérrez, A., Nieto, L., Santos, J. I., Jiménez-Barbero, J., Martínez, Á. T., and del Río, J. C. (2009b). "HSQC-NMR analysis of lignin in woody *(Eucalyptus globulus* and *Picea abies*) and non-woody (*Agave sisalana*) ball-

milled plant materials at the gel state," *Holzforschung* 63(6), 691-698. DOI: 10.1515/HF.2009.070

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

Wang, Z. G., Yokoyama, T., Chang, H. M., and Matsumoto, Y. (2009). "Dissolution of beech and spruce milled woods in LiCl/DMSO," *J. Agric. Food Chem.* 57(14), 6167-6170. DOI: 10.1021/jf900441q

Wen, J. L., Sun, Z., Sun, Y. C., Sun, S. N., Xu, F., and Sun, R. C. (2010). "Structural characterization of alkali-extractable lignin fractions from bamboo," *J. Biobased Mater. Bioenergy* 4(4), 408-425. DOI: 10.1166/jbmb.2010.1111

Wen, J. L., Sun, S. L., Xue, B. L. and Sun, R. C. (2013). "Recent advances in characterization of lignin polymer by solution-state nuclear magnetic resonance (NMR) methodology," *Materials* 6(1), 359-391. DOI: 10.3390/ma6010359

Wu, S., and Argyropoulos, D. S. (2003). "An improved method for isolating lignin in high yield and purity," *J. Pulp Paper Sci.* 29(7), 235-240.

Wu, W. J., Wang, Z. G., Jin, Y. C., Matsumoto, Y., and Zhai, H. (2014a). "Effects of LiCl/DMSO dissolution and enzymatic hydrolysis on the chemical composition and lignin structure of rice straw," *Biomass Bioenerg.* 71, 357-362. DOI: 10.1016/j.biombioe.2014.09.021

Wu, W. J., Wang, Z. G., Jin, Y. C., Matsumoto, Y., and Zhai, H. M. (2014b). "Isolation of cellulolytic enzyme lignin from rice straw enhanced by LiCl/DMSO dissolution and regeneration," *BioResources* 9(3), 4382-4391. DOI: 10.15376/biores.9.3.4382-4391

Xu, F., Jiang, J. X., Sun, R. C., Tang, J. N., Sun, J. X., and Su, Y. Q. (2008). "Fractional isolation and structural characterization of mild ball-milled lignin in high yield and purity from *Eucommia ulmoides* Oliv." *Wood Sci. Technol.* 42(3), 211-226. DOI: 10.1007/s00226-007-0162-5

Yamamoto, E., Bokelman, G. H., and Lewis, N. G. (1989). "Phenyl propanoid metabolism in cell walls," in: *Plant Cell Wall Polymers, Biogenesis and Biodegradation*, N. G. Lewis and M. G. Paice (eds.), American Chemical Society, Washington, DC. pp. 68-88.

Yu, H. M., Hu, J., Fan, J., and Chang, J. (2012). "One-pot conversion of sugars and lignin in ionic liquid and recycling of ionic liquid," *Ind. Eng. Chem. Res.* 51(8), 3452-3457. DOI: 10.1021/ie2025807

Zavrel, M., Bross, D., Funke, M., Büchs, J., and Spiess, A. C. (2009). "High-throughput screening for ionic liquids dissolving (ligno-)cellulose," *Bioresour. Technol.* 100(9), 2580-2587. DOI: 10.1016/j.biortech.2008.11.052

Zhang, A., Lu, F., Sun, R. C., and Ralph, J. (2010). "Isolation of cellulolytic enzyme lignin from wood preswollen/dissolved in dimethyl sulfoxide/N-methylimidazole," *J. Agric. Food Chem.* 58(6), 3446-3450. DOI: 10.1021/jf903998d

Zhu, Y., Yang, L., Wu, W., Wang, Z., and Jin, Y. (2016). "Complete dissolution of ball-milled Masson pine using an aqueous sodium hydroxide solvent," *BioResources* 11(3), 6017-6025. DOI: 10.15376/biores.11.3.6017-6025

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