Characterization of Lignin Isolated from Wheat Leaf Based on LiCl/DMSO Dissolution and Regeneration

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The isolation of lignin is of great importance to understand its structural characteristics. A lithium chloride/dimethyl sulfoxide (LiCl/DMSO) solvent system has been developed for the dissolution of lignocellulose and for the isolation of lignin for this purpose. In this work, ball-milled wheat leaf (sheath included) was dissolved in the LiCl/DMSO solvent system and then regenerated in water. Two lignin preparations, cellulolytic enzyme lignin from the ball-milled leaf (CEL) and from the regenerated leaf (RCEL), were obtained through a cellulolytic enzyme lignin procedure. The RCEL and CEL were comparatively investigated by the use of wet chemistry and spectroscopic methods. The results indicate that the effects of ball milling and regeneration on the aromatic structure and β-O-4' linkages of lignin were not significant. The RCEL had a higher isolation yield and purity, but a similar structure with the corresponding CEL. The RCEL can be used for structural analysis.

Keywords: Wheat leaf; Ball milling; LiCl/DMSO solvent system; Regeneration; Cellulolytic enzyme lignin (CEL); Structural characteristics

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INTRODUCTION

Lignocellulose, which is mainly composed of cellulose, hemicellulose, and lignin, is the most abundant and renewable biomass in the biosphere. China is an agricultural country with rich non-wood resources including corn, rice straw, and wheat straw. However, most of these agricultural residues are burned, causing air pollution and creating the risk of a spreading fire. Wheat straw is one of the most important graminaceous biomass materials and is considered an attractive feedstock within the papermaking industry. The wheat stem is the main raw material used in the pulping and papermaking industry, while the wheat leaf (sheath included) is discarded as residues in the process of straw handling. These residues have not yet been reasonably and economically applied because of their lack of abundance of cellulose content and their high amount of parenchymal cells. The lignin content in wheat leaf is lower than that in the stem, and the enzymatic hydrolysis yield of sugars is superior to the wheat stem after an appropriate pretreatment (Jin *et al*. 2013).

It is impossible to isolate native lignin from the plant cell wall without any chemical or physical change (Lu and Ralph 2010). Currently, the most widely used lignin preparations for structure studies are milled-wood lignin (MWL) (Björkman 1954) and cellulolytic enzyme lignin (CEL), with the CEL being structurally similar to the MWL (Chang *et al*. 1975). Fasching *et al*. (2008) developed a novel method for lignin isolation based on a liquid-liquid extraction. The ball-milled wood meal was completely dissolved

in the dimethyl sulfoxide/N-methylimidazole (DMSO/NMI) solvent system, and then the solution was extracted with dioxane/water to obtain isolated lignin. The lignin sample isolated by this method is structurally similar to MWL. However, the product from this method contained a relatively low yield of Klason lignin (41%), with a rather long milling time of 48 h to dissolve the wood meal. Petrus *et al*. (1995) found that DMSO is able to dissolve cellulose oligomers, and an addition of lithium chloride increases the solubility of cellulose in DMSO. Wang *et al*. (2009) first reported that ball-milled wood (beech and spruce) can be completely dissolved in 6% LiCl/DMSO system. Based on this solvent system, Capanema *et al*. (2014) and Wu *et al*. (2014) isolated the regenerated CEL preparations (RCEL) from red alder and rice straw, respectively, with a very high yield and purity, as well as minimal structural degradation.

Wheat leaf has long been considered as residues and has not been utilized as a natural resource. Fully understanding the structure of wheat leaf lignin will be helpful for the more productive use of wheat leaf. In this work, CEL and RCEL were isolated from ball-milled and regenerated LiCl/DMSO dissolved wheat leaf. The structure characteristics of RCEL and CEL were comparatively investigated by wet chemistry and spectroscopy.

EXPERIMENTAL

Materials

The wheat straw (*Triticum aestivum*) used in this work was collected in Yancheng, Jiangsu, China, in May 2011. The wheat leaf (sheath included) was obtained by manual classification. The leaf was cut to a length of 3 cm to 5 cm and then ground using a Wiley mill. The particles that passed through a 20-mesh (0.85 mm) sieve were collected. The leaf meals were extracted with an ethanol/benzene solution (1:2, v/v) for 48 h to obtain extractive-free samples. No specific step was carried out to remove the protein. The main structural components of the leaf were 62.6% carbohydrate (including 35.1% of glucan, 22.7% of xylan, and 4.8% of arabinan), 16.6% total lignin (14.2% Klason lignin and 2.4% acid-soluble lignin), and 11.3% ash.

Cellulases from *Trichoderma reesei* (NS-50013, 84 FPU/mL), β-glucosidase from *Aspergillus niger* (NS-50010, 350 CBU/mL), and xylanase (NS-50014, 850 FXU/mL) generously provided by Novozymes (Novo Nordisk A/S, Demark) were applied for enzymatic hydrolysis. The chemicals used in this study were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and/or Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan) and were used as received without further purification.

Methods

Ball milling

Two grams of the vacuum dried sample were subjected to ball milling for 4 h in a planetary mill (QM-3SP2, Nanjing Nanda Instrument Plant, Nanjing, China) at a fixed frequency of 600 rpm. Two 100 mL zirconium dioxide bowls with 16 zirconium dioxide balls (1 cm in diameter) in each bowl were used in the milling. A 5 min interval was set between every 15 min of milling to prevent overheating. After ball milling, the leaf powder was carefully collected and dried under a vacuum for further use.

DMSO/LiCl dissolution and regeneration

The ball-milled wheat leaf was dissolved in 8% LiCl/DMSO and regenerated in Milli-Q water, according to the method described by Gu *et al*. (2015). The solid recovery of the regenerated leaf was 77.1%. The carbohydrate and lignin contents of the regenerated leaf were 63.3% (including 40.3% glucan, 20.0% xylan, and 3.0% arabinan) and 16.0% (13.9% Klason lignin and 2.1% acid-soluble lignin), respectively. The ash content was 11.7%. The regenerated sample was freeze-dried for further treatment.

Preparation of CEL and RCEL

The CEL and RCEL preparations were isolated according to the methods given by Gu *et al*. (2015), as shown in Fig. 1. The enzymatic hydrolysis of the ball-milled and regenerated leaf was carried on in an incubator (DZH-2102, Jinghong, Shanghai, China) at 180 rpm and 50 °C for 72 h. The charge of mixed enzyme (NS 50013:NS 50014:NS 50010 = 1 FPU:1.2 FXU:1 CBU) based on cellulase activity was 60 FPU/g-cellulose.

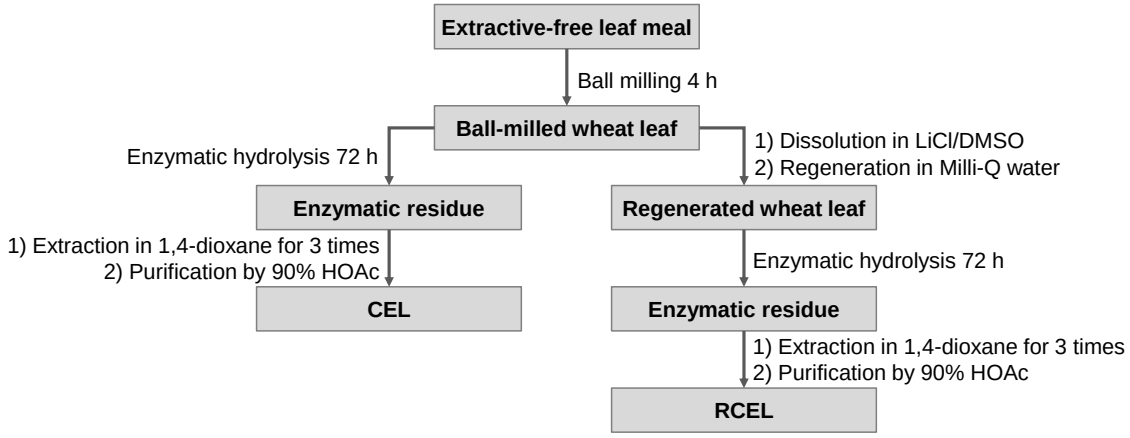

Fig. 1. The preparation procedure of CEL and RCEL

Analytical Methods

The lignin and the carbohydrate content of the extractive-free leaf and the pretreated samples were analyzed as previously described (Sluiter *et al*. 2008). The Klason lignin (KL) content was taken as the ash-free residue after acid hydrolysis. The hydrolysate of the KL procedure was collected to analyze the acid-soluble lignin (ASL) and the structural sugars. The ASL was measured by absorbance at 205 nm in a UV-vis spectrometer (TU-1810, Puxi, Beijing, China). The monomeric sugars were quantitatively measured with high-performance liquid chromatography (HPLC, Agilent 1200 Series, Santa Clara, CA, USA), equipped with a refractive index detector (RID). The HPLC analysis was carried out using a BioRad Aminex HPX-87H 20n exclusion column (300 mm × 7.8 mm, Bio-Rad Laboratories, Hercules, California, USA) with a Cation-H Refill Cartridge guard column (30 mm × 4.6 mm, Bio-Rad Laboratories, Hercules, California, USA). The ash content was determined by combustion at 575 °C.

Alkaline nitrobenzene oxidation (NBO) and ozonation were carried out according to the procedure reported by Chen (1992) and Akiyama *et al*. (2002), respectively.

The obtained CEL and RECL were acetylated according to the method described by Lu and Ralph (2010), and the resultant acetylated lignin samples were used for ^{1}H NMR and ^{1}H-^{13}C HSQC NMR analysis, respectively. The NMR experiments of the samples were carried out on a JEOL Alpha 500 spectrometer (500 MHz, JEOL, Japan)

with a cryogenically cooled 5 mm inverse geometry gradient probe according to the method described by Gu *et al*. (2015). About 80 mg acetylated lignin sample was dissolved in 0.5 mL deuterated chloroform ($CDCl_3$). The central solvent peak was used as the internal reference (δ_C/δ_H 77.2/7.26).

RESULTS AND DISCUSSION

Effect of Dissolution-Regeneration on Lignin Structure

Nitrobenzene oxidation (NBO) and ozonation are the two important wet chemistry methods for the structural analysis of the aromatic part and the side chain of lignin *in situ*. The side chain of the noncondensed lignin is oxidized, and the properties of the aromatic ring are preserved when lignin reacts with nitrobenzene in alkaline conditions (Chen 1992). The main NBO products of gramineous lignin are vanillin, syringaldehyde, and *p*-hydroxybenzaldehyde, as well as small amounts of vanillic acid, syringic acid, and *p*-hydroxybenzoic acid. Ozone can completely destroy the double bonds and aromatic parts of lignin. The erythronic and threonic acids are formed from the corresponding isomers, *i.e.*, *erythro* and *threo* forms of β-aryl ethers (Akiyama *et al*. 2002). Thus, the stereo chemical configuration of the side chain carbons in lignin is retained in the oxidation products.

The product yields of NBO and ozonation, which are represented as millimoles per gram of KL, of ball-milled and regenerated wheat leaf are given in Table 1. The NBO products yield decreased 6.4% after ball milling, while the yield dropped 14.4% in the regenerated leaf. However, the changes of the NBO products ratio (S/V/H) of lignin in ball-milled and regenerated leaf were rather little. This means that the condensation degree of wheat leaf lignin increased slightly, and the ratio of the structural units of noncondensed lignin remained constant. The loss of the lignin was 23% after the LiCl/DMSO dissolution and regeneration, which implies that the lignin fraction with a low condensation degree was probably easy to dissolve in diluted LiCl/DMSO during the regeneration process. The ozonation products yields of the three samples were almost the same, however, the *erythro* conformation of the lignin decreased slightly after ball milling and regeneration. It seems that the *threo* conformation is more stable than the *erythro* conformation. Compared with the wheat stem, the wheat leaf showed a lower ozonation products yield and a lower *E*/*T* ratio (Gu *et al*. 2015). This should be caused by low syringyl units in the leaf lignin, as stated by Akiyama *et al*. (2002), as the amount of β-O-4’ linkages and their *E*/*T* ratio depends on the content of the syringyl lignin groups.

Table 1. NBO and Ozonation Products Yields of Ball-milled and Regenerated Wheat Leaf

Samples	Nitrobenzene Oxidation		Ozonation	
	Yield (mmol·g^{-1})	S/V/H [a]	Yield (mmol·g^{-1})	*E*/*T* [b]
Wheat leaf	1.87± 0.03	29/59/12	0.38± 0.05	1.29± 0.03
Ball-milled wheat leaf	1.75± 0.03	29/58/13	0.38± 0.03	1.23± 0.10
Regenerated wheat leaf	1.60± 0.01	30/58/12	0.40± 0.05	1.18± 0.04

[a] S: syringaldehye + syringic acid; V: vanillin + vanillic acid; H: *p*-hydroxybenzaldehyde + *p*-hydroxybenzoic acid;
[b] *E*: erythronic acid; *T*: threonic acid

Effect of Dissolution-Regeneration on the Isolation of CEL

The CEL and RCEL were both extracted by a 96% dioxane/water solution from the corresponding enzymatic residues of ball-milled and regenerated wheat leaf. The isolation yields and the main chemical composition of the lignin preparations are given in Table 2. Compared with CEL, the RCEL exhibited higher purity due to the higher lignin content (RCEL 91.2% and CEL 85.6%) and the lower carbohydrate content (RCEL 6.0% and CEL 8.4%). Furthermore, the actual final lignin yield of the RCEL (56.8% × 91.2% = 51.8%) was about 10% higher than that of the CEL (48.7% × 88.0% = 41.7%). The yield was up to 65.7% on the basis of lignin in the regenerated wheat leaf. Capanema *et al.* (2014) reported that the yield of the RCEL extracted with 80% aqueous dioxane from 3 h ball-milled hardwood was about 4.5 and 2.5 times higher than the yields of the traditional MWL and CEL preparations. The RCEL obtained from the wheat stem also showed a higher yield and better purity than that of the CEL (Gu *et al.* 2015). The xylan/glucan ratio of the ball-milled and regenerated leaf was about 0.6 and 0.5, and it rose to 5.7 and 5.3 for the CEL and the RCEL, respectively. This result was attributed to the fact that the lignin is combined together with hemicelluloses not only through physical interaction (including van der Waals forces and hydrogen bonds), but also through covalent bonds (Overend and Johnson 1991). Thus, LiCl/DMSO dissolution and regeneration noticeably improved the final yield and purity of the isolated CEL.

The analytical results of the NBO and ozonation products of CEL and RCEL are listed in Table 3. Both the CEL and the RCEL showed higher yields of NBO and ozonation products than lignin in the original leaf. Guaiacyl lignin groups, with a high condensation degree, are harder to extract from the enzymatic residue than the syringyl lignin (Ralph *et al.* 2004); therefore, the obtained lignin preparations contained more syringyl units than the guaiacyl units. Meanwhile, about 90% of syringyl units can be oxidized into syringaldehye after nitrobenzene oxidation, and the conversion of guaiacyl units to vanillin is only 30% (Sarkanen and Hergert 1971). As a result, the S/V ratios of CEL and RCEL were higher than that of the lignin in wheat leaf.

Table 2. Main Chemical Components of CEL and RCEL Preparations of Wheat Leaf

	Yield [a] (%)	Carbohydrate (%)				Lignin (%)			Ash (%)
		Glucan	Xylan	Arabinan	Total	KL	ASL	Total	
CEL	48.7	1.1± 0.0	6.3± 0.0	1.0± 0.0	8.4± 0.1	82.3± 1.0	3.3± 0.1	85.6± 0.7	0.2± 0.1
RCEL	56.8	0.8± 0.1	4.2± 0.1	1.0± 0.0	6.0± 0.3	88.0± 1.1	3.2± 0.0	91.2± 1.0	0.1± 0.1
[a] The yield of lignin preparations were based on lignin in raw wheat leaf									

Table 3. NBO and Ozonation Products Yields of CEL and RCEL Preparations of Wheat Leaf

	Nitrobenzene Oxidation		Ozonation	
	Yield (mmol·g^{-1})	S/V/H [a]	Yield (mmol·g^{-1})	*E*/*T* [b]
CEL	2.21± 0.06	41/51/8	0.47± 0.14	1.18± 0.03
RCEL	2.23± 0.03	42/50/8	0.55± 0.01	1.17± 0.02
[a] S: syringaldehye + syringic acid; V: vanillin + vanillic acid; H: *p*-hydroxybenzaldehyde + *p*-hydroxybenzoic acid; [b] *E*: erythronic acid; *T*: threonic acid				

The ozonation products yield of CEL and RCEL was higher than that of the raw material, indicating that the β-O-4' linkages increased in the isolated lignin. This is confirmed with the result of the NBO analysis. However, the *E*/*T* ratio of the CEL and RCEL changed little compared with that of the lignin in the wheat leaf.

Spectral analysis of wheat leaf CEL and RCEL

Fourier transform infrared spectroscopy of the CEL and RCEL preparations and the ^{1}H NMR spectra of the acetylated lignin samples are shown in Figs. 2 and 3, respectively. The peaks at 1595 cm^{-1}, 1510 cm^{-1}, 1459 cm^{-1}, and 1423 cm^{-1} in the infrared spectra were corresponding to the skeletal vibrations of the aromatic ring (Karmanov and Derkacheva 2013), and their intensities were similar, which meant that the aromatic structures in the CEL and RCEL preparations were similar. The strength of the peaks at 1361 cm^{-1} and 1126 cm^{-1} were assigned to the vibrations of the syringyl units, and at 1264 cm^{-1} were assigned to the vibrations of the guaiacyl units, were also very similar for CEL and RCEL.

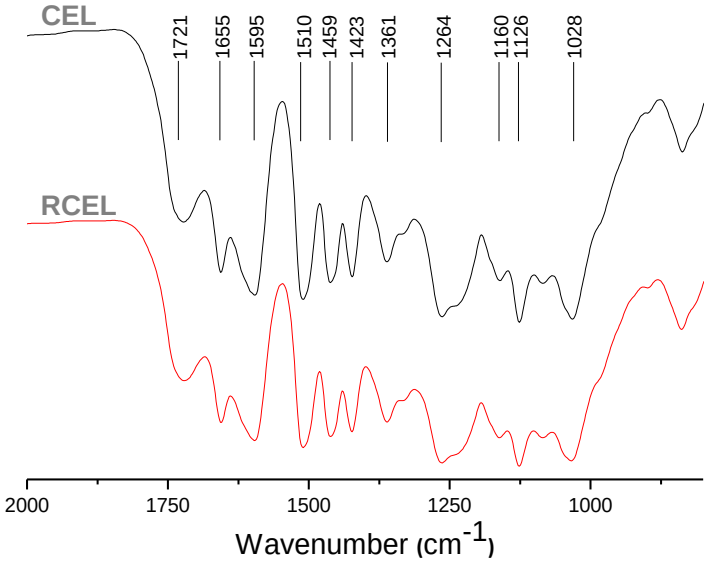

Fig. 2. The FTIR spectra of CEL and RCEL preparations

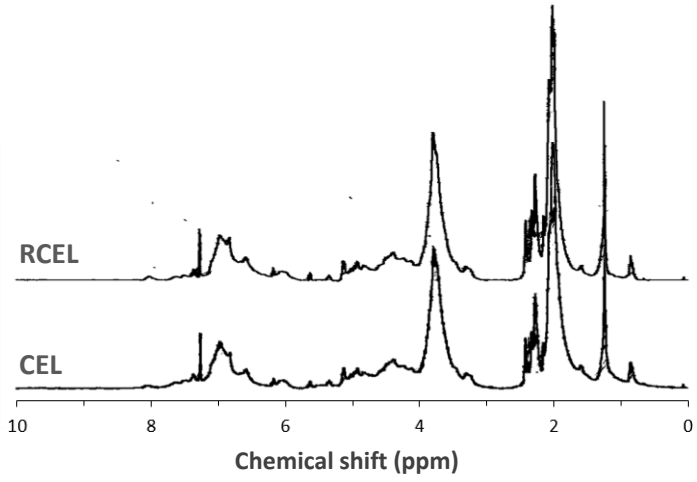

Fig. 3. The ^{1}H NMR spectra of acetylated lignin samples

The ^{1}H NMR spectra also revealed similar general structures of the acetylated CEL and RCEL. Protons at 6.25 ppm to 7.90 ppm were assigned to the aromatic ring and protons at 3.00 ppm to 4.02 ppm were of methoxyl. Signals at 2.10 ppm to 2.52 ppm and 1.60 ppm to 2.10 ppm were protons in the aromatic acetates and aliphatic acetates, respectively. Differences in the absorption of functional groups were not observed from the spectra of the acetylated CEL and RCEL.

The acetylated CEL and RCEL preparations from wheat straw were characterized by 2D HSQC NMR. The obtained 2D NMR spectra (δ_C/δ_H 140-50/8.0-2.5) and the major lignin substructures are illustrated in Fig. 4.

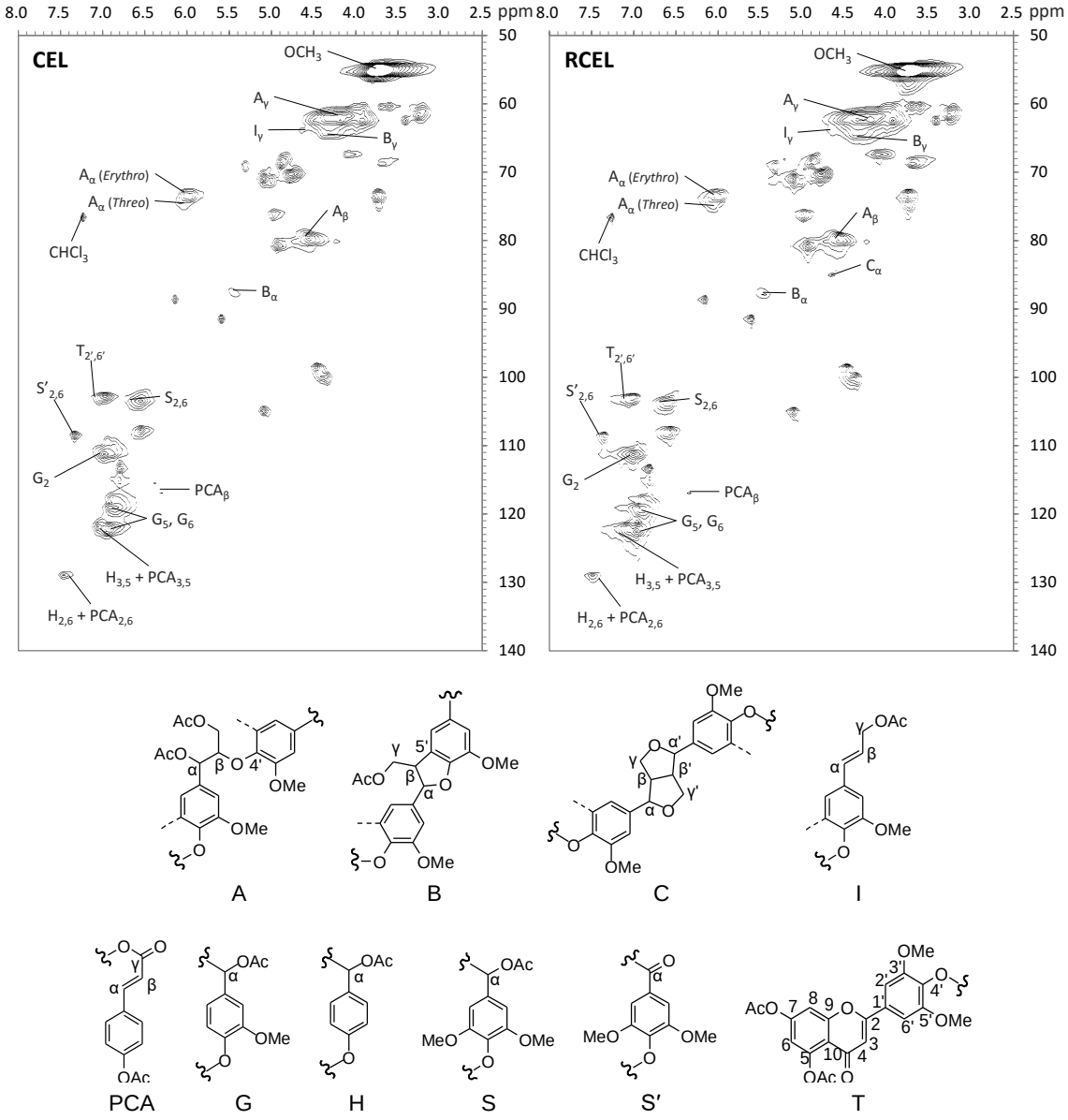

Fig. 4. The ^{1}H-^{13}C HSQC NMR spectra and the main substructures of acetylated CEL and RCEL: (A) β-O-4' alkyl-aryl ethers; (B) phenylcoumarans; (C) resinols; (I) cinnamyl alcohol end-groups; (T) tricin units; (PCA) p-coumarates; (G) guaiacyl units; (H) p-hydroxyphenyl units; (S) syringyl units ; (S′) oxidized syringyl units bearing a carbonyl at C_α

The side-chain region of the spectra gives important information about the different interunit linkages occurring in the structure of the lignin polymer. The spectra of the CEL and the RCEL show prominent signals corresponding to the β-O-4' aryl ether linkages. The structures of the C_α-H_α correlations in the β-O-4' substructures linked to the guaiacyl (G) or syringyl (S) units were seen at δ_C/δ_H 73.1/5.81 and 75.2/5.98, respectively. The β-O-4' structures linked to the G units were observed in the C_β-H_β correlations at δ_C/δ_H 80.0/4.51, and the β-O-4' structures linked to the S units were found in the C_β-H_β correlations at δ_C/δ_H 81.5/4.90. The C_γ-H_γ correlations of the β-O-4' substructures were observed at δ_C/δ_H 62.5/4.20, superimposed to the C_γ-H_γ correlations signals of the cinnamyl alcohol end-groups (I) and phenylcoumarans (B) substructures (del Río *et al.* 2012). The intensity of the other signals was weak relative to those of the phenylcoumaran and resinol substructures, which means there were small quantities of the β-5' and β-β' linkages.

The main cross-signals in the aromatic regions of the HSQC spectra corresponded to the different lignin and *p*-hydroxycinnamate units. Signals from the *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) units were observed clearly both in the isolated CEL and the RCEL. In addition, the prominent signals corresponding to the *p*-coumarate (PCA) structures were observed, which were typically identified in the gramineous plant lignin. Like other monocot samples, the signals of tricin were found, a flavone that is considered to be an authentic lignin monomer and be involved in the lignification process. Lan *et al.* (2015) pointed out that the tricin is fully compatible with the lignification reactions because it can only start a lignin chain and functions as a nucleation site for lignification in monocots. Comparatively, the HSQC NMR analysis showed that the dissolution/ regeneration process does not change the structure of lignin because no significant structural difference was found between the RCEL and the CEL from the wheat leaf. Moreover, the RCEL had a higher isolation yield and purity than that of the CEL. Therefore, the RCEL is good enough for structural analysis.

CONCLUSIONS

1. The analytical results of the NBO and ozonation showed that the LiCl/DMSO dissolution and regeneration had little effect on the condensation degree and the β-O-4' linkages of lignin in the regenerated wheat leaf.
2. The steps of dissolution in LiCl/DMSO and regeneration in water prior to enzymatic hydrolysis improved the removal of carbohydrate and enhanced the isolation of lignin with a high yield and purity.
3. The RCEL showed a slightly higher condensed degree and more amount of β-O-4' linkages than the lignin in the original leaf, as did the results of the CEL. The FTIR, ^1H, and ^1H-^{13}C spectra of the RCEL were identical with those of the CEL. The wet chemistry and spectroscopy analysis showed that the RCEL has a similar structure to the corresponding CEL, and it can be used for structural analysis.

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REFERENCES CITED

Akiyama, T., Sugimoto, T., Matsumoto, Y., and Meshitsuka, G. (2002). "*Erythro*/*threo* ratio of beta-O-4 structures as an important structural characteristic of lignin. I: Improvement of ozonation method for the quantitative analysis of lignin side-chain structure," *J. Wood Sci.* 48(3), 210-215. DOI: 10.1007/BF00771369

Björkman, A. (1954). "Isolation of lignin from finely divided wood with neutral solvents," *Nature* 174(4440), 1057-1058.

Capanema, E., Balakshin, M., Katahira, R., Chang, H. M., and Jameel, H. (2014). "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., and Brown, W. (1975). "Comparative studies on celluloytic enzyme lignin and milled wood lignin of sweetgum and spruce," *Holzforschung* 29, 153-159. DOI: 10.1515/hfsg.1975.29.5.153

Chen, C. L. (1992). "Nitrobenzene and cupric oxidations," in: *Methods in Lignin Chemistry*, S. Y. Lin and C. W. Dence (eds.), Springer, New York, pp. 301-321.

del Río, J. C., Rencoret, J., Prinsen, P., Martínez, Á. 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. Agric. Food Chem.* 60(23), 5922–5935. DOI: 10.1021/jf301002n

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

Gu, F., Wu, W. J., Wang, Z. G., Yokoyama, T., and Jin, Y. C. (2015). "Effect of complete dissolution in LiCl/DMSO on the isolation and characteristics of lignin from wheat straw internode," *Ind. Crop. Prod.* 74, 703-711. DOI: 10.1016/j.indcrop.2015.06.002

Jin, Y. C., Huang, T., Geng, W. H., and Yang, L. F. (2013). "Comparison of sodium carbonate pretreatment for enzymatic hydrolysis of wheat straw stem and leaf to produce fermentable sugars," *Bioresource Technol.* 137, 294-301. DOI: 10.1016/j.biortech.2013.03.140

Karmanov, A. P., and Derkacheva, O. Y. (2013). "Application of Fourier transform infrared spectroscopy for the study of lignins of herbaceous plants," *Russ. J. Coord. Chem.* 39(7), 677-685. DOI: 10.1134/S1068162013070066

Lan, W., Lu, F. C., Regner, M., Zhu, Y. M., Rencoret, J., Ralph, S. A., Zakai, U. I., Morreel, K., Boerjan, W., and Ralph, J. (2015). "Tricin, a flavonoid monomer in monocot lignification," *Plant Physiol.* 167(4), 1284-1295. DOI: 10.1104/pp.114.253757

Lu, F. and Ralph, J. (2010). "Lignin," in: *Cereal Straw as a Resource for Sustainable Biomaterials and Biofuels: Chemistry, Extractives, Lignins, Hemicelluloses, and*

Cellulose, R. C. Sun (ed.), Elsevier Press, Amsterdam, pp. 169-200.

Overend, R. P. and Johnson, K. G. (1991). "Lignin-carbohydrate complexes from poplar wood-isolation and enzymatic degradation," *ACS Symp. Ser.* 460, 270-287.

Petrus, L., Gray, D. G., and BeMiller, J. N. (1995). "Homogeneous alkylation of cellulose in lithium chloride/dimethyl sulfoxide solvent with dimsyl sodium activation. A proposal for the mechanism of cellulose dissolution in LiCl/Me_2SO," *Carbohyd. Res.* 268(2), 319-323. DOI: 10.1016/0008-6215(94)00330-l

Ralph, J., Lundquist, K., Brunow, G., Lu, F. C., Kim, H., Schatz, P. F., Marita, J. M., Hatfield, R. D., Ralph, S. A., Christensen, J. H., and Boerjan, W. (2004). "Lignins: Natural polymers from oxidative coupling of 4-hydroxyphenylpropanoids," *Phytochem. Rev.* 3 (1-2), 29-60. DOI: 10.1023/B:PHYT.0000047809.65444.a4

Sarkanen, K. V. and Hergert, H. L. (1971). "Classification and distribution," in: *Lignins: Occurrence, Formation, Structure, and Reactions*, K. L. Sarkanen and C. H. Ludwig (eds.), Wiley-Interscience, New York, pp. 43-94.

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, Golden, CO, USA.

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

Wu, W. J., Wang, Z. G., Jin, Y. C., Matsumoto, Y., and Zhai, H. M. (2014). "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

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