Deep Eutectic Solvent for Separation and Extraction of High Purity Lignin from *Salix babylonica* and its Characteristics

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A choline chloride-based deep eutectic solvent (DES) system utilizing oxalic acid and citric acid as hydrogen bond donors was used for lignin extraction from *Salix babylonica* biomass under controlled thermal conditions (130 °C). In choline chloride-oxalic acid system, lignin yield increased from 17.2% (1 h) to 66.1% (9 h) and was stabilized after 7 h of extraction. The optimal samples (DES-E1 and DES-E7, 130 °C/1 h and 7 h) showed high purity lignin (> 89%) while maintaining structural integrity. Both DES systems achieved recovery rates (63.3% and 53.6%) of natural lignin content after 7 h, respectively. Spectral characterization identified selective breaking of the β-O-4 bond between the syringyl (S) and guaiacyl (G) units, indicating DES-mediated bond modification. Furthermore, it was found that the carbon-rich macromolecular structure showed a gradual increase in C/O ratio with prolonged reaction time. This investigation provided a thermal regulation strategy for sustainable lignin extraction while establishing a new pathway for the utilization of *Salix babylonica* biomass through DES driven structural customization.

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INTRODUCTION

Lignocellulose is recognized as the most abundant renewable biological resource on earth, composed of cellulose, hemicellulose, and lignin. It is regarded as a promising clean energy alternative to fossil fuels and has been utilized for producing biofuels, biochemicals, and bioenergy (Guo *et al.* 2019). Lignin, a natural polyphenolic polymer, is ubiquitously present in plant cell walls as a structural component. This biopolymer content in terrestrial plants is second to cellulose, accounting for about one-third of the plant biomass; it serves the functions of keeping the plant body upright and preventing pests and diseases (Huang *et al.* 2024). Traditionally, lignin is often regarded as a low value by-product in the pulp industry. It is often casually discharged during the production process, or subjected to evaporation and concentration of the resulting black liquor for utilization as fuel in thermal energy production (Ponnusamy *et al.* 2019).

However, the high C/O ratio and polyphenol structure of lignin can allow it to serve as a precursor for manufacturing high-value products, such as adsorbents, carbon fibers, phenolic compounds, various oxidation products, and biofuels (Upton and Kasko 2016). Lignin contains three types of structural monomers, which are classified as G/S/H-type (guaiacyl, syringyl, and a small amount of p-hydroxyphenyl), with its structure that changes due to different pretreatment processes for biomass and the presence of functional groups (Bajwa *et al.* 2019). The complex structure of lignin arises primarily from intermonomeric linkages between monolignols through ether and carbon-carbon bonds. Some of the more common bond types include β-O-4 (β-aryl ether), β-β (resinol), and β-5 (phenylcoumaran). Analytical studies have indicated that lignin structure contains rich hydroxyl, methoxy, carbonyl, carboxyl and other different functional groups, making it a potential high polymer for various applications (Tarasov *et al.* 2018; Ma *et al.* 2022).

Given the potential product application value of lignin, it is essential to carry out biomass pretreatment steps before the product conversion process to promote its dissociation and fractionation. The fractionation of biomass components with high purity and structural completion is of great significance for the development of unique biopolymers. The primary challenge of efficient fractionation process lies in the structural rigidity of lignin-carbohydrate complexes (LCC), and the heterogeneity of lignin monomers and complexity of interunit connecting bonds in lignin. These molecular-level features collectively govern the difficulties in separation and extraction of high-purity lignin (Zhao *et al.* 2018).

Considering the serious pollution, high cost, and toxicity to living organisms caused by traditional chemical methods, green solvents have been introduced into the field of biomass processing (Sosa *et al.* 2020). Among these developed green biomass pretreatment methods, deep eutectic solvents (DES) have garnered significant attention because of their advantages, including low chemical cost, ease of synthesis, simple operation, low/non-toxic properties, and environmental friendliness (Lynam *et al.* 2017; Park *et al.* 2022). The DES are synthesized through stirring and mixing hydrogen bond acceptors (HBA) and hydrogen bond donors (HBDs) under mild heating conditions. In such systems, the HBAs and HBDs can self bind to form a homogeneous medium and low melting point of the eutectic mixture, resulting in a homogeneous liquid phase. The melting point of this simple solvent formed through hydrogen bonding interactions is much lower than the melting points of its components (Wang *et al.* 2020).

This study developed a feasible method for DES to selectively separate and extract high-purity lignin from *Salix babylonica*. During the pretreatment process, two distinct DES were prepared with choline chloride (ChCl) as the HBA and oxalic acid (OA) or citric acid (CA) as the HBDs. The effects of two DES systems on lignin extraction efficiency under different conditions were compared. The influence of treatment time on lignin extraction rate was investigated, leading to the identification of optimal dissolution conditions. Moreover, the structural properties of the recovered lignin were comprehensively characterized through Fourier transform infrared spectroscopy (FT-IR), gel permeation chromatography (GPC), and nuclear magnetic resonance (NMR). These analyses provided a practical reference for high-value utilization applications of the DES-derived lignin.

EXPERIMENTAL

Materials

In this study, the *Salix babylonica* wood chips employed were procured from a local industry in Shandong province, China. Prior to the experiment, the raw materials were thoroughly rinsed to remove impurities and let them naturally air dry. The dried raw materials were cut into small strips and then ground into 40 to 60 mesh powder in a grinder. To eliminate lipid interference, the powdered materials were extracted in an extractor with ethanol at 100 ℃ for 12 h. The purified material components were as follows: cellulose (43.8%), hemicellulose (22.8%), acid-insoluble lignin (25.7%), and acid-soluble lignin (1.9%). All chemical reagents used in the experimental process were sourced from Shanghai Macklin Biochemical Co., Ltd.

Synthesis of DES

Two different binary DES systems were prepared as ChCl-OA and ChCl-CA (molar ratio 1:4). The preparation process involved continuous mechanical agitation at 400 rpm in an oil bath at 60 ℃ for 1 h, until homogeneous liquid phases were obtained without visible particulate residues (Li *et al.* 2017). The mixture was cooled to room temperature and placed in a drying oven to remove any moisture from the DES system.

DES Treatment

A total of 4 g of raw material was mixed with 80 g of DES (biomass/DES ratio= 1:20) in a pressure resistant bottle and homogenized. The mixture was placed in an oil bath maintained at 130 °C with continuous magnetic stirring at 400 rpm for the set time. Upon completion of the thermal treatment, the pretreated solid-liquid mixture was cooled to room temperature and subsequently subjected to solid-liquid separation using a high-speed centrifuge operated at 8000 rpm for 10 min to achieve solid-liquid phase separation. Then, the solid residue was washed three times with deionized water and centrifuged through successive centrifugation cycles three times, then dried and weighed to determine the mass. The centrifuged liquid was combined with 800 mL of deionized water and allowed to stand for 12 h. Upon completion, the precipitate was centrifuged and circulated three times with deionized water. Finally, absolutely dry samples were obtained by freeze-drying for subsequent lignin analysis and characterization. For ease of documentation, the lignin samples derived from the DES synthesized by ChCl/OA with molar ratio of 1:4 and processed at 130 °C for 1 h and 7 h were labeled as DES-E1 and DES-E7, respectively. The ChCl/CA system reacted under the same conditions for 1 h and 7 h was labeled as DES-C1 and DES-C7.

Determination of Lignin Purity

The purity of the extracted lignin was measured through quantitative determination of acid soluble lignin (ASL) and acid insoluble lignin (AIL) by the National Renewable Energy Laboratory standard procedure (Sluiter *et al.* 2008).

$$\text{Lignin purity (100\%)} = \frac{\text{AIL weight+ ASL weight}}{\text{lignin weight}} \times 100\% \qquad (1)$$

Characterization of Lignin

The FT-IR spectrometer (Thermo Fisher, Waltham, MA, USA) was used to analyze the structure of lignin fractions, and the fractions were prepared by pressing potassium bromide tablets (mass ratio 1:100).

The molecular weight distribution of lignin fractions was determined by gel permeation chromatography (GPC) (Agilent, Santa Clara, CA, USA). Firstly, at room temperature, lignin was acetylated in a solution of acetic anhydride/pyridine (1/1, v/v) for 24 h, resulting in 50 mg of acetylated lignin. Then, the acetylated lignin (2 mg) was completely dissolved in tetrahydrofuran and filter through a 0.22-μ m filter.

The mass contents of carbon (C), hydrogen (H), nitrogen (N), and sulfur (S) in lignin fractions were analyzed and determined by a Vario EL Cube elemental analyzer (Elementar, Germany). The content of oxygen (O) was calculated using the difference method.

The information of lignin aromatic groups and side chain groups was obtained through 2D ^{1}H-^{13}C HSQC NMR analysis. A total of 80 mg of lignin fractions were completely dissolved in 0.5 mL of deuterated dimethyl sulfoxide (DMSO). The NMR spectra of lignin components were recorded at 25 ℃ on a 400 MHz spectrometer (AVIII400, Bruker, Germany) (Faleva *et al.* 2020). The scanning time for each group of lignin was 12 h.

The thermal stability analysis of lignin was conducted using a synchronous thermal analyzer (DTG-60, Shimadzu, Japan). The experiment conducted a thermal analysis study on the sample with the temperature of room temperature to 800 ℃, and was heated at a rate of 10 ℃/min.

RESULTS AND DISCUSSION

Lignin Yield, Solid Residue, and Lignin Purity of *Salix babylonica* Treated by ChCl-OA DES System

The effects of DES and pretreatment time (1 to 9 h) on lignin extraction were systematically studied under fixed molar ratio and temperature conditions (1:4, 130 ℃). The yield of lignin, solid residue, and lignin purity are summarized in Fig. 1.

The results indicated that the yield of lignin largely depends on the composition and duration of DES pretreatment. Specifically, in the ChCl-OA DES system, the lignin yield increased from 17.2% after 1 h of pretreatment to 66.1% after 9 h. However, the lignin yield remained stable for 7 h after pretreatment. In contrast, the solid residue yield showed a decreasing trend under the same pretreatment conditions, reaching 78.5% (1 h) and 50.4% (7 h), respectively.

Lignin fractions showed a trend of high purity (> 89%). The lignin extracted *via* the two DES methods exhibited significantly higher purity compared to that obtained through the conventional alkaline method (Cassoni *et al.* 2022). Given the structural integrity of lignin, lignin yield, and solid residue content, lignin obtained under reaction conditions at 130 °C for 1 h and 7 h were used for subsequent characterization and labeled as DES-E1 and DES-E7.

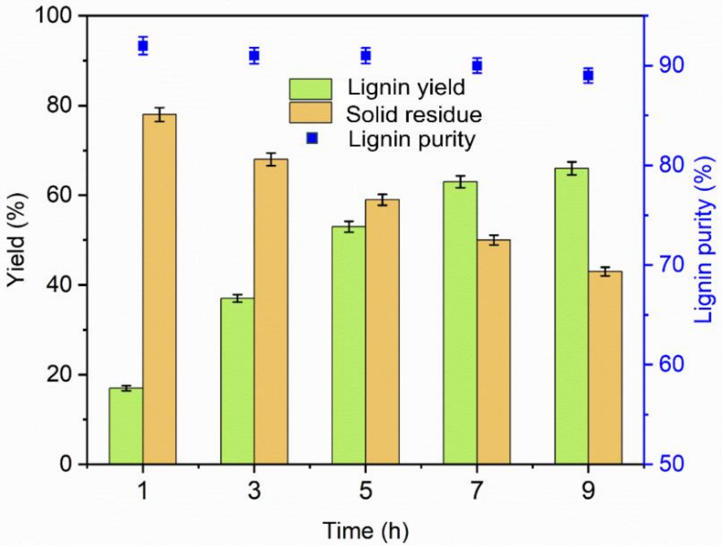

Fig. 1. Lignin yield, solid residue, and purity treated by ChCl-OA DES system

Lignin Yield, Solid Residue, and Lignin Purity of *Salix babylonica* Treated by ChCl-CA DES System

Lignin yield and solid residue yield of *Salix babylonica*, as well as lignin purity are presented in Fig. 2.

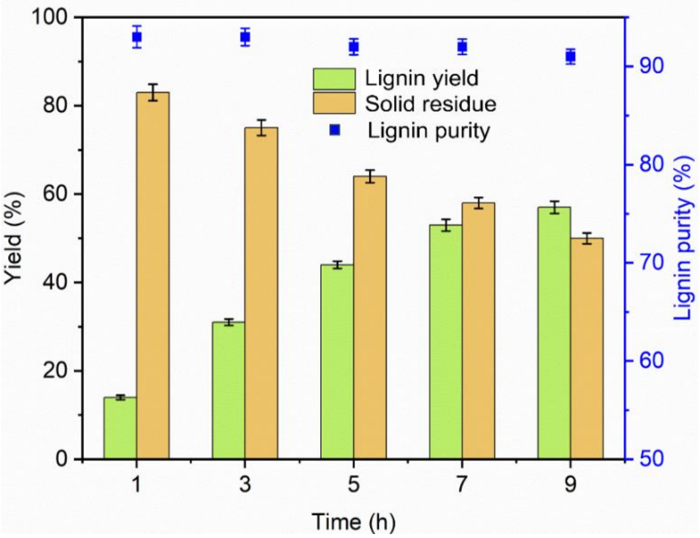

Fig. 2. Lignin yield, solid residue, and purity treated by ChCl-CA DES system

As shown, the lignin yield obtained from raw material under these conditions was clearly weaker than that under ChCl-OA conditions. The lignin yield exhibited a gradual increase with prolonged treatment time, reaching maximum values in the DES system after 9 h of treatment. The yield of lignin and solid residue were recorded as 61.2% and 51.3%, respectively. Notably, both lignin and solid residue yield remained stable after 7 h of treatment. As the treatment time was prolonged, the production of lignin had slightly increased, while the solid residue further decreased. These findings suggested that optimal treatment involved a molar ratio of 1:4 and extraction time of 7 h to achieve high lignin yield. Due to the formation of LCC complexes between lignin and carbohydrates, DES can only selectively extract lignin from raw materials (Lou *et al.* 2019). As a comparison with lignin obtained from the ChCl-OA DES system, DES-C1 and DES-C7 lignin were used for subsequent structural characterization.

FT-IR Analysis

The functional groups present in lignin fractions confirmed by the peak assignments in the FT-IR spectra were in close agreement with previous work (Li *et al.* 2016; Chen *et al.* 2019). The FT-IR spectra of all lignin fractions are shown in Fig. 3. The absorbance peaks at 1722 cm^{-1} were attributed to the C-O stretching of the unconjugated ketone and carbonyl groups of the lignin fractions (Muley *et al.* 2019). All lignin fractions exhibited bands at 1451 cm^{-1}, corresponding to C-H deformations (CH and CH_2) from phenol rings (de França Serpa *et al.* 2020). The band at 1216 cm^{-1} was assigned to the C-O group. Similarly, C-O vibration and C-O stretching peaks at 1266 and 1221 cm^{-1} demonstrated the presence of G/S units. The wideband near 831 cm^{-1} implied the presence of C-H out of plane deformation in G units, confirming the part of lignin studied in this work belonged to the G type (Morozova *et al.* 2024). The lignin obtained after DES treatment retained the integrity of its aromatic structure, with clear G/S type characteristic peaks observed, demonstrating that these two DES systems can effectively extract structurally intact lignin from raw materials under relatively mild conditions.

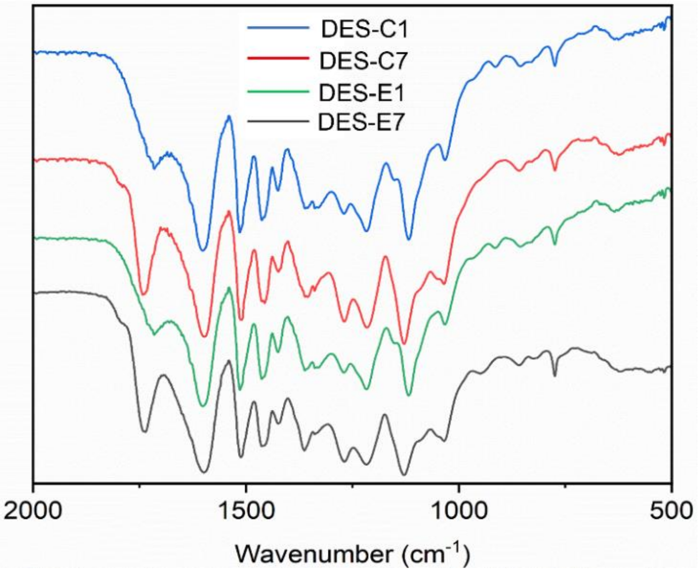

Fig. 3. FT-IR spectra of lignin fractions

Molecular Weight Analysis

The values of the average molecular weights (M_w and M_n) and polydispersity index (PDI) of recovered lignin are summarized in Fig. 4. Notably, the lignin treated with the ChCl-OA DES system exhibited higher molecular weight than lignin treated with the ChCl-CA system. Specifically, in the ChCl-CA system, the Mw value increased from 1612 g/mol after 1 h of pretreatment to 3897 g/mol after 7 h. In contrast, the ChCl-OA system showed an increase in molecular weight, with M_w value reaching 2045 g/mol (1 h) and 4327 g/mol (7 h) under the same pretreatment conditions. The lignin obtained from the DES system exhibited a marginally higher PDI (> 2) after being treated for 7 h, indicating relatively poor uniformity of lignin with prolonged reaction time. That disparity suggested that compared to systems based on ChCl-CA, DES based on ChCl-OA may induce different structural modifications of lignin through different depolymerization recondensation mechanisms. At equivalent time points, the higher M_w values in samples treated with ChCl-OA may be attributed to the stronger acidity of oxalic acid, leading to component mediated enhanced recombination reactions (Wang *et al.* 2020).

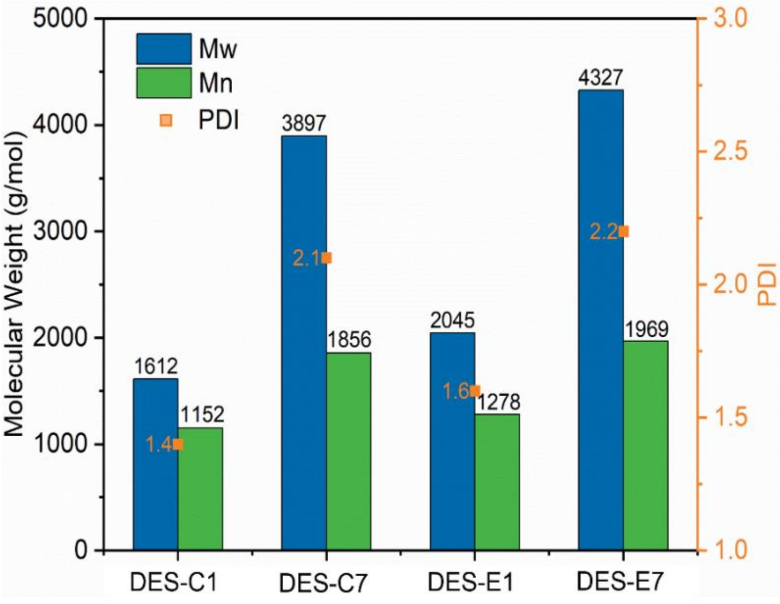

Fig. 4. Molecular weights and polydispersity of lignin fractions

Elemental Analysis

The lignin elemental content extracted by DES is shown in Table 1. According to previous research, the content of carbon and hydrogen in the original lignin was 50 to 65% and 5.0 to 6.5%, respectively (Li *et al.* 2020; Lu *et al.* 2022). In this work, it can be observed that all DES lignin fractions exhibited relatively high carbon content (60.6 to 64.4%) and low levels of hydrogen content (5.29 to 6.21%) and oxygen content (29.9 to 33.4%), confirming that lignin was a macromolecular compound characterized by high C/O ratios. Meanwhile, the C/O ratio of lignin in both systems increased with the prolongation of reaction time. The reason for this difficulty was attributed to condensation reactions initiated at 130 °C, where prolonged thermal exposure promoted carbon enrichment through structural reorganization.

Table 1. Elemental Analysis of Lignin Fractions

Sample	Elemental Composition (wt%)					C/O	C9 Formula
	C	H	O	N	S		
DES-C1	60.56	5.54	33.37	0.41	0.12	1.81	$C_9H_{9.82}O_{3.72}N_{0.05}$
DES-C7	63.53	6.21	29.91	0.33	0.02	2.12	$C_9H_{10.47}O_{3.18}N_{0.04}$
DES-E1	61.27	5.92	32.44	0.31	0.06	1.89	$C_9H_{10.36}O_{3.58}N_{0.04}$
DES-E7	64.42	5.29	29.88	0.28	0.13	2.16	$C_9H_{8.81}O_{3.13}N_{0.03}$

2D-NMR Analysis

The 2D-NMR spectroscopy can resolve overlapping signals, thereby offering more crucial structural information on lignin. Only the lignin obtained under optimal conditions was subjected to analysis. The side chain (δC/δH 50 to 90/2.5 to 6.0) and aromatic (δC/δH

100 to 150/6.0 to 8.0) regions of the 2D-NMR spectra of lignin fractions are shown in Fig. 5, and the structures of the main lignin substructures are depicted in Fig. 6. The signals were assigned according to previous literatures (Jensen *et al.* 2017).

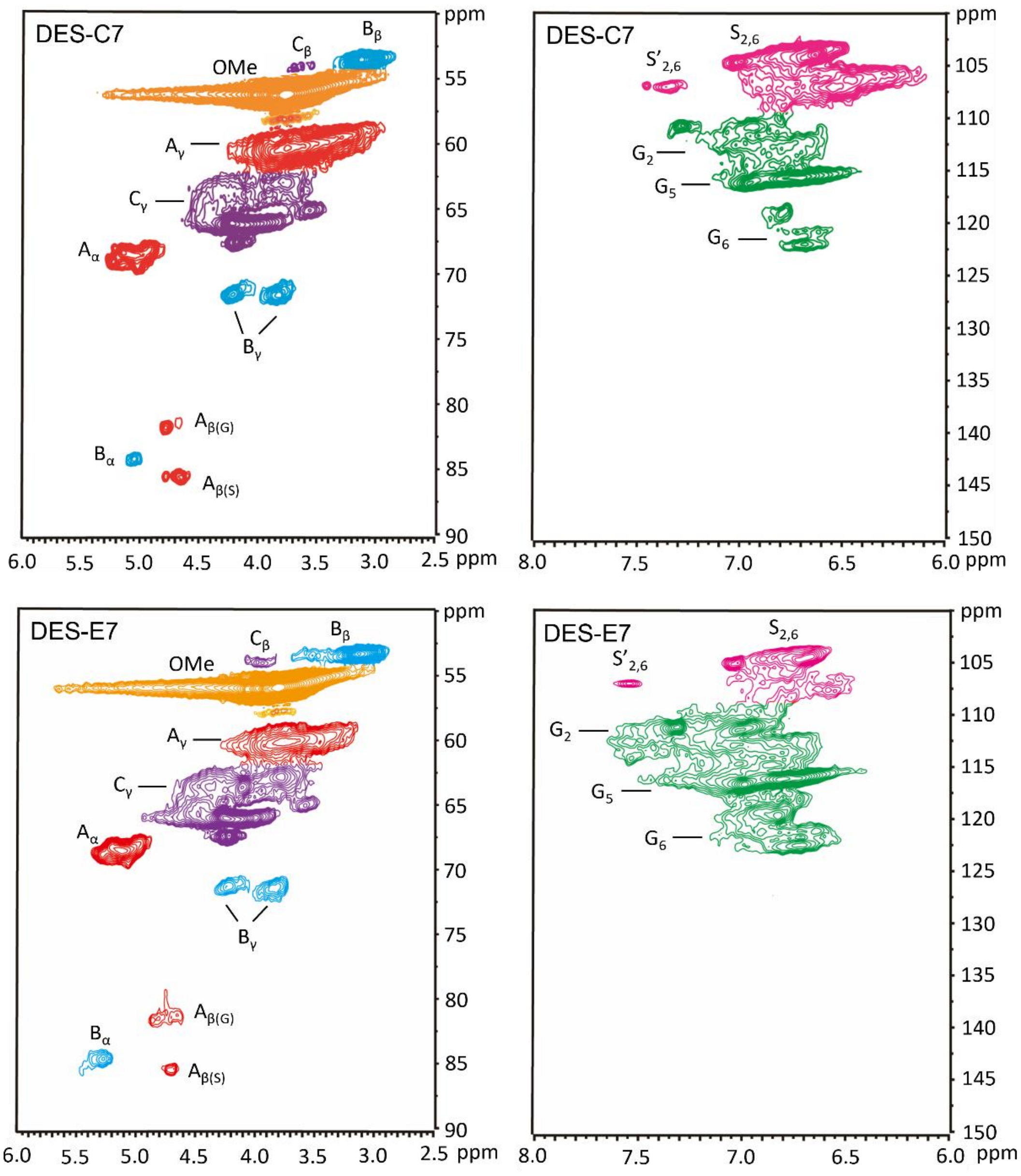

Fig. 5. Side chain regions and aromatic regions in the 2D-NMR spectra of lignin fractions

As illustrated in the side chain region of lignin samples, the intense signals of methoxyls located at δC/δH 55.6/3.71 were presented, indicating that abundant S and G units were present in the lignins fractionated from *Salix babylonica*. The most obvious linkages were methoxy groups (OMe, δC/δH 56.29/3.75) and β-O-4 ether bonds (A) (He *et al.* 2022). In general, the signals at δC/δH 71.69/4.75, 84.13/4.41, and 60.27/3.73 were related to C_α-H_α, C_β-H_β, and C_γ-H_γ of β-O-4 substructures, respectively. Additionally, β-β bonds in resinol (B) were also detected in the side chain region (Rencoret *et al.* 2015). The C_β-H_β linkages observed at δC/δH 84.1/4.23 [$A_{\beta(G)}$] and δC/δH 86.4/4.12 [$A_{\beta(s)}$] for β-O-4′

substructures were assigned to G and S units, respectively. The signals of C_β-H_β in the A substructure became weak, thereby indicating the oxidation of C_β and the cleavage of β-O-4 linkages. The signal for the C_α-H_α of phenylcoumaran substructures (C) correlation was discovered at δC/δH 87.5/5.56 in lignin fractions (Jensen *et al.* 2017). The signal for the C_β-H_β of (C) correlation was only discovered at δC/δH 53.05/3.72 in lignin (Wang *et al.* 2020).

In the aromatic regions (δC/δH 100.0 to 150.0/6.00 to 8.00), syringyl (S), guaiacyl (G) unit signals were unambiguously identified. For G units, the signals were all strong, including C_2-H_2 (δC/δH 111.5/6.96, G_2), C_5-H_5 (δC/δH 115.4/6.65, G_5), and C_6-H_6 (δC/δH 119.5/6.85, G_6). Additionally, the weak signals of the S′ at δC/δH 102.1/6.52 ($S'_{2,6}$) oxidized syringyl units were also observed in the 2D-HSQC spectra of lignin fractions (Rencoret *et al.* 2015).

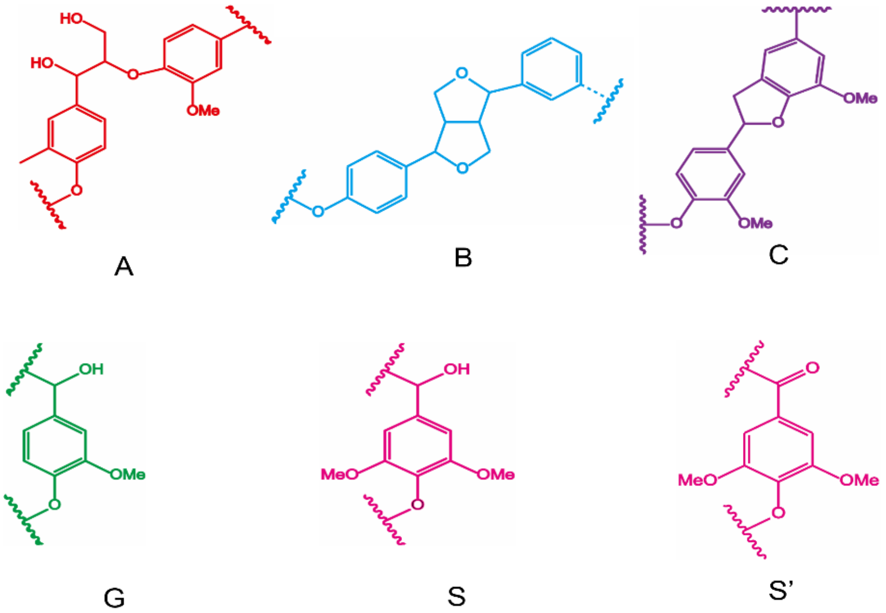

Fig. 6. Main structures observed of lignin fractions

Thermal Analysis

The results of lignin thermogravimetric analysis are shown in Fig. 7. The curves recorded the stability, residual carbon content, and maximum thermal degradation temperature (T_M) of lignin heated from room temperature to 800 °C. Lignin fractions experienced a similar trend of thermal degradation. The first stage of weight loss occurred below 130 ℃, which can be attributed to the evaporation of moisture in the sample at relatively low temperatures. The second stage of weight loss occurred in all samples within the temperature range of 150 to 500 ºC. Lignin was thermally degraded into small molecule phenolic compounds and charcoal, and the maximum thermal weight loss temperature of lignin occurs within this temperature range (Sun *et al.* 2019). The final stage occurred at 600 to 800 ℃, which was mainly due to the thermal decomposition of aromatic rings to produce carbon monoxide, as well as the formation of carbon through crosslinking of aromatic compounds (Ramakoti *et al.* 2019).

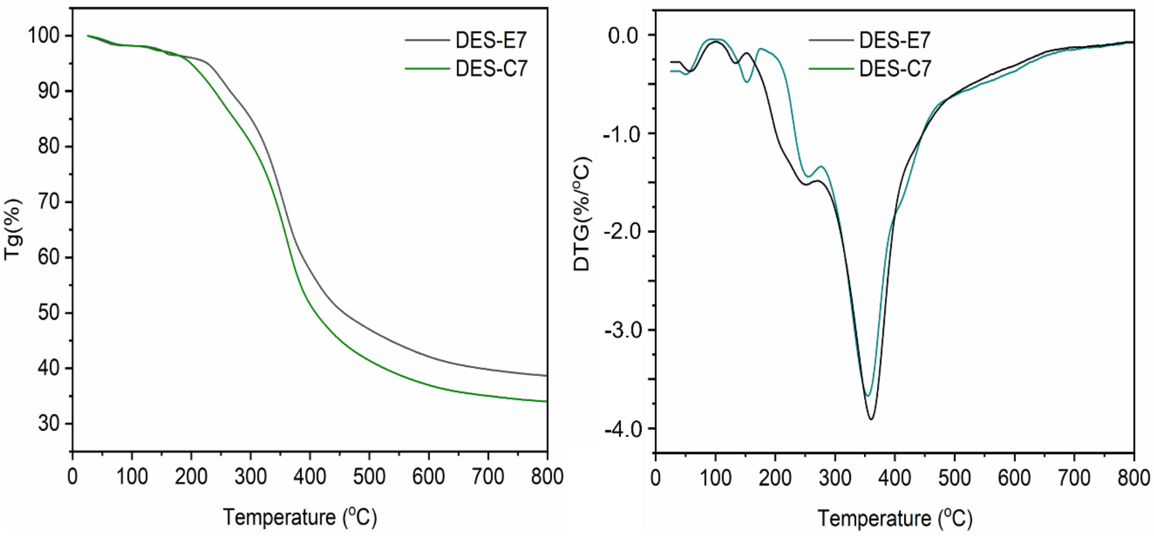

Fig. 7. TG curves and DTG curves of lignin fractions

CONCLUSIONS

1. The two systems achieved lignin recovery percentages of 63.3% and 53.6% relative to the original lignin content, respectively, after 7 h of pretreatment. Meanwhile, the choline chloride-oxalic deep eutectic solvent (DES) system exhibited superior performance on materials compared to the choline chloride-citric acid system.
2. The experimental results indicated that both types of lignin exhibited high purity (> 89%) with preserved structural integrity.
3. Lignin exhibited a carbon-oxygen macromolecular architecture with clearly elevated C/O ratios (60.6 to 64.4%), and the C/O ratio increased with the prolongation of reaction time.
4. The β-O-4 bond connecting the S and G groups in the DES lignin structure was most affected. Considering the high purity and high C/O ratio of lignin.

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REFERENCES CITED

Bajwa, D. S., Pourhashem, G., Ullah, A. H., and Bajwa, S. G. (2019). "A concise review of current lignin production, applications, products and their environmental impact," *Industrial Crops and Products* 139, article ID 111526. DOI: 10.1016/j.indcrop.2019.111526

Cassoni, A.C., Mota, I., Costa, P., Vasconcelos, M.W., and Pintado, M. (2022). "Effect of alkaline and deep eutectic solvents pretreatments on the recovery of lignin with antioxidant activity from grape stalks," *International Journal of Biological Macromolecules* 220, 406-414. DOI: 10.1016/j.ijbiomac.2022.07.233

Chen, Y., Zhang, L., Yu, J., Lu, Y., Jiang, B., Fan, Y., and Wang, Z. (2019). "High-purity lignin isolated from poplar wood meal through dissolving treatment with deep eutectic solvents," *Royal Society Open Science* 6(1), article ID 181757. DOI: 10.1098/rsos.181757

de França Serpa, J., de Sousa Silva, J., Borges Reis, C. L., Micoli, L., Alexandre E Silva, L. M., Canuto, K. M., Casimiro De Macedo, A., and Ponte Rocha, M. V. (2020). "Extraction and characterization of lignins from cashew apple bagasse obtained by different treatments," *Biomass and Bioenergy* 141, article ID 105728. DOI: 10.1016/j.biombioe.2020.105728

Faleva, A. V., Kozhevnikov, A. Y., Pokryshkin, S. A., Falev, D. I., Shestakov, S. L., and Popova, J. A. (2020). "Structural characteristics of different softwood lignins according to 1D and 2D NMR spectroscopy," *Journal of Wood Chemistry and Technology* 40(3), 178-189. DOI: 10.1080/02773813.2020.1722702

Guo, Z., Zhang, Q., You, T., Ji, Z., Zhang, X., Qin, Y., and Xu, F. (2019). "Heteropoly acids enhanced neutral deep eutectic solvent pretreatment for enzymatic hydrolysis and ethanol fermentation of *Miscanthus* x *giganteus* under mild conditions," *Bioresource Technology* 293, article ID 122036. DOI: 10.1016/j.biortech.2019.122036

He, M., He, Y., Li, Z., Zhao, L., Zhang, S., Liu, H. and Qin, Z. (2022). "Structural characterization of lignin and lignin-carbohydrate complex (LCC) of sesame hull," *International Journal of Biological Macromolecules* 209, 258-267. DOI: 10.1016/j.ijbiomac.2022.04.009

Jensen, A., Cabrera, Y., Hsieh, C., Nielsen, J., Ralph, J., and Felby, C. (2017). "2D NMR characterization of wheat straw residual lignin after dilute acid pretreatment with different severities," *Holzforschung* 71(6), 461-469. DOI: 10.1515/hf-2016-0112

Li, H., Deng, Y., Liang, J., Dai, Y., Liu, B., Ren, Y., Qiu, X., and Li, C. (2016). "Direct preparation of hollow nanospheres with kraft lignin: A facile strategy for effective utilization of biomass waste," *BioResources* 11(2), 3073-3083. DOI: 10.15376/biores.11.2.3073-3083

Li, T., Lyu, G., Liu, Y., Lou, R., Lucia, L., Yang, G., Chen, J., and Saeed, H. (2017). "Deep eutectic solvents (DESs) for the isolation of willow lignin (*Salix matsudana* cv. *Zhuliu*)," *International Journal of Molecular Sciences* 18(11), article ID 2266. DOI: 10.3390/ijms18112266

Li, T., Ma, H., Wu, S., and Yin, Y. (2020). "Effect of highly selective oxypropylation of phenolic hydroxyl groups on subsequent lignin pyrolysis: Toward the lignin valorization," *Energy Conversion and Management* 207, article ID 112551. DOI: 10.1016/j.enconman.2020.112551

Lou, R., Ma, R., Lin, K., Ahamed, A., and Zhang, X. (2019). "Facile extraction of wheat straw by deep eutectic solvent (DES) to produce lignin nanoparticles," *ACS Sustainable Chemistry and Engineering* 7(12), 10248-10256. DOI: 10.1021/acssuschemeng.8b05816

Lu, C., Xu, J., Xie, J., Zhu, S., Wang, B., Li, J., Zhang, F., and Chen, K. (2022). "Preparation, characterization of light-colored lignin from corn stover by new ternary deep eutectic solvent extraction," *International Journal of Biological Macromolecules* 222, 2512-2522. DOI: 10.1016/j.ijbiomac.2022.10.035

Lynam, J. G., Kumar, N., and Wong, M. J. (2017). "Deep eutectic solvents' ability to solubilize lignin, cellulose, and hemicellulose; thermal stability; and density," *Bioresource Technology* 238, 684-689. DOI: 10.1016/j.biortech.2017.04.079

Ma, S., Chen, B., Zeng, A., Li, Z., Tang, X., Sun, Y., Lin, L., and Zeng, X. (2022). "Chemical structure change of lignin extracted from bamboo biomass by maleic acid," *International Journal of Biological Macromolecules* 221, 986-993. DOI: 10.1016/j.ijbiomac.2022.09.002

Morozova, O., Vasil Eva, I., Shumakovich, G., Khlupova, M., Chertkov, V., Shestakova, A., and Yaropolov, A. (2024). "Green extraction of reed lignin: The effect of the deep eutectic solvent composition on the UV-shielding and antioxidant properties of lignin," *International Journal of Biological Macromolecules* 25(15), article ID 8277. DOI: 10.3390/ijms25158277

Muley, P. D., Mobley, J. K., Tong, X., Novak, B., Stevens, J., Moldovan, D., Shi, J., and Boldor, D. (2019). "Rapid microwave-assisted biomass delignification and lignin depolymerization in deep eutectic solvents," *Energy Conversion and Management* 196, 1080-1088. DOI: 10.1016/j.enconman.2019.06.070

Park, C. W., Han, S. Y., Park, J. S., Lee, E. A., Bandi, R., Dadigala, R., and Lee, S. H. (2022). "Deep eutectic-like solvent-assisted isolation of lignin from *Pinus densiflora* and its characteristics," *BioResources* 17(4), 5600-5611. DOI: 10.15376/biores.17.4.5600-5611

Ponnusamy, V. K., Nguyen, D. D., Dharmaraja, J., Shobana, S., Banu, J. R., Saratale, R. G., Chang, S. W., and Kumar, G. (2019). "A review on lignin structure, pretreatments, fermentation reactions and biorefinery potential," *Bioresource Technology* 271, 462-472. DOI: 10.1016/j.biortech.2018.09.070

Ramakoti, B., Dhanagopal, H., Deepa, K., Rajesh, M., Ramaswamy, S. and Tamilarasan, K. (2019). "Solvent fractionation of organosolv lignin to improve lignin homogeneity: Structural characterization," *Bioresource Technology Reports* 7, article ID 100293. DOI: 10.1016/j.biteb.2019.100293

Rencoret, J., Prinsen, P., Gutiérrez, A., Martínez, Á. T., and Del Río, J. C. (2015). "Isolation and structural characterization of the milled wood lignin, dioxane lignin, and cellulolytic lignin preparations from brewer's spent grain," *Journal of Agricultural and Food Chemistry* 63(2), 603-613. DOI: 10.1021/jf505808c

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.

Sosa, F. H. B., Abranches, D. O., Da Costa Lopes, A. M., Coutinho, J. A. P., and Da Costa, M. C. (2020). "Kraft lignin solubility and its chemical modification in deep eutectic solvents," *ACS Sustainable Chemistry and Engineering* 8(50), 18577-18589. DOI: 10.1021/acssuschemeng.0c06655

Sun, Y., Liu, X., Wang, T., Xue, B., and Sun, R. (2019). “Green process for extraction of lignin by the microwave-assisted ionic liquid approach: Toward biomass biorefinery and lignin characterization,” *ACS Sustainable Chemistry and Engineering* 7(15), 13062-13072. DOI: 10.1021/acssuschemeng.9b02166

Tarasov, D., Leitch, M., and Fatehi, P. (2018). “Lignin-carbohydrate complexes: Properties, applications, analyses, and methods of extraction: A review,” *Biotechnology for Biofuels* 11(1), article 269. DOI: 10.1186/s13068-018-1262-1

Upton, B. M., and Kasko, A. M. (2016). “Strategies for the conversion of lignin to high-value polymeric materials: Review and perspective,” *Chemical Reviews* 116(4), 2275-2306. DOI: 10.1021/acs.chemrev.5b00345

Wang, H., Liu, Z., Hui, L., Ma, L., Zheng, X., Li, J., and Zhang, Y. (2020). “Understanding the structural changes of lignin in poplar following steam explosion pretreatment,” *Holzforschung* 74(3), 275-285. DOI: 10.1515/hf-2019-0087

Wang, Y., Meng, X., Jeong, K., Li, S., Leem, G., Kim, K. H., Pu, Y., Ragauskas, A. J., and Yoo, C. G. (2020). “Investigation of a lignin-based deep eutectic solvent usingp-hydroxybenzoic acid for efficient woody biomass conversion,” *ACS Sustainable Chemistry and Engineering* 8(33), 12542-12553. DOI: 10.1021/acssuschemeng.0c03533

Wang, Z., Hong, S., Wen, J., Ma, C., Tang, L., Jiang, H., Chen, J., Li, S., Shen, X., and Yuan, T. (2020). “Lewis acid-facilitated deep eutectic solvent (DES) pretreatment for producing high-purity and antioxidative lignin,” *ACS Sustainable Chemistry and Engineering* 8(2), 1050-1057. DOI: 10.1021/acssuschemeng.9b05846

Zhao, B., Xu, J., Chen, B., Cao, X., Yuan, T., Wang, S., Charlton, A., and Sun, R. (2018). “Selective precipitation and characterization of lignin-carbohydrate complexes (LCCs) from eucalyptus,” *Planta* 247(5), 1077-1087. DOI: 10.1007/s00425-018-2842-9

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