Microwave-assisted Deep Eutectic Solvent Pretreatment of *Salix babylonica* for High-Purity and Antioxidative Lignin Production

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Two kinds of deep eutectic solvents (DES) were synthetized and utilized microwave-assisted technology to rapidly disrupt the recalcitrance of lignocellulosic biomass, thereby further enhancing the yield of lignin and obtaining lignin with high-purity and notable antioxidant properties. The DES system, synthetized with choline chloride/formic acid and choline chloride/tartaric acid in a molar ratio of 1:6, was carried out at 140 °C for efficient lignocellulosic biomass separation. Surprisingly, the application of microwave-assisted DES for extracting lignin from *Salix babylonica* wood was able to substantially shorten the conventional 12 h extraction process to 30 min while significantly improving the separation efficiency. Especially for the DES system synthetized with choline chloride and formic acid, after pretreatment for 30 min, the lignin yield was 70.8%. The lignin fractions had high purity (>88%) and low molecular weight (M_w 1756 to 2546 g/mol). Infrared spectroscopy and two-dimensional nuclear magnetic resonance revealed that the recovered lignin components retained intact aromatic structures, which showed typical structure of G/S-type. Furthermore, the DES lignin fractions exhibited excellent antioxidant properties compared with butyl hydroxyanisole (BHA), thereby laying a foundation for the value-added utilization of lignin.

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Keywords: Deep eutectic solvents; Microwave-assisted; Lignin; Antioxidant

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INTRODUCTION

Rapid industrialization has driven a substantial increase in the consumption of non-renewable resources, and energy shortages are an imminent concern (Cheng *et al.* 2022). Biomass resources are sustainable alternatives to address the limitations of non-renewable energy. Wood, as the most abundant biomass resource on earth, is mainly composed of cellulose, hemicellulose, and lignin (Bajwa *et al.* 2019). The lignin macromolecule has a three-dimensional network aromatic structure composed of three phenylpropanoid monomers (S, G, and H unit), with coupled aryl ether bonds (β-O-4) and carbon-carbon bond (β-β) (Zhang and Naebe 2021). Lignin is characterized by its high molecular weight with high C/O ratio and rich aromatic skeleton (Lobato-Peralta *et al.* 2021). The presence of abundant -OH, -COOH, and other active groups in lignin make it a valuable material for biochemical products (Li *et al.* 2016). Its active functional groups have unique antioxidant, UV resistant, and antibacterial properties, making it applicable across diverse fields including food, cosmetics, and agriculture. Lignin is superior to synthetic antioxidants in several aspects, including safety, effectiveness, and simple extraction process (Lu *et al.* 2022). Lignin, being a polyphenolic compound, can serve as a natural antioxidant to replace synthetic antioxidants in packaging applications. Its antioxidant activity depends on biomass source and extraction method, with higher phenolic hydroxyl content and lower molecular weight favoring enhanced performance. Chemical modifications including organic scavenger incorporation, methoxylation, amination, and hydroxymethylation can improve lignin's antioxidant capacity (Lu *et al.* 2022; Matos *et al.* 2021). Despite the notable reactivity of functional groups in lignin, challenges persist in its isolation from lignocellulosic biomass due to structural complexities, including intricate ether/carbon-carbon bond networks and resilient lignin-carbohydrate complexes (LCCs) (Huang *et al.* 2020).

Although there are many lignin separation techniques, such as acid pretreatment, alkali pretreatment, organic solvent treatment, and steam explosion, significant technical limitations persist in reagent preparation and application (Huang *et al.* 2020). Before these methods are widely applied, the issues should be addressed including cost pressure, mechanical stability, biodegradability of organisms, and especially the impact on the environment. Consequently, there is an urgent need for more effective ways to separate lignin from biomass. Deep eutectic solvents (DES) are an innovative class of solvents. It is expected that the primary bonds within lignin-carbohydrate complexes (LCC), including phenolic alkyl-aryl ether bonds (β-O-4′), are cleaved during DES pretreatment. This results in the separation of lignin from lignocellulosic biomass, while the major portion of carbohydrates is retained. This phenomenon may be attributed to the distinctive strong hydrogen bonding interactions characteristic of DES (Dai *et al.* 2014).

DESs are formed through hydrogen bond donors (HBD) and hydrogen bond acceptors (HBA) (Huang *et al.* 2020). These solvents exhibit significantly reduced melting points compared to their constituent components, and they are characterized by simple preparation, degradability, low cost, and renewability (Ruesgas-Ramón *et al.* 2017). Due to the excellent physicochemical profiles, DESs have been applied to wood fiber component separation and subsequent high-value utilization, including antioxidant and antibacterial properties (Anushikha and Gaikwad 2024; Yang *et al.* 2021). When DES is used for lignin separation from biomass, high-purity lignin is recovered, but there are serious drawbacks during pretreatment, such as low efficiency, long reaction times, and high processing temperature (Shen *et al.* 2019; Wang *et al.* 2025). Therefore, a thorough solution to the limitations of DES separation of biomass is necessary.

This study proposed an innovative solvent system employing microwave-assisted to achieve rapid separation of lignin from biomass while optimizing energy consumption and enhancing process efficiency. Two binary DES system of choline chloride (ChCl) combined with formic acid (Fa) and ChCl combined with tartaric acid (Ta) were designed at a molar ratio of 1:6 (based on preliminary investigation), through microwave-assisted pretreatment, was developed for extracting high-purity lignin from *Salix babylonica*. The influence of lignin antioxidant by microwave-assisted was compared to commercial antioxidants of butyl hydroxyanisole (BHA). To elucidate the functional groups and structure, the recovered DES lignin were comparatively studied by FT-IR, GPC, 2D-HSQC techniques to characterize the structural features. The results showed that microwave-assisted extraction of lignin achieved a remarkable enhancement in separation efficiency, while preserving a substantial proportion of structural integrity within the extracted components. Concurrently, these lignin fractions exhibited prominent antioxidant capacity compared with commercial antioxidants.

EXPERIMENTAL

Materials

Salix babylonica wood chips were obtained from a local industry in Shandong Province, China. Wooden strips were cut into cubic blocks (2 cm× 2 cm× 0.5 cm), then ground and sieved to a particle size of 40- to 60-mesh using high-speed grinder. Wood powder was extracted with ethanol to remove lipid substances under reflux conditions at 100 ℃ for 12 h. The chemical compositions of *Salix babylonica* were as follows: acid-insoluble lignin 25.71%, acid-soluble lignin 1.89%, cellulose 43.81%, hemicellulose 22.75%, and ash 1.35%. All chemical reagents used in the experimental process were purchased from Shanghai McLean Chemical Reagent Company.

Preparation of DES

The ChCl (dried in vacuum for 12 h) was combined with Fa and Ta in a certain molar ratio (1:6) in a single port flask to synthesize DES system. The reaction was carried out in an oil bath, maintaining a temperature of 60 °C with continuous stirring at 400 rpm for 1.5 h, resulting in the formation of a transparent and homogeneous liquid. Upon completion, the mixture was allowed to cool to room temperature.

DES Treatment

The DES treatment of materials were carried out in a single reactor with a mixture ratio of 1:20 (4.0 g dry materials and 80.0 g DES). Using the MICROSYNTH microwave accelerated reaction system (Milestone Srl, Sorisole, Italy), the pretreatment temperature was raised from room temperature to 140 ℃ under 400 W microwave radiation for 1 min while maintaining the set pretreatment time. After the DES pretreatment, the reaction mixture was diluted with 1 L deionized water, then left to settle for 12 h. The precipitated lignin was obtained by centrifuging the mixture.

Determination of Lignin Purity

To assess the lignin purity after DES pretreatment, both acid-soluble lignin (ASL) and acid-insoluble lignin (AIL) were determined according to the standard from National Renewable Energy Laboratory procedure (Sluiter *et al.* 2008). Determination of ASL components at 205 nm by UV-VIS spectrophotometry (UV-3600Plus, Shimadzu, Japan).

Characterization of Lignin

FT-IR (Thermo, Waltham, MA, USA) was used to characterize the structure of DES lignin fractions. The sample was prepared using KBr compression method. Mixing lignin powder with KBr and compressed it in a weight ratio of 1:100 to prepare a transparent film for characterization.

The molecular weight distribution of lignin was determined with use of a Shodex KF804L column (Japan). Tetrahydrofuran (THF) was used as the mobile phase, and the flow rate of THF was 0.5 $mL\cdot min^{-1}$, and the temperature was 25 ℃.

The 2D-NMR of lignin was recorded at 25 ℃ on a 400 MHz NMR spectrometer

(AVIII400, Bruker, Germany). About 60 mg of lignin fractions were dissolved in 0.5 mL of DMSO-d6, and the scanning time for each group of lignin samples was 12 h (Cui *et al.* 2024).

The thermal stability of the lignin was determined by using a thermogravimetric analyzer, TG 209 F Tarsus (Netzsch Instruments) under controlled conditions. The temperature range of 25 to 800 °C was employed, with a heating rate of 10ºC/min under N_2 atmosphere.

Assessment of Antioxidant Capacity

The inhibitory ability of lignin on DPPH free radicals was determined using a UV spectrophotometer (UV-3600Plus, Shimadzu, Japan). The lignin fractions were completely dissolved in a water/dioxane mixture (1:9, v/v). Then, added 0.1 mL of sample solution to 3.9 mL of 25 mg/L DPPH ethanol solution and let it stand at room temperature in the dark for 30 min. The UV absorption wavelength of the solution was measured at 517 nm (Li *et al.* 2023). The DPPH radical inhibitory activity of lignin was calculated using formula:

$$\text{DPPH scavening activity (\%)} = \frac{A_m - A_n}{A_m} \times 100\% \tag{1}$$

where A_m is the absorbance of sample without lignin, and A_n is the absorbance of lignin.

RESULTS AND DISCUSSION

Solid Residue, Lignin Yield, and Purity of Materials Treated by DES

Based on the lignin content in raw materials, the following parameters were determined after DES pretreatment: the yield of lignin, the purity of lignin, and the yield of solid residue, as shown in Fig. 1.

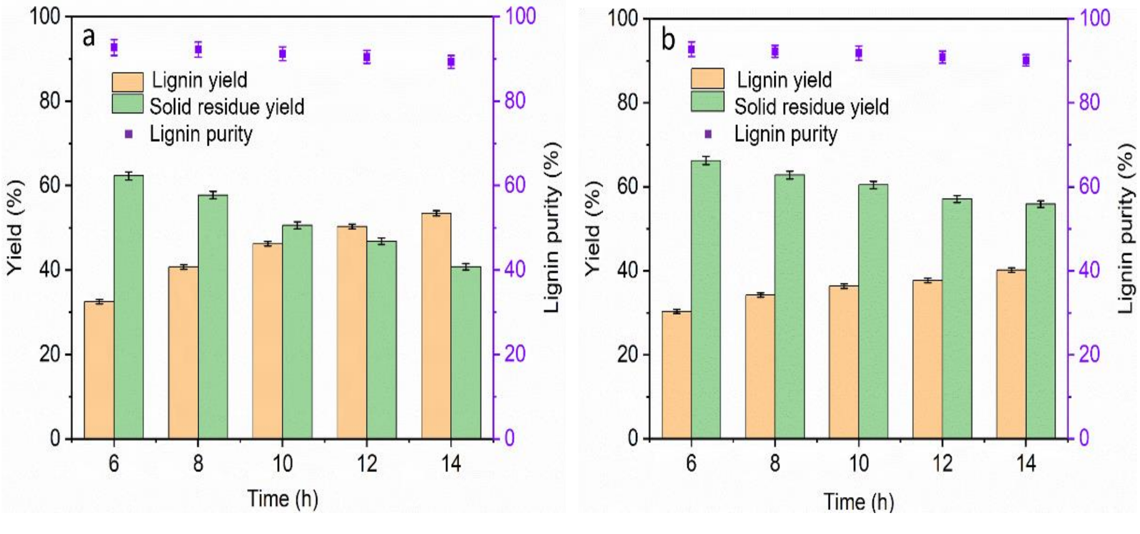


Fig. 1. DES lignin yield, solid residue, and purity of lignin under the condition of 140°C, 1:6; (a) the choline chloride - formic acid system; (b) the choline chloride - tartaric acid system

Without microwave-assisted treatment, it took 12 h of pretreatment to observe the obvious degree of extraction, while the lignin yield was 50.3% of ChCl-Fa DES system. From Fig. 1(a, b), after 14 h of pretreatment, the lignin extraction extents of ChCl-Ta and

ChCl-Fa DES systems were 40.2% and 53.5%, and solid residues were 55.9% and 40.2%, respectively. The recovered lignin had a high purity (>89%). The yield of lignin did not significantly increase when the pretreatment time exceeded 12 h. Therefore, the lignin obtained from DES synthetized with ChCl-Fa under the condition of 12 h was taken as the reference in the subsequent analysis and characterization, which was labeled as DES-F.

Microwave-Assisted Enhanced Separation of Lignin by DES

The lignin yield and its parameters obtained from microwave-assisted DES treatment of materials are shown in Fig. 2(c, d). When microwave was applied, significantly enhanced lignin removal efficiency in the pretreatment process. For the DES system synthetized with ChCl and Fa, after 40 min pretreatment, the yield of lignin and solid residue were 71.5% and 35.8%, respectively. For ChCl-Ta DES system, it also showed a high lignin extraction efficiency, with lignin yield and solid residue was 64.8% and 37.4%. The microwave-assisted DES system could significantly improve the extraction efficiency of lignin and shorten the pretreatment time. Although significant separation effects were observed after 40 min of pretreatment, the yield of lignin remained stable after 30 min of pretreatment. Therefore, the lignin fractions obtained through 30 min pretreatment using ChCl-Fa and ChCl-Ta based DES were designated as a reference sample for subsequent analysis and characterization, which were labeled as DES-W-F and DES-W-T, respectively.

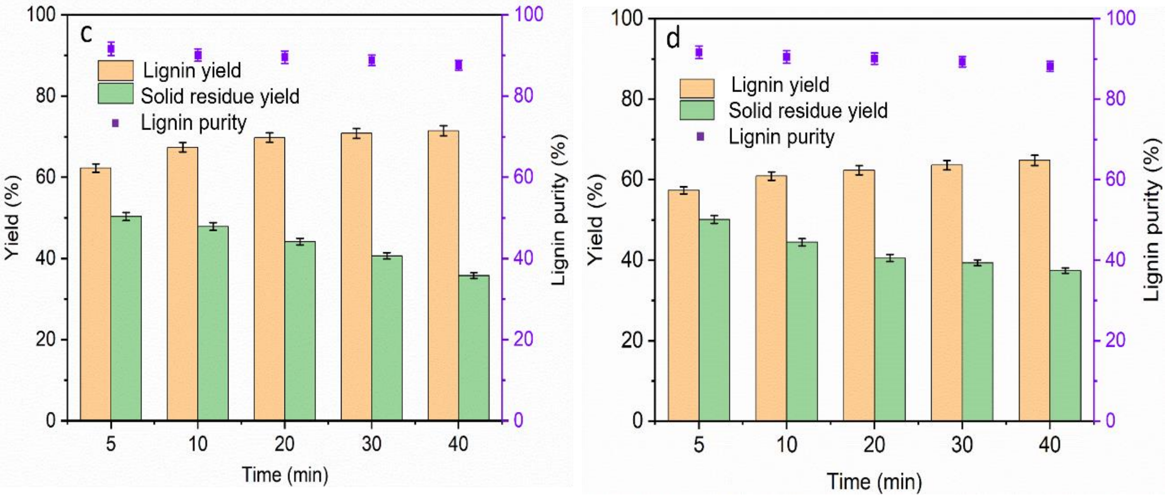


Fig. 2. DES lignin yield, solid residue, and purity yield of lignin with microwave-assisted under the condition of 140 °C, 1:6; (a) the choline chloride - formic acid system; (b) the choline chloride - tartaric acid system

FT-IR Spectra Analysis

The analytical characterization of lignin was performed using fractions obtained under optimal conditions, which included DES-F, DES-W-T, and DES-W-F. The chemical structure of three lignins fractions were analyzed by FT-IR. The spectra are shown in Fig. 3. All samples presented a similar spectra profile, the typical characteristic peak occurred at 1591 cm^{-1} were corresponded to aromatic skeletal vibrations and C=O stretching (Sun *et al.* 2021). The bands at 1516, and 1427 cm^{-1} corresponded to aromatic skeletal vibrations, and narrow band at 1459 cm^{-1} was caused by the C-H bending and C-C stretching of methoxyl (Liu *et al.* 2024). The findings demonstrated that the aromatic structure of lignin remained largely unaltered, indicating that microwave-assisted pretreatment of biomass can effectively preserve the integral structure of lignin. The strong band observed at 1121, 1267, and 1332 cm^{-1} were attributed to vibrations in syringyl and guaiacy units which appeared in all lignin samples (Nie *et al.* 2024). The tiny absorption peaks at 916 cm^{-1} represented the C–H out-of-plane deformation.

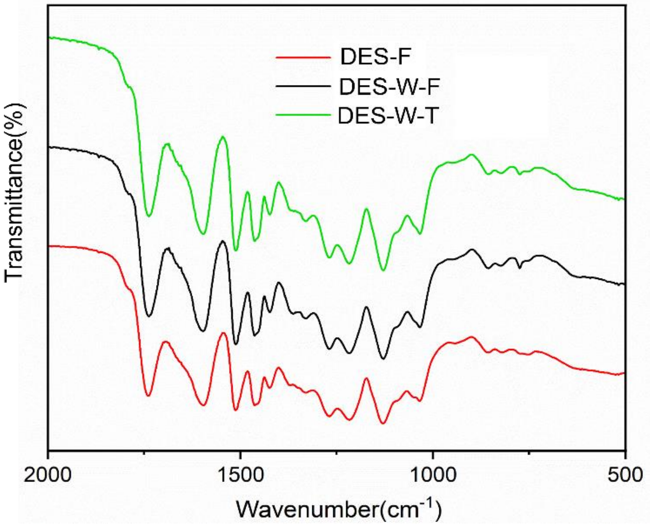


Fig. 3. FT-IR spectra of the fractionated lignin

Molecular Weight Analysis

The molecular weight (M_w and M_n) and polydispersity index (PDI) of DES-F, DES-W-F, and DES-W-T were analyzed by GPC, and the results are shown in Fig. 4. The results showed that the molecular weight of DES extracted lignin could be reduced by using microwave-assisted extraction.

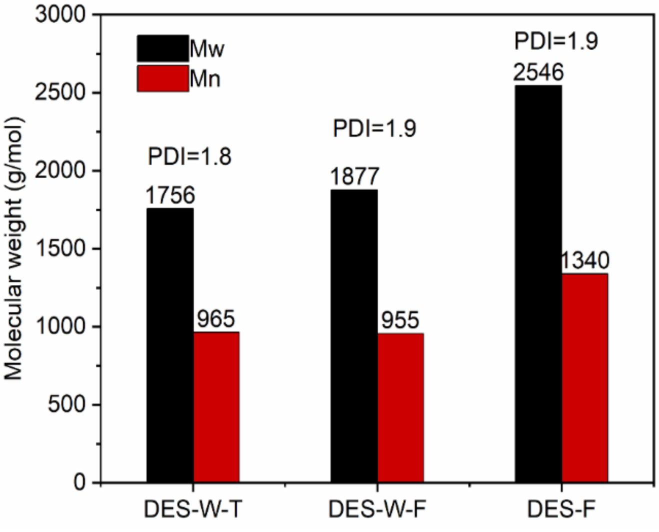
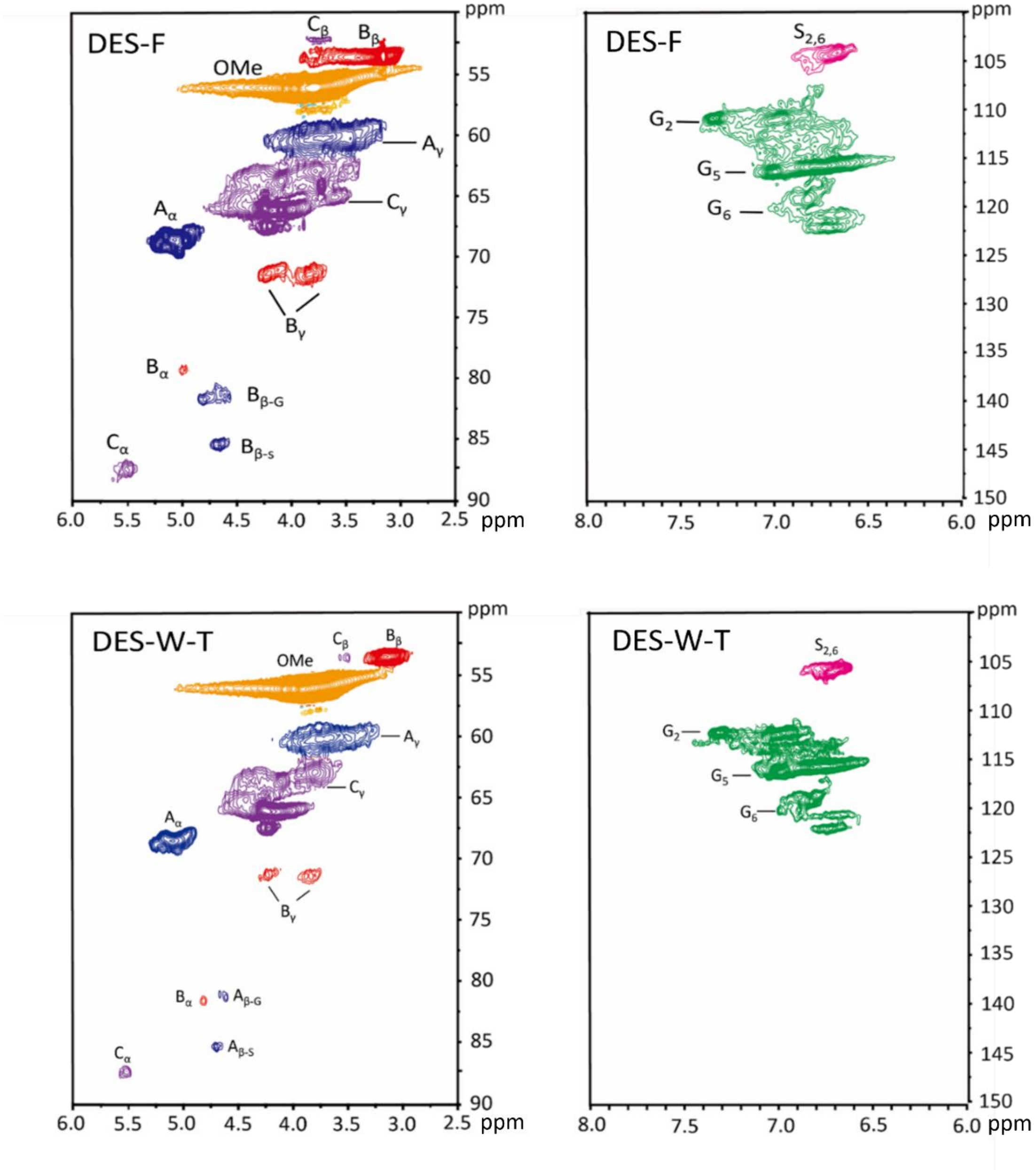


Fig. 4. Molecular weight of the fractionated lignin

The M_w of DES-W-T and DES-W-F lignin increased from 1756 to 1877 g/mol, but the values were significantly smaller than DES-F lignin (2546 g/mol). The M_n of three lignin fractions also showed a similar trend. The M_n of DES-W-T, DES-W-F and DES-F were 965, 955, 1340 g/mol, respectively. The results showed that the PDI of lignin ranged from 1.8 to 1.9, indicating that the molecular weight dispersion range of DES-treated lignin was relatively wide. This may be due to the rapid separation of lignin from the raw materials by microwave treatment, which promoted the condensation reaction of lignin and increased its molecular weight under high-temperature acidic conditions.

2D-NMR Analysis

The method of 2D-HSQC NMR elaborated important information in the expression of aromatic structure and side chain characteristics of lignin, including unit connectivity and unit types. The spectra information of the lignin fractions in the side-chain regions (δC/δH 90-50/6.0-2.5) and aromatic regions (δC/δH 150-100/8.0-6.0) are depicted in Fig 5. The HSQC spectra of DES-F, DES-W-T and DES-W-F and the key substructures in lignin are shown in Fig. 6. The ^{13}C-^{1}H signal assignments was based on previous literature reports as references (Wen *et al.* 2014).



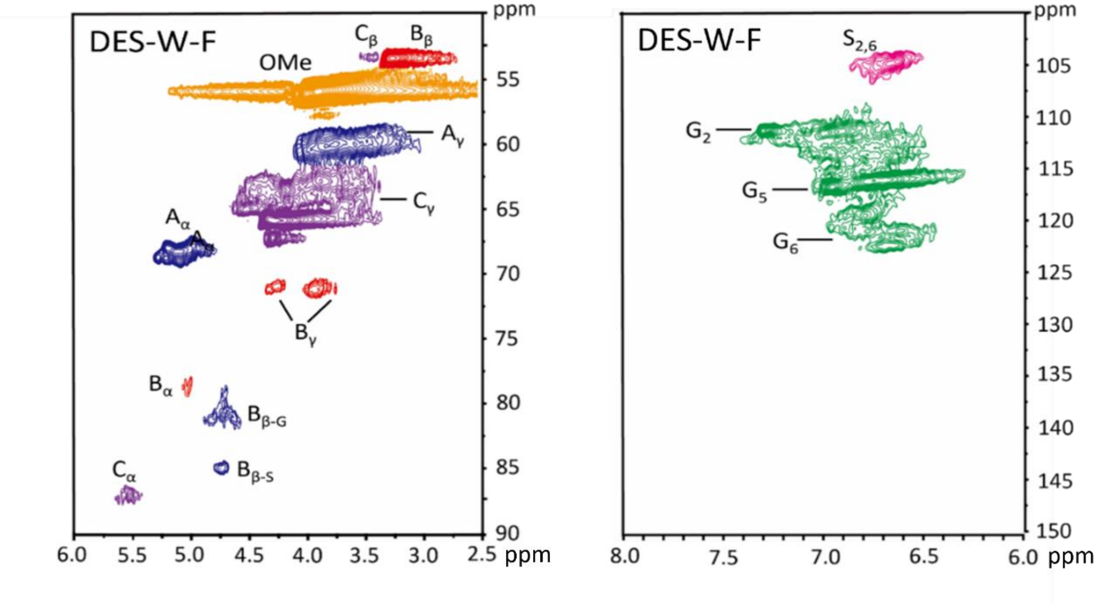
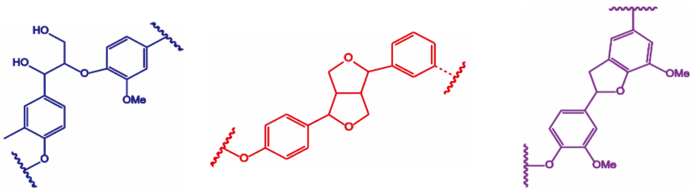


Fig. 5. 2D-NMR spectra of the fractionated lignin



A
β-aryl-ether units

B
Resinol substructures

C
Phenylcoumaran substructures

G
Guaiacyl unit

S
Syringyl unit

Fig. 6. Main structures observed in lignin fractions

In the side-chain region, the obvious signals of methoxyls identified at δC/δH 56.8/ 3.85 ppm were detected, indicating that abundant S and G units were present in the fractionated lignin. The aliphatic region of lignin of β-O-4′ (A) were linked at δC/δH 69.6/5.12 (C_α-H_α, A unit), and δC/δH 60.1/3.82 (C_γ-H_γ, A unit) (Li *et al.* 2021). The signals belonging to inter-unit linkages β-β (B) at δC/δH 82.1/4.64 (C_α-H_α, B unit) and δC/δH 71.1/3.81 (C_γ-H_γ, B unit) were also clearly recognized (Wang *et al.* 2022). The δC/δH 85.5/4.58 and δC/δH 71.2/4.21 band was assigned to the inter-unit linkages β-5 (C) of C_α-H_α, C unit and C_γ-H_γ, C unit, respectively (Wang *et al.* 2020). The intensity signal of classical units connected with inter-units, β-aryl ether (A), phenyl coumaran (B), and resinol (C) were clearly appeared, that indicating the complete structure of DES-F, DES-W-T and DES-W-F lignin.

In the aromatic regions, the cross-signals from basic structural detected at δC/δH

104.8, /6.74, ($S_{2,6}$, S unit), δC/δH 110.9/6.91 (G_2, G unit), 115.7/6.61 (G_5, G unit), and 118.1/6.80 (G_6, G unit) coincided with the correlations of syringyl (S) and guaiacyl (G), respectively (Hong *et al.* 2020).

Thermal Stability Analysis

To understand the thermal properties of lignin and master the maximum weight loss temperature range, the lignin fractions were characterized by thermogravimetric analysis (TGA), and the curves are shown in Fig. 7. The thermogravimetric analysis curves of the three lignin fractions showed similar three-stage thermal decomposition process, but the characteristics were different. The tiny thermogravimetric phase of lignin, occurring at 25 to 150 °C, was mainly due to the evaporation of water (Guo *et al.* 2019). The second, more pronounced weight loss stage in the three lignin samples occurred between 160 and 500 °C, which was mainly due to the pyrolysis of lignin (Li *et al.* 2021). The main products involved in this stage were phenolic compounds, organic substances, and char. The DTG curves showed the thermogravimetric rate of DES-W-F and DES-W-T lignin was higher than that of DES-F, and the maximum decomposition temperature (T_M) occurred in the range 330 to 380 °C (Song *et al.* 2024). The third, and slower, pyrolysis stage occurred after 500 °C. The residual char yield of DES-F lignin was slightly higher when compared to that two of lignin recovered from microwave-assisted DES. All lignins resulted in a notable amount of ash residue, accounting for about 36.9 wt.% of DES-F, 33.5 wt.% of DES-W-T and 31.8 wt.% of DES-W-F, respectively.

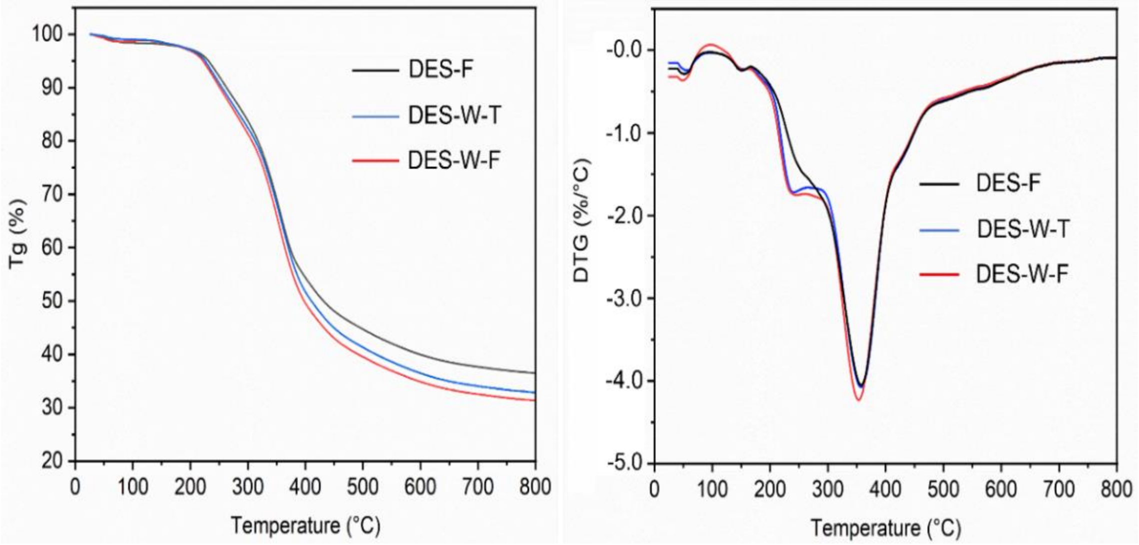


Fig. 7. Thermal stability analysis of the fractionated lignin

Antioxidant Activity Analysis

The antioxidant activity of lignin fractions were evaluated by using DPPH radical scavenging assays, and the results are shown in Fig. 8. All lignin fractions extracted through DES exhibited the ability to scavenge DPPH, and radical scavenging rate was increased with increasing lignin concentration. The free radical scavenging ability of lignin was significantly higher than that of commercial BHA. Among three lignin fractions, DES-F lignin achieved the most significant scavenging ability. When lignin concentration was 0.05 mg/mL, the inhibition percentages of DES-F, DES-W-F and DES-W-T fractions were 41.3%, 36.5%, and 32.1%, respectively. When the concentration of lignin increased to 0.5

mg/mL, the inhibition percentages of DES-F, DES-W-F and DES-W-T fractions were 83.4%, 80.2%, and 77.3 %, which were significantly higher than BHA (69.1%). In addition, the IC50 data revealed that DES-F lignin exhibited the lowest value (IC50=0.12), demonstrating the most potent antioxidant activity among the three fractions. The results showed that the antioxidant activity of all lignin fractions was significantly higher than that of BHA. Moreover, it was also superior to that of alkali-extracted sugarcane lignin (Kaur and Uppal 2015). Therefore, microwave-assisted extracted lignin could be further developed into a potential antioxidant packaging material for food.

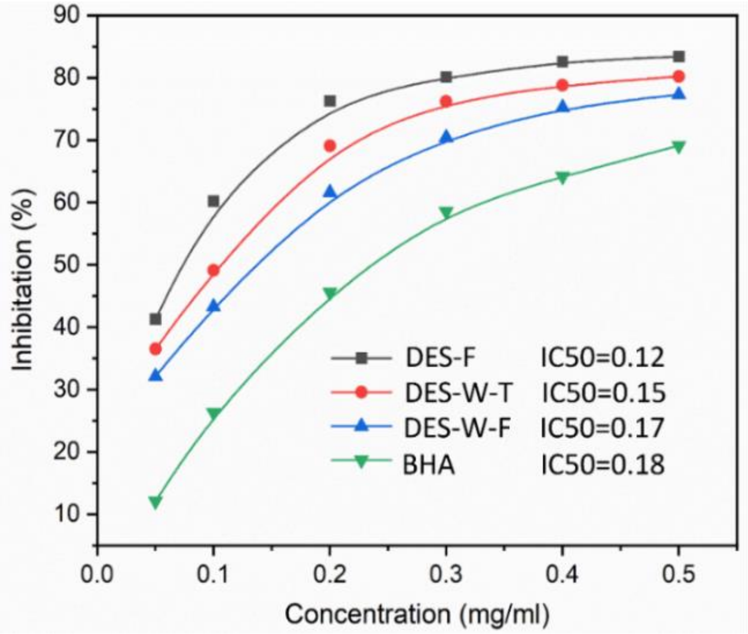


Fig. 8. Antioxidant activity of the fractionated lignin

CONCLUSIONS

1. The results indicated that the extraction efficiency of lignin was shortened from 12 h to 30 min in the process of microwave-assisted extraction with deep eutectic solvent (DES) pretreatment, achieving a lignin yield of 70.8% with the purity of 88.3% under 30 min processing conditions.
2. The isolated lignin demonstrated exceptional purity exceeding 88% accompanied by a relatively low weight-average molecular weight (M_w 1756 to 2546 g/mol).
3. Structural characterization manifested well-preserved aromatic architectures in lignin fractions, with typical guaiacyl to syringyl (G/S) subunit composition confirmed by 2D-NMR analysis.
4. The recovered lignin exhibited significantly superior radical scavenging capacity compared to commercial butylhydroxyanisole (BHA), demonstrating its potential for high-value utilization.

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REFERENCES CITED

Anushikha, and Gaikwad, K. K. (2024). "Lignin as a UV blocking, antioxidant, and antimicrobial agent for food packaging applications," *Biomass Conversion and Biorefinery* 14 (15), 16755-16767. DOI: 10.1007/s13399-022-03707-3

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 111526. DOI: 10.1016/j.indcrop.2019.111526

Cheng, J., Huang, C., Zhan, Y., Han, S., Wang, J., Meng, X., Yoo, C. G., Fang, G., and Ragauskas, A. J. (2022). "Effective biomass fractionation and lignin stabilization using a diol DES system," *Chemical Engineering Journal* 443, article 136395. DOI: 10.1016/j.cej.2022.136395

Cui, J., Chen, R., Lei, L., and Hou, Y. (2024). "Green wood pulping processes with high pulp yield and lignin recovery yield by deep eutectic solvent and its aqueous solutions," *Biomass Conversion and Biorefinery* 14(13), 13987-14001. DOI: 10.1007/s13399-022-03498-7

Dai, Y., Verpoorte, R., and Choi, Y.H. (2014). "Natural deep eutectic solvents providing enhanced stability of natural colorants from safflower (*Carthamus tinctorius*)," *Food Chemistry* 159, 116-121. DOI: 10.1016/j.foodchem.2014.02.155

Guo, Z., Zhang, Q., You, T., Zhang, X., Xu, F., and Wu, Y. (2019). "Short-time deep eutectic solvent pretreatment for enhanced enzymatic saccharification and lignin valorization," *Green Chemistry* 21(11), 318-399. DOI: 10.1039/c9gc00704k

Hong, S., Shen, X., Pang, B., Xue, Z., Cao, X., Wen, J., Sun, Z., Lam, S.S., Yuan, T., and Sun, R. (2020). "In-depth interpretation of the structural changes of lignin and formation of diketones during acidic deep eutectic solvent pretreatment," *Green Chemistry* 22(6), 1851-1858. DOI: 10.1039/d0gc00006j

Huang, D., Li, R., Xu, P., Li, T., Deng, R., Chen, S., and Zhang, Q. (2020). "The cornerstone of realizing lignin value-addition: Exploiting the native structure and properties of lignin by extraction methods," *Chemical Engineering Journal* 402, article 126237. DOI: 10.1016/j.cej.2020.126237

Kaur, R., and Uppal, S. K. (2015). "Structural characterization and antioxidant activity of lignin from sugarcane bagasse," *Colloid and Polymer Science* 293(9), 2585-2592. DOI: 10.1007/s00396-015-3653-1

Li, H., Li, X., You, T., Li, D., Nawaz, H., Zhang, X., and Xu, F. (2021). "Insights into alkaline choline chloride-based deep eutectic solvents pretreatment for *Populus deltoides*: Lignin structural features and modification mechanism," *International Journal of Biological Macromolecules* 193, 319-327. DOI: 10.1016/j.ijbiomac.2021.10.134

Li, P., Lu, Y., Long, G., Li, S., Li, K., Jiang, B., and Wu, W. (2023). "Structural characterization of acid DES-modified alkaline lignin and evaluation of antioxidant properties," *Forests* 14(3), article 550. DOI: 10.3390/f14030550

Li, S., Liu, S., Colmenares, J.C., and Xu, Y. (2016). "A sustainable approach for lignin valorization by heterogeneous photocatalysis," *Green Chemistry* 18(3), 567-594. DOI: 10.1039/c5gc02109j

Li, X., Guo, T., Li, M., and Peng, F. (2021). "Comparison of structure, thermal stability, and pyrolysis products of lignin extracted with ChCl-formic acid/lactic acid systems," *Journal of Materials Research and Technology* 14, 841-850. DOI: 10.1016/j.jmrt.2021.07.017

Liu, Z., Hou, Y., and Hu, S. (2024). "Rapid microwave-assisted pretreatment using a ternary deep eutectic solvent for lignin fractionation of eucalyptus," *Industrial Crops and Products* 212, article 118297. DOI: 10.1016/j.indcrop.2024.118297

Lobato-Peralta, D. R., Duque-Brito, E., Villafán-Vidales, H. I., Longoria, A., Sebastian, P. J., Cuentas-Gallegos, A. K., Arancibia-Bulnes, C. A., and Okoye, P. U. (2021). "A review on trends in lignin extraction and valorization of lignocellulosic biomass for energy applications," *Journal of Cleaner Production* 293, article 126123. DOI: 10.1016/j.jclepro.2021.126123

Lu, X., Gu, X., and Shi, Y. (2022). "A review on lignin antioxidants: Their sources, isolations, antioxidant activities and various applications," *International Journal of Molecular Sciences* 210, 716-741. DOI: 10.1016/j.ijbiomac.2022.04.228

Matos, M., Claro, F. C., Lima, T. A. M., Avelino, F., Hansel, F. A., Maciel, G. M., Lomonaco, D., and Magalhães, W. L .E. (2021). "Acetone: Water fractionation of pyrolytic lignin improves its antioxidant and antibacterial activity," *Journal of Analytical and Applied Pyrolysis* 156, article 105175. DOI: 10.1016/j.jaap.2021.105175

Nie, K., Liu, S., Zhao, T., Tan, Z., Zhang, Y., Song, Y., Li, B., Li, L., Lv, W., Han, G., and Jiang, W. (2024). "Efficient fractionation of biomass by acid deep eutectic solvent (DES) and rapid preparation of lignin nanoparticles," *Biomass Conversion and Biorefinery* 14(22), 28755-28765. DOI: 10.1007/s13399-022-03496-9

Ruesgas-Ramón, M., Figueroa-Espinoza, M.C., and Durand, E. (2017). "Application of deep eutectic solvents (DES) for phenolic compounds extraction: Overview, challenges, and opportunities," *Journal of Agricultural and Food Chemistry* 65(18), 3591-3601. DOI: 10.1021/acs.jafc.7b01054

Shen, X., Wen, J., Mei, Q., Chen, X., Sun, D., Yuan, T., and Sun, R. (2019). "Facile fractionation of lignocelluloses by biomass-derived deep eutectic solvent (DES) pretreatment for cellulose enzymatic hydrolysis and lignin valorization," *Green Chemistry* 21(2), 275-283. DOI: 10.1039/C8GC03064B

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.

Song, K., Yu, L., Yang, S., Cao, Y., Li, L., Wu, Z., Shi, H., and Ma, Q. (2024). "Insights into the chemical structure and antioxidant activity of lignin extracted from bamboo by acidic deep eutectic solvents," *ACS Omega* 9(39), 40956-40969. DOI: 10.1021/acsomega.4c06259

Sun, S., Yang, H., Yang, J., and Shi, Z. (2021). "Structural characterization of poplar lignin based on the microwave-assisted hydrothermal pretreatment," *International Journal of Biological Macromolecules* 190, 360-367. DOI:

10.1016/j.ijbiomac.2021.08.230

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, L., Li, X., Jiang, J., Zhang, Y., Bi, S., and Wang, H. (2022). "Revealing structural and functional specificity of lignin from tobacco stalk during deep eutectic solvents deconstruction aiming to targeted valorization," *Industrial Crops and Products* 180, article 114696. DOI: 10.1016/j.indcrop.2022.114696

Wang, Q., Cao, K., Yan, F., Duan, X., and Shi, J. (2025). "Alkaline deep eutectic solvent for separation and extraction of lignin from lignocellulose biomass," *Biomass Conversion and Biorefinery* 15(1), 1-18. DOI: 10.1007/s13399-023-05108-6

Wen, J., Yuan, T., Sun, S., Xu, F., and Sun, R. (2014). "Understanding the chemical transformations of lignin during ionic liquid pretreatment," *Green Chemistry* 16(1), 181-190. DOI: 10.1039/C3GC41752B

Yang, W., Ding, H., Qi, G., Li, C., Xu, P., Zheng, T., Zhu, X., Kenny, J. M., Puglia, D., and Ma, P. (2021). "Highly transparent PVA/nanolignin composite films with excellent UV shielding, antibacterial and antioxidant performance," *Reactive and Functional Polymers* 162, article 104873. DOI: 10.1016/j.reactfunctpolym.2021.104873

Zhang, Y., and Naebe, M. (2021). "Lignin: A review on structure, properties, and applications as a light-colored UV absorber," *ACS Sustainable Chemistry and Engineering* 9(4), 1427-1442. DOI: 10.1021/acssuschemeng.0c06998

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