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Green Fractionation and Structural Characterization of Lignin Nanoparticles via Carboxylic-Acid-Based Deep Eutectic Solvent (DES) Pretreatment

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Abstract: Green fractionation and a comprehensive overview of lignin molecular structures during the DES (deep eutectic solvent) pretreatment are very important for lignin valorization and the whole biorefinery process. Herein, intractable woody biomass (poplar wood) was pretreated with five types of carboxylic-acid-based DESs (acetamide served as an HBA (hydrogen bond acceptor), propanedioic acid, tartaric acid, malic acid, glutaric acid, and succinic acid served as HBDs (hydrogen bond donors)) under the optimized pretreatment conditions. Results showed that the optimal delignification ratio was achieved for tartaric-acid-based DES at 140 °C for 20 min under microwaveassisted heating. Two-dimensional HSQC NMR data demonstrated that the isolated poplar DES lignin consisted mainly of β - β , β -O-4 (normal and acylated forms), β -5, and esterified *p*-hydroxybenzoates (PBs) in different contents. Especially, the contents of β -O-4 in the isolated DES lignin fractions varied based on the pretreatment temperature and different chemical compositions of the DES. The antioxidant activity (2,2-diphenyl-1-picrylhydrazyl, DPPH analysis), microstructure (scanning electron microscope, SEM), and molecular weights (gel permeation chromatography, GPC) of the DES lignin fractions demonstrated that the DES delignification promoted the rapid assembly of lignin nanoparticles (LNPs) and could yield homogeneous lignin (1.23 < PDI < 1.58) with controlled nanometer size (30–170 nm) and good antioxidant activity. This study will improve the knowledge of structural changes of lignin during the different carboxylic-acid-based DES pretreatments and maximize the lignin valorization.

Keywords: DES pretreatment; NMR analysis; lignin characterization; lignin nanoparticles

1. Introduction

Owing to the gradual depletion of nonrenewable petrochemical resources and the increasing global environmental problems, researchers are required to develop alternative renewable resources to sustain the development of human society as soon as possible [1,2]. As the most plentiful renewable feedstock on the Earth, lignocellulosic biomass consisting of lignin, hemicelluloses, and cellulose can be utilized as biofuels, biomaterials, and biochemicals to support sustainable development [3–5]. Nowadays, the separation and utilization of the carbohydrate components (hemicellulose and cellulose) in lignocellulosic biomass are major concerns; these components are mainly converted into the fuel ethanol, oligosaccharides, cellulosic materials, etc. [6]. However, as the most abundant renewable aromatic resource in lignocellulose biomass, lignin is often used as a by-product and has not been properly developed and utilized due to the heterogeneity and complexity of the macromolecular structure, leading to a severe loss of biomass resources [7–9].

Recently, as promising green solvents for lignin separation from biomass, deep eutectic solvents (DESs) have received a lot of attention from researchers because of their renewable



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Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). and natural components, simple preparation, wide range of raw materials, low cost, and degradability [10,11]. DESs contain at least one hydrogen bond donor (HBD) and one hydrogen bond acceptor (HBA), which are synthesized from renewable and natural ingredients [12–15]. According to the different compositions, these solvents can be classified as basic DESs, neutral DESs, and acid DESs. Thereinto, the acid DESs are most widely applied for their excellent lignocellulose disassembly ability, which contributes to their outstanding biomass pretreatment performance. However, some challenges remain with DES-based biorefinery strategies [16,17]. One of the major challenges is the lack of applications for lignin, which could lead to a reduction in the overall economic benefits of biorefining. Moreover, the structural heterogeneity of lignin and its low delignification performance also limit the subsequent valorization of lignin [18–20]. Therefore, it is important to comprehensively understand the deconstruction mechanism and structural characteristics of lignin during DES pretreatment, which can provide theoretical guidance and data support for the high-value application of lignin and the full utilization of biomass resources. However, further research is needed to optimize the pretreatment conditions and to develop efficient and selective methods for lignin extraction and functionalization.

Previous studies have shown that the traditional oil-bath heating method is not energy efficient due to the long heating time and low efficiency. As a result, microwave radiation has become a popular alternative in many fields due to its fast heating speed, simple operation, and environmental friendliness [21–23]. This method not only increases the polarity of the DES molecules but also maximizes their ionic polarity, resulting in a significant reduction in reaction time and temperature requirement. During microwaveassisted DES pretreatment, the oscillating electromagnetic waves interact with polarity, leading to molecular heat loss through molecular friction, which creates "hot spots" and rapid expansion capable of destroying the crystalline structure of cellulose and lignin. Furthermore, the destruction of the crystalline structure of cellulose and lignin during microwave-assisted DES pretreatment can improve the accessibility of enzymes and other catalysts to the biomass, leading to increased yields and higher-quality products [24,25]. This means that the microwave-assisted DES pretreatment can greatly enhance the efficiency of the biomass-conversion processes, leading to energy savings and lower costs, which make it a more sustainable and economically viable option. Overall, microwave-assisted DES pretreatment has the potential to further diminish biomass recalcitrance and to yield lignin with a tailored structure by reducing the reaction time and temperature. This method is a promising alternative to traditional oil-bath heating and has the potential to revolutionize the field of biomass pretreatment.

To address the challenges mentioned above, a novel approach is proposed in this study, which combines carboxylic-acid-based deep eutectic solvents (DESs) with microwave radiation to achieve efficient and rapid dismantling and utilization of all components in the lignocellulosic biomass. This study evaluated the performance of different types of carboxylic-acid-based DESs and revealed the influence of DES characteristics on biomass pretreatment. Meanwhile, in order to indicate the disassembling mechanism and structural transformation of lignin during DES pretreatment, our research focused on the structural characteristics of lignin macromolecules in different DES pretreatment processes, which could provide some useful enlightenment for the separation of high-quality lignin with a specific structure and excellent chemical reactivity via DES pretreatment. Moreover, the radical scavenging test using 1,1-diphenyl-2-picrylhydrazyl (DPPH) was applied to evaluate the antioxidant capability of the obtained lignin. In addition, the morphological characteristics of lignin nanoparticles and the influences of DES pretreatment on subsequent fractionation and valorization were also investigated. Overall, this research aims to comprehensively elucidate the deconstruction mechanism and structure characteristics of lignin in the carboxylic-acid-based DES pretreatment, which could provide theoretical guidance and data support for improving the overall economic benefits of biorefinery and the subsequent high-value-added utilization of DES lignin and realize the full utilization of biomass resources.

2. Materials and Methods

2.1. Materials

Poplar (*Populus* × *canadensis* Moench) was obtained from Shandong Province (China). The 40–60 mesh sawdust was obtained by grinding in a laboratory pulverizer. The NREL method was used to analyze the chemical composition. In this study, all the chemicals (acetamide, propanedioic acid, tartaric acid, malic acid, glutaric acid, and succinic acid) were purchased from Sigma Chemical Co., Ltd. (Shanghai, China) and were of analytical-grade purity.

2.2. Preparation of Polycarboxylic-Acid-Based DES

A molar ratio of 1:1 was used with acetamide (AR, 99%) as an HBA and propanedioic acid (PA, AR, 99%), succinic acid (SA, AR, 99%), glutaric acid (GA, AR, 99%), malic acid (MA, AR, 99%), and tartaric acid (TA, AR, 99%) as HBDs, and the DES was synthesized under reduced pressure at 80 °C for 1 h. The synthesized polycarboxylic-acid-based DESs were stored in the desiccator before use.

2.3. Polycarboxylic-Acid-Based DES Pretreatment

First, 70.0 g of DES and 7.0 g of biomass were mixed, and then, the DES pretreatments were implemented in a 150 mL round-bottom flask. The pretreatment temperature was raised from room temperature to 120–150 °C within 2 min and held for 20 min, using a MICROSYNTH microwave-accelerated reaction system (Milestone Srl, Sorisole, Italy) under 400 W of microwave radiation. The microwave reactor had a wavelength of 122 mm and a frequency of 2450 MHz. After DES pretreatment, a dark-brown liquid was obtained, and then the antisolvent (acetone/water, 1:1 v/v) was added to it. The solid substrates (named P_{-SA120}, P_{-SA130}, P_{-SA140}, P_{-SA150}) were separated by filtration through a Buchner funnel. All the filtrates were collected, removed from acetone, and centrifuged to obtain DES lignins, which were named L_{-SA120}, L_{-SA130}, L_{-SA140}, and L_{-SA150}. Further, to investigate the performance of different polycarboxylic-acid-based DESs for biomass pretreatment and their effect on the lignin structure, different polycarboxylic acid DES lignins (L_{-PA140}, L_{-GA140}, L_{-MA140}, L_{-TA140}) and substrates (P_{-PA140}, P_{-GA140}, P_{-MA140}, P_{-TA140}) were obtained using the same method as in the above-mentioned procedures.

2.4. Analysis Methods

The antioxidant activity of the lignin macromolecule was evaluated using the 2,2diphenyl-1-picrylhydrazyl (DPPH, AR, 99%) methodology in a methanol solution according to the previous literature [25,26]. The composition of control and cellulose residues was analyzed using the NREL method. The number-average (M_n) and weight-average (M_w) molecular weights of the lignin fractions were measured using GPC (Agilent 1200, Santa Clara, CA, USA) [27]. The lignin was quantitatively analyzed by ³¹P NMR and 2D-HSQC using a Bruker AVIII 400 MHz spectrometer (Bruker; Fallanden, Switzerland) based on the previous literature [28,29]. The SEM analysis of the samples was performed using the S-3400NII SEM. The nanoparticle size analysis of DES lignin was achieved through the dynamic light scattering technique (Anton Paar Litesizer 500, Turin, Italy) using water as the dispersing medium. The detailed procedures of the analysis method are listed in the ESI section.

3. Results and Discussion

In the present study, the biomass was subjected to different carboxylic-acid-based DES pretreatments, which could destroy the cellular structure and achieve total component dissociation/separation of the main three components. Five types of DES were synthesized using acetamide as an HBA and PA (propanedioic acid), TA (tartaric acid), MA (malic acid), GA (glutaric acid), and SA (succinic acid) as HBDs Figure 1. As hydrogen bond donors, the number of carbon atoms in the main chain of the chemical structure of PA, SA, and GA increases by one, which in turn increases the length of the carbon chain. Based on the chemical

ical structure of SA, a hydroxyl group is added to the chemical structure of MA and TA, in turn. In this study, the influences of different DESs on the structure characteristics of lignin macromolecules were emphatically explored. Meanwhile, the dissociative mechanism and structure transformations of lignin during the DES pretreatment were investigated systematically, which will provide some useful inspiration for the separation of high-quality lignin with a specific structure and chemical reactivity by the DES pretreatment.



Figure 1. The hydrogen bond acceptor (HBA) and hydrogen bond donors (HBDs) of the DES.

3.1. Pretreatment Performance Analysis

To evaluate the effects of different DES pretreatments under rapid microwave heating, the chemical compositions of substrates and the lignin and hemicellulose removal ratio were analyzed and compared (Table 1 and Figure 2). In view of the microwave heating method used in the present study, the effects of pretreatment temperature were first evaluated. As the temperature increased from 120 °C to 150 °C, the solid yield decreased from 70.41% to 51.48% after DES pretreatment. Meanwhile, the solid yield of the different types of acidic DESs also decreased from 63.45% to 47.64%. The minimum solid yield was 47.64% at 140 °C by the TA-DES pretreatment. In the pretreated residues, the contents of residual hemicelluloses (mainly xylan) and residual lignin dramatically declined, which decreased from 19.30% to 3.51% and 24.36% to 4.30%, respectively. These results suggested that plenty of lignin and hemicelluloses were removed during the different DES biorefinery strategies. Meanwhile, it was observed that the removal ratio of lignin and hemicelluloses rose as the temperature increased from 120 °C to 150 °C.

The removal ratio of hemicelluloses was significantly enhanced from 74.93% at 120 °C to 87.44% at 150 °C, while the delignification remarkably increased from 70.40% at 120 °C to 86.42% at 150 °C, which confirmed that lignin and hemicelluloses were effectively removed under high temperature. This phenomenon was in accordance with the component analysis. Meanwhile, with an increase in the carbon chain, the ability of DES to form hydrogen bonds was weakened in the DES system, while an increase in the hydroxyl group enabled the DES to form hydrogen bonds more easily [16]. This phenomenon revealed that the effects of the different DES pretreatments were likely related to the hydrogen-bonding ability of the DES. Remarkably, when the temperature was 120–140 °C, the delignification and removal ratio of hemicelluloses signally increased from 74.93% to 85.30% and from 70.40% to 82.07%, respectively. While the temperature was higher than 140 °C in the DES pretreatment, the removal ratio of hemicelluloses and lignin was relatively stable. Therefore, in terms of energy saving, 140 °C was selected as the uniform temperature for the subsequent different

DES pretreatments. The removal ratio of hemicelluloses and lignin both decreased under the same temperature as the carbon chain in the hydrogen bond donor grew. When the number of hydroxyl groups in the hydrogen bond donor increased, the delignification and removal ratio of hemicelluloses rose. The maximum values of the hemicellulose removal ratio and the delignification ratio (R_{TA140}) were 91.34% and 90.93%, respectively, indicating that the performance of the pretreatment improved remarkably as the number of hydroxyl groups increased. The different degrees of removal ratio of hemicelluloses and lignin under the same pretreatment temperature were mainly relevant to the composition and properties of the DES. Based on the aforementioned data, it was shown that the TA-DES (140 °C, 20 min) was the optimal pretreatment condition for separating the three main components of biomass. These results suggest that the lignin and hemicelluloses were effectively removed and that the DES pretreatment has an effective ability to deconstruct the main components of poplar wood.

Table 1. Chemical compositions of substrates after different DES pretreatments (%).

Sample	Solid Yield	Xyl ^a	Glu	AL	KL	Total
Control	_ b	19.30 ± 0.17	48.59 ± 0.78	2.05 ± 0.06	24.56 ± 0.21	94.50 ± 1.64
R-SA120	70.41 ± 0.23	6.88 ± 0.14	67.76 ± 0.81	1.38 ± 0.02	9.81 ± 0.02	85.83 ± 0.99
R-SA130	62.41 ± 0.21	6.95 ± 0.17	71.31 ± 0.81	1.29 ± 0.03	9.70 ± 0.05	89.26 ± 1.07
R-SA140	59.42 ± 0.24	4.78 ± 0.15	79.84 ± 0.74	1.07 ± 0.01	6.97 ± 0.03	92.65 ± 0.96
R-SA150	51.48 ± 0.19	4.72 ± 0.11	79.38 ± 0.76	0.81 ± 0.01	6.23 ± 0.02	91.15 ± 0.93
R-PA140	52.26 ± 0.13	4.11 ± 0.14	81.08 ± 0.73	0.64 ± 0.03	4.97 ± 0.02	90.80 ± 0.98
R-GA140	63.45 ± 0.15	7.90 ± 0.19	75.27 ± 0.80	1.84 ± 0.02	9.67 ± 0.06	94.68 ± 1.07
R-MA140	58.02 ± 0.22	3.94 ± 0.18	78.56 ± 0.78	1.40 ± 0.03	5.33 ± 0.17	89.23 ± 1.16
R-TA140	47.64 ± 0.19	3.51 ± 0.16	85.45 ± 0.68	0.76 ± 0.02	4.30 ± 0.01	94.03 ± 0.87

^a Xyl: xylan, Glu: glucose; AL: acid-soluble lignin, KL: Klason lignin. ^b N.D.: not detected.



Figure 2. (**a**) The removal ratio of hemicellulose after DES pretreatment; (**b**) the delignification ratio after DES pretreatment.

3.2. Structural Analysis of the DES Lignin Fractions

To characterize the structural characteristics of DES lignin and illustrate the structural variations of lignin after the proposed pretreatment, the DES lignin macromolecules and native lignin (DEL) were compared using the 2D-HSQC NMR technique based on previous publications [17,21,27]. Indeed, the well-characterized lignin fractions can boost the further value-added utilizations of these lignin macromolecules. Figures 3 and 4 showed the aromatic and side-chain regions in the 2D-HSQC spectra, and the main structures were also identified and are shown in Figures 3 and 4. In fact, the structure changes of DES lignin are mainly attributed to the detailed DES pretreatment conditions.





Figure 3. Two-dimensional HSQC spectra (side-chain regions) of the DES lignins.

Different signals associated with the linkages (substructures) such as acylated β -aryl ethers (β -O-4, A'), β -aryl ethers (β -O-4, A), phenyl coumarin (β -5, C), methoxy (Ome) resin alcohols (β - β , B), and *p*-hydroxycinnamyl end groups (I) could be identified in the side-chain region of the 2D-HSQC spectrum (Figure 3) [30,31]. Generally, the appearance of acylated β -O-4 linkages in native lignin from poplar wood demonstrated that the lignin has the acylation γ position of *p*-hydroxybenzoate (PB), which is a typical unit in the DEL from poplar wood. In this study, an in-depth analysis of the 2D-HSQC NMR spectra was performed. The results suggested that the proposed DES pretreatment could lead to some variations in the lignin structures due to the appearance of some new signals in the NMR spectra of DES lignin. After the polycarboxylic acid DES pretreatment, novel β -O-4-linked structures (A'_{\beta(G)}) and A'_{\beta(S)}) emerged in all the DES lignin. This observation suggested that normal β -O-4 linkages might undergo different reactions, such as chemical modification, cleavage, and some possible changes. Indeed, these reactions often took place under the acidic pretreatment [32], which is highly in agreement with the polycarboxylicacid-based pretreatment. The emerging signal located near 80.2/4.6 ppm also appears in recent studies on DES preprocessing, suggesting that the novel β -O-4 linkages are the esterifiable form as a result of the reaction between the carboxylic acid of the DES and the hydroxyl group of the lignin [32]. As shown in Table S2, the quantification data of the 2D-HSQC spectra displayed that the relative content of β -O-4 linkages in the DES lignin fractions ranged from 9.53/100Ar to 38.17/100Ar, which were lower than that of DEL (59.86/100Ar), indicating that the cleavage of aryl ethers was the main reaction in the current DES pretreatment. To reveal the influence of temperature on the structural variation of lignin, the β -O-4 linkages in the lignin were quantified and obviously dropped from 35.91/100Ar in L_{-SA120} to 15.87/100Ar in L_{-SA150} when the pretreatment temperature

increased from 120 °C to 150 °C. Considering the different DES pretreatments, the minimum amount of β -O-4 linkage was 9.53/100Ar under the TA-DES pretreatment condition. This fact suggested that the β -O-4 linkages of DES lignin were also related to the delignification performance of the DES pretreatment. In summary, the structural characterization of lignin during the DES pretreatment provides important insights into the sustainable development and utilization of lignin from lignocellulosic biomass.



Figure 4. Two-dimensional HSQC spectra (aromatic regions) of the DES lignin fractions.

The correlated signals from guaiacyl (G), *p*-hydroxybenzoic acid (PB), and syringyl (S) were distinctly shown in the aromatic region of the 2D-HSQC spectra of the DES lignin (Figure 4). These phenomena indicate that not only are the typical substructural bonds (main aryl ether bonds) in lignin cleaved and degraded under the condition of acid DES pretreatment, but the different G-type lignin units may also undergo condensation reactions with each other, resulting in a reduction in the C-H correlation signal of the G unit in the 2D-HSQC spectra [33,34] Figure 5. Moreover, the DES lignin fractions (L-SA-120, L_{SA-130}, L_{GA-140}) contained abundant PB units, suggesting that the mild conditions of DES pretreatment could efficiently preserve the lignin structure. However, the PB units in the DES lignin fractions obtained from harsh conditions (L-SA-140, L-SA-150, L-PA-140, L-MA-140, L-TA-140) were lower than that of the DEL, indicating that PB units in the DES lignin macromolecules decreased with the enhancement of pretreatment intensity. The S/G ratio is an important parameter for assessing the structural characteristics of different lignin components obtained from the same raw material. Simultaneously, the S/G ratios in all lignin macromolecules were quantified by the integration of the correlation signals by dividing $0.5S_{2,6}$ by the G₂ position (Table S2). In fact, the structural changes of the

acidic DES lignin could be more effectively described via the changes in the S/G ratio of lignin fractions from the same feedstock [17]. The S/G ratio of DEL was 2.75, which is in accordance with a previous publication [21]. Nevertheless, the S/G ratios of lignin after the polycarboxylic acid DES pretreatment showed an increase in the S/G ratio of the DES lignin fraction from 1.18 for L_{SA140} to 1.49 for L_{SA140} and 2.20 for L_{TA140}. This ascending S/G ratio suggested that the G-type lignin was more readily decomposed during the DES pretreatment, while the resulting DES lignin macromolecules contained a significant amount of S-type lignin units. Actually, this phenomenon also took place in different hydrothermal pretreatments [35–37] and in dilute acid pretreatment [38,39]. Another important reason for the different S/G ratios of lignin from the same poplar wood is due to the different yields of lignin. In general, the lignin fragments with low yield are mainly from the middle lamella (ML) layer of the plant cell wall, which is rich in G-type lignin units. As lignin production in the lignin fraction increased, the lignin rich in S-type lignin from the secondary wall (SW) continued to be dissolved, which also resulted in the phenomenon of high-yield S-rich lignin units.



Figure 5. Quantitative results obtained from 2D-HSQC NMR spectroscopy (X/100Ar).

In short, it was concluded that DES pretreatment could effectively alter the structure of lignin, leading to changes in the content and distribution of different linkages and PB units. The signal integration of the cleavage of β -O-4 linkages and the degradation of carbon–carbon linkages in lignin molecules was also observed under the carboxylic-acid-based DES pretreatment. The content of PB units in the DES lignin fractions was found to be decreased with the enhancement of pretreatment intensity, while increasing the S/G ratio with increasing pretreatment temperature. These changes in the lignin structure have important implications for the value-added utilization of lignin macromolecules, as they can affect the properties and potential applications of lignin during DES pretreatment, the 2D-HSQC NMR technique could contribute invaluable information for optimizing the pretreatment conditions and developing novel lignin-based products.

3.3. Molecular Weights and FT-IR Analysis of the DES Lignin Macromolecules

To further investigate the depolymerization pattern of different DES pretreatments, the molecular weight (M_n , M_w) variations of the lignin were systematically estimated (Figure 6a). For evaluating the M_n and M_w of the DES lignin, the double enzymatic lignin

(DEL) sample was also isolated according to a previous report [40], which could be representative of native lignin in the biomass feedstock. For the native lignin, the molecular weights (M_w, M_n) were 7230 \pm 60 g/mol and 3820 \pm 40 g/mol, respectively. After the DES pretreatment, the molecular weights (M_w, M_n) of the DES lignins were 1210–1980 g/mol and 970-1250 g/mol, respectively. These results showed that the molecular weights (M_w , M_n) were lower than that of the DEL from raw material. In general, the molecular weights decreased under acidic conditions [41]. The observed decrease in molecular weights was a clear indication of successful lignin depolymerization. A key role in lignin depolymerization under acidic conditions is played by the cleavage of the ether bonds (mainly β -O-4 linkages) in lignin. It was noticed that the M_w of all the SA-DES lignin samples markedly decreased as the temperature increased from 120 $^{\circ}$ C (1980 \pm 20 g/mol) to 140 $^{\circ}$ C (1290 \pm 20 g/mol). However, the M_w slightly increased under the harsh pretreatment condition (L-SA150), suggesting that the condensation and fragmentation reactions of lignin occurred simultaneously under the given pretreatment conditions. When the DES pretreatment temperature exceeds 150 °C, condensation might predominate, which is not beneficial for producing the homogeneous lignin macromolecules and lignin utilization, which is in agreement with the above analysis. Additionally, the effect of the different structures of acidic DES on the molecular weights of lignin was investigated at the optimal pretreatment temperature (140 °C). Further analysis of the data revealed that the molecular weights of DES lignins were also related to the DES delignification performance. When the DES pretreatment performance was weak, the depolymerization for lignin was also limited, and the molecular weight of L_{GA140} (M_w, 1570 \pm 30 g/mol) was larger than that of L_{-SA140} (M_w, 1290 \pm 20 g/mol). Furthermore, the M_w of L_{-MA140} (1240 \pm 20 g/mol) and L_{-TA140} (1280 \pm 20 g/mol) were slightly increased. The increase in molecular weight might be related to the chemical modification during the DES pretreatment, possibly the binding of the HBDs (such as propanedioic acid, tartaric acid, malic acid, glutaric acid, and succinic acid) of the DES to lignin. In addition, the polydispersity index (PDI, M_w/M_n) of lignin is a representation of the molecular weight distribution of polymers, which is essential to evaluate the degree of molecular homogeneity of lignin. The PDI of the native DEL was higher (PDI, 1.89) than that of the DES lignin (PDI, 1.23–1.58). With an increase in the pretreatment temperature from 120 °C to 150 °C, the lignin macromolecules were rendered more uniform, suggesting that the high-value-added conversion and utilization of these DES lignins could be favorable. Overall, these results suggested that the DES pretreatment temperature and the DES types had significant effects on the molecular weight and polydispersity of lignin, which could improve the efficiency of lignin utilization and contribute to the development of a sustainable bioeconomy.



Figure 6. (a) Molecular weights of the lignin fractions; (b) the free radical scavenging index (RSI).

To further study the structure variations of DES lignin, the DES lignin fraction was investigated using the FT-IR technique. Figure 7 shows the FT-IR spectra of DES lignin. According to a previous publication [42], the main assignments were assigned (Table S5). As shown in Figure 7, the absorption band at 3410–3430 cm⁻¹ is a wide and strong absorption band due to the stretching of aliphatic OH groups. The absorbance at 2940 cm⁻¹ and 2850 cm⁻¹ corresponds to the stretching vibration of C-H in methyl and methylene. The C-H bonds at 1600, 1508, 1420, and 1267 cm⁻¹ are related to the vibration of the aromatic framework, and the C-H bond deformation at -CH₃ and -CH₂ is related to the vibration of the aromatic ring at 1460 cm⁻¹. Interestingly, it was noted that the vibratory peaks of the carbonyl group derived from the ester group (C = O, 1730 cm⁻¹ in the ester group) were significantly enhanced with increasing pretreatment temperature. The results showed that, with increasing pretreatment temperature, the absorption peak (1167 cm⁻¹) was represented as HGS lignin in reference [42], which further proved that all these lignins belong to HGS-type lignin.



Figure 7. FT-IR spectra of DES lignin.

3.4. DPPH Antioxidant Activity and Morphology Analysis of the DES Lignin

The antioxidant activity of lignin is crucial for further utilization [43]. Numerous studies have shown that the content of phenolic OH groups in lignin is positively correlated with its antioxidant activity [21,22]. In order to assess the antioxidant activity of polycarboxylicacid-based DES lignin, an industrial antioxidant (BHT) was used as a control. The results showed that the industrial antioxidant BHT (RSI = 1.98) had significantly lower antioxidant activity than that of all the DES lignin fractions (RSI = 12.46–21.55), indicating that the obtained DES lignin had the potential to be used as the effective antioxidant. Furthermore, it was found that, as the pretreatment severity increased, the antioxidant activity of the DES lignin fractions also increased. This was demonstrated by the fact that the antioxidant activity of the extracted DES lignins increased from 12.46 in L_{-SA120} to 20.24 in L_{-SA150} with the increasing pretreatment temperature, as shown in Figure 6b and Table S4. This was mainly due to the breakage of the β -O-4 aryl ether bond, which led to an increase in the phenolic hydroxyl content of lignin. This positive correlation between antioxidant activity and phenolic OH group content is consistent with previous publications. The antioxidant activity of lignin is mainly attributed to the existence of phenolic OH groups, which can scavenge free radicals and prevent oxidative damage. The excellent performance of the DES lignins as antioxidants is noteworthy, as it was found to be 6.29–10.22 times higher than of BHT (RSI = 1.98). This fact demonstrates that these lignins have the potential to be used as environmentally friendly antioxidants in various industrial applications, providing a sustainable alternative to conventional synthetic antioxidants. Furthermore, the high antioxidant activity of DES lignin makes it a valuable resource for various value-added applications in different fields, such as food, pharmaceuticals, and cosmetics. With regard to the increased antioxidant activity of DES lignin with elevated pretreatment severity, the pretreatment process can be optimized to obtain lignin with desired properties for specific applications. This is important because different applications might require lignin with different structural characteristics and properties, and the ability to tailor the properties of DES lignin through DES pretreatment could provide a more versatile and efficient material for various applications. In conclusion, the antioxidant activity of lignin is critical for its value-added applications, and DES lignin has shown great potential as a green and efficient alternative to commercial antioxidants. The ability to optimize the pretreatment process to obtain lignin with desired properties further enhances their versatility and potential for various industrial applications.

The size distribution and morphological characteristics of the DES lignin extracted from poplar were observed using scanning electron microscopy (SEM) and nanoparticle size analysis, which is conducive to the development of lignin nanoparticle (LNP)-based biomaterials [22,44]. Thus far, many methods have been used for obtaining LNPs; DES pretreatment is a potential and simple preparation method for LNPs [17,41]. As shown in Figure 8, the DES lignin component (L_{TA140}) is a typical LNP with a regular spherical shape. According to the size distribution, the size of the LNPs was mainly concentrated in the range of 40–90 nm, accounting for 80% of the total size distribution. In conclusion, the DES pretreatment can effectively yield LNPs, and smaller LNPs are preferred and can improve performance in downstream value-added applications.



Figure 8. (a) SEM images of the LNPs; (b) dimensional distribution of the DES lignin.

3.5. Structural Changes of Lignin Macromolecule during the Polycarboxylic-Acid-Based DES Pretreatment

To fully comprehend the potential for the value-added utilization of lignin, it is essential to elucidate the structure transformations during the pretreatment process. By employing a combination of NMR and GPC techniques, this study could identify structural transformation during the polycarboxylic-acid-based DES pretreatment. The results indicated that there were three main reactions during the DES pretreatment process: (1) the condensation reaction, which was the dominant reaction as evidenced by 2D-HSQC NMR and molecular weight analysis (the harsh conditions during the DES pretreatment promoted the occurrence of condensation reaction); (2) the depolymerization reaction, which competed with condensation reaction during pretreatment, as observed by the GPC and NMR analysis; and (3) the demethylation reaction, which occurred in lignin during the pretreatment process, as confirmed by 2D-HSQC NMR analysis. On the basis of the above discussions, the proposed structural changes of lignin during the polycarboxylic-acid-based DES pretreatment process are illustrated in Figure 9.



Figure 9. Proposed structure evolution of lignin during the integrated process.

4. Conclusions

In short, the significant effects of the different DES types and pretreatment temperatures on the subsequent poplar wood deconstruction and delignification, antioxidant activity, and chemical structures of the DES lignin fractions have been demonstrated in the present study. The highest removal ratio of hemicelluloses (91.34%) and an excellent delignification ratio (90.93%) can be realized under the optimal condition (140 °C, 20 min). GPC and NMR analysis suggested that the typical linkages in lignin (such as β - β , β -O-4 and β -5 and PB linkages) could be largely retained, but the β -O-4 linkages were varied with different pretreatment conditions and more liable to cleavage under sufficient

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delignification. In short, DES lignin fractions have relatively well-preserved structures, small molecular weight with less polydispersity, and more uniform morphological characteristics than DEL. Moreover, the structural changes of lignin macromolecules during the polycarboxylic-acid-based DES pretreatment were quantified. In conclusion, the proposed DES pretreatment is an effective delignification method, and these DES lignins are promising raw materials for downstream applications, such as natural antioxidants and lignin-based functional materials.

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