Structural Elucidation of Lignins from Corncob Acid Hydrolysis Residue by Enzymatic Mild Acidolysis and Deep Eutectic Solvent Pretreatment

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Achieving mild and efficient extraction of high purity lignin from corncob acid hydrolysis residue is essential for efficient lignin application. In this study, enzymatic/mild acidolysis lignin (EMAL) and deep eutectic solvent (DES)-lignin were extracted from corncob acid hydrolysis residue. The structural features of the two lignin fractions were investigated by Fourier transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), gel permeation chromatography (GPC), and ^{31}P-nuclear magnetic resonance (^{31}P-NMR). The highest DES-lignin yield of 58.8 wt% was achieved at 120 °C with a choline chloride-to-lactic acid (ChCl-to-Lac) molar ratio of 1:10 and a reaction time of 12 h. The FTIR analysis indicated a higher amount of guaiacyl units in EMAL than DES-lignin. Furthermore, condensation and fragmentation occurred simultaneously under DES pretreatment, but the fragmentation reaction was dominant. The structural characteristics investigated will allow for more effective lignin usage.

Keywords: Corncob acid hydrolysis residue (CAHR); Lignin; Deep eutectic solvent (DES); Structural characterization

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INTRODUCTION

Alternative, sustainable fuel sources have received increased attention due to concerns of petroleum shortages and global warming (Ten and Vermerris 2013; You *et al.* 2016). Bio-ethanol produced from lignocellulose is attracting worldwide attention, as lignocellulose is the world's most abundant renewable resource (Zhang *et al.* 2012; Wang *et al.* 2018). Corncob, a common agricultural waste product, is the main feedstock for the production of bio-ethanol and xylose by acid hydrolysis. Acid hydrolysis residue (AHR) is a by-product in the hydrolysis process, and its availability has risen due to increased bio-ethanol and xylose production (Matsushita and Yasuda 2005). Lignin is one of the main components of AHR (Wang *et al.* 2014). Traditionally, AHR has been considered a waste material or low-value by-product and is usually burned for heat or power. The effective utilization of AHR is essential, as it can reduce production costs and prevent pollution caused by AHR discharge (Huang *et al.* 2012).

Lignin is the most abundant renewable resource composed of aromatic units in the plant world (Li *et al.* 2015). Lignin is mainly composed of three phenylpropane units, which are the *p*-hydroxyphenyl (H) units, guaiacyl (G) units, and syringyl (S) units (Shao *et al.* 2018). The specific chemical structure of lignin makes it a sustainable alternative feedstock for the production of aromatic chemicals and performance products, such as phenol, BTX (benzene, toluene, and xylene) (Zakzeski *et al.* 2010), phenolic resins (Da Silva *et al.* 2013), polyurethanes (Pan and Saddler 2013), and carbon quantum dots (Wang *et al.* 2019). Despite its potential, the current industrial utilization of lignin is still limited due to its purity, yield, and complex chemical structure. In addition, the low solubility and functional group heterogeneity of lignin are major drawbacks to its use in polymer synthesis (Mbotchak *et al.* 2015). Due to the complicated linkages between lignin and other components in plants, the isolation of lignin from lignocellulosic biomass is challenging. Several processes for isolating lignin from lignocellulosic feedstocks have been developed. All such isolation methods have the same goals of obtaining high yield, high purity, and homogeneous lignin. In addition, lignins extracted by different methods exhibit different structural characteristics, which influence how the material can be used. A combination of enzymatic and mild acid hydrolysis can isolate high yield lignin (Wu and Argyropoulos 2003). Furthermore, enzymatic mild acidolysis lignin (EMAL) is more representative of the lignin typically present in milled lignin (Guerra *et al.* 2006). To find a better strategy to isolate lignin, deep eutectic solvent (DES) was investigated. Formed by mixing hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA), DES has low volatility, nontoxicity, non-flammability, biocompatibility, and low cost (Zhang *et al.* 2016; Lynam *et al.* 2017; Liu *et al.* 2019). Tan *et al.* (2019) extracted 60 wt% of lignin from empty fruit bunches using acidic deep eutectic solvent. Additionally, lignin extracted by DES treatment has been reported to have high purity, high yield, and low molecular weight, which would be conducive to future utilization.

The comparable structural alterations of lignin from different integrated biorefineries have not been clearly determined due to the chemical technology and raw materials. Therefore, prior to utilization of lignin to chemicals and materials, comparative characterization of lignin obtained from different technologies is essential. In this study, EMAL and DES-lignin were obtained from corncob acid hydrolysis residue (CAHR). Two lignin fractions were characterized by Fourier transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), gel permeation chromatography (GPC), and ^{31}P-nuclear magnetic resonance (^{31}P-NMR). Comparative characterization of lignin from CAHR will facilitate faster development of chemicals and materials from lignin by-product.

EXPERIMENTAL

Materials

The CAHR, obtained from Shandong Longlive Bio-Techbology Co., Ltd., Dezhou, China, was collected for feedstock. The corncob was treated hydrothermally to convert hemicellulose into oligosaccharides, producing a CAHR residue. The main compositions of CAHR were determined using the NREL Laboratory Analytical Procedure (Sluiter *et al.* 2008). The CAHR, which was dried prior to use, contained 25.85 wt% lignin, 62.34 wt% cellulose, and 6.12 wt% hemicellulose.

Isolation of Lignins

The EMAL was isolated from CAHR following the procedure described by Wu and Argyropoulos (2003).

The DES-lignin was obtained by pretreatment of CAHR with prepared DES. The CAHR and DES were combined with a 1:30 ratio by weight. To remove extractives, CAHR was first extracted with mixed benzene and 95% ethanol for 8 h. Then, DES was prepared by mixing choline chloride (ChCl) and lactic acid (Lac). The impact of the molar ratio of ChCl:Lac, reaction temperature, and reaction time were investigated. The DES pretreatment procedure is shown in Fig. 1. The yields of solid residue and extracted DES-lignin were calculated using Eq. 1 and Eq. 2, respectively,

$$\text{Yield of solid residue (wt\%)} = W_{\text{SR}} \,/\, W_{\text{CAHR}} \times 100 \qquad (1)$$

$$\text{Yield of DES-lignin (wt\%)} = W_{\text{DES–lignin}} \,/\, W_{\text{lignin in CAHR}} \times 100 \qquad (2)$$

where W_{SR} (g) is the weight of solid residue , W_{CAHR} (g) is the weight of CAHR, $W_{\text{DES-lignin}}$ (g) is the weight of extracted DES-lignin, and $W_{\text{lignin in CAHR}}$ (g) is the weight of lignin in CAHR.

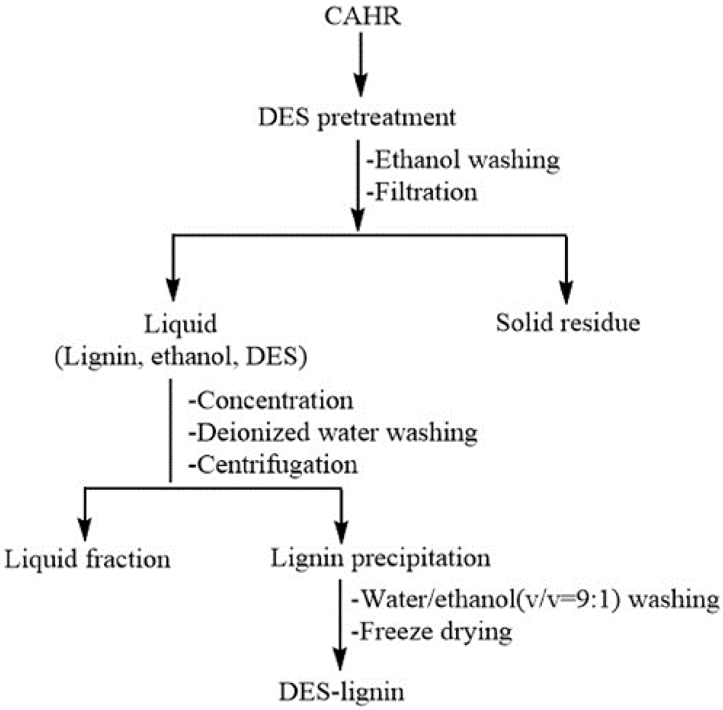

Fig. 1. The DES pretreatment procedure

Methods

FTIR analysis

Fourier-transform infrared spectra analysis of lignins was performed with a VERTEX70 instrument (Bruker, Karlsruhe, Germany) using a KBr pellet procedure. Lignins were scanned in the range of 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1}.

Thermal stability

Thermal stability of lignins were investigated on a STA449F3 simultaneous thermal analyzer (Netzsch, Selb, Germany). Approximately 10 mg of lignin was placed in an alumina crucible and heated from ambient temperature to 800 °C with a heating rate of 10 °C/min. High-purity nitrogen was used as the carrier gas with a flow rate of 40 mL/min.

Molecular weight determination

The molecular weight distributions of the lignins were determined using an Alliance e2695 gel permeation chromatograph (Waters, Milford, MA, USA). Lignin was dissolved in tetrahydrofuran (THF), and 10 µL of solution was injected into the column calibrated with polystyrene standards at a flow rate of 1 mL/min.

^{31}P-NMR analysis

Quantitative ^{31}P-NMR spectra of lignins were acquired according to published procedures (Argyropoulos 1994; Granata and Argyropoulos 1995). Forty mg of lignin was completely dissolved in 650 µL of pyridine and deuterated chloroform (1.6:1, v/v). Next, 50 µL of N-hydroxy-5-norbornene-2,3-dicarboximide (80 mg/mL) was added as an internal standard, and 50 µL of chromium(III) acetylacetonate (11 mg/mL) was added as a relaxation regent. Finally, 100 µL of 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholate was added to the NMR tube for subsequent NMR analysis.

RESULTS AND DISCUSSION

Yield Analysis

The yield of EMAL, based on the initial lignin in CAHR, was 22.34 wt%.

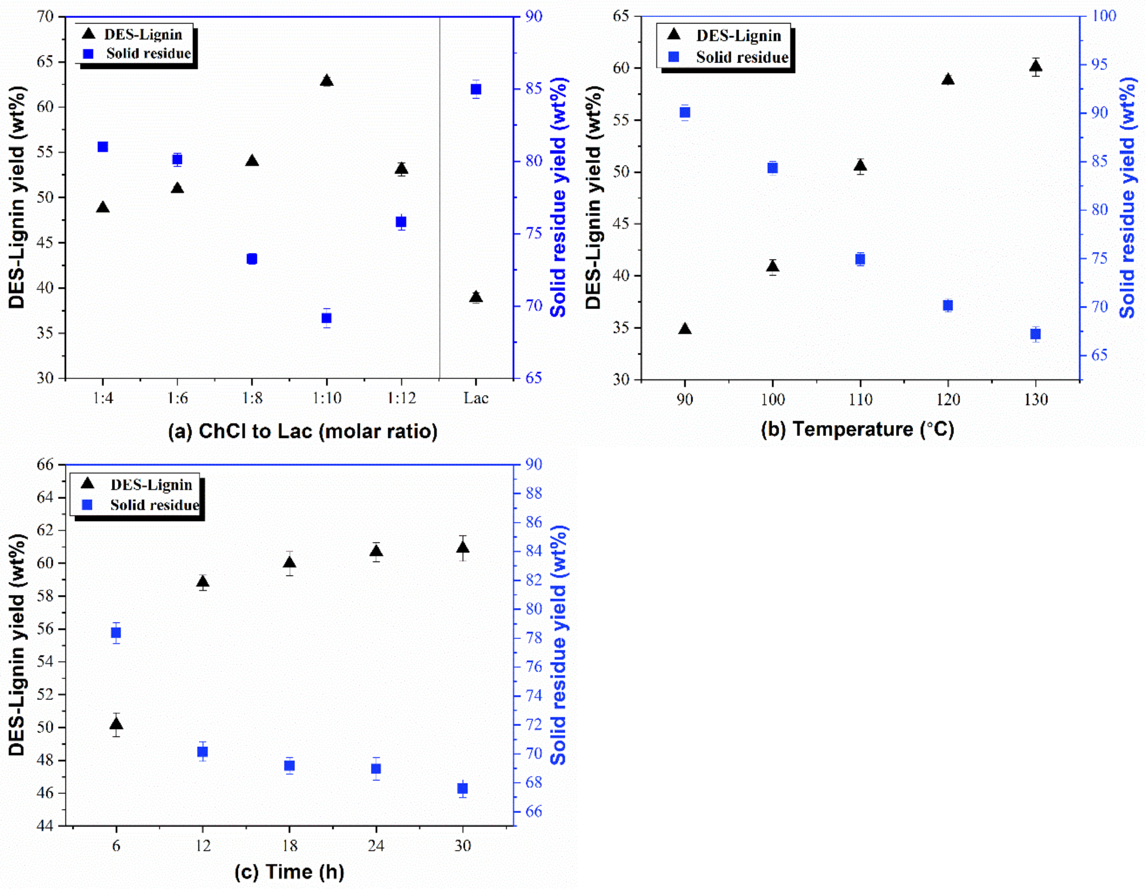

Fig. 2. Effects of DES composition, temperature, and time on the extraction of DES-lignin. Condition: (a) Temperature, 120 °C; time, 12 h (b) ChCl-to-Lac molar ratio, 1:10; time, 12 h (c) ChCl-to-Lac molar ratio, 1:10; temperature, 120 °C

The effects of DES composition, temperature, and time on the extraction of DES-lignin are shown in Fig. 2. Five different molar ratios of ChCl-to-Lac (1:4, 1:6, 1:8, 1:10, and 1:12) were investigated. As the ChCl-to-Lac molar ratio increased from 1:4 to 1:10, the yield of DES-lignin gradually increased, and the solid residue yield decreased. The highest DES-lignin yield of 58.8 wt% with the lowest solid residue yield of 70.2 wt% was obtained with a ChCl-to-Lac molar ratio of 1:10. Increasing the lactic acid ratio decreased the DES-lignin yield to 53.1 wt% and increased solid residue yield. This might have been caused by an excess of lactic acid, which reduced the solubility of lignin in solvent. The result of the pure lactic acid pretreatment also supported this explanation. Figure 2b shows the effect of temperature on the DES-lignin and solid residue yields. The yields of DES-lignin and solid residue were highly temperature dependent. There were substantial yield changes in the temperature range of 90 °C to 120 °C. The yield of DES-lignin increased from 34.8 to 58.8 wt% as temperature increased from 90 °C to 120 °C, and solid residue yield decreased from 90.1 to 70.2 wt%. When the temperature was increased to 130 °C, the yield of DES-lignin increased slowly. Therefore, 120 °C was the most appropriate temperature for DES pretreatment. Figure 2c shows the effect of time on DES pretreatment. The yield of DES-lignin increased as time increased from 6 h to 12 h. With a pretreatment time of 12 h, a 58.8 wt% yield of DES-lignin was obtained. Small variation in lignin yield was observed when the pretreatment time was greater than 12 h. Thus, 12 h was the most suitable pretreatment time for obtaining high lignin yield. Based on the above findings, the optimum condition for ChCl-to-Lac pretreatment of CAHR was a ChCl-to-Lac molar ratio of 1:10, a temperature of 120 °C, and a time of 12 h. These conditions resulted in a 58.82 wt% DES-lignin yield.

FTIR Analysis

Figure 3 shows the FTIR spectra of the EMAL and DES-lignin obtained from CAHR.

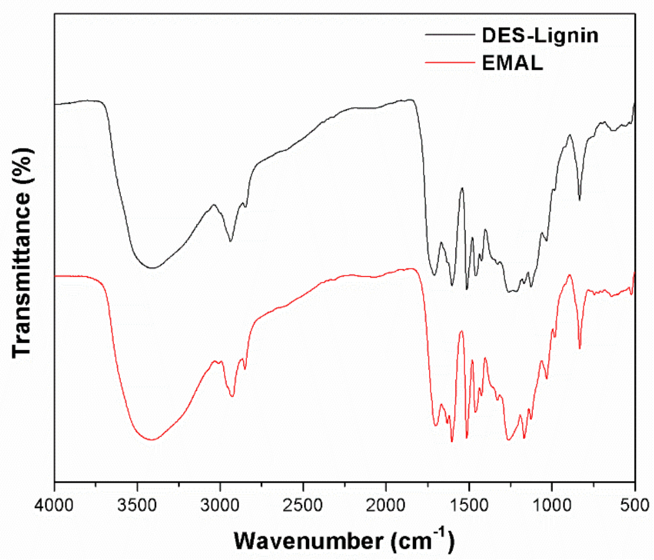

Fig. 3. The FTIR spectra of EMAL and DES-lignin

Table 1 lists the band assignments (Shao *et al.* 2017). The spectra of the EMAL and DES-lignin were similar except for the intensities of the absorption bands. A wide absorption band at 3420 cm^{-1} was assigned to the O-H stretching vibration in aliphatic and aromatic OH groups, and the bands at 2930 and 2845 cm^{-1} represent the C-H vibrations in the methyl and methylene groups. The band at 1710 cm^{-1} was attributed to unconjugated carbonyl stretching vibration in ketone or aldehyde groups. The spectrum of EMAL showed a stronger absorbance at 1630 cm^{-1}, which was assigned to -COO- antisymmetric stretching of glucuronic acid or glucuronic acid carboxylate. This indicated that EMAL contained a high amount of glucuronic acid or glucuronic acid carboxylate. The bands at 1605, 1510, and 1425 cm^{-1} are attributed to aromatic skeletal vibrations (Wen *et al.* 2013). The bands at 1167 cm^{-1} indicated that the two lignin fractions were HGS-type lignins. The band at 1265 cm^{-1} (C-O stretching of guaiacyl ring) showed a stronger absorbance in EMAL, suggesting that it contained a higher amount of guaiacyl units than DES-lignin.

Table 1. Band Assignments for FTIR Spectrum of Lignin

Wavenumber (cm^{-1})	Band Assignment
3420	O-H stretching vibration in hydroxyl groups
2930, 2845	C-H stretching vibration in methyl and methylene groups
1710	C=O stretching vibration in unconjugated ketone and carbonyl groups
1630	COO- stretching vibration
1605, 1510, 1425	Aromatic skeletal vibrations
1265	C-O stretching of guaiacyl ring
1167	C–O–C symmetrical stretching vibration

Thermal Stability Analysis

The thermal properties of lignin have important implications for its utilization. The thermogravimetric (TG) and different thermogravimetric (DTG) curves of lignins are shown in Fig. 4. The thermal degradation behavior of these two lignin fractions was consistent. The thermal degradation process could be divided into three stages. The initial stage before 160 °C was mainly attributed to the release of water and some organic constituents. The second stage between 160 °C and 600 °C belonged to fast degradation stage in which lignin was converted into volatiles; this part would become bio-oil using condensation technology. The third stage from 600 °C to 800 °C belonged to a slow degradation stage, and char was the main product instead of bio-oil. In the fast degradation stage, the maximum rate of mass loss occurred at 374 °C. Further, a 41.7 wt% char yield char was obtained from DES-lignin, whereas a 36.7 wt% char yield was obtained from EMAL, which could have been due to the difference in HGS units (Chen *et al.* 2015). This explanation is consistent with the FTIR results.

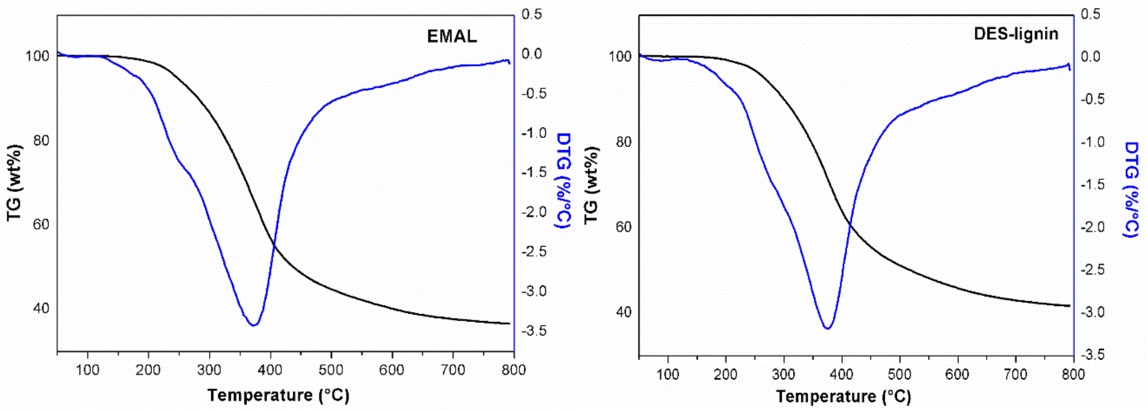

Fig. 4. TG and DTG curves of EMAL and DES-lignin

Molecular Weight Distribution

The molecular weight distribution of lignin was obtained by gel permeation chromatography (You *et al.* 2015a). The number-average molecular weight (M_n), weight-average molecular weight (M_w), and polydispersity (PDI, M_w/M_n) of EMAL and DES-lignin are shown in Table 2. The weight-average molecular weight and number-average molecular weight of DES-lignin were 2601 and 1281 g/mol respectively, which were substantially lower than 3384 and 1404 g/mol of EMAL (Lyu *et al.* 2018). Lignin molecular weight is closely related to the amount of structural units. Therefore, the above result might have been caused by the breakage of lignin linkages during DES pretreatment, resulting in the formation of smaller fragments. It can be seen from Table 2 that the polydispersity value of DES-lignin was lower than that of EMAL, which indicated that lignin produced under DES pretreatment was relatively homogeneous. This was conducive to the more stable development of lignin into high value-added products. Furthermore, the condensation reaction would result in a heterogeneous lignin structure with higher molecular weight, whereas the fragmentation reaction would lead to a decrease in lignin molecular weight (You *et al.* 2015b). The results indicate that the fragmentation reaction was dominant over the condensation reaction in the DES pretreatment.

Table 2. Molecular Weight Distribution of Lignin Samples

Lignin Sample	Molecular Weight (g/mol)		PDI (M_w/M_n)
	M_w	M_n	
EMAL	3384	1404	2.4101
DES-Lignin	2601	1281	2.0302

^{31}P-NMR Analysis

^{31}P-NMR was used to differentiate and quantify the hydroxyl groups (phenolic, aliphatic, and carboxylic) in lignin (Pu *et al.* 2011). The contents of different types of hydroxyl groups, which were determined by integration of the corresponding peaks with respect to the internal standard, are listed in Table 3. The quantitative results showed that aliphatic hydroxyl dominated the hydroxyl groups. With respect to phenolic hydroxyl groups in EMAL, p-hydroxyphenyl phenolic hydroxyl was predominant, followed by guaiacyl and syringyl phenolic hydroxyl. The findings were similar to those in previous studies, indicating that the content of p-hydroxyphenyl phenolic hydroxyl in corncob or corn stalk lignin presented a high proportion among the phenolic hydroxyl groups (Liu *et al.* 2018; You *et al.* 2019). In contrast to the EMAL, the DES pretreatment led to a decrease in aliphatic hydroxyl content in DES-lignin. The content of aliphatic hydroxyl in DES-lignin decreased to 1.17 mmol/g. This reduction can be attributed to the dehydration reaction during the breakage of the β-O-4 linkage in the DES pretreatment process. The increase of syringyl phenolic hydroxyl content indicated that the syringyl unit in lignin underwent a greater extent of ether linkage cleavage during the DES pretreatment. This increase of S units could be attributed to the lack of association of lignin-carbohydrate complex in CAHR (Sun *et al.* 2015). In addition, an increase in condensed phenolic hydroxyl in DES-lignin occurred. Acidic conditions facilitate the recondensation of lignin (Gierer 1986). Consequently, lignin recondensation occurred in DES pretreatment. Meanwhile, the stable content of total phenolic hydroxyl meant that the lignin structure framework did not change. The COOH content increased slightly during DES pretreatment, indicating that an oxidation reaction occurred.

Table 3. Quantification of Functional Groups in EMAL and DES-lignin by ^{31}P-NMR

Chemical Shift (ppm)	Assignment	Content (mmol/g)	
		EMAL	DES-lignin
134.4 to 137.0	Carboxyl (COOH)	0.52	0.63
137.0 to 138.4	p-hydroxyphenyl phenolic hydroxyl (H)	1.03	0.81
138.4 to 140.2	Guaiacyl phenolic hydroxyl (G)	0.96	0.85
142.4 to 143.6	Syringyl phenolic hydroxyl (S)	0.32	0.43
140.2 to 142.4/ 143.6 to 144.4	Condensed phenolic hydroxyl	0.35	0.52
145.6 to 150.0	Aliphatic hydroxyl	1.71	1.17
-	Total phenolic hydroxyl	2.66	2.61
-	Total hydroxyl	4.37	3.90

CONCLUSIONS

1. The enzymatic/mild acidolysis lignin (EMAL) and deep eutectic solvent (DES) lignin were extracted from corncob acid hydrolysis residue. A 58.8 wt% yield of DES-lignin was obtained under the optimum pretreatment conditions (ChCl-to-Lac molar ratio of 1:10, 120 °C, and 12 h).
2. The FTIR spectra indicated that DES-lignin had a higher amount of guaiacyl units than the EMAL. In contrast with the EMAL, DES pretreatment led to decreases in the molecular weight and aliphatic hydroxyl content of the DES-lignin.
3. The GPC and ^{31}P-NMR results indicated that both condensation and fragmentation reactions occurred under DES pretreatment, with the fragmentation reaction being the dominant reaction.

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