

Deep Eutectic-like Solvent-Assisted Isolation of Lignin from *Pinus densiflora* and its Characteristics

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Lignin, the most abundant aromatic biopolymer on Earth, has great potential to replace petrochemical-based polymers in the production of value-added products. However, lignin is difficult to extract from lignocellulose because of the recalcitrance of the latter. Herein, the extraction of lignin from lignocellulose using deep eutectic-like solvents (DESs) as green solvents was investigated. Three types of DESs were used, and the effects of treatment temperature (100, 110, and 130 °C) and time (6, 12, and 24 h) on lignin yield and its characteristics were studied. For each DES, the yield of DES-lignin increased with reaction temperature and time. At the same time, the lignin yield obtained using different DESs decreased in the order of choline chloride/lactic acid > betaine/lactic acid > K₂CO₃/glycerol. At higher temperatures and longer reaction times, lignin with a high molecular weight was produced, which was attributed to the recondensation of lignin fragments. Furthermore, the methoxyl and hydroxyl group contents decreased with increasing reaction temperature and time. Thermal stability studies revealed that the increased molecular weight of DES-lignin enhanced its thermal resistance.

DOI: 10.15376/biores.17.4.5600-5611

Keywords: Deep eutectic-like solvent (DES); Lignin recondensation; Lignin extraction; Red pine

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INTRODUCTION

Lignin is the most abundant aromatic biopolymer on Earth and accounts for 20% to 40% of lignocellulose. It consists of three basic phenylpropane units: *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol (Zhang *et al.* 2010; Shuai *et al.* 2016; Ma *et al.* 2018), which are randomly cross-linked with β -O-4, β -5, and β - β linkages (Zhang *et al.* 2010; Shuai *et al.* 2016). Lignin possesses several valuable properties, including biodegradability, biocompatibility, non-toxicity, low density, high carbon content, thermal resistance, and oxidation resistance (Li *et al.* 2016). Because of these properties, it has great potential for use in bioplastics, films, carbon fibers, and various chemicals (Sadeghifar *et al.* 2017; Schutyser *et al.* 2018; Park *et al.* 2019; Chen *et al.* 2021). Lignin is primarily produced using pulping processes such as kraft pulping, soda pulping, and sulfite pulping (Kong *et al.* 2015). Technical lignin can be recovered from black liquor with a recovery yield of approximately 30% through regeneration by precipitation. During the precipitation of lignin, depolymerized lignin fragments are to some extent recondensed (Tumbalam Gooty *et al.* 2014; Lancefield *et al.* 2018), which can lead to the formation of recalcitrant condensed units and structural heterogeneity (Li *et al.* 2018). This results in lower hydroxyl

group content and reduced reactivity, thereby limiting its potential for further utilization. The process of technical lignin precipitation requires toxic chemicals and large quantities of water. Various purification procedures are required to depollute this wastewater (Mahmood *et al.* 2013; He and Fatehi 2015). Recently, the isolation of lignin using green solvents, such as ionic liquids and deep eutectic-like solvents (DES), has shown promise.

A DES is an ionic liquid composed of molten salts with organic cations and anions at a eutectic point (Abbott *et al.* 2004; Lynam *et al.* 2017; Ling *et al.* 2019). Though the term eutectic implies a minimization of melting temperature of mixtures, in practice DES formulations do not need to match a eutectic condition. A DES can be facilely synthesized by mixing a hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD). Choline chloride (ChCl), betaine (BE), and alanine are representative HBAs, and lactic acid (LA), glycerol (GLY), oxalic acid, formic acid, and urea have been widely used as HBDs for DES synthesis (Liu *et al.* 2017b; Loow *et al.* 2017; Tan *et al.* 2020). The DESs offer various advantages, such as facile preparation at low cost, low vapor pressure, non-flammability, sustainability, biodegradability, and biocompatibility (Tan *et al.* 2020; Wang *et al.* 2020a). Moreover, they can efficiently dissolve lignocellulose constituents, particularly lignin. Using DES, lignin can be effectively extracted from lignocellulose without posing any environmental problems (Lyu *et al.* 2018; Malaeke *et al.* 2018b; Ji and Lv 2020). Furthermore, the isolation of lignin using DES is simple, and the regenerated lignin can have a high assay because no other salts are produced during regeneration (Alvarez-Vasco *et al.* 2016; Shen *et al.* 2019; Wang *et al.* 2020b). It is known that DES-isolated lignin (DL) has a higher hydroxyl group content without sulfur groups and a molecular structure similar to that of protolignin (Di Marino *et al.* 2016; Kumar *et al.* 2016). The isolation yield of lignin and its structural properties can vary depending on the DES type and reaction conditions (Malaeke *et al.* 2018a); therefore, it is essential to investigate the effects of these phenomena on the yield and characteristics of lignin.

In this study, wood powder was treated with ChCl-, BE-, and potassium carbonate (PC)-based DESs, and lignin was isolated by adding an anti-solvent to the mixture. The effects of the DES type and reaction conditions on the yield, molecular weight, and structural characteristics of the obtained lignin were investigated.

EXPERIMENTAL

Materials

Korean red pine (*Pinus densiflora* S. et Z.) used as a raw material for lignin isolation was obtained from the experimental forest, Kangwon National University (Chuncheon, Republic of Korea). A disk with a thickness of 8 cm was ground to 40–80 mesh size. The degreased wood powder was prepared using an ethanol/benzene (1/2, v/v) solution in a Soxhlet extractor operating at 90 °C for 6 h. The chemicals ChCl, BE, PC, LA, GLY, dimethylformamide, tetrahydrofuran (THF), and 1 M HCl were all purchased from Daejung Chemical & Metals (Siheung, Republic of Korea).

DES Treatment and Isolation Of Lignin

The DESs of ChCl/LA, BE/LA, and PC/GLY were obtained by mixing respective chemicals at molar ratios of 1/1, 1/1, and 1/4, respectively. The mixtures were stirred at 80 °C until clear liquids were obtained. Wood powder was added to each DES at a concentration of 2 wt% and stirred at 400 rpm for 24 h at 100 °C and 120 °C, and for 6, 12, and 24 h at 130 °C. After that the content was centrifuged at 4,000 g for 20 min; thereafter, the DES-soluble fraction and DES-insoluble residue were separated. The DES was

completely washed from the DES-insoluble residue by vacuum filtration with a 1,4-dioxane/water (4:1) solution.

The DES filtered with dioxane/water was evaporated using a rotary evaporator to remove dioxane. The DES-soluble fraction was then dropped in water, and the pH was adjusted to 2 using 1 M HCl to regenerate lignin. After stirring for 24 h, the regenerated lignin was filtered using vacuum, washed with distilled water, and freeze-dried for 24 h at $-55\text{ }^{\circ}\text{C}$ using a freeze-dryer. Lignin yield was calculated by comparing the weight of the obtained lignin with that of the raw material.

Isolation of Milled Wood Lignin

Milled wood lignin (MWL) was isolated from wood powder according to the method described by Björkman (1954). Wood powder (10 g) was added to a 400-mL zirconium oxide jar with 15 zirconium oxide balls (15-mm diameter) and ball-milled at 450 rpm for a total of 3 h by alternating milling and cooling stages, each lasting for 10 min. The milled wood powder was added to 1,4-dioxane/water (96/4, v/v) (200 mL) and stirred for 24 h. Next, the soluble fraction and residue were separated by vacuum filtration, and the 1,4-dioxane/water filtrate was concentrated using a rotary evaporator. The condensed soluble fraction was transferred to water and maintained for 24 h to regenerate the MWL. Crude MWL (~1 g) was dissolved in 90% acetic acid (30 mL) and then added dropwise to distilled water (300 mL) while stirring to precipitate lignin, which was further washed after filtration under vacuum with distilled water and freeze-dried. Dried lignin was dissolved in 30 mL of 1,2-dichloroethane/ethanol (2:1, v/v). The soluble fraction was added to anhydrous ethyl ether (300 mL) to precipitate purified lignin. After washing with ether, the purified MWL was freeze-dried at $-55\text{ }^{\circ}\text{C}$.

Fourier Transform Infrared Analysis

Fourier transform infrared (FT-IR) analysis was conducted using a Nicolet iS10 (Thermo Fisher Scientific, Waltham, MA, USA) equipped with an attenuated total reflectance attachment. A total of 128 scans were performed per sample in the range of 4000 to 400 cm^{-1} .

Thermogravimetric Analysis

Thermogravimetric analysis (TGA) was performed using a Q500 instrument (TA Instruments New Castle, DE, USA) to study the mass-loss characteristics during the thermal decomposition of lignin. The samples (15 to 20 mg) were heated in a platinum pan under nitrogen atmosphere. The scanning temperature range was 25 to $800\text{ }^{\circ}\text{C}$, and the heating rate was $5\text{ }^{\circ}\text{C}/\text{min}$. Derivative thermogravimetry (DTG) was performed by measuring mass loss against time.

Gel Permeation Chromatography (GPC)

To perform GPC, the acetylated lignin (1 mg) was dissolved in THF (1 mL) and filtered using a syringe filter. The number average molecular weight (M_n), weight average molecular weight (M_w), and polydispersity index (PDI) were measured using a Prominenece-I GPC system (Shimadzu, Kyoto, Japan) equipped with ultraviolet (UV) and refractive index detectors. The columns were connected to a PLgel 5 μm mixed-C,-D, and PLgel 3 μm mixed-E (Agilent Technologies, Santa Clara, CA, USA), and the oven temperature was set to $40\text{ }^{\circ}\text{C}$. The injection volume used was 100 μL , and the wavelength of the UV detector was set at 280 nm. The THF was used as the mobile phase at a flow rate of 100 mL/min. The calibration curve was prepared using polystyrene in the range of 1480 to 1,233,000 g/mol.

Gas Chromatography (GC) with Flame Ionization Detection (FID)

The methoxyl content of lignin was determined using GC with FID (GC/FID). The samples for analysis were prepared according to the method proposed by Baker (1996). Lignin (30 mg) was added to hydroiodic acid (4 mL) and allowed to react at 130 °C for 30 min. An internal standard of 200 µL (ethyl iodide in pentane, 300 mg/mL) was added to the solution, followed by 3 mL pentane. An aliquot of the pentane phase was then separated. Methyl iodide was analyzed *via* GC/FID (7890 B, Agilent Technologies, Santa Clara, CA, USA) equipped with a DB-624 column (30 mm × 0.25 mm i.d.). The flow rate used was 1.1 mL/min, and the split ratio was 1/120. The injector and detector temperatures were 200 and 230 °C, respectively. The GC oven temperature was maintained at 40 °C for 5 min and then increased to 180 °C at 10 °C/min.

Proton Nuclear Magnetic Resonance (¹H-NMR) Analysis

¹H-NMR analysis of lignin was conducted using a Bruker Avance 600 MHz spectrometer (Bruker, Ettlingen, Germany). Acetylated lignin (10 mg) was dissolved in CDCl₃ (0.75 mL) and filtered using a syringe filter. The content of hydroxyl groups was determined by integrating the hydroxyl and methoxyl groups in the ¹H-NMR data. After calculating the ratio of the hydroxyl groups based on the integral value of the methoxyl group, the quantitative value of each hydroxyl group was calculated by substituting the quantitative value of the methoxyl group into Eq. 1:

$$\text{Hydroxyl group (mmol/g)} = \text{Methoxyl group (mmol/g)} \times \text{Ratio (integral value of hydroxyl group/integral value of methoxyl group)} \quad (1)$$

RESULTS AND DISCUSSION

Table 1 presents the yield of DL based on the raw material after ChCl/LA (1:1), BE/LA (1:1), and PC/GLY (1:4) DES-treatments at different reaction temperatures and time. In all samples, the DL yield increased with increasing reaction temperature and time. The yield of DL isolated using the treatment with ChCl/LA was higher than that of the BE/LA and PC/GLY treatments under the same reaction conditions.

Table 1. Yields of DL Based on Raw materials at Different Reaction Conditions

DES Treatment	Temperature (°C)	Reaction Time (h)	Yield of DL (%)*
ChCl/LA (1:1)	100	24	17.9
	120	24	21.7
	130	6	20.3
		12	25.4
		24	29.0
BE/LA (1:1)	100	24	3.8
	120	24	9.0
	130	6	3.6
		12	7.1
		24	10.4
PC/GLY (1:4)	100	24	11.0
	120	24	17.7
	130	6	11.1
		12	15.7
		24	20.8

Note: *Yield of DL is the weight percentage of DL based on the weight of the raw material

The amount of lignin extracted from wood powder by DESs increased with temperature and reaction time. Furthermore, the amount of lignin dissolved decreased in the order of ChCl/LA > PC/GLY > BE/GLY under the same reaction conditions. Therefore, as the amount of lignin dissolved in the DESs increased, so did the DL yield from the DES-soluble fraction. Zhang *et al.* (2016) investigated the yield of DL extraction using ChCl/LA from corncobs. The DL yield obtained using ChCl/LA (1:2) treatment for 24 h increased from 18.1% to 95.5% as the reaction temperature was increased from 70 to 110 °C.

The M_n , M_w , and PDI of MWL and DL samples prepared *via* DES treatment with ChCl/LA (1:1), BE/LA (1:1), and PC/GLY (1:4) under different reaction conditions are summarized in Table 2. The molecular structure of MWL is similar to that of proto-lignin; thus, the characteristics of DL samples were compared with those of MWL. The M_n and M_w of MWL were 2011 and 4612 g/mol, respectively, with a PDI of 2.3. The M_w of DLs isolated by ChCl/LA and BE/LA treatments were in the range of 2505 to 6580 and 4060 to 6369 g/mol, respectively, and they increased with reaction temperature and time. This was attributed to the increased temperature and time promoting the recondensation of lignin fragments during DES treatment. However, the M_w of the DLs isolated by PC/GLY treatment at 130 °C marginally decreased with increasing reaction time, whereas higher M_w values were obtained at higher temperatures. In addition, the DLs from PC/GLY at 100 and 120 °C had PDI values of 9.6 and 6.8, respectively.

Figure 1 shows the FT-IR spectra of the MWL and DLs isolated using ChCl/LA (1:1), BE/LA (1:1), and PC/GLY (1:4) at 100 and 130 °C for 24 h. In all samples, the peaks at 3430 and 2934 cm^{-1} , corresponding to O–H and C–H stretchings, respectively, were prominent. The strong bands in the range of 1710 to 1750 cm^{-1} were attributed to C=O stretching. The aromatic skeletal vibrations were observed at 1602, 1507, and 1418 cm^{-1} , which are typical bands of lignin. The peak at 1455 cm^{-1} correspond to the C–H deformations in methyl and methylene carbons. The C–O stretching in the guaiacyl ring was observed at 1272 cm^{-1} , and aromatic C–H deformation was apparent at 1129 to 1034 cm^{-1} . The DLs exhibited typical FT-IR spectra of the MWL molecular structure, which is known to be the form of lignin that is most similar to the native lignin, indicating that there was no damage or minimal damage occurred to the lignin structure during DES treatment.

Table 2. M_n , M_w , and PDI of MWL and DLs Prepared *via* DES Treatment at Different Reaction Conditions

Sample	DES Type	Temperature (°C)	Reaction Time (h)	M_n (g/mol)	M_w (g/mol)	PDI (M_w/M_n)
MWL	-	-	-	2011	4612	2.3
DLs	ChCl/LA (1:1)	100	24	1595	2505	1.6
		120	24	2340	5074	2.2
		130	6	1953	4321	2.2
			12	2021	5453	2.7
			24	1943	6580	3.4
		BE/LA (1:1)	100	24	1256	4060
	120		24	934	4711	3.9
	130		6	1338	4549	3.4
			12	1432	5312	3.7
			24	1731	6369	3.7
	PC/GLY (1:4)		100	24	607	5833
		120	24	1059	7186	6.8
		130	6	2016	9694	4.8
			12	2389	8994	3.8
24			1950	7436	3.8	

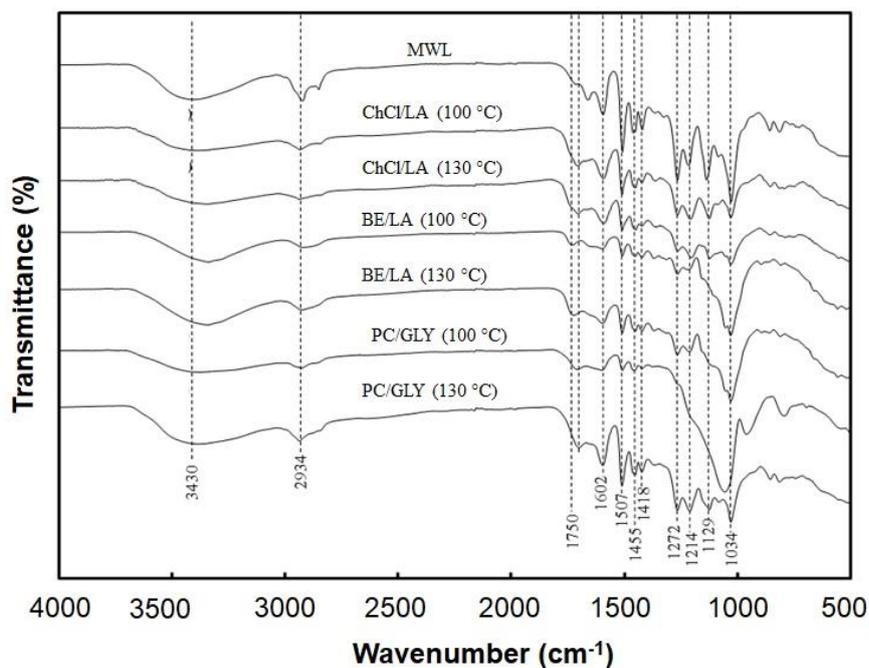


Fig. 1. FT-IR spectra of the MWL and DLs isolated using ChCl/LA (1:1), BE/LA (1:1), and PC/GLY (1:4) at 100 °C and 130 °C for 24 h

Figure 2 presents the ¹H-NMR spectra of the MWL and DLs isolated using ChCl/LA (1:1), BE/LA (1:1), and PC/GLY (1:4) at 100 °C for 24 h.

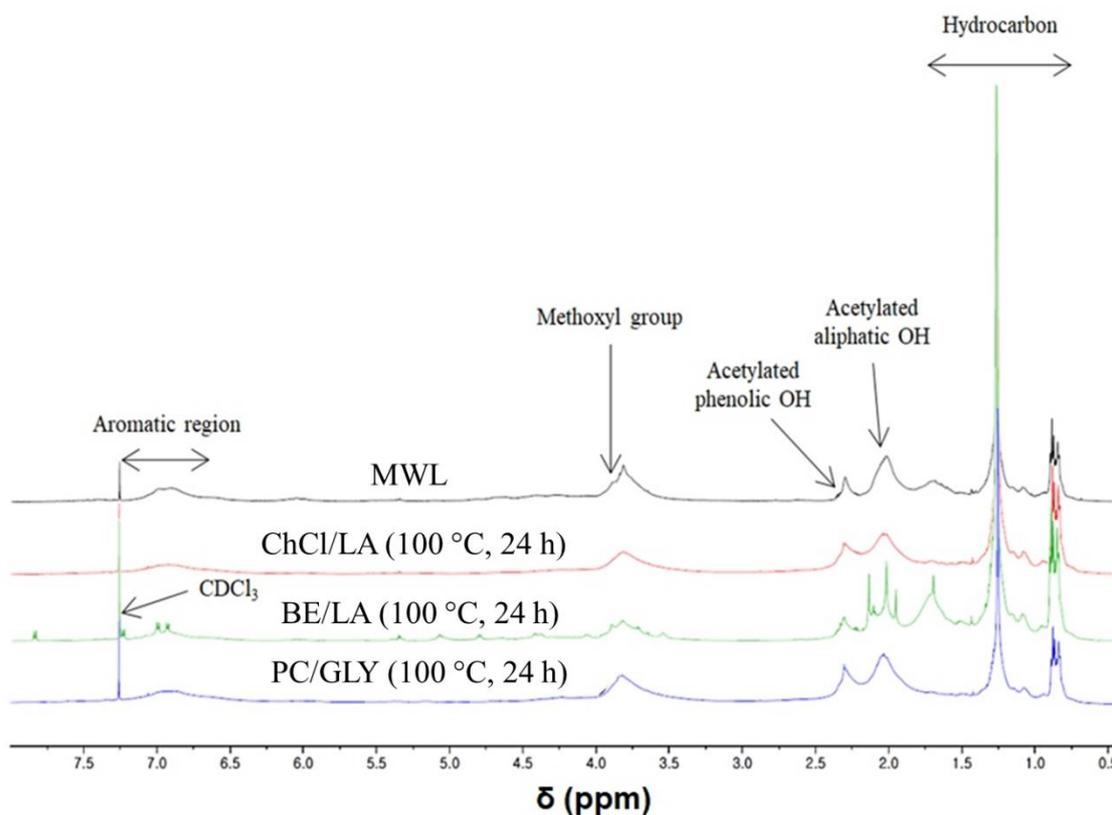


Fig. 2. ¹H-NMR spectra of the MWL and the lignins isolated using ChCl/LA (1/1), BE/LA (1/1), and PC/GLY (1/4) at 100 °C for 24 h

The chemical shifts in the range of δ 7.1 to 6.5 ppm are ascribable to the aromatic region of lignin, while the weak signals in the range of δ 5.8 to 4.2 ppm are attributed to aliphatic protons. The signal corresponding to the methoxyl groups is found in the range of δ 3.9 to 3.4 ppm. The chemical shifts, in the ranges of δ 2.4 to 2.2 and 2.2 to 1.7 ppm, are assigned to the acetylated phenolic hydroxyl and acetylated aliphatic hydroxyl groups, respectively. The signal observed at approximately δ 1.3 ppm is assigned to hydrocarbons. The ratios of the phenolic and aliphatic hydroxyl groups were calculated by setting the integral value of the methoxyl group region to 1. Then, the hydroxyl group contents were calculated based on the methoxyl group content obtained from the GC/FID analysis (Table 3).

Table 3 shows the contents of methoxyl group, as well as the phenolic and aliphatic hydroxyl groups in MWL and DL samples isolated using ChCl/LA (1:1), BE/LA (1:1), and PC/GLY (1:4) DES treatments under different reaction conditions. The contents of methoxyl, phenolic, and aliphatic hydroxyl groups in MWL were 3.12, 1.00, and 3.68 mmol/g, respectively. The methoxyl group content of DLs isolated by ChCl/LA and BE/LA treatments decreased with increasing reaction temperature and time, indicating a lower hydroxyl group content. The decrease in the methoxyl and hydroxyl contents might be caused by the recondensation of lignin fragments with the increasing reaction temperature and time. Liu *et al.* (2017a) isolated lignins using ChCl/oxalic acid with molar ratios of 1:2, 1:1, 2:1, 3:1, and 4:1 at 80 °C for various reaction time. With the increase in reaction time to 10 h, the methoxyl content was decreased. The authors attributed this phenomenon to the condensation reaction of degraded lignin with the extension of reaction time. The lignin isolated from PC/GLY had lower methoxyl and hydroxyl group contents than the lignins isolated using ChCl/LA and BE/LA treatments, which is in accordance with their high molecular weights.

Table 3. Contents of Methoxyl, Phenolic, and Aliphatic Hydroxyl Groups in the MWL and DLs at Different Reaction Conditions

Sample	DES Type	Temp. (°C)	Reaction Time (h)	Methoxyl Group (mmol/g)	Phenolic OH Group (mmol/g)	Aliphatic OH Group (mmol/g)	Total OH Group (mmol/g)
MWL		-	-	3.12	1.00	3.68	4.68
DLs	ChCl/LA (1:1)	100	24	2.67	1.87	3.24	5.11
		120	24	2.63	1.80	2.89	4.69
		130	6	2.59	1.14	2.79	3.93
			12	2.34	1.13	2.64	3.77
			24	2.23	1.08	2.34	3.42
	BE/LA (1:1)	100	24	2.49	1.47	2.24	3.71
		120	24	2.47	1.39	2.10	3.49
		130	6	2.27	1.30	2.08	3.38
			12	2.25	1.22	1.98	3.20
			24	2.08	1.18	1.84	3.02
	PC/GLY (1:4)	100	24	1.36	1.00	0.99	1.99
		120	24	1.49	1.03	1.22	2.25
		130	6	1.96	1.11	2.24	3.35
			12	1.84	1.08	2.10	3.19
			24	1.83	1.19	2.15	3.41

Figure 3 shows the TGA and DTG curves of MWL and DLs treated with DES combinations of ChCl/LA (1:1), BE/LA (1:1), and PC/GLY (1:4) under various conditions. Thermal decomposition of MWL occurs in the range of 210 to 450 °C.

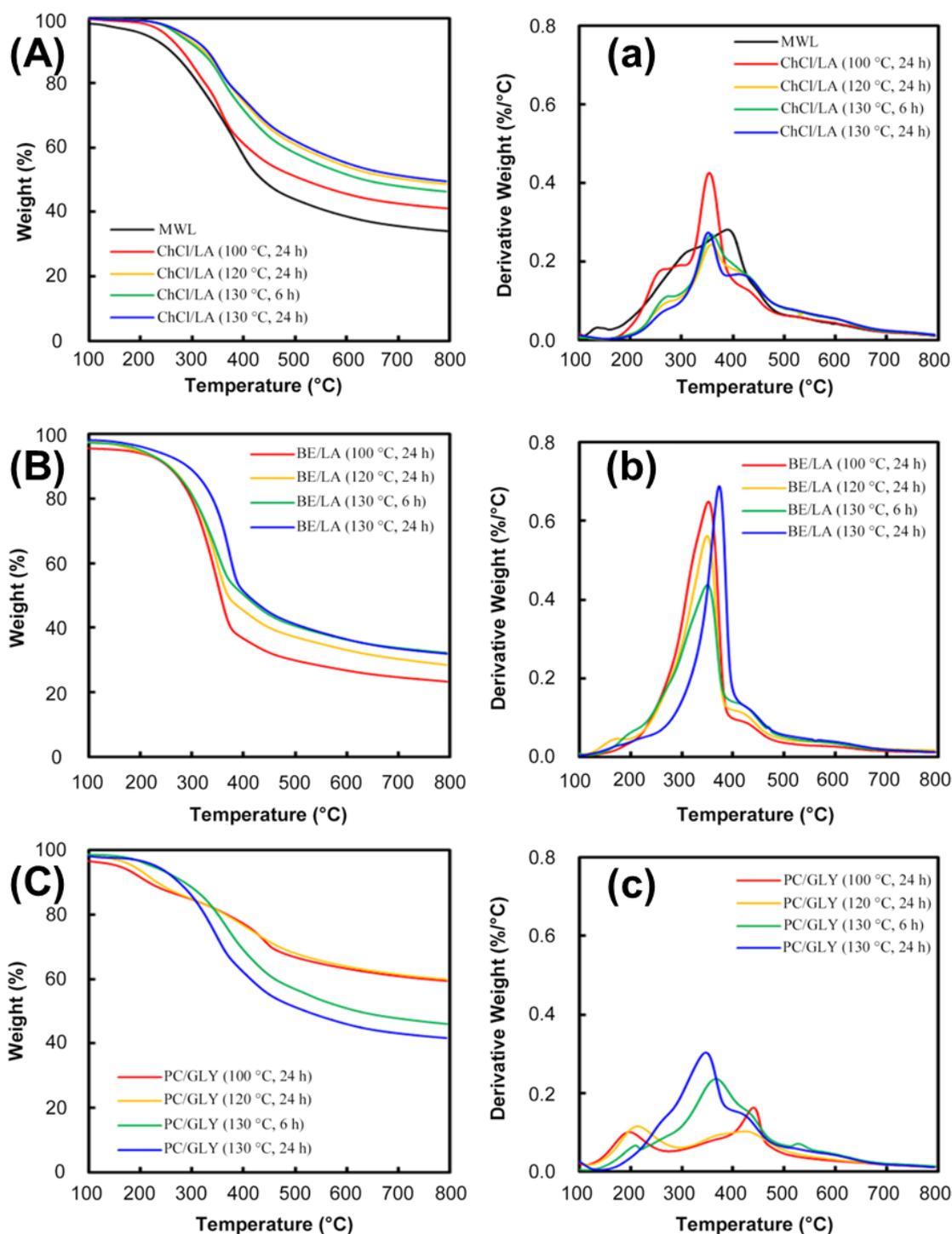


Fig. 3. TGA (A, B, C) and DTG (a, b, c) curves of MWL and DLs obtained using ChCl/LA (A and a), BE/LA (B and b), and PC/GLY (C and c) DES combinations under different reaction conditions

The ash content of MWL at 800 °C was approximately 35%, which is lower than that of lignins treated using ChCl/LA. For the lignins treated with ChCl/LA and BE/LA, the temperature at which thermal degradation started, as well as char residues, increased with increasing reaction temperature and time. This could be attributed to the recondensation of lignin fragments with increasing reaction temperature and time. The DTG curves of the DL treated with ChCl/LA showed three peaks at 260, 350, and 435 °C, resulting from the thermal degradation of the β -O-4, C-C, and β - β linkages, respectively

(Moustaqim *et al.* 2018). The height of the peak at 260 °C, corresponding to the degradation of the β -O-4 linkage, increased with increasing reaction temperature and time. While the DL treated with BE/LA exhibits only two peaks at 350 to 370 °C and 430 to 450 °C, there was no peak found at 260 °C. For lignin treated with PC/GLY, despite high M_w observed at low temperatures, thermal degradation started at a relatively low temperature of approximately 200 °C. As shown in Table 2, the lignins treated with PC/GLY at 100 and 120 °C for 24 h exhibited an extremely high PDI. This implies that the M_w of lignin is widely distributed and that lignins with a relatively low M_w thermally decompose first.

CONCLUSIONS

1. Lignin was successfully isolated from red pine using three types of deep eutectic-like solvents (DESs), and all of the obtained DES-isolated lignins (DLs) exhibited Fourier-transform infrared (FT-IR) peaks similar to those of milled wood lignin (MWL), indicating a similar structure to MWL.
2. For a particular DES, the DL yield increased with increasing reaction temperature and time. Based on DES type used, the DL yield is in the order of choline chloride/lactic acid (ChCl/LA) > betaine/actic acid (BE/LA) > K₂CO₃/glycerol (PC/GLY).
3. Higher treatment temperature and time resulted in DL with higher M_w because of the recondensation of lignin fragments.
4. Methoxyl and hydroxyl groups contents of DL decreased as the reaction temperature and time was increased.
5. Thermal stability studies revealed that the increased molecular weight of DL enhanced its thermal resistance.

ACKNOWLEDGMENTS

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (No. 2018R1A6A1A03025582).

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Article submitted: June 20, 2022; Peer review completed: July 23, 2022; Revised version received and accepted: August 2, 2022; Published: August 5, 2022.
DOI: 10.15376/biores.17.4.5600-5611