# Treatment Effects of Choline Chloride-based Deep Eutectic Solvent on the Chemical Composition of Red Pine (*Pinus densiflora*)

Gu-Joong Kwon,[a,b] Bong-Suk Yang,[c] Chan-Woo Park,[d] Rajkumar Bandi,[b] Eun-Ah Lee,[d] Ji-Soo Park,[d] Song-Yi Han,[b] Nam-Hun Kim,[b,d] and Seung-Hwan Lee [b,d,*]

Effects of deep eutectic solvent (DES) treatment were determined for the chemical composition of the sapwood and heartwood of red pine (*Pinus densiflora*). Two DES systems were made from the mixture of choline chloride (ChCl) and two different hydrogen bond donors (HBDs), namely, lactic acid (LA) and glycerin (GLY), with different molar ratios (1:2, 1:6, and 1:10). The yield of the solid residue after DES treatment decreased with an increase in the HBD concentration and treatment time, indicating that the water-soluble fraction was increased. The amount of solid residue was lower in the DES with LA than in DES with GLY, and higher in sapwood than in heartwood during both DES treatments. There was no substantial change in the lignin content of the samples, each being 24.7 to 29.5 wt.%, based on the mass of the treated product. Similar to the yield of the solid residue, cellulose and hemicellulose content in the treated product decreased with an increase in the HBD concentration and treatment time, and it was higher in sapwood than in heartwood. The cellulose crystallinity exhibited a slight increase with increasing treatment time, but there was no difference among the treatments using different molar ratios and between sapwood and heartwood.

*Keywords: Deep eutectic solvent; Red pine; Choline chloride; Lactic acid; Glycerin; Chemical composition*

*Contact information: a: Kangwon Institute of Inclusive Technology, Kangwon National University, Chuncheon-Si, 23341, Republic of Korea; b: Institute of Forest Science, Kangwon National University, Chuncheon-Si, 24341, Republic of Korea; c: College of Life Science and Biotechnology, Dongguk University, Goyang-Si, 10326, Republic of Korea; d: Department of Forest Biomaterials Engineering, College of Forest and Environmental Sciences, Kangwon National University, Chuncheon-Si, 24341, Republic of Korea;*
*\* Corresponding author: lshyhk@kangwon.ac.kr*

## INTRODUCTION

In recent years, discovering renewable feedstock to produce fine chemicals, materials, and fuels has become important due to increasing energy demands and environmental issues (Hou *et al*. 2017a; Ho *et al*. 2019). Lignocellulosic biomass is the most abundant, low-cost, and renewable biomass that can be utilized for conversion into a valuable chemical or materials (Isikgor and Becer 2015; Capolupo and Faraco 2016; Ahorsu *et al*. 2018; Lee *et al*. 2019). The biorefinery conversion of lignocellulosic materials is a developing process for efficient and economically viable utilization as a pretreatment to obtain high-value products. Solvents for the separation and extraction of lignocellulosic materials include organic solvents (Espinoza-Acosta *et al*. 2014; Zhang *et al*. 2016). However, many organic solvents have adverse effects on the environment and the human body (Agbor *et al.* 2011; Kucharska *et al*. 2018).

An ionic liquid refers to a substance that exists in a liquid state at room temperature

and forms ionic bonds. The ionic liquids are considered as being environmentally friendly or green solvents because they have favorable properties, including high solvent capacity towards both organic and inorganic materials and negligible vapor pressure, which reduces the volatile organic compound emission (Zhao 2003; Sirviö *et al*. 2016). Furthermore, it is possible to appropriately select cations and anions according to need, as well as control their characteristics (Sun *et al*. 2009; Espinoza-Acosta *et al.* 2014; Han *et al.* 2017; Merza *et al.* 2018). The pretreatment of woody biomass by an ionic liquid increases the accessibility of enzymes during saccharification by reducing the degree of crystallization of cellulose and selectively separating the constituents (Sathitsuksanoh *et al.* 2012; George *et al*. 2015; Han *et al*. 2020).

However, the manufacturing of ionic liquids is cumbersome. They are very expensive; some ionic liquids are harmful to the human body and can also cause environmental hazards. Deep eutectic solvents (DESs) have been developed as new alternative solvents to resolve these problems (Shamsuri and Abdullah 2010; Dai *et al*. 2013). DESs are of increasing interest in science and industry because they are simple to use, safe, cost-effective, and do not present problems in purification or disposal (Yadav *et al.* 2014). These solvents are generally environmentally friendly, biocompatible compounds that are composed of a hydrogen bond acceptor (HBA) such as quaternary ammonium salts and a hydrogen bond donor (HBD) such as amides, carboxylic acids, and alcohols. When released, they are not environmentally hazardous. Their synthesis is economically feasible and allows the incorporation of the components of green chemistry (Malaeke *et al.* 2018). Typically, deep eutectic solvents consist of large, non-symmetric ions that have low lattice energy and hence, low melting points. They are usually prepared by mixing an HBA and HBD at moderate temperatures to form eutectic mixtures. Hydrogen bonding results in charge delocalization or self-association occurring between the HBA and HBD, and consequently the melting point of the eutectic mixture is much lower as compared to the individual compounds (Satlewal *et al*. 2018). The H bonding is the key for DESs forming. The H bonding is very unique compared with other bonding types. H bonding can have different bonding forces and lengths depending on the ambient conditions for the same component (Kadhom *et al*. 2017). Choline chloride is the most commonly used HBA because it is low cost, biodegradable, safe, non-toxic, and extractable from biomass. And, DESs mixtures formed on the basis of choline chloride tend to be controlled by the stability of carboxylic acids, amides, or alcohol.

In view this, previous studies for DESs have been focused on the physicochemical characterization, fractionation efficiency, pre-treatment for nanofibrillation, extraction bioactive substances, and delignification at constant molar ratios, temperature and time conditions using DES formed by combining with various HBDs (Sirviö *et al*. 2015; Hiltunen *et al*. 2016; Loow *et al*. 2018; Ong *et al*. 2019; Sakti *et al*. 2019; Smink *et al*. 2019; Yu *et al*. 2019). However, the effect of DES pH was not taken into consideration in most of these reports. On the basis of pH, Skulcova *et al*. (2018) classified DESs into two groups, *i.e.* organic acid as HBD (pH 0 to 3.0), and polyol as HBD (pH 4.0 to 4.5), and they emphasized the importance of efficient DES application and suggested that the DES pH value can greatly affect the chemical reaction with the components of biomass. In this context, in order to take DES pH into account, two representative HBDs, *i.e.* lactic acid (LA) for organic acid and glycerol (GLY) for polyol were selected. The main aim of this study was to investigate the effect of DES treatment time and mole ratio of HBDs with different pH values on the chemical composition of sapwood and heartwood of the red pine.

## EXPERIMENTAL

## Materials

The red pine was provided by the Research Forest of Kangwon National University in Korea. The sapwood and heartwood were separated by identifying the color difference in the cross-sectional disk. The percentage of sapwood and heartwood was 80.5% and 19.5%, respectively. Each sample was cutter-milled to a specific size (40- to 80-mesh). The milled red pine was extracted with acetone before DES treatment. Choline chloride (ChCl), LA, and GLY were purchased from Daejung Chemical Co., Ltd., Siheung, Korea. All other reagents were of analytical grade and used without further purification.

## Methods

### DES treatment

Two DES systems were prepared using the heating method by mixing ChCl and two hydrogen bond donors, *i.e.*, LA and GLY, with molar ratios of 1:2, 1:6 and 1:10, respectively. The two components of each mixture were placed in a round-bottom flask with magnetic stirring for 10 min at 100 °C until a clear colorless liquid was obtained. Wood powder (1 g, oven-dry) was added to the DESs (20 g) and heated at 100 °C. The ratio of the sample and the DESs was 1:20 (w/w). The treatment times were 2 h, 4 h, 6 h, and 12 h. After the treatment, the sample was filtered. The solid residue was washed using distilled water/acetone (1:1) two times, washed with water, and dried at 105 °C to obtain the DES treatment sample.

### Chemical composition measurement

The chemical composition of the sample was determined using a scaled-down version of the Klason protocol of TAPPI T222 om-88 (2011). Briefly, 0.2 g of sample was prepared at room temperature with 3 mL of 72% $H_2SO_4$ for 2 h. The reaction was diluted using 112 mL of deionized water. The solution was autoclaved at 121°C for 1 h. The solid was filtered through a 1G4 glass filter and dried overnight at 105 °C. The acid-insoluble lignin content was determined by the ratio between the weights of the solid residue and the initial amount of the sample. The acid soluble lignin was determined using a UV-vis spectrophotometer (Lamda 35, PerkinElmer, Waltham, MA, USA) at a wavelength of 205 nm.

The sugar analysis of samples was performed using Bio-LC (ICS-3000, Dionex, Sunnyvale, CA, USA) according to the NREL analytical procedure (Sluiter *et al*. 2008). High-performance anion-exchange chromatography (HPAEC) was connected with electrochemical detection using pulsed amperometry (gold electrode), which was used for quantitation of neutral sugar. The sample was chromatographed on a CarboPac PA-1 column. The system was operated in isocratic mode at a flow rate of 1.0 mL/min with a mixture of 250 mM of sodium hydroxide (20%) and deionized water (80%). Five types of sugars (glucose, xylose, arabinose, galactose, and mannose) were calculated using Chromeleon software (Version 6.8, Dionex).

### Characterization techniques

The X-ray diffraction patterns of the DES treatment sample were recorded with an X-ray diffractometer (DMAX 2100V, Rigaku, Akishima, Japan) operated at 40 kV and 30 mA. Scattered radiation was detected in the range of $2\theta = 10$ to $35°$ at a scan rate of 1°/min. The crystallinity (*CrI*) was calculated using the peak intensity method,

$$CrI\,(\%) = (I_{200}\text{-}I_{am}/I_{200}\,) \times 100$$

where $I_{200}$ is the diffraction intensity of (200) ($2\theta = 22.5°$) and $I_{am}$ is the diffraction intensity of the amorphous region ($2\theta = 18°$).

The Fourier transform infrared spectroscopy (FTIR) spectra of the DES-treated samples were recorded on a Fourier transform infrared instrument (Nicolet 10, Thermofisher Scientific, Waltham, MA, USA) in the range from 600 to 4000 cm$^{-1}$ with a resolution of 4 cm$^{-1}$. The attenuated total reflection (ATR) method was used for the measurement. A total of 32 scans were taken for each sample.

The morphologies of the samples, with or without DES treatment, were examined by scanning electron microscopy (S-4800, Hitachi, Ltd, Japan) at a 5 kV accelerating voltage.

## RESULTS AND DISCUSSION

### Yield of the Solid Residue

The yield of the solid residue after DES treatments is summarized in Table 1. With increasing molar ratios of ChCl and HBDs, *i.e.*, the amount of LA and GLY in DES, the yield of solid residue decreased in both sapwood and heartwood, indicating that the water-soluble fraction was increased. The yield in sapwood was higher than in heartwood. In DES with LA, the yield was in the range of 56.4 to 79.7% and 50.6 to 73.6% in sapwood and heartwood, respectively, for all molar ratios. These values were lower than the range of 83.0 to 92.4% and 79.0 to 87.8% in sapwood and heartwood, respectively, in DES with GLY.

**Table 1.** Yield of the Solid Residue Obtained after DES Treatments at 100 ℃

| Sample Molar ratio of ChCl/HBDs and Time | | DES with LA | | DES with GLY | |
|---|---|---|---|---|---|
| | | SW (%) | HW (%) | SW (%) | HW (%) |
| 1:2 | 2 h | 79.7 | 73.6 | 92.4 | 87.8 |
| | 4 h | 75.6 | 69.7 | 90.1 | 84.8 |
| | 6 h | 72.6 | 66.8 | 89.7 | 82.7 |
| | 12 h | 62.7 | 60.0 | 84.5 | 80.5 |
| 1:6 | 2 h | 73.8 | 68.5 | 83.5 | 79.6 |
| | 4 h | 67.7 | 61.8 | 85.2 | 78.1 |
| | 6 h | 62.7 | 56.8 | 84.4 | 74.9 |
| | 12 h | 56.4 | 51.8 | 84.6 | 73.0 |
| 1:10 | 2 h | 70.7 | 64.9 | 84.5 | 80.1 |
| | 4 h | 67.6 | 62.3 | 85.9 | 80.6 |
| | 6 h | 57.5 | 55.7 | 84.8 | 79.8 |
| | 12 h | 56.4 | 50.6 | 83.0 | 79.0 |

SW: Sapwood, HW: Heartwood

### Chemical Compositions

Table 2 shows the chemical composition of untreated material, *i.e.* sapwood and heartwood of red pine. The chemical properties of the sapwood and heartwood of different wood species have been reviewed by several authors. Generally, sapwood contains more cellulose than heartwood (Benouadah *et al*. 2019). For hemicellulose, only small

differences have been observed between heartwood and sapwood. Concerning lignin, it has been reported to be present in slightly lesser amounts in heartwood than in sapwood (Li *et al*. 2019). The total sugar amount of 72.3% in the sapwood was lower than the 77.5% in the heartwood. The total lignin content of sapwood was slightly higher than that of heartwood. In this result, cellulose showed the opposite trend from the previous results, but hemicellulose and lignin were similar.

**Table 2.** Chemical Composition of the Raw Material

| | Composition (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Glu | Xyl | Man | Ara | Gal | Total Sugars | AIL | ASL | Total lignin |
| SW | 46.5 | 6.3 | 13.9 | 2.0 | 3.7 | 72.3 | 28.7 | 0.5 | 29.2 |
| HW | 48.9 | 8.7 | 12.4 | 2.5 | 4.9 | 77.5 | 27.1 | 0.5 | 27.6 |

SW: Sapwood, HW: Heartwood, Glu: Glucose, Xyl: Xylose, Man: Mannose, Gal: Galactose, AIL: Acid insoluble lignin, ASL: Acid soluble lignin

Figures 1 and 2 show the changes in the amounts of cellulose (expressed as glucose), hemicellulose (expressed as the sum of xylose, mannose, arabinose and galactose) and lignin (expressed as the sum of acid insoluble lignin and acid soluble lignin) in the treated product by varying the molar ratio of ChCl and HBDs (LA and GLY) and treatment time. With increasing molar ratio and treatment time, the amount of cellulose and hemicellulose decreased in both sapwood and heartwood. In particular, the amount of cellulose (Fig. 1. C) was remarkably decreased by increasing the amount of LA in DES, exhibiting the minimum value of 20.9% in heartwood with a molar ratio of 1:10. This value was almost half of the cellulose content of 48.9% of the untreated material. The amount of hemicellulose (Fig. 1.H) was not substantially different in any samples. Hou *et al*. (2017b) reported that treatment using ChCl-based DES with oxalic acid can degrade 95.8% of hemicellulose at 120 °C for 4 h. Lignin content (Fig. 1. L) was not significantly different among treated products. Even compared to the lignin content (29.2% and 27.6% in sapwood and heartwood, respectively) of the untreated product, the range of 24.5 to 26.5% in the treated product was slightly changed. In a previous study where the lignocellulosic biomass of rice straw was treated with ChCl-LA DES, the solubility of pure lignin, cellulose and hemicellulose was found to be selective, and lignin dissolved more (Kumar *et al*. 2016). However, in the present study more cellulose and hemicellulose was removed than lignin, indicating the possible difference in the interaction of DESs with different biomass materials.

The amount of cellulose and hemicellulose (Fig. 2.C, H) by treatment using DES with GLY decreased in both sapwood and heartwood with increasing molar ratio and treatment time. The decrease in cellulose was significant, but the amount of hemicellulose was not significantly different. Compared with the treatment using DES with LA, the reduction rate of hemicellulose by treatment using DES with GLY was less. This could have been caused by the strong acidic properties of LA. The change in the amount of lignin (Fig. 2. L) was not significant. Alvarez-Vasco *et al*. (2016), suggested that hardly any wood components were removed by ChCl-glycerol treated with different temperature. The results showed that the cellulose and hemicellulose was decreased by increasing the molar ratio and treatment time.

Overall, the heartwood exhibited a greater decreasing rate than the sapwood. The choline chloride based acidic solvents contain a high abundance of nonsymmetric chloride

ions and carboxylic acid molecules, possessing the features of both ionic liquids and organic solvents. The strong hydrogen bond interactions in cellulose may be weakened because of competing hydrogen bond formation between the chloride ions in DES and the hydroxyl groups in carbohydrates, consequently breaking the intramolecular hydrogen bond network (Liu *et al*. 2017). In addition to their internal hydrogens bonds, DESs can also form hydrogen bonds with cellulose and dissolve cellulose. Hence, studying the H bond type and the ability of H bond formation is crucial for gaining insights into the dissolution of cellulose. In the ChCl-based DESs, one carbonyl oxygen can form two hydrogen bonds, and one hydroxyl oxygen can form one hydrogen bond with cellulose. The ChCl can accept three $H^+$ ions. Hence, two hydrogen bonds can be formed between ChCl and LA. ChCl can form a hydrogen bond with cellulose, and LA molecules can form 4 hydrogen bonds with cellulose, giving a total of five bonds with cellulose. GLY can form three hydrogen bonds with ChCl and three with cellulose. Therefore, the number of hydrogen bonds with cellulose was relatively small for ChCl/GLY compared to ChCl/LA, and hence the dissolution of cellulose is more in LA than GLY (Zhang *et al*. 2020). Furthermore, increase in the reaction time and HBD ratio also resulted in the increased dissolution, in accordance with the literature reports. Chen *et al*. (2019) reported that under the same temperature, the solubility of poplar wood meal in the DES showed an upward trend with the increase of the dissolving time. van Osch *et al*. (2017) observed that the dissolvability of the cellulose was increased with increasing HBD ratio. Therefore, the heartwood with a high cellulose and hemicellulose content was decreased more than sapwood, and the yield of the heartwood (shown in Table 1) was lower than that of the sapwood.
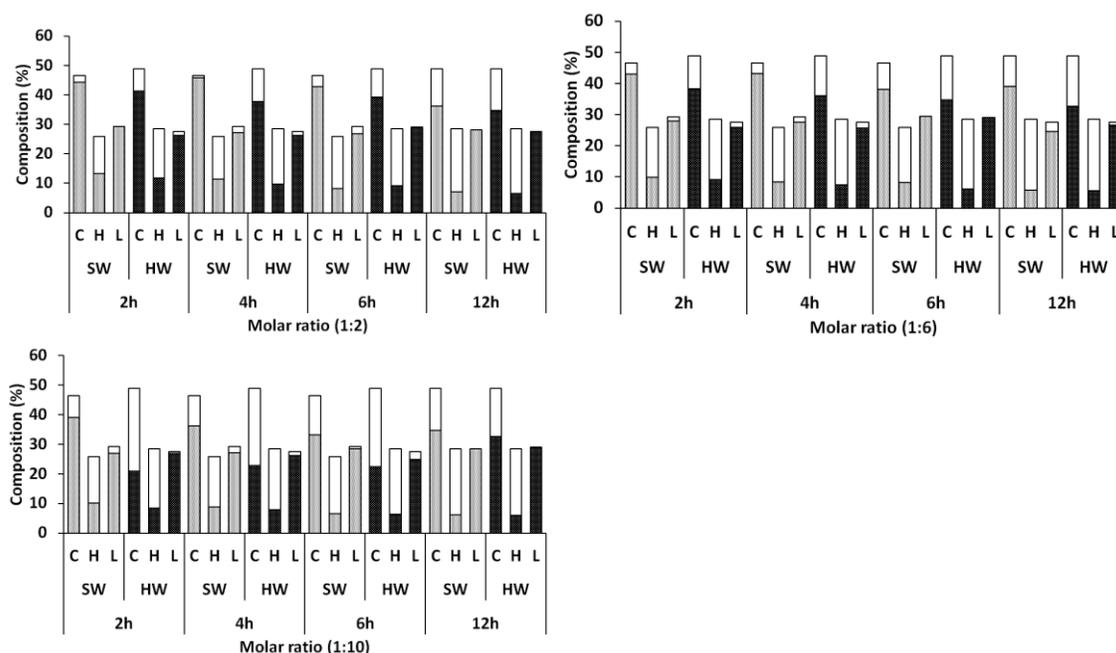


**Fig. 1.** Chemical compositions(C: Cellulose, H: Hemicellulose, L: Lignin) of the soluble fraction(☐) and solid residue (▨:Sapwood (SW), ■: Heartwood (HW) after DES with LA with different molar ratios and treatment time at 100 °C
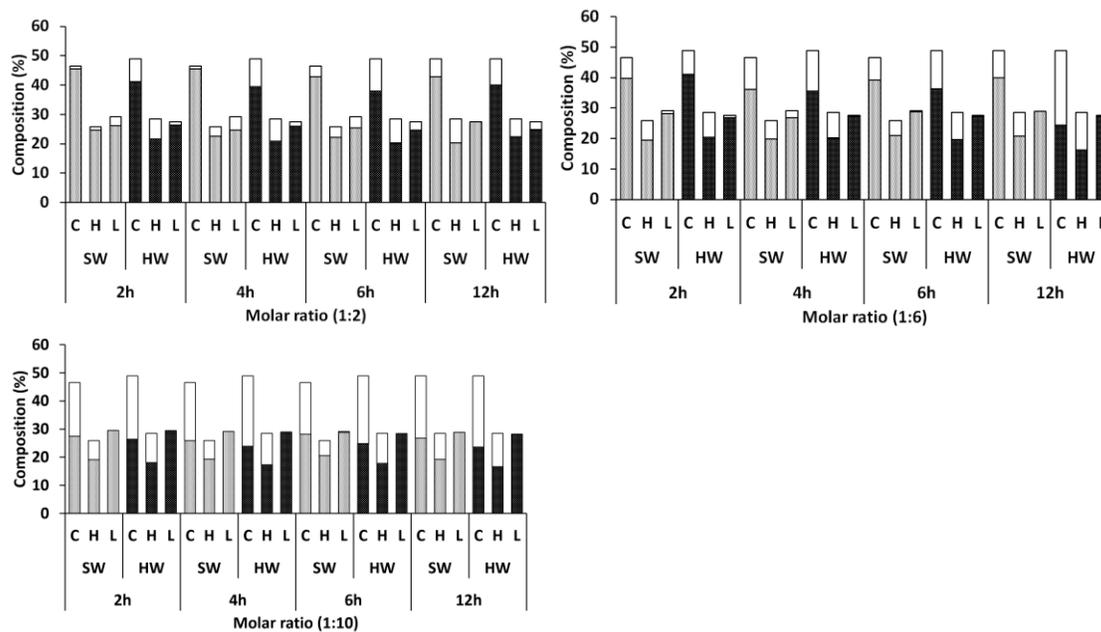
**Fig. 2.** Chemical compositions(C: Cellulose, H: Hemicellulose, L: Lignin) of the soluble fraction(☐) and solid residue (▨:Sapwood (SW), ▦: Heartwood (HW) after DES with GLY with different molar ratios and treatment time at 100 °C

## X-ray Diffraction (XRD) of DES-treated and Untreated Samples

Figure 3 shows the XRD patterns of the DES-treated and untreated sapwood. All samples exhibited the typical diffraction pattern of the cellulose I polymorph, indicating that the treatment using DES with LA and GLY did not affect the cellulose structure regardless of the molar ratio and treatment time. The crystallinity values in all samples are summarized in Table 3.

The crystallinity of the untreated material was 46.2% and 53.8% in sapwood and heartwood, respectively. The crystallinity values of the treated products using DES with LA were higher than those using DES with GLY under all treatment conditions. This may have been caused by the lower yield of the solid residue in the treated product using DES with LA.

Procentese *et al.* (2015) reported that the increase in the degree of cellulose crystallinity after treatment using ChCl-based DES with GLY, urea, and imidazole was mainly caused by the removal of the amorphous hemicellulose and lignin. However, there was little difference with various molar ratios and treatment times in all samples. Overall, the crystallinity of both sapwood and heartwood treated with both DES with LA and GLY was somewhat higher than that of the untreated material. Relatively, the wood samples (both sapwood and heartwood) treated by DES with LA were more crystalline than that of DES with GLY. Moreover, with both types of DESs heartwood appeared less crystalline than sapwood.

Satlewal *et al.* (2018) reported that the crystallinity of biomass increased after treatment of DESs, usually due to the removal of lignin as well as amorphous hemicellulose. Hence, it can be expected that, compared to the DES with LA, DES with GLY has the less efficiency of removing amorphous hemicellulose and also, the less crystallinity of the heartwood is thought to be due to this difference.
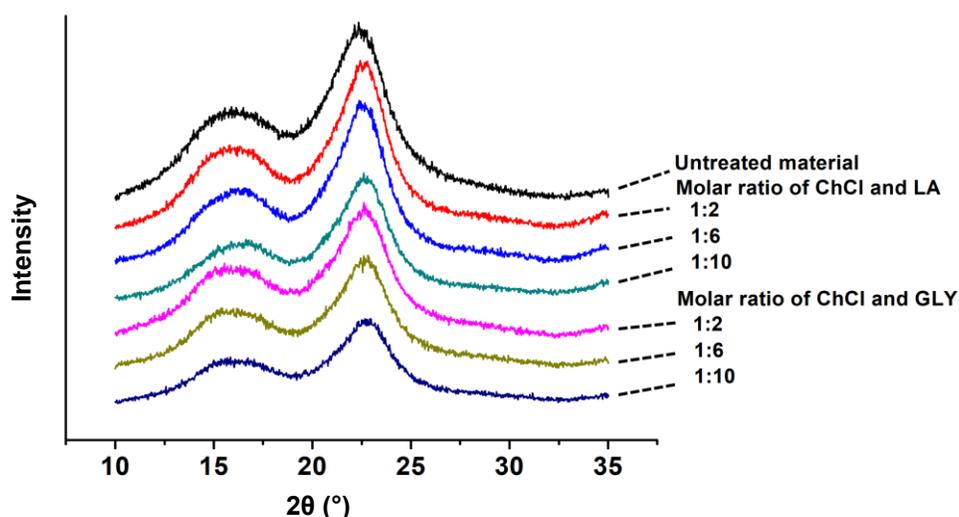
**Fig. 3.** XRD patterns of the untreated material and DES-treated products with different molar ratios of ChCl and HBDs for 12 h at 100 °C using red pine sapwood

**Table 3.** Summary of the Crystallinity of DES-treated Products at 100 °C

| Sample | | DES with LA | | DES with GLY | |
|---|---|---|---|---|---|
| Molar ratio of ChCl/HBDs and Time | | SW (%) | HW (%) | SW (%) | HW (%) |
| Untreated material | | 46.2 | 48.7 | - | - |
| 1:2 | 2 h | 57.3 | 54.1 | 52.5 | 51.6 |
| | 4 h | 58.2 | 55.7 | 53.0 | 52.5 |
| | 6 h | 57.7 | 57.7 | 53.1 | 52.9 |
| | 12 h | 59.7 | 58.5 | 53.3 | 53.1 |
| 1:6 | 2 h | 57.9 | 56.6 | 51.4 | 52.2 |
| | 4 h | 57.4 | 56.7 | 50.0 | 51.3 |
| | 6 h | 58.3 | 56.8 | 52.5 | 52.6 |
| | 12 h | 60.9 | 60.0 | 51.0 | 51.6 |
| 1:10 | 2 h | 56.1 | 54.5 | 51.4 | 52.4 |
| | 4 h | 56.5 | 54.8 | 50.5 | 50.0 |
| | 6 h | 57.7 | 57.3 | 50.6 | 51.0 |
| | 12 h | 57.9 | 57.3 | 50.7 | 50.6 |

SW: Sapwood, HW: Heartwood

## Functional Group Analysis of the DES-treated and Untreated Samples

The FTIR spectra of the untreated and DES-treated sapwood with different molar ratios of ChCl and LA or GLY are shown in Fig. 4. There was no significant change in any of the spectra because the DES-treated products contained all components of cellulose, hemicellulose, and lignin.

The broad absorbance region in the range of 3300 to 3500 cm⁻¹ corresponds to the stretching of hydroxyl groups of cellulose, hemicellulose, and lignin. The absorbance in the range of 2800 to 2900 cm⁻¹ is caused by CH stretching in the crystalline region of cellulose. The individual band in the region 1800 to 800 cm⁻¹ is related to the absorbed O-H vibration, CH deformation, C-O stretch lignin, and carbohydrates (Gupta *et al.*, 2015). In particular, the intensity of the peak at 1104 cm⁻¹ caused by the vibration of cellulose

*bioresources.com*

alcohol was very small in the untreated sample, whereas it increased in the DES-treated products. This result indicated that lignin removal by DES treatment exposed cellulose on the surface of the DES-treated products.
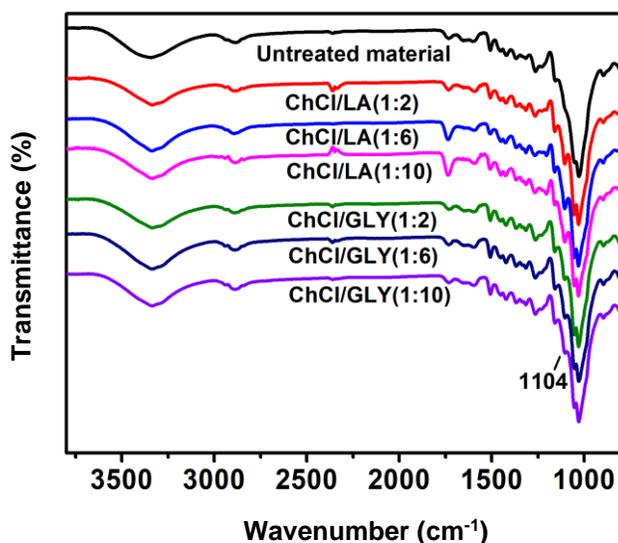


**Fig. 4**. FT-IR spectra of the untreated material and DES-treated products with different molar ratios of ChCl and HBDs for 12 h at 100 °C using red pine sapwood

## SEM Observation of the DES-treated and Untreated Samples

Figure 5 shows the SEM images of red pine sapwood samples, including untreated and treated specimens with DES at different HBD ratios for 12 h at 100 °C. The untreated material (Fig. 5a) displays a rigid and rough surface morphology. By contrast, the surfaces of the DES treated products (Fig. 5b-g) were broken into separate fibers or fiber bundles and became smoother in comparison to the untreated material. These results suggest that DES treatment significantly removed the chemical components of red pine to different extents. However, there seems to have been little difference according to the molar ratio.
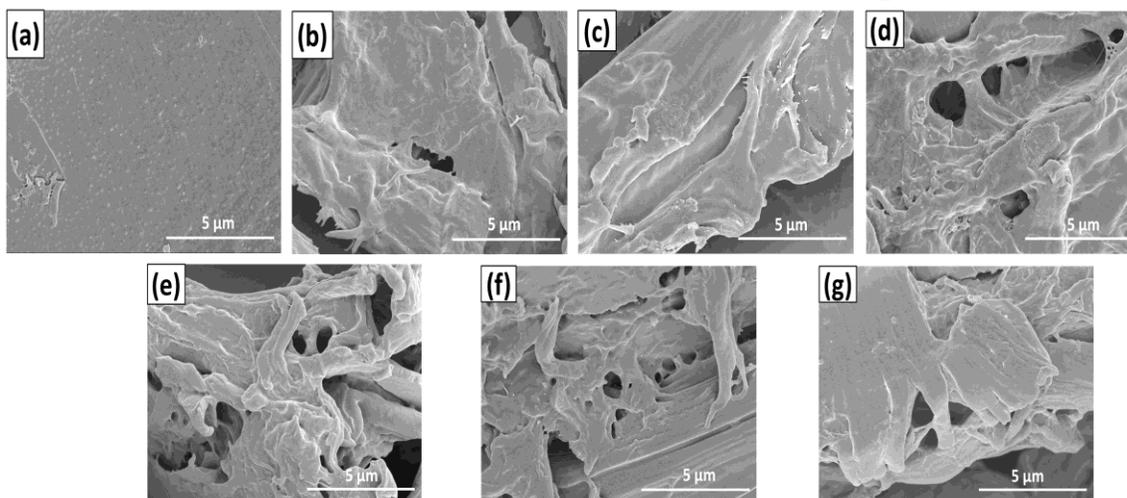


**Fig. 5**. SEM image illustrating the morphology of the untreated material (a) and DES-treated products (ChCl/LA 1:2(b), ChCl/LA 1:6(c), ChCl/LA 1:10(d), ChCl/GLY 1:2(e), ChCl/GLY 1:6 (f) and ChCl/GLY 1:10(g)) with different molar ratios of ChCl and HBDs for 12 h at 100 °C using red pine sapwood.

*bioresources.com*

## CONCLUSIONS

The effects of various molar ratios and treatment time with different pH DES buffer systems made from a mixture of choline chloride (ChCl) and two hydrogen bond donors (HBDs) lactic acid (LA) and glycerin (GLY) on the chemical composition of sapwood and heartwood of red pine was studied.

1. Deep eutectic solvent (DES) treatment significantly decreased the yield of solid residue with an increase in the HBD (LA, GLY) molar ratio and treatment time.

2. Chemical composition analysis of residues revealed that the content of total sugars was gradually decreased as the treatment time and HBD molar ratios were increased, and were higher in sapwood than in heartwood.

3. ChCl/LA solvent with a low pH value dissolved hemicellulose a lot regardless of the treatment time and molar ratio, which resulted in high crystallinity. In addition, heartwood with high cellulose content was more soluble than sapwood, and these results were greatly affected by treatment time and molar ratio.

4. The pH value of DES in the process of DES treatment based on choline chloride at 100 °C was a key factor affecting the cellulose and hemicellulose solubility of biomass. Overall, the low pH DES, *i.e.* ChCl/LA, is promising with the mole ratio of 1:2 and treatment time of 6 h for the pretreatment of red pine.

## ACKNOWLEDGMENTS

## REFERENCES CITED

Agbor, V. B., Cicek, N., Sparling, R., Berlin, A., and Levin, D. B. (2011). "Biomass pretreatment: Fundamentals toward application," *Biotechnology Advances* 29(6), 675-685. DOI: 10.1016/j.biotechadv.2011.05.005

Ahorsu, R., Medina, F., and Constantí, M. (2018). "Significance and challenges of biomass as a suitable feedstock for bioenergy and biochemical production: A review," *Energies* 11(12), Article 3366. DOI: 10.3390/en11123366

Alvarez-Vasco, C., Ma, R., Quintero, M., Guo, M., Geleynse, S., Ramasamy, K. K., Wolcott, M., and Zhang, X. (2016). "Unique low-molecular-weight lignin with high purity extracted from wood by deep eutectic solvents (DES): A source of lignin for valorization," *Green Chemistry* 18(19), 5133-5141. DOI: 10.1039/c6gc01007e

Benouadah, N., Aliouche, D., Pranovich, A., and Willför, S. (2019). "Chemical characterization of *Pinus halepensis* sapwood and heartwood," *Wood Material Science & Engineering,* 14(3), 157-164. DOI: 10.1080/17480272.2018.1448436

*bioresources.com*

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

Capolupo, L., and Faraco, V. (2016). "Green methods of lignocellulose pretreatment for biorefinery development," *Applied Microbiology and Biotechnology* 100(22), 9451-9467. DOI: 10.1007/s00253-016-7884-y

Dai, Y., Witkamp, G. J., Verpoorte, R., and Choi, Y. H. (2013). "Natural deep eutectic solvents as a new extraction media for phenolic metabolites in *Carthamus tinctorius* L.," *Analytical Chemistry* 85(13), 6272-6278. DOI: 10.1021/ac400432p

Espinoza-Acosta, J. L., Torres-Chávez, P. I., Carvajal-Millán, E., Ramírez-Wong, B., Bello-Pérez, L. A., and Montaño-Leyva, B. (2014). "Ionic liquids and organic solvents for recovering lignin from lignocellulosic biomass," *BioResources* 9(2), 3660-3687. DOI: 10.15376/biores.9.2.3660-3687

George, A., Brandt, A., Tran, K., Zahari, S. M. S. N. S., Klein-Marcuschamer, D., Sun, N., Sathitsuksanoh, N., Shi, J., Stavila, V., Parthasarathi, R., Singh, S., Holmes, B. M., Welton, T., Simmons, B. A., and Hallett, J. P. (2015). "Design of low-cost ionic liquids for lignocellulosic biomass pretreatment," *Green Chemistry* 17(3), 1728-1734. DOI: 10.1039/C4GC01208A

Gupta, B. S., Jelle, B. P., and Gao, T. (2015). "Wood facade materials ageing analysis by FTIR spectroscopy," *Proceedings of the Institution of Civil Engineers - Construction Materials* 168(5), 219-231. DOI: 10.1680/coma.13.00021

Han, S. Y., Park, C. W., and Lee, S. H. (2017). "Preparation of lignocellulose nanofiber by mechanical defibrillation after pretreating using cosolvent of ionic liquid and DMF," *Journal of the Korean Wood Science and Technology* 43(3), 268-277. DOI: 10.5658/WOOD.2017.45.3.268

Han, S. Y., Park, C. W., Park, J. B., Ha, S. J., Kim, N. H., and Lee, S. H. (2020). "Ethanol fermentation of the enzymatic hydrolysates from the products pretreated using [EMIM]Ac and its co-solvents with DMF," *Journal of Forest and Environment Science* 36 (1), 62-66. DOI: 10.7747/JFES.2020.36.1.62

Hiltunen, J., Kuutti, L., Rovio, S., Puhakka, E., Virtanen, T., Ohra-Aho, T., and Vuoti, S. (2016). "Using a low melting solvent mixture to extract value from wood biomass," *Scientific Reports* 6(1), 32420. DOI: 10.1038/srep32420

Ho, M. C., Ong, V. Z., and Wu, T. Y. (2019). "Potential use of alkaline hydrogen peroxide in lignocellulosic biomass pretreatment and valorization – A review," *Renewable and Sustainable Energy Reviews* 112, 75-86. DOI: 10.1016/j.rser. 2019.04.082

Hou, Q., Ju, M., Li, W., Liu, L., Chen, Y., and Yang, Q. (2017a). "Pretreatment of lignocellulosic biomass with ionic liquids and ionic liquid-based solvent systems," *Molecules* 22(3), 490. DOI: 10.3390/molecules22030490

Hou, X. D., Feng, G. J., Ye, M., Huang, C. M., and Zhang, Y. (2017b). "Significantly enhanced enzymatic hydrolysis of rice straw *via* a high-performance two-stage deep eutectic solvents synergistic pretreatment," *Bioresource Technology* 238, 139-146. DOI: 10.1016/j.biortech. 2017.04.027

Isikgor, F. H., and Becer, C. R. (2015). "Lignocellulosic biomass: A sustainable platform for the production of bio-based chemicals and polymers," *Polymer Chemistry* 6(25), 4497-4559. DOI: 10.1039/C5PY00263J

Kadhom, M. A., Abdullah, G. H., and Al-Bayati, N. (2017). "Studying two series of

ternary deep eutectic solvents (choline chloride–urea–glycerol) and (choline chloride–malic acid–glycerol), synthesis and characterizations," *Arabian Journal for Science and Engineering* 42(4), 1579-1589. DOI: 10.1007/s13369-017-2431-4

Kucharska, K., Rybarczyk, P., Hołowacz, I., Łukajtis, R., Glinka, M., and Kamiński, M. (2018). "Pretreatment of lignocellulosic materials as substrates for fermentation processes," *Molecules* 23(11), 2937. DOI: 10.3390/molecules23112937

Kumar, A. K., Parikh, B. S., and Pravakar, M. (2016). "Natural deep eutectic solvent mediated pretreatment of rice straw: Bioanalytical characterization of lignin extract and enzymatic hydrolysis of pretreated biomass residue," *Environmental Science and Pollution Research* 23(10), 9265-9275. DOI: 10.1007/s11356-015-4780-4

Lee, S. H., Kim, H. J., and Kim, J. C. (2019). "Nanocellulose applications for drug delivery: A review," *Journal of Forest and Environmental Science* 35(3), 141-149. DOI: 10.7747/JFES.2019.35.3.141

Li, Y. F., Deng, X. W., Zhang, Y. F., Huang, Y. Q., Wang, C. Y., Xiang, W. H., Xiao, F. M., and Wei, X. C. (2019). "Chemical characteristics of heartwood and sapwood of red heart Chinese fir (*Cunninghamia lanceolata*)," *Forest Products Journal* 69(2), 103-109. DOI: 10.13073/FPJ-D-18-00042

Liu, Y., Guo, B., Xia, Q., Meng, J., Chen, W., Liu, S., Wang, Q., Liu, Y., Li, J. and Yu, H. (2017). "Efficient cleavage of strong hydrogen bonds in cotton by deep eutectic solvents and facile fabrication of cellulose nanocrystals in high yields," *ACS Sustainable Chemistry & Engineering* 5(9), 7623-7631. DOI: 10.1021/ acssuschemeng.7b00954

Loow, Y.-L., Wu, T. Y., Yang, G. H., Ang, L. Y., New, E. K., Siow, L. F., Jahim, J. M., Mohammad, A. W., and Teoh, W. H. (2018). "Deep eutectic solvent and inorganic salt pretreatment of lignocellulosic biomass for improving xylose recovery," *Bioresource Technology* 249, 818-825. DOI: 10.1016/j.biortech.2017.07.165

Malaeke, H., Housaindokht, M. R., Monhemi, H., and Izadyar, M. (2018). "Deep eutectic solvent as an efficient molecular liquid for lignin solubilization and wood delignification," *Journal of Molecular Liquids* 263, 193-199. DOI: 10.1016/j.molliq.2018.05.001

Merza, F., Fawzy, A., AlNashef, I., Al-Zuhair, S., and Taher, H. (2018). "Effectiveness of using deep eutectic solvents as an alternative to conventional solvents in enzymatic biodiesel production from waste oils," *Energy Reports* 4, 77-83. DOI: 10.1016/j.egyr.2018.01.005

Ong, V. Z., Wu, T. Y., Lee, C. B. T. L., Cheong, N. W. R., and Shak, K. P. Y. (2019). "Sequential ultrasonication and deep eutectic solvent pretreatment to remove lignin and recover xylose from oil palm fronds," *Ultrasonics Sonochemistry* 58, 104598. DOI: 10.1016/j.ultsonch.2019.05.015

Procentese, A., Johnson, E., Orr, V., Garruto Campanile, A., Wood, J. A., Marzocchella, A., and Rehmann, L. (2015). "Deep eutectic solvent pretreatment and subsequent saccharification of corncob," *Bioresource Technology* 192, 31-36. DOI: 10.1016/j.biortech.2015.05.053

Sakti, A. S., Saputri, F. C., and Mun'im, A. (2019). "Optimization of choline chloride-glycerol based natural deep eutectic solvent for extraction bioactive substances from *Cinnamomum burmannii* barks and *Caesalpinia sappan* heartwoods," *Heliyon* 5(12), e02915. DOI: 10.1016/j.heliyon.2019.e02915

Sathitsuksanoh, N., George, A., and Zhang, Y. H. P. (2012). "New lignocellulose pretreatments using cellulose solvents: A review," *Journal of Chemical Technology & Biotechnology* 88(2), 169-180. DOI: 10.1002/jctb.3959

Satlewal, A., Agrawal, R., Bhagia, S., Sangoro, J., and Ragauskas, A. J. (2018). "Natural deep eutectic solvents for lignocellulosic biomass pretreatment: Recent developments, challenges and novel opportunities," *Biotechnology Advances* 36. 2032-2050. DOI: 10.1016/j.biotechadv.2018.08.009

Shamsuri, A. A., and Abdullah, D. K. (2010). "Ionic liquids: Preparations and limitations," *Makara Sains* 14(2), 101-106. DOI: 10.7454/mss.v14i2.677

Sirviö, J. A., Visanko, M., and Liimatainen, H. (2015). "Deep eutectic solvent system based on choline chloride-urea as a pre-treatment for nanofibrillation of wood cellulose," *Green Chemistry* 17(6), 3401-3406. DOI: 10.1039/c5gc00398a

Sirviö, J. A., Visanko, M., and Liimatainen, H. (2016). "Acidic deep eutectic solvents as hydrolytic media for cellulose nanocrystal production," *Biomacromolecules* 17(9), 3025-3032. DOI: 10.1021/acs.biomac.6b00910

Skulcova, A., Russ, A., Jablonsky, M., and Sima, J. (2018). The pH behavior of seventeen deep eutectic solvents," *BioResources* 13, 5042-5051. DOI: 10.15376/biores.13.5042-5051

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., and Crocker, D. (2008). *Determination of Structural Carbohydrates and Lignin in Biomass* (NREL/TP-510-42618), National Renewable Energy Laboratory, Golden, CO, USA.

Smink, D., Juan, A., Schuur, B., and Kersten, S. R. A. (2019). "Understanding the role of choline chloride in deep eutectic solvents used for biomass delignification," *Industrial & Engineering Chemistry Research* 58, 16348-16357. DOI: 10.1021/acs.iecr.9b03588

Sun, N., Rahman, M., Qin, Y., Maxim, M. L., Rodríguez, H., and Rogers, R. D. (2009). "Complete dissolution and partial delignification of wood in the ionic liquid 1-ethyl-3-methylimidazolium acetate," *Green Chemistry* 11(5), 646-655. DOI: 10.1039/B822702K

Van Osch, D. J. G. P., Kollau, L. J. B. M., van den Bruinhorst, A., Asikainen, S., Rocha, M. A. A., and Kroon, M. C. (2017). "Ionic liquids and deep eutectic solvents for lignocellulosic biomass fractionation," *Physical Chemistry Chemical Physics* 19(4), 2636-2665. DOI: 10.1039/c6cp07499e

Yadav, A., Trivedi, S., Rai, R., and Pandey, S. (2014). "Densities and dynamic viscosities of (choline chloride+glycerol) deep eutectic solvent and its aqueous mixtures in the temperature range (283.15–363.15) K," *Fluid Phase Equilibria* 367, 135-142. DOI: 10.1016/j.fluid.2014.01.028

Yu, W., Wang, C., Yi, Y., Zhou, W., Wang, H., Yang, Y., and Tan, Z. (2019). "Choline chloride-based deep eutectic solvent systems as a pretreatment for nanofibrillation of ramie fibers," *Cellulose* 26, 3069-3082. DOI: 10.1007/s10570-019-02290-7

Zhang, H., Lang, J., Lan, P., Yang, H., Lu, J., and Wang, Z. (2020). "Study on the dissolution mechanism of cellulose by ChCl-based deep eutectic solvents," *Materials* 13(2), 278. DOI: 10.3390/ma13020278

Zhang, K., Pei, Z., and Wang, D. (2016). "Organic solvent pretreatment of lignocellulosic biomass for biofuels and biochemicals: A review," *Bioresource Technology* 199, 21-33. DOI: 10.1016/j.biortech.2015.08.102

Zhao, H. (2003). "Review: Current studies on some physical properties of ionic liquids," *Physics and Chemistry of Liquids* 41(6), 545-557. DOI: 10.1080/00319103100011 7319