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*Published in:*  
Chemical Engineering Journal

*DOI:*  
[10.1016/j.cej.2025.159232](https://doi.org/10.1016/j.cej.2025.159232)

Published: 01/02/2025

*Document Version*  
Final published version

*Document License*  
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*Please cite the original version:*

Xu, J., Si, C., Dai, L., Hemming, J., Pranovich, A., & Xu, C. (2025). Microwave-assisted deep eutectic solvent extraction of lignin from spruce heartwood and sapwood, targeting the comparison of different biorefinery concepts. *Chemical Engineering Journal*, 505, Article 159232. <https://doi.org/10.1016/j.cej.2025.159232>

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# Microwave-assisted deep eutectic solvent extraction of lignin from spruce heartwood and sapwood, targeting the comparison of different biorefinery concepts

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## ARTICLE INFO

### Keywords:

Biomass fractionation  
Deep eutectic solvent  
Microwave-assisted  
Heartwood (HW)  
Sapwood (SW)  
Lignin

## ABSTRACT

Lignin-first fractionation (microwave-assisted deep eutectic solvent fractionation, MW-DES) and carbohydrate-first fractionation (hot water extraction followed by MW-DES, HWE-MW-DES), were compared while pretreating spruce heartwood (HW) and sapwood (SW) for lignin extraction. The lignin recovery yield was 80 % for HW and 74 % for SW, while SW demonstrated high selectivity in delignification, achieving 86 % removal of lignin with only minor cellulose loss (3.6 %) treated with ChCl/LA (1:2) at 145 °C for 10 min. The carbohydrate-first approach gives an opportunity to obtain lignin with a lower carbohydrate content (~0.5 %) and higher lignin purity (88 %). The structure of lignin exhibited no significant differences after two different fractionation approaches, with the phenolic hydroxyl content in lignin in the amount of 2.59/2.73 mmol/g (MW-DES) and 2.54/2.74 mmol/g (HWE-MW-DES) from HW/SW. The above provides a theoretical basis for developing and optimizing the valorization of lignin from spruce HW/SW, employing either lignin-first or carbohydrate-first fractionation approaches of the sustainable biorefinery processes.

## 1. Introduction

The utilization of lignocellulosic biomass offers a considerable opportunity for the sustainable production of biofuels and biobased materials. However, the recalcitrance of lignocellulosic biomass presents major obstacles in terms of separating and effective utilization of the primary components of lignocellulosic biomass. To solve this problem and optimize the utilization of components in lignocellulosic biomass, scientists have proposed various approaches for fractionation in recent decades, including the utilization of organic solvents or ionic liquids [1]. However, given the growing awareness of environmental contamination and energy shortage, current investigations are focused on developing environmentally friendly and sustainable methods for fractionation.

In recent years, researchers have demonstrated a high interest in deep eutectic solvents (DES) due to their convenient preparation technology, biocompatibility and biodegradability. DES have physicochemical properties comparable to ionic liquids but offer the advantage of lowering processing costs [2]. Though numerous previous studies

have shown that acidic DES has an excellent delignification ability [3], there are still several challenges and limitations that need further investigation, particularly regarding the efficiency of extraction. DES-based biomass fractionation often requires a long reaction time (6–24 h) to remove lignin effectively. For example, the previous studies have demonstrated that lignin extraction from Willow can be achieved by the use of DES (choline chloride/lactic acid, ChCl/LA) at a temperature 120 °C for 6–24 h [4]. Alvarez-Vasco et al. employed DES (ChCl/LA, 1:2) to extract lignin from poplar and D. fir under 145 °C for 6 h and 9 h, respectively [5]. Similarly, Catarina Fernandes et al. used DES (ChCl/LA, 1:2) at 150 °C for 2 h to extract lignin from pine [6].

Combining DES with several other fractionation methods (e.g. microwave) can overcome many economic, technical, and environmental problems in the biorefining process of lignocellulosic raw materials and improve the effect of biomass fractionation considerably [7,8]. Unlike conventional external heating, which is either conductive or convective, microwave heating heats a solution using microwave radiation [9]. During the fractionation process, microwave radiation can effectively

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<https://doi.org/10.1016/j.cej.2025.159232>

Received 2 July 2024; Received in revised form 12 December 2024; Accepted 1 January 2025

Available online 2 January 2025

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**Table 1**

The composition of raw materials based on the extractive-free raw material (%).

Sample name	cellulose	hemicellulose	lignin
Heartwood (HW)	40.89	22.68	28.21
Sapwood (SW)	43.10	22.22	28.09
Residue of heartwood (RHW) <sup>a</sup>	50.90	9.69	33.04
Residue of sapwood (RSW) <sup>a</sup>	52.72	8.84	30.51

<sup>a</sup> Residue wood after hot water extraction.

enhance the ionic properties of connective tissue, increase its molecular polarity, and reduce processing time [10,11]. The microwave-assisted deep eutectic solvents (MW-DES) have been successfully employed in several studies to achieve efficient lignin removal from various lignocellulosic biomasses, including poplars [12], corn stover [13], switchgrass, *Miscanthus* [10], and pinewood sawdust [14].

The techniques for lignocellulosic biomass fractionation can be divided into two groups. The first group comprises methods aimed at releasing lignin from the biomass matrix (i.e. lignin first fractionation), while the second group focuses on to maximizing the utilization of lignocellulosic biomass (e.g. carbohydrate first fractionation). The differences in fractionation technologies have been reported in some studies [15]. For instance, Sun et al. employed the combined hydrothermal-alkaline DES fractionation approach for bamboo and particularly emphasized the valorization of carbohydrate (including hemicellulose and cellulose) [16]. They highlighted that hemicellulose is selectively converted into functional xylooligosaccharides (XOS) during the hydrothermal treatment stage and presents highly effective enzymatic hydrolysis. The tandem method, consisting of hydrothermal and DES pretreatment, was discussed by Xu et al. for the hierarchical fractionation of xylan and lignin from poplar [17]. Their primary focus was on producing prebiotic XOS, useful lignin and highly digestible cellulose. However, the MW-DES lignin extraction is a complex process that comprises various components and reactions. It requires more studies of different raw materials and conditions for its successful implementation and better utilization of lignin with specified parameters.

In this study, the objective is to incorporate MW-DES in the extraction of lignin from different morphological parts of spruce wood, i.e. heartwood (HW) and sapwood (SW). Additionally, a comparison for lignin deconstruction process will be made between the lignin extractions obtained from the lignin-first fractionation process and the carbohydrate-first fractionation process. The lignin-first fractionation

process involved the direct extraction of lignin from HW/SW using the MW-DES approach. The carbohydrate-first fractionation process was accomplished by integrating hot water extraction (HWE) and MW-DES fractionation from HW/SW.

## 2. Materials and methods

### 2.1. Materials

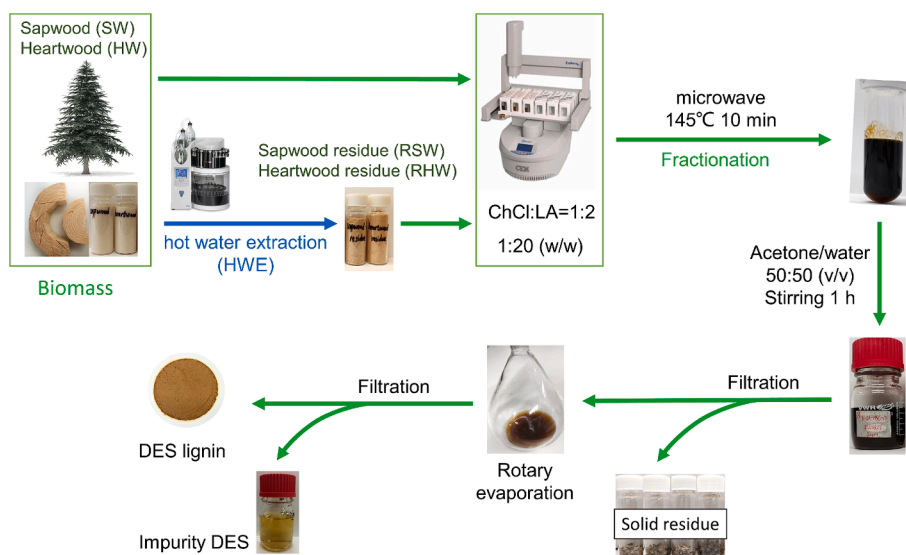
Based on moisture content on the cross-section surface of the unfrozen trunk [18], the starting materials were the heartwood (HW) and sapwood (SW) of the Norway spruce tree from Southwest Finland. The wood was cut into small pieces and then ground, avoiding heating. To obtain extractive-free material, the 60 mesh ground wood particles were extracted with acetone for 10 h in a Soxhlet apparatus (about 3 percolations per hour), then dried before further fractionation. One part of HW and SW was first treated with sequential hot water extraction (HWE) with accelerated solvent extractor (ASE). During the HWE, hemicelluloses, mainly galactoglucomannan (GGM), were extracted from wood in the structurally preserved, i.e. polymeric and acetylated form [19]. The solid residue was obtained for further MW-DES fractionation, and the latter were named RHW and RSW, respectively. The HWE temperature was set at 160 °C for 20 min, followed by another 160 °C for 40 min, and finally increased to 180 °C for an additional 10 min according to the previous work [20]. The composition of HW, SW, RHW and RSW was analysed by TAPPI standard, see Table 1. The milled wood lignin (MWL) was prepared for comparison of lignin properties according to the classical method [21]. Choline chloride (ChCl) and lactic acid (LA) were acquired from Aladdin Industrial Corporation.

### 2.2. DES preparation

The DES mixtures were prepared using ChCl/LA at molar ratio of 1:2. Briefly, ChCl and LA were mixed and heated at 70 °C until a transparent state without solid particles was obtained.

### 2.3. Microwave-assisted deep eutectic solvent (MW-DES) fractionation

A schematic representation of MW-DES fractionation is given in Fig. 1. 1 g of spruce sawdust was mixed with 20 g DES and then pre-stirred for 2 min in a microwave digestion system (Discover SP-D, CEM). The MW-DES fractionation was finished in the same microwave digestion system. The fractionation temperature was elevated from

**Fig. 1.** Schematic of Norway spruce fractionation with MW-DES and HWE-MW-DES.

**Table 2**

The conditions of ChCl/LA based DES for lignin extraction investigated in this work and presented in the published references.

Solvent	Biomass	Extraction conditions	Lignin purity (%) <sup>a</sup>	Lignin yield (%) <sup>b</sup>	Reference	
ChCl/LA (1:2)	HW	145 °C; 10 min	86	80	This work	
	SW		84	74		
	RHW		88	59		
	RSW		88	64		
	poplar	145 °C; 6 h	66	78.5		[5]
	Douglas fir	145 °C; 9 h	88	58.2		
	Maritime Pine	150 °C; 2 h	68.3 ± 2.8	76.7 ± 0.9		[6]
	Wheat straw	150 °C; 6 h	~ 90	81.24		[32]
	Coconut coir	150 °C; 5 min	n.d.	35.1		[31]
		150 °C; 10 min	n.d.	42.4		
	150 °C; 20 min	n.d.	73.9			
	150 °C; 30 min	n.d.	56			
	150 °C; 40 min	n.d.	53			

n.d.: not defined in the literature.

<sup>a</sup> lignin purity = (AIL + ASL)/DES lignin × 100 %, where AIL & ASL refer to acid insoluble lignin and acid soluble lignin.

<sup>b</sup> lignin yield = DES lignin/lignin in biomass × 100 %, where biomass refer to HW/SW/RHW/RSW.

ambient to 145 °C for 3 min and kept for 10 min.

After the MW-DES fractionation treatment, 50 mL acetone/water (50/50, v/v) was added to the slurry and stirred for 1 h at ambient temperature. The extracted biomass was vacuum filtered with a G3 glass filter and rinsed with 100 mL acetone/water (50/50, v/v). The filtrate was collected and rotary evaporated at low pressure to remove acetone. Then, more water was added to the filtrate (bringing the total volume to 300 mL) and left overnight to precipitate the lignin. The precipitated lignin was filtered under vacuum using a Nylon membrane filter (0.22 μm, Whatman™) and subsequently freeze-dried for further analysis. The cellulose-rich residue was vacuum dried at 40 °C and stored at room temperature until further analysis.

## 2.4. Analytical methods

### 2.4.1. Lignin purity and carbohydrate analysis

Lignin purity was determined by quantifying the combined amounts of acid soluble lignin (ASL) and acid insoluble lignin (AIL) in lignin samples determined according to a modified Klason lignin method [22]. Non-cellulosic carbohydrates content including hemicellulose and pectin were determined by GC-FID after acid methanolysis and silylation (see details in Supporting Information) [23,24].

### 2.4.2. FTIR analysis

2 mg Powder samples were mixed with 200 mg of KBr powder and pressed into a pellet. The FTIR spectrum was recorded from 400 cm<sup>-1</sup> to 4000 cm<sup>-1</sup> at the resolution of 4 cm<sup>-1</sup> using the FTIR microscope

**Table 3**

Carbohydrates composition in MWL and DES lignin samples (mg/g of lignin sample).

Sample	Ara <sup>a</sup>	Xyl	Gal	Glc	Man	Rha	GalA	MeGlcA	SUM
MWL	1.70	1.94	2.72	1.51	1.99	0.94	2.14	2.11	15.05
HW	–	1.97	0.71	1.00	1.89	0.89	2.06	3.06	11.58
SW	–	1.88	0.61	0.80	1.74	0.85	1.94	2.92	10.75
RHW	–	0.91	0.29	0.46	0.85	0.36	0.49	1.55	4.91
RSW	–	0.91	0.29	0.48	0.87	0.45	0.53	1.54	5.07

<sup>a</sup> Arabinose (Ara), xylose (Xyl), galactose (Gal), glucose (Glc), mannose (Man), rhamnose (Rha), galacturonic acid (GalA), 4-O-methyl-D-glucuronic acid (MeGlcA).

(Thermo Scientific FTIR Nicolet iS50, USA)

### 2.4.3. Thermogravimetric (TG) analysis

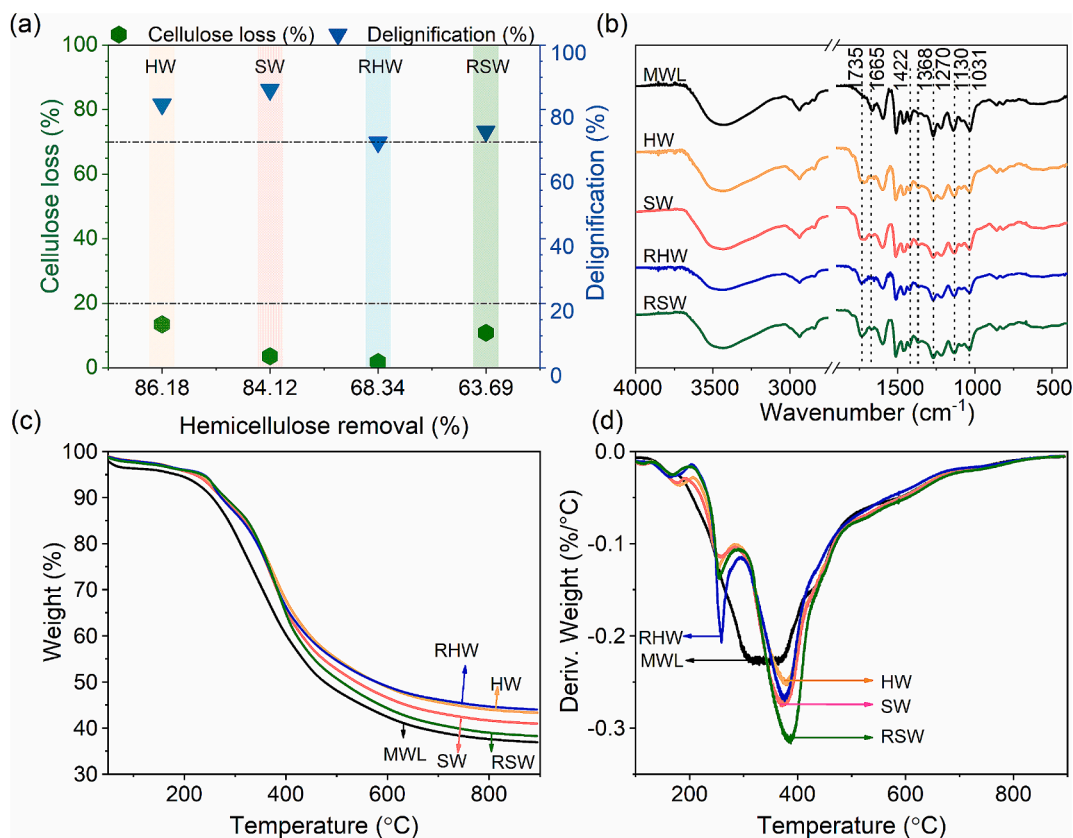
Thermogravimetric analysis was conducted using a Discovery SDT 650 thermogravimetric analyzer (TA instruments, USA). The MF samples were heated from ambient temperature to 900 °C at the heating rate of 10 °C/min in the nitrogen atmosphere at the flow rate of 100 mL/min.

### 2.4.4. Molar mass analysis

The molar mass characteristics of lignin samples were determined with a size exclusion chromatography (HPLC-SEC) instrument equipped with a differential refractive index concentration detector (RI) and a multiangle light scattering detector (MALS) using a Jordi X-stream guard column (50 × 10 mm) and Jordi X-stream column (250 × 10 mm) (Jordi Labs, MA, USA). The column temperature was 60 °C. 0.05 M LiBr/DMSO as an eluent and the eluent flow rate was 0.5 mL/min. The injection volume was 75 μL of 2.5 mg/mL lignin sample. The ASTRA software (version 7.3.2) was employed for data analysis. A dn/dc value of 0.1050 mL/g [25] and 0.15 mL/g [26] was used for MWL and DES lignin calculation respectively.

### 2.4.5. NMR analysis

2D HSQC NMR, quantitative <sup>13</sup>C NMR and quantitative <sup>31</sup>P NMR spectra of the samples were acquired using a Bruker 500 MHz spectrometer following the previous method [27,28]. For 2D HSQC, 80 mg of lignin was dissolved in 0.7 mL of DMSO-*d*<sub>6</sub> and transferred to a 5 mm NMR tube, with the referenced set at 39.5/2.5 ppm. The experiments utilized the Bruker standard pulse program hsqcedetgpcisp 2.3. The δC<sub>2</sub>-H<sub>2</sub> correlation of guaiacyl (G<sub>2</sub>) was used as an internal standard for the quantitative analysis of 2D-HSQC cross-signals, and the G<sub>2</sub> amount represents the aromatic C9 unit number in lignin [29,30]. The content of internal linkages is calculated as: I<sub>x</sub>% = I<sub>x</sub>/IC<sub>9</sub> × 100 %, where I<sub>x</sub> is the integral value of the α-position of A (β-O-4), B (β-5), C (β-β), and α-O-Alk. The Bruker Topspin-NMR software (version 4.1.4) was employed for data processing analysis and the integration was performed at same contour level. For <sup>31</sup>P NMR, 20 mg of lignin was dissolved in 400 μL of anhydrous pyridine/deuterated chloroform (CDCl<sub>3</sub>) (1.6:1, v/v) with continuous stirring. This solution was then mixed with the addition of 100 μL of *endo*-N-hydroxyl-5-norbornene-2,3-dicarboximide (0.12 M in pyridine/CDCl<sub>3</sub> 1.6:1, v/v) as an internal standard and 50 μL of chromium (III) acetylacetonate solution (11.4 mg/mL in pyridine/CDCl<sub>3</sub> 1.6:1, v/v) as a relaxation reagent. Finally, 100 μL of phosphorylating reagent (2-chloro-1,3,2-dioxaphospholane) was added, and the mixture was allowed to react for approximately 30 min before the analysis. For <sup>13</sup>C NMR, about 170 mg of lignin sample was dissolved in 550 μL DMSO-*d*<sub>6</sub> (containing Chromium (III) acetylacetonate (Cr(acac)<sub>3</sub>) using the Bruker Advanced Cryo 500 MHz spectrometer for quantitative <sup>13</sup>C NMR (298 K) analysis. The concentration of Cr(acac)<sub>3</sub> in the NMR solvent (550 μL) was 0.016 M which was applied to provide complete relaxation of all the nuclei. The quantitative <sup>13</sup>C NMR analysis employed a 2 s relaxation delay, 1.1 s acquisition time, and the inverse-gated decoupling pulse (zgif) with 20,000 scans.



**Fig. 2.** (a) The cellulose loss, hemicellulose removal and delignification of HW, SW, RHW and RSW during MW-DES fractionation process; (b) FTIR spectra of MWL and lignin samples; (c) TG and (d) DTG spectra of MWL and lignin samples after MW-DES and HWE-MW-DES fractionation. Cellulose loss =  $1 - (\text{cellulose in residue} / \text{cellulose in biomass}) \times 100\%$ , Delignification =  $1 - (\text{lignin in residue} / \text{lignin in biomass}) \times 100\%$ , Hemicellulose removal =  $1 - (\text{hemicellulose in residue} / \text{hemicellulose in biomass}) \times 100\%$ , where biomass refer to HW/SW/RHW/RSW.

### 3. Results and discussions

#### 3.1. Lignin yield, purity and carbohydrate composition

The two major indexes used to evaluate the efficiency of lignin extraction were the yield and purity of lignin obtained after MW-DES fractionation. The yield and purity of freeze-dried DES lignin after MW-DES fractionation were analyzed, and the corresponding results are presented in Table 2. The purity levels of the four DES lignins from HW/SW/RHW/RSW exhibit slight variations, yet they all demonstrate a high degree of purity (84 %–88 %). Hereby, we compared and discussed our DES lignin purity and yield with DES lignin extracted in similar conditions in the literature (Table 2). The maximum lignin yield (80 %) was obtained from HW after 10 min extraction at 145 °C using MW-DES. Alvarez-Vasco and colleagues reported comparable results: 78.5 % lignin yield was extracted from poplar at 145 °C after 6 h treatment whereas only 58.2 % was extracted from Douglas fir at 145 °C in 9 h [5]. The highest lignin yield (73.9 %) was obtained from coconut coir during 20 min treatment at 150 °C in using MW-DES, which is comparable to the lignin yield obtained from SW (74 %), but still lower than that from HW (80 %) using MW-DES at 145 °C for 10 min [31]. The lignin yield of RHW or RSW was relatively low when compared to HW or SW, indicating that the hemicellulose removal during the HWE stage may not be beneficial for delignification. Nevertheless, MW-DES fractionation achieved lignin yields of 59 % and 64 % from RHW and RSW, respectively, within only 10 min at 145 °C. Remarkably, these yields surpassed the traditional heating method's lignin yield of 58.2 % obtained from Douglas fir after the extensive 9 h-treatment at the same temperature [5]. To conclude, the MW-DES technique enables a short processing time and facilitates the production of lignin with enhanced purity and yield,

regardless of whether it is obtained through lignin-first or carbohydrate-first fractionation.

To further verify the purity of lignin obtained after MW-DES fractionation, the non-cellulosic carbohydrates of DES lignin were analysed using methanolysis and GC, see Table 3. It can be seen from the results that the non-cellulosic carbohydrates in the DES lignin extracted from HW and SW are 1.2 % and 1.1 %, respectively. The slight difference may be due to the fact that HW contains more glucuronoxylan than SW [33]. The content of non-cellulosic carbohydrates in the DES lignin extracted from RHW and RSW was only 0.5 %. This is another proof that DES lignin has a relatively high purity, see Table 2. Before MW-DES fractionation, HWE is beneficial for improving the purity of lignin due to its relatively low non-cellulosic carbohydrate content.

#### 3.2. Lignin characterization

To evaluate the fractionation efficiency of the MW-DES fractionation, the composition of solid residue was determined. The cellulose loss, delignification and hemicellulose removal during the MW-DES fractionation process were calculated according to the remaining components in HW/SW/RHW/RSW and the results are presented in Fig. 2a. It was shown that the cellulose loss of HW is approximately 3.8 times higher than the cellulose loss of SW after the MW-DES fractionation, indicating that the MW-DES fractionation towards SW has a good selectivity toward delignification while preserving cellulose. This phenomenon can be explained by the decrease in the skeletal density of HW under high-intensity microwaves [34]. It may allow the liquid to penetrate and react more easily causing a greater loss of cellulose in the HW compared to SW. The delignification degree of HW is lower than that of SW, which aligns with the findings from the previous research



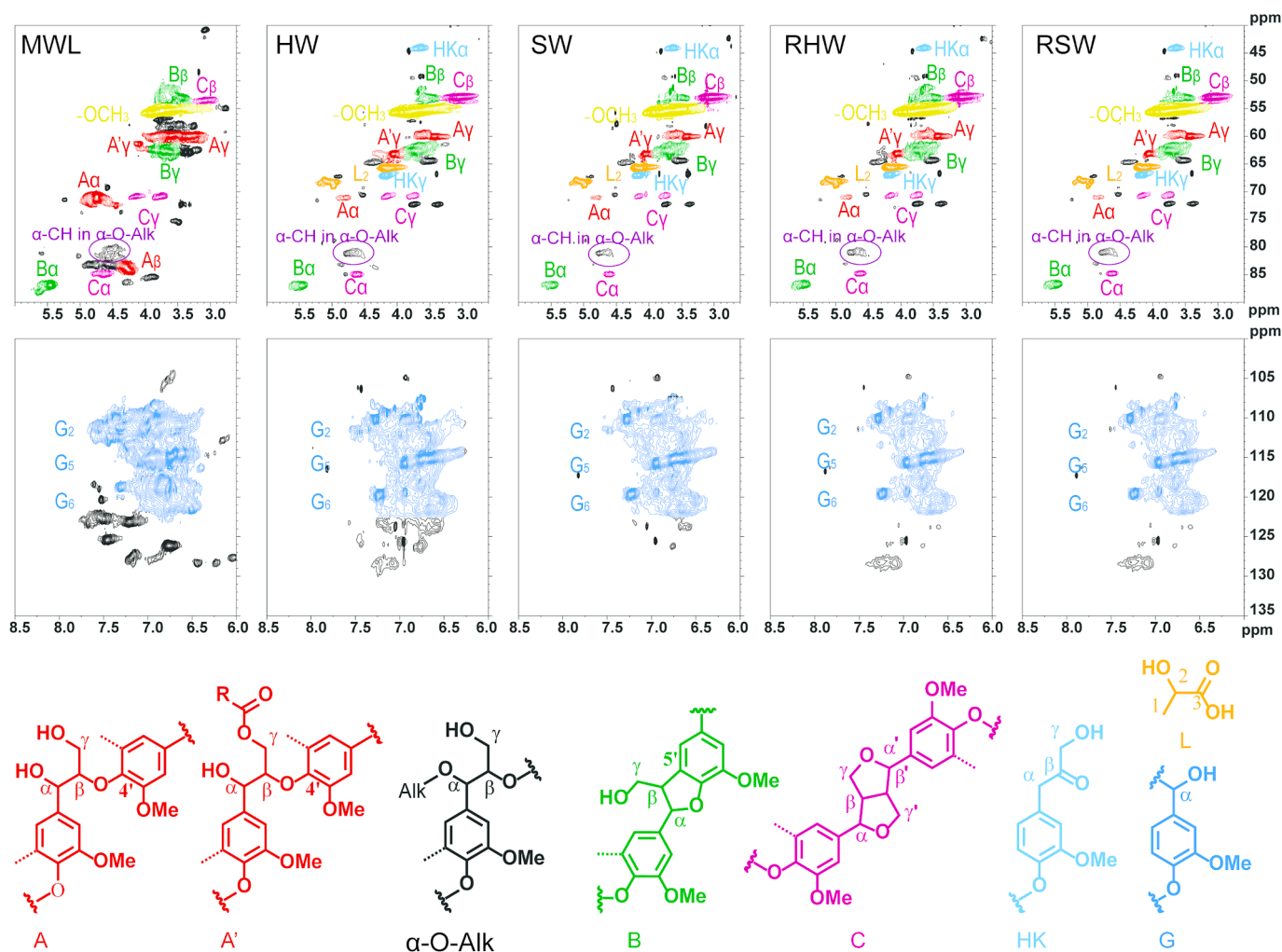


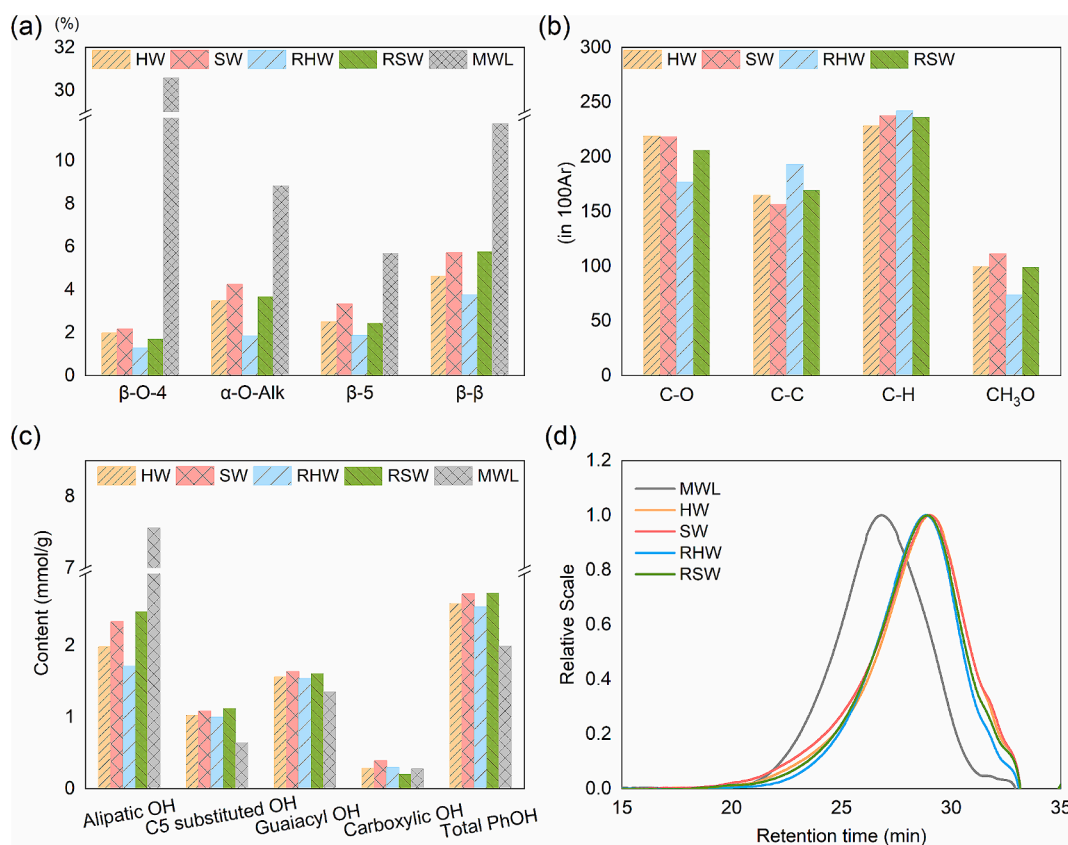
Fig. 3. The HSQC spectra of the side chain (top) and aromatic region (bottom) of MWL and lignin fractions after MW-DES fractionation.

indicating the greater difficulty in lignin removal from HW compared to SW [35]. However, it is of interest to note that despite the extensive delignification of SW, the lignin yield from SW (74 %) remains relatively low compared to HW (80 %). This probably means that a certain amount of lignin removed from the SW is water-soluble lignin with a low molar mass which cannot be collected through water precipitation.

The FTIR was conducted to identify the changes of the HW, SW, RHW and RSW after the MW-DES fractionation and the results are shown in Fig. 2b. Regardless of the used materials, all the studied DES lignins exhibit a similar structure. The band at  $1270\text{ cm}^{-1}$  represented the specific C-O vibration of the guaiacyl (G) subunit, which is a lignin subunit mainly present in softwoods [36]. Compared with MWL, all the DES lignins have a new absorption peak at  $1735\text{ cm}^{-1}$  which is produced by the C = O stretching vibration of non-conjugated ketones, carbonyls and esters, highlighting some newly formed substance during the fractionation process. The appearance of this peak probably indicated that carbohydrates were partly converted into pseudo-lignin during the deep eutectic solvent fractionation [37,38]. The absorption peak of  $1665\text{ cm}^{-1}$ , attributed to the conjugated carbonyl group in all the DES lignin, was no longer observed. This phenomenon can be elucidated by Chen and co-authors who suggested that the disruption of the C $\alpha$  double bond in lignin during DES fractionation consequently led to the destruction of its conjugated structure [39]. This is typical for lignin depolymerization when lignocellulosic raw materials are treated under acidic conditions. The peak of  $1031\text{ cm}^{-1}$  is assigned to the stretching vibrations of C-O in cellulose, hemicellulose, and lignin [40]. In comparison with MWL, the

reduction of the  $1031\text{ cm}^{-1}$  peaks for the DES lignin samples showed that there was a bigger amount of hemicellulose removed from the raw material, which correlates with the carbohydrate compositional analysis reported in Table 3.

The determination of the thermochemical degradation properties of the DES lignin helps to understand the correlation between its chemical structure and properties during the MW-DES fractionation process. The thermogravimetric (TG) and differential thermogravimetric (DTG) curves of the lignin obtained after the MW-DES treatment are shown in Fig. 2c and Fig. 2d. The TG curves of the four DES lignins exhibit a high degree of consistency before reaching  $400\text{ }^{\circ}\text{C}$ , indicating that the thermochemical degradation properties of these lignins are fundamentally similar. However, notable distinctions can be observed when comparing them with MWL. Specifically, these four DES lignins undergo thermal decomposition at an earlier stage. The thermochemical degradation process of the DES lignin can be roughly divided into three stages: the initial stage ( $100\text{--}200\text{ }^{\circ}\text{C}$ ), the main degradation stage ( $200\text{--}500\text{ }^{\circ}\text{C}$ ), and the final carbonization stage (after  $500\text{ }^{\circ}\text{C}$ ). The initial stage mainly refers to the evaporation of the absorbed water from lignin. The DES lignin from RHW had the highest content of residual char compared to MWL and the DES lignin from RSW, as shown in TG (Fig. 2c). This can be attributed to the fact that the DES lignin from RHW has less ether bonds and more condensed linkages [41]. The DES lignin from HW exhibited a higher residual char content compared to MWL and the DES lignin from SW, indicated a similar trend. The above results show that the residual char content of lignin obtained from HW is higher than that obtained



**Fig. 4.** (a) Quantification of the lignin inter-unit linkages (% of 100 aromatic units) by 2D HSQC NMR; (b) Quantification of lignin samples by quantitative  $^{13}\text{C}$  NMR (% of 100 aromatic units); (c) Quantification of the hydroxyl groups (mmol/g) in lignin samples by  $^{31}\text{P}$  NMR; (d) HPLC-SEC of MWL and lignin samples after MW-DES fractionation.

**Table 4**

Weight-average molecular weights ( $M_w$ ) and number-average molecular weights ( $M_n$ ), and polydispersity ( $D_M$ ,  $M_w/M_n$ ) of the lignin samples.

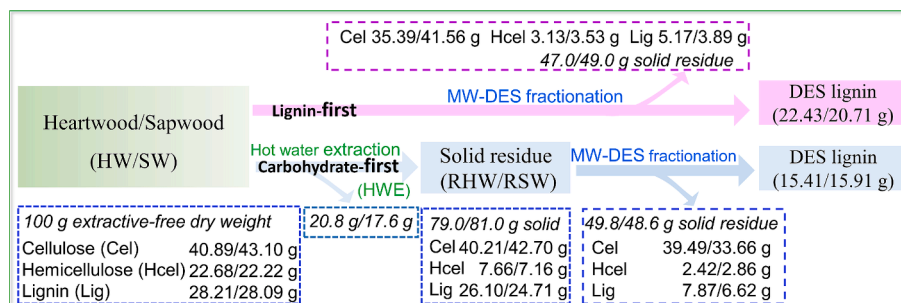
Samples	$M_w$	$M_n$	$D_M$
MWL	14,500	6700	2.16
HW	9700	3500	2.77
SW	10,200	3400	3.00
RHW	8600	3700	2.32
RSW	9700	3800	2.55

from SW under the same treatment conditions. This occurs regardless of whether it is through the MW-DES fractionation, HWE-MW-DES fractionation, or only the HWE process as mentioned in the previous research [20].

The DES lignin samples were characterised by 2D HSQC NMR, and their major substructures are presented in following the literature

[42–44] and given in Fig. 3. To enable comparison of the structural changes during microwave-assisted deep eutectic solvent (MW-DES) fractionation, MWL from Norway spruce was used.

In the side-chain ( $\delta_C\text{-}\delta_H$  90–40/6.0–2.5), the MWL exhibited a high abundance of  $\beta$ -O-4 ether linkages (A) simultaneously had small amounts of phenyl-coumarans ( $\beta$ -5, B) and resinols ( $\beta$ - $\beta$ , C) which can be seen in Fig. 3. The above is in agreement with the other studies [45]. The signal at  $\delta_C/\delta_H$  66.0/4.21 ppm and  $\delta_C/\delta_H$  68.2/4.95 ppm for lactic acid protons can be observed in the DES lignin [46]. The presence of the signal at  $\delta_C/\delta_H$  81/4.6 ppm for CH- $\alpha$  in  $\alpha$ -O-Alk moieties has been observed in both the MWL and DES lignins [44]. Compared to the MWL, the lower number of  $\alpha$ -O-Alk linkages and  $\beta$ -O-4 linkages in the DES lignin supports the possibility of  $\beta$ -O-4 cleavage during the MW-DES fractionation. The other two additional signals visible in the DES lignin are the signal at  $\delta_C/\delta_H$  44.6/3.64 ppm for HK (Hibbert ketone)  $\alpha$ -protons and the signal at  $\delta_C/\delta_H$  67.6/4.17 ppm for HK  $\gamma$ -protons. The results indicate that with the attenuation of  $\beta$ -ether units, some new



**Fig. 5.** Mass Balance of HWE-MW-DES and MW-DES fractionation. (Mass balanced adjusted to 100 g starting biomass).

chemical structures (HK) were released after the MW-DES fractionation [47,48]. The comparison of the integrated data in Fig. 4a with the MWL also supports this conclusion, as it reveals a significant reduction in the number of  $\beta$ -O-4 and  $\alpha$ -O-Alk linkages. This can be attributed to the susceptibility of  $\beta$ -O-4 linkages to cleavage under acidic conditions [49]. In addition to the cleavage of the  $\beta$ -O-4 bond, there is also probably partial disruption of the other C-C bonds. The quantity of both lignin  $\beta$ - $\beta$  and  $\beta$ -5 linkages exhibited a decrease during the MW-DES fractionation process (Fig. 4a).

To provide a preliminary estimation of the aromatic carbon-carbon (C-C), aromatic carbon-hydrogen (C-H), and aromatic carbon-oxygen (C-O) bonds present in the DES lignin, the quantification analysis of  $^{13}\text{C}$  NMR was performed. This analysis involved normalizing the resonance of aromatic lignin moieties (including the  $\alpha$ -carbonyl) in the spectra (ca. 163–102 ppm) to a value of 612 per 100 Ar [44], as shown in Fig. 4b and Fig. S1. It was noticed that the C-O content in lignin for RHW/RSW decreased compared to HW/SW while the C-C content increased, and this was consistent with the aforementioned 2D HSQC NMR results. The cleavage of additional  $\beta$ -O-4 linkages in the DES lignin from RHW/RSW provides an opportunity for a reduction in the C-O content and an increase in the C-C content. Moreover, the amount of  $-\text{OCH}_3$  of RHW/RSW was decreased compared to HW/SW, indicating that the initial HWE step enhances subsequent demethoxylation during MW-DES fractionation [43].

It can be seen from the  $^{31}\text{P}$  NMR spectra (Fig. S2), and the result of MWL (Fig. 4c) that the main type of hydroxyl groups in spruce lignin is aliphatic hydroxyl groups. After the MW-DES treatment of HW, SW, RHW and RSW, the aliphatic hydroxyl groups content of lignin decreased significantly compared to MWL, only 26 %, 31 %, 23 % and 33 % of aliphatic hydroxyl groups respectively remained in the DES lignin. This could potentially be attributed to the elimination of  $\gamma$ -OH as formaldehyde and OH group on C $\alpha$  to form ketone types of structures [50]. The increased presence of phenolic OH groups (including C5 substituted OH and guaiacyl OH) in comparison with MWL suggests the occurrence of  $\beta$ -O-4 linkage cleavage during the MW-DES fractionation process. The DES lignin from RHW/RSW shows only a slight decrease in  $\beta$ -O-4 linkages and no noticeable changes in phenolic OH groups compared to the DES lignin from HW/SW (Fig. 4a and Fig. 4c). The results suggest that the lignin obtained through the sequential process of hot water extraction for carbohydrate isolation followed by MW-DES fractionation exhibits high reactivity similar to direct MW-DES fractionation. The carboxylic group in the DES lignin compared to MWL, only the carboxylic group in lignin from SW increased slightly. This result suggests that microwave-assisted DES treatment primarily induces dehydration and de-acylation reactions rather than oxidation reactions on the aliphatic hydroxyl groups of lignin [43].

Further investigation into the change in lignin molar mass during MW-DES fractionation could offer valuable insights into lignin depolymerization and structural change. HPLC-SEC results of MWL and the lignin samples after the MW-DES fractionation are given in Fig. 4d. Weight-average molecular weights ( $M_w$ ), number-average molecular weights ( $M_n$ ) and molar mass dispersity ( $D_M$ ) measured are summarized in Table 4. Compared to MWL, the DES lignin with long retention time shows a lower molar mass, which indicates depolymerization during the fractionation process [5]. In comparison with lignin from HW/SW, lignin from RHW/RSW has a slightly lower molar mass, which can be interpreted by the first step with HWE removal of some lignin or lignin carbohydrate complex (LCC).

### 3.3. Deconstruction process mass balance

During the MW-DES fractionation, lignin was efficiently extracted from spruce HW and SW. To compare HW and SW in respect of MW-DES fractionation, the mass balance was calculated (based on 100 g extractive-free dry weight of biomass) and shown in Fig. 5. The focus of the mass balance was on the flow of the main components of the solid

part including raw materials and solid residue after the HWE and MW-DES fractionation. It is well acknowledged that the carbohydrate-first fractionation method can prevent hemicellulose from further dehydrating into furfural during HWE stage [17,51]. The results show that hemicellulose removal during the HWE step for HW and SW can reach 66 % and 68 %, respectively. For example, the MW-DES fractionation method directly yielded 22.43 g of the DES lignin from the initial amount of 28.21 g of DES lignin in HW. After the HWE-MW-DES fractionation, the yield of the DES lignin reduced by 31 % (from 22.43 g to 15.41 g). Additionally, the total delignification of HW decreased by 12 % (from 82 % to 72 %) when calculated based on the final solid residue component. These results show that HWE is not always beneficial for subsequential MW-DES delignification [32]. However, it is to be noted that compared to the lignin-first fractionation method, the lignin obtained by the carbohydrate-first fractionation method has a similar phenolic hydroxyl content (Fig. 4c), and the carbohydrates in the lignin are halved (Table 3), resulting in a higher purity (Table 2). Overall, the carbohydrate-first fractionation method will create the possibilities to obtain carbohydrates (i.e. structure preserved polymeric hemicelluloses) beneficial to a high-value utilization during the first HWE stage without loss of the chemical activity of lignin extracted in the subsequent MW-DES fractionation stage. The choice can be made between the lignin-first or the carbohydrate-first fractionation approaches, depending on the specific focus of the value-added application. If highest lignin yield is not the goal, a carbohydrate-first approach is a good option, allowing for the utilization of hemicellulose, lignin, and cellulose-rich residues, respectively.

## 4. Conclusions

The MW-DES fractionation method was evaluated for direct lignin extraction from HW/SW and compared to the lignin obtained using a combined approach (i.e., HWE-MW-DES fractionation) from HW/SW. The lignin recovery yield of 80 %/74 % and the lignin purity of 86 %/84 % can be obtained from HW/SW by MW-DES fractionation. While HWE-MW-DES fractionation resulted in a higher lignin purity of 88 % from both HW and SW, with a low carbohydrate content of less than 0.5 %. MW-DES fractionation enables SW to achieve higher selectivity in delignification compared to HW, resulting in the preservation of cellulose. Regardless of whether the lignin-first fractionation (MW-DES fractionation) or the carbohydrate-first fractionation (HWE-MW-DES fractionation) methods is used, DES lignin exhibits a higher phenolic hydroxyl content due to the shorter completion time of MW-DES fractionation.

### CRedit authorship contribution statement

**Jiayun Xu:** Writing – original draft, Investigation, Data curation, Conceptualization. **Chuanling Si:** Visualization. **Lin Dai:** Visualization. **Jarl Hemming:** Resources, Methodology. **Andrey Pranovich:** Writing – review & editing, Supervision, Conceptualization. **Chunlin Xu:** Writing – review & editing, Supervision, Resources.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Acknowledgements

Jiayun Xu acknowledge the financial support from the China Scholarship Council (No. 202008120139) and EU-funded Biodemo project. Parts of research used Research Council of Finland Research Infrastructure "Printed Intelligence Infrastructure" (PII-FIRI).



## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cej.2025.159232>.

## Data availability

Data will be made available on request.

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