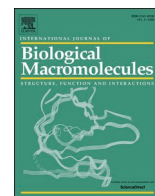


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Multiproduct biorefinery of Paulownia wood by synergy of hydrothermal and deep eutectic solvents (DES) pretreatments for polymers isolation and various cellulose applications

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ABSTRACT

This study highlights the efficiency of using coupled pretreatments to fractionate Paulownia wood (PW) into separated streams of high-added value products, including hemicelluloses, lignin, phenolic compounds, bioethanol, succinic acid, and cellulose nanocrystals (CNCs), following a green biorefinery approach. The sequential process began with a hydrothermal treatment (at 203 °C under non-isothermal regime), enabling the solubilization of the hemicellulosic fraction and achieving a high recovery of xylooligosaccharides (66.5 %). Subsequently, deep eutectic solvents (DES) were applied, resulting in a cellulose-enriched solid (81 %) and high-purity lignin recovery (85 %) under optimized conditions (130 °C, 1 h, choline chloride:lactic acid, 1:9 molar ratio, 8 mL/g liquid-to-solid ratio). The DES treatment also yielded a lignin-free black liquor rich in residual carbohydrates and phenolic compounds (2.70 g/100 g initial PW). The autohydrolyzed and DES-delignified PW was then subjected to three different types of valorizations: (i) bioethanol production, reaching 41.79 g/L (80 % yield), (ii) succinic acid production, achieving 32.02 g/L (0.76 g of succinic acid per g of glucose), and (iii) CNCs with an average aspect ratio of 17.71 (length: 90–558 nm, width: 11–23 nm), demonstrating the potential of coupling hydrothermal and DES pretreatments to produce high-value products from lignocellulosic biomass.

1. Introduction

The growing global requirement for renewable energy sources and sustainable materials that replace conventional fossil resources has driven significant research into the valorization of lignocellulosic biomass (LB) [1]. Thus, the use of LB is promoted as raw material for efficient biotechnological processes, such as biorefineries [2]. Among the various types of biomasses, *Paulownia* sp., a hardwood genus native to China, has been widely planted in several countries owing to its rapid growth, high biomass production, and ability to thrive in a diversity of soil and climatical conditions [3,4]. Besides, Paulownia wood (PW) is rich in cellulose, hemicelluloses and lignin, making it an outstanding raw material for manufacturing other products [4,5] like bioethanol or succinic acid. However, the efficient conversion of PW into these products requires effective pretreatment to surpass the recalcitrance of

its lignocellulosic structure [6] since lignin acts as a shield against chemical and enzymatic attacks [7].

Despite being a copious and renewable resource, lignocellulosic biomass holds great potential to produce biochemicals and biofuels [8], its conversion into these products generally involves several key steps: pretreatment, hydrolysis, fermentation, and product recovery [9,10]. Pretreatment is, therefore, a crucial step for the valorization of LB, and it enhances the accessibility of cellulose and hemicelluloses for enzymatic hydrolysis by disrupting the complex lignin-carbohydrate matrix [11].

Traditional pretreatment methods are well understood and function appropriately but many, such as acid hydrolysis, often require harsh conditions and can provoke the formation of inhibitory by-products that affect downstream processes [12,13]. Hereby, the development of efficient and sustainable methods to pretreat lignocellulosic biomass that can substitute or complement traditional ones is essential for the

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economic feasibility of many industrial processes such as the production of biofuels and biochemicals, while addressing environmental concerns and reducing dependence on fossil-based resources [14]. In this sense, deep eutectic solvents (DES) have gained attention as a novel class of green solvents with potential applications in biomass pretreatment [15].

DES are formed by the complexation of a hydrogen bond donor (HBD) and a hydrogen bond acceptor (HBA), obtaining a eutectic mixture with a melting point significantly lower than that of its individual components [16]. DES pose several advantages when compared to conventional solvents, such as low toxicity, biodegradability, and adjustable physicochemical properties [17,18]. Another significant benefit is the possibility of recovering these solvents, which would further reduce process costs. One of the most common techniques for DES recycling is the anti-solvent precipitation method, which consists of adding an anti-solvent (such as water, acetone, or ethanol) to the DES solution, inducing the precipitation of the target components [19]. These features make DES attractive for the selective fractionation of lignocellulosic biomass into its constituent components [18].

Among the diverse type of DES, those based on choline chloride (ChCl) and natural carboxylic acids, such as lactic acid, have shown promise for biomass fractionation due to their ability to solubilize lignin and hemicellulose while maintaining the cellulose unaltered [20,21]. This capability not only reduces energy requirements, and the environmental impact compared to conventional methods [18] but also facilitates the production of diverse value-added products.

However, it is worthy to note that despite the use of DES alone has shown great potential for LB pretreatment, their combination with other methods could offer a synergistic approach to an even more efficient fractionation [6,22]. For example, the use of DES could benefit from a previous autohydrolysis (AH) of the LB. AH is a method that involves the sole use of water at high temperature and pressure to chemically break down the hemicelluloses fraction of biomass into its constituent sugars, solubilizing it and disrupting the lignin structure [23]. This integrated approach can benefit from both strategies, enabling the efficient separation of biomass components but also facilitates their valorization into high-value products. For example, lignin and hemicellulose derivatives obtained through these processes can be utilized to produce bioactive compounds with functional properties [24], while the preserved cellulose can be converted into cellulose nanocrystals (CNCs) [25], a material with applications in composite reinforcements, packaging, and biomedical fields. Additionally, fermentable sugars can be biotransformed into bioethanol, a renewable energy source for transportation and power, or into succinic acid, a versatile precursor for biodegradable polymers, food additives, and pharmaceuticals [26,27].

This study explores the use of DES for the delignification of autohydrolyzed PW, a critical step to enhance enzymatic susceptibility and recover high-value lignin derivatives as co-products [28]. By integrating autohydrolysis and DES delignification, this work establishes a framework for efficiently fractionating lignocellulosic biomass into hemicelluloses, bioethanol, succinic acid, CNCs, and lignin. These findings aim to contribute to the advancement of multiproduct biorefineries, paving the way for scalable and sustainable solutions to biomass valorization.

2. Materials and methods

2.1. Reagents

The following reagents, assumed to be pure unless otherwise noted, were utilized: acetic acid (96 %), formic acid, lactic acid (90 %), glycerol, ethanol, ethylene glycol, sulfuric acid (72 % and 98 %), disodium hydrogen phosphate, and dimethyl sulfoxide, all sourced from Carlo Erba; arabinose, glucose, xylose, and sodium chloride obtained from Panreac; furfural, hydroxymethylfurfural, levulinic acid, and Trolox provided by Acros Organics; choline chloride acquired from Alfa Aesar; citric acid and sodium chloride supplied by Scharlau; thymol and Brain

Heart Infusion (BHI) medium from VWR; sodium hydroxide from ThermoScientific; peptone from Labkem; yeast extract from Cultimed; sodium citrate, heavy magnesium carbonate, and gallic acid from Sigma-Aldrich; cysteine from TCI; and ethyl acetate from Supelco.

The enzymes employed included Cellic-CTec2 (Novozymes). The microorganisms used were (i) a commercial strain developed by Fermentis S.L. Lesaffre for the ethanol production sector *Saccharomyces cerevisiae* Ethanol Red®, and (ii) *Actinobacillus succinogenes* (DSM 22257), supplied by the German Collection of Microorganisms and Cell Cultures GmbH (DSMZ).

2.2. Raw material and chemical characterization

The wood of *Paulownia elongata x fortunei* was sourced from a local supplier, Maderas Álvarez Oroza S.L., located in the Northwest of Spain. This wood underwent a process of stripping, air drying, and was then ground to achieve particle sizes smaller than 8 mm with Retsch SM 300 equipment. The resulting particles were kept in plastic bags at room temperature, in conditions of darkness and dryness to maintain their integrity.

The milled wood was prepared to ensure adequate carbohydrate hydrolysis [29] and was subjected to analyses to determine moisture content [30], ethanol-based extractives [31], and ash content [32]. The extractive-free PW, milled to a particle size of <1 mm, was subjected to quantitative acid hydrolysis to quantify its polymeric content [33]. The solid fraction obtained was used to determine Klason lignin (acid-insoluble lignin), while the hydrolyzed fraction was assessed for monosaccharide content using HPLC. This was performed on an Agilent 1200 series, equipped with a Rezex ROA-Organic acid H+ column (Phenomenex) maintained at 60 °C, a refractive index detector at 40 °C, and a mobile phase of 3 mM H₂SO₄ at 0.6 mL/min. Additionally, uronic acids were quantified following the method developed by Blumenkrantz et al. [34]. Protein content was measured using the Kjeldahl method, with a correction factor of 6.25 applied for lignocellulosic materials based on the nitrogen content [35]. All experiments were conducted in triplicate to ensure accurate results.

Following these procedures, the chemical composition of the untreated PW, expressed on a dry basis as g of component per 100 g of raw material, resulted as follows: 42.27 ± 0.14 g of glucan, 17.32 ± 0.02 g of xylan, 0.82 ± 0.00 g of arabinan, 3.71 ± 0.36 g of acetyl groups, 20.70 ± 0.57 g of Klason lignin, 5.12 ± 0.04 g of ethanol-based extractives, 0.40 ± 0.01 of ashes, 1.25 ± 0.01 of proteins and 6.56 ± 0.10 of uronic acids measured as galacturonic acid equivalents.

2.3. Autohydrolysis of PW

For the autohydrolysis treatment, PW and distilled water were combined in a pressurized Büchiglasuster versoclave of 1.6 L reactor able to control and measure the temperature. It is also equipped with an external fabric mantel to heat the media and internal water flow to cool it. The assay was performed, founded on a previous study by the authors [5], using a LSR of 6 mL/g, at 203 °C under non-isothermal regime and 500 rpm. The harshness of the pretreatment corresponds to a severity (S₀) of 3.98, defined by the following equation:

$$S_0 = \log \left(\int \exp \left(\frac{T(t) - 100}{14.75} \right) \cdot dt \right) \quad (1)$$

where S₀ is the severity, T(t) is the temperature on the heating and cooling stages, 100 °C is the reference temperature, and 14.75 °C is the common value for the empiric parameter concerning activation energy.

The resulting autohydrolyzed Paulownia wood (labelled as AH-PW) was chemically characterized using previously stated NREL analytical protocols for polymeric content (see Section 2.2). Following that procedure, the chemical composition of the AH-PW, expressed on a dry basis as g of component per 100 g of PW, resulted as follows: 41.86 ±

0.14 g of glucan, 3.92 ± 0.04 g of xylan, 0.67 ± 0.10 g of acetyl groups, 23.72 ± 0.14 g of Klason lignin. The arabinan was totally solubilized during this process.

2.4. Deep eutectic solvents (DES) synthesis and delignification procedure

The deep eutectic solvents (DES) used were synthesized based on the procedure outlined by Martín et al. [36], with some modifications. The process involved combining a hydrogen bond acceptor (HBA), specifically choline chloride in all cases, with a hydrogen bond donor (HBD), such as various carboxylic acids and alcohols. These solvents were then stirred magnetically and warmed to approximately 80 °C for 2 h to ensure entire homogenization. The mixtures were composed of choline chloride as HBD, and lactic acid, glycerol, ethylene glycol, formic acid or acetic acid as HBA, evaluating molar ratios (HBD:HBA) from 1:2 to 1:9.

Regarding the delignification procedure, AH-PW and the selected DES were mixed at a selected liquid-to-solid ratio (LSR) (8 to 15 mL/g) and heated to a set temperature (110 to 130 °C) during a certain residence time (0.5 to 2 h), evaluating different DES molar ratio (1:2 to 1:9). The effectiveness on the removal of lignin was optimized using a one-factor-at-a-time (OFAT) approach, which involves systematically varying a single parameter while maintaining all other parameters constant, as previously determined, to assess their individual impacts on the process. After treatment, the solid and liquid fractions were separated using vacuum filtration.

The solid fraction was collected and washed with warm 50 % (v/v) ethanol followed by sodium hydroxide (evaluating from 0.1 % to 1 % w/w) to prevent lignin precipitation and remove any residual DES, before washing with distilled water and labelled as AH + DES-PW.

Both the liquid hydrolysate and the pretreated biomass were stored at 4 °C and analyzed. The pretreated biomass was analyzed following the procedures detailed in Section 2.2. The liquid hydrolysate (which lignin was prior precipitated as stated in Section 2.8) was analyzed by direct injection into an HPLC system for monomer quantification, in addition to being subjected to acid post-hydrolysis with 4 % H₂SO₄ for 20 min at 121 °C before HPLC injection to quantify oligomers content. Additionally, the acid-soluble lignin content of the hydrolysate was determined by measuring the absorbance of the resultant liquor at 240 nm using a ONDA UV-20 spectrophotometer after appropriate dilution [37].

2.5. Bioethanol production

2.5.1. Microorganism and inoculum preparation

The strain *Saccharomyces cerevisiae* Ethanol Red® was used for ethanol fermentation. The yeast was cultured at 30 °C with shaking at 200 rpm for 24 h in a sterile medium containing 20 g/L peptone, 20 g/L glucose, and 10 g/L yeast extract. After incubation, the yeast cells were collected by centrifugation at 4000 rpm (4200 ×g) for 10 min using a Hettich Rotixa 50 S centrifuge. The harvested cells were diluted in a 0.9 % NaCl to achieve a final approximately 8 g of fresh *S. cerevisiae*/L, equivalent to around 1.5 g/L of dry *S. cerevisiae*.

2.5.2. Pre-saccharification and simultaneous saccharification and fermentation (PSSF)

After the optimization of delignification parameters, AH-PW treated under optimal variables (and sterilized at 121 °C for 15 min) was subjected to Pre-saccharification and simultaneous saccharification and fermentation (PSSF) assays. The tests comprised the use of Cellic CTec2 (116 FPU/mL of final activity measured by Filter Paper Assay [38]) at a cellulase to substrate ratio (CSR) of 20 FPU per g of dry solid, setting a controlled temperature of incubation of 50 °C at 160 rpm of agitation for 72 h within the enzymatic hydrolysis stage, using LSR ranging 8 and 10 mL/g. The glucose manufacture was assessed as glucose concentration (g/L) and as glucan to glucose conversion (GGC) percentage using Eq. (2):

$$GGC (\%) = \frac{(Glucose_t - Glucose_{t_0})}{\frac{Gn \cdot 180}{100} \cdot \frac{\rho}{162 \cdot LSR + 1} - \frac{KL}{100}} \quad (2)$$

In this equation, *Glucose_t* and *Glucose_{t₀}* represent the glucose concentration (g/L) at time (t) and initial time. Gn denotes the g of glucan per 100 g of pretreated PW, 180/162 is the glucan hydration upon hydrolysis (based on stoichiometry), ρ is the medium's density (fixed at 1005 g/L), LSR indicates the liquid-to-solid ratio (mL/g), and KL corresponds to the g of Klason lignin per 100 g pretreated solid.

Upon saccharification completion, the fermentation stage commenced using *S. cerevisiae* at 35 °C under 120 rpm of agitation. A nutrient solution was added to the medium, resulting in final concentrations of 20 g/L peptone and 10 g/L yeast extract. Samples were withdrawn at selected times, centrifuged at 15,000 rpm (22,000 ×g) and the supernatant was injected into HPLC to quantify glucose and ethanol. All experiments were conducted in two replicates.

Ethanol production was expressed in both concentration (g/L) and yield percentage using Eq. (3):

$$Ethanol \ yield (\%) = \frac{(EtOH_t - EtOH_{t_0})}{0.51 \cdot \frac{Gn \cdot 180}{100} \cdot \frac{\rho}{162 \cdot LSR + 1} - \frac{KL}{100}} \quad (3)$$

where *EtOH_t* and *EtOH_{t₀}* denote the ethanol concentration in g/L at time (t) and at the initial time, respectively. The factor 0.51 accounts for the stoichiometric conversion of glucose to ethanol, with other parameters defined as in Eq. (1).

2.6. Succinic acid (SA) production

2.6.1. Microorganism and inoculum preparation

The strain *Actinobacillus succinogenes* (DSM number 22257), supplied by the German Collection of Microorganisms and Cell Cultures GmbH, was selected based on other succinic production articles [39,40]. *A. succinogenes* was maintained in 3.5 g/L Brain Heart Infusion (BHI) medium:glycerol (1:1 v/v) at -80 °C. The composition of BHI medium was: 17.5 g/L of brain heart infusion solids, 10.0 g/L of peptone, 2.0 g/L of glucose, 5.0 g/L of sodium chloride and 2.5 g/L of disodium hydrogen phosphate. For inoculum preparation, the strain was cultivated in bottles previously purged with N₂ for 20 min with BHI medium at 37 °C and 200 rpm for 20 h.

2.6.2. Separate hydrolysis and fermentation (SHF)

First, the PW hydrolysates were prepared through enzymatic hydrolysis. The saccharification of AH + DES-PW was performed in as explained in Section 2.5.2. Upon completion, the reaction mixture was centrifuged under sterile conditions, the solid was discarded, and the hydrolysate was preserved at a temperature of -18 °C for further fermentation. The fermentation assays were conducted in 100 mL bottles with a working volume of 50 mL. The PW hydrolysate was mixed with magnesium carbonate salt added at 100 % w/w of the carbon source to act as a pH buffer and CO₂ source. The medium was also supplemented with nutrient concentrate, inoculated with *A. succinogenes* at 10 % v/v, and cysteine was added as a reducer to reinforce anaerobiosis. The fermentation was carried out under anaerobic conditions at 37 °C and 180 rpm for 72 h [40,41]. The nutrient formulation, in g/L in the final medium, was: 5 g/L sodium chloride, 2.5 g/L sodium dihydrogen phosphate, and 10 g/L peptone.

2.7. Cellulose nanocrystal (CNC) production

The method used to produce cellulose nanocrystals (CNCs) was an adaptation of Morales et al. [42] and Barbosa et al. [43], consisting of a bleaching treatment followed by acid hydrolysis. In the bleaching stage, 5 g of AH + DES-PW were mixed with 160 mL of distilled water, 1 mL of acetic acid, and 5.2 mL of sodium chlorite (25 %). The mixture was

stirred continuously on a thermostatic stirrer plate at 75 °C for 2 h. The bleached material was vacuum filtered, washed with distilled water until neutral pH, and dried at 50 °C.

In the acid hydrolysis stage, the dried bleached pulp was treated with 50 % sulfuric acid at LSR of 15 mL/g. The reaction was carried out in an ultrasonic bath at 60 °C for 1 h. Afterward, the reaction was stopped by adding distilled water, and the mixture was vacuum filtered. The solid phase, corresponding to cellulose nanocrystals (CNCs), was washed with distilled water until neutral pH and dried at 50 °C.

To confirm the presence of CNCs, the material was resuspended in water at a 1:10 weight ratio and sonicated. For Atomic Force Microscopy (AFM) analysis, the CNC suspension was deposited onto a mica substrate, dried, and analyzed using a Veeco's Multimode 8 Nanoscope in peak force tapping mode.

2.8. Lignin isolation and characterization

Following the selection of optimal conditions to delignify AH-PW, the resulting black liquor was treated with acidified water using sulfuric acid (pH = 2) at a volume ratio of 3:1 (acidified water to black liquor). This mixture was mixed for 15 min and refrigerated at 4 °C overnight. The obtained lignin was vacuum filtered, carefully washed with distilled water, and dried completely at a temperature of 50 °C in an oven. The lignin's purity was assessed through quantitative acid hydrolysis, as explained in Section 2.2.

Characterization of the lignin was conducted using Fourier-Transform Infrared (FTIR) Spectroscopy on a PerkinElmer Spectrum 100 spectrometer with an attenuated total reflectance (ATR) accessory. Measurements included eight scans per sample over a spectral range of 4000–650 cm⁻¹.

Proton Nuclear Magnetic Resonance (¹H NMR) spectroscopy of the extracted lignin was conducted at 25 °C using a Bruker Neo 400 spectrometer operating at 400 MHz, with the lignin dissolved in dimethyl sulfoxide (DMSO).

The thermal behavior of the lignin was analyzed using a Perkin Elmer TGA 4000. Samples were subjected to heating up to 800 °C using a rate of 10 °C/min under a nitrogen flow of 20 mL/min.

2.9. Influence of the lignin precipitation over the black liquors' total phenolic content and antioxidant capacity

The black liquor obtained at optimized conditions for DES delignification of AH-PW was analyzed for antioxidant capacity using DPPH, ABTS, and FRAP assays, both before and after lignin precipitation.

Antioxidant capacity was evaluated through DPPH, ABTS, and FRAP assays, and trolox was used as the standard equivalent. All the methods are based on the originals developed by Brand-Williams [44], Re et al. [45] and Benzie & Strain [46], respectively.

2.10. Determination of phenolic profile in DES black liquor post-lignin precipitation

Phenolic compounds were extracted from the DES black liquor using ethyl acetate in a 1:1 ratio. This mixture was magnetically stirred for 15 min at room temperature and then allowed to settle in a separatory funnel. The ethyl acetate phase was collected, and the extraction process was repeated two more times under identical conditions. The ethyl acetate collected from all three extractions was removed by rotary evaporation at 40 °C, and the resulting matter was dissolved in methanol and injected into HPLC equipped with an AB SCIEX Triple Quad 3500 detector (AB Sciex, Foster City, USA) with an electrospray ionization (ESI) source using a Luna C18 column (Phenomenex) as stated in an earlier work by the authors [47].

2.11. Statistical and mathematical methods

Results from DES screening underwent statistical analysis using R (version 4.1.0). Significant differences among samples were identified through a one-way ANOVA with Tukey's post hoc test, considering $p < 0.05$ as the threshold for significance.

3. Results and discussion

3.1. Autohydrolysis pretreatment of Paulownia wood

The autohydrolysis of Paulownia wood was conducted at 203 °C with a LSR of 6 mL/g, yielding a solid fraction of 74.26 %. This process effectively targeted the solubilization of hemicellulosic fractions (see Table 1), with xylooligosaccharides (XOs) as the primary product, resulting in a recovery of 11.51 g per 100 g of initial PW, which corresponds to 67 % of the original xylan content. Comparable studies in the field have demonstrated similar efficiencies, showing consistent potential across different lignocellulosic feedstocks to selectively isolate hemicellulose-derived oligosaccharides. For instance, a two-stage autohydrolysis of Paulownia under optimized conditions achieved an 85 % xylan conversion, producing high yields of xylose in the liquid

Table 1

Phenolic content, antioxidant capacity and phenolic and carbohydrate profiles of autohydrolysis liquor (230 °C, LSR = 6 mL/g) and black liquor (ChCl:LA (1:9), 130 °C, 1 h, LSR = 8 mL/g) before and after precipitation, of Paulownia wood.

	Autohydrolysis liquor	Black liquor	Black liquor after lignin precipitation
Phenolic content and antioxidant capacity (mg equivalents/g initial PW)			
DPPH (mg TE/g)	6.61 ± 0.70	77.44 ± 5.97	13.54 ± 0.36
ABTS (mg TE/g)	10.77 ± 0.89	67.85 ± 1.81	12.32 ± 0.32
FRAP (mg TE/g)	51.94 ± 0.39	272.62 ± 0.67	71.62 ± 2.17
Phenolic profile (µg/g initial PW)			
3,4-dihydroxybenzoic acid	13.57	–	1767.24
4-hydroxybenzoic acid	0.37	–	86.20
Caffeine	0.02	–	–
Epicatechin	0.21	–	–
Ethylvanilline	0.08	–	–
Ferulic acid	3.95	–	51.62
Gallic acid	0.14	–	7.86
Luteolin	0.02	–	59.97
Naringenin	0.02	–	–
<i>p</i> -coumaric acid	0.77	–	34.76
<i>p</i> -hydroxybenzaldehyde	–	–	67.08
Phthalic acid	–	–	148.64
Quercetin	0.03	–	–
Salicylic acid	0.07	–	–
Syringaldehyde	56.20	–	44,651.82
Syringic acid	4.28	–	1124.14
Theobromine	2.14	–	–
Vanillic acid	5.70	–	1553.44
Vanillin	88.92	–	29,015.62
Monomers and oligomers content (g/100 g initial PW)			
Glucose	1.15	–	0.57
Xylose	2.16	–	0.58
Arabinose	0.29	–	0.00
Acetic acid	0.70	–	0.00
Hydroxymethylfurfural	0.00	–	0.07
Furfural	0.00	–	0.39
Glucosaccharides	1.41 ± 0.02	–	0.50 ± 0.08
Xylooligosaccharides	11.51 ± 0.02	–	0.54 ± 0.01
Arabinooligosaccharides	0.26 ± 0.01	–	0.00 ± 0.00
Acetyl groups linked to oligosaccharides	2.40 ± 0.04	–	2.49 ± 0.06

phase [48] and other hardwoods like beech [49] reported up to 65 % XOs recovery from xylan content under hydrothermal pretreatment.

The removal of acetyl groups (AGs) is notable after the autohydrolysis, with 2.40 g/100 g of initial PW, which results in a recovery rate of 65 %. These data are consistent with other works. For example, a study carried out by Neto et al. [50] using *Eucalyptus* wood achieved a removal of acetyl groups ranging from 54 to 90 % in the mildest and harshest conditions evaluated, respectively.

On the other hand, in the autohydrolysis processing, the recovery of glucooligosaccharides (GOs) is always lower than AGs and XOs [51] and, in this case, it was quantified at 1.41 g/100 g of initial PW, corresponding to a 3.34 % recovery. This performance is consistent with studies on other lignocellulosic compounds like the one performed by Torrado et al. [52], in which the recovery of GOs from the autohydrolysis pine nut shells accounted for 5 % of the total oligosaccharides produced, which is significantly lower than the primary product, XOs (95 %).

Regarding the phenolic profile of the AH-PW liquor observed in Table 1, it presented antioxidant capacities measured at 6.61 mg TE/g for DPPH, 10.77 mg TE/g for ABTS, and 51.94 mg TE/g for FRAP. These values suggest some bioactive potential for applications in antioxidant supplementation, food preservation, or polymer stabilization. Among the number of compounds found, the most prominent were vanillin (88.92 µg/g) and syringaldehyde (56.20 µg/g), with lower proportions of compounds like 3,4-dihydroxybenzoic acid (13.57 µg/g) and ferulic acid (3.95 µg/g), all known for their bioactive properties [53–55].

These results align with findings from hydrothermal treatments on other woods. For instance, *Eucalyptus globulus* [56] and *Robinia pseudoacacia* [57] have also yielded notable amounts of vanillin, gallic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid and quercetin, all of them present in the AH-PW liquor of this study.

In summary, the phenolic composition and antioxidant activity of the AH liquor underscore its potential for high-value applications. The selective release of key phenolics through autohydrolysis offers a practical and sustainable route for valorizing wood biomass in biorefinery contexts, with promising applications across health-related industries.

3.2. Deep eutectic solvents (DES) screening

To evaluate the effectiveness of DES treatments on AH-PW, five DES were tested (see Section 2.4). In all cases, ChCl was used as the HBA, combined with different HBD including 3 carboxylic acids (formic,

acetic, and lactic acid) and 2 alcohols (glycerol, and ethylene glycol) at a molar ratio of 1:2. The treatment conditions were fixed at 120 °C for 60 min at LSR of 15 mL/g.

Fig. 1 presents the recovery or removal of the main constituents of AH + DES-PW (glucan, hemicelluloses, and lignin), as well as the solid yield obtained from each DES treatment. Solid yield provides an important measure of efficiency of the treatment. For DES composed of lactic acid (LA), a solid yield of 72.13 % was achieved, which was similar to that from DES formed by formic acid (71.29 %) or acetic acid (73.53 %). In contrast, the DES formulated with glycerol and ethylene glycol gave the highest solid yields at 88.54 % and 87.21 %, respectively, reflecting their lower effectiveness in biomass breakdown.

Glucan recovery, which is crucial for subsequent biomass bioconversion applications, was consistently high across all DES treatments, ranging from 95 % to almost 100 %.

Hemicelluloses solubilization showed little variation regarding the DES composed of alcohols (glycerol and ethylene glycol), whereas a slightly higher solubilization was reached for DES synthesized with carboxylic acids. In spite of this, the hemicelluloses were already highly solubilized in the previous pretreatment (autohydrolysis), so the remaining fraction is residual.

However, in terms of delignification, the DES formed by LA and formic acid performed particularly well, achieving delignification ratios of 68 % and 72 %, respectively. On the other hand, delignification with DES synthesized with glycerol and ethylene glycol did not surpass 30 %.

Taking into account the previous results, DES composed of lactic acid and formic acid presented the most positive performances with the highest delignification ratios, retaining the great majority of glucan in the solid fraction (>96 % regarding initial values). Apart from their performance, cost is another critical factor in selecting the optimal DES for efficiency purposes. Among the most economical options, ChCl:LA (1:2) is priced at 1.80 €/kg using reagents from an industrial supplier (Alibaba) and 35.00 €/kg from a laboratory supplier (Sigma Aldrich), whereas formic acid DES costs 2.01 €/kg (industrial supplier) and 44.33 €/kg (laboratory supplier) [47]. Thus, ChCl:LA (1:2) DES is approximately 10–21 % cheaper than that composed of formic acid, providing a significant economic advantage without compromising efficiency.

Furthermore, prior studies have shown the suitability of LA-based DES for the delignification of various lignocellulosic biomasses, demonstrating both high efficiency and economic viability. For instance, in a study on spruce wood delignification, an ChCl:LA DES achieved significant lignin removal (around 90 %), enabling efficient lignin

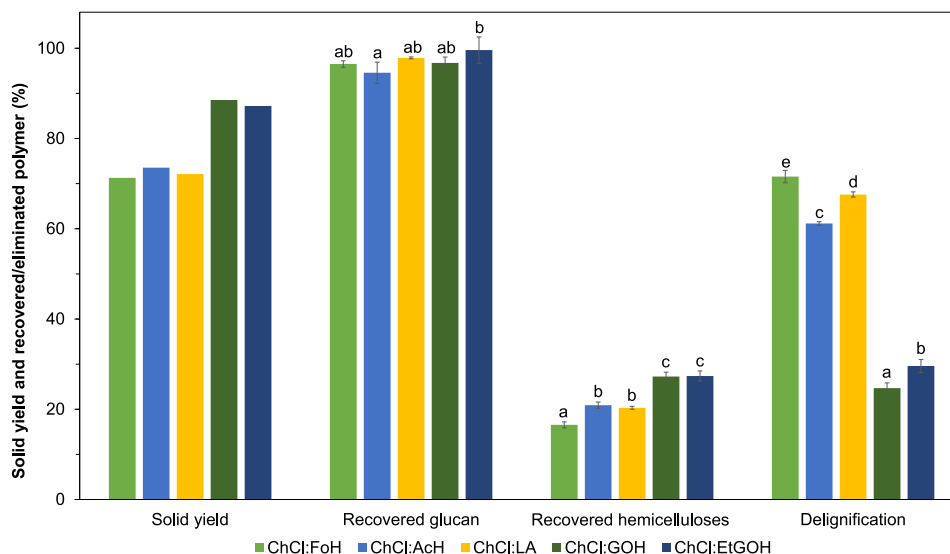


Fig. 1. Recovered solid fraction and polysaccharides and lignin removal with various DES (120 °C for 60 min). Abbreviations: AcH - acetic acid; ChCl - choline chloride; EtGOH - ethylene glycol; FoH - formic acid; GOH - glycerol; LA - lactic acid. Different letters indicate statistically significant differences at $p < 0.05$.

recovery while preserving other valuable biomass components [58]. Similarly, for mosso bamboo, this DES formulation removed an impressive 94.39 % of lignin, effectively isolating cellulose and allowing the production of nanocellulose, which highlights the broad applicability of this DES in lignocellulosic processing [59].

These previous findings along with its performance (high delignification, coupled with minimal glucan loss) and low cost support the use of ChCl:LA DES for further optimization of delignification process of AH-PW presented in this study.

3.3. Effect of ChCl:LA DES on the fractionation of AH-PW

After selecting the DES composed of ChCl:LA for the subsequent experiments, different variables (namely reaction temperature, reaction time, molar ratio of the DES and LSR) were studied following the one-factor-at-a-time (OFAT) method. The selected conditions studied and the results of the 12 runs can be consulted in Table 2.

Throughout the first 3 experiments, the factor studied was the temperature, varying between 110 and 130 °C, while maintaining the time (1 h), molar ratio (1:2) and LSR (15 mL/g) invariable. The effect of the treatment is reflected in the yield of the solid phase, i.e., the increase of temperature (up to 130 °C) promoted the solubilization of components, whereas a lower reaction temperature (110 °C) exhibited an inferior liberation of said components. In this case, the cellulosic fraction varied in a narrow range of 41.37–42.08 g glucan/100 g initial PW, which corresponds to practically quantitative glucan recovery regarding values from initial PW. Similarly, the hemicelluloses were also minimally affected by this treatment, for instance, only up to 22 % of the remaining xylan after autohydrolysis was solubilized. On the other hand, the most affected component was lignin, reaching delignification ratios (regarding initial lignin values) of 61 % at the mildest condition (110 °C) and up to 84 % at the harshest condition (130 °C). Considering the previous results, the highest temperature was selected for the subsequent runs. Other authors using DES for delignification processes found similar tendencies. For instance, a study conducted on microwave-autohydrolyzed *Robinia pseudoacacia* wood using ChCl:LA resulted in up to 18 % more lignin solubilization when using 130 °C rather than 110 °C [60]. Similar results were found after processing cassava residue with ternary DES (ChCl:LA: citric acid, 1:10:1), increasing the lignin removal from 23 % at 70 °C up to 91 % at 130 °C (reaction time to 3 h) [61]. In addition, other authors reported comparable delignification rates, achieving up to 72 % of lignin removal from poplar wood while using ChCl:LA at a temperature of 120 °C for a duration of 3 h [62].

Table 2

Treatment yield and chemical characterization of the solid phase after sequential autohydrolysis and delignification with DES studying different variables using an OFAT method.

Run	1	2	3	4	5	6	7	8	9	10	11	12
Reaction temperature (°C)	110	120	130	130	130	130	130	130	130	130	130	130
Reaction time (h)	1	1	1	0.5	1.5	2	1	1	1	1	1	1
Molar ratio	1:2	1:2	1:2	1:2	1:2	1:2	1:3	1:5	1:7	1:9	1:9	1:9
LSR (mL/g)	15	15	15	15	15	15	15	15	15	15	8	11.5
Solid yield (g/100 g autohydrolyzed PW)	79.57	72.13	68.71	70.30	68.43	69.38	67.49	68.04	67.23	66.93	69.47	70.77
Solid phase (g/100 g initial PW)												
Glucan	41.76 ± 0.24	41.37 ± 0.09	42.08 ± 0.34	40.98 ± 0.39	41.51 ± 0.87	40.71 ± 0.14	41.46 ± 0.23	42.57 ± 0.13	41.53 ± 0.13	41.42 ± 0.06	41.73 ± 0.16	42.11 ± 0.19
Xylan	3.70 ± 0.10	3.29 ± 0.06	3.04 ± 0.06	2.74 ± 0.04	2.57 ± 0.09	2.40 ± 0.02	2.44 ± 0.08	2.37 ± 0.04	2.31 ± 0.04	2.32 ± 0.01	2.22 ± 0.10	2.25 ± 0.02
Arabinan	0.52 ± 0.02	0.75 ± 0.05	0.35 ± 0.07	0.15 ± 0.01	0.14 ± 0.01	0.19 ± 0.02	0.08 ± 0.07	0.08 ± 0.07	0.28 ± 0.05	0.12 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
Acetyl groups	0.73 ± 0.07	0.40 ± 0.03	0.52 ± 0.11	0.45 ± 0.06	0.40 ± 0.09	0.39 ± 0.05	0.25 ± 0.03	0.22 ± 0.03	0.18 ± 0.02	0.19 ± 0.03	0.17 ± 0.03	0.18 ± 0.01
Klason lignin	8.09 ± 0.57	6.71 ± 0.12	3.38 ± 0.54	5.87 ± 0.26	3.18 ± 0.10	3.39 ± 0.01	2.78 ± 0.14	2.16 ± 0.09	2.24 ± 0.03	2.00 ± 0.07	2.31 ± 0.24	3.19 ± 0.04

It is, however, noteworthy to say that some authors have investigated higher temperatures, yielding different outcomes. For instance, a study conducted by [63], in which delignification was evaluated at three different temperatures (60, 115, and 150 °C) using agro-industrial food waste resulted in the highest production of fermentable sugars at 115 °C rather than at the highest temperature. In contrast, [64] found that when delignifying Acacia wood using various NaDES the highest lignin extraction yielded at elevated temperatures (140 °C). However, they also found that further temperature increases rendered the process unsuitable for lignin recovery due to the formation of a black, foam-like mixture, likely caused by the degradation of DES components and their crosslinking with the biomass. Therefore, future studies could consider evaluating the effect of increasing the selected temperature to further enhance the delignification performance, although 130 °C is considered a safe and effective temperature for lignin removal in lignocellulosic biomass.

The following experiments performed (runs 3–6) enabled to comprehend the effect of the reaction time (varying between 0.5 and 2 h) on the solubilization of the polymers of AH-PW. Firstly, the increase on the treatment's reaction time thoroughly affected the solid yield, decreasing to values of around 68 % at residence times higher than 1 h, but with little variation if compared with longer periods (1.5 or 2 h). This is also reflected in the solubilization of the polymers, for instance, glucan was maintained in values higher than 96 % in all cases. Regarding the xylan, higher reaction times enabled to solubilize almost 40 % of the remaining xylan after autohydrolysis, whereas the delignification ratio was similar (84–85 %) for reaction times equal or larger than 1 h and lower (72 %) for reaction time of 0.5 h. In this sense, the reaction time was set at 1 h since it was the minimum necessary time to reach high delignification ratios without a great removal of the glucan. Similarly, Pradhan et al. [65] processed barley straw with ChCl:oxalic acid (2:1) by ultrasonication (25 kHz), reaching higher removal of lignin (86 %) and hemicelluloses (87 %) when rising the reaction time from 3 to 5 h at 80 °C.

The molar ratio was the next studied variable (runs 3 and 7 to 10), with increasing values from 1:2 to 1:9. In this case, the solid yield was similar (67–69 %) for all the runs. The cellulosic fraction was retained in the solid with almost quantitative values, while the xylan solubilization reached values of around 40 % when using molar ratios higher than 1:3. Meanwhile, the removal of lignin was increased at molar ratios of 1:5, 1:7 and 1:9, reaching values of around 90 %. All this, added to the lessen in the price of DES when the amount of lactic acid increases [47], resulted in the molar ratio of 1:9 being chosen as optimal. This effect was

also studied by Li et al. [66] on willow, increasing the delignification ratio up to 67 % when utilizing a molar ratio of 1:10 with ChCl:LA. Similarly, almost the double delignification ratio (61 %) was acquired by Tan et al. [67] when processing oil palm empty fruit bunch with ChCl:LA (1:15) rather than 1:1.

Finally, the relationship between DES and solid employed (LSR) was assessed since the use of the minimum necessary amount of DES would imply a drastic reduction in the price of the process [68]. For that reason, LSR of 8 and 11.5 mL/g were evaluated. In this regard, very similar results were obtained for runs 10, 11 and 12 reaching higher cellulose recovery than 98 %, delignification ratio of 85–90 % and hemicelluloses removal of 57–59 % and a measure. Considering these results, the lower LSR (implying the use of less amount of DES) was selected as optimal. Summarizing, after processing AH-PW with ChCl:LA, the optimized values for the variables were: temperature of 130 °C, reaction time of 1 h, molar ratio of 1:9 and LSR of 8 mL/g.

3.4. Hydrolysis and fermentation strategies for bioethanol and succinic acid production

The enzymatic digestibility of AH + DES-PW under optimized conditions was evaluated, testing different NaOH concentrations to wash the solid and increase its enzymatic susceptibility (see Fig. 1S). A concentration of 0.1 % NaOH was enough to reach almost quantitative glucan to glucose conversions, as already stated previously by the authors [47].

The production of bioethanol was conducted proposing an enzymatic-fermentation scheme which envisages the study of two distinct LSR (8 and 10 g liquid/g AH + DES-PW) and the addition of commercial nutrients at 20 g/L of yeast extract and 10 g/L of peptone, as observed in previous studies. The results obtained are presented in Fig. 2a. After 72 h of enzymatic hydrolysis, the achieved glucose concentration was 62.0 g/L and 78.5 g/L glucose for LSR 10 and 8, respectively. At that time, the yeast was inoculated, and the ethanol reached its maximum value at fermentation times of 6 to 9 h, with a concentration value of 32.63 g/L (77 % yield) at an LSR of 10 mL/g and 41.79 g/L (80 % yield) at an LSR of 8 mL/g.

The results demonstrated a slight improvement in comparison to those observed in similar studies utilizing alternative feedstock. For instance, banana peel waste treated with ChCl:urea (1:2) achieved an ethanol efficiency of 59.2 % [69]. Conversely, Yadav et al. [70], adopted a pre-treatment scheme involving the raw material (cocoa pod husks) and a combination of microwave (600 W) and the DES CHCl: citric acid (1:2), resulting in a 67.17 % ethanol yield at 72 h. However, the results achieved in this work are analogous to those obtained in a previous

study by Rodríguez-Rebello et al. [47] on the same feedstock (Paulownia wood) with direct delignification with DES (ChCl:LA, 1:9), where up to 43.61 g ethanol/L (89.7 % of ethanol yield) were obtained at LSR 8 mL/g. Similarly, the work carried out by Jose et al. [71] with Napier grass, where a yield of 0.335 g/g was obtained following a one-pot fermentation scheme that permitted continuous exposure of the biomass to DES (ChCl:urea (1:2)). Furthermore, the study conducted with the same raw material only pretreated by autohydrolysis by Domínguez et al. [72] reported significantly lower ethanol concentrations compared to those observed in the present study, with a severity factor close to 4.19, yielding over 16 g/L of ethanol using SSF and around 24 g/L using SHF processes. This finding further highlights the crucial role of the combined action of the two pretreatments applied in this work.

On the other hand, the production of succinic acid (SA) was also explored using the cellulosic-rich substrate of AH + DES-PW and the results are exhibited in Fig. 2b. The hydrolysis of the process was set at 48 h and 72 h. Specifically, GGC after 48 h and 72 h of saccharification reached 78 % (33.10 g/L) and 99 % (42.07 g/L), respectively. Regarding the fermentation process, similar values of SA were obtained, reaching up to 29.64 and 32.02 g of SA/L, which yielded 0.90 and 0.76 g of SA/g of glucose, respectively. Regarding the generation of co-products, FoH yielded up to 0.12–0.13 g/g of glucose in both strategies, whereas AcH yielded up to 0.22 (7.28 g/L) and 0.38 (15.85 g/L) g/g of glucose at final time of the fermentation in SHF using 48 h and 72 h of saccharification respectively. The higher production of AcH may explain the lower yield on SA production in the SHF strategy which set the enzymatic hydrolysis at 72 h. Even so, the low concentration of FoH, which is stated as the main inhibitor [40], does not affect the fermentation, since the production of all SA, AcH and FoH yielded 1.25 g/g of glucose using either of the strategies. Additionally, the productivity of SA attained 0.41–0.44 g/(L*h).

Analogously, Filippi et al. [73] developed a biorefinery to process grape pomaces and stalks with ChCl:LA (1:10) for 2 hat 100 °C, submitting the spent solid to SA production. From a glucose-rich hydrolysate (62.9 g/L), up to 36 g SA/L, with a yield of 0.62 g SA/g of total sugars was obtained, with a simultaneous production of 5.8 g acetic acid /L and 2.6 g formic acid/L after 55 h of fermentation. Additionally, a similar yield of SA was obtained by Niglio et al. [74] who reached up to 0.84 g SA/g of glucose, although lasting 140 h of fermentation to achieve a concentration of 20.8 g/L with a productivity of 0.15 g/(L*h).

3.5. Characterization of cellulose nanocrystals-CNC by AFM

Besides producing different biotechnological products like bioethanol and succinic acid, the cellulose-rich fraction of AH + DES-PW

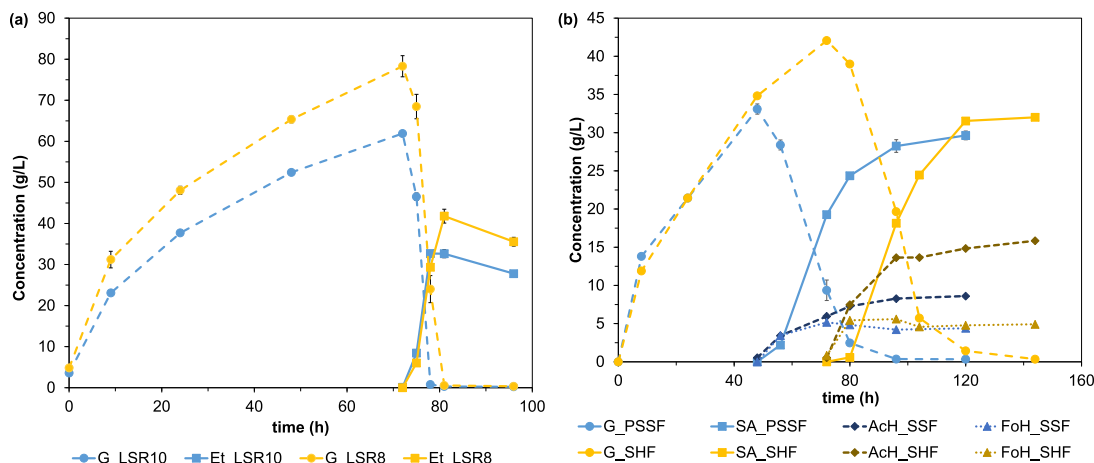


Fig. 2. Hydrolysis and fermentation time course of AH + DES-PW at optimized conditions for (a) bioethanol production using PSSF strategy and LSR of 10 and 8 mL/g, and (b) succinic acid production using PSSF and SHF strategies and LSR of 20 mL/g.

was subjected to further processing to produce precursors for bio-materials synthesis. In this context, cellulose nanocrystals (CNC) were analyzed by AFM, and one of the images obtained is displayed in Fig. 3. It exhibits elongated structures with rod-shape morphology, and the measured dimensions are length ranging 90–558 nm, width (diameter) between 11 and 23 nm, and height ranging 1.506 to 9.557 nm, which may correspond to an individual nanocrystal and to a piling and accretion of multiple nanocrystals, respectively. The small size and high specific surface area of the crystals may be the cause of forming aggregations owing to the generation of Van der Waals and hydrogen bonds among them [75].

Another important factor to be considered on CNC regarding their application is the aspect ratio (length/diameter). Specifically, the current study obtained an average aspect ratio of 17.71, which is significantly higher than that obtained for other feedstock like *Linum usitatissimum* with a value of 6.06 [76] or walnut shells with 10.77 [77]. On the other hand, other study by the authors reached values of up to 41.21 [60] when using autohydrolyzed and DES-delignified Robinia wood. As a general trend, CNC presenting higher aspect ratios than 10 are deemed as potential material to be used as reinforcing agent, for instance for polymer-based films synthesis [75].

3.6. Lignin characterization

After the processing of PW-AH with DES at optimal conditions, the lignin was precipitated by mixing the black liquor with a water/sulfuric acid solution to reach a pH of 2 and submitted to further characterization. The purity of the sample was determined by quantitative acid hydrolysis as stated in a previous section, reaching a value of 85.29 % (accounted as Klason lignin) with small amounts of polysaccharides, specifically 1.61 % of glucan and 0.33 % of xylan. Similar lignin purities (84–88 %) were obtained by other authors in analogous processes [47,62]. The acid-soluble lignin remaining in the black liquor accounted for 1.41 ± 0.04 g per 100 g of initial PW.

The different analysis carried out on lignin can be consulted in Fig. 4. The ^1H NMR spectrum of lignin is depicted in Fig. 4a. The analysis reveals chemical shifts indicative of aliphatic -CH₂ and -CH₃ groups at δ_{H} values of 1.2, 1.1, and 9.9 ppm. Furthermore, the peaks at δ_{H} 3.4 and 3.7

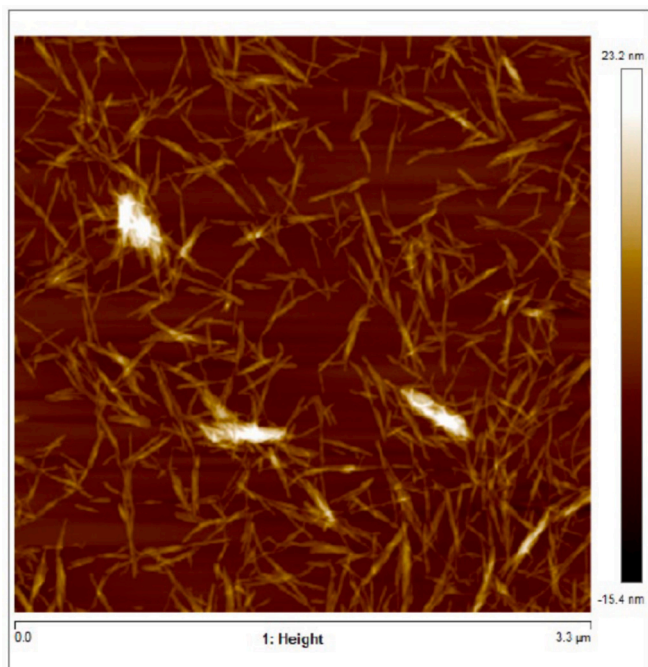


Fig. 3. AFM topographic 2D image of PW CNC.

ppm are attributed to methoxy groups, while only a small amount of aromatic hydrogen is detected within the range of 6.4–7.4 ppm [78].

To get more information, the 2D HSQC spectra was obtained and is illustrated in Fig. 4b. The sample predominantly displayed signals in the aliphatic-oxygenated and aromatic/unsaturated regions, specifically within the ranges of $\delta\text{C}/\delta_{\text{H}}$ 50–90/2.6–5.2 ppm and $\delta\text{C}/\delta_{\text{H}}$ 100–125/6.0–7.3 ppm. In the aliphatic-oxygenated region, a prominent signal corresponding to methoxyl groups (OMe, $\delta\text{C}/\delta_{\text{H}}$ 56/3.7 ppm) was observed, alongside signals indicative of resinol (B_{β} , $\delta\text{C}/\delta_{\text{H}}$ 56/3.7 ppm and B_{γ} , 70/4.1 ppm) and phenylcoumaran (C_{γ} , 61/3.7 ppm) linkages. Additionally, signals associated with β -O-4 ether bonds were detected in this region (A regions corresponding to A_{γ} 60/3.3 ppm, A_{α} 69–73/4.8–4.9 ppm, $\text{A}_{\beta(\text{S})}$ 88/4.3 ppm) [79–82]. In the aromatic section of the spectra, signals related to guaiacyl (G) and syringyl (S) units were present; however, hydroxyphenyl (H) units were absent. This observation aligns with findings from Domínguez et al. [82], who reported only trace amounts of H-units in the milled wood lignin derived from Paulownia wood. No signals attributed to carbohydrates, such as acetylated xylopyranoside at $\delta\text{C}/\delta_{\text{H}}$ 100/4.5, were also noted, indicating a high purity level of lignin. The limited presence of carbohydrates, coupled with reduced signals for G2 and G6 compared to G5, suggested a lower degree of lignin condensation overall. These results are in accordance with the obtained results by the authors when processing Paulownia wood (without the hemicellulosic extraction by autohydrolysis) with DES [47].

The FTIR spectra of obtained lignin can be observed in Fig. 4c. The bands observed between 1450 and 1600 and 1000–1300 cm^{-1} can be ascribed to the C–O stretching and aromatic skeletal vibrations of lignin and the stretching of C–O in ester groups and deformation of C–O for secondary alcohol and aliphatic esters [83–85]. Moreover, the band at 4000 cm^{-1} may be ascribed to the stretching vibration of hydroxy groups [86,87]. Additionally, the presence of guaiacyl and syringyl (that were already observed in HSQC spectra) can be noted due to the breathing in C–O stretching and the C–H deformation, corresponding to 1213 and 1269 cm^{-1} for the former and 813, 1114, 1320 cm^{-1} for the latter [83,87].

The lignin was also submitted to TGA to assess its thermic stability, while the derivative of TGA (DTG) was also determined (see Fig. 4d). A small peak can be observed in DTG plot at a lower temperature than 100 °C which may correspond to the removal of remaining water in the sample (<5 % of the total weight) [88]. Around 280 °C, the degradation of residual polysaccharides, namely glucan and xylan, may take place as observed by the reduction of around 15 % of the total weight, whereas the greater peak at 380 °C may reflect the thermal decomposition of lignin. However, the curves generated during each phase can be viewed as the simultaneous breakdown of multiple compounds [89]. At 800 °C, the lignin maintained 34.5 % of the total mass which may correspond to the inorganic matter, perceiving a positive thermic stability that can be comparable to other lignins in the bibliography [90,91].

3.7. Chemical characterization of liquor after lignin precipitation

The phenolic content and antioxidant capacity of the black liquor, before and after the precipitation of lignin, were also evaluated and can be consulted in Table 1. After lignin precipitation, there is a general decrease antioxidant capacity in the remaining liquor.

Regarding antioxidant capacity, all DPPH, ABTS and FRAP assays showed a major decreasing in antioxidant capacity with values dropping from 77.44 mg TE/g to 13.54 mg TE/g, 67.85 to 12.32 ± 0.32 mg TE/g, and 272.67 to 71.62 mg TE/g, respectively. These values would therefore tend to indicate that even with the removal of lignin by precipitation, there was still some antioxidant capacity present in the liquor.

Assuming this, the phenolic profile of the black liquor (after lignin precipitation) was carried out and can be observed in Table 1. Among them, syringaldehyde and vanillin appeared in higher concentrations: 44651.82 $\mu\text{g/g}$ and 29,015.62 $\mu\text{g/g}$, respectively. Other major phenolics

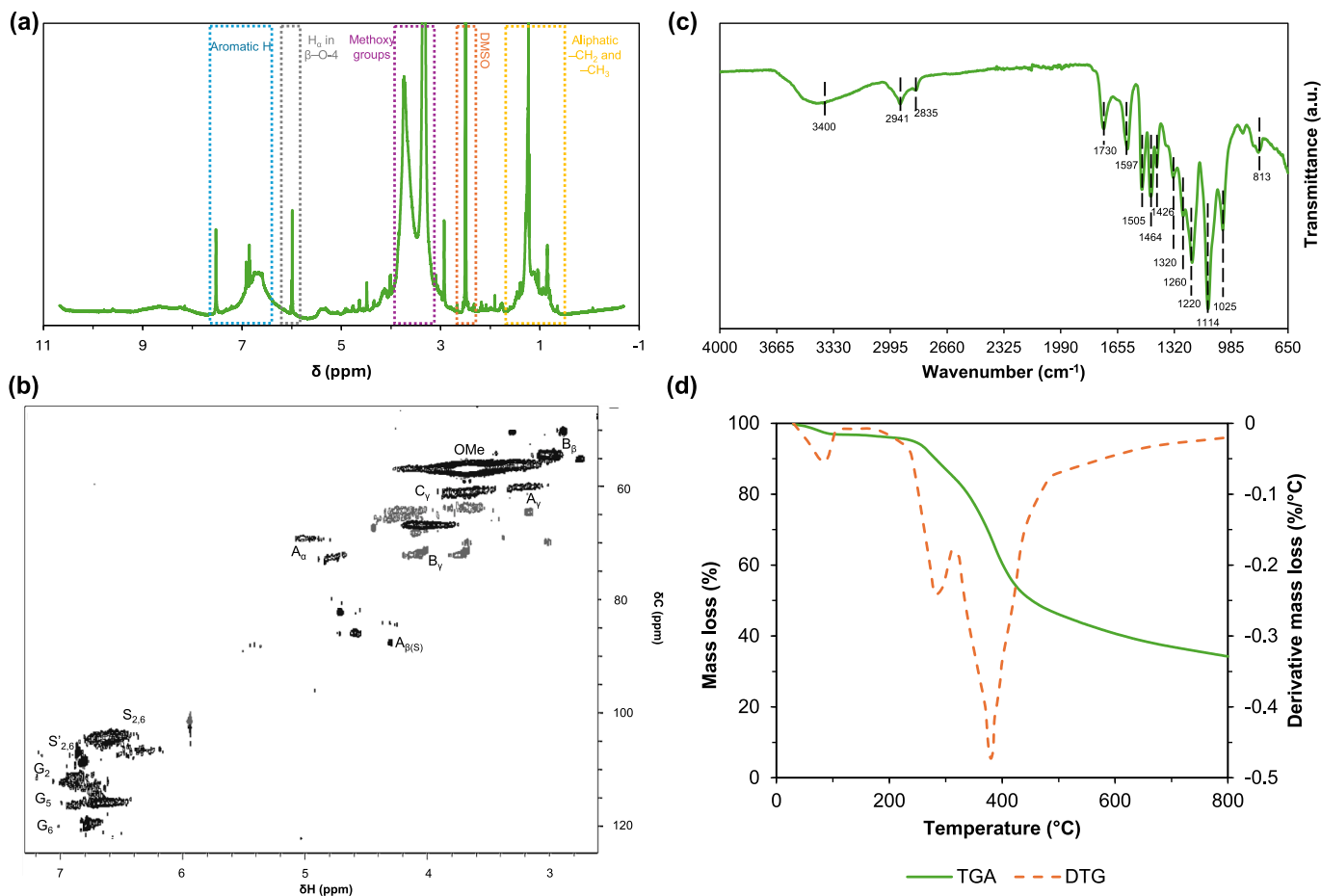


Fig. 4. Characterization of lignin obtained after DES-delignification of PW-AH at selected conditions (temperature-130 °C, reaction time-1 h, molar ratio-1:9 and LSR-8 mL/g), comprising: (a) ¹H NMR, (b) 2D HSQC, (c) FTIR, and (d) TGA.

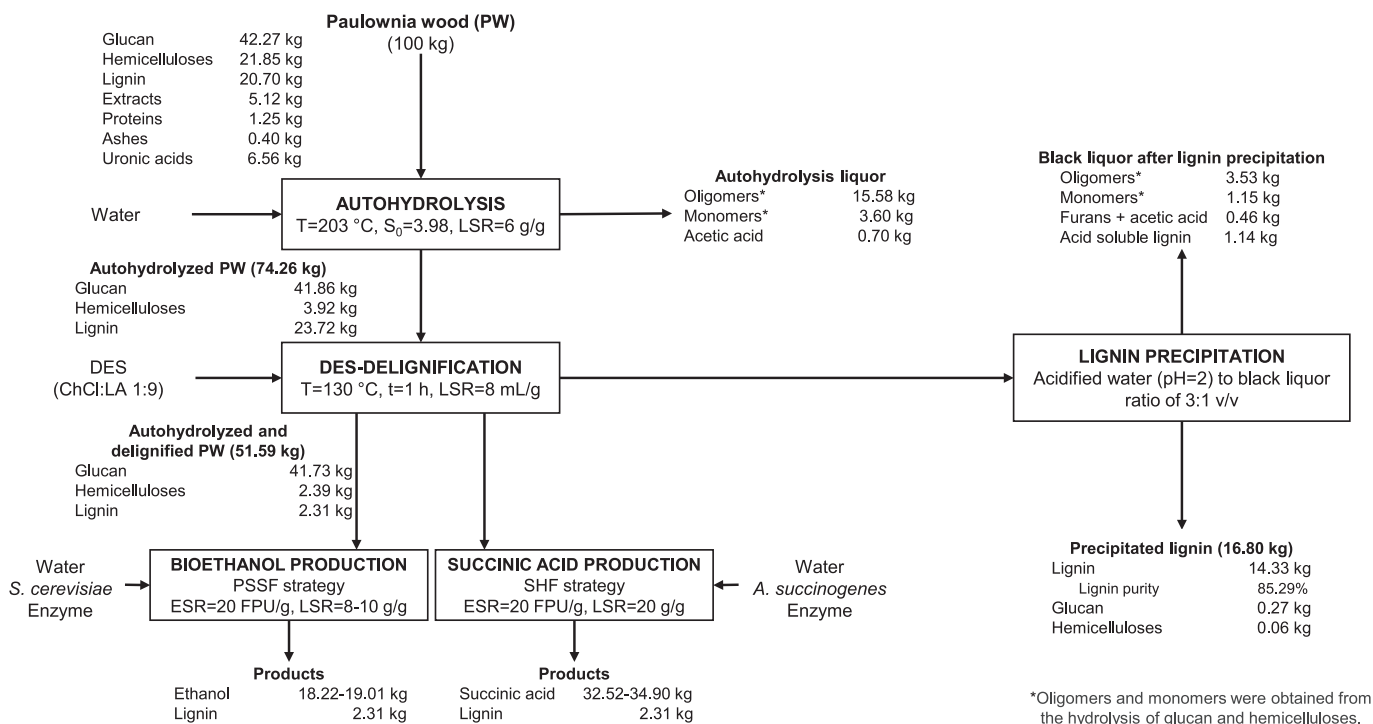


Fig. 5. Mass balance of the multiproduct processing of Paulownia wood via autohydrolysis followed by DES-delignification.

included syringic acid (1124.14 µg/g), 3,4-dihydroxybenzoic acid (1767.24 µg/g), and vanillic acid (1553.44 µg/g). In all cases, this high content in phenolic compounds is intimately related to the unprecipitated lignin.

The liquor also contained minor quantities of monomers and oligomers, including glucose xylose, glucooligosaccharides, and xylooligosaccharides, being in all cases lower than 0.60 g/100 g. On the other hand, the amount of acetyl groups linked to oligosaccharides was as high as 2.49 g/100 g initial PW.

3.8. Mass balance of the whole process

In order to get a full understanding of the processing of PW in a multiproduct biorefinery (for hemicelluloses, lignin and bioethanol obtainment), a mass balance was performed, and it can be consulted in Fig. 5.

Starting from 100 kg of PW, hydrothermal treatment was carried out to remove the hemicellulosic fraction without a thorough alteration of the cellulosic and lignin fractions. In this sense, only 3.92 kg of hemicelluloses were retained in the solid fraction, while the great majority were solubilized into the autohydrolysis liquor, mainly in the form of oligomers (15.58 kg). The liquid phase mainly comprised oligomers, accounting for a 78 % of the carbohydrates, and the main components were the xylooligosaccharides (56 % of the total of carbohydrates in the liquor).

Subsequently, the delignification was performed using the DES formed by ChCl:LA at optimal conditions, attaining a removal of 18.40 kg of lignin with residual amounts of oligomers (3.53 kg) and monomers (1.15 kg) in the black liquor. The lignin was then precipitated, recovering a sample of 16.80 kg that presented a purity of 85 % in Klason lignin (14.33 kg). On the other hand, the black liquor after lignin precipitation retained the carbohydrates and phenolic compounds (2.70 kg).

Conversely, the solid phase after autohydrolysis and DES-delignification (AH + DES-PW) was chiefly composed of glucan (81 %) and was subjected to enzymatic-fermentative processes, to produce ethanol (between 18.22 and 19.01 kg per 100 kg of initial PW) and succinic acid (between 32.52 and 34.90 kg per 100 kg of initial PW). These ethanol values are in the range of other work by the authors where the DES-delignification on raw PW was studied [47]. In addition, the ethanol obtained in this work was higher than that by Wu et al. [92] who attained 9.45 kg of ethanol per 100 kg of initial biomass after sequential alkali and ChCh:LA processing of sorghum straw. Alternatively, 14.9 kg of ethanol/100 kg were obtained by Xu et al. [93] after processing corn stover using ChCl:GOH (1:2) DES. Similarly, higher succinic acid production was reached in the current work regarding the study by [73], who obtained up to 20.08 g of succinic acid from the mixture of 100 kg of grape stalks and 80.5 g of extracted grape pomace.

Considering the abovementioned, the studied processing of PW enables to obtain the three main components of lignocellulosic biomass in different streams following a multi-product biorefinery with high selectivity treatments, recovering 15.58 kg of hemicellulosic oligomers, 41.73 kg of cellulose (that may be transformed into 19.01 kg of bioethanol or 34.90 kg of succinic acid), and 16.80 kg of lignin.

4. Conclusions

The sequential application of autohydrolysis and deep eutectic solvents (DES) pretreatments on PW demonstrated a promising route for efficient biomass fractionation in a multiproduct biorefinery approach. Autohydrolysis efficiently solubilized hemicelluloses, predominantly as xylooligosaccharides, while the use DES at optimized conditions (130 °C, 1 h reaction time, ChCl:LA (1:9) and a LSR of 8 mL/g), achieved high delignification (up to 89 %) with minimal glucan loss (<1.3 %). Subsequently, cellulose was processed to produce bioethanol (up to 41.79 g/L, yield of 80 %), succinic acid (32.02 g/L, yield of 0.76 g of

succinic acid per g of glucose), and cellulose nanocrystals (average aspect ratio of 17.71). The obtained lignin presented a high purity (85 %), and the phenolic compounds isolated from the black liquor, including high concentrations of syringaldehyde and vanillin, underscore the potential for added-value applications in antioxidant production. This study demonstrates the effectiveness of combining autohydrolysis and DES for the valorization of Paulownia wood, enabling the production of high value bioproducts such as hemicellulosic oligosaccharides, bioethanol, and lignin. These findings highlight the approach's strong potential for scalable and sustainable biorefinery applications.

CRedit authorship contribution statement

Fernando Rodríguez-Rebello: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Conceptualization. **Beatriz Rodríguez-Martínez:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Pablo G. Del-Río:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Maurice N. Collins:** Writing – review & editing, Validation, Supervision, Methodology, Funding acquisition. **Beatriz Gullón:** Writing – review & editing, Validation, Supervision, Methodology, Funding acquisition, Conceptualization.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijbiomac.2025.144385>.

References

- [1] J.A. Okolie, S. Nanda, A.K. Dalai, J.A. Kozinski, Chemistry and specialty industrial applications of lignocellulosic biomass, Waste Biomass Valorization 12 (2021) 2145–2169, <https://doi.org/10.1007/s12649-020-01123-0>.
- [2] N.A.K. Khairil Anwar, N. Hassan, N. Mohd Yusof, A. Idris, High-titer bio-succinic acid production from sequential alkaline and metal salt pretreated empty fruit bunch via simultaneous saccharification and fermentation, Ind. Crops Prod. 166 (2021) 113478, <https://doi.org/10.1016/j.indcrop.2021.113478>.
- [3] A. Palma, J.M. Loaiza, M.J. Díaz, J.C. García, I. Giráldez, F. López, Tagasaste, leucaena and paulownia: three industrial crops for energy and hemicelluloses

- production, *Biotechnol. Biofuels* 14 (2021) 89, <https://doi.org/10.1186/s13068-021-01930-0>.
- [4] L.Q. Lee, H. Zhao, J. Ge, Y. Zhou, H. Li, Valorization of fast-growing Paulownia wood to green chemicals and green hydrogen, *Green Chem.* 26 (2024) 1949–1963, <https://doi.org/10.1039/d3gc03458e>.
- [5] P.G. del Río, A. Pérez-Pérez, G. Garrote, B. Gullón, Manufacturing of hemicellulosic oligosaccharides from fast-growing Paulownia wood via autohydrolysis: microwave versus conventional heating, *Ind Crops Prod* 187 (2022) 115313, <https://doi.org/10.1016/j.indcrop.2022.115313>.
- [6] R. Rai, V. Kumar, P. Dhar, Recalcitrance of lignocellulosic biomass and pretreatment technologies: A comprehensive insight, in: P. Verma (Ed.), *Thermochemical and catalytic conversion technologies for future biorefineries: volume 1*, Springer Nature Singapore, Singapore, 2022, pp. 13–52, https://doi.org/10.1007/978-981-19-4312-6_2.
- [7] R. Ceaser, S. Rosa, D. Montané, M. Constantí, F. Medina, Optimization of softwood pretreatment by microwave-assisted deep eutectic solvents at high solids loading, *Bioresour. Technol.* 369 (2023) 128470, <https://doi.org/10.1016/j.biortech.2022.128470>.
- [8] C. Kole, C.P. Joshi, D.R. Shonnard, *Handbook of bioenergy crop plants*, CRC Press, 2012.
- [9] S. Beluhan, K. Mihajlovski, B. Šantek, M. Ivancić Šantek, The production of bioethanol from lignocellulosic biomass: pretreatment methods, fermentation, and downstream processing, *Energies (Basel)* 16 (2023) 7003, <https://doi.org/10.3390/en16197003>.
- [10] G. Brodeur, E. Yau, K. Badal, J. Collier, K.B. Ramachandran, S. Ramakrishnan, Chemical and physicochemical pretreatment of lignocellulosic biomass: a review, *Enzyme Res.* 2011 (2011) 787532, <https://doi.org/10.4061/2011/787532>.
- [11] A.A. Awoyale, D. Lokhat, Experimental determination of the effects of pretreatment on selected Nigerian lignocellulosic biomass in bioethanol production, *Sci. Rep.* 11 (2021) 557, <https://doi.org/10.1038/s41598-020-78105-8>.
- [12] H.A. Ruiz, M. Conrad, S.-N. Sun, A. Sanchez, G.J.M. Rocha, A. Romani, E. Castro, A. Torres, R.M. Rodríguez-Jasso, L.P. Andrade, R.-C. Sun, A.S. Meyer, Engineering aspects of hydrothermal pretreatment: from batch to continuous operation, scale-up and pilot reactor under biorefinery concept, *Bioresour. Technol.* 299 (2020) 122685, <https://doi.org/10.1016/j.biortech.2019.122685>.
- [13] S. Akizuki, H. Suzuki, M. Fujiwara, T. Toda, Impacts of steam explosion pretreatment on semi-continuous anaerobic digestion of lignin-rich submerged macrophyte, *J. Clean. Prod.* 385 (2023) 135377, <https://doi.org/10.1016/j.jclepro.2022.135377>.
- [14] Z. Guo, Q. Zhang, T. You, X. Zhang, F. Xu, Y. Wu, Short-time deep eutectic solvent pretreatment for enhanced enzymatic saccharification and lignin valorization, *Green Chem.* 21 (2019) 3099–3108, <https://doi.org/10.1039/c9gc00704k>.
- [15] C. Picot-Allain, M.F. Mahomoodally, G. Ak, G. Zengin, Conventional versus green extraction techniques — a comparative perspective, *Curr. Opin. Food Sci.* 40 (2021) 144–156, <https://doi.org/10.1016/j.cofs.2021.02.009>.
- [16] P. Gullón, B. Gullón, A. Romani, G. Rocchetti, J.M.J.M. Lorenzo, Smart advanced solvents for bioactive compounds recovery from agri-food by-products: a review, *Trends Food Sci. Technol.* 101 (2020) 182–197, <https://doi.org/10.1016/j.tifs.2020.05.007>.
- [17] I. Pacheco-Fernández, V. Pino, Green solvents in analytical chemistry, *Curr. Opin. Green Sustain. Chem.* 18 (2019) 42–50, <https://doi.org/10.1016/j.cogsc.2018.12.010>.
- [18] Y. Guo, J. Zhang, C. Wang, M. Liu, J. You, L. Yin, M. Shi, Green pretreatment of lignocellulosic biomasses via deep eutectic solvents, *Sustain. Chem. Pharm.* 39 (2024) 101569, <https://doi.org/10.1016/j.scp.2024.101569>.
- [19] A. Lobato-Rodríguez, B. Gullón, A. Romani, P. Ferreira-Santos, G. Garrote, P. G. Del-Río, Recent advances in biorefineries based on lignin extraction using deep eutectic solvents: a review, *Bioresour. Technol.* 388 (2023) 129744, <https://doi.org/10.1016/j.biortech.2023.129744>.
- [20] G. Zeng, L. Zhang, B. Qi, J. Luo, Y. Wan, Cellulose esterification with carboxylic acid in deep eutectic solvent pretreatment inhibits enzymatic hydrolysis, *Bioresour. Technol.* 380 (2023) 129085, <https://doi.org/10.1016/j.biortech.2023.129085>.
- [21] J. Sonyeam, R. Chaipanya, S. Suksomboon, M.J. Khan, K. Amatariyakul, A. Wibowo, P. Posoknistakul, B. Charmonk, C.G. Liu, N. Laosiripojana, C. Sakdaronnarong, Process design for acidic and alcohol based deep eutectic solvent pretreatment and high pressure homogenization of palm bunches for nanocellulose production, *Sci. Rep.* 14 (2024) 7550, <https://doi.org/10.1038/s41598-024-57631-9>.
- [22] A.K. Kumar, S. Sharma, Recent updates on different methods of pretreatment of lignocellulosic feedstocks: a review, *Bioresour. Bioprocess.* 4 (2017) 7, <https://doi.org/10.1186/s40643-017-0137-9>.
- [23] Y. Yuan, B. Jiang, H. Chen, W. Wu, S. Wu, Y. Jin, H. Xiao, Recent advances in understanding the effects of lignin structural characteristics on enzymatic hydrolysis, *Biotechnol. Biofuels* 14 (2021) 205, <https://doi.org/10.1186/s13068-021-02054-1>.
- [24] C. Espro, E. Paone, F. Mauriello, R. Gotti, E. Uliassi, M.L. Bolognesi, D. Rodríguez-Padrón, R. Luque, Sustainable production of pharmaceutical, nutraceutical and bioactive compounds from biomass and waste, *Chem. Soc. Rev.* 50 (2021) 11191–11207, <https://doi.org/10.1039/D1CS00524C>.
- [25] P. Kumar, K. Miller, A. Kermanshahi-pour, S.K. Brar, R.F. Beims, C.C. Xu, Nanocrystalline cellulose derived from spruce wood: influence of process parameters, *Int. J. Biol. Macromol.* 221 (2022) 426–434, <https://doi.org/10.1016/j.ijbiomac.2022.09.017>.
- [26] S. Beluhan, K. Mihajlovski, B. Šantek, M. Ivancić Šantek, The production of bioethanol from lignocellulosic biomass: pretreatment methods, fermentation, and downstream processing, *Energies (Basel)* 16 (2023) 7003, <https://doi.org/10.3390/en16197003>.
- [27] S. Zhou, M. Zhang, L. Zhu, X. Zhao, J. Chen, W. Chen, C. Chang, Hydrolysis of lignocellulose to succinic acid: a review of treatment methods and succinic acid applications, *Biotechnol. Biofuels Bioprod.* 16 (2023) 1, <https://doi.org/10.1186/s13068-022-02244-5>.
- [28] F. Cotana, G. Cavalaglio, A. Nicolini, M. Gelosia, V. Coccia, A. Petrozzi, L. Brinchi, Lignin as co-product of second generation bioethanol production from lignocellulosic biomass, *Energy Procedia* 45 (2014) 52–60, <https://doi.org/10.1016/j.egypro.2014.01.007>.
- [29] B. Hames, R. Ruiz, C. Scarlata, A. Sluiter, J. Sluiter, D. Templeton, Preparation of samples for compositional analysis (NREL/TP-510-42620), 2008.
- [30] A. Sluiter, B. Hames, D. Hyman, C. Payne, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, J. Wolfe, Determination of total solids in biomass and total dissolved solids in liquid process samples (NREL/TP-510-42621), 2008.
- [31] A. Sluiter, R.O. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, D. of Energy, Determination of extractives in biomass (NREL/TP-510-42619), 2008.
- [32] A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, Determination of ash in biomass (NREL/TP-510-42622), 2008.
- [33] A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Crocker, Determination of structural carbohydrates and lignin in biomass (NREL/TP-510-42618), 2008.
- [34] N. Blumenkrantz, G. Asboe-Hansen, New method for quantitative determination of uronic acids, *Anal. Biochem.* 54 (1973) 484–489, [https://doi.org/10.1016/0003-2697\(73\)90377-1](https://doi.org/10.1016/0003-2697(73)90377-1).
- [35] J.B. Sluiter, R.O. Ruiz, C.J. Scarlata, A.D. Sluiter, D.W. Templeton, Compositional analysis of lignocellulosic feedstocks. 1. Review and description of methods, *J. Agric. Food Chem.* 58 (2010) 9043–9053, <https://doi.org/10.1021/jf1008023>.
- [36] M.I. Martín, I. García-Díaz, M.L. Rodríguez, M.C. Gutiérrez, F. del Monte, F. A. López, Synthesis and properties of hydrophilic and hydrophobic deep eutectic solvents via heating-stirring and ultrasound, *Molecules* 29 (2024) 3089, <https://doi.org/10.3390/molecules29133089>.
- [37] A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, D. Crocker, Determination of structural carbohydrates and lignin in biomass (NREL/TP-510-42618), 2011.
- [38] B. Adney, J. Baker, Measurement of cellulase activities: laboratory analytical procedure (LAP). technical report: NREL/TP-510-42628, 2008.
- [39] K. Filippi, H. Papapostolou, M. Alexandri, A. Vlysidis, E.D. Myrtilis, D. Ladakis, C. Pateraki, S.A. Haroutounian, A. Koutinas, Integrated biorefinery development using winery waste streams for the production of bacterial cellulose, succinic acid and value-added fractions, *Bioresour. Technol.* 343 (2022) 125989, <https://doi.org/10.1016/j.biortech.2021.125989>.
- [40] I.A. Escanciano, V.E. Santos, Á. Blanco, M. Ladero, Bioproduction of succinic acid from potato waste. Kinetic modeling, *Ind. Crops Prod.* 203 (2023) 117124, <https://doi.org/10.1016/j.indcrop.2023.117124>.
- [41] M.V. Guettler, D. Rumler, M.K. Jain, *Actinobacillus succinogenes* sp. nov., a novel succinic-acid-producing strain from the bovine rumen, *Int. J. Syst. Evol. Microbiol.* 49 (1999) 207–216, <https://doi.org/10.1099/00207713-49-1-207>.
- [42] A. Morales, J. Labidi, P. Gullón, Hydrothermal treatments of walnut shells: a potential pretreatment for subsequent product obtaining, *Sci. Total Environ.* 764 (2021) 142800, <https://doi.org/10.1016/j.scitotenv.2020.142800>.
- [43] A. Barbosa, E. Robles, J. Ribeiro, R. Lund, N. Carreño, J. Labidi, Cellulose nanocrystal membranes as excipients for drug delivery systems, *Materials* 9 (2016) 1002, <https://doi.org/10.3390/ma9121002>.
- [44] W. Brand-Williams, M.E. Cuvelier, C. Berset, Use of a free radical method to evaluate antioxidant activity, *LWT Food Sci. Technol.* 28 (1995) 25–30, [https://doi.org/10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5).
- [45] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans, Antioxidant activity applying an improved ABTS radical cation decolorization assay, *Free Radic. Biol. Med.* 26 (1999) 1231–1237, [https://doi.org/10.1016/S0891-5849\(98\)00315-3](https://doi.org/10.1016/S0891-5849(98)00315-3).
- [46] I.F.F. Benzie, J.J. Strain, Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration, *Methods Enzymol.* 299 (1999) 15–27, [https://doi.org/10.1016/S0076-6879\(99\)99005-5](https://doi.org/10.1016/S0076-6879(99)99005-5).
- [47] F. Rodríguez-Rebello, B. Rodríguez-Martínez, P.G. Del-Río, M.N. Collins, G. Garrote, B. Gullón, Assessment of deep eutectic solvents (DES) to fractionate Paulownia wood within a biorefinery scheme: cellulose bioethanol production and lignin isolation, *Ind. Crops Prod.* 216 (2024) 118761, <https://doi.org/10.1016/j.indcrop.2024.118761>.
- [48] E. Domínguez, T. Nóvoa, P.G. del Río, G. Garrote, A. Romani, Sequential two-stage autohydrolysis biorefinery for the production of bioethanol from fast-growing Paulownia biomass, *Energ. Convers. Manage.* 226 (2020) 113517, <https://doi.org/10.1016/j.enconman.2020.113517>.
- [49] C.K. Nitsos, T. Choli-Papadopoulou, K.A. Matis, K.S. Triantafyllidis, Optimization of hydrothermal pretreatment of hardwood and softwood lignocellulosic residues for selective hemicellulose recovery and improved cellulose enzymatic hydrolysis, *ACS Sustain. Chem. Eng.* 4 (2016) 4529–4544, <https://doi.org/10.1021/acssuschemeng.6b00535>.
- [50] F.S.P.P. Neto, I.U.M. Roldán, J.P.M. Galán, R. Monti, S.C. de Oliveira, F. Masarin, Model-based optimization of xylooligosaccharides production by hydrothermal pretreatment of Eucalyptus by-product, *Ind. Crops Prod.* 154 (2020) 112707, <https://doi.org/10.1016/j.indcrop.2020.112707>.

- [51] Y. Lu, Q. He, G. Fan, Q. Cheng, G. Song, Extraction and modification of hemicellulose from lignocellulosic biomass: a review, *Green Processes Synth.* 10 (2021) 779–804, <https://doi.org/10.1515/gps-2021-0065>.
- [52] I. Torrado, B.G. Neves, M. da Conceição Fernandes, F. Carvalho, H. Pereira, L. C. Duarte, Microwave-assisted hydrothermal processing of pine nut shells for oligosaccharide production, *Biomass Convers. Biorefinery* 14 (2024) 20751–20760, <https://doi.org/10.1007/s13399-023-05244-z>.
- [53] A. Olatunde, A. Mohammed, M.A. Ibrahim, N. Tajudeen, M.N. Shuaibu, Vanillin: a food additive with multiple biological activities, *Eur. J. Med. Chem. Rep.* 5 (2022) 100055, <https://doi.org/10.1016/j.ejmc.2022.100055>.
- [54] J. Wu, Y.S. Fu, K. Lin, X. Huang, Y. Jing Chen, D. Lai, N. Kang, L. Huang, C.F. Weng, A narrative review: the pharmaceutical evolution of phenolic syringaldehyde, *Biomed. Pharmacother.* 153 (2022) 113339, <https://doi.org/10.1016/j.biopha.2022.113339>.
- [55] C. Perez-Terreno, C.M. Werner, A.G. Nickel, M.D. Herrera, M.J. Motilva, M. Böhm, M. Alvarez de Sotomayor, U. Laufs, Ferulic acid, a bioactive component of rice bran, improves oxidative stress and mitochondrial biogenesis and dynamics in mice and in human mononuclear cells, *J. Nutr. Biochem.* 48 (2017) 51–61, <https://doi.org/10.1016/j.jnutbio.2017.06.011>.
- [56] D.M. Neiva, R.A. Costa, J. Gominho, S. Ferreira-Dias, H. Pereira, Fractionation and valorization of industrial bark residues by autohydrolysis and enzymatic saccharification, *Bioresour. Technol. Rep.* 11 (2020) 100441, <https://doi.org/10.1016/j.biteb.2020.100441>.
- [57] A. Pérez-Pérez, B. Gullón, Á. Lobato-Rodríguez, G. Garrote, P.G. del Río, Microwave-assisted extraction of hemicellulosic oligosaccharides and phenolics from *Robinia pseudoacacia* wood, *Carbohydr. Polym.* 301 (2023) 120364, <https://doi.org/10.1016/j.carbpol.2022.120364>.
- [58] M. Gholami, J.M. Tjburg, B. Schuur, Continuous liquid–liquid extraction to recover lignin and furanics from lactic acid: choline chloride deep eutectic solvent after cooking of spruce, *Sep. Purif. Technol.* 338 (2024) 126526, <https://doi.org/10.1016/j.seppur.2024.126526>.
- [59] Q. Liu, T. Yuan, Q. jin Fu, Y. yuan Bai, F. Peng, C. li Yao, Choline chloride-lactic acid deep eutectic solvent for delignification and nanocellulose production of moso bamboo, *Cellulose* 26 (2019) 9447–9462, <https://doi.org/10.1007/s10570-019-02726-0>.
- [60] A. Pérez-Pérez, P.G. Del-Río, Á. Lobato-Rodríguez, G. Garrote, B. Gullón, Synergistic effect of hydrothermal and deep eutectic solvents (DES) pretreatments on Robinia wood fractionation for the manufacture of bioethanol and cellulose nanocrystals, *Ind. Crops Prod.* 203 (2023) 117157, <https://doi.org/10.1016/j.indcrop.2023.117157>.
- [61] Y. Xiong, W. Li, Z. Qin, T. Su, X. Xie, H. Ji, A green extraction technology of lignocellulose from cassava residue by mechanical activation-assisted ternary deep eutectic solvent, *Int. J. Biol. Macromol.* 281 (2024) 136339, <https://doi.org/10.1016/j.ijbiomac.2024.136339>.
- [62] C. Alvarez-Vasco, R. Ma, M. Quintero, M. Guo, S. Geleynse, K.K. Ramasamy, M. Wolcott, X. Zhang, Unique low-molecular-weight lignin with high purity extracted from wood by deep eutectic solvents (DES): a source of lignin for valorization, *Green Chem.* 18 (2016) 5133–5141, <https://doi.org/10.1039/C6GC01007E>.
- [63] A. Procentese, F. Raganati, G. Olivieri, M.E. Russo, L. Rehmann, A. Marzocchella, Deep eutectic solvents pretreatment of agro-industrial food waste, *Biotechnol. Biofuels* 11 (2018) 1–12, <https://doi.org/10.1186/S13068-018-1034-Y/TABLES/4>.
- [64] C. Fernandes, M.J. Aliaño-González, L. Cid Gomes, D. Bernin, R. Gaspar, P. Fardim, M.S. Reis, L. Alves, B. Medronho, M.G. Rasteiro, C. Varela, Lignin extraction from acacia wood: crafting deep eutectic solvents with a systematic D-optimal mixture-process experimental design, *Int. J. Biol. Macromol.* 280 (2024) 135936, <https://doi.org/10.1016/J.IJBIOMAC.2024.135936>.
- [65] D. Pradhan, S. Jaiswal, B.K. Tiwari, A.K. Jaiswal, Choline chloride – oxalic acid dihydrate deep eutectic solvent pretreatment of Barley straw for production of cellulose nanofibers, *Int. J. Biol. Macromol.* 281 (2024) 136213, <https://doi.org/10.1016/j.ijbiomac.2024.136213>.
- [66] T. Li, G. Lyu, Y. Liu, R. Lou, L.A. Lucia, G. Yang, J. Chen, H.A.M. Saeed, Deep eutectic solvents (DESs) for the isolation of willow lignin (*Salix matsudana* cv. zhuliu), *Int. J. Mol. Sci.* 18 (2017) 2266, <https://doi.org/10.3390/ijms18112266>.
- [67] Y.T. Tan, G.C. Ngoh, A.S.M. Chua, Effect of functional groups in acid constituent of deep eutectic solvent for extraction of reactive lignin, *Bioresour. Technol.* 281 (2019) 359–366, <https://doi.org/10.1016/j.biortech.2019.02.010>.
- [68] P.G. del Río, B. Gullón, J. Wu, J. Saddler, G. Garrote, A. Romani, Current breakthroughs in the hardwood biorefineries: hydrothermal processing for the co-production of xylooligosaccharides and bioethanol, *Bioresour. Technol.* 343 (2022) 126100, <https://doi.org/10.1016/j.biortech.2021.126100>.
- [69] H. Manivannan, B.L. Anguraj, Valorization of fruit waste using DES pretreatment and hydrolysis over a heterogeneous catalyst for bioethanol production, *Biomass Convers. Biorefin.* 13 (2023) 5731–5741, <https://doi.org/10.1007/s13399-021-01669-6>.
- [70] A. Yadav, C. Di Dong, D. Sharma, M.L. Tsai, P.P. Sun, P. Nargotra, C.W. Chen, K. Choure, V. Sharma, Integrated choline chloride/citric acid-microwave pretreatment for efficient nanolignin extraction and bioethanol production from cocoa pod husk waste, *Energy Environ.* 0 (2024) 1–18, <https://doi.org/10.1177/0958305X241270269>.
- [71] D. Jose, S. Vasudevan, P. Venkatchalam, S.K. Maity, A.A. Septevani, M. Gupta, P. Tantayotai, H. El Bari, M. Sriaryanun, Effective deep eutectic solvent pretreatment in one-pot lignocellulose biorefinery for ethanol production, *Ind. Crop. Prod.* 222 (2024) 119626, <https://doi.org/10.1016/j.indcrop.2024.119626>.
- [72] E. Domínguez, A. Romani, L. Domingues, G. Garrote, Evaluation of strategies for second generation bioethanol production from fast growing biomass *Paulownia* within a biorefinery scheme, *Appl. Energy* 187 (2017) 777–789, <https://doi.org/10.1016/j.apenergy.2016.11.114>.
- [73] K. Filippi, E. Stylianou, C. Pateraki, A. Koutinas, D. Ladakis, Pretreatment of grape pomaces and stalks using deep eutectic solvents for succinic acid production integrated in a biorefinery concept, *Waste Biomass Valoriz.* 14 (2023) 2857–2872, <https://doi.org/10.1007/s12649-023-02047-1>.
- [74] S. Niglio, A. Procentese, M.E. Russo, G. Sanna, A. Marzocchella, Investigation of enzymatic hydrolysis of coffee silverskin aimed at the production of butanol and succinic acid by fermentative processes, *Bioenergy Res.* 12 (2019) 312–324, <https://doi.org/10.1007/s12155-019-09969-6>.
- [75] Y. Xiao, Y. Liu, X. Wang, M. Li, H. Lei, H. Xu, Cellulose nanocrystals prepared from wheat bran: characterization and cytotoxicity assessment, *Int. J. Biol. Macromol.* 140 (2019) 225–233, <https://doi.org/10.1016/j.ijbiomac.2019.08.160>.
- [76] M. Mujtaba, A.M. Salaberria, M.A. Andres, M. Kaya, A. Gunyakti, J. Labidi, Utilization of flax (*Linum usitatissimum*) cellulose nanocrystals as reinforcing material for chitosan films, *Int. J. Biol. Macromol.* 104 (2017) 944–952, <https://doi.org/10.1016/j.ijbiomac.2017.06.127>.
- [77] A. Morales, J. Labidi, P. Gullón, Hydrothermal treatments of walnut shells: a potential pretreatment for subsequent product obtaining, *Sci. Total Environ.* 764 (2021) 142800, <https://doi.org/10.1016/j.scitotenv.2020.142800>.
- [78] B. Soares, A.M. da Costa Lopes, A.J.D. Silvestre, P.C. Rodrigues Pinto, C.S.R. Freire, J.A.P. Coutinho, Wood delignification with aqueous solutions of deep eutectic solvents, *Ind. Crops Prod.* 160 (2021) 113128, <https://doi.org/10.1016/j.indcrop.2020.113128>.
- [79] S. Rivas, L. López, C. Vila, J.C. Parajó, Organosolv processing of vine shoots: fractionation and conversion of hemicellulosic sugars into platform chemicals by microwave irradiation, *Bioresour. Technol.* 342 (2021) 125967, <https://doi.org/10.1016/j.biortech.2021.125967>.
- [80] J. Rencoret, A. Gutiérrez, E. Castro, J.C. Del Río, Structural characteristics of lignin in pruning residues of olive tree (*Olea europaea* L.), *Holzforchung* 73 (2019) 25–34, <https://doi.org/10.1515/hf-2018-0077>.
- [81] R. Martín-Sampedro, J.I. Santos, Ú. Fillat, B. Wicklein, M.E. Eugenio, D. Ibarra, Characterization of lignins from *Populus alba* L. generated as by-products in different transformation processes: Kraft pulping, organosolv and acid hydrolysis, *Int. J. Biol. Macromol.* 126 (2019) 18–29, <https://doi.org/10.1016/j.ijbiomac.2018.12.158>.
- [82] E. Domínguez, P.G. del Río, A. Romani, G. Garrote, P. Gullón, A. de Vega, Formosolv pretreatment to fractionate *Paulownia* wood following a biorefinery approach: isolation and characterization of the lignin fraction, *Agronomy* 10 (2020) 1205, <https://doi.org/10.3390/agronomy10081205>.
- [83] R.J. Sammons, D.P. Harper, N. Labbé, J.J. Bozell, T. Elder, T.G. Rials, Characterization of organosolv lignins using thermal and FT-IR spectroscopic analysis, *Bioresources* 8 (2013) 2752–2767, <https://doi.org/10.15376/biores.8.2.2752-2767>.
- [84] A. Morales, J. Labidi, P. Gullón, Impact of the lignin type and source on the characteristics of physical lignin hydrogels, *Sustain. Mater. Technol.* 31 (2022) e00369, <https://doi.org/10.1016/j.susmat.2021.e00369>.
- [85] E. Pérez, N. Abad-Fernández, T. Lourençon, M. Balakshin, H. Sixta, M.J. Cocero, Base-catalysed depolymerization of lignins in supercritical water: influence of lignin nature and valorisation of pulping and biorefinery by-products, *Biomass Bioenergy* 163 (2022) 106536, <https://doi.org/10.1016/j.biombioe.2022.106536>.
- [86] A. Morales, J. Labidi, P. Gullón, Influence of lignin modifications on physically crosslinked lignin hydrogels for drug delivery applications, *Sustain. Mater. Technol.* 33 (2022) e00474, <https://doi.org/10.1016/j.susmat.2022.E00474>.
- [87] I. Dávila, B. Gullón, J. Labidi, P. Gullón, Multiproduct biorefinery from vine shoots: bio-ethanol and lignin production, *Renew. Energy* 142 (2019) 612–623, <https://doi.org/10.1016/j.renene.2019.04.131>.
- [88] A. Del Castillo-Llamas, B. Rodríguez-Martínez, P.G. del Río, G. Eibes, G. Garrote, B. Gullón, Hydrothermal treatment of avocado peel waste for the simultaneous recovery of oligosaccharides and antioxidant phenolics, *Bioresour. Technol.* 342 (2021) 125981, <https://doi.org/10.1016/j.biortech.2021.125981>.
- [89] D.S. Monje, K.M. Chacon, I.C. Galindo, C. Castaño, L.M. Ballesteros-Rueda, G. Valencia, M.C. Gonzalez, D.F. Mercado, Carbon dots from agroindustrial residues: a critical comparison of the effect of physicochemical properties on their performance as photocatalyst and emulsion stabilizer, *Mater. Today Chem.* 20 (2021) 100445, <https://doi.org/10.1016/j.mtchem.2021.100445>.
- [90] A. Morales, J. Labidi, P. Gullón, Integral valorisation of walnut shells based on a three-step sequential delignification, *J. Environ. Manage.* 310 (2022) 114730, <https://doi.org/10.1016/j.envman.2022.114730>.
- [91] I. Dávila, P. Gullón, M.A. Andrés, J. Labidi, Coproduction of lignin and glucose from vine shoots by eco-friendly strategies: toward the development of an integrated biorefinery, *Bioresour. Technol.* 244 (2017) 328–337, <https://doi.org/10.1016/j.biortech.2017.07.104>.
- [92] M. Wu, L. Gong, C. Ma, Y.C. He, Enhanced enzymatic saccharification of sorghum straw by effective delignification via combined pretreatment with alkali extraction and deep eutectic solvent soaking, *Bioresour. Technol.* 340 (2021) 125695, <https://doi.org/10.1016/j.biortech.2021.125695>.
- [93] F. Xu, J. Sun, M. Wehrs, K.H. Kim, S.S. Rau, A.M. Chan, B.A. Simmons, A. Mukhopadhyay, S. Singh, Biocompatible choline-based deep eutectic solvents enable one-pot production of cellulosic ethanol, *ACS Sustain. Chem. Eng.* 6 (2018) 8914–8919, <https://doi.org/10.1021/acssuschemeng.8B01271>.