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Assessment of deep eutectic solvents (DES) to fractionate Paulownia wood within a biorefinery scheme: Cellulosic bioethanol production and lignin isolation

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ABSTRACT

Five deep eutectic solvents (DES) were evaluated to disrupt Paulownia wood structure to produce bioethanol and lignin. The DES formulated with choline chloride:lactic acid provided the most promising result. Temperature (110–130 °C), residence time (30–120 min), molar ratio (1:2–1:9), and liquid-to-solid ratio (8–15 mL/g) were optimized for cellulose recuperation (93% retained in the solid phase) and lignin removal (94% delignification yield). The spent solid was used for bioethanol production, achieving up to 43.6 g ethanol/L (89.7% ethanol yield). Lignin (84% of purity) was isolated from the black liquor and thoroughly characterized by FTIR, ^{1}H NMR, TGA-DTG and SEM, while the liquor after lignin precipitation was chemically characterized for monomers/oligomers, total phenolic content, antioxidant activity and phytochemical profile (highlighting the presence of 25.06, 10.21 and 2.51 mg of syringaldehyde, vanillin and 3,4-dihydroxibenzoic acid per g of initial biomass). Overall, this study show that DES pre-treatment is a promising strategy for simultaneous lignin extraction and cellulose digestibility enhancement.

1. Introduction

In the last century, the increasing world population coupled with the intensive exploitation of natural resources has raised global environmental concerns regarding the release of pollutants and greenhouse gases (GHG). These emissions threaten the environment, contributing to global warming, ecological imbalances, water pollution and ocean acidification, just to name a few, they endanger the wellness of the planet, biodiversity, human health and the development of future generations (Lopes, 2021; Pérez-Pérez et al., 2023a). To fight this situation, in recent years, the production of biofuels from green and renewable resources that would replace conventional ones has been promoted by the use of biorefineries (del Río et al., 2022b; Lopes, 2021).

One of the feedstock for biorefineries is lignocellulosic biomass (LB), that comprises of a group of non-edible plant materials (Morais et al., 2020; Wang and Lee, 2021) mostly composed of polymers such as cellulose (30–50%), hemicelluloses (20–30%), and lignin (15–30%) Among their features, some of the most advantageous are their abundance, renewability, relatively low price, biodegradability and non-competitiveness with feedstock as a source of production (Malaeke et al., 2018; Soto-Salcido et al., 2020; Zhou et al., 2022). In consequence, LB has subsequentially emerged as a good source of chemicals and energy (Muddasar et al., 2023; Soto-Salcido et al., 2020; Xiang et al., 2022) that can reduce the dependence of society on the oil industry (Malaeke et al., 2018; Soto-Salcido et al., 2020). Particularly, Paulownia is a deciduous fast-growing short-rotation tolerant to abiotic stress tree. These characteristics allow for the obtainment of value-added products due to the production of a large amount of biomass (up to 50 t/(ha·year)), which explains the interest in it as an industrial feedstock to produce bioethanol (del Río et al., 2022b).

Due to the recalcitrant nature of lignocellulose, one or several pretreatments would need to be carried out to valorize the three main components and increase cellulose accessibility (Nagoor Gunny et al., 2019). The removal of lignin alters the structure of the lignocellulose,

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increasing the accessibility of enzymes that improve the effectiveness of enzymatic hydrolysis (Gundupalli et al., 2022; Guo et al., 2019). One alternative to the traditional methods is the use of deep eutectic solvents (DES) (Nagoor Gunny et al., 2019).

DES are low-cost, easily synthesized, safe, simple to purify and environmentally friendly solvents that can be prepared with the combination of at least one hydrogen bond acceptor (HBA) and one hydrogen bond donor (HBD). When combined at appropriate conditions, this combination forms a eutectic mixture (Almeida et al., 2022; Gundupalli et al., 2022; Guo et al., 2019; Nagoor Gunny et al., 2019) that has a lower melting point than the individual compounds since a delocalization of charge occurs through the hydrogen bonds between HBA and HBD (Almeida et al., 2022; Gundupalli et al., 2022). DES have been efficiently used for the extraction of hemicelluloses and lignin from LB owing to their higher selectivity for this polyphenol and various studies suggest that DES have the unique ability to dissolve lignin from lignocellulosic materials thanks to the interaction of HBD and HBA (Alvarez-Vasco et al., 2016; Fernandes et al., 2021; Guo et al., 2019).

Lignin is a three-dimensional macromolecule that is a precursor to aromatic alcohols. Furthermore, it is a natural and renewable raw material with interesting physical-chemical properties such as its defined plasticity or its precipitation in aqueous medium and solubility in organic solvents. Among the many applications of lignin is may be used as an emulsifier, dyes, binders, fuels, etc (Watkins et al., 2015; Zhu et al., 2023). The reason lignin must be removed from the LB is that the saccharification of the LB is crucial for ethanol production by fermentation (Nawaz et al., 2022). These two steps are usually considered as a whole and three widely used strategies for obtaining bioethanol can be distinguished: separate hydrolysis and fermentation-SHF, simultaneous saccharification and fermentation-SSF and pre-saccharification and simultaneous saccharification and fermentation-PSSF (Mithra et al., 2018; Nawaz et al., 2022). On the other hand, the other two methods combine the enzymatic hydrolysis of cellulose together with the concurrent fermentation of the monosaccharides (SSF will be called PSSF if a prior short pre-saccharification step is contemplated). In general, PSSF usually overcomes almost completely the SHF disadvantages since the presence of ethanol decreases the chances of contamination, and the continuous consumption of sugars avoids their accumulation and, therefore, the inhibition of the enzyme (Mithra et al., 2018). However, it has the major disadvantage that, because the saccharification and the fermentation occur simultaneously, both are performed at suboptimal conditions (Mithra et al., 2018).

The objective of this study is the evaluation of DES to fractionate PW in a multi-product biorefinery scheme to mainly obtain lignin and bioethanol. Different DES formulations and pretreatment conditions were assessed to reach high delignification ratios and increase the enzymatic susceptibility in the solid phase (enriched in cellulose). Finally, the conversion of PW to ethanol by PSSF was evaluated, and the lignin fraction and the liquor obtained after lignin precipitation were thoroughly characterized.

2. Materials and methods

2.1. Raw material

Paulownia elongata x fortunei wood was obtained from a local distributor (Maderas Álvarez Oroza S.L., NW Spain). The *Paulownia* wood (PW) was stripped, air dried and ground to a particle size below 8 mm. The recovered homogeneous PW was stored in plastic bags at room temperature in a dark dry environment.

PW was chemically characterized employing the NREL analytical methodology to determine the moisture percentage (Sluiter et al., 2008a), ethanol extractives (Sluiter et al., 2008c) and ash (Sluiter et al., 2008b). The extractives-free PW (milled to a particle size below 1 mm) was summited to quantitative acid hydrolysis for hemicelluloses, glucan, and lignin quantification. The resulting solid fraction was weighed and determined as Klason lignin and the hydrolyzed fraction was (i) injected in HPLC (Agilent 1200 series equipped with a Rezex ROA-Organic acid H+ column (Phenomenex) at 60 °C, refractive index detector at 40 °C and mobile phase consisting of 3 mM H_2SO_4 at 0.6 mL/min) for monosaccharides determination and (ii) used for the quantification of uronic acids by the methodology proposed by (Blumenkrantz and Asboe-Hansen, 1973). While protein content was quantified by the Kjeldahl method, applying a rectification factor for lignocellulosic materials of 6.25 on the obtained nitrogen (Sluiter et al., 2010). All procedures were carried out in triplicates.

2.2. Reagents and chemicals

Chemicals and reagents, which are pure if not specified, were: acetic acid (96%), formic acid, lactic acid (90%), glycerol, ethanol, ethylene glycol and sulfuric acid (72 and 98%) provided by Carlo Erba; arabinose, glucose and xylose belonged to Panreac; furfural, hydroxymethylfurfural and levulinic acid purchased to Acros organics; choline chloride supplied by Alfa Aesar, and citric acid, sodium hydroxide and thymol were provided by Scharlau, VWR and ThermoScientific, respectively. Finally, peptone and yeast extract were supplied by Labkem and Cultimed, respectively. The enzyme used was Cellic-CTec2 from Novozymes and the microorganism was the *Saccharomyces cerevisiae* EthanolRed®, a commercial strain obtained from Fermentis, S.I. Lesaffre to use in the ethanol industry.

2.3. Deep Eutectic Solvents (DES) synthesis

The DES employed in this work were prepared based on the method described by (García et al., 2016) with slight differences. The synthesis consists of merging a hydrogen bond acceptor (HBA), in this case, choline chloride for all mixtures, with a hydrogen bond donor (HBD) such as carboxylic acids, alcohols, etc. in a capped recipient. The mixtures were magnetic stirred and heated up to a temperature close to 80 °C for 120 min to achieve a complete homogenization of the solutions. In Table 1 the different DES formulations used in this work are shown along with the sources from which they were selected.

2.4. Delignification procedure and DES selection

A delignification pretreatment was applied to the PW to remove the lignin fraction and, consequently, improve the saccharification

Table 1
List of the DES synthesized in this study.

HBA	HBD	MR	Price (€/kg) Ind/Lab	Reference
Choline chloride	Formic acid	(1:2)	2.01/ 44.33	(Chen and Mu, 2019)
Choline chloride	Acetic acid	(1:2)	1.85/ 41.72	(Chen and Mu, 2019)
Choline chloride	Lactic acid	(1:2)	1.80/ 35.00	(Loow et al., 2017)
		(1:3)	1.59/ 29.85	(El Kantar et al., 2019)
		(1:5)	1.37/ 24.26	(Kumar et al., 2016)
		(1:7)	1.25/ 21.29	(Jablonský and Jančíková, 2023)
		(1:9)	1.18/ 19.45	(Van Osch et al., 2017)
Choline chloride	Glycerol	(1:2)	1.87/ 50.07	(Van Osch et al., 2017)
Choline chloride	Ethylene glycol	(1:2)	1.84/ 58.74	(Zhang et al., 2016)

HBA- hydrogen bond acceptor; HBD- hydrogen bond donor; MR- molar ratio; Ind/Lab- Industry (Alibaba)/ Laboratory (Merck).

efficiency of the remaining cellulose.

As a previous experiment, the solid was mixed in an LSR of 15 mL/g and pretreated for 60 min at 120 °C with five different DES (Table 1). The remaining solid and liquid hydrolysate were separated by vacuum filtration. Finally, the solid fraction was collected and washed using warm ethanol 50% (v/v), to avoid the precipitation of the lignin on the solid, warm sodium hydroxide (0.1–2% w/w) (both at a ratio of 10 mL per g of pretreated PW) and distillate water, until neutral pH, to eliminate DES traces. After the washing, the resulting pretreated biomass was stored at 4 °C. An aliquot of the solid was dried and characterized as explained in Section 2.1. by quantitative acid hydrolysis (QAH). The liquid hydrolysate was characterized by direct injection in HPLC (monomers quantification) and acid posthydrolysis (H_2SO_4 4%, 121 °C for 20 min) before injection in HPLC (oligomers quantification) as explained in Section 2.1.

2.5. *Optimization of delignification parameters*

The efficiency of the delignification pretreatment was optimized using a one-factor-at-a-time (OFAT) design. This procedure consists of altering one parameter while the rest of them are kept as chosen in a previous step with the purpose of evaluating the individual impact of all of them into the process. Employing this method, and considering the operational costs, the optimal temperature (110–130 °C), reaction time (30–120 min), DES molar ratio (1:2–1:9) and LSR (8–15 mL/g) were determined to obtain an efficient lignin removal.

2.6. *Enzymatic susceptibility of the pretreated solid phase*

After the evaluation of the pretreatment parameters, PW processed under selected conditions was submitted to enzymatic saccharification. The selected conditions were a temperature of 50 °C, agitation of 160 rpm and cellulase to substrate ratio (CSR) of 20 FPU/g of solid (dry basis). The enzymatic cocktail used was Cellic CTec2, kindly supplied by Novozymes (Denmark) and the cellulase activity was measured according to the Filter Paper assay (Adney and Baker, 2008) and expressed in terms of Filter Paper Units (FPU), achieving a final activity of 116 FPU/mL.

The final glucose production was calculated as glucose concentration (g/L) and as glucan to glucose conversion (GGC) (%) using the Eq. (1):

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

Where $Glucose_t$ and $Glucose_{t_0}$ correspond to the glucose concentration in g/L reached at time t and initial time, respectively, Gn describes the glucan content of pretreated PW (g glucan/100 g pretreat solid), 180/162 represents the stoichiometric factor for glucan hydration upon hydrolysis, ρ is the density of the medium (fixed in 1005 g/L), LSR describes the liquid-solid ratio and KL is related to the Klason lignin content of pretreated PW (g Klason lignin/100 g pretreated solid, oven dry basis).

2.7. *Microorganism and inoculum preparation*

For fermentation, the *Saccharomyces cerevisiae* Ethanol Red® yeast strain was employed. This yeast was grown at 30 °C and 200 rpm for 24 h in a sterilized medium with a concentration of peptone, glucose, and yeast extract of 20, 20 and 10 g/L, respectively. After this time, cells were centrifuged at 4000 rpm (4200xg) for 10 min (Hettich Rotixa 50 S) to recover the biomass, which was then re-suspended in a 0.9% NaCl solution to obtain a final concentration of 8 g of fresh yeast/L (approximately 1.5 g of dry yeast per L).

2.8. *Pre-saccharification and simultaneous saccharification and fermentation (PSSF)*

In PSSF assays, first, PW was sterilized at 121 °C for 15 min and was subjected to pre-saccharification, as described in Section 2.5. The pre-saccharification occurred for 72 h following previous conditions from Section 2.5., and when finished, the fermentation step was performed with *S. cerevisiae* at 35 °C, 120 rpm. A nutrient solution (final concentration of 20 g/L of peptone and 10 g/L of yeast extract) was also added to the medium. Conditions were set as follows: ESR (enzyme to substrate ratio) of 20 FPU/g, LSR varied between 6 and 10 g/g. The PSSF was followed by sampling the experiments throughout the course of the process. Samples were centrifuged at 15,000 rpm (22,000×g) (Hettich Universal 320) and filtered through 0.2 μm membranes. This supernatant was analyzed using high-performance liquid chromatography (HPLC) for monosaccharides, acetic acid, and ethanol quantification. All the experiences were done in duplicate.

Finally, the produced ethanol was expressed in terms of concentration (g/L) and yield (%) employing the following equation (Eq. (2)):

$$Ethanol\ yield(\%) = \frac{\left(EtOH_t - EtOH_{t_0}\right)}{0.51\cdot\frac{Gn}{100}\cdot\frac{180}{162}\cdot\frac{\rho}{LSR+1-\frac{KL}{100}}} \tag{2}$$

where $EtOH_t$ and $EtOH_{t_0}$ corresponds to the ethanol concentration in g/L achieved at time t and time zero, respectively, 0.51 is related to the stoichiometric glucose to ethanol conversion factor and the rest of parameters were explained in Eq. (1).

2.9. *Isolation and characterization of lignin*

After the pretreatment conditions to delignify PW were selected, the obtained black liquor was precipitated using water acidified with sulfuric acid (pH=2) at a ratio of acidified water/black liquor of 3:1 v/v and kept at 4 °C overnight after magnetic stirring for 15 min. The precipitated lignin was filtered, carefully rinsed with distilled water, and let dry in an oven at 50 °C. The purity of the lignin was obtained by quantitative acid hydrolysis following the method explained in Section 2.1.

The lignin was subjected to Fourier-Transform Infrared (FTIR) Spectroscopy, conducted in a PerkinElmer (Waltham, MA, USA, USA) Spectrum 100 spectrometer equipped with an attenuated total reflectance (ATR) accessory conducting 8 scans per sample and using a range of 4000–650 cm^{-1}.

Proton Nuclear Magnetic Resonance (^1H NMR) of the extracted lignin was performed at 25 °C on a Bruker Neo 400 spectrometer (Bruker, Billerica, MA, USA) functioning at 400 MHz. Lignin was dissolved in dimethyl sulfoxide (DMSO).

A Perkin Elmer TGA 4000 was used to assess the thermal stability of the lignin. Lignin was heated from room temperature to 800 °C at a constant increase of 10 °C/min in a nitrogen atmosphere (20 mL/min).

HSQC 2D NMR of lignin was performed in a Bruker Advance prepared with a z-gradient 5 mm QNP probe, at 25 °C functioning at 400 MHz. Lignin was dissolved in dimethyl sulfoxide (DMSO).

Scanning Electron Microscopy (SEM) of the lignin was carried out employing Hitachi SU-70 SEM at a voltage of 5.0 kV. The sample was sputter coated with gold before SEM.

2.10. *Total phenolic content and antioxidant capacity before and after lignin precipitation*

Black liquor after DES delignification at selected conditions was submitted to total phenolic content (TPC) and antioxidant capacity assays (DPPH, ABTS and FRAP) prior and after lignin precipitation.

For TPC, the method described by Singleton et al. (1999) was followed with minor modifications, using gallic acid as standard (measured as mg of gallic acid equivalent (GAE)/g of PW). Briefly, 2.5 mL of 10 times diluted Folin–Ciocalteu reagent were combined with 0.3 mL of extracts and 2 mL of 7.5% w/v Na_2CO_3 solution. Samples were shaking and incubated for one hour at room temperature and their absorbance was measured at 760 nm.

The antioxidant capacity tests performed were DPPH, ABTS and FRAP. DPPH or α,α-Diphenyl-β-picrylhydrazyl radical scavenging assay methodology was reported by von Gadow et al. (1997). Shortly, 2 mL of methanolic dilution of DPPH was mixed with 0.2 mL of extract and, after 16 minutes, the decrease in absorbance at 515 nm was read. On the other hand, 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical cation decolorization assay was realized, first, diluting an $ABTS^{\bullet+}$ solution with phosphate buffer saline to an absorbance of 0.700 at 734 nm at 25°C. Then, 20 μL of diluted extracts was added to 2 mL of diluted $ABTS^{\bullet+}$ solution and after 6 min, the absorbance was recorded (Re et al., 1999). Finally, ferric reducing antioxidant power (FRAP) method was described by Benzie and Strain (1999) and FRAP reagent was prepared mixing acetate buffer (300 mM, pH 3.6), TPTZ solution (10 mM) and $FeCl_3 \cdot 6\,H_2O$ solution (20 mM) in a volumetric ratio of 10:1:1. Subsequently, 0.1 mL of diluted extracts will be added to FRAP reagent and the absorbance was measured after 6 min of incubation at 593 nm.

2.11. Phytochemical profile of phenolic compounds in DES after lignin precipitation

The phenolic compounds were separated from PW DES liquor by liquid-liquid extraction (1:1 ratio) with ethyl acetate. The mixture was magnetically stirred for 15 min at room temperature and let separate in a decanting ampoule. The ethyl acetate was retrieved, while the DES was afterwards extracted again with ethyl acetate at the same conditions twice more. The ethyl acetate recovered after the three extractions was rotatory evaporated at 40 °C and the obtained substance was resuspended in methanol.

A volume of 5 μL of sample was injected HPLC (Agilent 1260 series, Palo Alto, CA, USA) with AB SCIEX Triple Quad 3500 detector (AB Sciex, Foster City, CA, USA) and equipped with an electrospray source of ionization (ESI). Two mobile phases (0.1% formic acid and acetonitrile with 0.1% formic acid), at a flow rate of 300 μL/min, were employed with a Luna C18 column (Phenomenex). A positive/negative source of ionization with turbo V™, with nitrogen as nebulizer and collision gas were used. Multiple reaction monitoring (MRM) was employed to obtain the data.

3. Results and discussion

3.1. Raw material characterization

Paulownia elongata x fortunei wood (PW) used in this study was thoroughly characterized, and its composition is as follows (g/100 g PW): glucan, 42.27 ± 0.14; Klason lignin 20.70 ± 0.57; hemicelluloses 21.85 ± 0.39 (containing xylan 17.32 ± 0.02, arabinan 0.82 ± 0.00, acetyl groups 3.71 ± 0.36); extractives 5.12 ± 0.00, proteins 1.25 ± 0.01, ashes 0.40 ± 0.00; uronic acids (galacturonic acid equivalent) 6.56 ± 0.10; others (by difference) 3.10.

The obtained polymers composition is similar to other hardwoods such as *Eucalyptus regnans* (Penín et al., 2022), or *Acacia dealbata* (Portela-Grandío et al., 2021). Additionally, similar values of cellulose and lignin (40.17 and 23.48 g/100 g) were found by Esteves et al. (2021) when characterizing *Paulownia tomentosa* wood, although presenting a higher hemicelluloses content (36.34 g/100 g). Other components (extractives, ashes, and uronic acids) were found in lesser amount, which is in accordance with the composition of other similar feedstock such as vine shoots (Dávila et al., 2016) or *Eucalyptus globulus* wood (López et al., 2020).

3.2. Deep eutectic solvents (DES) screening

In order to process PW, five different DES were studied (see Table 1). For all of them, choline chloride was used as hydrogen bond acceptor (HBA) in combination with diverse hydrogen bond donors (HBD) such as formic acid, acetic acid, lactic acid, glycerol and ethylene glycol at a molar ratio of 1:2 (HBA:HBD). Temperature and time were fixed at 120 °C and 60 min.

Fig. 1 displays the recovery (in the pretreated solid) or elimination (from the pretreated solid) of the main components of PW (glucan, hemicelluloses, and lignin) and the solid yield achieved. Firstly, the solid yield can aid to recognize the effectiveness of the treatment. PW processed with DES containing glycerol and ethylene glycol (mixed with choline chloride) showed a poor performance, reaching solid yield values higher than 93%. Solid yield after processing with acetic acid-DES resulted in a similar value of 87%. On the other hand, DES comprising formic acid and lactic acid were more effective, reaching a solid yield of 59 and 64%, respectively. Regarding the glucan recovery, all treatments enabled a higher recovery than 86%, with slight differences among the DES employed. The recovery of hemicelluloses presented a similar trend to that obtained for solid yield, reaching higher hemicelluloses solubilization for the DES containing formic acid and lactic acid (up to 71% of hemicelluloses solubilization). Finally, DES composed with acetic acid, glycerol or ethylene glycol showed little effect on the solubilization of lignin (between 0% and 13%), whereas those composed with formic acid or lactic acid reached values between 54% and 60%.

Regarding the abovementioned, DES containing formic acid or lactic acid seemed the most effective considering the higher solubilization of hemicelluloses and lignin, while maintaining a low glucan loss. Among the effect of both DES, there are no statistical differences regarding the solubilization of lignin (at a 95% significance) while the recovery of glucan and hemicelluloses showed significant differences ($p<0.05$). Additionally, if the price to produce both DES is considered, the value for choline chloride:formic acid (1:2) was 2.01 €/kg with reagents from AliBaba (http://www.alibaba.com) and 44.33 €/kg with reagents from Merck (http://www.sigmaaldrich.com), while for choline chloride:lactic acid (1:2) the price would be 1.80 €/kg and 35.00 €/kg, respectively. In this sense, the lactic acid DES was about 10–21% cheaper than that formed by formic acid. Hence, the DES formed by lactic acid seemed a more interesting mixture to continue this study.

Pérez-Pérez et al. (2023a) compared the effect of three different choline chloride-DES combined with lactic acid (1:3), glycerol (1:1) and oxalic acid (1:1) on *Robinia pseudoacacia* wood, reaching similar

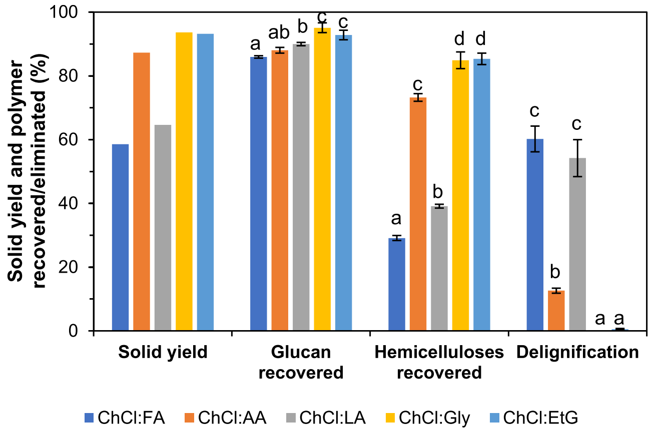

Fig. 1. Solid yield, glucan and hemicelluloses recovery and delignification obtained after treatment with different DES at 120 °C for 60 min. AA- acetic acid; ChCl- choline chloride; EtG- ethylene glycol; FA- formic acid; Gly- glycerol; LA- lactic acid. Different letters (a, b, c, d) mean statistical differences at $p<0.05$.

conclusions to that obtained in this study: the choline chloride:lactic acid DES enabled a higher delignification (78%) with lower glucan loss (10%) than the other DES at a temperature of 120 °C for 60 min. On the other hand, Poy et al. (2023) pretreated rice straw with choline chloride: lactic acid (1:5) at 121 °C for 1 h, reaching slightly higher glucan and xylan recoveries (99 and 59%, respectively) than in this work, but lower delignification ratios of about 30% comparatively to delignification of 54% reached in this study at same temperature and residence time. Similar results were obtained when processing hydrothermally extracted (180 °C for 4 h) moso bamboo powder with chloride:lactic acid (1:1) at 110 °C for 4 h, reaching a delignification of around 70% (Zhang et al., 2023). Additionally, Zhu et al. (2023) also selected ChCl:LA (1:4) as a better choice than other DES to reach higher lignin solubilization on grapevine.

3.3. *One-factor-at-a-time method*

After the selection of the most interesting DES to reach higher delignification with little glucan loss (in this case, choline chloride:lactic acid), a one-factor-at-a-time (OFAT) method was employed to study other variables of the process, such as temperature (110–130 °C), time (30–120 min), molar ratio (1:2–1:9), and liquid to solid ratio-LSR (8–15 mL/g). Table 2 shows the main results after the processing of PW at different conditions within the OFAT method.

Experiments 1–3 (see Table 2) display the three different temperatures tested in this study (110, 120 and 130 °C), setting the other variables as follows: time of 60 min, molar ratio of 1:2 (ChCl:LA), and LSR of 15 mL/g. First, the solid yield mirrored the effectiveness of the process. In this case, it varied from 86.78% at the mildest condition to 53.58% at the harshest condition, comprising a higher solubilization of polymers at the highest temperature. Regarding the cellulose, it ranged 45.03–72.04 g glucan/100 g of pretreated PW, corresponding to a recovery between 90% and 93% (in relation to initial glucan). Hemicelluloses were more intensely affected by this process, reaching a xylan solubilization of up to 68% (concerning xylan in the initial biomass) at temperature of 130 °C. Finally, the lignin was the most affected polymer, and the delignification ratio varied in a wide range of 24% at 110 °C to 81% at 130 °C. In this context, it was proved that higher temperatures enabled a more interesting behavior for DES to achieve higher delignification ratios without a great loss of glucan.

Other works revealed similar results. For example, (Okur and Eslek Koyuncu, 2020) explored the lignin removal of paddy husks using citric acid:ethylene glycol DES, obtaining a delignification close to 50% at 120 °C for 1 h, while the same solubilization was obtained at 90 °C but increasing the residence time to 24 h. Another study by Kohli et al. (2020) exhibited that the solubilization of lignin from birch wood and miscanthus increased while the temperature augmented from 60 up to 130 °C. However, a higher temperature (150 °C) resulted in lower lignin extraction, which may be owing to the onset of thermal degradation of lower molecular weight lignin (Chen et al., 2019).

When comparing experiments 3–6 (Table 2), temperature was now fixed at 130 °C while time varied between 30 and 120 min. The solid yield decreased when the residence time augmented, reaching close to 50% of solubilization. Glucan was majorly recollected in the solid phase recovering 92% concerning glucan in the raw material, as average. The hemicelluloses, especially xylan, fluctuated in a fine range of 8.38–10.72 g xylan/100 g pretreated PW, corresponding to a recovery of 25–41% regarding initial xylan. The solubilization of lignin was around 49% at 30 min; however, little differences (81–87% of delignification) were found at higher times (60–120 min). In this sense, only 3% higher delignification was found between 60 and 90 min, and between 90 and 120 min. Although there were statistical differences on the delignification at 60 and 120 min (at a 95% significance), there is no point in doubling the residence time to increase the lignin solubilization by 6%, hence, 60 min was set as fixed residence time for the following experiments.

Similar conclusions were stated by Kohli et al. (2020) when pretreating birch wood with choline chloride:oxalic acid DES and microwave heating, reaching higher delignification ratios at conditions of 130 °C for 60 min. Alternatively, *Populus europaeus* was treated at 120 °C using ChCl:LA (1:10) showing significant differences when augmenting the reaction time. In this case, the delignification was doubled (from around 45% to about 85%) when increasing the reaction time from 3 to 12 h. However, from an economic perspective, the intensification of lignin solubilization does not justify a 4-fold higher residence time, with a consequent increase in consumption of energy (Li et al., 2019).

The variation of molar ratio was studied in experiments 3, and 7–10 (Table 2), maintaining the temperature at 130 °C and the time at 60 min. Analogous to previous steps, the solid yield varied in a narrow range (50.04–53.58%), the glucan loss was low (<9% regarding initial glucan), and the xylan was highly solubilized, retaining about 22–32% in the solid phase, concerning xylan in the raw material (corresponding to 7.09–10.33 g of xylan/100 g pretreated PW). Regarding the delignification, the increase on lactic acid implied a higher lignin solubilization, reaching values between 96% and 98% (retaining in the solid phase as little lignin as 0.85 g lignin/100 g pretreated PW) when using higher molar ratios than 1:5 (ChCl:LA). The highest delignification ratio occurred at molar ratio of 1:7 with a value of 98%; however, only 1% less delignification was achieved at molar ratio of 1:9. In fact, those two values were not statistically different ($p>0.05$). Be that as it may, the rise

Table 2
Solid yield and solid phase characterization after delignification with choline chloride:lactic acid DES varying the temperature, time, molar ratio and liquid-solid ratio following the one-factor-at-a-time method.

Experiment number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Temperature (°C)	110	120	130	130	130	130	130	130	130	130	130	130
Time (min)	60	60	60	30	90	120	60	60	60	60	60	60
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
Liquid to solid ratio (mL/g)	15	15	15	15	15	15	15	15	15	15	8	11.5
Solid yield (g/100 g raw material, o. d. b.)	86.78	64.64	53.58	66.40	53.23	51.78	50.82	52.83	50.04	52.54	52.44	51.45
Solid phase composition (g/100 g pretreated PW, o. d. b.)												
Glucan	45.03 ± 0.73	58.74 ± 0.34	72.04 ± 0.89	59.50 ± 1.18	71.32 ± 0.49	74.61 ± 0.10	85.53 ± 1.06	83.60 ± 1.79	80.52 ± 1.47	73.30 ± 1.52	75.32 ± 0.37	77.92 ± 0.26
Xylan	13.24 ± 0.25	10.53 ± 0.07	10.33 ± 0.29	10.72 ± 0.31	8.79 ± 0.14	8.38 ± 0.15	9.44 ± 0.13	8.46 ± 0.36	8.17 ± 0.21	7.09 ± 0.19	8.32 ± 0.03	7.40 ± 0.02
Arabinan	0.62 ± 0.14	0.83 ± 0.05	0.64 ± 0.35	0.15 ± 0.03	0.28 ± 0.02	0.49 ± 0.34	0.44 ± 0.27	0.50 ± 0.30	0.40 ± 0.14	0.76 ± 0.07	0.00 ± 0.00	0.00 ± 0.00
Acetyl groups	3.11 ± 0.14	1.90 ± 0.10	1.84 ± 0.22	2.26 ± 0.11	1.63 ± 0.18	1.40 ± 0.11	1.35 ± 0.13	1.05 ± 0.12	0.99 ± 0.03	0.82 ± 0.07	0.88 ± 0.05	0.76 ± 0.04
Klason lignin	18.14 ± 0.23	14.66 ± 0.12	7.51 ± 0.72	13.93 ± 0.23	6.23 ± 0.98	5.14 ± 0.48	2.67 ± 0.21	1.48 ± 0.34	0.85 ± 0.16	1.20 ± 0.27	2.39 ± 0.35	1.51 ± 0.34

of lactic acid in the DES implied lowering its price by an average of 13% from 1:2–1:3, from 1:3–1:5, and so on. Moreover, increasing its content from 1:2–1:9 would reduce almost to the half the DES synthesis cost. The prices of reagents were extracted from Merck (http://www.sigmaaldrich.com). In this sense, the molar ratio selected for following experiments was 1:9 since allowed a high solubilization of lignin, with little loss of glucan, and reducing the DES synthesis cost.

Tan et al. (2019) observed a significant effect of the molar ratio of the ChCl:LA DES regarding the solubilization of lignin. In this case, the increase of lactic acid from 1:1 up to 1:15 resulted in an increase of delignification from 33.5% to 61%. Other authors varied the amount of lactic acid in the DES ChCl:LA from 1:2–1:10 when processing willow for 6 h at 120 °C, increasing the lignin solubilization ratio from 52% to 67% (Li et al., 2017).

Finally, three different LSR (from 8 to 15 mL/g) were evaluated in experiments 10–12 (Table 1). The reduction of the LSR may imply mass transfer issues due to the lower amount of liquid in the media, although presenting a paramount advantage of using less reagent to obtain similar results, reducing the cost as well (del Río et al., 2022a). Concerning these experiments, little differences on solid yield (51.45–52.54%), glucan recovery (91–95%), xylan recovery (22–25%) and delignification (94–97%) were found. For that reason, 8 mL/g was selected as desired LSR to maximize the glucan recovery and delignification of PW while employing almost half of the reagents regarding a LSR of 15 mL/g.

3.4. *Enzymatic hydrolysis*

A set of five trials was performed to choose and assess the influence of the percentage of NaOH, added during the solid washing process, on the enzymatic susceptibility of the PW. Fig. 2a displays the values of GGC (Eq. (1)) for the delignified PW experiments.

Although the PW used contains a high glucan content (75.32 g/100 g pretreated PW) and is, therefore, a potential feedstock for glucose production, the GGC results obtained in the enzymatic hydrolysis only reached a conversion value of 44% for the pretreated PW without NaOH washing. This limited yield might be provoked by the barrier effect of the residual lignin contained in the solid after delignification, or the remains of DES embedded in the raw material when it is not washed.

By the same token, Zhou et al. (2021) studied that a NaOH post-pretreatment (NaOH 1%, LSR=80 g/g during 2 h at ambient temperature) after DES-delignification increased the enzymatic digestibility of processed poplar sawdust and herbaceous miscanthus. A study by Yao et al. (2022) confirmed that the EtOH-washing of spent solid from DES-delignification increased the glucan digestibility of pine, corn stover and *Broussonetia papyrifera*. Shen et al. (2019) proceeded in a similar way washing the residual solid after DES-delignification with acetone-water (50%) three times. Hence, a washing or a post-treatment stage may be crucial to obtaining highly enzymatically digestible cellulose after DES-delignification.

For all these reasons, in the current study, NaOH washing of the delignified solid with concentrations between 0.1% and 2% was proposed. In Fig. 2a it can be seen that only with the minimum concentration of NaOH (0.1%) employed the percentage of GGC increased from 44% to 93% at 72 h, which leads to a 2.1-fold higher glucose concentration (from 17.69 to 37.47 g/L of glucose). On the other hand, the experiments with higher concentrations of NaOH had faster kinetics and were able to reach a glucose yield of 100% in the same amount of time.

These data were notably superior to those obtained in prior studies for the same biomass, such as the one carried out by Ye et al. (2016), where the enzymatic hydrolysis efficiencies had a peak of 88.5% at 120 h (alkali pretreatment with 10% NaOH). Conversely, Wang et al. (2022), studied different pretreatments on PW to achieve a higher glucose yield, observing positive results when the pretreatment of 5% acetic acid and 4% NaSO$_3$ is combined with 1% NaOH, thus increasing the yield at 72 h from 59% to 77%.

In the current research, the 0.1% NaOH treatment, despite not reaching the highest yield, reported acceptable results with a maximum digestibility of 93%. Therefore, this NaOH concentration was set as the most desirable considering economic and environmental criteria.

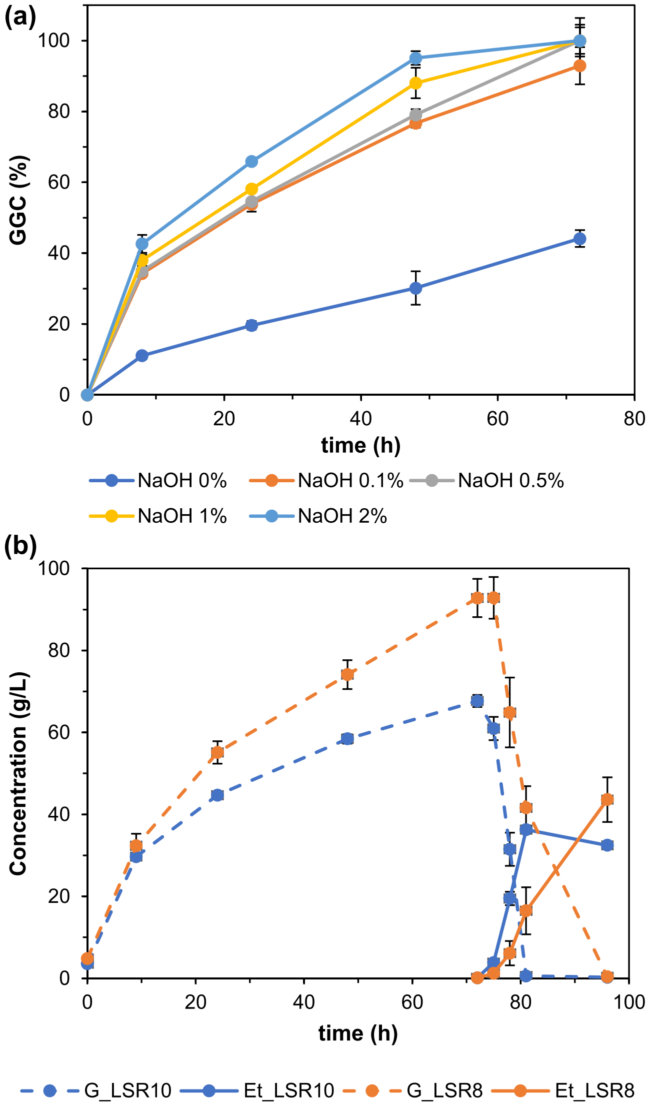

Fig. 2. (a) Enzymatic saccharification of PW pretreated with DES after NaOH washing at different concentrations (0–2%); and (b) ethanol-Et and glucose-G concentrations during PSSF at different LSR (10 and 8 g/g) of PW after DES delignification at selected conditions (ChCl:LA (1:9) at 130 °C for 60 min, LSR=8 mL/g).

3.5. *Pre-saccahrification and simultaneous saccharification and fermentation (PSSF) of DES-delignified Paulownia wood*

As a final stage, PSSF of pretreated PW was carried out at LSR of 8 and 10 g liquid/g of pretreated PW, selecting a presaccharification of 72 h with the addition of commercial nutrients (peptone and yeast extract at a final concentration of 20 and 10 g/L). Industrially, those commercial nutrients could be substituted by other nitrogen source such as cheese whey (Cunha et al., 2021), whey powder or whey protein concentrate (Cunha et al., 2018). The results are displayed in Fig. 2b. As a general trend, 72 h were enough to reach a GGC of 83–93%, corresponding to glucose concentrations of 64.10 and 87.98 g/L at LSR of 10 and 8 g/g, respectively. Once the inoculum was introduced in the media, the trial assessed at LSR of 10 g/g reached a maximal concentration of 36.29 g ethanol/L (being equivalent to 91% of ethanol yield) at 9 h of fermentation, whereas the experiment performed at LSR of 8 g/g delayed 24 h of fermentation to reach a maximum value of 43.61 g ethanol/L (ethanol yield of 89.7%).

Similar results were obtained by other authors like (Isci et al., 2020) who reached an ethanol yield of 72% after ChCl:glycerol pretreatment at 100 °C during 6 h. On the other hand, wheat straw treated with ChCl: formic acid allowed to achieve up to 81.5% of ethanol yield (Isci et al., 2020). Conversely, Pérez-Pérez et al. (2023a) pretreated Robinia wood with ChCl:LA, and the PSSF of that solid enabled the obtainment of 40.3–53.3 g ethanol/L (81–83% ethanol yield).

3.6. Lignin characterization

After the delignification process at selected conditions (ChCl:LA 1:9, 130 °C, 60 min, LSR=8 mL/g), lignin was recovered by precipitation with acidic water (pH=2) and subjected to several analysis. The first one was related to the purity of the lignin (which was attained by acid hydrolysis, see Section 2.1). In this case, 83.89% of the lignin was accounted as Klason lignin, while the carbohydrate content was lower than 2%, implying a lignin recovery yield of 84% (17.42 kg per 100 kg of initial PW). The lignin obtained in this work may be compared to that attained by Dávila et al. (2017) after autohydrolysis and delignification (using 12% NaOH, during 105 min at 124 °C) of vine shoots, with an approximate purity of 80%. In that range, Alvarez-Vasco et al. (2016) obtained up to 88.4% of purity in the lignin extracted from Douglas fir with ChCl:LA DES (145 °C, 9 h), although showing a lower delignification ratio (up to 58%).

Fig. 3a depicts the FTIR of the lignin that aids to identify specific molecular structures. The band of 1030 cm^{-1} may be ascribed to the bending and stretching vibration of C-O, C-C and C-OH and glycosidic C-O-C that could be an indicative of the presence of residual amounts of hemicelluloses (Dávila et al., 2019; Sun et al., 2012).

The bands between 1000 and 1300 cm^{-1} and the bands of 1464, 1505 and 1597 cm^{-1} can represent to C-O stretching and aromatic skeletal vibrations, respectively (Pérez et al., 2022). In this sense, the bands of 1080 and 1152 cm^{-1} (shoulders) may be ascribed to the C-O stretch vibration of ester groups and the C-O deformation in secondary alcohol and aliphatic esters (Morales et al., 2022a; Sammons et al., 2013).

Regarding the breathing with C-O stretching and the C-H in-plane and out of plane deformation of the syringyl ring can be observed in bands 1320, 1114 and 813 cm^{-1} (Dávila et al., 2019). The guaiacyl presence can be noted in bands 1213 and 1269, corresponding to the guaiacyl ring breathing (Dávila et al., 2019) and the C-O stretch (Sammons et al., 2013).

The existence of the band in 1718 cm^{-1} may reflect the oxidation of side chains of -OH, in addition to the presence of some impurities (Morales et al., 2022a, 2022b; Sammons et al., 2013). The stretching vibration of C-H (CH$_3$, CH$_2$ and CH) between lignin and polysaccharides can be seen in the range of 2835–2941 cm^{-1} (Morales et al., 2022a). The stretching vibration of hydroxy groups may be reflected in band at 3392 cm^{-1} (Dávila et al., 2019; Morales et al., 2022b).

Fig. 3b exhibits the ^{1}H NMR of the lignin. In this case, the chemical shifts characteristic of aliphatic -CH$_2$ and -CH$_3$ (δH, 1.23, 1.13, 9.86 ppm) can be observed. On the other hand, δH, 3.43 and 3.74 ppm

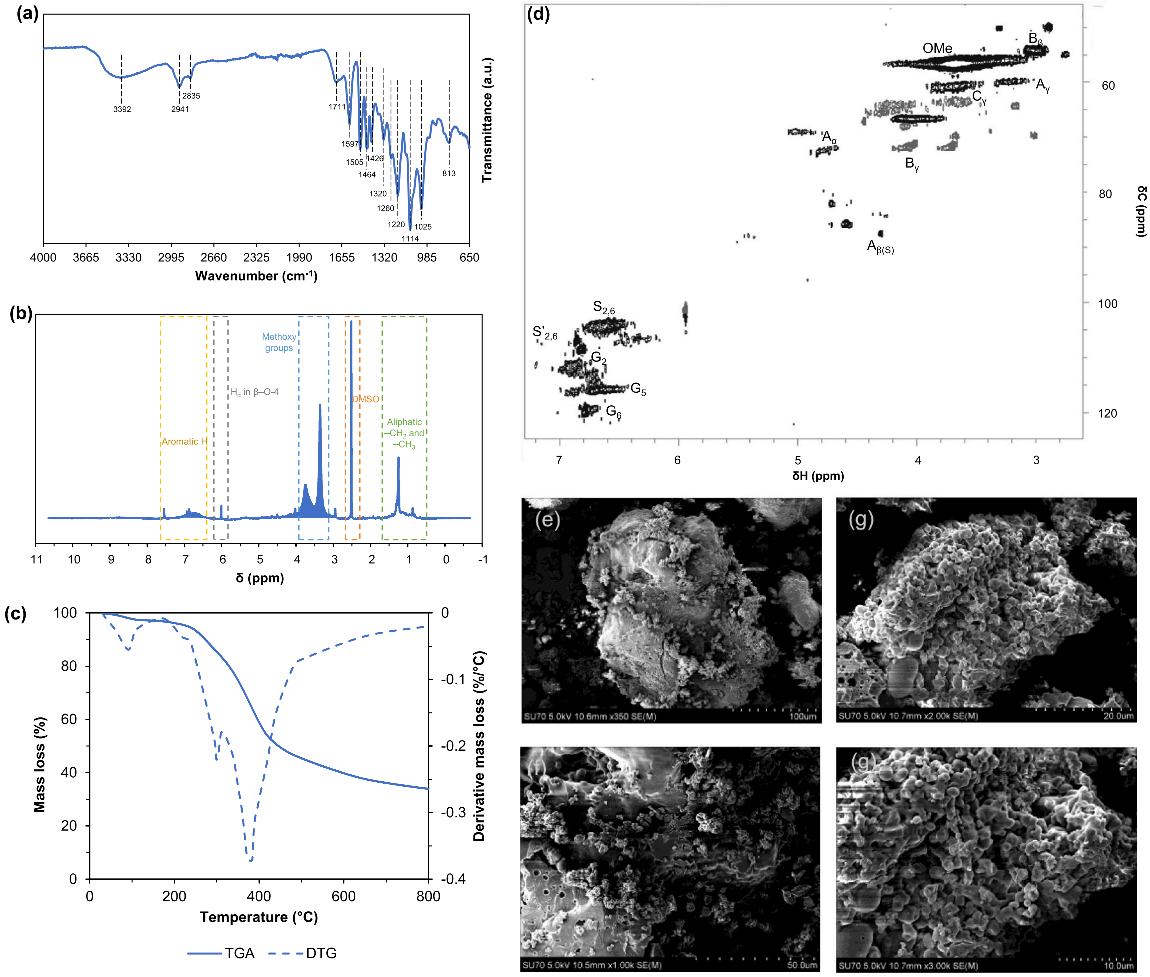

Fig. 3. Lignin characterization via (a) FTIR spectroscopy, (b) ^{1}H NMR, and (c) TGA-DTG, (d) HSQC 2D NMR and (e, f, g, h) SEM micrographs at different optical magnifications.

represent the methoxy groups while the little presence of aromatic H is observed at 6.5–7.5 ppm (Soares et al., 2021).

Finally, in Fig. 3**c** exhibits plotted the TGA-DTG obtained from the lignin. Firstly, the peak at 90 °C represents the water evaporation, corresponding to a mass loss lower than 3%, which agrees with the bibliography (Ankona et al., 2021; Bhaumik et al., 2014). The following peak at 300 °C may represent the degradation of residual hemicelluloses (Gullón et al., 2018), corresponding to a mass loss of around 15%. However, low-carbohydrate samples may also exhibit this peak because the weak chemical linkages (β-O-4'bonds) of the lignin structure are decomposing (Dávila et al., 2019). Finally, the degradation of lignin can be observed in the peak at 350 °C, representing a great mass loss of around 35%. This lignin presented a good thermal stability, maintaining 34% of mass (likely mostly composed of inorganic matter) at 800 °C, showing an analogous value than that obtained for pine straw lignin with 29.45% (Watkins et al., 2015).

Most signals in 2D HSQC spectra (^{13}C–^{1}H) were exhibited in the aliphatic-oxygenated and aromatic/unsaturated regions, corresponding to δC/δH 49–89/2.7–5.5 ppm and δC/δH 100–135/5.9–7.5 ppm respectively, and can be consulted in Fig. 3d. Signals were ascribed based on bibliographic data from Rivas et al. (2021), Rencoret et al. (2019) and Martín Sampedro et al. (2019). Concerning the aliphatic-oxygenated region, it can be highlighted the intense signal of methoxyl groups (OMe), although also presenting signals of resinol (B_β and B_γ) and phenylcoumaran (C_γ) bonds, corresponding to signals at δC/δH 54.3/3.0 ppm, 71.8/3.9–4.2 ppm and 63.8–3.6 ppm, respectively. Additionally, β-O-4 ether bonds-related signals were also found (A regions). Regarding the aromatic region, the spectra exhibit signals related to syringyl (S) and guayacil (G) units but not presenting hydroxyphenyl (H) units. This fact was also stated by Domínguez et al. (2020), who found only minor amounts of H-units in milled wood lignin of Paulownia wood. Very minor signals of carbohydrate (acetylated xylopyranoside at δC/δH 100/4.5) were found, exhibiting a high lignin purity. This low carbohydrate presence, in addition to the lower signals of G_2 and G_6 regarding G_5, indicated a minor lignin condensation.

Fig. 3e-h exhibits the SEM micrographs of the PW lignin obtained after precipitation from DES. Higher magnifications show that the lignin is comprised of agglomeration of interconnected spherical-like particles. Likewise, Shen et al. (2020) obtained spherical-like particles after the use of bio-based DES on lignin macromolecules reaching 50–100 nm-particles, which is a smaller size than the particles observed in this work. Other work studied the effect of the alkali DES composed of choline chloride:ethanolamine (1:6) on corncob lignin to obtain lignin nanoparticles with spheric morphology (Luo et al., 2021). The particles obtained in this work were present in a range of around 1–6 µm. Analogous sizes (microparticles) were obtained by Gomide et al. (2020) when ultrasonicating kraft lignin from eucalyptus wood, although presenting an irregular morphology.

3.7. Chemical characterization of liquor after lignin precipitation

Lignin presents phenolic hydroxyl groups and may have some antioxidant capacity that would make it appropriate as additive versus oxidation and biological degradation or photodegradation in polymers mixtures, cosmetics, and drugs (García et al., 2017; Kai et al., 2016). In this case, Table 3 displays data regarding the phenolic content and antioxidant capacity of the black liquor at selected conditions (ChCl:LA 1:9, 130 °C, 60 min, LSR=8 mL/g) before and after lignin precipitation. As expected, the TPC was higher before the precipitation of lignin, although still presenting around a 37% of phenols after the process. In this case, lignin would represent around 47.93 mg GAE/g. Regarding the antioxidant capacity, the black liquor showed values up to 49.19, and 58.85 mg TE/g PW for DPPH and ABTS. It has to be highlighted the FRAP value, reaching up to 244.33 mg TE/g before the lignin isolation, and 75.28 mg TE/g after its precipitation.

Regarding the phytochemical profile, 12 phenolic compounds (8 phenolic acids, 2 phenolic aldehydes and 1 flavonoid) were identified. It stands out the high concentration of syringaldehyde (25.06 mg/g), vanillin (10.21 mg/g) and 3,4-dihydroxibenzoic acid (2.51 mg/g, which come, more than likely, from the unprecipitated lignin.

Table 3
Total phenolic compounds (TPC), antioxidant capacity (DPPH, ABTS and FRAP), phytochemical profile and carbohydrate content of the DES black liquor (at selected conditions of ChCl:LA 1:9, 130 °C, 60 min, LSR=8 mL/g) before and after lignin precipitation. GAE-gallic acid equivalents, TE-Trolox equivalents.

	Black liquor	Black liquor after lignin precipitation
Phenolic content and antioxidant capacity (mg equivalents/g initial PW)		
TPC (mg GAE/g)	81.10 ± 0.35	30.33 ± 0.17
DPPH (mg TE/g)	52.10 ± 1.79	14.12 ± 0.06
ABTS (mg TE/g)	62.34 ± 1.61	21.21 ± 0.20
FRAP (mg TE/g)	258.82 ± 2.00	79.74 ± 1.73
Phenolic profile (µg/g initial PW)		
3,4-dihydroxibenzoic acid	-	2511.02
4-hydroxybenzoic acid	-	110.51
Ferulic acid	-	82.65
Gallic acid	-	193.22
Luteolin	-	27.29
p-coumaric acid	-	57.65
p-hydroxybenzaldehyde	-	151.19
Phthalic acid	-	354.15
Syringaldehyde	-	25059.39
Syringic acid	-	622.29
Vanillic acid	-	998.31
Vanillin	-	10211.89
Monomers and oligomers content (mg/g initial PW)		
Glucose	-	24.36
Xylose	-	40.97
Arabinose	-	0.00
Acetic acid	-	53.07
Hydroxymethylfurfural	-	6.40
Furfural	-	18.09
Glucooligosaccharides	-	6.53 ± 0.15
Xylooligosaccharides	-	53.66 ± 0.22
Arabinooligosaccharides	-	2.82 ± 0.07
Acetyl groups linked to oligosaccharides	-	6.95 ± 0.01

Conversely, the monomers and oligomers content of the black liquor is, as well, presented in Table 3. This liquor is mainly composed of xylooligosaccharides and xylose, which corresponded to a recuperation of 54% of the xylan concerning the quantity in the initial material. That value can be compared to other pretreatments which were more selective for the recovery of hemicellulosic compounds such as autohydrolysis. For instance, Pérez-Pérez et al. (2023b) subjected Robinia wood to microwave-assisted autohydrolysis, reaching a maximal recovery of 79% of the xylan y the form of xylooligosaccharides (230 °C and 0.25 min). The other carbohydrates appeared in lower amounts, while acetic acid reached a high value of 8.78 g/L, probably as a result of degradation procedures.

3.8. Mass balance of the whole process

Fig. 4 displays the mass balance of the multiproduct process carried out in this work. The figure shows the inputs and outputs per 100 kg of initial PW after pretreatment with ChCl:LA DES (1:9) at selected conditions (130 °C, 60 min, LSR=8 mL/g).

After the delignification process with DES, almost 53 kg of PW were recovered, mainly composed of glucan (38.52 kg), retaining a 93% of the initial glucan, while the 78% of hemicelluloses (17.02 kg) and 94% of lignin (21.45 kg) were solubilized in this process. In that range, Tan et al. (2018) reached a lignin removal of up to 88% (corresponding to 15.75 kg of lignin per 100 kg raw material) when using choline chloride:

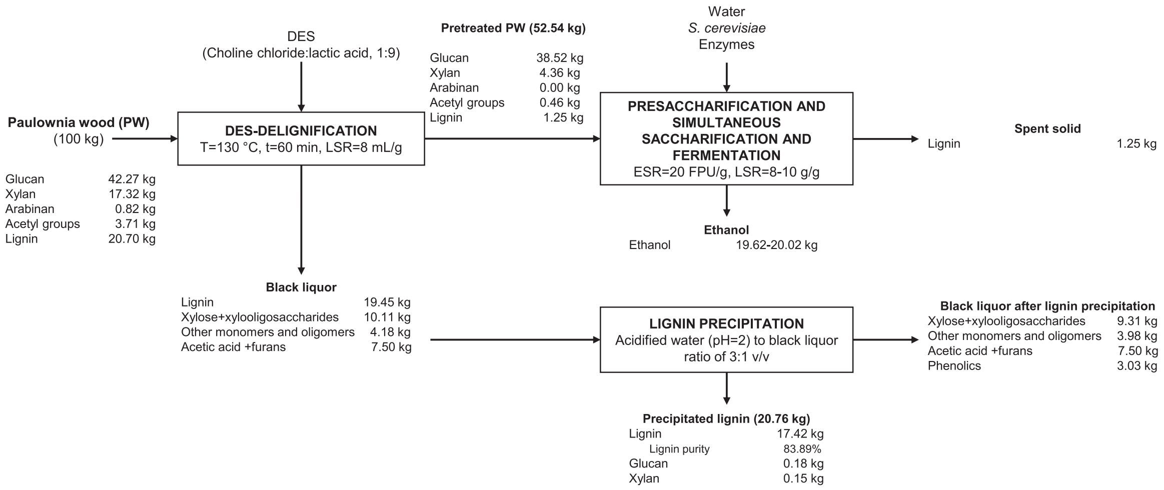

Fig. 4. Mass balance of Paulownia wood biorefinery process based on 100 kg of initial wood.

lactic acid (1:5) at 120 °C for 8 h on oil palm empty fruit bunch. Analogous results were found by Xu et al. (2023) when processing bamboo shoot shells with benzyl triethylammonium chloride and formic acid (1:1) with 10% water in microwave at 120 °C for 20 min, reaching delignification ratios up to 88.6% (about 23.75 kg of lignin per 100 kg of raw material). Conversely, Ceaser et al. (2023) reached interesting delignification (90%), hemicellulose removal (96%) and glucan retention (93.5%), corresponding to 26.37, 17.38 and 42.2 kg per 100 kg of raw material respectively, when using choline chloride:formic acid (1:4) on milled softwood mixture (majorly composed of *Pinus insignis*) at 140 °C for 14 min.

After that, different PSSF assays at an enzyme to substrate ratio (ESR) of 20 FPU/g and LSR of 8 and 10 g/g were performed on the cellulose-rich solid, attaining close to 20 kg of ethanol for both cases, while the residual lignin (1.25 kg) was retained in the spent solid. This may be positively compared to other bibliographic results. For instance, 9.45 kg of ethanol were obtained from sorghum straw after alkali extraction (NaOH 0.75%, 121 °C during 1 h) and DES (ChCl:LA 1:1 at 140 °C during 40 min) pretreatment (Wu et al., 2021).

Regarding the black liquor obtained after the DES-delignification, it was mainly composed of lignin, but also comprised xylan derivatives (especially xylose and xylooligosaccharides) accounting for 9.31 kg, other monomers and oligomers coming from glucan and arabinan (3.98 kg) and acetic acid and furfural (7.50 kg). In order to separate the lignin to get a thorough analysis, it was precipitated by acidified water (pH=2), recovering 20.76 kg that was mainly composed of lignin (17.42 kg, corresponding to a lignin purity of almost 84%), and residual amounts of glucan and xylan (0.18 and 0.71 kg respectively). Regarding the black liquor after lignin precipitation, it retained around 3.03 kg of phenolics (measured as gallic acid equivalents), probably coming from the unprecipitated lignin, especially as syringaldehyde (2.51 kg) and vanillin (1.02 kg).

In this context, this process enabled the recovery of ethanol, lignin and residual monomers and oligomers (especially xylose and xylooligosaccharides) by a single pretreatment based on the use of the DES choline chloride:lactic acid, creating a new pathway to reach multi-product biorefineries with a one-step pretreatment.

4. Conclusions

Paulownia wood was submitted to delignification, evaluating different DES and experimental conditions. The use of ChCl:LA (1:9) at 130 °C for 60 min and LSR of 8 mL/g were selected as positive conditions to remove 94% of lignin, retaining 93% of glucan in the solid phase. The spent solid was submitted to saccharification and fermentation, achieving 43.61 g ethanol/L (89.7% yield). The lignin and residual liquor were structurally and chemically characterized (for carbohydrate and phenols determination). This process involved a one-step delignification with DES to obtain highly susceptible glucan and highly pure lignin (around 84%) within a biorefinery scheme.

CRediT authorship contribution statement

Fernando Rodríguez-Rebelo: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Conceptualization. **Beatriz Rodríguez-Martínez:** Writing – review & editing, Writing – original draft, Visualization, 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. **Gil Garrote:** 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.

Data availability

Data will be made available on request.

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