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Strategies for vine shoots valorization using hydrothermal treatment and delignification using deep eutectic solvents within a biorefinery scheme

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ABSTRACT

Vine shoots (VS) are one of the main residues generated by winemaking, usually disposed of by open-field burning, which contributes to environmental pollution. Owing to their high content of lignin, cellulose and hemicelluloses, these residues represent a valuable feedstock for the bio-based products production. This study assessed environmentally friendly pretreatment strategies based on deep eutectic solvents (DES) and water to enable the valorization of vine shoots within a biorefinery framework. Two processing routes were compared: direct DES delignification and a sequential configuration combining autohydrolysis followed by DES delignification. The eutectic mixture of choline chloride and formic acid (ChCl:FA) at 130 °C for 60 min was identified as the most suitable condition for full valorization of vine shoots. Direct DES delignification yielded a delignified VS with low lignin content (5.41 kg of lignin per100 kg VS) and glucan recovery yield of 68 %. In contrast, the sequential strategy enabled the recovery of a hemicelluloses-rich aqueous stream containing 43.8 g/L of oligosaccharides. Although glucan retention in the solid was slightly lower, 25 % of glucan was additionally recovered as glucooligosaccharides and 55 % of lignin was removed. Overall, the combined autohydrolysis-DES approach proved to be more suitable for an integrated biorefinery, allowing milder conditions, ~50 % delignification efficiency, and multiproduct recovery including cellulose-rich solids, hemicellulosic oligosaccharides, and high-purity lignin.

1. Introduction

The viticulture industry is considered one of the main agro-industrial sectors worldwide, with a production of 26 million m^3 of wine in 2022, more than 60 % of which was generated within the European Union (EU). In addition, Spain leads Europe in terms of vineyard area with 9620 km^2 according to data from 2021 (OIV, 2022). This large-scale production generates substantial amounts of residues, the most significant being vine shoots (VS) from pruning, which may imply up to 200 tons per km^2 per year (Duarte et al., 2024; Rabelo et al., 2023). VS are normally burned *in situ* or left in the field provoking serious environmental issues. For that reason, the use of this residual biomass as source in a biorefinery approach may provide not only a reduction of contamination regarding its burn or lack of removal, but also an economic benefit providing a profitable product following the circular economy concept (Jesus et al., 2019; Madadi et al., 2025a and Madadi et al., 2025b).

To fully valorize biomass, it is essential to select an appropriate pretreatment that enables the separation of the main components of vine shoots, lignin and polysaccharides (hemicelluloses and cellulose), while employing green solvents and sustainable processing methods. In recent years, the use of deep eutectic solvents (DES) for lignocellulosic biomass has attracted significant attention as promising substitutes for ionic liquids in biomass fractionation (Fu et al., 2024) due to their remarkable selectivity for lignin solubilization. DES are mixtures of two or more components, typically comprising at least one hydrogen bond donor and one acceptor, that are cost-effective and exhibit low vapor pressure and toxicity (Ge et al., 2024; Mattonai et al., 2024; Singh et al., 2024). DES are considered innovative and environmentally friendly solvents, offering an alternative to traditional lignin delignification methods, which

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are typically carried out using alkalis or organic solvents (Jamaldheen et al., 2022; Lobato-Rodríguez et al., 2023). Moreover, their performance can be effectively optimized using machine learning tools (Madadi et al., 2025c). Recently, DES have been employed to process VS for different applications, for instance, VS were treated with DES in combination with ultrasonics (Duarte et al., 2024) or microwaves (Mattonai et al., 2024) for polyphenols extraction. On the other hand, Cañadas et al. (2024) used choline chloride-based DES to valorize both the lignin and cellulosic fractions of VS. However, although the lignin fraction is highly removed using DES, the hemicellulosic fraction may be only partially solubilized.

In this sense, a previous solubilization of hemicelluloses would be an interesting step to consider in order to valorize all the fractions from VS. Several studies demonstrated the suitability of using hydrothermal treatment or autohydrolysis for the recovery of hemicellulosic compounds, such as oligosaccharides, and for cellulosic ethanol production from vine pruning residues (Davila et al., 2016; Jesus et al., 2017; Dávila et al., 2019). Autohydrolysis consists in using water at high temperatures as a reaction medium to favor the removal of water-soluble compounds as a result of the self-ionization of water (Rahmati et al., 2023). This method is considered environmentally friendly, as it eliminates the need for harsh chemicals and minimizes waste generation (Díaz et al., 2021).

Achieving integral valorization of lignocellulosic biomass with a single treatment remains challenging for the development of second-generation biorefineries (Del Río et al., 2022). Therefore, at least two sequential treatments are typically employed, each targeting specific fractions of interest to enable selective separation. Several authors have applied the biorefinery concept in cascade, generally using hydrothermal treatment to solubilize hemicelluloses, followed by a delignification process to remove lignin and recover a cellulose-enriched solid residue (Romaní et al., 2011; Del Río et al., 2020). Usually, the delignification process is based on alkaline reagents, which require additional washing and neutralization steps (Argun and Onaran, 2015; Dávila et al., 2018 and 2019). To the best of the authors' knowledge, this study represents the first time VS have been processed using sequential hydrothermal (autohydrolysis) and DES treatments, being the latter employed as an alternative delignification method.

In this sense, the aim of this work is the green processing of VS residue through (i) a single step pretreatment using DES or (ii) the integration of sequential pretreatments using autohydrolysis and DES treatments. Different conditions of autohydrolysis and various choline chloride-based DES were evaluated to valorize the separate fractions. Additionally, the enzymatic saccharification of the obtained solid phase was also assessed to verify its digestibility, and the recovered lignin was further characterized. This study represents a step forward to a greener processing of lignocellulosic residues following a biorefinery approach.

2. Material and methods

2.1. Vine shoots as raw material

VS used in this work were kindly provided by Bodegas Gómez Sanmartín (O.D. O Ribeiro, NW Spain). The feedstock was dried at 60 ºC, milled to a particle size lower than 8 mm, and put in storage in a dark and dry place until use. A chemical characterization was performed for the determination of VS composition as follows: moisture and ash content were determined following the protocols described by NREL procedures (Sluiter et al., 2008a, 2008b). Firstly, VS was subjected to Soxhlet extraction to quantify ethanol extractives. VS free-extractives was submitted to acid quantitative hydrolysis to determine the structural polysaccharide and lignin content (Sluiter et al., 2008c, 2008d). Briefly, the sample was submitted to acid hydrolysis using 72 % of H_2SO_4 (30 °C for 1 h) to break out polysaccharides into oligomers. In the second stage, hydrolysis was carried out at 4 % H_2SO_4 (121 °C for 1 h) to convert oligomers into monomers. Insoluble acid lignin (Klason lignin) was subsequently determined gravimetrically using crucible Gooch nº3, while the liquid phase was analyzed by HPLC for monomers quantitation, corresponding to structural polysaccharides. Another aliquot of this liquid phase was used for the determination of acid soluble lignin (measured by 198 nm in UV-Visible spectrophotomer). The uronic acids were determined using a colorimetric method applied to the liquid obtained in the acid hydrolysis (Blumenkrantz and Asboe-Hansen, 1973).

2.2. Vine shoot processing

2.2.1. Autohydrolysis treatment

VS and water were mixed at a solid to liquid ratio (LSR) of 4 g/g, dry basis in a Büchiglasuster versoclave reactor with 1.6 L of capacity. Agitation of the biomass was maintained at 250 rpm throughout the process. VS were subjected to autohydrolysis at several temperatures (195 °C, 200 °C, and 205 °C) under non-isothermal regime, which correspond with the severities (S_0) of 3.71, 3.92, and 4.05, respectively, to determine the most suitable temperature for extracting the hemicelluloses fraction. These treatment temperatures were selected based on previous autohydrolysis studies of vine pruning residues (Jesus et al., 2017, Pinheiro et al., 2019).

$$S_0 = \log R_0 = \log(R_{0HEATING} + R_{0cOOLING}) = \log\left(\left[\int_0^{tMAX} \exp\left(\frac{T(t)-T}{\omega}\right)\cdot dt\right] + \left[\int_0^{tMAX} \exp\left(\frac{T(\acute{t})-T_{REF}}{\omega}\right)\cdot dt\right]\right)$$

Once the target temperature was achieved, cold water (20–25 °C) was circulated through the reactor's inner jacket to rapidly cool the system. The resulting mixture was subsequently vacuum filtered to separate the solid and liquid phases. The solid fraction was washed with water to remove residual autohydrolysis liquor, then weighed to calculate the solid yield (SY) of the treatments. The solids were air-dried at room temperature, and an aliquot was collected for analysis as described in previous Section 2.1. The liquid phase (or autohydrolysis liquor) was analyzed by triplicate for oligosaccharides determination by posthydrolysis (4 % of H_2SO_4, 121° C for 30 min). Monosaccharides (glucose, xylose and arabinose), acetic acid, hydroxymethylfurfural and furfural were quantified by HPLC.

2.2.2. Delignification treatment with deep eutectic solvents

In this study, a total of four deep eutectic solvents (DES) were prepared using choline chloride (ChCl) as the hydrogen bond acceptor (HBA) and three organic acids, lactic acid (LA), formic acid (FA), and acetic acid (AA) as hydrogen bond donors (HBD). All DES were synthesized using a 1:5 molar ratio (HBD:HBA). Additionally, a ChCl:LA mixture at molar ratio of 1:10 was prepared to assess the effect of molar composition on delignification performance. The synthesis of these solvents was carried out using magnetic stirring at a temperature close to 80 °C for 1–4 h, until a stable and homogeneous mixture was obtained. These eutectic mixtures were used for delignification experiments, using either untreated VS or autohydrolyzed VS as lignocellulosic biomass. The experimental parameters assessed included temperature, reaction time, type of DES, and the molar ratio of DES components. Delignification processes were performed in an autoclave at 121 or 130 ºC, with a DES-to-solid ratio fixed at 10:1 (g/g).

After DES delignification, the solid and liquid streams were separated by vacuum filtration. The liquid fraction, also known as black liquor and containing solubilized lignin, was stored at 4 ºC for further precipitation. The recovered solid phase was employed for solid yield determination and for evaluating enzymatic digestibility. For this purpose, a washing step was carried out following the methodology described by Rodríguez-Rebelo et al. (2024). The DES-pretreated VS was washed with 50 % (w/w) ethanol, followed by 0.1 % NaOH to remove any residual lignin, and then washed with distilled water until pH (~7) was reached. The pretreated VS were dried and stored for subsequent enzymatic hydrolysis. In addition, an aliquot of the solid phase was

withdrawn for chemical composition analysis.

2.2.3. Enzymatic saccharification

Samples obtained from autohydrolysis or from autohydrolysis followed by DES delignification were subjected to enzymatic saccharification in 0.05 N citric acid-sodium citrate buffer (pH 4.8). The assays were performed in Erlenmeyer flasks (100 mL) in an orbital shaker at 50 °C for 72 h, adding the commercial enzyme Cellic® Ctec2 (kindly provided by Novozymes, Denmark), with an activity of 120 FPU/mL. The solid loading was 5 % (w/w) pretreated biomass, and the enzyme to substrate ratio (ESR) was 20 Filter Paper Units (FPU)/g. Samples were collected at 0, 4, 8, 24, 48, and 72 h, and centrifuged at 4500 rpm for 10 min. The supernatants were analyzed by HPLC for glucose quantification.

2.3. Lignin recovery and characterization

Lignin was recovered from the liquid phase (black liquor) containing ChCl:FA solvent from the delignification process by precipitation with sulfuric acid solution (pH= 2). Black liquor was gradually added to the acid solution under stirring at a liquor-to-acid ratio of 1:10. The mixture was left at room temperature for 24 h, after which the lignin and supernatant were separated by filtration. The lignin fractions were washed with distilled water, frozen, and lyophilized.

The lyophilized lignin was subjected to quantitative acid hydrolysis, Fourier-Transform Infrared (FTIR), Thermogravimetric analysis (TGA), Proton Nuclear Magnetic Resonance (^{1}H NMR) and 2D HSQC NMR analyses. FTIR spectroscopy was performed using a PerkinElmer Spectrum 100 equipped with an ATR accessory. Each spectrum was obtained by averaging eight scans within the range 4000–650 cm^{-1} . TGA of the lignin was conducted on a PerkinElmer TGA 4000, where samples were heated to 800 °C at a constant rate of 10 °C/min under a nitrogen atmosphere flowing at 20 mL/min. Lignin samples dissolved in dimethyl sulfoxide (DMSO were used for ^{1}H NMR and HSQC ^{2}D NMR analyses, performed on a Bruker Neo 400 spectrometer operating at 400 MHz and a Bruker Advance with a z-gradient 5 mm QNP probe, respectively, both operating at 400 MHz and 25 °C.

2.4. Analytic methods

Samples from quantitative acid hydrolysis, post-hydrolysis, and enzymatic saccharification were analyzed by High Performance Liquid Chromatography (HPLC, Agilent 1200 series, CA, USA), using a Rezex ROA-Organic acid H^+ column (Phenomenex) at 60 ºC, with a refractive index detector at 40 ºC and 0.03 M H_2SO_4 as the mobile phase at a flow rate of 0.6 mL/min.

3. Results and discussion

3.1. Characterization and processing of vine shoots

The residue generated annually during vine pruning shows variable chemical composition depending on variety, region, climatic conditions, and soil characteristics (Cebrián-Tarancón et al., 2019). As a lignocellulosic biomass, VS are mainly constituted of main structural polymers: cellulose, hemicelluloses, and lignin, along with non-structural components such as water-extractives, which in some cases can reach up to 20 % (w/w) (Ioannidou et al., 2022). In this study, the chemical composition of VS, determined as g per 100 g of solid on a dry basis ± standard deviation, was as follows: 33.35 ± 0.08 g of cellulose (measured as glucan), 12.79 ± 0.09 g of xylan, 0.90 ± 0.06 g of arabinan, 1.17 ± 0.32 g of acetyl groups, 22.27 ± 0.31 g of Klason lignin (or acid insoluble lignin), 3.30 ± 0.03 g of acid soluble lignin; 12.53 ± 0.15 g of uronic acids, 10.84 ± 2.15 g of extractives, and 2.98 ± 0.14 g of ashes. These results are comparable with those reported by other authors, who found cellulose contents of 28–32 %, hemicelluloses (mainly xylan) of 18–23 %, and lignin of 15–25 % (Ioannidou et al., 2022). By contrast, extractives content appears more variable, ranging from 3.9 % to 13.3 % (Rivas et al., 2021; Jesus et al., 2019).

Fig. 1 presents the two alternative processing schemes evaluated in this study, including the operational conditions and the main fractions recovered.

3.1.1. Hydrothermal treatment of vine shoots

In this work, autohydrolysis was proposed for hemicelluloses extraction at temperatures of 195, 200, and 205 ºC, as shown in Table 1, including the main composition of solids and liquors after hydrothermal treatment. Temperatures (or severities) of treatments were selected based on previous works in which high concentrations of oligosaccharides were obtained (Dávila et al., 2016; Jesus et al., 2017). The solid phase after treatment was composed mainly of glucan and Klason lignin, with a lower xylan content. Table 1 shows that as the temperature rises, the amount of glucan increases up to 40.81 g/100 g of autohydrolyzed VS, while the amount of xylan decreases to 6.52 g/100 g of autohydrolyzed VS. This behavior is usual when acidic or autocatalytic treatments are used on lignocellulosic materials such as agricultural residues, hardwoods and softwoods (Bassani et al., 2020; Nitsos et al., 2016). Regarding glucan, more than 70 % (73.72–78.13 %) was recovered in the solid fraction. When considering both glucan and glucooligosaccharides and glucose present in autohydrolysis liquors, up to 99.95 % of glucan in raw material can be recovered after hydrothermal treatment at 200ºC. This value was slightly higher than obtained at 205 ºC and 195 ºC. Conversely, Klason lignin does not vary substantially, reaching values between 38.9 and 43.10 g/100 g of autohydrolyzed VS. The increase in glucan and lignin content with respect to the same compounds in the raw material is due to the treatment's selective capacity to solubilize part of the polysaccharides, namely, hemicelluloses (Dávila et al., 2016; Jesus et al., 2019). Regarding the recovery of lignin, higher 100 % of lignin was recovered at T > 200 ºC, which could indicate repolymerization or condensation reactions. This fact can be due to pseudo-lignin can be formed by the interactions between carbonium ions and nucleophiles, after acid treatments or hydrothermal treatments (Trajano et al., 2013).

On the other hand, autohydrolysis liquors (Table 1) were mainly composed of oligosaccharides, achieving the highest concentration of 19.38 g/L of glucooligosaccharides (GOS) and 24.29 g/L of xylooligosaccharides (XOS) at 200 ºC, which corresponded to 25.39 % and 82.97 % of glucan and xylan recoveries in the liquid phase, respectively. XOS were the predominant sugars present in liquor, accounting for 39.7–41.7 % of identified compounds. The treatment was performed at high solid loading (LSR 4 g/g, corresponding to 20 % of solid), which enabled the recovery of hemicelluloses derivatives (mainly as oligosaccharides) in a more concentrated form while reducing water consumption during the process (Yuan et al., 2021). The content of degradation products of hexoses and pentoses (hydroxymethylfurfural and furfural, respectively) slightly increased with the severity of the treatment, reaching values of 0.41 and 0.61 g/L, respectively.

Interestingly, the xylan recovery as XOS was comparable to that reported by other researchers using lower solid loadings (Dávila et al., 2016; Jesus et al., 2017), indicating that mass transfer limitations were not significant even under high solid loading conditions in the hydrothermal treatment. Considering the high recovery of hemicelluloses as oligosaccharides obtained at 200 °C and its prebiotic potential previously reported by Dávila et al. (2019), this temperature was selected for the subsequent delignification step using deep eutectic solvents.

3.1.2. Delignification treatment based on choline chloride mixed with carboxylic acids

In this study, several eutectic mixtures, selected based on previous literature (Oh et al., 2020; Wang and Lee, 2021), were evaluated. Table 2 summarizes the experiments performed at 130 °C for 120 min, using untreated VS (runs D1–D4) or autohydrolyzed VS (runs AD1–AD4)

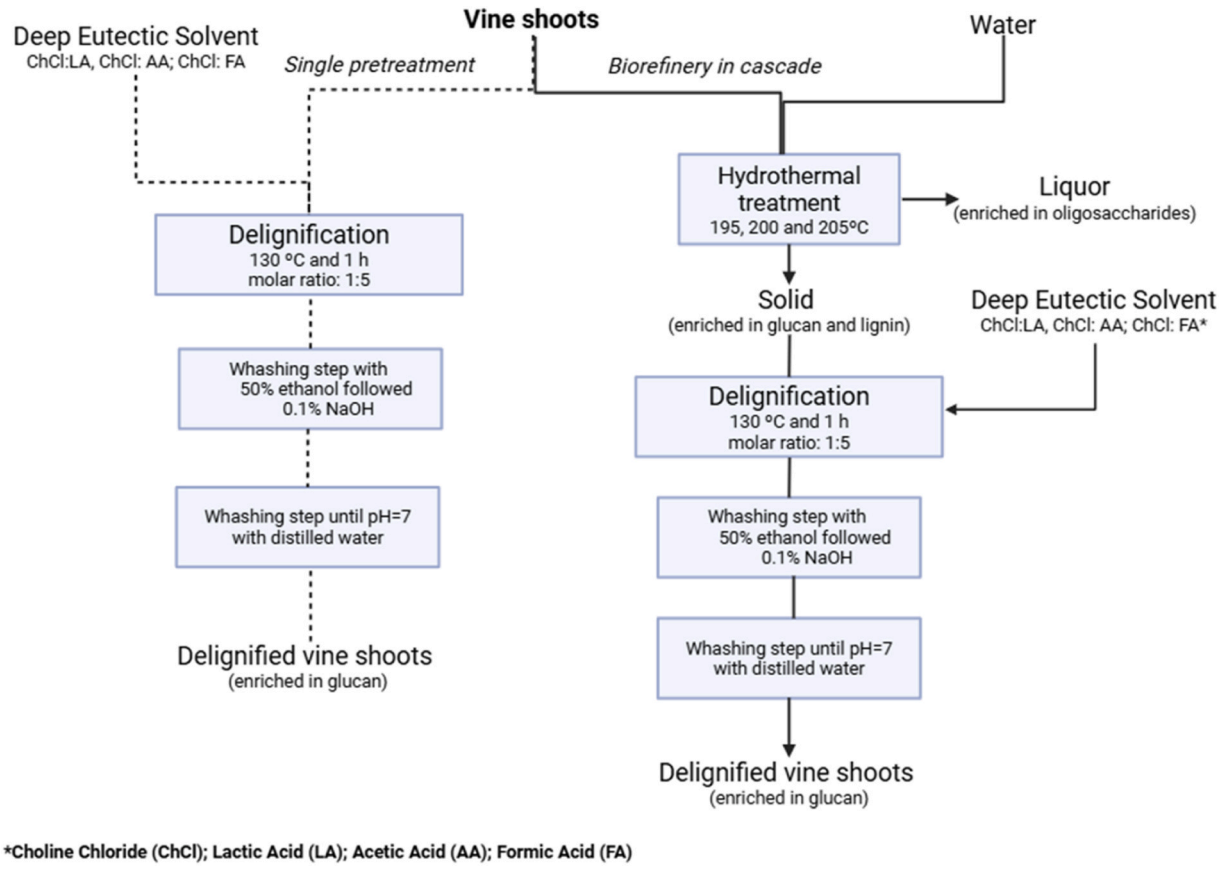

Fig. 1. Flowchart of alternatives valorization ways of vine shoots using a delignification process based on deep eutectic solvents.

Table 1
Chemical composition of solid and liquor fractions from vine shoots after autohydrolysis treatment.

T_{max} (°C) (or S_0)	**195 (S_0=3.71)**	**200 (S_0=3.92)**	**205 (S_0=4.05)**
Solid Yield (g/100 g raw material)	64.07	63.09	61.93
*a) Solid composition in g/100 of autohydrolyzed raw material (% Recovery)**			
Glucan	38.37 ± 2.32 (73.71 %)	39.15 ± 1.59 (74.06 %)*	40.81 ± 1.15 (75.78 %)
Xylan	7.1 ± 0.92 (35.56 %)	6.8 ± 0.24 (33.54 %)	6.52 ± 0.58 (31.57 %)
Acetyl groups	1.17 ± 1.06	1.05 ± 0.97	0.94 ± 0.07
Lignin Klason	38.90 ± 2.00 (97.47 %)	43.69 ± 0.61 (107.8 %)	43.10 ± 0.32 (104.4 %)
b) Liquor composition (g/L)			
Glucose	1.86	2.68	2.40
Xylose	1.31	1.84	2.37
Arabinose	1.30	2.40	0.42
Acetic acid	3.35	5.19	5.14
Hydroxymethylfurfural	0.24	0.41	0.45
Furfural	0.32	0.41	0.61
Glucooligosaccharides (GOS)	18.57	19.38	17.91
Xylooliosaccharides (XOS)	22.52	24.29	23.05
Arabinooligosaccharides (ArOS)	1.04	0.14	0.78
Acetyl groups (AG)	4.18	4.41	3.77

* % Recovery was calculated as g of component (glucan, lignin or xylan) in raw material after treatment/100 g of glucan or lignin or xylan in vine shoots

as feedstocks. The eutectic mixtures were prepared from choline chloride (ChCl) combined with three carboxylic acids: lactic acid (LA), acetic acid (AA), and formic acid (FA).

The selection of mixtures and the molar ratio were based on previous studies on delignification with DES using wood species, such as eucalyptus and birch, using choline chloride-acid mixtures at molar ratios ranging from 1:1–1:10 (Wang et al., 2021). In this study, the delignification yields (as percentages of the initial lignin content) ranged from 36 % to 93 % for eucalyptus and 13–85 % for birch (Wang et al., 2021). Based on these findings, the first part of this work assessed the effect of choline chloride-lactic acid mixtures at molar ratios of 1:5 and 1:10 on both biomasses (runs D1, D2, AD1, and AD2; Table 2).

The solid yield after autohydrolysis followed by DES treatment ranged from 65.65 % to 68 %, whereas VS subjected only to DES treatment showed lower yields, varying from 40.70 % to 50.03 %, which could be related to higher xylan solubilization achieved vine shoot without authydrolysis treatment. Similar behavior was observed using hydrothermally treated sugarcane bagasse and delignification treatment with Ch:LA (1:2) at 130 °C for 90 min (Gundapalli et al., 2022). The glucan content remained nearly unchanged, with an average value of 52.4 % (runs D1–D2 and AD1–AD2, Table 2). In contrast, the xylan content decreased compared to the raw material, from 12.79 g/100 g of VS to 8.15 or 6.70 g/100 g of delignified VS (Table 2), indicating that the treatment enhanced hemicelluloses solubilization (up to 73.5 % xylan solubilization) when using ChCl:LA at a 1:5 molar ratio.

Regarding lignin, both strategies resulted in similar contents, with lignin representing the second major fraction. Delignification yields obtained (measured as lignin removal with respect to lignin in the raw material) were 58.7 % and 59.4 % for experiments D1 and D2, respectively. However, the lignin content in the biomass delignified after autohydrolysis was slightly higher than in D1 and D2, achieving nearly 32 % of Klason lignin/100 of autohydrolyzed VS. The percentage of delignification with respect to vine shoots was lower in the combined process (47.74 and 47.56 % for AD1 and AD2, respectively), likely due to lignin changes such as condensation phenomena during the

Table 2
Conditions of delignification process with deep eutectic mixtures at 130 ºC and 120 min and main results obtained (chemical composition and solid yield).

Raw Material	Vine Shoot (g component/100 g of vine shoot)				Autohydrolyzed Vine shoot (g component/100 g of autohydrolyzed vine shoot)			
Code run	D1	D2	D3	D4	AD1	AD2	AD3	AD4
DES	[ChCl:LA]	[ChCl:LA]	[ChCl:AA]	[ChCl:FA]	[ChCl:LA]	[ChCl:LA]	[ChCl:AA]	[ChCl:FA]
Molar ratio	1:5	1:10	1:5	1:5	1:5	1:10	1:5	1:5
Solid Yield	41.51	40.94	50.03	40.70	65.65	67.81	68.00	67.62
Glucan	51.6 ± 0.87	50.61 ± 1.06	37.63 ± 2.29	55.96 ± 1.52	54.32 ± 1.02	52.99 ± 0.94	54.03 ± 1.07	54.55 ± 0.14
Xylan	8.15 ± 0.36	9.88 ± 0.37	9.78 ± 0.29	3.09 ± 0.34	6.65 ± 0.24	6.7 ± 0.37	6.67 ± 0.47	3.3 ± 0.42
Klason Lignin	25.45 ± 0.54	25.36 ± 1.41	24.4 ± 1.34	26.96 ± 0.89	32.26 ± 0.73	32.37 ± 0.57	30.32 ± 0.79	33.81 ± 0.57

hydrothermal treatment. During the delignification process, bonds within the lignin structure can be cleaved, leading to the formation of new linkages or larger lignin molecules, which could increase the lignin content (Trajano et al., 2013). Nevertheless, DES-delignification process in both biomasses with respect of native raw material showed similar effect achieving higher 50 % of delignification (Fig. 2a).

Based on these results, the 1:5 molar ratio was selected for further work, as it requires less lactic acid. In addition to the eutectic mixtures with lactic acid, experiments were performed with acetic acid and formic acid at the same molar ratio (1:5) to assess the effect of varying the hydrogen bond donor (D3, D4, AD3 and AD4, in Table 2).

For clarity in interpreting the delignification process of both biomasses (VS and autohydrolyzed VS), Fig. 2 presents the recoveries of xylan and glucan, together with the delignification percentages obtained in this study. Glucan recoveries ranged from 56.46 to 68.30 % for VS and 67.47–69.79 % for autohydrolyzed VS (Fig. 2a). Among the eutectic mixtures, the ChCl-formic acid system showed the highest glucan recoveries and the maximum xylan solubilization. Furthermore, after autohydrolysis, subsequent delignification with different eutectic mixtures produced similar results, with no notable differences under the studied conditions. Delignification yields, relative to the raw material, varied between 43.6 % and 58.7 % across all conditions (Fig. 2a; Table 2).

To further assess the delignification process, the eutectic mixture with formic acid was selected for additional experiments at shorter treatment times (30 and 60 min) and at a lower temperature (121 °C). These experiments (denoted D5–D7 and AD5–AD7) evaluated the effect of temperature and time on delignification with ChCl:FA at a 1:5 molar ratio. Table 3 summarizes the composition of the delignified solids obtained under these conditions. As observed, experiment D5 (121 °C, 120 min) exhibited a lower solids yield than experiment D7 (130 °C, 30 min), despite being conducted at a lower temperature. This finding

Table 3
Main results from vine shoots processing after DES treatment with choline chloride and formic acid (molar ratio 1:5), with or without prior autohydrolysis at Tmax of 200 ºC.

Raw Material	Vine Shoot (g component/100 g of vine shoot)			Autohydrolyzed Vine shoot (g component/100 g of autohydrolyzed vine shoot)		
Code run	D5	D6	D7	AD5	AD6	AD7
Temperature (ºC)	121	130	130	121	130	130
Time (min)	120	60	30	120	60	30
Solid Yield	33.9	32.28	39.9	59.2	57.5	58.4
Glucan	51.79 ± 0.63	62.36 ± 0.34	59.6 ± 1.71	49.57 ± 0.49	51.3 ± 0.51	49.58 ± 0.83
Xylan	4.6 ± 0.35	6.33 ± 0.07	6.39 ± 0.43	3.56 ± 0.24	4.56 ± 0.49	5.34 ± 0.31
Klason Lignin	24.32 ± 0.83	16.76 ± 0.15	18.88 ± 0.78	36.43 ± 1.97	31.36 ± 0.44	32.36 ± 0.18

indicates that treatment time shows a more pronounced effect than temperature on biomass solubilization under the evaluated conditions. Similar behavior has been reported in the literature, where prolonged treatment times lead to decreased solid recovery due to the solubilization and degradation of polysaccharides present in the biomass (Xu et al., 2020).

The glucan content increased at shorter treatment times; however, lowering the temperature to 121 °C (run D5) did not result in notable changes in VS composition compared with 130 °C (run D4, Table 2). Regarding results showed in Fig. 2b, the highest glucan recovery (71.4 %) was obtained at 130 °C for 30 min (run D7), in which the xylan recovery also reached its highest value (19.9 %). When compared with the combined treatments, the xylan recoveries obtained were slightly

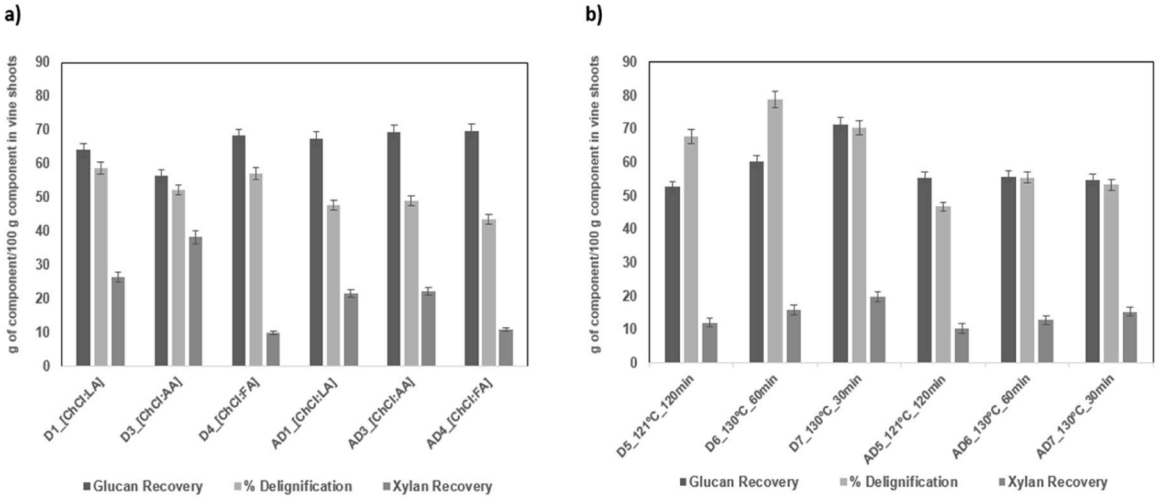

Fig. 2. Glucan and xylan recoveries and delignification of vine shoot after DES treatment or autohydrolysis followed by DES treatment: a) eutectic mixtures with lactic acid, acetic acid and formic acid at 130 ºC for 120 min and b) eutectic mixture with formic acid.

lower than those achieved with the direct DES delignification treatment. This behavior may be attributed to the fact that autohydrolysis previously removed part of the more soluble xylan fraction. Regarding lignin removal, experiments carried out for 60 min of treatment (78.8 % in D6 and 55.5 % in AD6 of % delignification) enabled 1.16 and 1.18-fold higher delignification, respectively, compared with the results obtained at 121ºC for 120 min, indicating that a temperature of 130 ºC had a more significant effect.

In addition to the effect of DES treatment on VS fractionation, pretreatment of lignocellulosic materials is crucial to improve the enzymatic saccharification of cellulose (Rodríguez-Rebelo et al., 2025). To further evaluate the impact of DES treatment combined with autohydrolysis, the enzymatic susceptibility of AD4 and AD6 were assayed considering the high glucan recovery obtained for AD4 (69.8 %) and high degree of delignification achieved for AD6 (55.5 %). Fig. 3 compares the time course of enzymatic hydrolysis of vine shoots subjected to autohydrolysis alone (200 °C) with those treated sequentially by autohydrolysis followed by DES delignification (AD4 and AD6). As shown, glucan-to-glucose conversion was markedly enhanced in the delignified samples, resulting in a 45 % improvement in enzymatic saccharification of glucan to glucose. After 72 h, conversion reached 72.3 % in AD6 and 63.8 % in AD4, compared with only 33 % in the samples treated solely by autohydrolysis. These findings confirm that DES delignification substantially improves enzymatic digestibility by removing lignin and increasing glucose accessibility; 60 min of treatment was sufficient to achieve this effect. The results obtained in this work compare favorably with those reported by other authors for VS biomass treated with a mixture of choline chloride and lactic acid (1:5) at 120 ºC for 6 h, which achieved 56 % cellulose conversion and 77 % xylan conversion (Cañadas et al., 2024). Moreover, vine pruning pretreated through sequential autohydrolysis stages was also employed for ethanol production by separate hydrolysis and fermentation, increasing the solid loading up to 16 %, which resulted in a lower overall glucan conversion compared to a solid loading of 4 % (Jesus et al., 2017). Increasing the solid loading in the saccharification process is necessary to reduce distillation costs, which can improve large-scale implementation (Modenbach and Nokes, 2013). Nevertheless, if the treatment effect is not properly optimized, the efficiency may be reduced (Romaní et al., 2014).

3.2. Lignin characterization from pretreated vine shoots

Taking into account the highest delignification percentages obtained in both process configurations, 78.8 % in D6 and 60.3 % in AD6, these conditions were selected for further lignin characterization. The lignin obtained after the processing of VS (run D6) and autohydrolyzed VS using DES (run AD6) presented a purity of 84.67 % and 85.77 %, respectively, presenting minor amounts of polysaccharides. The similar purity of lignin after DES pretreatment of both raw and autohydrolyzed biomass is due to the solvent's selective extraction mechanism, which is largely independent of prior autohydrolysis. These results can be compared to analogous processing of Douglas fir wood (Alvarez-Vasco et al., 2016) and autohydrolyzed Paulownia wood (Rodríguez-Rebelo et al., 2025) employing DES. Negligible amounts of acid-soluble lignin remained in the black liquor after the lignin precipitation. Lignin was further characterized by TGA, FTIR, ^{1}H NMR and 2D HSQC NMR, and the results obtained are depicted in Fig. 4.

The thermal stability of the lignin samples was evaluated by TGA (see Fig. 4a). Firstly, 2–7 % of mass loss occurred prior to around 100 °C, reflecting the loss of water in the sample as stated by other authors (Ankona et al., 2021). The degradation of residual polysaccharides happened at 260 °C with a mass variation of around 11 %, which is consistent with previous results of Paulownia wood lignin (Rodríguez-Rebelo et al., 2024). Subsequently, the greater mass loss occurred at 350–360 °C by losing 30–32 % of the total weight, which represents the degradation of the lignin. Nevertheless, the curves observed for polysaccharides and lignin may reflect the concurrent degradation of several compounds (Monje et al., 2021). Finally, at 800 °C, only 44–50 % of the lignin remained, corresponding to the inorganic matter. This value is higher than that found for other lignin samples in the bibliography, showing the higher thermal stability of the recovered lignin from VS evaluated in this work.

FTIR spectra of the recovered lignin samples are shown in Fig. 4b. The weak band at 1030 cm^{-1} resulting from bending and stretching vibrations of C–O, C–C, C–OH and glycosidic C–O–C indicate present hemicelluloses. The 1000–1300 cm^{-1} region together with bands at 1457, 1504 and 1594 cm^{-1} is mainly attributed to C–O stretching and aromatic skeleton vibrations. The region contains as shoulder bands at 1087 and 1174 cm^{-1} ascribable to C–O stretching of ester functionalities

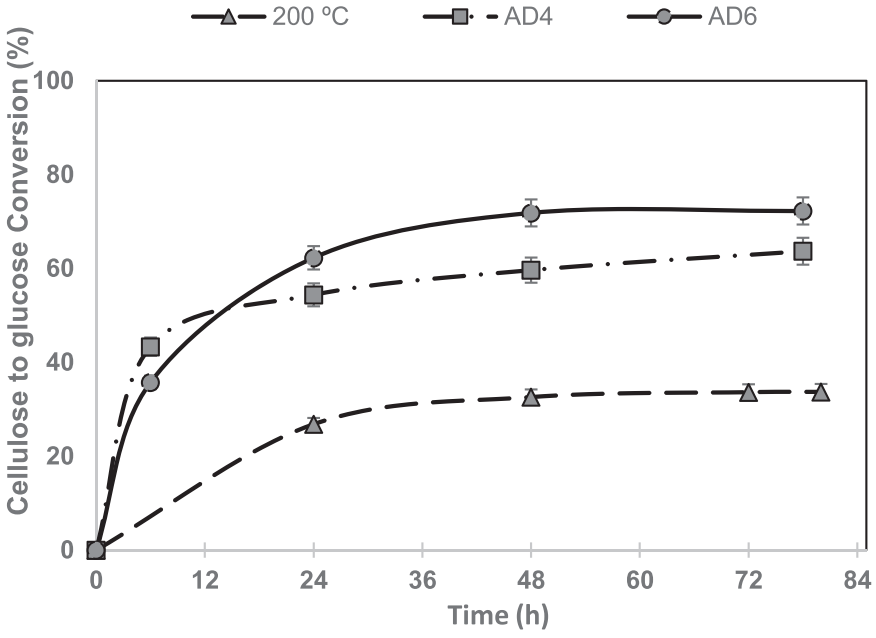

Fig. 3. Enzymatic saccharification of autohydrolyzed vine shoots at 200 ºC and delignified vine shoots after DES treatment with ChCl:FA at 130 ºC for 120 min (AD4) and 60 min (AD6).

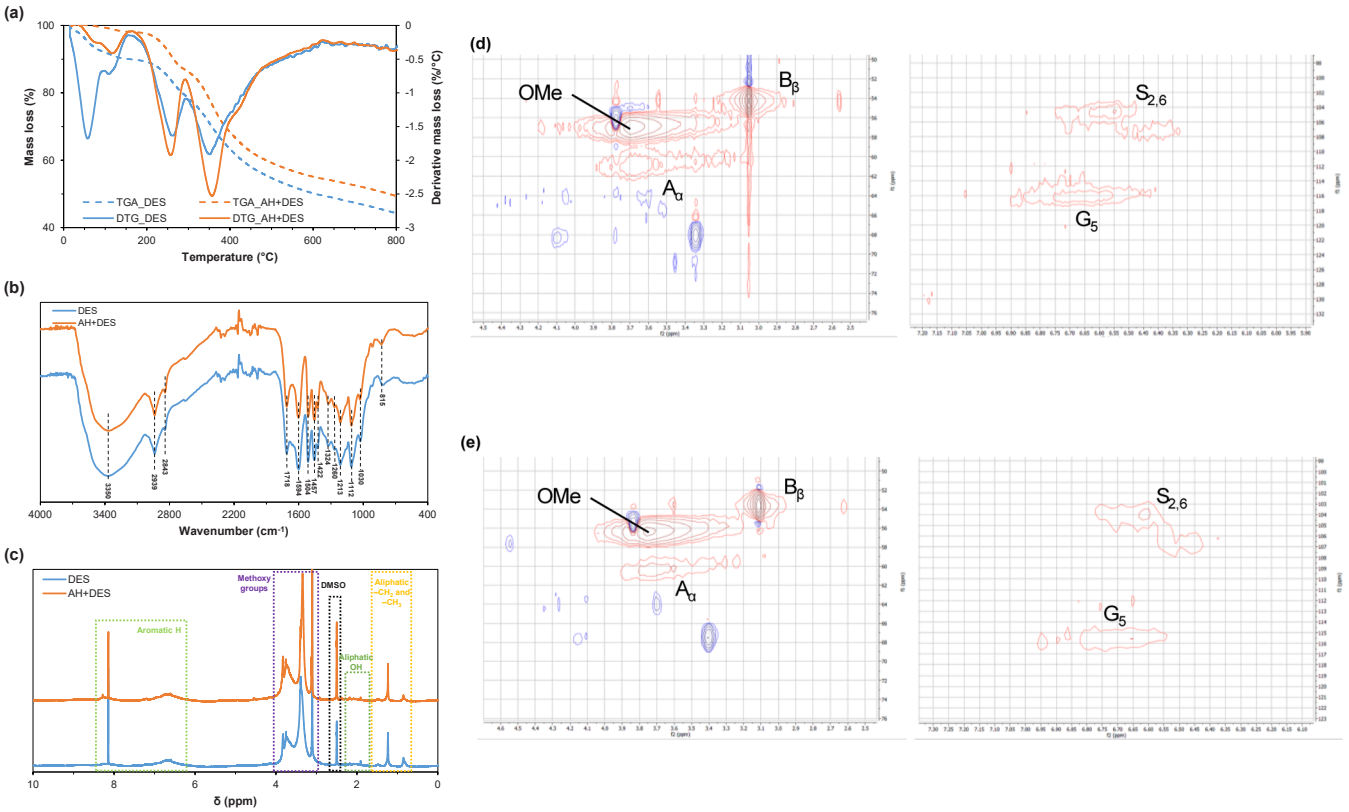

Fig. 4. Lignin characterization obtained after delignification with DES (ChCl:FA, 1:5) of raw (DES) and autohydrolyzed (AH+DES) vine shoots, comprising: (a) TGA, (b) FTIR, (c) ^{1}H NMR, and 2D HSQC of (d) DES and (e) AH+ DES.

and C–O deformation of secondary alcohols and aliphatic esters. The syringyl ring specific vibrations, C–O stretching and C–H in-plane/out-of-plane deformations are at 1324, 1112 and 815 cm^{-1} (Dávila et al., 2019; Rodríguez-Rebelo et al., 2024), whereas guaiacyl units are identified by bands at 1213 cm^{-1} (Dávila et al., 2019) and 1260 cm^{-1} (Sammons et al., 2013), corresponding with the ring breathing and C-O stretch, respectively. The oxidation of side-chain hydroxy groups and possible impurities are indicated by the band at 1718 cm^{-1} (Sammons et al., 2013). The 2835–2941 cm^{-1} region reveals C–H stretching (CH_3, CH_2 and CH) by lignin as well as by polysaccharides, and the broad band at 3350 cm^{-1} is ascribable to O–H stretching vibrations (Dávila et al., 2019; Rodríguez-Rebelo et al., 2024).

The ^{1}H NMR spectra (see Fig. 4c) reflected analogous chemical shifts for both lignin samples, indicating the presence of aliphatic groups (-CH2 and -CH3) at δH values of 0.9, 1.2 and 1.5 ppm, whereas small peaks of aliphatic hydroxyl groups were found between 1.6 and 2.4 ppm. On the other hand, several different peaks attributed to methoxy groups can be observed between 3.1 and 3.9 ppm. Finally, only a small amount of aromatic hydrogen is detected within the range of 6.4–7.4 ppm (Ee et al., 2023; Soares et al., 2020).

Fig. 4d-e represent the 2D HSQC NMR spectra of VS lignins displaying the main structural features typically described for hardwood lignins. In the side-chain region (δC 50–80/δH 2.5–5.0 ppm), a clear resonance at δC ~55.4/δH ~3.8 ppm confirmed the presence of methoxyl groups, which are characteristic of syringyl and guaiacyl units, in both lignin samples (Cortés-Triviño et al., 2024). Other characteristic signals detected in the aliphatic-oxigenated area were attributed to β-O-4 linkages, which are the most common interunit structures in native lignins (Kim and Ralph, 2010), indicating the presence of A_γ (δC 61.0/δH 3.7 ppm) and resinol linkages-B_β (δC 54.3/δH 3.1 ppm) (Rodríguez-Rebelo et al., 2025). Besides, the aromatic/unsaturated area (δC 100–150/δH 6.0–8.0 ppm) was dominated by syringyl (S) units, identified through C2–H2 and C6–H6 correlations at δC ~104 ppm/δH ~6.6 ppm ($S_{2,6}$), together with guaiacyl (G) units showing C5–H5 (δC ~115–116 ppm/δH ~6.7 ppm) (G_5) (Cortés-Triviño et al., 2024). Conversely, the absence of signals in the polysaccharides region (δC 90–110/δH 3.5–6.0 ppm) (Kim and Ralph, 2010) added to the higher presence of well-defined G and S units revealed a high purity in the lignin samples and a low degree of condensation in the lignins (Rodríguez-Rebelo et al., 2025).

3.3. Overall balance of the two process configurations

Based on the results of this study, the eutectic mixture of choline chloride and formic acid at 130 °C for 60 min was identified as the most suitable condition for VS valorization, considering the high lignin removal achieved in both process configurations evaluated. Accordingly, mass balances were established for these selected conditions (Fig. 5). In the direct DES delignification (D6, Fig. 5a), 100 kg of vine shoots yielded 32.28 kg of delignified solids, while most hemicelluloses and lignin were solubilized into the black liquor. In contrast, the sequential configuration combining autohydrolysis and DES delignification (AD6, Fig. 5b) produced a slightly higher yield of delignified solids (36.27 kg) and enabled recovery of a hemicelluloses-rich autohydrolysis liquor containing mainly oligosaccharides. Lignin removal was substantially higher in D6 (final lignin of 5.41 kg) compared with AD6 (11.38 kg). Regarding glucan recovery, both strategies yielded substrate remained similar glucan amount (~20 %). Although glucan recovery was higher in D6 (68 %) in comparison with sequential pretreatments, 25 % of the glucan was recovered as glucooligosaccharides

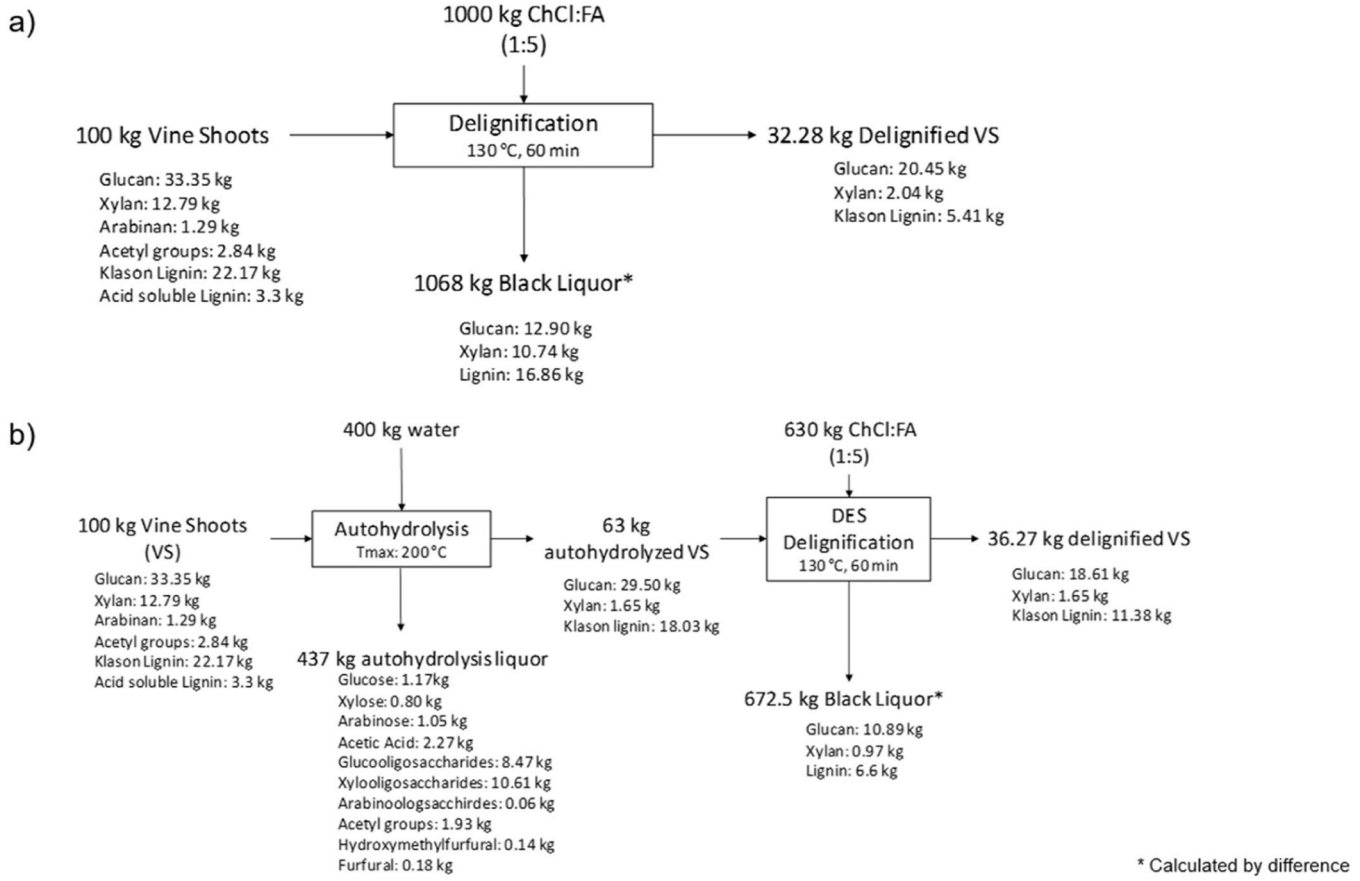

Fig. 5. Overall balance of VS processing by: a) DES delignification with choline chloride: formic acid (1:5) at 130 ºC for 60 min, and b) autohydrolysis at 200 ºC followed by DES delignification with choline chloride: formic acid (1:5) at 130 ºC for 60 min.

in the autohydrolysis liquor, while 48 % of the cellulose was retained in the solid phase. Overall, the AD6 strategy proved more effective within a biorefinery concept, as it generated three distinct and valuable product streams: cellulose-rich solids, hemicellulosic derivatives, and lignin. In this sense, recent techno-economic studies of several biorefinery scenarios have shown that hemicellulose valorization, particularly through the recovery of XOS, can significantly improve overall process feasibility and energy efficiency due to their high market value (Gomes et al., 2021; van Heerden et al., 2023). Nevertheless, for a complete evaluation of DES on biorefineries, the recovery and recycling of DES should be also taken into account (Lobato et al., 2023). In fact, this step is considered one of the most important challenges and a critical point in the reduction of operational costs (Contreras et al., 2023). The main strategies to recover DES from the target product (mainly lignin) involves the use an anti-solvent (such as water, acetone or ethanol) and subsequent evaporation of the anti-solvent for DES separation, which requires high energy consumption. In consequence, alternative methods such as membrane technology and liquid-liquid extraction have been also explored (Smink et al., 2020; Liang et al., 2023). Therefore, the economic feasibility of lignocellulosic biorefineries, the large-scale implementation of DES still depends on developing more efficient solvent recovery and recycling strategies.

The results obtained in this work highlight the advantages of the sequential autohydrolysis-DES configuration (AD6) compared with direct DES delignification (D6), achieving performance comparable to that reported in the literature. While single-step alkaline, organosolv, or DES treatments can achieve significant delignification, they often require harsher conditions, longer residence times, or the use of less sustainable reagents (Argun and Onaran, 2015; Cañadas et al., 2024; Cardoza et al., 2024). On the hand, several cascade biorefinery strategies have been reported in the literature, combining hydrothermal, chemical, or organosolv treatments with subsequent delignification steps. For instance, Dávila et al. (2019) applied hydrothermal treatment followed by simultaneous saccharification and fermentation (SSF) and alkaline delignification, achieving 65.2 % delignification and ethanol production of 13.3 g/L. Senila et al. (2020) combined autohydrolysis with acid chlorite delignification, obtaining 80 % delignification but requiring long reaction times and corrosive reagents. Cardoza et al. (2024) reported a two-step acid and organosolv sequence with high carbohydrate recovery, though delignification was limited to 43.4 %.

4. Conclusions

Under the conditions evaluated in this study, the use of ChCl:FA (1:5) resulted in glucan recovery higher than 68 % compared with eutectic mixtures containing acetic acid or lactic acid. A pretreatment time of 60 min and 130 ºC led to higher lignin solubilization (nearly 12 % in D6 and AD6) than shorter treatments (30 min, in D7 and AD7). Although single-step pretreatment achieved higher lignin removal (78 %, D6), the sequential approach, combining hydrothermal treatment with DES delignification, proved more effective within a biorefinery framework. This strategy enabled (i) milder operating conditions, (ii) approximately 50 % delignification efficiency, and (iii) multiproduct recovery, including oligosaccharides and high-purity lignin. Overall, DES-based delignification coupled with autohydrolysis demonstrated high potential for integration into industrial biorefineries.

CRediT authorship contribution statement

Beatriz Gullón: Writing – review & editing, Funding acquisition, Formal analysis, Conceptualization. **Romani Aloia:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization. **Meirielly Jesus:** Writing – review & editing, Formal analysis. **Alexandre Rubira:** Writing – original draft, Methodology, Formal analysis. **Pablo G. del Río:** Writing – review & editing, Writing – original draft, Methodology.

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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Data availability

Data will be made available on request.

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