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ORIGINAL ARTICLE

Synergy of ball milling, microwave irradiation, and deep eutectic solvents for a rapid and selective delignification: walnut shells as model for lignin-enriched recalcitrant biomass

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Abstract
The combination of ball milling (BM), microwave irradiation (MI), and deep eutectic solvents (DES) results synergistic for an efficient, selective, and very rapid (10 min) delignification of materials with high lignin content (ca. 50 wt%) such as walnut shells (WS). Lignin is dissolved in the DES, whereas the polysaccharide fractions remain suspended with limited degradation, due to the rapid pretreatment. After ball milling procedure (3 h), biomass loadings in the range of 100–200 g L^{-1} are selectively delignified in 10 min at 150 °C by using choline chloride:formic acid DES (1:2 molar ratio), rendering lignin yields of 60–80% (ca. ~ 40–60 g lignin L^{-1}). Ball milling, microwave irradiation, and DES systems are much more efficient than ball milling, conventional heating, and DES system. The obtained lignins exhibited similar Fourier-transform infrared spectroscopy (FTIR) profile to that of milled wood lignin (MWL), indicating minimal functional group changes.

Keywords Walnut shell · Deep eutectic solvents · Ball milling · Microwave processes · Lignin

1 Introduction

Lignin, a major biopolymer in lignocellulosic biomass, may serve for the production of aromatic chemicals and BTX-fuels in biorefineries (Scheme 1). Under oxidative conditions, highly functionalized valuable compounds, such as vanillin, are produced from lignin. Under reductive conditions, alkylphenolics and BTX fractions predominate [1]. Alkylphenols, including 4-propylphenol, *p*-cresol, 4-propylphenol, and 4-ethylphenol, are relevant building blocks utilized in the production of surfactants, synthetic resins, and a variety of other specialty chemicals [2]. Valorization of lignocellulosic biomass via an integrated biorefining process has rekindled interest in environmentally friendly and sustainable chemistry [3, 4]. Walnut shells (WS) are typically discarded as agricultural feedstock or used in low-value, large-volume applications such as abrasives and heating. Walnuts (in shells, WS) are produced in ca. 3.8 million metric tons annually [5]. With a high lignin content (49–52%) [6, 7], WS have the potential to be an appealing raw material, particularly when it comes to "lignin-first" biorefinery concepts, in which the potential of lignin is put forefront, and prior to the subsequent valorization of the polysaccharide fractions [8]. For instance, the viability of lignin biorefineries was evaluated by process simulation utilizing 700 t/y of kraft lignin as feedstock, where 0.3 kg aromatic/kg net lignin was attainable. At a 10% interest rate assumption on a 10-year plant lifespan, an encouraging 14% return on investment could be generated [9, 10]. In more classic approaches for biomass valorization, emphasis lies on the obtention of fermentable sugar streams for biofuel production, while lignin is underutilized (e.g., combustion) [11, 12]. Importantly, lignin, an integral component of the cell wall of plant fibers [13], remains elusive to be extracted in high purity while preserving the native structure [14]. Pretreatment

Highlights
- High lignin yield of 60–80%.
- Formic acid:choline chloride DES was most efficient.
- Workable DES system at 20 wt%.
- Efficient lignin deconstruction in 10 min.

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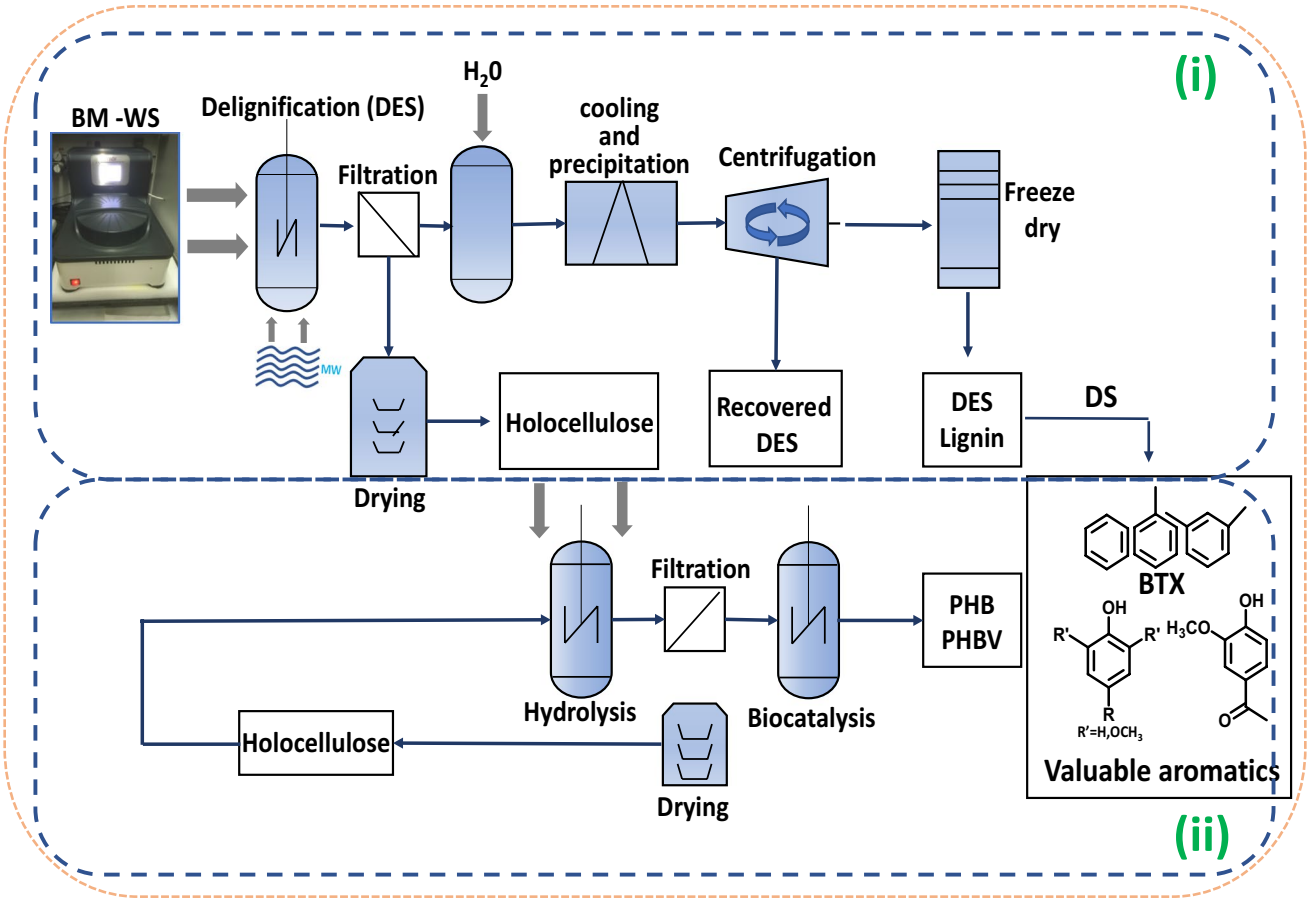

Scheme 1 Envisioned flow to integrate a synergistic multiple pretreatment process as biorefinery, using WS as prototypical biomass with high recalcitrance due to their high lignin content (route [i]). The polysaccharide fractions are envisioned as fermentable sugars to produce polyhydroxybutyrate, but other valorization routes can be considered as well (route [ii]). DS, downstream process; BM, planetary ball mill; WS, walnut shell; MW, microwave; BM, ball milling; DES, deep eutectic solvent; PHB, polyhydroxy butyrate

techniques to deconstruct biomass include steam explosion, diluted acid, ionic liquids, and alkaline-assisted hydrolysis. The recently introduced OrganoCat approach aims at processing lignocellulose while addressing environmental concerns associated with other methods [15].

When emphasis is put on a (non-reductive) "lignin-first" approach, pretreatments for selective delignification, while leaving the polysaccharide fractions as intact as possible, are desired. Recently, deep eutectic solvents (DES) derived from the combination of hydrogen-bond donors (HBDs) and hydrogen-bond acceptors (HBAs) have garnered interest in biomass delignification [11, 16], being DES typically cost-effective, recyclable, and biodegradable solvents [17–20]. Also, lignin recovery from DES has received some significant attention with several techniques such as anti-solvent addition, crystallization, membrane filtration, solid–liquid extraction, liquid–liquid extraction, short path distillation, supercritical fluid extraction, or separation due to density differences [21]. The choice of a recovery method depends ultimately on other considerations such as the qualities of the DES, the characteristics of the target compound/product, the amount of energy required, the type of the conversion/extraction process, and the cost of the equipment [21]. DES trigger the partial cleavage of ether bonds among lignin phenylpropane units, contributing to the depolymerization [22], and generating narrower molecular weight lignin distributions, while maintaining many structural bonds of native lignin. Jablonsky et al. successfully employed several DES such as lactic acid:alanine (9:1 molar ratio), lactic acid:glycine (9:1 molar ratio), lactic acid:betaine (2:1 molar ratio), choline chloride:ethylene glycol (1:2 molar ratio), or choline chloride:glycolic acid (1:3 molar ratio) at 60 °C for 24 h on beechwood and straw. Of all of them, lactic acid:alanine (9:1 molar ratio) and choline chloride:glycolic acid (1:3 molar ratio) DES resulted the most efficient ones for broad delignification [23]. Recently, higher biomass loadings (20 wt% and 27 wt%) in an acidified choline chloride:ethylene glycol (1:2 molar ration) DES system showed that lignin removal was

affected with by the biomass loading [24]. In another example, three DES, namely formic acid:choline chloride (FA:CC), lactic acid:choline chloride (LA:CC), and acetic acid:choline chloride (AA:CC) were successfully investigated on the lignin removal of herbaceous biomass [25]. In general, the lignin yield and purity depended on the reaction temperature, (long) residence times, DES composition, and type of biomass.

It must be noted, however, that the delignification of other more recalcitrant (but potentially useful) derivatives containing higher lignin contents (e.g., walnut shells), have been scarcely assessed with DES [26]. Those derivatives are typically non-edible and present a high energy density, which may be advantageous for future second-generation biorefineries [26]. Envisioning the need for more efficient approaches using DES for delignification, and considering the potential of valorizing recalcitrant materials, this paper explores strategies with largely diminished residence times (ultimately, reducing them to a matter of minutes), with relevant biomass loadings (100–200 g L^{-1}), and valorizing raw materials with high lignin content (WS), see Scheme 1 (route i). If successful, those approaches may pave the way for continuous delignification processes, which could certainly improve the economic figures and overall sustainability. To that end, a combination of a ball milling (BM) step, together with microwave irradiation (MI), and DES-mediated delignification is demonstrated (Scheme 1). To the best of our knowledge, the joint combination of BM with MI in DES has never been assessed in the area, and only one paper describes, with that triple combination, the separation of polyprenyl acetates from *Gingko biloba* leaves [27]. Mechanical approaches can improve performance in biomass pretreatment [28–30]. With respect to the use of MI with DES, some publications evidence the residence time reduction, yet typically using lower biomass loadings [31, 32], and materials with less lignin contents. MI has the following advantages over conventional heating during the pretreatment: (i) heating is non-contact, and volumetric; (ii) energy is transferred; (iii) energy is conserved; (iv) heating is rapid and efficient; and (v) material is selectively heated [33].

2 Experimental section

Materials: Choline chloride (CC) (≥ 98% purity), formic acid (FA) (98% purity), DL lactic acid (98% purity), furfural (99% purity), and glycolic acid (GA) (99% purity), levulinic acid (98% purity), arabinose (≥ 99% purity), acetic acid (≥ 99% purity), D-Xylose (≥ 99% purity) were purchased from sigma Aldrich. Glucose (99% purity, Panreac), L-alanine (AL) (≥ 98% purity), and nylon filter were purchased from Scharlab.

2.1 Walnut shell preparation

The walnut used in this study was grown in Southern California. The shells were reduced using a small blade coffee blender (KunFT GTM-8803120 W, 30 g capacity). The NREL sample preparation approach was applied [34]. Subsequently, a knife blender (milling) was used in the comminution of walnut shell and segregated into assorted sizes by sieve. The sieve brand cisa was applied and arranged or placed in vertical position in this order. (top) 1 mm > 0.5 mm > 0.4 mm > 0.3 mm > 0.2 mm > 0.1 mm, and collector (bottom). Collector was placed at the bottom to collect fine powder samples. So, the sieve's mesh retained the appropriate walnut shell diameter while smaller ones passed through. WS were dried in an oven at 105 °C for 24 h until constant weight. A moisture content of less than 2% was obtained for all WS, which were kept in an airtight vial stored in a desiccator for further usage.

2.2 DES preparation

Each DES system was prepared by weighing the required quantity computed from their molar ratios. Each system was heated at 60 °C for 24 h. A clear mixture formed was cooled down to room temperature in desiccator. Three different DES systems were prepared in various molar ratios lactic acid:alanine (9:1), formic acid:choline chloride (2:1), and glycolic acid:choline chloride (3:2).

2.3 Procedure for WS delignification via DES

Extract-free WS of 10 wt% (100 g/1000 g) or 20 wt% (200 g/1000 g) were studied. The appropriate amount of WS of various diameters (0.1 mm, 0.3 mm, and 0.5 mm) was placed in a clear mixture and reacted at 60 °C, 90 °C, 120 °C, and 150 °C either with conventional heating (oil bath) or microwave reactor (milestone). Milestone synthWave microwave reactor controlled by panel with easyControl software was employed. It contains a close chamber that has the ability to maintain the temperature under consideration. Extractive free ball-milled WS were also tested at 60 °C, 90 °C, 120 °C, and 150 °C. In microwave heating, temperature was elevated from room temperature to 60 °C, 90 °C, 120 °C, and 150 °C with a power of 1000 W over 5 min to reach set-point temperature and held at set-point temperature for the appropriate residence time (either 5, 15, 35, and 55 min). 1000 W was used to reach the set-point temperature and it automatically dropped to 500–600 W until the reaction ended. After each reaction with DES-WS system, the system was cooled down. 0.5 mL of distilled water was added and stirred vigorously for 5 min and then filtered through nylon filter. Lignin was then precipitated from the liquid fraction by addition of 20 mL of distilled

water. The precipitated lignin was separated by centrifugation at 5000 rpm and subsequent decantation. The lignin was lyophilized for 24 h, and the yield (recovered lignin) was calculated as reported elsewhere [35]. The solid fraction was washed with distilled water until neutral pH was reached and dried at 105 °C for 16 h. The yield of residual solids was calculated as reported in the literature [35].

2.4 Biochemical analysis of solid residue

Soxhlet extraction was used to remove extractives, and ethanol-toluene (1:2, v/v) was utilized as the solvent in line with the American Society for Testing Materials (ASTM) D 1107–84 protocol. The pretreated WS residue was analyzed for carbohydrates and Klason lignin with protocol ASTM D 1106–84 [36]. Analysis was performed by quantitative hydrolysis of the residue in 72% wt H_2SO_4 at 30 °C for 60 min. The solution was then diluted to 4% wt H_2SO_4, further hydrolysis at 121 °C for 60 min in an autoclave. A sample of this acidic solution was filtered through a 0.45-mm nylon syringe filter. HPLC analyses were performed with an Agilent 1100 series chromatograph using an ICSep ICE-COREGEL 87H3 (Column serial n_12525124). The temperature of the column was 50 °C, and the mobile phase was a solution of 5 mM H_2SO_4 at a flow rate of 0.6 mL min^{-1}. An Agilent 1100-DAD ultraviolet, diode-array (UV) detector, and an Agilent 1100-RID refractive index (RI) detector were connected in series to quantify furfural, HMF, and carbohydrate/carboxylic acids respectively. Klason lignin content was estimated as the residue after sulfuric acid hydrolysis of the pre-extracted material, corrected for ash.

2.5 Milled wood lignin (MWL) and mild acid lignin (MAL)

MWL (milled wood lignin) was isolated with Björkman method [37]. For 24 h, the ball-milled wood sample was suspended in 96% dioxane at a solid-to-liquid ratio of 1:10 (g/mL) and stirred continuously at room temperature. Filtration and washing with the same solvents were performed until the filtrate was clear. These operations were carried out twice. MAL and purification steps were adapted from the literaure [38].

2.6 FTIR (Fourier transform infrared spectroscopy)

It was used to study and interpret the chemical changes that occurred in pretreated WS and lignin obtained. The FTIR spectra were recorded with a Jasco FT/IR-600 Plus equipped with ATR Specac Golden Gate, which directly measures the sample. The sample spectra were recorded with 32 scans using 2 cm^{-1} resolution. Baseline normalization of all spectra was carried out in origin 9 software and plotted.

2.7 ESEM (environmental scanning electron microscopy with Quanta 600) was used to evaluate the morphology of pretreated WS

Samples were sputtered with gold nanoparticles before the analysis. Images were taken at 3 kV.

2.8 Viscosity measurement

Calibrated Ubbelohde viscometer ($K = 0.09989$ cst/s, 10–100 mm^2/D) was applied to measure the kinematic viscosity of each DES. The viscometer was charged with 20 mL DES and placed in a temperature bath for 20 min to allow for DES to adjust the bath temperature. Three valve rubber suction bulb was applied to raise the DES to the small upper resevoir. The efflux time was recorded when the DES traveled between the set-marked distances. Kinematic viscosity (v) = C*t, where C denotes the viscometer constant. t denotes flux time. The density of each DES was measured using a pycnometer at different temperatures (25 °C, 60 °C, and 90 °C). The dynamic viscosity was calculated from the relation $v = \eta / \rho$, where v is kinematic viscosity, η is dynamic viscosity and ρ is density.

3 Results and discussion

The first set of experiments was conducted to evaluate the viscosity of the different DES. The diffusivity of a solvent is critical to the process's efficiency in biomass deconstruction, and thus it is desirable to use DES with low viscosity. Several DES were assessed (Table 1): GACC (glycolic acid:choline chloride 3:1 molar ratio) exerted the highest viscosity (326 cP at 25 °C), while FACC (formic acid:choline chloride 2:1 molar ratio) had the lowest one (26 cP at 25 °C). At 90 °C, all DES had viscosities less than 20 cP (see Fig. 1a), creating potential as delignifyers because DES possess less viscosity with increasing ion mobility, consistent with the literature [39–41]. Subsequently, a screening of WS with different particle sizes (0.1–0.5 mm) in the three DES was conducted at several processing times (8 h, 16 h, and 24 h) at 60 °C and 10 wt% WS loadings (Fig. 1b-d). All experiments were conducted with conventional heating. Once the delignification occurred, the work-up was performed by adding first 0.5 mL water (to 10 mL of reaction media), to reduce the viscosity and facilitate filtration—recovering the suspended polysaccharide fractions—, and then 20 mL of deionized water were added to the filtered DES liquid (ca. 5.5–7.0 mL, [Fig S1) to precipitate the lignin. All recovered lignin are referred to as DES-lignin while lignin quantified from solid residue after DES treatment is refered to as acid-insoluble lignin (AIL). As observed (Fig. 1b–c), in all cases delignification occurred, regardless of the DES and the reaction time. The

Table 1 Experiments performed with WS in different DES and conditions, including conventional heating, microwave irradiation, and ball milling. Processing time varied from hours to just minutes, when synergies appear and processing fast results (BM + MI + DES)

DES	Temp (°C)	**Time (minutes)**	Heating source	Acronym
Lactic acid: alanine (LAAL)	60	480 min (8 h)	Conventional	LAAL-8
Formic acid: choline chloride (FACC)	60	480 min (8 h)	Conventional	FACC-8
Glycolic acid: choline chloride (GACC)	60	480 min (8 h)	Conventional	GACC-8
Lactic acid: choline chloride (LAAL)	60	960 min (16 h)	Conventional	LAAL-1
Formic acid: choline chloride (FACC)	60	960 min (16 h)	Conventional	FACC-16
Glycolic acid: choline chloride (GACC)	60	960 min (16 h)	Conventional	GACC-16
Lactic acid: alanine (LAAL)	60	1440 min (24 h)	Conventional	LAAL-24
Formic acid: choline chloride (FACC)	60	1440 min (24 h)	Conventional	FACC-24
Glycolic acid: choline chloride (GACC)	60	1440 min (24 h)	Conventional	GACC-24
Formic acid: choline chloride (FACC)	60	60 min	Microwave	M6060
Formic acid: choline chloride (FACC)	90	20 min	Microwave and ball milled	M(B)9020
Formic acid: choline chloride (FACC)	90	40 min	Microwave and ball milled	M(B)9040
Formic acid: choline chloride (FACC)	90	60 min	Microwave and ball milled	M(B)9060
Formic acid: choline chloride (FACC)	90	20 min	Conventional and ball milled	C(B)9020
Formic acid: choline chloride (FACC)	90	40 min	Conventional and ball milled	C(B)9040
Formic acid: choline chloride (FACC)	90	60 min	Conventional and ball milled	C(B)9060
Formic acid: choline chloride (FACC)	120	10 min	Microwave and ball milled	M(B)12,010
Formic acid: choline chloride (FACC)	150	10 min	Microwave and ball milled	M(B)15,010

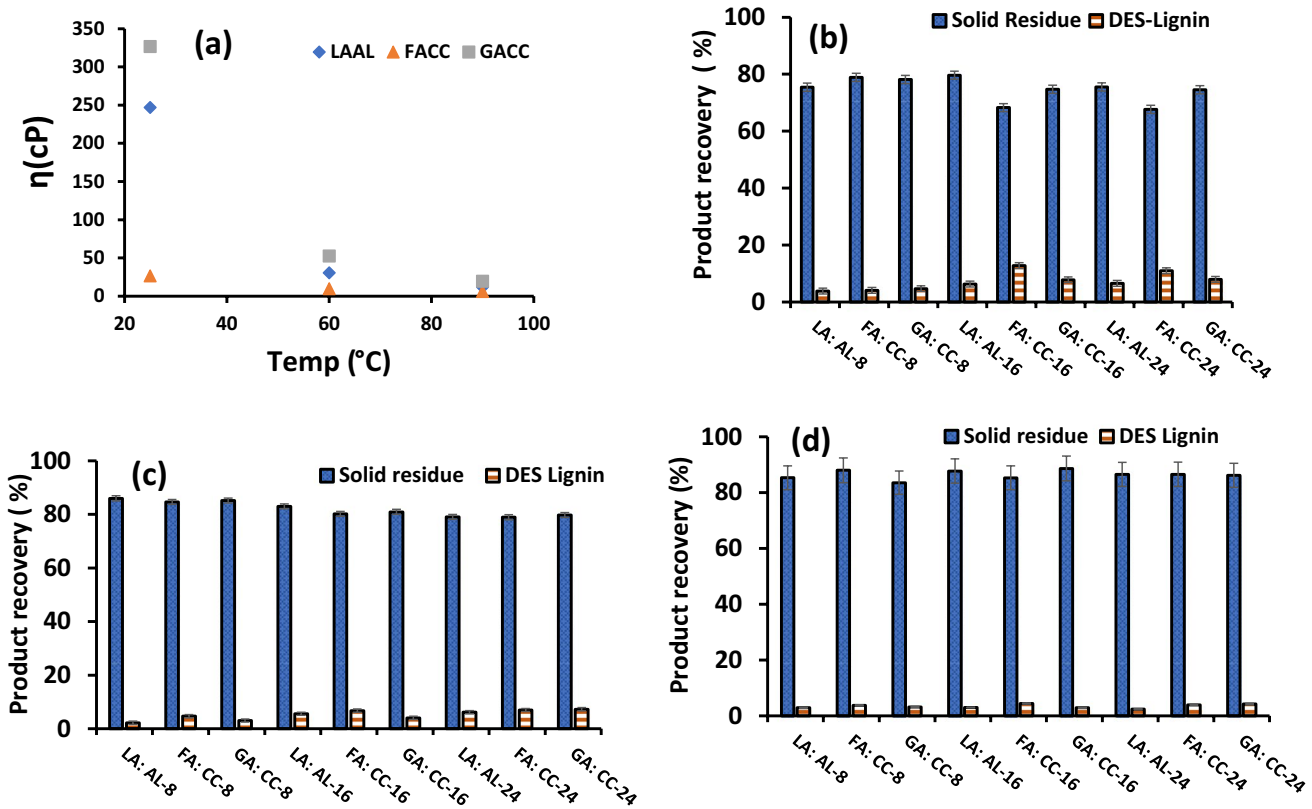

Fig. 1 **a** DES viscosity profiles as a function of temperature. **b–d** DES lignin (recovered) yield and solid residue upon delignification with DES. **b** 0.1 mm. **c** 0.3 mm. **d** 0.5 mm. Constant temperature (60 °C) and different processing times (8 h, 16 h, and 24 h)

best results were obtained with WS of 0.1 mm operating under FACC (formic acid:choline chloride) DES, yielding 13.6% lignin (ca. 27% of the original lignin content OLC) (Fig. 1b). Conversely, lower lignin yields were obtained for 0.5 mm WS operated under LAAL system (Fig. 1d). A higher lignin yield was obtained with WS with a diameter of 0.1 mm, presumably due to the high surface-to-solvent contact ratio compared to the 0.5-mm WS-DES system with the lowest surface-to-solvent contact ratio. Thus, subsequent experiments were conducted with WS of 0.1 mm.

WS were then subjected to planetary ball milling for 3 h at a rotational speed of 600 rpm and were then treated with different DES at varying residence times (8, 16, and 24 h) (Fig. 2).

When ball-milled WS was treated with FACC DES at 60 °C using conventional heating, lignin yield of 19.8% (ca. 40% of OLC) after 8 h was achieved (Fig. 2). The surface-to-solvent ratio was not the only factor improving lignin yield, but also the DES and the mechanical disruption of molecular structure in biomass (WS) caused by ball milling [42]. Other authors have reported no significant cleavage of β-O-4 bonds upon 48 h of only planetary ball milling of different lignocellulosic biomass [43]. FACC and GACC contain monocarboxylic acids with different alkyl chain lengths, modulating the solvent's acidic strength that may determine the efficiency of DES in the delignification [44]. Thus, the weak acidity of GACC DES might lead to low lignin yields compared to the lignin yield of FACC-DES (Fig. 2). LAAL DES led to a lignin yield of ca.17% (ca.34.0% OLC), which is comparable to GACC's yield of 16% (ca. 32% OLC) after 8 h at 60 °C, (Fig. 2). When the reaction time was increased to 16 h and 24 h, no significant improvement in lignin yield was observed in any of the DES. Therefore, the focus of subsequent experiments was put on temperature increment with the shortest reaction time possible. FACC was selected as promising prototypical DES for further investigations. At 90 °C, experiments were conducted at 20, 40, and 60 min on both non-milled and milled WS. At all processing times, the ball-milled derivative was much more processable than untreated samples, demonstrating the utility of mechanical approaches for delignification. Ball milling of walnut shell caused disruption of biomass bond architecture thus making it less crystalline and enabling the accessibility of mobile ion to cleave bonds (see also Sect. 3.3). For instance, lignin yield reached 15.8% (ca.32% OLC) after 60 min of reaction of ball-milled WS (conventional heating + ball milled + 90 °C + 60 min [CB9060]) while non-ball-milled WS (conventional + 90 °C + 60 min [C9060]) yielded 11.2% of lignin (ca. 22% of OLC) (Fig. 3).

Having successfully tested the use of DES with BM—showing a consistent improved delignification (Fig. 3)—, subsequent experiments focused on incorporating microwave irradiation instead of conventional heating. Experiments were performed at 60 °C, 90 °C, 120 °C, and 150 °C using FACC-DES coupled with microwave heating, and/or incorporating ball milling (Fig. 4).

Preliminary, the effect of the microwave was examined at 60 °C (Fig. 4a), illustrating the synergy created with BM, MI, and DES. Thus, the ball-milled WS and microwave-assisted DES system led to ca. twofold higher lignin yield than when conventional heating was applied (ca.14% vs. 7%). Results with microwave at 60 °C and 1 h were comparable to the obtained with the FACC-16-h system (ca. 14%, Fig. 1b). The temperature was then increased up to 90 °C, and reaction times of 20, 40, and 60 min were set, using both milled and non-milled WS (Fig. 4b). The results show the improved performance (delignification) when BM and MI are applied together with DES. For example, after only 40 min of reaction at 90 °C with microwave heating, microwave in combination with ball milling (MB9040) yielded

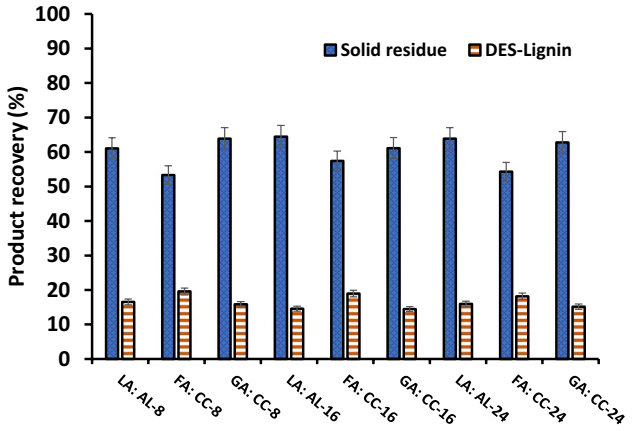

Fig. 2 DES-lignin (recovered) yield and solid residue of ball-milled WS at 60 °C for 8 h, 16 h, and 24 h. Conventional heating was applied in all cases

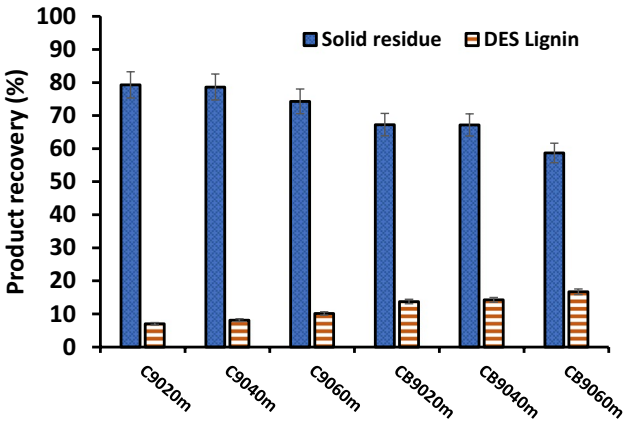

Fig. 3 DES-lignin (recovered) yield and solid residue of ball-milled and unmilled WS at 90 °C for 20, 40, and 60 min. Conventional heating was applied in all experiments

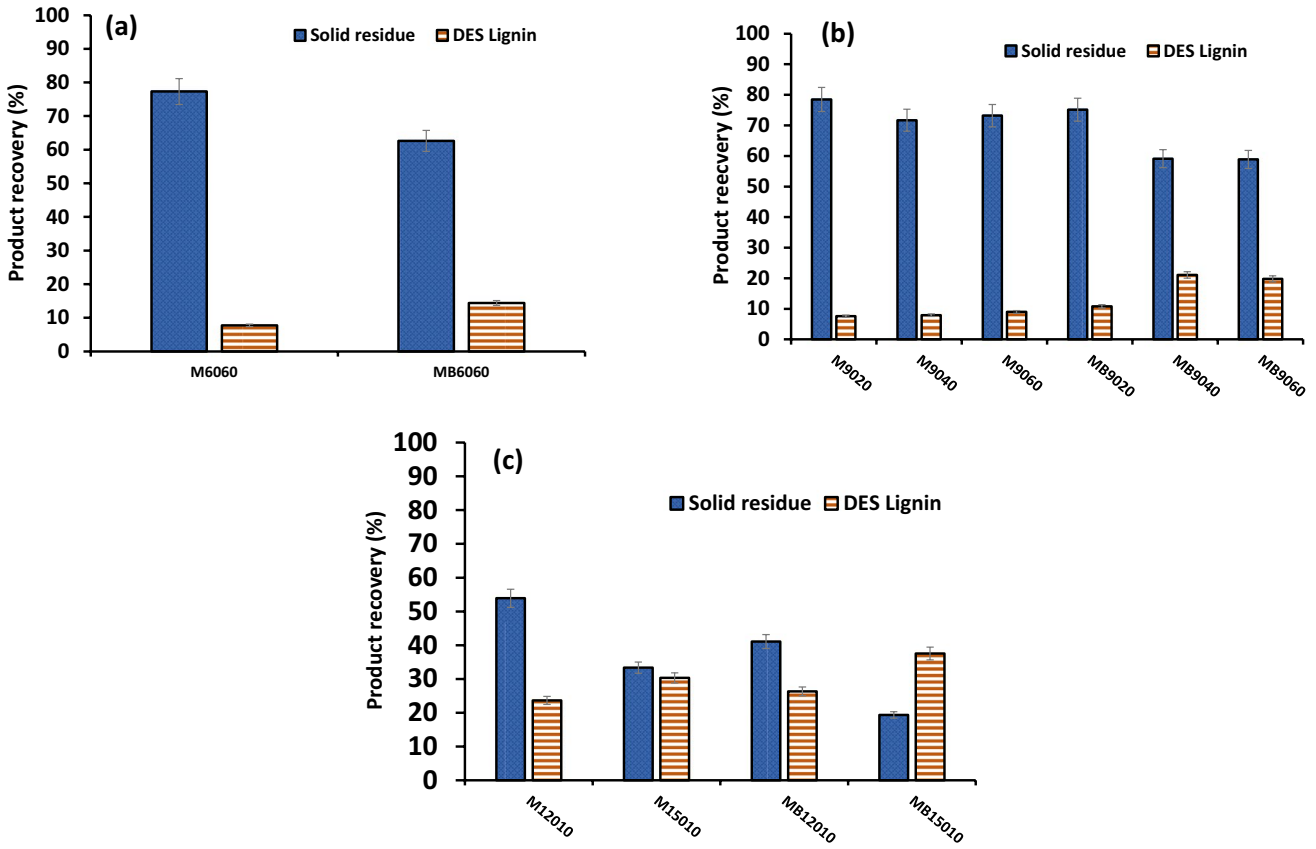

Fig. 4 DES-lignin (recovered) yield and solid residue. (**a**) MI at 60 °C, 60 min, with or without ball milling (denoted by the "B"). (**b**) MI at 90 °C, 20–60 min, with or without ball milling (denoted by the "B"). (**c**) MI at 120 °C and 150 °C for 10 min and loading capacity of 10 wt%, with or without ball milling (denoted by the "B")

24.2% lignin (ca 50% of OLC) (Fig. 4b). Stimulated with the promising results, the processing temperature was elevated to examine its impact on lignin yield, and entries at 120 and 150 °C were recorded, while reducing residence times to 10 min (Fig. 4c). MB15010 (ball milling + microwave + 150 °C + 10 min) led to 37.7% lignin yield (ca 75.4% OLC at a conversion rate of 100% of the original 49.2% lignin content). DES treatment of ball-milled WS at 120 °C for 10 min, yielded 27.9% lignin (ca. 56% OLC). Lignin yields were lower for non-ball-milled WS (Fig. 4c).

When water was added to the DES, high-purity lignin was precipitated, as reported by other groups [45]. Triggered by the promising concept which enables extensive delignification of materials with high lignin content, we decided to increase the WS loadings up to 20 wt% (200 g L^{-1}), to further explore the potential (or limitations) of the strategy (Fig. 5).

As depicted (Fig. 5), after 10 min of reaction at 150 °C with ball-milled WS, a DES-lignin yield of 31.7% (ca. 63% OLC) was obtained, which represents a production of ~ 60 g Lignin L^{-1} (under non-optimized conditions). By comparison, WS that had not been ball milled yielded 15.4% of DES

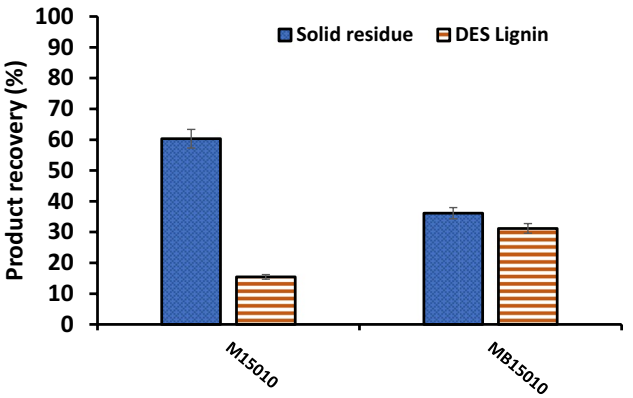

Fig. 5 DES-lignin (recovered) yield and solid residue obtained when using MI at 150 °C for 10 min and loading capacity of 20 wt%. Comparison between ball-milled and unmilled samples

lignin (ca. 30% OLC) (Fig. 5). The observed large delignification at relatively high biomass loadings (20 wt%) further reinforces the potential of the synergy for processing recalcitrant biomasses with high lignin content. The low residence time indicates that probably the set-up of continuous

delignification processes is feasible, which could significantly improve yields and environmental impact of the pretreatment. At higher biomass contents (40 wt%), however, the delignification was not successful as too much solid content was present in the DES-biomass mixture (Fig. S2).

3.1 Analysis of solid residue and composition

The solid residue was analyzed to determine the proportion of the remaining biopolymers (glucan, xylan, and acid-insoluble lignin [AIL]). Both conventional and microwave processes on ball-milled WS resulted in a markedly reduced level of AIL (Fig. 6), fully consistent with a large delignification, when the synergy BM + MI + DES was applied for 10 min. All ball-milled DES-system exhited increased acid-soluble lignin (ASL) (Fig. S4), indicating the impact of milling on the physical distruption WS molecular structure.

Even though the recovered lignin from DES reached 75.4% (OLC), some lignin losses were observed, as also reported elsewhere when choline chloride:oxalic acid DES was applied in the delignification of rice straw [46]. Herein, a trade-off between reaction time and temperature with respect to lignin yield may be established, as it has been shown for *Eucalyptus* biomass with choline chloride:lactic acid (1:2 molar ratio) at 100 °C for various reaction times [47].

The glucan and xylan fractions were analyzed as well. The proportion of both polysaccharides in the original material resulted ca. 1:1 (Table S1, and Fig. 6). This profile remained when delignifications at short reaction times (10 min) were conducted (Fig. 6). However, different glucan-xylan proportions were obtained at longer reaction times (Fig. S3). The glucan yields of non-ball-milled WS with microwave irradiation at 90 °C at 20, 40, and 60 min remained at 39–41%, analogous to the profiles of non-ball-milled WS (38–44%). Likewise, xylan yields of these fractions resulted similar as well, 20–22% for all samples either with microwave irradiation or with conventional heating (Fig. S3). Overall, it leads to a glucan/xylan proportion of 2:1, which suggests a partial degradation of the xylan fraction (which is amorphous and more prone to acid-mediated depolymerization at high temperature and longer residence times). The proportion of glucan/xylan was further enhanced when ball milling was incorporated to the delignification process. Thus, all samples that were ball milled led to glucan amounts of 70% at lower reaction times (20–40 min), decreasing to 55% when 60 min were applied with conventional heating (this effect was not so significantly observed for MI samples). At longer times, glucose is also released and dehydrated to HMF. Likewise, xylan proportions remained constant at 20–22% leading to glucan/xylan proportions of 3:1 (Fig. S3). A plausible explanation is the degradation of polysaccharides (hydrolysis, dehydration) at high temperature, long residence time, and acidic media, which will be more severe when ball milling is applied (as it decreases the cellulose crystallinity). To shed light on this, the crystallinity index of different samples (at 90 °C and 60 min, with or without ball milling and microwave) was assessed (Fig. S7). After 3 h of BM WS, the CrI index for RawW decreased by 25.21%. On the other hand, pretreated residue from non-ball-milled WS exhibited a higher crystallinity than residue from ball-milled WS (Fig. S7a). This demonstrates that the low crystallinity of ball-milled WS contributes significantly to the increase in lignin yield, observed with both microwave and conventional heating at mild temperatures of 60 °C and 90 °C.

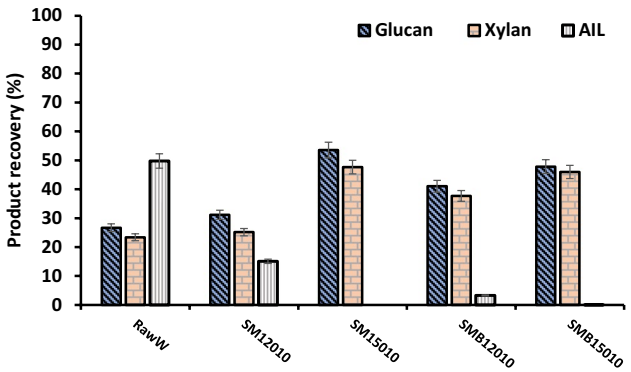

Fig. 6 Biopolymer profile solid residue after microwave heating at 120 °C and 150 °C for 10 min and loading capacity of 10 wt%. AIL is reduced depending on the severity of the delignification, while polysaccharide fractions remain constant if no significant degradation of them proceeds. SM, solid material

The glycan/xylan proportion remained 1:1 when higher temperatures were set (120 or 150 °C) at lower reaction times (10 min). Therefore, the synergy of BM + MI + DES allows not only large delignification, but also a limited degradation of the polysaccharide fractions, due to the low reaction times applied (Fig. 6). This aspect may have profound implications for future biorefineries, as all raw materials can be rapidly obtained with high purity and with high biomass loadings. Surely, a compromise could be found, by optimizing aspects like the time for ball milling, microwave irradiation (time and power), residence time (in the range of minutes), and temperature (120–150 °C), to obtain high lignin yields with non-degraded polysaccharide fractions.

3.2 Fourier transform infrared (FTIR) spectroscopy

To gain a better understanding of the properties associated with the obtained lignin through the DES digestion, milled wood lignin (MWL) [37] and mild acid lignin (MAL) [38] were prepared from WS and used as controls in FTIR analysis (Fig. S5). The effect of BM and temperature on the various DES-lignins was monitored.

Major peaks were assigned based on previous research (Table S2) [48–52]. As observed (Fig. 7), MWL, MAL, and DES-lignins all exhibited significant absorption near 3460 cm^{-1} due to the O–H stretching vibrations of phenolic OH and aliphatic alcohol hydroxyl constituents. At 1754 cm^{-1}, the absorption band corresponds to the C = O stretching of carbonyl/ester constituents or unconjugated ketones [53]. The distinctive absorption bands at 1600 cm^{-1}, 1500 cm^{-1}, and 1452 cm^{-1} may be related to aromatic vibrations [54] detected in MWL, MAL, and all DES-lignins (see Fig. 7a, and b). At 1640 cm^{-1}, the absorption band is associated with the unconjugated carbonyl group's stretching vibrations [55] (Fig. 7a, b, c). MWL, MAL, and DES-lignins exhibited C–O breathing of the guaiacyl ring at 1254 cm^{-1} and in-plane aromatic C–C deformation vibration at 1028 cm^{-1} [51, 55]. At 1154 cm^{-1}, the band corresponded to ether linkages. There are few differences in the absorption bands of some functional groups identified in MWL, MAL, and DES-lignin when compared to published values, which could be due to variation in the WS composition and DES used [45, 55]. A thorough examination of MWL and MAL revealed that MWL had a negligible absorption fingerprint at 1754 cm^{-1}, whereas MAL had a discernible absorption at 1754 cm^{-1} (see Fig. 7a, b, c). The absorption band at 1640 cm^{-1} exhibited a wide range of intensity variations depending on the reaction conditions and system under consideration. 1640 cm^{-1} absorption band was visible in both MWL and MAL, as well as in all ball-milled WS that yielded lignins (MB9060, MB9040, MB9020, CB9060, CB9040, and CB9060), but was absent in lignins (M9020, M9040, M9060, C9020, C9040, and C9060) obtained from non-ball-milled WS using both microwave and conventional heating (Fig. 7a and b). When the reaction temperature was increased to 120 °C and 150 °C, both milled- and non-milled-WS DES-lignins exhibited a strong absorption band of the conjugated carbonyl vibrations at 1640 cm^{-1}. In those cases, lignins seemed to retain their conjugated carbonyl vibrations without any adverse effects from DES presumably due to the short processing times applied. Also other authors reported the disappearance of the absorption band 1640 cm^{-1} following DES pretreatment

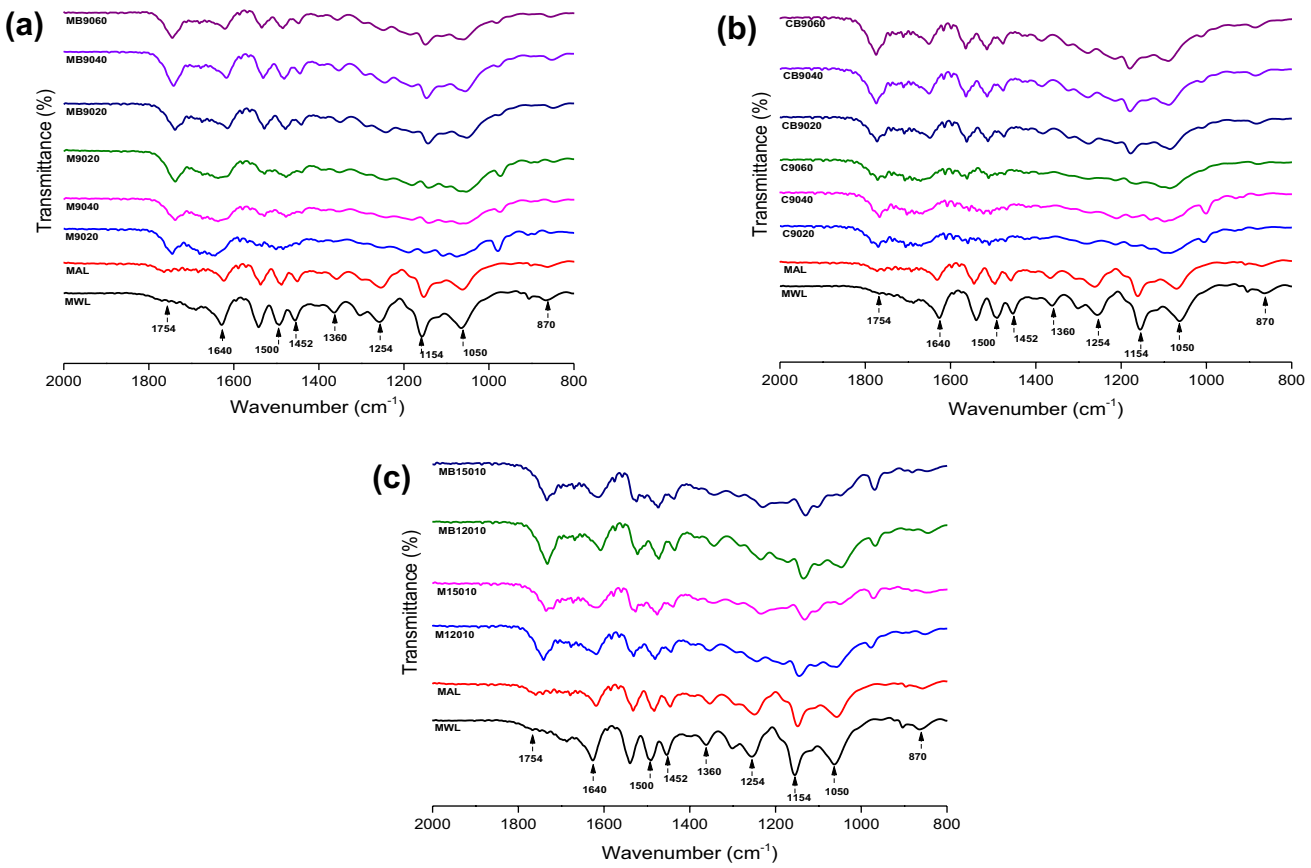

Fig. 7 FTIR of MWL, MAL, and DES lignins. (**a**) Recovered after 90 °C on ball-milled and un-milled WS with microwave irradiation. (**b**) Recovered after 90 °C reaction temperature on ball-milled and un-milled WS with conventional heating. (**c**) Recovered after 120 °C and 150 °C on ball-milled and un-milled WS microwave heating

of non-ball-milled poplar wood after 6 h of processing [45]. This discrepancy could be explained by either the biomass type or the DES type and composition, as well as the residence time ([h] vs. [min]). All lignins obtained after DES pretreatment exhibited an intense absorption band at 1754 cm^{-1}, originating from conjugated carbonyl (see Fig. 7a, b, and c), which is found in the literature [45]. At 1154 cm^{-1}, ether linkages appear to remain defined in lignins obtained from ball-milled WS at 90 °C with microwave and conventional heating sources. At 120 and 150 °C, both ball-milled and non-ball-milled WS DES-lignins exhibited a distinct and pronounced absorption band at 1154 cm^{-1}. This finding reveals the presence of β-O-4 linkages, which could serve as an entry point for lignin valorization [56]. At 1254 cm^{-1}, an intense guaiacyl ring absorption was detected in MWL and MAL while the intensity of this absorption band was moderate in DES-lignin (MB9020-60, CB9020-60). Moreover, the absence of carbohydrate fingerprints at 1426 cm^{-1} and 1373 cm^{-1} attests to the purity of the lignin obtained, reflecting the selectivity of DES to delignify recalcitrant biomass [57]. Overall, the synergy of mechanical force and microwave irradiation at a mild temperature (90 °C) in the presence of FACC-DES resulted in lignins with analogous primary functional constituents to native lignin (MWL). At 120 °C and 150 °C, microwave-assisted FACC-DES delignification of non-ball-milled WS also resulted in neat lignin with similar functional constituents to MWL.

The FTIR analysis of the solid residue revealed a significant intensity of cellulose and hemicellulose at 1056 cm^{-1} for the C–O stretching vibration [57] (Fig. S6) (where SMB12010 denotes a solid recovered from pretreated ball-milled WS at 120 °C for 10 min with MI, SM15010 denotes a solid recovered from pretreated non-ball-milled WS at 150 °C for 10 min with MI, and SM12010 denotes a solid recovered from pretreated non-unball-milled WS at 120 °C for 10 min with microwave. OB denotes "only ball-milled WS", and RawW denotes "Untreated WS"). Guaiacyl ring breathing coupled with carbonyl stretching at 1256 cm^{-1} [58] was intense in OB, but decreased significantly in solid residues analyzed following DES pretreatment of both ball-milled and non-ball-milled WS except OB (Fig. S6a, S6b, and S6c). Overall, this indicates that lignin was largely removed during the pretreatment process. The aromatic skeletal vibration of lignin and the carbonyl/acetyl constituent of hemicellulose were detected in the solid residue at 1598 cm^{-1} and 1750 cm^{-1}, respectively (Fig. 7a–c). However, the absorption intensity at 1750 cm^{-1} was greater in OB, SM12010, SM15010, and SMB12010 than in RawW. Lignin's skeletal vibration at 1598 cm^{-1} was absent from all solid residues except OB and RawW. These observations at 1056 cm^{-1} and 1750 cm^{-1} confirm the presence of a carbohydrate-rich solid residue. Examination of OB and RawW revealed that mechanical forces significantly affected the WS. Following only ball milling (OB), the intensity of all major functional groups and fingerprint constituents increased (Fig. S6a-S6c).

3.3 Morphology of pretreated residue

Environmental scanning electron microscopy (ESEM) analysis of the solid residues SM9060, SMB9060, SC9060, SCB9060, SM12010, SMB12010, and SM15010 revealed that DES treatment altered the morphology of the cells compared to Raw WS and OB (only ball milled) (Fig. 8c, e, and g). The edges of the solid residues from conventional and microwave heating of WS that were not ball-milled demonstrated ridges and holes. Conversely, pretreated solid residue from ball-milled WS appeared to be as compact as raw WS, RawW (Fig. 8b, d, f, and h). Despite the compact morphology of ball-milled pretreated WS, XRD analysis and CrI calculations revealed that SCB9060 and SMB9060 have a lower crystallinity index than SC9060 and SM9060 (Fig. S7a, and S7b). As a result, it can be predicted that pretreated SCB9060 and SMB9060 residues will be more amenable to further enzymatic hydrolysis than SM9060 and SC9060 residues.

4 Conclusions

A synergistic concept incorporating ball milling (BM), microwave irradiation (MI), and DES has demonstrated the ability to delignify biomasses with high lignin content (WS) at biomass loadings in the range of 10–20 wt% in a matter of minutes (after 3 h of ball milling). Lignin yields of 37.7% (ca 75.4% of the original content, OLC) and 31.7% (ca. 63% OLC) were obtained for biomass loadings of 10 wt% and 20 wt%, respectively. The three investigated DES were able to delignify walnut shells, with FACC being the most effective. Lignin yields are in the same range of other studies assisted by microwave and performed with biomasses with less lignin content, such as miscanthus and birchwood, in choline chloride:formic acid and choline chloride:oxalic acid (82% lignin extraction after 60 min of microwave irradiation) [59]. The herein presented concept may help valorize large volumes of recalcitrant biomasses with high lignin contents, which otherwise would have to be subjected to low-value applications (e.g., incineration). The obtained lignin preserves the most important chemical bonds. More in-depth characterization analyses are needed to calibrate the actual degradation of the lignin, to put forth reaction conditions that could reduce it. Thus, the further fine-tuning of the BM and MI conditions, together with DES versatility, may enhance the yields and the lignin qualities. The short

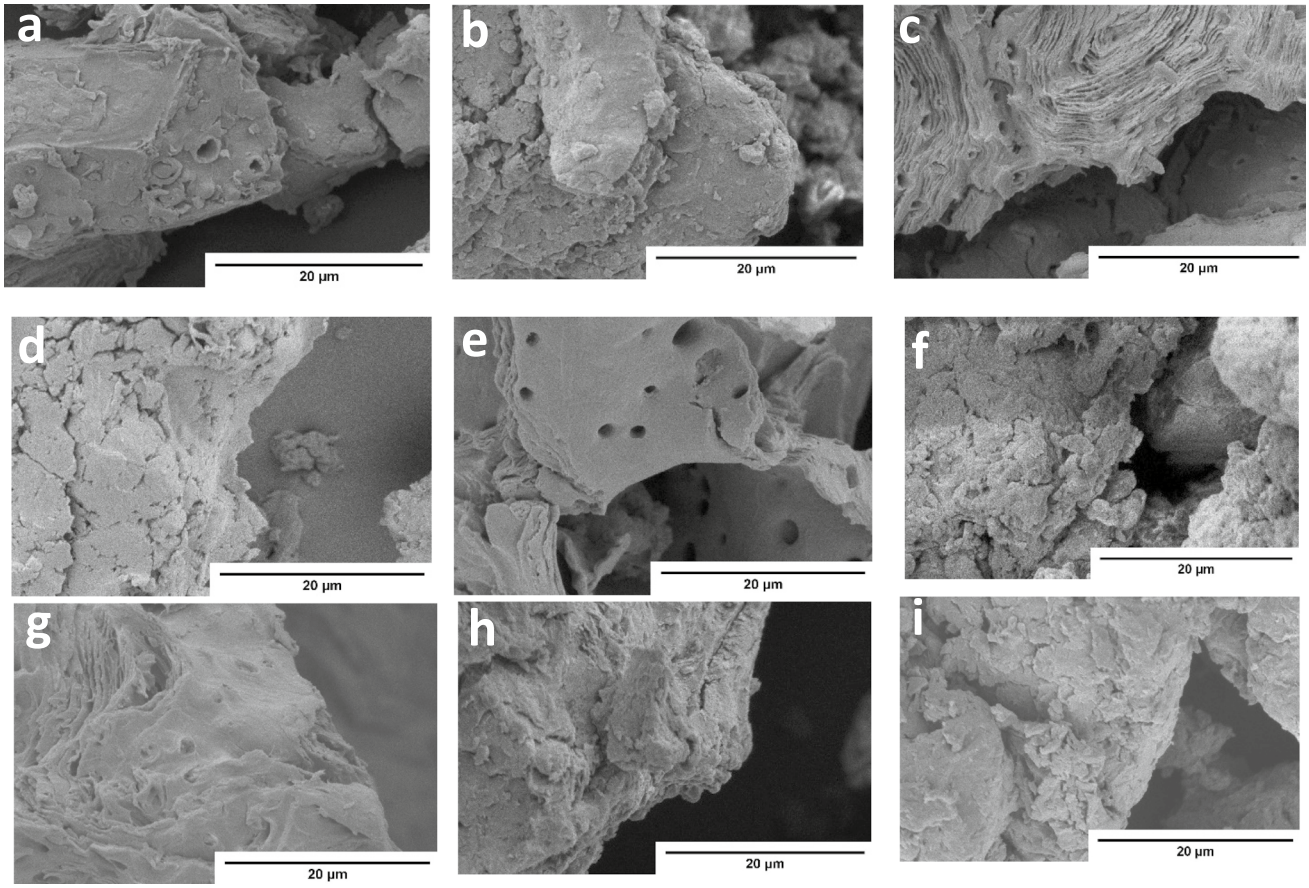

Fig. 8 ESEM images of (**a**) RawW, (**b**) OB, (**c**) SM9060, (**d**) SMB9060, (**e**) SC9060, (**f**) SCB9060, (**g**) SM12010, (**h**) SMB12010, and (**i**) SM15010

processing digestion times suggest that continuous delignification can be implemented, and that degradation of the raw materials can be reduced significantly, as exposure times to severe processing conditions can be minimized. Recent findings on bleached kraft pulp, microcrystalline cellulose, and xylans at 130° in cholinium chloride:lactic acid (1:10) DES system revealed that treatment at long residence reaction times (> 4 h) led to significant modifications of polysaccharides fractions [60].

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Author contribution R. Ahorsu: data curation, investigation, and writing—original draft. M. Constanti: supervision, resources, review, and editing. P. Domínguez de María: conceptualization, writing, review, and editing. F. Medina: conceptualization, resources, supervision, review, and editing. All authors read and approved the final manuscript.

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Data availability The corresponding author is available to provide dataset and analyses used in this study upon valid request.

Declarations

Ethical approval Ethical approval is not applicable due to this study's exclusion of human or animal trials.

Conflict of interests The authors declare no competing interests.

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