Acacia Wood Fractionation Using Deep Eutectic Solvents: Extraction, Recovery, and Characterization of the Different Fractions

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ABSTRACT: The selective extraction and recovery of different lignocellulosic molecules of interest from forestry residues is increasing every day not only to satisfy the needs of driving a society toward more sustainable approaches and materials (rethinking waste as a valuable resource) but also because lignocellulosic molecules have several applications. For this purpose, the development of new sustainable and ecologically benign extraction approaches has grown significantly. Deep eutectic solvents (DESs) appear as a promising alternative for the processing and manipulation of biomass. In the present study, a DES formed using choline chloride and levulinic acid (ChCl:LA) was studied to fractionate lignocellulosic residues of acacia wood (*Acacia dealbata* Link), an invasive species in Portugal. Different parameters, such as temperature and extraction time, were optimized to enhance the yield and purity of recovered cellulose and lignin fractions. DESs containing LA were found to be promising solvent systems, as the hydrogen bond donor was considered relevant in relation to lignin extraction and cellulose concentration. On the other hand, the increase in temperature and extraction time increases the amount of extracted material from biomass but affects the purity of lignin. The most promising DES system, ChCl:LA in a ratio of 1:3, was found to not significantly depolymerize the extracted lignin, which presented a similar molecular weight to a kraft lignin. Additionally, the ^{31}P NMR results revealed that the extracted lignin has a high content of phenolic OH groups, which favor its reactivity. A mixture of ChCl:LA may be considered a fully renewable solvent, and the formed DES presents good potential to fractionate wood residues.

1. INTRODUCTION

Over the past few years, the production of chemicals from lignocellulosic biomass has been increasingly followed by the scientific community to couple with the concepts of sustainable and circular economy in different industrial sectors.[1,2] Biomass is a great alternative to fossil resources, and the main classes of feedstock that can be used for new chemical products are triglycerides, lignocellulose, and saccharides.[3,4] The transformation of these raw materials into novel value-added materials is made by transformations of several biomass compounds, such as saccharides (glucose and xylose), polyols (sorbitol, xylitol, and glycerol), furans (furfural and 5-hydroxymethylfurfural), and organic acids (succinic, levulinic, and lactic acids).[5] An important advantage of biomass-derived chemicals is their chemical structure richness consisting of functionalized compounds. This allows such a biomass platform to be further converted into more valuable chemicals compared to compounds derived from fossil sources.[3] Lignocellulosic biomass and its main components (i.e., cellulose, hemicellulose, and lignin) represent a very appealing platform for numerous applications, including biofuels, cosmetics, detergents, adhesives, biomedical and

pharmaceutical devices, flocculants, and food additives.[6,7] The range of applications is wide with the native biopolymers but also with its derivatives.[8,9]

Acacia wood has high potential as a source of biopolymers due to its high level of silvicultural performance and capacity to grow on marginal lands, being considered an invasive species.[10,11] The genus *Acacia*, from the subfamily *Mimosoideae*, includes about 1200 species, the majority of them native of Australia and Southern Africa.[10] Some of these species were introduced in Portugal at the beginning of the 20th century and comprise several of the worst invasive plant species in the world.[10] Due to the rapid growth rate and relatively high carbohydrate content, they can be easily supplied as a raw material.[12] Its composition is mainly based on carbohydrate polymers (e.g., cellulose and hemicellulose), aromatic poly-

mers (lignin), and other minor components, such as resins, lipids, pectins, proteins, and alkaloids usually known as extractives.[12]

Efficient extraction and isolation methods to obtain purified raw materials are crucial for their subsequent transformation into value-added materials. Due to the increasing environmental concerns, there is an urge in developing ecofriendly and efficient solvent systems that allow the sustainable extraction of lignocellulosic materials with high yields and purity.

Deep eutectic solvents (DESs) have been considered the "solvents of the future" due to their excellent performance in many different processes including the extraction of lignin and dearomatization, among other related applications.[13,14] DESs are typically formed by an eutectic mixture of Brønsted or Lewis acids (hydrogen bond donors, HBDs) with quaternary ammonium salts (hydrogen bond acceptors, HBAs), for instance, choline chloride (ChCl). Figure 1 illustrates the
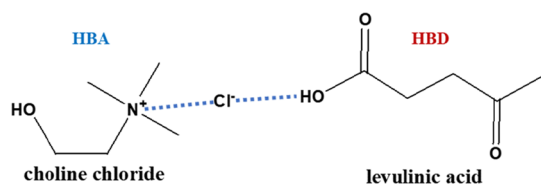

Figure 1. Schematic representation of structures of ChCl and LA, showing the hydrogen bonding responsible for the formation of the DES.

formation of a DES.[15,16] When mixed at a certain molar ratio, the melting point of the mixture becomes significantly lower than that of the original individual compounds.[13] DESs share many physicochemical features with common ionic liquids (ILs), such as viscosity, melting point and volatility, high thermal stability (low vapor pressure and nonflammability), conductivity, and surface tension.[15,17] Depending on the applications, their properties can be fine-tuned by changing the structure of the HBDs or HBAs.[15] Moreover, these solvent systems are easy to prepare at a low cost, without requiring the presence of any cosolvent and producing no waste. Mixtures of natural biosourced cations and anions are often used for DESs formation highlighting their low toxicity and biodegradability profiles.[18]

In the literature, several studies can be found on the application of DESs in the extraction of aromatic hydrocarbons from alkanes or in the gasoline purification process.[8,15] Other studies have focused on the extraction of lignin and purification of cellulose.[19] Recently, the effects of different wood origins (such as pine, acacia, sugarcane bagasse, corn stover, etc.) and extraction methods have been explored.[8,9]

In particular, some of us have demonstrated that a levulinic acid (LA)-based solvent displays superior affinity toward lignin leading to its high extraction efficiency from pine wood.[20] LA is fully biodegradable and nontoxic and can be sourced from biomass itself.[9] Therefore, given the enhanced performance of LA in lignin fractionation, in this study, a less-explored DES was developed combining LA with ChCl and its fractionation performance was evaluated regarding the recovery yield and purity of the different biopolymers from acacia residues. To our knowledge, there is only one report addressing the ChCl−LA mixture, but since the fractionation process was not fully optimized, and therefore, the lignin yield and purity were not impressive.[21] Like LA, ChCl is also nontoxic and biodegrad-

able, and it can be obtained at a very low cost. Therefore, the mixtures formed are expected to be sustainable and fully renewable.

In the present study, 1:2 and 1:3 molar ratios of ChCl:LA were used; the 1:1 mixture was excluded because a solid white crystal formed at room temperature.[16] In addition, the LA-based DESs systems were compared with other DES formed by ChCl and imidazole (IM) $(3:7)^{22}$ in terms of fractionation efficiency and selectivity for lignin extraction.[17,23] One of the major challenges faced in biorefinery is related to efficient biomass fractionation, since the structure of the plant cell wall and the high crystallinity of cellulose makes the separation of the raw material into its components a complex process. It is also important to keep in mind that the structure of the isolated lignin is affected by the extraction methods and thus high-value products with different properties are possible to be formed.[24,25] The acid-based DESs are effective in dissolving technical lignins and can also lead to successful biomass delignification. The acidity of these solvents is crucial for the breakage of the biomass matrix and lignin chemical bonds, particularly β-O-4 ether bonds, leading to the selective separation of lignin from cellulose structures.[19] This study aims at the selective extraction and characterization of cellulose and lignin that result from fractionation of acacia biomass with different green DES-based solvents. The relationship between the DES composition and chemical features and their extraction/selectivity performance will be further addressed.

2. METHODS

2.1. Materials. The *Acacia dealbata* Link wood used in this study was collected and harvested in Midões (Tábua), a Portuguese parish in the district of Coimbra, Portugal, and its main components are presented in Table 1. The raw material

Table 1. Composition of the Acacia Wood in dry basis

humidity of biomass (%)	composition (%)		
	lignin	cellulose	xylose
7.36	22.20 (±0.30)	47.41 (±0.90)	18.79 (±0.25)

consisted of waste branches, which were finely milled at the laboratory mill (cross beater mill, Retsch) and screened in a mechanical sieve shaker (Thomas Scientific, USA). The sawdust sample with a particle size between 0.25 and 0.84 mm was selected for the subsequent pretreatment.

LA (98 w/w%), ChCl (99 w/w%), and crystalline IM (99 w/w%) were purchased from Acros Organics. Sodium hydroxide pearls were purchased from Labkem. Formic acid (99 w/w %) was purchased from CARLO ERBA. Dichloromethane was purchased from Sigma-Aldrich and 4-nitroanisol was purchased from DAGMA. Microcrystalline cellulose, Avicel PH-101, with an average particle size of 50 mm and a degree of polymerization of ca. 260, was purchased from Sigma-Aldrich. Kraft lignin, 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane acid (95 w/w%), and pyridine (99.8 w/w%) were purchased from Sigma-Aldrich. For the viscometry estimation of the molecular weight of lignin, *N,N*-dimethylformamide (DMF) (≥99.8% ACS Reagent, reag. Ph. Eur. Grade, Honeywell) was used as the solvent. Sulfuric acid purchased from Chem-Lab with 72% purity was used for lignin quantification. Deionized water was used for the preparation of all solutions.

2.2. Synthesis of DESs. ChCl and LA were used as received. The 1:2 molar ratio DES was prepared by mixing 46.54 g of ChCl and 77.41 g of LA, while the DES with a 1:3 molar ratio was prepared by mixing 46.54 g of ChCl and 116.11 g of LA.[16] In both cases, the resulting mixtures were stirred for 2 h with a magnetic stirrer at room temperature. For comparison, a mixture of ChCl with IM in a 3:7 molar ratio was prepared by mixing 53.85 g of ChCl and 61.27 g of IM and then stirred for 1 h using a magnetic stirrer at 100 °C. Table 2 summarizes the composition of the prepared eutectic solvents.

Table 2. Chemical Structures and Composition of the Studied DESs

hydrogen bond acceptor (HBA)	hydrogen bond donors (HBD)	abbreviations and (HBA:HBD) molar ratios
		ChCl:LA (1:2)
		ChCl:LA (1:3)
		ChCl:IM (3:7)

2.3. Characterization of the Prepared DESs. The viscosity properties of the formed DESs were investigated by rheological measurements; the experiments were conducted using a HAAKE MARS rheometer (Thermo Fisher Scientific, Germany) set with a plate-plate geometry (20 mm and 0.2 mm gap). A water recirculatory bath (HAAKE Phoenix II, Germany) was used for temperature control, set to 20.0 ± 0.1 °C. The water content of the DES was assessed by Karl Fischer volumetric titration using a Metrohm 890 Karl Fischer Titrando apparatus equipped with an 803 Ti Stand to 0.4% accuracy in water mass content. The refractive indices were measured at the sodium D line (589.3 nm) using an ATAGO RX-5000α refractometer at 25 °C. Table 3 summarizes the most relevant properties obtained for the different DESs prepared. Note that the DESs were also evaluated 30 days after preparation to infer on the aging effect.

2.4. Lignin Extraction and Cellulose Recovery. It is important to choose a selective method for biomass fractionation to achieve highly valuable and pure compounds, such as cellulose and lignin. Some important operational parameters must be considered in the optimization of the extraction process, such as temperature, time, and solvent composition. Moreover, the ratio between the biomass and solvent should also be considered. In this study, different ratios (w/w) were initially evaluated, and it was found that the ideal ratio for the conditions used is 1.5:10 (biomass dry weight to solvent weight). This ratio was kept constant during all the experiments. The vessel consists of a Teflon container with a volume of 15 mL inside a stainless steel vessel with a thickness of 3 mm, well-sealed and watertight. Due to the simplicity of the reactor, it is not possible to measure or control the pressure generated inside. After the extraction, the resulting solid residue (cellulose-rich fraction) was separated from the DES by vacuum filtration. A NaOH (aq) solution (10%, w/w) was used to wash the cellulose-rich fraction and enhance its purity. On the other hand, lignin was obtained from the liquid fraction (filtrate), and water was used as an antisolvent to drive its precipitation (as described in the Supporting Information). In the case where the DES formed by ChCl:IM was used, the cellulose-rich fraction was first mixed with methanol and then washed with water. The precipitation of lignin from the liquid fraction was triggered by the addition of a large excess of water after previous evaporation of methanol.

The lignin and cellulose fractions were then oven-dried and weighed. Extraction yields were calculated as the percentage of extracted material/biomass on dry weight basis.[9]

$$\text{extraction yield, \%}$$
$$= \frac{\text{biomass weight} - \text{extracted biomass weight}}{\text{biomass weight}} \times 100 \tag{1}$$

2.5. Determination of Lignin in Biomass (Original and after Extraction). The lignin content was estimated using the National Renewable Energy Laboratory (NREL) protocol.[26] A total of 300 mg of dried extracted material was hydrolyzed with 3 mL of 72% of a sulfuric acid (12 M) solution for 60 min at 30 °C. The reaction between the acid and the sample proceeded for 60 min with intermittent stirring. Then, the hydrolysates were diluted to obtain a sulfuric acid solution of 4%, autoclaved at 121 °C for 60 min and left to cool to room temperature. The autoclaved mixtures were vacuum-filtered using weighted filtering crucibles, and the acid-insoluble lignin fraction was gravimetrically determined. After washing the insoluble material, samples of the filtrates were diluted and used for the determination of the acid-soluble lignin by measuring the absorbance of the solution at 205 nm using a UV−vis spectrometer (JASCO V650 spectrophotometer). The insoluble and soluble lignin fractions (expressed in %) are calculated via eqs 2 and 3, respectively. The total lignin content is simply the arithmetic sum of both fractions (eq 4).

$$\text{insoluble lignin (\%)} = \frac{m_{\text{solid-material}}}{m_{\text{dry-sample}}} \times 100 \tag{2}$$

$$\text{soluble lignin (\%)} = \frac{\text{ABS} \times \text{FD} \times V_{\text{hydrolysate}}}{\varepsilon\left(\frac{L}{g\ cm}\right) \times b\ (\text{cm}) \times m_{\text{sample}}(g)}$$
$$\times 100 \tag{3}$$

$$\text{total lignin} = \text{insoluble lignin} + \text{soluble lignin} \tag{4}$$

Table 3. Physical−Chemical Parameters of the Different DESs

	ChCl:LA (1:2)	ChCl:LA (1:3)	ChCl:IM (3:7)	ChCl:LA (1:2)	ChCl:LA (1:3)	ChCl:IM (3:7)
		0 days			30 days	
density (g/mL)	1.136	1.130	1.082	1.136	1.133	1.136
refractive index (nD) at 25 °C	1.466	1.461	1.493	1.467	1.462	1.466
viscosity (mPa s) at 20 °C	153.0	87.9	Solid	188.6	130.6	solid
water, %	1.1	1.3	1.5	1.4	1.5	1.1

In eq 3, $V_{hydrolysate}$ is the volume of the liquid fraction where the soluble lignin is dissolved (supernatant, L), m_{sample} is the mass of the initial sample (dry basis), ABS is the absorbance value of the diluted supernatant at 205 nm, FD is the dilution factor to obtain absorbance of the supernatant at 205 nm in the range between 0.2 and 0.8, ε is a special extinction coefficient for lignin (110 L/(g.cm)), and b is the optical path (1 cm).[2]

The contents in lignin of the treated wood samples were obtained according to the analytical procedure of the National (US) Renewable Energy Laboratory "Determination of Structural Carbohydrates and Lignin in Biomass" (NREL/TP-510-42618),[26] as shown in eq 5.

$$\text{delignification, \%} = \left[\text{total lignin}_{in\text{-}sawdust} - \text{total lignin}_{in\text{-}extracted\text{-}biomass} \right.$$
$$\left. \times \frac{100 - \text{extracted yield, \%}}{100} \right] / [\text{total lignin}_{in\text{-}sawdust}] \times 100 \quad (5)$$

2.6. Statistical Analysis.
Extraction yields were calculated as the average (duplicate) of extracted biomass, and the total lignin recovery was calculated based on the extraction yield and lignin purity.[8]

A statistical analysis was performed using one-way ANOVA (α = 0.05) to evaluate the significant differences between the extraction yields.

2.7. Characterization of Lignin.
Nuclear magnetic resonance (NMR) techniques allow analyzing the molecular structure of the different lignins. To do so, samples should be solubilized in deuterated solvents. In this case, the choice of the appropriate solvent, considering its polarity and aromaticity, is an essential factor in the optimization of spectral resolution and signal separation as it leads to considerable changes in chemical displacement, standard coupling, signal dispersion, and baseline pattern as well as in global complexity of the ^{31}P NMR spectrum.[27] In order to separate overlapping resonances and to avoid the solvent effect, usually caused by the solute−solvent interaction, anisotropy of the solvent molecules and van der Waals strength, the type of solvent, or the pH value of the solution can be changed. Additionally, auxiliary reagents such as cyclodextrins or lanthanide displacement reagents can be used.[28] In the present case, the extracted lignin was reacted with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane; after the phosphorylation of lignin, the different samples were dissolved in a mixture of pyridine and dichloromethane. Pyridine is used to promote the dissolution of phosphorylated lignin, and deuterated dichloromethane allows us to obtain a signal with enhanced resolution.

Quantitative determination of the hydroxyl groups was made using 1D ^{31}P NMR spectroscopy, and the lignin structural features were characterized by heteronuclear single quantum coherence spectroscopy (HSQC), two-dimensional (2D) [^{1}H−^{13}C] HSQC. Spectra were obtained using a Bruker Avance III 400 MHz spectrometer (Bruker, Hamburg, Germany), operating at frequencies of 400.13 MHz (^{1}H), 100.62 MHz (^{13}C), and 161.99 MHz (^{31}P). The selected 1D ^{31}P pulse sequence with decoupling in ^{1}H was zgpg30, with a spectral window of 406.12 ppm and a total of 128 k points. The acquisition time was 0.99 s with a relaxation delay (d1) of 2 s for a total of 6144 scans. All the spectra were obtained at 25 °C.

The average molecular weight (MW) of the extracted lignin was estimated based on the intrinsic viscosity according to the Huggins equation; the intrinsic viscosity $[\eta]$ can be related to the MW of the polymer by the semi-empirical Mark−Houwink−Sakurada equation[29]

$$[\eta] = K \, (MW)^{\alpha} \quad (6)$$

where K and α are specific constants dependent on the determination conditions (in this case, α = 0.11 and K = 2.51[29]). Lignin was dissolved in DMF at a concentration of 5 g/L at 25 °C. The solution was stirred for 2 h and allowed to stand for 24 h. It was then filtered with a 0.45 μm nylon syringe filter (FiltraTech). Successive lower concentrations, used for the $[\eta]$ determination, were obtained by automatic volumetric dilution in a Viscologic TI1viscometer (Sematech, Nice, France).

2.8. Determination of Cellulose Purity in the Cellulose-Rich Fractions.
Samples of cellulose-rich fractions were hydrolyzed following the same procedure used for lignin determination (Section 2.5, NREL protocol) and characterized by high-performance liquid chromatography (HPLC). After hydrolysis, liquor aliquots were neutralized with calcium carbonate, filtered with a 0.2 μm syringe filter, and analyzed for the monosaccharides by HPLC in a Knauer equipment with a refractive index (RI) detector. The operating conditions in all HPLC analyses were as follows: injection of a volume of 20 μL in a Rezex ROA-Organic acid column (kept at 40 °C) with the respective guard column (room temperature), mobile phase with a flow rate of 0.6 mL/min, and use of a RI detector at room temperature. The mobile phase used was a 0.0025 M H_2SO_4 solution previously filtered with a 0.2 μm nylon membrane filter (Fioroni).

3. RESULTS AND DISCUSSION

The sawdust of the acacia wood was found to have, in average, ca. 22.2% of lignin.

The reaction conditions are one of the most important steps in the biomass fractionation process. Therefore, the extraction yield was first evaluated by combining two acids, LA and formic acid (FA).[9] The molar ratio between the acids was fixed to 1:1 (LA:FA), and different reaction times and temperatures were evaluated, as shown in Figure 2.

As it can be observed, at low extraction times, up to 4 h, the obtained extraction yields at 160 °C were relatively higher than those for the other two temperatures, which are relatively similar in terms of the extraction yield. Moreover, the extraction yield was found to increase with time, for extraction times up to 4 h. No substantial improvement in the extraction
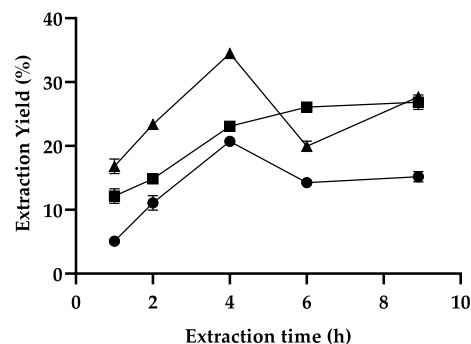
Figure 2. Extraction yield of *Acacia dealbata* Link wood using LA:FA (1:1) as a function of reaction time at three different temperatures: 120 °C (solid circle), 140 °C (solid square), and 160 °C (solid triangle).

process was observed for longer extraction times (above 4 h). In fact, the extraction yield decreases when the extraction time increases from 4 to 6 h (except at 140 °C, where a more common behavior was exhibited, the values reaching a plateau after 6−8 h). The highest increase in the extraction yield occurred between 1 and 4 h of the reaction.

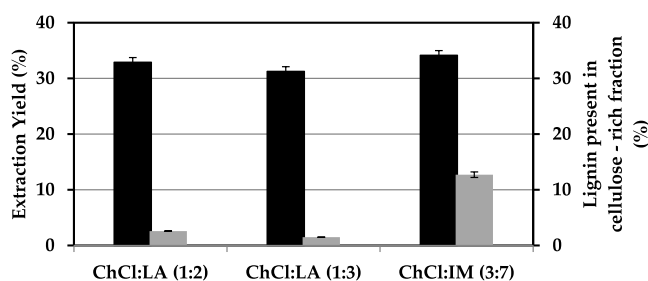Based on the data presented in Figure 3, the conditions of 4 h and 160 °C were selected in the forthcoming trials,

Figure 3. Extraction yield (black color) and lignin content (gray color) after fractionation of acacia wood at 160 °C for 4 h. According to the one-way ANOVA test ($P \leq 0.05$), all solvents are significantly different.

particularly for the DES composed of LA and ChCl and for the DES composed of ChCl and IM, a combination already reported in the literature.[30] Generally, the principles behind the DES formation are based on a large decrease in the melting point of the mixture of the components, when compared to the isolated substances, and thus most DESs are liquid between 25 °C and 70 °C.[31] For example, all DESs containing LA are liquid at room temperature, unlike the DES containing IM, which is liquid typically above 50 °C.[22] Furthermore, DESs are prone to absorb water quickly, as seen in Table 3. The main objective of this study is to extract and isolate cellulose and lignin as pure as possible. It was found that the solid residue (cellulose-rich fraction) is mainly composed of cellulose and the filtrate contains both lignin and hemicellulose, revealing a good fractionation ability of the studied DES (see Figure 3 and Table 4).

Table 4. Composition of the Cellulose-Rich Fractions Obtained after Acacia Wood Fractionation with DESs (160 °C for 4 h)

	ChCl:LA (1:2)	ChCl:LA (1:3)	ChCl:IM (3:7)
cellulose (%)	94.79 (±0.7)	96.06 (±0.07)	69.32 (±1.05)
hemicellulose (%)	2.57 (±0.002)	2.56 (±0.07)	16.96 (±0.41)
lignin (%)	2.64 (±0.18)	1.38 (±0.08)	13.72 (±0.51)
delignification (%)	95.31 (±0.48)	95.77 (±0.08)	58.72 (±2.48)

According to the literature, acid-based DESs have a greater efficiency in the extraction of lignin[8] when compared to basic and neutral DESs.[14] The results obtained in this study demonstrate that there is an evident trend in the pretreatment performance among the same groups of solvents containing LA: all cellulose-rich fractions exhibited a lignin content lower than 5%. The development of green and efficient solvents for wood fractionation is a priority to pursue sustainability. The ones used in the present study perfectly fit this purpose, demonstrating a great ability to fractionate lignocellulosic biomass, without the presence of harmful compounds, and

using mainly components from lignocellulose biorefinery, such as LA.

In addition, the obtained yields suggest that the functional groups of the acid affect the extraction capacity of DES, in agreement with previous studies.[32] The presence of a short alkyl chain, OH group, and double bond in HBD carboxylic acid was found to improve the selectivity and the performance of the DES. These results clearly demonstrate the importance of selecting the constituents based on criteria to produce a DES with excellent lignin selectivity.

The data in Table 4 indicate that the proposed fractionation processes based on less-explored DESs provide cellulose-rich fractions with a high cellulose yield and purity. These high yields of cellulose reveal the strong potential of acacia biomass as a source of cellulose, contributing to green economic growth due to its large availability. According to the results presented above, the lignin content of the cellulose fraction is very low, comparable with the lignin content of pulps obtained by the kraft pulping process.[9]

Scanning electron microscopy (SEM) was used to characterize the microscopic morphological features of acacia wood before and after fractionation with ChCl:LA (1:2) and (1:3) for 4 h at 160 °C (Figure 4). The starting acacia sawdust material exhibits a dense structure, where characteristic "pits" with different sizes are observed. Interestingly, some longitudinal fractures between the fibers can be observed, probably due to the mechanical cut of the biomass. After extraction, the materials presented a well-defined fiber shape, thus revealing good fractionation of the acacia wood; lignin was successfully removed (Figure 4b,c). Once lignin was removed, the fiber bundles were disrupted thus exposing the cellulose fibrils; high deterioration of the cell walls resulted in an increase of the surface area.[33]

3.1.1. Characterization of the Lignin-Rich Fractions. ^{31}P-NMR, HSQC NMR, and capillary viscometry were used for a deeper characterization of the extracted lignin. For comparison purposes, commercial kraft lignin was used as a reference material.

In Table 5, the intrinsic viscosity of extracted lignin (dissolved in DMF) and estimated MW are reported. These parameters are known to be strongly dependent on the extraction conditions, which may cause depolymerization.[9]

According to the Mark−Houwink−Sakurada relation, the lower the MW, the lower the expected intrinsic viscosity.[29,34] As can be observed, the increase in LA induces a subtle decrease in lignin MW. Nevertheless, the extracted lignins present an average MW similar to the MW of commercial kraft lignin.

The phenolic OH groups of the extracted and reference lignins can be quantified by NMR after phosphorous derivatization.[35] In Figure 5, the ^{31}P NMR spectra obtained for the different lignin samples are presented.

The peaks in the range of 150 and 145 ppm were assigned to the aliphatic hydroxyl groups.[36,37] The condensed phenolic hydroxyl groups normally appear in the range of 140.5 and 136 ppm.[37] In short, the aliphatic OH became the predominant hydroxyl signal in the case of kraft lignin, the obtained ^{31}P-NMR spectra of kraft lignin being in good agreement with the literature; the chemical shifts reported for the aliphatic OH are between 151.0 and 144.7, for the 5-substituted OH between 144.0 and 142.3, for guaiacyl OH between 142.5 and 141.5, for p-hydroxyphenyl between 141.5 and 141.1, and for the carboxylic acids between 141.1 and 135.9.[38] On the other
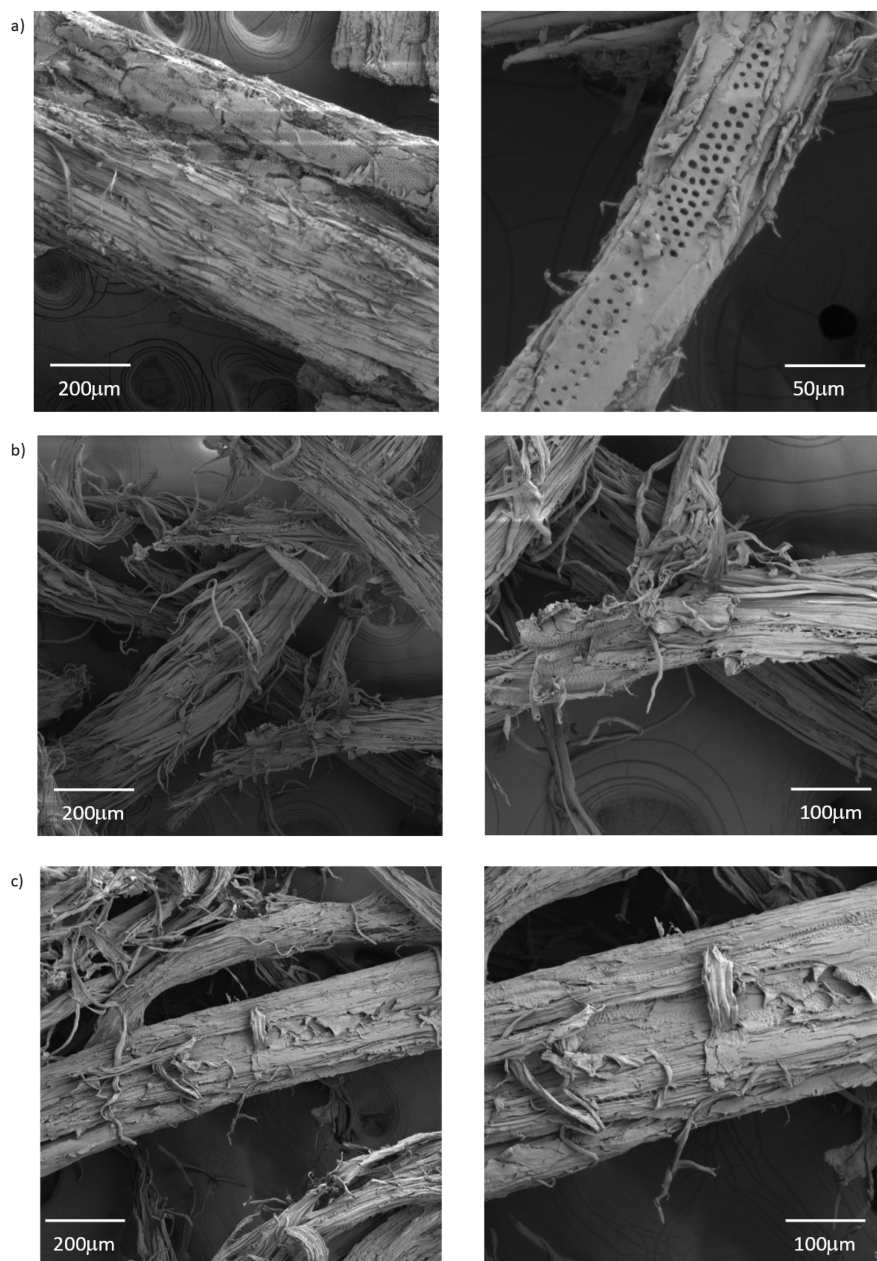
Figure 4. SEM images of the starting acacia sawdust (a) and the cellulose-rich materials obtained after fractionation with ChCl:LA (1:2) (b) and ChCl:LA (1:3) (c).

Table 5. Intrinsic Viscosity [η] and MW of the Extracted Lignin Fractions Obtained after Acacia Wood Fractionation with Different DESs (160 °C for 4 h)[a]

	ChCl:LA (1:2)	ChCl:LA (1:3)	kraft lignin
$[\eta]$ (mL/g)	32.17 (±0.68)	30.80 (±0.68)	46.40 (±3.11)
correlation	0.93 (±0.03)	0.82 (±0.02)	0.90 (±0.13)
lignin MW (g/mol)	9600 (±0.08)	9440 (±0.08)	11100 (±0.28)

[a]Commercial kraft lignin is added for comparison.

hand, the C_5-subtituted signal, mainly from syringyl units,[39] has more intense peaks for lignin extracted using the ChCl:LA 1:2- and 1:3-based DESs. Also, it is important to note that the relative abundance of the different units is very different when comparing kraft and lignin extracted with the ChCl:LA-based DES. For the kraft lignin case, for each carboxylic OH unit, the sample presented 5.5 phenolic units (3.5 C_5-substituted OH and 2 guaiacyl + p-hydroxyphenyl) and 4.7 aliphatic OH groups. Conversely, the lignin extracted using the ChCl:LA-based DES presented a higher density of phenolic OH groups and a low density of aliphatic OH. For the lignin extracted using the DES ChCl:LA of a 1:2 ratio, for each carboxylic acid OH group, it presented 7.7 phenolic units (4.7 C_5-substituted OH and 3 guaiacyl + p-hydroxyphenyl) and only 0.4 aliphatic OH groups; the sample obtained by extraction with the 1:3 DES presented 12.7 phenolic OH groups for each carboxylic acid OH group (8 C_5-substituted OH and 4.7 guaiacyl + p-hydroxyphenyl) and only 0.5 aliphatic OH groups. The higher content of phenolic OH groups can be possibly associated with the cleavage of the β-aryl ether linkages.[37] Nevertheless, the carboxylic acid OH can be caused by the cleavage of the ether linkages in lignin, forming acids during the pretreatment. The
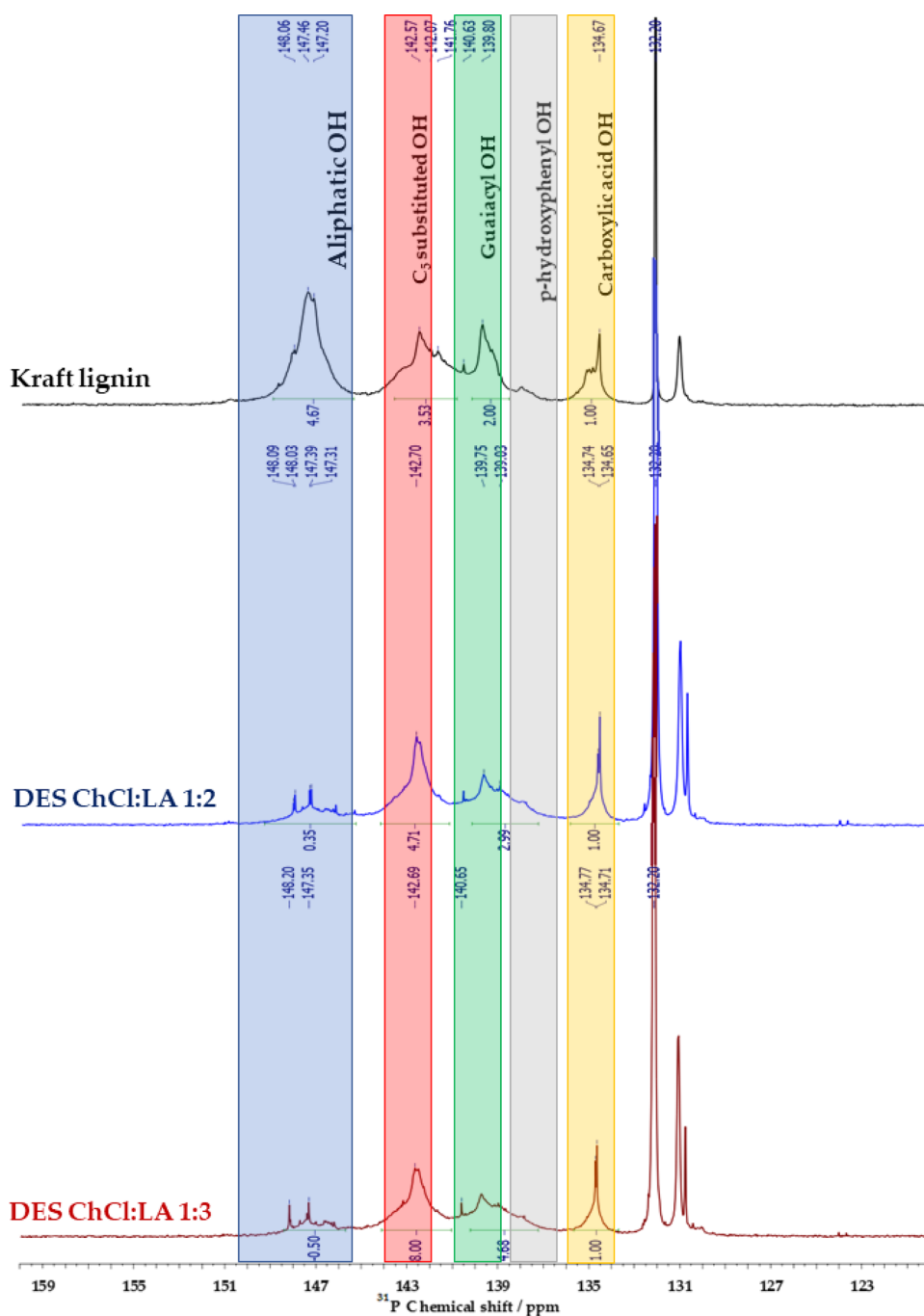
Figure 5. 1D ^{31}P NMR spectra of the different lignin samples in pyridine/CDCl$_3$ at 25 °C.

obtained results suggest that the ChCl:LA-based DES extracted lignin with a higher potential reactivity compared with kraft lignin due to the presence of higher amounts of phenolic groups.[40]

Bidimensional NMR was also applied to further characterize the structural features of the obtained lignins. Three main regions were identified in the 2D [^{1}H−^{13}C] HSQC NMR spectrum (Figure 6). Nonoxygenated and oxygenated aliphatic side chains appeared at δ^{13}C/δ^1H 50.0−80.0/2.8−4.2 ppm, and the aromatic region appeared with C−H correlation signals δ^{13}C/δ^1H 100.0−120.0/6.0−7.5 ppm, in good agreement with the literature.[38] The spectrum exhibited intense signals at 56.0/3.7, corresponding to methoxyl groups and side chains in −O−4− structures. The signal at δ^{13}C/δ^1H 60.0/3.7

ppm is attributed to the gamma-C−H of gamma-acylated lignin units. A prominent region is located at δ^{13}C/δ^1H 105.0−115.0/6.3−7.0 ppm, which can be correlated with C−H aromatic signals from guaiacyl units (G units), whereas signals higher than 120 ppm are related to aromatic C−H signals from *p*-hydroxyphenyl units (H units).

4. CONCLUSIONS

In this study, ChCl-based DESs were evaluated regarding their performance toward the fractionation of lignocellulosic residues of acacia wood (*Acacia dealbata* Link). The results obtained demonstrated the influence of the solvent composition on the fractionation efficiency; the DES containing LA showed high selectivity, leading to good extraction yields and
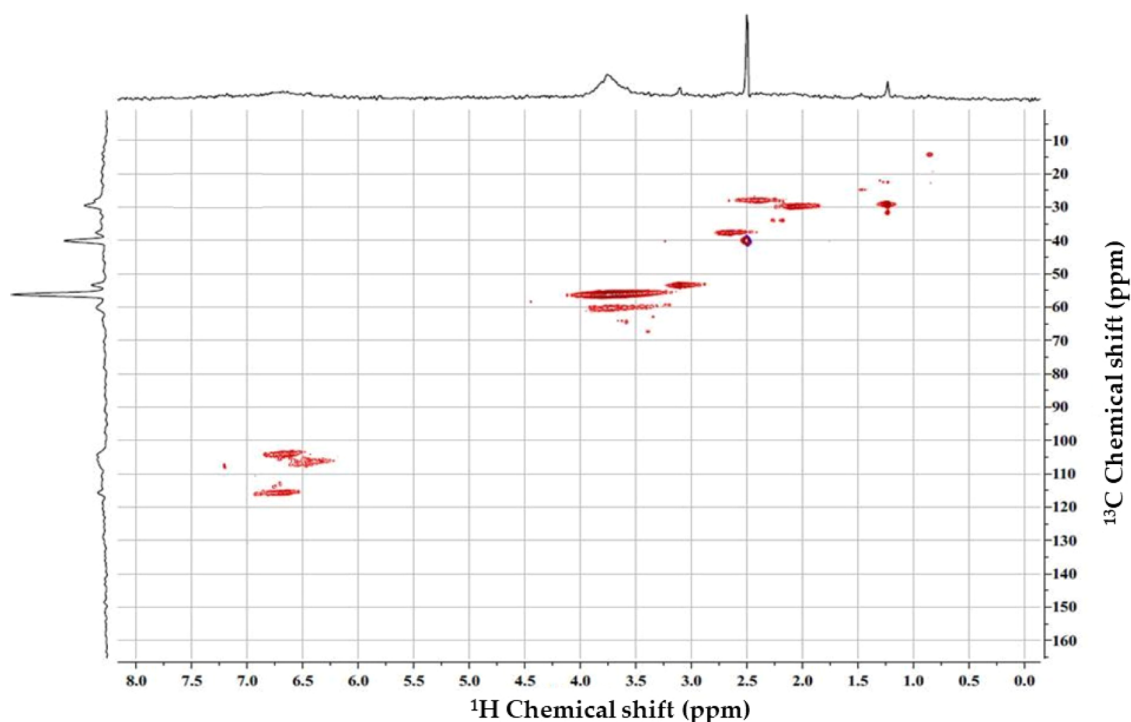
Figure 6. [^{1}H–^{13}C] HSQC NMR spectrum of lignin purified using ChCl:LA (1:2).

purity of the isolated materials; the lignin content of the cellulose fraction was remarkably low. This selectivity was also visible in the SEM images since the fiber bundles were clearly disrupted after fractionation (removal of cross-linking structures between hemicellulose and lignin, exposing the cellulose fibrils).

Regardless of the DES used, the extracted lignins displayed a rather similar MW among them, which was similar to the MW of commercial kraft lignin. As elucidated by NMR, the molecular structures of the lignin samples extracted seem to be not substantially affected by the solvent/conditions. Nevertheless, it should be highlighted that a higher content of phenolic groups was observed in the DES system composed of ChCl:LA. This higher content of OH enhances reactivity, which might be appealing for different applications. Considering the green characteristics of the studied LA-based systems and the remarkable extraction performance and selectivity toward lignin, one can consider that the LA-based DES explored in this study is highly promising for future large-scale applications, contributing to valorize lignocellulose biomass into novel chemicals and biomaterials.

ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c07380.

> Lignin extraction and purification procedures, efficiency of the washing procedure, HSQC NMR spectrum of lignin extracted from ChCl:LA of 1:2 ratio, ^{13}C and ^{1}H NMR spectra of the ChCl:LA solvent of 1:2 ratio, and HPLC spectra of the extracted cellulose-rich fraction and ChCl:LA solvent of 1:2 ratio (PDF)

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S.M., A.M., R.A., L.A., B.M., A.R., J.A.F.G., and M.G.R were involved in conceptualization of the study; S.M. and A.M conducted the experiments; S.M. was involved in original draft preparation; and all authors were involved in writing the manuscript (preparation, review, and editing).

Notes
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