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Catarina Santos Fernandes

FOLLOWING GREEN CHEMISTRY PRINCIPLES FOR THE VALORIZATION OF LIGNOCELLULOSE WASTE

ON THE EXTRACTION AND CHARACTERIZATION OF LIGNIN FROM MARITIME PINE SAWDUST WITH NOVEL DEEP EUTECTIC SOLVENTS

Dissertação no âmbito do Mestrado em Química, ramo de Química Avançada e Industrial orientada pelo Professor Doutor Filipe João Cotovio Eufrásio Antunes e pelo Doutor Bruno Filipe Figueiras Medronho e apresentada ao Departamento de Química da Faculdade de Ciências e Tecnologia da Universidade de Coimbra.

Novembro de 2020

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Abstract

As the most abundant renewable resource on Earth, lignocellulosic biomass has a great potential as a sustainable supply to produce biofuels and chemicals. Lignocellulosic materials are mainly composed of cellulose, hemicellulose and lignin. Cellulose is the most abundant polymer comprised in lignocellulose and it is widely used in diverse applications. On the other hand, in the recent years, lignin has received increased attention due to its attractive properties. It is the most abundant aromatic feedstock, and its efficient extraction could expand the utilization of biomass and reduce the dependence on fossil fuels. Lignin can be removed from biomass by several extraction processes, but its complex molecular structure makes its isolation from the other biomass components non-trivial. Somehow, an efficient and sustainable extraction method is still lacking. Besides, the increasing environmental concerns lead to the search of environmentally friendly systems. In this work, maritime pine (*Pinus pinaster*) sawdust was initially characterized for its lignin and extractives (mainly hydrophilic) content and the extractives were characterized. It was found that the extractive removal does not affect the lignin extraction yield. Additionally, deep eutectic solvents (DESs) were prepared, characterized, and screened for the lignin extraction from maritime pine sawdust. The lignin extraction performance of different binary DESs with varied compositions was evaluated. The effect of cosolvents and the development of new ternary DESs was also tested regarding the improvement of the extraction capacity. The results show that the novel DES composed of lactic acid, tartaric acid and choline chloride, in a molar ratio of 4:1:1, is capable of extracting 95 wt.% of the total lignin present in pine sawdust with a purity of 89% (at optimized conditions). Moreover, the developed DES can be recycled and reused without compromising its performance for, at least, two additional cycles.

Abstract

The superior performance of the prepared DES and its "green" features makes the process highly appealing for biomass fractionation.

Keywords: Biomass fractionation; deep eutectic solvents (DESs); lignin extraction; *Pinus pinaster*.

Resumo

Enquanto fonte renovável mais abundante na Terra, a biomassa lignocelulósica tem um elevado potencial como fonte sustentável para a produção de biocombustíveis e outros produtos químicos. Os materiais lignocelulósicos são maioritariamente compostos por celulose, hemicelulose e lignina. A celulose é o polímero mais abundante presente na lignocelulose e é amplamente utilizado em diversas aplicações. Nos últimos anos, a lignina tem recebido uma acrescida atenção devido às suas propriedades atrativas. A lignina é a maior fonte renovável de compostos aromáticos e a sua eficiente extração permitirá, não só ampliar a utilização da biomassa como reduzir a dependência nos combustíveis fósseis. A lignina pode ser extraída da biomassa por diversos processos de extração, mas a sua complexa estrutura molecular dificulta a tarefa de isolá-la dos outros componentes da biomassa, sendo que, atualmente, não existe um verdadeiro método sustentável (do ponto de vista económico e ambiental) e eficiente para extração de lignina. Neste trabalho, a serradura de pinho bravo (*Pinus pinaster*) foi inicialmente caracterizada quanto ao seu teor de extrativos e lignina. Estes extrativos são essencialmente de natureza hidrofílica e, dado o seu baixo teor, a sua remoção não teve influência no rendimento de extração de lignina. Solventes eutéticos profundos (DESs) foram preparados, caracterizados e testados para a extração de lignina a partir de serradura de pinho. O rendimento da extração da lignina foi avaliado usando diferentes DESs binários com variadas composições. Foi ainda avaliado o efeito da adição de cosolventes e a performance de novos DESs ternários na capacidade de extração de lignina. Os resultados revelam que o novo DES composto por ácido lático, ácido tartárico e cloreto de colina, na proporção molar 4:1:1, é capaz de extrair 95% da lignina presente na serradura de pinho com uma pureza de 89% (condições operacionais otimizadas). Além disso, foi ainda observado que o DES desenvolvido pode ser reciclado e reutilizado,

sem comprometer a sua eficácia, durante pelo menos dois ciclos adicionais. O excelente desempenho de extração aliado às favoráveis características ambientais, sugerem que o novo DES pode ser um processo altamente apelativo para o futuro fracionamento da biomassa.

Palavras-chave: Fracionamento de biomassa; solventes eutéticos profundos; extração de lignina; *Pinus pinaster*.

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List of abbreviations

Acet	acetic acid
AIL	acid-insoluble lignin
ASE	accelerated solvent extraction
ASL	acid-soluble lignin
BSTFA	N,O-bis(trimethylsilyl)trifluoroacetamide
ChCl	choline chloride
Cit	citric acid
DES	deep eutectic solvent
FTIR	Fourier transform infrared spectroscopy
G unit	guaiacyl unit
GC	gas chromatography
GC–MS	gas chromatography–mass spectrometry
Gly	glycolic acid
H unit	*p*-hydroxyphenyl unit
HBA	hydrogen-bond acceptor
HBD	hydrogen-bond donor
ILs	ionic liquids
Lact	lactic acid
NADES	natural deep eutectic solvents
Prop	propionic acid
S unit	syringyl unit
SEC	size-exclusion chromatography
SEM	scanning electron microscopy
SFE	supercritical fluid extraction

List of abbreviations

Tart	tartaric acid
TGA	thermogravimetric analysis
UV-Vis	ultraviolet-visible

Chapter 1

Introduction

1.1 Problem statement

Currently, most of the chemicals and energy are produced from fossil fuel-based resources [1]. The concern about fossil fuels depletion and the environmental issues associated to its large-scale use led to the assessment of renewable and environmentally friendly alternative resources. Besides this, the world population growth causes an increase in waste generation that results in the appearance of concepts, such as "circular economy" and "zero waste" that aim to use waste as raw feedstock for the production of new materials of added value [2,3]. The production of biofuels and valuable chemicals and materials from lignocellulose not only contributes to the mitigation of the waste generation problem (lignocellulose can be obtained from agricultural, industrial, or municipal solid waste) but also allows the creation of whole new "green" business opportunities [1]. Lignocellulosic biomass has a great potential as a sustainable supply since it is the most abundant renewable resource on Earth, with an estimated annual production worldwide of ca. 1.3×10^{10} metric tons [4].

Lignocellulosic materials are mainly composed of three structural polymers: cellulose, hemicellulose, and lignin. Carbohydrate-based products have been widely used for industrial applications as in food, materials, medical, and pharmaceutical

industries [5]. For example, cellulose fibers are used as a substitute of synthetic fibers for the reinforcement of bioplastics [6]. In papermaking industry, cellulose is isolated and used for the pulp and paper production. In most of applications, biomass is fractionated to isolate cellulose and lignin is generated as a by-product. Regarded as a waste-product, ca. 90% of lignin is directly burned without further use [7]. For example, from the 50 million tons of lignin extracted in pulp and paper industry in 2010, only 2% was commercialized for further applications as adhesives and surfactants [8]. There is a great interest in lignin conversion into value added products because its valorization would maximize the utilization of the biomass. In addition, lignin has some valuable inherent properties, such as polyphenolic structure, high thermal stability, biodegradability, and antimicrobial and antioxidant activities that encourage its application in several different areas, such as to produce dyes, emulsifiers or polymer composites. However, the complex molecular structure of lignocellulose caused by the intricate interactions between lignin and the carbohydrates hinders the fractionation efficiency of the biomass and makes it difficult to isolate pure lignin from the other biopolymers. Additionally, the method employed to extract lignin typically has a strong influence on its final structural features and properties. Consequently, a selective and efficient extraction process is required to obtain valuable lignin. Several solvents have been developed for biomass fractionation but the demand for environmentally friendly and economically viable methods led to the search for novel non-toxic, inexpensive, and recyclable systems. In this respect, deep eutectic solvents (DESs) have been employed in biomass fractionation to replace organic solvents or ionic liquids due to their apparent favorable properties, such as ease of preparation and biodegradability combined with its high efficiency and selectivity for lignin extraction [9].

1.2 Aims

The main aim of this project was to evaluate the suitability of binary and ternary DESs systems as a sustainable and renewable alternative for lignin extraction from maritime pine (*Pinus pinaster*) sawdust while exploring a plethora of different conditions (e.g., DES composition, temperature, extraction time, co-solvent effect, etc.).

1.3 Dissertation structure

In chapter 2, the readers can find a detailed introduction mainly focused on lignocellulosic feedstock, their composition and fractionation methods. The motivation for the selection of DESs as "green" solvents for lignin extraction and the current state-of-the-art of biomass fractionation using DESs are also described. Chapter 3 focus on wood pretreatments for the removal of extractives and their characterization. In chapter 4, a screening of different acidic DESs for lignin extraction and the optimization of the extraction conditions are presented. Lignin characterization is also described in this section. Finally, chapter 5 outlines the main conclusions of this work with further suggestions for future work.

The list of all chemicals used in this work, as well as the procedures and characterization techniques, are described in chapter 6.

Chapter 2

Literature review

2.1 Lignocellulosic material

Lignocellulose is mainly constituted by a mixture of three biopolymers: cellulose, hemicellulose and lignin [10]. Besides the structural polymers, lignocellulosic feedstock contains other minor compounds, such as pigments, resins and phenolics, named extractives [11,12].

- **Cellulose**

Cellulose is a linear polysaccharide with high degree of polymerization, composed of *β*-D-glucopyranose units linked by *β*-1,4 linkages (**Figure 2.1**) [7,13]. It is the major component of lignocellulosic materials and accounts for ca. 30–50 wt.% of the dry biomass [7]. The high number of hydroxyl groups in cellulose are responsible for the strong and extended hydrogen-bonding network between cellulose chains to form sheets [14,15]. The three-dimensional structure of cellulose is stabilized by hydrophobic interactions between these sheets into a crystal-like stacked structure [15]. Cellulose is present in the plant cell walls and is responsible for its structural support due to its superior mechanical features [16].

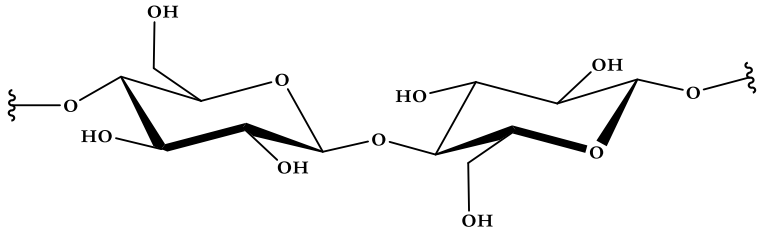

Figure 2.1. Schematic representation of cellulose linear structure (cellobiose unit).

- **Hemicellulose**

Hemicellulose is another polysaccharide present in the cell walls of lignocellulosic plants and accounts for ca. 20–35 wt.% of biomass [7]. In contrast to cellulose, hemicellulose has a random and amorphous structure composed of different sugar units. The most common sugar residue in hemicellulose is D-xylose but several other are present, such as D-glucose, D-galactose and L-arabinose [16]. These residues link to each other to form a branched polymer chain with little mechanical strength (**Figure 2.2**).

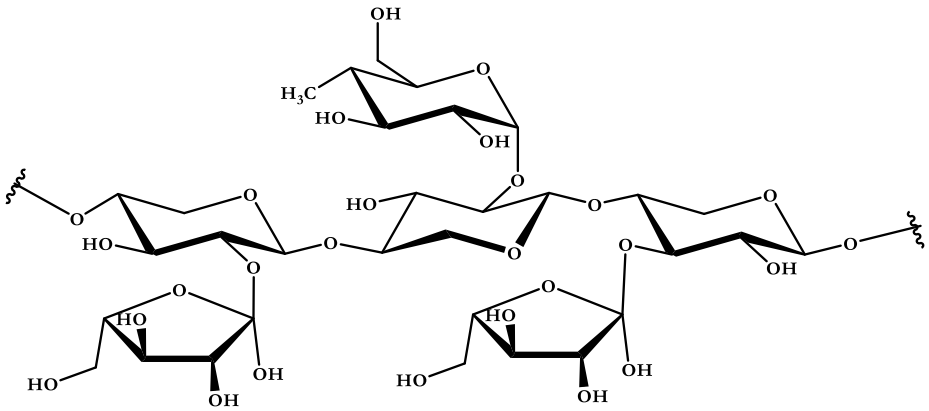

Figure 2.2. Schematic representation of hemicellulose branched structure.

- **Lignin**

Lignin is the only non-polysaccharide structural polymer comprised in lignocellulosic materials. It is a branched aromatic polyphenol polymer and it is more hydrophobic than the other two biopolymers [17]. Lignin structure, functions and properties will be briefly reviewed in **section 2.1.2**.

- **Extractives**

Extractives are a group of compounds present in lignocellulosic materials that have a non-structural function. Fats and phenolic compounds are some examples of

extractives whose function in plants are mainly related to energy storage and antifungal and antimicrobial activities [11]. In **Section 2.1.3** we will further elaborate on this subject.

Wood and agricultural wastes are some examples of lignocellulosic biomass. Thus, several sources of lignin can be found in nature, including cereal straws, sugarcane bagasse and wood [18]. Among these sources, wood has the highest content of lignin, ca. 15–30 wt.% [19], in contrast to other sources, such as corn cobs and rice straws that typically contain ca. 3–25 wt.% [18,20]. In **Table 2.1**, the content of extractives and polymers of several lignocellulosic materials is listed.

Table 2.1. Cellulose, hemicellulose, lignin, and extractives content, expressed as wt.% of dry mass, for some selected lignocellulosic resources. Data collected from [21,22].

Source	Cellulose	Hemicellulose	Lignin	Extractives
Wood	39–54	11–37	16–31	1–8
Sugarcane bagasse	40–41	27–37	10–20	10
Corn cobs	34–45	32–36	6–16	5
Cotton fiber	95	2	1	0.4
Wheat straw	30–50	24–50	9–17	5

2.1.1 Wood

Wood is the major source of lignin. In fact, the word lignin derives from *lignum*, the Latin word for wood [23]. The presence of lignin in the cell walls of vascular plants allowed the appearance of larger plants, such as trees, due to its stability and strength and the efficient transport of water [23,24]. The first trees occurring in nature were gymnosperms, that are non-flowering plants. Conifers belong to this group of plants and are the woody plants that origin the so called "softwood". On the other hand, "hardwood" classification derives from flowering plants, named angiosperms. This group of plants represent more than 90% of the existing plant species [23]. Douglas fir, pine, spruce, fir, and larch are some examples of "softwoods" whereas eucalyptus, aspen, birch, oak, poplar, and willow belong to "hardwoods" group. These two classes of wood differ from each other, not only by the nature of the tree (flowering or not) but also by their

chemical composition. Other factors that also affect the chemical composition of wood include the age of the tree, species and even the position inside the tree [11,25-27]. Differences can be expressed as the relative content of the structural polymers, water content in the cells, and percentage of extractives. Generally, softwoods contain more extractives than hardwoods [28]. Lignin content and structure varies between hardwoods and softwoods, with the latter generally showing a higher content, but also varies in plants from the same genus. For example, within the *Pinus* genus, the lignin concentration may vary from 25% for "*Pinus monticola*" to 30% for "*Pinus palustris*" [25]. According to Miranda et al., the lignin content of "*Quercus faginea*" is higher in the outer part of the wood, sapwood, than in the inner part, heartwood (**Figure 2.3**) [27]. This difference depends on the age of the tree, with older trees showing more pronounced difference in composition between the two parts of the plant [27]. However, extractive-free wood does not show significant differences regarding to the lignin content in these two parts. This suggests that the differences in lignin content between heartwood and sapwood are caused by the higher content of extractives present in the heartwood [27,29].

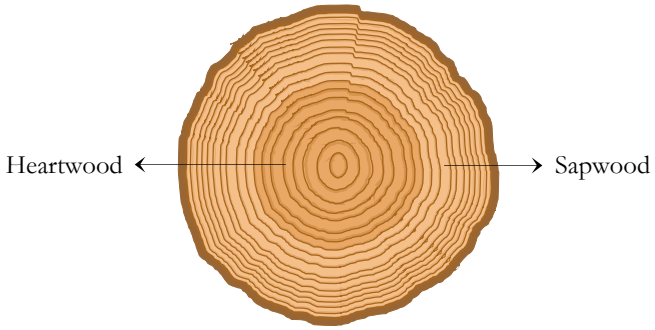

Figure 2.3. Representation of heartwood and sapwood in a wood cross-section.

Besides these original properties, it is necessary to consider that, after felling, wood properties may change. Acquired properties will depend on wood transport, seasoning, size and storage [30]. A decrease in microbial activity, moisture content and extractive content can occur during these processes [30,31]. Thus, before any application or biomass fractionation, it is necessary to know in detail the composition of the raw material. Several analytical techniques and standard procedures for biomass characterization are described in the literature and the main ones used in this work will be further discussed in subsequent sections.

2.1.2 Lignin

Lignin is a branched phenolic heteropolymer composed of a complex network mainly created by three different monolignols monomers: *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol (**Figure 2.4**) [32]. The three-dimensional structure of lignin occurs by dehydration polymerization of these monolignols, forming a network comprising three units, linked by ether and C−C chemical bonds: *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) units, differ from each other in the number of methoxy groups in C3 and C5 atoms of the aromatic ring [21,33].

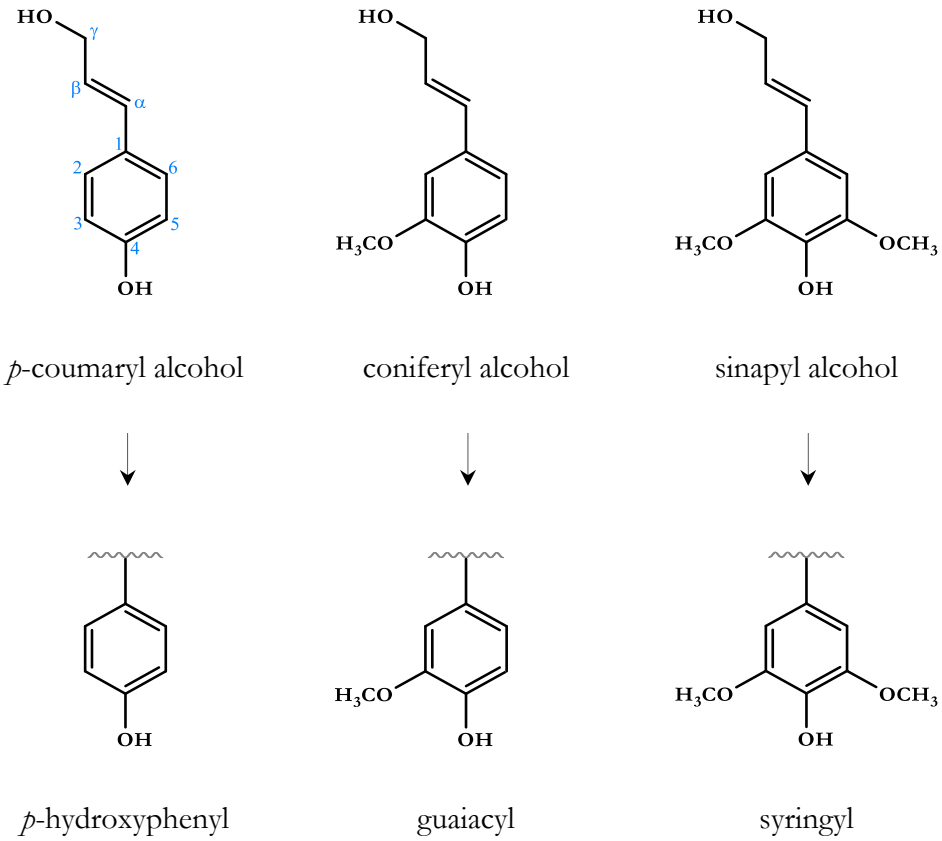

Figure 2.4. Representation of the molecular structures of lignin monolignol monomers: *p*-coumaryl, coniferyl and sinapyl alcohols and respective units: *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S).

The proportion of lignin monomers is different in the various sources of lignocellulosic biomass. For example, lignin in grasses has a more complex structure than in woods due to the presence of a mixture of all three units, H, G and S [32,34]. Hardwood lignin is essentially composed of G and S units in a ratio of 1:2 and trace

amounts of H units whereas, in softwoods, it is mostly composed of G units (ca. 95%) and some minor H units [18,25].

Polymerization occurs by random coupling of monolignol radicals, forming dilignols that undergo coupling with other mono-, di- or ololignol radicals, creating a three-dimensional cross-linked network [35]. Linkages between monolignols may occur as C−C or ether bonds at different positions, as represented in **Figure 2.5**. The most frequent bond in lignin structure is *β*−O−4, accounting for around 50% of the total lignin coupling bonds [18,35]. Due to the high content of G units that can link to each other by 5−5 bonds (**Figure 2.5**), lignin in softwoods has a more branched and condensed structure [25,36]. In contrast, as the major monomer in hardwoods, S units contribute to improve lignin processibility by reducing branching reactions [21]. Due to the great diversity of linkages and monomer relative composition, lignin cannot be described as a unique compound but rather as a class of natural phenolic polymers with diverse compositions [34].

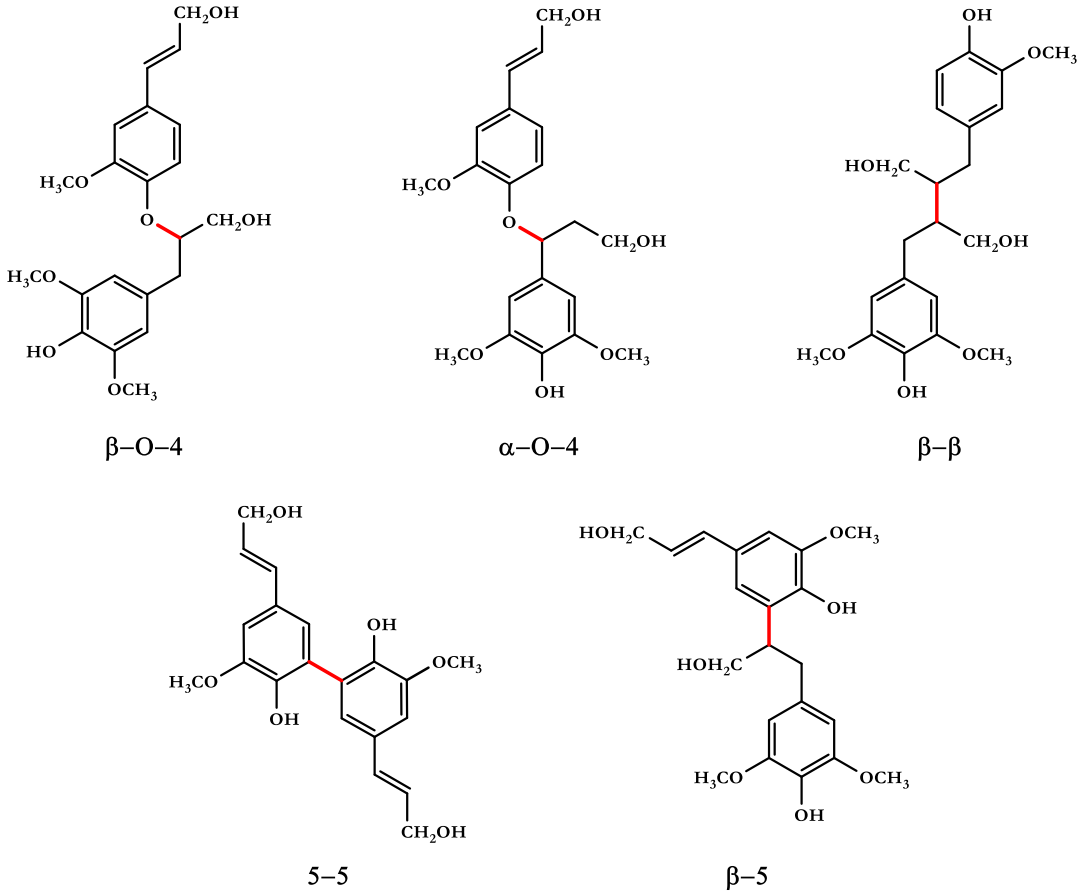

Figure 2.5. Examples of some monomer coupling linkages occurring in lignin.

Besides its monolignol network, lignin binds to the structural carbohydrates (i.e., cellulose and hemicellulose), through ether and/or ester chemical bonds, depending on the functional groups of the monosaccharides, forming lignin–carbohydrate complexes [18,33,37]. The linkages present in such complex network are strongly responsible for the structural stability and resilience to degradation of lignocellulosic materials [33,38].

The high number of aromatic groups present in lignin makes this macromolecule the most hydrophobic of all the structural polymers in lignocellulosic biomass [17,39] and it is responsible for waterproofing the tissues [32,40,41]. Lignin provides mechanical stability (strength and rigidity) to the cell-walls and plays an important role in the thermal stability of the plants [18]. Additionally, its antimicrobial and antifungal activities prevent the attack of pathogens [42].

As a natural polyphenol, lignin can be regarded as a sustainable substitute for petroleum-based phenol [43]. It finds potential applications in different areas, such as paints, thermosets, emulsifiers, dyes, polymer composites, aerogels, and adhesives [18,44,45]. In the food industry, lignin can be used as an antioxidant or an additive for color or taste stabilization [25]. The wide availability, biodegradability and good mechanical properties of lignin have encouraged its use, for instance, as a substitute of synthetic fibers in bioplastics reinforcement [6].

2.1.3 Wood extractives

Extractives are non-structural compounds present in lignocellulosic materials as phenols, sugars, waxes, fatty acids, fatty alcohols, steroids, resin acids, terpenes and inorganic compounds [11,46]. The type and relative amount of extractives varies from wood type and species. Extractives content varies between hardwoods and softwoods, with the latter typically showing a higher content [28]. Different compounds are found in the two wood types; in contrast to hardwoods, that have a small amount of terpenes, pine and other softwoods have high content of these compounds [47]. As mentioned before, different parts of wood have different composition and heartwood contains more extractives than sapwood [27]. The content of extractives in the bark is usually high and it is a great source of resin [11]. Opposite to lignocellulosic polymers, extractives have

non-structural functions but provide additional resistance to the tree besides conferring taste, color and odor [47]. For example, polyphenols and other aromatic compounds are responsible for the decay resistance of the bark and heartwood because of their antifungal and antimicrobial activities whereas fats play a role in energy storage [11,47]. Function and wood type occurrence of some extractives are summarized in **Table 2.2**.

Table 2.2. Classification and function of different extractives typically found in wood. Adapted from [48].

Extractives	Subclasses	Function	Tree type
Terpenoids	Monoterpenoids Resin acids Other terpenoids	Protection	Softwoods
Fats	Triglyceride Fatty acids Steryl esters Sterols	Physiological	All wood species
Phenolic substances	Lignans Flavonoids Stibenes Tannins	Protection	All wood species, specially softwoods
Carbohydrates	Glycosides Sugars Starch Proteins Gum Pectins	Biosynthesis Nutrient reserve Protection	All wood species
Inorganic	Various salts	Photosynthesis Biosynthesis	All wood species

The presence of extractives may cause an overestimation of lignin content, thus resulting in inaccurate biomass analysis [49]. These compounds are also reported to negatively affect biomass utilization in different applications. For instance, the production of cement boards from woods containing high content of carbohydrates and starch, results in poor mechanical properties of the final boards [13]. Biomass pyrolysis is also affected by the presence of extractives: organic extractives, such as resin acids promote clogging in the reactor and produce low-quality oil, whereas the inorganic extractives are responsible for the decrease of the sugar yield and for the modification of

the properties of lignin-based products [11]. The mechanical durability of biofuel wood pellets produced from pine sawdust was observed to increase with storage time, caused by the decrease in extractives content over the time [31]. It was reasoned that extractives molecules block the binding sites in wood surface and therefore less bonds between wood particles are established [31]. In pulp and paper industry, extractives affect the production line by deposition of pitch on the equipment [30,50]. Quality of the pulp and paper are also affected by the presence of extractives. They can decrease the bonding area between the fibers and, consequently, reduce the paper strength and influence the bonding of toner particles to the printing paper [50]. Furthermore, colored extractives may impart color to pulp and result in darker paper with visible defects [50,51]. **Table 2.3** summarizes some of the negative effects caused by extractives in papermaking industry.

Table 2.3. Effect of fatty and resin acids on the papermaking industry [52].

Component Groups	Effect
Resin acids	Paper machine runnability, deposits
	Odor
	Allergic reactions (oxidized products)
	Effluent and sediment toxicity
Fatty acids	Paper machine runnability, deposits
	Odor
	Lower sheet strength, friction
	Toxicity (unsaturated fatty acids)
Fatty and resin acid soaps	Foaming
	Deposits

Extractives have low to moderate molecular weight and can be easily removed from biomass by extraction with aqueous or organic solvents [13,47]. As discussed above, the removal of these compounds is advantageous as it avoids several negative effects. On the other hand, valuable compounds, such as fatty and resin acids that can be isolated during pretreatments, can be used and valued in other applications. In kraft pulping, the volatile monoterpenes dissolved in the black liquor can be isolated to obtain turpentine that is widely used for the production of solvents, paints and varnishes [11,50]. Pine species are

a great source of terpenes and, consequently, turpentine [47]. The yield of isolated terpene can reach ca. 10 kg/ton of pulp [50]. The non-volatile fraction of the extractives present in the black liquor is composed of fatty and resin acids that originate the tall oil, a source for the production of ink and adhesives [50,53]. Pine pulping results in tall oils yields of ca. 50 kg/ton of pulp [50].

From this brief analysis it becomes clear that detailed information on extractives content and composition is essential to evaluate the presence of valuable products and avoid undesirable side effects [53].

a) Extractives removal

As mentioned above, extractives can be removed using different solvents, such as ethanol–benzene, ethanol–toluene, ether, acetone, ethanol and water [46]. Due to the great diversity of compounds present in wood, sequential extractions with different solvents are often employed to eliminate both lipophilic and hydrophilic extractives. Some standardized procedures are available for the preparation of extractives-free wood [54-57]. In the past, benzene used to be applied for extractives removal because of the high extraction yields, but this solvent was gradually replaced by other less toxic solvents, such as ethanol or acetone [26]. The ASTM D1105-96 standard, for the preparation of extractives-free wood, describes a two-step Soxhlet extraction using ethanol–toluene and ethanol, followed by a three-step reflux extraction using water [57]. Ethanol–toluene mixture is used to remove waxes, fats, resins and wood gums, whereas hot water removes hydrophilic compounds, such as tannins, gums, sugars, starches and pigments [26,57,58].

Alternative methods to Soxhlet have been suggested to reduce the long extraction times and solvent volumes. One of the alternatives is Soxtec, where the samples are directly immersed in the vessel that contains the solvent. This method is faster than Soxhlet and requires considerably less solvent volume[59]. The Scandinavian standard for the determination of acetone-soluble matter describes Soxhlet and Soxtec techniques as identical alternative methods for extractives determination in wood chips [54]. Soxtec is performed in automated apparatus that are more expensive than Soxhlet and restricts its use. On the other hand, Soxhlet is an inexpensive method and has the advantage of the

sample being constantly in contact with fresh solvent [48]. Accelerated solvent extraction (ASE) and supercritical fluid extraction (SFE) have also been proposed as viable alternatives to the Soxhlet method [59,60]. In ASE, high temperatures and pressures are required to obtain subcritical fluid conditions for extractions. ASE yields are similar to those obtained with Soxhlet, but higher lignin content was found in the extractives [60]. SFE uses supercritical fluids to improve mass transfer but the expensive equipment required and energy consumption hinders its application [59].

b) Extractives analysis

The analysis of extractives can be made in a qualitative and/or quantitative approach. Usually, the total extractive content of the sample is determined by gravimetry after proper separation of the wood from the solvent and its evaporation. This method gives information about the total extractives present in the sample and can be used to compare the efficiency of different solvents for the extractives removal, but no information about its composition is provided. When successive extractions with solvents with different polarities are performed, it is possible to assess information about the lipophilic and hydrophilic extractives content. Gravimetrical analysis is generally sufficient for quality control in routine processes, but for a more detailed information about biomass composition, qualitative analysis must be performed [59]. Qualitative analysis allows for the determination of the different biomass component groups or even the individual compounds [59]. Chromatographic techniques are used for qualitative and quantitative analysis of the component groups of biomass: gas chromatography (GC), high performance liquid chromatography and size-exclusion chromatography [48]. Fourier transform infrared (FTIR) is a spectroscopic technique used for the determination of functional groups and it has also been applied to collect information about wood extractives composition [49,61-65]. Identification of the individual compounds is mostly achieved by the combination of GC and mass spectrometry (GC–MS) techniques [59].

2.2 Lignin extraction

Biomass fractionation consists in the separation of biomass components (i.e., cellulose, hemicellulose, and lignin). The goal is to break the covalent bonds formed between lignin and carbohydrates in order to enable their isolation. During lignin extraction, lignin intramolecular bonds are also partially cleaved and thus changes in polymer structure may occur [66]. The cleavage of intramolecular covalent bonds of lignin causes an increase in solubility of the polymer due to a decrease of molecular weight [25,67].

Since the extraction process involves interaction between the solvent and the lignin molecules, the surface properties of the raw material influence the extraction rate and the final characteristics of the extracted lignin. These properties are dependent on the biomass source and composition. Extractives content of the lignocellulosic material or application of any pretreatments for their removal also affects the surface properties of biomass and are expected to influence lignin extraction. As alluded above, the extraction method employed in the fractionation of biomass to isolate lignin may strongly affect its final properties. Different fractionation approaches lead to lignins with different structure, solubility, molecular weight and mechanical performance [25,66-68]. In paper and pulping industry, the most common process is kraft pulping [69]. Kraft lignin is soluble in alkaline solutions whereas lignosulfonate, lignin obtained from sulfite pulping, is water soluble [21,68]. So, in order to achieve highly pure and valuable lignin, a selective solvent and adjusted extraction conditions (i.e., time and temperature) are essential [67,70]. An excessively long extraction time or high temperature may compromise the selectivity of the extraction and, consequently, increase the impurities of extracted lignin [69].

As mentioned above, lignin binds to both cellulose and hemicellulose by chemical and physical interactions creating an entangled network. Due to the heterogeneity and complexity of these structures, the delignification process is complicated and a selective and efficient separation is difficult [71].

Lignin dissolution depends on both entropic and enthalpic contributions during the separation. In one hand, the entropic term always favors the mixing and it is easily

increased by rising the temperature [72]. On the other hand, chemical interactions are responsible for the enthalpic factor and can be related to the so called "Hansen solubility parameters" [73]. According to Hansen, solubility depends on three different parameters: dispersion (δ_d), polarity (δ_p), and hydrogen bonding (δ_H) between the solute and the solvent. The highest solubility is achieved when the term given by the equation (1) is minimized [74]. This occurs when similar interactions are present in the solute and solvent [74]. Thus, to achieve lignin dissolution, chemical similarity between the solvent and the polymer is required.

$$\left[\left(\delta_{d,solute} - \delta_{d,solvent}\right)^2 + \left(\delta_{p,solute} - \delta_{p,solvent}\right)^2 + \left(\delta_{H,solute} - \delta_{H,solvent}\right)^2\right]^{1/2} \quad (1)$$

The three Hansen parameters show a synergistic effect in solubility. In a work conducted by Duval et al., a linear dependency of the solubility with solvent polarity (δ_p) was observed with polar solvents showing full solubility of kraft lignin, but some exceptions were noted, proving that there are other parameters to take into account [75]. The hydrogen bond parameter (δ_H) seems to account for the fact that alcohols with shorter chain and, therefore, greater hydrogen bond capacity, lead to a higher lignin solubilization, but it is not a suitable parameter to evaluate the solubility of lignin in ketones [75]. Thus, this approach can be useful to predict the solubility of a polymer in a certain solvent, but it should not be regarded as an universal principle [72].

Numerous methods are employed in biomass fractionation to separate lignin from carbohydrates. Some of them were designed to directly extract lignin whereas others originate lignin as a by-product. It is also possible to classify the methods by the type of lignin extracted, e.g., sulfur lignin and sulfur-free lignin [8]. Generally, paper and pulping processes for biomass fractionation are methods where sulfur lignin is obtained as a by-product [76]. These were the first methods used for lignin isolation and include kraft and sulfite processes. Other methods include acid pretreatment, organosolv, ionic liquids and, more recently, deep eutectic solvents.

2.2.1 Kraft process

The kraft process is the main method used in paper and pulping industry. This process involves cooking the biomass for 2 h at a temperature range of 150–180 °C using sodium hydroxide (NaOH) and sodium sulfide (Na_2S), solutions and originates lignin as a by-product in the extracted "black liquor" [77]. Kraft lignin typically contain 1–3 wt.% of sulfur in form of thiol groups [78].

2.2.2 Sulfite process

Lignosulfonate is a by-product of sulfite extraction process, where wood is treated with sulfur dioxide solution (SO_2) and sulfite (SO_3^{2-}) or bisulfite (HSO_3^-) ions [78]. Sodium, calcium, magnesium, ammonium and potassium are some cations used in this process [77]. Wood treatment in sulfite pulping usually occurs in a temperature range from 120 to 180 °C during 1–5 h [79]. Lignosulfonates contain a larger amount of impurities, such as ash and carbohydrates, when compared to Kraft lignin and has higher average molecular weight [77,78]. During this process, sulfonate groups (SO_3^-) are incorporated in lignin structure [77,78]. Most of commercially available lignin is in the form of lignosulfonate [77].

2.2.3 Organosolv

Organosolv is a fractionation process that promotes the dissolution of lignin and hemicellulose from biomass using organic solvents or a mixture of water and organic solvents, in the presence or absence of a catalyst [74,77]. Lignin is then separated from hemicellulose by precipitation with addition of excess water [80]. Organic solvents include short-chain aliphatic alcohols (e.g., methanol, ethanol), polyols (e.g., glycerol, ethylene glycol, triethylene glycol), organic acids (e.g., formic and acetic acid), alkylene carbonates, acetone, dioxane and phenol [80,81]. As short-chain aliphatic alcohols have a great potential in lignin extraction, ethanol has been widely used because of its lower toxicity compared to methanol [74,82]. The temperature range for extraction varies from 100 to 250 °C according to the selected solvent [77]. Lower temperatures can be applied

in peracid fractionation, that can be conducted at room temperature, but requires a long period of extraction (typically, between 1 to 7 days) [83-85]. In several organosolv processes, biomass fractionation at high temperatures (185–210 °C) does not require the use of an external catalyst due to the extraction of organic acids from biomass which, themselves, can act as catalysts in lignin–carbohydrate bonds rupture [83]. Organic solvents promote the cleavage of these type of bonds, improving the process selectivity and originating lignin with small modifications and high molecular weight [77,80]. However, addition of an acid catalyst leads to rupture of ether bonds in the lignin structure and promotes intramolecular condensation reactions [77]. Organosolv lignin has high purity, is more condensed and has a lower sulfur content than lignin from kraft and sulfite processes. However, the high temperatures required during processing and the low recyclability compromises the economic viability of the process [74,81].

2.2.4 Ionic liquids

Ionic liquids (ILs) are organic salts formed by a combination of organic and inorganic cations and anions which, due to their asymmetry, typically present a melting point below 100 °C and some of them are liquid at room temperature [86,87]. They show some favorable properties, such as high thermal stability and low flammability and vapor pressure [86]. These properties have attracted the interest to use ILs as substitutes to traditional volatile organic solvents [86]. The suitability of ILs as solvent for polymers dissolution, as cellulose and hemicellulose, led to the investigation on their performance for lignin dissolution and its extraction from biomass [88]. Due to the great variety of cations and anions that can be combined to prepare ILs, it is possible to obtain ILs with tunable properties and the ionic components will impact the lignin extraction efficiency. According to Pu et al., lignin solubility is principally affected by the nature of the anions [89]. ILs with large size and non-coordinating anions, such as BF_4 and PF_6, were found unsuitable for lignin dissolution [89]. Similar trend was observed by Lee et al. [90]. The authors found that ILs containing BF_4 and PF_6 are not able to extract lignin from maple wood flour [90]. On the other hand, extraction with ILs based on Cl^- anion show high lignin extraction, but a higher solubility of cellulose was also observed [90]. As a good hydrogen-bond acceptor, Cl^- can interact with hydroxyl groups of cellulose [88].

The ionic liquid 1-ethyl-3-methylimidazolium acetate ([Emim][CH_3COO]) shows a good lignin extraction and a poor cellulose solubility, making it a suitable solvent for lignin extraction [90]. Lignin is also efficiently extracted from sugarcane bagasse using 1-ethyl-3-methylimidazolium alkylbenzenesulfonate ([Emim][ABS]) at atmospheric pressure and high temperatures (170–190 °C) [91]. Extraction conditions also affect lignin recovery yield. The lignin recovery yield increases when raising the temperature from 170 to 190 °C and the extraction time from 30 to 120 min [91].

Although lignin extracted with ILs is similar to lignins obtained by organosolv extraction, ILs pretreatments are not implemented at a large scale because of their high price and low availability [77]. Furthermore, some studies report toxicity and poor biodegradability as inherent characteristics of ILs [92-94].

2.2.5 Deep eutectic solvents (DESs)

Deep eutectic solvents (DESs) can be regarded as a great alternative to common ILs since they share some important properties, such as low melting point and low vapor pressure. However, DESs show additional favorable properties, such as their ease of preparation and lower cost compared to ILs [95,96]. Besides, DESs can be non-toxic and biodegradable when prepared with natural compounds [95]. DESs are formed by the combination of a hydrogen-bond acceptor (HBA) and a hydrogen-bond donor (HBD), in an appropriate ratio, that results in a homogenous mixture with a melting point significantly lower than the melting points of each individual component on their own [70,97,98]. In **Figure 2.6**, it is represented a schematic phase diagram of generic mixture of two components, HBA and HBD, forming an eutectic mixture. The molar composition with the lowest melting temperature corresponds to the eutectic composition.

A wide variety of compounds can be used to prepare natural deep eutectic solvents (NADES) including sugars, carboxylic acids, amines, polyalcohols and amino acids [99,100]. These compounds can form a hydrogen-bond network that is responsible for the decreasing of the melting point [101,102]. In **Figure 2.7**, some of the most common HBA and HBD used in DES synthesis, are summarized. Whereas a great variety

of HBD are found in literature, choline chloride (ChCl) is frequently used as HBA for DES preparation.

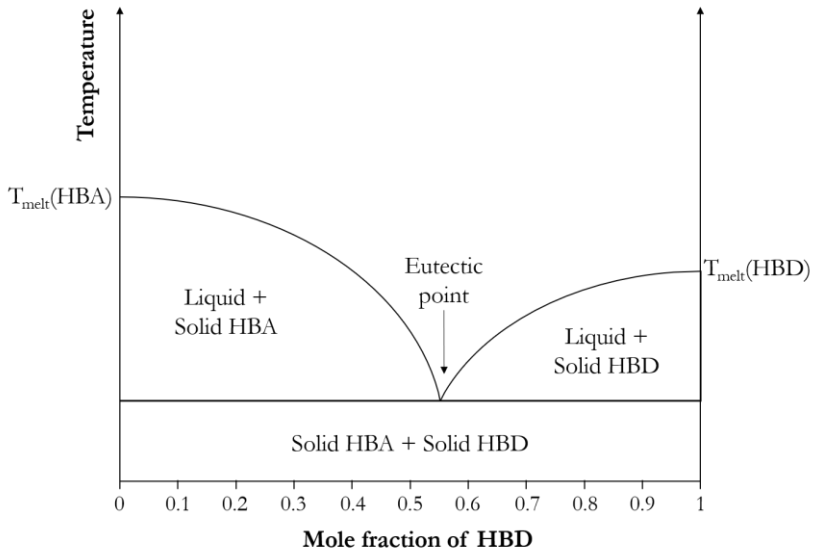

Figure 2.6. Schematic representation of a phase diagram and eutectic point of a generic two-component (HBA and HBD) mixture.

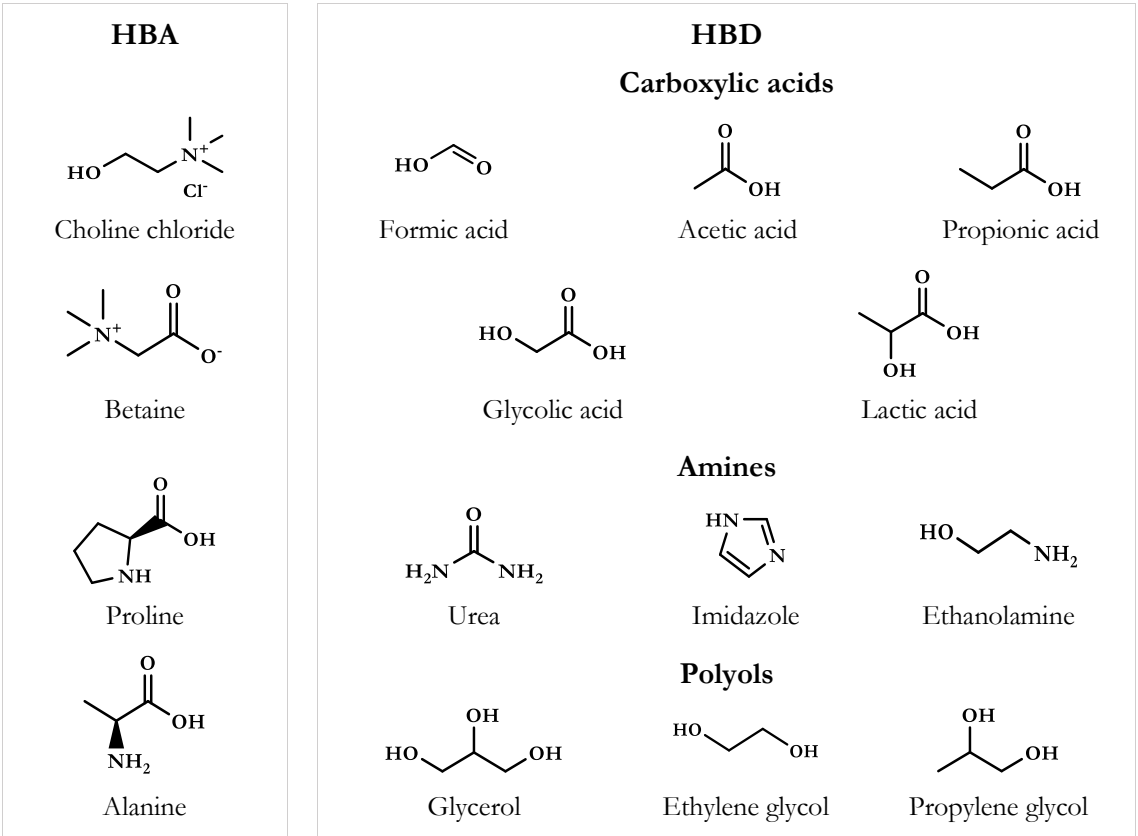

Figure 2.7. Molecular structures of typical hydrogen-bond acceptors (HBA) and donors (HBD) used in DES preparation.

Implementation of different DESs in biomass fractionation has been extensively studied during the last years and several reviews are available [5,98,103-105]. Generally, DESs are observed to show selective lignin dissolution and poor cellulose solubility [9]. This has been attributed to the strong hydrogen-bonding network present in both cellulose and DES which dissociation and reorganization are not thermodynamically favorable [106]. Acid-based DESs are highly efficient for lignin dissolution when compared to other DESs [98]. Nevertheless, both HBA and HBD affect lignin extraction yield and selectivity. Francisco et al. have evaluated the effect of 26 different DESs in biopolymer solubility in order to infer on their suitability for biomass fractionation [9]. The authors found that lactic acid–choline chloride mixtures provide high lignin solubilization with poor starch and cellulose dissolution; a higher acid ratio improves lignin dissolution [9]. However, for malic acid–proline, an opposite trend was observed; lower acid content favors lignin solubility, while an increase in cellulose and starch was also detected, suggesting that proline improves cellulose solubility [9].

Tan et al. conducted a study on the effect of different functional groups of acid HBD of choline chloride-based DESs for lignin extraction [107]. The authors found that, for linear saturated acids with similar functional groups, short alkyl chain acids exhibit higher performance for lignin dissolution: choline chloride–formic acid mixtures achieve higher lignin extraction yield than choline chloride–butanoic acid, with 61.9% and 14.3% lignin yield, respectively [107]. Similar results were found by Lynam et al. which reported a higher lignin solubility in DESs containing formic acid than DESs containing lactic or acetic acid [108]. This behavior results from the fact that the solvent ability to donate protons in solvent–solute hydrogen-bonding networks decreases with the increase in the alkyl chain length of the acid [109]. For both alpha-hydroxy acids and linear saturated acids, monocarboxylic acids show higher extraction yield than di- and tricarboxylic acids. The presence of additional carboxylic groups impairs the extraction due to the physical entanglement caused by the formation of extensive chains of dimers [110]. Such dimerization restricts the mobility of solvent molecules, resulting in poorer interactions between solvent and solute [107]. When comparing acids with similar chain length and same number of carboxylic groups, it was found that extra hydroxyl groups in alpha-hydroxy acids facilitates lignin extraction due to their higher polarity. Double

bonded unsaturated acids exhibit lower extraction performance than alpha-hydroxy acids, but higher than linear saturated acids [107].

DESs have the ability to accommodate a certain amount of water without either destroying its structure or compromising its dissolution performance. Hammond et al. studied the effect of water in a mixture of ChCl–Urea and concluded that the nanostructure of the eutectic system is not affected with a hydration level up to 42 wt.% but, at 51 wt.% H_2O, the DES molecular structure is disrupted [111]. A similar study conducted by Dai et al. about the changes in physicochemical properties and structure of several DESs due to the dilution effect revealed that the hydrogen-bonding network disappears when the water content is above 50% (v/v) [112]. In such cases, the systems are no longer regarded as "pure" DESs, but rather as an aqueous solution where the dominant interactions are water–water and water–DES [111,112]. Furthermore, the addition of water affect DESs properties, such as density, viscosity, polarity and conductivity [95,112,113]. The ability to change the viscosity of a DES is sought after because most of these solvents are highly viscous, making the extraction processes more difficult and time-consuming, mainly due to mass transfer limitations [112]. The addition of water (< 40–50%) to DESs can promote a decrease in the viscosity, thus favoring the extraction [112]. DES polarity can also be tuned according to the water content and such effect can be either positive or negative, depending on the polarity of the DES components. A non-polar DES will exhibit an increase in polarity with the addition of water, while a highly polar DES is expected to have the opposite response. The DES polarity will be closer to that of water as its water content increases [112,114]. Thus, water can be an effective tuning agent to adjust DESs properties and improve their extraction or dissolution performance. Nevertheless, it should be stressed that the addition of water to DESs may cause a beneficial change in viscosity but impair the solubility, due to changes in the solvent polarity.

One of the advantages of using DESs as solvent for lignin extraction is that they are easily recovered at the end of the process and can be reused for additional extractions. This is an important aspect fitting in the "green chemistry" principles and makes DESs an environmentally friendly option for biomass fractionation [115]. In contrast to lignin, DESs components are water soluble. This means that water can be used as an excellent

antisolvent to precipitate and isolate lignin and posteriorly removed from DES by evaporation [115]. It has been reported that DESs can exhibit an efficiency of, at least, 80% of that of the freshly synthesized solvent even after three consecutive extractions [115].

Chapter 3

Pine sawdust analysis and pretreatment

Prior to any processing of biomass, detailed information about the feedstock material is important to better understand its properties and avoid possible problems in line production or final product quality due to the presence of undesirable compounds or unexpected side reactions [46,53].

Wood analysis consists in the quantification of the polymeric components (i.e., cellulose, hemicellulose, and lignin), extractive content, ash or moisture determination [46]. There are several standard methods available describing the sample preparation and quantification. During the sample collection and preparation, it is necessary to ensure the representativity of the sample. For component analysis of wood as lignin or extractives content determination, wood should be dried until constant mass is achieved [46].

3.1 Moisture content of pine sawdust

Wood has a hygroscopic nature as it is a porous material that can absorb water from the air [116]. The moisture content is dependent on the surrounding conditions as relative humidity, temperature, and pressure [116]. In addition, in woods with lower extractives content, the equilibrium moisture content is usually higher than in woods with higher quantity [117]. Extractive-free woods hold more empty space in cell walls to

accommodate water [118]. Since cut and seasoned wood presents a lower content of extractives than the living tree [31,48], they will exhibit higher water uptake. Therefore, the influence of the moisture content is expected to be greater in these samples [119-121]

For a proper analysis of the biomass, moisture content should be previously determined, and a complete dryness should be ensured to avoid inaccurate determinations.

The most common approach to remove water from the wood is the oven-dry method. It is a well-known procedure widely described in several standards [122-124]. In this method, the wood is oven-dried at 101 to 105 °C and the moisture is determined as the ratio between the mass loss and the mass of the wood sample [116,125]. However, it has some disadvantages, such as the overestimation of the true moisture content if the sample contains volatile compounds, such as oils and resins, that evaporate in the drying process [125,126]; in addition, oven-dry wood easily absorbs moisture from the atmosphere [125]. Because of this, a second drying procedure has also been tested, submitting the samples to freeze-drying, also known as lyophilization. In **Figure 3.1**, the moisture contents determined for pine sawdust by the oven-drying and freeze-drying methods, are presented. The moisture content can be expressed in a wet basis, in which the moisture content is given by the ratio of the mass loss and the initial mass of the sample or in a dry basis, where it is calculated by the ratio of the mass loss and the dry weight of the sample. The results show that freeze-dried sawdust exhibit a significantly higher moisture content for both wet and dry basis determinations. This suggests that freeze-drying is capable of a more efficient removal of the water present in the sawdust. The greater standard errors observed in oven dried samples also support the idea that, in such method, it is more difficult to avoid moisture. For these reasons, the freeze-drying-based procedure was considered the most efficient method to dry the sawdust and ensure complete (or close to it) dryness of the samples. Thus, before any analysis or pretreatment, all wood samples were dried overnight by freeze-drying.

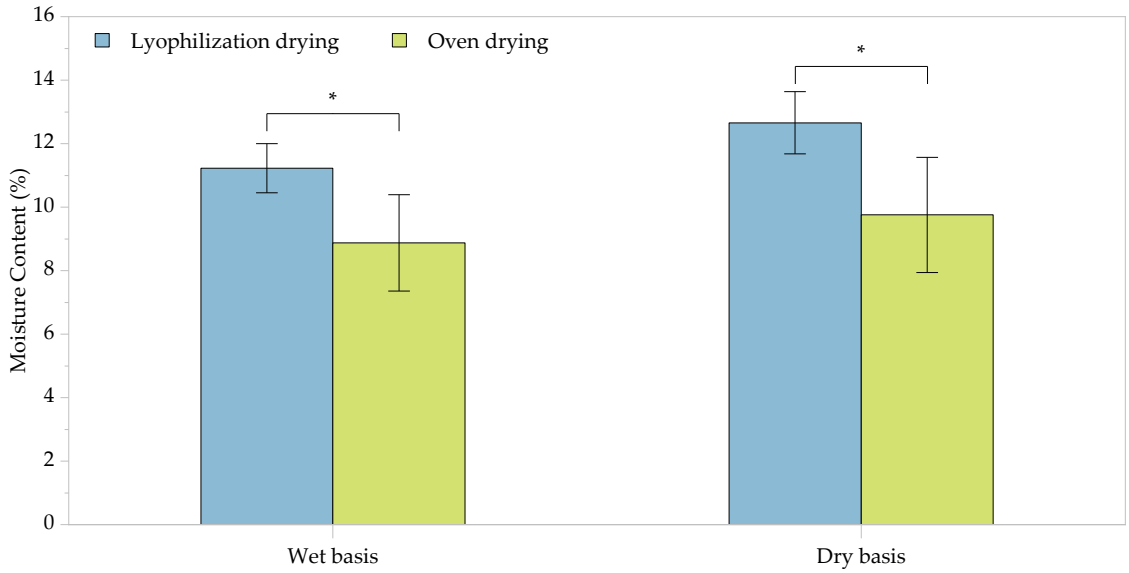

Figure 3.1. Moisture content of pine sawdust determined using two different drying methods: lyophilization and oven drying. * Indicates significant differences between the methods (P≤0.05).

3.2 Removal of extractives

The ASTM D1105-96 standard procedure, for the preparation of extractives-free wood, describes a three-step extraction using three different solvents (i.e., ethanol–toluene, ethanol, and water) for sequential removal of both lipophilic and hydrophilic fractions of extractives. Because of the toxicity of toluene, it is aimed to find less harmful solvents that can remove the same compounds as the ethanol–toluene solvent with similar yields. Acetone has been suggested as alternative to remove the lipophilic fraction of wood extractives [26,55]. Thus, some different solvents were used to extract pine sawdust extractives, and their suitability as viable alternatives to the toxic ethanol–toluene mixture was evaluated. Solvents with different polarities were chosen to evaluate the polarity in extractives removal. The effect of the extraction time was tested for three of the studied solvents and the results are presented in **Figure 3.2**.

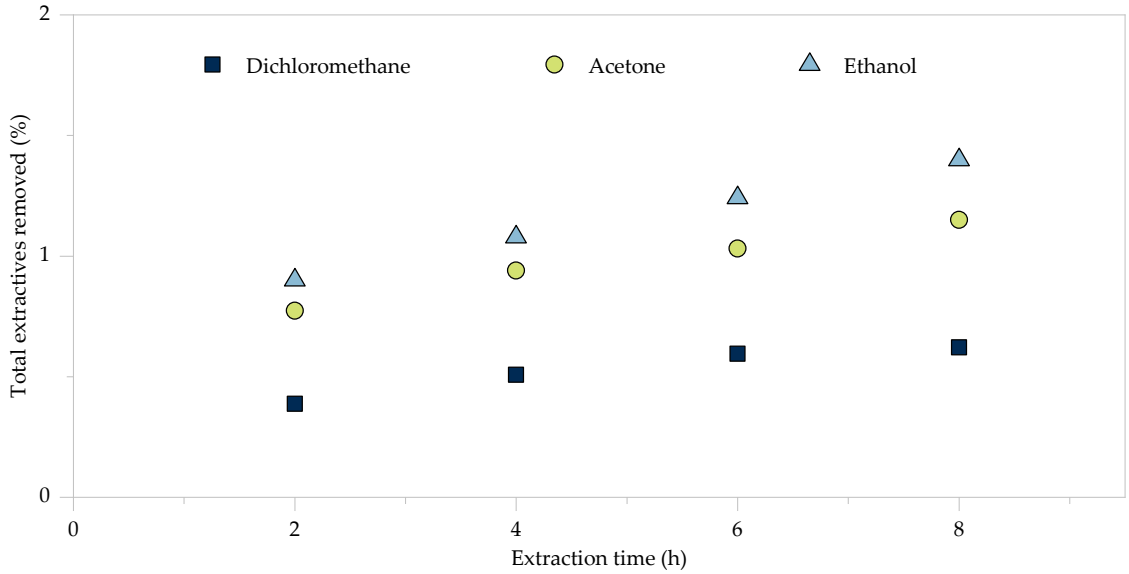

Figure 3.2. Effect of extraction time in the yield of extractives removal. The extractions were performed in a Soxhlet apparatus and the temperature was adjusted for each solvent to allow an extraction rate of not less than four siphonings per hour.

The increase of the extraction time results in an increase of the extractives removal yield and, for all extraction times, ethanol is the solvent with the highest extraction performance. After 2 h, extraction of 0.90 wt.% extractives was achieved using ethanol; after 8 h, 1.40 wt.% yield is achieved. In contrast, the lowest removal was obtained with the dichloromethane extraction, with yields below 50% of those obtained with ethanol ranging from 0.39 wt.% to 0.62 wt.%. These results suggest that solvents of higher polarity are more efficient for extractives removal from pine sawdust. Although a continuous increase in the extractive's removal yield is observed when raising the extraction time from 2 h to 8 h, at 4 h of extraction it is already possible to achieve ca. 80% of the removal obtained for 8 h for all three solvents. Besides, ASTM D1105-96 standard defines 4 h as the extraction period for Soxhlet extractions with ethanol–toluene (7:3) and ethanol. So, in order to avoid long extraction times and maintain a good extraction performance with comparable parameters to the standard procedure, the extraction time was set at 4 h. **Table 3.1** indicates the extractives removal yields for 4 h extractions using ethanol, water, acetone and dichloromethane and for the ASTM sequential extractions (i.e., 4 h ethanol–toluene, 4 h ethanol and 3 × 1 h water).

Table 3.1. Extractives yield for 4 h extraction with different solvents.

Solvent		Extractives removal yield (%)
ASTM standard[a]	1. Ethanol-toluene	0.9 ± 0.1
	2. Ethanol	0.30 ± 0.09
	3. Water[b]	2.1 ± 0.3
Water		1.37 ± 0.03
Ethanol		1.2 ± 0.1
Acetone		0.9 ± 0.2
Dichloromethane		0.6 ± 0.1

[a]Ethanol–toluene, ethanol and water extractions are sequential extractions performed to the same wood sample in that order.

[b]Extraction time: 3 h (3 sequential 1 h extractions), according to ASTM standard. See experimental section (6.2.2) for more detailed information.

The yield of extractives removal resulting from the different extraction approaches reveals that the major extraction is achieved when water is used as solvent. It is important to notice that water extractions were not performed in a Soxhlet apparatus. For these extractions, the wood was directly placed in the flask and magnetically stirred. The constant mixing and the permanent contact between the wood and the solvent may contribute for the increase of the extraction yield. Nonetheless, these results may also suggest that maritime pine sawdust contains a high content of hydrophilic compounds, capable of being removed by hot water treatment. This idea is additionally supported by the trend observed between the solvent polarity and the removal yield. Changing from a highly polar solvent, such as water, to solvents with lower polarity (e.g., ethanol, acetone, and dichloromethane) results in a decrease of the extraction efficiency, being the lowest when using dichloromethane. The observed trend is in accordance with the TAPPI standard for extractives removal where is reported that, generally, dichloromethane extraction results in lower extractives removal than either acetone or ethanol–benzene mixtures [55].

Comparing the extraction efficiency of the sequential extractions of the ASTM D1105-96 procedure with the individual extractions, it is possible to notice that the three sequential extractions are capable of extracting a significantly higher amount of extractives. While the maximum yield obtained for a single extraction was 1.37 wt.% for

water extraction, the combination of three solvents results in a removal of 3.30 wt.% of extractives, in agreement with data found in literature (2.6–4.5 wt.%) [127-129]. This reveals that a great diversity of compounds is present in the maritime pine sawdust and single-step extraction does not allow the removal of the total extractives. The combination of different sequential extractions, using solvents with a wide range of polarities, seems to be advantageous for both extraction of lipophilic and hydrophilic fractions. The hot water extraction allows the removal of hydrophilic compounds, such as tannins, gums, sugars, starches and pigments, while ethanol–toluene and ethanol extractions are preferred for the removal of more hydrophobic extractives, such as waxes, fats, resins and wood gums [57].

3.3 Extractives analysis

The qualitative analysis of the biomass is a complementary approach to the gravimetric yield to evaluate the extraction performance of a solvent. FTIR analysis was carried to assess the information on the functional groups and classes of compounds present in the samples. The information of the individual compounds extracted from each solvent was further elucidated by GC–MS.

3.3.1 FTIR analysis

To compare the removal of extractives with different solvents in a qualitative way, all samples were analyzed by FTIR to evaluate the main functional groups present in each sample.

The FTIR spectra of the extractives removed by five different solvents — water, ethanol–toluene, ethanol, dichloromethane, and acetone — are illustrated in **Figure 3.3**. More detailed band assignment for each curve is given in **Table 3.2**.

Some wood samples were extracted with more than one solvent, in sequential extractions. The spectra of those extractives' samples are not illustrated because they were similar to those obtained for the extraction of untreated sawdust with the respective solvent, differing only in the intensity of the bands.

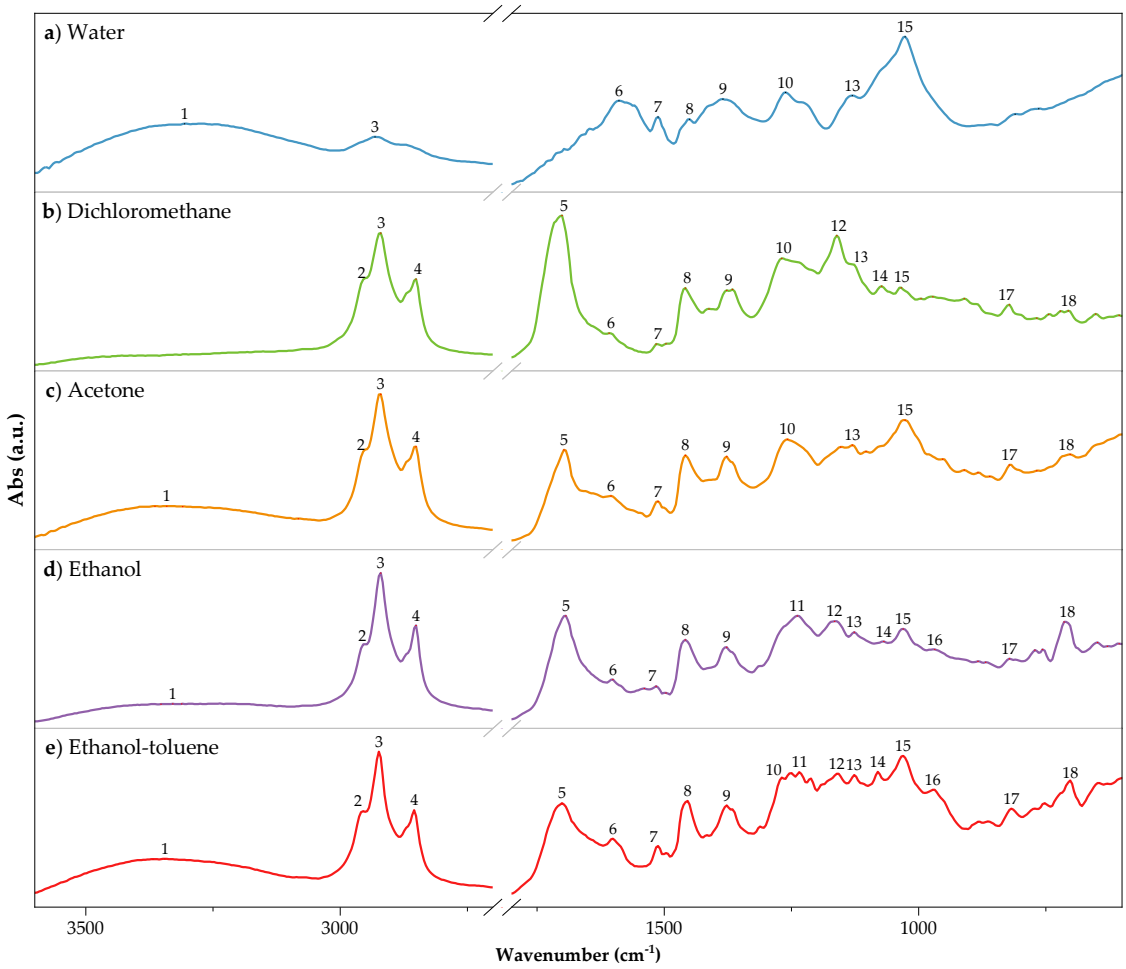

Figure 3.3. FTIR spectra of the extractives removed by a) water; b) dichloromethane; c) acetone; d) ethanol and e) ethanol–toluene. The numbers above each spectrum represent a certain band whose assignment is further discussed in the text.

The different spectra show some similarities, as some of the main bands are present in all five spectra. Except for the extractives removed using dichloromethane, all the other spectra show a broad band in the region of 3344–3306 cm^{-1} (peak no.1) corresponding to O−H and N−H stretching vibration modes [63]. The regions of 2955, 2930–2920 and 2850 cm^{-1} (peaks nos. 2, 3 and 4) show intense bands that are attributed to aliphatic C−H stretching vibration of CH, CH_2 and CH_3, respectively, except for water extractives, that only shows the peak no. 3 and with a lower intensity [49,63-65]. These bands may suggest the presence of fatty acids, fatty esters and lipophilic alcohols [130]. A very strong carbonyl (C=O) band appears around 1700 cm^{-1} (peak no. 5) in all samples whose solvent used was different than water. This band is characteristic of resin acids [131]. At 1601–1512 cm^{-1} (peaks nos. 6 and 7), water extractives show two moderate

absorbance bands representative of C=C and C−C stretching in aromatic rings [65,131], while the other samples have low intensity bands at these wavenumbers. These findings are compatible with the fact that biomass pretreatment with hot water extracts tannins that comprises aromatic units [65]. Around 1455 cm^{-1} (peak no. 8), all samples exhibit a band that could be assigned to C−C aromatic stretching [49] or to CH_2 scissoring and CH_3 asymmetrical bending [65]. The differences in relative intensity of the peaks 6, 7 and 8, may suggest that the structure of the aromatic compounds varies according to the solvent that was used; in addition to this, the higher intensity of the peak no. 8 is possibly due to the presence of more methyl and methylene groups in extractives removed by a less polar solvent. The symmetrical bending vibration of CH_3 appears at 1377 cm^{-1} (peak no. 9). Since the band for water extractives is broad, this peak may be attributed to in-plane C−OH bending [63]. The peaks 10 to 15 in the region of 1269–1026 cm^{-1} may be ascribed to C−O vibrations for ether, ester and alcohol groups and are characteristic of carbohydrates [63,132,133]. Ethanol–toluene extractives show a band at 972 cm^{-1} (peak no. 16) that can be attributed to =CH out-of-plane deformation (trans) [134,135]; this band is present in ethanol extractives but as a weak band. At 822–702 cm^{-1}, peaks nos. 17 and 18, correspond to C−H bending vibration [136] and rocking vibration CH_2 [137,138].

Table 3.2. FTIR band assignment of pine extractives according to literature data.

Band no.	Wavenumber (cm^{-1})					Band assignment
	W	DCM	Ac	EtOH	E-T	
1	3306	absent	3340	3329	3344	O−H stretching; N−H stretching [63]
2	absent	2955	2950	2955	2955	C−H stretching in CH [64]
3	2931	2920	2920	2920	2924	C−H stretching in CH_2 [49,63,65]
4	absent	2850	2850	2850	2854	C−H stretching in CH_3 [49,63,65]
5	absent	1701	1697	1693	1701	C=O stretching in acids [136,139]; C=N stretching [63]
6	1589	1608	1605	1601	1601	C−C stretching of aromatic ring [65]; C=C stretching of aromatic ring [63,132]; C−H deformation [134]

Table 3.2. (Cont.).

Band no.	Wavenumber (cm^{-1})					Band assignment
	W	DCM	Ac	EtOH	E-T	
7	1512	1516	1512	1516	1512	C−H deformation; C=C stretching of aromatic ring; C=O vibration [134]
8	1450	1458	1458	1458	1454	Aromatic C−C stretching [49,63]; CH_2 scissoring [63,134]; CH_3 asymmetrical bending [63]
9	1385	1377	1377	1377	1377	CH_3 symmetrical bending [63,140]; C−OH bending [63]
10	1261	1269	1257	absent	1269	C−O vibration [63]
11	absent	absent	absent	1238	1234	C−O−C asymmetric stretching [141]
12	absent	1161	absent	1165	1161	C−H in-plane deformation [134] C−O frequency from ester groups [136]
13	1130	1126	1130	1126	1126	C−OH stretching [63]
14	absent	1080	absent	1080	1080	C−O deformation in secondary alcohols and aliphatic ethers [134]
15	1026	1034	1026	1030	1030	C−C stretching [134]; C−O stretching [63,134]
16	absent	absent	absent	968	972	=CH out-of-plane deformation (trans) [134,135]; C−O stretching of ester groups [136]
17	absent	822	822	822	818	C−H bending vibration [136]
18	absent	706	702	714	702	Rocking vibration CH_2 [137,138]

W: water; **DCM**: dichloromethane; **Ac**: acetone; **EtOH:** ethanol; **E-T**: ethanol–toluene

3.3.2 GC-MS analysis

The chromatograms of the extractives removed with the different solvents are depicted in **Figure 3.4**. The regions of some classes of compounds found in the extractives are highlighted in the chromatograms and the detailed assignment of the peaks is presented in **Table 3.3**. A clear difference can be noticed between the various chromatograms. The hydrophilic water extractives are mainly composed of

carbohydrates (i.e., arabinose and ribose derivatives) and levulinic acid. With the exception of the peak corresponding to palmitic acid, the peaks attributed to fatty and resin acids have low intensity, suggesting a poor removal of these lipophilic compounds. These results are in accordance with those observed in the FTIR analysis since the carbonyl band characteristic of the resin acids was not observed in the water extractives. Dichloromethane and ethanol–toluene chromatograms show a small number of peaks revealing their affinity to a reduced number of compounds. Ethanol–toluene and dichloromethane extractives are essentially composed of resin acids, such as pimaric and abietic acids, fatty acids and some other phenolic compounds (e.g., isovanillate, oxanilic acid, 2,6-di-tert-butylphenol). The ethanol and acetone extractives exhibit similar chromatograms that clearly show the evident presence of resin acids, some carboxylic acid, glycerol, and some low intense peaks from fatty acids and carbohydrates. In ethanol extractives it is also noticed two intense bands from benzoic acid and tetradecane, not present in acetone extractives. The ASTM ethanol extractives fraction, that is, extractives obtained in ethanol extraction performed after ethanol–toluene extraction, exhibit a chromatogram with similar profile to the ethanol extractives. The major difference between these two samples is in the region of the resin acids. As ethanol–toluene extracts a greater content of resin acids, additional extraction with ethanol results in extractives with low content of these compounds and only the band correspondent to isopimaric acid is evident.

Pine wood usually has a high content of monoterpenes, such as α- and *β*-pinene [142]. However, monoterpenes were not detected during GC-MS analysis of the different fractions of extractives. The absence of these compounds may be reasoned by the fact that monoterpenes are volatile compounds and its content is known to decrease during the storage of the wood [59].

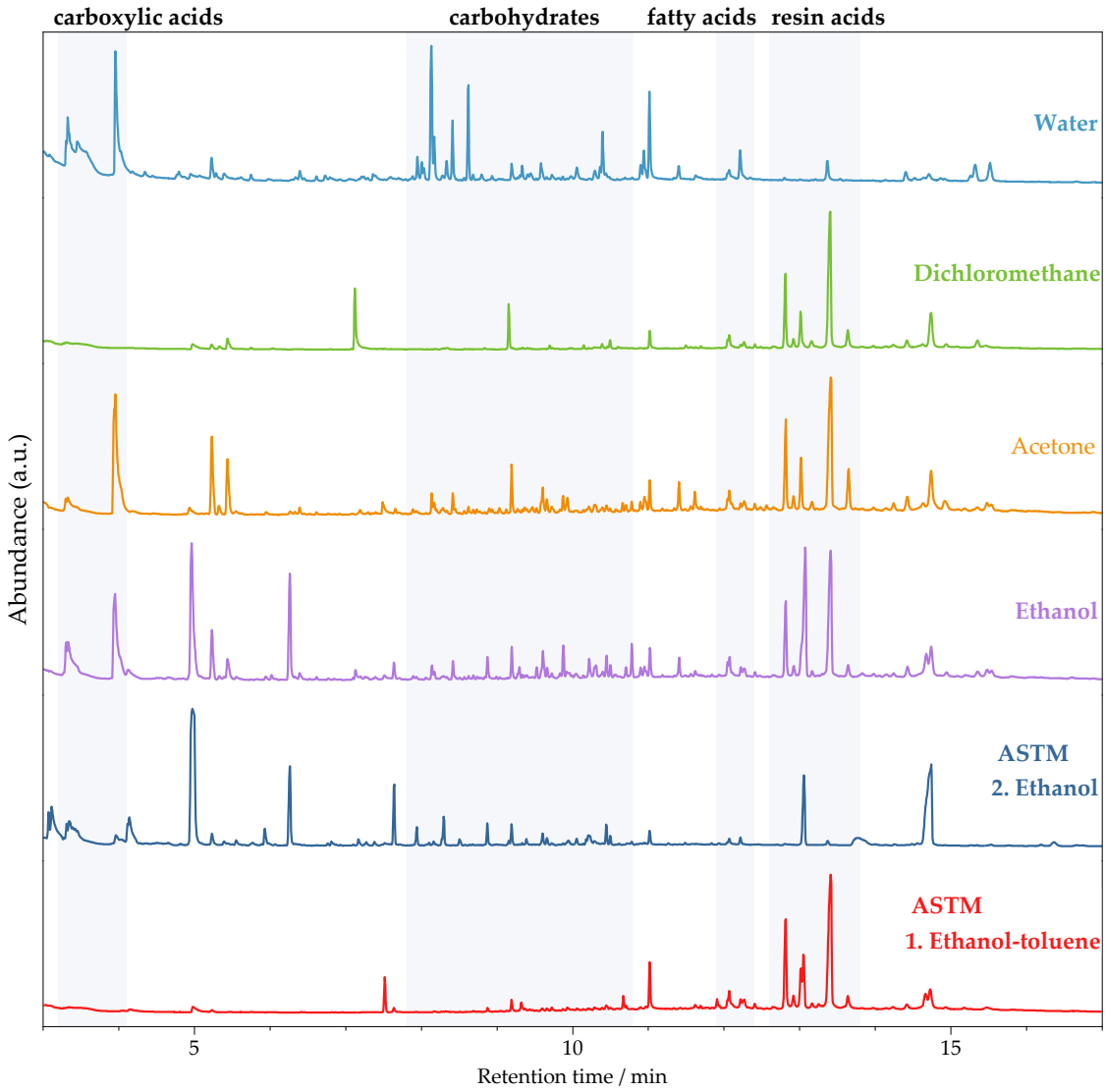

Figure 3.4. Chromatograms of the pine sawdust extractives removed with different solvents.

Table 3.3. Maritime pine extractive compounds identified by GC–MS.

Retention time (min)	Identified compound	ET	E1	E2	A	D	W
3.303	Propanoic acid	–	+	+	+	–	–
3.956	Levulinic acid	–	++	+	++	–	++
4.14	phenylmethanol	–	–	+	–	–	–
4.977	Benzoic acid	–	++	++	–	–	–
5.232	Glycerol	–	+	+	++	–	+
5.434	Pentenoic acid	–	+	–	++	+	–
5.932	Nonanoic acid	–	–	+	–	–	–
6.259	Tetradecane	–	++	++	–	–	–
7.120	N,N-dimethyldodecan-1-amine	–	–	–	–	++	–

Table 3.3. (Cont.)

Retention time (min)	Identified compound	ET	E1	E2	A	D	W
7.517	2,6-di-tert-butylphenol	+	−	−	−	−	−
7.636	Oxanilic acid	+	+	++	−	−	−
7.938–8.140; 8.295; 8.621	Arabinofuranose	−	+	+	+	−	++
8.170; 8.419	Ribose	−	+	−	−	−	+
8.330	Arabinopyranose	−	−	−	−	−	+
8.413 8.419	Arabinose	−	−	−	+	−	++
8.876; 9.197; 12.075	Glucopyranose	−	+	+	−	−	−
9.191	Isovanillate	+	−	+	++	++	−
9.606	Benzoic acid	−	+	+	+	−	−
9.660	Fructose	−	−	−	+	−	−
9.879	Galactofuranose	−	+	−	−	−	−
10.147; 10.776	Glucose	−	+	−	+	−	−
10.295	Glucopyranosiduronic acid	−	−	−	−	−	+
10.396	5-allyl-3-methoxybenzene-1,2-diol	−	−	−	−	−	+
10.444	Ribitol	−	+	+	−	−	−
10.669	1-Docosene	+	−	−	−	−	−
10.894	3-(4-hydroxy-3-methoxyphenyl)propanoic acid	−	−	−	−	−	+
11.019	Palmitic acid	++	+	+	+	+	++
11.405	2-hydroxy-2-(4-methoxyphenyl)propanoate	−	+	−	+	−	+
12.069–12.224	Stearic acid	+	+	−	+	+	+
12.817	Pimaric acid	++	++	−	++	++	−
13.013	Isopimaric acid	++	++	++	++	+	−
13.405	Dehydroabietic acid	++	++	−	++	++	+
13.637	Abietic acid	+	+	−	+	+	−
14.740	Diisooctyl phthalate	+	+	+	+	+	−
15.322; 15.518	(3-Hydroxy-4-methoxyphenyl)ethylene glycol	−	−	−	−	−	+

ET: Ethanol–toluene; **E1**: Ethanol; **E2**: Ethanol (ASTM); **A**: Acetone; **D**: Dichloromethane; **W**: Water

− Not detected; + detected, low content; ++ detected.

3.4 Lignin content of pine sawdust

The lignin content was determined in raw pine sawdust and in the pretreated wood samples where the highest yields of extractives removal were observed, that is, wood pretreated as described in ASTM D1105-96 standard. Lignin content was calculated as the sum of the acid-insoluble lignin (AIL) and acid-soluble lignin (ASL) and the average results are listed in **Table 3.4**. The removal of extractives is usually recommended before wood analysis, but the lignin content determination for the untreated pine sawdust and extractive-free sawdust resulted in similar contents, with 29 wt.% lignin for both samples. These values are in agreement with those reported in literature [143].

Table 3.4. Lignin content in untreated and extractive-free maritime pine sawdust.

Pretreatment	AIL (wt.%)	ASL (wt.%)	Total lignin (wt.%)
Untreated	28.9 ± 2.8	0.5 ± 0.1	29.4 ± 2.8
ASTM extraction	28.3 ± 4.0	0.3 ± 0.2	28.7 ± 3.9

3.5 Effect of extractives removal on lignin extraction

The influence of the extractives removal on the lignin extraction yield was evaluated for three different pretreatments: ASTM standard procedure, 4 h ethanol Soxhlet extraction and 4 h water extraction. **Figure 3.5** presents the extraction yields and lignin purity obtained for untreated pine sawdust and the pretreated samples. Although the results are not significantly different, ASTM pretreatment leads to a small increase in the extraction yield from 31 wt.% to 35 wt.% and the total amount of lignin present in the wood is removed but a loss in purity is also observed. The lowest extraction yield (28 wt.%) is achieved when extracting lignin form pine sawdust pretreated with ethanol. From this analysis it was concluded that the extractives removal does not show any significant improvement in the lignin extraction process. The extracted lignin structure is also not affected by the pretreatment as will be discuss in section 4.5.1.

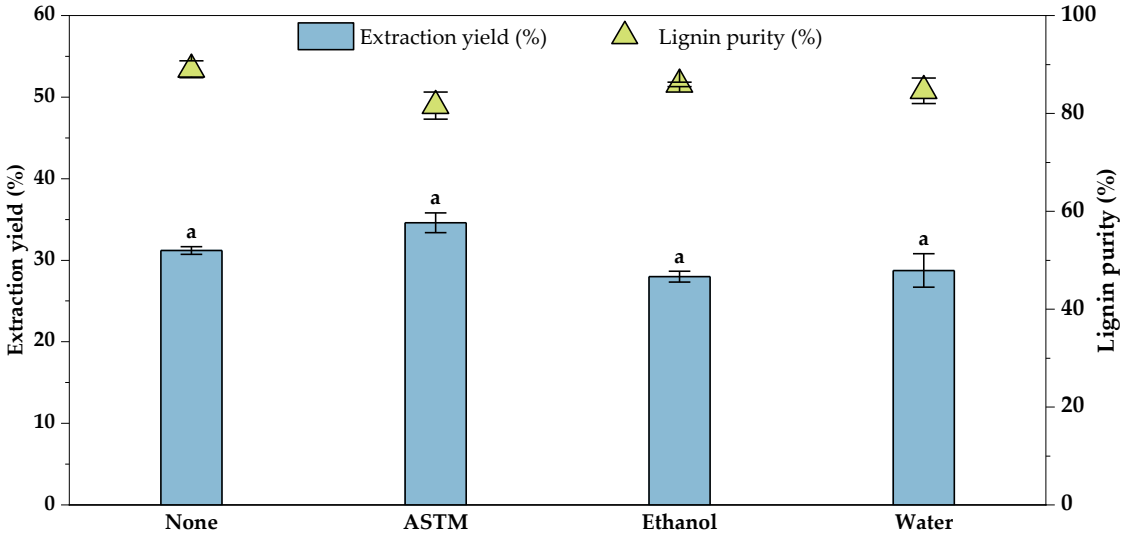

Figure 3.5. Effect of different pretreatments for the removal of extractives on the extraction yield (bars) and lignin purity (triangles) resulting from pine fractionation with Lact:Tart:ChCl (4:1:1). The same letter above the bars indicates no significant differences ($P \leq 0.05$). Extraction conditions: 1 h; 175 °C.

Chapter 4

Lignin extraction and characterization

4.1 DESs characterization

The solvent performance for lignin extraction is dependent on its physicochemical properties. Detailed characterization of the prepared DESs is useful to predict and understand its extraction or dissolution performance and thus contributing to superior solvent design.

4.1.1 Water content and refractive index

The water content determination is important for DES characterization since its presence can change the DES properties, such as density, polarity and viscosity, thus impairing their capacity for lignin dissolution and extraction. The water content of the DESs used in this work was determined by the Karl Fischer titration method. The results presented in **Table 4.1** show that all DESs have a water content below 10 wt.%, similar to the values found in literature [144].

The DES Acet:ChCl (5:1) shows the lowest water content from all ChCl-based DESs, bellow 2%, much lower than for the (2:1) ratio. The same trend appears to occur for Lact:ChCl, with higher water content for (2:1) than for (5:1) DES, thus suggesting that higher molar fraction of ChCl promotes water uptake. This is expected because of

the strong hygroscopic nature of ChCl. Lact:Betaine (2:1) and Prop:Urea (2:1) have the highest and lowest water contents of all DES, respectively, supporting the idea that the HBA affects the DES water uptake.

The refractive indices (**Table 4.1**) were also obtained and are similar to those found in the literature, ranging from 1.42 to 1.49 [145]. The DES Acet:ChCl (5:1) has the lowest refractive index and an increase in the acid molar ratio, for both Lact:ChCl and Acet:ChCl, contributes for lowering the refractive index.

Table 4.1. Physicochemical characterization of acidic DESs.

DES	Water content (wt.%)	Refractive index[a]
Acet:ChCl (2:1)	6.0 ± 0.3	1.460
Acet:ChCl (5:1)	1.9 ± 0.1	1.423
Gly:ChCl (1:1)	5.0 ± 0.3	1.480
Prop:ChCl (2:1)	3.5 ± 0.1	1.450
Lact:ChCl (2:1)	5.2 ± 0.1	1.466
Lact:ChCl (5:1)	4.87 ± 0.02	1.454
Tart:ChCl (1:2)	3.9 ± 0.5	1.497
Cit:ChCl (1:1)	5.8 ± 0.3	1.493
Lact:Betaine (2:1)	8.93 ± 0.02	1.457
Prop:Urea (2:1)	0.954 ± 0.006	1.428
Lact:Tart:ChCl (0.67:0.33:1)	6.0 ± 0.4	1.481
Lact:Tart:ChCl (0.75:0.25:1)	7.2 ± 0.8	1.477
Lact:Tart:ChCl (1.5:0.5:1)	9.0 ± 0.4	1.471
Lact:Tart:ChCl (4:1:1)	6.28 ± 0.08	1.460
Gly:Cit:ChCl (0.5:0.5:1)	8.1 ± 0.8	1.480

a) The standard deviation is lower than 0.07%

4.1.2 Viscosity

The performance of DES as an extraction solvent strongly depends on its ability to interact with the desired compound. These interactions are affected not only by the chemical nature of the solvent but also by its viscosity [104,107]. Solvent viscosity has a direct impact on the mass transfer phenomenon and, thus, highly viscous solvents can

hinder extraction efficiency [112]. Some DESs are reported to be highly viscous as a result of their strong hydrogen-bonding networks that restrict the mobility of the free species, limiting their ability to act as extraction media [146]. DESs viscosities are dependent on HBA and HBD nature, molar ratios, and temperature [147]. Although a great diversity of DESs is reported to have a Newtonian behavior, some of them act as non-Newtonian fluids and their viscosities are also dependent on the applied shear stress [148]. The rheological studies of the prepared DESs are thus important to understand their flow properties and their dependence on the operational conditions (e.g., temperature and pressure).

DESs were characterized for their rheological behavior. In **Figure 4.1**, selected flow curves of four of the DESs prepared in this work are depicted. Note that these flow curves are representative of the general behavior and the individual flow curves of all DESs are collected in **Appendix A**. All DESs exhibit a Newtonian behavior for the tested shear stress range since their viscosities are constant and independent of the applied shear stress. Similar behavior was reported by Altamash et al. for DESs composed of ChCl and carboxylic acids: lactic, citric, and malic acids. Although the DESs viscosities are not dependent on the testing conditions (i.e., shear stress and shear rate), a huge dependence of the viscosity on the composition is observed.

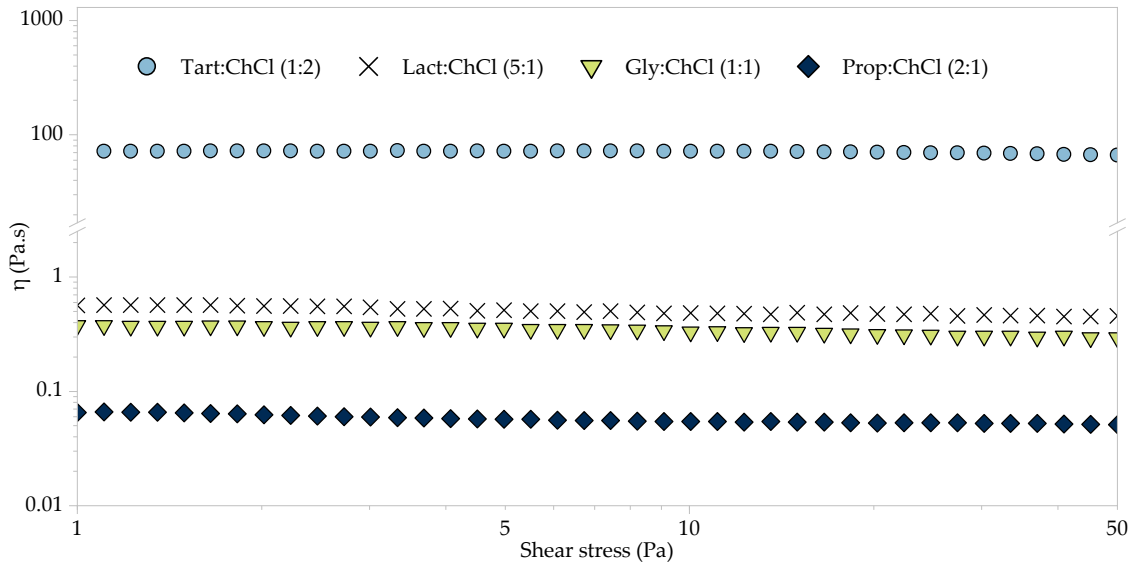

Figure 4.1. Flow curves of selected DESs: Tart:ChCl (1:2), Lact:ChCl (1:5), Gly:ChCl (1:1) and Prop:ChCl (2:1).

Considering that lignin extraction requires the use of high temperatures, the influence of temperature in DES viscosity, was evaluated from 25 °C to 150 °C. **Figure 4.2** represents the apparent dynamic viscosities of some selected DESs and the results obtained for the remaining DESs can be found in **Appendix B**. All DESs show similar trends with viscosity decreasing as temperature rises. The viscosities of the studied DESs at 25 °C and 150 °C are reported in **Table 4.2**.

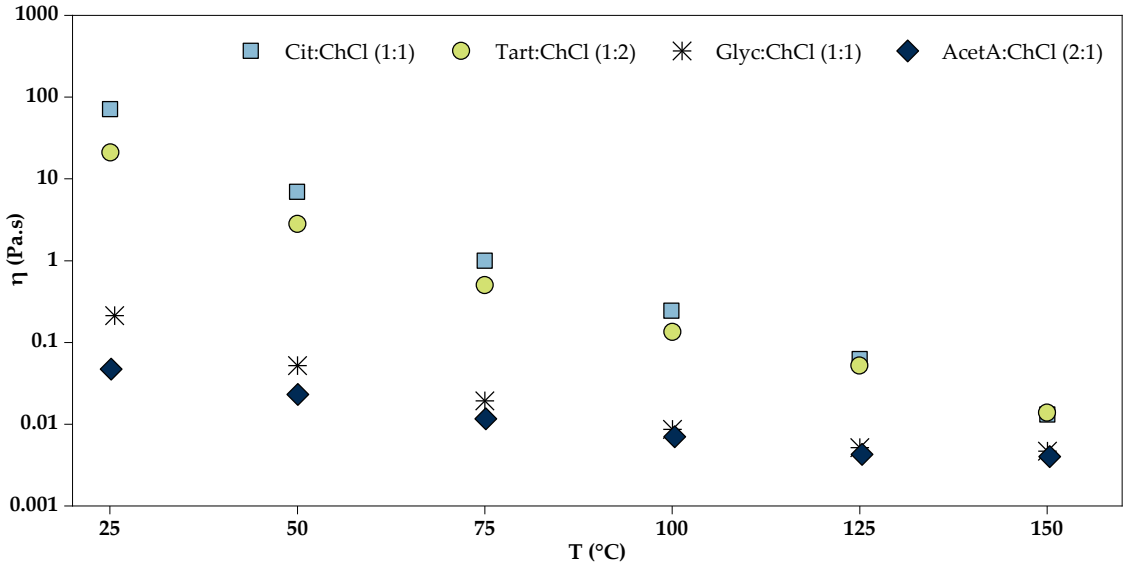

Figure 4.2. Effect of temperature on the dynamic viscosities of different binary DES.

For all DES, higher temperatures weaken the attractive forces by promoting molecular dynamics and increasing the kinetic energy [150]. At 25 ºC, citric and tartaric-based DESs are highly viscous, with a viscosity of 70.90 Pa.s and 21.00 Pa.s, respectively, compared to the others ChCl-based DES with viscosities ranging from 0.016 to 0.291 Pa.s. Citric and tartaric acid are tri- and dicarboxylic acids and, thus, have a large number of carboxylic groups capable of forming extra hydrogen-bonds [150,151]. For the same reason, extra hydroxyl groups may be responsible for the slight superior viscosities observed in alpha-hydroxy acids, Gly:ChCl and Lact:ChCl, when compared to Acet:ChCl and Prop:ChCl [150]. Comparing the viscosities at extraction temperature (i.e., 150 °C), the differences are more subtle than those at 25 °C, with viscosities below 0.015 Pa.s for all binary DESs. For DESs containing two acidic HBD, the addition of a co-HBD contributes to a decrease of the viscosity compared to binary DESs composed of tartaric or citric acid and ChCl, showing viscosities below 2.2 Pa.s. Similar effects have been reported in literature; for example, the addition of glycerol [152] and acetic acid [150] to

binary DESs have also been reported to lower solvent viscosity. Although at 25 ºC, a decrease in viscosity is observed with the addition of a third component, at extractions conditions, Cit:ChCl and Tart:ChCl are less viscous, except for Lact:Tart:ChCl in a molar ratio of 4:1:1.

Table 4.2. Apparent Newtonian viscosities of binary DESs, ternary DESs and DESs combined with cosolvents (i.e., water, DMSO, and levulinic acid)

Class	DES	Viscosity / Pa.s	
		25 °C	150 °C
Binary DES	Acet:ChCl (2:1)	0.047	0.004
	Acet:ChCl (5:1)	0.016	0.003
	Gly:ChCl (1:1)	0.213	0.005
	Prop:ChCl (2:1)	0.038	0.004
	Lact:ChCl (2:1)	0.291	0.004
	Lact:ChCl (5:1)	0.050	0.003
	Tart:ChCl (1:2)	20.999	0.014
	Cit:ChCl (1:1)	70.902	0.013
	Lact:Betaine (2:1)	0.813	0.007
	Prop:Urea (2:1)	0.018	0.002
Ternary DES	Gly:Cit:ChCl (0.5:0.5:1)	1.595	0.050
	Lact:Tart:ChCl (0.67:0.33:1)	1.887	0.031
	Lact:Tart:ChCl (0.75:0.25:1)	0.954	0.027
	Lact:Tart:ChCl (1.5:0.5:1)	2.177	0.020
	Lact:Tart:ChCl (4:1:1)	1.005	0.001
DES combined with cosolvents	Cit:ChCl (1:1); 10% Water	0.318	0.006
	Cit:ChCl (1:1); 20% Water	0.021	0.001
	Cit:ChCl (1:1); 10% DMSO	23.765	0.005
	Cit:ChCl (1:1); 20% DMSO	3.591	0.007
	Cit:ChCl (1:1); 10% Levulinic acid	10.340	0.070
	Cit:ChCl (1:1); 20% Levulinic acid	3.989	0.006

4.2 Lignin extraction

A selective method for biomass fractionation is crucial to achieve highly valuable and pure lignin. Some important parameters to consider in the optimization of the

extraction process are temperature, time, solid to liquid ratio (S/L), and the solvent. In this work, S/L was not evaluated, and its value was kept constant at 1:10 during all the experiments. Since DESs were used in this work, additional parameters can be studied when compared to traditional solvents, such as different HBA/HBD pairs, different molar ratios of HBA:HBD, binary or ternary mixtures, and the use of a cosolvent. All these extra parameters were evaluated.

4.2.1 Effect of HBA on lignin extraction

Testing the effect of different HBA in lignin extraction yield, at same extraction conditions (i.e., 150 °C and 2 h), ChCl-based DESs resulted in a more efficient extraction, presenting higher lignin recovery than DESs containing betaine or urea (**Figure 4.3**). For pine sawdust treated with Lact:Betaine (2:1) and Prop:Urea (2:1), it was not possible to determine the lignin purity due to the small amount of residue obtained, much lower than the minimum required in the standard procedure for lignin quantification [153]. At these extraction conditions, these solvents prove to be inefficient for pine sawdust fractionation, with extraction yields of ca. 2 wt.%. Using ChCl as HBA combined with these same acids as HBD, a significant improvement in the extraction yields was observed. For these solvents, the residue yields were 13% for Prop:ChCl (2:1) and 32 wt.% for Lact:ChCl (2:1), with a lignin purity of ca. 70% for both lignins obtained.

Similar results were reported by Kumar et al. for lignin extraction from rice straw; 60 wt.% of lignin extraction was achieved with lactic acid:ChCl whereas only 52 wt.% was extracted with lactic acid:betaine [154]. Additionally, the dissolution measurements of pure lignocellulosic components conducted by Lynam et al. show a greater solubility of lignin when using lactic acid combined with ChCl than with betaine [108]. Prop:Urea does not seem to be an ideal solvent for pine sawdust fractionation and this can be explained by its incapacity to cleave the $\beta-O-4$ ether bonds present in lignin structure [155]. Based on these results, ChCl was selected as the most suitable HBA for lignin extraction and was further used in the following experiments.

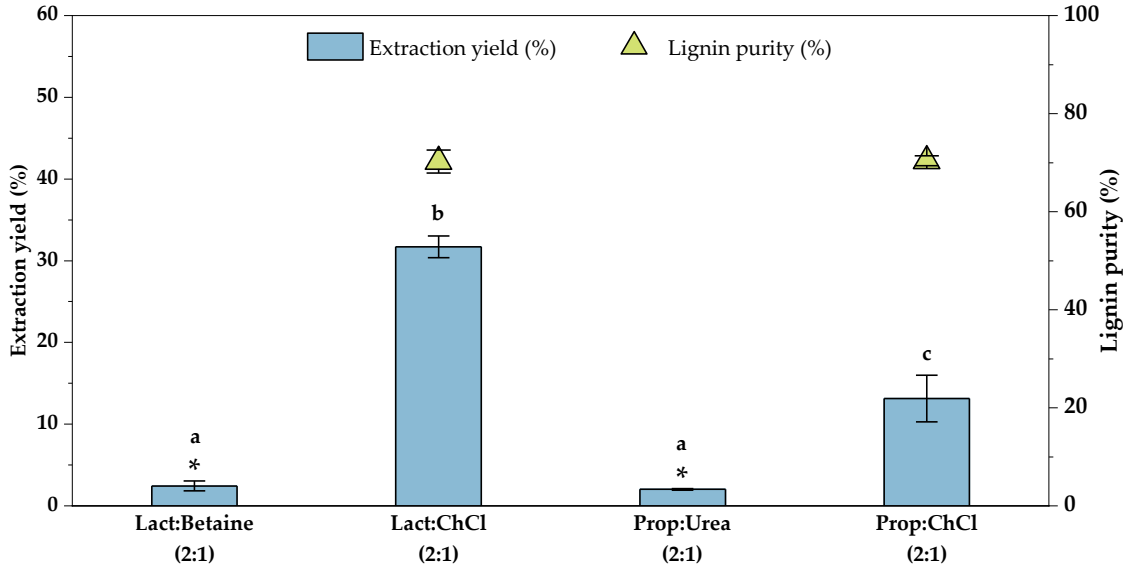

Figure 4.3. Influence of different HBA on the extraction yield (bars) and lignin purity (triangles) resulting from pine fractionation with acidic DES. The same letter above the bars indicates no significant differences (P≤0.05). For bars marked with *, purity of the samples was not determined due to the low yields below the minimum required by the standard norms. Extraction conditions: 2 h, 150 °C.

4.2.2 Effect of HBD chain features on lignin extraction

Figure 4.4 illustrates the extraction yield and lignin purity for pine sawdust fractionation using different acid ChCl-based DESs at the same extraction conditions (i.e., 2 h and 150 °C). Lact:ChCl (2:1) is the DES that shows the highest extraction yield (ca. 32 wt.%) from all series, while the purest lignin, ca. 87%, is achieved when Tart:ChCl (1:2) is used. This may be due to the fact that tartaric acid has extra hydroxyl groups that can establish more favorable interactions with lignin, turning this system more selective for lignin than for carbohydrates. However, the high viscosity of this DES may impair the mixture between pine sawdust and solvent and limit the extraction process. Cit:ChCl (1:1) shows similar results to Tart:ChCl (1:2) and this is expected since they are both alpha-hydroxy acids and highly viscous. Nevertheless, the former shows lower extraction yield because of the higher entanglement promoted by the presence of the additional carboxylic groups in the tricarboxylic acid that reduces the solvent–solute interactions [107,110].

These results reveal that changing from Acet:ChCl (2:1) to Gly:ChCl (1:1) and from Prop:ChCl (2:1) to Lact:ChCl (2:1), the lignin recovery is slightly improved. This corresponds to the replacement of the HBD from a linear monocarboxylic acid with an alpha-hydroxy acid with the same chain length, suggesting that alpha-hydroxy acids have higher performance for lignin dissolution. These findings are in agreement with a study conducted by Tan et al., where a higher polarity of alpha-hydroxy acids was observed to enhance lignin extraction when compared to linear saturated acids for oil palm empty fruit bunch fractionation with acidic DES [107]. The authors also observed an improvement in lignin recovery when shorter chain acids are used, in agreement with Lynam et al. findings, where formic acid:ChCl exhibits the highest extraction yield [108]. In the present work, this tendency is not visible; however, a slight increase in lignin purity is noted when using DESs containing acetic or glycolic acids compared to those comprising propionic or lactic acids.

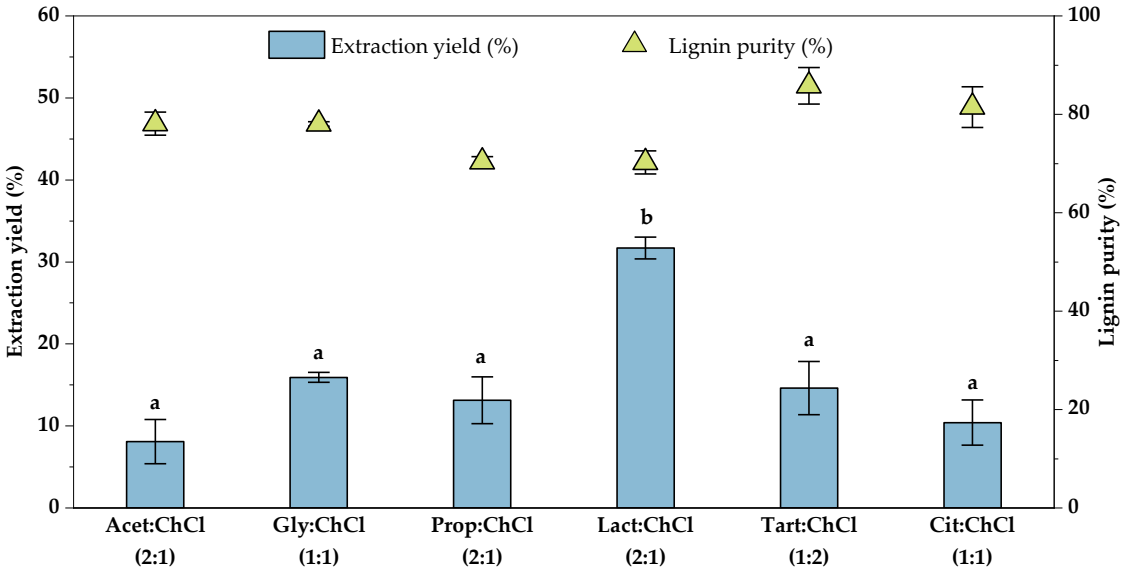

Figure 4.4. Influence of the acid chain length and functional groups on the extraction yield (bars) and lignin purity (triangles) resulting from pine fractionation. The same letter above the bars indicates no significant differences (P≤0.05). Extraction conditions: 2 h; 150 °C.

4.2.3 Effect of cosolvent addition on lignin extraction

ChCl DESs containing tartaric or citric acid proved to be the most selective for lignin extraction at experimental conditions (i.e., 150 °C and 2 h), but their high viscosities

hinder the process, resulting in lower extraction yields. Reduction of the viscosity of these solvents can improve the lignin recovery, while maintaining the lignin selectivity of the system. The addition of a cosolvent is expected to induce a decrease in DES viscosity and it can tune the DES properties. As mentioned before, besides the viscosity, cosolvent polarity needs to be considered to avoid a significant shift in the polarity of the system that may compromise the selectivity. **Figure 4.5** shows the effect of different cosolvents (i.e., water, levulinic acid, and DMSO) added to Cit:ChCl (1:1) for pine sawdust fractionation. When water is added to the system, the extraction yield decreases but not significantly. Lignin recovery yield and purity could not be determined for systems containing water due to the small residue obtained. Although water can reduce DES viscosity, it is not an ideal cosolvent because lignin is not water-soluble and the change in polarity negatively affects lignin dissolution. Moreover, water may compete with DES hydrogen-bonding network, decreasing DES performance towards lignin. In Kumar et al. work, lignin extraction from rice straw was enhanced when 5% (v/v) water was added to acidic DES, but for water contents above 5%, the extraction yield was observed to be considerably worse [154]. New et al. observed an improvement in delignification of oil palm fronds with ChCl:urea when water is added up to 30% (v/v) [156]. Data was rationalized as the possible enhancement of ChCl:urea penetration into the biomass, promoted by the observed decrease in solvent viscosity.

When levulinic acid is used as cosolvent, an increase in the extraction yield occurs and, consequently, in the lignin recovery. A higher acid content also seems to be favorable for lignin extraction. In this case, an increase in extraction yield from 10 to 18% is observed when the cosolvent was added up to 20 wt.%. Contrary to what happens with water, the presence of a carboxylic acid as cosolvent seems not to cause such a drastic change in the environment properties of an acidic DES.

The use of DMSO as cosolvent also boosts pine fractionation. In this case, the use of a small amount of cosolvent does not show a substantial improvement in extraction yield. Nevertheless, when 20 wt.% DMSO is added to the mixture, a significant improvement in the extraction yield from 10% to 21% is observed. The lignin recovery yield increases remarkably to a total of 60%.

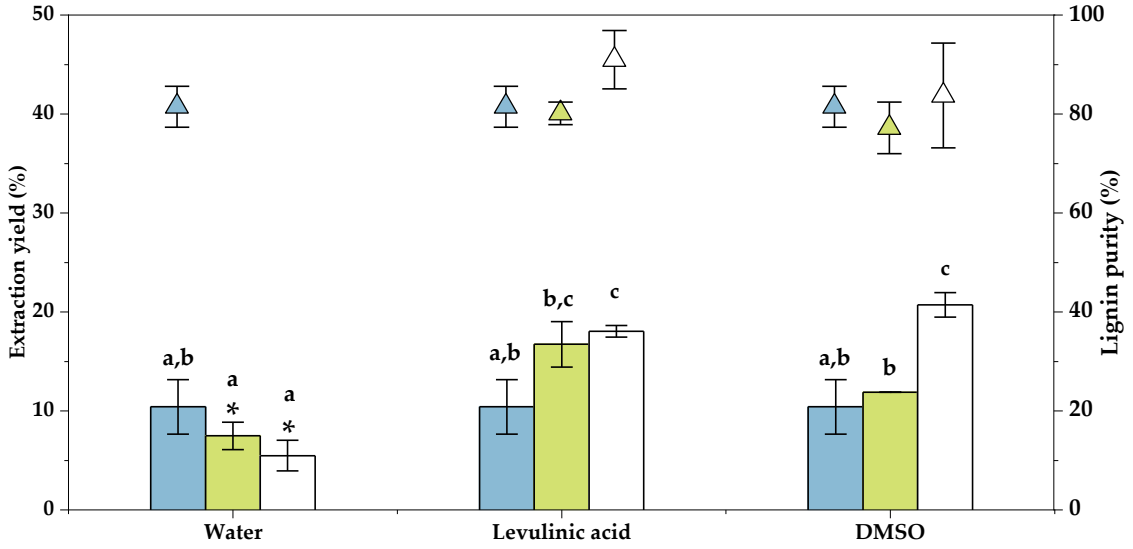

Figure 4.5. Effect of adding 0 (blue), 10 (green) and 20 (white) wt.% of different cosolvents to Cit:ChCl (1:1) on the extraction yield (bars) and lignin purity (triangles) resulting from pine fractionation. The same letter above the bars indicates no significant differences (P≤0.05). For bars marked with *, purity of the samples was not determined due to the low yields below the minimum required by the standard norms. Extraction conditions: 2 h; 150 °C.

4.2.4 Ternary DESs for lignin extraction

From the screening of different acidic ChCl-based DESs, it was noticed that Lact:ChCl was the system capable of extracting the highest lignin content and that the purest lignin is achieved with Tart:ChCl. Thus, it was hypothesized that preparing a new DES, combining these two acids as HBD with ChCl, would lead to an enhancement of the extraction performance, combining both selectivity and efficiency in a single and novel DES system. For that reason, two types of ternary DES were prepared, one combing lactic acid and tartaric acid (Lact:Tart:ChCl) and another one containing glycolic acid and citric acid (Gly:Cit:ChCl), that presented similar results in both lignin recovery and purity. In **Figure 4.6**, the purity and extraction yield for ternary and binary mixtures containing the same acids are presented. The ternary DES of Gly:Cit:ChCl does not show any significant improvement for the delignification of pine sawdust compared to binary DES, composed of those acids, as they show similar efficiency and selectivity. In contrast, when mixing tartaric and lactic acids with ChCl in 4:1:1 molar ratio, a remarkable increase is observed in the extraction yield, in comparison to Tart:ChCl (1:2), while still preserving its high purity. This mixture combines the properties of both corresponding binary

DESs, having great potential for future applications. Some authors report the use of a third component in eutectic solvents to form ternary DES with enhanced performance in lignocellulosic deconstruction. Xing et al. tested five different dihydrogen-bonding DES for rice straw pretreatment and concluded that formic acid:acetic acid:ChCl mixture improves biomass fractionation in comparison to both formic acid:ChCl and acetic acid:ChCl binary system, achieving the higher total sugar content and delignification [157]. A ternary DES of guanidine hydrochloride, ethylene glycol and *p*-toluenesulfonic acid proved to be highly efficient for lignin removal from switchgrass [158]. $AlCl_3 \cdot 6H_2O$ has been used in combination with ChCl and glycerol to form a ternary DES with an active acidic site holder able to enhance the cleavage of lignin–carbohydrate complexes linkages [159,160].

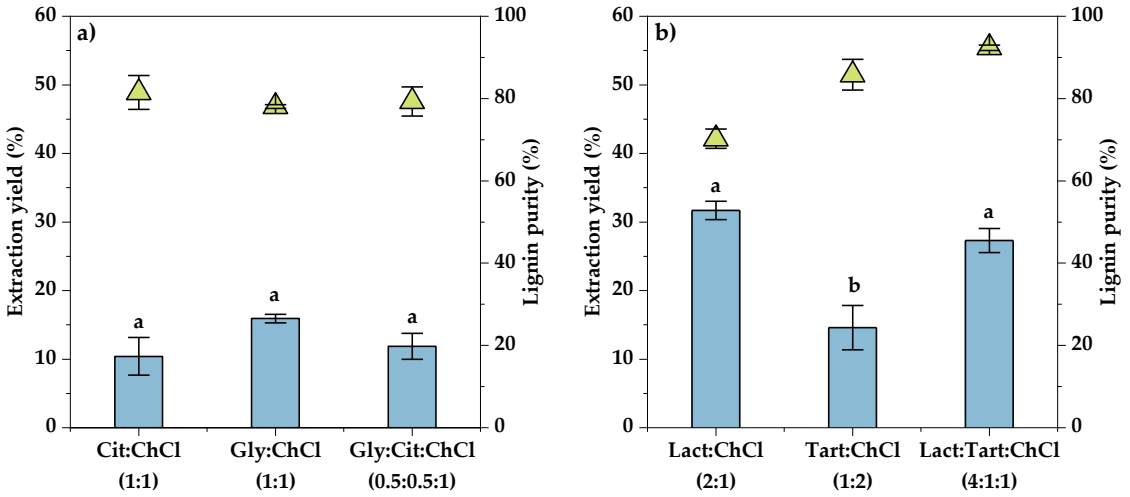

Figure 4.6. Extraction yields (bars) and lignin purity (triangles) resulting from pine fractionation with binary and ternary ChCl-based DES containing: a) citric acid and glycolic acid and b) lactic acid and tartaric acid. The same letter above the bars indicates no significant differences (P≤0.05). Extraction conditions: 2 h; 150 °C.

4.2.5 Effect of HBD:HBA molar ratio on lignin extraction

DES components have a great impact on its suitability for lignin solubilization, but the ratio between the HBA and HBD also contributes to its affinity to the target solutes. The most efficient binary and ternary DESs were prepared in different ratios to evaluate HBD:HBA ratio influence on its extraction performance. For binary DES, Lact:ChCl was selected and two different proportions were compared, (2:1) and (5:1). For ternary

Lact:Tart:ChCl, both HBD:HBA and HBD:HBD ratios were evaluated. Although not statistically different, the lignin extraction yield trend (**Figure 4.7a**) for binary solvents seems to increase when the acid content increases from (2:1) to (5:1) for pine sawdust fractionation with Lact:ChCl, in agreement with related studies using willow [97] and poplar wood pretreatments [70].

The results presented in **Figure 4.7b** show that all Lact:Tart:ChCl mixtures are capable of a highly selective lignin extraction. The lignins resulting from these pretreatments show a purity above 80%, with a maximum purity of ca. 93% for the 4:1:1 mixture ratio, proving that the addition of tartaric acid improves the selectivity of the process. For a HBD to HBA ratio of 1:1, a higher amount of lactic acid is beneficial. Using lactic acid and tartaric acid in a molar ratio of 3:1 results in a higher extraction yield than a ratio of 2:1. This could be explained by the fact that the smaller amount of the dicarboxylic acid decreases the entanglement caused by the extra carboxylic groups, promoting the mixing. As observed for the binary DESs, a higher acid content enhances pine sawdust fractionation. The extraction yield increases from 13% to 27% when changing the ratios from 0.67:0.33:1 to 4:1:1. Comparing the binary and ternary DESs, extraction with Lact:ChCl results in higher residue yield, between 32% and 35%, higher than the lignin content in pine wood (i.e., 29%) and, consequently, a purity of ca. 70% is reached. A notable improvement in lignin recovery and purity occurs when tartaric acid is used as a second HBD. Lact:Tart:ChCl (4:1:1) shows an extraction yield of 27% and lignin with 93% purity, resulting in 86% of lignin recovery. Thus, this novel solvent proved to be the most efficient DES used in this work for lignin extraction from maritime pine sawdust.

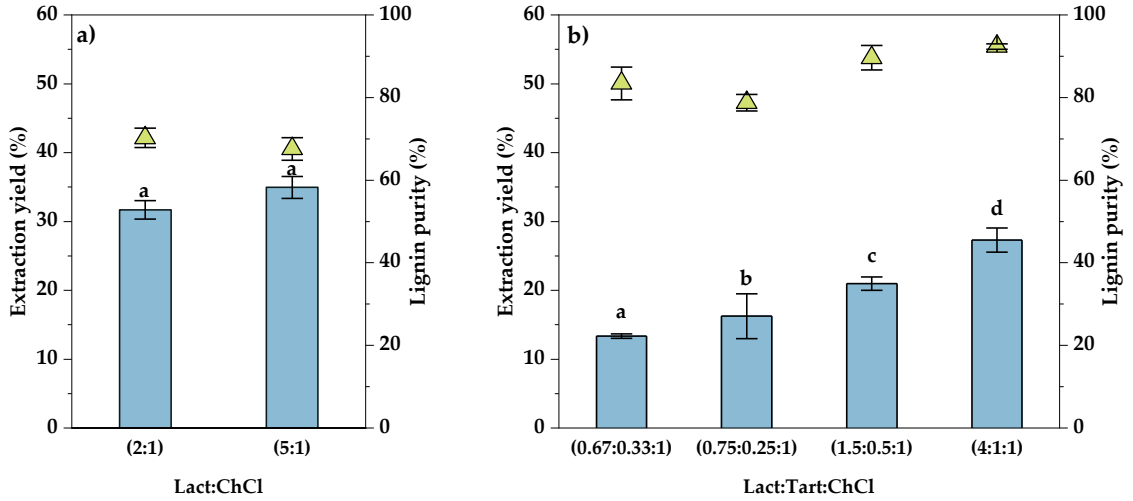

Figure 4.7. Influence of HBD to HBA molar ratio on the extraction yield (bars) and lignin purity (triangles) resulting from pine fractionation with: a) binary DESs and b) ternary DESs. The same letter above the bars indicates no significant differences (P≤0.05). Extraction conditions: 2 h, 150 °C.

4.3 Optimization of extraction conditions

As mentioned before, Lact:Tart:ChCl (4:1:1) proved to be the best solvent for the extraction of highly pure lignin. To verify the optimal conditions of the extraction process, four different extraction times (i.e., 0.5, 1, 2 and 4 h) and different temperatures (i.e., 100, 135, 150 and 175 °C) were tested. From the results shown in **Figure 4.8a**, it is possible to conclude that the extraction temperature has a great impact on the lignin extraction yield. Lignin extraction significantly increases when higher temperatures are employed, although it was not statistically meaningful when raising the temperature from 150 °C to 175 °C; the lignin recovery at 175 °C was ca. 89%. At 100 °C, almost no fractionation occurs and, therefore, it was not possible to evaluate the purity of the residue. Regarding the effect of the extraction time (keeping the temperature at 175 °C), no major differences were noticed (**Figure 4.8b**) except when increasing the extraction time from 0.5 to 1 h. Therefore, at this temperature, 1 h of extraction is enough for an efficient extraction, being possible to extract ca. 95% of the lignin present in the pine with 89% purity.

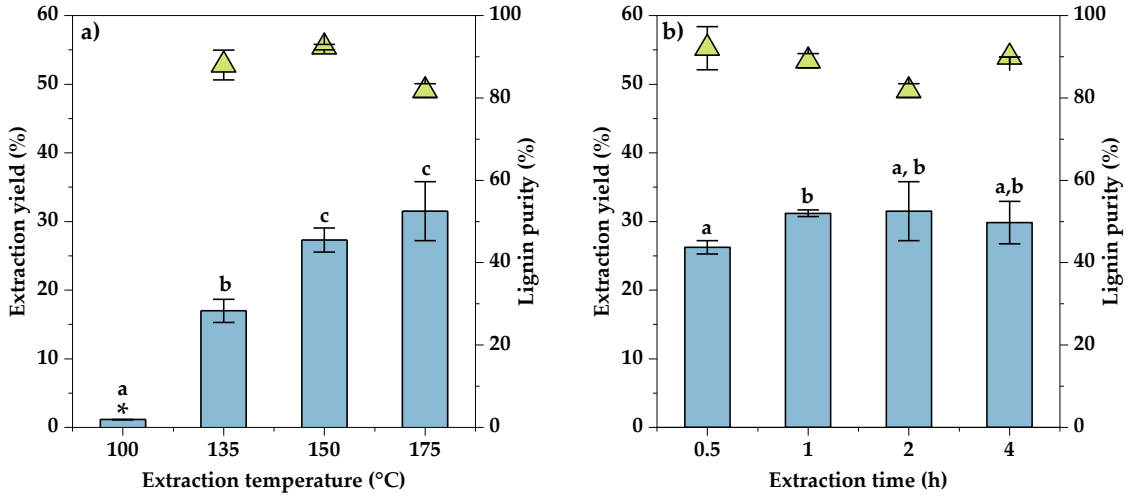

Figure 4.8. Effect of extraction (a) temperature and (b) time on residue yield (bars) and purity (triangles) for pine fractionation with Lact:Tart:ChCl (4:1:1). Extraction conditions: 2h for temperature experiments and 175 °C for time experiments. The same letter above the bars indicates no significant differences (P≤0.05). For bars marked with *, purity of the samples was not determined due to the low yields below the minimum required by the standard norms.

4.4 DES recycle and reutilization

The recycle of the DESs and sequent reutilization in new extractions without compromising the efficiency of the system is desirable for the development of an environmental unhazardous process. The recovery of DESs after lignin extraction has been reported in the literature to be easily achieved. Moreover, it is further suggested that the recovered solvents can be reused three to five times without a great loss of the extraction efficiency with yields of ca. 80% (in comparison to the first extraction yield) [115,161].

The water solubility of the DES components and water insolubility of lignin makes water a good antisolvent to drive lignin precipitation that allows their separation by filtration. The DES can be easily recovered after water evaporation. The Lac:Tart:ChCl (4:1:1) reusability at optimized conditions was tested for a cycle of 3 consecutive extractions. The extraction performances of the reused DES are represented in **Figure 4.9** and their visual appearance is illustrated in **Figure 4.10**. No significant differences were observed between the extraction yields of the freshly synthesized DES and those obtained for the second and third extractions. The selectivity of the process is still

preserved in the sequent extractions, with all three extracted lignins showing a similar purity. The extraction yields of the recovered DESs were slightly higher than that for the fresh DES, resulting in lignin recovery yields above 100%: 116 and 117 wt.% for the second and third cycle, respectively. The high yields may be due to some remaining lignin present in the DES after the first extraction that is further recovered in the following extractions. Besides, the recovered DES were freeze-dried before new extractions to eliminate the water used for the lignin precipitation and may have lower water content that could result in a superior extraction.

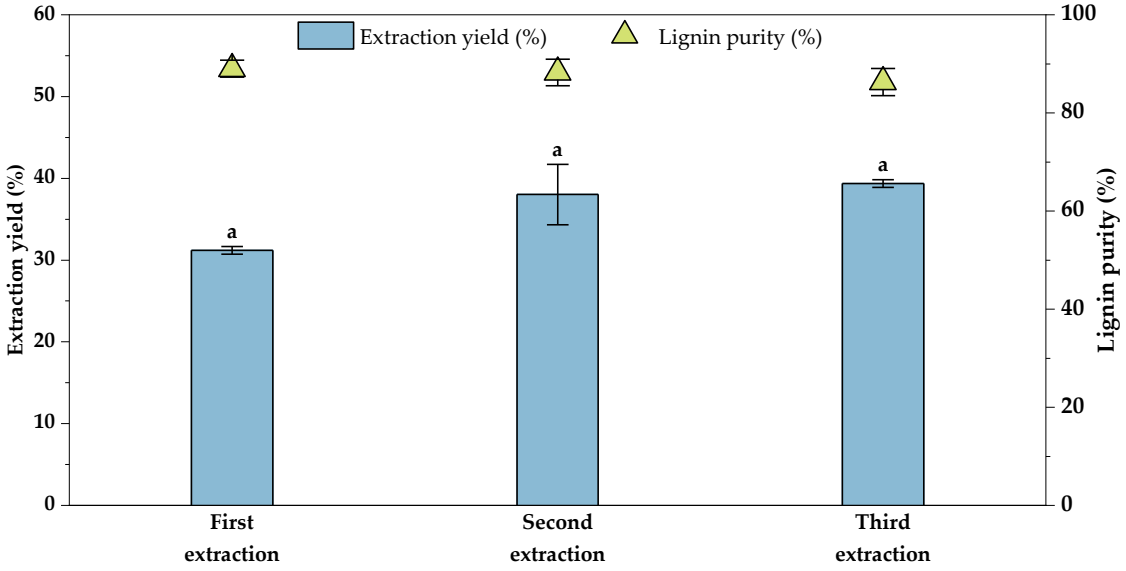

Figure 4.9. Extraction yield (bars) and lignin purity (triangles) resulting from pine fractionation with freshly synthesized and recovered Lact:Tart:ChCl (4:1:1). The same letter above the bars indicates no significant differences (P≤0.05). Extraction conditions: 1 h; 175 °C.

Figure 4.10. Lact:Tart:ChCl (4:1:1) before use (left), after 1 extraction process (middle) and after 2 extraction cycles (right).

4.5 Lignin characterization

Biomass fractionation promotes the cleavage of the lignin–carbohydrate linkages and the separation of the biopolymers. The structure and properties of the wood components are considerably affected by the fractionation method employed.

From the images depicted in **Figure 4.11**, there are clear differences in the visual appearance of the raw pine sawdust and the cellulosic-rich and lignin fractions. The fractionation of the pine sawdust at optimal conditions using Lact:Tart:ChCl (4:1:1) results in the solubilization of the lignin polymer and a fine brown powder is obtained. The insoluble fraction, mainly composed of cellulose, has a milled fiber appearance that reveal the successful disruption of the lignin–carbohydrates complexes.

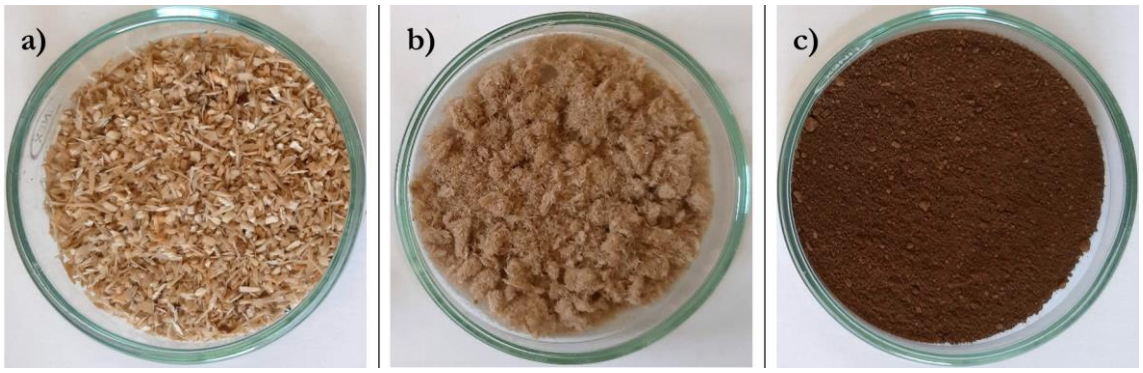

Figure 4.11. Images of a) untreated pine sawdust; b) cellulosic solid residue and c) extracted lignin obtained in Lact:Tart:ChCl (4:1:1) extraction at optimal conditions (i.e., 1 h and 175 °C).

Lignin was characterized for its chemical structure and functional groups by FTIR spectroscopy. The thermal properties were assessed by thermogravimetric analysis (TGA) and scanning electron microscopy (SEM) was used to evaluate lignin morphology.

4.5.1 Lignin structural analysis

The recovered lignin samples were analyzed by FTIR spectroscopy. All samples presented a similar spectra profile, differing from each other only in the relative band intensity, except for a few samples, where an extra band (i.e., 1184–1170 cm^{-1}) was found in the form of a "shoulder". The FTIR spectra of the lignins extracted with binary and ternary DESs, composed of lactic and tartaric acid combined with ChCl, are depicted in

Figure 4.12. The detailed band assignment is compiled in **Table 4.3**. The FTIR spectra of all lignins extracted with the studied DESs are collected in **Appendix C**.

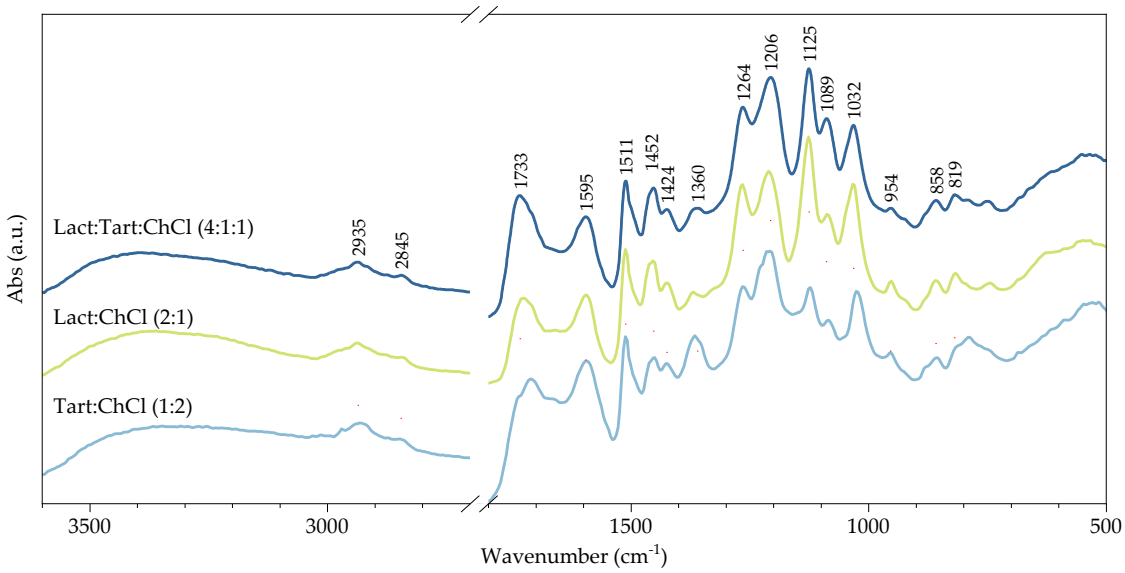

Figure 4.12. Normalized FTIR spectra of lignin extracted with Lact:Tart:ChCl (4:1:1), Lact:ChCl (2:1) and Tart:ChCl (1:2). The main bands are highlighted with the corresponded wavenumber.

In the region of 3500–2800 cm^{-1}, all samples exhibit a broad band, at around 3400 cm^{-1}, attributed to phenolic and aliphatic O−H stretching and two bands at 2930 and 2850 cm^{-1} assigned to C−H stretching in methyl and methylene and in aromatic methoxyl groups [162]. In the so called "fingerprint region", the band at 1728–1709 cm^{-1} corresponds to C=O stretching from a nonconjugated ketone, for example the guaiacyl acetone or the keto form of α-hydroxy coniferyl alcohol [163]. This band can also be attributed to ketone, carbonyl, and ester groups from carbohydrates that can suggest the presence of holocellulose [164]. Aromatic skeletal vibrations and C=O stretching appear at 1597–1593 cm^{-1} [164]. Since lignin from softwoods is composed of 95% guaiacyl (G) units [18], pine lignin exhibits a curve characteristic of G type lignin; the band observed at 1508 cm^{-1} is usually more intense than those at 1593 and 1462 cm^{-1}. The most intense peaks occur at 1265, 1211, 1138, and 1026 cm^{-1} and are attributed to vibrations in G units. In some samples, a shift from 1138 to 1122 cm^{-1} was observed. The presence of small amounts of syringyl units is usually responsible for the shift to lower wavenumbers of vibrational mode [164,165]. Lignins extracted by Prop:ChCl (2:1) and Cit:ChCl (1:1) exhibit a "shoulder" at 1184–1170 cm^{-1}, attributed to C=O in ester groups [164,165] and ring breathing of *p*-hydroxyphenyl units [166]. This band usually appears in HGS lignin

and it is rarely seen in other lignin spectra without deconvolution [165]. The relative intensity of the bands at 1026 and 1211 cm^{-1} and the presence of the peaks at 856 and 806 cm^{-1} associated with the vibration modes of the G units also confirms the G type profile of pine lignin. Regardless of the DES system used, all the extracted lignins studied exhibit a similar structure.

Table 4.3. FTIR assignment of lignin according to literature data.

Band no.	Wavenumber (cm^{-1})	Band assignment	Reference
1	3400–3300	O−H Stretch	[164]
2	2939–2924 and 2850–2837	C−H stretching in methyl and methylene groups and in aromatic methoxyl groups	[162,164]
3	1728–1705	C = O Stretch in unconjugated ketone, carbonyl and in ester groups (frequently of carbohydrate origin)	[164]
4	1665–1659	C = O stretching in conjugated *p*-subst. aryl ketones	[164]
5	1597–1593	Aromatic skeletal vibrations plus C=O stretching	[164]
6	1512–1508	Aromatic skeletal vibrations	[164]
7	1462–1450	C–H deformations in methyl and methylene	[167]
8	1427–1419	Aromatic skeletal vibrations combined with C−H in-plane deformation	[164]
9	1369–1358	Aliphatic C−H stretching in methyl and phenolic OH	[164]
10	1265–1261	G ring breathing plus C = O stretching	[164,165]
11	1219–1203	C−C plus C−O plus C = O stretching	[164]
12	1184–1170	C = O in ester groups (conjugated) (typical for HGS lignins)	[164]
13	1138–1122	Aromatic C−H in-plane deformation plus secondary alcohols plus C—O stretch	[164]
14	1088–1080	C−O deformation in secondary alcohols and aliphatic ethers	[164]
15	1034–1026	Aromatic C−H in-plane deformation, plus C−O deformation in primary alcohols, plus C = O Stretch (unconjugated)	[164]
16	953	−HC = CH-out-of-plane deformations (trans)	[164]
17	860–52 and 818–806	C−H out-of-plane in positions 2,5, and 6 of G units	[164]

From the analysis of the effect of removal of extractives on lignin extraction yield, it was observed that performing time-consuming pretreatments to the wood does not result in any significant improvement of the extraction yield or purity of the extracted lignin. However, the structure of the extracted lignins could be affected by the implementation of a pretreatment before the biomass fractionation. Lignin extracted from untreated pine sawdust and extractive-free pine sawdust pretreated with ethanol–toluene, ethanol and water, following the ASTM procedure were analyzed by FTIR. The spectra depicted in **Figure 4.13** confirm the results obtained for the extraction yields since the two samples exhibit a similar profile. No extra bands or significant shifts in the wavenumber were observed between pretreated and not pretreated wood. Consequently, we can conclude that there are no benefits from performing a pretreatment procedure before the lignin extraction regarding both purity and recovery yield.

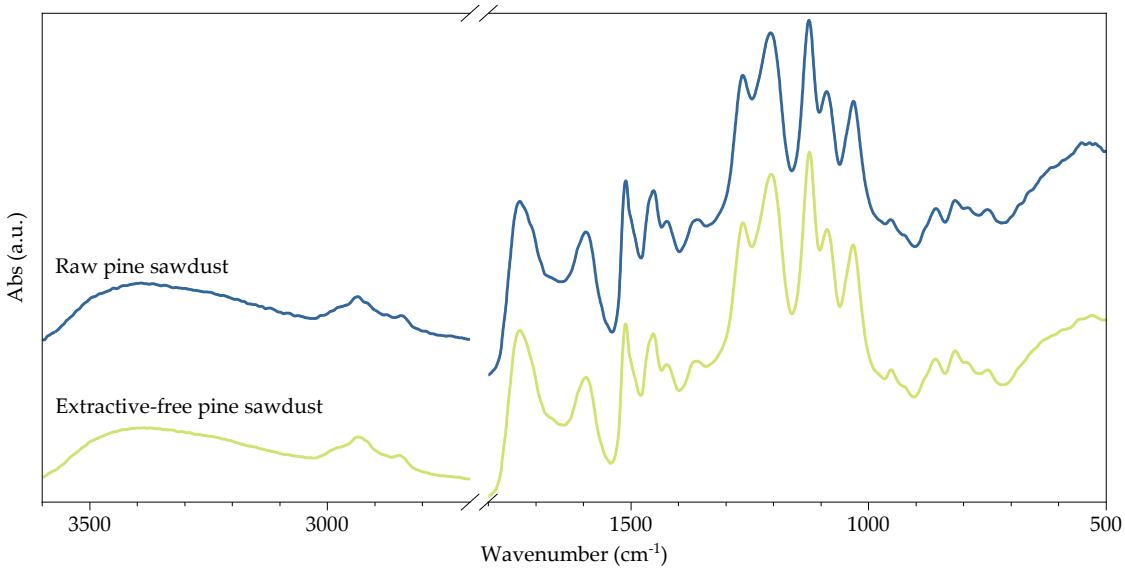

Figure 4.13. Normalized FTIR spectra of lignin extracted with Lact:Tart:ChCl (4:1:1) from raw pine sawdust and pretreated extractive-free sawdust (ASTM standard procedure).

The reutilization of the developed Lact:Tart:ChCl (4:1:1) proved to be efficient for sequential lignin extractions without compromising the extraction yield or the purity of the recovered lignin. The evaluation of the effect of DES recycle and reutilization on the lignin structure was assessed by FTIR. The spectra profile of lignins extracted with freshly synthesized Lact:Tart:ChCl and with the recovered DES, depicted in **Figure 4.14**, shows that there are no different bands present in the lignins extracted with fresh and recycled

DESs. Therefore, the Lact:Tart:ChCl is capable of being successfully recovered and reused for, at least, two more extraction cycles without loss of selectivity or efficiency loss and without affecting the resultant lignin structure.

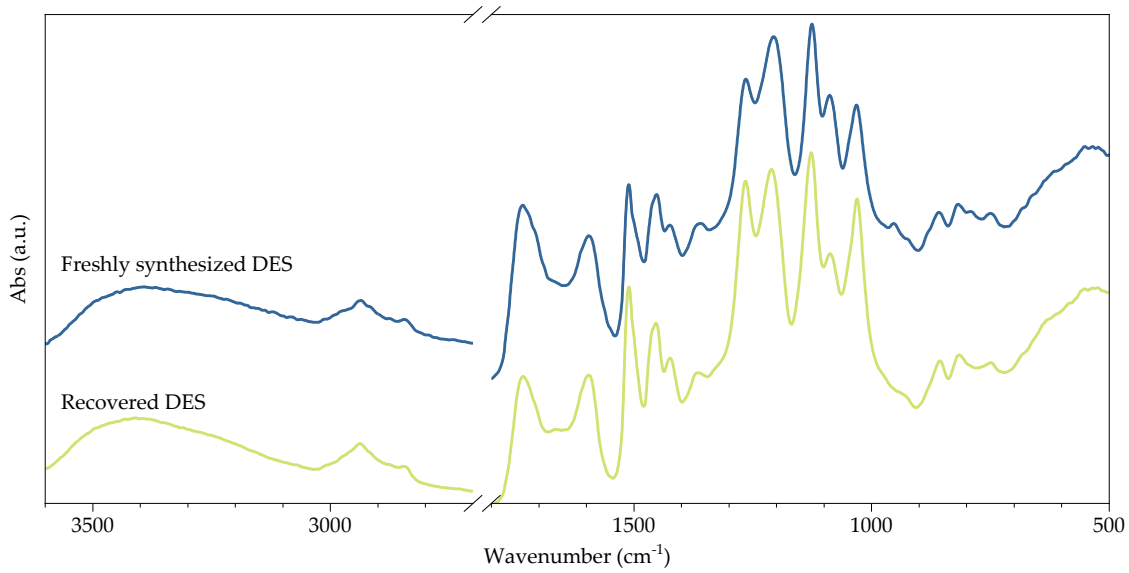

Figure 4.14. Normalized FTIR spectra of lignin extracted with freshly synthesized and recovered Lact:Tart:ChCl (4:1:1) at optimal conditions.

4.5.2 Lignin thermal analysis

Thermal properties of lignin can be studied by TGA [168]. This technique is useful to assess information about the lignin thermal decomposition from the evaluation of the weight loss with the temperature increase. The TGA and derivative thermogravimetry (DTG) curves obtained for pine lignin extracted with Lact:Tart:ChCl (4:1:1) are represented in **Figure 4.15**. The thermogravimetric profile of the extracted lignin exhibits four stages of weight loss. The stages in the range of 25–150 °C correspond to the elimination of moisture and volatile compounds with low molecular weight [169,170]. Lignin degradation occurs in two steps. The first weight loss at 200–300 °C, with a maximum weight loss at 217 °C, corresponds to the degradation of lignin's side chains and smaller lignin molecules [171]. The last stage, from 300 to 450 °C, exhibit the maximum weight loss at 360 °C and is caused by the cleavage of lignin internal linkages [171].

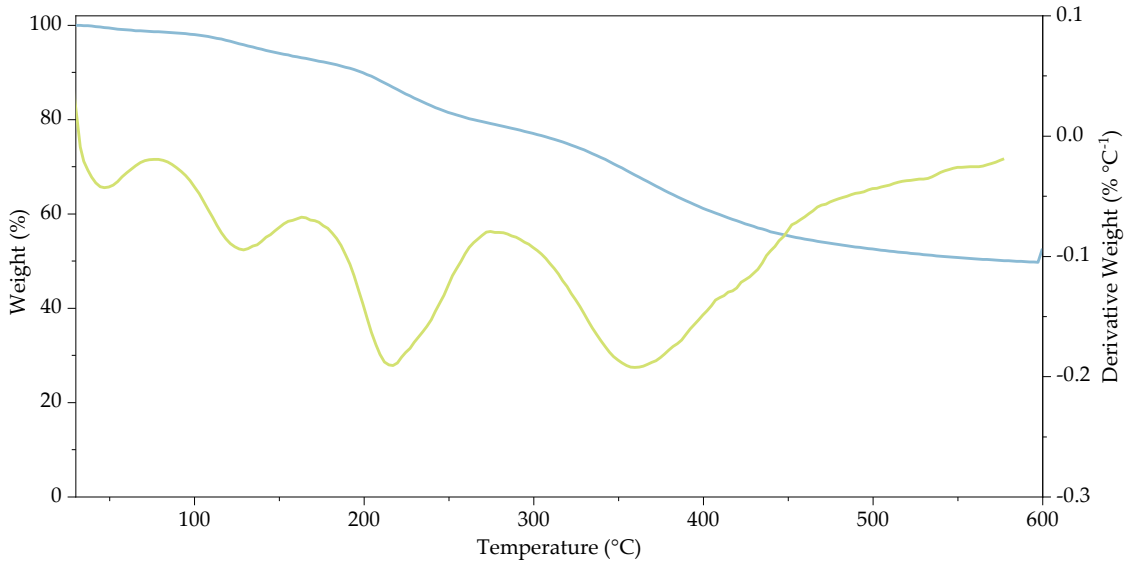

Figure 4.15. TGA (blue) and DTG (green) curves of the lignin extracted with Lact:Tart:ChCl (4:1:1) at optimal conditions.

4.5.3 Lignin morphological analysis

The morphology of the initial pine sawdust, cellulosic solid residue and extracted lignin was analyzed by SEM and the images are compiled in **Figure 4.16** at different magnifications. The pine sawdust has an ordered fibrillar structure characterized by the presence of pits that are responsible for the transport of water and minerals [172]. The cellulose structure is essentially defined by the presence of loose rod-like fibers and the organized structure of the pine sawdust was broken. This supports the high lignin removal yields observed. Nonetheless, the wood pits are still preserved in the cellulose fibers. The lignin structure is substantially different from the others fractions: there is no tubular fibers present in the extracted lignin, thus suggesting a good separation of the wood components as proved by the high lignin removal yield and purity obtained for the optimized extraction system.

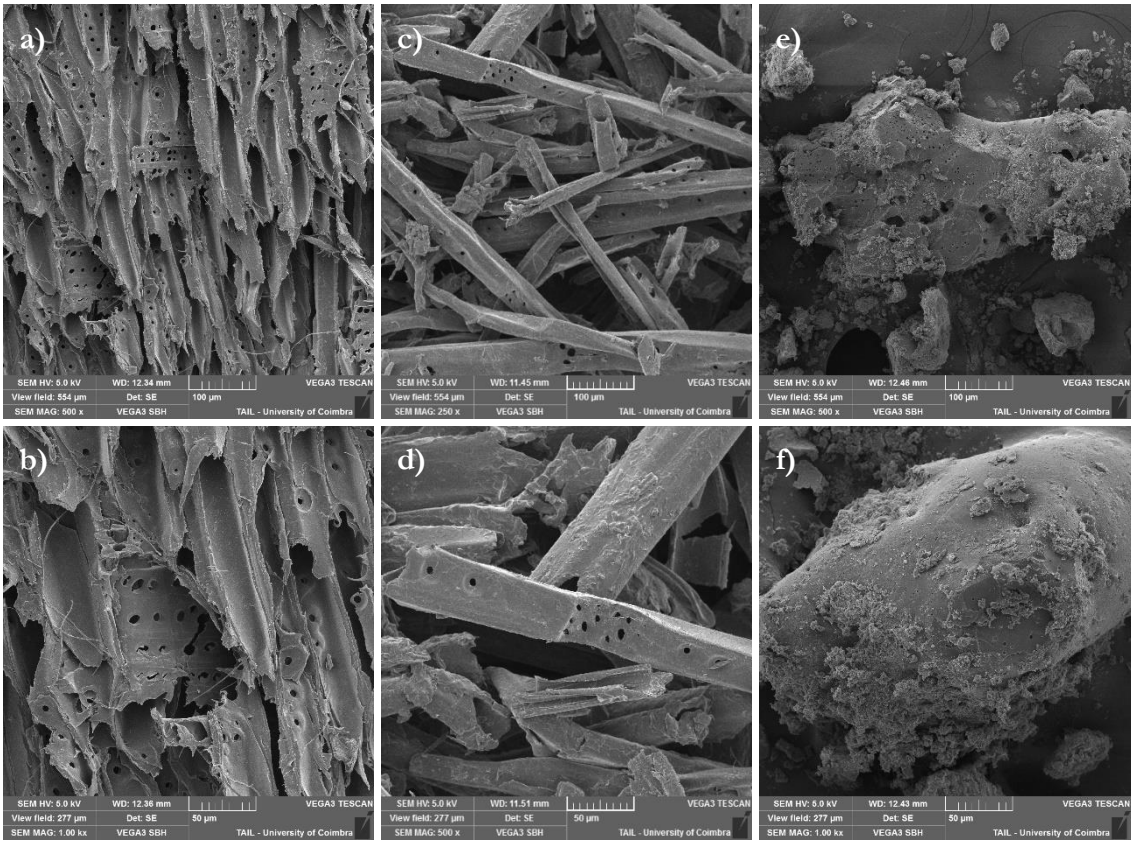

Figure 4.16. SEM images of a,b) initial raw pine sawdust; c,d) fractionated pine sawdust (cellulose-rich fraction); and e,f) extracted lignin. Extractions conditions: 1 h; 175 °C. Magnification of 250x and 500x.

Chapter 5

Conclusion

Lignin is a natural aromatic polymer that has tremendous potential as a renewable and sustainable feedstock to produce fuels, chemicals and biomaterials. However, selective and efficient extraction is difficult due to its complex structure. The aim of this work was mainly related to the development and characterization of novel "greener" methods, based on natural solvents, capable of a superior efficient and selective extraction of lignin from maritime pine sawdust.

The raw pine sawdust was characterized for its moisture and lignin content and pretreated with different solvents to remove wood extractives. The performance of individual solvents with different polarities was evaluated and compared to a standard procedure for the preparation of extractives-free wood. The results show that the extractives content removed for the sequential extractions is higher than those obtained for individual extractions and water is the solvent that is able to remove the highest amount of extractives. That is indicative of a high content of hydrophilic compounds in maritime pine sawdust. The extractives were characterized by FTIR and GC-MS. The water extractives clearly show a distinct composition from those extracted with less polar solvents. Water extractives are mainly composed of carbohydrates and some short chain organic acids whereas the fractions resulting for ethanol–toluene, ethanol, acetone or dichloromethane have a high content of resin acids.

For the lignin extraction, DESs were chosen as extraction solvent due to their biodegradability, low cost and tunability. Several acidic DESs were prepared in this work, combining different HBA and HBD, and characterized for their physicochemical properties. A remarkable dependency of the DESs properties on their composition was observed, namely water content and viscosity. DESs composed of di- and tricarboxylic acids are highly viscous whereas the ones comprising short monocarboxylic acids have low viscosities. Notwithstanding, the differences at extraction temperatures are not as pronounced as at room temperature. Regarding their rheological behavior, all DESs exhibit a Newtonian behavior. The DESs were screened for the fractionation of maritime pine sawdust to infer on their suitability for an efficient and selective extraction of lignin from biomass. The results suggest that the extraction capacity is greatly affected by the DES composition and both HBA and HBD play an important role in the DESs tailoring process. DES containing ChCl as HBA result in a significantly higher lignin extraction compared to urea or betaine combined with the same acids. For the screening of HBDs, different organic acids were used, varying the acid chain length and functional groups, namely carboxylic and hydroxyl groups. DESs comprising alpha-hydroxy acids seem to be more suitable for lignin extraction than linear carboxylic acids. Di and tri-carboxylic acids show high selectivity but their high viscosities limit mass transfer. Consequently, Lact:ChCl was the solvent capable of extracting the highest lignin content and the purest lignin was achieved with Tart:ChCl. The addition of levulinic acid and DMSO as cosolvents to DES allow an increase of the extraction yield, while the addition of water is not beneficial.

New ternary DESs, composed of two acids as HBDs and ChCl as HBA, were prepared to achieve a DES with enhanced properties and superior extraction performance. Lact:Tart:ChCl, in a molar ratio of 4:1:1, is the most efficient DES for a selective lignin extraction and recovery. Extraction temperature and time were also optimized for this process, revealing that 1h at 175 °C allowed to recover ca. 95% of the total lignin present in pine sawdust with a purity of ca. 89%.

Recycle and reutilization of DESs was successfully accomplished after sawdust fractionation. DES can be easily separated from the extracted lignin using excess water and recovered by evaporation. The performance of the recycled DES is not significantly affected in three consecutive cycles of extractions.

Lignin extraction was carried in different pretreated pine woods to investigate the influence of extractives presence on the lignin extraction yield. According to the results, no significant improvement was observed for any pretreated wood and it was demonstrated that it is not necessary to perform time-consuming extractions, that sometimes requires the use of harmful solvents, to develop an efficient and selective extraction system for lignin.

The FTIR analysis demonstrated that, independently of the extraction yield, there are no significant differences in lignin structure using different DESs. Lignin was further characterized for its thermal and morphological properties. The extracted lignin presents a high thermal stability, and the SEM analysis suggests a good separation of the wood components.

The extraction method developed in this work using the novel ternary DES proved to be highly efficient for lignin extraction from maritime pine sawdust. The fractionation of other lignocellulosic materials could be considered for future research to test the ability of this new eco-friendly system for the extraction of pure lignin from other biomass sources. Some additional characterization could be also performed for more detailed information about the structure and properties of the extracted lignin, such as molecular weight and mechanical properties assays. In addition, the study of potential applications of the extracted lignin should be conducted. Lignin has some attractive properties that motivate its application for the production of bioplastics since it can reduce the production cost, improve the plasticization of the material and reduce the water uptake. Future research could focus on blending lignin with other bioplastics.

Chapter 6

Materials and methods

6.1 Chemicals and raw materials

Maritime pine (*Pinus pinaster*) sawdust was provided from Valco – Madeiras e Derivados, S.A (Leiria, Portugal). The sawdust was sieved to mesh size between 0.7 and 1.4 mm and freeze-dried before use.

DL–Tartaric acid (Tart) (≥ 99%, Sigma), acetic acid glacial (Acet) (99–100%, Cham-Lab), glycolic acid (Gly) (70%, DuPont), propionic acid (Prop) (99%, Aldrich), lactic acid (Lact) (90%, VWR), dimethyl sulfoxide (DMSO) (≥ 95%, VWR), citric acid monohydrate (Cit) (> 99%, José Manuel Gomes dos Santos, Lda.), levulinic acid (≥ 98%, Merck), urea (≥ 99%, Merck), betaine anhydrous (> 97%, TCI), hydranal composite 5 (Fluka), dichloromethane (> 99%, Sigma-Aldrich) toluene (> 99%, Sigma-Aldrich), N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) (> 98%, Alfa Aesar), sulfuric acid (96%, Panreac) and acetone (José Manuel Gomes dos Santos, Lda.) were used as received. Choline chloride (ChCl) (≥ 98%, Sigma) was oven-dried (100 ºC) before use to remove the residual water.

6.2 Methods

6.2.1 Wood moisture content determination

The moisture content of the pine sawdust was determined by gravimetric method. This method consists of weighing a sample of the wood material, removing the water from the sample and then weighing the sample again until a constant mass is achieved. The moisture content is given by the ratio between the mass loss and the weight of the sample, calculated using the equation (2) which can be expressed either on a wet basis, $m_X = m_w$; or on a dry basis, $m_X = m_d$ [125].

$$\text{Moisture content } (\%) = \frac{m_w - m_d}{m_X} \times 100 \qquad (2)$$

- Where m_w and m_d are the masses of the wood before and after the drying process, respectively.
- m_X assumes m_w value when the moisture content is calculated on a wet basis and the m_d value when calculated on a dry basis.

The dry basis method is commonly used in the timber industry while the wet basis method is used in chemistry processing industries [125].

a) Oven-dry method

Samples containing ca. 5 g of pine sawdust were placed in an oven and weighed until a constant mass was obtained. A constant mass is achieved when the mass difference between two successive weighings is less than 0.2% [125], meaning that the mass change cannot be more than 0.01 g for the samples used. The tested was performed in triplicate.

b) Freeze-dry method

Three samples of ca. 6.5 g of sawdust were tared to sample tubes and submitted to lyophilization for a period of 24 h. After the drying process was completed, the sample was weighed again, and the mass loss calculated.

6.2.2 Extractives removal procedure

Sawdust extractions were performed with different solvents (i.e., ethanol–toluene, ethanol, acetone, dichloromethane, and water) to evaluate the extractive content removal of each solvent in the sawdust pretreatment.

- **Ethanol, acetone, and dichloromethane Soxhlet extractions**

Ethanol, acetone, and dichloromethane extractions were performed in a Soxhlet apparatus. Briefly, ca. 2.5 g of dry sawdust were weighed to a thimble and placed in the Soxhlet apparatus and then extracted with the solvent of interest. The volume of the solvent was ensured to be higher than the volume of the extraction chamber and the extraction temperature was adjusted for each solvent to allow an extraction rate of not less than four siphonings per hour as suggested by some standards [55,57]. A schematic representation of the Soxhlet apparatus is illustrated in **Figure 6.1a**. To evaluate the impact of the extraction time in the removal of extractives, the solvent was collected from the flask every 2 h and replaced by fresh solvent. The solvent was then evaporated in a rotary evaporator and the extractives were freeze-dried and weighed.

- **Water reflux extraction**

Extraction with water was performed in a reflux apparatus (**Figure 6.1b**). The sample (ca. 2.5 g sawdust) was placed in a round bottom flask containing 125 mL of water and magnetically stirred at 100 °C for 4 h in a oil bath. The sawdust was separated from the solvent by low pressure filtration using a Büchner funnel and the solvent was evaporated. The extractives were weighed after freeze-drying of the samples.

- **ASTM standard extraction**

The ASTM D1105-96 standard procedure [57] was followed for comparison of the individual solvents with the standard removal process. This standard describes two sequential Soxhlet extractions using ethanol–toluene (7:3) and ethanol, followed by a three-step reflux extraction using water. Initially, sawdust (ca. 2.5 g) was extracted for 4 h using ethanol–toluene solution as described above for Soxhlet extractions. After the extraction, the cartridge and sawdust were washed with ethanol and oven dried. The cartridge was placed again in the Soxhlet apparatus and extracted with ethanol for 4 h.

After washing and drying, the sawdust was placed in a round bottom flask and extracted 3 times with 200 mL of water in an oil bath at 100 °C in reflux with constant magnetic stirring. After the extractions, the solvents were evaporated under a rotary evaporator and the extracts were freeze-dried and weighed.

Extractive content was calculated as the percentage of the extracted mass compared to the dry mass of the wood.

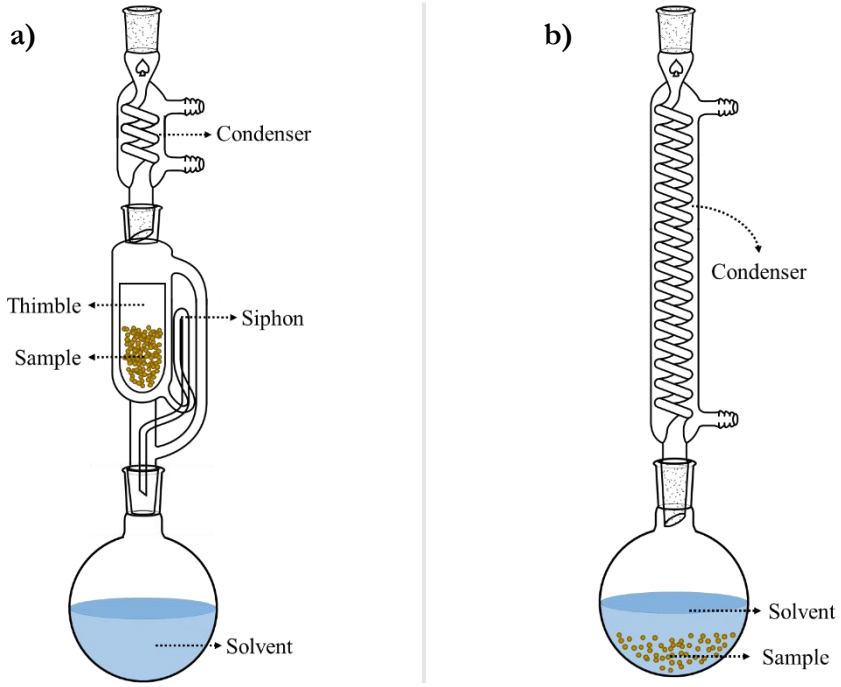

Figure 6.1. Schematic representation of a) Soxhlet extraction apparatus and b) traditional reflux extraction apparatus.

6.2.3 DESs preparation

Several DESs were prepared combining different carboxylic acids with ChCl, betaine or urea. The components were weighed according to the respective molar ratios (**Table 6.1**), and the mixtures were stirred with a magnetic stirrer in an oil bath at 80 °C for 1 h, except for those prepared from two solid compounds (i.e., citric and tartaric acid with ChCl), where a temperature of 100 °C was used to improve dissolution. All mixtures resulted in clear and homogeneous liquids, and no precipitation was observed after cooling to room temperature. The carboxylic acids and the molar ratios used to prepare

the DESs are reported in **Table 6.1** and the acids structures are represented in **Figure 6.2**.

For the Cit:ChCl DES mixtures with water, levulinic acid and DMSO, 10 wt.% or 20 wt.% of each cosolvent were added to the DES before extraction.

Table 6.1. HBA, HBD, molar ratios and temperature used for DESs preparation.

DES name	HBA	HBD	Molar ratio	Temperature (°C)
Acet:ChCl	Acetic acid	ChCl	2:1 and 5:1	80
Gly:ChCl	Glycolic acid	ChCl	1:1	80
Prop:ChCl	Propionic acid	ChCl	2:1	80
Lact:ChCl	Lactic acid	ChCl	2:1 and 5:1	80
Tart:ChCl	Tartaric acid	ChCl	1:2	100
Cit:ChCl	Citric acid	ChCl	1:1	100
Lact:Betaine	Lactic acid	Betaine	2:1	80
Prop:Urea	Propionic acid	Urea	2:1	80
Lact:Tart:ChCl	Lactic acid + tartaric acid	ChCl	0.67:0.33:1, 0.75:0.25:1, 1.5:0.5:1 and 4:1:1	100
Gly:Cit:ChCl	Glycolic acid + citric acid	ChCl	0.5:0.5:1	100

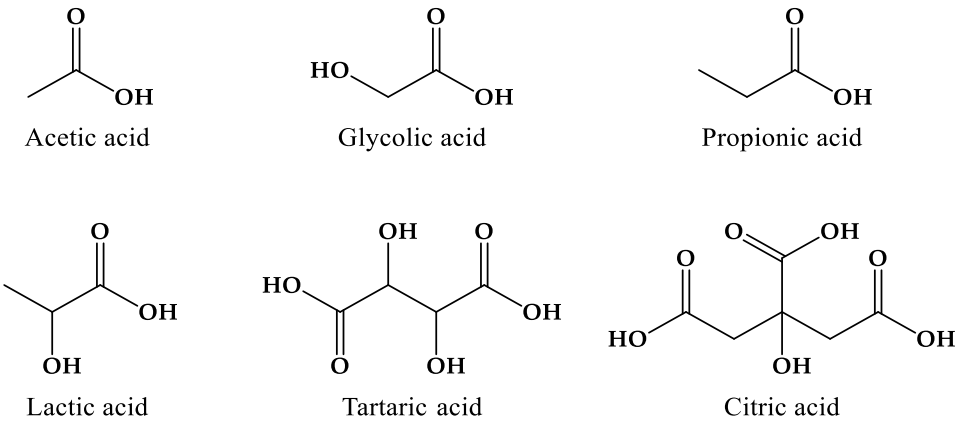

Figure 6.2. Structures of the carboxylic acids used in DES preparation.

6.2.4 Lignin extraction procedure

Several acidic DESs were screened for lignin extraction from pine sawdust to evaluate the influence of DES components and ratio on the extraction yield. Briefly, ca. 1 g of pine sawdust was placed in a round-bottom flask containing the DES of interest (solute-to-solvent ratio of 1:10) and stirred in reflux in an oil bath at 150 °C for 2 h, unless mentioned otherwise. After the extraction, the solid residue was separated from the DES via low-pressure filtration using a Büchner funnel and the residue was washed with acetone. The solid residue is mainly composed of cellulose and the filtrate contains both lignin and hemicellulose. To obtain the lignin from the solution, water was used as antisolvent to trigger lignin precipitation and its subsequent separation by centrifugation. The recovered lignin was then freeze-dried and weighed. To recover the DESs after extraction, the water was removed using a rotary evaporator and the remain residual water eliminated by freeze-drying. The regenerated DESs were used again in new extractions to evaluate the recyclability of these novel solvents.

Statistical analysis was performed using one-way ANOVA (α=0.05) to evaluate significant differences between the extraction yields resulting from the different DES.

a) Determination of extraction yield

The lignin extraction yield was calculated according to the equation (3):

$$\text{Extraction yield (\%)} = \frac{\text{Extracted mass}}{\text{Sample initial dry mass}} \times 100 \qquad (3)$$

This yield accounts for all the residue extracted during the extraction process and does not represent the lignin content extracted since this residue may contain cellulose or/and hemicellulose and wood extractives as well if the sample was not pretreated or if the extractives were not fully removed.

b) Determination of lignin content

Besides the ability of the method to fractionate the biomass, it is important to measure its selectivity for the desired polymer. The selectivity is measured from the lignin

purity, that is, the lignin content in the extracted residue, calculated according to the equation (4):

$$\text{Lignin purity (\%)} = \frac{\text{Mass of lignin in the extracted fraction}}{\text{Total extracted mass}} \times 100 \qquad (4)$$

To evaluate the lignin content present in pine sawdust and in the residue obtained after the extraction, both acid-soluble lignin (ASL) and acid-insoluble lignin (AIL) were determined as described in the NREL standard procedure [153]. The ASL fraction was accessed by UV-vis spectrophotometry (Shimadzu UV–2450) at a wavelength of 205 nm.

c) Lignin recovery yield

Using both extraction yield and purity, it is possible to calculate the total amount of lignin that is removed from the wood. Lignin recovery yield is determined dividing this value by the lignin content of pine sawdust (equation 5).

$$\text{Lignin recovery (\%)} = \frac{\text{Extracted residue} \times \text{Lignin purity}}{\text{\% Lignin in wood}} \times 100 \qquad (5)$$

6.3 Characterization techniques

6.3.1 Karl Fisher titration

Karl Fischer titration is an analytical method for the determination of water content. This technique allows the quantification of the water present in a sample based on its reaction with iodine in an alcoholic solution, according to the following two-step chemical reaction [173,174]:

$$CH_3OH + SO_2 + R_3N \longrightarrow R_3NH^+ + CH_3OSO_2^-$$

$$R_3NH^+ + CH_3OSO_2^- + I_2 + H_2O + 2\,R_3N \longrightarrow 3\,R_3NH^+ + CH_3OSO_3^- + 2\,I^-$$

Where R_3N is a base (e.g., imidazole).

The water content of all DESs was determined by Karl Fischer titration using Hydranal composite 5 in a Metrohm 890 Titrando apparatus equipped with 803 Ti Stand. The Tiamo 2.5 software was used to control automatic titrations. All samples were measured in triplicate after apparatus calibration with Milli-Q water. The DESs were stored in an oven at 50 °C before performing the analysis to avoid moisture uptake.

6.3.2 Refractometry

The incidence of light in a surface results in its reflection and refraction (**Figure 6.3**).

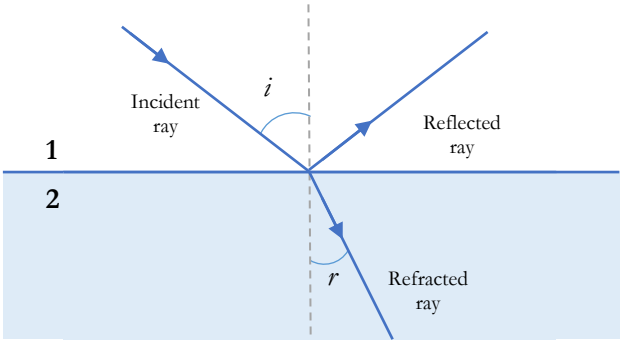

Figure 6.3. Schematic representation of the refraction and reflection of the incident radiation.

If the two media have different composition, they will present different refractive indices (n). Refractive index is the ratio between the speed of the light in vacuum and in a given medium (equation (6)) [175]. The degree of refraction that occurs between two media depends on the light speed in each medium and, consequently, on their refractive indices (equation (7))[176].

$$n_1 = \frac{c_{\text{vac}}}{c_1} \tag{6}$$

$$n_1 \sin(i) = n_2 \sin(r) \tag{7}$$

The refractive index of a mixture varies with the mole fraction of its components [176]. Thus, refractometry is an important tool for the characterization of a sample.

Refractive indices of the prepared DESs were measured at the sodium D line (589.3 nm) using an Atago RX–5000 α refractometer at 25 °C. The data is presented as the mean of two measurements for each sample.

6.3.3 Fourier transform infrared spectroscopy (FTIR)

FTIR is a spectroscopic technique that use infrared radiation to identify functional groups present in a molecule. A molecule that shows variations in the dipole moment with the bonds vibrations is an infrared-active molecule and it will absorb infrared radiation during the vibrations of its bonds. Shining the sample with infrared radiation results in absorption of the radiation that has the same frequency of the vibration modes of the molecules and results in a spectrum with characteristic bands at these frequencies. The spectrum can be presented in % transmittance or in absorbance [177].

Absorbance FTIR spectra were recorded in a Thermo Nicolet 380 FT-IR spectrophotometer from Thermo Scientific equipped with an ATR Smart Orbit apparatus in a spectral range of 4000–400 cm^{-1} with a resolution of 8 cm^{-1} and 64 scans. Background spectra were collected before every analysis.

6.3.4 Gas chromatography–mass spectrometry (GC-MS)

GC-MS combines the gas chromatography and mass spectrometry in one technique that allows the separation and qualitative and quantitative analysis of the samples. In gas chromatography, the sample is injected into an injector oven at high temperature to ensure its volatilization. The gaseous sample is then transported through the chromatographic column by a carrier gas, usually He, N_2 or H_2 to the detector [178]. The separation occurs by the differences in the interactions between the solutes and the stationary phase. Solutes with greater affinity to the stationary phase are eluted more slowly and reach the detector later [178]. In GC-MS, mass spectrometry is used as detector to identify the masses of atoms or fragments of the molecules [178]. The analytes are ionized and separated by their mass-to-charge ratio.

Previous to the analysis, some samples need to be chemically modified (i.e., derivatized) in order to decrease the boiling point of non-volatile compounds, enhance

the analytes thermal stability and/or to improve the interactions between analytes and stationary phase in GC, resulting in a better separation [179]. Derivatization usually consists in the acylation, alkylation or silylation of the compounds [48].

Derivatization of the samples was carried by adding 100 μL of the derivatization agent, BSTFA, to 2 mg of sample. The samples were heated at 60 °C for 15 min and then 1 mL of dichloromethane was added.

Volatile compounds were determined by GC-MS. The analysis was performed in a 7820A GC system (Agilent Technologies), equipped with a mass spectrometer MSD 5975 (Agilent Technologies). The compounds were separated in a capillary column HPS-MS of 30 m × 250 μm × 0.25 μm. Helium was used as the carrier gas at a flow rate of 1.33 mL min^{-1}. Injections were performed in the splitless mode and the temperatures of the injector, interface, quadrupole, and ion source were 250, 300, 150, and 230 ºC, respectively. The following temperature program was used. Temperature was raised from 70 to 250 °C at 15 °C min^{-1} and hold for 10 min. Next, temperature was further increase to 290 °C at a rate of 5 °C min^{-1} and hold for 2 min. The compounds were identified as silylated derivatives (in-house Spectral Library and the commercial Wiley 10th/NIST 2012 spectral library).

6.3.5 Rheometry

Rheology consists in the investigation of the deformation and flow of matter. Fluids can be classified according to their flow behavior. The behavior of a fluid is assessed by plotting the shear stress as a function of the deformation (shear rate). When the shear stress and shear rate are linearly proportional, the fluid has a Newtonian behavior [180]. Non-Newtonian fluids have a viscosity response dependent on the applied shear stress and can be divided into several categories based on this response, as represented in **Figure 6.4** [181].

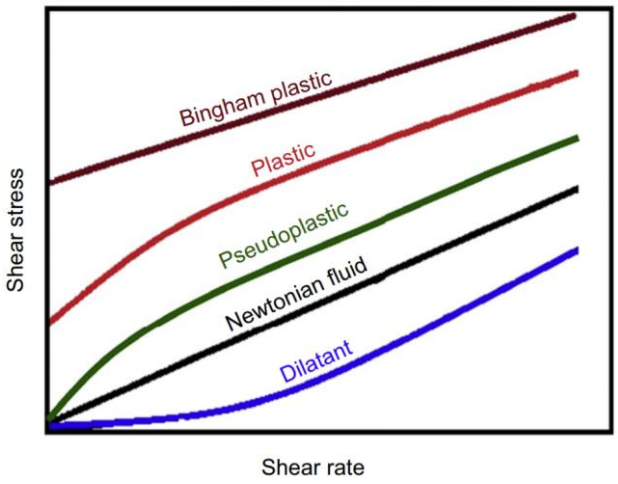

Figure 6.4. Rheological behavior of different materials [181].

The viscosities of all DES were measured at different temperatures. The viscosities were obtained in a temperature range from 25–150 °C with 25 °C steps, on a HAAKE MARS III rheometer from Thermo Fisher Scientific using a cone-plate geometry (35 mm, 1°, 0.052 mm gap). A Peltier unit was used for strict temperature control.

Rotational tests were performed at 20 °C in a shear stress range of 0.1–200 Pa, dependent on the samples.

6.3.6 Thermogravimetric analysis (TGA)

TGA is used to assess the thermal stability of materials. This technique consists in placing a sample in a furnace equipped with a sensitive scale and heating it. The weight loss is measured during the heating and moisture and volatiles content and degradation temperature can be determined [182].

Thermograms were measured using a thermogravimetric analyzer, TG 209 F Tarsus (Netzsch Instruments). The samples, containing ca. 3 mg, were weighed in alumina pans and heated from 25 to 600 ºC at a heating rate of 10 C.min^{-1} under N_2 atmosphere (flow rate of 50 mL.min^{-1}).

6.3.7 Scanning electron microscopy (SEM)

SEM is a microscopic technique that allows the analysis of the materials surface. Prior to the analysis, the non-conductive samples must be sputtered with a conductive material, usually gold [182]. In SEM, the sample is scanned by an electron beam that results in the emission of electrons and photons that are collected to form an image of the surface [182].

The morphology of the extracted lignin, pine sawdust and cellulosic solid residue was assessed by a scanning electron microscope VEGA3 SBH from TESCAN. The samples were freeze-dried before the analysis and then deposited directly over the carbon tape on the support and sputtered with an approximately 6 nm thin Au/Pd film, by cathodic pulverization using a SPI Module Sputter Coater, during 90 s at a current of 15 mA. The accelerating voltage used ranged from 5 to 15 kV, and the work distance (WD) was set to 10 mm.

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Appendices

Appendix A – Flow curves of the prepared DESs

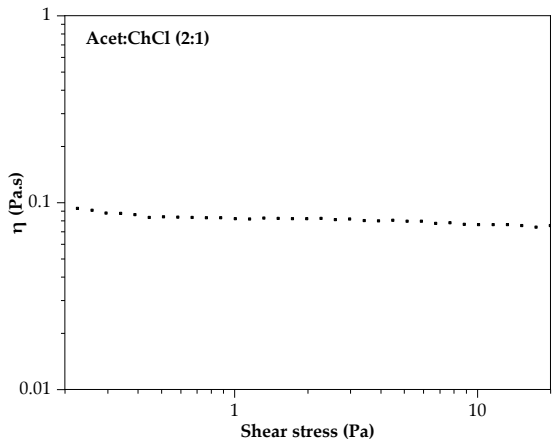

Figure A.1. Flow curve of Acet:ChCl (2:1) DES

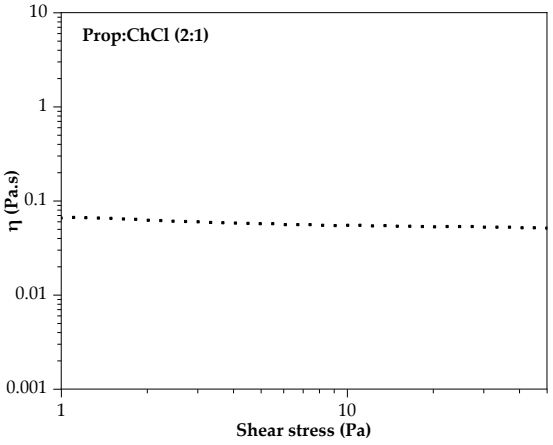

Figure A.2. Flow curve of Prop:ChCl (2:1) DES

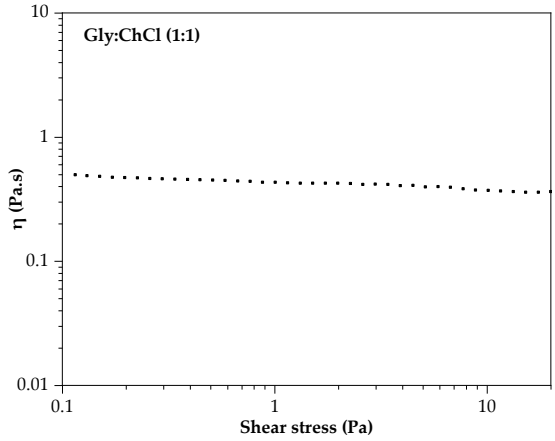

Figure A.3. Flow curve of Gly:ChCl (1:1) DES

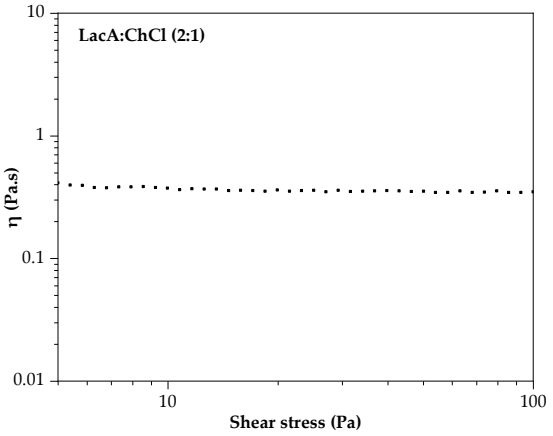

Figure A.4. Flow curve of Lact:ChCl (2:1) DES

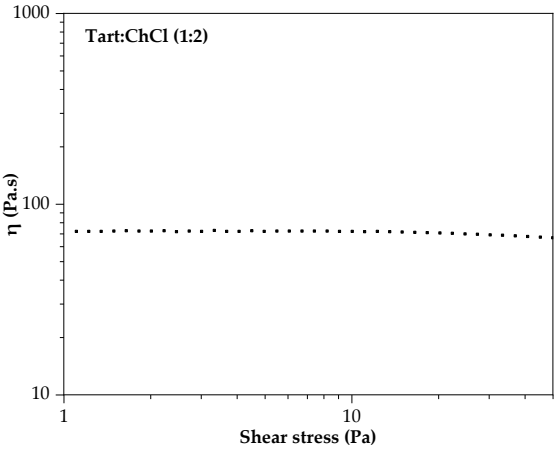

Figure A.5. Flow curve of Tart:ChCl (1:2) DES

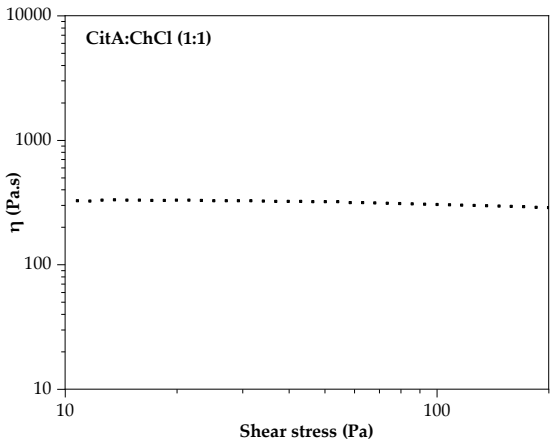

Figure A.6. Flow curve of Cit:ChCl (1:1) DES

Appendix B – Viscosities of DESs at different temperatures

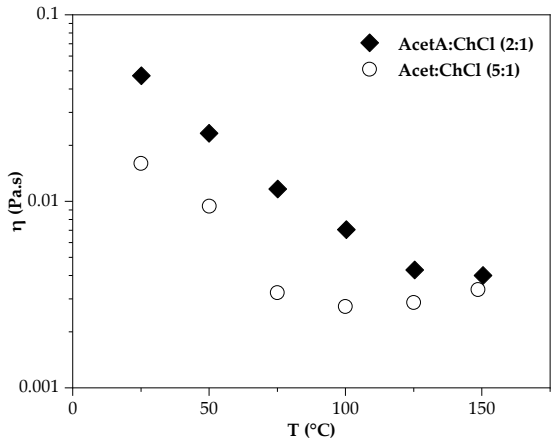

Figure B.1. Dynamic viscosity of Acet:ChCl (2:1) and (5:1) DESs

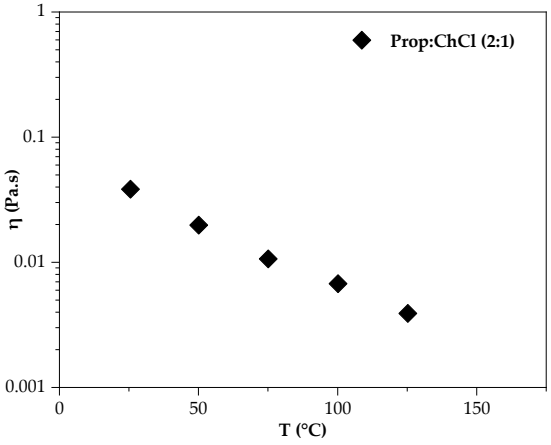

Figure B.2. Dynamic viscosity of Prop:ChCl (2:1) DES

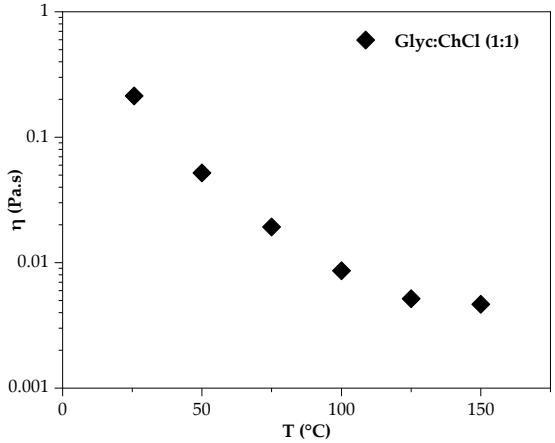

Figure B.3. Dynamic viscosity of Gly:ChCl (1:1) DES

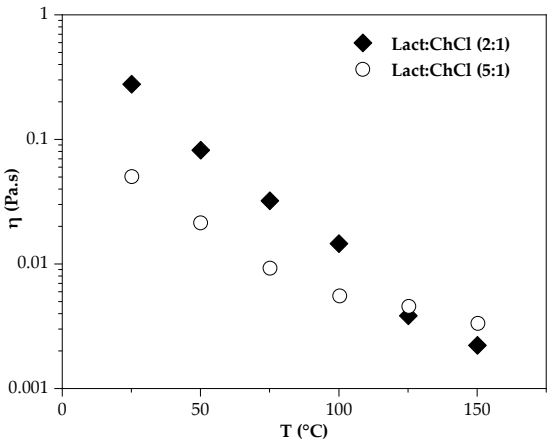

Figure B.4. Dynamic viscosity of Lact:ChCl (2:1) and (5:1) DESs

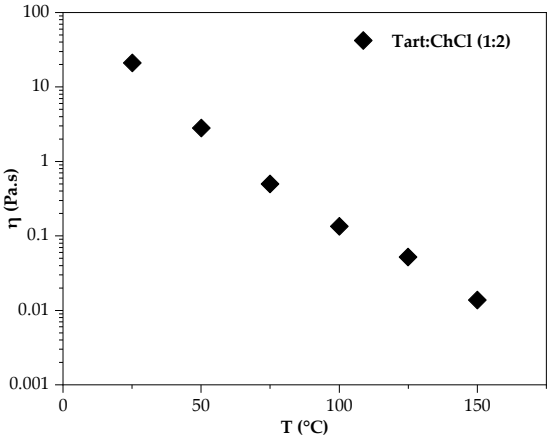

Figure B.5. Dynamic viscosity of Tart:ChCl (1:2) DES

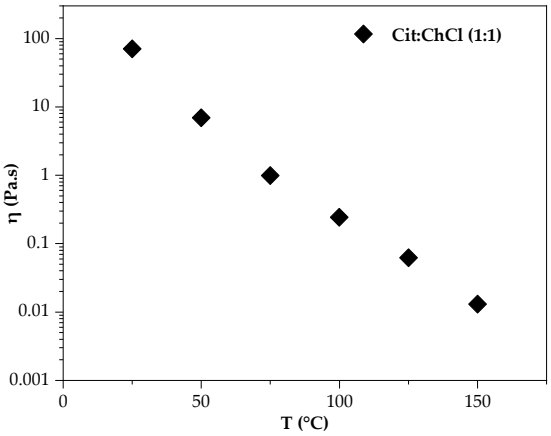

Figure B.6. Dynamic viscosity of Cit:ChCl (1:1) DES

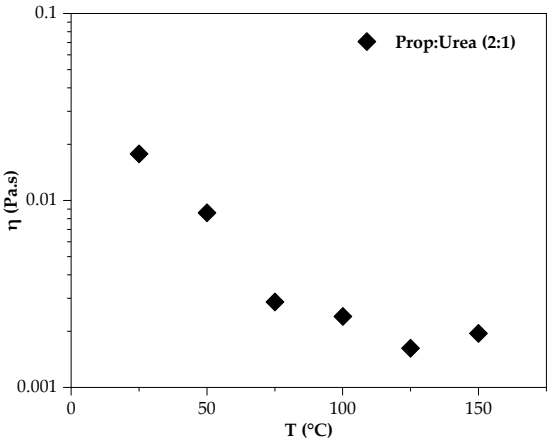

Figure B.7. Dynamic viscosity of Prop:Urea (2:1) DES

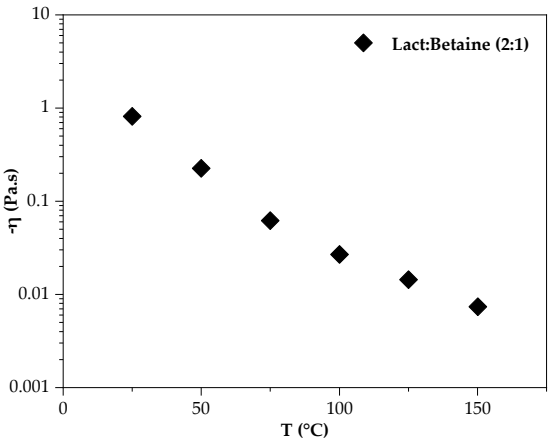

Figure B.8. Dynamic viscosity of Lact:Betaine (2:1) DES

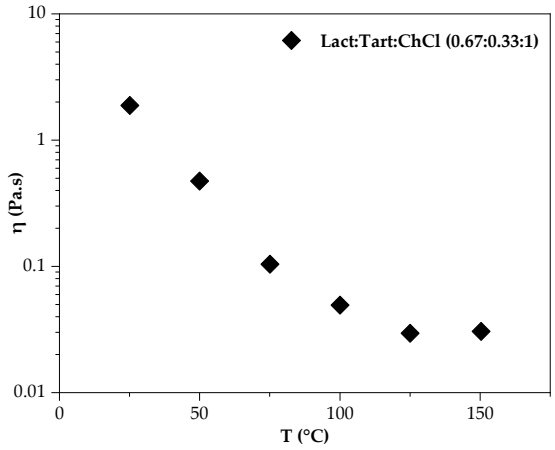

Figure B.9. Dynamic viscosity of Lact:Tart:ChCl (0.67:0.33:1) DES

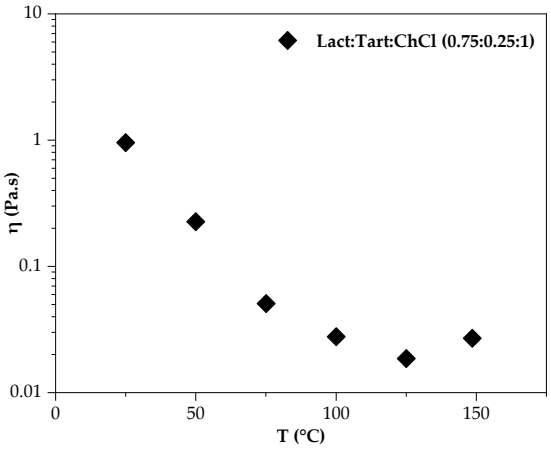

Figure B.10. Dynamic viscosity of Lact:Tart:ChCl (0.75:0.25:1) DES

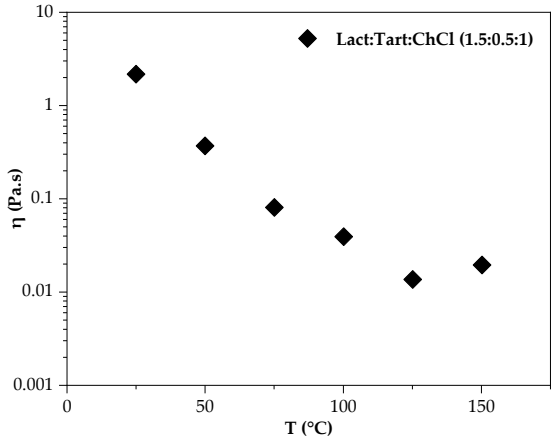

Figure B.11. Dynamic viscosity of Lact:Tart:ChCl (1.5:0.5:1) DES

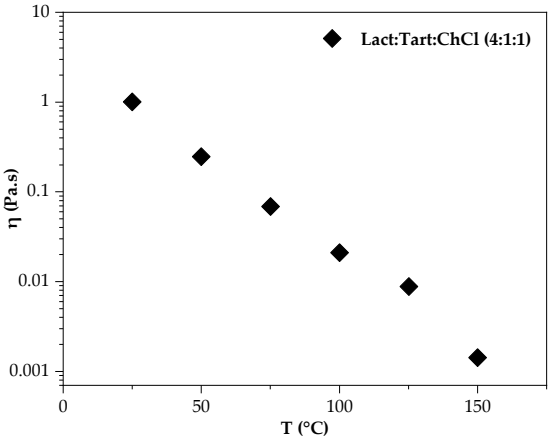

Figure B.12. Dynamic viscosity of Lact:Tart:ChCl (4:1:1) DES

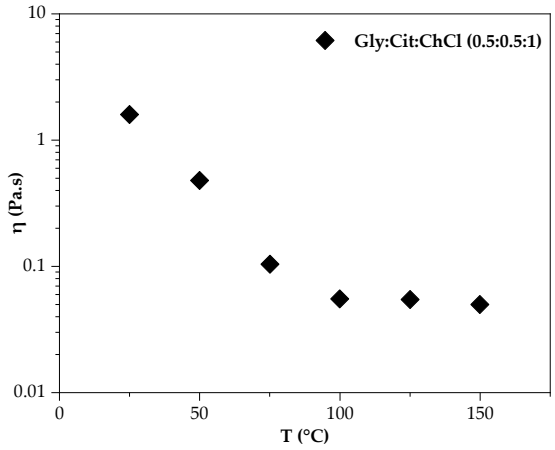

Figure B.13. Dynamic viscosity of Gly:Cit:ChCl (0.5:0.5:1) DES

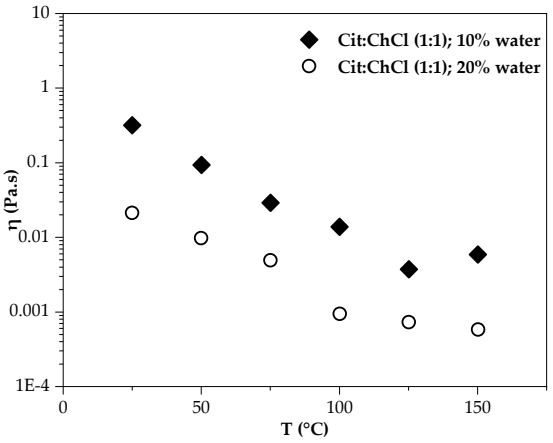

Figure B.14. Dynamic viscosity of Cit:ChCl (1:1) DES with water

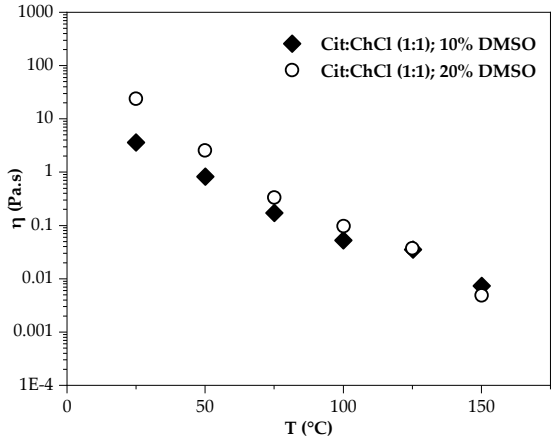

Figure B.15. Dynamic viscosity of Cit:ChCl (1:1) DES with DMSO

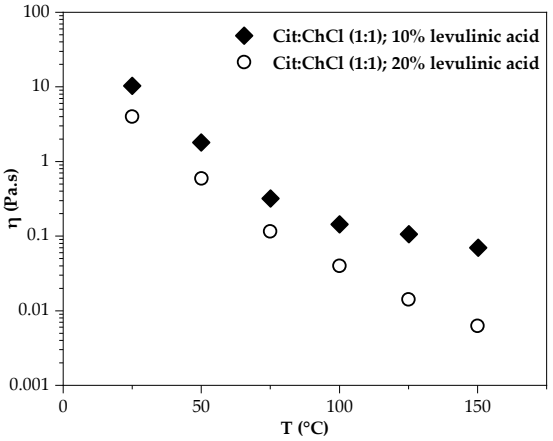

Figure B.16. Dynamic viscosity of Cit:ChCl (1:1) DES with levulinic acid

Appendix C – FTIR spectra of extracted lignins

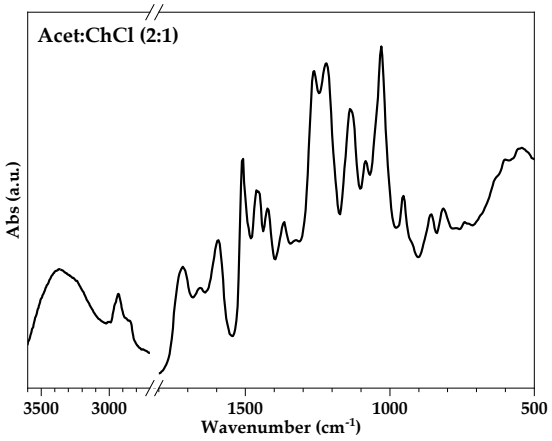

Figure C.1. FTIR spectrum of lignin extracted with Acet:ChCl (2:1)

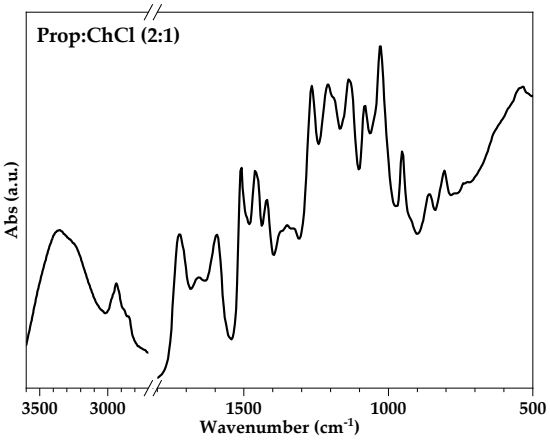

Figure C.2. FTIR spectrum of lignin extracted with Prop:ChCl (2:1)

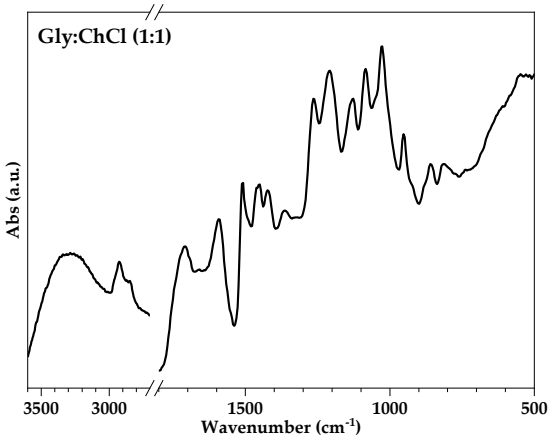

Figure C.3. FTIR spectrum of lignin extracted with Gly:ChCl (1:1)

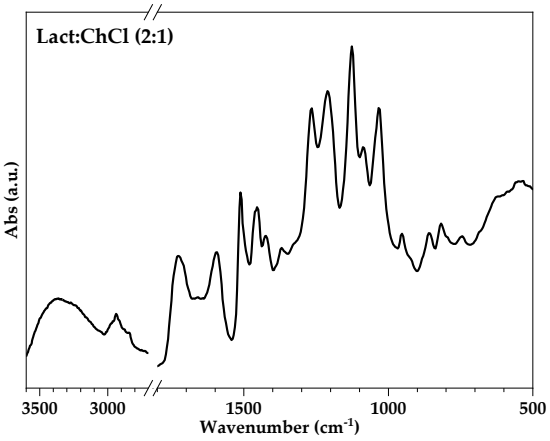

Figure C.4. FTIR spectrum of lignin extracted with Lact:ChCl (2:1)

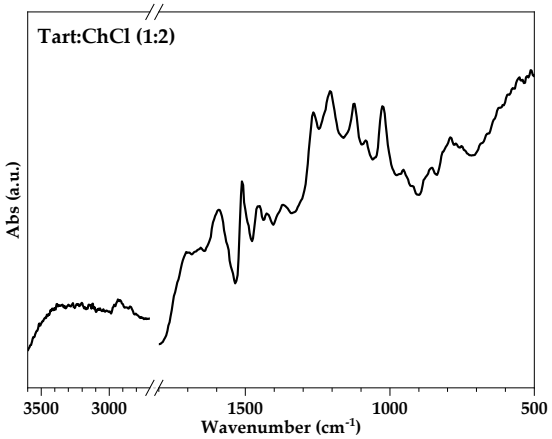

Figure C.5. FTIR spectrum of lignin extracted with Tart:ChCl (1:2)

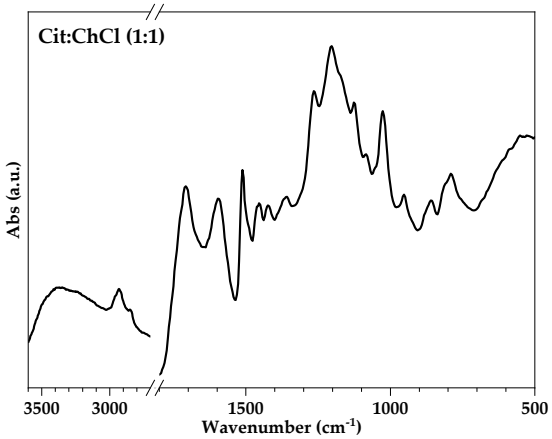

Figure C.6. FTIR spectrum of lignin extracted with Cit:ChCl (1:1)

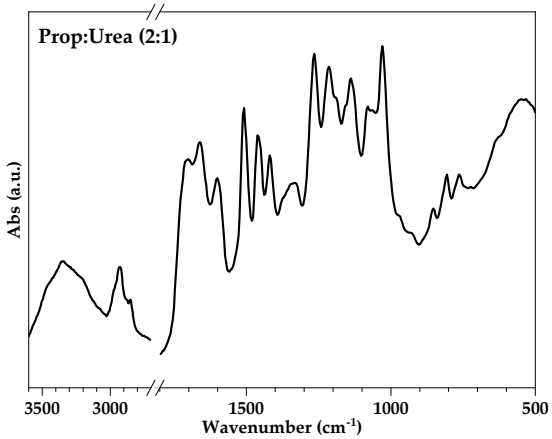

Figure C.7. FTIR spectrum of lignin extracted with Prop:Urea (2:1)

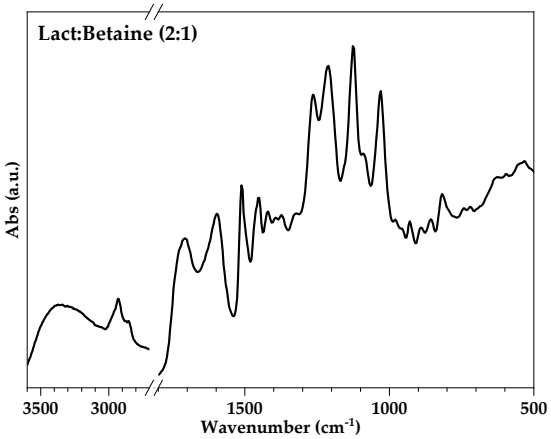

Figure C.8. FTIR spectrum of lignin extracted with Lact:Betaine (2:1)

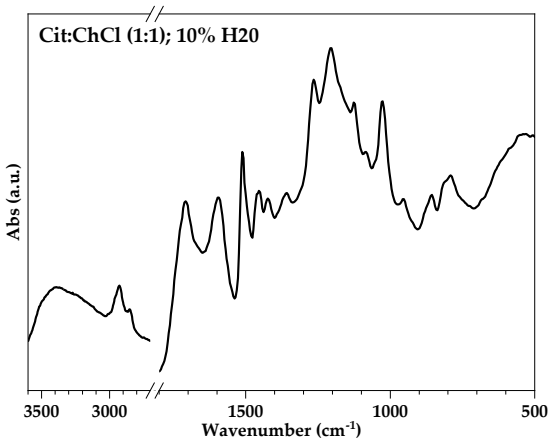

Figure C.9. FTIR spectrum of lignin extracted with Cit:ChCl (1:1); 10% H_2O

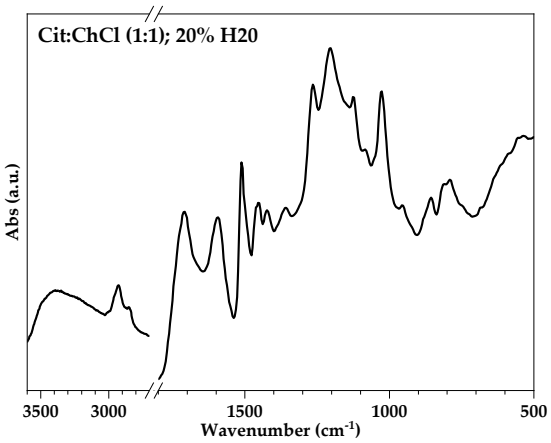

Figure C.10. FTIR spectrum of lignin extracted with Cit:ChCl (1:1); 20% H_2O

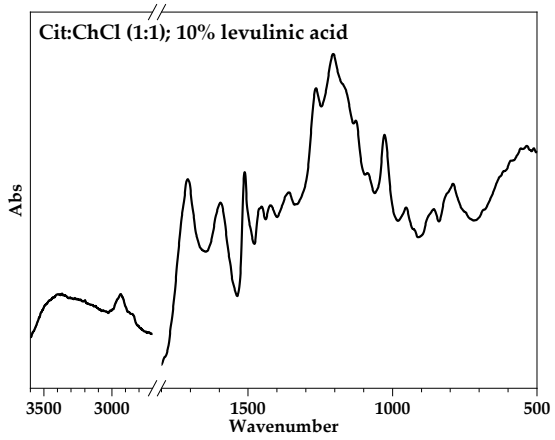

Figure C.11. FTIR spectrum of lignin extracted with Cit:ChCl (1:1); 10% levulinic acid

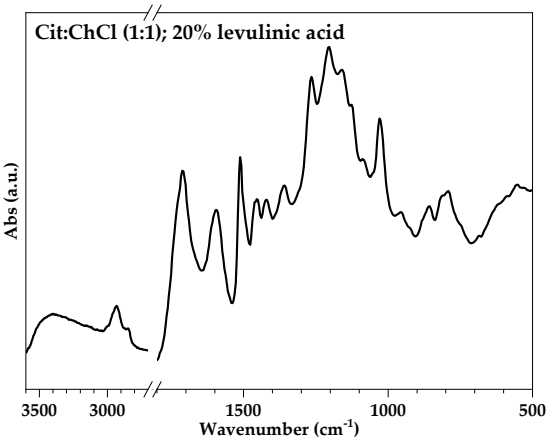

Figure C.12. FTIR spectrum of lignin extracted with Cit:ChCl (1:1); 20% levulinic acid

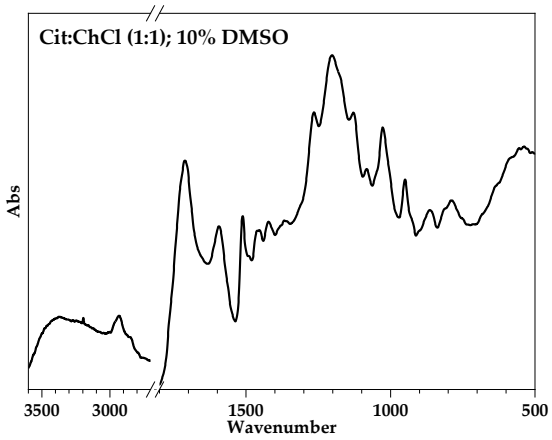

Figure C.13. FTIR spectrum of lignin extracted with Cit:ChCl (1:1); 10% DMSO

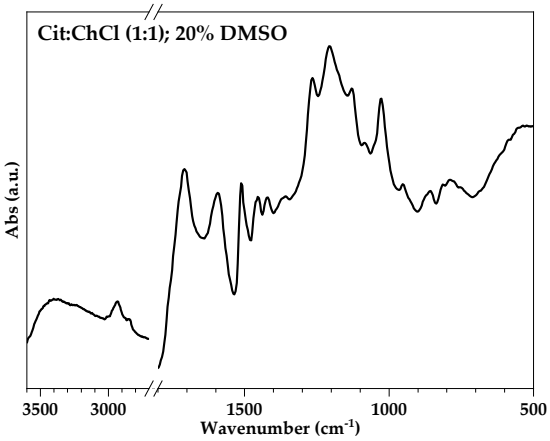

Figure C.14. FTIR spectrum of lignin extracted with Cit:ChCl (1:1); 20% DMSO

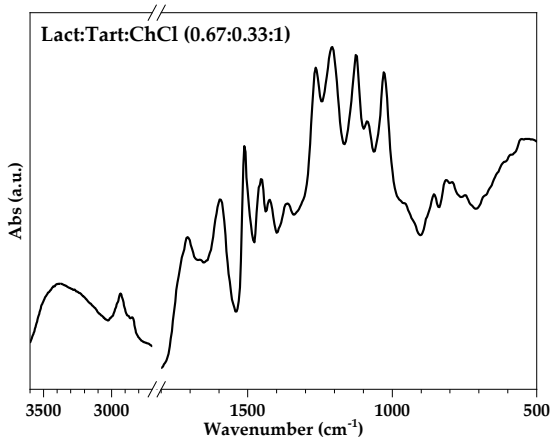

Figure C.15. FTIR spectrum of lignin extracted with Lact:Tart:ChCl (0.67:0.33:1)

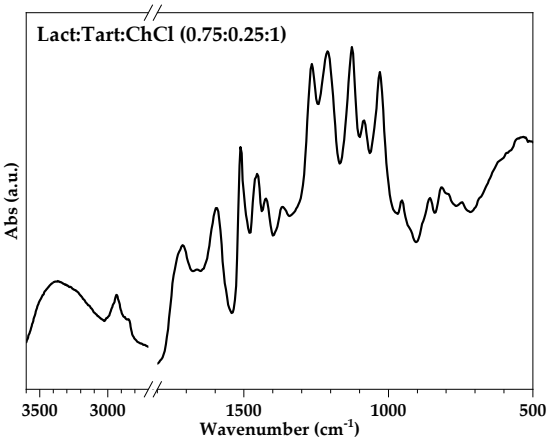

Figure C.16. FTIR spectrum of lignin extracted with Lact:Tart:ChCl (0.75:0.25:1)

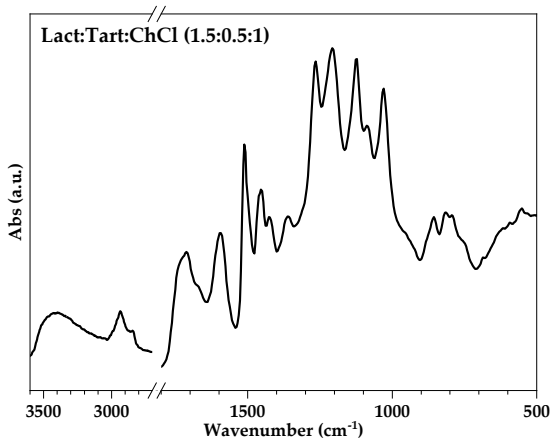

Figure C.17. FTIR spectrum of lignin extracted with Lact:Tart:ChCl (1.5:0.5:1)

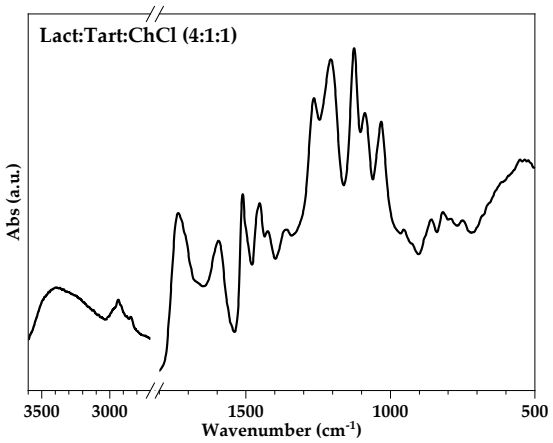

Figure C.18. FTIR spectrum of lignin extracted with Lact:Tart:ChCl (4:1:1)

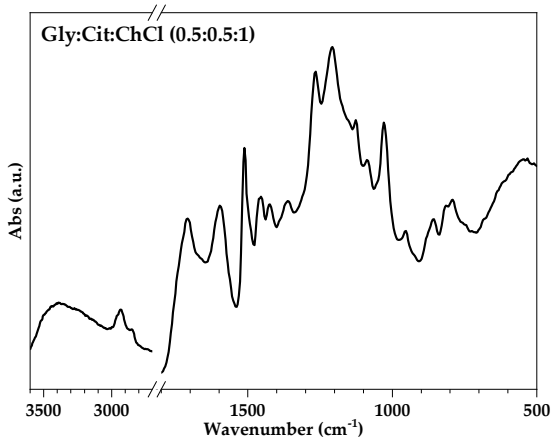

Figure C.19. FTIR spectrum of lignin extracted with Gly:Cit:ChCl (0.5:0.5:1)