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André Gabriel Ferreira Simões

**PRECIPITATION OF LIGNIN AND  
HEMICELLULOSES FROM BLACK LIQUOR: A  
ROUTE TOWARDS THEIR VALORIZATION**

**Dissertação no âmbito do Mestrado Integrado em Engenharia  
Química, orientada pelo Doutor Licínio Manuel Gando de Azevedo  
Ferreira, pela Doutora Patrícia de Jesus Pinto Alves e apresentada  
no Departamento de Engenharia Química da Faculdade de  
Ciências e Tecnologia da Universidade de Coimbra**

Outubro de 2021



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This work was prepared under the Inpactus project - Innovative Products and Technologies from Eucalyptus, Project No. 21 874 funded in Portugal 2020 through the Regional Development Fund (FEDER), Compete 2020, No. 246/Axis II/2017.

Cofinanciado por:



UNIÃO EUROPEIA  
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de Desenvolvimento Regional



## **Acknowledgments**

Esta dissertação marca a conclusão de uma das etapas da minha vida. Este é o meu agradecimento a todos aqueles que me apoiaram.

Agradeço aos meus orientadores, Doutor Licínio Ferreira, por todo o conhecimento transmitido e apoio dado durante toda a dissertação, à Doutora Patrícia Alves pelo acompanhamento e sugestões de melhoria no desenvolvimento da dissertação e à Mestre Manorma Sharma pelo tempo dedicado e por toda a paciência durante este trabalho.

Agradeço ao RAIZ pelo licor negro disponibilizado, a matéria-prima do meu trabalho e também ao Departamento de Química da Universidade de Coimbra pelo apoio prestado na técnica de RMN.

Agradeço a toda a minha família, em especial à minha mãe, por todo o carinho e por ter sempre as palavras certas no momento certo, ao meu pai pela compreensão, apoio e força e ao mano por sempre acreditar em mim.

Agradeço aos meus amigos que me acompanharam nesta jornada, Azeiteiro, Porco, Abrantes, Pato, Serafim, Nelson, Mauro, Civil, Rafa, Pinguim, Rui Costa, Megafone, Ângelo, Bea Marona, Hugago, Presidente Lopes, Catarina Sofia, Dani e Paula, Paredes, Salgueirão e Beatriz Ribeiro vocês sabem que sem vocês este curso não tinha sido o mesmo.

A todos vocês o meu sincero obrigado!



## Resumo

A hipótese da integração da lenhina, bem como das hemiceluloses na área da biorrefinaria tem sido estudada ao longo dos anos existindo inúmeros artigos científicos sobre esta temática. O método mais utilizado no isolamento da lenhina é a precipitação através da acidificação do licor negro (BL). Através desta tecnologia é possível extrair lenhina para produzir materiais de valor acrescentado, como por exemplos as fibras de carbono, carvão ativado, nano partículas, entre outros. O trabalho desenvolvido no âmbito desta dissertação tem como objetivo principal o isolamento da lenhina e das hemiceluloses a partir do licor negro proveniente do cozimento kraft de *Eucalyptus globulus*.

Inicialmente, recuperou-se a lenhina através da acidificação do licor negro (pH inicial na ordem dos 13) até um pH entre 3 e 4,5. Para tal, recorreu-se a diferentes ácidos orgânicos, dos quais se destacam o ácido acético e láctico, visto que se obteve o melhor rendimento, na ordem dos 54 %. A recuperação das hemiceluloses foi realizada através da acidificação do licor negro até um pH entre 2 e 3. Para além dos diferentes ácidos orgânicos utilizados, dos quais o ácido acético foi o que obteve melhores resultados, com um rendimento de 21 %, utilizou-se um solvente verde.

De seguida, a recuperação da lenhina foi estudada utilizando o *software* JMP<sup>®</sup> PRO, de modo a escolher uma gama de valores para as variáveis de operação. Estas são a temperatura, que variou entre 30 e 60 °C, o pH, que variou entre 3,5 e 4,5, e os ácidos com melhor rendimento, que neste caso foram o acético e o láctico. Para as condições ótimas de operação, T = 45 °C; pH = 3,5 e ácido láctico, obteve-se um rendimento de 48 %.

Na recuperação das hemiceluloses foram utilizados dois solventes, acetona e solvente verde. Relativamente à acetona, os melhores resultados foram obtidos para as amostras de hemiceluloses isoladas com acetona a amostra 1 (AHC 1) e a 4 (AHC 4), quando o teor de lenhina foi de aproximadamente 67 % e o volume de acetona adicionado foi 90 % do volume de sobrenadante. Com estes resultados obteve-se um rendimento de 44 % para as duas amostras e uma pureza de 41 e 44 % para a amostra AHC 4 e AHC 1, respetivamente. Para o solvente verde as condições operacionais com melhor rendimento, na ordem dos 58 e 68 % de pureza, foram obtidas para as amostras de hemiceluloses

isoladas com solvente verde, a amostra 10 (CPME10), para as seguintes condições: T = 60 °C; pH = 2,6 e CPME / BL = 2.

Foi realizado um teste com o solvente mais utilizado na literatura, o dioxano, nas mesmas condições operacionais da amostra CPME10, onde os valores de rendimento e pureza foram de 63 e 41 %, respectivamente.

A técnica de HPLC foi utilizada para determinar a concentração dos compostos nas amostras com maior e menor grau de pureza. No caso das amostras de lenhina, estas são compostas por 96 a 98 % de lenhina, 2 a 3 % de polissacarídeos e 1 % de inorgânicos, dependendo do tipo de ácido utilizado. As amostras de hemiceluloses são compostas maioritariamente por xilanas, aproximadamente 45 %, por lenhina, cerca de 40 % e por impurezas polisacarídicas e de inorgânicos.

Por último, as técnicas de FTIR e RMN foram realizadas, de forma a conhecer a estrutura das amostras. Através destas técnicas e por comparação dos picos obtidos com artigos da literatura, foi possível concluir que o grau de pureza das amostras reflete o que era esperado, isto é, nas amostras onde se pretendia isolar a lenhina, o precipitado era constituído maioritariamente por lenhina e o mesmo aconteceu com as amostras de hemiceluloses.

**Palavras-chave:** Biorrefinaria, lenhina, hemiceluloses, licor negro e precipitação.

## Abstract

The hypothesis of integrating lignin, as well as hemicelluloses in the biorefinery field has been studied over the years and there are numerous scientific papers on this topic. The most used method for lignin isolation is precipitation through acidification of the black liquor (BL). This technology, allows the extraction of lignin to produce value-added materials, such as carbon fibers, activated carbon, nanoparticles, among others. The main objective of the work developed in this thesis is the isolation of lignin and hemicelluloses of black liquor obtained from the kraft cooking of *Eucalyptus globulus*.

Initially, the lignin was recovered through the acidification of the black liquor (initial pH around 13) to a pH between 3 and 4.5. For this, different organic acids were used, of which acetic and lactic acid stand out, as the best yield was obtained, around 54 %. The recovery of the hemicelluloses was performed through the acidification of black liquor until a pH between 2 and 3. Besides the different organic acids used, of which acetic acid was the one that obtained the best results with a yield of 21 %, a green solvent was used.

Moreover, lignin recovery was studied using the JMP<sup>®</sup> PRO software, to choose a range of values for the operation variables. These are the temperature, which varied between 30 and 60 °C, the pH, which ranges between 3.5 and 4.5, and the acids with the best yield, which in this case were acetic and lactic acids. For the optimal operating conditions, T = 45 °C; pH = 3.5 and lactic acid, a yield of 48 % was obtained.

In the recovery of hemicelluloses two solvents were used, acetone and green solvent. Regarding acetone, the best results were obtained for hemicelluloses samples isolated with acetone, sample 1 (AHC 1) and 4 (AHC 4), for a lignin content of approximately 67 % and the volume of acetone added was 90 % of the volume of supernatant. These results led to a yield of 44 % for both samples and a purity of 41 and 44 % for samples AHC 4 and AHC 1, respectively. The operational conditions with the best yield (in the order of 58 and 68 % of purity) obtained for hemicelluloses samples isolated with green solvent, sample 10 (CPME 10), were the following: T = 60 °C; pH = 2.6 and CPME / BL = 2.

A test with the most widely used solvent in the literature, dioxane, was also performed under the same operating conditions of sample CPME10, and the yield and purity values obtained were 63 and 41 %, respectively.

The HPLC technique was used to determine the concentration of the compounds in the samples with higher and lower purity degrees. In the case of lignin samples, these were composed of 96 to 98 % lignin, 2 to 3 % polysaccharides, and 1 % inorganics, depending on the type of acid used. The hemicelluloses samples were composed mostly of xylans, approximately 45 %, about 40 % of lignin, and by polysaccharide and inorganic impurities.

Finally, FTIR and NMR techniques were performed to infer about the structure of the samples. Through these techniques and by comparison of the peaks obtained with literature information, was possible to conclude that the degree of purity of the samples reflects what was expected, that is, in the samples where it was intended to isolate lignin, the precipitate consisted mostly of lignin and the same occurred with the hemicelluloses samples.

**Keywords:** Biorefinery, lignin, hemicelluloses, black liquor, and precipitation.

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## List of symbols or abbreviations (nomenclature)

### Acronyms

<b>AA</b>	Acetic acid
<b>BL</b>	Black liquor
<b>CA</b>	Citric acid
<b>CPME</b>	Cyclopentyl methyl ether
<b>CPME+PA</b>	CPME and phosphoric acid
<b>CPME+GAA</b>	CPME and glacial acetic acid
<b>CPME+HA</b>	CPME and hydrochloric acid
<b>CPME+LA</b>	CPME and lactic acid
<b>GAA</b>	Glacial acetic acid
<b>HA</b>	Hydrochloric acid
<b>IC</b>	Inorganic content
<b>LA</b>	Lactic acid
<b>NaOH</b>	Sodium hydroxide
<b>OC</b>	Organic content
<b>PA</b>	Phosphoric acid
<b>SA</b>	Sulfuric acid
<b>SA+AA</b>	Sulfuric acid and acetic acid
<b>SC</b>	Silica crucible
<b>TOC</b>	Total organic content
<b>TSC</b>	Total solid content



# 1. INTRODUCTION

## 1.1 Scope and Motivation

Nowadays, people are increasingly concerned about the environment and a short supply of non-renewable resources. The treatment and recycling of potential waste and the application of renewable resources are becoming more important. Thus, the conception of biorefinery has emerged, which includes the processing of renewable feedstock/biomass to produce energy, fuel, chemicals, and materials. In the pulp and paper industry, large amounts of wood are processed to produce paper and also to generate energy in a sustainable way from its lignocellulosic residues. But in the present scenario, recycling these lignocellulosic byproducts and residues to produce high value products is imperative to maintain the established concept of biorefining in these industries [1].

In pulp production, the main process is kraft pulping, where wood chips are cooked in a digester to obtain cellulose fibers by removing lignin from wood. The residue of this process is called black liquor, which is composed by a mix of organic and inorganic components and has the color dark due to the presence of high concentrations of lignin. This black liquor – coming from the digester is called weak black liquor – has a total dissolved solid content of ~ 17 %. Then it is concentrated in a series of multi-effect evaporators up to a total solids content of 60 - 70 %. This contains organic matter consisting of lignin, hemicelluloses and some dissolved residual cellulose, is burned in the recovery boiler and used as energy to generate steam and power. On the other hand, the inorganic part is causticized, recovered and recycled in the process, making the pulping process economically viable [2,3].

Lignin is a carbon rich, polyphenolic biopolymer and can be used to produce various value-added products. But due to a complex and robust structure, its industrial scale applications still remain a challenge. In recent decades, researchers have explored the possibilities to manufacture lignin-based additives, resins, adhesives, dispersants, surfactants, etc. . Thus, despite multiple existing studies developed for this purpose, lignin isolation is still a problem in this industry. In addition to lignin, the second most abundant biopolymer in black liquor are hemicelluloses, which can also be used in value-added products, such as hydrogels, thermoplastics and paper additives [4,5].

The aim of this work is to develop effective methodologies to recover lignin and hemicelluloses from kraft black liquor, as well as to analyze the feasibility of their

valorization. Lignin precipitation by acidification is the most common method to separate lignin from kraft black liquor, where generally sulfuric acid is used as an acidification agent. Due to environmental and process outgrowths associated with sulfuric acid precipitation, alternative precipitating agents were studied as potential replacements for sulfuric acid, such as organic acids, along with the effect of precipitation temperature to optimize the separation efficiency and yield [6]. Furthermore, a similar study was performed for the separation of hemicelluloses from black liquor aiming to exploit the complete potential of available organic components of black liquor for biorefinery applications [7].

## **1.2 Objective**

The main objective of this study is to optimize lignin precipitation and its recovery using different types of acids for acidification; and hemicelluloses separation using an organic solvent and antisolvents from kraft black liquor. Shortly, the specific objectives of this work are:

- To characterize the kraft black liquor through appropriate analytical techniques;
- To investigate more sustainable organic acids as alternative acidifying agents for lignin precipitation (compared with sulfuric acid which is currently used);
- To investigate the influence of some important process parameters, such as pH and temperature, on the separation efficiency and yield;
- To investigate a more sustainable organic solvent as an alternative for hemicellulose separation compared to dioxane;
- To qualitative and quantitative characterize the separated lignin and hemicelluloses using appropriate analytical techniques.

## **1.3 Organization of the dissertation**

The present thesis is divided into six chapters. The first includes the introduction where the scope and motivation of the work are discussed along with the objectives and structure of the thesis. The second chapter discusses the theoretical foundations associated with the subject, such as wood chemistry, the kraft pulping process, the black liquor recovery cycle and, the lignin isolation methods with special emphasis on precipitation.

The third chapter consists on a review of the recent literature related to the lignin and hemicelluloses precipitation processes, as well as, lignin valorization applications. In Chapter 4, the materials and methodology for experimental work and analytical techniques are described and the results are presented and discussed in Chapter 5. Finally, conclusions and some suggestions for future work are presented in Chapter 6.



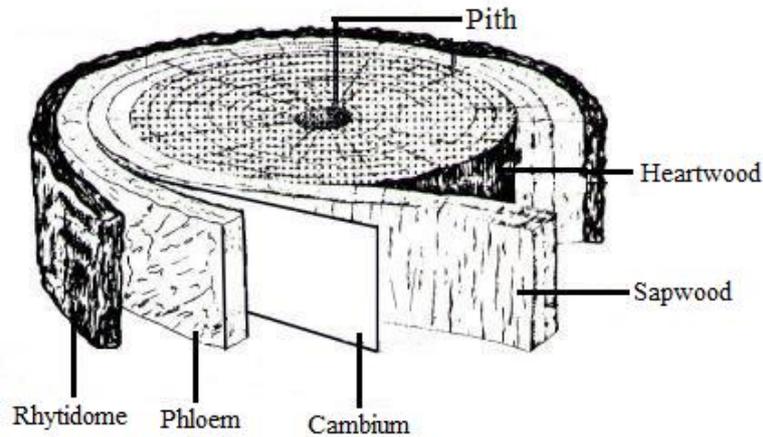
## 2. THEORETICAL BACKGROUND

### 2.1 Structure and Chemical Composition of Wood

Pulp & paper production uses cellulose as the main raw material. There are many types of plants and trees in nature with lignocellulose as the main component, however, depending on the type of tree, the wood presents different characteristics and compositions, which will affect the quality and specifications of the pulps obtained at the end of the cooking process. Therefore, it is necessary to have a good knowledge of the raw materials in the pulping production [8].

Regardless of the plant species, the cellular structure of wood is very diverse, which makes it a suitable raw material for the most diverse applications. For pulp production in the paper industry, the commonly used fibers derive from trees called gymnosperms and dicotyledonous angiosperms, better known as softwoods and hardwoods, respectively. The fibers of softwoods, usually called long fibers, are between 2 and 5 mm long, and hardwoods, also called short fibers, are between 0.5 and 2 mm long. Each type of fiber offers different characteristics for paper. In comparison with short fibers, long fibers have higher mechanical strengths but have lower printability and smoothness properties [3,9].

In Portugal, there is a large supply of the hardwood tree species *E. globulus*, from the *Eucalyptus* genus, which is the main raw material in the national pulp and paper industry. The wood from *E. globulus* has excellent papermaking properties, largely due to the quantity and quality of the fibers in its trunk [3,9]. Figure 1 shows the cross-section of a tree trunk where six types of tissues can be distinguished: rhytidome, phloem, cambium, sapwood, heartwood, and pith.



**Figure 1** - Cross section of a tree trunk. Adapted from [10].

Wood consists of about 50 % carbon, 43 % oxygen, 6 % hydrogen, and 1 % nitrogen, and these are distributed in high molecular weight and low molecular weight compounds. The first includes polysaccharides (hemicelluloses and cellulose) and lignin, while the latter include organic substances (extractives) and inorganic substances (ash) [3,9]. In Table 1, it is possible to see the chemical composition of hardwoods, softwoods, and the particular case of *E. globulus*, the species of interest [11].

**Table 1** - Chemical composition of hardwood, softwood and *E. globulus* [11].

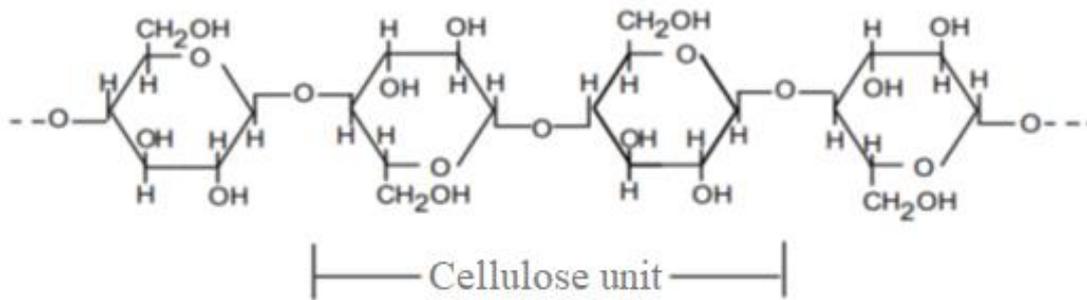
<b>Component</b>	<b>Hardwoods (%, w/w)</b>	<b>Softwoods (%, w/w)</b>	<b><i>E. globulus</i> (%, w/w)</b>
<b>Cellulose</b>	42-49	41-46	47-58
<b>Hemicelluloses</b>	23-34	25-32	14-22
<b>Lignin</b>	20-26	26-31	20-24
<b>Extractives</b>	3-8	10-25	1-3
<b>Ash</b>	0.2-0.8	0.2-0.4	0.3-1

### 2.1.1 Cellulose

Cellulose is the elemental structure of plant cells and is the most important natural substance distributed in all plants, from the most developed trees to the most primitive plants [8].

Cellulose constitutes almost half of the composition of wood and it is a linear, unbranched, homopolymer composed of anhydro-D-glucopyranose units joined together by  $\beta(1\rightarrow4)$  type glycosidic bonds. The repeating structural unit in the cellulose macromolecule is cellobiose, a disaccharide of glucose monomer. The molecules establish hydrogen bonds with each other, both intramolecular and intermolecular, and Van Der Waals forces, giving rise to a generally crystalline structure. The combination of several cellulose molecules results in structures called microfibrils composed of amorphous and crystalline zones. The degree of crystallinity influences the chemical and physical properties of fibers, and therefore the resulting pulp and paper [3,9].

The chemical formula of cellulose is  $C_{12}H_{22}O_{11}$ , where the average degree of polymerization, can vary with both wood and cellulose fibers, depending also on the species, wall layer, and nature of the cells [3,9]. The molecular structure of cellulose is represented in Figure 2.



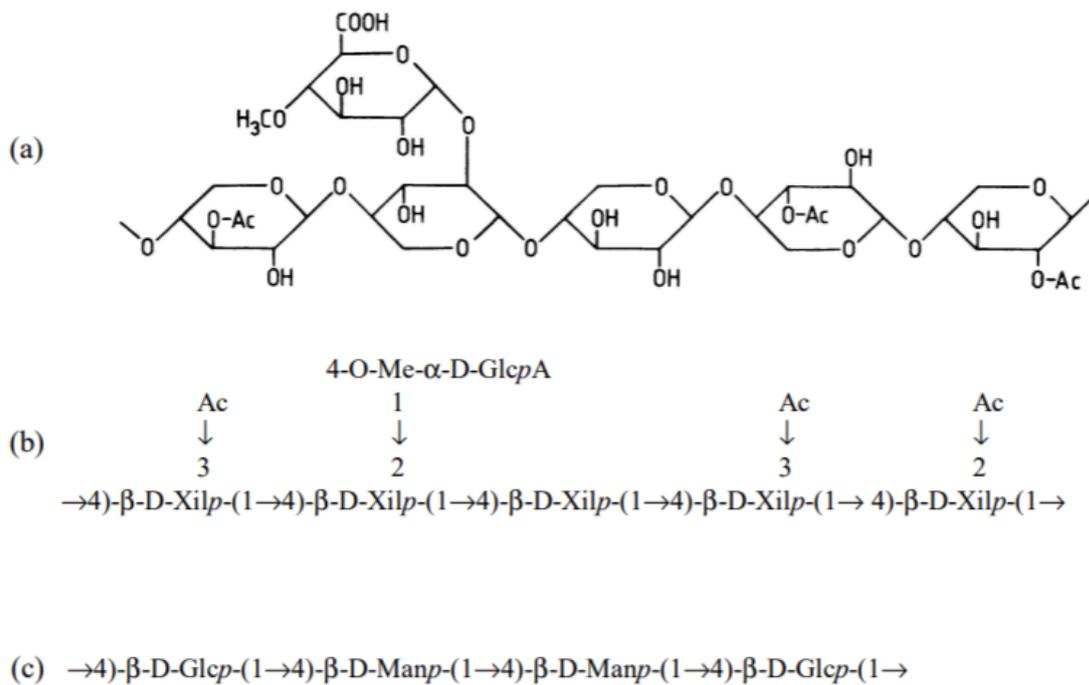
**Figure 2** - Cellulose molecular structure (central part of the chain) Adapted from [12].

### 2.1.2 Hemicelluloses

Hemicelluloses, as well as cellulose and pectins, belong to the structuring components of the cell walls of developed plants, where they are associated with proteins and phenolic compounds. Initially, it was thought that the role of hemicelluloses was to establish bonds between cellulose and lignin, but today it is thought that it functions as controllers of cellulose aggregation [8].

Hemicelluloses are macromolecules made up of sugar units, hexoses (D-glucose, D-mannose, or D-galactose), in hexosans, or pentoses (D-xylose or L-arabinose), in pentosans. These are linked to each other by glycosidic bonds and are distinct from cellulose due to the aforesaid units, their amorphous structure, and generally short branched chains [3,9].

The hemicelluloses amount, composition, degree of polymerization, and molar ratio varies among the different plant species depending on cell type and location in the cell wall. In coniferous trees, hemicelluloses present are arabinoglucuronoxylans and galactoglucomannans, in which the later shows a high preponderance. In hardwoods, the hemicelluloses (Figure 3 (a)) that mainly compose the total amount in wood are called xylans (pentosans are the most repeated structural unit is xylose) and glucomannans (hexosans that release glucose and mannose during hydrolysis). The predominant hemicelluloses is the glucuronoxylan (15 to 30 %), represented in Figure 3 (b), followed by the glucomannans (2 to 5 %), represented in Figure 3 (c) [3,9].

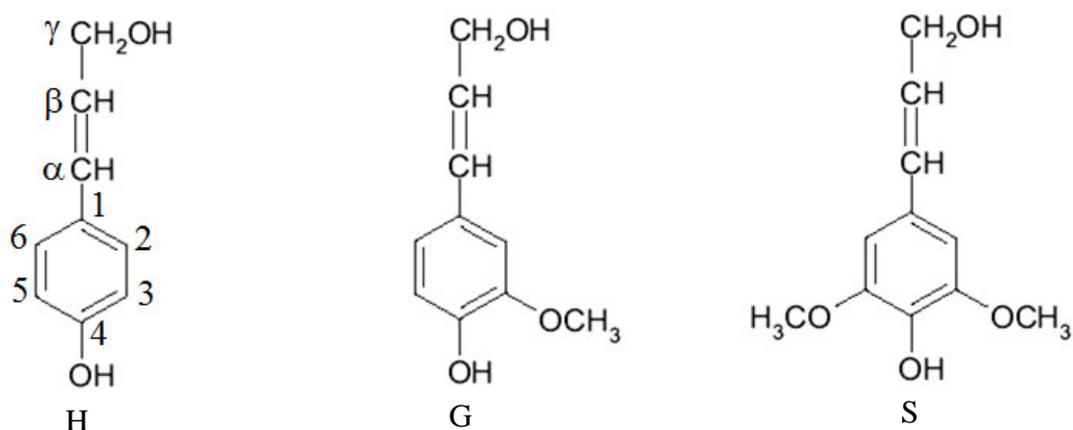


**Figure 3** – (a) General structure of hemicelluloses; (b) abbreviated formula of glucuronoxylan (O-acetyl-4-O-methylglucurono-β-D-xylan); (c) abbreviated formula of glucomannan. Xilp - xylopyranose, Ac - acetyl group, Me-GlcpA - 4-O-methyl-α-D-glucopyranose-uronic acid, Manp - mannopyranose, Glcp - glucopyranose, Ramp – raminopyranose. Adapted from [13].

### 2.1.3 Lignin

Lignin, after cellulose, is the most abundant and important polymeric component of plants. It provides mechanical strength to plant tissues and is responsible for the cohesion of the fibers in wood. Its composition in wood can vary from 20 - 40 % depending on the species [14,15].

Lignin is a considerably branched, complex, amorphous aromatic macromolecule consisting of phenylpropane units linked together primarily by ether bonds and by less abundant carbon-carbon bonds. It can be defined as a polyphenolic material resulting from the polymerization of its three precursors-p-coumaryl, coniferyl, and sinapyl alcohols (see Figure 4). The designation assigned to each atom of the phenylpropane unit is identified in Figure 4 [14,15].



**Figure 4** -Lignin precursors. H - p-coumaryl alcohol, G - coniferyl alcohol, S - sinapyl alcohol [8].

Hardwood lignin, designated as guaiacyl-seringyl lignin, or the G/S type, is composed by units gathered primarily from coniferyl and sinapyl alcohols with a varied composition in their ratios (40 – 80 % of S units, 20 - 60 % of G units, and 4 – 10 % of H units) [13,16,17].

The lignin of *E. globulus* shows some differences, at the structural level, that distinguish this tree from other hardwoods. The bonds involved in the lignins are generally of the β-O-4 type, the hardwoods with 50 – 60 % of these bonds and *Eucalyptus globulus* with 58 %, the ratio of non-condensed/condensed units is about 78/22, and the S:G:H ratio is approximately 84:15:1, i.e., the lignin of *E. globulus* is of the S type [18].

Lignin in softwoods are known as guaiacyl, or G- type, lignins, since they're derived mainly from coniferyl alcohol (95 – 98 % of G units and 2 - 5 % H units) [8].

Lignin is known to be occasionally linked to polysaccharides (lignin-carbohydrates complexes), mainly through arabinose, xylose, and galactose. It is the stability of these bonds that justifies the difficulty in removing residual lignin from the pulps [15,19,20].

The dominant bonds in hardwood and softwood lignin are  $\alpha$ -aryl ether, in phenolic units, and  $\beta$ -aryl ether, in phenolic and non-phenolic units, and it is through the breaking of these bonds that the degradation of lignin in the cooking liquor occurs. The  $\beta$ -O-4 bonds are the predominant bonds of both coniferous and hardwood lignin, being about 50 % of all coniferous lignin bonds and practically 60 % of all hardwood lignin bonds (see Table 2) [8].

**Table 2** - Types and abundances of the most important linkages in softwood and hardwood lignin [8].

<b>Bond Type</b>	<b>Softwood (%)</b>	<b>Hardwood (%)</b>
<b><math>\beta</math>-O-4</b>	45-50	50-60
<b><math>\alpha</math>-O-4</b>	6-8	6-8
<b><math>\beta</math>-5</b>	9-15	4-6
<b><math>\beta</math>-1</b>	2-3	5-15
<b>5-5</b>	10-11	2-5
<b>4-O-5</b>	2-4	2-3
<b><math>\beta</math>-<math>\beta</math></b>	2-4	3-7

### 2.1.4 Low molecular weight compounds

Besides cellulose, hemicelluloses, and lignin, there are smaller quantities of other substances in the wood, called low molecular weight compounds. These include both organic and inorganic compounds, the quantity of which depends on species, origin, and age of the plant [3].

The organic compounds include a variety of fatty acids and alcohols, polyphenolics, etc. Their quantities are determined based on their solubility in organic solvents, and so they are called extractives. The pulps with higher extractive content have low yields, with the particularity that they are soluble in liquors and claim higher consumption of reagents [3].

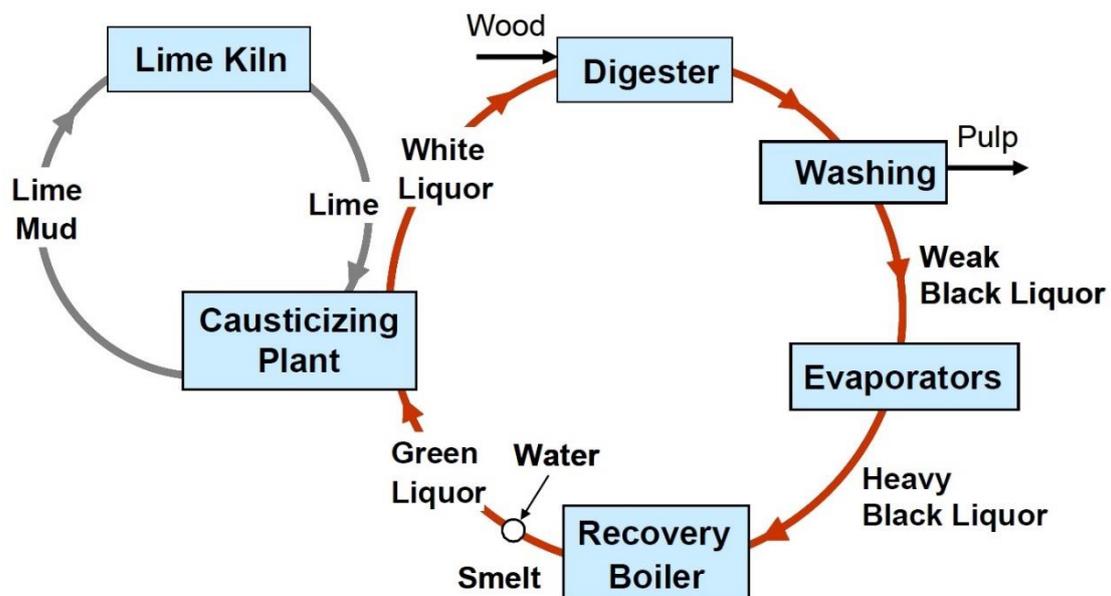
Finally, inorganic compounds have a truly small presence in wood, about 1 %, and are normally referred to as ash. These include calcium sulfates, phosphates, oxalates, carbonates, and silicates, as well as potassium and magnesium [3].

## 2.2 The Kraft Process

The main objective of cooking wood is the separation of cellulosic fibers and consequent removal of lignin. Several mechanical or chemical processes can be used for lignin removal and separation of cellulosic fibers. The chemical processes can be acid - sulfite process, or alkaline - soda process, and sulfate or kraft process [8].

The kraft process is worldwide, and especially at the national level, the most used pulping process for chemical pulp production. This process offers many advantages over other chemical processes such as being a flexible in the sense that it can be applied to a great variety of woods, it allows the recovery of the used pulping chemicals and it is performed for shorter cooking times, which results in preserving the characteristics of the wood fibers. In general, the papers produced by this process have better physical-mechanical properties [15].

The kraft process (Figure 5) has evolved a lot since when it was carried out in autoclave type digesters in a batch process. Nowadays, the cooking is carried out in continuous digesters, optimizing the dead times between discharges and fills in the batch digester [21].



**Figure 5** - Scheme of the kraft pulping process. Adapted from [22]

The continuous process allows to obtain better pulp quality with higher yields, to minimize the consumption of steam for heating, and to minimize the quantities of alkali for the same quantity of processed wood. This procedure is divided into several phases, such as feeding and pre-impregnation, heating and impregnation, pre-cooking, cooking, counter-current washing, and discharge. In the early 1980s, extended cookstoves that take advantage of the counter-current zone emerged. In addition to washing, they create conditions to extend the cooking, allowing more favorable conditions to be used in the upper part of the digester [21,23,24].

The cooking itself can be separated into several phases: the initial phase, where the heating period of the digester and the temperature reach values around 140 °C, the main phase, which includes the end of the heating period and the temperature plateau around 160-170 °C – phase where most of the delignification reactions occur – and the residual phase, the final cooking phase [15].

### **2.2.1 Pulp cycle**

The wood is received in the pulp industry in the form of logs, with or without bark, which is subsequently reduced to wood chips so that the reaction is easier and faster. These wood chips are exposed to a screening process to obtain similar sizes, resulting in a more uniform cooking. Wood chips with larger sizes give origin to badly separated fibers, called the uncooked ones, due to the poor impregnation of the cooking liquor in them. On the other hand, small wood chips that suffer total impregnation result in pulps with low yields and strengths [3,22].

The cooking stage aims at degrading the non-cellulosic constituents of wood, mainly lignin. The duration of this stage depends on the degree of delignification, which is related to the residual lignin content. The wood chips are cooked, normally up to 3 hours, with white liquor in a digester, under high pressure and temperature conditions (from 155 to 180 °C). The reagents that constitute the cooking liquor, or white liquor, are sodium hydroxide (NaOH) and sodium sulfide (Na<sub>2</sub>S), and small quantities of sodium salts (such as carbonate and sulfate) that come essentially from incomplete reactions of the black liquor recovery cycle [3,22,25,26].

Before entering the digester, the wood chips suffer vaporization where they are heated with the use of water vapor, approximately at atmospheric pressure, to remove the trapped air and to allow better and effective absorption of the liquor. Next, the wood chips

enter a high-pressure feeder, where the cooking liquor circulates, and are transported to the digester. The impregnation takes place for about 45 min at 130 °C, to facilitate the subsequent cooking, where the liquor heating takes place in external heat exchangers. Then the cooking zone follows, where the temperature is kept constant and approximately equal to the maximum of the heating zone. Once cooking is complete, the resulting black liquor is directed to the recovery cycle, through screens located on the perimeter of the digester. At the base of the digester, cold washing liquor is added, coming from the pulp washers, circulating in countercurrent in relation to the wood chips for greater efficiency. This step is preponderant to have good removal of the remaining dissolved solids in the pulp, otherwise, they interfere in the bleaching stage, in the paper production, and lead to increased prices in these processes. Thus, it is necessary to repeat the washing procedure several times [22,25,26].

The resulting brown-colored pulp is then bleached to achieve the standards of whiteness required. Afterward, the pulp is drained to eliminate excess water, pressed, and then dried. Finally, it is cut and packed to be used in papermaking [22,25,26].

### **2.2.2 Black liquor recovery cycle**

The kraft process includes a recovery cycle for the resulting liquid, called weak black liquor. In this, the inorganic chemicals are recovered and recirculated to the process, and the dissolved organics are used as fuel in the production of steam and electricity [27].

The weak black liquor from the digester is concentrated, up to 65 % solids, in multiple effect evaporators, so that it can be effectively burned in a recovery boiler. The inorganic part, consisting essentially of sodium sulfide ( $\text{Na}_2\text{S}$ ) and calcium carbonate ( $\text{CaCO}_3$ ), commonly known as smelt, enters a tank where it is dissolved in water, forming green liquor. This is sent for caustification resulting in sodium hydroxide from the reaction of calcium oxide or lime ( $\text{CaO}$ ) with calcium hydroxide ( $\text{Ca}(\text{OH})_2$ ). The precipitated calcium carbonate ( $\text{CaCO}_3$ ), commonly known as the lime mud, is separated through a clarifier, and then calcined to return to the process as a causticizing agent for the green liquor. In this way, the white liquor is regenerated and can be returned as feed to the digester [27,28].

## 2.3 Black Liquor

Black liquor is a very viscous liquid, and it is believed that the greater the amount of lignin and hemicelluloses present, the greater the viscosity, since these together form the most part of black liquor with bulky molecules [29]. It is obtained from the digester and pulp washing in the chemical pulping process. It is an aqueous solution consisting of organic residues such as lignin, polysaccharides, carboxylic acids, and extractives, as well as the spent cooking chemicals used in the process. The chemistry and properties of black liquor are directly correlated to these components. The organic materials in black liquor have a latent heat that can be recovered during combustion in a recovery boiler, with lignin being the major constituent. The chemical and elemental compositions of a typical kraft black liquor are shown in Table 3 and Table 4, respectively [30].

**Table 3** - Chemical species found in kraft black liquors from north American wood species) [31].

<b>Chemical species in kraft black liquor</b>	<b>Content (%)</b>
<b>Alkali lignin</b>	30-45
<b>Carbohydrate degradation products</b>	
<b>-Hydroxyl acids</b>	25-35
<b>-Acetic acid</b>	5
<b>-Formic acid</b>	3
<b>Extractives</b>	3-5
<b>Methanol</b>	1
<b>Sulphur</b>	3-5
<b>Sodium</b>	15-20

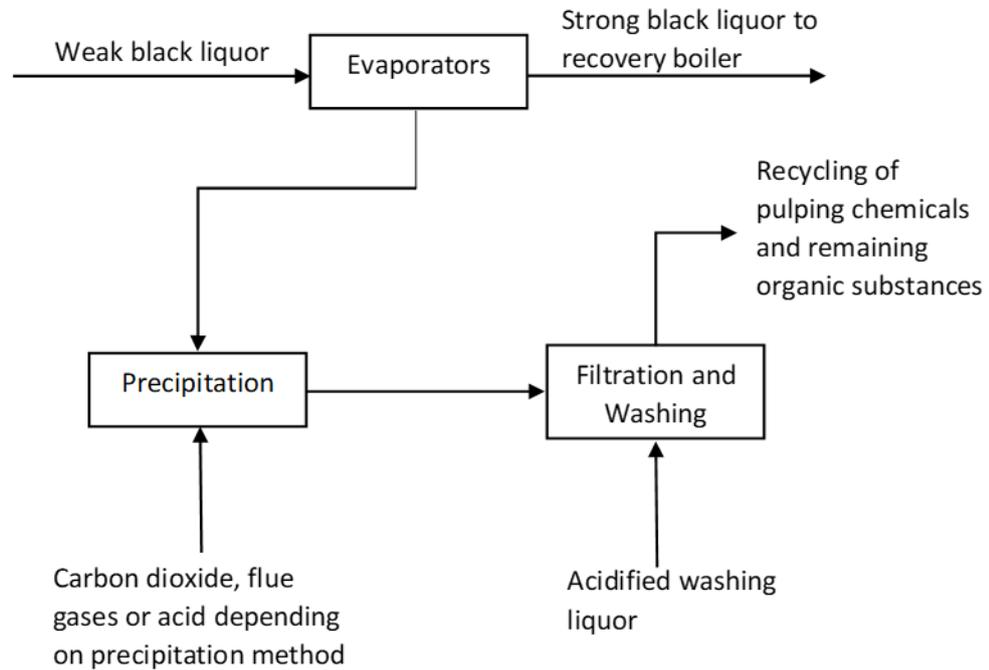
**Table 4** - Typical elemental composition of virgin black liquor from Scandinavian wood species [32].

Elemental Composition	Content (%)			
	Softwood (pine)		Hardwood (birch)	
	Typical	Range	Typical	Range
<b>Carbon</b>	35.0	32-37	32.5	31-35
<b>Hydrogen</b>	3.6	3.2-3.7	3.3	3.2-3.5
<b>Nitrogen</b>	0.07	0.04-0.11	0.1	0.14-0.2
<b>Oxygen</b>	33.9	33-36	35.5	33-37
<b>Sodium</b>	19.0	18-22	19.8	18-22
<b>Potassium</b>	2.2	1.5-2.5	2.0	1.5-2.5
<b>Sulphur</b>	5.5	4-7	6.0	4-7
<b>Chlorine</b>	0.5	0.1-0.8	0.5	0.1-0.8
<b>Inert</b>	0.2	0.1-0.3	0.2	0.1-0.3

## 2.4 Lignin and hemicelluloses Isolation Strategies and Applications

### 2.4.1 Lignin precipitation

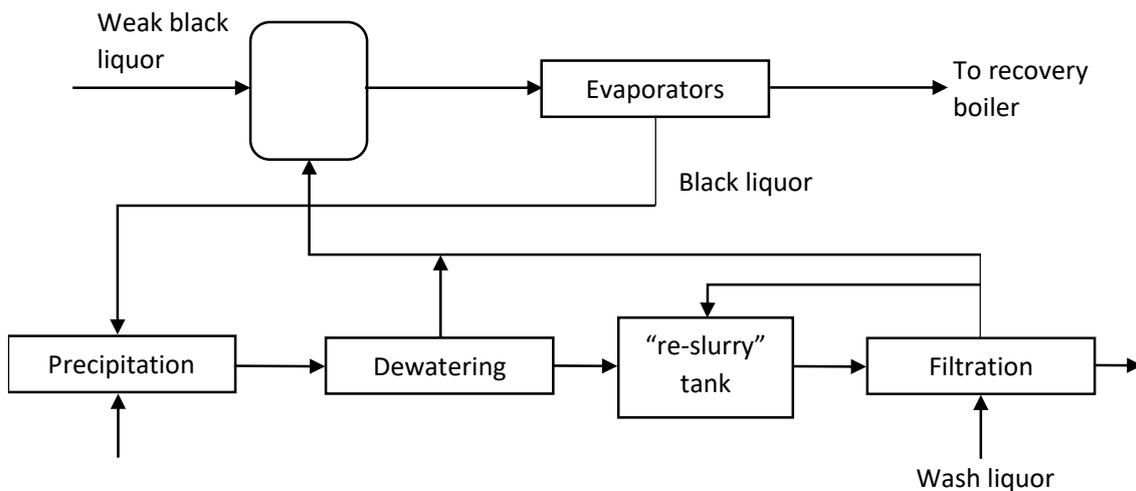
The most traditional and employed method for lignin isolation from black liquor is the acidification, forming lignin particles that can be later separated through filtration (see Figure 6). Normally, this method begins with the use of carbon dioxide to reduce the pH of the black liquor from 13 to 9, this is filtered and precipitates are washed with sulfuric acid and acidic water (pH ~ 2). However, there is a great difficulty in filtering the lignin from the acidified black liquor, due to the high size of the particles formed and consequent lengthy filtration flow. In addition, separated lignin, particularly the kraft lignin, still contains many impurities, such as carbohydrates and inorganics, which remove the possibility of it being a ready-to-use compound. Other disadvantages of this strategy are the high quantities of acid required and the formation of extremely unpleasant smelling compounds, with negative effects on human health and other forms of life [33].



**Figure 6** – Technical procedure for lignin extraction from black liquor precipitation.  
Adapted from [34].

Another obstacle in the use of kraft lignin is its intense dark color, which can restrict its application. This color is due to the presence of chromophore groups in its structure, which absorb light. The hypothesis to solve the problem would be bleaching with ozone and/or hydrogen peroxide, as it is done with pulp, but this would affect the final product [10].

As mentioned earlier, the precipitation of lignin by acidification of black liquor despite having a relatively low total solid content (TSC) and high ash content, also results in serious problems such as high levels of impurities in the lignin. Then, a new two-host washing/dewatering process called "*LignoBoost*" was developed to effectively separate the lignin from the black liquor (see Figure 7). Lignin with a low ash content but a high TSC content and to be produced on a large industrial scale [35].



**Figure 7** - Schematic diagram of the *LignoBoost* process adapted from [34].

In the *LignoBoost* process, a stream of black liquor is taken from the evaporation plant. The BL is then acidified at 60 to 80 °C and the lignin is precipitated. The *LignoBoost* process differs from the single-phase lignin separation process in that the lignin (filter cake) is re-dispersed in the re-slurry tank rather than being washed directly after filtration. When the lignin is re-dispersed, the pH is controlled to approximately the same as the final pH of the wash liquor, meaning it will be a pH in the range of 2 to 4. Therefore, the change in pH, and hence lignin solubility, takes place mainly in the re-dispersion tank and not in the final lignin washing stage. The resulting slurry is then filtered and washed by displacement washing [36].

The main advantages of the *LignoBoost* process can be considered to be higher lignin yield obtaining a Lignin with lower ash and carbohydrate content and higher TSC, lower investment costs due to reductions in filter area size and acid wash volume, and lower operating costs due to a reduction in the amount of H<sub>2</sub>SO<sub>4</sub> required [37].

Lignin has a variable reactivity due to its uncertain and irregular structure, something that becomes an obstacle in its application. Several attempts have been made to improve the reactivity of lignin as a substitute for phenol in resins, for example. One method is to modify the chemical structure of lignin to increase its reactive potential by inserting functional groups into its molecules. Other strategies such as reduction, oxidation, and hydrolysis have also been studied to improve the reactivity of lignin, as well as to produce phenolic compounds from it [38].

As for the effects of process variables on lignin precipitation, temperature, pH, and residence time on recovery stand out. The factor that has the most influence on lignin recovery through acidification is solution pH, followed by residence time and ultimately temperature, all of which have a positive correlation with recovery yield. Regarding pH, as it is reduced, the concentration of aliphatic hydroxyls in the purified lignin increases, the molecular weight decreases, and the polydispersity of the molecules increases. The same effect as the residence time increases. This means that lower molecular weight samples, which in turn will be lighter in color, are recovered under these conditions. Regarding temperature, as the temperature increases, the molecular weight increases, causing samples recovered at higher temperatures to be darker. Similarly, below pH 4, the concentration of alkene in the black liquor and ester bonds in the recovered lignin increased with decreasing pH, suggesting condensation and acid-catalyzed esterification reactions occur [39].

#### **2.4.2 Hemicelluloses precipitation**

During the final stage of cooking there is a decrease in the concentration of xylans in the cooking liquor and a slight increase of these in the pulp, thus decreasing the number of hemicelluloses recovered. This fact results from the higher degradation of xylans and/or their adsorption on the fibers. At the end of the cooking process, the pulp surface becomes richer in xylose. The surface layer of the kraft pulp fibers has a higher xylan composition than the inner layer, and the degree of substitution in uronic acids of the surface xylans is lower than that of the xylans in the innermost fiber layers. This may result either from the migration of xylans from the fiber interior to the exterior or from the precipitation of xylans. In addition to xylans, a small amount of glucomannan is also found, resulting from its precipitation on the fibers, although this is to a much lesser extent [15,40,41].

As a result of changes in the chemical composition of the fibers, the lowering of the alkalinity of the liquor at the end of cooking, and structural changes in the xylans, mainly their lower substitution, there is an increase of xylans on the fiber surface from partial adsorption of the xylans dissolved in the liquor. The substitution patterns and the degree of substitution of xylans are the factors that most influence their solubility in the solution [40,42–50].

Alkaline saponification of acetyl groups and uronic esters in xylans also contribute to their deposition on the fibers as a result of their partial crystallization and aggregation. The removal of the acidic groups from the xylan chain results in a decrease in the charge density. Thus, there is a decrease in electrostatic repulsion and a lower solubility and, consequently, a greater aptitude for xylan aggregation due to the greater ease of xylan interaction and association. Furthermore, after the removal of the substituent groups from the xylans, the affinity between xylans and cellulose increases. The xylans then have a stereochemistry that is more favorable for hydrogen bridging and are arranged to make these bonds, and they are most likely to adopt a chain conformation identical to that of cellulose. The precipitation of xylans is an irreversible process [19,49,51–56].

The degree of precipitation of xylan on fibers depends on a variety of parameters. The substrate on which adsorption studies are being carried out, as well as concentration, temperature, and reaction time, are important factors. The pH on the other hand has little influence, especially for relatively low values. Non-soluble xylans form agglomerates that adsorb to the pulp in larger quantities than soluble xylans. The latter change the surface morphology to a greater extent than the soluble xylans [46,51,54,55].

## **2.5 Opportunities for lignin valorization**

Biorefinery has become an increasingly attractive option in the pulp and paper industry due to the need and emergency for environmental protection, as well as for economic reasons. One scenario is the production of value-added products from the lignin generated during the pulping process, rather than using it as fuel. Despite being a compound with a high energy potential due to the presence of active groups, its applicability reaches an immense and varied range of products [57–59].

There are several types of industrial lignins, depending on the used separation processes, differing in terms of composition, reactivity, molecular weight, and structure. The most widely available is kraft lignin, presenting high quality making it the most versatile. Among the various products made from lignin, there are biodispersants, carbon fibers, polyurethane foams, resins, and thermoplastic materials. Table 5 is a summary of possible applications of lignin, depending on its type and purity level [57–59].

**Table 5** - Types of technical lignins, characteristics and their applications [57–59].

Parameter	Lignin type*				
	SL	KL	HL	OSL	LS
<b>Purity Level</b>	Very high	High	Low	High	Average
<b>Ash (%)</b>	0.7 - 2.3	0.5 - 3	4 - 9	7.5	5.8
<b>Carbohydrates (%)</b>	1.5 - 3	1 – 2.3	10 – 22.4	1 - 3	-
<b>Moisture (%)</b>	2.5 - 5	3 - 6	4 - 9	7.5	5.8
<b>Acid soluble lignin (%)</b>	1 - 11	1 – 4.9	2.9	1.9	-
<b>Nitrogen (%)</b>	0.2 - 1	0.05	0.5 – 1.4	0 - 0.3	0.02
<b>Sulphur (%)</b>	0	1 - 3	0 - 1	0	3.5 - 8
<b>Molecular weight (g/mol)</b>	1000 - 3000	1500 - 5000	5000 - 10000	500 - 5000	1000 - 50000
<b>Applications</b>	Carbon fibers, vanillin, phenol derivatives	Asphalt, refinery, cement additives, biofuel, activated carbon, phenolic resins, carbon fibers, vanillin, benzene, toluene, xylene and phenol	Energy and refinery	Activated carbon, phenolic resins, carbon fibers, vanillin, phenol derivatives	Refinery and cement additives

\*SL - Soda lignin; KL - Kraft lignin; HL - Lignin from hydrolysis; OSL - Organosolve lignin; LS - Lignin-sulfonates.

However, lignin isolation from the black liquor is still challenging due to its amorphous and robust structure. Thus, incorporation of lignin isolation processes into the pulp industry can make it a more viable and industrially attractive branch of industry [60].

### **3. STATE OF ART**

Lignin is an important renewable source due to its versatility in value-added product applications such as dispersants, emulsifiers, resins, adhesives. Although its isolation is quite challenging and complex due to its amorphous and robust structure. There are several studies with different proposals for this purpose in the literature. The kraft lignin is obtained from the sulfate (kraft) cooking process and this type of lignin corresponds to 85 % of the total global production of lignin [5,61–63].

#### **3.1. Methods for recovery of lignin and hemicelluloses from black liquor**

Regarding methods to extract lignin from black liquor, we have that the simplest and most used method is precipitation, through acidification (usually with sulfuric acid) of black liquor, forming lignin particles that can be separated. However, this separation can be complicated due to the larger size of some particles [5,62].

Sulfuric acid has been the acid of choice for lignin precipitation due to its low cost and non-introduction of any foreign elements since it is compatible with the pulp chemicals. Notwithstanding, a two-stage process showed better lignin yields after the precipitation process. This process involved precipitation by both carbon dioxide and sulfuric acid, where the black liquor was first treated with carbon dioxide to lower the pH to near 9, and finally with sulfuric acid to a pH in the range of 4 to 2. The approach was found to be more economically viable when compared to using sulfuric acid alone [64,65].

Although sulfuric acid contains elements that can be comparable to slurry chemicals, it can be argued that its use in an integrated biorefinery could disrupt the sulfur balance in the factory. In addition, the introduction of more sulfur could also be of environmental concern due to the release of dangerous gases such as SO<sub>2</sub> and/or H<sub>2</sub>S during the precipitation experiment. So, it is worth exploring other acids that could have a resembling precipitating effect without any adverse effects on the pulping process. For example, CO<sub>2</sub> has already been found to be a good precipitating agent up to certain pH limits [66–68].

Organic acids could also play a similar function while being able to overcome the limitations experienced using only CO<sub>2</sub>. There are some studies in the literature, where

authors have explored the use of organic acids, for the extraction of lignin from both wood and black liquor [69,70].

As organic acids, acetic acid was found to be a good precipitating agent for the lignin acquired from the hardwood / bamboo black liquor mixture. The lignin precipitated with acetic acid showed higher yields of lignin and better thermal stability for precipitation up to pH 9 when compared to  $H_3PO_4$ ,  $H_2SO_4$ , and HCl. The studies also demonstrate that an increase in the number of hydrogen atoms in the acid resulted in higher lignin yields [69].

Other types of organic acids such as oxalic and citric acids were also studied with acetic acid being used in the precipitation of lignin from bamboo soda black liquor, using sulfuric acid as a control. All the organic acids used showed lower yields than sulfuric acid, with citric acid being the most suitable precipitating agent among the three organic acids. The authors concluded that increasing the number of carboxylic groups in citric acid caused a complete electrophilic substitution reaction [70].

Another alternative to extract lignin from the black liquor is using selective solvents, both soluble and insoluble. Otherwise, for this alternative, at the industrial level, there must be a solvent recovery unit, since large quantities are required, which could make this hypothesis unfeasible [5,62].

A good option for extracting lignin from black liquor is electrolysis. In this system, we have an anode and a cathode separated from an ion exchange resin. In the anode section, there are generation of oxygen that cause a drop in pH and consequent formation of lignin. At the cathode, sodium hydroxide and hydrogen are produced. This method has been widely studied as an option in hydrogen production due to its competitive energy efficiencies. However, it has a disadvantage of high membrane start-up and difficulties in removing lignin from the system [5,71].

Finally, the most promising and efficient technology is that of membranes, with studies using several approaches. The most used method is ultrafiltration, through polymers or ceramic membranes, of which the goal is low retention of lignin and high retention for high molecular weight hemicelluloses. Through this type of process, the molecular weight control of the lignin fraction is possible, depending on the MWCO (molecular weight cut-off) values, and these range from 1 to 400 kDa, achieving recovered lignin with high purity [5,72].

Although the previously described methods such as precipitation, ultrafiltration (using membranes), and electrochemical methods are simpler methods of lignin recovery,

these are not currently of industrial relevance. As such, it has that the methods used at the industrial level are, from the oldest to the most recent, WestVaco, LignoBoost, LignoForce, and Sequential Liquid-Lignin Recovery and Purification (SLRP).

Table 6 gives an overview of lignin isolation processes on industrial and pilot scales. WestVaco is the longest running process. The two processes of LignoBoost and the LignoForce System™, both have inserted an additional process step to improve the filterability of the precipitated lignin, and the processing of pure lignin is also claimed. These two processes are also operated at an industrial scale. The SLRP process, there is no industrial-sized application is currently known [73].

**Table 6** - Overview of the lignin isolation processes on industrial and pilot scale.

<b>Lignin Isolation Process</b>	<b>Operating conditions</b>			<b>Performance</b>		<b>Ref</b>
	<b>TSC (%)</b>	<b>Acid type</b>	<b>pH</b>	<b>Temperature (°C)</b>	<b>Yield (%)</b>	
<b>WestVaco</b>	20 – 40	CO <sub>2</sub>	9	71 – 93	~70	[73,74]
<b>LignoBoost</b>	20 – 40	CO <sub>2</sub> + H <sub>2</sub> SO <sub>4</sub>	13.5 - 10	60 - 80	~70	[35,75]
<b>LignoForce</b>	20 – 40	CO <sub>2</sub> + H <sub>2</sub> SO <sub>4</sub>	13 – 10	80 - 90	~70	[57]
<b>SLRP</b>	20 – 40	CO <sub>2</sub> + H <sub>2</sub> SO <sub>4</sub>	9 – 10	n.d.	~55	[76]

Regarding the WestVaco method, Kienberger et al., 2021 [73] state that the operating conditions translate as the use of CO<sub>2</sub> as a form of acidification of the black liquor, being carried out at a temperature in the range of 71 to 93 °C with a pH of 9. Years later, Ball et al., 1962 [77] claimed a continuous process using sulfuric acid as an acidifying agent in combination with a black liquor which leads to the creation of new types of methods since it leads to high yields of precipitated lignin.

For the LignoBoost process the authors Öhman et al., 2007 [75] and Zhu, 2015 [35] describe as the operating conditions a temperature of 60 to 80 °C, a pH in the range of 13.5 to 10 with acidification using CO<sub>2</sub>. This method has a filter cake, consisting of precipitated lignin, residual lignin lean liquor, inorganic salts, and hemicelluloses, and it is then necessary to use H<sub>2</sub>SO<sub>4</sub> until a pH between 2 and 4 is reached. The difference presented from the LignoBoost process to LignoForce is described by the author Kouisni et al., 2012 [57] this being the introduction of two stages, a filtration stage, and a black liquor oxidation stage are introduced before the precipitation of lignin

Finally, the newer process, SLRP, is described by Lake et al., 2014 [76] as being a process that operates at elevated temperature and pressure, unlike the WestVaco, LignoBoost, and LignoForce processes, which leads to the formation of lignin in the liquid state.

Regarding the methods of extracting hemicelluloses from black liquor, the method described by some authors, using black liquor precipitated polysaccharides (BLPS), is the commonly used one, through acidification of black liquor, usually with an organic solvent (e.g., tetrahydrofuran (THF) or dioxane) and an organic acid (e.g., acetic acid), forming hemicelluloses particles that can be centrifuged [7,78].

Acidic dioxane/water solution have been reported to extract the hemicelluloses with high yield/purity. Nevertheless, the toxicity of acid dioxane does not satisfy the principles of green chemistry. Therefore, sustainable alternatives are being sought, such as cyclopentyl methyl ether (CPME), which is a hydrophobic ether solvent with a high boiling point, obtained from renewable resources, and offers benefits such as safer handling [79,80].

## **3.2. Valorization of technical lignins and hemicelluloses**

### **3.2.1. Lignin as a source of materials**

The earlier development of lignin as valuable material saw lignin being used as a reinforcement for rubber materials, because of its low density and lighter color, which brings about lighter colored rubber materials [81–83].

In one of the more interesting studies, lignin was modified into nano-lignin, and co-precipitation of the colloidal lignin cationic polyelectrolyte complexes and rubber latex resulted in the successful preparation of lignin/rubber nanocomposites [84].

The incorporation of lignin into polyolefins has also been shown to give these polymers with enhanced performance [85,86]. Lignin has been prepared for conversion into carbon fibers with thermal spinning by hydrocracking, phenolate, or hydrogenolysis. Kadla et al., 2002 [87], reported that they were the first to produce lignin-based carbon fibers from commercial hardwood kraft lignin utilizing the thermal extrusion process. The carbon fibers produced were found to have suitable properties for general performance rating.

### **3.2.2. Lignin as a source of fuel**

Due to its complexity, lignin needs to be depolymerized to unlock its full chemical potential. Several methods of depolymerization have been studied, and these include enzymatic, thermal, or catalytic degradation [88].

Depolymerized lignin would allow for its use in different valorization products, such as fuel or as fuel additives and chemical precursors. Currently, the pulp and paper industry already utilize lignin as a fuel through the combustion of the process liquor in the recovery unit operations to produce electricity. However, this method of lignin utilization has fallen out of favor due to the foreseen under-exploitation of such a rich carbon source. Therefore, it is important to find alternative methods of modifying lignin into fuel. For example, a well-established method such as gasification could be a way for lignin to be converted into syngas [6].

Lignin-derived syngas has the potential to produce green fuel which has and still is advancing technologies in this field. Syngas also has the potential to produce mixed alcohols and high-value chemicals. Pyrolysis is also another method that has been extensively researched to produce lignin bio-oils [89–91].

### **3.2.3. Lignin as a source of chemicals**

Pollutants removal has in recent years been at the forefront of many discussions for the moderation of effects to the environment. Raw lignin has been successfully utilized as heavy metal adsorbent, where it was found to have a high affinity for heavy metals with considerably higher adsorption capacities [92]. It was also observed that lignin contains two sites of adsorption, the carboxylic- and phenolic-type surface group, where the latter was observed to have a higher affinity for metal ions [93]. Lignin-epoxy compounds can also be applied for metal adsorption, as shown by Gabaldón et al., 2000 [94].

### **3.2.4. Lignin as a source of activated carbon**

In general, activated carbon is known as the oldest adsorbents, due to their very porous structure possessing high internal surface area. As adsorbents, activated carbon has been used mainly in the removal of a variety of contaminants, both organic and inorganic [95–97].

Studies on lignocellulosic materials have shown that the lignin component is the main source of coal formation during the process of producing activated carbon [98–100]. The hemicelluloses and cellulose components are volatile fractions [98]. Hence, lignin is a good precursor for activated carbon.

Activated carbon produced from lignin as a raw material have been extensively studied [101–104]. It was also reported by the authors that there was an increased development of microporosity, as well as micropore widening in the activated carbons. In addition to the widening of the micropores, CO<sub>2</sub> was also responsible for the opening of the blocked micropores as well as the creation of new ones [101].

Reactivity studies of the gasification reaction showed that the reaction proceeds as a combination of catalyzed and uncatalyzed reactions [103]. The catalyzed reactions were reported at higher conversions, where it was proposed that the distribution of sodium on the lignin matrix caused this effect, while at lower conversions during the gasification reaction the uncatalyzed reactions occur.

Chemical activation has been shown to have higher surface area activated carbon compared with those from physical activation [104–106]. The surface areas of chemically activated carbon were found to be affected by an increase in temperature (350 – 500 °C). Activation with alkali metals hydroxides is preferable to ZnCl<sub>2</sub>, as the latter was found to be toxic [105].

### **3.2.5. Hemicelluloses as a source in biorefineries**

The depletion of natural fossil fuels has increased the interest to valorize lignocellulosic biomass to power biorefineries. The term “biorefinery” encompasses biofuels such as biodiesel and biochar, energy and heat [107,108]. Production of cereal by product-based bioethanol is cost-effective and provides a clean alternative, but has not been industrialized [107]. On the other hand, the production of biogas is mostly from cellulose and hemicellulose fractions of biomass, such as cereal straw [109]. The ideal method to efficiently valorize cereal straws for biorefineries purposes is organosolv fractionation where the cereal straws are treated with mixtures of water with organic solvents such as acetone or acids [110]. Biochar is also gaining attention as it can replace the existing active carbon and enhance soil structure by increasing soil productivity such as increasing soil’s water holding capacity [111]. Caban et al., 2019 [112], confirmed that the surface of cereal-based biochar is full of polar moieties which are beneficial to remove

polar pollutants. The method to create biochar is straightforward and impure fractions of hemicellulose can be used. The steps involved pre-treatment of the cereal grains and straws with tap water and distilled water, followed by oven drying for 24 h. Finally, the cereal grains and straws were burned at 650 °C in a furnace for an hour [112].

### **3.2.6. Hemicelluloses as a source of nano-materials**

Sarker et al., 2020 [113], utilized chemically modified wheat bran (by alkaline extraction followed by ethanol precipitation and centrifugation) to develop functional nano-materials for nucleic acid delivery in plants. Ngu et al., 2016 [114], converted rice husk into fluorescent carbon nanoparticles using a thermally-assisted carbonization technique under high acidic conditions using sulfuric acid. According to the authors, the work found that the water solution of the carbon nanoparticles can emit very bright blue luminescence at 439 nm, and the higher the concentration of Sn(II) ions in the carbon nanoparticles, the lower the fluorescence capacity.



## 4. MATERIALS AND METHODS

### 4.1. Materials

#### 4.1.1. Reagents

Table 7 shows all chemical reagents used with their chemical formula and supplier.

**Table 7** - Reagents used, supplier and their chemical formula.

<b>Name</b>	<b>Purity (%)</b>	<b>Chemical formula</b>	<b>Supplier</b>
<b>Acetic acid</b>	99-100	CH <sub>3</sub> COOH	<i>Chem-Lab</i>
<b>Acetone</b>	99.8	C <sub>3</sub> H <sub>6</sub> O	<i>Fisher Chemical</i>
<b>Citric acid</b>	99-100	C <sub>6</sub> H <sub>8</sub> O <sub>7</sub>	<i>LabChem</i>
<b>CPME</b>	-	C <sub>6</sub> H <sub>12</sub> O	<i>Sigma-Aldrich</i>
<b>Dioxane</b>	-	C <sub>4</sub> H <sub>8</sub> O <sub>2</sub>	<i>Sigma-Aldrich</i>
<b>Ethanol</b>	99.9	C <sub>2</sub> H <sub>5</sub> OH	<i>Carlo Erba Reagents</i>
<b>Hydrochloric acid</b>	37	H <sub>2</sub> O:HCl	<i>Fisher Chemical</i>
<b>Lactic acid</b>	80	C <sub>3</sub> H <sub>6</sub> O <sub>3</sub>	<i>Labkem</i>
<b>Phenol</b>	99.5	C <sub>6</sub> H <sub>5</sub> OH	<i>ITW Reagents</i>
<b>Phosphoric acid</b>	85	H <sub>3</sub> PO <sub>4</sub>	<i>Panreac</i>
<b>Sulfuric acid</b>	95-97	H <sub>2</sub> SO <sub>4</sub>	<i>Honeywell</i>

#### 4.1.2. Black Liquor

The industrial kraft black liquor used in this study, whose raw material is *Eucalyptus globulus*, came from a paper mill, located in Central Portugal. This black liquor is called weak since it was collected before entering the evaporation stage. It was also used a black liquor obtained from the ultrafiltration, called retentate, through the use of membranes where the concentration of lignin in this liquor is higher than the weak black liquor. These were kept in the refrigerator until all the experiments planned for the development of the work had been carried out.

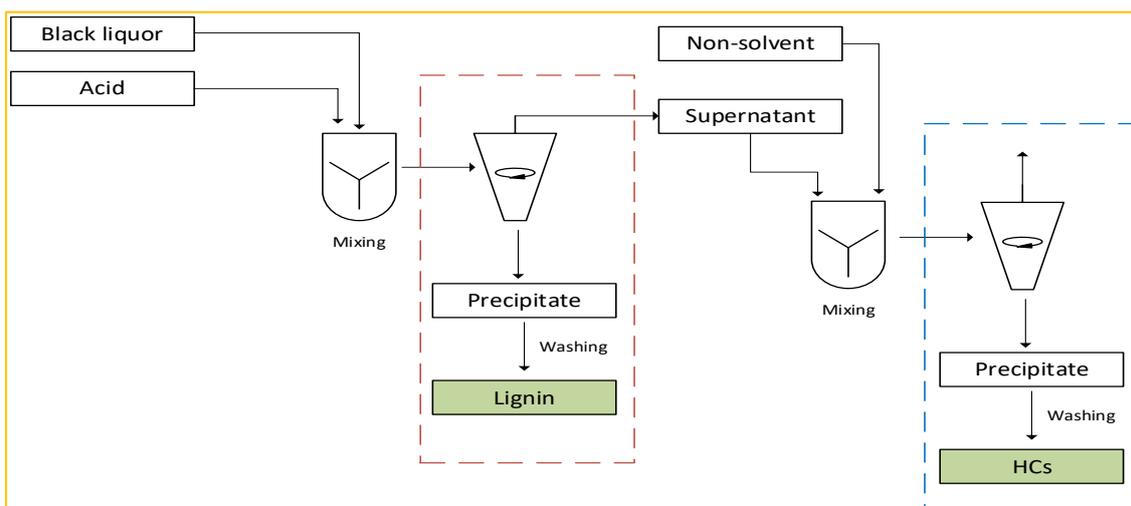
## 4.2. Methods

### 4.2.1. Precipitation and isolation of lignin

The lignin content in the black liquor was determined utilizing UV-Vis spectroscopy at a wavelength equal to 296 nm (see Figure 8),

Using an initial volume of black liquor (initial pH  $\approx$  13) on the order of 50 mL, lignin was precipitated, using different acids, such as lactic, acetic and phosphoric acid, until a pH of 3.5 - 4.5. When the target pH was reached, the washing step was performed using acidified water, with a pH of 3 to 3.5. The washing step was performed 3 times where a centrifugation for 15 min at 6000 rpm was used. After washing, the samples were left to dry in an oven, they were crushed, weighed, and left once again in an oven so that the samples were completely dry, after which the samples were collected and placed in properly labeled flasks. The same sample conditions were run twice in order to be able to make a comparison between them.

From the obtained samples, 25 mg were diluted in 4 mL of sodium hydroxide. Then, in a goblet, 100  $\mu$ L of each sample and 400  $\mu$ L of NaOH 0.5 M was added. Afterwards, in another goblet, 150  $\mu$ L of each sample and 2850  $\mu$ L of NaOH 0.5 M were added. But if the sample presented a light initial coloration, the dilutions to be performed will have a lower total factor, that is, in one goblet there will be 300  $\mu$ L of each sample plus 2700  $\mu$ L of NaOH 0.5 M, this being the first dilution to be done. In another goblet, there will be 1500  $\mu$ L of each sample obtained from the first dilution plus 1500  $\mu$ L of NaOH 0.5 M in order to obtain the final diluted sample. The absorbance of these samples was measured in a quartz cell. The absorbance values were converted to concentrations using the lignin calibration curve, performed with standard solutions, see Figure A1 (Appendix A).



**Figure 8** - Lignin separation process.

#### 4.2.2. Precipitation and isolation of hemicelluloses

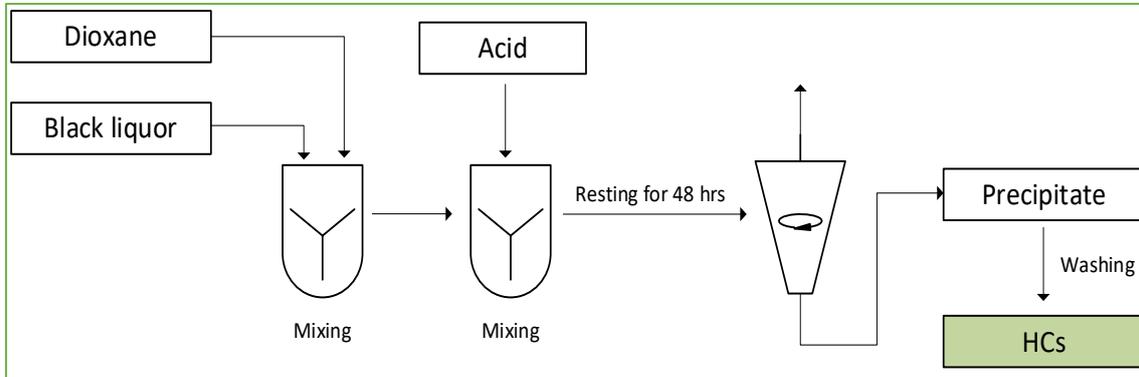
##### Quantification of hemicelluloses content

The hemicelluloses content of the black liquor was determined using the phenol-sulfuric colorimetric method (see Figure 9).

First, 100 mL of a black liquor (initial pH  $\approx$  13) were evaporated to approximately 50 mL. Then, 100 mL of CPME were added and the mixture was precipitated until it reached a pH of 2-3, using different acids, such as lactic, acetic and phosphoric acid, and left for approximately 48 h in the refrigerator. After this step the precipitate was washed with four liquids, first with CPME and water, second with CPME followed by ethanol and acetone. Each washing step of the precipitate (performed 3 times) was performed by centrifugating the samples for 15 min at 6000 rpm. After washing, the samples were left to dry, then crushed and the mass was registered, the samples were again placed in an oven until constant weight was achieved and finally placed in properly labeled flasks. The same sample conditions were only checked once due to the limited volume of the available CPME.

From the obtained samples, 25 mg were diluted in 4 mL of sodium hydroxide. After that, 500  $\mu$ L of each sample and 500  $\mu$ L of distilled water was added in a goblet. Then, in a test tube was added 50  $\mu$ L of each sample, 450  $\mu$ L of distilled water and 250  $\mu$ L of phenol. The samples were shaken in a vortex. Then, 1.5 mL of sulfuric acid was added, shaken again, and placed in a bath with stirring at 30  $^{\circ}$ C for 30 min. The blank

sample consisted of 500  $\mu\text{L}$  of distilled water, along with the same amounts of the previously mentioned reagents. Finally, absorbance was measured by setting the wavelength at 480 nm on a Spectrophotometer UV-VIS. Each sample measured had three replicates, and the absorbance values were converted into concentrations, through the calibration curve performed for hemicelluloses, see Figure A2 (Appendix A).



**Figure 9** - Hemicelluloses separation process.

#### 4.2.3. Characterization of extracted lignin and hemicelluloses

The generic equations used in the calculation of both yield and purity are shown in equations 1 and 2:

$$\text{Yield} = \frac{M_{\text{psi},j}}{M_{\text{psBLi},j}} * 100 \quad (1)$$

The yield was calculated as the mass that precipitated in the sample over the original mass (i: lignin or j: hemicelluloses) in the black liquor sample, in percentage.  $M_{\text{psi},j}$  is the mass that precipitated (i: lignin, j: hemicelluloses) in the sample, in g, and  $M_{\text{psBLi},j}$  the original mass (i: lignin, j: hemicelluloses) in the black liquor sample, in g.

$$\text{Purity} = \frac{M_{\text{ps}}}{M_{\text{psBLi}} + M_{\text{psBLj}}} * 100 \quad (2)$$

Purity was calculated as the mass that precipitated in the sample over the mass that precipitated (i: lignin and j: hemicelluloses) in the black liquor sample. Where  $M_{\text{ps}}$  is the mass that precipitated in the sample, in g;  $M_{\text{psBLi}}$  is the mass of lignin in the black liquor sample that precipitated, in g and  $M_{\text{psBLj}}$  is the mass that precipitated of hemicelluloses in the black liquor sample, in g.

## Method for the determination of purity

As for the equations used to determine the purity of lignin, inorganic content (IC) must be calculated first in order to know the organic content (OC) since this is directly related to the purity of lignin. So, the mass of inorganics was determined according to equation 3.

$$IC = M3 - M1 \quad (3)$$

Where IC is the organic content, in g, M3 is the mass of crucible plus sample after being incinerated, in g, and M1 is the mass of crucibles in g.

The mass of inorganics was then converted to percentage using equation 5, because the purity must to be obtained in percentage. Through a direct relationship between the inorganics and organics, were obtain the total organic content (equation 6) which translates the purity (equation 7).

$$TIC = \frac{IC}{M2 - M1} * 100 \quad (5)$$

$$TOC = 100 - TIC \quad (6)$$

$$\text{Purity} = TOC \quad (7)$$

Where TIC is the total inorganic content, in percentage, M2 is the mass of the crucible plus the mass of the sample before incineration, in g, M1 is the mass of the crucibles, in g, and TOC is the total organic content, in percentage, and sample purity, in percentage.

Regarding the purity of hemicelluloses, this is obtained by HPLC where the compounds present in the precipitated sample were analyzed, such as xylose, arabinose, or uronic acids. Using equation 8 [115], can be obtained the concentration of hemicelluloses (polymeric form).

$$\text{Hemicelluloses} = 0,88 * X + 0,88 * A + 0,72 * A + UA \quad (8)$$

Where Hemicelluloses is the concentration of compounds present in the sample, in g/L, X the amount of xylose present in the sample, in g/L, A the amount of arabinose present in the sample, in g/L, AA the amount of acetic acid present in the sample, in g/L, and UA the amount of uronic acids (for example glucuronic or galacturonic acids) present in the sample, in g/L.

Then to determine the purity, the hemicelluloses value is divided by the stock solution concentration (CS) as shown in equation 9:

$$\text{Purity} = \frac{\text{Hemicelluloses}}{\text{CS}} * 100 \quad (9)$$

Where the purity of the sample obtained is in percentage, hemicelluloses is the concentration of compounds present in the sample, in g/L, and CS is the concentration of the stock solution, in g/L.

### **Method for the determination of yield**

As for the equations used to determine the yield, first, the total solid content was calculated using equation 10:

$$\text{TSC} = \frac{\text{Ms}}{\text{Vi BL}} * 100 \quad (10)$$

Where TSC is the total solid content, a Ms the final sample mass obtained, and Vi BL the initial volume of black liquor.

The value of the total solid content multiplied by the purity obtained previously, gives the concentration of lignin or hemicelluloses present in the sample (equation 11).

$$\text{Cps } i,j = \frac{\text{TSC} * \text{Purity}}{100} \quad (11)$$

Where CPS *i,j* is the contraction of either *i* - lignin - or *j* - hemicelluloses - in the final sample, the TSC is the total solid content and purity of the sample obtained, in percentage.

With this value and considering that 1 L is used as reference,  $C_{psi,j}$  is equal to  $M_{ps i,j}$ . With this, all the variables present in equation 1 can be obtained and it is possible to determine the yield.

## Density

The density of the black liquor was determined using a pycnometer, the first step was to weigh its mass. As reference, distilled water was used and the pycnometer was filled with this liquid, weighed and the same procedure was repeated for the black liquor. Both densities were measured for the liquids at a temperature of 21 °C ( $\rho_{water} = 998,02$  g/L) and the density of the black liquor was calculated according to equation 12.

$$\rho_{BL} = \frac{(M3p - M1p)}{(M2p - M1p)} \times \rho_{water} \quad (12)$$

Where  $\rho_{BL}$  is the density of the black liquor, in g/L,  $M1p$  is the pycnometer mass, in g,  $M2p$  is the pycnometer mass with distilled water, in g,  $M3p$  is the pycnometer mass with black liquor, in g, and  $\rho_{water}$  is the water density, in g/L.

## 4.2.4. Characterization of samples by FTIR, HPLC and NMR measurements

### Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR-ATR analysis was performed for the lignin and hemicelluloses precipitates to identify the existing functional groups. For this purpose, a FT/IR-4200 equipment from JASCO was used for an analysis region of 4000 to 550  $cm^{-1}$ , resolution of 4  $cm^{-1}$ , and 128 scans.

### High-performance Liquid Chromatography (HPLC)

The HPLC analysis was performed for hemicelluloses precipitates and some lignin samples to analyze the concentration of different polysaccharides. For sample preparation, the BL samples were acid hydrolyzed with 6 %  $H_2SO_4$ , followed by autoclaving them for 2.5 h at 105 °C. Afterwards, samples were neutralized to a pH range,

suitable for equipment using CaCO<sub>3</sub> powder, and filtered before injection to the HPLC column.

The equipment used in the analysis was a High-performance liquid chromatographer (HPLC, Knauer), equipped with a refractive index (RI) detector and a Rezex ROA-Organic acid H<sup>+</sup> column with the respective guard column (Phenomenex). The operating conditions in all HPLC analyses were, injection volume of 20 μL, the flow rate of 0.6 mL/min, column temperature of 40 °C, and the detector was at room temperature. The mobile phase used in the HPLC analyses was ultrasonicated H<sub>2</sub>SO<sub>4</sub> solution previously filtered.

### **Nuclear Magnetic Resonance (NMR)**

The samples collected were subjected to NMR analyses, which is performed by the Nuclear Magnetic Resonance Laboratory of the Chemistry Department of Coimbra. From this analysis, is obtained a spectrum for each sample, from which the sample composition, degree of polymerization, and molecular weight can be determined.

The analysis will be applied to the nuclei of the <sup>1</sup>H atoms present in the molecules, acquiring a spectrum for each sample. Each peak presented in the spectrum corresponds to a hydrogen atom, each having a corresponding value. After determining the peaks corresponding to each chemical species, it's possible to quantify the composition of the sample by peak area. For this analysis, the samples were dissolved in Deuterium Oxide, D<sub>2</sub>O, and Sodium deuterioxide, Na(OD). The quantification was performed using the MestReNova<sup>®</sup> software.

#### **4.2.5. Statistical analysis**

Lignin and hemicelluloses optimization was carried out using the software JMP<sup>®</sup> Pro software in order to determine the best operating conditions.

The effect of two operating variables (temperature and pH) at three levels and one variable (acid type) at two levels, on the removal of lignin was examined according to a 3x3x2 full-factorial design of experiments (DOE) (see Table 8).

**Table 8** - Factors and levels used in the experimental design of lignin isolated using organic acids.

<b>Factors</b>	<b>Level</b>		
	1	2	3
<b>Temperature</b>	30	45	60
<b>pH</b>	3.5	4.0	4.5
<b>Acid type</b>	AA	LA	-

The effect of two operating variables (lignin content and supernatant acetone ratio) on the removal of hemicelluloses using acetone as solvent, was examined according to a  $2^2$  (two factors at two levels) full-factorial design of experiments (DOE), including two replicates at central point (see Table 9)

**Table 9** – Factors and levels used in the experimental design of hemicelluloses isolated using acetone.

<b>Factors</b>	<b>Level</b>		
	-1	0	+1
<b>Lignin content (%)</b>	67.3	71.3	84.5
<b>Supernatant acetone ratio (%)</b>	50	90	130

The effect of three operating variables (temperature, pH and CPME/BL) on the removal of hemicelluloses using CPME as solvent, was examined according to a  $2^3$  (three factors at two levels) full-factorial design of experiments (DOE), including two replicates at central point (see Table 10).

**Table 10** – Factors and levels used in the experimental design of hemicelluloses isolated using CPME.

<b>Factors</b>	<b>Level</b>		
	-1	0	+1
<b>Temperature</b> (°C)	30	45	60
<b>pH</b>	2.6	2.9	3.2
<b>CPME/BL</b>	1	1.5	2

## 5. RESULTS AND DISCUSSION

In this section, firstly was intended to select the organic acid that gives the best yield to carry out the recovery of lignin and hemicelluloses by precipitation, and then optimize this recovery using the best organic acid.

### 5.1. Screening of organic acids suitable for precipitation of lignin and hemicelluloses

For isolation of lignin samples different organic acids were tested (see Table 11), and the best acids were screened based on the composition of extract in terms of yield and purity. The method of analysis of lignin samples is described in chapter 4. The formulations were prepared in duplicate.

**Table 11** - Experimental conditions and chemical characteristics of lignin obtained after extraction from different acids.

Acid used	pH	Volume of acid used (mL)	TSC (g/L)	TOC (g/L)	Purity (%)	Lignin Yield (%)
SA	3.21	2.00	55.88	52.90	94.67±0.05	63.47±2.32
SA+AA	3.26	1.50+5.00	56.58	52.73	93.20±0.25	65.05±2.75
PA	4.30	3.00	46.90	45.26	96.51±0.24	49.92±1.41
CA	4.63	5.04*	37.70	36.75	97.48±0.29	39.37±0.65
AA	4.63	6.00	43.78	41.88	95.67±0.25	54.03±1.47
LA	4.55	5.50	54.76	49.82	90.98±0.24	53.69±3.35

\*CA was used in powder form so instead of volume its content is reported in mass (mg).

From Table 11, it is possible to notice that concerning lignin precipitation by both strong and weak acids, strong acids, namely sulfuric acid (SA) and the mixture of sulfuric acid and acetic acid (SA+AA) have a higher difference between total solid content (TSC) and total organic content (TOC), which means that it has a lower organic content when compared to that of weak acids such as phosphoric acid (PA), citric acid (CA), and acetic acid (AA). The difference between using only SA or SA+AA is not significant in terms

of yield, which means that using only SA will be better. Finally, the acids with the best TSC yield are SA+AA followed by SA, PA, and lastly, AA.

For isolation of hemicelluloses samples, the performance of different organic acids along with the green solvent, CPME, were analyzed. The experimental conditions, type of acid and the characteristics of separated hemicelluloses samples is presented in Table 12. The preliminary study for the selection of organic acid was performed on the weak black liquor sample. But the optimization studies for hemicelluloses separation were performed over concentrated black liquor, which was collected from ultrafiltration of weak black liquor to concentrate the hemicelluloses in the retentate stream. This concentrated liquor is referred as UF retentate.

**Table 12** - Experimental conditions and chemical characteristics of hemicelluloses obtained after extraction from different acids.

<b>Acid used</b>	<b>pH</b>	<b>Volume of acid used (mL)</b>	<b>TSC (g/L)</b>	<b>TOC (g/L)</b>	<b>Purity (%)</b>	<b>Hemicelluloses Yield (%)</b>
<b>CPME+LA</b>	2.52	65.00	3.75	3.16	29.03	7.99
<b>CPME+HA</b>	2.26	27.75	3.47	3.23	8.78	2.24
<b>CPME+PA</b>	2.37	24.60	11.97	10.95	7.36	6.47
<b>CPME+AA</b>	2.86	125.00	7.31	6.05	40.72	21.84

Analyzing Table 12, it can be seen that although phosphoric acid (PA) has the best values for total solid content, TSC (12 g/L), and total organic content, TOC (11 g/L), the purity obtained is very low, approximately 7 %, which leads one to believe that the final precipitated content contains many impurities, which could be lignin or inorganic.

It can also be seen that there is a tendency in the increase of the yield of the samples the higher the volume of acid used, this happens because the final precipitate is less thick, making it easier to centrifuge which leads to that the purity of the sample also increasing, that is why the acetic acid (AA) is the one with the highest purity (41 %) and highest yield (22 %).

## 5.2. Optimization of lignin and hemicelluloses recoveries from black liquor

### 5.2.1. Lignin Precipitation

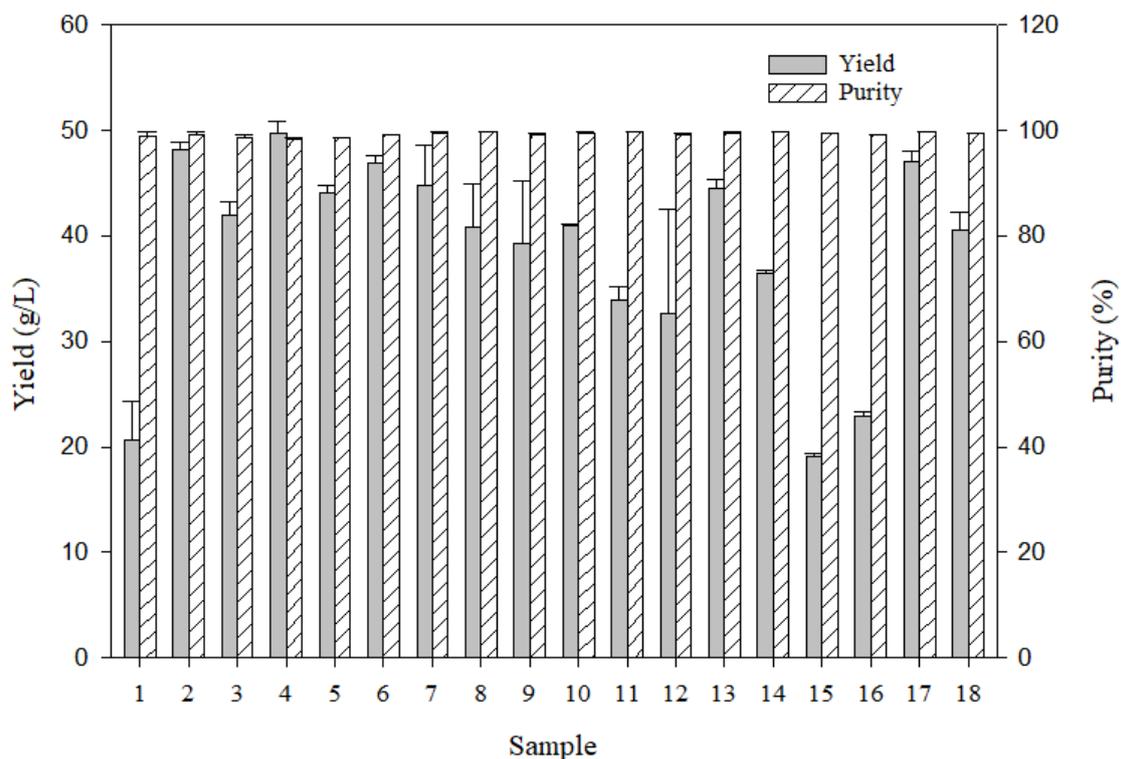
Precipitation of lignin from BL was performed using the factors temperature, pH, and type of acid.

Figure 10 identifies itself as a Pareto chart. It is pictured as a simple bar chart that ranks the related measures in descending order of occurrence. The purpose of a Pareto diagram is to separate the significant aspects of a problem from the trivial ones. This type of Pareto analysis is used to find out which category occurs most frequently, in this work, the three variables considered show good significance, with the type of acid having the most influence followed by pH, and finally temperature [116].

Source	LogWorth		PValue
Acid type	4,463		0,00003
pH*Acid type	4,455		0,00004
pH(3,5,4,5)	3,254		0,00056
Temperature(30,60)	2,634		0,00232
Temperature*Acid type*pH	0,501		0,31555
Temperature*Acid type	0,293		0,50887
Temperature*pH	0,262		0,54663

**Figure 10** - Summary of the effect of independent variables and their interactions.

The yield and purity values obtained for the precipitated lignin can be seen in Figure 11. It can be noticed that the purity remains constant, and the determining factor for choosing the optimal conditions must be the yield. As the purity has values close to 100 %, the hemicelluloses present in the samples will not be significant and it was considered that the organic content is mainly lignin.



**Figure 11** - Yield and purity for lignin samples isolated using AA and LA.

The best yields, around 48 %, for the lignin recovery were achieved with lactic acid in the samples 2 ( $T = 45\text{ }^{\circ}\text{C}$ ;  $\text{pH} = 3.5$ ), 4 ( $T = 30\text{ }^{\circ}\text{C}$ ;  $\text{pH} = 3.5$ ) and 17 ( $T = 45\text{ }^{\circ}\text{C}$ ;  $\text{pH} = 4.5$ ). Therefore, it can be concluded that the optimal conditions for precipitation are: temperature  $\approx 45\text{ }^{\circ}\text{C}$ ,  $\text{pH} \approx 3.5$  and use of lactic acid, see also Table B1 (Appendix B).

The lignin precipitate and supernatant obtained after centrifugation is shown in the Figure 12.



**Figure 12 - Lignin precipitate.**

### **5.2.2. Hemicelluloses Precipitation**

The precipitation of hemicelluloses from UF retentate by two different approaches (a) using acetone as antisolvent and (b) using acetic acid in combination with CPME.

#### **a) Precipitation of HC using acetone**

To analyze the precipitation of hemicelluloses with acetone, two parameters were taken into account, (a) lignin content in the used supernatant and (b) ratio of acetone to the supernatant. The results are shown in Table 13.

**Table 13** - Values of response variables obtained in the experimental design for acetone.

Experiments number	Lignin content (%)	Supernatant acetone ratio (%)	Response	
			Yield (%)	Purity (%)
AHC 1	67.3	90	44.26	43.56
AHC 2	71.3	130	43.75	33.35
AHC 3	71.3	50	32.54	41.11
AHC 4	67.3	90	44.23	41.31
AHC 5	84.5	130	54.37	38.17
AHC 6	84.5	50	43.88	38.74

Analyzing Table 13, it was found that there is a maximum level of yield in the sample AHC (hemicellulose sample isolated with acetone) 5 around 54 % however it is the sample with the lowest level of purity, around 38 %, despite being the sample where the initial lignin content is the highest, around 85 %. The parameter that makes this sample not viable is the ratio of acetone in the supernatant since it is quite high (130 %), which implies a high use of acetone.

Then, the best samples are AHC 1 and AHC 4 where despite reaching a yield around 44 %, these obtained a purity percentage between 41 and 44 % where only the lignin content in the initial sample is 67.3 % and the supernatant to acetone ratio is lower (90 %).

#### **b) Precipitation of HC using green solvent (CPME)**

To analyze the precipitation of hemicelluloses with acetone three parameters were taken into account, temperature, pH and black liquor to solvent ratio.

For comparison purposes, precipitation of hemicelluloses with dioxane was also performed since the literature only mentions the use of this solvent in the precipitation and isolation of hemicelluloses. This was performed under the following conditions: dioxane/BL ratio of 2, the temperature of 60 °C, and pH of 2.6 [7,117].

The optimal conditions were those obtained in experiment 10 (see Table 14), where the best yield and purity values were obtained under the following conditions: temperature of 60 °C, pH of 2.6 and CPME/BL ratio of 2.

**Table 14** - Values of response variables obtained in the experimental design for the CPME.

Experiments number	Pattern	Response	
		Yield (%)	Purity (%)
CPME 1	+ - -	43.51	48.81
CPME 2	0 0 0	17.62	31.53
CPME 3	0 0 0	20.49	29.02
CPME 4	- + +	54.23	44.46
CPME 5	+ + +	50.87	56.38
CPME 6	---+	36.48	38.85
CPME 7	---	40.72	47.80
CPME 8	++-	27.03	69.11
CPME 9	-+-	44.05	52.16
CPME 10	+++	57.71	68.09

Finally, through a comparative analysis between the yield and purity of the solvents, dioxane and CPME, under the same operating conditions, in terms of yield and purity, CPME obtained better values being 58 and 46 % and dioxane obtained values of 68 and 41 %, respectively. It can be said that CPME is a good alternative to dioxane since under the same conditions it obtained better results and it is also not harmful to humans, as previously mentioned [118].

The hemicelluloses precipitate and supernatant obtained after centrifugation is shown in the Figure 13.



**Figure 13** - Hemicelluloses precipitate.

### **5.3. Chemical composition**

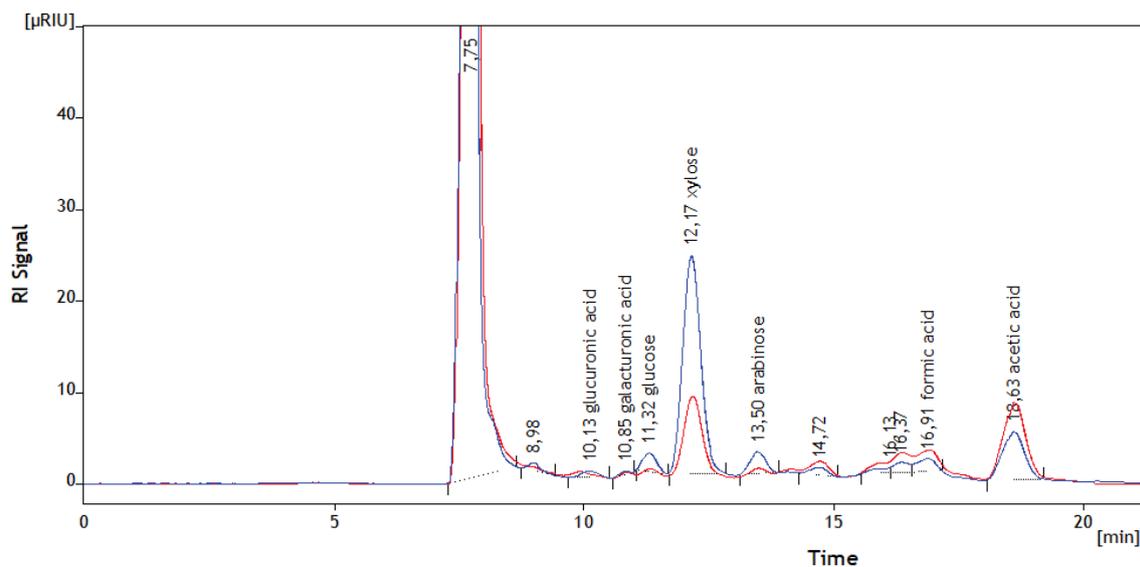
#### **5.3.1. High Performance (pressure) Liquid Chromatography (HPLC) analysis**

The purity of precipitated hemicelluloses samples was evaluated considering the concentration of arabinose, xylose, uronic acid, acetic acid and formic acid. The total amount of hemicelluloses was determined after anhydro corrections of 0.88 for pentose and 0.72 for the acetyl group and 0.9 for hexose. The quantification method is described in chapter 4.

First, the tests were performed on the type of black liquor used in this work, the weak black liquor and the concentrated black liquor (UF retentate) (see Figure 14). After obtaining these curves for comparison, tests were performed on the precipitates using dioxane, CPME and acetone, and finally, tests were chosen on four precipitated lignin samples, two with the higher purity value for both acids, acetic and lactic acid, and two with the lowest purity value. The purpose of this selection was to find out the difference

in percentage in terms of lignin obtained after precipitation since this technology allows us to know the composition of the samples.

As for the hemicelluloses samples, the selected samples were those that contained the best degree of purity for the three solvents used. This technology will allow us to check which of the solvents precipitates the highest amount of hemicelluloses.



**Figure 14** - HPLC spectra for weak black liquor (red line) and UF Retentate (blue line).

Analyzing the spectra, it can be seen that the retentate spectrum has better characteristics for use in hemicelluloses precipitation, since the xylose peak has a higher intensity in this spectrum, which allows the conclusion that isolated hemicelluloses have a higher level of purity. It is then from the ultrafiltration retentate that the study of hemicelluloses isolation will be treated in this dissertation.

In Table 15, the three samples analyzed are that of dioxane, CPME, and acetone. These samples were the ones selected in this analysis since they present the best values in terms of yield and purity.

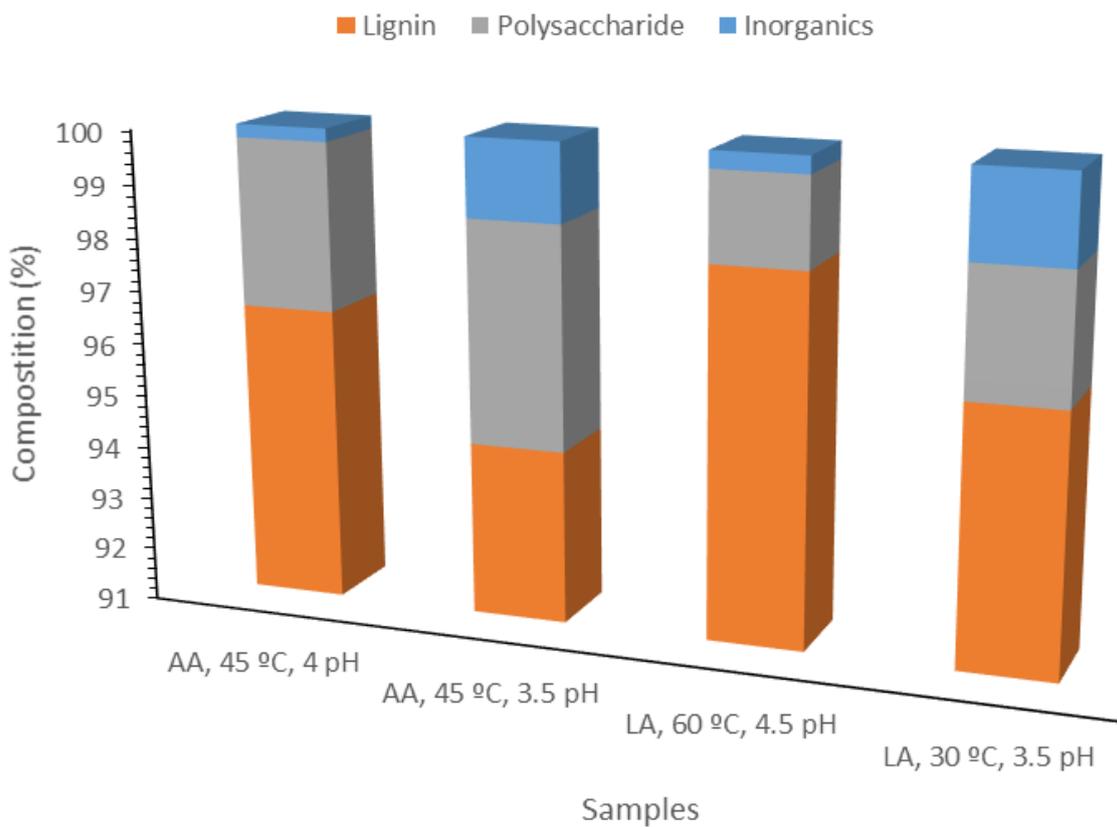
**Table 15** - Composition of compounds of recovered hemicelluloses using dioxane, CPME and acetone through HPLC.

Compounds	Amount (%)		
	Dioxane	CPME	Acetone
<b>Xylose</b>	75.7	77.3	65.2
<b>Glucose</b>	8.8	7.8	7.7
<b>Cellobiose</b>	3.8	2.2	0
<b>Glucuronic acid</b>	2.5	2.2	2.5
<b>Galacturonic acid</b>	1.2	0.8	2.9
<b>Arabinose</b>	3.2	3.7	4.3
<b>Formic acid</b>	3.3	2.8	0
<b>Acetic acid</b>	1.5	3.2	17.4

By direct observation of Table 15, it can be stated that they all have the highest concentration of xylose because the black liquor came from the eucalyptus-based pulp, and the CPME, with a percentage of approximately 77 %, is the solvent where the hemicelluloses recovery was the highest. There are other relevant compounds such as uronic, glucuronic, and galacturonic acids, and also the presence of arabinose, glucose, acetic acid, and formic acid that are in line with the compounds visible in the spectrum of the black liquor shown in Figure 14, so it can be claimed that the isolated hemicelluloses were obtained in the final precipitate.

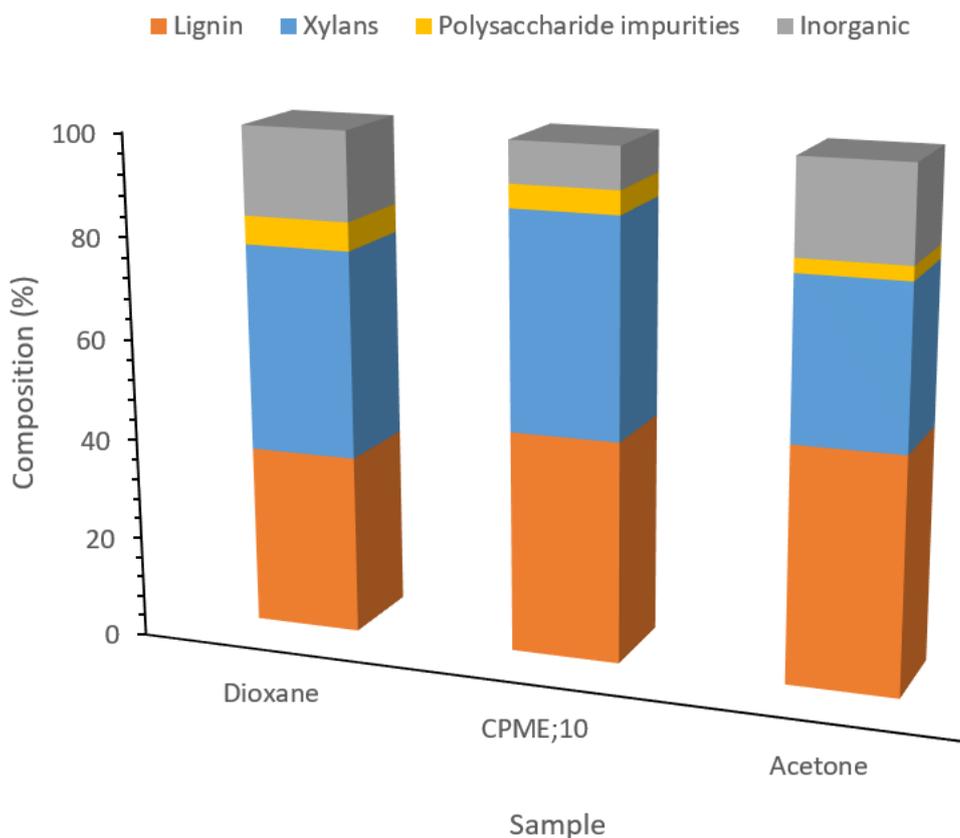
The composition of lignin samples in terms of inorganic, lignin, polysaccharides and impurities is shown in Figure 15. The polysaccharide impurities are reported considering all sugars detected in HPLC (see Figure 14).

By direct observation of Figure 15, the samples with the highest amount of lignin are AA, 45 °C, 4 pH and LA, 60 °C, 4.5 pH with around 98 and 97 %, respectively. It is also visible that the AA samples have a higher percentage of polysaccharides, since these are associated with the formation of formic acid due to the use of acetic acid in the lignin precipitation. It can then be concluded that the acid with the highest percentage in lignin isolation is lactic acid.



**Figure 15** – Organic and inorganic composition analyzed by HPLC for isolated lignin.

From the analysis of Figure 16, the organic fraction (xylan, lignin, and polysaccharide impurities) is the one with the biggest percentage in the precipitation and isolation of hemicelluloses samples when using different solvents.



**Figure 16** - Composition of isolated hemicelluloses samples analyzed by HPLC.

Comparing the solvents to evaluate the one that presents the best performance for the isolation of hemicelluloses, it can be observed that the CPME solvent led to better results where an organic fraction of the order of 92 % was obtained with approximately 43 % of xylans. The worst result was obtained with acetone, where an organic content around 82 % was quantified, with only about 32 % of xylans. Thus, CPME presents good arguments to be treated as a good alternative to dioxane. This test also showed that some xylans were composed of polysaccharide impurities that can be identified as other sugars, such as glucose or cellobiose.

## **5.4. Fourier transform infrared spectroscopy – attenuated total reflectance (FTIR–ATR) analysis**

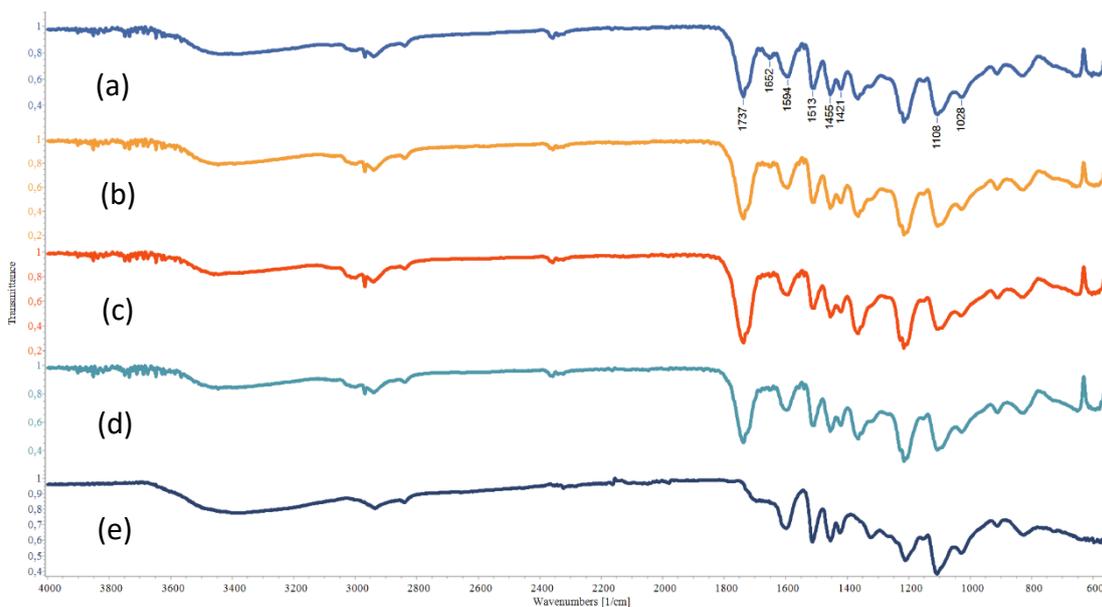
Lignin and hemicelluloses characterization was performed using the FTIR-ATR method, as described in chapter 4.

### **5.4.1. Lignin characterization**

The samples chosen in the lignin isolation are two using acetic acid with lower (a) and higher percentage of purity (b), other two using lactic acid also with lower (c) and higher percentage of purity (d) and other with commercial lignin (e).

As shown in Figure 17, the FTIR spectra of the different samples show peaks in the same zone and after comparison with literature values of lignin spectra and with the (e) spectra of commercial lignin, there are five peaks as being the most important, and that allows us to conclude that the precipitated lignin had a good quality because when compared to commercial lignin, the peaks of the five spectra are comparable.

The peaks located at 1737 and 1652  $\text{cm}^{-1}$  are assigned to the C=O stretching not conjugated to the aromatic ring; around at 1594 and 1513  $\text{cm}^{-1}$  correspond to aromatic skeleton vibrations; around 1455 and 1421  $\text{cm}^{-1}$  are assigned to C-H deformation vibrations. The band corresponding to the aromatic skeletal vibration is found around 1108; Last but not least, all samples show a vibration band at 1028 that can be assigned to the C-O deformation belonging to the primary alcohols [119].



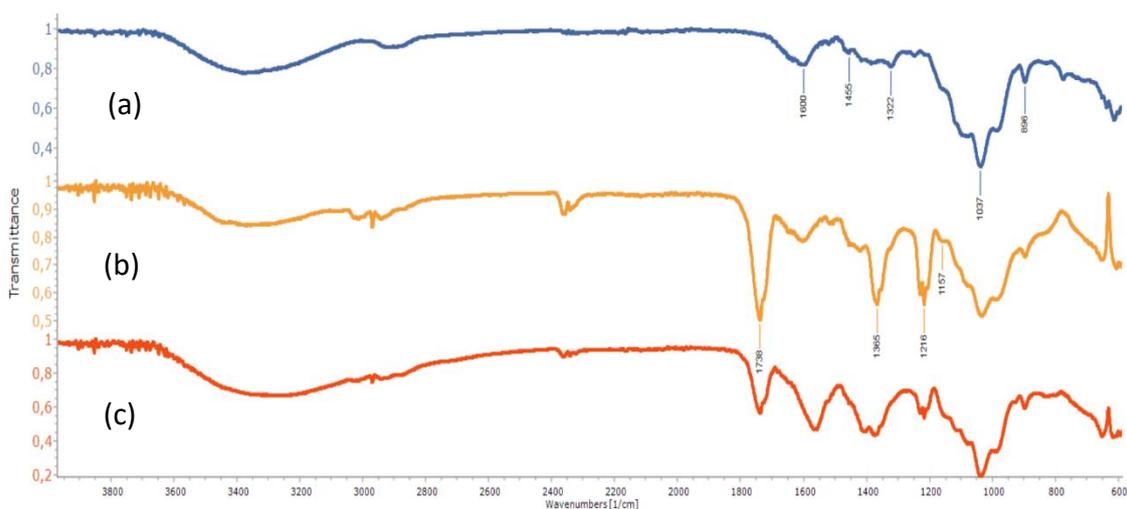
**Figure 17** - FTIR spectra for lignin precipitated using acetic acid (a,b) and lactic acid (c,d) with purity of (a) 98.5 %, (b) 99 %, (c) 98.8 % and (d) 99.7 % and commercial lignin (e).

#### 5.4.2. Hemicelluloses characterization

The samples chosen in the isolation of hemicelluloses are dioxane (a), CPME (b) and acetone (c).

As shown in Figure 18, all the spectra showed the same pattern and were similar to those of typical hemicelluloses, indicating a similar structure of the polymers [117,120].

These are the prominent band at  $1037\text{ cm}^{-1}$ , which is uniquely assigned to C–O, C–C stretching, or C–OH bending in xylan, the band at  $1365\text{ cm}^{-1}$ , is mainly ascribed to the in-plane bending vibrations of the  $\text{CH}_2$  groups and the third peak is in the band of  $1157\text{ cm}^{-1}$ , is mainly due to stretching of C–O bonds. The  $1455\text{ cm}^{-1}$  band is mainly ascribed to the in-plane bending vibrations of the  $\text{CH}_2$  groups. The  $895\text{ cm}^{-1}$  band is associated with  $\beta$ -glycosidic bonding between xylose units (typical for xylan). As for the  $1600$  and  $1738\text{ cm}^{-1}$  bands these relate to carbonyl stretching of glucuronic acid and acetyl groups, respectively. The transmittances at  $1322$  and  $1216\text{ cm}^{-1}$  are also associated with hemicelluloses groups [117,120].



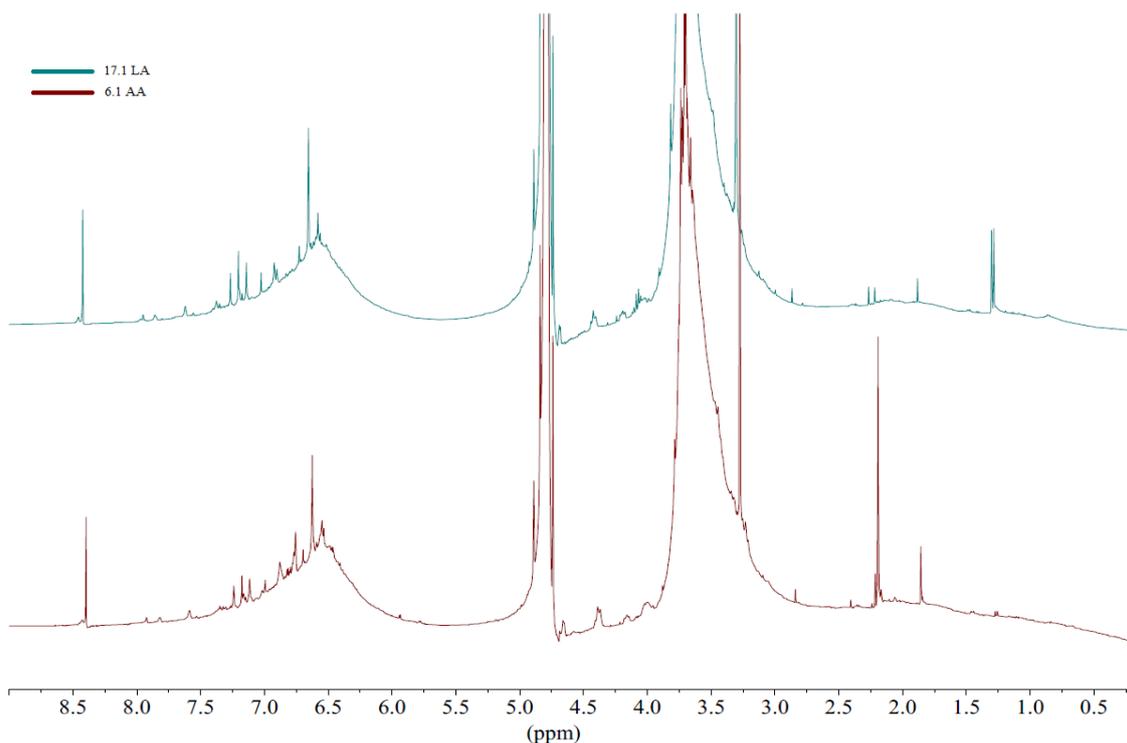
**Figure 18** - FTIR spectra for hemicelluloses precipitated using: (a) dioxane; (b) CPME; (c) acetone.

Considering data from the literature, can be concluded that isolated hemicelluloses did indeed precipitate. As the purity only reaches values close to 50 %, some peaks are relatable to the lignin spectrum, which indicates that there was also lignin in the final precipitate.

## 5.5. Nuclear Magnetic Resonance (NMR) analysis

### 5.5.1. NMR spectrum for lignin samples

For this type of test two samples were also analyzed (see Figure 19), differing in the type of acid used in the precipitation of lignin, one with acetic acid (AA), 6.1, the other where the acid used was lactic acid (LA), 17.1.



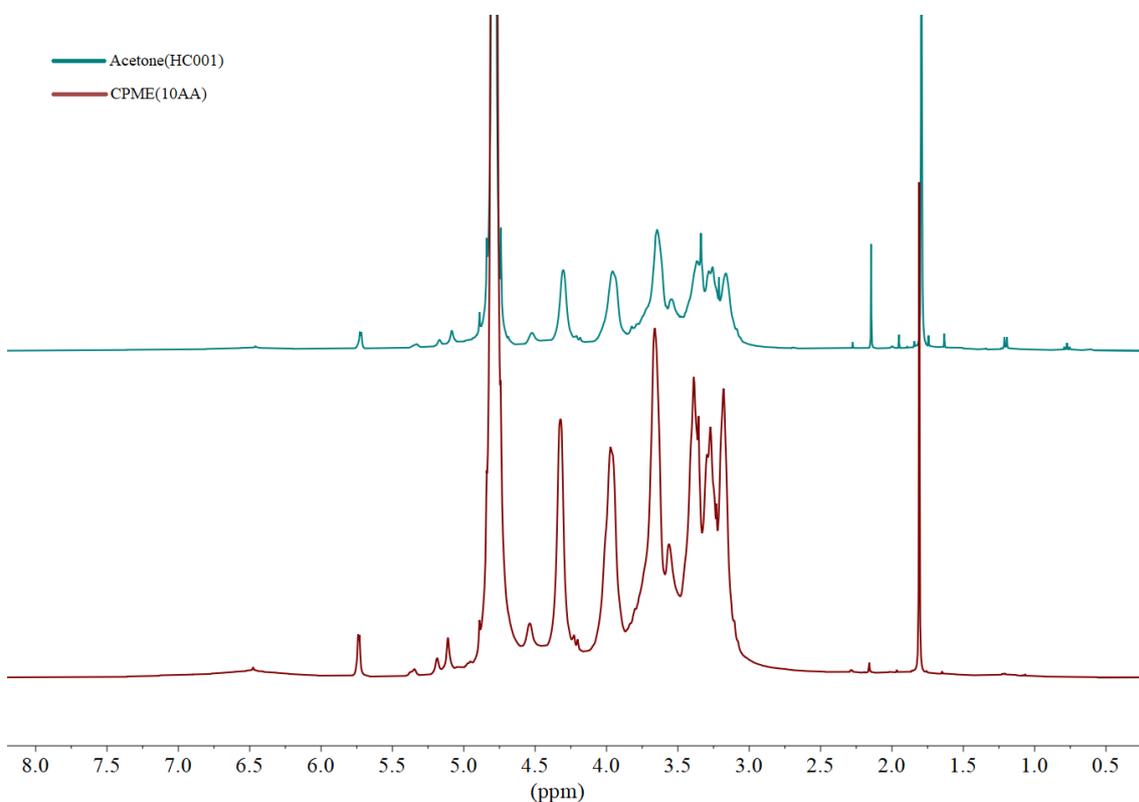
**Figure 19** -  $^1\text{H}$  NMR spectrum of the precipitated lignin isolated with AA and LA.

Analyzing Figure 19 for the  $^1\text{H}$  NMR spectrum and comparing it with literature values, can be seen that in both spectra, peaks in the order of 1.26, 2.17, and 4.80 ppm are represented, and they concern about hydrocarbon contaminant, aliphatic acetate, aromatic acetate, benzylic protons in  $\beta$ - $\beta$  structures of secoisolariciresinol type, benzylic protons in 3-aryl-1-propanol units, Inflection possibly due to  $\text{H}\alpha$  in pinoresinol units and  $\text{H}\beta$  in noncyclic benzyl aryl ethers (threo forms), respectively. The peaks at 3.81, 4.39, 6.53, and 7.53 ppm correspond to protons in methoxyl groups,  $\text{H}\gamma$  in, primarily,  $\beta$ -0-4 structures (erythro forms) and  $\beta$ -5 structures, aromatic protons (certain vinyl protons) and aromatic protons in benzaldehyde units, respectively. There is also a notable presence of a sharp peak at 4.7 ppm which relates to the solvent used, deuterium oxide. [16,18].

In conclusion, it can be stated that the samples analyzed are related to precipitated lignin.

### 5.5.2. NMR spectrum for hemicelluloses samples

For this analysis, two samples were selected: one of hemicellulose precipitated with CPME and one of Acetone, sample 10AA and HC001, respectively (see Figure 20).



**Figure 20** - <sup>1</sup>H NMR spectrum of the precipitated hemicellulosic fraction isolated with CPME and Acetone.

Analyzing the <sup>1</sup>H NMR spectrum of the precipitated hemicellulosic fraction isolated with CPME or Acetone presented in Figure 20, it can be seen that both spectra have some peaks in common being the most relevant ones, according to the literature [7,117], around 5.2 and 5.0 ppm, for the  $\alpha$ -anomeric arabinose proton substituted at C-3 and C-2 (disubstituted) of the xylose backbone, indicating a significant amount of substituted xylose residues. Disubstituted  $\beta$ -D-xylopyranose residues with  $\alpha$ -L-arabinofuranose substituents at C-2 and C-3 are therefore also present. The peak at around 4.2 ppm is attributed to anomeric  $\beta$ -D-xylose protons substituted at C-3 residues (monosubstituted), and the region between 4.1 and 4.5 ppm corresponds to the  $\beta$ -configuration and the region 4.9-5.6 ppm corresponds to the  $\alpha$ -configuration. Signals for other arabinose and xylose protons can be observed in the region of 3.0-3.5 ppm, centered

at 3.3 ppm. Resonance signals from phenolic compounds around 6.7 ppm are also visible in the spectrum. The peak for the reference solvent is present around 4.7 ppm and relates to deuterium oxide as stated in the materials and methods chapter as being the solvent used for the NMR analysis [7,117].

In summary, and after comparison with the literature, it can be stated that the analyzed samples present peaks with values close to dioxane isolation, making CPME and acetone viable alternatives.

## 6. Conclusions and future work

### 6.1. Conclusions

In this work, the isolation of lignin from black liquor was performed by the precipitation process using different types of acids and the isolation of hemicelluloses by the use of a green solvent, CPME, and organic acids.

Initially, lignin isolation was carried out through the acidification of the black liquor using organic and non-organic acids at a pH of 3 to 4.5. Among the organic acids studied, acetic acid and lactic acid were the ones that led to the best yields, both around 54 %.

Next, for the lignin isolation, acidification was performed using the two selected acids and the operating conditions were defined using a statistical tool, where the temperature range was defined as 30 to 60 °C and the pH as 3.5 to 4.5. The optimum operating conditions of temperature was defined as approximately 45 °C and of pH around 3.5 using lactic acid, in order to obtain the best yield, around 48 %.

As for the isolation of hemicelluloses, their precipitation was performed by acidifying the black liquor using a solvent (acetone and CPME) and organic acids to a pH of 2 to 3. Among the organic acids evaluated, acetic acid was the one that obtained the best yield, of 21 %.

Regarding acetone, a maximum yield value was obtained, in the order of 44 %, with a degree of purity between 41 and 44 %. For CPME, the best yield values were in the order of 58 % with a purity of 68 %. A test was performed with the solvent most reported in literature, dioxane, where yield values of 63 % and 41 % of purity were obtained under the same operating conditions used for the best results obtained with CPME. The results showed that CPME led to better results concerning hemicelluloses precipitation.

Characterization of lignin and hemicelluloses was performed using HPLC. Through this technique, it was possible to confirm that the lignin precipitate recovered is composed of 96 to 98 % lignin, 2 to 3 % of polysaccharides, and 1 % of inorganics, thus confirming that a good level of purity was obtained using this method.

Regarding the recovered hemicelluloses precipitate, taking into account the three type of solvents, it was possible to verify that the purity of the hemicelluloses samples, despite containing xylans, also contain polysaccharides impurities. It was also verified

that the CPME sample, despite obtaining 40 % of lignin, obtained a percentage of xylans in the order of 45 %, concluding that CPME may be a good alternative to dioxane.

Finally, FTIR and NMR techniques were performed to evaluate the structure of the samples. Through these techniques it was possible to conclude that the purity level in the samples reflects what was expected, that is, in the samples where it was intended to isolate lignin, the precipitate consisted mostly of lignin and in the samples where hemicelluloses were to be isolated, the precipitate consisted mostly of hemicelluloses.

## **6.2. Suggestions for future work**

Currently, the concept of the biorefinery is essential in most industries, including the pulp and paper industry. In this sense, the valorization of lignin is fundamental. As mentioned in this thesis, lignin presents a high potential for application, however, the complexity and robustness of its structure is still a major obstacle. Therefore, it is important to investigate techniques capable of a high-efficiency extraction and isolation of lignin, so that it can be applied in value-added products of higher quality than the existing ones.

After the development of this thesis, some aspects and alternatives should be considered in the follow-up of this work, such as:

- Investigate further the effect of CPME compared to other organic solvents used in hemicelluloses isolation;
- Study the recovery of CPME after the precipitation of hemicelluloses;
- Use of recovered lignin for production of nanoparticles.

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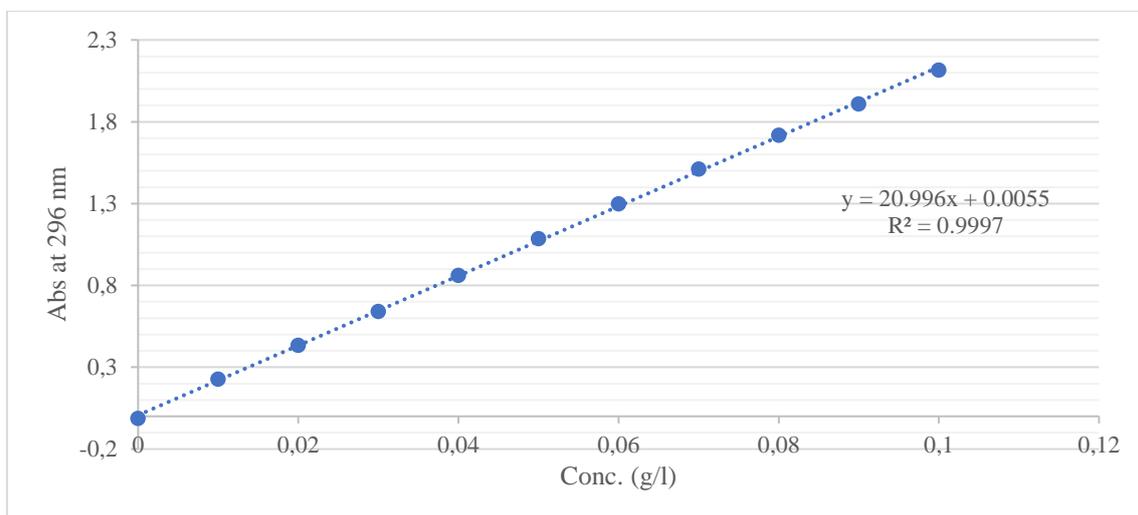
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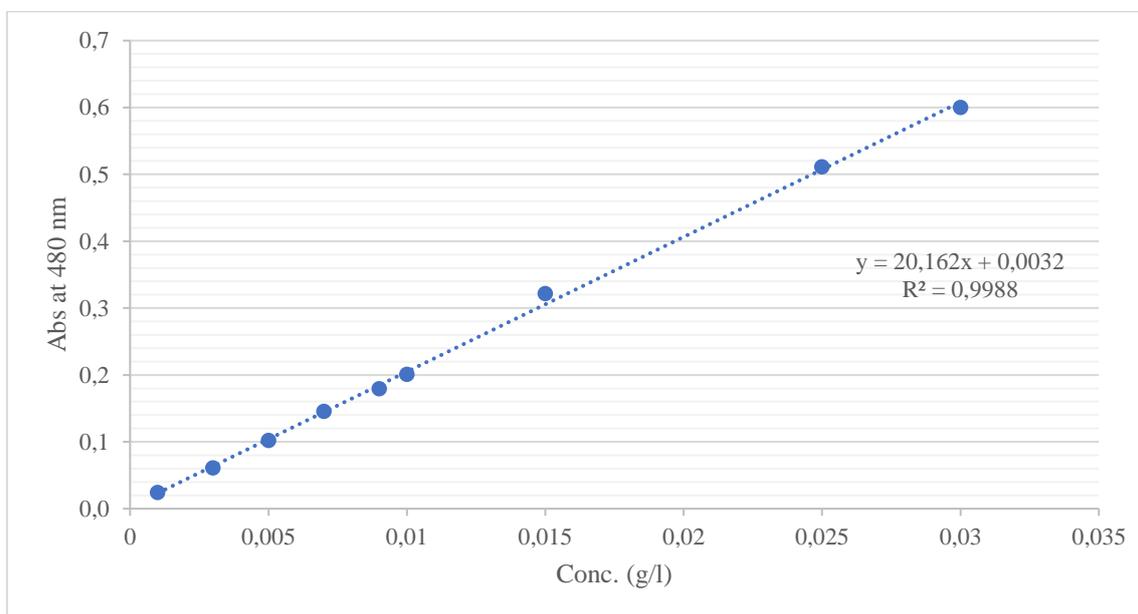


# APPENDIX

## Appendix A – Standard curves



**Figure A1** - Standard curve for lignin



**Figure A2** - Standard curve for hemicelluloses

## Appendix B – Experimental results

**Table B1** - Values of response variables obtained in the experimental design for lignin.

Experiments number	Temperature (°C)	pH	Acid type	Response	
				Yield (%)	Purity (%)
1	45	3.5	AA	20.62±3.72	99.01±0.70
2	45	3.5	LA	48.16±0.74	99.25±0.59
3	30	4.5	LA	42.02±1.20	98.76±0.58
4	30	3.5	LA	49.69±1.10	98.42±0.12
5	30	4.5	AA	44.05±0.73	98.59±0.11
6	45	4.5	AA	46.86±0.80	99.10±0.11
7	45	4	LA	44.75±3.83	99.59±0.12
8	60	4.5	LA	40.86±4.01	99.67±0.00
9	30	4	LA	39.29±5.89	99.09±0.34
10	60	4.5	AA	41.01±0.13	99.50±0.24
11	60	4	AA	33.92±1.24	99.67±0.00
12	30	4	AA	32.64±9.84	99.17±0.23
13	60	3.5	LA	44.49±0.83	99.50±0.23
14	45	4	AA	36.40±0.29	99.63±0.18
15	60	3.5	AA	19.07±0.36	99.50±0.00
16	30	3.5	AA	22.97±0.31	99.27±0.00
17	45	4.5	LA	47.08±1.01	99.67±0.00
18	60	4	LA	40.52±1.77	99.42±0.12