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DAVI RABELO DE OLIVEIRA

VALORIZATION OF *EUCALYPTUS UROGRANDIS* LIGNIN: MECHANISTIC APPROACHES OF DIFFERENT DELIGNIFICATION METHODS AND CHEMICAL MODIFICATIONS VIA SUSTAINABLE METHODOLOGY

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Orientador: Prof. Dr. Diego Lomonaco Vasconcelos de Oliveira.
Coorientadora: Profª. Dra. Selma Elaine Mazzetto

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BANCA EXAMINADORA

Prof. Dr. Diego Lomonaco Vasconcelos de Oliveira (Orientador)
Universidade Federal do Ceará (UFC)

Prof. Dr. Claudenilson da Silva Clemente
Universidade Federal do Ceará (UFC)

Profª. Dra. Davila de Souza Zampieri
Universidade Federal do Ceará (UFC)

Profª. Dra. Maria Teresa Salles Trevisan
Universidade Federal do Ceará (UFC)

Dr. Adriano Lincon Albuquerque Mattos
Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA)

Dedicado a todos que me inspiraram e me incentivaram a sempre continuar buscando conhecimento.

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"Eu poderia viver recluso numa casca de noz e me considerar rei do espaço infinito"

Shakespeare. Hamlet, Ato 2, Cena 2

ABSTRACT

Lignin, a complex and abundant aromatic biopolymer, represents a renewable resource that is still underutilized in the concept of biorefineries. The effective valorization of lignin depends critically on its chemical structure, which is strongly influenced by the extraction method employed, and on chemical modifications that modulate its properties for specific applications. However, traditional extraction methods can lead to altered structures, and chemical modifications often involve dangerous reagents and long reaction times. This thesis had the following two objectives: (1) to comparatively investigate the impact of the Kraft, Rapid Pyrolysis (FP) and Microwave-Assisted Organosolv (MWAOD) delignification methods on the structural, thermal and antioxidant characteristics of *Eucalyptus urograndis* lignin, using Milled Wood Lignin (MWL) as a reference; and (2) to develop and evaluate a sustainable and selective acetylation method for Kraft lignin, employing acetic acid as a reagent under microwave irradiation. The lignins were extensively characterized by FTIR, NMR (2D HSQC, ^{31}P), GPC, TGA, DSC, and antioxidant activity assay (DPPH). The results of delignification revealed that the methods produced lignins with varying yields (33-69%), and high purities (76-93%). The severity of the process (FP>Kraft>MWAOD) has been shown to directly influence the structure of the lignin, resulting in lower molecular weights (657-1959 g/mol), higher degree of condensation (β-5', 5-5' bonds) and higher content of phenolic hydroxyls, with consequent changes in thermal properties and antioxidant capacity. The modification study demonstrated that microwave-assisted acetylation with acetic acid is a fast and efficient alternative, allowing partial and selective acetylation (preferably in phenolic hydroxyls) of Kraft lignin, modifying its solubility, thermal stability and antioxidant activity in a controlled manner. With the results obtained, it was possible to conclude that the selection of the extraction method is fundamental to define the properties of technical lignin and that innovative and sustainable modification approaches, such as microwave acetylation with acetic acid, are promising to adapt lignin to higher value-added applications, contributing to the circular economy and the development of biorefineries.

Keywords: delignification; *Eucalyptus urograndis*; acetylation; microwave; acetic acid.

RESUMO

A lignina, um biopolímero aromático complexo e abundante, representa um recurso renovável ainda subutilizado no conceito de biorrefinarias. A valorização eficaz da lignina depende criticamente de sua estrutura química, a qual é fortemente influenciada pelo método de extração empregado, e de modificações químicas que modulem suas propriedades para aplicações específicas. Contudo, métodos tradicionais de extração podem levar a estruturas alteradas e modificações químicas frequentemente envolvem reagentes perigosos e longos tempos de reação. Esta tese teve como duplo objetivo: (1) investigar comparativamente o impacto dos métodos de deslignificação Kraft, Pirólise Rápida (FP) e Organosolv Assistido por Micro-ondas (MWAOD) nas características estruturais, térmicas e antioxidantes da lignina de *Eucalyptus urograndis*, utilizando a Lignina de Madeira Moída (MWL) como referência; e (2) desenvolver e avaliar um método de acetilação sustentável e seletivo para a lignina Kraft, empregando ácido acético como reagente sob irradiação de micro-ondas. As ligninas foram caracterizadas extensivamente por FTIR, RMN (2D HSQC, ^{31}P), GPC, TGA, DSC e ensaio de atividade antioxidante (DPPH). Os resultados da deslignificação revelaram que os métodos produziram ligninas com rendimentos variados (33-69%), e purezas elevadas (76-93%). A severidade do processo (FP>Kraft>MWAOD) demonstrou influenciar diretamente a estrutura da lignina, resultando em menores pesos moleculares (657-1959 g/mol), maior grau de condensação (ligações β-5', 5-5') e maior teor de hidroxilas fenólicas, com consequentes alterações nas propriedades térmicas e na capacidade antioxidante. O estudo de modificação demonstrou que a acetilação assistida por micro-ondas com ácido acético é uma alternativa rápida e eficiente, permitindo a acetilação parcial e seletiva (preferencialmente em hidroxilas fenólicas) da lignina Kraft, modificando sua solubilidade, estabilidade térmica e atividade antioxidante de forma controlada. Com os resultados obtidos, foi possível concluir que a seleção do método de extração é fundamental para definir as propriedades da lignina técnica e que abordagens de modificação inovadoras e sustentáveis, como a acetilação por micro-ondas com ácido acético, são promissoras para adequar a lignina a aplicações de maior valor agregado, contribuindo para a economia circular e o desenvolvimento de biorrefinarias.

Palavras Chave: deslignificação; *Eucalyptus urograndis*; acetilação; micro-ondas; ácido acético.

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SUMMARY

1 GENERAL INTRODUCTION

In recent decades, a growing and undeniable global concern regarding environmental issues has been observed, driven by the adverse impacts of anthropogenic activities, notably the excessive dependence on fossil resources. Greenhouse gas emissions, pollution, and the depletion of non-renewable raw materials demand an urgent transition towards more sustainable economic and technological models.

In this context, the principles of Green Chemistry and Green Engineering gain prominence, advocating for the development of processes and products that minimize or eliminate the use and generation of hazardous substances. One of the cornerstones of this transition lies in the valorization of biomass, an abundant and diverse renewable source (CASAU et al., 2022; NING et al., 2021).

Lignocellulosic biomass, derived from sources such as agricultural, forestry, and industrial residues, emerges as a promising alternative to petrochemical feedstocks for the production of energy, fuels, and a wide range of value-added chemicals, aligning with the concepts of biorefinery and circular economy (CASAU et al., 2022)

Lignocellulosic biomass is primarily composed of three biopolymers: cellulose, hemicelluloses, and lignin. While cellulose and hemicelluloses have been extensively exploited, lignin, the second most abundant organic polymer on Earth after cellulose, remains largely underutilized (ALAM et al., 2024; SHOREY et al., 2024). Structurally, lignin is a complex, amorphous, and three-dimensional aromatic polymer resulting from the radical polymerization of three main phenylpropanoid monomers: p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol (LAURICHESSE; AVÉROUS, 2014a; XU; FERDOSIAN, 2017). Its primary function in plants is to provide structural rigidity to the cell wall, impermeability to water, and resistance against microbial attacks (BILAL et al., 2017). Despite often being treated as a residue in pulp and paper production processes (where it is mostly burned for energy generation), lignin's structure, rich in phenolic and aliphatic units, endows it with intrinsic potential to be converted into a myriad of aromatic chemicals, building blocks, resins, adhesives, composites, and other high-value-added materials (BAJWA et al., 2019). The effective valorization of lignin is, therefore, a crucial step for the economic viability and sustainability of lignocellulosic biorefineries.

The separation of lignin from other biomass components, a process known as delignification, is a fundamental step for its subsequent utilization. Various pretreatment and extraction methods have been developed, such as the Kraft process, widely used in the pulp industry, which employs alkaline conditions and sulfide, resulting in lignin with incorporated sulfur and structural modifications, and the Organosolv process, which uses organic solvents and produces lignins with higher purity and potentially better-preserved structure(ALAM et al., 2024). Other processes, including Soda, steam explosion, and emerging methods using ionic liquids or deep eutectic solvents, are also explored(SAADAN et al., 2024). It is crucial to emphasize that the delignification method profoundly influences the final chemical structure of the isolated lignin, affecting molecular weight, polydispersity, functional group content, and degree of condensation (SAADAN et al., 2024). These structural differences, in turn, dictate lignin's physicochemical properties (solubility, thermal stability, reactivity) and, consequently, its suitability for specific application. A detailed understanding of this structure-property-process relationship is essential.

Although lignin possesses intrinsic potential, its native properties such as heterogeneity, low solubility, and variable reactivity often limit its direct application. Chemical modification emerges as a powerful strategy to customize lignin's properties, making it more processable and compatible (KOMISARZ; MAJKA; PIELICHOWSKI, 2023). Among the various possible reactions (phenolation, urethanization, etc.), esterification, particularly acetylation, has received considerable attention. Acetylation, by introducing acetyl groups (CH3CO-), typically increases solubility in a wider range of organic solvents, reduces polarity, and can improve compatibility with polymers and thermal stability (JOHANSSON et al., 2023a; SHOREY et al., 2024; SZABÓ et al., 2021; TREJO-CÁCERES; SÁNCHEZ; MARTÍN-ALFONSO, 2023). Traditionally performed with reagents like acetic anhydride, the search for greener alternatives is growing.

Despite advancements, a detailed understanding of how the extraction method affects the fine structure of lignin from specific sources, such as Eucalyptus urograndis, and the development of greener and more selective chemical modification methods, are fundamental. Controlled acetylation, aiming for partial and selective modification, and the use of milder reagents like acetic acid, represent promising and less explored approaches, especially for industrial Kraft lignin.

In this context, the main objectives of this doctoral thesis are: 1) To investigate and compare the effect of different delignification methods on the structural and physicochemical characteristics of lignin isolated from Eucalyptus urograndis; and 2) To explore the partial and potentially selective acetylation of Eucalyptus urograndis Kraft lignin, employing acetic acid as a green acetylating agent, investigating the effect of reaction conditions on the degree of substitution, selectivity, and properties of the resulting material. It is expected that the results obtained will contribute to a better understanding of eucalyptus lignin structure and its relationship with the extraction method, as well as provide valuable insights into more sustainable chemical modification routes for this important renewable biopolymer.

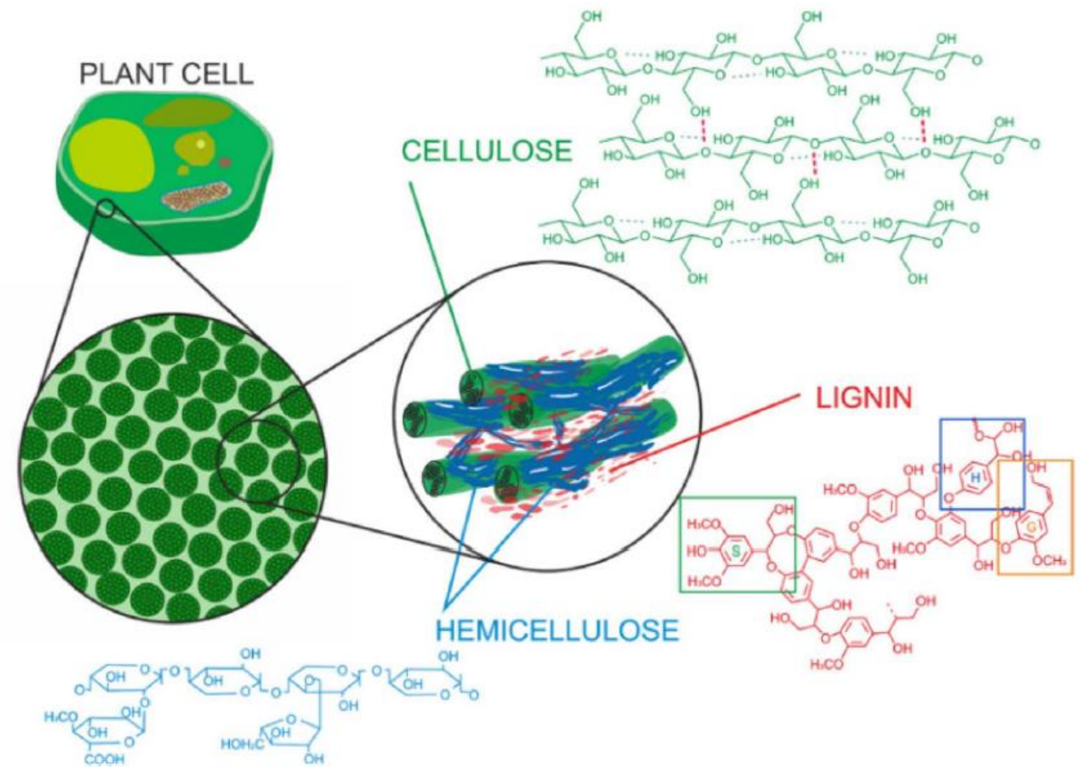
2 LITERATURE REVIEW

2.1 Lignin: Structure, function, and natural variation

2.1.1 Definitions, biosynthesis, and biological roles.

Lignin stands as one of the principal structural components within the secondary cell walls of terrestrial vascular plants (KATAHIRA; ELDER; BECKHAM, 2018; LIU; LUO; ZHENG, 2018). It is formally defined as a complex, high-molecular-weight, three-dimensional, amorphous biopolymer characterized by its alkyl-aromatic nature (KATAHIRA; ELDER; BECKHAM, 2018; LIU; LUO; ZHENG, 2018; MA, 2024; TANIS et al., 2024). Lignin is part of the lignin-carbohydrate complexes (LCC) that are found in cell walls of plants and woody materials, as illustrated in Figure. 1.

Figure 1 - Composition of lignocellulosic biomass



Source: RUWOLDT; BLINDHEIM; CHINGA-CARRASCO, 2023

Following cellulose, lignin is the second most abundant natural organic polymer on Earth, constituting a significant fraction of lignocellulosic biomass, typically ranging from 15% to 35% by dry weight, and even up to 50% in specialized tissues like fruit endocarps. This abundance translates to lignin representing approximately 30% of the organic carbon in the biosphere, making it the planet's largest renewable reservoir of aromatic structures (DEMUNER, I. F., COLODETTE, J. L., DEMUNER, A. J., & JARDIM, 2019; LI et al., 2018; LIU; LUO; ZHENG, 2018; RALPH; LAPIERRE; BOERJAN, 2019; TANIS et al., 2024).

The biosynthesis of lignin is an intricate process originating from the phenylpropanoid metabolic pathway, utilizing the amino acid phenylalanine (and tyrosine in certain pathways) as the primary precursor (LIU; LUO; ZHENG, 2018). This pathway can be broadly divided into three sequential stages. First, the synthesis of monomeric precursors, known as monolignols (specifically, p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol), occurs within the cytoplasm through a series of enzymatic reactions including deamination, hydroxylation, methylation, and reduction. These monolignols are often glycosylated, rendering them water-soluble and less toxic for transport. Second, these monomers are transported from the cytoplasm across the plasma membrane to the apoplast, the region outside the cell membrane where cell wall construction takes place (DIXON; BARROS, 2019; LIU; LUO; ZHENG, 2018) . The exact mechanism of transport remains under investigation, potentially involving passive diffusion targeted to specific polymerization sites (DIXON; BARROS, 2019) . Third, within the secondary cell wall, the monolignols undergo oxidative polymerization (LIU; LUO; ZHENG, 2018). This crucial step involves the generation of monolignol radicals through the action of oxidative enzymes, primarily cell wall-bound peroxidases (POD) and laccases (LAC), utilizing hydrogen peroxide as an oxidant (DEMUNER, I. F., COLODETTE, J. L., DEMUNER, A. J., & JARDIM, 2019; RALPH; LAPIERRE; BOERJAN, 2019). These radicals then couple in a combinatorial, seemingly random fashion ('radical-radical coupling') to form the growing lignin polymer network, creating a diverse array of intermolecular linkages (LIU; LUO; ZHENG, 2018).

This biosynthetic process results in a polymer of remarkable heterogeneity and complexity, which underpins both its vital biological functions and its notorious recalcitrance to degradation (KATAHIRA; ELDER; BECKHAM, 2018; LIU; LUO; ZHENG, 2018; MA, 2024) . The random coupling of different monomers via various linkage types creates a highly cross-linked, amorphous structure that provides plants with essential attributes but poses significant challenges for industrial processing (KATAHIRA; ELDER; BECKHAM, 2018).

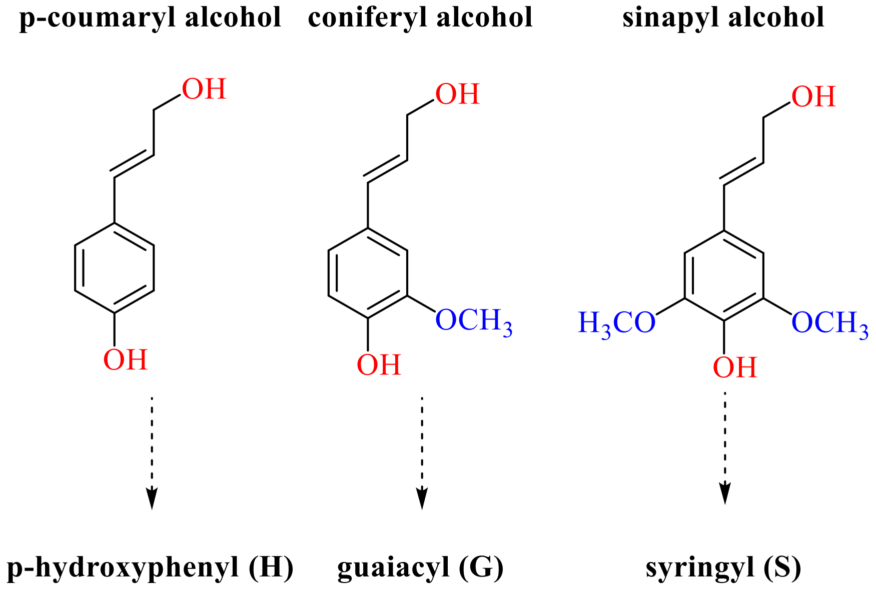
In planta, lignin fulfills several indispensable biological roles. Its primary function is structural: it imparts rigidity, stiffness, and compressive strength to secondary cell walls, providing mechanical support that allows terrestrial plants to grow upright against gravity and resist physical stresses like wind (lodging resistance). Lignin's inherent hydrophobicity is critical for water transport; by cross-linking with hydrophilic cell wall polysaccharides (cellulose and hemicellulose) and filling the spaces between them, it creates a waterproof barrier within the xylem vessels, enabling the efficient conduction of water and dissolved nutrients throughout the plant. Furthermore, lignin contributes significantly to plant defense mechanisms. It forms a durable physical barrier that protects cell wall polysaccharides from enzymatic degradation by microbial pathogens and deters herbivores. Its accumulation at sites of infection or stress is a common plant defense response, contributing to resistance against both biotic and abiotic stresses (LIU; LUO; ZHENG, 2018; PERACCHI et al., 2024).

2.1.2 Macromolecular architecture: monomers and linkages.

The complex macromolecular structure of lignin arises from the polymerization of three primary phenylpropanoid monomers, commonly referred to as monolignols: p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol. These precursors differ structurally in the number of methoxy groups attached to the C3 and C5 positions of their aromatic rings: p-coumaryl alcohol has no methoxy groups, coniferyl alcohol has one at C3, and sinapyl alcohol has two, at C3 and C5. Once incorporated into the lignin polymer via oxidative radical coupling, these monolignols give rise to distinct structural units: p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) units, respectively (KATAHIRA; ELDER; BECKHAM, 2018).

Figure 2 shows the structures of the three primary phenylpropanoid monomers and their corresponding H, G and S structural units.

Figure 2 - Structures of the three primary phenylpropanoid monomers and their corresponding H, G and S structural units.



Source: The author

The polymerization process connects these H, G, and S units through a diverse array of stable intermolecular bonds, primarily involving the β-carbon of the side chain and the aromatic ring or phenolic oxygen of adjacent units (LIU; LUO; ZHENG, 2018; RALPH; LAPIERRE; BOERJAN, 2019). These linkages can be broadly classified into two main types: ether bonds (C–O–C) and carbon–carbon bonds (C–C) (WANG et al., 2023).

The most prevalent inter-unit linkage across all lignin types is the β-O-4 aryl ether bond, which typically accounts for 40% to over 60% of all linkages, depending on the biomass source. This linkage involves an ether bond between the β-carbon of one unit's side chain and the phenolic oxygen (at C4) of another unit. Its relative abundance and comparative lability make it a primary target in chemical delignification and depolymerization processes (DEMUNER, I. F., COLODETTE, J. L., DEMUNER, A. J., & JARDIM, 2019; KATAHIRA e colab., 2018; LI, Wenqi e AMOS e e colab., 2018).

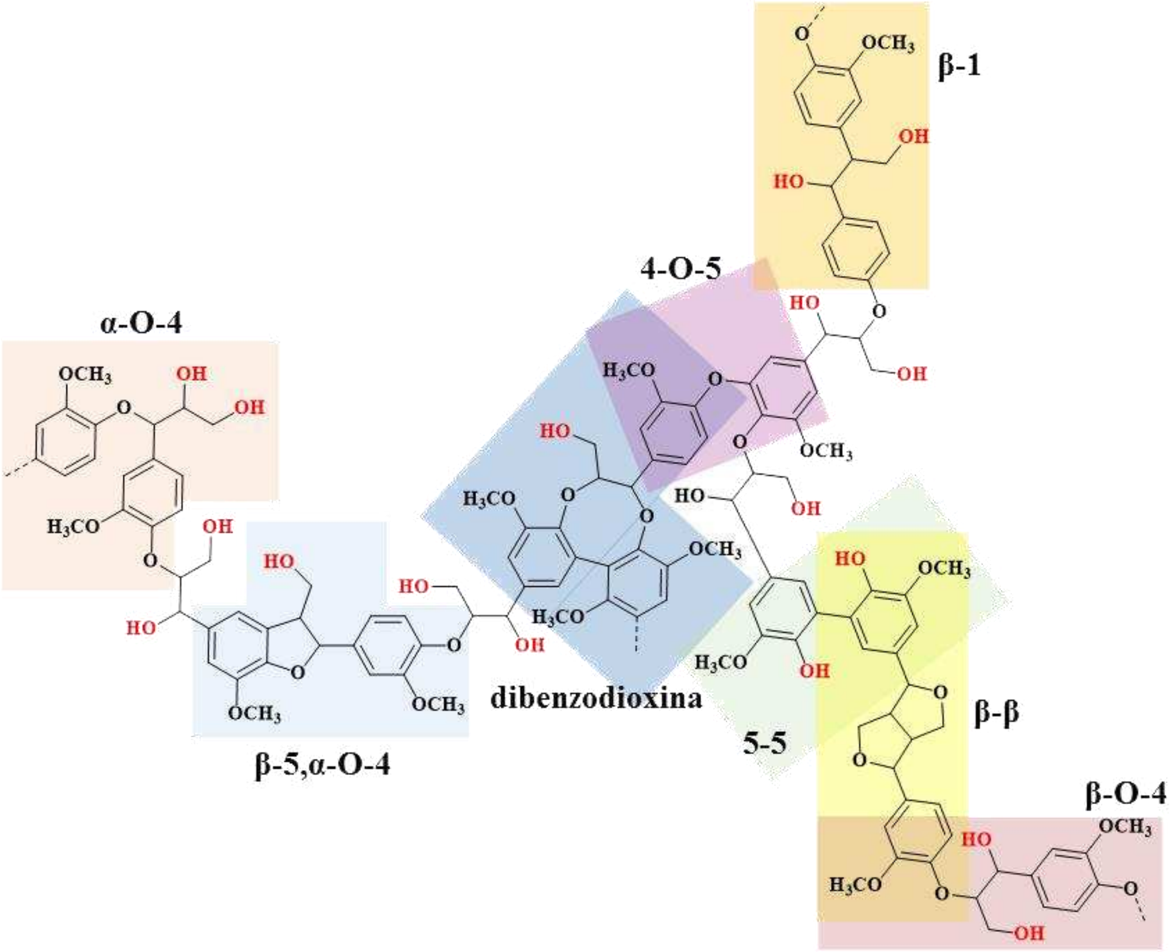
Other significant linkages contribute to the three-dimensional network structure (KATAHIRA; ELDER; BECKHAM, 2018):

- **β-5 (Phenylcoumaran):** A C–C bond between the β-carbon of one unit and the C5 position of an adjacent aromatic ring.
- **5-5' (Biphenyl):** A direct C–C bond linking the C5 positions of two aromatic rings.
- **β-β (Resinol):** A C–C bond formed by the coupling of the β-carbons of two units.

- **4-O-5 (Diaryl ether):** An ether bond connecting the C4 oxygen of one unit to the C5 position of another aromatic ring.
- **β-1 (Spirodienone):** Involving linkage between the β-carbon of one unit and the C1 position of another, often existing as a spirodienone intermediate.
- **Dibenzodioxocin:** A more complex structure involving both 5-5' and β-O-4 type linkages within an eight-membered ring.

Figure 3 represents some of inter-unit linkages found in lignin, forming its complex structure

Figure 3 - Some of inter-unit linkages found in lignin



Source: The author

In addition to the backbone structure formed by these linkages, lignin possesses various peripheral functional groups that influence its chemical reactivity and physical properties. These include phenolic hydroxyl groups (Ar–OH), aliphatic hydroxyl groups (primarily at the α and γ positions of the side chain), methoxyl groups (–OCH_3) attached to the aromatic ring, and smaller amounts of carbonyl and carboxyl groups. The relative abundance and accessibility of these groups, particularly the hydroxyl groups, are critical for chemical modification strategies (KARTHÄUSER et al., 2021; SAADAN et al., 2024).

The specific combination of monomers and the resulting linkage patterns profoundly influence lignin's overall architecture and properties. The relative proportion of easily cleaved ether bonds versus more recalcitrant C–C bonds is a key determinant of lignin's behavior. Ether linkages, especially the β-O-4 type, are generally less stable and more susceptible to chemical cleavage under various pulping or depolymerization conditions (LI et al., 2018a; SUN et al., 2018; WANG et al., 2023). Carbon-carbon linkages like β-5 and 5-5' require significantly higher bond dissociation energies to break, contributing to lignin's recalcitrance. Monomer composition dictates the potential linkage types: S units, being methoxylated at both C3 and C5, primarily participate in β-O-4 linkages as the C5 position is blocked for C-C coupling, leading to more linear polymer chains. G units, with a free C5 position, can form β-O-4, β-5, and 5-5' linkages, resulting in more branched and condensed structures. H units, lacking methoxy groups, tend to favor C-C bond formation. Consequently, lignins rich in S units (like hardwoods) tend to be more linear and have a higher proportion of cleavable β-O-4 bonds compared to G-rich lignins (like softwoods), which are more condensed and branched. These structural differences directly impact physical properties like rigidity and chemical properties like degradability and suitability for specific valorization pathways (LI et al., 2018a; RESE et al., 2025; SUN et al., 2018; WANG et al., 2023).

Furthermore, the inherent randomness of the radical coupling polymerization process results in significant structural heterogeneity and polydispersity, even within lignin isolated from a single plant source. Unlike the precise, template-driven synthesis of proteins or DNA, lignin polymerization lacks a defined sequence or repeating unit (HASANOV; RAUD; KIKAS, 2020; JASIUKAITYTĖ-GROJZDEK et al., 2025; JÕUL et al., 2022; RIDDELL et al., 2024; WANG et al., 2020). This leads to a distribution of polymer chains with varying molecular weights (high polydispersity index, PDI) and diverse arrangements of monomers and linkages. This intrinsic variability presents major challenges for detailed structural characterization and contributes to batch-to-batch inconsistencies in technical lignins derived from industrial processes, thereby hindering the development and deployment of reproducible, high-value applications (GONÇALVES et al., 2021; SONG et al., 2024) .

2.1.3 Influence of Biomass Source on Lignin Structure (Softwood, Hardwood, Grasses)

The botanical origin of lignocellulosic biomass profoundly influences the structure and composition of its native lignin. The relative proportions of the H, G, and S monomer units, as well as the prevalence of different inter-unit linkages, vary significantly among major plant groups, primarily softwoods (gymnosperms), hardwoods (angiosperms), and grasses (herbaceous angiosperms) (KATAHIRA; ELDER; BECKHAM, 2018; RALPH; LAPIERRE; BOERJAN, 2019).

Lignin from softwood species (e.g., pine, spruce, fir) is characterized by its overwhelming predominance of guaiacyl (G) units, typically constituting over 95% of the monomers. It contains only minor amounts of *p*-hydroxyphenyl (H) units and is virtually devoid of syringyl (S) units. Due to the high G-unit content and the availability of the C5 position on the guaiacyl ring for coupling, softwood lignin features a significantly higher proportion of resistant carbon-carbon linkages, such as β-5 (phenylcoumaran) and 5-5' (biphenyl), compared to hardwood lignin. This results in a more branched and highly condensed three-dimensional structure. The β-O-4 aryl ether linkage, while still present, constitutes a lower proportion (approximately 50%) compared to hardwood lignin (DEMUNER, I. F., COLODETTE, J. L., DEMUNER, A. J., & JARDIM, 2019; LI, Wenqi e ZHANG e e colab., 2018).

Hardwood species (e.g., birch, poplar, oak, eucalyptus) typically possess lignin composed of both guaiacyl (G) and syringyl (S) units, with S units often being more abundant than G units (S/G ratio > 1 in many cases). The presence of methoxy groups at both C3 and C5 positions in S units sterically hinders coupling at the C5 position. Consequently, hardwood lignin exhibits a lower frequency of C-C linkages (β-5, 5-5') and is dominated by the β-O-4 aryl ether linkage, which can account for 60% or more of the inter-unit bonds. This leads to a more linear and less condensed polymer structure compared to softwood lignin. Some hardwood lignins may also incorporate minor amounts of ester-linked *p*-hydroxybenzoate units. The S/G ratio itself can vary significantly even among different hardwood species (DEMUNER, I. F., COLODETTE, J. L., DEMUNER, A. J., & JARDIM, 2019; LI, Wenqi e ZHANG e e colab., 2018)..

Lignin found in grasses and other herbaceous plants (e.g., corn stover, wheat straw, sugarcane bagasse, bamboo) displays the greatest compositional complexity. It incorporates all three primary monolignols, resulting in H, G, and S units within the polymer structure. A distinctive feature of grass lignin is the significant incorporation of p-coumaric acid and, to a lesser extent, ferulic acid. These hydroxycinnamic acids are typically attached to the lignin polymer via ester linkages at the γ-carbon of the side chain, predominantly on S units, although they can also form ether linkages or act as bridges between lignin and hemicelluloses (forming lignin-carbohydrate complexes, LCCs). Furthermore, grass lignins often incorporate flavonoids, particularly tricin, which can act as nucleation sites for lignin polymerization. These additional components contribute to a highly branched and complex structure (KATAHIRA; ELDER; BECKHAM, 2018; LI et al., 2018b; LIU; LUO; ZHENG, 2018; PERACCHI et al., 2024).

These inherent structural variations based on biomass source have significant consequences for the physicochemical properties of lignin, including its molecular weight distribution, solubility, thermal stability, chemical reactivity, and susceptibility to different delignification or depolymerization strategies. For instance, the higher β-O-4 content in hardwood lignin generally makes it more amenable to depolymerization strategies targeting these linkages, potentially yielding higher monomer yields compared to softwood lignin. Conversely, the condensed nature of softwood lignin might make it more suitable for applications requiring high thermal stability or carbon yield. The presence of ester linkages in grass lignins introduces additional points of chemical lability (LI et al., 2018a; RALPH; LAPIERRE; BOERJAN, 2019; WANG et al., 2023; ZHOU et al., 2016a).

Table 1 - Comparative structural features of lignins from different biomass sources

Feature	Softwood	Hardwood	Grass
Predominant Monomer(s)	Guaiacyl (G)	Syringyl (S) and Guaiacyl (G)	Hydroxyphenyl (H), Guaiacyl (G), Syringyl (S)
Typical S/G/H Ratio	~0: 95-100: <5	~45-75: 25-50: ~0	β-O-4, Ester linkages

Dominant Linkage Type(s)	β-O-4, β-5, 5-5'	β-O-4	β-O-4, Ester linkages
Relative β-O-4 Abundance	~50%	~60%	Variable, often high
Condensation/ Branching	High / Branched	Moderate / Linear	High / Branched
Other Key Features	Condensed C–C linkages	More linear chains	*p*-Coumarates, Ferulates (ester-linked), Tricin

Source:DEMUNER, I. F., COLODETTE, J. L., DEMUNER, A. J., & JARDIM, 2019; KARTHÄUSER et al., 2021; KATAHIRA; ELDER; BECKHAM, 2018; LI et al., 2018b; RESE et al., 2025; ZHOU et al., 2016b

2.2 Isolation of lignin: delignification processes and lignin properties

2.2.1 Overview of delignification strategies and challenges

Delignification refers to the process of separating lignin from the other major components of lignocellulosic biomass, namely cellulose and hemicellulose (SAADAN et al., 2024; TANIS et al., 2024; TOFANI et al., 2023). This separation is a critical step in various industrial processes, including the production of pulp for paper manufacturing, the liberation of carbohydrates for conversion into biofuels (like cellulosic ethanol) and biochemicals, and the isolation of lignin itself for potential valorization into value-added products (SAADAN et al., 2024; TANIS et al., 2024).

The primary challenge in delignification lies in the inherent recalcitrance of the lignocellulosic matrix. Lignin forms a complex, amorphous, and highly cross-linked network that encrusts and interpenetrates the polysaccharide components. Furthermore, lignin is covalently linked to hemicelluloses, forming lignin-carbohydrate complexes (LCCs), which further impede the separation of individual biopolymers. Effective delignification requires methods capable of cleaving these linkages and breaking down the lignin structure sufficiently to allow its solubilization and removal, ideally without causing excessive degradation of the desired cellulose fraction or the lignin itself if it is targeted for valorization (GIERER, 1985; TANIS et al., 2024; WANG et al., 2023).

Another significant challenge arises from the structural heterogeneity of lignin, which varies depending on the biomass source (softwood, hardwood, grass) and even within different parts of the same plant. This variability makes it difficult to develop universally applicable delignification processes and leads to inconsistencies in the properties of the isolated lignin (SAADAN et al., 2024).

Furthermore, economic viability and environmental sustainability are crucial considerations. Delignification processes often require significant inputs of energy and chemicals, and the recovery and recycling of solvents or catalysts are essential to minimize costs and environmental impact (MIKULSKI; KŁOSOWSKI, 2022).

A wide array of delignification strategies has been developed to address these challenges, often employed as a pretreatment step prior to further biomass conversion (D'ORSI et al., 2023; TANIS et al., 2024). These methods can be broadly categorized based on their operating principles:

- **Chemical Pulping:** Industrial processes like Kraft, sulfite, and soda pulping use chemical reagents (alkalis, sulfides, sulfites) under harsh conditions (high temperature, pressure) to chemically degrade and solubilize lignin(JOSÉ BORGES GOMES et al., 2020; MIKULSKI; KŁOSOWSKI, 2022; SAADAN et al., 2024).
- **Organosolv Processing:** Uses organic solvents (often with water and catalysts) at elevated temperatures to selectively dissolve lignin (JASIUKAITYTĖ-GROJZDEK et al., 2025; SAADAN et al., 2024).
- **Hydrothermal Treatments:** Employ hot water or steam (e.g., steam explosion, autohydrolysis) under pressure to hydrolyze hemicellulose and modify lignin structure.(JACQUET et al., 2015; LI; GELLERSTEDT; TOVEN, 2009; MA et al., 2022; YU et al., 2022)
- **Thermochemical Conversion:** Processes like fast pyrolysis rapidly heat biomass in the absence of oxygen to produce bio-oil, char, and gas, fundamentally altering the lignin structure. (ECHRESH ZADEH; ABDULKHANI; SAHA, 2020; LENG et al., 2017a)
- **Novel Solvent Systems:** Emerging technologies utilize ionic liquids (ILs) or deep eutectic solvents (DES) to dissolve biomass components selectively under potentially

milder conditions (HASANOV; RAUD; KIKAS, 2020; LI et al., 2018b; MIKULSKI; KŁOSOWSKI, 2022; TANIS et al., 2024).

- **Biological Treatments:** Utilize lignin-degrading enzymes or microorganisms, though typically slower and less efficient for bulk delignification.(D’ORSI et al., 2023; FIGUEIREDO et al., 2018a)

A critical distinction exists between the lignin present in its native state within the plant cell wall and the "technical lignins" obtained after industrial or laboratory isolation processes (LI; TAKKELLAPATI, 2018; SAADAN et al., 2024; WANG et al., 2020). Most commercially available lignin falls into the latter category. The chemical and thermal conditions employed during isolation inevitably alter the lignin's structure through reactions like bond cleavage (especially β-O-4 ethers), condensation (formation of new C-C bonds), fragmentation, functional group modification, and, in some cases, incorporation of elements like sulfur . Consequently, technical lignins (e.g., Kraft lignin, lignosulfonates, soda lignin, organosolv lignin) possess distinct molecular weights, polydispersity, solubility profiles, and reactivity compared to the idealized native lignin structure. This structural modification during isolation is a fundamental factor influencing the suitability and performance of technical lignins in high-value applications, often necessitating further fractionation or chemical modification (SAADAN et al., 2024).

Recognizing the limitations imposed by lignin degradation during conventional processing, particularly when polysaccharide recovery is the primary goal, has spurred the development of "lignin-first" biorefining strategies (KARTHÄUSER et al., 2021; SUN et al., 2018). These approaches prioritize the selective extraction or depolymerization of lignin under conditions designed to preserve its structural integrity and inherent value early in the biomass fractionation process, before subjecting the carbohydrate fractions to potentially harsher treatments (KARTHÄUSER et al., 2021; SUN et al., 2018; TOFANI et al., 2023). Methods like organosolv processing, and potentially IL or DES treatments, are often associated with this philosophy (LI; GELLERSTEDT; TOVEN, 2009; LI; TAKKELLAPATI, 2018; LI et al., 2018a). The underlying driver for lignin-first approaches is the economic imperative of integrated biorefineries, which must efficiently valorize all major biomass components—cellulose, hemicellulose, and lignin—to achieve overall process profitability and sustainability. Traditionally, lignin has been significantly undervalued, often combusted merely for process heat, representing a loss of potential revenue from this abundant aromatic resource (KATAHIRA; ELDER; BECKHAM, 2018).

2.2.2 Industrial chemical pulping methods

Chemical pulping processes, developed primarily for the paper industry, remain the largest source of technical lignins globally. These methods utilize harsh chemical conditions to dissolve lignin and liberate cellulose fibers.

Kraft process

The Kraft process is the most widely used chemical pulping method worldwide, particularly for wood biomass.

- **Mechanism:** It employs a highly alkaline solution (pH ~13-14) of sodium hydroxide (NaOH) and sodium sulfide (Na_2S), known as "white liquor," at elevated temperatures (typically 155-175°C) and pressures for several hours. The strong alkaline conditions promote the cleavage of α-aryl and, more significantly, β-aryl ether bonds (β-O-4) in the lignin structure. The hydrosulfide ions (HS^-) from Na_2S act as potent nucleophiles, accelerating the cleavage of these ether linkages and limiting undesirable lignin condensation reactions, thus enhancing the rate and selectivity of delignification compared to the soda process. The fragmented lignin macromolecules become soluble in the alkaline cooking liquor, forming "black liquor". Lignin is typically recovered from the black liquor by precipitation through acidification, often in sequential steps using carbon dioxide followed by a stronger acid like sulfuric acid, as implemented in commercial processes like LignoBoost and LignoForce. A crucial aspect of the Kraft process is the chemical recovery cycle, where the inorganic chemicals (NaOH, Na_2S) are regenerated from the black liquor through combustion and subsequent reactions, allowing for process sustainability (FABBRI et al., 2023; HU; ZHANG; LEE, 2018; SAADAN et al., 2024).
- **Lignin Characteristics:** Kraft Lignin (KL) is characterized by the presence of covalently bound sulfur, typically 1-3% by weight, incorporated during the pulping reactions. It generally possesses a relatively low weight-average molecular weight (Mw) compared to native lignin, often reported in the range of 1.5–5 kDa, although values up to 25 kDa have been cited, reflecting significant fragmentation. However, KL exhibits a broad molecular

weight distribution, indicated by a high polydispersity index (PDI or Mw/Mn) typically between 2.5 and 3.5. The cleavage of numerous ether bonds results in a high concentration of free phenolic hydroxyl groups. Despite the action of sulfide in mitigating condensation, the harsh alkaline and thermal conditions promote side reactions leading to the formation of new, stable C-C bonds, resulting in a more condensed lignin structure compared to native lignin. Kraft lignin is generally insoluble in water and acidic solutions but soluble in alkaline aqueous solutions (typically pH > 10.5) and polar organic solvents like dimethyl sulfoxide (DMSO) and dimethylformamide (DMF). Ash content can vary depending on the recovery process (HU; ZHANG; LEE, 2018; JARDIM et al., 2020; SAADAN et al., 2024).

- **Economics**: As the dominant pulping technology, the Kraft process generates the largest volume of technical lignin globally. However, this lignin is predominantly utilized as a low-value fuel source, burned within the pulp mill's recovery boiler to generate energy and recover pulping chemicals. While commercial lignin separation technologies (LignoBoost, LignoForce) are now operational in some mills , the widespread valorization of Kraft lignin into higher-value products remains challenging. Key hurdles include its structural complexity, condensed nature, relatively low reactivity, inherent heterogeneity, and the presence of sulfur. The sulfur content, in particular, is detrimental as it can poison catalysts used in many downstream chemical conversion processes and may be undesirable in certain material applications. This limitation drives significant interest in developing sulfur-free delignification methods or effective desulfurization techniques for Kraft lignin. Furthermore, the economic motivation for installing lignin separation units in Kraft mills is often linked to increasing pulp production capacity by reducing the thermal load on the recovery boiler, rather than solely relying on the revenue from lignin sales. The separated lignin is frequently still used as fuel, for example, replacing fossil fuels in the mill's lime kiln. Despite these challenges, the sheer scale of Kraft lignin availability makes it a crucial target for biorefinery research and development (EKIELSKI; MISHRA, 2021; FABBRI et al., 2023; FORSSELL et al., 2025; LI; TAKKELLAPATI, 2018; MORE; ELDER; JIANG, 2021).

Sulfite process

The Sulfite process represents an older generation of chemical pulping technology, though it remains significant due to the established market for its lignin byproduct, lignosulfonates.

- **Mechanism:** This process utilizes aqueous solutions containing sulfur dioxide (SO_2) and a sulfite or bisulfite salt based on calcium, magnesium, sodium, or ammonium. The pulping can be conducted under acidic, neutral, or alkaline conditions, typically at temperatures ranging from 120°C to 180°C. The core chemical reaction is sulfonation, where sulfonate groups ($–SO_3H$ or $–SO_3^-$) are introduced onto the lignin structure, primarily at the α-carbon position of the propane side chain. This reaction facilitates the cleavage of native lignin linkages (including α- and β-aryl ethers) and renders the resulting lignin fragments highly water-soluble. Acid hydrolysis of lignin and hemicelluloses also occurs, particularly under acidic pulping conditions. The dissolved lignosulfonates are separated from the cellulose pulp in the spent sulfite liquor (SSL). Recovery often involves techniques like ultrafiltration (to separate based on molecular size) or chemical precipitation methods (e.g., the Howard process involving lime precipitation) (MILES-BARRETT et al., 2017; SAADAN et al., 2024; WANG et al., 2020).
- **Lignin Characteristics:** The product, Lignosulfonates (LS), are anionic polyelectrolytes characterized by their high water solubility across a wide pH range, due to the abundant sulfonate and carboxylic acid groups. They possess a high sulfur content, typically ranging from 3.5% to 8%. Lignosulfonates generally exhibit a high weight-average molecular weight (Mw often 20–50 kDa, but can exceed 100 kDa) and significant polydispersity (PDI typically 4–8), reflecting both fragmentation and condensation reactions during pulping. The structure is considerably altered from native lignin, often highly condensed with a low residual content of easily cleavable β-O-4 linkages due to the harsh processing conditions. LS typically have a high ash content (4–8%) and may contain significant amounts of residual sugars from hemicellulose co-extraction, depending on the purification process. Their structure imparts amphiphilic properties, with hydrophilic anionic groups and hydrophobic aromatic/aliphatic moieties (KARTHÄUSER et al., 2021; MILES-BARRETT et al., 2017; SAADAN et al., 2024; WANG et al., 2020).
- **Economics:** Although largely superseded by the Kraft process for papermaking , the Sulfite process remains relevant due to the large, established market for lignosulfonates. LS constitute the vast majority (~90%) of the commercially traded technical lignin volume, estimated at over 1 million tons annually. Their primary applications leverage their properties as water-soluble dispersants, binders, emulsifiers, and chelating agents.

Major uses include concrete plasticizers (water reducers), dispersants for drilling muds, pesticides, and dyes, binders for animal feed pellets and roads, and emulsion stabilizers. The well-established market and relatively low cost provide a baseline for lignin valorization efforts. However, the high water solubility and structural modifications (sulfonation, condensation, low β-O-4 content) limit the use of lignosulfonates in applications requiring organic solvent solubility, compatibility with hydrophobic polymers, or specific native lignin structures targeted by some advanced valorization routes (GONÇALVES et al., 2021; LI; TAKKELLAPATI, 2018; MILES-BARRETT et al., 2017; WANG et al., 2020).

Soda process

The Soda process is the oldest chemical pulping method and operates under alkaline conditions without the use of sulfur compounds.

- **Mechanism:** Delignification is achieved using primarily sodium hydroxide (NaOH) as the active chemical, typically at high temperatures (140-170°C). The alkaline conditions promote the hydrolytic cleavage of ether bonds (α-O-4, β-O-4) in lignin, breaking it down into smaller, alkali-soluble fragments. To enhance delignification efficiency and, importantly, to minimize the degradation of cellulose and hemicellulose that occurs under harsh alkaline conditions, anthraquinone (AQ) is often added in small amounts (0.1-1%) as a catalyst (Soda/AQ process). AQ participates in a redox cycle, protecting carbohydrates from peeling reactions while promoting lignin fragmentation. The process is particularly suited for non-wood biomass sources like agricultural residues (straw, bagasse) and grasses, which generally have lower lignin content and different lignin structures compared to wood. Lignin is recovered from the spent liquor (black liquor) via acid precipitation (JOSÉ BORGES GOMES et al., 2020; MARÍA E. EUGENIO , RAQUEL MARTÍN-SAMPEDRO , JOSÉ I. SANTOS, 2021; SAADAN et al., 2024).
- **Lignin Characteristics:** Soda lignin is distinguished by being sulfur-free, a significant advantage for applications where sulfur is detrimental. It typically exhibits a low molecular weight (Mw often 800–3000 Da) similar to or slightly lower than Kraft lignin, with moderate polydispersity (PDI ~2.5-3.5). Due to the extensive cleavage of ether bonds, soda lignin possesses a high content of phenolic hydroxyl groups. Its structure is

significantly modified and degraded compared to native lignin. The purity can be variable; soda lignins derived from non-wood sources often contain higher levels of impurities such as residual carbohydrates (from hemicellulose breakdown) and inorganic components like ash or silicates. Soda lignins may also have a higher residual sodium content compared to other technical lignins (GONÇALVES et al., 2021; MARÍA E. EUGENIO , RAQUEL MARTÍN-SAMPEDRO , JOSÉ I. SANTOS, 2021; SAADAN et al., 2024).

- **Economics:** While historically important, the Soda process is less common for wood pulping today due to lower pulp yields and slower delignification rates compared to the Kraft process. However, its suitability for non-wood biomass makes it strategically relevant for biorefineries utilizing agricultural residues or dedicated energy crops. The annual production volume of soda lignin is considerably smaller than that of Kraft lignin or lignosulfonates. Its sulfur-free nature, combined with low molecular weight and high phenolic content, makes it a potentially valuable precursor for applications like substitution in phenolic resins, production of polymers, or as a substrate for chemical modifications targeting hydroxyl groups. However, the variability in purity, particularly the presence of ash and carbohydrate contaminants from non-wood feedstocks, can pose challenges for consistent performance in high-value applications and may necessitate additional purification steps (EKIELSKI; MISHRA, 2021; MARÍA E. EUGENIO , RAQUEL MARTÍN-SAMPEDRO , JOSÉ I. SANTOS, 2021).

Organosolv process

Organosolv processing represents a category of delignification methods that utilize organic solvents, often in combination with water, to extract lignin from biomass.

- **Mechanism:** The process typically involves treating lignocellulosic biomass with an organic solvent (e.g., ethanol, methanol, acetone, acetic acid, formic acid, ethylene glycol, 2-methyltetrahydrofuran (MeTHF)) mixed with water at elevated temperatures (ranging from 150°C to over 200°C). Catalysts, either acidic (e.g., sulfuric acid, formic acid) or basic (e.g., NaOH, ammonia), are often added to enhance the process, although catalyst-free systems also exist. The organic solvent system effectively cleaves lignin-carbohydrate bonds and hydrolyzes internal lignin ether linkages (primarily α-O-4 and β-O-4), solubilizing the lignin fragments. The severity of the process (temperature, time, catalyst concentration) dictates the extent of lignin fragmentation versus potential lignin re-condensation reactions. After the reaction, the solubilized lignin is typically recovered from the solvent mixture by reducing the solvent concentration (e.g., adding water as an anti-solvent) or by solvent evaporation, causing the lignin to precipitate. This method allows for the co-production of a relatively pure cellulose-rich solid fraction and a hemicellulose-derived sugar stream (JASIUKAITYTĖ-GROJZDEK et al., 2025; KARTHÄUSER et al., 2021; LATIF et al., 2021; SAADAN et al., 2024) .

- **Lignin Characteristics:** A key advantage of Organosolv lignin (OSL) is that it is typically sulfur-free and possesses high purity, characterized by low ash and residual carbohydrate content. Compared to Kraft and Sulfite lignins, OSL generally has a lower weight-average molecular weight (Mw typically 500–5000 Da) and a narrower molecular weight distribution (PDI often 1.3–4.0). The structure of OSL is considered to be less degraded and closer to that of native lignin, especially when extracted under milder conditions, potentially retaining a higher proportion of reactive β-O-4 linkages. OSL exhibits good solubility in a wide range of organic solvents but is generally insoluble in water, reflecting its hydrophobic character. Depending on the alcohol solvent used (e.g., ethanol), some hydroxyl groups in the lignin may become etherified during the process, forming alkoxy groups (e.g., ethoxy groups). The relatively preserved structure and functional group availability often result in higher chemical reactivity compared to more condensed technical lignins (JASIUKAITYTĖ-GROJZDEK et al., 2025; JÕUL et al., 2022; SAADAN et al., 2024).
- **Economics:** Organosolv processing is often associated with the "lignin-first" biorefinery concept, aiming to produce high-quality streams of all major biomass components (cellulose, hemicellulose sugars, and lignin) for integrated valorization. While several pilot and demonstration plants exist, large-scale commercial implementation is less widespread than Kraft or Sulfite pulping. A major economic challenge is the cost associated with the organic solvents and the energy-intensive requirement for their efficient recovery and recycling (often >99% needed) to ensure process viability. However, the potential to produce high-purity, reactive, sulfur-free lignin suitable for higher-value chemical and material applications makes the Organosolv process highly attractive for future biorefineries moving beyond traditional pulp production. The inherent "tunability" of the Organosolv process is a significant advantage. By carefully selecting the solvent system (type of organic solvent, water

ratio), catalyst (type and concentration), temperature, and reaction time, it is possible to tailor the delignification process to specific biomass feedstocks and, crucially, to control the properties of the extracted lignin. This allows for the production of lignin fractions with targeted characteristics, such as specific molecular weight ranges, polydispersity, functional group content (e.g., ratio of phenolic to aliphatic hydroxyls), purity, and solubility, making them potentially more suitable for demanding downstream applications compared to the less controllable outputs of traditional pulping methods. This flexibility is key to realizing the potential of lignin as a versatile platform chemical. However, realizing this potential on an industrial scale hinges critically on overcoming the economic barrier posed by solvent management. Efficient solvent recovery and recycling systems are not just desirable but essential for the commercial feasibility of Organosolv processes, representing a major focus for process engineering and optimization efforts (DE LA TORRE et al., 2013a; SAADAN et al., 2024; TOFANI et al., 2023).

2.2.3 Thermochemical and physical pretreatment methods

Beyond chemical pulping, other methods employ heat or physical force, often as pretreatments to enhance downstream processing rather than as primary lignin isolation techniques.

Fast Pyrolysis

Fast pyrolysis is a thermochemical conversion process that rapidly decomposes biomass in the absence of oxygen.

- **Mechanism:** Biomass, or isolated lignin, is heated very quickly to high temperatures (typically 450-550°C, though ranges up to 800 K are mentioned) with very short vapor residence times (usually 1-2 seconds). The intense heat breaks down the complex biopolymers (cellulose, hemicellulose, lignin) into smaller volatile molecules. These vapors are then rapidly cooled (quenched) to condense into a liquid product known as bio-oil or pyrolysis oil. Non-condensable gases (e.g., CO, CO_2, H_2, light hydrocarbons) and a solid residue (char) are also produced. Catalytic fast pyrolysis (CFP) incorporates catalysts (e.g., zeolites) either within the pyrolysis reactor (in-situ) or in a secondary reactor (ex-situ) to upgrade the pyrolysis vapors before condensation, aiming to improve bio-oil quality (BEIS et al., 2010; ECHRESH ZADEH; ABDULKHANI; SAHA, 2020; RANZI; DEBIAGI; FRASSOLDATI, 2017).

- **Product Characteristics (Bio-oil):** Fast pyrolysis bio-oil is a highly complex mixture, containing hundreds of different organic compounds derived from the degradation of all biomass components. Key characteristics include: high water content (20-30%, from biomass moisture and dehydration reactions) ; high acidity (pH 2-3) due to the presence of carboxylic acids (mainly acetic and formic acid) ; high oxygen content (35-40% or higher) distributed across various functional groups (hydroxyls, carbonyls, carboxyls, ethers) ; and consequently, a lower heating value (typically 13-19 MJ/kg LHV) compared to conventional fossil fuels. The oil is dense (~1.15-1.2 kg/L), has variable viscosity, and is immiscible with hydrocarbon fuels due to its polarity. A major issue is its instability; reactive components like aldehydes, ketones, unsaturated compounds, and phenolics can undergo polymerization and condensation reactions over time, especially upon heating, leading to increased viscosity, phase separation, and gum/solid formation. Bio-oil derived specifically from lignin is rich in phenolic compounds (e.g., phenol, guaiacol, syringol derivatives, catechols) reflecting the aromatic nature of the precursor. The specific phenolic profile depends on the lignin source (G-phenols from softwoods; S and G phenols from hardwoods). CFP generally produces bio-oils with lower oxygen content, lower acidity, higher heating value, increased aromatic hydrocarbons, and improved stability compared to non-catalytic pyrolysis oils. Lignin typically yields less bio-oil (e.g., 16-22% reported in one study) compared to whole biomass (up to 75-80%) (ECHRESH ZADEH; ABDULKHANI; SAHA, 2020; RANZI; DEBIAGI; FRASSOLDATI, 2017) .
- **Economics/Application:** Fast pyrolysis is primarily viewed as a route to convert solid biomass into a liquid intermediate (bio-oil) that can be more easily transported and potentially upgraded into transportation fuels, chemicals, or materials. However, the poor quality of raw bio-oil (instability, acidity, high oxygen/water, immiscibility) necessitates significant and costly upgrading steps (e.g., hydrodeoxygenation, esterification, catalytic cracking) before it can be used as a drop-in fuel or feedstock. Fractionation techniques, such as solvent extraction, can be employed to isolate specific valuable chemical groups like phenolics from the complex mixture. The overall economic feasibility depends heavily on the efficiency and cost of both the pyrolysis

process and the subsequent upgrading or separation steps, as well as the market value of the final products. Fundamentally, fast pyrolysis represents a *conversion* technology that breaks down lignin's polymeric structure into a diverse array of smaller molecules, rather than an *isolation* method aimed at preserving the macromolecule like pulping or organosolv processes. While the phenolic compounds derived from lignin are valuable components within the bio-oil, representing a potential pathway to renewable aromatics , their efficient separation and purification from the highly complex, reactive, and acidic bio-oil matrix remain significant technological and economic challenges(ECHRESH ZADEH; ABDULKHANI; SAHA, 2020; LI; TAKKELLAPATI, 2018; RANZI; DEBIAGI; FRASSOLDATI, 2017).

Steam explosion

Steam explosion is a widely applied hydrothermal pretreatment method used to disrupt the structure of lignocellulosic biomass and improve its accessibility for subsequent processing.

- **Mechanism:** The process involves exposing biomass to high-pressure saturated steam (typically 1-3.5 MPa) at elevated temperatures (180-240°C) for a short duration (seconds to minutes). This is followed by an instantaneous, explosive release of pressure down to atmospheric levels. The mechanism combines two primary effects: (1) **Thermochemical treatment:** The high temperature and steam pressure promote autohydrolysis reactions. Acetic acid released from hemicellulose acetyl groups acts as an *in situ* acid catalyst, accelerating the hydrolysis of glycosidic bonds, primarily in hemicellulose, breaking it down into soluble oligosaccharides and monosaccharides. Lignin also undergoes chemical modifications, including softening, partial depolymerization through cleavage of labile ether bonds (like β-O-4) and ester linkages, and potential re-condensation reactions. (2) **Physical disruption:** The rapid decompression causes the superheated water trapped within the biomass structure to instantly vaporize and expand violently. This explosive force physically tears apart the fiber structure, separating fiber bundles and increasing the porosity and accessible surface area of the material. The severity of the treatment (controlled by temperature, pressure, and residence time) determines the extent of chemical degradation and physical disruption. Acid catalysts (e.g., SO_2, H_2SO_4) can be impregnated into the biomass prior to steam treatment to enhance hemicellulose hydrolysis and allow for lower operating temperatures (D'ORSI et al., 2023; JACQUET et al., 2015; LI; GELLERSTEDT; TOVEN, 2009; MA et al., 2022).

- **Structural Effects & Lignin Properties:** Steam explosion significantly alters the biomass ultrastructure. It effectively removes a large portion of hemicellulose and disrupts the lignin-hemicellulose matrix, thereby increasing the accessibility of the remaining cellulose to enzymatic hydrolysis. Lignin undergoes complex changes: it softens due to heating above its glass transition temperature, potentially flows and redistributes onto fiber surfaces, undergoes partial depolymerization through cleavage of ether and ester bonds, leading to an increase in phenolic hydroxyl groups, but can also undergo significant re-condensation reactions, especially at higher severities, forming more recalcitrant structures often referred to as "pseudo-lignin". Lignin isolated *after* steam explosion (typically via subsequent solvent or alkaline extraction) reflects these modifications, often exhibiting reduced molecular weight but potentially increased condensation compared to native lignin (D'ORSI et al., 2023; JACQUET et al., 2015; LI; GELLERSTEDT; TOVEN, 2009; MA et al., 2022). .
- **Economics/Advantages:** Steam explosion is considered an effective, relatively low-cost, and environmentally friendly pretreatment technology, requiring minimal or no external chemical input (autohydrolysis relies on *in situ* generated acids). It generally has lower energy consumption compared to methods like extensive milling. The process is industrially scalable and widely implemented as a pretreatment for various applications, including: enhancing enzymatic saccharification for bioethanol and biogas production; improving the properties of biomass pellets for combustion (increasing durability, hydrophobicity, and heating value); and potentially facilitating subsequent chemical pulping or extraction processes. Its primary role is thus to *prepare* biomass for further conversion by overcoming its structural recalcitrance, rather than to selectively *isolate* a high-quality lignin fraction directly. The lignin remaining after steam explosion is significantly modified, and its suitability for valorization depends on the severity of the pretreatment and the intended application. Careful optimization of process conditions is necessary to maximize the benefits (e.g., increased cellulose digestibility, improved pellet quality) while minimizing undesirable side effects such as the formation of fermentation inhibitors (e.g., furfural, HMF from sugar degradation) or excessive lignin condensation, which could hinder subsequent lignin utilization

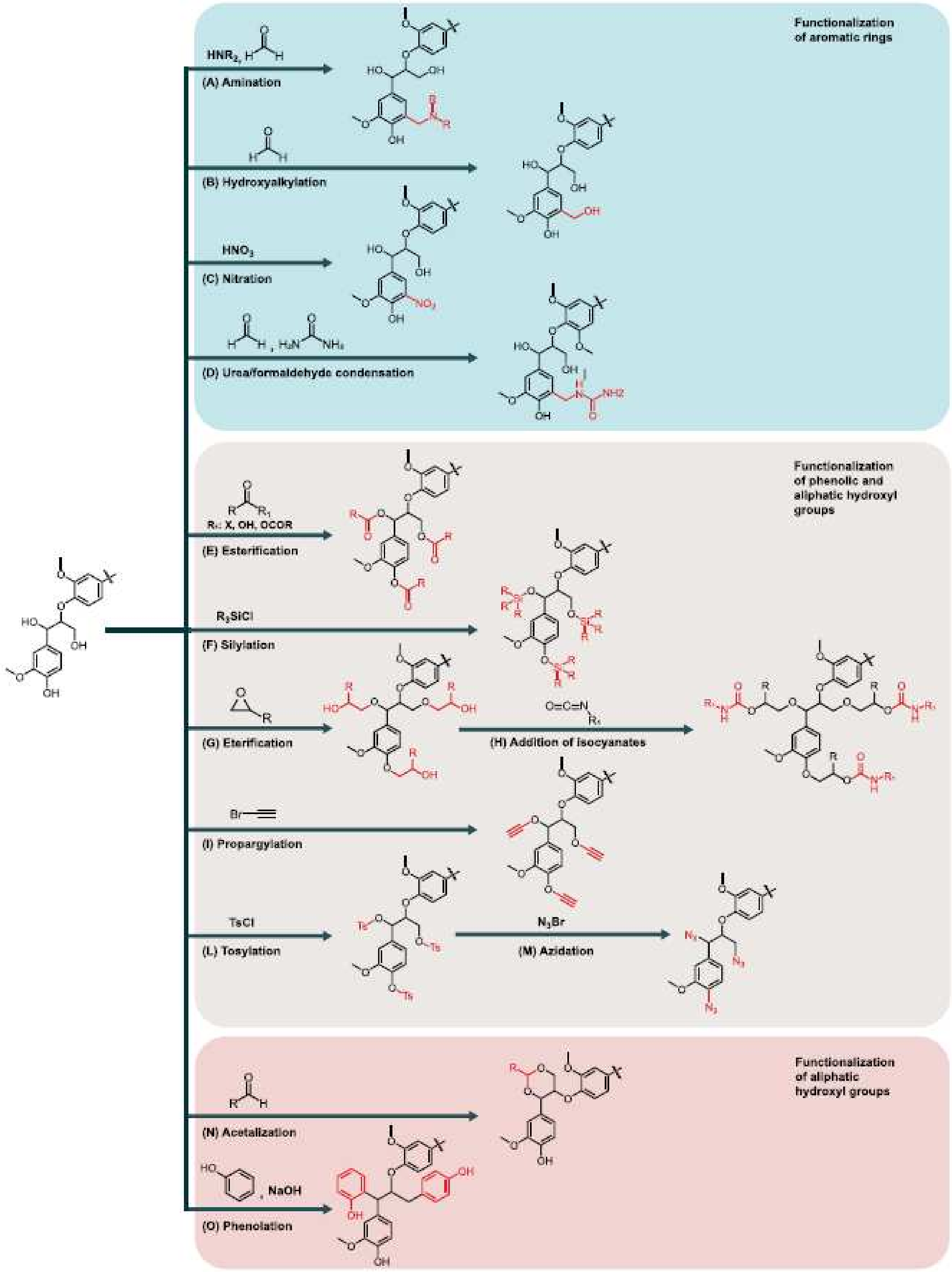
(D’ORSI et al., 2023; JACQUET et al., 2015; LI; GELLERSTEDT; TOVEN, 2009; MA et al., 2022).

2.3 Overview of lignin chemical modifications

Chemical modification is a versatile toolbox for tailoring lignin's properties. The primary targets for these reactions are the abundant hydroxyl groups (both phenolic and aliphatic) distributed throughout the lignin macromolecule, although other functionalities like carboxyl or carbonyl groups, or even the aromatic ring itself, can be involved The overarching goal is typically to enhance lignin's performance in specific applications by improving its solubility, processability, compatibility with other materials (especially polymers), thermal stability, reactivity for further transformations, or by introducing entirely new functional characteristics (KOMISARZ; MAJKA; PIELICHOWSKI, 2023; KUMAR et al., 2020; LI; GELLERSTEDT; TOVEN, 2009; LIBRETTI; SANTOS CORREA; MEIER, 2024; MA et al., 2022; PODSCHUN et al., 2015; RUWOLDT; BLINDHEIM; CHINGA-CARRASCO, 2023; SUOTA et al., 2021; TANG et al., 2020).

Figure 4 illustrates some of the various possibilities of chemical modifications of lignin at different reactive sites present in its structure. Some of these modifications are discussed later in this text.

Figure 4 - Some of possible chemical modifications of the lignin backbone.



Source: BERTELLA; LUTERBACHER, 2020

2.3.1 Esterification

Esterification involves the reaction of lignin's hydroxyl groups with various acylating agents to form ester linkages. Common reagents include carboxylic acid anhydrides (e.g., acetic, propionic, butyric, maleic, succinic, phthalic), acyl chlorides (e.g., oleoyl chloride, undecenoyl chloride), carboxylic acids (e.g., lactic acid, fatty acids like oleic acid), and lactones (e.g., butyrolactone) (CHEN et al., 2014; KOMISARZ; MAJKA; PIELICHOWSKI, 2023; LIBRETTI; SANTOS CORREA; MEIER, 2024; LISÝ et al., 2022; RUWOLDT; BLINDHEIM; CHINGA-CARRASCO, 2023; SUOTA et al., 2021).

Reaction conditions vary widely, utilizing temperatures from ambient to over 120°C, reaction times spanning hours to days, and employing solvents such as pyridine, dioxane, or acetone, or sometimes proceeding neat (solvent-free) (KOMISARZ; MAJKA; PIELICHOWSKI, 2023; RESENDE et al., 2024; SUOTA et al., 2021). Catalysts like 1-methylimidazole, 4-dimethylaminopyridine (DMAP), sulfuric acid, or basic catalysts may be used to facilitate the reaction (CHEN et al., 2014; KOMISARZ; MAJKA; PIELICHOWSKI, 2023; RESENDE et al., 2024). Microwave assistance has also been explored to potentially enhance reaction rates (SUOTA et al., 2021).

The introduction of ester groups generally increases lignin's hydrophobicity, thereby improving its solubility in non-polar organic solvents and enhancing its compatibility with hydrophobic polymer matrices like polyethylene (PE), polypropylene (PP), or polylactic acid (PLA). This improved compatibility can lead to better dispersion and potentially enhanced mechanical properties (e.g., modulus, strength) in lignin-polymer composites. Thermal stability can be affected, sometimes increasing due to protection of hydroxyl groups, but sometimes decreasing depending on the nature and thermal lability of the introduced ester group (CHEN et al., 2014; KOMISARZ; MAJKA; PIELICHOWSKI, 2023; LIBRETTI; SANTOS CORREA; MEIER, 2024). Esterification with cyclic anhydrides (e.g., succinic, maleic) introduces pendant carboxylic acid groups, offering sites for further functionalization or crosslinking. Esterification with unsaturated reagents (e.g., acrylic acid, methacrylic anhydride, undecenoyl chloride) can introduce polymerizable double bonds for creating thermosets or graft copolymers (KOMISARZ; MAJKA; PIELICHOWSKI, 2023; LIBRETTI; SANTOS CORREA; MEIER, 2024) .

2.3.2 Etherification (Alkylation, Hydroxyalkylation, Oxyalkylation/Oxypropylation)

Etherification strategies focus on forming ether linkages (C-O-C) by reacting lignin's hydroxyl groups. This category includes several related modifications:

- **Alkylation/Arylation:** Reaction with alkyl or aryl halides (KOMISARZ; MAJKA; PIELICHOWSKI, 2023).
- **Hydroxyalkylation/Hydroxymethylation:** Reaction with aldehydes (e.g., formaldehyde, glyoxal, furfural) typically under basic conditions, or with cyclic carbonates (e.g., ethylene carbonate, glycerol carbonate). This introduces new hydroxyl-terminated side chains (KOMISARZ; MAJKA; PIELICHOWSKI, 2023; LISÝ et al., 2022).
- **Oxyalkylation/Oxypropylation:** Reaction with epoxides like ethylene oxide or propylene oxide, usually under alkaline catalysis at elevated temperatures and pressures. This grafts polyether chains onto the lignin (KUMAR et al., 2020; SUOTA et al., 2021; WESTWOOD; PANOVIC; LANCEFIELD, 2016).

Conditions often involve alkaline catalysts (NaOH, KOH) or sometimes acid catalysts, with temperatures ranging from moderate (30-90°C for hydroxymethylation) (KOMISARZ; MAJKA; PIELICHOWSKI, 2023) to high (>150°C for oxyalkylation with epoxides or cyclic carbonates) (SUOTA et al., 2021). Reactions with cyclic carbonates can potentially be performed solvent-free (LIBRETTI; SANTOS CORREA; MEIER, 2024).

These modifications can significantly alter lignin's properties. Hydroxyalkylation and oxyalkylation increase the number of reactive aliphatic hydroxyl groups, effectively transforming lignin into a polyol suitable for synthesizing polyurethanes (PU) (KOMISARZ; MAJKA; PIELICHOWSKI, 2023; KUMAR et al., 2020; LISÝ et al., 2022). This can also improve solubility and compatibility with polar polymers (KOMISARZ; MAJKA; PIELICHOWSKI, 2023; LISÝ et al., 2022). Oxyalkylation, in particular, can reduce the brittleness of lignin-based materials and improve viscoelastic properties (SUOTA et al., 2021). Hydroxymethylation has been shown to improve processability and compatibility, leading to enhanced mechanical and thermal properties in composites (KOMISARZ; MAJKA; PIELICHOWSKI, 2023).

2.3.3 Phenolation

Phenolation aims to increase the reactivity of lignin, particularly towards aldehydes like formaldehyde, by grafting phenol or related phenolic molecules (e.g., resorcinol) onto the lignin structure. This is typically achieved by reacting lignin with excess phenol under acidic catalysis (e.g., H_2SO_4) at elevated temperatures (e.g., 90-120°C), where phenol often serves as both reactant and solvent (KUMAR et al., 2020; PODSCHUN et al., 2015; SUOTA et al., 2021; WESTWOOD; PANOVIC; LANCEFIELD, 2016).

The primary outcome is an increase in the content of phenolic hydroxyl groups and the introduction of new, highly reactive ortho and para positions on the grafted phenol rings. This enhanced reactivity makes phenolated lignin a more viable substitute for petroleum-based phenol in the synthesis of phenol-formaldehyde (PF) resins, phenolic foams, or polybenzoxazines (KUMAR et al., 2020; LISÝ et al., 2022; PODSCHUN et al., 2015; RUWOLDT; BLINDHEIM; CHINGA-CARRASCO, 2023; SUOTA et al., 2021). Phenolation may also improve thermal stability and adsorption capabilities (PODSCHUN et al., 2015; SUOTA et al., 2021). However, the effectiveness of phenolation is highly dependent on the type of lignin; organosolv and sulfite lignins often show good results, whereas soda lignins from grasses or softwood Kraft lignin may exhibit poor activation potential (PODSCHUN et al., 2015).

2.3.4 Amination

Amination introduces nitrogen-containing functional groups into the lignin structure. The most common method is the Mannich reaction, where lignin reacts with formaldehyde and a primary or secondary amine (e.g., dimethylamine, ethylenediamine, diethylenetriamine) under typically alkaline conditions (pH ~10) and moderate temperatures (50-90°C). Other routes include reaction with aminosilanes or reacting epoxidized lignin with diamines (KOMISARZ; MAJKA; PIELICHOWSKI, 2023; LIBRETTI; SANTOS CORREA; MEIER, 2024; LISÝ et al., 2022; RUWOLDT; BLINDHEIM; CHINGA-CARRASCO, 2023; SUOTA et al., 2021).

Amination can impart hydrophilicity, potentially rendering lignin water-soluble (KOMISARZ; MAJKA; PIELICHOWSKI, 2023). The introduced amino groups are reactive sites for further functionalization or crosslinking. In acidic media, these groups can become protonated, creating cationic polymers useful as flocculants, emulsifiers (e.g., for asphalt), or dye/metal ion adsorbents. Aminated lignins have also been reported to enhance thermal stability, mechanical properties, aging resistance, and potentially flame retardancy when incorporated into polymer composites (KOMISARZ; MAJKA; PIELICHOWSKI, 2023; LISÝ et al., 2022).

2.3.5 Sulfonation and Sulfomethylation

These modifications introduce sulfur-containing acidic groups, primarily to enhance water solubility and dispersancy.

- **Sulfonation:** Directly introduces sulfonic acid ($-SO_3H$) groups, typically onto the α-carbon or aromatic ring. This is the key reaction in the sulfite pulping process, yielding water-soluble lignosulfonates (FABBRI et al., 2023; KOMISARZ; MAJKA; PIELICHOWSKI, 2023; LIBRETTI; SANTOS CORREA; MEIER, 2024). Technical lignins can also be sulfonated post-extraction using reagents like concentrated sulfuric acid (often at <40°C) or sodium sulfite (LISÝ et al., 2022; SUOTA et al., 2021).
- **Sulfomethylation:** Introduces methylene sulfonate ($-CH_2SO_3^-$) groups via reaction with formaldehyde and sodium sulfite or metabisulfite, usually under alkaline conditions (pH 7-13) at elevated temperatures (60-160°C) (LISÝ et al., 2022; RUWOLDT; BLINDHEIM; CHINGA-CARRASCO, 2023; SUOTA et al., 2021).

Both modifications significantly increase hydrophilicity and impart anionic character in aqueous solutions. Sulfonated lignins (lignosulfonates) and sulfomethylated lignins are widely used as dispersants (e.g., in concrete, dyes, pesticides), emulsifiers, binders, and water-reducing agents (LISÝ et al., 2022; SUOTA et al., 2021).

2.3.6 Other Relevant Modifications

Several other chemical strategies are employed for specific purposes:

- **Oxidation:** Uses various oxidizing agents (e.g., O_2, H_2O_2, peracids, catalytic systems) to introduce carbonyl or carboxyl groups, modify reactivity, or achieve oxidative depolymerization into smaller aromatic chemicals.(FIGUEIREDO et al., 2018a; TANG et al., 2020; WESTWOOD; PANOVIC; LANCEFIELD, 2016).

- **Reduction/Hydrogenolysis:** Employs reducing agents (e.g., $NaBH_4$) or catalytic hydrogenation/hydrogenolysis, often aiming to stabilize lignin, reduce carbonyl groups, or cleave linkages for depolymerization into monomers or oligomers (FIGUEIREDO et al., 2018a; SUN et al., 2018; WESTWOOD; PANOVIC; LANCEFIELD, 2016).
- **Grafting (Copolymerization):** Attaches synthetic polymer chains (e.g., polyesters like PLA, PCL; polyacrylates; polyethers) onto the lignin backbone using techniques like ring-opening polymerization (ROP), atom transfer radical polymerization (ATRP), or click chemistry (KOMISARZ; MAJKA; PIELICHOWSKI, 2023; TANG et al., 2020; WANG et al., 2020). This is a powerful method to compatibilize lignin with specific polymer matrices or introduce desired functionalities (e.g., biocompatibility, stimuli-responsiveness). Grafting can occur "from" the lignin backbone (initiating polymerization from lignin) or "to" the backbone (attaching pre-formed polymers) (KOMISARZ; MAJKA; PIELICHOWSKI, 2023).
- **Silylation:** Reacts lignin hydroxyl groups with organosilane compounds (e.g., tert-butyldimethylsilyl chloride, aminosilanes like APTES) (BUONO et al., 2016a; KOMISARZ; MAJKA; PIELICHOWSKI, 2023; SUOTA et al., 2021). This modification significantly increases hydrophobicity, enhances thermal stability, and improves compatibility with non-polar polymers (like PE) and inorganic fillers like silica. Silylation is often compared directly with acetylation for compatibilization purposes. (BUONO et al., 2016a; KOMISARZ; MAJKA; PIELICHOWSKI, 2023)
- **Phosphorylation:** Introduces phosphorus-containing groups using reagents like phosphorus oxychloride ($POCl_3$), phosphoric acid, or phosphorus pentoxide (P_2O_5) (KOMISARZ; MAJKA; PIELICHOWSKI, 2023; RUWOLDT; BLINDHEIM; CHINGA-CARRASCO, 2023; SUOTA et al., 2021) . The primary goal is usually to impart flame retardant properties to lignin and lignin-based materials, often accompanied by enhanced thermal stability (KOMISARZ; MAJKA; PIELICHOWSKI, 2023).
- **Nitration:** Reaction with nitric acid, often in the presence of acetic or sulfuric anhydride, introduces nitro groups onto the aromatic rings. Nitrolignin has been explored for use in polyurethanes, where it can affect mechanical and thermal properties (LISÝ et al., 2022; SUOTA et al., 2021).
- **Demethylation/Methylation:** These reactions target the methoxy groups or hydroxyl groups, respectively. Demethylation (using reagents like sulfur compounds, indium

triflate, or HBr) increases the phenolic hydroxyl content, thereby enhancing reactivity for subsequent reactions like epoxy or polyester synthesis. Methylation (using reagents like dimethyl sulfate or dimethyl carbonate) masks hydroxyl groups, increasing solubility in organic solvents and protecting these groups during other transformations (SUOTA et al., 2021).

It is important to recognize that many chemical modification strategies involve inherent trade-offs. Enhancing one property may negatively impact another. For example, modifications that increase hydrophilicity, such as sulfonation or amination, improve water solubility and dispersibility but simultaneously reduce compatibility with hydrophobic polymer matrices (LISÝ et al., 2022; PRAUCHNER; RODRÍGUEZ-REINOSO, 2012). Conversely, modifications that increase hydrophobicity, like esterification or silylation, enhance compatibility with non-polar polymers but decrease water solubility (BUONO et al., 2016a). The choice of modification strategy is therefore dictated by the target application and the desired balance of properties. This principle arises directly from the fundamental changes induced in lignin's polarity and hydrogen bonding capacity. Introducing polar functional groups enhances interactions with polar media like water, while replacing polar hydroxyl groups with less polar moieties favors interactions with non-polar environments (BUONO et al., 2016a; CHEN et al., 2014; KOMISARZ; MAJKA; PIELICHOWSKI, 2023; LISÝ et al., 2022; SAMENI; KRIGSTIN; SAIN, 2017a).

Furthermore, the sustainability of lignin modification processes is becoming an increasingly critical consideration. While lignin itself is a renewable resource, many conventional modification procedures utilize hazardous reagents (e.g., toxic solvents like pyridine, corrosive acids, carcinogenic compounds like formaldehyde or epichlorohydrin) or require energy-intensive conditions (high temperatures, pressures) (LIBRETTI; SANTOS CORREA; MEIER, 2024; MORENA et al., 2022; RESENDE et al., 2024). This can potentially offset the environmental benefits of using a bio-based starting material. Consequently, significant research efforts are now directed towards developing greener modification pathways. This includes exploring the use of less hazardous reagents, employing bio-based catalysts or enzymatic methods (e.g., using laccases or peroxidases) (FABBRI et al., 2023; FIGUEIREDO et al., 2018a; GIANNÌ; LANGE; CRESTINI, 2020; MORENA et al., 2022) , utilizing greener solvents like ionic liquids (ILs) or deep eutectic solvents (DES) (FIGUEIREDO et al., 2018a; SONG et al., 2024), developing solvent-free reaction conditions, and employing energy-efficient techniques like microwave or ultrasound assistance. Evaluating the overall environmental impact using metrics like the E-factor (Environmental factor) is also gaining (LIBRETTI; SANTOS CORREA; MEIER, 2024)traction. This shift reflects a necessary evolution in the field, aiming to ensure that lignin valorization strategies are truly sustainable from raw material to final product.

2.4 Lignin acetylation: in-depht analysis

Among the various chemical modifications applied to lignin, acetylation stands out as one of the most extensively studied and frequently employed methods. It involves the esterification of lignin's hydroxyl groups specifically with acetyl moieties ($-COCH_3$). This section provides a detailed examination of lignin acetylation, covering the underlying rationale, common methodologies, characterization techniques, and the impact on key properties relevant to material applications.

2.4.1 Rationale and Objectives

The primary motivation behind acetylating lignin is to alter its physicochemical properties, primarily by modifying its polarity and hydrogen bonding capacity, to make it more suitable for specific applications, particularly in polymer blends and composites (BUONO et al., 2016a; CHEN et al., 2014; DIMENT et al., 2024; KOMISARZ; MAJKA; PIELICHOWSKI, 2023; TREJO-CÁCERES; SÁNCHEZ; MARTÍN-ALFONSO, 2023). Key objectives include:

- **Improving Solubility in Organic Solvents:** Unmodified technical lignins often exhibit limited solubility in common, relatively non-polar organic solvents used in polymer processing (e.g., tetrahydrofuran (THF), chloroform, ethyl acetate, acetone). Acetylation replaces the polar, hydrogen-bond-donating hydroxyl groups with less polar, non-hydrogen-bond-donating acetyl groups. This reduction in polarity and disruption of intermolecular hydrogen bonding significantly enhances lignin's solubility in these solvents, facilitating solution-based processing or characterization. For instance, studies have shown that while various unmodified lignins were only slightly or partially soluble in solvents like THF, chloroform, or ethyl acetate, their acetylated counterparts became almost completely soluble (BUONO et al., 2016b; SAMENI; KRIGSTIN; SAIN, 2017a).

- **Enhancing Polymer Compatibility:** The poor miscibility between hydrophilic lignin and hydrophobic polymer matrices (like polyolefins or polyesters) often leads to phase separation, aggregation of lignin particles, and weak interfacial adhesion in blends or composites. Acetylation increases the hydrophobicity of lignin, making it more compatible with these matrices. This improved compatibility promotes better dispersion of lignin within the polymer, enhances interfacial interactions, and can lead to improved mechanical properties, particularly toughness and ductility. The acetyl groups might also exert an internal plasticizing effect, further modifying mechanical behavior (CHEN et al., 2014; JOHANSSON et al., 2023a; TREJO-CÁCERES; SÁNCHEZ; MARTÍN-ALFONSO, 2023) .
- **Modifying Thermal Properties:** Acetylation influences both the thermal stability (degradation behavior) and the glass transition temperature (Tg) of lignin. By blocking reactive hydroxyl groups, acetylation can sometimes increase the onset temperature of major thermal degradation compared to unmodified lignin. However, the acetyl groups themselves are susceptible to thermal cleavage (deacetylation), typically occurring in the range of 230-250°C, introducing a new degradation step. The effect on overall thermal stability in composites can be positive, with acetylated lignin blends sometimes showing improved stability compared to blends with unmodified lignin. Acetylation consistently tends to *lower* the Tg of lignin. This is attributed to the disruption of intermolecular hydrogen bonds and the increased free volume introduced by the bulkier acetyl groups, which enhances segmental mobility of the lignin chains. A lower Tg can be advantageous for thermoplastic processing, improving melt flow and processability. (BUONO et al., 2016a; CHEN et al., 2014; DIMENT et al., 2024; JOHANSSON et al., 2023b)
- **Increasing Hydrophobicity and Reducing Moisture Uptake:** The replacement of hydrophilic -OH groups with hydrophobic acetyl groups significantly reduces lignin's affinity for water. This leads to reduced moisture absorption in acetylated lignin and its composites, improving dimensional stability and potentially enhancing durability in humid environments. Studies have reported significantly delayed moisture absorbance and maintenance of hydrophobic surface characteristics (contact angles) for materials

containing acetylated lignin (BUONO et al., 2016a; CHEN et al., 2014; JOHANSSON et al., 2023a).

- **Impact on Antioxidant Activity:** Lignin's intrinsic antioxidant properties are primarily attributed to its phenolic hydroxyl groups, which can readily donate a hydrogen atom to scavenge free radicals (Hydrogen Atom Transfer mechanism, HAT). Acetylation directly blocks these crucial phenolic hydroxyls. Consequently, a significant **trade-off** of acetylation is the substantial reduction or complete elimination of lignin's natural antioxidant activity. This must be considered in applications where antioxidant functionality is desired (SADEGHIFAR; ARGYROPOULOS, 2015; SADEGHIFAR; RAGAUSKAS, 2025).

Therefore, the decision to acetylate lignin often prioritizes improvements in processability (solubility, lower Tg) and compatibility with hydrophobic materials over preserving its inherent antioxidant capacity. The enhanced interfacial adhesion and dispersion achieved through acetylation frequently translate into better mechanical performance (especially impact strength and elongation) in polymer composites, which is a critical requirement for many structural or packaging applications. While the loss of antioxidant activity is a drawback, the gains in compatibility and processability often justify the modification for these specific end-uses.

2.4.2 Acetylation methodologies

The acetylation of lignin is typically achieved via esterification using an acetylating agent, often in the presence of a catalyst and/or solvent.

- **Reagents:** Acetic anhydride (Ac_2O) is by far the most commonly used acetylating agent due to its reactivity and availability. Acetyl chloride is another possibility but generates HCl as a byproduct, requiring an acid scavenger. Acetic acid itself has also been used, sometimes facilitated by microwave heating. More recently, N-acetylimidazole has been employed in mechanochemical (ball milling) approaches (SHEN; XIE; WANG, 2017; SUOTA et al., 2021; TREJO-CÁCERES; SÁNCHEZ; MARTÍN-ALFONSO, 2023; ZHAO et al., 2017).

- **Catalysts and Solvents:**

- *Pyridine:* Historically, pyridine has been widely used, often serving as both a basic catalyst and a solvent for the reaction. It effectively catalyzes the reaction and neutralizes the acetic acid byproduct formed from the anhydride. However, pyridine is toxic and poses significant environmental and health concerns, driving the search for alternatives. Furthermore, studies have indicated that even with pyridine, complete acetylation may not always be achieved under standard conditions, suggesting limitations in its effectiveness or the need for careful optimization (RESENDE et al., 2024; SAMENI; KRIGSTIN; SAIN, 2017a; SHEN; XIE; WANG, 2017; TREJO-CÁCERES; SÁNCHEZ; MARTÍN-ALFONSO, 2023).
- *Other Catalysts:* Alternatives to pyridine have been explored. 4-Dimethylaminopyridine (DMAP) is a highly effective acylation catalyst. Sodium acetate and sulfuric acid have also been tested, exhibiting different catalytic activities and potentially different selectivities towards phenolic versus aliphatic hydroxyl groups. For instance, DMAP was reported to favor phenolic hydroxyl acetylation, while sodium acetate or sulfuric acid favored aliphatic hydroxyl acetylation under specific conditions (ZHAO et al., 2017).
- *Solvent-Free and Alternative Solvents:* Concerns about pyridine and other volatile organic solvents have spurred interest in catalyst-free acetylation at elevated temperatures (e.g., 120°C), relying on the thermal reactivity of acetic anhydride. Acetic anhydride itself can sometimes serve as the reaction medium. Toluene has been used as a non-participating solvent with pyridine added solely as an acid capturer. Solid-state acetylation using ball milling offers a solvent-free route. Pretreatment of lignocellulosic fibers with ionic liquids (ILs) has been shown to significantly enhance the efficiency of subsequent acetylation, potentially by improving reagent accessibility (SHEN; XIE; WANG, 2017; ZHAO et al., 2017).

- **Reaction Conditions:**
- *Temperature:* Acetylation has been performed across a broad temperature range, from room temperature (often requiring longer reaction times or highly effective catalysts) up to 120-160°C. Higher temperatures generally accelerate the reaction but may also increase the risk of lignin degradation or side reactions (BORRERO-LÓPEZ et al., 2023; DIMENT et al., 2024).
- *Time:* Reaction durations reported in the literature vary significantly, from a few hours to multiple days (24h, 48h, 72h). Optimizing reaction time is important, as excessively long times may not necessarily increase the degree of substitution (DS) and could potentially

  lead to degradation or even cleavage of the formed ester bonds (reverse reaction) under certain conditions (CHEN et al., 2014; SAMENI; KRIGSTIN; SAIN, 2017a).
- *Assisted Methods*: To improve efficiency and potentially reduce reaction times or temperatures, energy-assisted methods have been investigated. Microwave irradiation has been used to control the DS based on reaction temperature (130-160°C) and improve the properties of resulting lignin-based oleogels. Ultrasonication applied as a pretreatment has been shown to reduce lignin particle size, thereby increasing the specific surface area and enhancing the rate and efficiency of the subsequent acetylation reaction, even preventing DS reduction at longer times (AVELINO et al., 2018c; BORRERO-LÓPEZ et al., 2023; DE OLIVEIRA et al., 2017).

The prevalence of the acetic anhydride/pyridine method highlights its status as a benchmark procedure, but its acknowledged drawbacks concerning efficiency and sustainability are evident. This motivates the ongoing exploration of alternative catalysts , catalyst-free thermal methods , greener solvent systems or pretreatments like ILs , and energy-assisted techniques such as microwave or ultrasound. This transition reflects a broader trend in chemical synthesis, seeking to balance reaction effectiveness with the principles of green chemistry, which is particularly relevant when valorizing a bio-based resource like lignin.

2.4.3 Characterization and Effectiveness

Confirming the success of acetylation and quantifying its extent (degree of substitution, DS) are crucial steps. Various analytical techniques are employed for this purpose.

- **Spectroscopic Confirmation (Qualitative):**
  - *Fourier Transform Infrared Spectroscopy (FTIR):* FTIR is a rapid and widely used technique for qualitatively confirming acetylation. The key spectral changes include a significant decrease or disappearance of the broad O-H stretching vibration band (around 3400 cm^{-1}) and the concomitant appearance or intensification of characteristic ester carbonyl (C=O) stretching bands (typically strong peaks around 1735-1765 cm^{-1}). Often, two carbonyl peaks are observed, attributed to acetyl groups attached to aliphatic (~1735 cm^{-1}) and phenolic (~1765 cm^{-1}) hydroxyls. Strong C-O stretching bands associated with the ester group also appear or intensify around 1220-1240 cm^{-1} and 1040 cm^{-1}. Aromatic ring vibrations (e.g., ~1510 cm^{-1}, ~1600 cm^{-1}) often remain

unchanged and can be used for spectral normalization. (Figure 7 compares typical FTIR spectra of unmodified and acetylated lignin, highlighting these key changes) (BUONO et al., 2016a; CHEN et al., 2014; EL MANSOURI; YUAN; HUANG, 2011; TREJO-CÁCERES; SÁNCHEZ; MARTÍN-ALFONSO, 2023; VALLEJOS et al., 2011).

- *Nuclear Magnetic Resonance (NMR) Spectroscopy:* NMR (^{1}H, ^{13}C, 2D HSQC) provides more detailed structural information confirming acetylation. In ^{1}H NMR, the appearance of a strong singlet peak around 1.8-2.3 ppm is characteristic of the methyl protons (-$COCH_3$) of the acetyl groups. In ^{13}C NMR, signals corresponding to the acetyl methyl carbon (~20-21 ppm) and the ester carbonyl carbon (~169-171 ppm) appear. 2D HSQC NMR can reveal changes in the chemical environment of carbons bearing hydroxyl groups (e.g., Cα, Cγ) upon acetylation. (CHEN et al., 2014; LAHTINEN et al., 2021; TREJO-CÁCERES; SÁNCHEZ; MARTÍN-ALFONSO, 2023)

- **Quantification of hydroxyl groups and degree of substitution (DS)**:

Determining the extent of acetylation (DS), often defined as the fraction or percentage of hydroxyl groups that have been acetylated, is critical for understanding structure-property relationships

- *Titration Methods:* Traditional methods sometimes involve acetylating the lignin, hydrolyzing the unreacted acetic anhydride, and then titrating the total acetic acid formed. The difference between this and a blank titration (without lignin) is used to estimate the amount of anhydride consumed by the lignin hydroxyl groups. However, these methods are prone to inaccuracies, especially if the acetylation reaction is incomplete or if side reactions occur, and their reliability has been questioned .
- *Phosphorus-31 NMR (^{31}P NMR):* This technique has become the gold standard for accurate quantification of various types of hydroxyl groups (aliphatic, different types of phenolic OH based on substitution pattern - G, S, H) and carboxylic acid groups in lignin. The method involves derivatizing the lignin sample with a phosphorus-containing reagent (commonly 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane, TEPP) in the presence of an internal standard. The different derivatized hydroxyl groups give distinct, well-resolved signals in the ^{31}P NMR spectrum, allowing for precise integration and quantification. By comparing the ^{31}P NMR spectra of lignin before and after acetylation, the reduction in the signals corresponding to hydroxyl groups directly indicates the conversion percentage for each type of hydroxyl group, allowing for a detailed calculation of DS. This method is

considered significantly more reliable and informative than titration methods. (Figure 8 conceptually compares ^{31}P NMR spectra before and after acetylation, showing the decrease in OH signals) (BUONO et al., 2016a; DIMENT et al., 2024; FIGUEIREDO et al., 2018a; GRANATA; ARGYROPOULOS, 1995a; SUGIARTO et al., 2022).

- *Proton NMR (^{1}H NMR):* The DS can sometimes be estimated by integrating the signal of the acetyl protons (~1.8-2.3 ppm) relative to the integral of aromatic protons in the lignin structure. However, this requires accurate integration, knowledge of the average number of aromatic protons per lignin unit (which varies with source and isolation method), and assumes complete dissolution and uniform relaxation times, making it generally less precise than ^{31}P NMR.

- **Factors Influencing DS**

The achieved DS is a function of multiple parameters, including the reaction time, temperature, concentration and ratio of reagents (lignin:anhydride:catalyst), the type of catalyst used, the specific structure and reactivity of the starting lignin (influenced by source and isolation method), and any pretreatments applied (e.g., particle size reduction via ultrasonication, swelling or activation via IL treatment). Under optimized conditions, near-complete acetylation (DS approaching 100% conversion of all hydroxyl groups) can be achieved, as confirmed by ^{31}P NMR showing disappearance of OH signals. Achieving selective acetylation (e.g., acetylating only phenolic OH while leaving aliphatic OH intact, or vice versa) is generally difficult using standard acetic anhydride methods, as both types of hydroxyl groups tend to react, although potentially at different rates depending on conditions and catalysts (BUONO et al., 2016a; DIMENT et al., 2024; SHEN; XIE; WANG, 2017; ZHAO et al., 2017).

2.4.4 Impact on properties

Acetylation leads to measurable changes in various lignin properties, influencing its behavior both as a standalone material and as a component in formulations.

- **Solubility:** As previously noted, acetylation significantly enhances solubility in many common organic solvents. Acetylated Kraft, soda, and organosolv lignins were found to be almost completely soluble (>95 mg/100 mL or >9.5 mg/mL) in solvents like THF, chloroform, dichloromethane, acetone, ethyl acetate, pyridine, and DMSO, whereas the corresponding unmodified lignins showed much lower and variable solubility in most of these solvents (except DMSO and pyridine). This improved solubility is crucial for solution processing techniques like solvent casting or electrospinning, and for analytical methods like Gel Permeation Chromatography (GPC) which often require acetylated samples for accurate molecular weight determination. The solubility behavior is related to the solubility parameters of the lignin and the solvent, although prediction based solely on these parameters can be poor for unmodified lignin due to complex interactions like hydrogen bonding, which are reduced upon acetylation (SAMENI; KRIGSTIN; SAIN, 2017a).

- **Polymer Blend Performance:** The improved compatibility resulting from acetylation often translates to enhanced performance in polymer blends.
    - *Mechanical Properties:* Compared to blends with unmodified lignin, incorporating acetylated lignin into matrices like PLA or PP often leads to significant improvements in impact strength and elongation at break (ductility). For instance, a PLA blend with 20 wt% acetylated lignin showed a 74% increase in Charpy impact strength compared to the blend with 20 wt% unmodified lignin. This indicates better stress transfer and energy absorption due to improved interfacial adhesion. Effects on tensile strength and Young's modulus are more variable. While acetylated lignin blends sometimes show slightly higher tensile strength than unmodified lignin blends , both often result in lower strength and modulus compared to the neat polymer matrix, especially at higher loadings. However, acetylation can mitigate the reduction in properties compared to unmodified lignin. The optimal loading level for achieving desired properties needs careful consideration, as high loadings (>5-10 wt%) can sometimes lead to property deterioration even with modification (JOHANSSON et al., 2023a).

- **Antioxidant Activity:** As established, the blocking of essential phenolic hydroxyl groups during acetylation leads to a significant loss of lignin's intrinsic radical scavenging ability. Studies comparing unmodified lignin fractions with their methylated (another OH-blocking modification) counterparts showed that the modified lignins lost their antioxidant capacity in stabilizing polyethylene against thermal oxidation. Therefore, if antioxidant properties are a key requirement for the final application (e.g., active packaging, stabilization of lubricants), acetylation is generally counterproductive , and alternative modifications or the use of unmodified lignin (perhaps fractionated to enrich phenolic content) should be considered (DIAS et al., 2017; JEDRZEJCZYK et al., 2021; SADEGHIFAR; ARGYROPOULOS, 2015).

- **Hydrophobicity:** Acetylation demonstrably increases lignin's hydrophobicity. This is reflected in reduced moisture uptake over time and potentially higher water contact angles on surfaces of materials containing acetylated lignin. This property is beneficial for applications requiring water resistance or dimensional stability under humid conditions (JOHANSSON et al., 2023a; LISÝ et al., 2022).

It is plausible, though not always explicitly demonstrated in the reviewed literature, that the degree of substitution (DS) achieved during acetylation significantly influences the magnitude of these property changes. A higher DS, meaning more hydroxyl groups are converted to acetyl groups, would logically lead to greater increases in hydrophobicity, organic solvent solubility, and compatibility with non-polar matrices, along with a more pronounced decrease in Tg and antioxidant activity. Some studies hint at this correlation; for example, controlling DS via microwave temperature influenced the viscoelastic properties of oleogels (BORRERO-LÓPEZ et al., 2023) , and achieving "complete" acetylation led to a substantial Tg drop (DIMENT et al., 2024). Similarly, using acetylated lignin with "medium" DS was found effective for thickening lubricants (TREJO-CÁCERES; SÁNCHEZ; MARTÍN-ALFONSO, 2023). However, systematic studies directly correlating specific DS values (differentiating between phenolic and aliphatic OH conversion) with quantitative changes in thermal, mechanical, and solubility properties are still needed for a more predictive understanding. This represents an important area for future research to enable fine-tuning of lignin properties through controlled acetylation.

3 UNVEILING THE MECHANISTIC ASPECTS OF DIFFERENT DELIGNIFICATION METHODS AND ITS EFFECTS ON THE STRUCTURE OF TECHNICAL EUCALYPTUS LIGNINS

Note: This chapter is based on the following published article:
OLIVEIRA, D. R. de; MATOS, M.; SILVA, K. T.; MAZZETTO, S. E.; MAGALHÃES, W. L. E.; LOMONACO, D.; AVELINO, F. Unveiling the mechanistic aspects of different delignification methods and their effects on the structure of technical Eucalyptus lignins. Biomass Conversion and Biorefinery, [S.l.], 2025. https://doi.org/10.1007/s13399-025-06502-y.

RESUMO

A aplicação correta da lignina está diretamente relacionada à sua estrutura, que é fortemente influenciada pelo método de extração. Neste trabalho, três ligninas técnicas foram obtidas de *Eucalyptus urograndis* por meio dos métodos Kraft, pirólise rápida (FP) e deslignificação organossolvassistida por micro-ondas (MWAOD), e suas propriedades estruturais e antioxidantes foram avaliadas e comparadas com as da lignina de madeira moída (MWL). As amostras foram caracterizadas por FTIR, NMR 2D e ^{31}P, GPC, TGA, DSC e ensaio DPPH. As ligninas técnicas foram obtidas com rendimentos elevados (33 – 68,5%) e altas purezas (76 – 93%). A combinação de diferentes técnicas analíticas mostrou que quanto maior a severidade, menores os pesos moleculares (657 – 1959 g mol-1), maior a quantidade de estruturas condensadas (β-5’ e β-β’) e maior a concentração de hidroxilas fenólicas. Durante o processo MWAOD, ocorreu acetilação parcial das hidroxilas alifáticas da OL sob condições brandas. A estabilidade térmica e a mobilidade molecular das ligninas também foram afetadas pela natureza do método de extração. O ensaio DPPH mostrou que as características estruturais das ligninas também afetaram suas atividades antioxidantes, uma vez que os valores de IC_{50} variaram de 13,2 a 19,3 µg mL^{-1}. Dessa forma, três ligninas técnicas com características estruturais diferentes foram obtidas da mesma biomassa através de processos distintos, oferecendo diferentes possibilidades para sua valorização.

Palavras-chave: madeira de lei; valorização da lignina; ligninas técnicas.

ABSTRACT

The correct application of lignin is directly related to its structure which is strongly affected by the extraction method. In this work, three technical lignins were obtained from *Eucalyptus urograndis* by Kraft, fast pyrolysis (FP), and microwave-assisted organosolv delignification (MWAOD), and their structural and antioxidant properties were evaluated and compared to those of milled wood lignin (MWL). The samples were characterized by FTIR, 2D and ^{31}P NMR, GPC, TGA, DSC and DPPH· assay. The technical lignins were obtained with elevated yields (33 – 68.5 %) and high purities (76 – 93 %). The combination of different analytical techniques showed that the higher the severity the lower the molecular weights (657 – 1959 g mol^{-1}), the higher the quantity of condensed structures (β-5' and β-β'), and the higher the concentration of phenolic hydroxyls. During MWOAD process, partial acetylation of aliphatic hydroxyls of OL occurred under mild conditions. The thermal stability and the molecular mobility of lignins were also affected by the nature of the extraction method. The DPPH· assay showed that the structural features of lignins also affected their antioxidant activities, since the IC$_{50}$ values varied from 13.2 to 19.3 μg mL^{-1}. Therefore, three technical lignins with different structural features were obtained from the same biomass through distinct processes, offering different possibilities for their valorization.

Keywords: hardwood; lignin valorization; technical lignins.

3.1 Introduction

Lignin is the most abundant natural polyphenol on Earth and holds significant potential as a source of building block compounds that can replace petroleum-derived materials in various technologies. Its complex structure arises from the random free radical polymerization of three monolignols: syringyl (S), guaiacyl (G), and p-hydroxyphenyl (H). These monolignols are interconnected through ester, ether, and carbon-carbon bonds, resulting in a three-dimensional amorphous polymer. However, the structural complexity of lignin poses challenges for its valorization, as its composition can vary widely depending on the biomass source and the methods used for extraction and isolation. (FIGUEIREDO et al., 2018).

Key structural features of lignin, such as molecular weight distribution, lignin-carbohydrate complexes (LCC), condensed units, and ash and sulfur contents, are influenced by process parameters including temperature, reaction time, and pH (WANG, PU, et al., 2019). Therefore, selecting appropriate applications for lignin requires a thorough investigation of its structural characteristics.

Lignin can be sourced from various materials, including both wood and non-wood resources. Among these, wood-derived lignin has emerged as a promising alternative to petroleum-based compounds due to its abundance and versatility (TRIBOT et al., 2019). *Eucalyptus* species, particularly *Eucalyptus urograndis*, are widely utilized in the pulp and paper industries, making them an attractive source for lignin extraction (PIGHINELLI et al., 2014a; PIGHINELLI; SCHAFFER; BOATENG, 2018a). Despite its potential, most research focuses on the pulping and bleaching aspects of *Eucalyptus urograndis* to obtain high-quality cellulose (DA SILVA MORAIS; SANSÍGOLO; DE OLIVEIRA NETO, 2016; HÄNNINEN et al., 2008; MARTINO et al., 2013; NEIVA et al., 2015), leaving a gap in the literature concerning its use for lignin extraction (SANTOS et al., 2011; SILVA et al., 2012).

The pulp and paper industry is the main producer of lignin through the Kraft process, which is carry out in an aggressive environment, the “white liquor”, composed by sodium sulfide under alkaline medium and temperatures ranging from 150 to 180 °C for 1-3 h (CRESTINI et al., 2017; GALKIN; SAMEC, 2016; RINALDI et al., 2016a). The lignin obtained by this process has specific features, such as high ash, sulfur and condensed units contents, which decrease its reactivity and limit its application (GALKIN; SAMEC, 2016). Although Kraft lignin (KL) has these drawbacks, it is crucial to explore and understand its structure in order to find a suitable application for it, since its production exceeds 70 million tons per year, being 95 % of that burned in the boilers to produce steam and energy (LAURICHESSE; AVÉROUS, 2014b).

Certain applications require lignin with higher purity standards. An alternative to obtain high-quality, sulfur-free lignin is the organosolv process, which utilizes organic solvents (such as formic acid, acetic acid, methanol, or ethanol) catalyzed by inorganic acids to dissolve lignin during biomass hydrolysis. This method typically requires elevated temperatures (130 – 205 °C) and extended reaction times (60-120 minutes), leading to high energy consumption (AVELINO et al., 2018a; DE LA TORRE et al., 2013b; EL HAGE et al., 2010; GALKIN; SAMEC, 2016; PINHEIRO et al., 2017). Recently, our research group has developed an eco-friendly technique called microwave-assisted organosolv delignification (MWAOD), which operates under mild conditions (110°C and atmospheric pressure) for only 20 minutes, producing high-purity lignin with elevated yields and lower molecular weights—essential for scalable production and effective valorization. (AVELINO et al., 2018b).

Another process that can be used to fractionate the biomass is the fast pyrolysis (FP), which has been explored to produce fuels and several interesting coproducts for the chemical industry. This process can use several types of feedstocks, such as wood fines (MATOS et al., 2020a), however the use of *Eucalyptus urograndis* is particularly interesting since its remarkable features, such as bulky density and high calorific power (PIGHINELLI; SCHAFFER; BOATENG, 2018b). FP submits the biomass to high temperature (≈500 °C) during short residence times (≈2 s) under an inert atmosphere, characterizing it as a high severity process (BRIDGWATER, 2012; TORRI et al., 2016).

FP yields a solid (bio-char), liquid (bio-oil) and gaseous phases (syngas) that have interesting technological applications (CHENG et al., 2017; PIGHINELLI et al., 2014b; PIGHINELLI; SCHAFFER; BOATENG, 2018b). The bio-oil has been used in its crude form for different purposes (KOSINSKI LIMA et al., 2018), however, since it is a complex mixture of phenolic and carbohydrate-derived compounds, the bio-oil can be fractionated in order to maximize the utilization of its fractions (LENG et al., 2017b). The water insoluble fraction is known as pyrolytic lignin (PL), which can be transformed on monophenols through a depolymerization process or directly used as raw material in chemical industries by different technological routes (LENG et al., 2017b).

Given the limited research on lignin extraction from *Eucalyptus urograndis* wood (EUW), this study aims to evaluate the structural characteristics of three technical lignins obtained from this species, comparing them to those of their respective protolignin, milled wood lignin (MWL). This comparison seeks to establish a structure-property relationship that could unveil new opportunities for lignin valorization.

3.2 Experimental Section

3.2.1 Materials

The following chemicals were used as received: glacial acetic acid (Synth), sulfuric acid (H2SO4, 98 %, Vetec), toluene (99.5 %), ethanol (99.5 %), 1,4-dioxane (99.8 %), dichloroethane (99.0 %), diethyl ether (99.0 %), petroleum ether, deuterated dimethyl sulfoxide (DMSO-d6) (99.96 %, Sigma-Aldrich), tetrahydrofuran (THF, HPLC-grade, Sigma-Aldrich), deuterated chloroform (99.96 %, Sigma-Aldrich), pyridine (99 %, Neon), chromium (III) acetylacetonate (99.99 %, Sigma-Aldrich), cyclohexanol (99 %, Sigma-Aldrich) and 2-chloro-4,4,5,5 – tetramethyl-1,3,2 – dioxaphospholane (TMDP-Cl, 95 %, Sigma-Aldrich).

3.2.2 Extraction of Eucalyptus lignins using different methods

Milled wood lignin (MWL) was isolated according to the method described by Guerra et al. (2004) and Holtman et al. (2006) with minor modifications (GUERRA et al., 2004; HOLTMAN et al., 2006). Kraft lignin (KL) was gently donated by Suzano Papel e Celulose S/A (Suzano, Brazil). Pyrolytic lignin (PL) was obtained from the bio-oil of the fast pyrolysis of eucalyptus wood fines as described by the methodology developed by our group (MATOS et al., 2020b). Organosolv lignin (OL) was obtained through microwave-assisted organosolv delignification (MWAOD) process as previously reported by our group (AVELINO et al., 2018b). The experimental details about the extraction processes are described in the supporting information (Appendix A). The lignin yield, for all obtained lignin samples, was calculated according Eq. (1):

$$\eta = \frac{m_{LigIsol}}{m_{LigEUW}} \quad \text{Eq. (1)}$$

Where η is the lignin yield (%); mLigIsol is the mass of lignin obtained from each different extraction process (g); and mLigEUW is the mass of lignin present in EUW (g) as previously determined by TAPPI standards.

3.2.3 Structural characterization of Eucalyptus lignins

The purity of lignin samples was evaluated according to TAPPI T222 om-2 method with some modifications. The acid-soluble lignin was determined by UV-Vis spectroscopy using the first filtrate obtained in the acid-insoluble lignins (Klason lignin) experiments. The sum of acid-insoluble and acid-soluble lignins (total lignin content) was reported as the purity of the samples.

The content of carbon, hydrogen, nitrogen and sulfur (CHNS analysis) of each lignin was determined using a Perkin Elmer 2400Series II CHN-S/O analyzer according standard procedures. The percentage of oxygen was calculated by subtracting the C, H,N and S contents from 100%.

Fourier transform infrared spectroscopy (FTIR) analyses were performed using a spectrometer (PerkinElmer, FT-IR/NIR FRONTIER) using an attenuated total reflectance (ATR) accessory with ZnSe crystal surface. The analyses were recorded between 4000 and 550 cm^{-1} with spectral resolution of 4 cm^{-1} using the average of 32 scans.

^{1}H-^{13}C heteronuclear single quantum coherence (^{1}H-^{13}C HSQC) experiments were performed in a Bruker Avance DPX 300 operating at 300 and 75 MHz for ^{1}H and ^{13}C nuclei, respectively. Samples (30 mg) were completely solubilized in 500 µL of DMSO-d6, and the peak related to the residual DMSO was used as internal reference (δ_H/δ_C 2.50/39.5).

^{31}P NMR experiments were carried out after phosphytilation of lignins, according to Granata and Argyropoulos (1995) with slight modifications (GRANATA; ARGYROPOULOS, 1995b) for determining the different types of hydroxyl groups present in the lignins. The spectra were acquired on a Bruker Avance DPX500 spectrometer operating at 202.46 MHz for ^{31}P nucleus.

Gel permeation chromatography (GPC) analyses were performed in a liquid chromatograph (Shimadzu LC-20AD) at 40 °C using a setup comprising two analytical GPC columns in series (Phenogel 5µ 50Å and Phenogel 5µ 103Å, 4.6 mm x 300 mm, Phenomenex) and HPLC-grade THF as mobile phase at flow rate of 0.35 mL min^{-1}. The samples were monitored by UV-Vis detector (Shimadzu SPD-M20A) at 280 nm. The calibration curve was constructed using polystyrene standards with M_w values ranging from 162 to 1.3×10^{5} g mol^{-1}.

3.2.4 Thermal behavior and antioxidant activity of Eucalyptus lignins

Thermogravimetric analyses (TGA) were performed using a thermal analyzer (Mettler-Toledo, TGA/SDTA 851e), in which 10 mg of sample was heated from 30 to 900 °C under nitrogen atmosphere (50 mL min-1) at a heating rate of 10 °C min-1.

Differential scanning calorimetry (DSC) analyses were carried out in a calorimeter (Mettler-Toledo, DSC 823e), in which 10 mg of lignin was placed in a closed aluminum crucible with a lid centrally punctured. Samples were submitted to an annealing procedure, where they were heated from 25 to 90°C, where temperature was kept constant at 90 °C for 10 minutes, then cooled from 90 to 0°C, where temperature was kept constant at 0 °C for 3 minutes and heated from 0 to 250°C, under a nitrogen atmosphere (50 mL min^{-1}) at a heating rate of 20 °C min^{-1}.

The DPPH free radical scavenging assay was performed using a UV-Vis spectrophotometer (Cary 60, Agilent Technologies) and an experimental protocol previously reported by our group (AVELINO et al., 2019).

3.3 Results and discussion

3.3.1 General overview of the processes by wet analysis and CHNSO analysis

The conditions of temperature, pressure, reaction time, pH and catalyst used to promote the fractionation of biomass severely affect the structure of its components, such as the lignin. Two important parameters for evaluating the efficiency of the delignification process are the lignin yield and purity. Table 2 shows the acid insoluble lignin (Klason lignin), acid soluble lignin, purity and yield values, as well as elemental analysis (CHNSO) for MWL, KL, PL and OL, respectively.

Table 2 - Wet analysis and CHNSO data for *Eucalyptus* lignins.

Content/Sample	**MWL**	**KL**	**PL**	**OL**
wet analysis				
Acid insoluble lignin (%)	90.0 ± 2.0	86.5 ± 3.1	90.0 ± 0.1	72.7 ± 0.4
Acid soluble lignin (%)	9.0 ± 2.0	7.1 ± 0.5	6.0 ± 0.1	3.51 ± 0.1

Purity (%)	99.0	93.6	96.0	76.2
Yield (%)	2.0 ± 0.3	50	33 ± 1.0	68.5 ± 5.7
CHNSO analysis				
Carbon (%)	56.63± 0.01	60.95 ± 0.01	65.00 ± 0.33	60.45 ±0.04
Hydrogen (%)	6.17 ±0.00	6.12±0.01	6.3±0.03	5.83±0.03
Nitrogen (%)	0.39±0.02	0.42±0.11	0.75±0.01	0.30±0.03
Sulphur (%)	1.24±0.07	2.92±0.49	1.34±0.04	1.07±0.05
Oxygen (%)	35.58±0.08	29.61±0.59	26.62±0.27	32.36±0.01

Source: The author

Table 2 shows that all technical lignins had high purities, although OL presented a lower value compared to the others. This abnormal behavior can be attributed to the amount of water in the acetosolv solution, since water molecules are responsible for the cleavage of ester bonds in LCC. Therefore, their amount in the medium could acted as a limiting factor in the separation of lignin from the carbohydrates. This fact combined to the mild conditions employed in the MWAOD process can contributed to this unusual purity value. On the other hand, KL and PL were obtained from harsh processes which employed high temperatures and pressures, favoring the extensive cleavage of ester bonds between lignin and carbohydrates and, consequently, yielding high purity lignins.

It is also possible to observe from **Table 2** that all technical lignins presented high yields, which were considerable higher than that of MWL. This is directly related to the reaction parameters of the processes, since the conditions used for MWL extraction are not aggressive enough to promote an extensive delignification. However, the other processes are harsh enough to cause the deconstruction of the biomass, yielding considerable amounts of lignin per extraction.

It is worth mentioning that PL had the lowest yield value although it was obtained from the most aggressive process. The high temperature and pressure used in FP can favor the formation of small molecules, such as monophenols which cannot be recovered in the isolation step, since they are water-soluble, diminishing the lignin yield. However, despite that significant amount of water-insoluble lignin can be recovered by this process, showing its feasibility.

The elemental composition (CHNSO analysis) of four lignin samples was analyzed to assess the impact of different extraction methods on lignin structure. While all samples share a substantial carbon content (ranging from 56.63% to 65.00%), significant variations were observed in oxygen contents, reflecting the distinct chemical modifications imparted by each extraction method (Table 2).

The most notable differences lie in the oxygen content. MWL, representing a relatively unmodified lignin structure, exhibits the highest oxygen content (35.58%). This is consistent with the presence of abundant oxygenated functional groups in native lignin. Conversely, PL shows the lowest oxygen content (26.62%), a direct consequence of the pyrolysis process, which leads to the significant removal of oxygenated functional groups through decarboxylation and dehydration reactions, resulting in a more condensed and aromatized structure. The KL sample, extracted under strongly alkaline conditions, displays an intermediate oxygen content (29.61%), suggesting that while some oxygenated groups are removed during kraft pulping, the degree of modification is less extensive than in pyrolysis. The OL sample presents an oxygen content (32.36%) slightly lower than MWL, indicating that while the organosolv method is considered milder, some structural modifications still occur, leading to a reduction in oxygenated functional groups compared to the native lignin.

All samples exhibited generally low nitrogen content. As expected, KL samples showed significantly higher sulfur content (2.92%), a result of sulfur-containing chemicals used in the kraft pulping process.

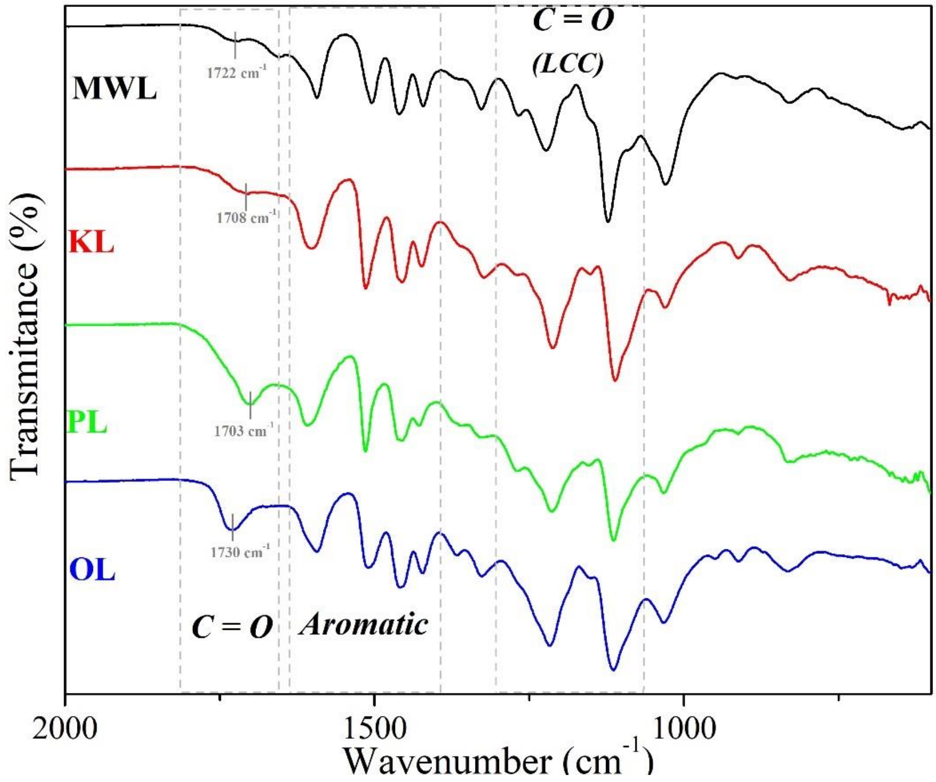
The differences in elemental composition (especially oxygen) among the lignin samples clearly demonstrate the substantial impact of different extraction methods. The data suggests that while MWL retains a relatively native structure, the kraft, organosolv, and pyrolysis methods induce varying degrees of structural modification, affecting the overall oxygen content of the final lignin product.

3.3.2 Fourier transform infrared spectroscopy (FTIR)

The main functional groups in the lignins and any modification in its structure resulted from the different extractions were monitored by FTIR. The peak assignments were conducted based on those made by Faix (1991) (FAIX, 1991). Figure 5 shows that all Eucalyptus lignins spectra presented similar spectral patterns with some differences in their intensities. The region comprised between 1800 – 550 cm^{-1} is known as fingerprint, which has valuable information about the main characteristic absorption bands of functional groups present in the lignin structure. The full FTIR spectra of Eucalyptus lignins are shown in Figure S1 (Appendix A)

.

Figure 5 - Fingerprint region of Eucalyptus lignins FTIR spectra.



Source: The author

Among these bands, some of them deserve a special attention, such as those at 1602, 1514 and 1505 cm-1 related to C=C stretching of the aromatic ring skeleton; 1460 and 1423 cm-1 related to C-H deformations and aromatic skeletal vibrations coupled with C-H in plane deformation, respectively. The presence of these bands in all lignins means that their backbone was sustained after the biomass fractionation, despite the severity of the processes.

The absorption bands at 1705, 1269, 1153 and 1121 cm^{-1} were assigned to C-O stretching, which is commonly attributed to carbohydrates linked to lignin as a lignin-carbohydrate complexes (LCC). These bands can be used as a way to estimate the efficiency of the delignification process and the purity of the lignin, since they are an indicative of residual carbohydrates in the lignins, which were not cleaved during the extraction (LIU et al., 2017).

Therefore, the existence of absorption bands related to LCC in all lignin spectra combined to the Klason lignin and purity values shown in **Table 2** suggests that the linkage between carbohydrates and lignin is a little recalcitrant, because even when high severity processes were used, significant amount of residual carbohydrates were detected by both methods.

A remarkable difference can be observed in the intensities and wavenumbers of the absorption bands related to C=O stretching for each lignin, which are 1722, 1708, 1703 and 1730 cm^{-1} for MWL, KL, PL and OL, respectively. This behavior suggests that depending on how harsh the extraction process is, different oxygenated species could be formed.

In the case of KL and PL that were obtained by high severity processes, the oxidative environment could favor the formation of ketones through the oxidation of secondary alcohols in the phenylpropanoid chains, which explains the low wavenumbers in relation to that of MWL. On the other hand, OL that was extracted under mild conditions, presented the band at a higher wavenumber in comparison to the other lignins, specifically in the region related to aliphatic esters. This could be explained by the occurrence of a partial acetylation of the OL's aliphatic hydroxyls during the hydrolysis, since it was carried out using acetic acid as solvent.

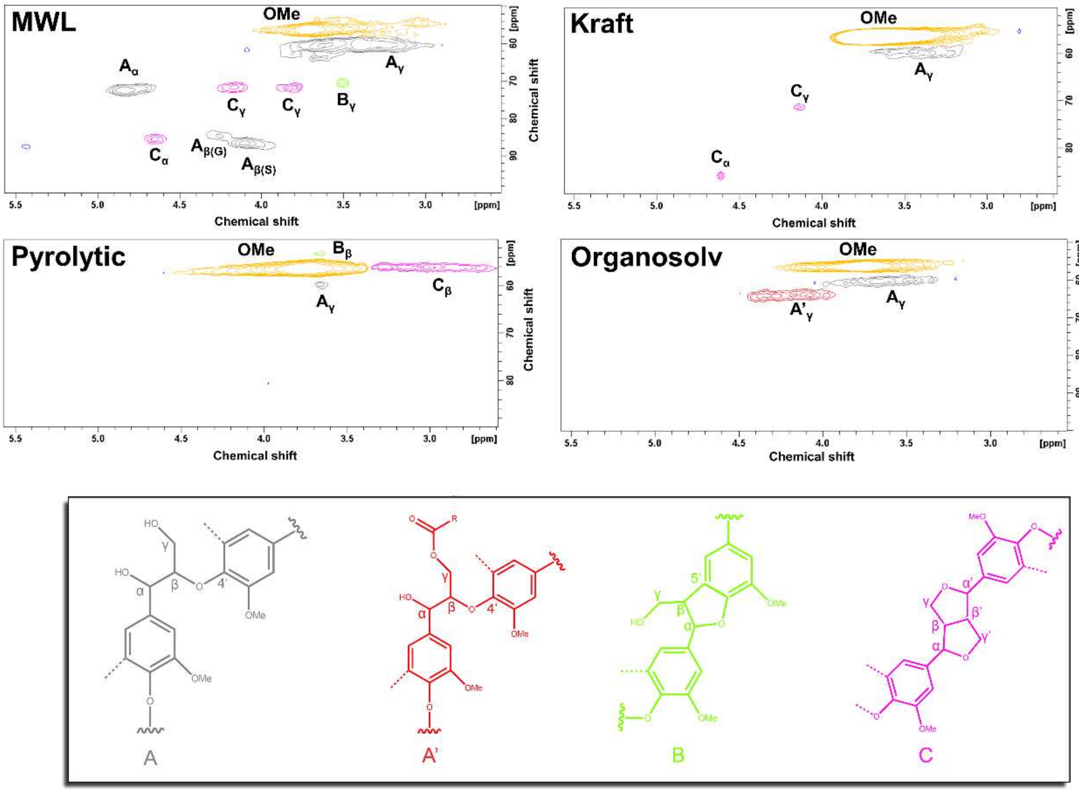
3.3.3 ^{1}H-^{13}C HSQC NMR

The main structural modifications resultant from the use of processes with different severity degrees were assessed by the ^{1}H-^{13}C HSQC NMR, in which the changes in the interunit linkages (δC/δH 50 – 95 ppm/2.5 – 5.5 ppm) and monolignol composition (δC/δH 100 – 150 ppm/6.0 – 8.0 ppm) were accompanied and compared to those of MWL, as show in figures 6 and 7, respectively. These figures also show the main depicted substructures identified in the lignins' spectra.

In addition, the changes in the intensity of the cross-peaks were evaluated by using the relative proportion of interunit linkages (RPIL) and monomers (RPM), which were calculated by the integration of the cross-peaks in the oxygenated aliphatic and aromatic regions, as shown in Tables 2 and 3, respectively. The assignment of the main cross-peaks in

1H-13C HSQC spectra have made based on previous reports in the literature (LENG et al., 2017c; PEREIRA et al., 2017; ZHOU et al., 2016c) and they are listed in Table S1.

Figure 6 - Oxygenated aliphatic region of 1H-13C HSQC spectra of Eucalyptus lignins.



Source: The author

Table 3 - Relative proportion of interunit linkages of Eucalyptus lignins.

Sample	A (%)	A' (%)	B (%)	C (%)
MWL	86.4	-	1.9	11.6
KL	82.0	-	-	18.0
PL	6.9	-	3.9	89.2
OL	48.4	51.6	-	-

Source: The author

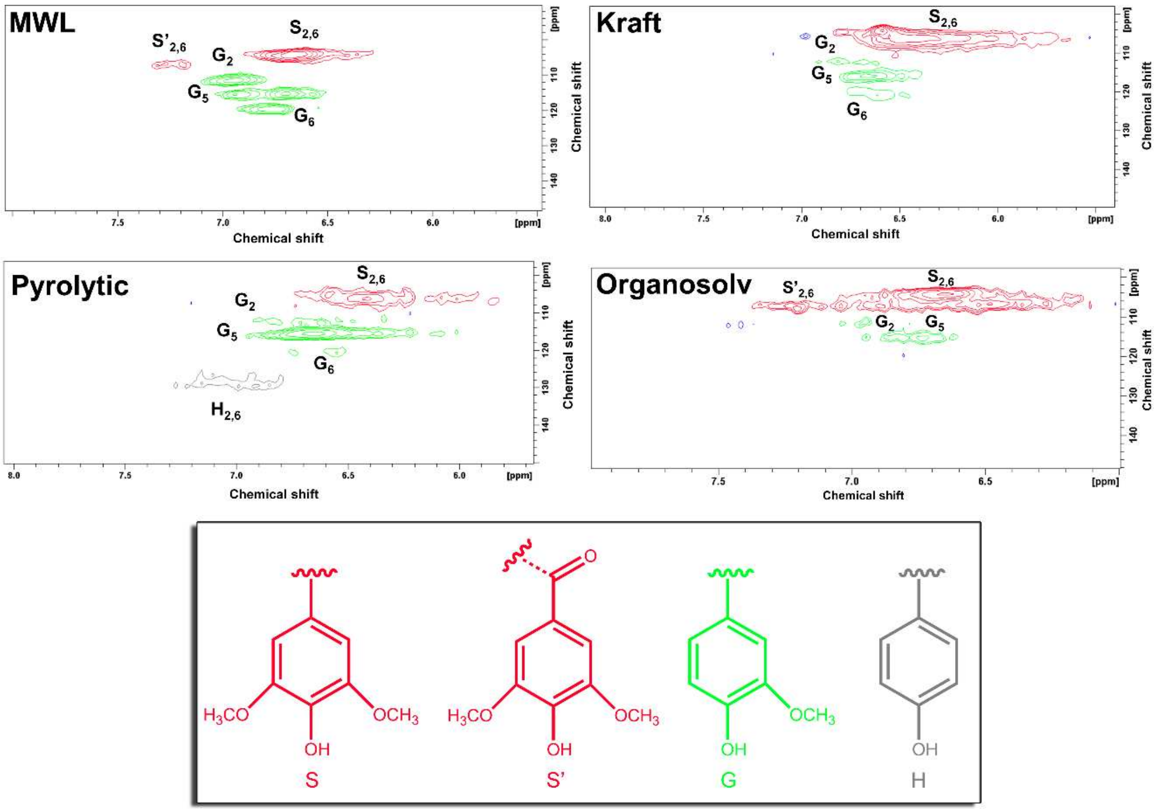
Figure 6 and Table 3 show that the most abundant linkage present in native lignin (MWL) is the ether bonds, specifically the β-O-4 ones, substructure A. The most used process in industrial scale, Kraft, is commonly carry out under harsh conditions (1 mol L-1 NaOH and 0.25-0.70 mol L-1 Na2S aqueous solution at 165-175°C during 1-2h), yielded a lignin with high β-O-4 and condensed units in comparison with the native lignin (RINALDI et al., 2016a).

The high content of β-O-4 bonds in KL could be attributed to the occurrence of parallel reactions, such as the cleavage of α-O-4 bonds in phenylcoumaran (**B**) structures, since it has a bond dissociation energy (BDE) of 50-56 kcal mol^{-1} against 54-72 kcal mol^{-1} for β-O-4 bonds (RINALDI et al., 2016b). The harsh conditions employed in the Kraft process could favor the cleavage of ether bonds in **B** substructure to the detriment of those in **A**. Another parallel reaction is the release of formaldehyde from the elimination of γ-CH_2OH groups, which would also justify the increase in the resinol (C) content from MWL to KL, since formaldehyde could favor the occurrence of recondensation reactions, inducing the formation of carbon-carbon (C-C) bonds (RINALDI et al., 2016b).

On the other hand, PL was obtained by a higher severity process that employs high temperature under inert atmosphere, and presented low content of **A** substructures, indicating that the major units (ether bonds) in the native lignin were cleaved. In addition, the harsh conditions of pyrolysis favored the increase of the amount of condensed units, such as **B** and **C**, through the formation of C-C bonds (β-5' and β-β', respectively), as a result of the thermal degradation of phenylpropanoid chains. The presence of condensed structures can offer interesting thermal properties to PL and can play a key role in its performance depending on the application.

OL was obtained under mild conditions (atmospheric pressure and short reaction time), and presented a significant decrease in the content of **A** structures. This shows the potentiality of microwave-assisted organosolv delignification (MWAOD), since it has the lowest severity degree compared with Kraft and pyrolysis processes and it was able to promote the cleavage of β-O-4 in a great extension. In addition, this process also allows the occurrence of the partial acetylation of aliphatic hydroxyls during the delignification, mainly in γ-position, generating the A'γ substructure. This kind of feature can be very valuable in some applications that require a balance of polarity, such as in the compatibilization of lignin with thermoplastics (LI; SARKANEN, 2002, 2005) and in the synthesis of lignin micro/nano-particles (GUPTA; WASHBURN, 2014; LI; GE; WAN, 2015; NYPELÖ; CARRILLO; ROJAS, 2015; OGUNKOYA et al., 2015).

Figure 7 - Aromatic region of 1H-13C HSQC spectra of Eucalyptus lignins.



Source: The author

Table 4 - Relative proportion of monomers (RPM) of Eucalyptus lignins.

Sample	S (%)	G (%)	H (%)	S/G ratio	H/G ratio
MWL	46.5	53.5	-	0.9	-
KL	71.4	28.6	-	2.5	-
PL	32.4	54.3	13.3	0.6	0.3
OL	89.1	10.9	-	8.2	-

Source: The author

Considering the general classification, Eucalyptus belongs to the class of hardwoods, yielding lignins composed mainly by guaiacyl (G) and syringyl (S) units, Figure 7.

Table 4 shows that MWL has almost the same S and G proportion, while that varies in the technical lignins depending on the harshness of the process.

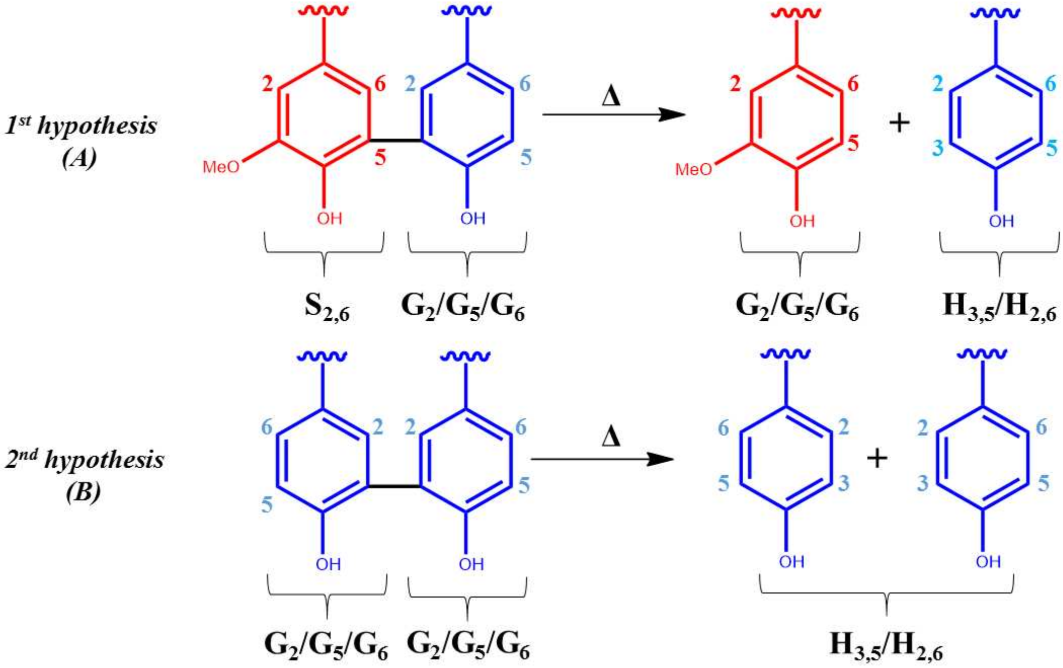
Therefore, a considerable increase of S and decrease of G occurred in Kraft and organosolv processes. In both cases, this fact suggests the occurrence of recondensation reactions between G units by the 5 position, forming C-C bonds, such as 5-5’ in biphenyl structures, which is a very strong and recalcitrant linkage (BDE: 70-118 kcal mol^{-1}) (HEITNER; DIMMEL; SCHMIDT, 2010).

In addition, the release of formaldehyde from the elimination of γ-CH_2OH groups can occur in both processes. This would also favor a recondensation reaction in the 5 position of G ring forming methylene bridges (BDE: 86-91 kcal mol^{-1}) (CRESTINI et al., 2017; RINALDI et al., 2016b), decreasing the C_5-H_5 correlation of G and increasing the $C_{2,6}$-$H_{2,6}$ of S ring.

However, among the technical lignins, PL had an interesting and remarkable behavior, which was the appearance of a cross-peak related to the $C_{2,6}$-$H_{2,6}$ correlation of **H** units. As mentioned before, the hardwood lignins are major composed of **G** and **S** units, as shown in the MWL, KL and OL spectra. Nevertheless, since the pyrolysis process employs high temperatures, some linkages with high BDE values such as C-C could be broken, justifying the presence of a cross-peak related to H unit.

Therefore, according to the MWL spectrum the first possibility would be the cleavage of a C-C bond of a condensed structure formed by S and G units, yielding a G and H units, **Scheme 1(A)**. On the other hand, another possible cleavage could be the C-C bond of a condensed structure formed by two G units, generating two H units, **Scheme 1(B)**. This structural feature could be very useful in applications that require a large amount of free ortho positions in relation to the phenolic hydroxyls, such as in the synthesis of phenolic resins, since the other technical Eucalyptus lignins (KL and OL) have presented high amount of S units, which limits its use for such application.

Scheme 1 - Possible pathways for some cleavage of condensed structures for pyrolytic lignin, yielding (A) G and H and (B) H units.

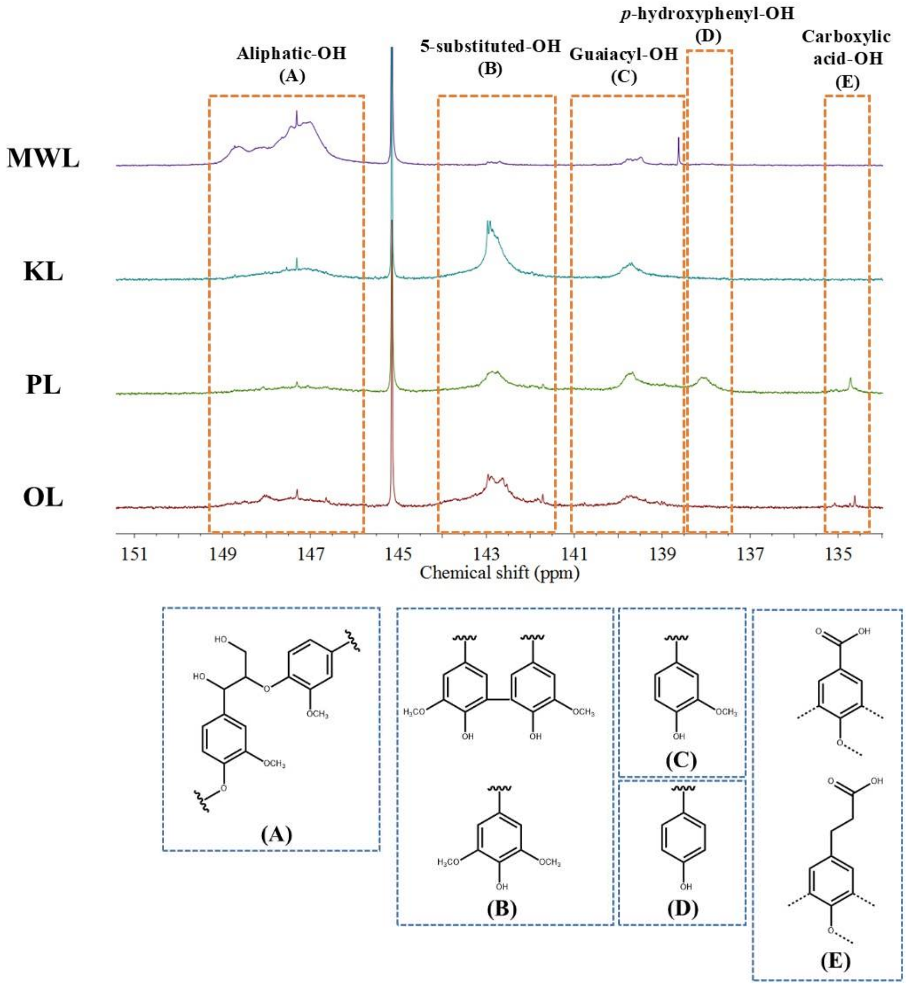


Source: The author

3.3.4 ^{31}P NMR

^{31}P NMR analysis provides valuable information about the amount of different types of hydroxyls groups present in the lignins' structure, such as aliphatic, phenolic (5-substituted-OH, G-OH, H-OH and carboxylic acid-OH), as well as their monolignol composition, allowing the elucidation of insights about the influence of the severity of the lignin extraction processes on their structures. Figure 8 shows the 31P NMR spectra of Eucalyptus lignins, in which the identified substructures are depicted. Table 5 summarizes the quantification data of the hydroxyl groups present in the Eucalyptus lignins.

Figure 8 - ^{31}P NMR spectra of Eucalyptus lignins and their identified substructures.



Source: The author

Table 5 - Quantification data of hydroxyl groups of *Eucalyptus* lignins.

Label/Sample	MWL	KL	PL	OL
Aliphatic-OH (mmol g^{-1})	4.05	0.70	0.54	0.71
5-substituted-OH (mmol g^{-1})	0.26	1.33	0.92	1.15
Guaiacil-OH (mmol g^{-1})	0.60	0.42	0.72	0.40
***p*-hydroxyphenyl-OH (mmol g^{-1})**	-	-	0.34	-
Carboxylic acid-OH (mmol g^{-1})	-	-	0.15	0.05
OH phenolic (mmol g^{-1})	0.86	1.75	1.98	1.55
Total OH groups' content (mmol g^{-1})	4.91	2.45	2.67	2.31

Source: The author

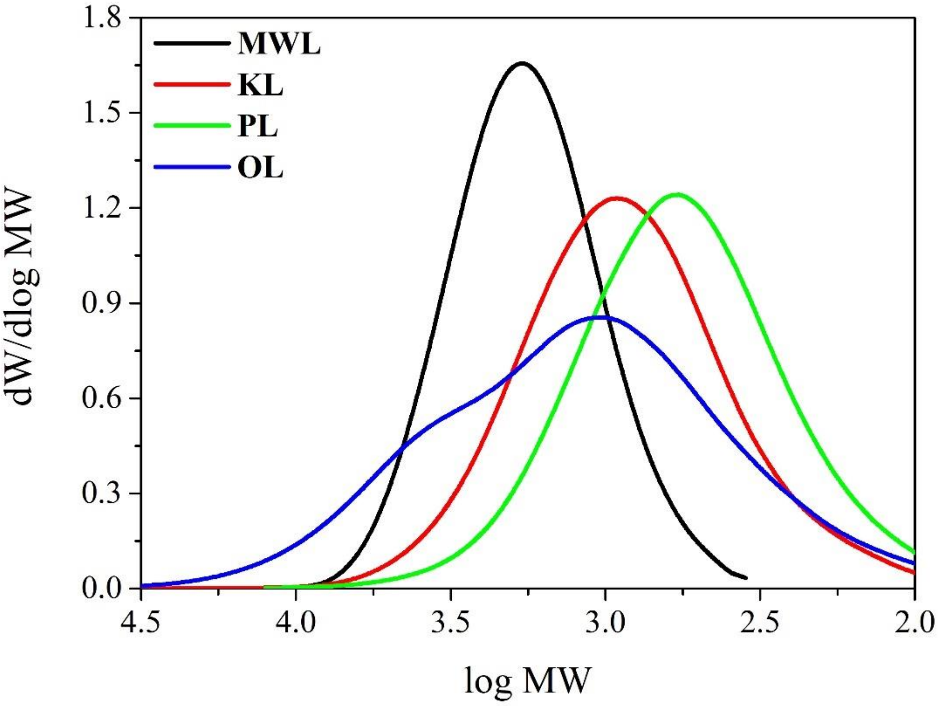
The results obtained from the 31P NMR spectra showed that the monolignol composition of Eucalyptus lignins corroborated with those provided by their 1H-13C HSQC spectra (Figure 8 and Table 5). It is possible to notice from Table 5 that the increase in the severity of the processes generated lignins with higher concentration of phenolic hydroxyls, probably due to the more extensive cleavage of β-O-4 linkages.

Figure 8 and **Table 5** also suggest that PL undergone severely structural changes, due to its highest values of phenolic and carboxylic acid hydroxyls, as well as the appearance of *p*-hydroxyphenyl units in its structure, indicating the extensive cleavage of C-C bonds between S and G units, as shown previously in **Scheme 1**. Therefore, these differences in the hydroxyl groups content offer a wide range of applicability for those lignins, since they probably will have different reactivity as well.

3.3.5 Gel permeation chromatography (GPC)

The extent of the cleavage of lignin linkages by using different extraction methods was evaluated by GPC. Figure 5 shows the molecular weight distribution curves of MWL, KL, PL and OL, respectively, and Table 5 summarizes their number-average (Mn), weight-average (Mw) molecular weights and polydispersity index (PDI).

Figure 9 - Molecular weight distribution curves of Eucalyptus lignins obtained by GPC.



Source: the author

Table 6 - Molecular weight data of *Eucalyptus* lignins obtained by GPC.

Sample	**M_w (g mol^{-1})**	**M_n (g mol^{-1})**	**M_w/M_n**
MWL	1959	1467	1.34
KL	1093	656	1.67
PL	657	433	1.52
OL	1805	600	3.01

Source: The author

Figure 9 shows that all lignins had different molecular weight distributions, which corroborates with their Mw, Mn and PDI values shown in Table 6. The observed behavior can be attributed to the severity of the processes, since parameters such as temperature, pressure, reaction time and pH severely affect the type of bonds that will undergo cleavage.

Therefore, as expected PL presented the lowest M_w and M_n values, since the FP process has the highest severity factor than the others used in this work. Thus, PL was obtained under high temperature, which favored the cleavage of several types of bonds in lignin structure, including the C-C bonds, yielding low molecular weight fragments. According to the M_w ranges in the literature (LENG et al., 2017c; WANG et al., 2014), PL would be form by tetramers or pentamers, which represent an important feature for several applications, since low molecular weight fragments tend to have high solubility in organic solvents and in another systems, such as fuels allowing its use as an additive.

Among the technical lignins, the one that had the highest M_w value was the OL, which was an expected result, since it was obtained under mild conditions in comparison to the others. However, it is important to consider the occurrence of recondensation and acetylation reactions during the organosolv pulping, as showed by FTIR and ^{1}H-^{13}C HSQC spectra of OL (**Figures 5, 6 and 7**), which can contribute to the increase in the M_w. Although high molecular weight fragments will have limited solubility in organic solvents and reactivity due to steric hindrance, some structural features such as partial acetylation of aliphatic hydroxyls can represent a great advantage for some purposes, especially in polymer chemistry.

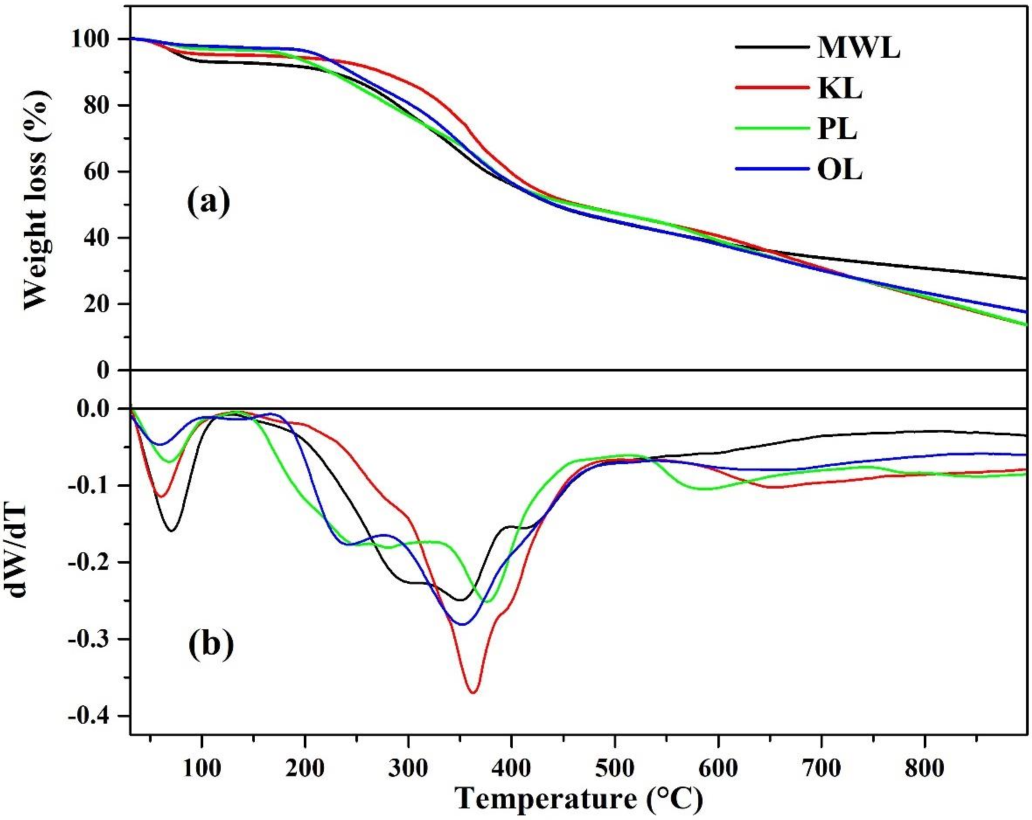
KL was the technical lignin that presented an intermediary behavior compared to PL and OL. The low M_w can be attributed to the high severity of the Kraft process, although recondensation reactions can also occur mainly due to the alkaline medium. Despite the low M_w of KL, it is worth mentioning some structural features that make it less noble than PL and OL, such as the sulfur and ash contents, which depending on the application could be a potential issue.

3.3.6 Thermal behavior

Given the numerous potential applications of lignin in materials that undergo thermal treatments during processing, a substantial interest exists in characterizing its thermal properties. As demonstrate previously in this work, structural features of lignin are affected by the extraction methods, and consequently, it is reasonable to anticipate that these varying extraction techniques will impart distinct thermal properties to lignin. Therefore, the thermal properties of Eucalyptus lignins were evaluated by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC).

Figures 10(A) and **10(B)** shows the TGA and DTG curves of MWL, KL, PL and OL, respectively. The values of initial degradation temperature (T_{onset}), maximum degradation temperature (T_{max}) and char yield are summarized in **Table 7**. The literature reports that thermal degradation of lignin can be often divided in three main stages (TEJADO et al., 2007; WATKINS et al., 2015). The first stage usually occurs between 30 – 120 °C and the weight loss is due to the evaporation of water absorbed. The second one usually takes place around 180 – 350 °C and it is attributed to the degradation of carbohydrates in the LCC. Finally, the third stage occurs over a wide range of temperatures above 350 °C and it is attributed to the degradation and vaporization of lignin-derived products, including phenolics, alcohols, aldehydes and carboxylic acids. As can be seen in DTG curves, MWL, PL and OL exhibit those three degradation events quite clearly. In KL, however, the second event seems to be overlaid by the third event, and it does not appear so clearly.

Figure 10 - (A) TGA and (B) DTG curves of Eucalyptus lignins obtained from different extraction methods



Source: The author

Table 7 - Thermal data of *Eucalyptus* lignins obtained by TGA and DSC analyses

Sample	T_{onset} (°C)	T_{max} (°C)	Char yield (%)	T_g (°C)
MWL	241	350	27.7	164
KL	291	360	13.5	149
PL	197	376	13.8	79 - 120

OL	211	353	17.7	108

Source: The author

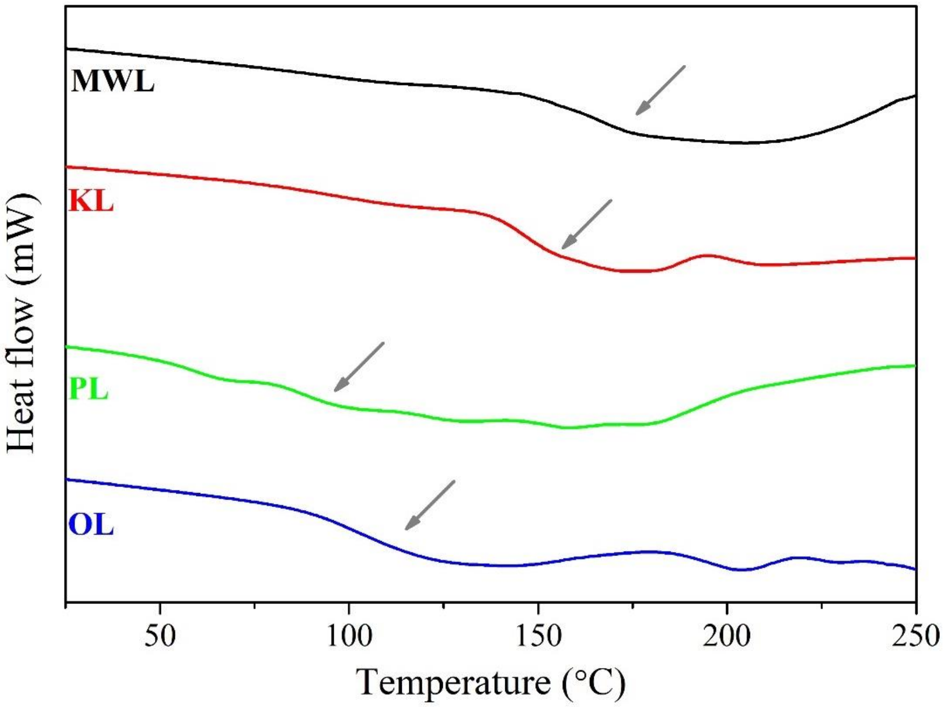
Concerning to the thermal stability of the lignins, as can be seen in Table 7, PL exhibited the lowest value of T_{onset}. This behavior can be related to the fact that this sample presented the lowest Mw value (section 3.3.5), which facilitates the volatilization of the compounds that result in the initial weight loss. In the other lignins, different factors appear to affect the thermal stability.

MWL and OL have greater Mw values than KL but their Tonset values are lower than that of KL. This can be attributed to their structure, since MWL and OL are composed of a larger number of substructures A (section 3.3.3), which have thermally labile β-O-4 linkages (KIM et al., 2014), resulting in a decrease of thermal stability. On the other hand, the higher T_{onset} value of MWL in relation to OL may be associated to its higher molecular weight combined to the fact that it has a larger number of condensed structures (substructures B and C), which are linked by C–C linkages, requiring more energy to be cleaved (RINALDI et al., 2016b).

The overlapping thermal events observed in the DTG curve of KL may result from the process's harsh conditions. The high proportion of condensed structures in KL, consequence of complex reactions at high temperature and alkalinity, might lead to less well-defined thermal degradation stages compared to other lignins. The presence of sulfur-containing compounds in KL, a byproduct of the process, could further contribute to this overlap, adding complexity to the thermal degradation profile. PL, while also containing many condensed structures, appears to exhibit a clearer TGA profile with less event overlap. This could be due to the more homogeneous nature of condensed structure formation during pyrolysis, a process involving more uniform thermal fragmentation. Therefore, the observed differences in TGA profiles may be explained by the distinct mechanisms and resulting structural heterogeneity of condensed units in KL. Further research is needed to confirm these hypotheses.

DSC measurements were carried out to determine the glass transition temperature (T_g) of lignins, which is reached when the polymeric chains have enough energy to rotate. At first, the polymeric chains in lignin vibrate when they receive energy, and the rotation of the lignin creates a free volume. Low molecular weight fragments have high speed mobility, requiring less energy to rotate the chain (SA'DON; RAHIM; HUSSIN, 2017). In **Figure 11**, the T_g*´s* of *Eucalyptus* lignins are indicated by arrows and their values are summarized in **Table 7**.

Figure 11 - DSC curves of Eucalyptus lignins.



Source: The author

As expected, PL presented the lowest value of Tg since it had the lowest Mw value, corresponding to shorter polymeric chains, which require less energy to allow their mobility. On the other hand, MWL with the highest Mw value needs more energy to rotate the polymeric chains and consequently had the higher Tg value. In this sense, OL was expected to have a higher Tg value than KL, but this was not observed. This behavior can be probably explained by the fact that KL is composed by a larger number of condensed structures (substructure C) than OL, which hinder the mobility of polymeric chains and consequently increases its Tg value. However, the presence of substructure A' in OL increased the degree of freedom, since the acetyl groups increase the free volume and also decrease the occurrence of hydrogen bondings, facilitating the mobility of the chains and decreasing its Tg.

Therefore, the severity of the extraction process will dictate the extent of cleavage of bonds and consequently the lignin structure, which includes its main substructures and functional groups, as well as its molecular weight distribution. All these parameters combined will affect directly the processability of the lignin. This is a very important characteristic for several applications, mainly for those in polymer chemistry, since it is related to the range of temperature in which the material gains molecular mobility.

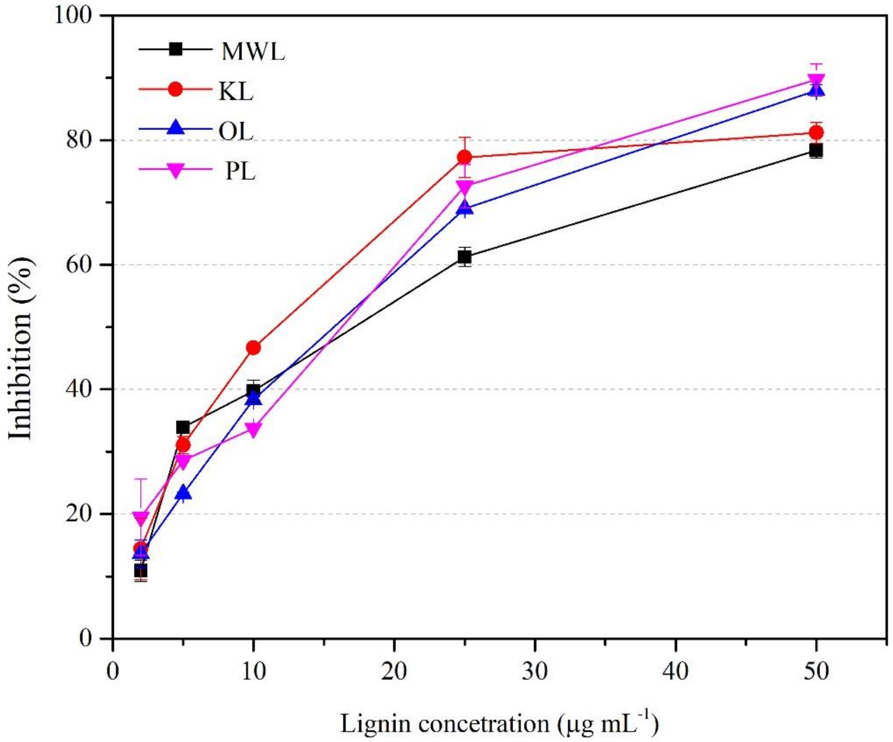
3.3.7 Antioxidant Activity

Another of the main suitable characteristics of lignin is its capacity to scavenge free radicals and, therefore, act as a promising natural antioxidant. Studying the effect of extraction methods on the antioxidant activity of lignin is essential for optimizing its use in various applications.

The antioxidant activity of lignins is often directly related to the presence of phenolic hydroxyl groups, due to their ability to form stable phenoxyl radicals after donation of a hydrogen atom (AVELINO, 2024). As observed in **section 3.3.4** of this study, the quantity of these phenolic groups is affected depending on the extraction method employed to obtain *Eucalyptus* lignins.

Therefore, in order to assess the effects of the extraction methods on the antioxidant activity of *Eucalyptus* lignins, their ability to neutralize DPPH radicals (DPPH•) was measured. The DPPH• scavenging curves are depicted in **Figure 13**. The results are expressed in terms of percent inhibition of the DPPH radical *versus* the antioxidant concentration. In addition, **Table 8** summarizes the calculated IC_{50} values for the lignin samples.

Figure 12 - DPPH free radical scavenging capacity of of Eucalyptus lignins.



Source: The author

Table 8 - DPPH free radical scavenging capacity (IC_{50}^* value) of Eucalyptus lignins.

Sample	IC_{50} (µg mL^{-1})
MWL	19.25 ± 0.25
KL	13.50 ± 0.01
OL	16.38 ± 0.01
PL	13.17 ± 0.49

*IC_{50} value is defined as the antioxidant concentration necessary to decrease the initial DPPH• concentration by 50% inhibition. The lower the IC_{50} value, the greater the antioxidant capacity of the sample.

Source: The author

In general, based on the obtained IC50 values, it was observed that lignins with higher quantities of phenolic OH groups (Table 8) exhibited greater capacity to inhibit DPPH radicals, in which the PL has presented the highest antioxidant capacity, followed by KL, OL, and finally, MWL. The performance of these lignins probably was influenced by several structural features, such as phenolic and total hydroxyl concentrations, molecular weight distribution (Mn and Mw values), amount of residual carbohydrates, etc (AVELINO, 2024). This further underscore the influence of the severity of the extraction processes on lignin properties.

Understanding the antioxidant capacity of lignin and its modulation through using different extraction techniques contributes to the broader efforts to harness natural and sustainable resources for applications in food, pharmaceuticals, and materials science, promoting both environmental sustainability and human health.

3.4 Conclusions

The present work presented different methods to promote the delignification of *Eucalyptus urograndis*, yielding lignins with remarkable and peculiar structural features. The technical lignins were obtained with elevated yields and high purities, which suggest that they can be produced in large scale for further valorization. Furthermore, combining different analytical techniques a deep structural characterization of those lignins revealed interesting features directly linked to the severity of the processes. The use of processes with different harshness provided the generation of a variety of lignins in terms of structural features, in which the harsher the process the lower the molecular weight and the higher the amount of condensed structures linked by C-C bonds. Furthermore, a mild condition process (MWAOD) was capable of promoting the delignification of the raw material concomitantly to the partial acetylation of the aliphatic hydroxyls, yielding a lignin with unique characteristic for some purposes. Therefore, the three processes (Kraft, FP and MWAOD) showed to be feasible to obtain lignin in significant amounts for further applications. Since each process yielded a lignin with some structural peculiarities, the choice of the most suitable one will depend on its application, always respecting the structure – performance relationship.

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4 MICROWAVE-ASSISTED SELECTIVE ACETYLATION OF KRAFT LIGNIN: ACETIC ACID AS A SUSTAINABLE REACTANT FOR LIGNIN VALORIZATION

Note: This chapter is based on the following published article:
OLIVEIRA, D. R.; AVELINO, F.; MAZZETTO, S. E.; LOMONACO, D. Microwave-assisted selective acetylation of Kraft lignin: Acetic acid as a sustainable reactant for lignin valorization. International Journal of Biological Macromolecules, v. 164, p. 1536–1544, 2020. https://doi.org/10.1016/j.ijbiomac.2020.07.216.

RESUMO

A acetilação da lignina, uma das modificações químicas mais difundidas para melhorar a solubilidade deste biopolímero em solventes orgânicos e aumentar a compatibilidade polímero-lignina, tem sido realizada por décadas utilizando metodologias demoradas e agentes de acetilação com sérias desvantagens. Além disso, as reações de acetilação tradicionais geralmente levam à acetilação não seletiva de grupos alifáticos e fenólicos. Neste trabalho, demonstramos que a acetilação parcial e seletiva da lignina Kraft pode ser realizada por meio de um processo assistido por micro-ondas mais ecológico, simples e rápido, utilizando ácido acético como solvente e agente de acetilação. A caracterização estrutural via FTIR, ^{1}H–^{13}C HSQC e RMN de ^{31}P demonstrou que a reação de acetilação ocorre seletivamente apenas nas hidroxilas alifáticas, preservando as hidroxilas fenólicas. As condições ótimas de reação foram obtidas utilizando 1% (v/v) de H2SO4 como catalisador e apenas 5 minutos de tempo de reação. A lignina Kraft acetilada (AKL) obtida apresentou maior solubilidade em solventes orgânicos (acetato de etila, clorofórmio e diclorometano) em comparação com a lignina Kraft não modificada (KL) e capacidade antioxidante quase 8 vezes maior que um antioxidante comercial BHT. Essas características tornam a lignina Kraft parcialmente e seletivamente acetilada um potencial aditivo antioxidante verde para ser utilizado em blends de polímeros.

Palavras-chave: acetilação da lignina; ácido acético; assistido por microondas; seletividade.

ABSTRACT

Lignin acetylation, one of the most widespread chemical modifications used for improve the solubility of this biopolymer in organic solvents and increase polymer-lignin compatibility, has been performed for decades using time-consuming methodologies and acetylating agents with serious drawbacks. Moreover, traditional acetylation reactions generally conduce to non-selective acetylation of both aliphatic and phenolic groups. In this work, we demonstrated that partial and selective acetylation of kraft lignin can be carried out through a greener, simple and fast microwave-assisted process using acetic acid as solvent and acetylating agent. Structural characterization via FTIR, ^{1}H-^{13}C HSQC and ^{31}P-NMR demonstrated that acetylation reaction occurs selectively only in aliphatic hydroxyls, preserving the phenolic hydroxyls. Optimal reaction conditions were obtained using 1% (v/v) of H_2SO_4 as catalyst and only 5 minutes as reaction time. The acetylated Kraft lignin (AKL) obtained, have enhanced solubility in organic solvents (ethyl acetate, chloroform and dichloromethane) compared to unmodified Kraft lignin (KL) and antioxidant capacity almost 8 times higher than a commercial antioxidant BHT. These characteristics makes the partially and selectively acetylated Kraft lignin a potential green antioxidant additive to be used in polymers blends.

Keywords: Lignin acetylation; Acetic acid; Microwave-assisted; Selectivity.

4.1 Introduction

Lignin is a polyphenol with a complex and amorphous three-dimensional structure, found in the cell wall of plants, being the second most abundant biopolymer in the planet, constituting between 15-30% of the lignocellulosic biomass. It is also the largest natural and renewable source of aromatic compounds, representing a sustainable alternative to petrochemicals, which makes the appreciation of lignin attractive from the point of view of the sustainable development and has called the attention of several research groups and industries (BILAL et al., 2017; XU, FERFOSIAN, 2017).

Currently, around 50-70 million tons of lignin are produced worldwide as a by-product of the pulp and paper industries, and it is expected that in the future this amount will keep rising as a consequence of the production of biofuels and chemicals from renewable sources (BAJWA et al., 2019).

Despite its wide availability and attractive chemical characteristics, lignin is still considered a low-value product, with more than 95% of its production being burned in industrial furnaces and boilers for energy production (GROSSMAN, VERMERRIS, 2019).

Aiming to valorize this biopolymer and make better use of its potentialities, many works have been developed using lignin and its derivatives. With emphasis on its wide application in the field of polymer chemistry, lignin has been used as a building block in copolymers (ARGYROPOULOS et al., 2014; ARSHANITSA et al., 2016; AVELINO et al., 2018a; LI et al., 2017; Lou et al., 2018; PAS and TORR, 2017) and as a filler or stabilizer (antioxidant) in different types of polymers (AVELINO et al., 2019a; GORDOBIL et al., 2015; KUN and PUKÁNSZKY, 2017; OLIVEIRA et al., 2017). In addition, several studies have shown that lignin plays an important role in the thermal and mechanical properties of these materials (DEHNE et al., 2016; MALDHURE and EKHE, 2017; SEN et al., 2015; Ye et al., 2018).

The valorization of lignin in new biomaterials, however, often requires, as part of the manufacturing process, its solubilization in organic solvents, as well as a good compatibility with the polymer matrix in which it is incorporated, which can be challenging due to the a large number of hydroxyl groups present in the lignin structure, which are centers of high polarity and may hamper their interaction with nonpolar polymers (OLIVEIRA et al., 2017; YE et al., 2018).

Chemical modifications of lignin, such as alkylation (LI AND SARKANEN, 2002; MALDHURE AND EKHE, 2017), silylation (BUONO et al., 2016) and esterification (KOIVU et al., 2016; MONTEIL-RIVERA and PAQUET, 2015; OLIVEIRA et al., 2017; SAMENI et al., 2017) have been employed as a strategy to overcome these limitations. Among these methods, the chemical modification of the hydroxyl groups through esterification, in particular through acetylation with acetic anhydride or acetyl chloride, has been used for decades as a way of improving lignin solubility in organic media. However, the use of such acylating agents presents serious drawbacks (YADAV, JOSHI, 2002).

Acetic anhydride is an expensive reagent and the atom economy of the reaction is poor because acetic acid is produced as co-product, which has to be recovered. Acetyl chloride, in turn, is very sensitive to water, demanding special care in its handling and produces HCl as the co-product of acetylation reaction, which is corrosive and has to be neutralized. These facts make the reactions with acetyl chloride more complicated and with a high cost, which difficult the industrial adoption.

In a previous work (AVELINO et al., 2019b), our group demonstrated that during acetosolv process a partial acetylation of lignin occurred. Based on this, from a green chemistry point of view, the use of acetic acid as acetylating agent represents an interesting alternative, since it is cheap, widely available, can be obtained from natural sources and produces water as co-product of esterification reaction.

The acetylation methods with acetic anhydride and acetyl chloride are also not selective and acetylation occurs in both aliphatic and phenolic hydroxyls, which, in the case of lignin, leads to loss of its antioxidant effect, since this activity is attributed to the presence of phenolic groups (NOGUEIRA et al., 2019; YE et al., 2018).

Thus, selective chemical modifications of the hydroxyl groups of lignin, modifying only the aliphatic hydroxyls and preserving the phenolic ones, are of great interest in the sense of improve processability in nonpolar solvents and polymer matrices as also maintaining its antioxidant effect, a highly desired property in polymer science. Moreover, the remaining phenolic hydroxyl groups are reactive sites that can act in metal adsorption or in further copolymerization reactions to synthetize polyesters or polyamides, for example (MONTEIL-RIVERA and PAQUET, 2015).

In the literature, only few papers describe selective chemical modifications of the lignin hydroxyls groups. In their work, Monteil-Rivera and Paquet (MONTEIL-RIVERA and PAQUET, 2015) report that through a microwave-assisted, solvent-free and catalyst-free process, maleic anhydride is able to react exclusively with the aliphatic hydroxyl groups of a Kraft softwood lignin. Ye et al. (YE et al., 2018) describe a selective aminolysis process of an acetylated Kraft lignin capable of releasing the phenolic hydroxyls while maintaining the acetylated aliphatic hydroxyl groups. Interestingly, in this work, the authors report that partially acetylated lignin simultaneously improves the thermo-oxidative stability of propylene and maintains its mechanical properties. The results presented in our previously work (AVELINO et al., 2019b) demonstrated that the acetylation during acetosolv process occurs only in aliphatic hydroxyl groups, which suggests that acetylation promoted by acetic acid can be used as a selective acetylation method.

Based on the above, this work describes a simple, efficient and solvent-free microwave-assisted selective acetylation process of the aliphatic hydroxyl groups of a Kraft lignin (KL) promoted by acetic acid.

Different catalyst concentrations and reaction times were studied in order to obtain an optimal acetylation condition of the aliphatic hydroxyls. Acetylated Kraft lignins (AKLs) were structurally characterized by FTIR, ^{1}H-^{13}C HSQC, ^{31}P-NMR spectroscopies and Gel permeation chromatography (GPC) in order to confirm the occurrence of acetylation reaction as well as provide insights about the selectivity of the acetylation reaction. The solubility of the AKL was compared with unmodified KL in a series of organic solvents. Finally, the antioxidant activity of the AKL was evaluated by the free radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl radicals (DPPH·) and compared with a commercial antioxidant (BHT).

4.2 Experimental Section

4.2.1 Materials

All chemicals were used as received: sulfuric acid (98 %, Synth, Brazil), glacial acetic acid (Synth, Brazil), HPLC-grade tetrahydrofuran (Sigma, Brazil), deuterated dimethysulfoxide (99.96 %, SigmaAldrich), pyridine (99%, Neon, Brazil), chromium (III) acetylacetonate (99.99 %, SigmaAldrich), cyclohexanol (99%, SigmaAldrich), 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (Cl-TMDP) (95%, SigmaAldrich). Kraft lignin (KL) was gently supplied by Suzano Papel e Celulose.

4.2.2 Microwave-assisted acetylation (MWAA)

The acetylation reactions were carried out in a Milestone microwave reactor (Sorisole, Italy), StartSYNTH model, operated in open-vessel configuration (2.45 GHz), and temperature measured by contactless infrared sensor. The maximum power applied was set to 500 W. A screening of reaction conditions was performed, in which the catalyst concentration and the reaction time were varied. KL (0.5 g) was dissolved in a solution (25 mL) formed by glacial acetic acid and H2SO4 (0, 0.5, 1.0 and 2.0 % v/v) at room temperature and magnetic stirring. The acetylation was performed at 110 °C during 5, 10 and 20 minutes under magnetic stirring and reflux. At the end of the reaction, the reaction mixture was poured in cold water (100 mL) and kept under magnetic stirring during 1 hour until lignin precipitation. The precipitate was vacuum filtered and washed with distilled water until pH 6.0, yielding the acetylated kraft lignins (AKLs).

4.2.2 Structural characterization of AKLs

4.2.2.1 Fourier transform infrared spectroscopy (FTIR)

FTIR analyses were carried out in a Perkin Elmer spectrometer (FT-IR/NIR FRONTIER) in the transmission mode by using KBr pellets containing 0.5% wt. lignin (KL and AKLs). The spectra were acquired in the range of 4000 and 400 cm^{-1} (resolution of 4 $cm^{-1)}$ using the arithmetic average of 32 scans.

4.2.2.1 ^{1}H-^{13}C HSQC NMR spectroscopy

^{1}H-^{13}C HSQC spectra were acquired at room temperature on a Bruker Avance DPX300, operating at 300 MHz for 1H nucleus. The sample preparation involved the solubilization of KL and AKLs (30 mg) in 0.5 mL of DMSO-d6. The residual solvent peak was used as an internal reference (δH/δC 2.50/39.5 ppm). The relative proportion of monolignols was calculated according methodology described elsewhere (AVELINO et al., 2018b).

4.2.2.2 ^{31}P NMR spectroscopy

The ^{31}P NMR spectra were acquired on a Bruker Avance DPX500 (operating at 202.4 MHz for ^{31}P nucleus). The signal related to the hydrolysis products of Cl-TMDP was used as an internal reference at 132.2 ppm. ^{31}P NMR experiments were carried out according to Granata and Argyropoulos (1995) (GRANATA; ARGYROPOULOS, 1995b) with some modifications (NOGUEIRA et al., 2019).

4.2.3 Gel permeation chromatography (GPC)

GPC measurements were performed in a Shimadzu LC-20AD (Kyoto, Japan) using a setup formed by two columns in series (Phenogel 5µ 50Å and Phenogel 5µ 103Å, 4.6 mm x 300 mm, Phenomenex, USA) and HPLC-grade THF as mobile phase (0.35 mL min^{-1}). A UV-Vis detector (Shimadzu SPD-M20A) at 280nm was used to monitor the samples. Lignin samples (1 mg mL^{-1}) were filtered using a 0.22µm PTFE filter and then were injected (20µL) into the system. The calibration was performed by using polystyrene standards (PSS) in the Mw range of 162 and 1.3 x 105 g mol^{-1}.

4.2.4 Solubility tests

The solubility of KL and AKL in a series of organic solvents was determined as described by Sameni et al (2017) (SAMENI; KRIGSTIN; SAIN, 2017b), with minor modifications. To 100 mg of a previously oven-dried KL or AKL, a total of 10 mL of organic solvent was added, at 23±5 °C. Samples were sonicated for 15 min in a water bath sonicator. The insoluble fraction was vacuum filtered on Buchner funnel (paper with 8µm average pore diameter). Samples were dried at 100°C during 1h and weighed. The soluble fraction was determined by subtracting the insoluble fraction from initial weight. The experiments were performed in duplicate.

4.2.5 Antioxidant activity

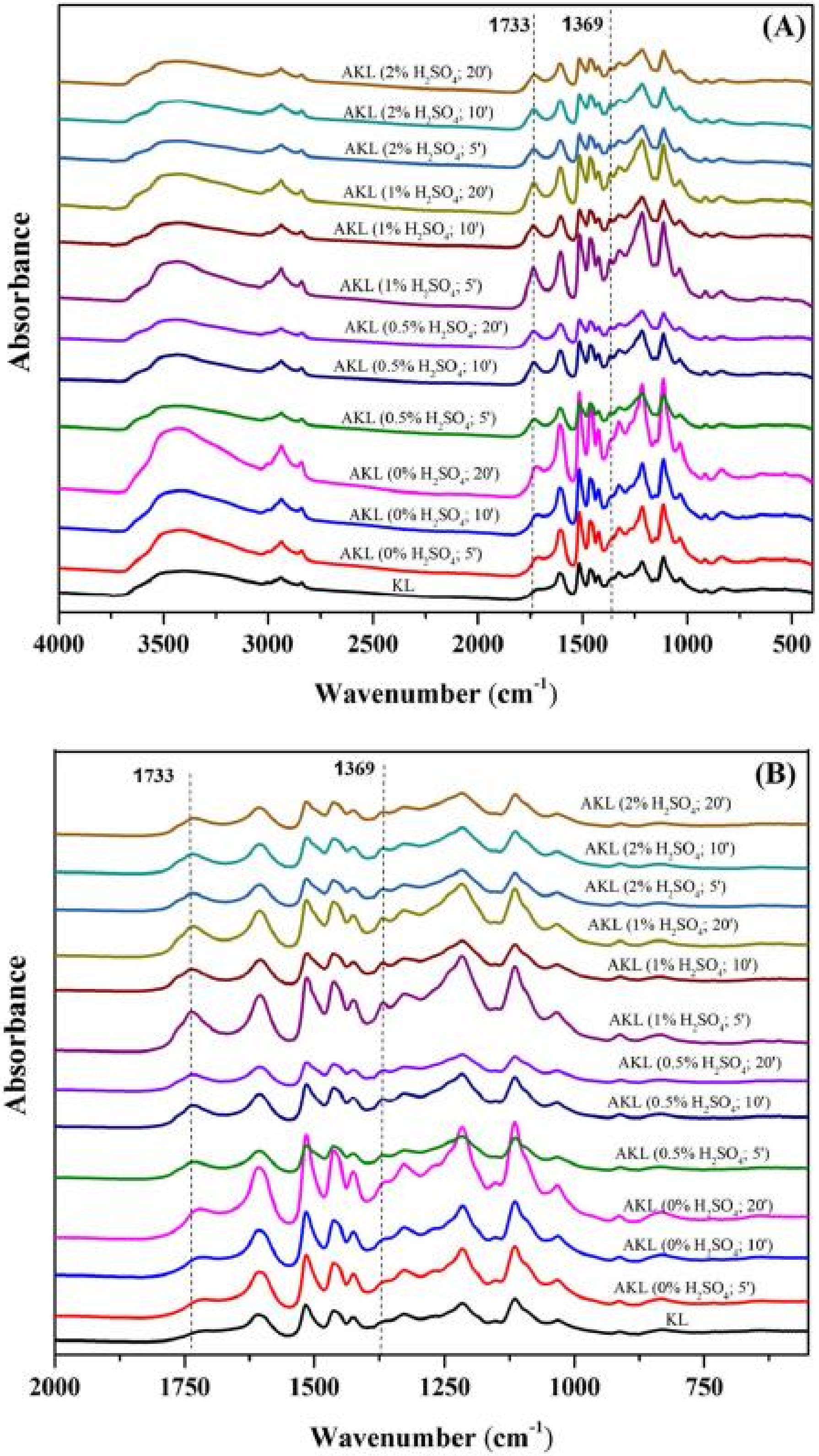
The DPPH free radical scavenging assay was determined using a Cary 60 spectrophotometer (Agilent Technologies), as previously reported (AVELINO et al., 2019).

4.3 Results and discussion

4.3.1 Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of unmodified KL and acetylated Kraft lignins (AKLs) obtained with different concentrations of H2SO4 and different reaction times are show in Figure 13.

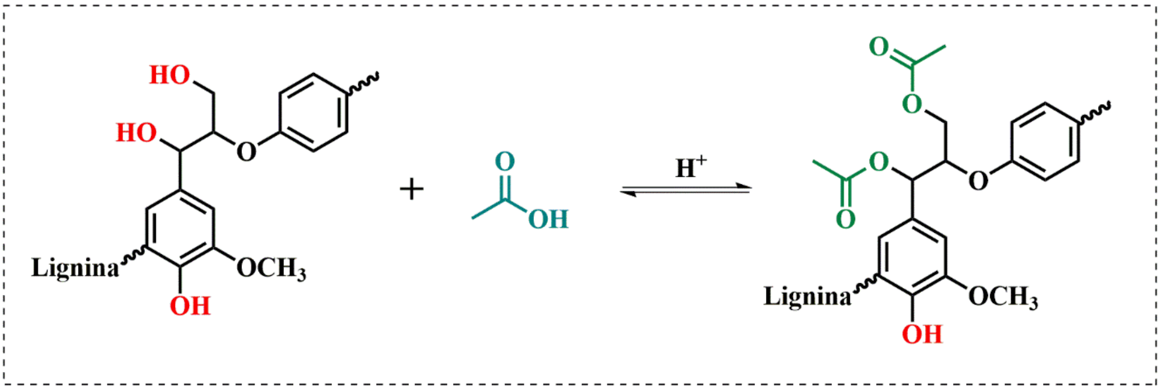
Figure 13 - (A) Full and (B) fingerprint region in FTIR spectra of unmodified KL and acetylated Kraft lignins (AKLs).



Source: The Author

Both unmodified LK and AKLs exhibit typical lignin bands at 3030-3681 (O-H stretch), 2793-3000 (C-H stretch), 1608, 1510, 1462, 1425 (aromatic skeletal vibrations), 1217 (C-O stretch) and 1033 (aromatic in plane C-H deformation) cm^{-1} (MONTEIL-RIVERA; PAQUET, 2015). The main evidences of acetylation in AKLs spectra can be observed from the appearance of a band centered at 1733 cm^{-1}, attributed to aliphatic C=O stretch in ester groups, as well as the band at 1369 cm^{-1}, related to C–H stretching of acetate methyl group. The appearance of these bands and the fact that the band attributed to O-H stretch is still apparent in spectra suggests that just a partial acetylation of lignin hydroxyl groups occurred in AKLs. In addition, it was not observed the appearance of the aromatic C=O stretch band, which is generally reported in the literature at wavenumber values greater than 1760 cm^{-1} (MONTEIL-RIVERA; PAQUET, 2015; OLIVEIRA et al., 2017; YE et al., 2018), which suggests a selective acetylation reaction just in the aliphatic hydroxyl groups. Based on this information, Schema 1 demonstrates the proposed reaction for partial and selective acetylation of kraft lignin with acetic acid:

Scheme 2 - Proposed reaction for partial and selective acetylation of kraft lignin with acetic acid



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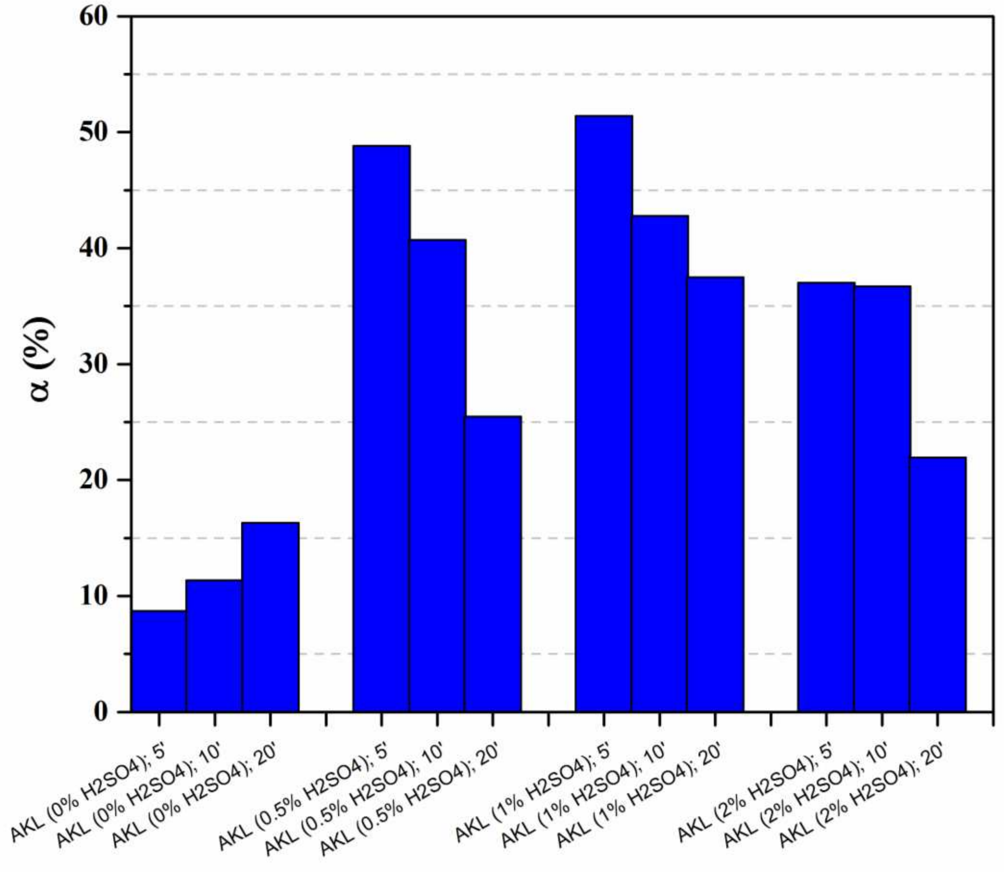
The esterification process efficiency can be quantified using FTIR by calculation of the degree of conversion (α) (OLIVEIRA et al., 2017; SARALEGI et al., 2013). For this, absorbances assigned to O-H stretch vibration (AOH) are measured and normalized with respect to the band at 1510 cm^{-1} (A_{ref}), related to aromatic skeletal vibrations of C-C bonds, that remains unchanged both in KL and in AKLs, as presented in Equation 2:

$$\alpha = 1 - [(A_{OH}/A_{ref})_{\mathbf{AKL}} / (A_{OH}/A_{ref})_{\mathbf{KL}}] \times 100 \qquad (2)$$

Based on this equation, the degree of conversion of AKLs was calculated in order to determine the reaction condition that promoted the highest acetylation of hydroxyl groups, which are shown in Figure 14:

Figure 14 - Degree of conversion (α) of AKLs.



Source: The author

As can be seen in Figure 14, the reaction condition that promoted the highest degree of conversion was that using 1.0 % v/v H2SO4 as catalyst during 5 minutes (α = 51.42%). The use of the same catalyst concentration combined to a higher reaction time (10’) or the use of a higher catalyst concentration (2.0 % v/v) combined to the same reaction time (5’) promoted a decrease in α values. This trend is extensively discussed in ^{31}P NMR section.

4.3.2 ^{1}H–^{13}C Heteronuclear single quantum coherence spectroscopy (1H–13C HSQC)

^{1}H–^{13}C HSQC spectroscopy can confirm the occurrence of the acetylation reaction through the appearance of characteristic signals of acetyl groups. In this sense, the analysis of two regions of the 1H – 13C HSQC spectra of KL and AKL is very important, such as the oxygenated aliphatic (δC/δH 50 – 90/ 3.0 – 5.5 ppm) and the aliphatic ones (δC/δH 0 – 50/ 0 – 3.0 ppm), which are shown in Figures 15 and 16, respectively. The aromatic region of 1H-13C HSQC spectra of KL and AKL is presented in Figure S1 (Appendix B), as well as their relative proportion of monolignols. The assignment of the most important cross-peaks was made based on a previous report (ABDELKAFI et al., 2011; CONSTANT et al., 2016; PEREIRA et al., 2017) and it is summarized on Table S2 (Appendix B).

Figure 15 - Oxygenated aliphatic region in ^{1}H–^{13}C HSQC spectra of KL and AKL

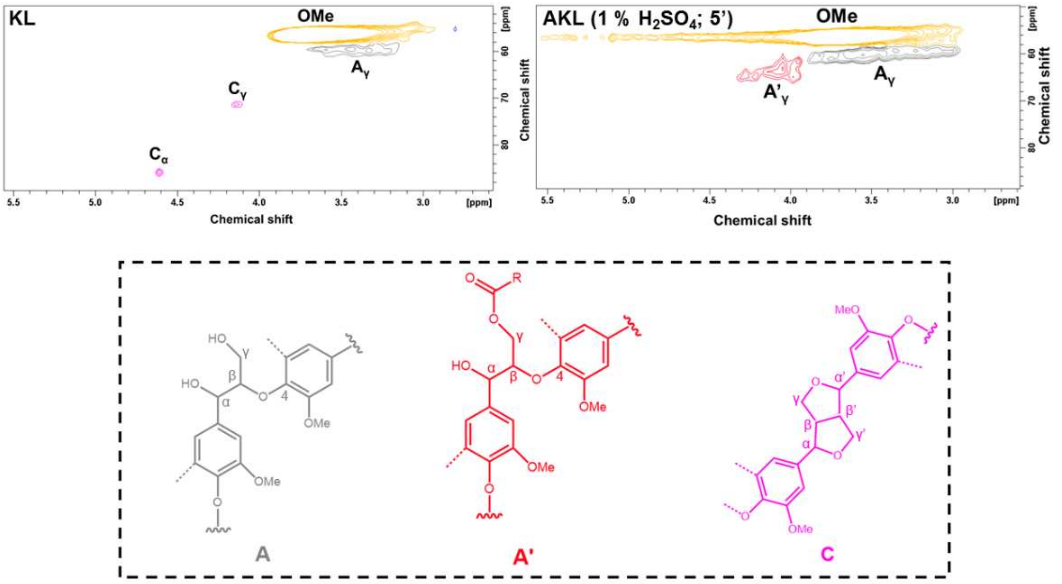


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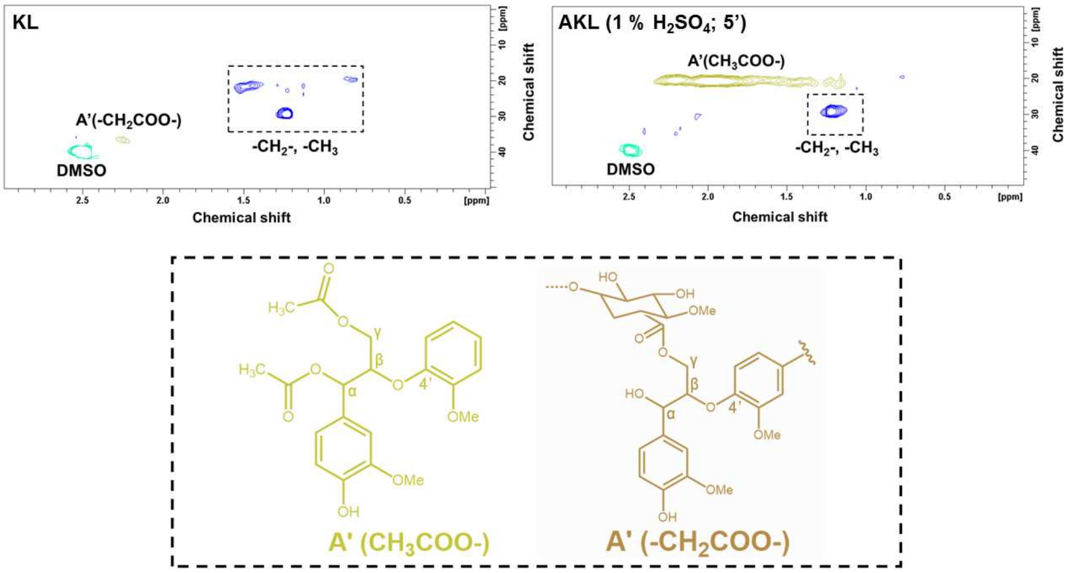
Figure 15 clearly shows the appearance of a cross-peak at 64.6/4.21 ppm in the AKL spectra, which is related to C – H correlation in the γ-positions of A' substructures. This behavior corroborates with the spectral differences between KL and AKL observed by their FTIR spectra, in which the absorption bands at 1733 cm-1 is related to C=O stretching of acetyl groups and that at 1369 cm-1 is related to C–H stretching of acetate methyl group, being both bands absent in the KL spectra.

Figure 16 - Aliphatic region in ^{1}H–^{13}C HSQC spectra of KL and AK



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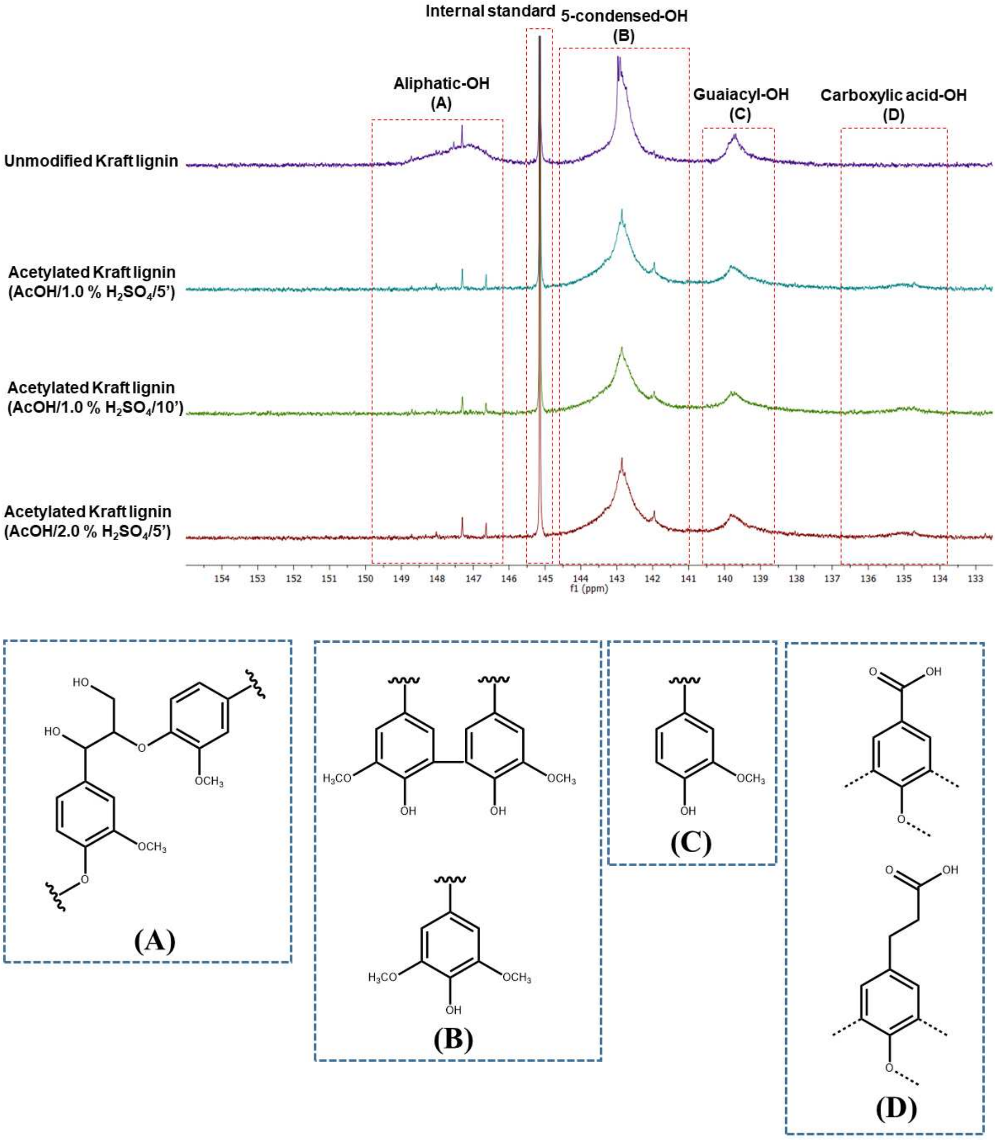
In order to confirm the occurrence of the acetylation reaction of KL, the analysis of the aliphatic region of KL and AKL spectra was also carried out (Figure 16). It can be seen that AKL has a broad and strong cross-peak ranging from 20.2/1.7 – 2.1 ppm related to acetyl groups incorporated to AKL structure, which is not observed in the KL spectra. Therefore, the results obtained by FTIR and ^{1}H – ^{13}C HSQC analyses showed that the chemical modification (acetylation) successfully occurred, however they do not provide any insights about the selectivity of this reaction, which is a very valuable structural feature for some applications and that need to be explored.

4.3.3 ^{31}P NMR

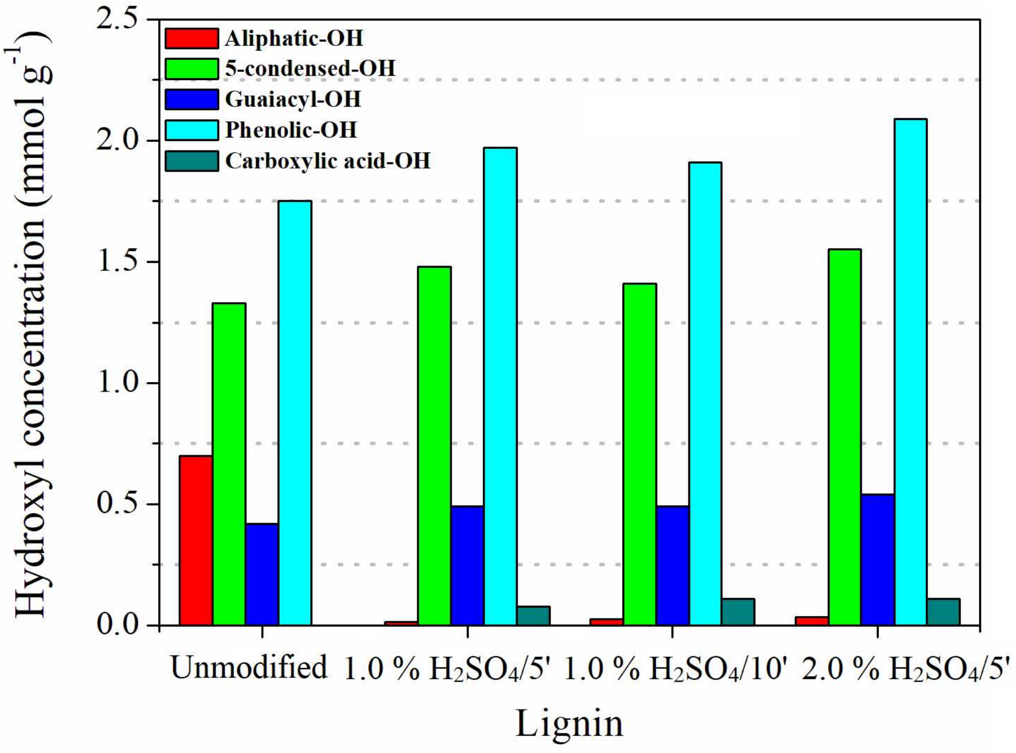
Quantification and identification of the different types of hydroxyl groups present in KL and AKL, such as aliphatic-OH, syringyl-OH/5-substituted-OH, guaiacyl-OH, *p*-hydroxyphenyl-OH and COOH, were performed by quantitative ^{31}P NMR analysis. This technique can also provide insights about the selectivity of the acetylation reaction, as well as the effects of the reaction conditions. **Figure 17** shows the ^{31}P NMR spectra of KL and AKL with the main substructures identified, while **Figure 17** and **Table S3 (Appendix B)** summarizes the quantification data of hydroxyl groups present in KL and AKL.

Figure 17 - ^{31}P NMR spectra of KL and AKL with the main identified substructures.



Source: The author

Figure 18 - Hydroxyl concentration data of KL and AKL obtained under different reaction conditions.



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Figures 17 and 18 show that all AKL presented a significant decrease in the aliphatic hydroxyls concentration in comparison to that of KL, while the phenolic hydroxyls concentration almost did not change. This behavior can be attributed to the selective acetylation of aliphatic hydroxyls due to their higher nucleophilic character in comparison to the phenolic ones.

In addition, the minor changes observed in the phenolic hydroxyls concentration can be explained by the high acidity of the medium, which could favor the occurrence of recondensation reactions, as suggested by the increase in the M_w value of AKL, as well as the increase in its concentration of 5-condensed-OH in relation to those of KL, suggesting the increase in the number of phenolic hydroxyls per mass unit.

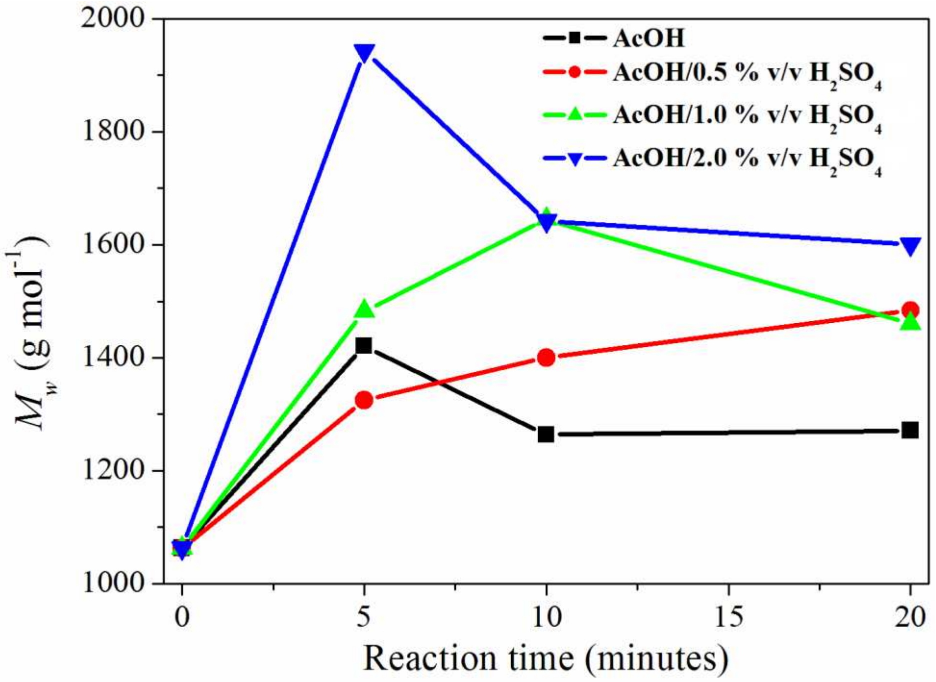
It is worth mentioning that the reaction condition that promoted a higher acetylation of aliphatic hydroxyls (partial acetylation) was that using 1.0 % v/v H_2SO_4 as catalyst during 5 minutes, as can be seen in **Figure 14**. In addition, the use of the same catalyst concentration combined to a higher reaction time (10’) or the use of a higher catalyst concentration (2.0 % v/v) combined to the same reaction time (5’) seems to affect the extent of the acetylation reaction. Probably, long reaction times and higher catalyst concentrations favor the cleavage of ether bonds present in the lignin structure, competing with the acetylation reaction.

Therefore, the use of such optimized condition (AcOH/1.0 % v/v H_2SO_4/5’) could be valuable for obtaining a partial acetylated lignin through an eco-friendlier methodology, in comparison with the state of the art, with a balanced polarity. This structural feature can play a key role in some applications, such as additives in thermoplastics, generating materials with interesting mechanical properties, as previous described by our group (AVELINO et al., 2019).

4.3.4 Gel permeation chromatography (GPC)

The effects of the acetylation conditions (catalyst concentration and reaction time) on the molecular weight distribution of AKLs and their weight-average and number-average molecular weights, Mw and Mn, respectively, were assessed by GPC analysis. The correlation between the Mw values AKLs with the reaction time is shown in Figure 19 and their molecular weight distribution curves are shown in Figure S2 (appendix B).

Figure 19 - Dependence of M_w values of AKLs with the reaction time.



Source: The author

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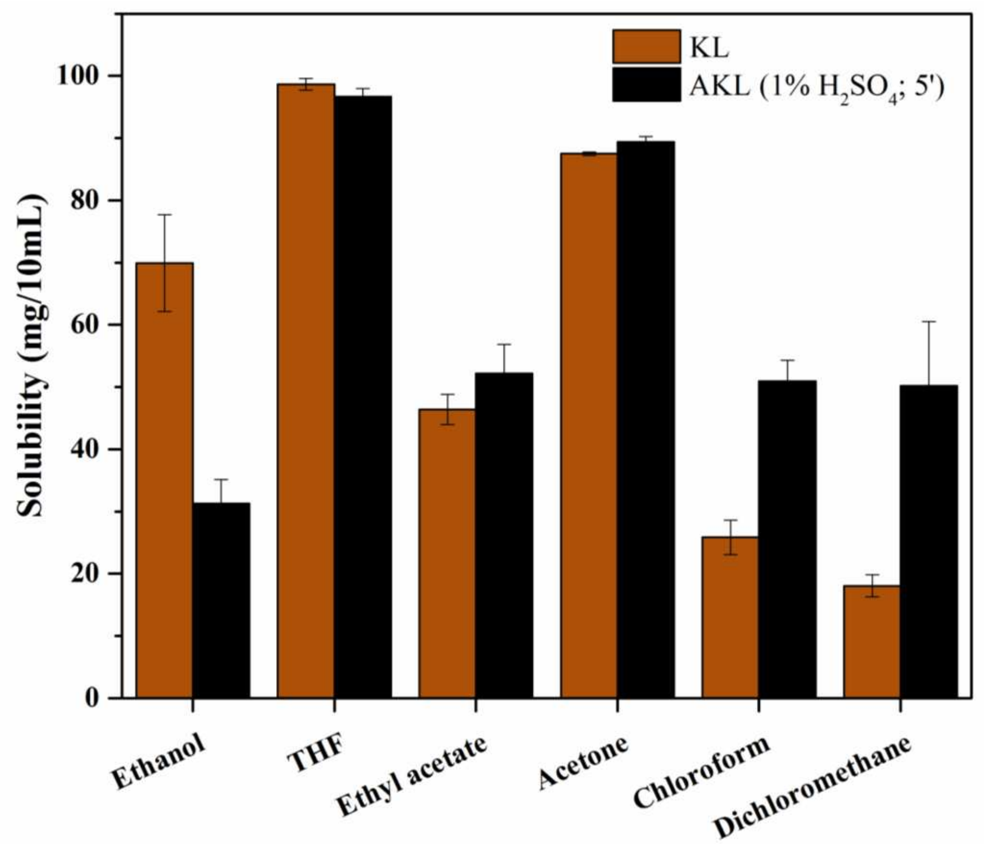
Figure 7 shows that there is not possible to establish a relationship between the degree of acetylation and the Mw values of AKLs, since the optimized condition found out by FTIR and 31P NMR analyses, namely AcOH/1.0 % H2SO4/5' had an intermediary Mw value, but the highest incorporation of acetyl groups into aliphatic hydroxyls. This can be attributed to the occurrence of parallel reactions that can compete with acetylation, such as the depolymerization/repolymerization of lignin structure by the cleavage of ether bonds, specifically the β-O-4 ones.

Evidence of this behavior is the increase of the catalyst concentration or the reaction time increase can cause an increase or a decrease in the M_w values of AKLs, which can be attributed to repolymerization and depolymerization reactions, respectively. Therefore, corroborating with the results of FTIR and ^{31}P NMR analyses, the long reaction times and high catalyst concentrations tend to decrease the extent of the partial acetylation of lignin.

4.3.5 Solubility of lignin samples in organic solvents

The solubility of lignin in organic solvents is an important parameter that must be taken into account when it is desired to increase its compatibility with polymeric matrices or in the development of new materials, since solubilization is an integral part of several manufacturing processes. In Figure 20, the solubility of AKL (1% H2SO4, 5') in several organic solvent with different polarities is compared to unmodified KL.

Figure 20 - Solubility of 100 mg of KL and AKL in 10 mL of different organic solvents.



Source: The author

As can be seen in Figure 20, both KL and AKL had great solubility in THF and acetone and the acetylation process did not cause significant changes in AKL solubility. As expected, the solubility of partially acetylated lignin decreased in ethanol, since the replacement of aliphatic hydroxyls by acetyl groups decreases the number of hydrogen bonds that this lignin is able to establish with the solvent, which is directly correlated to its solubility.

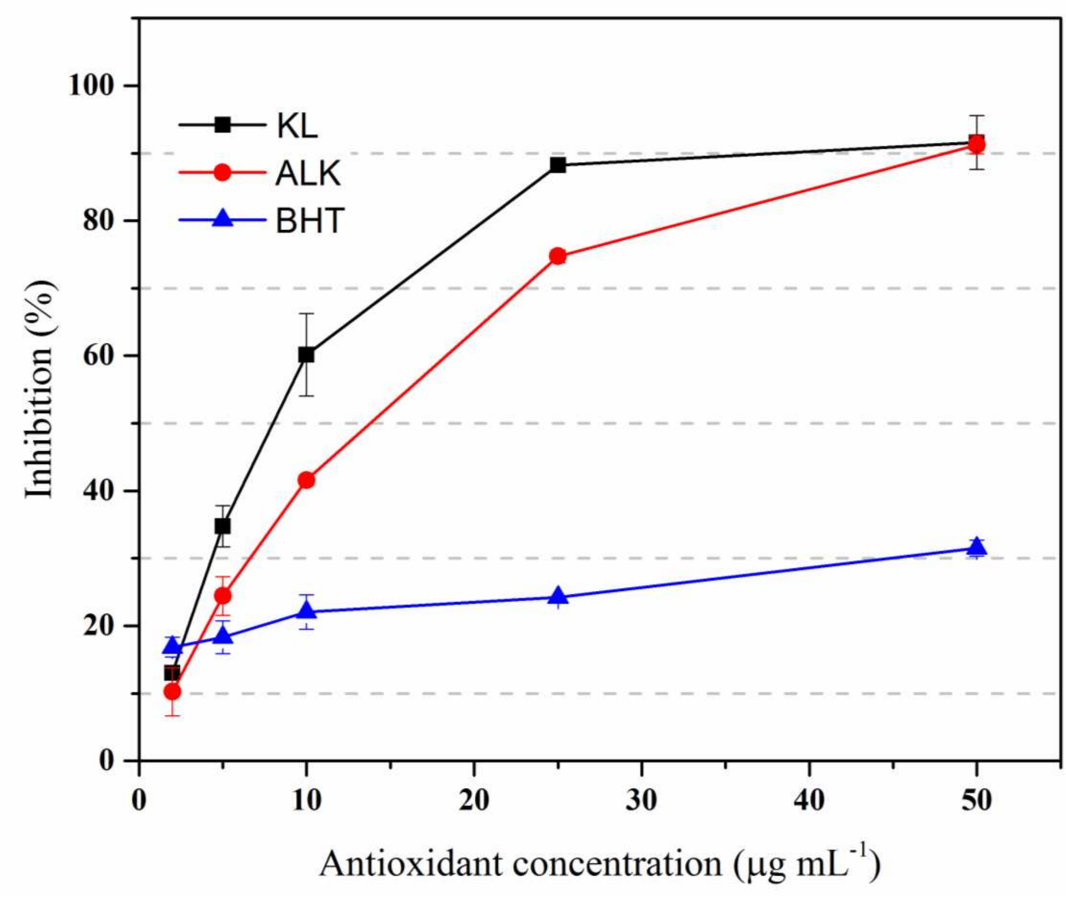
On the other hand, an increase in solubility was observed in solvents with intermediary polarities. In ethyl acetate the solubility was just moderately increased after partial acetylation, but in chlorinated solvents, such as chloroform and dichloromethane, a remarkable improvement was observed (97% and 177% of solubility improvement, respectively). This fact is interesting from the point of view of lignin valorization in new materials since these solvents are capable of solubilizing a wide variety of polymers and, consequently, the incorporation of partially acetylated lignin would be facilitated in these polymeric matrices.

4.3.6 Antioxidant activity

One of the main suitable characteristics of lignin is its capacity to scavenge free radicals and, therefore, act as a promising natural antioxidant. In order to evaluate the effect of partial and selective acetylation of lignin in this property, the ability of AKL to scavenge DPPH radicals (DPPH•) was compared to unmodified KL and a commercial antioxidant (BHT).

The DPPH• scavenging curves are depicted in **Figure 21**. The results are expressed in terms of percent inhibition of the DPPH radical *versus* the antioxidant concentration. In addition, **Table 9** summarizes the calculated IC_{50} values for the lignin samples, as well as for BHT.

Figure 21 - DPPH free radical scavenging capacity of LK, AKL and commercial antioxidant BHT.



Source: The author

Table 9 - DPPH free radical scavenging capacity (IC50 value) of AKL, KL and BHT.

Sample	IC_{50}^{*} (µg/ml)
KL	8.06 ± 0.65
AKL	15.26 ± 0.26
BHT	113.6 ± 1.51

* IC_{50} value is defined as the antioxidant concentration necessary to decrease the initial DPPH• concentration by 50% inhibition.

Source: The author

The antioxidant activity of lignins is often directly related to the presence of their phenolic hydroxyl groups, due their ability to form stable phenoxyl radicals after donation of a hydrogen atom. It was expected that AKL exhibited the higher antioxidant activity than KL, since AKL has higher amounts of phenolic hydroxyls (31P-NMR, Figure 17). The inhibition curves and the values of IC50, however, showed that KL have a higher antioxidant activity, which suggest that others factors can be involved in the radical scavenging mechanism besides the phenolic hydroxyls.

In fact, aliphatic hydroxyl groups can also participate in radical scavenging mechanism as hydrogen donators (DIZHBITE et al., 2004; MICHELIN et al., 2018), which can explain the fact that AKL has a lower antioxidant activity than KL, since in partially acetylated lignin almost all aliphatic hydroxyls are acetylated and cannot act as hydrogen donors to neutralize DPPH radicals.

It is worth mentioning that, even with an antioxidant activity lower than unmodified KL, the AKL has an elevated antioxidant activity, with an IC_{50} value almost eight times lower than commercial antioxidant BHT, which can be attributed to phenolic hydroxyl groups that are preserved during selective acetylation processes.

As discussed in previous works of our group (AVELINO et al., 2019; NOGUEIRA et al., 2019), the most efficient antioxidant activity of lignin samples compared to commercial antioxidants can be explained due to the greater number of phenolic hydroxyl groups per mass unit present in lignin. Moreover, the presence of methoxyl groups ($-OCH_3$) in the S and G units of lignin helps to stabilize the phenoxy radicals, favoring the process of DPPH• scavenging. In addition, conjugated double bonds in the side chain of lignin can provide additional stabilization of the phenoxy radicals through extended delocalization.

These facts combined with enhanced solubility in organic solvents reinforces the potential use of AKL as a natural additive with high antioxidant capacity and better compatibility with polymeric matrices.

4.4 Conclusions

Kraft lignin could be partially and selectively acetylated in aliphatic hydroxyl groups through a simple, fast and more eco-friendly, in comparison with the state of the art, microwave assisted methodology using acetic acid as acetylating agent. A series of experiments varying the catalyst concentration and the reaction time demonstrated that an optimal reaction condition can be obtained using 1.0 % v/v H2SO4 as catalyst and only 5 minutes of reaction time, in which almost all aliphatic hydroxyl groups were acetylated and just minor changes were observed in phenolic hydroxyls. The solubility of partially acetylated lignin was improved in organic solvents as ethyl acetate, chloroform and dichloromethane, a fact that can be valuable in the incorporation of AKL in polymeric matrices. The AKL showed high antioxidant activity, with IC50 values almost 8 times lower than commercial antioxidant BHT. Therefore, partially and selectively acetylated kraft lignin showed interesting characteristics to the development of new bio-based materials. Moreover, these results highlight that esterification of lignin can be performed in a much faster way using a greener esterification agent than traditional esterification reactions using anhydride and acyl chloride, paving the way to new studies in which a series of different carboxylic acids can be tested in the esterification of lignins using the conditions described here.

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5 CONCLUSIONS

This thesis addressed crucial challenges in lignin valorization by investigating how different extraction methods shape its structure and how sustainable chemical modifications can tailor it for future applications.

In the first research axis (Chapter 3), the impact of Kraft, Fast Pyrolysis (FP), and Microwave-Assisted Organosolv Delignification (MWAOD) processes on *Eucalyptus urograndis* lignin was comparatively evaluated. The results, corroborated by a robust set of analytical techniques (FTIR, 2D HSQC NMR, ^{31}P NMR, GPC, TGA, DSC, DPPH), confirmed that the severity of the extraction process (following the order FP > Kraft > MWAOD) decisively influences the structural characteristics of the technical lignin. A direct correlation was observed between higher severity and lower molecular weight, higher content of condensed structures (such as β-5' and 5-5'), and greater abundance of phenolic hydroxyl groups. These structural alterations were directly reflected in the thermal properties (Tg, decomposition profile) and antioxidant capacity of the obtained lignins.

The second axis (Chapter 4) focused on developing a greener and more efficient chemical modification method. The feasibility of Kraft lignin acetylation using acetic acid as both reagent and solvent under microwave irradiation was successfully demonstrated. This method proved not only rapid (significantly reducing reaction time compared to conventional methods) but also selective, preferentially modifying phenolic hydroxyl groups over aliphatic ones, as quantified by ^{31}P NMR. This controlled acetylation resulted in predictable changes in the solubility, thermal properties, and antioxidant activity of Kraft lignin. The integration of the two studies highlights that a deep understanding of how extraction defines the initial lignin structure is fundamental for effectively planning and executing subsequent chemical modifications in a targeted manner.

The findings of this thesis hold significant implications for the forest-based industry and the development of biorefineries. The ability to correlate extraction methods with specific properties allows for a more informed choice of the type of lignin to be produced or utilized for a given purpose (e.g., more condensed and phenolic lignins for resins or antioxidants; less modified lignins for other functionalizations). The microwave acetylation methodology with acetic acid represents a promising alternative for the industry, offering a faster, potentially more economical process aligned with green chemistry principles to improve lignin compatibility with polymer matrices or adjust its solubility and reactivity.

Perspectives are opened for exploring the combination of specific extraction methods (like MWAOD, which yields high-purity lignin) with subsequent modifications (such as selective acetylation) to create lignocellulosic materials with tailored properties.

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APPENDIX A - SUPPORT INFORMATION FOR CHAPTER 3: UNVEILING THE MECHANISTIC ASPECTS OF DIFFERENT DELIGNIFICATION METHODS AND ITS EFFECTS ON THE STRUCTURE OF TECHNICAL EUCALYPTUS LIGNINS

- **Extraction of Eucalyptus lignins using different methods**

For the MWL extraction, the wood of Eucalyptus urograndis was ground to pass through a 0.50 mm sieve. The milled wood (50 g) was treated with toluene/ethanol 2:1 (v/v) for 24 h in a Soxhlet apparatus to remove extractives. The extractive-free sample was dried, transferred to a porcelain jar and ground in a rotary ball. Before milling, 1000 g of porcelain beads and 200 ml of toluene were added. The grinding was carried out for 130 h. The sample was then dried to remove toluene and extracted three times with a solution of 1,4-dioxane/water (96% v/v) in a shaker at 190 rpm for 24 h in the dark. The 1,4-dioxane/water extracts were combined and the solvents were removed under reduced pressure until their volume reached 100 mL. This solution was added dropwise in deionized water (DI, 200 mL) under stirring and then lyophilized. The crude MWL was dissolved in 90% acetic acid (20 mL) and precipitated in DI water (400 mL). After centrifugation the solid was dissolved in 1,2-dichloroethane/ethanol (10 mL, 2:1 v/v) and precipitated in diethyl ether (200 mL). The obtained solid was then washed with petroleum ether and air dried at 50 °C for 24 h.

To obtain pyrolytic lignin, the bio-oil was obtained from a rapid pyrolysis plant (BIOWARE, Brazil) with a reactor operating in a fluidized bed (500 °C, for 5 s at 100 mmH_2O). The bio oil (10 g) was added dropwise to the ice water (1 L at 4 °C) under ultra-turrax (Ika, Germany) stirring (10,000 rpm), after addition stirring was continued for 10 min. Subsequently, the PL was filtered and dried under vacuum at 40 °C until it reached constant weight.

The MWAOD experiment was was carried out in a Millestone (Sorilose, Italy) microwave reactor, model StartSYNTH, operating in an open-vessel configuration at a frequency of 2.45 GHz. The temperature was controlled by a contact-less infrared sensor and the power applied was set to a maximum of 500 W. MWAOD extraction was performed using a procedure established by our group(AVELINO et al., 2018b), as described in the supporting information. Then, the feedstock (20 g) was introduced in a round-bottom flask containing an aqueous acetic acid solution (90 % v/v) and 2.0 % v/v H_2SO_4 in a fiber per solution ratio of 1:10 (g/mL). The extraction was carried out at 110 °C during 20 minutes under magnetic stirring and atmospheric pressure. The purification and isolation steps are described in our previous work elsewhere(AVELINO et al., 2018b).

- **Characterization of Eucalyptus lignins**

For determination of Klason lignin values of Eucalyptus lignins, a lignin sample (1.0 g) was mixed with 17 mL of H2SO4 (72 % wt.) at room temperature for 24 h. The solution was diluted with deionized water to 4 % wt. H2SO4 and refluxed for 4 h. After the end of the hydrolysis, the system was cooled for 30 minutes at room temperature. The acid-insoluble residue was isolated by vacuum filtration and washed with deionized water until the pH of the filtrate was equal to that of the deionized water. After the washings, the residue was dried at 105 °C for 24 h. The experiment was performed in triplicate.

For determination of acid-soluble lignin, the filtrate was diluted with 4 % wt. H2SO4 with 1:10 (v/v) ratio and the acid-soluble lignin was calculated from the UV absorbance at 215 and 280 nm. The experiment was performed in triplicate.

For ^{31}P NMR experiments, lignin (30 mg) was dissolved in 450 μL of a solvent mixture (C_5H_5N:$CDCl_3$, 1.6/1 v/v ratio). To this solution were added 100 μL of chromium (III) acetylacetonate solution (5.0 mg mL^{-1}) and 100 μL of cyclohexanol solution (10.85 mg mL^{-1}). Finally, 100 μL of 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (Cl-TMDP) was added to the mixture, followed to the addition of solvent mixture until the mark of 1 mL. Experiments were performed on a Bruker Avance DPX500 spectrometer operating at 202.46 MHz for ^{31}P nucleus. Chemical shifts reported are related to the hydrolysis reaction of Cl-TMDP, which generates a signal at 132.2 ppm. Quantitative analysis using cyclohexanol as internal standard was carried out based on previous reports (Granata and Argyropoulos, 1995; Pu et al., 2011). In order to establish a pattern of integration, signals were integrated according to the following chemical shifts: internal standard (145.39 – 144.97 ppm), aliphatic-OH (150 – 145.50 ppm), C_5-substituted-OH (144.5 – 141.2 ppm), guaiacyl-OH (141 – 138.50 ppm), *p*-hydroxyphenyl-OH (138.4 – 137.20 ppm) and COOH-OH (136.5 – 133.34 ppm).

For GPC analyses, lignin samples (2.0 mg) were dissolved in 2.0 mL HPLC-grade THF and then were filtered using a 0.22 μm PTFE filter. Thus, 20 μL of filtered solution was injected into GPC system at a flow rate of 0.35 mL min-1. Standard calibration was performed with polystyrene standards PSS (M_w range 172 – 1.3 x 10^5 g mol^{-1})

Table S1 - Assignment of the main cross-peaks of Eucalyptus lignins shown in their ^{1}H-^{13}C HSQC NMR spectra.

Label	δ_C/δ_H (ppm)	Assignment
-OMe	56.4/3.72	C – H in methoxyls (O-Me)
Aα	59.7/3.44	Cα – Hα in β – O – 4’ substructures (A)
$A_{\beta(G)}$	72.5/4.73	C_β – H_β in β – O – 4’ substructures (A) linked to a G unit
$A_{\beta(S)}$	86.03/4.06	C_β – H_β in β – O – 4’ substructures (A) linked to a S unit
Aγ	59.8/3.44 and 3.64	Cγ – Hγ in β – O – 4’ substructures (A)
A’γ	63.6/4.19	Cγ – Hγ in acylated β – O – 4’ substructures (A’)
B_β	53.04/3.64	C_β – H_β in β-β’ in phenylcoumaran substructures (B)
Bγ	70.3/3.50	Cγ – Hγ in β-β’ in phenylcoumaran substructures (B)
Cα	85.2/4.61	Cα – Hα in β – β’ resinol substructures (C)
C_β	55.5/3.12	C_β – H_β in β – β’ resinol substructures (C)
Cγ	71.4/3.8	Cγ – Hγ in β – β’ resinol substructures (C)
Cγ	72.01/4.18	Cγ – Hγ in β – β’ resinol substructures (C)
$S_{2,6}$	104.7/6.68	$C_{2,6}$ – $H_{2,6}$ in syringyl units (S)
$S'_{2,6}$	107.2/7.21	$C_{2,6}$ – $H_{2,6}$ in oxidized syringyl units (S)
G_2	110.1/6.97	C_2 – H_2 in guaiacyl units (G)
G_5	115.1/6.80	C_5 – H_5 in guaiacyl units (G)
G_6	119.1/6.75	C_6 – H_6 in guaiacyl units (G)
$H_{2,6}$	127.9/7.07	$C_{2,6}$ – $H_{2,6}$ in *p*-hydroxyphenyl units (H)

APPENDIX B - SUPPORT INFORMATION FOR CHAPTER 4: MICROWAVE-ASSISTED SELECTIVE ACETYLATION OF KRAFT LIGNIN: ACETIC ACID AS A SUSTAINABLE REACTANT FOR LIGNIN VALORIZATION

Table S2 – Assignment of the main cross-peaks of KL an AKL shown in their ^{1}H-^{13}C HSQC NMR spectra.

Label	δ_C/δ_H (ppm)	Assignment
-OMe	56.4/3.72	C – H in methoxyls (-OMe)
Aγ	59.8/3.44 and 3.64	Cγ – Hγ in β – O – 4' substructures (A)
A'γ	64.6/4.21	Cγ – Hγ in acylated β – O – 4' substructures (A')
Cα	85.2/4.61	Cα – Hα in β – β' resinol substructures (C)
Cγ	72.01/4.18	Cγ – Hγ in β – β' resinol substructures (C)
A' (CH_3COO-)	20.2/1.7 – 2.1	C– H in acetyl groups (CH_3COO-)

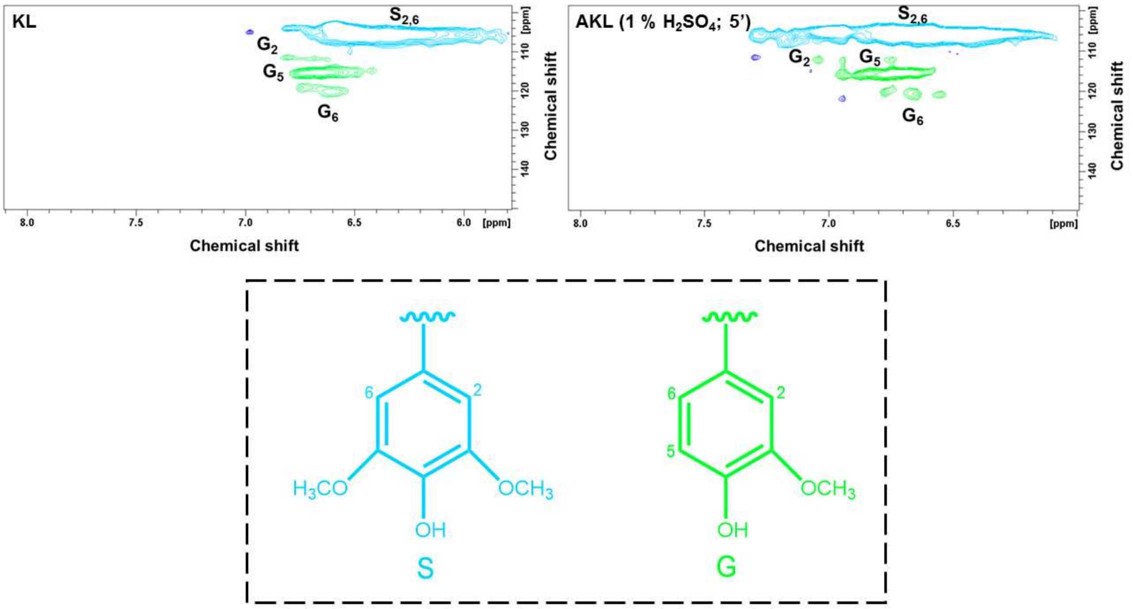
Table S3 – Relative proportion of monolignols (RPM) of KL and KL.

Sample	S (%)	G (%)
KL	73.6	26.4
AKL	76	24

Table S3 - Hydroxyl quantification data of unmodified and acetylated kraft lignins

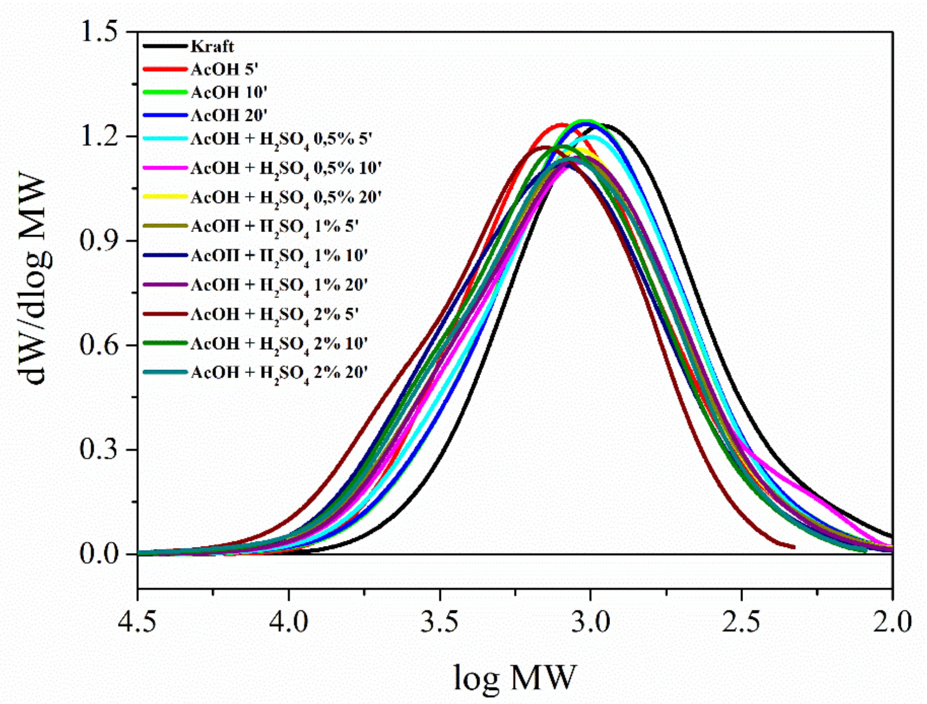
Index/Lignin	Unmodified	1.0 % H_2SO_4/5'	1.0 % H_2SO_4/10'	2.0 % H_2SO_4/5'
OH aliphatic content (mmol g^{-1})	0.70	0.013	0.025	0.034
C_5-substituted-OH	1.33	1.48	1.41	1.55
Guaiacyl-OH	0.42	0.49	0.49	0.54
p-Hydroxyphenyl-OH	-	-	-	-
OH phenolic content (mmol g^{-1})	1.75	1.97	1.91	2.09
COOH content (mmol g^{-1})	-	0.08	0.11	0.11
Total OH groups' content (mmol g^{-1})	2.45	2.07	2.04	2.24
$OH_{phen..}/OH_{total}$	0.71	0.95	0.93	0.93

Figure S1 – Aromatic region of ^{1}H-^{13}C HSQC spectra of KL and AKL.



Source: The author

Figure S2 – Molecular weight distribution curves of KL and AKLs.



Source: The author