ABSTRACT

MARANGON JARDIM, JULIANA. Insights Into Kraft Lignins. (Under the direction of Dr. Lucian Lucia, Dr. Hasan Jameel, and Dr. Peter W. Hart).

Technical lignins, *i.e.*, lignins isolated from biomass during chemical and mechanical processing, have attracted great attention in the past few decades. The biorefinery concept is an important driving force for such interest, where diverse products can be obtained from renewable materials. Kraft lignin (KL), a type of technical lignin, is relatively cheap and largely available; thus, it should be investigated as a feedstock for green materials. It is noteworthy that there are challenges to KL utilization due to its complex chemical structure, heterogeneity, lignin-carbohydrate complexes (LCCs), and modification upon processing and isolation. The present work reviews these challenges and provides a thorough elucidation of lignin precipitation, composition, and properties of very distinct kraft lignins obtained from pine, acacia, sweetgum, and eucalyptus black liquors from different stages of cooking during a time frame to produce bleachable-grade pulp. Detailed characterization and understanding of the chemical structure and composition of any technical lignin is crucial to efficient valorization strategies. Thus, this effort used a variety of wet chemistries, chromatography, and spectroscopy techniques to disclose the impact of kraft pulping and wood species upon kraft lignins. Lignin recovery performance and lignin properties varied greatly amongst the species. Specifically, different levels of contamination with carbohydrates; different LCC contents; wide range of molecular weight and functional groups were observed. Finally, the present work provides a better understanding of the differences between hardwood kraft lignins (HWKL) and softwood kraft lignins (SWKL), as well amongst HWKL with very distinct syringyl-to-guaiacyl (S/G) ratios, parameters of fundamental importance for future applications, and hence could greatly amplify the utilization of underutilized lignins.

Insights Into Kraft Lignins

by
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BIOGRAPHY

Juliana was born in Juiz de Fora, MG, Brazil, June 30th, 1991. In 1998, she moved to Viçosa – MG with her family, where she lived until 2016. In 2009, Juliana went to college to pursue her bachelor's degree in Forest Engineering at Universidade Federal de Viçosa, where she also earned her M.S. in Pulp and Paper Technology (2017). During her masters she was under the direction of Dr. Jorge Luiz Colodette and received excellent training in pulp and paper manufacturing, which led to her passion for scientific research on lignocellulosic processing. In 2016, Juliana had the opportunity to work as a visiting scholar in the Department of Forest Biomaterials at North Carolina State University on a project towards cost-effective wastewater decontamination via photocatalysis. In 2017 she started the doctoral program at the same university, under the supervision of Drs. Lucian Lucia, Peter W. Hart, Hasan Jameel, and Dr. Chang. During her time at NC State, she learned many different things, met incredible people, and grew personally and intellectually.

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1.1. Background

Technical lignins are lignins isolated from biomass during chemical and mechanical processes. For instance, kraft lignin (KL) is a technical lignins obtained as a by-product from the kraft pulping process. KL is an abundant, renewable, and relatively cheap feedstock that has shown great potential for bio-sourced energy, chemicals, and materials. Some opportunities for KL include dispersants, adsorbents, antioxidants, aromatic compounds (chemicals), briquettes, pellets, hydrogels, carbon fibers and polymer blends and composites (Jardim et al. 2020a). Yet, a gap exists between the kraft lignin producers and lignin users because current lignin processes have difficulty meeting quality control constraints and market specifications, which slows down lignin market penetration (Dessbesell et al. 2020). In addition, the value-added utilizations of technical lignin are restricted by its heterogeneous features, such as high polydispersity, complex functional group distribution, and ununiformed reactivity (Jiang et al. 2017).

1.2. Native lignin

Lignins represent 30% of terrestrial carbon in the biosphere and the largest source of renewable aromatic carbon (Boerjan et al. 2003). The lignin polymer can be composed of three phenylpropane units: syringyl (S), guaiacyl (G), and p-hydroxyphenyl (H). These three repeat units correspond to three *p*-hydroxycinnamyl monomers known as monolignols (*p*-coumaryl, coniferyl, and sinapyl alcohols). The monolignols and lignin units are shown in Fig. 1.1.

Despite having only three building blocks, the chemical structure of lignin is very complex. For instance, softwood lignins contain mainly G units and appears to vary little amongst species (Akiyama et al. 2005; Sarkanen et al. 1967); whereas hardwood lignins contain S and G units and have been reported to vary greatly between species due to the wide range of S/G ratio within its structure (Santos et al. 2012). In addition, the lignin structure can vary within a single plant and species depending on age, growth site, and season.

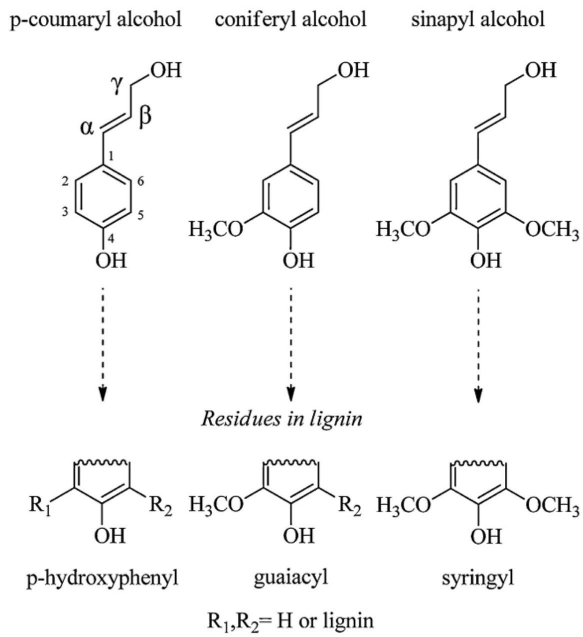

Figure 1.1. The three monolignols compounds and corresponding lignin units

Lignin is polymerized via native peroxidases through repetitive dehydrogenation and coupling of monolignols whose main interunit linkages are shown in Fig. 1.2.

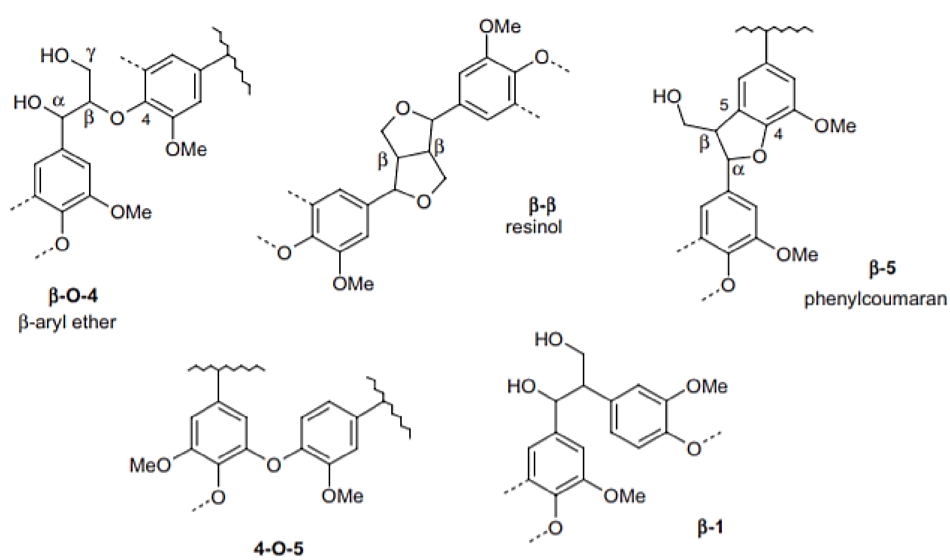

Figure 1.2. Main interunit linkages in lignin

Lignins are also covalently bonded to carbohydrates, forming lignin-carbohydrate complexes (LCCs). The three main types of LCCs in woody biomasses are γ-esters, benzyl ethers, and phenyl glycosides (Fengel *et al.* 1984; Koshijima *et al.* 2003; Zikeli *et al.* 2016). These structures are shown in Fig. 1.3. It is important to mention that even after ~ 150 years studying LCCs, its isolation, characterization, and quantification are still considered challenging (Giummaerlla *et al.* 2019).

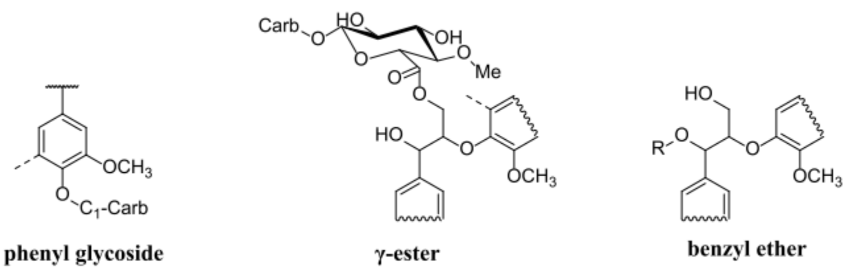

Figure 1.3. Lignin-carbohydrate complexes in woody biomasses

The present investigation focused on the characterization and comparison of kraft lignins obtained at different levels of pulping for pine, acacia, sweetgum, and eucalyptus, a consolidated effort not previously undertaken. During kraft pulping, the lignin structure undergoes intense modification as discussed in later sections.

1.3.Kraft pulping

Kraft is the dominant chemical pulping process in the world. It uses sodium hydroxide and sodium sulfide to deconstruct fibers, generating ~ 78 million tons of lignin annually (Miller and Faleiros 2016). In the course of pulping, lignin undergoes degradation reactions resulting from the liberation of phenolic hydroxyl groups with a simultaneous increase in hydrophilicity of lignin fragments (Hubbe et al. 2019). Lignin is degraded and reformed to be a more complex polymer than the native lignin through the following reactions: (1) hydrolysis of α-ethers and cleavage of β-aryl ether linkage in the phenolic unit; (2) demethylation; (3) formation of vinyl ethers and stilbene via γ-elimination of primary alcohol in the phenolic unit; and (4) condensation reaction at the C5 position in the aromatic ring (Marton 1971).

Furthermore, substantial changes take place in lignin structure during the kraft pulping process, such as an intense decrease in β-O-4, β-5, dibenzodioxocin, and aliphatic hydroxyl contents, a great increase in the phenolic hydroxyl content, and formation of enol ether and stilbene structures (Hu et al. 2016). The low aliphatic hydroxyl groups are attributed mainly to the formation of quinone methide intermediates and the subsequent splitting of the γ-hydroxylmethyl group in the form of formaldehyde. In addition, the large increase in the phenolic hydroxyl groups is due to hydrolysis of alkyl-aryl ether structures, especially among β-O-4 linkages. Another contribution to the higher phenolic hydroxyl groups in kraft lignin is formation of catechol groups, which are not found in native lignin (Hu et al. 2016). The formation of catechol groups results from nucleophilic substitution of methoxyl groups by the reduced sulphur species present in the kraft pulping liquor (hydrosulfide anion and methyl mercaptan anion) (Marton 1971). Also, its content has been reported to increase with decreasing molecular weight from degradation of lignin during kraft pulping (Jiang et al. 2017).

The black liquor (BL), or process (by-product) stream, generated in the kraft pulping process, is an aqueous solution consisting of organics including lignin, polysaccharides, carboxylic acids, extractives, and spent cooking chemicals. The chemistry and properties of BL are directly correlated to these components. Also, the proportion of the organic and inorganic components varies from mill to mill, from laboratory to laboratory, and from mill to laboratory, due to the natural variations of the organic constituents in wood species and the cooking conditions.

The BL, rich in lignin, is typically concentrated by evaporation and subsequently combusted in a recovery boiler to produce steam and energy for pulping operations (Gellerstedt et al. 2010). Although burning lignin is still a valuable contribution for reducing reliance on fossil sources, transforming lignin into value-added products is a key goal for creating economically feasible biorefinery processes (Rodrigues Pinto et al. 2011). In addition, it has been shown that 10–15% of the lignin in the BL can be isolated without adversely affecting the chemical recovery process (Hu et al. 2016).

1.4.Lignin precipitation

Lignin extraction from black liquor is an interesting option for pulp mills because it contributes to an increased production capacity without increasing recovery boiler load (Wallmo et al. 2007). Also, it allows for value creation, and enables the industry to transition to a biorefinery, in which multiple products from biomass are generated. Lignin yield and properties depend upon raw material used, pulping process, conditions applied, and extraction methods/conditions. Thus, every lignin is different; neglecting such fact gives rise to non-comparable works and irreproducible materials portfolios, which substantially hinder progress towards lignin-based commercial products (Schlee et al. 2019).

Obtaining lignin from BL generated during the kraft pulping can be industrially accomplished by acidification and ultrafiltration with membranes. The former is the main technique used and thus the one herein proposed. Fig. 1.4 depicts the LignoBoost process and how it is integrated into a kraft mill.

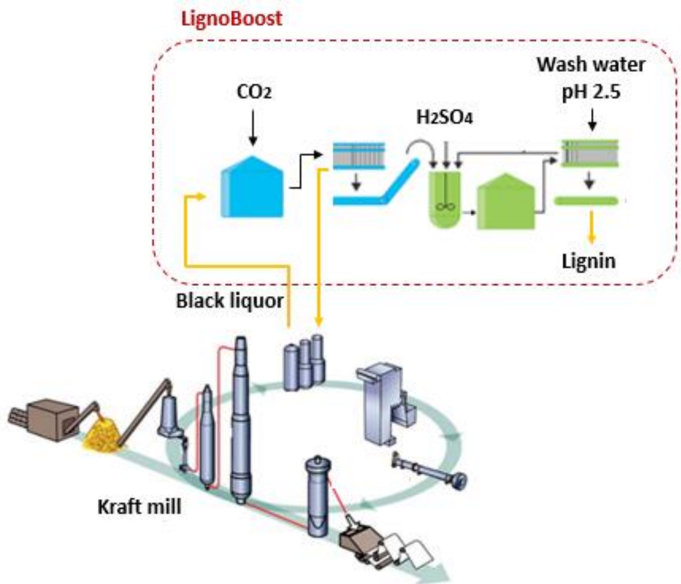

Figure 1.4. The LignoBoost process in a kraft mill (adapted from Björk et al. 2016)

This is the technology most widely employed for commercial kraft lignin separation. Dissolved carbon dioxide (CO_2) is sparged to the BL to decrease pH from ~ 13 to ~ 10. When acidified, phenoxide groups of the dissolved lignin become protonated and lignin solubility decreases, *i.e.*, the lignin begins to precipitate. After precipitation, the solids are separated by filtration, then subsequently re-suspended in water and sulfuric acid (H_2SO_4) to obtain a lower pH of ~ 2.5 to remove impurities (Wallmo et al. 2016).

The acidification technique used for lignin extraction is based on the dissociation equilibria of weak acid groups which affects the solubility behavior of lignin-related chemical species. pKa for phenolic groups in lignin varies between 6.2 and 11.3 depending on substitution pattern, while there is no difference between corresponding guaiacyl and syringyl structures (Ragnar et al. 2000). In addition, the pK_a for carboxylic groups varies from 3 to 5 and hence an additional pH decrease promotes better precipitation yields (Bertaud et al. 2014). Thus, the lower the pH, the higher is the lignin recovery yield. It is important to mention that lignin structure contain few or no carboxyl groups. These groups are mainly products of hemicellulose breakdown and they are either covalently bonded as lignin-carbohydrate complexes (LCC), adsorbed onto the lignin, or merely present with the lignin (Hubbe et al. 2019).

The equilibrium of dissociation of phenolic groups on lignin can be written chemically as (Zhu et al. 2015):

$$\mathrm{L-OH} \leftrightarrow \mathrm{L-O^-} + \mathrm{H^+} \qquad \text{(Eq1.1)}$$

where L is lignin and -OH is the phenolic group on the benzene ring of the lignin phenylpropane unit. The dissociation constant, K_a, of a phenolic group is a quotient of activities of {L-OH}, {L-O$^-$}, and {H$^+$}:

$$K_a = \frac{\{\mathrm{L-O^-}\}\{\mathrm{H^+}\}}{\{\mathrm{L-OH}\}} \qquad \text{(Eq1.2)}$$

CO_2 is the predominant acidifying agent. The reactions of CO_2 and hydroxide ions are described in reactions 1.3 to 1.5.

$$\mathrm{CO_2(g)} \leftrightarrow \mathrm{CO_2(aq)} \qquad \text{(Eq1.3)}$$

$$\mathrm{CO_2(aq)} + \mathrm{OH^-} \leftrightarrow \mathrm{HCO_3^-} \qquad \text{(Eq1.4)}$$

$$\mathrm{CO_2(aq)} + \mathrm{H_2O} \leftrightarrow \mathrm{HCO_3^-} + \mathrm{H^+} \qquad \text{(Eq1.5)}$$

$$\mathrm{HCO_3^-} + \mathrm{OH^-} \leftrightarrow \mathrm{CO_3^{2-}} + \mathrm{H_2O} \qquad \text{(Eq1.6)}$$

A key advantage of CO_2 is that it does not disturb the balance between sodium (Na) and sulfur (S) in the recovery cycle (Öhman et al. 2007a). The CO_2 concentration affects pH change significantly because a gas with lower CO_2 content decreases pH lowering rate (Wallmo et al. 2009a). Furthermore, CO_2 is effective for reducing pH in the approximate range of 8 to 10.5; thus, if lower values are desired, stronger acids are required.

Other acidifying agents have been reported in the literature, including sulfuric acid, hydrochloric acid, phosphoric acid, and organic acids. Even though they have been proven to be effective, some aspects must be taken into consideration, including the Na/S balance, cost, and corrosion (Hubbe et al. 2019).

pH has been found to be the most important process variable that highly affects the final product. However, the protonation of acidic groups in lignin occur in similar manner when comparing different acidifying agents and scales.

There are discrepancies regarding the effect of temperature on the process. A slight decrease in precipitation yield with increasing temperature (45 → 75 °C) has been reported (Zhu et al. 2014; Zhu and Theliander 2015), attributed to lower pK_a of lignin at higher temperatures, and thus higher solubility. In addition, increased temperatures decrease CO_2 solubility and absorption, resulting in higher final pH (Wallmo et al. 2007). On the other hand, increasing the acidification temperature has been reported to improve the lignin precipitation and filterability (Howell and Thring 2000). The maximum practical temperature was found to be 70 °C because lignin starts to form a tarlike substance at ~ 80 °C.

It has been demonstrated that the presence of specific anions bonded to the sodium ion (Na^+) may induce kraft lignin precipitation at different rates in a phenomenon known as the *salting-out effect* (Sewring and Theliander 2019). Overall, increasing the ion strength will reduce the range of repulsive interactions between lignin molecules after which lignin molecules come close enough for attractive forces to become dominant (Zhu and Theliander 2015).

The impact of ionic strength is less pronounced than the impact of final pH, yet when increased it improves lignin recovery. Also, different salts will affect the precipitation at different rates. So, the type of salt (anion) dissolved in the black liquor and proportions will alter precipitation conditions and hence final product.

Filtration is a challenging step in obtaining lignin due to lignin's colloidal nature. Partial plugging of filter cakes leads to extremely low flow that results in very high levels of impurities in the lignin (Öhman et al. 2007a). These effects are attributed to excessive pH level and ionic strength gradients in the lignin cake during washing. Moreover, filtration properties vary considerably between lignin particles precipitated from different black liquors under the same conditions. As a general rule, heat treatment improves filtration (Öhman et al. 2007b). Furthermore, the specific filtration resistance of precipitated lignin slurries subjected to a conditioning procedure prior to filtration is lower than slurries filtrated directly after precipitation, likely due to the agglomeration of precipitated particles (Wallmo et al. 2009a).

In addition, the average specific filtration resistance increases with increasing hemicellulose concentration (Wallmo et al. 2009b); however, size should be taken into consideration as carbohydrates of low-molecular mass do not affect the filtration properties. Another investigation has confirmed that the presence of carbohydrates hinders filtration and added that xylan is evenly distributed on the filter cake, thereby reinforcing xylans are adsorbed onto lignin (Durruty et al. 2017b). Moreover, the filtration resistance is significantly higher for HWKL than for SWKL due to higher concentration of hemicelluloses in hardwood lignin (Wallmo et al. 2009b).

An effective washing step is necessary after filtration because precipitated lignin contains Na^+, which must be washed out and returned to the mill to avoid upsetting the Na/S balance and hence necessitating an excessive demand of make-up chemicals (Öhman et al. 2007a). Also, some final uses for precipitated lignin require high purity. When the filter cake is re-dispersed at pH and temperature values approximately equal to those of the final washing liquor, the concentration gradients during the washing stage will be low; thus, the change in the pH level, ionic strength and any change in lignin solubility will take place in the slurry and not in the filter cake or in the filter medium during washing (Tomani 2010). Hence, washing efficacy is improved to obtain purer lignin.

Another motivation for redispersion is lignin recovery, a step shown to improve lignin yield from 6 to 7.5% (Öhman et al. 2007a). The losses experienced in the system without this step are attributed to large pH and ionic strength gradients that might lead to lignin re-dissolving. Moreover, improved recovery and lignin purity are expected in laboratory and commercial processes when redispersion and washing steps are performed.

The mixing speed may influence the absorption rate of the CO_2 because mixing speed alteration promotes changes in the size of the CO_2 bubbles and hence in the total mass transfer area (Wallmo et al. 2009a). Overall, higher mixing speeds improve absorption and increase the pH lowering rate. In addition, it can lead to increased specific filtration resistance possibly due to the disintegration of precipitated lignin particles or the fact that the high mixing speed hinders particles from agglomerating (Wallmo et al. 2009a). Another implication of high speeds is foaming, the formation of bubbles that are smaller and probably more stable, thus tending not to coalesce when reaching the surface (Wallmo et al. 2009a).

The stirring rate should be kept as slow as possible; however, some agitation is needed for uniformly mixing acid and black liquor (Howell and Thring 2000). Also, gentle agitation encourages collisions among droplets or particles of lignin, helping them to coagulate, while still allowing settling to occur (Hubbe et al. 2019).

The BL composition highly influences the process conditions during precipitation and, most importantly, filterability. The composition summarizes the influence of the wood source, the pulping process, the applied conditions, as well as the hemicellulose content, the dry substance content, and organic acids (Kienberger et al. 2021). The typical solids content of weak black liquor varies from 12 to 18% (Hubbe et al. 2019). Lignin constitutes 30-45 wt.% of organics (Belo et al. 2018), whereas the inorganic content is usually very high due to pulping chemicals and to the dissolution of inorganics from biomass representing about 28–44% of the total solids (Pinto et al. 2015).

Generally, the BL subjected to lignin precipitation is obtained from evaporators; thus, it exhibits higher solids content than weak BLs. The highest yield of precipitation are obtained at a solids content of 27-30% for pine kraft black liquors and 30-35% for birch kraft black liquors (Alén et al. 1985).

The presence of xylan causes a reduction in the further growth/agglomeration of particles due to increased electrostatic repulsive forces and/or steric effects resulting in smaller particles being formed when compared to the case without added xylan (Sewring et al. 2019). Another investigation dealing with the presence of xylan in the BL did not observe difference in the particle size; however, it was conjectured that xylan adsorbed at the surface of the lignin leads to an increase in contact area between solid and liquid thus making the particle structure more porous (Durruty et al. 2017a).

Traditionally, lignin precipitation from hardwood BL exhibits lower yields than the precipitation from softwood BL, and the precipitation from BL containing both lignins is lower than the yield of a pure softwood lignin at corresponding precipitation conditions (Zhu et al. 2014), which is explained by the different structures of softwood and hardwood lignins.

1.5.Research goals

Applied research on kraft lignin continues to increase; therefore, understanding the composition of KL obtained from different species and the structural modifications taking place in pulping and precipitation is crucial because such information supports lignin valorization and utilization. Thus, given the different behavior and performance of hardwood and softwood lignins throughout kraft pulping and lignin precipitation, the present study intended to interrogate such differences. This research also intended to expand the knowledge on hardwood kraft lignin (HWKL), and it was structured as follows:

Chapter 2 summarizes and critically reviews the research and development (R&D) on HWKL. It covers lignin structure and properties, as well as extraction, fractionation, and modification methods. Moreover, it reveals several interesting opportunities for this valuable material.

Chapters 3 and 4 deal with kraft lignins obtained from two wood species, pine (*Pinus taeda*), a softwood; and sweetgum (*Liquidambar styraciflua),* a hardwood. In chapter 3, the composition of pulp and kraft lignin samples obtained at different levels of the pulping process is determined. Moreover, a material balance accounting for solid and liquid flow is presented and the lignin recovery of two lignin fractions obtained at different pHs (9.5 and 2.5) is discussed. In chapter 4, the molecular weight of the pine and sweetgum kraft lignins is interrogated.

Chapter 5 establishes a correlative relationship on the influence of wood species upon lignin precipitation and lignin quality. Besides pine and sweetgum, acacia (*Acacia mangium*) and eucalyptus (*Eucalyptus globulus*) were also investigated. The study of these three hardwoods offers a great insight into the impact of S/G ratio upon lignin precipitation as it was shown that this parameter greatly affects pulping and lignin precipitation.

In chapter 6, a thorough characterization of the chemical structure of kraft lignins obtained from pine, acacia, sweetgum, and eucalyptus black liquor is presented. Such information amplifies the current knowledge on kraft lignins, which is vital for stablishing new lignin plants and future applications.

Chapters 7 and 8 overview the main conclusions drawn from this research and provide suggestions for future investigations, respectively.

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2. Chapter 2. Insights into the potential of hardwood kraft lignin to be a green platform material for emergence of the biorefinery

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Abstract

Lignin is an abundant, renewable, and relatively cheap biobased feedstock that has potential in energy, chemicals, and materials. Kraft lignin, more specifically, has been used for more than 100 years as a self-sustaining energy feedstock for industry after which it has finally reached more widespread commercial appeal. Unfortunately, hardwood kraft lignin (HWKL) has been neglected over these years when compared to softwood kraft lignin (SWKL). Therefore, the present work summarizes and critically reviews the research and development (R&D) dealing specifically with HWKL. It will also cover methods for HWKL extraction from black liquor, as well as its structure, properties, fractionation, and modification. Finally, it will reveal a number of interesting opportunities for HWKL that include dispersants, adsorbents, antioxidants, aromatic compounds (chemicals), and additives in briquettes, pellets, hydrogels, carbon fibers and polymer blends and composites. HWKL shows a great potential for all those applications, however more R&D is needed to make its utilization economically feasible and reach the levels in the commercial lignin market commensurate with SWKL. The motivation for this critical review is to galvanize further studies, especially increased understandings in the field of HWKL, and hence amplify much greater utilization.

2.1. Introduction

Lignin is the main source of renewable aromatic structures on Earth (Duval and Lawoko 2014; Kadla and Kubo 2003). The lignocellulose biorefinery is an interesting approach to maximize the value of lignin and its products. Lignin valorization for industrial applications depends on the development of novel technologies and approaches to overcome challenges mitigating conversion to valuable products (Wang et al. 2019a). Technical challenges include the need for efficient biomass pretreatment, lignin separation and purification technologies that can address lignin's diverse structure and complex chemistry. Despite recent advances, there is currently limited conversion of hardwood kraft lignin (HWKL) products into bulk or fine chemicals. Difficulties with the production of value added products from lignin can partly be attributed to the highly complex and condensed nature of kraft lignin, the prevalence of highly recalcitrant lignin linkages/bonding motifs, and a high amount of polydispersity in addition to a considerable sulfur content (Rinaldi et al. 2016).

Lignin can be utilized as an eco–friendly alternative to numerous petro–derived substances. Potential high-value products from isolated lignin include low- cost carbon fiber, engineering plastics and thermoplastic elastomers, polymeric foams and membranes, and a multitude of fuels and chemicals all currently sourced from petroleum (Ragauskas et al. 2014). A multitude of lignin applications have been explored at the research level. Although current industrial products are primarily produced from lignosulfonates and sulfonated kraft lignin such as dispersants, emulsifiers, raw material for vanillin and dimethyl sulfoxide (DMSO) production and road-dust suppression agents (Macfarlane et al. 2014). Kraft lignin production increased by 150% from 2014 to 2018, competing with lignosulfonates to some extent (Dessbesell et al. 2020). The kraft pulping process dominates the industry because of advantages in chemical recovery and pulp strength.

Sulfite pulps are not as strong, and the efficiency and effectiveness of the process is more sensitive to wood species characteristics than is the kraft pulping process. Thus, the use of sulfite pulping has declined in comparison to kraft pulping (Bajpai 2015).

Kraft mills account for ~ two-thirds of the global pulping industry (Magdeldin and Järvinen 2020), where ~ 78 million tons of lignin are generated annually (Miller and Faleiros 2016). Lignin is present in black liquor (BL) which is typically concentrated by evaporation before combustion in a recovery boiler to produce steam and a majority of the energy necessary for pulping operations (Gellerstedt et al. 2010). In the biorefinery concept, rather than just producing steam and energy, a portion of the black liquor would be utilized as a feedstock for production of value-added products. Removal of such a feedstock could be used for increasing pulp production since many recovery boilers are limited by total solids loading or steam generation (circulation limit), and hence they represent the capacity-limiting step in several pulp mills. Lignin isolation from black liquor is considered a potential method for reducing these process limitations (Öhman et al. 2007b). It has been estimated that the total amount of lignin produced by the pulp industry is 60% more than what is actually needed for supplying internal power (Sannigrahi et al. 2010).

Although burning lignin is still a valuable contribution for saving fossil sources, processing lignin into value-added applications is a key factor for creating economically feasible biorefinery processes (Rodrigues Pinto et al. 2011). Lignin is an outstanding feedstock for different materials because of its varied functional groups, aromaticity, renewability, biodegradability, nontoxicity, and relatively low cost. Yet, a gap exists between the kraft lignin producers and market off-takers, due to the current lignin processes having difficulties meeting stringent quality control constraints and market specifications which end-uses require. These constraints slow down lignin market penetration (Dessbesell et al. 2020).

Literature on the relation between the structure/chemistry of the lignin precursor and the resulting materials' properties is scarce. Since lignin is not a well-established chemical, but inherently a loosely defined precursor, it is crucial to characterize the lignin used as a precursor to better exploit its structural and chemical features. Every lignin is different and neglecting such a fact gives rise to a potpourri of non-comparable works and irreproducible materials portfolios which substantially hinder progress towards lignin-based commercial products (Schlee et al. 2019). This review is specifically focused on hardwood kraft lignin (HWKL) and critically discusses its structure and properties, as well as extraction, fractionation, and modification processes. Finally, HWKL applications are addressed.

2.2. Lignin extraction

The separation of lignin from the black liquor generated during kraft pulping can be achieved by acidification and ultrafiltration membranes. The acidification technique is based on the dissociation equilibria of weak acid groups which affects the solubility behavior of lignin-related chemical species. Membrane separation processes, on the other hand, can be efficient and cost-effective in many cases, however there are two key limitations: First, such processes get increasingly difficult to control as the concentration of the retained material increases. Second, the flux of permeate passing through a membrane tends to fall during continued usage due to such fouling phenomena as pore plugging and cake formation (Hubbe et al. 2019).

LignoBoost™ and LignoForce™ processes are the two main commercial technologies for precipitating lignin from black liquor (Dessbesell et al. 2020), and both can be used to obtain hardwood kraft lignin. The LignoBoost™ process uses dissolved carbon dioxide (CO_2) to decrease the pH of the process stream from ~ 13 to 10 (Figure 2.1). It is well known that when the BL is acidified, the phenoxide groups of the dissolved lignin become protonated and lignin solubility decreases, i.e., the lignin precipitates. After precipitation, the solids are separated by filtration, then subsequently re-suspended in water and sulfuric acid (H_2SO_4) to obtain a lower pH of ~ 2.5 to remove impurities (Wallmo et al. 2016).

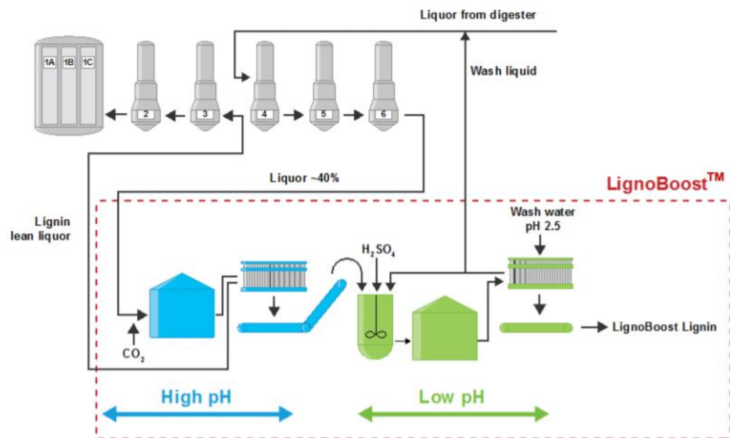


Figure 2.1. Interunit linkages and S/G ratio in pine, acacia, sweetgum, and eucalyptus native lignins

At pH values of less than 11, significant quantities of total reduced sulfur (TRS) compounds and other volatile sulfur species can be generated. Such compounds are strongly odorous with well-known negative effects on human health and other forms of life. Thus, LignoForce™ was created to address these issues (Kouisni et al. 2016). LignoForce™ is a commercialized technology that first oxidizes the black liquor with O_2 and then acidifies to pH ~ 9 with CO_2 (Fatehi and Chen 2016) (Figure 2.2).

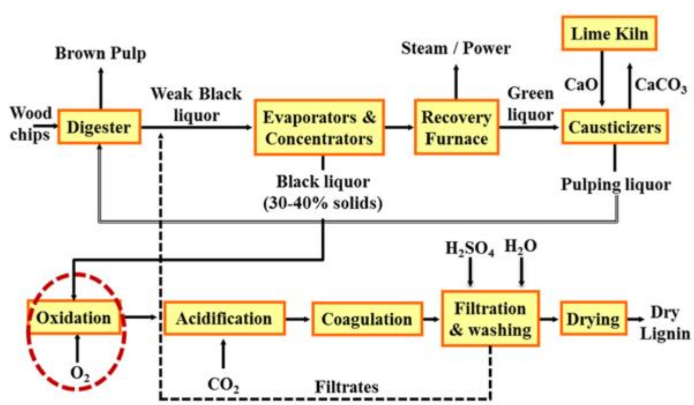


Figure 2.2. LignoForce™ System by FPInnovations and NORAM (Kouisni et al. 2016)

It is worth mentioning that lignin needs to be precipitated from pulping spent liquors effectively and selectively to have an economically feasible lignin production (Fatehi and Chen 2016). Unfortunately, acidic conditions used in lignin precipitation are known to cause some β-ether cleavage and lignin condensation (Li et al. 2018). Thus, the process conditions for lignin recovery from black liquor may interfere with its processing and use. The cleavage and condensation reactions are depicted in Figure 2.3.

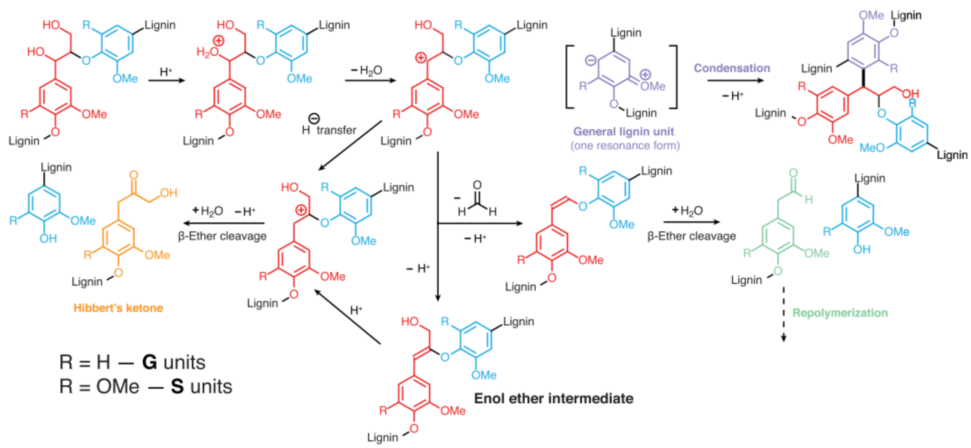


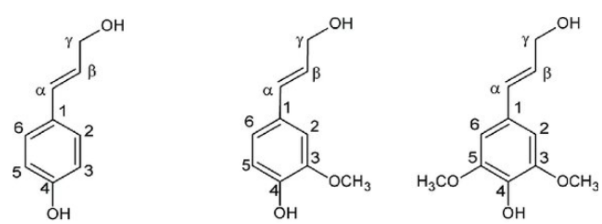
Figure 2.3. Lignin acidolysis and condensation routes (Li et al. 2018)

The use of extracted lignins rather than whole biomass can claim an advantage in that the material can be fully dissolved in organic solvents, facilitating recovery and continuous processing (Li et al. 2018) for diversification of products and hence value creation for the pulp and paper industry. However, it is important to mention that lignins can be extracted from the whole biomass. Lignins, in any form, are soluble in ionic liquids (ILs), which facilitates extraction from lignocellulose. The extraction can be performed with or without dissolution of the biomass (Yinghuai et al. 2012). ILs are considered green solvents due to their non-volatility and low flammability. Furthermore, ILs are not only used as solvents but also play an important part in catalytic cycles in pulping reactions (Peng et al. 2020). However, ILs have a major shortcoming as they are much more expensive when compared to common and traditional solvents. Thus, recoverability of ILs should be explored and emphasized. Due to the π-π interaction between ILs and lignin, removal of lignin from ILs are proven to be a complex process and, therefore, requires multiple steps (Zakzeski et al. 2010). This makes the recycling and regeneration of ILs, particularly in extremely large volumes, as cost inefficient (Mora-Pale et al. 2011).

Moreover, deep eutectic solvents (DESs) has been reported to completely isolate lignin from cellulosic biomass in one-pot procedure (Malaeke et al. 2018). DESs are green and inexpensive solvents, that have emerged at the beginning of this century to overcome the problems of ILs (Zhang et al. 2012). Similar to ILs, DESs have interesting properties including negligible volatility, non- flammability, and high conductivities (Domínguez de María and Maugeri 2011).

2.3. Structure and Properties

Lignin may be described as a complex, racemic, cross-linked, and highly heterogeneous aromatic macromolecule based on hydroxycinnamyl alcohol monomers with various degrees of methoxylation (p-coumaryl, coniferyl and sinapyl alcohol) (Fengel and Gerd 1984). Lignin building blocks are shown in Figure 2.4. These structural building blocks are zipped together by ether linkages such as β-O-4, 4-O-5, and by carbon-carbon bonds such as 5–5, β-5, β-1 and β–β linkages (Alén 2000).



H G S

Figure 2.4. Lignin building blocks: p-coumaryl alcohol (H), coniferyl alcohol (G), and sinapyl alcohol (S).

Hardwood lignin is made of coniferyl alcohol and sinapyl alcohol, forming the macromolecules guaiacyl (G) and syringyl (S), respectively. Softwood lignin contains only guaiacyl units in which one of the ortho-positions next to the phenol is free and hence more easily branched and/or crosslinked. In contrast, hardwood lignin that contains both guaiacyl and syringyl units, has a more linear structure compared to softwood (Nordström et al. 2013b). Softwood lignin appears to vary little amongst species (Akiyama et al. 2005; Sarkanen et al. 1967); whereas hardwood lignins vary greatly among species with a wide range of syringyl-to-guaiacyl ratio (Santos et al. 2012) within its structure.

Native lignin undergoes extensive chemical transformation during the pulping processes, such as fragmentation reactions, ring opening, and sulfonation reactions that facilitate its solubilization in black liquor. The extent of structural modification depends on the severity of the process, i.e., alkali charge, temperature and duration of cooking (Gellerstedt et al. 1984). Delignification leads to the modification of the frequency of interunit linkages (β-O-4 and C-C linkages), making changes in the frequency of the functional groups, like hydroxyl, carbonyl, and carboxyl groups (Rodrigues Pinto et al. 2011). The delignification reactions increase the number of phenolic hydroxides due the breakage of the β-aryl bonds and increase the number of condensed C–C bonds (Schutyser et al. 2018).

In addition, the hardwood kraft lignin properties depend upon the feedstock from which they are obtained (wood species), the extraction method employed, and downstream purification processes (Santos et al. 2012). The depolymerization of hardwood lignin generally results in high aromatic monomer yields because these feedstocks typically contain high S/G ratios (Sun et al. 2018).

2.3.1. Linkages

The most frequent lignin inter-unit linkage is the β–O–4- (β-aryl ether linkage), which is the linkage most easily cleaved chemically, providing a basis for industrial processes and several analytical methods (Ralph et al. 2007). The other linkages are all more resistant to chemical degradation. Table 2.1 shows the frequency of these linkages in the biomass.

Table 2.1. Frequency of lignin linkages in native hardwood species

Biomass	Linkage (%)						Reference
	β-O-4	β-5	α-O-4	β-β	5-5	4-O-5	
Hardwood	60-62%	3-11%		3-12%	<1%	2%	(Kumar et al. 2020)

Table 2.2 shows the frequency of linkages for different HWKL.

Table 2.2. Frequency of lignin linkages in hardwood kraft lignin

Lignin	Linkage (per 100 Ar)			
	β-O-4	β-5	β-β	Reference
Birch	2	2	3	(Berlin and Balakshin 2014a)
E. globulus	12	1	2	(Berlin and Balakshin 2014a)
E. grandis	5	2	3	(Berlin and Balakshin 2014a)

2.3.2. Molecular Weight

Molecular weight (MW) is an important chemical property for the characterization and utilization of lignin (Wang et al. 2019b). Reactivity and physico-chemical properties of lignins are partly governed by this property (Baumberger et al. 2007). For these reasons, MW is addressed in most of the papers dealing with lignin. Table 2.3 depicts the molecular weight distribution of several hardwood kraft lignins.

Table 2.3. Molecular weight distribution of hardwood kraft lignins

Sample	M_w (g/mol)	M_n (g/mol)	PDI	Method	Reference
HWKL	3900	1000	3.9	HPLC	(Asikkala et al. 2012)
HWKL	3300	1000	3.3	HPLC	(Asikkala et al. 2012)
Eucalyptus exserta	55500	31700	1.8	MALLS	(Wang et al. 2019b)
Indulin	3700	1300	2.3	GPC	(Glasser et al. 1993)
Eucalin	3900	1700	2.3	GPC	(Glasser et al. 1993)
Ligflow 401	2042	719	2.8	GPC	(Talyta Torrezan 2019)
HWKL	2400	1263	1.9	GPC	(Kadla and Kubo 2004)
HWKL	3290	1793	1.8	GPC	(Thielemans and Wool 2005)
European beech	1711	1044	1.6	GPC	(Ház et al. 2019)
Eucalyptus	4200	1273	3.3	HPLC	(Dehne et al. 2016)
Eucalyptus grandis	1740	910	1.9	GPC	(Zhao et al. 2019)

The non-uniformity of lignin precludes the characterization of a specific molecular weight (Tolbert et al. 2014). Therefore, it is necessary to characterize lignin in terms of average molecular weight. Two common averages used are number average molecular weight (M_n) and weight average molecular weight (M_w). The polydispersity index (PDI), defined as M_w/M_n represents the molecular weight distribution of the polymers (Lange et al. 2016). The magnitude of M_w and M_n results depends on the technique used for their determination; overall, values from light scattering are much larger than those obtained from conventional protocols (HPLC and GPC) (Wang et al. 2019b). In some instances, the expected range of molecular weight will dictate the technique used to measure the molecular weight. When the same sample is subjected to different measurement techniques, it is possible to obtain differing answers.

2.3.3. Thermo-mechanical properties

Lignin is moderately stable at elevated temperatures due to its highly aromatic backbone (Sen et al. 2015). Lignin thermally decomposes over a broad temperature range, because various oxygen functional groups from its structure have different thermal stability. Thus, their scissions occur at different temperatures. Furthermore, due to its complex composition and structure, the thermal degradation of lignin is strongly influenced by its nature, moisture content, reaction temperature, degradation atmosphere, and mass transfer processes with considerable effect on conversion and product yields, as well as on the physical properties and quality of the pyrolysis products (Brebu and Vasile 2009). In conventional thermogravimetric analysis (TGA) curve, main weight loss starts ~250 °C and continues to 600 °C. An inflection point of the main degradation step appears at 382 and 365 °C for SWKL and HWKL, respectively (Kubo and Kadla 2008). The same study evaluated Modulated TGA (MTGA) to study the kinetics of lignin pyrolysis. In this technique, a sinusoidal temperature wave is applied to a sample to calculate and display the kinetic parameters of decomposition on a continuous and real-time basis. Using a constant heating rate, the apparent activation energy (E_a) values were ∼230 and ∼210 kJ/mol for SWKL and HWKL, respectively.

Glass transition temperature (T_g) varies widely depending on the method of lignin isolation, adsorbed water, molecular weight and thermal history (Sarkanen and Ludwig 1971). T_g of isolated lignin samples is often difficult to determine due to their heterogeneous structures and broad molecular weight. In addition, T_g shifts to higher temperatures with increasing average molar mass (Laurichesse and Avérous 2014). Furthermore, it is well recognized that solubility generally follows T_g, with solubility (in organic solvents) increasing as T_g declines (Lora and Glasser 2002). In addition, large amounts of phenolic hydroxyl groups contribute towards higher T_g through the formation of intra-molecular hydrogen bonds, thereby creating a physically cross-linked structure (Sen et al. 2015).

Kraft lignins do not undergo a distinctive glass-to-rubber (or fluid) transition when heated (Glasser 1999). It has been reported that the T_g for eucalyptus kraft lignin is around 133 °C. Although, the authors claim that ~ 147 °C, all chains of its amorphous structure will acquire mobility (Talyta Torrezan 2019). For other hardwood kraft lignins, T_g values such as 108 °C (Kadla and Kubo 2004) and 138 °C (Ropponen et al. 2011) have been reported.

The aromatic structure of lignin provides rigidity and stiffness to materials (Jawerth et al. 2020). Also, lignin has been found to be incompatible with most aliphatic polyesters, thus deteriorating the mechanical properties of any resultant composites besides contributing to rougher and fractured surfaces (Park et al. 2019). Mechanical testing of 100% lignin fibers has revealed tensile strengths of 23 MPa and Young's modulus of 3.9 GPa (Kubo and Kadla 2005a) These values are comparable to those reported modified softwood kraft lignin thermoplastic (25 MPa and 1.5 GPa, respectively) (Li and Sarkanen 2002).

2.3.4. Functional groups

Functional groups in lignin include methoxyl-, carbonyl-, carboxyl-, and hydroxyl- linking to aromatic or aliphatic moieties (Lu et al. 2017). The amount and proportion of these functional groups will depend on the raw material, pulping conditions, and lignin extraction method. Table 2.4 shows the functional groups reported in the literature for different HWKL.

Table 2.4. Functional groups in hardwood kraft lignins

Sample	Functional group					Reference
	Methoxyl	Phenolic-OH	Aliphatic-OH	Carboxyl	Carbonyl	
Eucalyptus spp.	18.8%	3.16 mmol/g	1.54 mmol/g	-		(Dehne et al. 2016)
HWKL	-	1.93 mmol/g	1.56 mmol/g	0.39 mmol/g		(Husson et al. 2019)
HWKL	5.9 mmol/g	4.3 mmol/g	4.1 mmol/g	-		(Kubo and Kadla 2005b)
HWKL	-	1.45 meq/g		2.23 meq/g	0.13 meq/g	(Harmita et al. 2009)

2.3.5. Reactivity

HWKL reactivity depends on species, delignification method and its severity. These parameters influence the type and amount of functional groups, as well as key lignin linkages, S/G ratio and hence affects lignin reactivity (Kun and Pukánszky 2017; Schutyser et al. 2018). It should be noted that the different functional groups have different reactivities. The amount of hydroxyl groups (-OH), particularly phenolic-hydroxyls, is one of the most important parameters in lignin because it is an indicator of lignin reactivity and give lignin its potential to be utilized in a variety of technical applications (Goldmann et al. 2016).

The degree of condensation is an important lignin characteristic as it is often negatively correlated with lignin reactivity (Costa et al. 2014). Lignin condensation is a common phenomenon in various strategies of lignin processing/conversion. The increase of condensed structures largely reduces product yields since C-C bonds are more difficult to further decompose into aromatic monomers than structures connected by ether bonds (Liu et al. 2019). Figure 2.5 gives a simplified representation of the condensation process leading to a dead-end product for downstream processing.

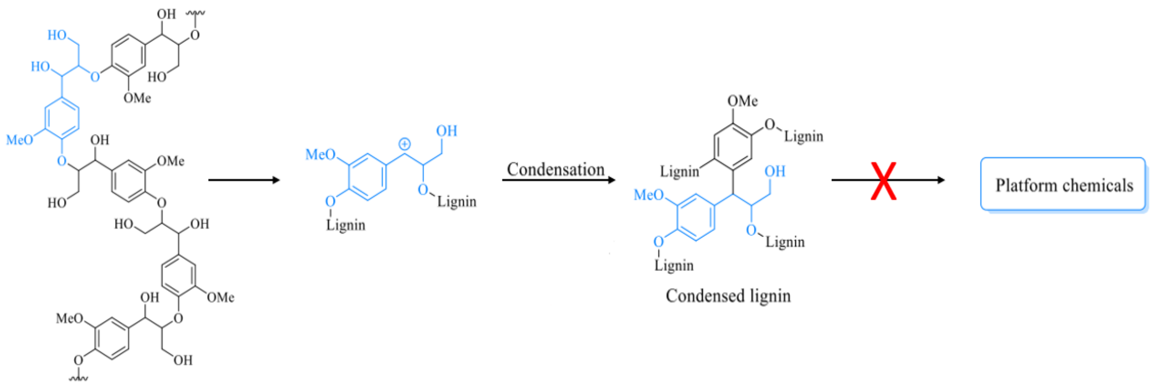


Figure 2.5. Schematic diagram of lignin condensation.

The β-O-4 linkage and the degree of condensation in lignin are closely related to the S/G ratio (Santos et al. 2012). The C-5 position of the syringyl aromatic ring is occupied by a methoxyl group and therefore it cannot be involved in condensation. Thus, hardwood lignins are less condensed than softwood lignins (Berlin and Balakshin 2014a), hence more reactive. A high lignin isolation yield usually corresponds with low reactivity towards depolymerization, and vice versa (Schutyser et al. 2018). Therefore, hardwood lignins are expected to show lower isolation yields than softwoods, which has been reported elsewhere (Jardim et al. 2020b).

2.3.6. Elemental composition

Table 2.5 shows the elemental composition of different hardwood kraft lignins. The differences in composition are related to their origins and to extraction processes (Schorr et al. 2014). The high carbon content makes HWKL very attractive as a source to a wide range of materials. Moreover, kraft lignin contains sulfur (S) and ash, which might limit its applications. Overall, an ideal kraft lignin has very low amounts of these components.

Table 2.5. Elemental composition of hardwood kraft lignins (wt%)

Sample	C	H	O	N	S	Na	Ash	Reference
Eucalyptus	63.2	5.1	-	1.7	1.4	1.2	3.2	(Mohan et al. 2006)
European beech	55.7	4.6	31.7	0.3	3.9	-	3.8	(Ház et al. 2019)
HWKL	62.5	5.7	29.0	0.3	2.9	-	0.5	(Schorr et al. 2014)

2.4. Lignin fractionation

One of the main issues limiting the application of technical lignins is their heterogeneity and high polydispersity (Duval and Lawoko 2014). Various types of C–O and C–C linkages with broad bond dissociation energy (BDE) linked between the aromatic centers of lignin lead to low selectivity in lignin depolymerization. Moreover, functional groups have an impact on lignin reactivity as previously discussed in § "Reactivity", and hence it affects lignin conversion. Lignin's relatively high molecular weight and amorphous structure results in limited solubility in most common organic solvents at standard temperature and pressure (STP.) In addition, the high reactivity of lignin degraded intermediates which are prone to side reactions makes it very difficult to produce a high yield of desired products from lignin (Wang et al. 2019a).

Fractionation based on size and/or composition is recommended for certain applications. A good fractionation method isolates lignin with high yield, high purity, and retain β-O-4 bonds in the technical lignins, which would be beneficial for subsequent conversion processes (Wang et al. 2019a). Fractionation enables development of a multi-stream integrated biorefinery since it creates multiple lignin streams with uniformity and specific chemical characteristics. These fractionated lignin streams could be used as starting materials for multiple biomanufacturing streams to make different value-added products (Li et al. 2017). Three common fractionation methods are fractionation with organic solvents, ultrafiltration, and acid precipitation.

2.4.1. Fractionation with organic solvents

Typically, this method starts from a state of complete dissolution of lignin in a polar solvent, from which specific lignin fractions can be precipitated upon gradual addition of a selected miscible but nonpolar solvent. In this way, the molecular composition of each lignin fraction can be manipulated by adjusting the solvent mixture along an almost continuous solvent gradient (Cui et al. 2014). This fractionation method has been widely used on an academic research level; however, the scale up of this technique seems compromised by the use of large quantities of organic solvents (Duval and Lawoko 2014). Fractionation of hardwood and softwood kraft lignins using dichloromethane, propanol and methanol has been reported. The solubility of HWKL was much higher (81%) than SWKL (34%) (Ponomarenko et al. 2014).

2.4.2. Fractionation with ultrafiltration membranes

Ultrafiltration is another fractionation technique, which uses a series of membranes that allows for a very efficient control of the molecular mass distribution of lignin fractions by the choice of different suitable membrane cut-offs (Sevastyanova et al. 2014). Ultrafiltrated lignin fractions are less contaminated with carbohydrates as compared to precipitated lignin (Toledano et al. 2010). This method has been shown to be effective for the fractionation of eucalyptus black liquor. The lignin fractions were heterogeneous not only with respect to their molecular weight, but also with respect to the type and frequency of functional groups (Costa et al. 2018). The decreasing molecular size was followed by a decrease in total phenolic and aliphatic hydroxyl groups. Also, the increase of membrane cut-off molecular weights led to fractions with a higher solids content and lower amounts of inorganics. Moreover, the highest content of non-condensed structures, i.e., highest content of β-O-4 structures (23 per 100 aromatic rings), was observed in the fraction with the smallest molecular weight.

2.4.3. Fractionation by acid precipitation

Acid precipitation is a common and simple method to obtain lignin fractions that uses strong acid to gradually decrease the pH of black liquor (García et al. 2009; Lourençon et al. 2015; Santos et al. 2014; Sewring et al. 2019). This technique is based on the dissociation equilibria of weak acid groups which affects the solubility behavior of lignin-related chemical species. Different pH intervals generate lignin fractions with different yields, composition and properties (García et al. 2009; Jardim et al. 2020b; Santos et al. 2014).

Acid precipitation has been shown to be an efficient method for fractionating hardwood and softwood kraft lignins into molecules with different relative molecular masses (Lourençon et al. 2015). Typically, the lignin fractions precipitated at higher pH show higher molecular weights, and as pH is lowered, lignin fractions with smaller molecular weights are precipitated.

2.5. Lignin Modification

Although lignin presents potential direct applications in the polymer industry, it can only be incorporated in small amounts, considering its thermal degradation and mechanical properties. The modification of lignin seems to be a way to overcome the limitations using this renewable product as a starting material for polymer and chemical synthesis (Duval and Lawoko 2014; Laurichesse and Avérous 2014).

Different types of modification have been proposed to increase its chemical reactivity, reduce the brittleness of lignin-derived polymers, increase its solubility in organic solvents, and improve the ease of processing the lignin. These modifications consist of increasing the reactivity of hydroxyl groups or changing the nature of chemical active sites, but it is always in view of synthesizing new, efficient and more reactive macromonomers (Laurichesse and Avérous 2014).

Modifications such as oxidative pre-treatment of hardwood kraft lignin has been reported to introduce oxygen in the material and to induce specific structural changes leading to much more stability against thermal degradation (Brodin et al. 2010). The oxidative pretreatment is performed in air, at temperatures around 250 °C, and at slow heating rates, which leads to an increase in oxygen content mostly due to carbonyl incorporation. There are several modification techniques reported in the literature, however the present review only addresses the most common and relevant methods to hardwood kraft lignin.

2.5.1. Sulfomethylation or sulfonation

The supply of lignosulfonate, lignin obtained from the sulfite pulping process, is limited by the small number of sulfite pulping processes currently operating in the world, but there is demand for it due to its water-solubility. Kraft lignins are not water-soluble; however, this can be achieved via sulfomethylation or sulfonation (Konduri and Fatehi 2015).

Sulfomethylated hardwood kraft lignin has been successfully prepared in an aqueous medium by using sodium sulfite and formaldehyde under alkali conditions as shown in Figure 2.6; the sulfomethylated lignin is formed via electrophilic substitution of the ring by a sodium methylsulfonate group (Konduri and Fatehi 2015).

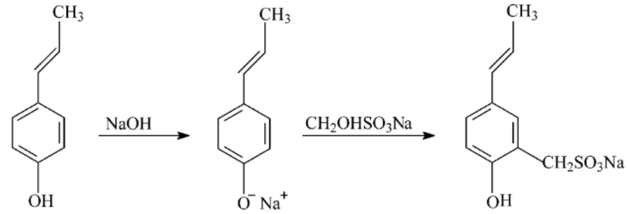


Figure 2.6. Sulfomethylation of lignin sodium sulfite and formaldehyde (Konduri and Fatehi 2015).

During sulfomethylation, introduction of sulfonate and methyl groups into lignin takes place, which leads to an increased water-solubility and an increase in thermal stability (Konduri and Fatehi 2015). In addition, the lignin modification increases molecular weight of the lignin due to the replacement of hydroxyl groups with sulfomethyl groups and possibly due to condensation reactions under alkaline conditions. Lignin extracted from birch has been converted to a water-soluble product via sulfonation with sodium sulfite under alkaline conditions (Watanabe et al. 1990).

2.5.2. Alkylation

Due to the presence of a very large amount of phenolic hydroxyl groups in kraft lignins, alkylation can be effortlessly carried out stoichiometrically through nucleophilic aromatic substitution reactions. These are well-acknowledged derivatization reactions that produce lignin ethers and hydroxyalkyl lignins which are used to prepare polymer blends for mixing with synthetic polymers (Sen et al. 2015).

Alkylation of hardwood kraft lignin by bromododecane (C12H23Br) is shown in Figure 2.7. This reaction is reported to improve the compatibility of HWKL when blended with polypropylene (Barkoula et al. 2008).

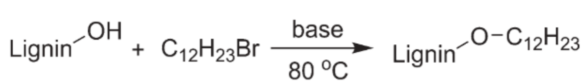


Figure 2.7. Reaction progress for the alkylation of lignin hydroxyl groups by bromododecane (Barkoula et al. 2008).

Alkylation completely removes the possibility of radical initiated self-polymerization of the kraft lignin, making it an important step for commercial processing of this natural polymer (Cui et al. 2013). However, after alkylation, the phenolic hydroxyl groups are converted to less acidic secondary aliphatic hydroxyl groups, which eventually reduce the extent of intra-molecular hydrogen bonding and thereby reduces T_g (Sen et al. 2015).

2.5.3. Methylation

Methylation is generally achieved by reacting lignin with diazomethane or dimethyl sulfate, as shown in Figure 2.8. Both reagents are highly toxic, which constitute a major drawback when considering the use of lignin in a more global green chemistry effort (Duval and Lawoko 2014).

After methylation of phenolic hydroxyl groups, a lower degree of hydrogen bonding between individual lignin macromolecules should be expected, which should facilitate macro-Brownian motion of lignin and thus its fusibility (Brodin et al. 2010). Also, the modified lignin form miscible blends with various aliphatic polyester.

Figure 2.8. Methylation of kraft lignin by dimethyl sulfate in aqueous NaOH (Sadeghifar et al. 2012).

Another effort verified that methylation of HWKL using dimethyl sulfate is selective toward the phenolic hydroxyl groups, allowing for a progressive methylation that can be adjusted by varying the feed ratio of dimethyl sulfate to the phenolic hydroxyl groups. The hardwood kraft lignin seemed to be relatively less reactive than softwood, which was attributed to relatively low reactivity of phenolic hydroxyl groups present in HWKL (Sadeghifar et al. 2012)..

2.5.4. Esterification

Lignin esterification has been studied as a strategy to improve compatibility between lignin and polyolefins in blends and composites (Dehne et al. 2017; Husson et al. 2019; Kumar and Alén 2015; Thielemans and Wool 2005). Figure 2.9 shows the esterification of lignin by acetic anhydride.

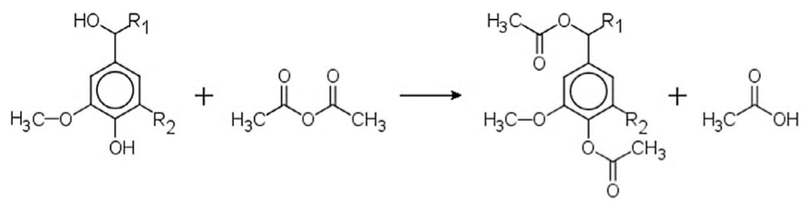


Figure 2.9. Esterification of lignin by acetic anhydride (Kun and Pukánszky 2017).

Esterified lignins from different sources, including HWKL (*Eucalyptus spp.*), have been investigated in lignin-polyethylene blends. The modification rendered purer lignin derivatives and less polar, as sugar and ash content were decreased and hydroxyl groups were completely esterified (Dehne et al. 2016). The researchers also mentioned modified lignins exhibited better miscibility with polyethylene than unmodified lignins.

2.5.5. Carboxymethylation

Carboxymethylation of mixed hardwood kraft lignin has been performed using sodium chloroacetate as a carboxylate group donor (Figure 2.10). Under alkali conditions, NaOH reacts with the hydroxyl group of the lignin's aromatic ring, generating a strong nucleophile. The alkoxide ion from the alkali lignin attacks the chloroacetate via an SN2 reaction resulting in the ether methyl carboxylation of lignin. The degree of carboxymethylation depends on the number of hydroxyl group substitutions with carboxymethyl groups (Konduri et al. 2015).

Carboxymethylated lignins are water soluble and show a relatively high charge density and low molecular weight, features that allow for the utilization as dispersant, for instance.

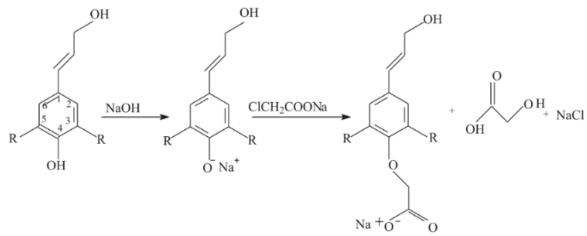


Figure 2.10. Carboxymethylation of lignin reaction scheme. ''R'' can be OCH3 or H (Konduri et al. 2015).

2.5.6. Grafting

Grafting attaches polymer chains to the lignin hydroxyl groups, thus resulting in a star-like branched copolymer that possess a lignin core. Two distinct routes can be used for the preparation of graft copolymers, and both have been applied to the manufacture of lignin graft copolymers, as depicted in Figure 2.11 (Duval and Lawoko 2014). "Grafting from" (Figure 2.11a): lignin plays the role a macro-initiator for the polymerization. A monomer first reacts with the lignin hydroxyls, and then the polymerization starts. The polymer chain is built on the lignin core. In the case of "grafting onto" (Figure 2.11b): the polymer chain is first synthesized, and functionalized at one end, to be able to react with the lignin OH functions. The polymer chains are then grafted to the lignin core (Duval and Lawoko 2014).

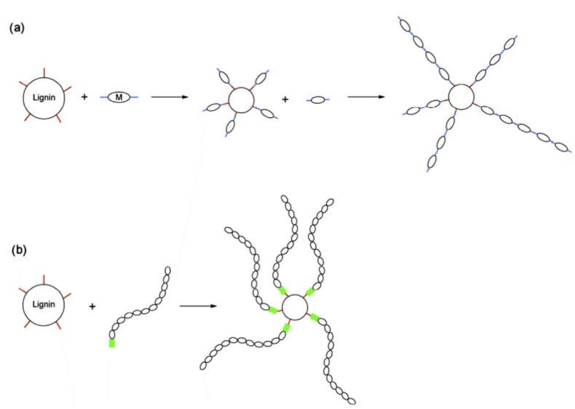


Figure 2.11. Schematic synthesis of lignin graft copolymers by "grafting from" (a) and "grafting onto" (b) techniques (Duval and Lawoko 2014)

Covalent grafting of a maleyl chain on HWKL and on other lignin types in acidic ionic liquid have been reported to be feasible (Husson et al. 2019). Lignin esterification of industrial lignins with maleic anhydride ($C_2H_2(CO)_2O$) were shown. Although the esterification induced a slight decrease in thermal stability, the modified lignin remained compatible with temperature conditions of extrusion processes for the generation of partially bio-sourced composites. Furthermore, the reaction significantly increased lignin solubility in polar and protic solvent probably due to the additional availability of carboxylic groups resulting from mono-acylation.

2.6. Lignin Applications

Major research & development findings for the applications of hardwood kraft lignins are addressed within this section.

2.6.1. Briquettes and pellets

The interest in renewable energy sources (RES) has been increasing worldwide as evidenced by the massive interest in pellets and briquettes. These two materials are fuels manufactured from biomass. European countries consumed 50% of the global wood pellets in 2018 (Jelonek et al. 2020). Also, the United States manufactured 8.2 million tons in the same year; being the second largest producer in the world only surpassed by China. It should be noted that the market continues to grow due to high demand from overseas markets.

Direct addition of 6% (w/w) of HWKL to briquettes has been reported to positively influence apparent density, energy density, heating value, and mechanical resistance of the material (Boschetti et al. 2019). Moreover, the addition of HWKL to pellets has been reported to be feasible. Improvements in physical and mechanical properties (density, mechanical durability, fines, and hardness) have been observed (Pereira et al. 2016). This study highlighted the importance of a lignin with low ash and moisture contents for briquette and pellet applications.

Although this application is still in the research level, the as mentioned promising results point to the high potential of briquettes and pellets to supply part of the energy consumed around the world, with a vast potential utilization curve for hardwood kraft lignin without concomitant fractionation and/or modification.

2.6.2. Dispersant

Dispersant is a term generically used to describe surfactants, plasticizers, or emulsifiers, depending on the field of application. Healthcare, food, civil construction, and agriculture greatly benefit from dispersants that enable the mixing of immiscible liquid phases and enhance the stability of particle suspensions. Dispersants lower the interfacial tension between immiscible liquids, as well as increase the repulsive forces between suspended particles and prevent settling and aggregation of phases, thus improving technical properties of the multiphase systems such as rheology, lifetime, and function (Österberg et al. 2020).

As discussed in previous sections, kraft lignins are not water-soluble. Thus, to use HWKL as a dispersant modification is required. A study of carboxymethylated hardwood kraft lignin shows that it can successfully be used as a dispersant for a clay suspensions (Konduri et al. 2015). The researchers added that it could potentially be applied in pesticide formulations, ceramic suspensions, and as a cement admixture. The optimal conditions for carboxymethylation were 1.5 M NaOH concentration, 3 mol/mol sodium chloroacetate (SCA)/lignin ratio, 40 °C, 4 h and 16.7 g/L lignin concentration. In addition, this modified lignin had a charge density and carboxylate group of 1.8 meq/g and 1.68 mmol/g, respectively.

In another study, sulfometylated HWKL was produced with formaldehyde and sodium sulfite under alkali conditions. The optimum conditions for the lignin modification were 0.5 M NaOH(aq), 0.9 mol/mol sodium hydroxymethyl sulfonate/lignin at 100 °C for 3 h, and 20 g/L lignin concentration (Konduri and Fatehi 2015). It was shown that the modified lignin had a charge density of −1.60 meq/g and sulfonate group content of 1.48 mmol/g. The sulfomethylated lignin was used as a cement dispersant, and the dispersibility of cement was increased from 60 to 155 mm by adding 1.2 wt % of modified lignin to cement. The researcher also evaluated the addition of unmodified lignin, which did not change the dispersibility of cement.

The majority of the industrially used dispersants are synthesized from non-renewable precursors and are not biodegradable, raising concerns over their sustainability (Österberg et al. 2020). Therefore, the development of lignin-based dispersants is an attractive solution. Besides, laboratory experiments have already shown that its manufacturing from modified HWKL is feasible.

2.6.3. Adsorbents

Lignin presents a good capacity to adsorb heavy metal ions because it possesses two types of acidic sites (carboxyl and phenol groups) that participate in the sorption mechanism. Thus, ion exchange using lignin has been studied as a potential low cost method for wastewater purification (Duval and Lawoko 2014; Mohan et al. 2006). Eucalyptus kraft lignin has been studied for the removal of Cu(II) and Cd(II) from water/wastewaters in single and multi-component systems (Mohan et al. 2006). The researchers highlighted a superior performance of HWKL as compared to most adsorbents, carbons, and biosorbents currently utilized. It was also mentioned that hardwood kraft lignin as an adsorbent is not commercial yet, however laboratory results show that it can be applied for development of large-scale systems.

Besides ion-exchange, lignin derivatives can efficiently capture metal ions through chelation and electrostatic interactions. A review paper about lignin application as adsorbent of heavy metal states that lignin can be modified by physical/chemical methods to fabricate desirable adsorbents with good sorption capacity and selectivity for the target metals (Ge and Li 2018). The researchers also mentioned that lignin-based materials have shown outstanding sorption for metals such as toxic metals (Hg), precious metals (Ag), and metal anions (Cr). Furthermore, it was recommended that specific emphasis should be placed on the lignin modification to design and develop advanced lignin-based adsorbents.

2.6.4. Hydrogels

Hydrogels are three-dimensional polymeric networks formed from cross-linked hydrophilic polymers. They are insoluble and capable of retaining a large amount of water in their swollen state. They are typically used for contact lenses, hygiene products, wound dressings, drug delivery and tissue engineering.

Hydrogels synthesis through radical polymerization of hardwood kraft lignin, N-isopropylacrylamide, and N,N′-methylenebisacrylamide is shown in Figure 2.12.

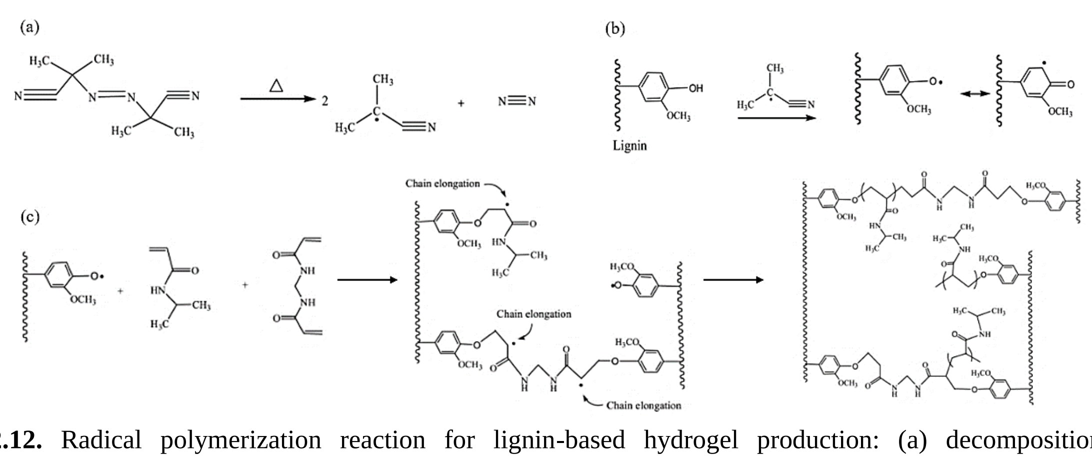


Figure 2.12. Radical polymerization reaction for lignin-based hydrogel production: (a) decomposition of Azobisisobutyronitrile (AIBN) initiator, (b) formation of phenoxy radicals, and (c) cross-linking reaction (Zerpa et al. 2018).

The reactions involved in the production of lignin-based hydrogels are well described elsewhere (Zerpa et al. 2018). The results of the study showed that lignin-based hydrogels exhibited less swelling affinity because it possessed a reduced surface area and a less porous structure than synthetic hydrogels. On the other hand, they were reported to be more thermally stable. The incorporation of lignin generated a less cross-linked hydrogel which tended to increase the rigidity and rheological stability of the hydrogel. It was also stated that when compared to synthetic hydrogels, lignin-based hydrogels exhibited less elastic behavior as temperature increased. This is the only study dealing with hardwood kraft lignin in lignin-based hydrogels.

2.6.5. Carbon fibers (CF)

CF are high-strength light-weight materials and their application in composites takes advantage of their strength, stiffness, low weight, fatigue characteristics, lack of corrosion and heat insulation (Gellerstedt et al. 2010). The main applications of CFs are in construction, electronics, transportation, and aviation. Currently carbon fibers are manufactured with polyacrylonitrile (PAN) and pitch, two non-renewable materials.

One of the key driving forces for the promotion of the CFs market is the potential for lightweight automobiles. However, the high cost (~ $ 35/kg) of CFs can inhibit their utilization for commercial applications (Fang et al. 2017). Lignin-based carbon fibers with its low-cost and sustainability appeal characterize a good alternative for the segment (Ragauskas et al. 2014). Besides, lignin is expected to offer additional benefits to CF, such as elimination of toxic substances involved in the preparation (Li and Takkellapati 2018), lower melting temperature and faster stabilization (Baker and Rials 2013) when compared with PAN- and pitch-based CFs.

To obtain lignin-derived CF, isolated lignin is first processed into fibers by extruding filaments from a melt or solvent swollen gel (spinning), and then the spun fibers are thermally stabilized in air where the lignin fiber is oxidized (stabilization). Afterwards, the fibers are subjected to pyrolysis under nitrogen or inert atmosphere, where fibers become carbonized through the elimination of hydrocarbon volatiles, their oxidized derivatives, carbon monoxide, carbon dioxide, and moisture (Ragauskas et al. 2014). Figure 2.13 provides a model workflow for the preparation of carbon fibers from lignin.

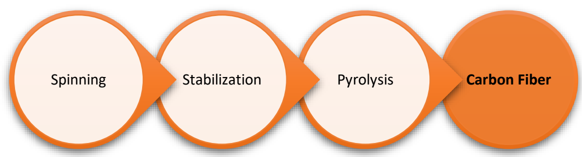


Figure 2.13. Workflow for the use of lignin as a precursor for carbon fiber.

CF from hardwood kraft lignin with mechanical properties suitable for general performance grades have been reported (Kadla et al. 2002). It was shown that thermally pretreating the lignin to remove volatile contaminants disrupts fiber integrity during subsequent thermal spinning and decreases hydroxyl content and subsequent intermolecular interactions by condensing the lignin aromatic nuclei.

The spinnability of lignin appears to be highly dependent on its structure. Hardwood lignins, whose structure is rather linear, can be melt spun without any additive (Duval and Lawoko 2014; Kadla et al. 2002). Blending HWKL with poly(ethyleneoxide) (PEO) produced miscible polymer blends which facilitated thermal spinning (Kadla et al. 2002). Furthermore, SWKL is reported to have spinning difficulties that can be overcome by addition of HWKL permeate as a softening agent (Nordström et al. 2013a).

It has been reported that HWKL can also be successfully transformed into CF by blending it with synthetic polymers such as poly(ethylene terephthalate) (PET) and poly(ethylene oxide) (PP), especially with the former (Kubo and Kadla 2005a). Both systems were easily spun into fibers and blend composition affected surface morphology of the carbon fibers. Immiscible lignin–PP fibers resulted in a hollow and/ or porous carbon fiber, whereas carbon fiber produced from miscible lignin–PET fibers tended to display a smooth surface.

Production of CF from HWKL copolymer with PAN has also been reported. The resulting copolymer was confirmed by a Fourier transform infrared (FTIR), 13C, and 1H nuclear magnetic resonance (NMR) spectroscopy, showing the presence of the C≡N group from PAN co-eluting with ether, hydroxyl, and aromatic groups that are attributed to lignin. The average tensile strength of the CF was 2.41 gf/den, a tensile strain of 11.04%, and a modulus of 22.92 gf/den (Maradur et al. 2012).

Stabilized hardwood and softwood kraft lignin CF have shown a skin-core structure similar to fibers made from pitch (Brodin et al. 2012). Also, pore creation in immiscible polymer blends of hardwood kraft lignin and PP occurs by a two-step process; oxidative degradation of the PP component followed by pyrolysis gasification of residual PP related components. Gasification is the main factor for pore growth. The internal surface area of the lignin-based CF (499 m2/g) was lower than that for commercial activated carbons (745 m2/g) (Kubo et al. 2007). However, the researchers assure that relatively simple processes, such as steam activation, could effectively activate these porous lignin carbon fibers and make them suitable for commercial applications.

Finally, a recent article demonstrated the development of activated carbon fiber electrodes produced from HWKL to fabricate electric double layer capacitors (EDLCs) with high energy and power densities using an IL electrolyte (Pakkang et al. 2020). A mixture solution of HWKL, polyethylene glycol as a sacrificial polymer, and hexamethylenetetramine as a crosslinker in dimethylformamide/acetic acid (6/4) was electrospun, and the obtained fibers were easily thermostabilized, followed by carbonization and steam activation to yield activated CF.

2.6.6. Antioxidants

Antioxidant properties of HWKL have been determined by a DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical assay. This technique is based on electron-transfer that produces a violet solution in ethanol. The radical scavenger activity is expressed in terms of the number of antioxidants necessary to decrease the initial DPPH absorbance by 50% (IC50). The inhibitory effect of HWKL samples was ~ 8.4 μg/mL, whereas the IC50 for commercial antioxidant BHT (butylated hydroxytoluene), ascorbic acid, and Trolox was 13.3 μg/mL, 2.9 μg/mL, and 3.4 μg/mL, respectively (da Silva et al. 2019), which shows the great antioxidant capacity of HWKL.

Overall, antioxidant activity increases with the phenolic hydroxyl content because they can scavenge free radicals, which is reduced with aliphatic hydroxyl content. Lignin with lower molecular mass and narrower molecular weight distribution seems to be beneficial (Ye et al. 2016) which also show the great potential of hardwood kraft lignins.

Another effort evaluated antioxidant activity of HWKL by the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay, which measures the relative ability of antioxidants to scavenge the ABTS generated in aqueous phase. The HWKL could oxidize ABTS to ABTS+ due to its reducing potential, which resulted in a color change (blue to green). (Santos et al. 2014).

Kraft lignins have shown potential to act as antioxidant for food, cosmetic and pharmaceutical industries instead of BHT, a synthetic resource. Lignin as a cosmetic or pharmaceutical product, is still not regulated because studies dealing with the safety of its use in humans are needed (da Silva et al. 2019). Recently, hardwood lignin nanoparticles were reported to enhance sunscreen performance. The best formulation had a UV transmittance of only 0.5-3.8%

over the entire UVA−UVB region compared to 2.7-51.1% of a commercial SPF 15 sunscreen (Widsten and Tamminen 2020).

2.6.7. Aromatic compounds – Chemicals

Lignin valorization in solvent systems to produce renewable aromatic chemicals has attracted great attention during recent years. The methodologies to obtain these compounds can be categorized as hydrolysis, hydrogenolysis, oxidation, and a two-step lignin depolymerization. Moreover, catalysis is a promising technique for lignin depolymerization to specific products (Cheng et al. 2017).

Benzene, toluene, and xylene (BTX) and phenols are high-value chemicals that can be more sustainably sourced from lignin than from fossil–based resources (Ponnusamy et al. 2019). BTX is the precursor for a series of materials such as resins, nylon fibers, polyurethane and polyester; thus, production of BTX from lignin could expand the utilization of lignin–based materials. It should be noted that when targeting chemicals from lignin, a key objective during the fractionation and depolymerization stage is to minimize lignin condensation as stated earlier in this review (Schutyser et al. 2018).

Other aromatic compounds that can be produced from lignin are syringaldehyde and vanillin. Eucalyptus and Northern European HWKL have been investigated to produce these compounds by oxidation with O2 in alkaline medium. The total yield of syringaldehyde was 14%, whereas for vanillin was 16% (Pinto et al. 2013). In another study of oxygen oxidation of eucalyptus kraft lignin, under optimum conditions, only a reduced number of phenolic aldehydes (4%) was obtained. In contrast, when in the presence of catalysts, the yield could be increased to 14% with nitrobenzene and to 8% with CuO (Villar et al. 2001). This was attributed to the low yield of transformation of the lignin / lignin oxidation products into low molecular weight acids.

Vanillin, the highest volume aroma chemical produced worldwide, is produced from a variety of sources, namely oil (85%), woody biomass (15%), and orchid pods (<1%). Around 20,000 tons of vanillin are produced per year, 15% of which coming from lignin (around 3000 tons/y) (Fache et al. 2016). Lignosulfonates are the main sources for its production; however, kraft lignin could also be used for this purpose. The use of lignin by chemistry and polymer industries represents an important field of research with major issues in terms of scientific, economic, and environmental point of views and it seems justified that lignin will become a promising renewable aromatic resource in subsequent years (Laurichesse and Avérous 2014). Syringaldehyde is another promising aromatic aldehyde that possesses worthy bioactive properties which can be used in pharmaceuticals, food, cosmetics, textiles, pulp and paper industries, and biological control applications.

2.6.8. Polymer blends and composites

Lignin has been added to several polymers for the express purpose of potentially providing valuable new composite properties. Because of their large number of polar functional groups, lignin molecules interact strongly with each other. Most polymers are immiscible with lignin because of weaker interactions between lignin and the matrix polymer than among lignin molecules. Therefore, competitive interactions determine the structure and properties of blends and composites (Kun and Pukánszky 2017).

The miscibility of synthetic polymers such as poly(ethyleneoxide) (PEO), poly(ethylene terephthalate) (PET) and poly(vinyl alcohol) (PVA) with HWKL has been investigated. Miscible blends were observed in lignin/PEO and lignin/PET blends, while immiscible blends were found in lignin/PP and lignin/PVA blends (Kadla and Kubo 2004). Those former polymers possess functional groups capable of interacting with lignin through secondary forces-bonding whereas the latter do not (Figure 2.14).

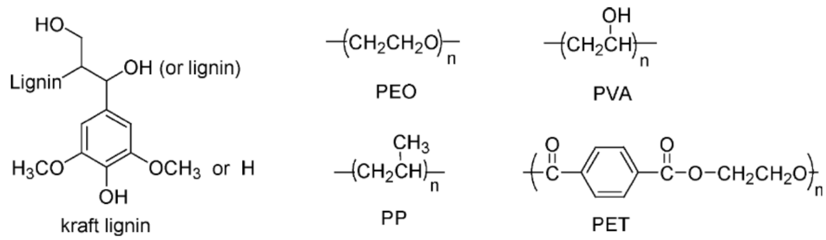


Figure 2.14. Structural representations of the various polymeric materials that can be blended with lignin (Kadla and Kubo 2004).

A possible approach towards lignin valorization is using it as a component in plastics. The development of lignin-based thermoplastics relies on altering the viscoelastic properties of lignin through chemical modification or polymer blending (Kadla and Kubo 2004). The latter is a convenient method to develop products with desirable properties. The chemical and physical properties of the blends/composites are dependent on monomer type(s), molecular weight, and distribution and composition of the respective polymers (Kadla and Kubo 2003, 2004).

Carbon-neutral thermoplastics have been successfully prepared by copolymerization of modified HWKL (oak variants) and dicarboxy-terminated polybutadiene (PBD-(COOH)2). Modified lignin (either by fractionation with methanol or by formaldehyde crosslinking) showed high molecular weight which facilitated preparation of free-standing films of lignin-based thermoplastic (Saito et al. 2012). Also, it enabled formation of a more continuous network with telechelic polybutadiene, whereas the very broad molecular weight distribution of unmodified lignin formed a poorly networked structure.

Lignin-based thermoplastic miscible blends have been studied using HWKL and PEO. Incorporation of small amounts of PEO (5-10% w/w) sufficiently disrupts the lignin supramacromolecular complexes leading to enhanced physical properties. Increasing PEO incorporation further disrupts the lignin structure and the observed physical properties become more influenced by the PEO component (Kadla and Kubo 2003).

It has been reported that olefinic thermoplastic polymer compositions comprising at least one polyolefin and eucalyptus kraft lignin, can successfully be manufactured (Torrezan et al. 2019). The patent assures increase in the following properties: flow index (MFI), thermo-oxidative resistance (OIT-oxidative induction time), heat deflection temperature (HDT), stiffness (elasticity module), breaking strength, and flexural strength. In addition, the material maintains hardness and tensile strength measured at the outflow.

Blends of eucalyptus kraft lignin and PBAT (a biodegradable polyester, produced by BASF, and based on the monomers 1,4-butanediol, adipic acid, and terephthalic acid) have been studied. The study showed that the addition of up to 20% of lignin generated miscible or partially miscible structured blends, where lignin acted as a lubricant. Furthermore, the bends reached technical requirements needed for sustainable solutions to rigid plastic apparatus in the agricultural segment, such as seedling tubes (Talyta Torrezan 2019).

The intermolecular interactions of HWKL and PVA blend fibers prepared by thermal extrusion have been studied (Kubo and Kadla 2003). Although the blend is immiscible (presents two distinct T_gs) some of the lignin was closely associated with the PVA in the PVA-rich phase. FT-IR analysis confirmed the formation of a relatively strong hydrogen bond between the hydroxyl groups of the short-chain PVA and lignin.

Petroleum-based polyol was replaced with hardwood kraft lignin preparing rigid polyurethane foam. The prepared foams contained from 9% to 28% (w/w) HKL (Pan and Saddler 2013). The addition of lignin reduced the density of the foams, which is desirable if the foam is used as packing or insulation material. Besides, the authors reported that the majority of the kraft lignin was chemically crosslinked, and the foams had satisfactory structure and strength up to 23% (w/w) addition when chain extender was applied.

Polyurethane–lignin copolymer has been produced by step-growth polymerization of modified eucalyptus kraft lignin with isocyanate and doped by multi-wall carbon nanotubes (MWCNTs) (F. Graça et al. 2012; Gonçalves et al. 2020; Rudnitskaya et al. 2013). Lignin possesses ion-exchange properties due to the presence of a variety of functional groups, which makes it an attractive active substance for chemical sensing. Co-polymerization allows fixing lignin inside a polymer matrix ensuring high stability of the resulting material (F. Graça et al. 2012). Furthermore, the electrical conductivity and impedance spectroscopy measurements revealed that the interaction between carbon nanotubes and lignin molecules in the polymer enhances its electrical conductivity (Gonçalves et al. 2020).

Lignin fractions having different molecular weights and varied chemical structures from HWKL (*Eucalyptus grandis*) were incorporated into a tunicate cellulose nanofibers (CNF)-starch mixture to prepare 100% bio-based composite films. In general, the addition of lignin led to a decrease in thermal stability and tensile strain and an increase in Young's modulus of the composite film (Zhao et al. 2019). Furthermore, some aggregates were observed in the composite films which may explain the lower tensile stress. The researchers pointed out that hardwood lignin with lower molecular weight and polydispersity reinforced the film structure.

The utilization of lignin in polyolefin blends is of growing research interest. However, the low compatibility of lignin and polyolefins restrains a satisfying blend production and leads to poor mechanical properties. It has been reported that it is possible to overcome these issues by modifying lignin prior to its incorporation into polymers to reduce its polarity (Dehne et al. 2016). The researchers performed esterification of eucalyptus kraft lignin and subsequently blended it with PE at a weight ratio of 1:1. They found a reduction of volume swelling and weight gain when modified lignin was used, which can be related to the esterification of hydroxyl groups as well as significant reduction of sugar and ash contamination. Also, they observed that the elongation at maximum strength decreased to 22% (εM = 11%), while for other technical lignins the decrease was of ~10%. Highest tensile strengths were observed for the hardwood blends (17 N/mm^2).

Copolymerization of N-isopropylacrylamide (NIPAM) with HWKL has been achieved by atomic transfer radical polymerization using a selectively modified lignin-based macroinitiator as shown in Figure 2.15. The thermal decomposition temperature of the lignin-polyNIPAM copolymers are reported to significantly increase with increasing degree of polymerization of NIPAM. The solubility of the lignin-polyNIPAM copolymers in water was dependent on copolymer structure. In both the water-soluble and suspended copolymers, at temperatures above 32°C, the component has been reported to undergo hydrophilic-to-hydrophobic transition, resulting in precipitation of the copolymer (Kim et al. 2010).

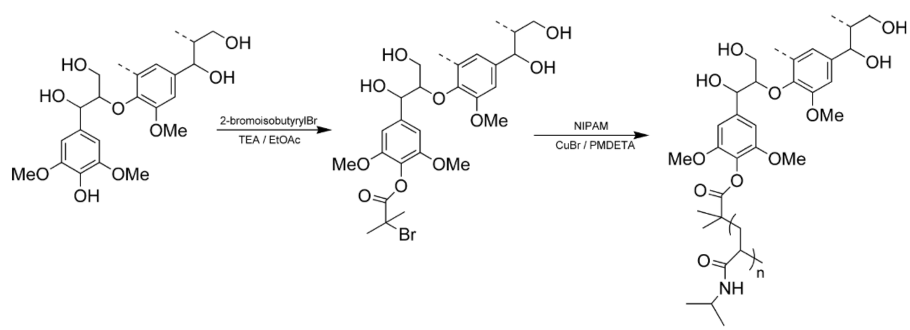


Figure 2.15. Scheme for the Preparation of Lignin-g-polyN-isopropylacrylamide (NIPAM) Copolymers (Kim et al. 2010).

Finally, lignin and acrylonitrile butadiene (ABS) blends and composites have received great attention recently. ABS is a very popular polymer material as shown by its widespread use as a thermoplastic resin in automotive, marine, home appliance, toys, and other industries. Lignin is generally incompatible with ABS polymers as shown by large phase separation domains with poor interfacial adhesion within the ABS matrix. This unfortunately leads to significant reductions in the strength and ductility of the resulting composite/blend, thereby limiting practical utility. A recent patent has addressed those issues and claims that by adding a compatibilizing agent, any lignin type, including HWKL, could be added to ABS plastics to provide increased stiffness and reduced cost (Webb et al. 2020). The inventors used compatibilizing agents such as, polyalkylene oxide, polyvinyl alcohol, polyvinyl acetate, ethylene vinylacetate copolymer, styrene-maleic copolymers, nitrile rubber, and others to assist in the dispersion and/or distribution and/or miscibility of lignin and ABS.

2.7. Conclusions

Due to lignin's high-energy density and intrinsic aromatic based structure, it is an ideal renewable feedstock that has tremendous potential in defining the modern biorefinery. Lignin I great potential to produce fuels, value-added chemicals, and functional materials, and ultimately reduce the environmental impact of their production. Although several studies have focused on converting lignin to valuable products, only a few of these efforts are commercially profitable mainly because of low yields and low quality of the final products (Wang et al. 2019a). It is unfortunate that most of lignin work deals with softwood kraft lignin, while hardwoods have been neglected. Because HWKL has a very different structure to SWKL, the knowledge acquired from the latter species cannot always be applied to the former. Thus, the intent of the present review was to provide a good basis for its possible valorization by overviewing the HWKL structure and properties. By appropriate understanding of the concepts in this review, it is the hope that utilization of this vast available and cheap aromatic source will be expanded to promote a firmer footing for a growing biorefinery.

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3. Chapter 3 - A quantitative comparison of the precipitation behavior of lignin from sweetgum and pine kraft black liquors

Jardim, J. M., Hart, P. W., Lucia, L., Jameel, H., and Chang, H-min (2020). BioResources, 15, 1–18.

Abstract

Lignin is a by-product of the pulp and paper industry that can be precipitated by acidification from black liquor as a potential feedstock for valuable green materials. Precipitation and quantification of lignin from softwood black liquors have been documented and commercialized with well-established methods; however, applying those methods to the precipitation of lignin from hardwood black liquors has produced low lignin yields. A need to understand the main differences between hardwood and softwood lignin precipitation prompted the current investigation. Multiple black liquor samples from sweetgum and pine pulping were obtained at different times of standard kraft cooks. Two lignin fractions were precipitated from each of these black liquors, one at pH 9.5 and one at pH 2.5. Detailed lignin and carbohydrate material balances were performed around each of the sample sets, starting with the wood and ending with precipitated lignin fractions. For all conditions tested, the amount and purity of pine lignin precipitated were superior to sweetgum lignin. The maximum recovery for sweetgum lignin was 69.7%, while for pine lignin, it was 90.9%.

3.1. Introduction

Kraft (or sulfate) pulp mills account for around two-thirds of the global pulping industry (Magdeldin and Järvinen 2020). The kraft process is based on the application of sodium hydroxide (NaOH) and sodium sulfide (Na_2S) to wood chips, under pressure and relatively high temperatures (150 to 170 °C), which targets lignin fragmentation/hydrolysis, dissolution, and subsequent fiber liberation while retaining the carbohydrates in a fibrous form. The process generates a residual process stream (spent liquor/black liquor) rich in organic degradation products (lignin and carbohydrates) and spent inorganics (*e.g.*, sulfides, sulfates, carbonates, hydroxides). Typically, this black liquor is subjected to a recovery system in which it is evaporated to a high solids content of 65 to 72 wt% and then combusted to smelt in a recovery boiler to recover the original chemicals (NaOH and Na_2S) with the concomitant production of steam and power from the dissolved organics.

Many recovery boilers are limited by total solids loading or steam generation (circulation limit), and hence they represent the capacity-limiting step in several pulp mills. Lignin isolation from black liquor is considered a potential method for reducing these process limitations (Öhman et al. 2007b). In fact, the creation of new economic downstream uses for the black liquor enables greater pulp production and an increase in total revenue (Klett 2017; Zhu et al. 2014) since the recovered lignin can serve as a feedstock for biomaterials and biochemicals.

Lignin is produced at large scale: approximately 78 million tons are generated globally each year by kraft pulp mills (Miller and Faleiros 2016). Additionally, research efforts also use lignin precipitation as a method to obtain selected, purified samples for lignin component analysis.

Lignin is a complex biopolymer biosynthesized by repetitive dehydrogenation and cross-coupling of three major phenylpropanoid (C6–C3) units, namely, sinapyl alcohol, coniferyl alcohol, and *p*-coumaryl alcohol; forming syringylpropane (S), guaiacylpropane (G) and *p*-hydroxyphenylpropane (H) units in lignin (Lin and Dence 1992). These structural building blocks are zipped together by ether linkages and carbon-carbon bonds (Alén 2000). Although the chemical structure of lignin has been extensively investigated by both wet chemical and spectroscopic methods, its structural complexity has never been fully elucidated (Ralph *et al.* 2004). However, it is well known that the structural characteristics of lignin from hardwoods and softwoods profoundly differ from each other. Guaiacyl lignin is typically found in softwoods and appears to vary little amongst species (Akiyama et al. 2005; Sarkanen et al. 1967). Guaiacyl-Syringyl lignin is typically found in hardwoods, and the S to G ratio tends to vary greatly between species (Santos *et al.* 2012).

Lignin properties depend on feedstock from which they are obtained, the extraction method employed, and downstream purification processes (Santos et al. 2014). LignoBoost and LignoForce processes are the two main commercial technologies for precipitating lignin from spent liquors (Dessbesell *et al*. 2020). LignoBoost is an industrial process that uses dissolved carbon dioxide (CO_2) to decrease the pH of the process stream from approximately 13 to 10. "LignoForce" is another commercialized technology that first oxidizes the black liquor with oxygen (O_2) and then acidifies to around pH 9 with CO_2 (Fatehi and Chen 2016). It is known that when the black liquor is acidified, the phenoxide groups of the dissolved lignin become protonated, and lignin solubility decreases, *i.e.*, the lignin precipitates. After precipitation, the solids are separated by filtration and subsequently resuspended in water and sulfuric acid (H_2SO_4) to obtain a lower pH of about 2.5 to remove impurities (Wallmo *et al.* 2016).

The bulk of the work on lignin precipitation from kraft black liquor has been performed on softwood, while very little has been published about hardwood black liquors (Zhu *et al.* 2014). It was initially thought that the lignin precipitation methods developed and proven to work for the majority of softwood species would be acceptable for hardwood species. However, investigations into various hardwood lignin compositions have used the softwood precipitation techniques and obtained unexpectedly low lignin yields (Giummarella et al. 2019; Gomes et al. 2018; Kumar et al. 2016; Santos et al. 2011, 2012). Lack of understanding of hardwood lignin precipitation is a significant hurdle toward fully characterizing and utilizing this raw material, which prompted this study.

The current work aimed at establishing a material balance over the kraft pulping process and lignin precipitation from hardwood and softwood black liquors obtained at different pulping extents. Quantification of lignin recovery was also intended. Thus, two fractions of sweetgum lignin were precipitated from black liquor at distinct pHs (pH 9.5 and 2.5), and a baseline comparison between these lignins and pine lignins was accomplished. The impact of H-factor on yield and purity of precipitated lignins was also investigated.

3.2. Experimental

3.2.1. Materials

Southern sweetgum (*Liquidambar styraciflua*), a hardwood, and Southern pine (*Pinus taeda*), a softwood, were the samples investigated. Wood logs were processed into chips and screened according to the standard method "Size distribution" (SCAN-CM 40:01, 2001).

3.2.2. Methods

3.2.2.1. *Kraft pulping*

Kraft pulping experiments were carried out in a 10 L laboratory pulping digester

(Greenwood Instruments, LLC; Andover, MA, USA) equipped with a temperature controller, liquor recirculation piping, and an indirect heating system. The general cooking conditions are shown in Table 3.1. Batch cooks were performed using 1000 g oven-dried (O.D.) chips. Pulped chips were placed in a stainless-steel box equipped with a 150-mesh screen, washed thoroughly with water, and disintegrated. The washed pulp was then centrifuged to approximately 30% consistency and subsequently stored in polyethylene bags for follow-up characterization. Pulp yield was gravimetrically determined. The black liquor was collected and characterized according to the protocols "Solids Content of Black Liquor" (TAPPI/ANSI T650 om-15) and "Residual Alkali" (SCAN-N 33:94). The black liquor samples were stored in sealed Nalgene bottles for lignin precipitation.

Table 3.1. Kraft Pulping Conditions

Parameter	Hardwood (Sweetgum)	Softwood (Pine)
Liquor/Wood ratio	4/1	4/1
Effective Alkali as Na_2O (%)	16	19
Sulfidity (%)	25	25
Temperature (ºC)	160	170

Pulping runs were performed at 30, 60, 90, 120, and 150 min to obtain different levels of H-factor covering an entire typical bleachable grade cook. All other pulping conditions remained constant (Table 3.1). H-factor and pulping temperature at the time of sampling are shown in Figure 3.1 for both the sweetgum and pine cooks. Each raw material generated five pulp samples and five black liquor samples. Additionally, a sample consisting of pure wood chips was also collected.

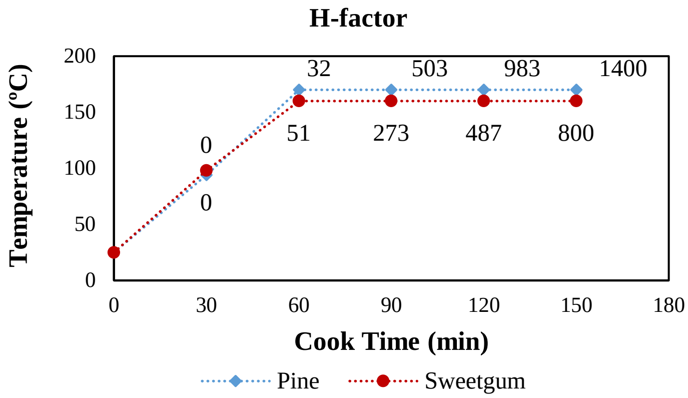

Figure 3.1. H-factor of each sample for the sweetgum and pine cooks.

3.2.2.2. Lignin precipitation

Collected black liquor samples were filtered over Whatman #3 filter paper to remove fines. Filtered black liquors were evaporated to a solids content of approximately 20% and used for precipitation experiments of two lignin fractions (pH 9.5 and 2.5). Concentrated black liquors were sparged with CO_2 at a low flow rate to attain a pH 9.5, which were allowed to equilibrate over 30 min. The precipitated materials were isolated from the liquors through centrifugation, and the supernatants were stored for further precipitation. The solids were washed with hydrochloric acid (HCl) to remove ash at pH 2.5 and subsequently washed with acetic acid to remove residual HCl at pH 3.5. After washing steps, the solids were isolated by centrifugation, suspended in deionized water, and freeze-dried. Typically, precipitated lignin is washed with H_2SO_4; however, in this work, washing was performed with HCl because it is reported to improve ash removal compared to sulfuric acid (Santos et al. 2014). Washing with acetic acid was carried out to remove residual HCl because it is less harmful to the freeze-drier vacuum pump compared to HCl. These solids, called lignin fraction at pH 9.5, were obtained according to commercially known lignin isolation protocols (*e.g.*, LignoBoost™).

The supernatants after carbonation were further acidified with 6 N HCl to pH 2.5 in order to precipitate a second lignin fraction, which was also washed with HCl, acetic acid, and freeze-dried. These solids were called lignin fraction at pH 2.5 and were obtained with the understanding that at pH 2.5, all remaining lignin or most of it would be precipitated.

Precipitation experiments were performed at room temperature, atmospheric pressure, and constant stirring. A schematic representation of the precipitation and purification processes is shown in Figure 3.2.

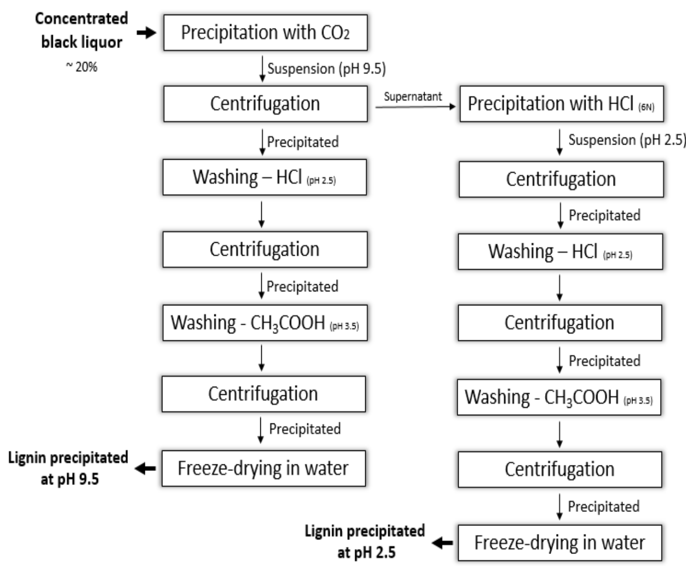

Figure 3.2. Schematic representation of the protocol used for lignin precipitation from kraft black liquor of two lignin fractions: pH 9.5 and pH 2.5.

The influence of pH, ionic strength, temperature, agitation speed, solids content, and atmosphere on precipitation have been well studied and reported before (Howell and Thring 2000; García *et al.* 2009; Wallmo *et al.* 2009; Theliander 2010; dos Santos *et al.* 2014; Zhu *et al.* 2014; Helander *et al.* 2016; Durruty *et al.* 2017; Sewring *et al.* 2019). The experimental conditions applied to sweetgum and pine black liquors in the present investigation were chosen to establish a fair comparison between the behaviors of both lignins over precipitation.

3.2.2.3.Characterization of wood, pulp, and precipitated lignin

The wood chips were ground in a Wiley mill. The resulting milled wood was screened, and the fraction, which passed through a 40-mesh screen and was retained on a 60-mesh screen was kept for analyses. The milled wood was extracted in a Soxhlet apparatus for 6 h using a benzene-ethanol mixture (2:1 v/v ratio) (TAPPI Standard Test Method T 264 cm-07 "Preparation of wood for chemical analysis"). Carbohydrates and lignin contents were quantified according to the Technical Report NREL/TP-510-42618 "Determination of structural carbohydrates and lignin in biomass." Ash content was measured according to "Ash in wood, pulp, paper and paperboard: combustion at 525 °C" (TAPPI Standard Test Method T 211 om-16). The same protocols were applied to the characterization methods for the lignin samples precipitated from the black liquors.

3.2.2.4.Material balance

Table 3.2 shows the template used for the material balance calculations.

Table 3.2. Material Balance of the Lignin Isolation from Sweetgum Black Liquor at 60 Min. Balance Assumes 100 O.D. g of Wood as the Starting Material

Sample Location	Inputs (g)		Intermediates (g)		Outputs (g)	
	Carbs	Lignin	Carbs	Lignin	Carbs	Lignin
Wood Chips	64.5	25.9	---	---	---	---
Pulp	---	---	---	---	47.7	10.8
Black Liquor	---	---	---	---	16.8	15.1
Black Liquor	16.8	15.1	---	---	---	---
Supernatant from CO_2 Precipitation	---	---	15.1	12.0	---	---
Supernatant from HCl Precipitation	---	---	14.8	8.8	---	---
Lignin @ pH 9.5	---	---	---	---	1.7	3.1
Lignin @ pH 2.5	---	---	---	---	0.4	3.2
% Recovery from Black Liquor	**---**	**---**	**---**	**---**	**12.2**	**41.8**

The starting wood chips were used as the input materials for the pulping process, while pulp and black liquor were the outputs. For the precipitation experiments, black liquors were the input, whereas the solids isolated were the outputs of the system. Solid and liquid flows were accounted for to determine lignin recovery (yield) over precipitation experiments. The calculations were based on the compositional analysis of wood, pulp, and solids precipitated from black liquor. In addition, the volume of white liquor and solids from the chemicals added to the pulping process were taken into consideration. The data in Table 3.2 were obtained for sweetgum pulp and liquor after 60 min of cooking time (51 H-factor).

The amount of carbohydrates and lignin in the sweetgum wood chips were 64.5 g and 25.9 g, respectively. After 60 min of pulping (at H-factor 51), the residual carbohydrates mass was 47.7 g, whereas the residual lignin mass was 10.8 g. The determination of carbohydrate and lignin contents in the wood and in the produced pulp allowed calculation of the amount of carbohydrates and lignin dissolved in the black liquor. Therefore, at an H-factor of 51, the amount of carbohydrates in the black liquor was 16.8 g (64.5 g – 47.4 g), and the amount of lignin was 15.1 g (25.9 g – 10.8 g). These values were used as inputs to calculate carbohydrate and lignin recovery.

After precipitation of both lignin fractions (pH 9.5 and 2.5), the solids isolated were characterized, and the values obtained were the outputs shown in the bottom part of Table 3.2. In the example, the lignin fraction isolated at pH 9.5 represented 1.7 g of carbohydrates and 3.1 g of lignin. In contrast, the lignin fraction obtained at pH 2.5 represented 0.4 g of carbohydrates and 3.2 g of lignin. The intermediate values were obtained by a simple subtraction of each compound dissolved in the black liquor minus the amount obtained in each step of the precipitation. Finally, the recovery of each compound (carbohydrates and lignin) was calculated based on its amount in the black liquor and in the solids precipitated.

3.3. Results and Discussion

3.3.1. Pulping

The present work evaluated pulp and black liquor samples of hardwood (sweetgum) and softwood (pine) at different points of kraft pulping. The cooks were performed to different levels by varying H-factor. Pulping results for sweetgum and pine are shown in Table 3.3.

Table 3.3. Pulping Results for Sweetgum and Pine: H-Factor, Pulp Yield, Residual Effective Alkali (rEA) and Percent of Solids in the Black Liquor

Cook Time (min)	Sweetgum				Pine			
	H-factor	Pulp Yield (%)	rEA (g/L)	Solids in BL (%)	H Factor	Pulp Yield (%)	rEA (g/L)	Solids in BL (%)
30	0	85.5	12.5	8.9	0	93.2	19.2	11.1
60	51	62.6	9.0	12.1	32	69.1	17.0	13.8
90	273	50.7	4.7	14.1	503	51.4	11.5	16.6
120	487	48.7	3.5	14.4	983	47.4	10.0	17.3
150	800	47.6	2.9	14.4	1400	43.5	5.9	16.6

Where BL is black liquor

Lower pulp yields were obtained for hardwood at the beginning of the process. Specifically, the total pulp yield of sweetgum after 30 min (0 H-factor; *i.e.* when the target temperature has not yet been reached) was 85.5%, whereas, for pine, it was 93.2%. The greater yield losses in sweetgum are because hardwoods are easier to pulp than softwoods, due to the former's lower lignin content and presence of syringyl units (Santos *et al.* 2011). In addition, at the beginning of conventional kraft cooking, xylan (main hemicellulose in hardwoods) is partly solubilized in the cooking liquor and many of the side groups and acetic acid residues are cleaved (Viikari *et al.* 2009). On the other hand, after 150 min, the pulp yield for sweetgum and pine were 47.6% (800 H-factor) and 43.5% (1400 H-factor), respectively. Even though initially sweetgum showed greater pulp yield losses, the final pulp yield was superior to pine also because of the relative ease in pulping hardwood compared to pine. Harsher pulping conditions were necessary to obtain pine pulp due to higher starting lignin content, which explains the lower yield.

The effective alkali (as Na_2O) for the sweetgum and pine cooks was 16% (20.5 g/L) and 19% (24.4 g/L) based upon O.D. wood, respectively, which accounts for most of the inorganics added to the process. A minor part of the inorganics was associated with the wood as Non-Process Elements (NPEs). The ions OH^- and HS^- are consumed by lignin and carbohydrate hydrolytic reactions; hence, the residual effective alkali (rEA) decreased over time. Within 150 min, the rEA was 2.9 g/L in the sweetgum black liquor (800 H-factor) and 5.9 g/L in the pine black liquor (1400 H-factor).

The method used to determine solids content in the black liquor accounts for the organic and inorganic fractions present in the liquor; thus, an increase over the process was expected because of the degradation and dissolution of wood compounds. The solids ranged from 8.9% (0 H-factor) to 14.4% (800 H-factor) in sweetgum and from 11.1% (0 H-factor) to 16.6% (1400 H-factor) in pine.

The goal of kraft pulping is to ensure carbohydrates content is as high as possible. Unfortunately, carbohydrates degrade as delignification reactions occur. The composition analysis of sweetgum and pine wood chips and pulps are shown in Table 3.4.

Table 3.4. Characterization of Sweetgum and Pine Wood Chips for Carbohydrates, Lignin and Ash at Time "0" Followed by their Resultant Pulps for All Other Times Throughout Kraft Pulping Of 100 g (O.D.) of Wood

Cook Time (min)	Parameter (g)							
	Cellulose	Xylan	Galactan	GM	Total Lignin	Klason Lignin	ASL	Ash
				Sweetgum				
0	43.5	14.8	1.2	5.1	25.9	23.5	2.4	0.4
30	38.4	12.3	0.8	3.6	21.6	19.1	2.5	1.2
60	36.0	9.9	0.7	1.2	10.8	9.5	1.3	0.8
90	33.5	8.7	0.3	0.9	2.9	2.1	0.8	0.6
120	33.5	8.5	0.3	0.9	1.4	0.8	0.7	0.6
150	33.2	8.6	0.3	1.1	1.2	0.5	0.7	0.6
				Pine				
0	40.0	6.1	3.3	16.4	31.7	30.9	0.8	0.7
30	38.7	5.8	2.3	14.6	28.7	28.1	0.6	1.8
60	36.1	4.9	1.4	6.4	19.2	18.8	0.4	1.4
90	35.1	3.6	1.0	6.2	6.7	6.3	0.4	0.9
120	35.4	3.5	0.9	5.1	3.9	3.5	0.3	0.9
150	32.8	3.4	0.7	4.6	3.3	3.0	0.3	0.7

Where GM is glucomannan, Total Lignin is a sum of Klason Lignin (acid-insoluble lignin) and acid-soluble lignin (ASL).

Time "zero" shows the characterization of the wood chips, while times 30 to 150 min are characterizations of the pulps produced. The residual carbohydrates content (*i.e.*, cellulose, xylan, galactan, and glucomannan), lignin, and ash in the pulp were calculated based on the content measured and pulp yield. Thus, the characterization presented in Table 3.4 depicts the degradation of each wood compound over the entire kraft pulping process.

The degradation of the main wood compounds of sweetgum and pine are graphically represented in Figure 3.3, where the values are the residual content (g) of lignin, cellulose, and hemicelluloses over the kraft pulping process.

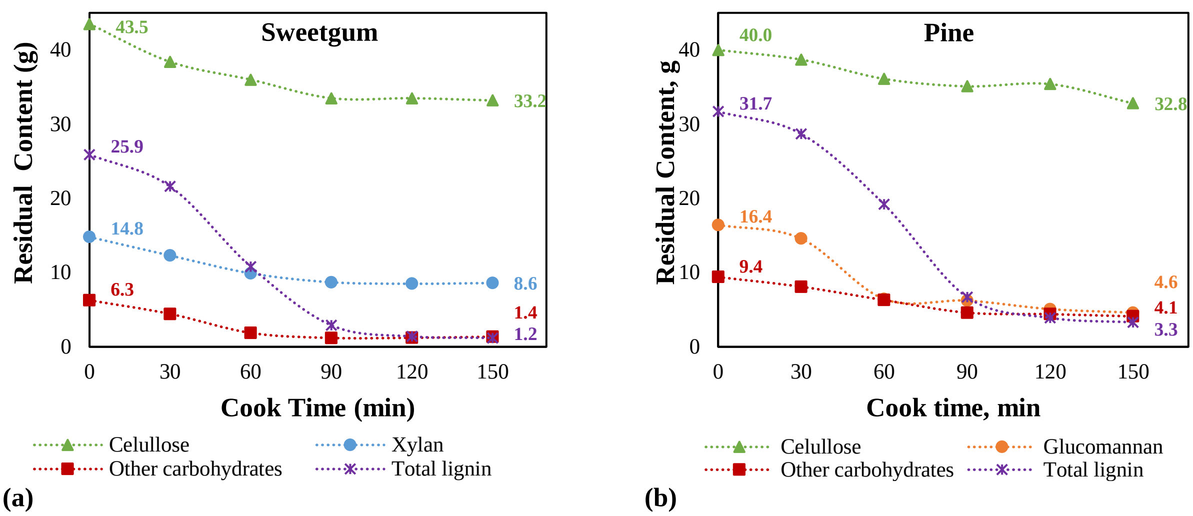

Figure 3.3. Residual lignin and carbohydrate contents (g) for (a) sweetgum and (b) pine throughout kraft pulping of 100 g (O.D.) of wood

A large fraction of hemicellulose components is degraded and dissolved in the black liquor throughout kraft pulping; however, xylan (main hemicellulose in hardwoods) and glucomannan (main hemicellulose in softwoods) behave differently. The glucomannan is rapidly degraded and dissolved in the black liquor, whereas xylan is more resistant (Gellerstedt 2009). For instance, xylan contents of 14.8 g and 6.1 g were found in 100 g of sweetgum and pine wood chips, respectively. At the end of the pulping process, the residual amount in sweetgum was 8.6 g, and in pine was 3.4 g. The total loss of xylan was 41.6% in sweetgum and 44.3% in pine. With respect to glucomannan, the starting amount in sweetgum was 5.1 g, whereas, in pine, it was 16.4 g. After pulping, the final contents were 1.1 g and 4.6 g in sweetgum and pine, respectively. The total loss of glucomannan was 79% in sweetgum and 72% in pine. Thus, Figure 3.3a shows how xylan is more resistant to the pulping process than the other hemicelluloses and Figure 3.3b shows how rapidly glucomannans are degraded through the process. Furthermore, in the early phases of kraft pulping, many of the xylan side groups are cleaved; these hydrolytic reactions consume alkali and contribute to carbohydrate loss *(*Viikari *et al.* 2009), which may account for the significant decrease in rEA (Table 3.1) and pulp yield (Figure 3.3).

On the other hand, the loss of cellulose was less prominent in both raw materials. It is well known that cellulose is resistant to kraft pulping because of its packing (crystallinity). In contrast, hemicelluloses are much less uniformly arranged (more exposed) and thus heavily degraded during pulping (Viikari *et al.* 2009). The predominant carbohydrate degradation reaction, called endwise peeling, consists of successive depolymerization of the carbohydrates (starting from the reducing end group). At elevated temperatures (between 160 and 170 °C), alkaline hydrolysis becomes important because it forms more reducing groups by the random cleavage of the glycosidic bonds in polysaccharides (Alén 2011a).

Moreover, cellulose has a much higher degree of polymerization than hemicelluloses (Sixta 2006). Thus, due to cellulose's higher molecular weight and crystalline structure, it is not as significantly degraded during the pulping process compared to the other carbohydrates. As a result, the retained carbohydrate yield of cellulose is significantly higher than that of the other carbohydrates (hemicelluloses). A cellulose loss of 23.7% was observed in sweetgum, whereas in pine the total loss was of 12.9%.

The delignification process over kraft pulping occurs in three distinct phases (initial, bulk, and residual) (LéMon and Teder 1973). The main delignification reactions/removal occur over the first two phases. Up to 120 min, approximately 97% of the sweetgum lignin was removed

(487 H-factor), while about 89% of pine lignin was removed (H-factor of 983). Even though the pulping conditions applied to pine were harsher than the conditions applied to sweetgum, the delignification of sweetgum was greater than pine, which reinforces the superior pulpability of hardwoods over softwoods. The residual phase, which is less selective towards lignin degradation, dominates after about 90 to 95% delignification (Sjödahl 2006). This phase was observed for both samples after approximately 20 min of cooking, in which less than 2% of lignin was removed.

3.3.2. Lignin Precipitation and Characterization

Two lignin fractions were precipitated from the black liquor generated; one was precipitated at pH 9.5 and the other at pH 2.5. Lignin precipitation is typically followed by filtration; however, due to its colloidal nature, the filtration step is a challenge. Complete or partial plugging of the filter cake has been reported, which requires large filter areas (Tomani 2010) and leads to high levels of impurities in the lignin (Öhman et al. 2007b). The separation of the lignin from the black liquor was carried out by centrifugation to mitigate this problem for laboratory studies.

During kraft pulping, lignin undergoes relatively drastic degradation reactions, for instance, cleavage of β-O-4 linkage (main linkage in lignin), which results in the liberation of phenolic hydroxyl groups. These groups increase the hydrophilicity of lignin fragments (Hubbe *et al.* 2019) and lead to lignin dissolution in the black liquor. It has been determined that the protonation of the phenolic hydroxyl groups occurs over about pH 9 to 11. So, under this pH range, lignin solubility decreases, resulting in precipitation. In addition, further lowering pH to around 2, the carboxylic acid groups in lignin (pK_a 3 to 5) are also protonated, which enhances precipitation (Alén 2011b). Therefore, lignin precipitation experiments are based on the concepts of pKa and protonation.

3.3.2.1. Sweetgum analysis

In addition to lignin, other degradation products are found in the black liquor that can also be co-precipitated with lignin as impurities. The characterization of wood chips and pulps has already been presented above; thus, it was possible to calculate the content of a given compound in the black liquor by the difference between its content in the wood and the pulp.

The material balance of the precipitation experiments for sweetgum is shown in Table 3.5. After pulping 100 g (O.D.) of wood chips, the solids in the black liquor were measured according to the standard "TAPPI/ANSI T 650 om-15" protocol and converted to a weight basis. Since the two lignin fractions (at pH 9.5 and 2.5) were obtained from the same black liquor sample, the solids in the black liquor presented in pH 2.5 section are a subtraction of the solids originally found in the black liquor and the solids precipitated at pH 9.5. The lignin in the black liquor was calculated by the difference between the lignin content in the wood and the pulp, at a given time/H-factor.

It was observed that the solids in the black liquor increased as pulping progressed, which was due to degradation and dissolution of wood compounds. After 30 min of pulping (0 H-factor), 38.9 g of solids were found in the sweetgum black liquor, which were a mixture of organic and inorganic compounds. Out of the 38.9 g, 4.3 g were accounted for by the difference between the lignin content in the wood and in the pulp. After 150 min (800 H-factor), an increased mass of solids was found (68.2 g,) and 24.7 g was accounted for by lignin.

Furthermore, the total amount of solids precipitated (lignin, carbohydrates, and ash) by acidification increased over the process. After 30 min (0 H-factor), only 0.6 g were precipitated during the carbonation of the sweetgum black liquor (acidification to pH 9.5), whereas at 120 min (487 H-factor), 14.7 g were precipitated. The same trend was observed throughout the precipitation of the lignin fraction at pH 2.5 (acidification with HCl). This increase was expected since more organic compounds were found in the black liquor throughout the process due to hydrolytic reactions.

Table 3.5. Material Balance of Lignin Precipitation from Sweetgum Black Liquors

Cook Time (min)	Parameter (g)								
	Solids in the BL	Lignin in the BL	Solids precipitated	Glucan	Xylan	Total Lignin	Klason Lignin	ASL	Ash
pH 9.5									
30	38.9	4.3	0.6	0.1	0.3	0.1	0.1	0.0	0.0
60	55.5	15.1	5.5	0.5	1.2	3.1	2.8	0.2	0.1
90	66.2	23.0	12.4	0.7	1.3	9.0	8.4	0.6	0.3
120	68.1	24.5	14.7	0.8	1.4	10.9	10.4	0.5	0.3
150	68.2	24.7	12.3	0.5	1.1	9.4	9.1	0.3	0.3
pH 2.5									
30	38.3	4.2	0.8	0.0	0.2	0.3	0.3	0.0	0.0
60	50.0	12.0	4.2	0.0	0.3	3.2	3.0	0.2	0.0
90	53.8	14.0	3.6	0.0	0.1	3.2	3.0	0.2	0.0
120	53.4	13.5	4.4	0.0	0.2	4.0	3.8	0.2	0.0
150	55.9	15.3	8.4	0.0	0.4	7.8	7.5	0.3	0.1

Weight based on pulping 100 g of wood chips. Where BL is black liquor, Total lignin is a sum of Klason lignin (acid-insoluble lignin) and acid-soluble lignin (ASL).

In general, at higher pH, the fraction of lignin that precipitates has a higher molecular weight, whereas, at lower pH, the lignin that precipitates has a lower molecular weight (García *et al.* 2009; dos Santos *et al.* 2014; Kumar *et al.* 2016). A decrease in the amount of lignin precipitated at pH 9.5 between 120 to 150 min (487 and 800 H-factor, respectively) was observed, followed by an increase in the fraction obtained at pH 2.5. This likely occurred due to further degradation of the lignin dissolved in the black liquor into smaller molecules and further lignin depolymerization over the pulping process.

Even though significant amounts of carbohydrates are not expected to precipitate with lignin, sweetgum lignins isolated at pH 9.5 were heavily contaminated with carbohydrates. For instance, at 30 min (0 H-factor), 0.6 g of solids were isolated after carbonation, of which 0.1 g was glucan, and 0.3 g was xylan, whereas only 0.1 g was found to be lignin. On the other hand, the lignin isolated at pH 2.5 showed superior purity, *i.e.,* less carbohydrates and ash. It is well known that lignin precipitated at higher pH is less degraded (higher molecular weight); therefore, it can be safely assumed that the carbohydrates associated with these lignins are also less degraded (Zhu *et al.* 2015). Thus, at pH 9.5, the carbohydrates are less degraded, less soluble, and hence they can be precipitated along with lignin. Whereas, at pH 2.5, less amount of carbohydrates is remaining, and they are more degraded/soluble and do not precipitate. Another possibility is the lignin and carbohydrates are chemically bonded (LCCs), which leads to precipitation of lignin and carbohydrates at the same time.

It is important to mention that higher lignin yields have been reported with straightforward filtration recovery when compared with centrifugal recovery; however, filtration takes a longer time due to the colloidal nature of the lignin precipitate (Namane *et al.* 2015).

3.3.2.2. Pine analysis and comparison with sweetgum

The present work intended to establish a baseline comparison between the precipitation of lignin from hardwood and softwood black liquors. Thus, the precipitation, characterization, and material balance for both hardwood and softwood black liquors were performed under similar operating conditions. The material balances for the precipitation experiments are shown in Table 3.5 for hardwood and Table 3.6 for pine.

During the pulping of pine, the solids content of black liquor increased, which is in accordance with the sweetgum experiments. After 30 min of the pulping of 100 g (O.D.) of pine wood chips (0 H-factor), 47.7 g of solids were found in the black liquor (organic and inorganic); while after 150 min (1400 H-factor), 83.0 g of solids were found. The solids content at 30 min (0

H-factor) was reasonably higher in pine black liquor (47.7 g) than in sweetgum (38.9 g). This surplus of solids has an inorganic origin (white liquor) since the alkali charge was greater, and not much degradation was observed in the pulped pine chips at this point.

Table 3.6. Material Balance of Lignin Precipitation from Pine Black Liquors

Cook Time (min)	Parameter (g)								
	Solids in the BL	Lignin in the BL	Solids precipitated	Glucan	Xylan	Total lignin	Klason Lignin	ASL	Ash
pH 9.5									
30	47.7	3.0	0.4	0.0	0.0	0.3	0.3	0.0	0.0
60	62.8	12.4	4.6	0.0	0.0	4.3	4.2	0.0	0.1
90	78.4	25.0	6.9	0.0	0.2	6.4	6.2	0.2	0.1
120	82.7	27.8	19.4	0.0	0.4	16.4	16.1	0.4	0.4
150	83.0	28.4	21.0	0.0	0.4	19.4	19.0	0.4	0.5
pH 2.5									
30	47.3	2.7	1.2	0.0	0.0	1.0	1.0	0.0	0.0
60	58.2	8.1	3.0	0.0	0.0	2.6	2.6	0.1	0.0
90	71.5	18.6	16.9	0.0	0.5	13.9	13.5	0.5	0.2
120	63.3	11.4	9.0	0.0	0.3	7.6	7.3	0.2	0.1
150	62.0	9.0	7.1	0.0	0.2	6.4	6.2	0.2	0.1

Where BL is black liquor, Total lignin is a sum of Klason lignin (acid-insoluble lignin) and acid-soluble lignin (ASL).

The total amount of solids precipitated from pine black liquor increased over the process, which is also in accordance with the sweetgum experiments. After 30 min of pulping (0 H-factor), 0.4 g of solids were obtained at pH 9.5, whereas at 150 min (1400 H-factor), same pH, the recovery was 21 g. In addition, the purity of the pine lignin was superior to the sweetgum lignin due to less carbohydrate and ash contamination. For instance, the total solids precipitated over the carbonation of sweetgum black liquor obtained at the end of the pulping process (800 H-factor, 150 min) was 12.3 g, where about 1.9 g was contamination (carbohydrates and ash). On the other hand, out of the 21 g of solids obtained at the end of the pulping of pine wood chips (1400 H-factor, 150 min), only around 0.9 g were accounted for by contamination. In addition, the increase in lignin recovery throughout the pulping process could also be explained by lignin concentration. In low lignin concentration samples, the lignin molecules are surrounded by significantly more water molecules than in high lignin concentration samples, which might influence lignin interactions/agglomerations (Helander *et al.* 2016) and hence affect lignin recovery. The results of lignin concentration are not shown, however the same liquor to wood ratio was applied to all pulping experiments and the collected black liquors were evaporated to 20% solids content. Thus, a higher lignin content in the black liquor translates to higher lignin concentration.

Overall, it was observed that the precipitation of the lignin fraction at pH 2.5 contributed to a considerable increase in lignin recovery (Figure 3.4). This is in agreement with earlier investigations, which have shown that yield of precipitated lignin increased by decreasing pH (García *et al.* 2009; Theliander 2010; dos Santos *et al.* 2014; Zhu *et al.* 2014: Sewring *et al.* 2019).

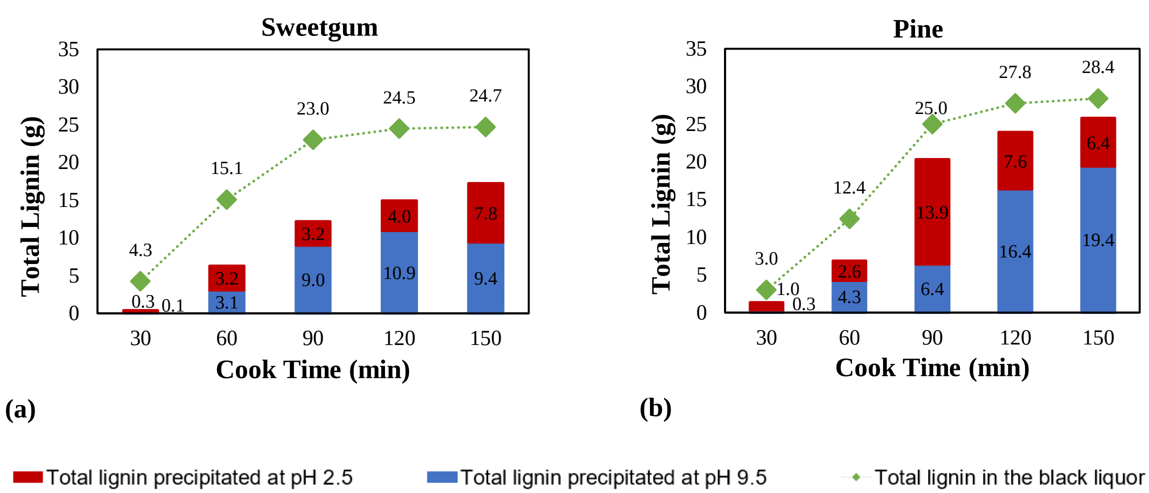

Figure 3.4. Total lignin content in the black liquor and Total lignin precipitated at pH 9.5 and 2.5 from (a) sweetgum and (b) pine samples throughout kraft pulping.

At the onset of the pulping, the yield of lignin fraction obtained at pH 2.5 was higher than the lignin fraction recovered at pH 9.5. It has been reported that lignin with high molecular weight precipitates at pHs around 8 to 11, and lignin with low molecular weights precipitate at lower pH (dos Santos *et al.* 2014; Kumar *et al.* 2016). In addition, it is known that mainly low molecular weight lignin is removed during the initial phase of delignification (Gellerstedt and Lindfors 1984). As the kraft pulping continues, lignin fractions of higher molecular weight are removed and solubilized in the black liquor (Gellerstedt *et al.* 1984). Moreover, it is known that condensation of lignin fragments occurs by repolymerization during the pulping process causing an increase in the molecular weight as the process continues (Shrotri *et al.* 2017). Therefore, initially, more lignin is obtained at pH 2.5 than at pH 9.5, and as the pulping proceeds, the yield of lignin obtained at pH 9.5 increases, while yield at pH 2.5 drops.

As softwood lignin precipitation methods have been well documented in both the literature and in commercial practice, it was assumed that those methods could be used as a template for hardwood lignin precipitation. However, this study showed that the behavior of sweetgum and pine lignin as a function of precipitation was considerably different. Firstly, the recovery of lignin from pine black liquors was greater than the recovery from sweetgum black liquor; which is in accordance with previous investigations that claims superior yields from softwoods over hardwoods (Öhman et al. 2007b; Santos et al. 2011, 2012; Zhu et al. 2014, 2015). Furthermore, Table 3.7 depicts the percentage of carbohydrates and lignin recovered via precipitation at both pH 9.5 and 2.5. As shown in the table, significant quantities of the sweetgum carbohydrates were precipitated, and relatively lower levels of lignin were obtained. For the pine liquor, only low amounts of the carbohydrates present in the black liquor were isolated with lignin via precipitation. For both the sweetgum and pine, the percentage of lignin precipitated increased as the cook progressed, but much superior lignin yields were obtained for the pine liquors at all conditions evaluated.

Table 3.7. Percentage of Carbohydrates and Lignin Precipitated from the Collected Sweetgum and Pine Black Liquors Throughout Cooking Process

Cook Time (min)	Sweetgum		Pine	
	Carbohydrates (%)	Total Lignin (%)	Carbohydrates (%)	Total Lignin (%)
30	6.6	9.3	0.5	45.5
60	12.2	41.8	0.6	55.4
90	10.1	52.9	4.0	81.4
120	11.2	61.1	3.7	86.2
150	9.5	69.7	2.5	90.9

The total lignin recovery presented in Table 3.7 was calculated based on the sum of the amount of lignin precipitated at pH 9.5 and 2.5 divided by the calculated lignin content in the black liquor. The maximum lignin recovery was obtained from black liquor samples generated at the end of the kraft pulping process. The total lignin recovery of sweetgum was 69.7% (800 H-factor), whereas the total lignin recovery of pine was 90.9% (1400 H-factor). Overall, the yield of lignin obtained from pine was greater than from sweetgum, mainly because part of the sweetgum lignin remained soluble in the supernatant due to the contamination with carbohydrates. The percentage of carbohydrates precipitated was calculated based on the amount of carbohydrates measured in the lignin fractions (pH 9.5 and 2.5), and the amount dissolved in the black liquor, which could be obtained by the difference between the residual content of carbohydrates in the produced pulp and the content in the black liquor.

The presence of lignin-carbohydrate complexes (LCCs) is a plausible explanation for the low yields achieved by sweetgum. Although these linkages exist in small amounts in lignocellulosic materials, it is speculated that almost all lignin is associated with polysaccharides (Lawoko *et al.* 2005; Du *et al.* 2013). The multiple –OH groups from carbohydrates will contribute to hydrophilicity, rendering the lignin more soluble in water (Hubbe *et al.* 2019). In addition, the LCCs are suggested to play a crucial role in recalcitrance during biomass processing and fractionation (Giummarella *et al.* 2019). For the analyses performed so far, it cannot be claimed that the carbohydrates that were precipitated with the sweetgum lignin were chemically bonded to it; however, it is plausible that they were chemically associated. Indeed, considerably more carbohydrates were detected in the sweetgum lignin versus pine. Moreover, the greater loss of cellulose by sweetgum during the kraft pulping might also be related to this association between carbohydrates and lignin. In addition, incorporation of xylan into lignin agglomerates as they form could cause a reduction in the further growth/agglomeration of particles due to increased electrostatic repulsive forces and/or steric effects, resulting in smaller particles being formed when compared to less xylan in the system (Sewring *et al.* 2019).

3.4. Conclusions

- Lignin recovery from black liquor and lignin purity increased with the increase of H-factor. The recovery at pH 2.5 was higher at the beginning of the process; however, as pulping proceeded, the recovery at pH 9.5 increased, while at pH 2.5, it decreased.
- The recovery of sweetgum lignin (hardwood) showed low yields since considerable levels were still soluble in the black liquor after acidification. In addition, these lignins presented low purity due to high contamination with carbohydrates.
- Pine lignin (softwood) was more easily precipitated and displayed superior purity.
- All lignin precipitated at pH 2.5 were purer than the fraction obtained at pH 9.5, and the former fraction contributed to a considerable recovery enhancement.
- The total recovery of the sweetgum lignin (summing both fractions) was 69.7% at 150 min (800 H-factor), whereas for pine was 90.2% at 150 min (1400 H-factor).
- Overall, the recovery of pine lignin was superior to the recovery of sweetgum lignin with respect to yield and purity.

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4. Chapter 4 - Probing the molecular weights of sweetgum and pine kraft lignin fractions

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Abstract

The present investigation undertook a systematic investigation of the molecular weight (MW) of kraft lignins throughout the pulping process to establish a correlation between MW and lignin recovery at different extents of the kraft pulping process. The evaluation of MW is crucial for lignin characterization and utilization since it is known to influence the kinetics of lignin reactivity and its resultant physico-chemical properties. Sweetgum and pine lignins precipitated from black liquor at different pHs (9.5 and 2.5) and different extents of kraft pulping (30-150 minutes) were the subject of this effort. Gel Permeation Chromatography (GPC) was used to determine the number average molecular weight (M_n), mass average molecular weight (M_w), and polydispersity of the lignin samples. It was shown that the MW of lignins from both feedstocks follow gel degradation theory, *i.e.*, at the onset of the kraft pulping process low molecular weight-lignins were obtained, and as pulping progressed, the molecular weight peaked and subsequently decreased. An important finding was that acetobromination was shown to be a more effective derivatization technique for carbohydrates containing lignins than acetylation, the technique typically used for derivatization of lignin.

4.1. Introduction

Lignin is the second most abundant terrestrial biopolymer, accounting for ~ 30% of the organic carbon in the biosphere (Ralph et al. 2007). Lignins are complex and stereochemically heterogeneous aromatic heteropolymers derived mainly from three hydroxycinnamyl alcohol monomers that differ in their degree of methoxylation. These monomers are *p*-coumaryl, coniferyl, and sinapyl alcohols. These monolignols produce, respectively, *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) phenylpropanoid units when incorporated into the lignin polymer (Fengel and Gerd 1984). During the final step of lignin biosynthesis, termed lignin polymerization, the monolignols are linked to the growing polymer by radical couplings that occur through "end-wise" polymerization steps (Tobimatsu and Schuetz 2019). Although exceptions exist, hardwood lignins consist principally of S and G units and traces of H units, softwood lignins are composed mostly of G units, whereas lignins from grasses present mainly H units (Boerjan et al. 2003).

Flory-Stockmayer Theory governs cross-linking and gelation of step-growth polymers (Flory 1941) which polymer science has invoked to describe lignin polymerization and degradation (Argyropoulos and Bolker 1987; Berry and Bolker 1986; Bolker and Brenner 1970; Leclerc and Olson 1992; Szabo and Goring 1968; Yan and Johnson 1981). As lignin polymerization initiates, monolignols are coupled forming a sol (a colloidal solution). The sol size increases stepwise until the polymerization reaches a gelation point characterized by a three-dimensional cross-linked polymer. Beyond the gelation point as the polymerization proceeds, the size of the sol decreases because it is depleted of high molecular weight moieties.

Lignin degradation processes such as chemical pulping and ethanol production have been reported to be a mirror phenomenon of lignification, *i.e.*, the reverse of polymerization. Thus, gel degradation theory treats these processes as de-gelation, in which crosslinking is reversed (Yan and Johnson 1981). In addition, it is well known that kraft pulp delignification occurs in three distinct phases (viz., initial, bulk, and residual) (LéMon and Teder 1973). During the initial phase, dissolution of up to ~20% of the lignin takes place and the lignin which goes into solution is of low molecular weight and highly phenolic (Gellerstedt and Lindfors 1984). As pulping proceeds, intense bond cleavage occurs, mainly of the β-O-4 linkage connecting the phenylpropane units together (Gellerstedt 2009), in order to detach larger fragments (Argyropoulos and Bolker 1987), which leads to an increase in the molecular weight of dissolved lignin. Subsequently, when these large lignin fragments have diffused out of the gel phase, they become available for further degradation (Leclerc and Olson 1992); afterwards, the molecular weight of dissolved lignins decreases.

Figure 4.1 is a schematic representation of lignin polymerization and degradation according to the Flory-Stockmayer Theory already mentioned.

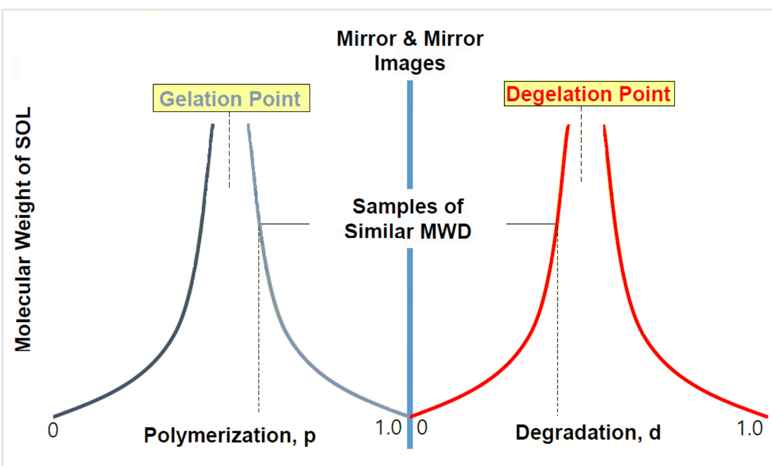


Figure 4.1. A qualitative plot of molecular weight versus polymerization and degradation (adapted from Argyropoulos and Bolker 1987).

The dotted lines define two points, one beyond the gel point and one before the degelation point, at which the sol's molecular species distributions are expected to be similar (Argyropoulos and Bolker 1987). The gelation point is observed during lignin polymerization (lignin biosynthesis), whereas the degelation point is observed during degradation (kraft pulping, process herein studied).

Molecular weight affects lignins reactivity and physico-chemical properties (Baumberger et al. 2007). Thus, the objective of the present work was (1) to interrogate the MW of kraft lignins, which is the most abundant source of technical lignin because ~78 million tons are generated annually (Miller and Faleiros 2016); and (2) to establish a correlation between MW and lignin recovery throughout pulping. The second objective was set to elucidate the behavior of very different lignin sources during acid precipitation, which is currently poorly understood.

4.2. Experimental

4.2.1. Material

Sweetgum and pine lignin fractions were obtained via acid precipitation from black liquor (pH ~13). Lignin fractions were precipitated at pHs 9.5 and 2.5 from kraft black liquors obtained after 30, 60, 90, 120, and 150 minutes of pulping. Thus, a total of 10 samples of each raw material were evaluated. Pulping and precipitation conditions, as well as yields and material balance have been reported elsewhere (Jardim et al. 2020b). Table 4.1 shows the composition of the studied lignins.

Table 4.1. Characterization of sweetgum and pine lignin fractions for total lignin, carbohydrates, and ash contents. Values in percentage (%)

Cook Time (min)	Sweetgum Lignin						Pine Lignin					
	Fraction at pH 9.5			Fraction at pH 2.5			Fraction at pH 9.5			Fraction at pH 2.5		
	Total Lignin	Carb.	Ash	Total Lignin	Carb.	Ash	Total Lignin	Carb.	Ash	Total Lignin	Carb.	Ash
30	14.9	59.3	2.5	39.6	35.0	1.0	92.4	1.3	1.8	84.3	1.5	1.6
60	56.0	31.4	2.6	77.4	8.5	1.1	92.9	1.0	1.3	88.0	1.8	1.1
90	72.4	16.0	2.4	88.0	4.7	1.0	85.4	2.9	1.9	82.4	3.4	1.3
120	74.4	14.8	2.1	90.6	5.5	0.9	84.9	2.5	2.0	83.7	3.3	1.2
150	76.3	13.2	2.6	92.4	5.1	1.3	92.3	2.0	2.5	91.0	2.6	1.1

4.2.2. Methods

4.2.2.1. Molecular Weight

Gel Permeation Chromatography (GPC) was used to determine the number average molecular weight (M_n) and mass average molecular weight (M_w), as GPC is the most conventional, comparable, and practical method for molecular weight determination (Huang et al. 2019). Lignin samples were acetylated before analysis to achieve total solubility in tetrahydrofuran (THF) our mobile phase of choice (Glasser et al. 1993). Acetobromination, another derivatization procedure, was also evaluated for sweetgum lignins, due to contamination with carbohydrates. This procedure was chosen since the reactivity of acetyl bromide in glacial acetic acid is significantly higher than in acetic anhydride/pyridine (Asikkala et al. 2012). ~10 mg of lignin was weighed into a reaction vessel, after which 2.3 mL of glacial (anhydrous) acetic acid was added. The reaction mixture was shaken gently and allowed to react for 1 h at room temperature. Finally, 0.25 mL (3.38 mmol) of acetyl bromide was added and the mixture was held at room temperature, but frequently stirred for 48 h. Acetic acid and excess acetyl bromide were evaporated in a rotary evaporator followed by vacuum drying. The GPC experiments were carried out using a Shimadzu liquid chromatograph equipped with a UV detector (set at 280 nm) at a flow rate of 0.7 mL/min at 35 °C. Two ultra styragel linear columns linked in series (Styragel HR 1 7.8 × 300 mm and Styragel HR 5E 7.8 × 300 mm) were used. The acetylated and acetobrominated lignins were dissolved into THF at a concentration of 1 mg/mL and filtered through 0.2 μm nylon filters. A series of monodisperse polystyrene standards were used as the calibration standards.

4.3. Results and Discussion

The non-uniformity of the chain lengths of lignin precludes characterization of a specific molecular weight (Tolbert et al. 2014). Therefore, it is necessary to characterize lignin in terms of average molecular weight. Two common averages used are number average molecular weight (M_n) and weight average molecular weight (M_w) shown in Eqs. (4.1) and (4.2). The polydispersity index (PDI) represents the molecular weight distribution of the polymers that is shown in Eq (4.3) (Lange et al. 2016).

$$M_n = \frac{\Sigma N_i M_i}{\Sigma N_i} \quad (4.1)$$

$$M_w = \frac{\Sigma N_i M_i^2}{\Sigma N_i M_i} \quad (4.2)$$

$$PDI = \frac{M_w}{M_n} \quad (4.3)$$

where the index number, i, represents the number of different molecular weights present in the lignin sample and N_i is the total number of moles with the molecular weight of M_i. M_w, M_n and PDI of acetylated sweetgum and pine lignin fractions are shown in Tables 4.2 and 4.3, respectively.

Table 4.2. M_w, M_n and PDI of acetylated sweetgum lignin fractions

Cook time (min)	**Sweetgum lignin at pH 9.5**			**Sweetgum lignin at pH 2.5**		
	M_w (g/mol)	M_n (g/mol)	PDI	M_w (g/mol)	M_n (g/mol)	PDI
30	1403	287	4.9	1704	567	3.0
60	4731	637	7.4	2390	712	3.4
90	4188	847	4.9	1868	631	3.0
120	3128	575	5.4	1680	485	3.5
150	2621	735	3.6	1667	606	2.8

Table 4.3. M_w, M_n and PDI of acetylated pine lignin fractions

Cook time (min)	**Pine lignin at pH 9.5**			**Pine lignin at pH 2.5**		
	M_w (g/mol)	M_n (g/mol)	PDI	M_w (g/mol)	M_n (g/mol)	PDI
30	4434	1361	3.3	1956	838	2.3
60	4782	1316	3.6	2556	1008	2.5
90	5656	1406	4.0	4710	1244	3.8
120	4953	1345	3.7	4251	1205	3.5
150	4750	1200	4.0	4092	1151	3.6

Also, the molecular weight distribution (MWD) for all samples herein studied are depicted in Figure 4.2. After 30 minutes of pulping, sweetgum lignin with an M_w of 1403 g/mol was obtained at pH 9.5 and M_w of 1704 g/mol at pH 2.5. Whereas for pine, the values were of 4434 g/mol and 1956 g/mol at pH 9.5 and 2.5, respectively. It has been reported that as the cook proceeds, the MWD curves move towards higher values (Gellerstedt and Lindfors 1984), *i.e.*, larger fragments are released. The maximum M_w of lignin fractions precipitated at pH 9.5 were observed at 60 minutes (51 H-factor) for sweetgum, and at 90 minutes (503 H-factor) for pine; with values of 4731g/mol and 5656 g/mol, respectively.

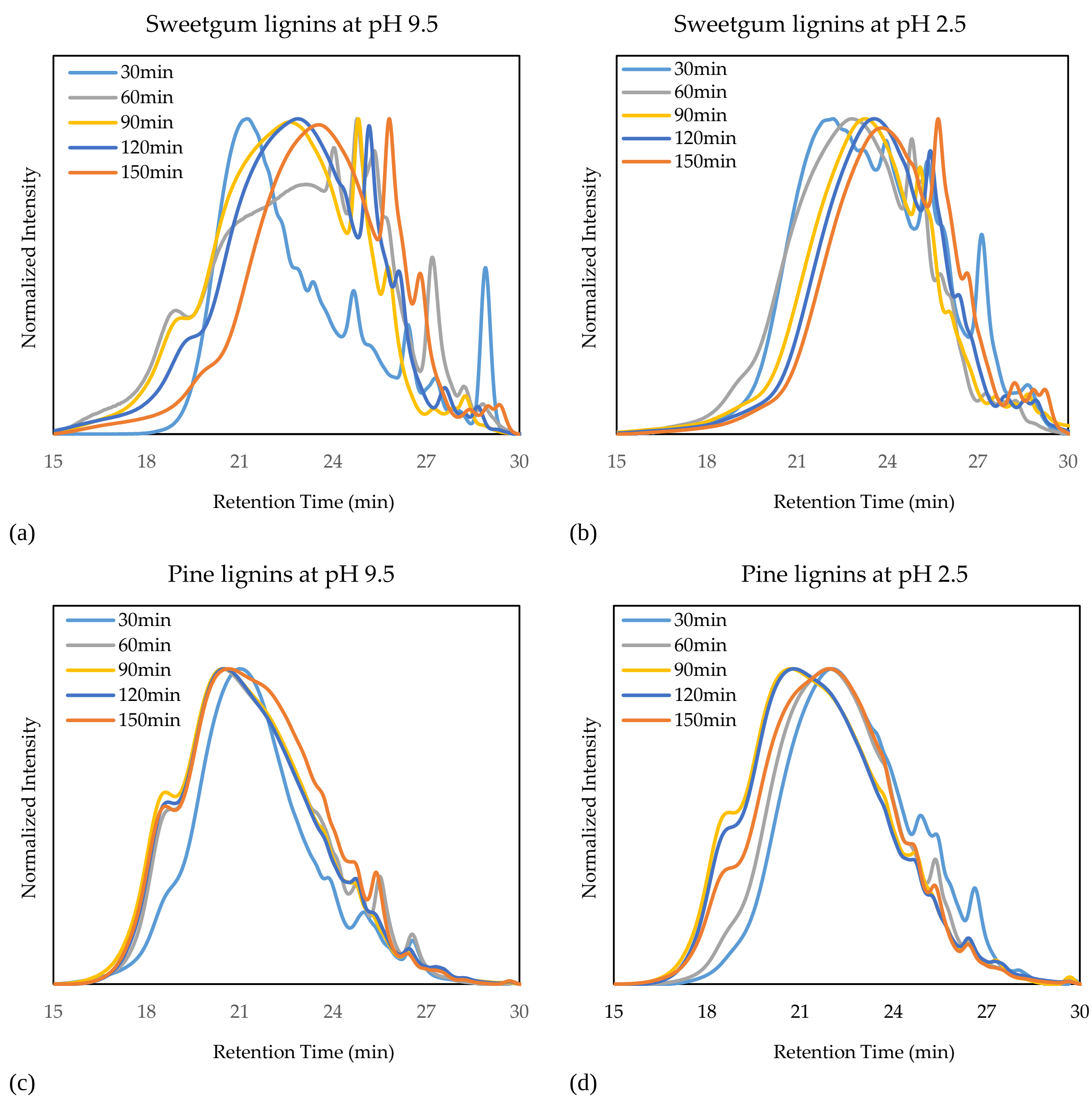


Figure 4.2. Molecular Weight Distribution of (a) sweetgum lignins obtained at pH 9.5, (b) sweetgum lignins obtained at pH 2.5, (c) pine lignins obtained at pH 9.5 and (d) pine lignins obtained at pH 2.5.

It is important to mention that under the kraft pulping conditions, condensation of dissolved lignin fragments occurs by repolymerization, which also contributes to increases in the molecular weight of lignins (Dimmel and Gellerstedt 2010; Rinaldi et al. 2016; Shrotri et al. 2017). Lignins undergo extensive chemical transformation as a result of pulping, with the extent of structural modification dependent upon the severity of the cooking conditions (*i.e.*, temperature, cooking time and chemical dosage (Gellerstedt et al. 1984)). Moreover, the same trend of M_w variation was observed for lignins precipitated at pH 2.5. A maximum M_w of 2390 g/mol was obtained at 60 minutes for sweetgum, while for pine it was 4710 g/mol at 90 minutes.

It is likely that because the structure and properties of hardwoods and softwoods lignins are different, they will show variations in performance in kraft pulping and precipitation. Additionally, the rates of increase toward larger molecular fragments throughout pulping are different for unique lignin networks (Leclerc and Olson 1992) which explains why pine and sweetgum lignins show increases in molecular weight at different extents of pulping.

As mentioned, lignin fragments in solution undergo condensation. Otherwise, further cleavage is prompted by pulping chemicals causing a drop in the weight average molecular weight (Argyropoulos and Bolker 1987). The final M_w for sweetgum at pH 9.5 was 2621 g/mol and 1667 g/mol at pH 2.5, while for pine the values were 4750 g/mol and 4092 g/mol at pH 9.5 and 2.5, respectively. Furthermore, in all cases, the M_w of pine lignin samples were greater than the M_w of sweetgum lignin samples in accordance with previous reports pointing to higher values of molecular weight for softwoods relative to hardwoods (Pakkanen and Alén 2012). Also, for both raw materials, the M_w of lignin samples precipitated at pH 9.5 were greater than lignin samples precipitated at pH 2.5 which is also in agreement with studies of gradient acid precipitation (García et al. 2009; Kumar et al. 2016; Santos et al. 2014). This difference is because the balance of attractive and repulsive forces depends on lignin molecular weight. Larger fragments are more easily destabilized when negative charges are protonated by acidification (H^+) than smaller fragments.

Overall, lignins precipitated at pH 9.5 presented higher PDI than lignins obtained at pH 2.5, revealing higher heterogeneity of lignin fractions obtained at higher pHs. It has been documented that carbonated lignin shows higher PDI than acidified lignins (Kumar et al. 2016) and low pH lignin fractions have shown lower polydispersity (Santos et al. 2014). In addition, the PDI of the majority of sweetgum lignins was greater than PDI of pine lignins.

In summary, results shown in Tables 4.2 and 4.3 reveal that at the onset of pulping lignin fractions showed low molecular weight; as the cook progressed, the molecular weight increased achieving a maximum M_w after which it underwent a decrease. These observations are in agreement with the assumption that lignin degradation is the reverse of lignin polymerization in line with the lignin degelation/gelation theory presented earlier (Argyropoulos and Bolker 1987; Berry and Bolker 1986; Bolker and Brenner 1970; Szabo and Goring 1968; Yan and Johnson 1981). Figure 4.3 shows the effect of cook extent on M_w of total lignin precipitated (both lignin fractions combined) and on delignification throughout the pulping process. Note that the M_w profile mimics the lignin degradation described by gel degradation theory (Figure 4.1).

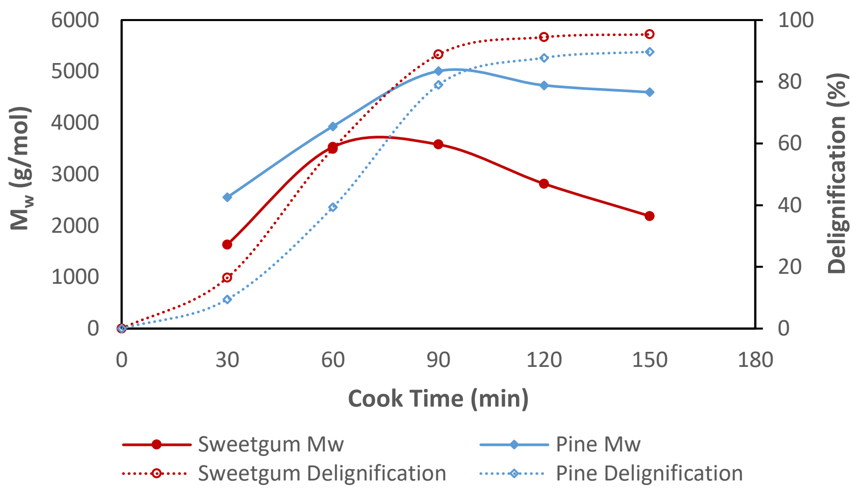
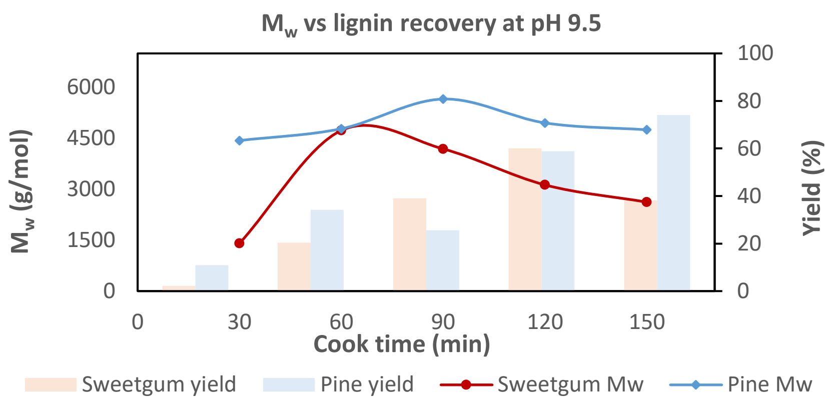


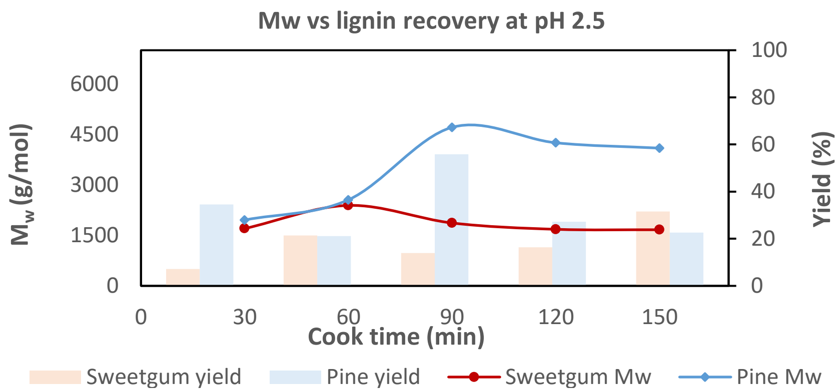
Figure 4.3. M_w and delignification of sweetgum and pine throughout the pulping process for total lignin precipitated.

Most of the lignin removal during kraft pulping takes place during the first two delignification phases (initial and bulk). It has been shown that up to 120 minutes, ~ 97% of the sweetgum lignin is removed, while ~ 89% of pine lignin is removed. Furthermore, the gel degradation theory predicts accelerated degradation rates as the degelation point is approached. This is because as degradation proceeds, a smaller number of bonds need to be cleaved to free larger fragments (Argyropoulos and Bolker 1987). Indeed, higher molecular weight-lignins were obtained at the accelerated delignification phase, *i.e.*, over the bulk phase (~ 60 to 90 minutes of pulping).

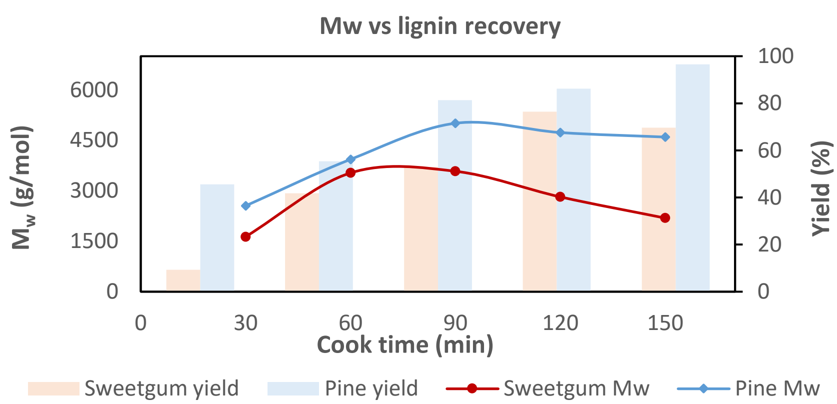
Figure 4.4 shows the M_w and lignin recovery yield at different extents of pulping. At the onset of kraft pulping, more lignin was obtained at pH 2.5 than at pH 9.5 (Fig. 4.4a and 4.4b). This is because high molecular weight-lignin precipitates at pHs ~ 8-11, whereas low molecular weight-lignin precipitates at lower pHs (García et al. 2009; Kumar et al. 2016; Santos et al. 2014); and mainly low molecular weight-lignin is removed during the initial phase of delignification (Gellerstedt and Lindfors 1984). As the pulping proceeds, the yield of lignin recovered at pH 9.5 increased due to the detachment of larger lignin fragments.



(a)



(b)



(c)

Figure 4.4. Mw and lignin recovery yield as a function of time for lignin fractions obtained at (a) pH 9.5, (b) pH 2.5, and (c) for both lignin fractions combined.

The amount of sweetgum lignin precipitated at pH 9.5 at 150 minutes was lower than the amount obtained at 120 minutes, which was unexpected. In addition, there was an increase in the amount of lignin obtained at pH 2.5 that is likely due to further degradation of lignin in the black liquor to smaller fragments and further lignin depolymerization (Jardim et al. 2020b).

Figure 4.4c depicts the variation of M_w and lignin recovery yield of total lignin (both lignin fractions) precipitated at different extents of pulping. Initially, the lignin recovery increases with increasing molecular weight for both feedstocks (sweetgum and pine). Towards the end of the pulping, the M_w of both lignins decreased, and the total recovery of sweetgum lignin decreased, whereas the total recovery of pine lignin kept increasing. This shows that molecular weight alone cannot explain the yield of lignin recovery from black liquor and highlights the different behavior of hardwood and softwood lignins. For instance, functional groups and contamination with carbohydrates may affect lignin recovery. In addition, the variation of M_w for sweetgum kraft lignins was more significant than for kraft pine lignins.

Figure 4.5 depicts the correlation between M_w and carbohydrates-to-lignin ratio (C/L ratio) of pine and sweetgum lignins precipitated from black liquor at different extents of the pulping process. A significant amount of carbohydrates was precipitated together with sweetgum lignins, especially at the onset of the kraft pulping process. Additionally, it was observed that as the pulping progressed, contamination decreased. For pine lignins, the impurities were much less significant. Thus, carbohydrate contamination is another indicator of divergent behavior between sweetgum and pine lignin precipitation.

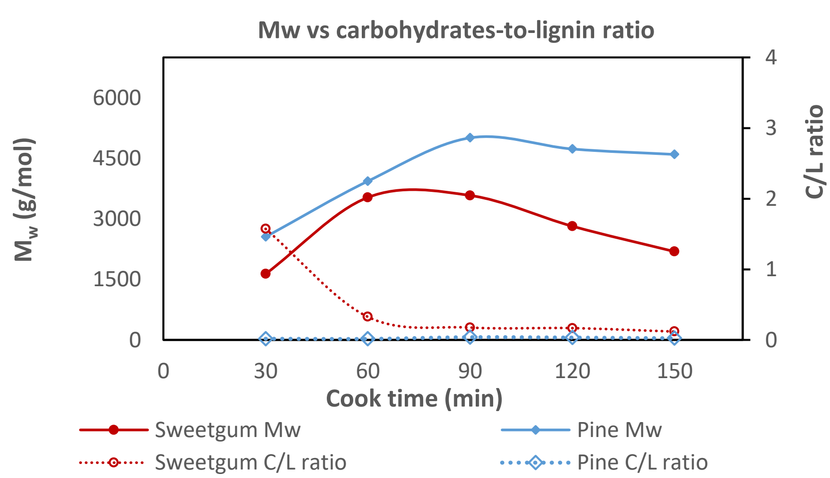


Figure 4.5. M_w and carbohydrates-to-lignin ratio throughout the pulping process for sweetgum and pine lignins.

The presence of lignin-carbohydrate complexes (LCCs) and/or lignin association are plausible explanations for the low yields shown in Fig. 4.4 and high carbohydrate contaminations depicted in Fig. 4.5 for sweetgum. Although LCCs exist in small amounts in lignocellulosic materials, it is speculated that almost all lignin is associated with polysaccharides (Du et al. 2013; Lawoko et al. 2005a). The structure of lignin bound to xylan (main hemicellulose in hardwoods) is different to lignin bound to glucomannan (main hemicellulose in softwoods) (Aminzadeh et al. 2017). For instance, the glucomannan-bound lignin seems to contain more condensed bonds and lower content of β–O-4 bonds than the xylan-bound lignin (Lawoko et al. 2005a). Thus, it has been suggested that the higher content of xylan and β–O-4 bonds in hardwoods may lead to a higher content of LCC (Aminzadeh et al. 2017).

The physical interactions between lignin and carbohydrates in the cell walls have not been fully understood yet. However, research conducted with non-woody biomasses has revealed that lignin self-aggregates in distinctive nanodomains with extensive surface contacts to hemicelluloses, mainly xylan (Kang et al. 2019). Also, the investigation showed that the number of physical contacts with carbohydrates correlates with the number of methoxyl groups in lignin and decreases in the order: S > G > H. Thus, this physical contact is more significant in sweetgum as hardwood lignins are composed of S and G units, whereas softwoods, such as pine, are composed of primarily G units.

In addition, the multiple –OH groups from carbohydrates contribute to hydrophilicity, rendering the lignin more solubility in water (Hubbe et al. 2019). Therefore, it can be inferred that at the onset of the pulping process, large moieties of lignin and carbohydrates were precipitated from sweetgum black liquors, which explains high contamination with carbohydrates. Moreover, towards the end of the pulping, those moieties were further degraded leading to a higher solubility of sweetgum lignins and hence lower yields.

All technical lignins contain impurities such as salts, carbohydrates, furans, and extractives to different extents, and often these impurities lead the MWD curves to tail into the low molecular weight ranges (Ringena et al. 2006). Also, the solubility of lignins containing carbohydrates is affected, causing low GPC recovery rates and unrepresentative analysis/results. These low molecular weight impurities have hardly any effect on the calculated M_w; nevertheless, they have great impact on the M_n and may result in very low number average molecular weights and increased polydispersities (Ringena et al. 2006). Sweetgum lignins had low M_n values (287 to 847 g/mol), as shown in Table 4.2. Furthermore, acetylated sweetgum lignins presented low solubility in THF, whereas acetylated pine lignins were fully dissolved in the same solvent. These observations can be attributed to the presence of carbohydrates because sweetgum lignins were highly contaminated with carbohydrates (13.2 to 59.3%), whereas in pine lignins contamination varied from 1.0 to 2.9% as evident in Table 4.1.

It has been suggested that there is a significant relationship between the PDI of a lignin sample and its M_w, although it is not always applicable to all lignins (Glasser et al. 1993). Additional variation may also result from topochemical effects that arise from different lignin sources (Leclerc and Olson 1992; Szabo and Goring 1968). Figure 4.6 shows plots of PDI *versus* M_w for all lignin samples.

A linear correlation between PDI and M_w was indeed observed for sweetgum lignins (Fig. 4.6a) and for pine lignins (Fig. 4.6c). The correlation was stronger for pine samples ($R^2 = 0.92$), which can be explained by the lower contamination with carbohydrates of these samples. Sweetgum lignins were highly contaminated with carbohydrates, especially at 30 min, pH 9.5 (Fig.

4.7). These impurities caused a significant drop in the M_n due to their low molecular weight while they had a minimum effect on the M_w measurement. Therefore, the presence of these impurities led to an increased PDI of the sweetgum lignins. Furthermore, when the most contaminated sweetgum sample was excluded from the plot (Fig. 4.6b) the correlation between PDI and M_w was improved. The R^2 for all sweetgum lignins was 0.63; whereas after removing the sweetgum lignin obtained at 30 min, pH 9.5, the R^2 = 0.84.

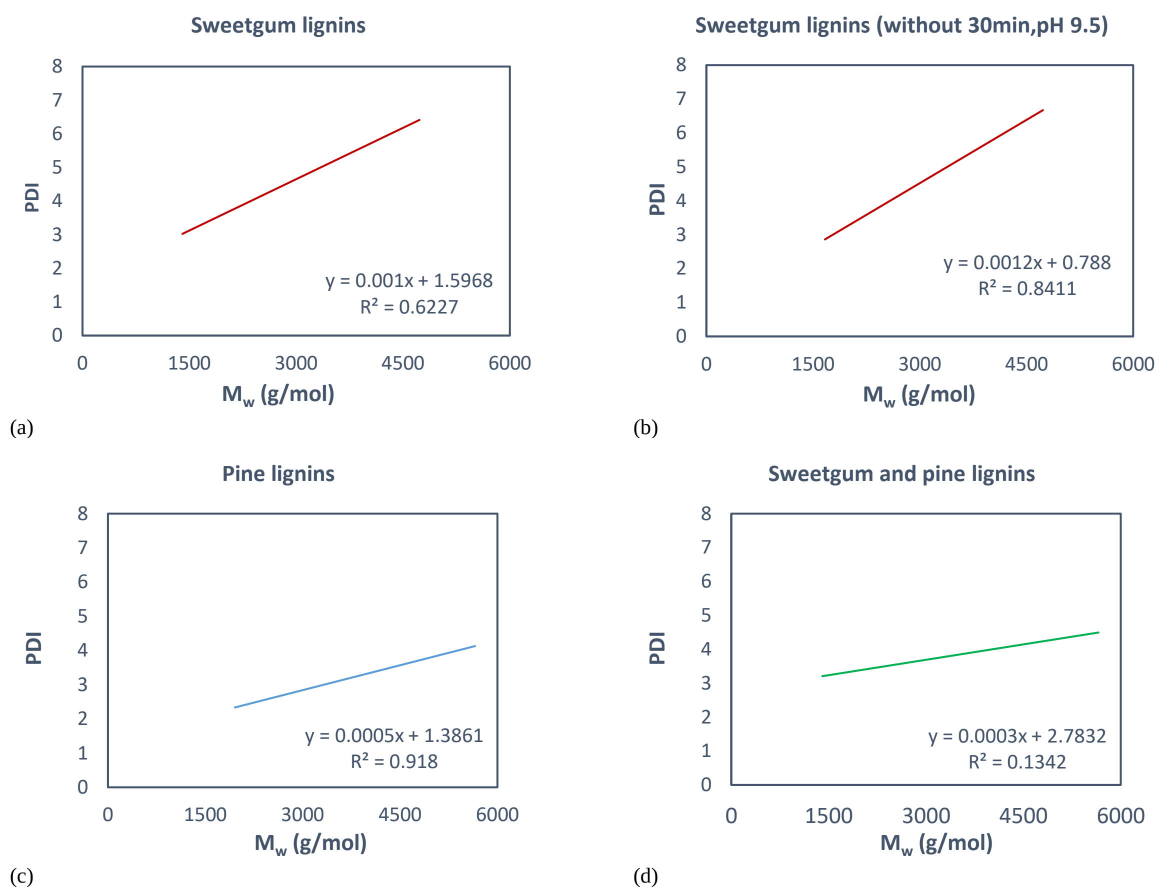


Figure 4.6. Correlation between PDI and M_w of (a) all sweetgum lignin samples; (b) sweetgum lignin samples excluding 30 min, at pH 9.5; (c) all pine lignins; and (d) all sweetgum and pine lignins.

When all sweetgum and pine lignin samples were combined (Fig. 4.6d) a very low correlation between PDI and M_n was observed. This is because hardwood and softwood lignins have very different chemical structures and hence show distinct behavior in pulping, acid precipitation, and in solution.

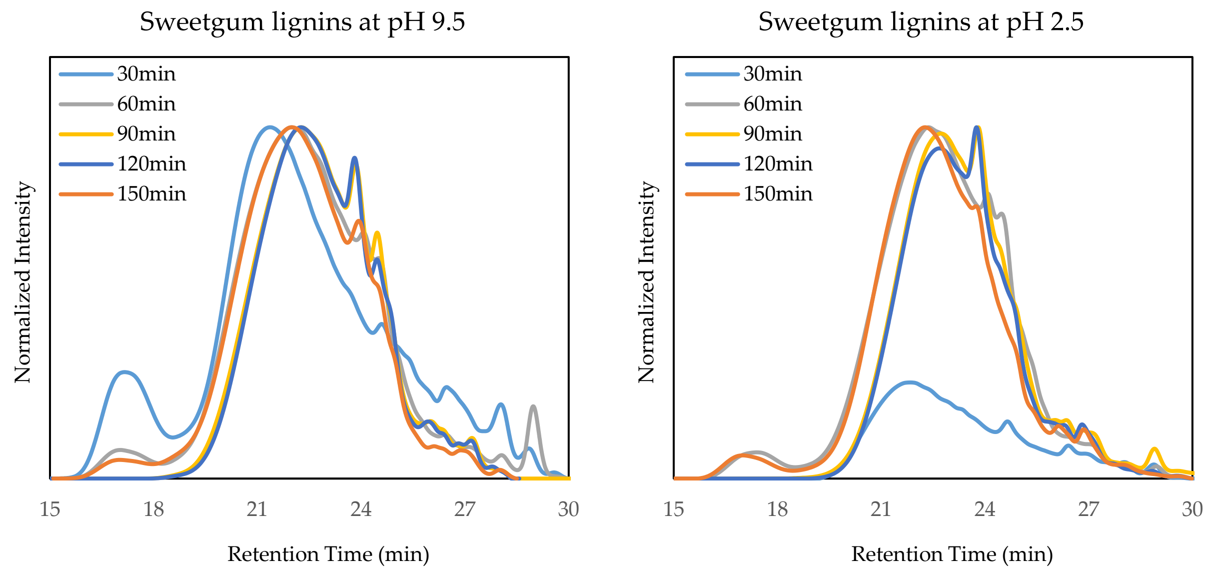
The low solubility of lignins containing carbohydrate impurities leads to low GPC recovery rates and unrepresentative MW analyses. A possible approach for obtaining a more accurate MWD of carbohydrates containing lignin is acetobromination. This technique was evaluated for sweetgum lignins and the results are shown in Table 4.4 and Figure 4.7.

Table 4.4. M_w, M_n and PDI of acetobrominated sweetgum lignin fractions

Cook time (min)	Fraction at pH 9.5			Fraction at pH 2.5		
	Mw (g/mol)	Mn (g/mol)	PDI	Mw (g/mol)	Mn (g/mol)	PDI
30	1662	517	3.2	1251	543	2.3
60	2942	748	3.9	2445	761	3.2
90	2834	794	3.6	1547	711	2.2
120	2811	745	3.8	1537	745	2.1
150	2765	763	3.6	1568	754	2.1

A comparison between acetylation and acetobromination as derivatization techniques for determining the MWD of milled wood lignin (MWL), hardwood kraft lignin (HWKL) and softwood kraft lignin (SWKL) has been reported (Asikkala et al. 2012). For MWL the results of MWD were significantly different between the two techniques. Only 60% of the acetylated MWL was dissolved in THF, whereas the acetobrominated MWL was fully dissolved. On the other hand, the results of MWD of both kraft lignins were nearly identical, which was attributed to the fact that technical lignins compared to MWL are structurally degraded during the kraft process and the molar mass is already so small that solubility is easily achieved.

It is noteworthy that acetylated sweetgum lignins were not fully dissolved in THF, whereas complete dissolution was achieved by acetobrominated sweetgum lignins. Thus, the relatively low molecular weight of technical lignins does not imply full dissolution in THF while the acetobromination technique was proven to be more effective for carbohydrate-contaminated lignins.



(a) (b)

Figure 4.7. Molecular Weight Distribution of acetobrominated lignins (a) obtained at pH 9.5 and (b) obtained at pH 2.5.

Finally, it is important to mention that the results of MWD for acetobrominated and acetylated lignin slightly differed, which was expected since when the same sample is subjected to different measurement techniques thus giving different results (Jardim et al. 2020a). Nonetheless, both techniques showed the same MWD trend, *i.e.*, the M_w profiles obeyed gel degradation theory.

4.4. Conclusions

It was proven that the molecular weight of sweetgum and pine lignins followed gel degradation theory. It could be concluded that the molecular weight of lignin samples in part explains lignin recovery yield; however, other parameters play important roles, such as the concentration and type of functional groups present. Furthermore, the high contamination with carbohydrates in sweetgum lignins obtained at the onset of the kraft pulping process greatly affected the M_n measurements leading to high PDI values. In addition, it was shown that acetobromination is a more effective derivatization technique for carbohydrates-contaminated lignins than acetylation.

4.5. References

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5. Chapter 5 -The effect of kraft pulping process, wood species, and pH upon lignin recovery from black liquor *(Submitted to Fibers)*

Abstract

Lignin has shown great potential to produce fuels, value-added chemicals, and functional materials due to its high-energy density and intrinsic aromatic based structure. As lignin is becoming more valorized into specialty products, it has become important to control precipitated lignin structures to reduce its polydispersity and achieve a more regular product, which avoids upsets in product performance. The present study investigated the precipitation and composition of forty (40) distinct kraft lignins obtained from pine, acacia, sweetgum, and eucalyptus black liquors. Two lignin fractions were precipitated at different pHs (9.5 and 2.5) from black liquors taken at different levels of pulping. Overall, lignin recovery increased with lignin concentration in the black liquor, i.e., higher amounts of lignin were obtained at higher levels of delignification. In addition, pine lignins showed superior yields than the hardwoods and were ~5 times purer. Among the hardwoods, eucalyptus showed the best performance, achieving the highest yields and purities. Moreover, the lignin recovery increased with S/G ratio of the native lignin. Finally, the present work provides a better understanding of the discrepancies observed between softwood and hardwood lignins behavior during lignin precipitation, which could greatly amplify the utilization of this diverse biopolymer as a green material.

5.1. Introduction

Lignin precipitation from kraft black liquor (BL) has been discussed for almost a century; however, the complexity of this wood-based polymer and its variation among species, growth site, and modifications upon processing hinder its understanding and hence its utilization. Nevertheless, obtaining lignin from black liquor has been shown to be commercially viable; thus, it is reasonable to expect increasing amounts of kraft lignin to be available in future years at relatively low cost (Hubbe et al. 2019). In addition, efforts to increase the fundamental knowledge of how pulping impacts hardwood lignin precipitation and the final properties are crucial. Softwood lignins have been well characterized and the precipitation behavior is relatively well understood. Besides, softwood lignin precipitation and lignin properties are not particularly species dependent.

Lignin extraction from BL is an interesting option for pulp mills as it allows for increased production capacity without increasing the recovery boiler load (Wallmo et al. 2007). It also enables the industry to become a biorefinery, generating multiple products from the biomass. Furthermore, it has been shown that 10–15% of the lignin dissolved in the BL can be isolated without adversely affecting the chemical recovery process (Hu et al. 2016).

Throughout kraft pulping, fibers are liberated via dissolution of the lignin into the spent liquor. The most common technique for lignin recovery is LignoBoost™, which is based on the dissociation equilibria of weak acid groups in lignin. Carbon dioxide (CO_2) is dissolved in the black liquor to decrease the pH of the BL from ~ 13 to ~10. A key advantage for using CO_2 is that it does not disturb the balance between sodium (Na) and sulfur (S) in the recovery cycle (Öhman et al. 2007a). The pKa value for phenolic groups in lignin vary between 6.2 and 11.3 depending on the substitution pattern (Ragnar et al. 2000). Thus, when the pH is reduced to ~10, these groups become protonated leading to lignin precipitation.

After precipitation, the solids are separated by filtration, then subsequently re-suspended in water and sulfuric acid (H_2SO_4) at pH ~ 2.5 to remove impurities (Wallmo et al. 2016). An effective washing step is necessary because precipitated lignin contains Na^+, which must be washed out and returned to the mill to avoid upsetting the Na/S balance and hence require an excessive demand of make-up chemicals (Öhman et al. 2007a). Besides, some final uses for precipitated lignin require high purity. It is important to mention that when the filter cake is re-dispersed, at pH and temperature values approximately equal to those of the final washing liquor, the concentration gradients will be low. Thus, the change in the pH level, ionic strength, and any change in lignin solubility will take place in the slurry, and not in the filter cake or in the filter medium during washing (Tomani 2010). Another motivation for redispersion is lignin recovery, as this step has been shown to improve lignin yield in a range of 6 to 7.5% (Öhman et al. 2007a). The losses experienced in the system without this step are attributed to the large pH and ionic strength gradients that lead to lignin re-dissolving. The final step in the precipitation process is another filtration to obtain solid lignin.

Lignin may be described as a complex, racemic, cross-linked, and highly heterogeneous aromatic macromolecule based on three major phenylpropanoids with various degrees of methoxylation (*p*-coumaryl, coniferyl and sinapyl alcohol) (Fengel and Gerd 1984). By repetitive dehydrogenation and cross-coupling syringyl (S), guaiacyl (G), and *p*-hydroxyphenyl (H) units are formed in lignin. These structural building blocks are zipped together by ether and by carbon–carbon linkages (Alén 2000). It is crucial to highlight that every lignin is different and neglecting such fact gives rise to non-comparable results and irreproducible materials portfolios, which substantially hinder progress towards a commercial lignin-based biorefinery (Schlee et al. 2019).

Softwood native lignins have been reported to contain mostly G units and vary little amongst species (Akiyama et al. 2005),(Sarkanen et al. 1967) . Thus, softwood lignin is much better understood, whereas hardwood native lignins vary greatly between species with a wide range of syringyl-to-guaiacyl ratios (S/G ratios) (Santos et al. 2012) within its structure. It has been shown that pine (softwood) and sweetgum (hardwood) behave very differently during lignin precipitation (Jardim et al. 2020b). Thus, the present investigation aimed at establishing a correlative relationship on the influence of wood species upon lignin precipitation and lignin quality. Several kraft lignins obtained from four different important species used extensively in the global pulp and paper industry were evaluated. In addition, because most of the lignin precipitation studies have been performed on pine black liquor samples, this species was treated as a control sample.

5.2. Experimental

5.2.1. Material

Pine (*Pinus taeda*), a softwood, and three hardwoods, acacia (*Acacia mangium*), sweetgum (*Liquidambar styraciflua*), and eucalyptus (*Eucalyptus globulus*) were investigated in the present study. Wood logs were processed into chips and screened according to the standard method "Size distribution" (SCAN-CM 40:01, 2001).

5.2.2. Methods

5.2.2.1. Kraft pulping

Kraft pulping experiments were carried out in a 10 L laboratory pulping digester (Greenwood Instruments, LLC; Andover, MA, USA) equipped with a temperature controller, liquor recirculation piping, and an indirect heating system. A liquor to wood ratio of 4 to 1 was applied for all cooks. The active alkali (as Na_2O) for pine was 19% and for hardwoods was 16% on oven dried (o.d.) wood. The sulfidity was 25% for all cooks and the temperature applied for pine cooks was 170 ^oC and 160 ^oC for hardwood cooks. Batch cooks were performed using 1000 g o.d. chips. Pulped chips were placed in a stainless-steel box equipped with a 150-mesh screen, washed thoroughly with water, and disintegrated. The washed pulp was then centrifuged to ~ 30% consistency and subsequently stored in polyethylene bags for follow-up characterization. Pulp yield was gravimetrically determined. The black liquor was collected and characterized according to the protocols “Solids Content of Black Liquor” (TAPPI/ANSI T650 om-15) and “Residual Effective Alkali” (rEA) (SCAN-N 33:94). The black liquor samples were stored in sealed Nalgene bottles for lignin precipitation.

Pulping runs were performed for 30, 60, 90, 120, and 150 minutes to obtain different levels of delignification. It is important to mention that the timing refers to the total cooking time and not time at temperature. Overall, the target temperature was achieved between 30 and 60 minutes after the start of the cook. Note that five pulp samples and five black liquor samples were generated from each wood species.

5.2.2.2.Lignin precipitation

Collected black liquor samples were filtered over Whatman #3 filter paper to remove fines. Filtered black liquors were evaporated to a solids content of ~20% and used for precipitation experiments of two lignin fractions (pH 9.5 and 2.5). Concentrated black liquors were sparged with CO_2 at a low flow rate to attain a pH 9.5 and allowed to equilibrate over 30 min. The precipitated materials were isolated from the liquors through centrifugation and the supernatants were stored for further precipitation. The solids were washed with hydrochloric acid (HCl) to remove ash at pH 2.5, and subsequently washed with acetic acid to remove residual HCl at pH 3.5.

After the washing steps, the solids were isolated by centrifugation, suspended in deionized water, and freeze-dried. Typically, precipitated lignin is washed with H_2SO_4; however, in this work, washing was performed with HCl because it has been reported to improve ash removal compared to sulfuric acid (Santos et al. 2014). Washing with acetic acid was carried out to remove residual HCl because it is less harmful to the freeze-drier vacuum pump compared to HCl. These solids, called lignin fraction at pH 9.5, were obtained according to commercially known lignin isolation protocols (e.g., LignoBoost™).

The supernatants after carbonation were further acidified with 6N HCl to pH 2.5 to precipitate a second lignin fraction, which was also washed with HCl, acetic acid, and freeze-dried. These solids were called lignin fraction at pH 2.5 and were obtained with the understanding that at pH 2.5 all remaining lignin or most of it would be precipitated.

Precipitation experiments were performed at room temperature, atmospheric pressure, and constant slow stirring. A schematic representation of the precipitation and purification processes is shown in Figure 5.1.

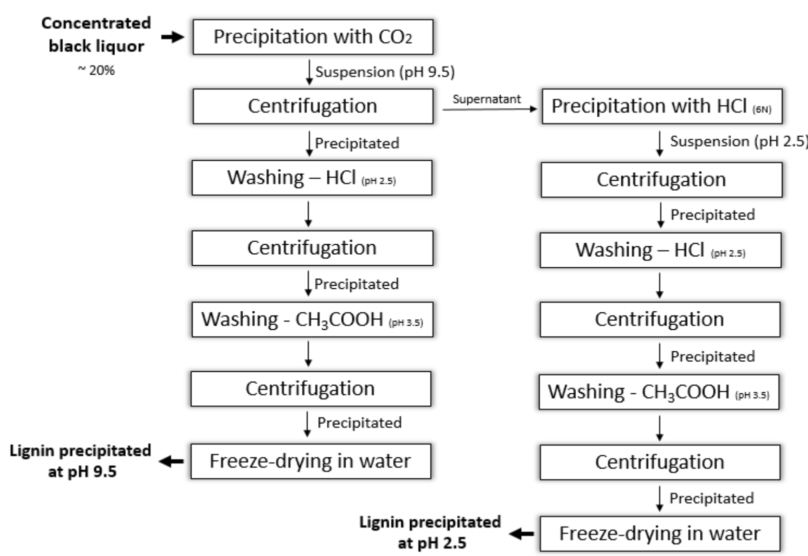

Figure 5.1. Schematic representation of the protocol used for lignin precipitation from kraft black liquor of two lignin fractions: pH 9.5 and pH 2.5.

The experimental conditions were kept constant for all precipitation experiments to establish a fair comparison between the behaviors of all lignin samples investigated.

5.2.2.3.*Characterization of wood, pulp, and precipitated lignin*

The wood chips were ground in a Wiley mill. The resulting milled wood was screened, and the fraction which passed through a 40-mesh screen and was retained on a 60-mesh screen was kept for analyses. The milled wood was extracted in a Soxhlet apparatus for 6 h using a benzene-ethanol mixture (2:1 v/v ratio) (TAPPI Standard Test Method T 264 cm-07 "Preparation of wood for chemical analysis"). Carbohydrates and lignin contents were quantified according to the Technical Report NREL/TP-510-42618 "Determination of structural carbohydrates and lignin in biomass". Ash content was measured according to "Ash in wood, pulp, paper and paperboard: combustion at 525°C" (TAPPI Standard Test Method T 211 om-16). The same protocols were applied to the characterization methods for the lignin samples precipitated from the black liquors. Syringaldehyde/vanillin (Sr/V) ratio of native lignins was determined according to the alkaline nitrobenzene oxidation (NBO) procedure reported in the literature (Chen 1992; Min et al. 2014a). As nitrobenzene oxidation accounts for only uncondensed structures, the equation below was used to obtain an accurate S/G ratio. This method was developed using a calibration line established by the S/V ratio obtained via nitrobenzene oxidation and the S/G ratio obtained via ^{13}C NMR of several milled wood lignins (MWLs) (Santos et al. 2012).

$$S/G = 0.806 * Sr/V \tag{5.1}$$

5.2.2.4.Material Balance

The characterization of wood, pulp, and lignin samples, as well as the determination of pulp yield and inorganics added into the process via white liquor allowed for a material balance.

Solid and liquid flows were accounted for to determine lignin yield over precipitation experiments. Also, the characterization of wood and pulps allowed for an estimation of the composition of black liquor used for lignin precipitation experiments.

5.3.Results and Discussion

5.3.1. Kraft pulping

Pine, acacia, sweetgum, and eucalyptus chips were each subjected to kraft pulping considered to be the dominant global chemical pulping process in which sodium hydroxide (NaOH) and sodium sulfide (Na_2S) (white liquor) are applied to wood chips under pressure and concomitant high temperatures, targeting lignin hydrolysis/dissolution and subsequent fiber liberation. The process generates pulp and a residual process stream (BL) that is rich in organic degradation products (lignin and carbohydrates) and spent inorganics. All four wood species were pulped at different levels of intensity with the results shown in Table 5.1.

Table 5.1. H-factor, pulp yield, residual effective alkali (rEA), and solids content of black liquor for pine, acacia, sweetgum, and eucalyptus

Species	Parameter	Cook Time (min)				
		30	**60**	**90**	**120**	**150**
Pine	H-factor	7	32	503	983	1400
	Pulp yield, %	93.2	69.1	51.4	47.4	43.5
	rEA, g/L	19.2	17.0	11.5	10.0	5.9
	Solids in BL, %	11.1	13.8	16.6	17.3	16.6
Acacia	H-factor	0	19	227	469	688
	Pulp yield, %	91.3	74.9	66.1	62.5	56.4
	rEA, g/L	26.4	17.3	11.8	8.9	7.3
	Solids in BL, %	9.0	11.1	12.8	13.6	13.8
Sweetgum	H-factor	0	51	273	487	650
	Pulp yield, %	85.5	62.6	50.7	48.7	47.6
	rEA, g/L	20.5	12.5	9.0	4.7	3.5
	Solids in BL, %	8.9	12.1	14.1	14.4	14.4
Eucalyptus	H-factor	0	15	216	442	648
	Pulp yield, %	93.8	67.6	52.0	48.7	48.1
	rEA, g/L	24.5	14.5	8.1	5.8	5.0
	Solids in BL, %	9.0	11.0	13.4	14.3	14.7

Overall, longer cooks (higher H-factor) yielded less pulp and the black liquor samples showed lower rEA and higher solid contents, which was expected. Throughout the pulping process, alkali is consumed in reactions with lignin, in dissolution of carbohydrates, in neutralizing various organic acids, both those present in the original wood and those produced during pulping, by the wood extractives, and to some extent, by adsorption on the fibers (Marton 1971), which decreases the residual effective alkali. In commercial cooks, the rEA is controlled to keep the dissolved lignin in solution. Moreover, the dissolution of degradation products is followed by an increase in the solids content of the BL. After 150 minutes of pulping, pine showed the lowest final pulp yield (43.5%), which is reasonable because much harsher conditions were applied to this species. Considering only the hardwoods, acacia showed the highest pulp yield (56.4%), whereas sweetgum and eucalyptus showed similar values (~48%). Note that at all levels of pulping the rEA for acacia was the highest among all four wood species, which could be attributed to its composition. Additionally, acacia has been shown to be harder to pulp in other investigations, mainly due to its lignin content and lignin chemistry (Almeida et al. 2015; Santos et al. 2011).

5.3.2. Composition of wood and pulps produced

Extractive-free wood chips and pulps were characterized for lignin and carbohydrate contents as shown in Table 5.2. The values for pulps obtained after 30, 60, 90, 120, and 150 minutes depict the residual content after the pulping of 100g (o.d.) of wood.

Pine and acacia wood chips showed the lowest contents of carbohydrates and the highest contents of lignin. Thus, it can be speculated that these two wood species are good candidates for lignin precipitation, as throughout the pulping process, there is more lignin to be dissolved in the black liquor. Even though sweetgum and eucalyptus wood chips showed lower lignin contents, they could also be considered suitable for lignin precipitation as most of the lignin was removed over the pulping process. After 150 minutes, the residual lignin content was 1.1 g for eucalyptus and 1.2 g for sweetgum, whereas for pine and acacia it was 3.3 g and 4.1 g, respectively.

Table 5.2. Composition analysis of pine, acacia, sweetgum, and eucalyptus wood chips and pulps

Species	**Compound**	**Sample / Cook Time (min)**					
		Wood*	**30**	**60**	**90**	**120**	**150**
Pine	Glucan, g	40.0	38.7	36.1	35.1	35.4	32.8
	Total Lignin, g	31.7	28.7	19.2	6.7	3.9	3.3
	Xylan, g	6.1	5.8	4.9	3.6	3.5	3.4
	Galac+Man+Arab, g	19.7	16.9	7.8	7.3	5.9	5.3
Acacia	Glucan, g	42.7	41.7	40.5	40.4	40.3	39.5
	Total Lignin, g	27.9	25.1	20.1	8.1	6.1	4.1
	Xylan, g	10.0	8.6	8.1	7.2	7.3	6.7
	Galac+Man+Arab, g	1.3	1.0	0.6	0.5	0.0	0.0
Sweetgum	Glucan, g	45.2	39.6	36.4	33.8	33.8	33.5
	Total Lignin, g	25.9	21.6	10.8	2.9	1.4	1.2
	Xylan, g	14.8	12.3	9.9	8.7	8.5	8.6
	Galac+Man+Arab, g	6.3	5.2	3.0	2.4	2.6	3.0
Eucalyptus	Glucan, g	44.6	41.9	31.9	31.7	30.6	30.4
	Total Lignin, g	24.8	24.5	13.8	3.0	1.7	1.1
	Xylan, g	13.1	11.2	7.5	7.8	7.0	7.4
	Galac+Man+Arab, g	4.0	3.4	1.3	0.9	0.5	0.5

where: galac is galactan, man is mannan, and arab is arabinan
* Content in 100g (o.d.)

Besides lignin content, the S/G ratio of lignin was also determined and the results are shown in Table 5.3. Note that pine lignin is not listed in that table as S is not one of the lignin building blocks of this species. This ratio was investigated because it is a key factor affecting the rate of hardwood kraft pulping (Santos et al. 2011). A lignin with a high syringyl content has a higher proportion of ether linkages because the C_5-position is blocked by a methoxyl group in S units. Therefore, species with high S/G ratio are easier to pulp (Santos et al. 2013a), as ether bonds are more easily broken than C-C bonds.

Table 5.3. S/V and S/G ratios of acacia, sweetgum, and eucalyptus

Species	**Sr/V ratio**	**S/G ratio**
Acacia	1.2	1.0
Sweetgum	2.8	2.3
Eucalyptus	4.5	3.6

In NBO, the three lignin building units p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) moieties are degraded into aldehydes: p-hydroxybenzaldehyde (B), vanillin (V), and syringaldehyde (Sr), and a small amount of their corresponding acids: p-hydroxybenzoic acid (Ba), vanillic acid (Va), and syringic acid (Sa), respectively (Min et al. 2014a). As the NBO quantifies only uncondensed structures an equation was used to obtain an accurate S/G ratio. Note that the highest S/G ratio was observed for eucalyptus, followed by sweetgum and acacia. This reinforces the effect of S/G ratio on kraft pulping process, as acacia (S/G ratio = 1.0) was much more difficult to pulp than the other hardwoods. In addition, acacia showed similar behavior to pine throughout the pulping process, to which harsher pulping conditions were applied because of the higher lignin content and for being composed of mainly G units.

Figure 5.2 depicts the degradation curves for wood compounds throughout the pulping process for all four wood species studied, including the delignification curve. The kraft pulping process undergoes three distinct phases as the wood chips are cooked into pulp. The phases are classically referred to as initial, bulk, and residual (Santos et al. 2013b). Most of the lignin is removed over the initial and bulk phases, whereas the residual phase is less selective and very low amounts of lignin are removed. This trend is observed independently of wood species, lignin content, and lignin type. Note that up to 90-120 minutes most of the delignification took place for all four wood species.

The eucalyptus delignification was faster than sweetgum, which was expected as the S/G ratio of the former is higher than the latter. However, given the difference one would expect a more significant difference. A possible explanation for this observation is the wood chips porosity. It has been reported that *E. globulus* shows a percentage of vessel of 8.5% and a vessel diameter average of 127.3 µm (Santos et al. 2011). On the other hand, the researchers reported a percentage of vessel of 35.9% for sweetgum and a vessel diameter average of 54.6 µm. It is likely that the higher porosity observed in sweetgum facilitated liquor impregnation and consequently enhanced the pulpability. It is important to mention that alkaline pulping is complex and affected by several factor such as morphological characteristics of the wood, the location of lignin in the woody tissue, the penetration pathway of the chemicals, the swelling or thermal coalescence of the lignin-containing interface, the cleavage of lignin-carbohydrate bonds, the chemical degradation of the lignin macromolecule, condensation reactions, stabilization of the degradation products via solvation and by means of electrostatic forces, the removal of degradation products from the reaction zone, and undesirable carbohydrates degradation reactions (Marton 1971).

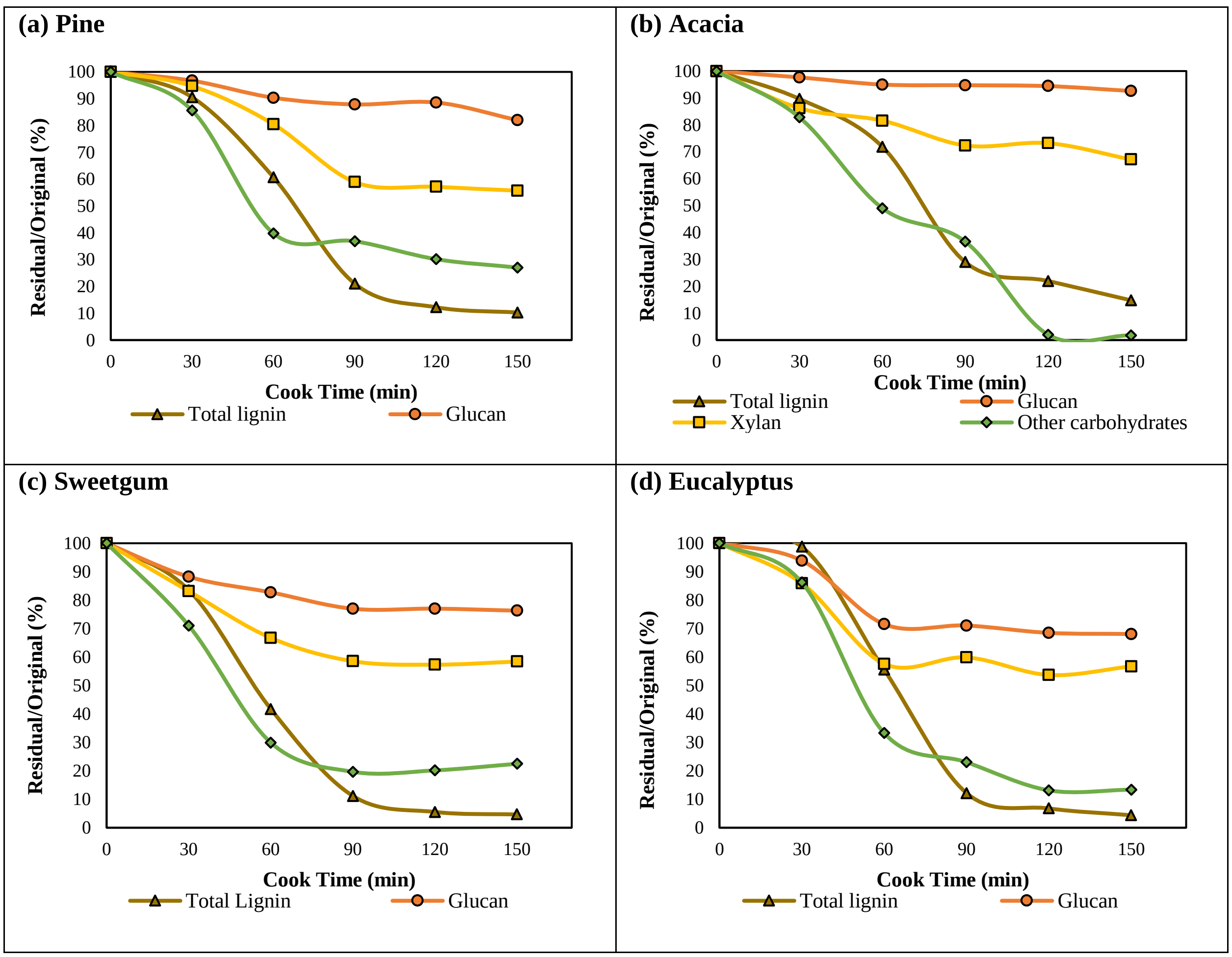

Figure 5.2. Lignin and carbohydrate degradation curves for (a) pine, (b) acacia, (c) sweetgum, and (d) eucalyptus

As the kraft pulping process is designed to promote delignification and preserve carbohydrates, the former is more degraded than the latter. The degradation profiles were herein observed. Among the carbohydrates, glucan and xylan were more resistant to the pulping process, while galactan, mannan and arabinan were more easily degraded. The great resistance of cellulose to degradation can be attributed to its crystallinity and high degree of polymerization; while the resistance of xylans to the pulping process is due to the presence of the lateral group called 4-O-methyl-α-D-glucuronic acid (MeGlcA) that protects the structure against the peeling reaction (Magaton et al. 2011). Additionally, the other hemicelluloses with lower molecular weight and more accessible chemical structure are more easily hydrolyzed and dissolved in the black liquor.

Moreover, xylan is the main hemicellulose in hardwoods, whereas glucomannan is the main hemicellulose in softwoods. Given the lower resistance of glucomannan to the pulping process, a greater loss of this wood compound is observed for pine. For instance, after 90 minutes of pulping, 19.8 g of carbohydrates were lost for pine, where 4.9 g were from cellulose and 14.9 g from hemicelluloses. Note that for eucalyptus, 23.6 g of carbohydrates were lost at this level of pulping, where 14.0 g were from cellulose and only 9.6 g were from hemicelluloses.

After every cook, the BLs were collected, filtered, and the solids content were adjusted to 20%. Then, these BL samples were acidified to obtain two lignin fractions: one at pH 9.5, where the acidifying agent was CO_2 and another at pH 2.5, where the acidifying agent was HCl. It is important to mention that both fractions were precipitated from the same BL sample, which was performed with the objective of characterizing all lignin dissolved in the BL. The protonation of the phenolic groups during the carbonation allows for precipitation of the lignin fraction at pH 9.5. When the pH was further acidified to 2.5 the carboxylic groups in lignin were also protonated (pK_a

3 to 5) leading to the precipitation of another lignin fraction. Thus, once the pH is lowered to 2.5 an increase in lignin recovery yield is observed.

The content of lignin dissolved at all levels of pulping, the total solids isolated at every precipitation experiment, as well as the characterization of the solids precipitated are shown in Table 5.4.

The lignin content in 100 o.d. grams of pine wood chips was 31.7 g and the residual content in the pulp was 3.3 g after 150 minutes of pulping; thus, the total amount of lignin dissolved in the black liquor was 28.4 g. Looking at the hardwoods, the total amount of lignin dissolved into the black liquor per 100 o.d. grams of chips, also after 150 minutes of pulping, was found to be 23.1 g for acacia, 24.7 g for sweetgum and 23.7 g for eucalyptus. Considering only lignin concentration in the BL, these results would suggest that sweetgum would be the best candidate for lignin precipitation given the highest concentration, followed by eucalyptus and acacia.

The total solids precipitated value also shown in the Table 5.4 represents the mass of material obtained after every precipitation experiment. For pine at 150 min, the total solids precipitated at pH 9.5 was 21.0 g, out of which 19.4 g was lignin, 0.4 g was carbohydrates, and 0.5 g was inorganics (ash). For the precipitation of the second lignin fraction (pH 2.5) the content of lignin in the black liquor was 9.0 g, the total solids precipitated was 7.1, out of which 6.4 g was lignin, 0.2 was carbohydrates and 0.1 was ash. Note that the solids precipitated contain other degradation products that were not herein determined, such as organic acids, extractives, and volatile compounds.

Table 5.4. Material balance of lignin precipitation from pine, acacia, sweetgum, and eucalyptus BLs

Species	pH	Parameter	Sample / Cook Time (min)				
			30	**60**	**90**	**120**	**150**
Pine	**9.5**	Lignin in the BL	3.0	12.4	25.0	27.8	28.4
		TS* precipitated	0.4	4.6	6.9	19.4	21.0
		Total lignin	0.3	4.3	6.4	16.4	19.4
		Carbs	0.0	0.0	0.2	0.5	0.4
		Ash	0.0	0.1	0.1	0.4	0.5
Pine	**2.5**	Lignin in the BL	2.7	8.1	18.6	11.4	9.0
		TS* precipitated	1.2	3.0	16.9	9.0	7.1
		Total lignin	1.0	2.6	13.9	7.6	6.4
		Carbohydrates	0.0	0.1	0.6	0.3	0.2
		Ash	0.0	0.0	0.2	0.1	0.1
Acacia	**9.5**	Lignin in the BL	2.8	8.4	19.3	21.1	23.1
		TS* precipitated	0.2	2.3	5.2	0.9	0.8
		Total lignin	0.1	1.8	4.3	0.4	0.3
		Carbohydrates	0.1	0.3	0.4	0.4	0.3
		Ash	0.0	0.0	0.0	0.0	0.0
Acacia	**2.5**	Lignin in the BL	2.8	6.6	14.9	20.8	22.7
		TS* precipitated	0.5	2.0	8.3	17.7	20.5
		Total lignin	0.4	1.8	7.2	15.8	18.7
		Carbohydrates	0.0	0.1	0.6	0.8	0.8
		Ash	0.0	0.1	0.1	0.4	0.2
Sweetgum	**9.5**	Lignin in the BL	4.3	15.1	23.0	24.5	24.7
		TS* precipitated	0.6	5.5	12.4	14.7	12.3
		Total lignin	0.1	3.1	9.0	10.9	9.4
		Carbohydrates	0.4	1.7	2.0	2.1	1.6
		Ash	0.0	0.1	0.3	0.3	0.3
Sweetgum	**2.5**	Lignin in the BL	4.2	12.0	14.0	13.5	15.3
		TS* precipitated	0.8	4.2	3.6	4.4	8.4
		Total lignin	0.3	3.2	3.2	4.0	7.8
		Carbohydrates	0.3	0.4	0.2	0.2	0.4
		Ash	0.0	0.0	0.0	0.0	0.1
Eucalyptus	**9.5**	Lignin in the BL	0.2	9.8	19.1	20.0	20.6
		TS* precipitated	0.0	2.5	9.8	13.5	13.6
		Total lignin	0.0	1.7	7.9	11.3	11.6
		Carbohydrates	0.0	0.5	1.1	1.4	1.3
		Ash	0.0	0.0	0.2	0.2	0.2
Eucalyptus	**2.5**	Lignin in the BL	0.1	8.1	11.2	8.7	9.0
		TS* precipitated	0.0	1.2	4.2	5.2	5.9
		Total lignin	0.0	1.0	3.5	4.7	5.4
		Carbohydrates	0.1	0.1	0.3	0.3	0.3
		Ash	0.0	0.0	0.0	0.0	0.0

* TS = Total solids

It was noteworthy that after 30 and 60 minutes of pulping very little lignin was obtained for all four wood species, and the recovery greatly increased with level of pulping; *i.e.*; the lignin recovery increased with lignin concentration. In low lignin concentrations (onset of kraft pulping), the lignin macromolecules are surrounded by significantly more water molecules than in high lignin concentration (offset of kraft pulping), which might influence lignin interactions/agglomerations (Helander et al. 2016) and hence precipitation.

Moreover, before the target pulping temperature was achieved (30 minutes), the amount of lignin recovered at pH 2.5 was higher than at pH 9.5 for all wood species studied. This can be attributed to the fact that mainly low molecular weight lignin is removed during the initial phase of delignification and that the lower molecular weight lignins are precipitated at lower pHs (Jardim et al. 2020b, 2021). Overall, as the pulping proceeds, larger lignin fractions are removed and condensation reactions taking place, leading to an increase in the molecular weight. Then, more lignin is recovered at pH 9.5 than at pH 2.5.

Figure 5.3 depicts lignin precipitation yields at the two different pHs and lignin-to-carbohydrate ratio (L/C ratio) of the solids precipitated, also at both pHs, as a function of pulping level for all four wood species.

It is noteworthy that hardwoods lignins showed much lower L/C ratio than pine, regardless of pH and pulping level. It is reasonable to assume that the presence of lignin-carbohydrate complexes (LCCs) and/or lignin association in hardwoods led to the co-precipitation of carbohydrates with the lignin. In addition, the structure of lignin linked to xylan is different than lignin linked to glucomannan (Aminzadeh et al. 2017), as the glucomannan-lignin seems to contain more condensed bonds and lower content of β–O-4 bonds than the xylan-lignin (Lawoko et al.

2005b). Thus, it has been suggested that the higher content of xylan and β–O-4 bonds in hardwoods may lead to a higher content of LCCs (Aminzadeh et al. 2017).

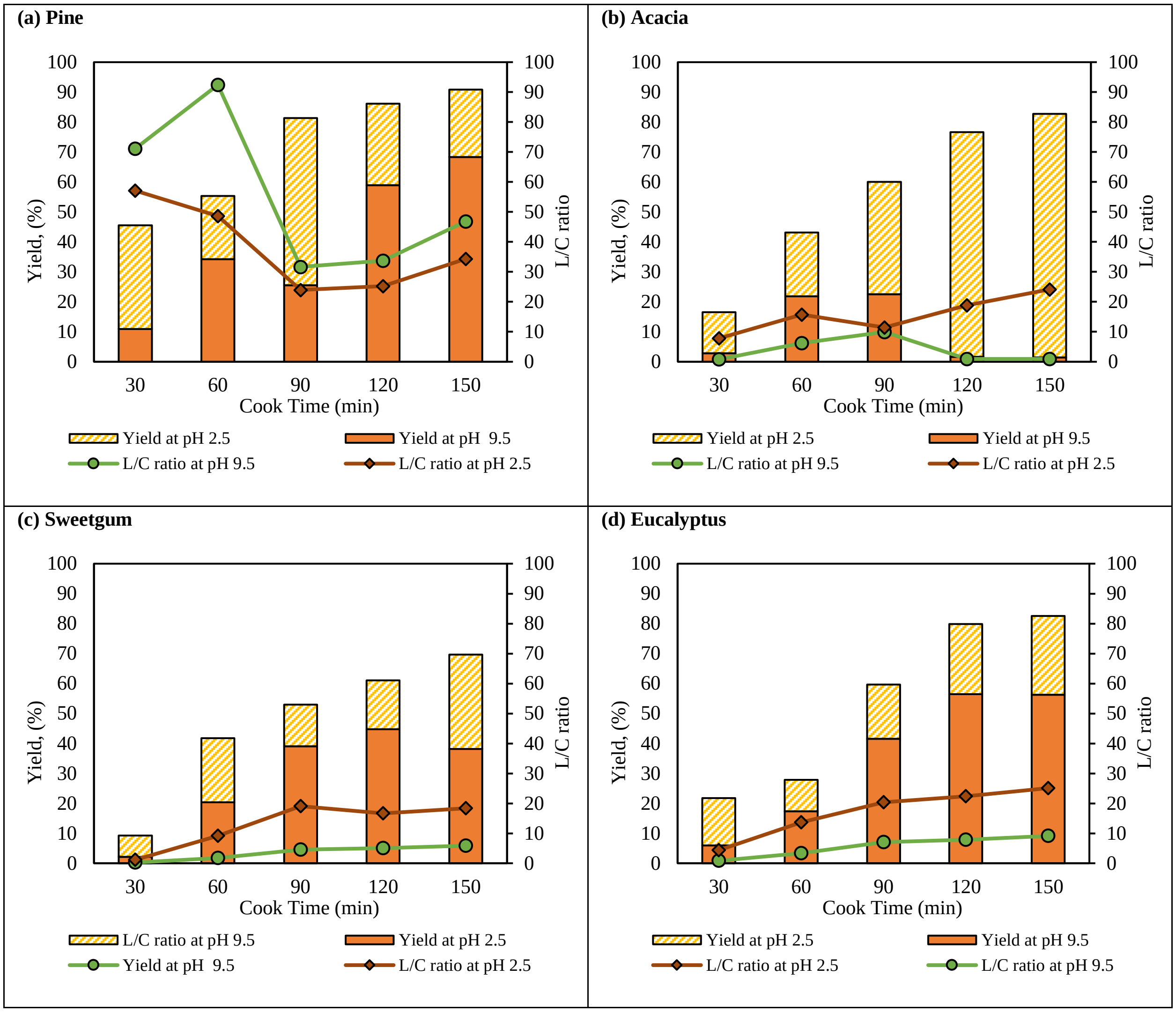

Figure 5.3. Lignin precipitations yield and L/C ratio as a function of pulping level for (a) pine, (b) acacia, (c) sweetgum, and (d) eucalyptus. Where L/C is the lignin-to-carbohydrate ratio in the solids precipitated.

The physical interactions between lignin and carbohydrates in the cell walls have not been fully understood. However, it has been shown that the number of physical contacts with carbohydrates correlates with the number of methoxyl groups in lignin and decreases in the order: S > G > H (Kang et al. 2019). Thus, this physical contact is more significant in hardwoods than softwoods that are composed of primarily G units.

Another investigation showed that S/G ratio and lignin content have influence on LCCs content (Min et al. 2014b). The research revealed that for samples with similar S/G ratio, the level of LCCs increased with increasing lignin content, and for samples with similar lignin contents, the level of LCCs increased with increasing S/G ratio. In the present investigation, it was not observed a clear relationship between L/C ratio and lignin content nor S/G ratio.

Finally, acacia lignins showed a unique behavior over the precipitation experiments than all the other three species herein investigated. For this species, very little amounts of lignin were precipitated at pH 9.5 at high pulping levels (120 and 150 minutes), whereas for the other species most of the lignin was obtained at this pH. The explanation for such discrepancy cannot yet be determined. Thus, a more complex characterization of the chemical structure of these lignins is recommended.

Additionally, an improved elucidation via determination of molecular weight distribution and characterization of functional groups and other chemicals structures would greatly contribute to understanding of this biomaterial and hence amplify the utilization as a green material. For instance, the linkage or association between lignin and carbohydrates should also be further investigated. Even though the LCC are suggested to play an important role in recalcitrance during biomass processing and fractionation (Giummarella et al. 2019), the LCCs have been associated with the expression of functionalities, such as antiviral, antioxidant, and antitumoral activities (Sakagami et al. 2005; Zhang et al. 2019). Such functionalities, however, are more likely due to the co-preservation of moieties of lignin and carbohydrates than to the LCC bonds themselves (Carvalho et al. 2020).

5.4.Conclusions

- Lignin content in the black liquor and the subsequent recovery increased with pulping level. In the initial phase, total lignin recovery ranged from ~ 9 to 55%, while in the later phase total lignin recovery varied from ~ 61 to 91%, depending on the species. The increased lignin recovery is due to the increasing in lignin concentration towards the end of the pulping process.
- Softwood showed higher lignin recovery and lower contamination with carbohydrates and ash than the hardwoods, regardless pH, and pulping level
- Hardwood lignin recovery was affected by S/G ratio, which resulted in higher yield at pH 9.5 with increasing S/G ratio
- Overall, more lignin was precipitated at pH 9.5 than at pH 2.5. However, lignin precipitation at pHs lower than 9.5 should be taken into consideration for samples that achieve low recovery yields. The precipitation of lignin fraction at pH 2.5 contributed with ~ 7 to 81% recovery increase depending on pulping level, S/G ratio, and species.
- Acacia lignins that showed the lowest S/G ratio amongst the hardwoods behaved very differently than the other species investigated, and the reasons are not yet fully understood. Further characterization of all lignins herein obtained will be performed using different spectroscopy and chromatography techniques, which will likely reveal the lignin characteristics that led to such discrepancies.

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6. Chapter 6. The effect of kraft pulping and wood species on the composition and chemical structure of technical lignins

Abstract

Technical lignins, *i.e.*, lignins isolated from biomasses during chemical and mechanical processes, have attracted great interest in the last several decades as a bio-based aromatic polymer feedstock (*e.g.*, polystyrene). The concept of a "biorefinery" is therefore an important driving force for such interest, where diverse products are obtained from renewable materials. Kraft lignins, a type of technical lignin, are relatively cheap and can become more widely available; thus, it should be investigated as a feedstock for green materials. The present study is part of a thorough investigation on lignin precipitation, composition, and properties of kraft lignins obtained from pine, acacia, sweetgum, and eucalyptus black liquors taken from different pulping levels. This effort investigated the impact of kraft pulping and wood species upon the chemical structure and composition of kraft lignins, aiming at determining the differences observed between the precipitation of hardwood and softwood lignins. The combination of less carbohydrate contamination, less LCCs, higher molecular weight, and high phenolic OH content explain the higher precipitation yields observed for pine lignin samples when compared to hardwoods. Among the hardwoods kraft lignins (HWKL), the S/G ratio of the native lignin was shown to play an important role on the characteristics of generated kraft lignins. Phenolic OH, methoxyl, and sulfur contents in HWKL increased with increasing S/G ratio, whereas molecular weight, carbohydrates contamination, and C/O ratio decreased. In addition, increasing pulping level was shown to increase the phenolic OH content, decrease the lignin interunit frequency, and increase the total lignin recovery yield. In summary, the kraft lignins generated in this study showed very distinct

characteristics and different behaviors during lignin precipitation; hence, it can be surmised that they will show different performances for lignin valorization.

6.1. Introduction

Lignins represent ~ 30% of terrestrial carbon in the biosphere which is the largest source of renewable aromatic carbon (Boerjan et al. 2003). Thus, it has attracted great attention over the last several decades given the increase of awareness and interest in sustainability. Hence, lignin-based materials industry's revenue is forecasted to rise from USD $730 million in 2018 to ~ USD $1 billion by 2025, which can be translated to an increase at a rate of 5.3% per year (Global Market Insights 2019).

The phenolic structure of lignin provides useful properties such as antioxidant ability, thermal stability, favorable brittleness, and high carbon content, which represent great potential in various fields of material science (Ragauskas et al. 2014). Therefore, it has been largely investigated as a renewable feedstock capable of completely or partially replacing petrochemical polymeric materials such as polystyrene due to their high aromatic nuclei content. Technical lignins can be obtained via any lignocellulosic processing and have been evaluated in such studies. For instance, kraft lignin (KL) is a technical lignin that represents a byproduct from kraft pulping, the dominant pulping process applied across the globe. KL is a highly abundant aromatic feedstock; however, it is still treated primarily as a source of heat and power, a default lower value application, which is attributed to its structural complexity, chemical recalcitrance, and dependency on feedstock and isolation process (Constant et al. 2016). It is important to mention that native lignin undergoes degradation and condensation reactions during the kraft process resulting in significant structure and composition modifications, and solubilization in the black liquor. Thus, it differs dramatically in properties from the native lignins found in plants (Berlin and Balakshin 2014).

A fundamental understanding of technical lignins is crucial for their post-processing and utilization in high-value applications because structural and composition studies will reveal critical data on lignin properties and their reactivity. This information enables processes optimization and hence allows for improvement of lignin as a feedstock and final products. For instance, information on residual sugars and ash is important as they may be detrimental for lignin valorization. The former could have an effect on lignin reactivity while the latter is undesirable due to accumulation of non-process minerals in the process and subsequent contamination and buildup (*e.g.*, scaling) on process equipment (*e.g.*, heat transfer systems) (Constant et al. 2016). In addition, molecular weight and functionalities will determine the reactivity of lignins during kraft pulping and lignin utilization.

There are two main types of kraft lignin readily available, softwood kraft lignin (SWKL) and hardwood kraft lignin (HWKL). Hardwood native lignins are composed of syringylpropane (S) and guaiacylpropane (G) units, whereas softwoods lignins are made up of G units and small levels of *p*-hydroxyphenylpropane (H) units. Therefore, hardwood and softwood lignins are highly different in their subunit compositions and as a consequence the interunit linkages and their subsequent reactivities also differ (Zhao et al. 2020). Monomer composition also affects molecular structure; for instance, in terms of branching of the polymer and the extent of cross-linking with the polysaccharide fraction (Constant et al. 2016).

Most of the comprehensive studies on kraft lignin structure and properties focuses on SWKL. Moreover, it has been shown that BCL and Indulin AT, two commercial SWKL, are structurally very similar, in spite of being produced under very different process conditions (Hu et al. 2016). This can be attributed to low variations within lignins from softwoods. On the other hand, hardwood lignins vary greatly among species (Santos et al. 2012); thus, it can be inferred that technical lignins obtained from these species also vary greatly.

Lignin isolation investigations have demonstrated that the softwood achieves higher yields and purities than hardwood; however, the reasons leading to these differences are not yet fully understood. Thus, the present work investigated the molecular features of pine, acacia, sweetgum, and eucalyptus kraft lignins, aiming at unraveling the chemical basis for these aforementioned discrepancies. These species were chosen based on their differences in the S/G ratio. Pine lignins were studied for comparison purposes because the precipitation and properties of SWKL are relatively well known. Acacia, sweetgum, and eucalyptus were chosen because they are industrially relevant hardwoods with very distinct S/G ratios, namely, 1.0, 2.3, and 3.6, respectively. Furthermore, the S/G ratio is known to greatly affect delignification during the pulping process (Santos et al. 2011); thus, it is conjectured that this parameter will also affect lignin precipitation and lignin properties.

6.2. Experimental

6.2.1. Materials

Pine (*Pinus taeda*), acacia (*Acacia mangium*), sweetgum (*Liquidambar styraciflua*), and eucalyptus (*Eucalyptus globulus*) lignin fractions precipitated from black liquor at different pHs and extents of the kraft pulping were studied. Lignin fractions at pH 9.5 and 2.5 were obtained after 30, 60, 90, 120 and 150 minutes of pulping. Thus, a total of 10 samples of each raw material were evaluated. Lignin recovery yield and composition of these lignin fractions are shown in Table 6.1. Pulping and precipitation conditions, as well as detailed discussion on lignin precipitation behavior have been reported in our previous study (Jardim et al. 2020).

Table 6.1. Lignin recovery yield and chemical composition of pine, acacia, sweetgum, and eucalyptus kraft lignins

Species	Cook time, min	pH	Yield, %	Lignin, %	Glu, %	Xyl, %	Gal, %	M+A, %	Ash, %
Pine	30	9.5	11.0	94.8	0.7	0.6	1.1	0.9	1.8
	60	9.5	34.3	95.7	0.2	0.9	1.1	0.9	1.3
	90	9.5	25.6	92.7	0.2	3.0	1.1	0.9	2.1
	120	9.5	58.9	93.0	0.2	2.5	1.1	0.9	2.2
	150	9.5	68.3	93.5	0.2	1.8	1.1	0.9	2.5
	30	2.5	34.6	94.4	0.5	1.1	1.2	0.9	1.8
	60	2.5	21.1	94.8	0.3	1.6	1.1	0.9	1.2
	90	2.5	55.8	92.5	0.3	3.6	1.2	1.0	1.5
	120	2.5	27.3	92.9	0.3	3.4	1.2	0.9	1.3
	150	2.5	22.6	94.2	0.3	2.4	1.1	0.9	1.1
Acacia	30	9.5	2.9	43.6	1.6	51.5	0.9	1.2	1.2
	60	9.5	21.9	85.7	0.5	11.3	1.1	0.7	0.7
	90	9.5	22.6	86.5	0.3	6.9	0.9	0.6	4.8
	120	9.5	1.7	48.3	4.2	45.0	1.2	0.8	0.5
	150	9.5	1.5	49.0	4.3	43.6	1.3	0.8	1.0
	30	2.5	13.7	88.1	0.5	9.1	1.1	0.6	0.7
	60	2.5	21.3	92.6	0.4	3.9	1.1	0.5	1.6
	90	2.5	37.4	91.2	0.3	6.0	1.2	0.5	0.8
	120	2.5	75.0	94.1	0.2	3.0	1.2	0.6	0.9
	150	2.5	81.2	95.5	0.2	2.5	0.9	0.4	0.5
Sweetgum	30	9.5	2.1	18.8	16.5	58.4	1.6	1.4	3.2
	60	9.5	20.3	60.7	10.1	24.0	1.1	1.3	2.8
	90	9.5	39.1	77.5	5.7	11.4	1.3	1.6	2.6
	120	9.5	44.7	79.7	5.5	10.3	1.1	1.2	2.3
	150	9.5	38.1	80.9	4.4	9.5	1.1	1.4	2.8
	30	2.5	7.2	50.9	7.7	37.2	1.6	1.4	1.3
	60	2.5	21.4	86.9	1.3	8.3	1.2	1.1	1.2
	90	2.5	13.9	91.3	0.6	4.2	1.3	1.5	1.0
	120	2.5	16.4	91.4	0.6	5.0	1.0	1.1	0.9
	150	2.5	31.6	91.4	0.5	4.5	1.1	1.2	1.3
Eucalyptus	30	9.5	6.0	45.3	3.3	49.8	0.0	0.0	1.6
	60	9.5	17.3	75.8	1.3	19.2	1.2	1.1	1.5
	90	9.5	41.5	85.9	0.4	10.2	0.9	0.7	1.9
	120	9.5	56.4	87.3	0.5	9.0	0.9	0.7	1.6
	150	9.5	56.2	88.4	0.6	7.5	0.9	0.6	1.9
	30	2.5	15.8	77.6	0.9	19.9	0.8	0.5	0.2
	60	2.5	10.5	91.0	0.6	7.0	0.7	0.5	0.2
	90	2.5	18.1	92.8	0.3	5.1	0.9	0.7	0.2
	120	2.5	23.5	93.2	0.3	4.8	0.7	0.6	0.4
	150	2.5	26.3	93.8	0.3	4.3	0.7	0.5	0.3

6.2.3. Methods

Elucidation of the chemical structure and properties of the lignin fractions was performed by different techniques because there is not a single technique that can provide adequate quantitative and qualitative information. It is valid to mention that among the variety of available analytical techniques, results can vary greatly among different studies depending upon the parameters and experimental conditions applied.

6.2.3.1. Gel Permeation Chromatography (GPC)

GPC was used to determine the number average molecular weight (M_n) and mass average molecular weight (M_w). Lignin samples were acetobrominated before analysis to achieve total solubility in tetrahydrofuran (THF), the mobile phase of choice (Asikkala et al. 2012). For the acetobromination, ~10 mg of lignin was weighed into a reaction vessel, after which 2.3 mL of glacial (anhydrous) acetic acid was added. The reaction mixture was shaken gently and allowed to react for 1 h at room temperature. Finally, 0.25 mL (3.38 mmol) of acetyl bromide was added and the mixture was held at room temperature, but frequently stirred for 2 h. Acetic acid and excess acetyl bromide were evaporated in a rotary evaporator followed by vacuum drying. The acetobrominated lignins were dissolved into THF at a concentration of 1 mg/mL and filtered through 0.2 μm nylon filters. The experiments were carried out using 1260 Infinity II LC System, a robust high performance liquid chromatography, equipped with UV (set at 280 nm) and RI detectors, two Waters Styragel™ columns linked in series (Styragel™ HR 1 7.8 × 300 mm and Styragel™ HR 5E 7.8 × 300 mm); at a flow rate of 0.7 mL/min at 30 °C. A series of monodisperse polystyrene standards were used as the calibration standards.

6.2.3.2. Elemental Analysis

Contents of carbon (C), hydrogen (H), nitrogen (N), oxygen (O), and sulfur (S) were determined by an atomic analyzer by Atlantic Microlab, in Norcross-GA. The oxygen content was calculated by difference and the values were corrected taking into consideration the carbohydrates content.

6.2.3.3. Catechol group

The amount of catechol groups was determined using a calorimetric method (Hu et al. 2016). Catechol and kraft lignin form a complex with iron (II) sulfate in the presence of tartrate at pH 8.1 and the complex has a blue color. 1 mL of sodium sulfite solution (5 mM), 1 mL of dimethylsulfoxide solution of kraft lignin (~ 0.05 M), 1 mL iron (II) tartrate solution (5 mM) and 2 mL of sodium phosphate buffer was added to a 10 ml volumetric flask and filled with water. The deionized water used to prepare all solutions was degassed before used and all processes was carefully exercised to avoid the presence of oxygen in the solutions. A lignin solution containing all chemicals except the iron (II) tartrate was used as a blank at the visible absorption spectrum. The catechol amount was estimated based on the difference in absorbance at 560 nm between equivalent concentration of lignin solution and catechol, pyrogallol-1-methyl ether, p-methylcatechol and 3,5-dimethylcatechol solutions. The UV spectra was recorded on a HP8453E UV−vis spectrophotometer (Hewlett-Packard Company, Palo Alto City, CA, USA) (Jiang et al. 2017).

6.2.3.4. Chemical structure and functional groups

The lignin chemical structure and functional groups were accessed via different NMR techniques. Data processing and analyzes were performed using Bruker Topspin 4.1.1 software.

^{31}P NMR

^{31}P-NMR analysis was conducted to determine the hydroxyl content following a reported method (Argyropoulos 1995; Balakshin and Capanema 2015; Granata and Argyropoulos 1995). Specifically, ~30 mg of lignin was completely dissolved in 500 µL of freshly prepared mixture of deuterated pyridine and chloroform (1.6:1, v/v). Subsequently, 100 µl of a solution with internal standard (N-hydroxy-5-norbornene-2,3-dicarboximide) and relaxation reagent (chromium acetylacetonate) was added and shaken vigorously. Finally, 100 μl of phosphitylating reagent (2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphopholane) was added to the mixture, shaken vigorously, and transferred into an NMR tube, and submitted for NMR acquisition. The ^{31}P-NMR spectrum was acquired using a Bruker 500 MHz spectrometer with a Quad probe.

^{13}C NMR and 2D HSQC NMR

^{13}C-NMR spectra were acquired on a 500 MHz Bruker Avance III NMR spectrometer, at 25 °C, using deuterated dimethyl sulfoxide (d6-DMSO) (99.9%, w/1%TMS) as the solvent. 0.016M Chromium (III) acetylacetonate (99.99%, Sigma– Aldrich, USA) was added as relaxation agent. The final lignin concentration was ~200 mg/mL. The experiments were performed on a 5mm BBO probe with Z-axis gradient and an inverse-gated proton de-coupling sequence was applied. Spectral acquisition time was of 1.1 s and relaxation delay of 2.0 s with 27648 scans. 2D HSQC NMR spectra were recorded on a Bruker Avance 700 MHz spectrometer equipped with a LHe Cryoprobe and the program selected was "hsqcedetgpsisp 2". Lignin samples were dissolved in d6-DMSO on a concentration of 200 mg/mL. Conditions for the HSQC analysis were as follows: at 25 °C, 2048 data points in F2 and 512 data points in F1 with 32 scans. This work was performed in part by the Molecular Education, Technology and Research Innovation Center (METRIC) at NC State University, which is supported by the State of North Carolina. Data processing was performed using Bruker Topspin-NMR software. The d6-DMSO peak was used as an internal chemical shift reference point (δC/δH 39.5/2.5).

The quantification of lignin structures was performed by combining ^{13}C and 2D HSQC NMR as follows:

$$X = \left(\frac{2D_X}{2D_{cluster}}\right) * \left(\frac{^{13}C_{cluster}}{^{13}C_{163-103}}\right) * A \tag{6.1}$$

where X is the substructure of interest; $2D_X$ is the integration of the signal of substructure X in 2D HSQC spectra; $2D_{cluster}$ is the integration of the whole region of the signal of substructure X in 2D HSQC spectra; $^{13}C_{cluster}$ and $^{13}C_{163-103}$ are the integration of the corresponding clusters in ^{13}C spectra; and A is the number of aromatic carbons in 100Ar or 100 monomeric lignin units (see discussion).

HSQC cross-signals of lignin and the associated carbohydrates were assigned by comparison with the literature (Balakshin et al. 2003; Constant et al. 2016; Giummarella et al. 2020; Jiang et al. 2020; Lancefield et al. 2018; Liitiä et al. 2003; Yuan et al. 2011; Zhao et al. 2020). The main lignin cross- signals are listed in Table 6.2.

Table 6.2. Chemical shift assignment of lignin substructures

Aliphatic oxygenated side chain (δC/δC 90-40/6.0-2.5)				**Aromatic region** (δC/δC 150-90/8.0-6.0)			
Label	Annotation	δC [ppm]	δH [ppm]	Label	Annotation	δC [ppm]	δH [ppm]
C'_β	Cβ-Hβ in β-β'	48.6/49.1	3.16/3.35	$S_{2,6}$	$C_{2,6}$-$H_{2,6}$ in S unit	101-108	6.06-7.43
B_β	Cβ-Hβ in β-5	53.1	3.49				
C_β	Cβ-Hβ in β-β	53.5	3.08	G_2	C_2-H_2 in G unit	109-113.5	6.62-7.55
C'_β	Cβ-Hβ in β-β'	54.2	2.79				
OMe	Methoxyl	55.7	3.76	G_5	C_5-H_5 in G unit	113.9/116.4	6.52/7.16
Aγ	Cγ-Hγ in β-O-4	59.5	3.43/3.74				
CAγ	Cγ-Hγ in cinnamyl alcohol	61.4	4.12	G_6	C_6-H_6 in G unit	117-122	6.5-7.1
Bγ	Cγ-Hγ in β-5	62.2	3.79				
C'γ	Cγ-Hγ in β-β'	69.0/69.6	2.80/4.16	V_6	C_6-H_6 in vanillin	123.3	7.60
Cγ	Cγ-Hγ in β-β	71.1	3.85/4.21				
Aα	Cα-Hα in β-O-4	71.9	4.89	SBα	Cα-Hα in stilbene	125.6	6.97
C'α	Cα-Hα in β-β'	81.3	4.76				
Cα	Cα-Hα in β-β	84.9	4.68	CA_β	C_β-H_β in cinnamyl alcohol	128.0	6.18
A_β	C_β-Hβ in β-O-4 in S units	85.8	4.15				
C'α	Cα-Hα in β-β'	87.2	4.35	CAα	Cα-Hα in cinnamyl alcohol	129.5	6.41
Bα	Cα-Hα in β-5	86.8	5.49				

The assignments of the carbohydrate and lignin-carbohydrate linkages cross- signals are listed in Table 6.3.

Table 6.3. Chemical shift assignment of LCCS and carbohydrates associated with lignin.

Label	Annotation	δC [ppm]	δH [ppm]
ARA_5	C_5-H_5 in α-L-arabinoside		
EST	γ-esters	62-65	4.0-4.5
X_5	C_5-H_5 in β-D-xylopyranoside	63.2	3.24/3.83
X_5	C_5-H_5 in β-D-xylopyranoside non-reducing end	65.9	3.71/3.09
MGU	4-*O*-methyl-α-D-Glc-UA	69.3	4.72
X_2	C_2-H_2 in β-D-xylopyranoside	72.8	3.16
X_3	C_3-H_3 in β-D-xylopyranoside	73.7	3.22
X_4	C_4-H_4 in β-D-xylopyranoside	75.4	3.60
MGU	4-*O*-methyl-α-D-Glc-UA	81.1	3.10
BE	C_α-H_α in benzyl ether LCC	81.3	4.65/5.07
MGU	4-*O*-methyl-α-D-Glc-UA	97.2	5.18
X_1	C_1-H_1 in β-D-xylopyranoside		
PhGly	Phenyl glycoside linkages	104-99	4.8-5.2

6.3.Results and Discussion

This effort used wet chemistry, chromatography, and spectroscopy techniques to evaluate the impact of kraft pulping with respect to wood species on the chemical structure and properties of kraft lignins. During pulping, lignin is both degraded and condensed to be a more complex polymer than the native lignin through the following reactions typical reactions: hydrolysis of α-ethers; cleavage of β-aryl ether linkage in the phenolic unit; demethylation reaction and formation of catechol; formation of vinyl ethers and stilbene via γ-elimination of primary alcohol in the phenolic units; and condensation reaction at the C5 position in the aromatic ring (Marton 1971). The following analyses will highlight such degradation reactions and modifications as they pertain to our study.

6.3.1. Molecular weight (MW)

The evaluation of MW is critical for proper lignin characterization and utilization because it influences the kinetics of lignin reactivity and its resultant physico-chemical properties (Baumberger et al. 2007). The weight average molecular weight (M_w), number average molecular weight (M_n), and polydispersity index (PDI) of acetobrominated lignin fractions are shown in Table 6.4. In addition, the molecular weight distribution of lignin samples obtained after 150 minutes of pulping are depicted in Figure 6.1.

Table 6.4. M_w, M_n, and PDI of acetobrominated pine, acacia, sweetgum, eucalyptus kraft lignins

Species	Cook time, min	pH	M_w, g/mol	M_n, g/mol	PDI
Pine	30	9.5	7607	996	7.6
	60	9.5	7346	1126	6.5
	90	9.5	6147	1260	4.9
	120	9.5	5826	1318	4.4
	150	9.5	5290	1224	4.3
	30	2.5	5353	1279	4.2
	60	2.5	5413	1413	3.8
	90	2.5	4896	1286	3.8
	120	2.5	5732	1235	4.6
	150	2.5	4414	1125	3.9
Acacia	30	9.5	3267	937	3.5
	60	9.5	3431	1093	3.1
	90	9.5	3495	1044	3.3
	120	9.5	3528	934	3.8
	150	9.5	3493	923	3.8
	30	2.5	2598	818	3.2
	60	2.5	2945	1004	2.9
	90	2.5	3070	999	3.1
	120	2.5	3935	1069	3.7
	150	2.5	3536	1008	3.5
Sweetgum	30	9.5	1954	650	3.0
	60	9.5	2284	802	2.8
	90	9.5	2430	875	2.8
	120	9.5	2597	876	2.9
	150	9.5	2599	843	3.1
	30	2.5	1990	551	3.6
	60	2.5	2185	821	2.7
	90	2.5	3186	899	3.5
	120	2.5	2579	873	2.9
	150	2.5	2337	801	2.9
Eucalyptus	30	9.5	2193	2193	3.7
	60	9.5	2031	2031	2.5
	90	9.5	1898	1898	2.4
	120	9.5	1792	1792	2.2
	150	9.5	1843	1843	2.2
	30	2.5	1711	1711	2.8
	60	2.5	1650	1650	2.1
	90	2.5	1559	1559	2.0
	120	2.5	1556	1556	2.0
	150	2.5	1600	1600	2.0

It was clear that the extent of pulping affected the molecular weight of the kraft lignins. The MW of pine and eucalyptus fractions, obtained at both pHs, decreased with increasing pulping level, whereas the MW of acacia and sweetgum, obtained at both pHs, slightly increased with increasing pulping intensity. The decrease in MW can be explained by lignin degradation and fragmentation throughout the pulping process. On the other hand, the slight increase in molecular weight could be attributed to condensation reactions. Thus, MW is dependent upon the balance between degradation and condensation reactions, which depends on pulping conditions and wood species. Moreover, the slight increase in the MWs for the kraft lignins from the acacia and sweetgum samples was not expected although we determined that the level of carbohydrate contamination in these samples may have influenced the extent of degradation.

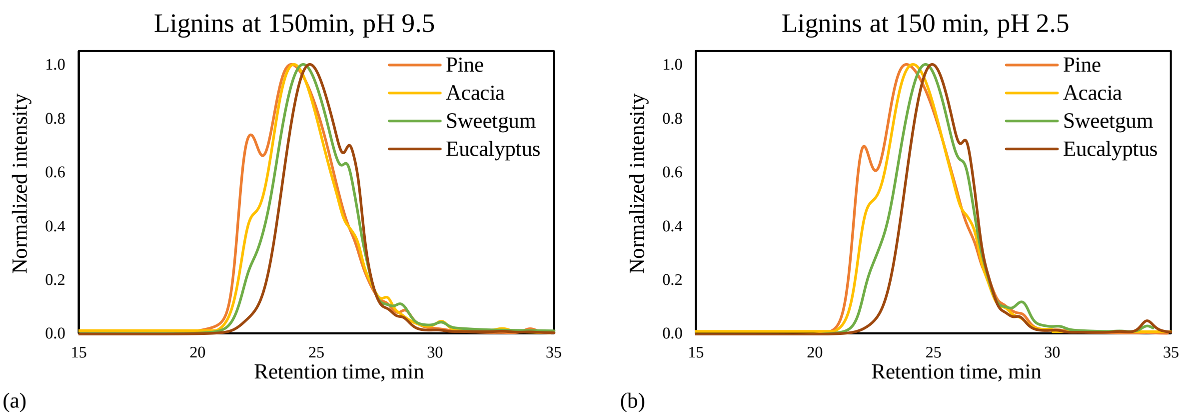


Figure 6.1. Molecular weight distribution of lignins obtained after 150 minutes at (a) pH 9.5 and (b) pH 2.5.

Overall, the MW of lignins precipitated at pH 9.5 was greater than lignins precipitated at pH 2.5, which is in agreement with studies of gradient acid precipitation (García et al. 2009; Kumar et al. 2016; Santos et al. 2014). This difference is because the balance of attractive and repulsive forces leads to these observations. Larger fragments are more easily destabilized when negative charges are neutralized by acidification (H^+) relative to smaller fragments; thus, larger fragments are more easily precipitated. There was no straightforward relationship between MW (Table 6.4) and lignin recovery yield (Table 6.1); thus, it can be inferred that MW is not the only lignin parameter affecting precipitation.

The MW of kraft lignin was highly affected by wood species and it varied as follows: pine lignin fragments were larger than acacia lignin fragments followed by sweetgum and eucalyptus. This trend was observed for lignin fractions obtained at both pHs. Therefore, it can be speculated that S/G ratio of lignin inversely affects MW, as the S/G ratio increases in this order: pine > acacia > sweetgum > eucalyptus. Therefore, the wood species with highest S/G ratio (eucalyptus) generated the smallest kraft lignin fragments (M_w =1556 - 2193 g/mol). This may be a reflection of the intrinsic (native) MW found in the wood and/or the lowest level of branching found in the highest S/G ratio wood (Dumitrache et al. 2016). In a more linear lignin polymer, the ease of MW loss is much greater than in a more branched lignin polymer such as found in pine.

A fractionation study demonstrated that smaller molecular weight lignins show higher methoxyl content and higher S/G ratio (Chen et al. 2020), which agrees with the trend observed in the present study. In addition, S/G ratio affects the abundance of β-O-4 linkages and hence the performance during the kraft process (Santos et al. 2011). Typically, samples with higher S/G ratio contain more β-O-4 linkages; thus, are easier to pulp. Therefore, it can be expected that technical lignins obtained from species with high S/G ratio will be much more susceptible to degradation and thus exhibit lower molecular weights.

Technical lignins are a mixture of lignin fragments of different molecular weights and polydispersities. M_w values varying from 500 to 150,000 and PDI values varying from 1.5 to 11.0 have been reported (Vishtal and Kraslawski 2011). Wood species, analysis method, and isolation procedure lead to such wide range of molecular weights and PDI. In addition, it has been confirmed that the chemical structure, thermal properties, and the contents of certain functional groups vary to some degree with molecular weight (Helander et al. 2013; Jiang et al. 2017; Yuan et al. 2009). Thus, given the significant variation in MW among the lignin evaluated in this study, it can be anticipated that they will show very different characteristics.

Finally, it is important to mention that the MW results presented for pine and sweetgum lignins slightly differ from our previous report (Jardim et al. 2021) that discussed Flory-Stockmayer Theory. This theory has been used to describe lignin polymerization and degradation. According to the theory, smaller lignin fragments should be obtained at the onset of the pulping process. As pulping progresses, the molecular weight should peak and subsequently decrease. In the present investigation this behavior was not obvious, which might be due to how the sampling was performed and pulping conditions applied. It is possible that this MW profile would be more evident if lignin samples were precipitated at more pulping levels and if different pulping conditions were applied. Furthermore, the differences between the results shown in the present and previous investigation can be attributed to differences in sample preparation and instrument set up. For this investigation, a different GPC was used, and the lignin samples were acetobrominated. This derivatization process is not as commonly used for determining the molecular weight of lignin as acetylation; however, it was a tradeoff we accepted in our study because it is much more useful for samples highly contaminated with carbohydrates.

6.3.2. Hydroxyl groups

The ^{31}P-NMR spectra for lignin samples obtained after 150 minutes of pulping, at pH 9.5, are shown in Figure 6.2, where the assignment of phosphitylating reagent (2-chloro-4,4,5,5-tetramethyl-1,3-2-dioxaphospholane - TMDP), internal standard (N-hydroxy-5-norbornene-2,3-dicarboximide - IS), aliphatic OH, phenolic OH, carboxylic OH, and TMDP + H_2O have been indicated.

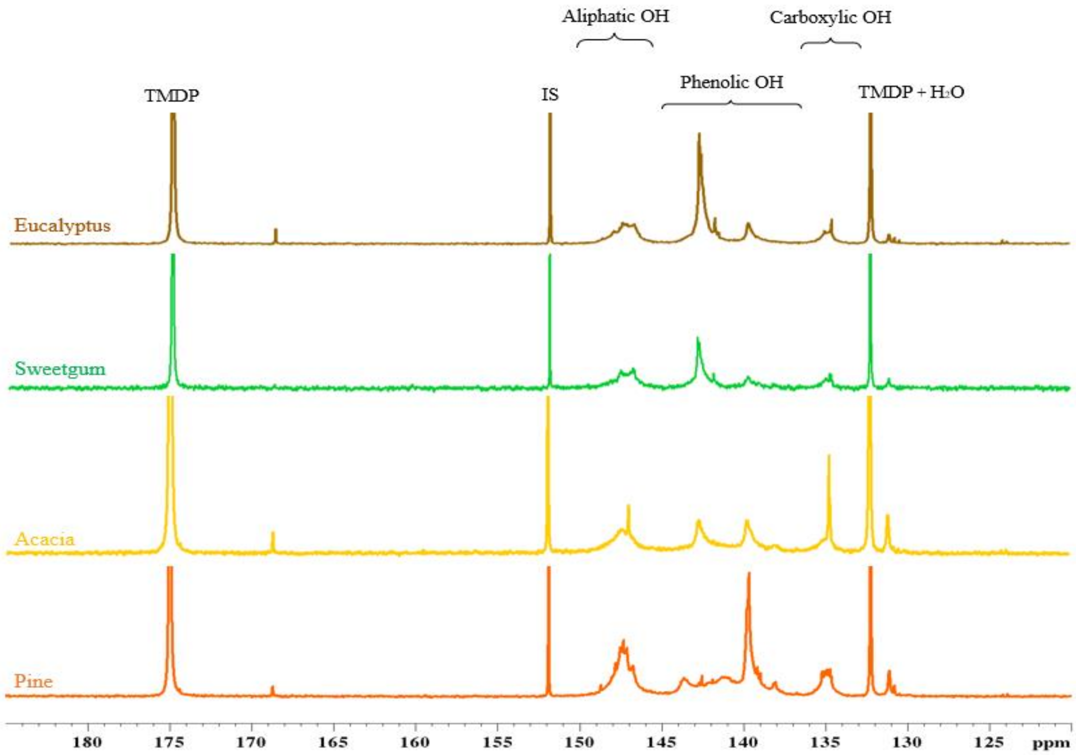


Figure 6.2. ^{31}P NMR spectra of pine, acacia, sweetgum, and eucalyptus lignins obtained after 150 minutes of pulping at pH 9.5.

The contents of various types of hydroxyl groups are shown in Table 6.5. Note that there was not a differentiation of various C_5-substituted phenolics because this is not appropriate for hardwood lignins (S/G- type). It has been shown that ~ 30% of condensed G-units are found in the region assigned to S-units; therefore, S-units would be overestimated and, correspondingly, G-condensed moieties would be underestimated (Balakshin and Capanema 2015). Also, hydroxyl contents of the sweetgum lignin obtained at 30 minutes, pH 9.5, could not be quantified because this lignin was extremely contaminated with carbohydrates and hence did not fully dissolve during sample preparation. It is important to mention that no further purification steps were applied because this investigation intended to evaluate all lignin fractions "as is", *i.e.*, modifying their structures as little as possible.

Table 6.5. Hydroxyl groups quantified by ^{31}P NMR expressed in mmol of functional group per g o.d. lignin*

Species	Cook time, min	pH	Aliphatic OH	C_5-substituted PhOH	C_5-free PhOH	Total PhOH	COOH
Pine	30	9.5	2.97	0.31	1.30	1.61	0.48
	60	9.5	2.52	0.65	1.68	2.33	0.40
	90	9.5	2.55	1.06	1.92	2.98	0.62
	120	9.5	2.21	1.19	2.02	3.21	0.66
	150	9.5	1.88	1.57	2.24	3.81	0.57
	30	2.5	0.22	0.92	0.28	1.20	1.19
	60	2.5	1.64	0.86	1.85	2.71	1.23
	90	2.5	2.37	1.03	1.92	2.94	0.82
	120	2.5	2.19	1.22	2.08	3.31	0.92
	150	2.5	1.53	1.49	2.09	3.58	0.88
Acacia	30	9.5	4.59	0.71	0.98	1.69	0.87
	60	9.5	2.91	1.10	1.42	2.52	0.82
	90	9.5	2.40	1.75	1.56	3.31	1.08
	120	9.5	1.61	1.19	0.81	1.99	0.98
	150	9.5	1.16	1.20	0.89	2.09	0.70
	30	2.5	0.74	0.24	0.93	1.17	0.89
	60	2.5	1.67	1.34	1.92	3.27	1.38
	90	2.5	2.19	1.75	1.20	2.95	1.20
	120	2.5	2.25	2.19	1.84	4.03	1.23
	150	2.5	1.96	2.34	1.91	4.25	1.23
Sweetgum	30	9.5	-	-	-	-	-
	60	9.5	2.07	1.28	0.55	1.83	0.06
	90	9.5	1.97	2.36	0.65	3.01	0.24
	120	9.5	1.73	2.40	0.64	3.04	0.29
	150	9.5	1.95	2.42	0.62	3.03	0.40
	30	2.5	0.26	0.48	0.63	1.11	0.30
	60	2.5	2.65	2.23	0.74	2.97	0.73
	90	2.5	1.42	2.65	0.66	3.31	0.84
	120	2.5	1.04	2.75	0.65	3.40	0.87
	150	2.5	1.36	2.36	0.56	2.92	0.64
Eucalyptus	30	9.5	15.04	0.57	0.64	1.21	0.51
	60	9.5	3.84	1.82	0.76	2.59	0.28
	90	9.5	2.26	2.55	0.69	3.24	0.31
	120	9.5	1.56	2.89	0.68	3.57	0.24
	150	9.5	1.29	3.23	0.72	3.96	0.31
	30	2.5	0.55	0.31	0.22	0.53	0.07
	60	2.5	3.44	2.71	0.97	3.68	1.31
	90	2.5	2.61	3.66	1.23	4.89	1.28
	120	2.5	1.38	3.78	0.78	4.56	1.08
	150	2.5	1.51	3.47	0.72	4.20	1.03

* Corrected for lignin purity

The aliphatic hydroxyl content decreased with increasing pulping level, which is in agreement with the literature (Hu et al. 2016). This decrease is suggestive of depolymerization and it is mainly due to the formation of the quinone methide intermediate and subsequent hydrolysis of the γ-hydroxylmethyl group as formaldehyde (Marton 1971). It is worth mentioning that carbohydrate hydroxyl groups also show signals at the region that is characterized as aliphatic OH in Fig. 6.2; thus, it is possible that purified samples would give different results. To address such interferences, lignin purity was taken into consideration in the calculation of the various hydroxyl groups in lignin.

The ^{31}P-NMR also allows for the quantification of phenolic OH, which is a critical functionality as it affects lignin's overall reactivity and physical-chemical properties (Meng et al. 2019). Moreover, free phenolic groups content reveals the extent of delignification because the cleavage of alkyl–aryl ethers, such as β-O-4, generate new phenolic moieties (Pinto et al. 2015). The increase in phenolic OH with increasing pulping level was evident for all wood species.

It is noteworthy that total lignin recovery yield was affected by phenolic OH content. Overall, the yield increased with increasing phenolic OH content. It is crucial to point out that acacia lignins obtained at pH 9.5 after 120 and 150 minutes of pulping showed demonstrably lower phenolic OH contents (~ 2.0 mmol/g of lignin) than any other lignin obtained at this pH and pulping intensities, which led to extremely low lignin recovery yield. These two samples also showed high MW, which theoretically would lead to high precipitation yields. Therefore, the fact that high molecular weight could not overcome the low phenolic OH content accounts for the finding that phenolic OH is a determining factor in lignin precipitation.

It has been reported that total phenolic OH content in kraft lignin relates with the abundance of H, G, and S units (Pinto et al. 2015); however, such a straightforward relationship was not evident in our investigation. Overall, the content of C_5-substituted phenolic OH was higher than C_5-free for pine, whereas for the hardwoods, the opposite trend was noted. The information on the substitution at the C-5 position is rather valuable because it indicates lignin reactivity towards reactions that require an available *o*-position on the aromatic ring (such as sulfomethylation, PF resin synthesis) (Balakshin and Capanema 2015). Moreover, the content of phenolic OH was reported to be inversely proportional to the molecular mass of the lignin (Wallmo et al. 2009), which was also observed in our investigation.

Another type of hydroxyl group identified in lignin is carboxylic OH. Native lignin contains few or no carboxylic OHs; thus, this carbohydrate component is either covalently bonded as a lignin-carbohydrate complex, or adsorbed onto the lignin, or merely present together with the lignin material (Hubbe et al. 2019). In general, the content of carboxylic OH was greater at pH 2.5 than at pH 9.5, which was expected because the pKa of such groups in lignin vary from 3 to 5 (Alén 2011). Thus, when pH was further lowered to 2.5 the carboxylic OH groups were also protonated, enhancing lignin precipitation. Moreover, amongst lignins precipitated at pH 9.5, acacia lignins showed the highest contents of carboxylic OH, which makes them more water soluble and hence more difficult to precipitate. This result is further evidence for the low yields observed for acacia lignins at pH 9.5.

A study comparing different hardwood kraft lignins has shown that the content of aliphatic OH varies from 1.12 to 2.58 mmol/ g lignin; whereas the phenolic OH content varies from 3.99 to 4.34 mmol/ g lignin; and the carboxylic OH content varies from 0.59 to 0.85 mmol/ g lignin (Balakshin and Capanema 2015). The same study showed that the content of aliphatic, total phenolic, and carboxylic hydroxyl groups for pine kraft lignin is 1.85, 4.30, and 0.71, respectively, in mmol/g lignin, determined via ^{31}P-NMR.

6.3.3. Elemental composition and methoxy analyses

Elemental and methoxyl analyses were performed to determine the molecular formula for key lignin samples and the results are shown in Table 6.6.

Table 6.6. Elemental and methoxyl analyses for pine, acacia, sweetgum, and eucalyptus kraft lignins.

Species	Cook time	pH	OCH_3, %	C, %	H, %	O, %	N, %	S, %	C9 formula	Unit weight
Pine	60	9.5	12.9	63.5	5.8	27.8	0.2	2.8	$C_9H_{8.3}O_{2.4}(OCH_3)_{0.77}S_{0.2}$	179
	90	9.5	12.2	61.9	5.5	30.1	0.2	2.3	$C_9H_{8.2}O_{2.8}(OCH_3)_{0.75}S_{0.1}$	184
	150	9.5	13.0	62.3	5.7	29.8	0.1	2.2	$C_9H_{8.3}O_{2.7}(OCH_3)_{0.79}S_{0.1}$	184
	60	2.5	12.6	60.4	5.5	29.6	0.4	4.0	$C_9H_{8.4}O_{2.8}(OCH_3)_{0.79}S_{0.2}$	186
	90	2.5	12.0	60.0	5.5	31.2	0.3	3.0	$C_9H_{8.5}O_{3.0}(OCH_3)_{0.75}S_{0.2}$	189
	150	2.5	11.1	62.8	5.7	28.4	0.2	2.9	$C_9H_{8.5}O_{2.6}(OCH_3)_{0.66}S_{0.2}$	179
Acacia	60	9.5	21.4	60.4	5.7	31.3	0.4	2.3	$C_9H_{7.5}O_{2.6}(OCH_3)_{1.43}S_{0.1}$	202
	90	9.5	21.0	60.8	5.5	31.1	0.4	2.2	$C_9H_{7.1}O_{2.6}(OCH_3)_{1.39}S_{0.1}$	200
	150	9.5	20.5	54.6	6.4	36.0	0.6	2.4	$C_9H_{10.1}O_{3.7}(OCH_3)_{1.53}S_{0.2}$	224
	60	2.5	13.8	58.5	5.1	29.9	0.5	6.1	$C_9H_{7.6}O_{2.9}(OCH_3)_{0.90}S_{0.4}$	190
	90	2.5	23.1	60.2	5.5	31.0	0.4	2.8	$C_9H_{6.9}O_{2.5}(OCH_3)_{1.57}S_{0.2}$	204
	150	2.5	16.9	61.7	5.4	30.0	0.4	2.6	$C_9H_{7.3}O_{2.6}(OCH_3)_{1.07}S_{0.2}$	190
Sweetgum	60	9.5	15.1	52.8	5.6	38.0	0.4	3.3	$C_9H_{9.4}O_{4.3}(OCH_3)_{1.12}S_{0.2}$	222
	90	9.5	23.3	58.9	5.6	32.5	0.2	2.7	$C_9H_{7.3}O_{2.8}(OCH_3)_{1.62}S_{0.2}$	210
	150	9.5	22.1	59.1	5.5	32.6	0.3	2.6	$C_9H_{7.2}O_{2.8}(OCH_3)_{1.52}S_{0.2}$	208
	60	2.5	20.1	57.1	5.1	33.9	0.5	3.4	$C_9H_{6.9}O_{3.2}(OCH_3)_{1.42}S_{0.2}$	210
	90	2.5	20.6	61.0	5.9	29.6	0.5	3.1	$C_9H_{7.9}O_{2.4}(OCH_3)_{1.35}S_{0.2}$	197
	150	2.5	23.4	59.6	5.5	31.5	0.3	3.0	$C_9H_{7.0}O_{2.6}(OCH_3)_{1.61}S_{0.2}$	206
Eucalyptus	60	9.5	28.0	54.4	5.4	21.5	0.2	6.1	$C_9H_{7.8}O_{3.3}(OCH_3)_{1.63}S_{0.4}$	220
	90	9.5	30.3	57.6	5.7	26.5	0.2	2.6	$C_9H_{7.1}O_{2.9}(OCH_3)_{1.96}S_{0.2}$	222
	150	9.5	23.1	59.7	5.6	32.3	0.2	2.3	$C_9H_{7.1}O_{2.7}(OCH_3)_{1.59}S_{0.2}$	208
	60	2.5	24.8	55.0	5.3	30.6	0.3	8.8	$C_9H_{6.8}O_{2.6}(OCH_3)_{1.90}S_{0.7}$	216
	90	2.5	23.4	57.2	5.5	32.2	0.3	4.8	$C_9H_{7.3}O_{2.8}(OCH_3)_{1.69}S_{0.3}$	213
	150	2.5	23.3	59.7	5.4	29.7	0.2	4.9	$C_9H_{6.7}O_{2.4}(OCH_3)_{1.60}S_{0.3}$	202

Traditional ^{13}C and 2D NMR methods report the results as total number of functional groups per aromatic ring (Ar), which can be considered as mol%, whereas ^{31}P-NMR, and most wet chemistry methods originally provide results in mmol/g (or mass %) units. Thus, a conversion factor based on the C9-formula is typically used to correlate these values (mmol/g and units/Ar) (Balakshin and Capanema 2015).

Methoxyl content is a fundamental characteristic of lignin; however, its determination is always accompanied by uncertainties. For instance, high levels of carbohydrates may lead to error in the determination via wet chemistry because carbohydrates decrease the effective proton concentration by acting as Lewis bases suppressing formation of methyl iodide from the methoxyl groups, which will result in an underestimation of methoxyl group content (Goto et al. 2006). Also, carbohydrates can produce methyl iodide of non-methoxyl group origin, which will result in an overestimation of the methoxyl group in lignin.

Another way to determine methoxyl groups is via ^{13}C-NMR, which was used in this investigation because this technique provides information on inter-monomeric linkage. In addition, twelve native hardwood lignins from alkaline pretreated wood with very low sugar content and a wide range S/G ratio (1.2 - 3.0) have been investigated by means of quantitative ^{13}C-NMR. Good correlations were found between methoxyl group determinations by wet chemistry and by ^{13}C-NMR spectroscopy (Balakshin et al. 2016). However, a drawback of using quantitative ^{13}C-NMR for lignin samples containing carbohydrate contamination is that the carbohydrates overlap with lignin signals and amplify estimation errors.

Overall, the methoxyl contents were higher for eucalyptus kraft lignin samples, followed by sweetgum, acacia, and pine, which was expected given the S/G ratio of the wood species. Moreover, the methoxy content decreased with increased pulping level, which was also expected given the demethoxylation reaction taking place throughout the process. Note that an increase in methoxyl content was occasionally observed that has been attributed to higher G units removal in the initial stage of the pulping (Pinto et al. 2002).

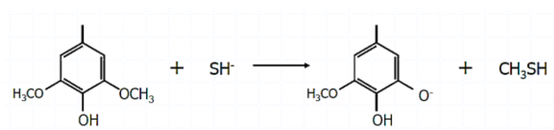
Generally, the carbon content was higher for pine than for the hardwood lignins, whereas the oxygen content was lower. This is obviously related to the abundance of S and G units, and consequently to the content of methoxyl groups in the lignin. Lignins with high methoxyl contents contain a lower percentage by weight of carbon and a higher percentage by weight of oxygen content (Sameni et al. 2016; Schorr et al. 2014).

The sulfur content (S%) was affected by pulping level and wood species. It decreased with increased pulping level. In addition, the highest values were observed for eucalyptus samples, followed by sweetgum, acacia, and pine. Thus, it can be inferred that this parameter is affected by S/G ratio and molecular weight. It has been reported that smaller lignin fragments contain a higher ratio of organically bound sulfur (Helander et al. 2013), which corroborates the results obtained in the present study.

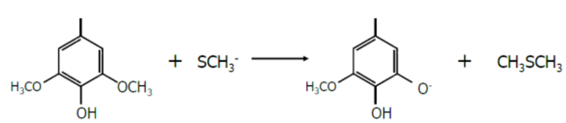
It is worth mentioning that determining C9-formula has challenges and it might be not accurate even for high-purity technical lignins. Lignin side chain is degraded to a certain extent during technical processing; therefore, the C-9 assumption becomes less precise (Balakshin and Capanema 2015). Additionally, lignin contamination from sugars, extractives, proteins, and ash, require further corrections, introduce extra sources of deviation, and decrease the accuracy of the results. It was observed that the overall unit weight is higher for hardwood lignins, given their higher methoxyl contents relative to softwood lignins.

6.3.4. Catechol groups

In kraft pulping, the strongly nucleophilic hydrosulfide ions cleave part of the methoxyl groups in the lignin structure generating catechol and methyl mercaptan (CH_3SH) as shown in Eq. 6.2. In addition, the anion of methyl mercaptan is an even stronger nucleophile that hydrosulfide, which also reacts with methoxyl groups, generating methyl sulfide and additional catechol groups (eq. 6.3) (Marton 1971).



(6.2)



(6.3)

The catechol content in key lignin samples evaluated in this study are shown in Table 6.7.

Table 6.7. Catechol content in pine, acacia, sweetgum, and eucalyptus lignins*

Cook time, min	Pine		Acacia		Sweetgum		Eucalyptus	
	pH 9.5	pH 2.5	pH 9.5	pH 2.5	pH 9.5	pH 2.5	pH 9.5	pH 2.5
60	5.3	7.2	14.7	18.1	5.2	5.2	5.6	9.2
90	5.2	4.6	8.8	9.1	6.5	6.3	6.1	5.1
150	10.3	12.2	7.4	6.7	7.4	6.6	7.1	7.3

* #/100 aromatic rings

The overall catechol content in lignins obtained at pH 2.5 was higher than the content in lignins obtained at pH 9.5, which can be attributed to the lower molecular weight and hence higher degradation levels for lignin samples obtained at pH 2.5. This observation is in agreement with a previous fractionation study, which has shown that low molecular weight lignin fractions undergo more demethylation during kraft pulping, highlighting that catechol increases with decreasing molecular weight (Jiang et al. 2017).

The amounts of catechol in BCL and Indulin AT, two commercial softwood kraft lignins, are 10.4% and 8%, respectively. The higher content in BCL is due to the more severe pulping conditions (Hu et al. 2016). Thus, when more severe pulping conditions are applied, higher catechol contents can be expected. Additionally, an increase in catechol content was expected with increasing pulping pulping level; however, such straightforward trend was not observed. In general, the catechol contents were lower than expected, which might be attributed to short period times under high temperatures for lignins obtained in the present study because it has been demonstrated that catechol and its derivatives are favored at long residence times (Petrocelli and Klein 1985).

Moreover, a pyrolysis study has shown that higher temperatures enhance the cleavage of methoxyl group on the aromatic rings (Zhao et al. 2014). The black liquors from which lignin samples were precipitated were collected after a given pulping level and immediately cooled down to room temperature. Thus, it is possible that the demethylation under laboratorial kraft pulping conditions take place at lower rate than the demethylation at industrial level, where the black is kept under higher temperatures for longer times, allowing for demethylation to occur.

6.3.5. ^{13}C and 2D HSQC NMR analyses

Two-dimensional Heteronuclear Single Quantum Coherence (2D HSQC) spectroscopic technique combines the sensitivity of ^{1}H-NMR with the high resolution of ^{13}C-NMR. Therefore, 2D HSQC NMR shows good signal separation revealing many lignin substructures. However, quantification of these structures is complicated by several factors, such as T1 and T2 relaxation rates, carbon purse offset effects, coupling constant deviations, and proton homo-nuclear coupling effects (Sette et al. 2011; Zhang and Gellerstedt 2007). On the other hand, ^{13}C-NMR provides quantitative data on quaternary moieties and on various lignin functional groups (Balakshin et al. 2016). Thus, the combination of 2D and quantitative ^{13}C-NMR constitute a very powerful approach in characterizing lignins.

Figure 6.3 shows the ^{13}C-NMR spectra of pine, acacia, sweetgum, and eucalyptus lignins obtained after 150 minutes of pulping, at pH 9.5, where carboxylic C; aromatic C and C=C bond; lignin side chain and carbohydrates; methoxyl (OMe); and dimethyl sulfoxide (DMSO) assignments have been indicated. The spectra highlights that the kraft lignins vary greatly amongst the lignins evaluated in this study. Specifically, the lower purities achieved by acacia and sweetgum kraft lignins are very evident in the region where carbohydrates show their signals.

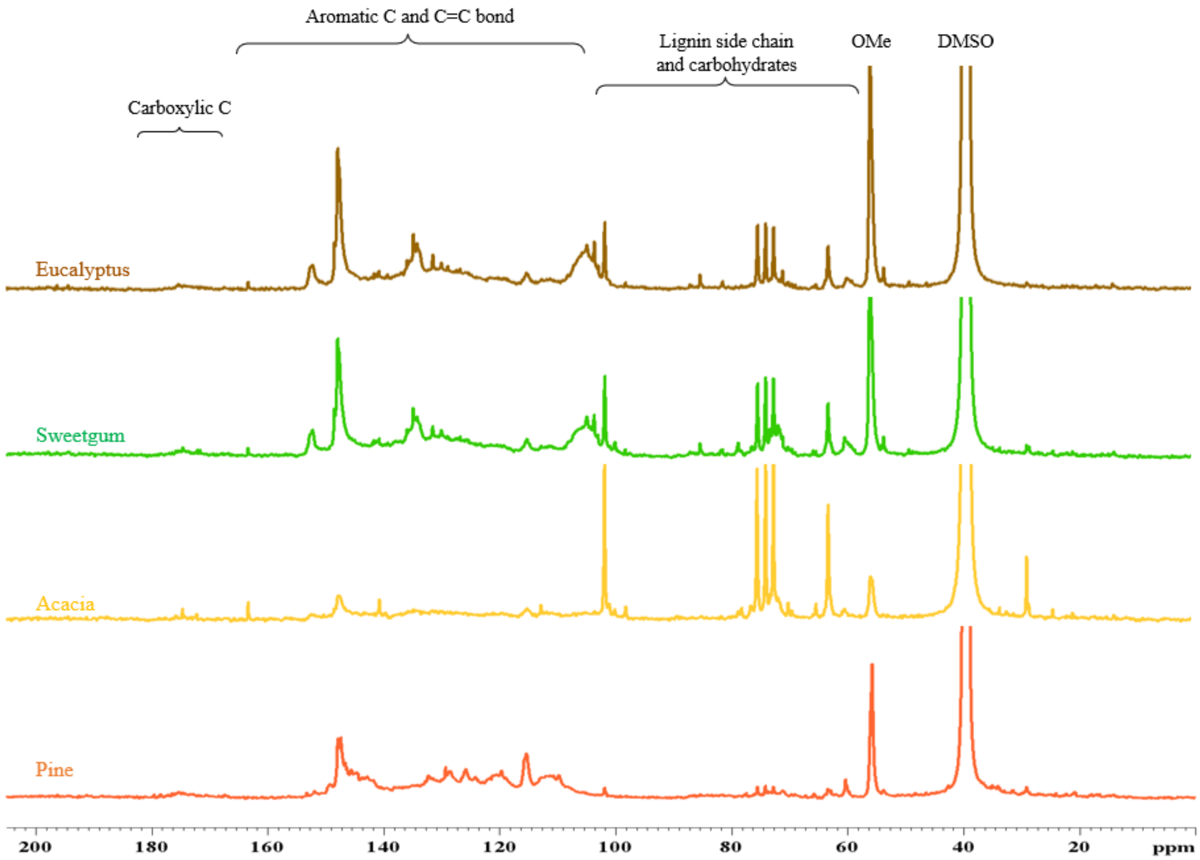


Figure 6.3. ^{13}C-NMR spectra of pine, acacia, sweetgum, and eucalyptus lignins obtained after 150 minutes of pulping, at pH 9.5

Figure 6.4 depicts the 2D HSQC NMR of eucalyptus lignin obtained after 150 minutes of pulping, at pH 9.5. Lignin interunits, such as β-O-4, β-β, β-5; carbohydrates moieties, such as xylan and arabinan; G and S units; and other lignin substructures are indicated. The assignment of mentioned structures was performed according to Tables 6.2 and 6.3.

The calculation of a given substructure was performed by taking the integration of the substructure of interest and the integration of the cluster in which the substructure resonates in the 2D NMR, as well as the integration of the aromatic carbons (163–103 ppm) and the region of where the structure resonates in the quantitative ^{13}C NMR (following the Eq. 6.1 in the experimental section). It is critical recognizing that the aromatic region contains vinylic carbons (Hu et al. 2016) and they were accounted for. The content of vinylic carbons per 100 aromatic units was determined by HSQC spectra and varied from 1 to 17. Thus, *A*, in Eq. 6.1, varied from 601 to 617.

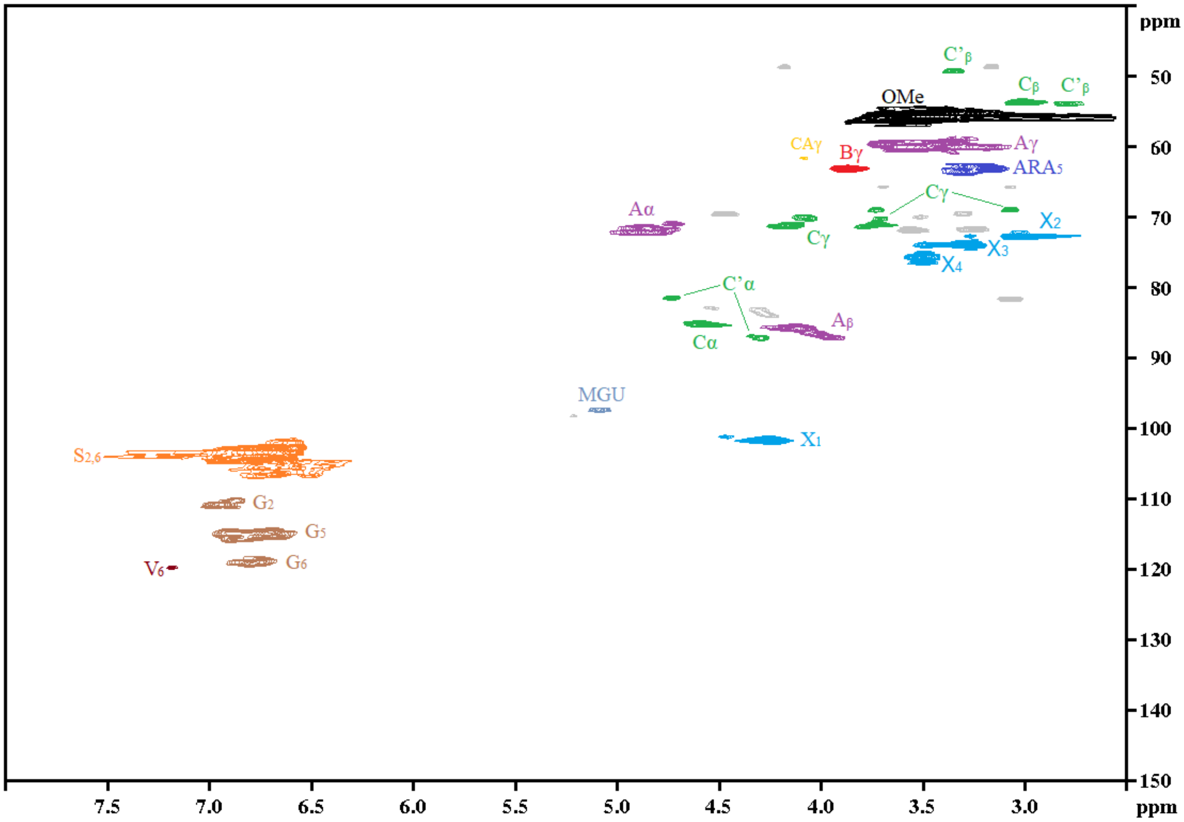


Figure 6.4. 2D HSQC NMR of eucalyptus lignin obtained at 150 minutes, pH 9.5.

The main interunit linkages in native lignins are β-O-4, β-β, β-5, and β-1 and their frequency are shown in Table 6.8. Note that most abundant linkage is β-O-4 and its frequency increases with increasing S/G ratio.

Table 6.8. Interunit linkages in pine, acacia, sweetgum, and eucalyptus native lignins

Characteristics	Pine	Acacia	Sweetgum	Eucalyptus
β-O-4'	27.5	45	49.1	50
β-β	4.4	11	7.7	14
β-5	9.8		3.6	1
β-1	-	-	2.5	3

During kraft pulping, lignin undergoes severe interunit cleavage and modifications on its structure. Although the relative proportions of the native structure decrease throughout the process, most of them are still present in technical lignins (Liitiä et al. 2003) and their frequency are shown in Table 6.9.

Table 6.9. Estimation of the contents of lignin interunit by ^{13}C NMR and 2D HSQC NMR*

Species	Cook time, min	pH	β-O-4	β-5	β-β
Pine	60	9.5	7.5	1.4	0.9
	90	9.5	4.0	1.4	1.2
	150	9.5	2.0	0.8	0.9
	60	2.5	5.0	0.9	0.7
	90	2.5	3.2	0.9	0.8
	150	2.5	0.7	0.2	0.3
Acacia	60	9.5	18.5	1.5	3.5
	90	9.5	6.2	0.8	2.4
	150	9.5	7.4	1.0	3.3
	60	2.5	11.1	0.6	2.1
	90	2.5	6.8	0.9	2.8
	150	2.5	3.3	0.5	2.3
Sweetgum	60	9.5	28.2	0.9	8.0
	90	9.5	10.8	0.5	5.4
	150	9.5	3.0	0.2	2.3
	60	2.5	10.9	0.3	3.2
	90	2.5	7.9	0.4	4.1
	150	2.5	2.0	0.1	1.4
Eucalyptus	60	9.5	13.6	0.3	2.2
	90	9.5	6.7	0.2	3.4
	150	9.5	2.0	0.1	1.5
	60	2.5	12.2	0.2	2.6
	90	2.5	7.5	0.2	3.5
	150	2.5	2.2	0.1	1.1

* Percent per 100 aromatic rings

It is noteworthy that proportionally much more β-O-4 linkages are cleaved with increasing pulping level than the C-C bonds due to the lower stability under alkali conditions of the former than the latter. Yet, the most abundant lignin interunit in the technical lignins is the β-O-4.

The overall linkage content per 100 aromatic rings was higher in lignin samples precipitated at pH 9.5. Moreover, these lignins showed higher molecular weights than the lignins precipitated at pH 2.5, which indicate that lignins obtained at pH 9.5 are indeed less degraded than lignins obtained at pH 2.5. Such observation is not without precedent and has also been reported elsewhere (Giummarella et al. 2020).

Besides the linkages shown in Table 6.8, new ones are identified in technical lignins such as epiresinols (β-β'), an alkali-catalyzed epimerization that occurs when the associated phenolic groups are free (Zhao et al. 2020). The content of such linkage, other lignin structures, and LCCs are shown in Table 6.10.

Table 6.10. Estimation of the contents of various lignin substructures and LCCs by ^{13}C NMR and 2D HSQC NMR*

Species	Cook time, min	pH	β-β'	Stilbene	Coniferyl alcohol	PhGly**
Pine	60	9.5	0.2	5.2	0.5	0.04
	90	9.5	0.3	3.5	0.3	0.07
	150	9.5	0.2	3.7	0.3	0.05
	60	2.5	0.1	3.7	0.3	0.07
	90	2.5	0.1	2.5	0.3	0.04
	150	2.5	0.1	3.1	0.2	0.01
Acacia	60	9.5	0.5	2.8	0.2	0.75
	90	9.5	0.2	1.8	0.2	0.21
	150	9.5	0.6	2.1	0.5	11.33
	60	2.5	0.3	3.8	0.1	0.08
	90	2.5	0.5	1.9	0.2	0.08
	150	2.5	0.2	1.6	0.1	0.01
Sweetgum	60	9.5	1.6	1.3	0.5	15.94
	90	9.5	1.2	1.4	0.5	3.88
	150	9.5	0.5	0.6	0.3	1.46
	60	2.5	0.7	1.8	0.2	0.80
	90	2.5	0.9	1.8	0.2	0.08
	150	2.5	0.3	1.0	0.2	1.01
Eucalyptus	60	9.5	0.1	0.6	0.1	0.01
	90	9.5	0.8	0.4	0.5	0.11
	150	9.5	0.0	0.3	0.1	0.13
	60	2.5	0.4	1.0	0.1	0.03
	90	2.5	0.8	0.9	0.2	0.16
	150	2.5	0.0	0.3	0.1	0.10

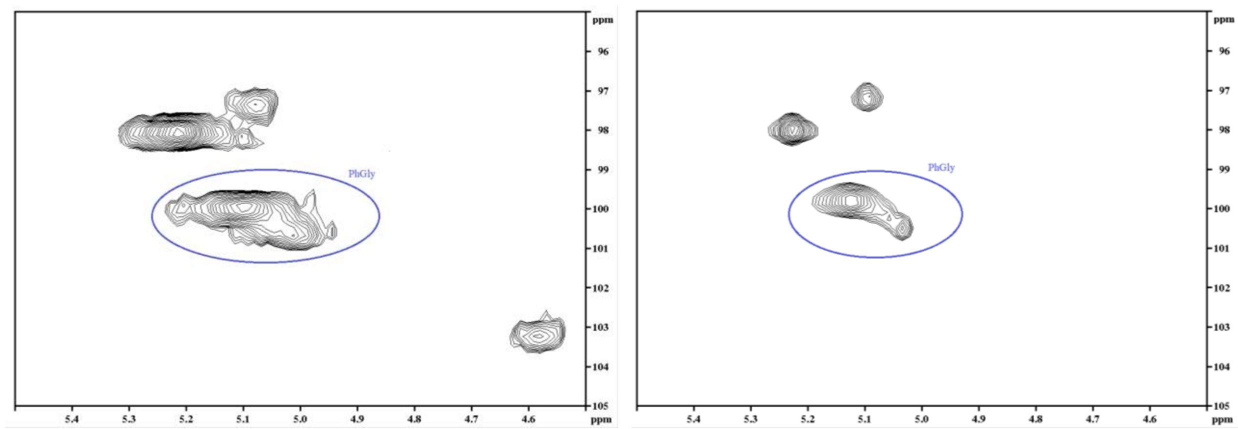
* Percent per 100 aromatic rings
** Phenyl glycoside

Coniferyl alcohol structures are formed under kraft pulping conditions, and they have been shown to stablish new C-C linkages between side chains, as well as to undergo various disproportionation reactions, forming lower molecular weight products with aliphatic side-chain structures (Gierer et al. 1973). Note that the frequency of this substructure is higher at less degraded lignins and they decreased with pulping level, indicating structural changes in lignin throughout kraft pulping.

Stilbene, another structure formed during kraft pulping, is generated mainly by cleavage of the α-aryl ether bond of β-5 structures (Liitiä et al. 2003). Its overall content was higher for lignin samples obtained at pH 9.5 than pH 2.5, indicating that stilbene is formed by degradation of lignin during kraft pulping.

The three main types of LCCs in woody biomasses are γ-esters, benzyl ethers, and phenyl glycosides. The phenyl glycoside (PhGly) is stable under alkaline conditions and were detected in all kraft lignins studied, varying from very low to high intensity, depending on wood species and pulping level.

The combination of ^{13}C and 2D HSQC NMR is crucial to elucidate and quantify LCCs. Figure 6.5. depicts a well resolved cross-signal of phenyl glycoside for two kraft lignins evaluated. When lignin samples are rich in associated carbohydrates, which was observed for most of the lignins herein studied, the quantification of lignin structure and LCC linkages with quantitative ^{13}C-NMR is challenging because the carbohydrates overlap in the side-chain region of the ^{13}C-NMR spectrum, which comprisea the integration and subsequent quantification of the lignin structures (Yuan et al. 2011). The advantage of using 2D NMR is that overlapping signals of protons directly linked to carbons having different chemical shifts are separated in the carbon dimension; similarly, in the proton dimension, carbons signals with similar chemical shift can be separated by the environment of the directly attached protons, which allows for deciphering simultaneously structural details of lignin skeleton and carbohydrates structure (Giummarella et al. 2019). Thus, by combining both techniques, more accurate values are obtained. Yet, the estimated values are relative.



(a) (b)

Figure 6.5. Phenyl glycoside in (a) acacia kraft lignin obtained at 150 minutes, pH 9.5, and in (b) sweetgum kraft lignin obtained at 150 minutes, pH 9.5.

According to Min *et al.* (2014), S/G ratio has influence on the LCCs content; the research reports that LCC amount increases with increasing S/G ratio. Such straightforward trend was not observed in the present investigation. The highest contents of phenyl glycoside were obtained for sweetgum lignins (intermediate S/G ratio), followed by acacia (low S/G ratio), eucalyptus (high S/G ratio), and pine. Moreover, there is no clear relationship between carbohydrate contamination and the amount of LCCs. However, the contamination with carbohydrates was shown to be affected by S/G ratio as it decreased with increasing S/G ratio. Finally, it is noteworthy that the lignin recovery yield increased with decreasing contamination with carbohydrates, not with LCC. Thus, the association/interaction between lignin and carbohydrates fragments are a determining factor for lignin precipitation.

6.4. Conclusions

- The elucidation of hardwood kraft lignins (acacia, sweetgum, and eucalyptus), was more challenging than the elucidation of softwood kraft lignin (pine) due to their lower purity, *i.e.*, higher contamination with carbohydrates. The contamination hinders lignin solubility and amplify errors in quantitative analyses.
- There is no straightforward relationship between lignin molecular weight and pulping level. The molecular weight is rather dependent on the balance between degradation and condensation reactions, which depends on pulping conditions and wood species. In addition, the molecular weight of kraft lignins was shown to decrease with increasing S/G ratio.
- The phenolic hydroxyl content in kraft lignin is a determining factor for lignin precipitation. Lignin samples with phenolic hydroxyl content lower than 3.0 mmol/g of lignin showed very poor recovery yields.
- The elemental composition of kraft lignins is dependent on species and pulping level. The carbon to oxygen ratio relates to S/G ratio, and consequently to the content of methoxyl groups. Lignins with high methoxyl contents contain a lower percentage by weight of carbon and a higher percentage by weight of oxygen content. In addition, given the higher content of methoxyl groups in hardwood lignins, the unit weight of these species is typically greater than softwood lignins.
- The abundance of native interunit linkages decreased with increasing pulping level. In addition, proportionally much more β-O-4 linkages were cleaved over the pulping due to its lower stability under alkali conditions. Yet, β-O-4 was the most abundant lignin interunit in the technical lignins evaluated.

- The overall linkage content per 100 aromatic was higher in lignin samples precipitated at pH 9.5. Moreover, these lignins showed higher molecular weight than the lignins precipitated at pH 2.5, and lower phenolic hydroxyl content which indicate that lignins obtained at pH 9.5 are indeed less degraded than the lignins obtained at pH 2.5
- Amongst the species investigated in the present study, there is no straight relationship between LCC abundance and S/G ratio. Rather, carbohydrate contamination in hardwoods was shown to increase with decreasing S/G ratio. Besides, such contamination was shown to be determining for lignin precipitation performance, as highly contaminated samples showed very low recovery yields.

6.5. References

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7. Chapter 7 – Conclusions

The evaluation of pine, acacia, sweetgum, and eucalyptus kraft lignins obtained at pHs 9.5 and 2.5 from different levels of the pulping process showed that lignin content in the black liquor and the subsequent recovery increased with pulping intensity. In the initial phase, total lignin recovery ranged from ~ 9 to 55%, while in the later phase total lignin recovery varied from ~ 61 to 91%, depending on species. The increased lignin recovery can be attributed to the increasing lignin concentration towards the end of the pulping process.

Softwood kraft lignin (SWKL), here represented by pine lignin, showed higher lignin recovery and higher purity than hardwood kraft lignin (HWKL), here represented by acacia, sweetgum, and eucalyptus. Such observation was due to lower contamination with carbohydrates, lower abundance of LCCs, high molecular weight, and high content of phenolic OH by SWKL when compared to HWKL. Besides, the elucidation of SWKL was shown to be less challenging than the elucidation of HWKL, given their higher purity, *i.e.*, less contamination with carbohydrates. The contamination hinders lignin solubility and amplify errors in quantitative analyses.

There is no straightforward relationship between lignin molecular weight and pulping level. The molecular weight is rather dependent on the balance between degradation and condensation reactions, which depends on pulping conditions and wood species. In addition, the molecular weight of kraft lignins was shown to decrease with increasing S/G ratio.

Overall, more lignin was precipitated at pH 9.5 than at pH 2.5. However, the precipitation of lignin fraction at pH 2.5 contributed with an increase of ~ 7 to 81% recovery depending on pulping level, S/G ratio, and species.

The phenolic hydroxyl content in kraft lignin was shown to be determining for lignin precipitation. The content should be at least 3.0 mmol/g of lignin, otherwise very poor recovery yields are observed, which was observed for acacia after 120-150 minutes of pulping at pH 9.5.

The elemental composition of kraft lignins is dependent on species and pulping intensity. The carbon to oxygen ratio relates to S/G ratio, and consequently to the content of methoxyl groups. Lignins with high methoxyl contents contain a lower percentage by weight of carbon and a higher percentage by weight of oxygen content. In addition, given the higher content of methoxyl groups in hardwood lignins, the unit weight of these species is typically greater than softwood lignins.

The abundance of native interunit linkages decreased with increasing pulping level. In addition, proportionally much more β-O-4 linkages were cleaved over the pulping due to its lower stability under alkali conditions. Yet, β-O-4 was the most abundant lignin interunit in the technical lignins herein evaluated.

The overall linkage content per 100 aromatic was higher in lignin samples precipitated at pH 9.5. Moreover, these lignins showed higher molecular weight than the lignins precipitated at pH 2.5, which indicates that lignins obtained at pH 9.5 are indeed less degraded than the lignins obtained at pH 2.5.

The overall linkage content per 100 aromatic and molecular weight were higher in lignin samples precipitated at pH 9.5, which indicates that lignins obtained at pH 9.5 are indeed less degraded than the lignins obtained at pH 2.5.

Amongst the species herein studied, there is no straight relationship between LCC abundance and S/G ratio. Rather, carbohydrate contamination was shown to increase with decreasing S/G ratio. Besides, such contamination was shown to be determining for lignin precipitation performance, as highly contaminated samples showed very low recovery yields.

8. Chapter 8 - Future Work

The present work evaluated pine, acacia, sweetgum, and eucalyptus kraft lignins obtained at pHs 9.5 and 2.5 from different levels of the pulping process. Lignin fraction at pH 9.5 was studied based on commercial processes for obtaining lignin. The fraction at pH 2.5 was evaluated to characterize most of the lignin dissolved in the black liquor throughout the pulping process. Given the lower yields obtained at pH 9.5 by some hardwood samples, the study of other final pHs is recommended based on dissolution of carbon dioxide (pH ~ 8 – 8.5). It is important to mention that more CO_2 will be required to decrease the pH to values below 9.5. Thus, it is also recommended to determine its feasibility via a techno-economic analysis.

After lignin precipitation experiments the solids were washed with sulfuric acid solution, but no further purification steps were applied to avoid further modifications on the composition and structure of the kraft lignins. However, the contamination with carbohydrates occasionally hindered the solubility of kraft lignin samples as well as data acquisition and interpretation. Thus, future investigations should consider purification steps with dioxane or enzymatic hydrolysis to mitigate such issues. It is important to mention that the conditions applied to such experiments should be carefully controlled to avoid significant losses of lignin structures/MWs and LCCs.

Studies on structural modifications taking place on residual lignins should also be considered. It is likely that it would provide a better understanding on lignin behavior during delignification, dissolution, and precipitation. Such studies have not yet been reported. It is valid to mention that residual lignins have already been studied but with the goal of either improving pulping or bleaching processes, not aiming at lignin precipitation.

Finally, the three hardwood lignins herein studied were chosen given their very distinct S/G ratio. Based on these three species, such parameter was shown to greatly affect lignin precipitation and lignin properties. Clear trends were observed and extensively discussed. However, other hardwoods should be investigated to prove that such trends were indeed due to S/G ratio and not intrinsic to any of the species. For instance, wood anatomy could affect the pulpability and hence the characteristics of technical lignins. Besides, lignin structure can vary within a single plant and species depending on age, growth site, and season.