UNIVERSIDADE FEDERAL DO PARANÁ

TAINISE VERGARA LOURENÇON

FRACTIONATION OF KRAFT LIGNIN AND POTENTIAL VALUE-ADDED APPLICATIONS

CURITIBA

2018

TAINISE VERGARA LOURENÇON

FRACTIONATION OF KRAFT LIGNIN AND POTENTIAL VALUE-ADDED APPLICATIONS

Thesis presented as partial requirement for obtaining the Doctor degree in Forestry Engineering, Wood Technology Area. Post-graduation Program in Forestry Engineering, Federal University of Paraná.

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CURITIBA

2018

Ficha catalográfica elaborada pela
Biblioteca de Ciências Florestais e da Madeira - UFPR

Lourençon, Tainise Vergara

Fractionation of kraft lignin and potential value-added applications / Tainise Vergara Lourençon. – Curitiba, 2018.

98 f. : il.

Orientadora: Prof.ª Dr.ª Graciela I. B. de Muñiz
Coorientadores: Prof. Dr. Washington L. E. Magalhães
Prof. Dr. Luiz Pereira Ramos

Tese (Doutorado) - Universidade Federal do Paraná, Setor de Ciências Agrárias, Programa de Pós-Graduação em Engenharia Florestal. Defesa: Curitiba, 19/02/2018.

Área de concentração: Tecnologia e Utilização de Produtos Florestais.

1. Polpação alcalina por sulfato. 2. Lignina. 3. Madeira – Química. 4. Resíduos vegetais. 5. Teses. I. Bolzón de Muñiz, Graciela Inés. II. Magalhães, Washington Luiz Esteves. III. Ramos, Luiz Pereira. IV. Universidade Federal do Paraná, Setor de Ciências Agrárias. V. Título.

CDD – 634.9
CDU – 634.0.813

MINISTÉRIO DA EDUCAÇÃO
SETOR SETOR DE CIENCIAS AGRARIAS
UNIVERSIDADE FEDERAL DO PARANÁ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO ENGENHARIA FLORESTAL

TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em ENGENHARIA FLORESTAL da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **TAINISE VERGARA LOURENÇON** intitulada: **FRACTIONATION OF KRAFT LIGNIN AND POTENTIAL VALUE-ADDED APPLICATIONS**, após terem inquirido a aluna e realizado a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

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ACKNOWLEDGMENTS

To Universidade Federal do Paraná (UFPR), specially to Programa de Pós-Graduação em Engenharia Florestal (PPGEF) for giving me the opportunity to study and to further develop myself.

To Embrapa Florestas, where I developed most of my experiments and spent most of my time as PhD student. I am thankful to all offered infrastructure and to the support given by the employees.

To CNPq for the financial support, substantial for the development of this work.

To PDSE sponsored by CAPES, for enabling the unique opportunity to develop part of my PhD at VTT (Technical Research Center of Finland).

To Suzano Pulp and Paper for donating the material to the development of this work.

To VTT for providing me free access to facilities and laboratories. For all personal support during my exchange in Finland. Special thanks to the researchers Dr. Tarja Tamminen (advisor in Finland) and Dr. Sami Alakurtti (supervisor in Finland) who I have learned a lot. I appreciated the time we shared. To Dr. Tiina Liitiä (team leader) who enabled my internship at VTT. To the others VTT researchers that made my time there unique and allowed me to improve my knowledge.

I want to thank my advisor Prof. Dr. Graciela I. B. de Muniz, for our conversations, for the advises, motherly affection and friendship.

My sincere gratitude to my co-advisor Dr. Washington L. E. Magalhães, for all advises and incentive during these years. For making me look to science with different perspectives.

I would like to express my gratitude to Prof. Dr. Luiz P. Ramos, for his valuable suggestions. Thanks for opening the doors of Chemistry department.

Special thanks to Embrapa's personal: Edson, Beth, Si, Pati, Cris and Day for the reception and friendship during these years. Specially to Tieli for helping me in all kind of situations and to Fabrício A. Hansel for the valuable suggestions and collaboration in my researches. Also, to Dr. Álvaro Santos and Caroline Buhrer from Phytopathology Department and to Dr. Léia Favaro from Embrapa Agroenergia.

To my friends from Forest Biomaterials & Systems (BioMaS) team, André, Débora, Pedro, Mailson, Francine and Aline, who shared their experiences with me. Thanks for the coffee moments and friendship.

I want to thank my brother Rangel for his understanding and love and I would like to show my greatest appreciation to my parents Lúcia and Telmo Lourençon. For all support, incentive, and unconditional love. "If I have seen further, it is because I have stood on the shoulders of giants".

I would like to thank my husband Bruno Mattos, for the uncountable conversations of professional or personal content, which encouraged me to always look forward and feel confident. For being my best friend and my love.

I want to thank to all who contributed somehow to the development of this work.

RESUMO

A lignina pode ser isolada da madeira, de plantas anuais ou de resíduos agrícolas por diferentes processos de extração. A principal fonte de lignina advém da recuperação do licor negro (LN) gerado em processos de polpação. O processo kraft é o principal método aplicado para polpação no mundo e o único no Brasil. A lignina gerada a partir desses processos é usualmente queimada para suprimento de energia interna da fábrica. No entanto, como a lignina apresenta uma estrutura química interessante – capaz de substituir recursos de origem fóssil – e está atualmente disponível em grandes quantidades, tem sido considerada uma realidade prospectiva para aplicações de maior valor agregado que não seja somente sua queima. As impurezas carregadas durante a recuperação, a alta polidispersividade e a grande composição de grupos químicos podem limitar suas aplicações. Para contornar essa situação, o fracionamento deste material, tornou-se não só desafiador, mas mandatório. Dessa forma, buscou-se como foco da pesquisa fracionar/purificar lignina de eucalipto (*hardwood*) e pinus (*softwood*) proveniente de LN do processo kraft, para obtenção de frações com diferentes e conhecidas propriedades, de maneira a potencializar suas aplicações nos mais variados campos. Primeiramente investigou-se o efeito dos ânions na precipitação da lignina através da adição de dois diferentes sais (cloreto de sódio e sulfato de sódio), onde foi verificado que o íon cloreto teve menor influência durante a precipitação. O ácido clorídrico foi então utilizado para uma precipitação ácida sequencial, em que os fatores pKa e tamanho molecular foram observados como principais fatores responsáveis pela precipitação. Foram obtidas frações com massas molares decrescendo conforme o pH decresceu. As frações provenientes da lignina de *hardwood* tiveram separação mais eficiente que a *softwood*. Dessa forma, o segundo foco do trabalho teve como principal objetivo buscar e indicar potenciais de aplicação para as frações de lignina de *hardwood* em duas grandes vertentes: testando suas atividades biológicas e testando a lignina como substituinte de fenol em resinas fenol formaldeído (FF). As frações obtidas nos pHs mais baixos apresentaram maior capacidade antioxidante e antifúngica. Tais atividades foram associadas a menor massa molar e maior conteúdo de lignina total (menor impureza) dessas frações. Além disso, todas as frações apresentaram atividade antibacteriana. As resinas FF testadas com as frações de lignina kraft de *hardwood* apresentaram performance de cola similar e até superior a resina comparativa utilizada contendo lignina de *softwood*, o que abre a oportunidade para novas discussões relacionadas as características da lignina (fatores estéricos, flexibilidade da macromolécula) que poderiam desempenhar um papel mais importante no desempenho das resinas. Além disso, com o trabalho foi provado que a lignina kraft de eucalipto tem, sim, potencial para ser utilizada em resinas FF. Tal resultado é de grande interesse e bastante vantajoso principalmente para o Brasil, onde a matéria-prima utilizada pelas indústrias de celulose e papel é 88% de eucalipto. Além disso, o estudo apresentou um método simples e barato de fracionamento e indicou potenciais de aplicação dessa lignina que ainda é pouco explorada e pouco utilizada comercialmente. A partir dos resultados apresentados, outros desdobramentos e investigações ainda poderão ser realizados tendo como base a tese desenvolvida.

Palavras-chave: Acidificação. Lignina técnica. Antioxidante. Antifúngico. Antibacteriano. Resina fenólica.

ABSTRACT

Lignin can be isolated from wood, annual plants or agricultural residues by different extraction processes. The most available resource of lignin are technical lignins, recovered as byproduct during pulp and paper production. Kraft process is the main method applied worldwide and the only one in Brazil. The recovered lignin is usually burned for internal energy production. As lignin presents a potential aromatic chemical structure - able to replace fossil resources – and is readily available in large amounts, it has been considered a realistic prospective to produce high value-added products. However, the presence of impurities - as inorganics compounds and traces of holocellulose - and the high polydispersity in the recovered lignin, can restrict its utilization. To overcome such heterogeneity, fragmentation and/or purification processes, became challenging but mandatory. Herein, this study focused on the fractionation and purification of kraft black liquor (BL) to obtain specific and well-defined lignin fractions to potentialize its application in many fields. Firstly, the effect of the anions on lignin precipitation were investigated through addition of two different salts (sodium chloride and sodium sulfate) in a hardwood and softwood kraft BL. It was verified that chloride ion had less influence during the precipitation. Hydrochloride acid was then used for sequential acid precipitation, in which the precipitation had been driven by pKa and molecular size over ion influence. From the acid fractionation different lignin fractions were obtained. It was observed that the average molar mass decreased as the pH decreased. Such fractionation process was considered more efficient to hardwood lignin than softwood lignin. Thus, the second aim of the work was to point potential applications for hardwood lignin fractions in two key fields: testing their bioactivities and testing lignin as phenol substitute in lignin phenol formaldehyde (LPF) resins. A greater antioxidant and antifungal activity was observed in those fractions obtained at lower pH. These results were associated to the higher content of phenolic compounds - due to higher content of total lignin - and to the lower molar mass of the fractions. Moreover, all fractions presented antibacterial activity. Regarding to the LPF resins, all hardwood lignin fractions showed similar and even higher capacity than the comparative LPF resin from softwood lignin. Thus, hardwood kraft lignin presents potential to be used as phenol replacement in phenol formaldehyde resins. This subject is of great importance as it opens opportunities for further discussions related to all characteristics (steric factors, flexibility of lignin macromolecule) that could play a more important role in LPF resins. Furthermore, this result is of big interest especially in Brazil, where hardwood (*Eucalyptus* genus) represents 88% of the used raw material during pulping process. Besides, this study presented a simple and cheap fractionation method and suggested potential applications of kraft lignin which still commercially little exploited. Also, other developments and investigations may be carried out based on the presented results.

Key-words: Acidification. Technical lignin. Antioxidant. Antifungal. Antibacterial. Phenolic resin.

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CHAPTER I: GENERAL INTRODUCTION AND LITERATURE REVIEW

I.1 GENERAL INTRODUCTION

In the plant cell wall of lignocellulosic materials, up to one-third is composed by lignin. This natural polymer is the most abundant aromatic renewable material in earth and can be isolated from wood, annual plants or agricultural residues by different extraction processes.

The most available resource of lignin are technical lignins, recovered as byproduct during pulp and paper production (LAURICHESSE; AVÉROUS, 2014). From 70 million tonnes of lignin produced annually in the world, only 5% is used in commercial applications, while 95% is burned for energy production (LAURICHESSE; AVÉROUS, 2014). Kraft process is the main method applied worldwide and the only one in Brazil (IBÁ, 2017). It has been considered a realistic prospective to produce high value-added products (SCHORR et al., 2014), highlighted by the fact that it forms a large proportion of the non-food biomass (DOHERTY et al., 2011). However, few or none studies exploited kraft lignin from hardwood type (*e.g.* eucalypt). In Brazil, *Eucalyptus* genus represent 88% of the used raw material (IBÁ, 2017). Thus, the valorization of this type of lignin became of big interest.

The total amount of produced lignin reaches 60% more than is actually needed for a pulp mill internal power supply (SANNIGRAHI et al., 2010; KOUISNI et al., 2012). In other words, 60% out of 70 millions of tons produced annually (LAURICHESSE; AVÉROUS, 2014) still can be used to obtain high value-added products without interfering in the pulp mills self-sufficiency energy supply.

Consequently, an efficient lignin utilization becomes economically required. The possibility of scarcity of current raw materials used in oil exploitation (LAURICHESSE; AVÉROUS, 2014), generated a space for the development of new products and processes based on aromatic renewable materials, such as lignin (NORBERG et al., 2013).

According to Sannigrahi and Ragauskas (2010), after a domination in biofuels research based on cellulose in the first decade of 2000's, the second decade will be surely dominated by lignin research as equal importance.

The large available technical lignins present non-uniform chemical structures (VISHTAL; KRASLAWSKI, 2011) and wide molar mass distribution (YOSHIDA et al.,

1987), which affect further solubility and reactivity. Besides, the presence of impurities, as inorganics compounds and traces of holocellulose, in the recovered lignin can restrict its utilization as high value-added products. To overcome such heterogeneity, fragmentation and/or purification processes, in order to obtain specific lignin fractions, became challenging but mandatory (STIEFEL et al., 2016)

In this scenario, this study had as first aim the fractionation and purification of kraft black liquor (BL) to obtain specific and well-defined lignin fractions. In a first place, the effects of chloride and sulfate anions upon precipitation of hardwood and softwood kraft lignin were investigated followed by the development of a sequential acid precipitation method. After obtainment of specific lignins, this work focused on the potential applications for such fractions, as biologic activity and polymer application.

This thesis is organized in chapters. Chapter I was built up of this general introduction and literature review addressing topics as lignin concepts and structure; main extraction processes; fractionation methods and emerging applications. Chapter II, shows the effects of chloride and sulfate anions upon precipitation of hardwood and softwood kraft lignins. Chapter III, refers to the fractionation of hardwood and softwood kraft lignin by a simple sequential acid precipitation. Chapter IV investigated the antioxidant capacity and bioactivity of the obtained lignin fractions. Chapter V presents the potential application of hardwood lignin fractions as phenol replacement in phenol formaldehyde resins.

I.2 LITERATURE REVIEW

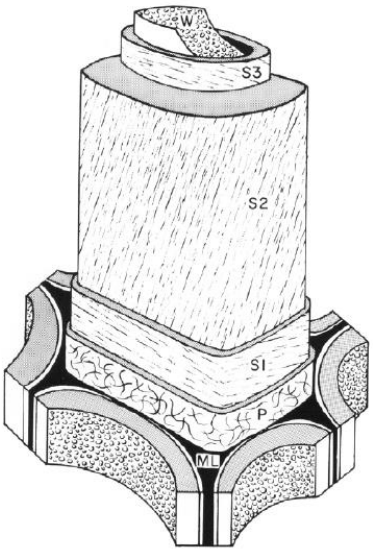
I.2.1 Lignin and its chemistry

By the year of 1838 Anselme Payen observed the disintegration of wood when treated with nitric acid. The solid and fibrous residue, was called cellulose. Further studies proved the presence of other polysaccharides in addition to cellulose. The dissolved material, with higher carbon content than the fibrous residue was termed lignin, from the Latin word for wood *lignum* (SJÖSTRÖM, 1993).

Lignin is the most abundant aromatic renewable material available in nature and the second most abundant organic polymer after cellulose (HATAKKA, 2001). In plant evolution, lignin was introduced some 440 million years ago when the group of vascular plants started to develop (GELLERSTEDT; HENRIKSSON, 2008).

The lignin is found in the vegetables cell wall and middle lamella (ML) (FENGEL; WEGENER, 2003). Despite a high concentration of lignin in the ML the predominant portion is located in the S2 layer of the secondary wall due to its large relative volume (FIGURE I.1). In its native state, structure in plant, lignin is referred as *protolignin* and is widely different from its structure after isolation/extraction.

FIGURE I.1- SCHEMATIC REPRESENTATION OF A WOOD CELL WALL: MIDDLE LAMELLA (ML): PRIMARY WALL (P): OUTER (S1), MIDDLE (S2), AND INNER (S3) LAYERS OF THE SECONDARY WALL: WARTY LAYER (W)

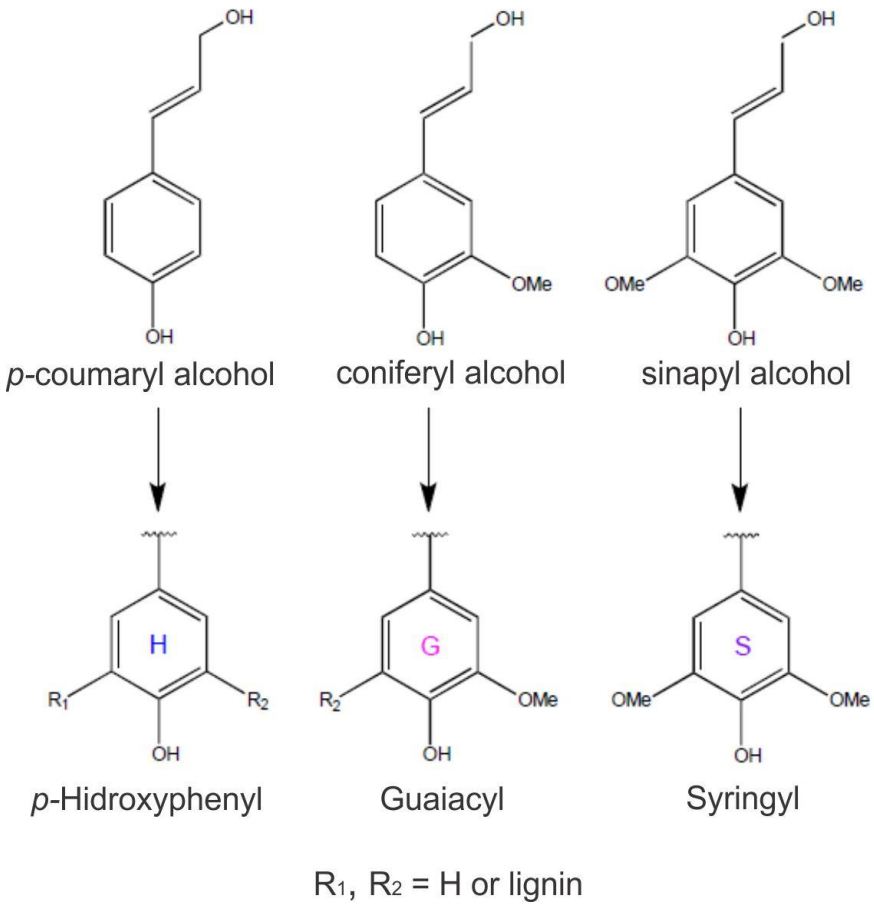


SOURCE: COTÉ (1967).

The most important physical property of this organic macromolecule in plant is its stiffness, which not only bring strength to the plant tissue, but also avoid water cell elements to collapse. Besides, lignin protects the plant against biochemical stress inhibiting enzymatic degradation of other components (GRABBER, 2005).

It has been consolidated that lignin consists of methoxylated phenylpropane units. FIGURE I.2 presents the three monolignol precursors to lignin formation in different species. According to the methoxylation degree, the aromatic group is named *p*-hydroxyphenyl (from *p*-coumaryl alcohol), guaiacyl (from coniferyl alcohol), or syringyl (from sinapyl alcohol). The first one is non-methoxylated while the two others have one and two adjacent methoxy groups in the phenolic hydroxyl group, respectively.

FIGURE I.2- MONOLIGNOL PRECURSORS AND THEIRS CORRESPONDING STRUCTURES IN LIGNIN POLYMERS



SOURCE: The author (2017).

The monolignol precursors are formed in the cytoplasm from D-glucose via the shikimate pathway, including a series of enzymatic reactions producing phenylalanine as key intermediate (SJÖSTRÖM, 1993). Through further enzyme mediated deamination, hydroxylation, reduction and methylation reactions, the lignin building blocks are formed (GELLERSTEDT; HENRIKSSON, 2008).

According to Ragauskas et al. (2014) in the biosynthetic route to form these building blocks of lignin structure, eleven enzymes are involved. The process was thought to be fully understood. However, due to its complexity, some reviews suggest new concepts to learn since new enzymes were found to be part of it (BONAWITZ et al., 2014; VANHOLME et al., 2013).

The chemical composition and amount of lignin characteristics are interfered by specie, cytologic origin, growth conditions and stage and development of the plant (FENGEL, D.; WEGENER, 2003).

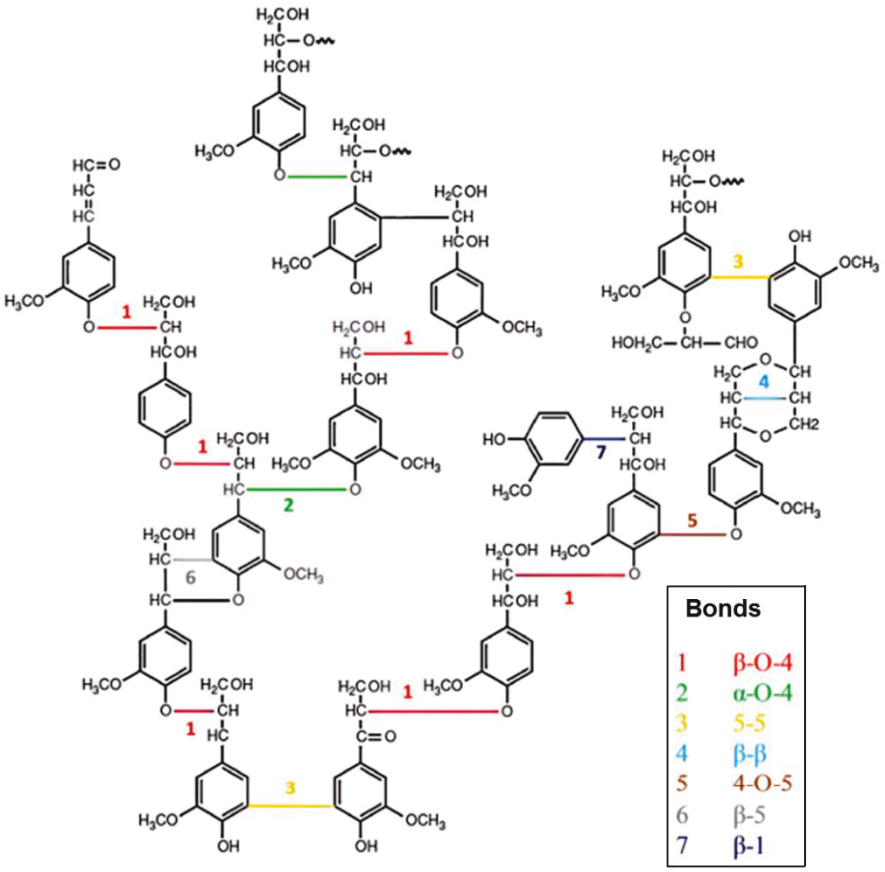
The lignin composition in the wood cell wall is recognized to contain in the S2 (FIGURE I.1) essentially lignin of syringyl (S) type, while the vessels and middle lamella present as predominance the guaiacyl (G) type (FIGURE I.2) (GELLERSTEDT; HENRIKSSON, 2008).

In the hardwoods, as eucalypt, are present S (50-75%) and G units (25-50%), with some traces of H (GELLERSTEDT; HENRIKSSON, 2008). In the softwoods, as pine, the structure is composed exclusively of G group with minor amounts of H (LIN; DENCE, 1992). While grasses present HGS lignin type, with similar levels of S and G and higher amounts of H (5-35%) when compared to *hardwood* and *softwood* (BOERJAN et al., 2003).

As a result of lignin monomeric units, a complex, three-dimensional and highly amorphous polymer are formed. Among the randomly distributed linkages in the lignin polymer, approximated two-thirds are ether bonds and one-third is carbon-carbon bonds (also known as condensed linkages). Lignin polymer is built up basically by phenol and condensed linkages in condensed clusters with no more than 4 units (HENRIKSSON, 2010).

During formation of lignin polymer, the linkage type is driven by the monolignol composition. The phenylpropane units present a series of characteristic linkages with each other and the most abundant is the β-O-4 type (FIGURE I.3).

FIGURE I.3- SOFTWOOD LIGNIN AND THE MAIN LINKAGES BETWEEN THE MOLECULE UNITS



SOURCE: WINDEISEN; WEGENER (2012).

The occurrence of β-O-4 type is further representative in hardwood lignins due the predominance of S type, which does not present the reactive carbon C5 available. In this unit composition, linkages of 5-5 and β-5 type cannot take place (GELLERSTEDT; HENRIKSSON, 2008). Thus, the radicals are unable to attach the 5-position of sinapyl alcohol unit during the biosynthesis polymerization of lignin making the radicals limited to react only with the covalent bonds in the side-chain. Besides, the methoxy group (OCH_3) at C5 makes syringyl lignin units less condensed, which increase their chemical reactivity and make hardwood lignins more susceptible to alkaline attack, becoming easier to remove and more degraded during kraft pulping process (DEL RÍO et al., 2005).

On the other hand, the available C5 in guaiacyl units, allow reactions with other aromatic phenylpropane structures. Hence, becoming harder to be broken. In addition, G lignin have higher molar mass and higher thermal stability, degrading at higher temperatures than S lignin (DEL RÍO et al., 2005). In this sense, different S/G ratios can be found in the cell wall.

The complexity of lignin structure is enhanced by the presence of several functional groups at such branched polymer. The mostly common are the methoxy group (92-96 per 100 phenylpropane units [PPU]), free phenolic hydroxyl groups (15-30 per 100 PPU), carbonyls (10-15 per 100 PPU), benzyl alcohol (15-20 per 100 PPU) and few terminal aldehyde groups (SANTOS et al., 2013).

Methoxyl group is more abundant in hardwood lignin than softwood lignin as it contains more sinapyl alcohol than softwood lignin. The functional groups affect directly or indirectly the final end of lignin as they are related to the molecule reactivity. The amount of phenolic groups, for instance, plays an important part in biodegradation and bleaching as it is the most reactive site of lignin. Only 10-13% of the aromatic rings are in free phenolic positions as the others form ether bonds (HENRIKSSON, 2009).

According to Gellerstedt and Henriksson (2008), lignin structure could be two different macromolecule types. The first one, a structure predominantly based on β-O-4’ linkages, both in G as in S units, with minor amounts of β-β’, β-5’ and β-1’ linkages. The number of 5-5´and 4-*O*-5´ linkages at this lignin is assumed to be very low or zero. As a consequence, the number of free phenolic hydroxyl group is also very low. In the second type of lignin, a more branched structure should prevail; 5-5´and 4-*O*-5´ would be present and the number of phenolic hydroxyl quite high.

The aromatic rings and hydroxyl groups of lignin allow the formation of linkages with hemicellulose and pectins (HENRIKSSON, 2010), forming the known Lignin Carbohydrate Complex (LCC). The LCC content is known to be responsible for the weak delignification and/or lignin that is difficult to remove during the residual stage of cooking (BALAKSHIN et al., 2007).

LCC limits many industrial application of plants, making necessary chemical processes to purification, but also displays unique and diverse pharmacological activities (YOU et al., 2015).

I.2.2 Lignin isolation methods

Among the non-technical lignin isolation methods, the Milled Wood Lignin (MWL) is widely used in an attempt to get close to *protolignin* and obtaining higher percental of isolated lignin. In this method, lignin is extracted after a mechanical disintegration by vibratory or rotatory mill followed by chemical extraction (GELLERSTEDT; HENRIKSSON, 2008). The MWL used to be obtained by employing long and sequential extraction times (*ca.* six weeks, using dioxane-water, 96:4). All isolation procedures tested so far, were looking for the most possible pure lignin obtainment; with less carbohydrates and more conserved structure (GELLERSTEDT; HENRIKSSON, 2008).

In these processes, the precede milling generates certain modifications on lignin structure, as the cleavage of β-O-4' linkages and the introduction of carbonyl groups. As a consequence, new phenolic lignin end-groups are formed with simultaneous fragmentation (GELLERSTEDT; HENRIKSSON, 2008). Thus, even those studies that drastically reduced the milling time (LAWOKO et al., 2006), could not obtain lignin as in it native form.

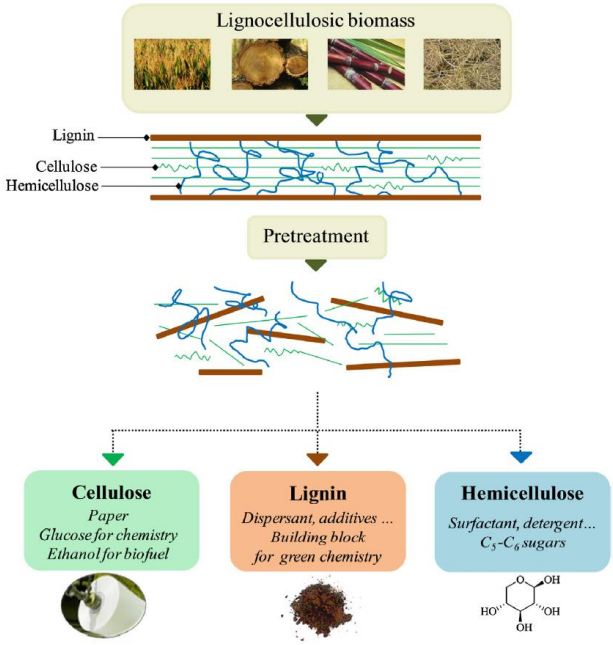
The lignin used as commercial end is not extracted as MWL, though. Nonetheless, all methods developed for deeply understanding about lignin structure and behavior are and will be fundamental for better use of isolated lignin, considering the complexity of such molecule (VISHTAL; KRASLAWSKI, 2011).

Even nowadays, lignin scarcely would be extracted from biomass as first component of interest, at least not for commercial purposes. Usually, lignin is a co-product in the pulp industry or a byproduct of pretreatment and saccharification of biomass (CARVAJAL et al., 2016).

The idea would be to use a known process and recovery lignin as part of this. Thus, being part of biorefinery concept, which has been defined by the National Renewable Energy Laboratory (NREL) as a "facility integrating biomass extraction and conversion processes and equipment to produce fuels, power, heat, and value-added chemicals".

In this framework, pulp mills can be considered fully integrated biorefinery, where wood can be converted into cellulose to produce paper and high value co-products as lignin and hemicellulose (FIGURE I.4), without degrading their functionality (LAURICHESSE; AVÉROUS, 2014).

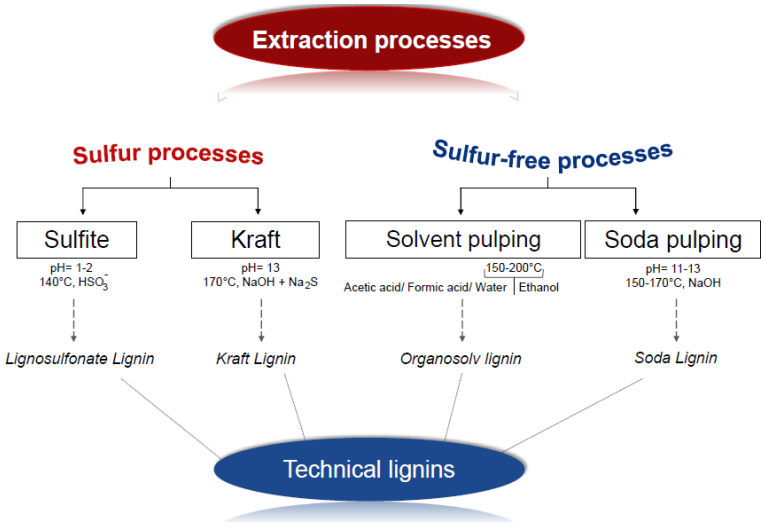
FIGURE I.4- SCHEMATIC CONCEPT OF BIOREFINERY BASED ON LIGNOCELLULOSIC BIOMASS



SOURCE: LAURICHESSE; AVÉROUS (2014).

The most common pulping processes are based on the cleavage of ether and ester bonds, resulting in lignins considerably different from *protolignin*. The available commercial processes that recovery lignin, can be divided in two main categories, sulfur and sulfur-free process (FIGURE I.5).

FIGURE I.5- EXTRACTION PROCESSES AND TECHNICAL LIGNINS



SOURCE: Adapted from Laurichesse; Avérous (2014).

These technical lignins present non-uniform chemical structures, which affect further solubility and reactivity. Besides, the presence of impurities (as inorganics compounds and traces of holocellulose) in the recovered lignin can restrict its utilization as high value-added products. Also, chemical and biochemical conversions become big challenges. On the other hand, technical lignins are readily available in large amounts (VISHTAL; KRASLAWSKI, 2011). In addition, lignin forms large proportion of non-food biomass under consideration for production of renewable fuels and chemical feedstocks (DOHERTY et al., 2011).

According to Heiningen (2006), decreasing in the demand for traditional pulp mills products, competition between emerging economies, limited oil supply as well as incentives to green products, increased the opportunities and urgency for pulp and paper industries to look for novel products from wood beyond paper.

I.2.2.1 Sulfur-free processes

Sulfur-free are an emerging class which present smaller molecular size after isolation. The structure is closer to the native lignin than kraft and lignosulfonate. It can offer attractive sources of phenol and aromatic compounds with low molarmass (LAURICHESSE; AVÉROUS, 2014).

- *Soda*

Soda lignin is based on the sodium hydroxide (NaOH) as delignifying agent. Some processes have used anthraquinone as auxiliary additive (soda-anthraquinone). This is an organic compound considered a redox catalyst during alkali pulping, able to increase the delignification rate, when added in small proportions.

This method presents low yields and lower quality of pulp when compared to kraft. Soda cooking is applied specially to annual plants or agricultural residues, which present low quantities of lignin (GARCÍA et al., 2014).

Lignin from soda cooking, besides sulfur-free characteristic is the closest to native structure of lignin among the technical lignins. However, as the source is usually from non-wood plants, its structure is highly different from lignin from wood, as it contains more *p*-hydroxyl units and carboxylic groups (WÖRMEYER et al., 2011).

Black liquor recovering from soda coking however, is not free from problems, as it comes from annual plants can bring high silicate contents (VISHTAL; KRASLAWSKI, 2011).

Soda lignin have been tested in areas which require high purity and biocompatibility such as phenolic resins (TEJADO et al., 2007), animal nutrition (BAURHOO et al., 2008) and dispersants (NADIF et al., 2002).

- *Organosolv*

During organosolv pulping process, a mixture of organic solvents and water are used in a medium cooking. The most common solvents are acetic acid, formic acid, ethanol and organic peroxide acid (VISHTAL; KRASLAWSKI, 2011).

Organosolv lignins usually present a high purity chemical structure, also considered with the highest quality. These lignins contain many reactive side chains available for further chemical reactions (MEISTER, 2002). Organosolv lignins are soluble in organic solvents and present poor solubility in water since they are very hydrophobic (LAURICHESSE; AVÉROUS, 2014). They are recovered from the solvent by precipitation, varying concentration, pH and temperature (VÁZQUEZ et al., 1997).

This type of lignin can have the same applications as soda and kraft lignin. Nevertheless, due its low molar mass applications in binders and adhesives is rather limited (VISHTAL; KRASLAWSKI, 2011).

I.2.2.2 Sulfur processes

Sulfur lignins are primarily from kraft and sulfite processes produced by pulp and paper industries and mainly correspond to the lignin extraction from cellulose. In both cases the recovering is based on acidifications of the produced black liquor (LAURICHESSE; AVÉROUS, 2014).

- *Sulfite*

About 80 years ago, sulfite was the dominant type among pulping processes. However, sulfite pulp has decreased over half of what was produced in the 1980s, and it is expected to continuing declining (LORA, 2008).

Wood cooking during sulfite is usually at 140-170ºC in an aqueous solution of a sulfite or bisulfite salt of sodium, magnesium, ammonium or calcium. Compared to kraft lignin, lignosulfonate lignin presents usually higher molar mass and broad polydispersity (LAURICHESSE; AVÉROUS, 2014).

Lignosulfonate lignin contains large amounts of sulfur on the aliphatic side chain in the form of sulfonate groups. These groups make lignosulfonates water-soluble and prevent its recondensation. Which would be not desirable during fiber isolation (LORA, 2008).

Water solubility is a remarkable characteristic of lignosulfonates, that mainly differ from kraft lignins and can be seen both as an advantage - when it makes easier further chemical procedures - and as a disadvantage since the remaining carbohydrates appear to be linked to lignosulfonate fractions. All these components are highly soluble in water, making their separation difficult by methods based on solubility differences (LORA, 2008).

As a function of such characteristics, lignosulfonates represent largest applications in many industrial segments such as, binders, dispersive agent, surfactant, adhesives and cement additives (VISHTAL; KRASLAWSKI, 2011).

Lignosulfonates are generally contaminated by the cations used during pulping and recovery processes. Such characteristic can limit their applications since the reactivity depends to some extend on the cation. Calcium and ammonium bases present lower and higher reactivity, respectively, while sodium and magnesium base present medium reactivity (NIMZ, 1983).

- *Kraft*

Kraft lignin represents, by far, the greatest amount of technical lignins produced (NORGREN; EDLUND, 2014; HADDAD et al., 2017). Although kraft has been known as a dominant process, its recovered lignin for conversion into chemicals is still in the first steps up to industrial scale. However, this scenario may change in the next years due to steady efficiency improvements, particularly in the Nordic countries (LORA, 2008). This brings new opportunities for the pulp and paper industries to evolve into modern biorefineries. Actually, the kraft process gained ground in relation to sulfite mainly due its versatility and production of more stronger pulps and more robust chemical recovery process (LORA, 2008).

In kraft pulping the fibrous raw material is disintegrated by a mixture of sodium hydroxide (NaOH) and sodium sulphide (Na_2S) at about 170ºC. In this process 90-95% of wood lignin is dissolved (CHAKAR; RAGAUSKAS, 2004). During the digestion many chemical reactions take place, as the cleavage of lignin-carbohydrate linkages, depolymerization of the lignin, its reaction with hydrosulphide ion and its recondensation (LORA, 2008).

According to Gierer (1986), in the delignification process, occur mainly two types of alteration on lignin structure that can be connected to each other. The first one is related to the cleavage of certain interunit bonds and the second type implies the introduction of hydrophilic groups in the polymer and its fragments.

Lignin depolymerization during kraft cooking occurs mainly by the cleavage of α and β aril ether linkages (SJÖSTRÖM, 1993). First in the phenolic units followed by cleavage in the non-phenolic units (LORA, 2008). These reactions generate free-phenolic groups, ionizable, which increases lignin solubility in the aqueous solution (NORGREN; LINDSTRÖM, 2000).

This is a complex process with severe conditions. Parameters as alkalinity, ionic force and temperature are important to lignin dissociation (NORGREN; LINDSTRÖM,

2000). To recover kraft lignin from the black liquor, the most applied method is to reduce water solubility of lignin as the pH is lowered. As the pH is reduced, the molecule ionization decreased, and self-aggregation takes place. Other components in the black liquor as carbohydrates, their products and inorganics are soluble in water over a wide pH range, making possible to recover lignin with relatively low in ash and carbohydrates (LORA, 2008).

Usually, kraft pulp mills recover lignin from the black liquor by two-step acidification. First adding carbon dioxide gas to pH 9-10 to precipitate lignin (*ca.* 75%) in the form of sodium salt. Next step is followed of heating to coagulate lignin, forming a more easily filterable material or the lignin cake is suspended in water and purified by addition of sufficient sulphuric acid to lower the pH below 3. Filtration, washing and drying processes may be applied (LORA, 2008; VISHTAL; KRASLAWSKI, 2011).

Among the most commercial applications of kraft lignin, the sulphonated products (water soluble) are widely used. This is considered a chemical derivative rather than lignin as such. The introduction of metasulphonated groups in the aromatic rings of lignin is normally accomplished by sulphomethylation. Major applications of these lignins include dyes and agrochemicals dispersants and asphalts emulsifiers. Another product from the black liquor which has commercial application are lignin amine derivatives (LORA, 2008).

The potential of high value-added applications of kraft lignin is uncountable (NORGREN; EDLUND, 2014), however is found under research or at most on a pilot scale (VISHTAL; KRASLAWSKI, 2011). Thus, the few latest mentioned commercial applications of lignin from kraft process, without any modification, are as anti-oxidant for fat during meat rendering and as carriers, adsorbents and UV screens for active compounds and in formulations of crop protection chemicals (LORA, 2008).

I.2.3 Physical properties of isolated lignins

Lignin is an amorphous polymer which behaves as a thermoplastic material; thus, it has a glass transition temperature T_g. According to the plant specie, isolation method, molar weight, thermal history, as well as sorbed water, T_g temperature can vary widely. It has been reported between 90-150ºC (IRVINE, 1984).

In this sense, water has been studied as a mechanism to plasticize lignin, in which small amounts are able to reduce T_g (HATAKEYAMA; HATAKEYAMA, 2005).

Then, chemical modifications on lignin hydroxyl groups, as esterification or alkylation, are strategies to increase solubility of lignin and its ability to undergo melt flow, both desirable in polymer processing and blending (LAURICHESSE; AVÉROUS, 2014).

Thermal decomposition of lignin is a complex process including competitive and/or consecutive reactions. Lignin degradation occurred in a rather broad temperature range, since the numerous oxygen-based functional groups have different stability. The decomposition starts at relatively low temperatures, 150-275 ºC (IRVINE, 1984) with the dehydration of hydroxyl groups located in benzyl group. Then, α- and β-O-4' linkages break between 150-300 °C. From around 300 °C, aliphatic side chains start to break down, up to the cleavage of more stable carbon-carbon bonds at 370-400 °C. Higher temperatures than 500-700 °C lead to complete rearranging of the lignin polymer structure as 30-50 wt% of the lignin becomes char. In addition, volatile products such as CO, CO_2, CH_4 and H_2 are formed (NASSAR; MACKAY, 1984).

I.2.4 Prospects and emerging applications in lignin field

Several efforts including chemical modifications of lignin structure and other simpler approaches as fractionation processes, have been done in an attempt to purify lignin. Among fractionation, methods as solvent fractionation (LINDBERG et al., 1964; LI; MCDONALD, 2014) and ultrafiltration (WALLBERG et al., 2003; TOLEDANO et al., 2010a) are commonly used. Fractionation by acid precipitation (WADA et al., 1962; GARCÍA et al., 2009; WANG; CHEN, 2013; SANTOS et al., 2014) is another modest method widely applied.

In solvent fractionation, usually a sequential extraction with selective organic solvents are used to obtain specific lignin fractions (DODD et al., 2015; WANG et al., 2010; YUAN et al., 2009). More recently, fractionation by antisolvent addition has also been introduced (JÄÄSKELÄINEN et al., 2017; LIANG et al., 2016; SADEGHIFAR et al.,2016) and was said to be a safe and non-toxic method (DOMÍNGUEZ-ROBLES et al., 2017).

Membrane technology allows to separate lignin molecules according to their molar mass, varying the selected cut-offs. Different membrane types and operation conditions distinguish the methods as microfiltration, ultrafiltration, nanofiltration and reverse osmosis (TOLEDANO et al., 2010b). This method presents the possibility of

directly application to spent liquors without pH or temperature adjusts; however, fouling of the membranes and high costs are major limitations.

Acid precipitation is a common method that extracts lignin from black liquor decreasing gradually the pH by the addition of a strong acid. Most acidifications are related to a selective pH, in which the precipitation is made by a specific pH with no sequential fractionation. More recently, a sequential precipitation at low pH (5, 4 and 2) from steam-exploded stalk was studied and, interestingly, the procedure was considered as a fractionation and purification method (WANG; CHEN, 2013).

The obtainment of more purified lignin fractions with well-defined properties allow potentialize its application in many fields. For instance, its antioxidant capacity and bioactivity that can be higher affected by the presence of impurities (inorganics compounds, traces of holocellulose, etc) and its original heterogeneity (DIZHBITE et al., 2004).

The increasing interest in the use of lignin antioxidant properties - due to the phenylpropanoic units and functional groups - is one promising application to lignin valorization. The mentioned structure can act as a neutralizer or inhibitor in oxidation process (GARCÍA et al., 2010). In this sense, lignin has been widely studied in the field of bioplastic (ZHANG et al, 2017), packaging (YANG et al., 2016), cosmetics as sun blocker (QIAN et al., 2015; 2017) and solar panels (GONG et al., 2017).

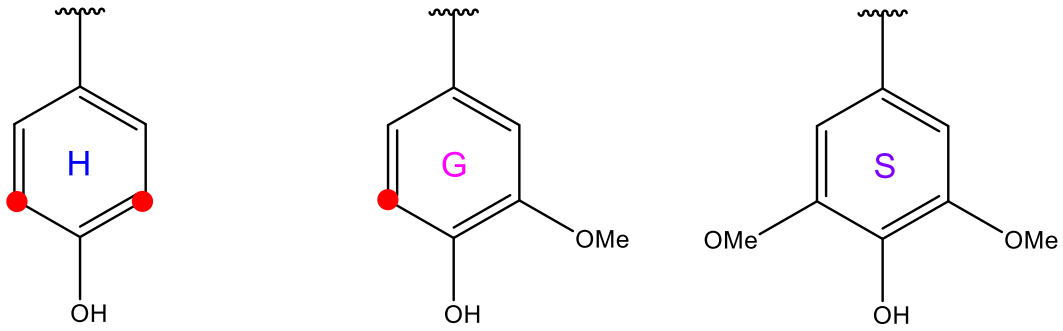
Such functional groups make lignin also of great interest for use in agriculture, in herbicides composition, fertilizers and biocides (WANG; ZHAO, 2013) as in the development of natural bioactive compounds to human health products (UGARTONDO et al., 2008; FIGUEIREDO et al., 2017).

Another potential application among the most promising final ends to lignin valorization is in the synthesis of new polymeric materials. This is the case of phenol formaldehyde (PF) resins in which phenol can be replaced by lignin due the similarity between the molecules (TEJADO et al., 2007). Besides the price and availability of phenol depending heavily on petroleum cost, the growing socio-environmental-economical concerns about scarcity of fossil feedstock, encourages the searching for petroleum replacement. Which justifies the researches highly focused on production of phenolic resins using the natural aromatic material, lignin, to replace phenol (MANSOURI; SALVADÓ, 2006; KALAMI et al., 2017; LORENTE et al., 2017). The replacement of a fossil resource by a natural polymer would be, by itself, enough to justify all efforts on this area; moreover, the prices of technical lignins are estimated to

be 600-800 € per ton while the price of phenol was 1350-1550 € per ton (ICIS, 2017), making the lower price another driving force in this scenario.

In lignin structure, phenolic hydroxyl groups, unsubstituted C3 and/or C5 positions and relatively low and narrow apparent molar mass, are some of the desirable properties for lignin-phenol-formaldehyde (LPF) resin synthesis. These characteristics are dependent on the source of wood used to isolate lignin. Thus, mostly of the studies have focused on the use of softwood or grasses in LPF resins, since G and H lignin present free C3 and/or C5 positions (FIGURE I.6), able to react during resin synthesis.

FIGURE I.6- REACTIVE SITES IN LIGNIN PHENYLPROPANE UNITS



SOURCE: The author (2017).

Moreover, kraft is the only pulping process used in Brazil and hardwood (*Eucalyptus* genus) represent 88% of the used raw material (IBÁ, 2017). Thus, the valorization of this type of lignin became of big interest.

CHAPTER II: EFFECTS OF CHLORIDE AND SULFATE ANIONS UPON PRECIPITATION OF HARDWOOD AND SOFTWOOD KRAFT LIGNIN

II.1 INTRODUCTION

Kraft process is the main method applied for pulping in the world (HADDAD et al., 2016). In this process wood chips are cooked in a digester with NaOH and Na_2S (white liquor) at about 170 °C to produce pulp, during the process the kraft lignin (KL) can be isolated from the spent liquor remaining. In these systems, the lignin depolymerization occurs manly by the cleavage of aryl-ether bonds between the lignin structure subunits in the wood cell wall (GELLERSTEDT; HENRIKSSON, 2008; NORGREN et al., 2002). Furthermore, the depolymerization process is well-known to be driven by several physicochemical phenomena (NORGREN et al., 2002).

KL are generally polydisperse with high molar mass (LOURENÇON et al., 2015; ZHU et al., 2015), and presents a few applications without any modification or purification; however, such processes have been used to improve quality of KL by decreasing its heterogeneity (STIEFEL et al., 2016). As a result, purified lignin could be easily applied for high added-value products such as carbon fiber, antimicrobial and antibacterial material and sun blocker (BAKER et al., 2012; QIAN et al., 2015; WANG et al., 2009).

The extraction of lignin with different organic solvents (LI; MCDONALD, 2014), its ultrafiltration by membrane technology (TOLEDANO et al., 2010a; WALLBERG et al., 2003) and fractionation by acid precipitation (GARCÍA et al., 2009; LOURENÇON et al. 2015; SANTOS et al., 2014; WADA et al., 1962; WANG; CHEN, 2013) are the mainly procedures for its purification.

Concerning to acid precipitation, two main inorganic acids have been used to fractionate/purify lignin through pH slowly reduction, *i.e.*, hydrochloric acid (HCl) (LOURENÇON et al., 2015; WANG; CHEN, 2013) and sulfuric acid (H_2SO_4) (HELANDER et al., 2013). When HCl is used to protonate KL and consequently to induce precipitation, the counter-ion sodium is released to the solution to balance the ion chloride added, which yields the formation of a sodium chloride in the solution ($NaCl_{aq}$). In case of H_2SO_4 addition, the sodium sulfate (Na_2SO_{4aq}) is formed in the solution. The effects of ions on chemical process in aqueous solution depend upon the

ions involved, since salts could induce the precipitation of KL as well (NORGREN; EDLUND, 2003).

Based on Hofmeister series (HOFMEISTER, 1888) it is suspected that sulfate ion (generated by precipitation with H_2SO_4) is more effective than chloride to destabilize lignin hydrophobic surfaces, which may cause a disorder during the KL purification. Since lignin composition differs from the start precursor, the effect of ions upon its recovery through precipitation process might be different. Thus, two different salts (Na_2SO_4 and NaCl) were added to precipitate both hardwood and softwood KL towards drawing conclusions about salting effect in lignin precipitation processes.

II.2 MATERIALS AND METHODS

II.2.1 Raw material

Eucalyptus sp. (hardwood) kraft black liquor was provided by Suzano Pulp and Paper Industry (São Paulo, SP, Brazil), while *Pinus* sp. (softwood) kraft black liquor was given by Cocelpa Pulp and Paper Industry (Curitiba, PR, Brazil).

The lignins used in this study were fractions precipitated from each black liquor. Briefly, diluted hydrochloric acid (12 mol L^{-1} and 1 mol L^{-1}) was slowly added to the black liquor until reach pH 9, then it was centrifuged at 3000 rpm for 15 min. The precipitated hardwood and softwood KL were filtered off, washed with acidified water (*ca.* pH 2), oven-dried (50ºC) and milled in a mortar.

II.2.2 KL characterization

The isolated KL were acetylated before analysis by gel permeation chromatography (GPC) in order to render them completely soluble in THF (5 µg mL^{-1}). GPC analysis were performed in a Waters 1515 HPLC equipped with a Waters 2707 autosampler and a MIL-S800i-v2 interface. Two Supelco TSK-HXL columns (G2000 and G1000) and a G1000Hxl-G4000Hxl guard column were used for separation at 40 °C under an isocratic THF flow rate of 0.8 mL min^{-1}. The injection volume was 20 µL, while the detection was carried out by differential refractometry (Waters 2414) and UV spectrophotometry at 254 nm (Waters 2487). Calibration was made using polystyrene standards.

Fourier transform infrared (FTIR) spectra of the isolated KL were acquired in a Bruker Tensor 37 spectrometer, in the direct transmittance mode. FTIR spectra were recorded from KBr discs containing 1 wt% of lignin in the range of 4000-400 cm^{-1} with a 4 cm^{-1} resolution and 32 scans.

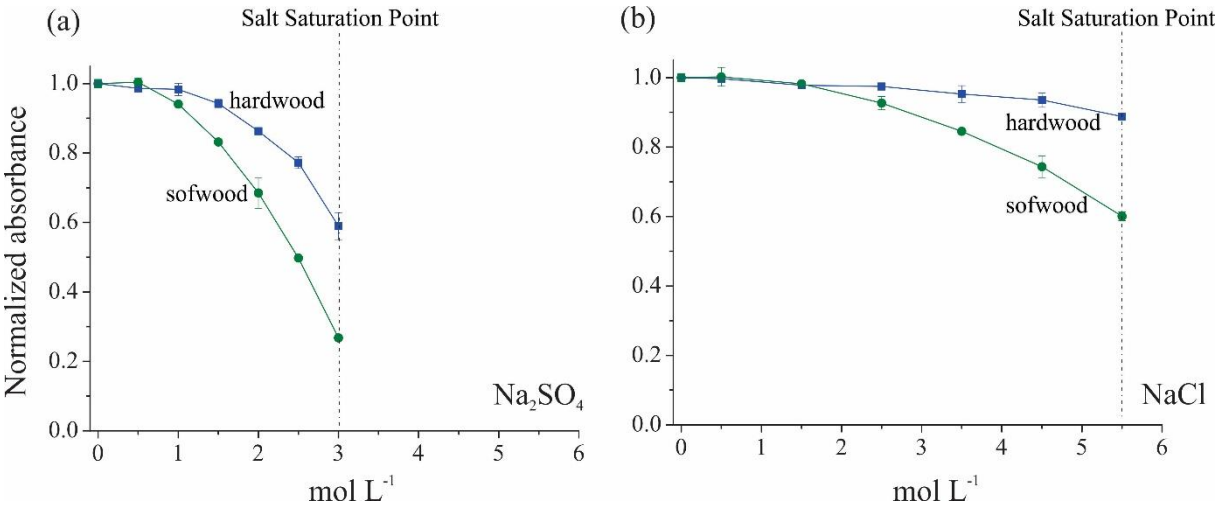
II.2.3 Salt induced precipitation

Stock solution (10 mg mL^{-1}) of the isolated KL were alkalinized with sodium hydroxide (2 mol L^{-1}) until achieve pH 11. Different salt amounts were added in an Eppendorf (2 mL) and diluted with KL stock solutions to achieve the following six concentrations of salts: Na_2SO_4 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mol L^{-1}; and NaCl 0.5, 1.5, 2.5, 3.5, 4.5 and 5.5 mol L^{-1}. The triplicated samples of each salt concentration were homogenized by ultrasonication (20 min, 60 °C), and let rested for 24 hours. The precipitated lignin was separated by centrifugation, then an aliquot (28 µL) from each supernatant was diluted to 10 mL using alkaline solution (sodium hydroxide, at pH 11) to further analyze it by using UV absorbance in the wavelength at 280 nm. Significance differences in each concentration were evaluated by analysis of variance (one-way ANOVA) and Bonferroni test at 95% of confidence level. The UV spectra are normalized against zero salt concentration.

II.3 RESULTS AND DISCUSSION

The absorbance at 280 nm was statistically different from the control in the Na_2SO_4 concentration of 2.5 mol L^{-1} ($p<0.001$) and 1.5 mol L^{-1} ($p<0.05$) for hardwood and softwood, respectively (FIGURE II.1a). A high concentration of NaCl was necessary to promote a significant lignin precipitation, 5.5 mol L^{-1} ($p<0.05$) for hardwood and 3.5 mol L^{-1} ($p<0.01$) for softwood (FIGURE II.1b). Therefore, the ion sulfate showed much more effectiveness than chloride to precipitate both KL (*i.e.* $Cl^- < SO_4^{-2}$). These ions belong to a group of polar water structure makers (kosmotropes) and they have high charge density (ZANGI; BERNE, 2006); thus, arranging a strong hydration complex away from the hydrophobic surfaces of KL resulting in a salting out on the negatively charge KL. From the results of the present investigation it is possible to affirm that the tested anions follows the Hofmeister series, which lists some ions ability in reducing the water activity solvation (HOFMEISTER, 1888).

FIGURE II.1- ABSORBANCE OF LIGNIN PRECIPITATED WITH Na_2SO_4 (a) AND NaCl (b)



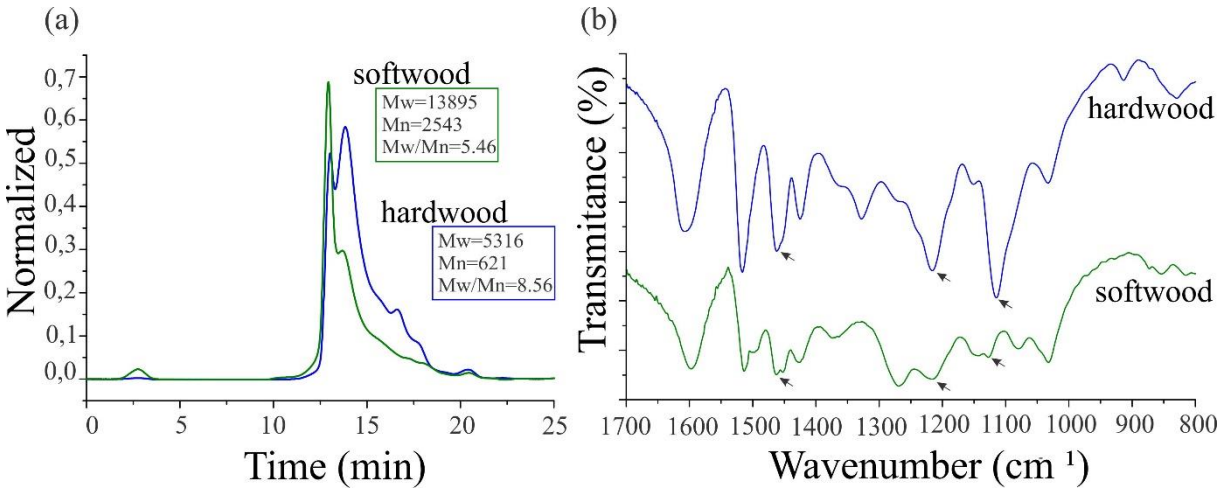
SOURCE: The author (2015).

During lignin precipitation at least two main factors are involved; apparent lignin pKa and molar mass (ZHU et al., 2015). In this work only molar mass could be evaluated, since the pH was kept constant (~11) throughout the experiment.

The two different lignins behaved very distinct concerning to salt induced precipitation (FIGURE II.1). Softwood KL presents bigger fragments and demanded a higher solvation force than hardwood. This destabilization promoted by the inorganic salts resulted in a partial precipitation. The increase of ionic forces into the solution due the salts addition makes many water molecules solvate the added ions; as a consequence, it decreased the available molecules to solvate lignin. If water has lower activity in solvating KL molecules, then more hydrophobic portions are attracted and consequently precipitated, and such behavior is facilitated with higher molar mass as presented by softwood KL (FIGURE II.1).

Hardwood KL showed a low molar mass (FIGURE II.2a) and more fragmented structure as observed by FTIR (FIGURE II.2b) through the accentuated vibrations at 1116-1126 cm^{-1} (related to C-O deformation in ester bond), 1217-1215 cm^{-1} (related to C-C plus C-O) and 1460-1458 cm^{-1} (related to deformation of methyl and methylene groups) (LI; MCDONALD, 2014). The greater amount of syringyl units present in hardwood KL explains the greater degradation of this lignin. These units are more reactive due the methoxy group at C5 and consequently easier to be the attacked by alkaline solution (RAGAUSKAS et al., 2014; DEL RÍO et al., 2005).

FIGURE II.2- MOLAR MASS DISTRIBUTION AND RESPECTIVE MASS-AVERAGE (MW), NUMBER AVERAGE (MN) AND POLYDISPERSITY (MW/MN) FROM GPC OF HARDWOOD AND SOFTWOOD KL (a) AND FTIR SPECTRA OF HARDWOOD AND SOFTWOOD KL (b).



SOURCE: The author (2015).

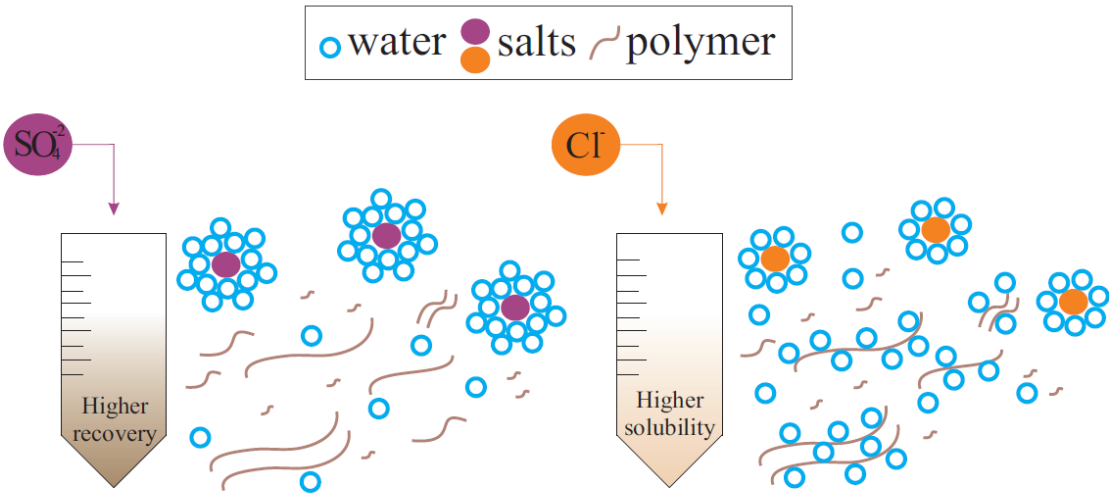
Some aspects should be considered regarding the salting out effects during purification of KL. Zhu et al. (2015), observed increase in recovery yield and a decrease in average molar mass by increasing Na_2SO_4 concentration in a fraction of KL. The lower molar mass may be related to the amount of small lignin subunit precipitated due to salting out effect of SO_4^{-2} anion. In our work, an increasing in the KL recovery was also observed with increasing of salt amount (FIGURE II.1), which was caused by the self-aggregation of small lignin fragments with kosmotropes salt additions.

A salting out effect (FIGURE II.3) is beneficial for a high recovery of KL. When an acid with a kosmotrope anion (*e.g.* H_2SO_4) is used, may yield a lignin precipitation by the parallel effects of decreasing pH and agglomeration. However, salting out effect may be interesting for KL purification since a controlled addition of kosmotropes salts (*i.e.* low concentration) yields a fraction of high molar mass lignin (ZHU et al., 2015). For example, when 2 mol L^{-1} of Na_2SO_4 was added, 50% of softwood was precipitated but only 20% of hardwood KL (FIGURE II.1a, based on UV signal). Thus, we suspect that with an addition of lesser effective salt (NaCl) a higher molar mass KL will be recovered, since only 30% of softwood and 10% of hardwood KL were recovered by addition of 5 mol L^{-1} of NaCl, respectively (FIGURE II.1b, based on UV signal).

In respect to KL purification, an acid addition which generates a water structure breaker - chaotrope anion as HNO_3 - may promote a salting in effect and helps in avoiding precipitation by self-aggregation (LUCK, 1980). Salting in occurs due to a

reduction of water-water hydrogen bonds, which results in more water molecules available to interact with KL polymers and consequently increasing their solubility (ROBERTS et al., 1996). Such approach is interesting for purification of high molar mass lignin (*e.g.* softwood versus hardwood, FIGURE II.1), in which a major KL solubilization may lead to a better separation of KL fragments via protonation with a less degree of agglomeration. Similar effect may be achieve by adding a salting in agent (*e.g.* urea) in the KL solution (MYRVOLD, 2013).

FIGURE II.3 - ILUSTRATIVE *SALTING OUT* EFFECT DURING BLACK LIQUOR PRECIPITATION



SOURCE: The Author (2015).

II.4 CONCLUSION

Hardwood and softwood kraft lignin were precipitated and analyzed after NaCl and Na_2SO_4 addition. The sulfate ion was more effective than chloride to precipitate lignin and a pronounced precipitation was observed for softwood lignin associated to its higher molar mass. Furthermore, controlled kosmotropes salts addition promote a fractionation of hardwood and softwood KL.

CHAPTER III: HARDWOOD AND SOFTWOOD KRAFT LIGNINS FRACTIONATION BY SIMPLE SEQUENTIAL ACID PRECIPITATION

III.1 INTRODUCTION

Lignin can be isolated from wood, annual plants or agricultural residues by different extraction types. The main methods are commercial extractions by the pulp and paper industry that recover lignin through sulfur (sulfite and kraft) and sulfur-free (organosolv and soda) processes (LAURICHESSE; AVÉROUS, 2014). The kraft process is the main method applied for pulping (SMOOK, 2002) and has been highlighted for biorefining purposes (HEININGEN, 2006). In addition, lignin from the kraft process is a realistic prospective to produce high value-added products from biomass (SCHORR et al., 2014) such as chemicals, polymers, adhesives, composites and carbon fibers.

The total amount of lignin produced by the pulp industry is 60% more than what is actually needed for internal power supply (SANNIGRAHI et al., 2010). Currently, 70 million tonnes of lignin are produced annually in the world and only 5% is used in commercial applications (additives, binders, surfactants or dispersants), while 95% is burned for energy production (LAURICHESSE; AVÉROUS, 2014). However, some studies have indicated changes in the near future (RAGAUSKAS et al., 2014) as efficient lignin utilization becomes economically necessary (KIM; DALE, 2004) , and in which biorefining concepts that consider the modification and/or incorporation of the components of lignin to obtain value-added products are an interesting alternative (RAGAUSKAS et al., 2014).

Research on the chemistry of lignin is not recent, but the possibility of scarcity of current raw materials used in oil exploitation (LAURICHESSE; AVÉROUS, 2014) has generated a space for the development of new products and processes based on aromatic renewable materials, such as lignin (NORBERG et al., 2013). It is well known that lignin has an amorphous and complex structure, and that its structure and properties change according to reactions during the pulping process (CHAKAR; RAGAUSKAS, 2004; DOHERTY et al., 2011). However, wide molar mass distribution (YOSHIDA et al., 1987) and wide chemical group composition (YOSHIDA et al., 1990) may be a problem in its utilization for biorefining. In this respect, a simple, easy and low cost fractionation process has been investigated in order to obtain different

fractions of lignin with specific characteristics to be explored as innovative products (LIN, 1992).

The main fractionation methods were related to the extraction of lignin with different organic solvents (LI; MCDONALD, 2014; LINDBERG et al., 1964), ultrafiltration by membrane technology (TOLEDANO et al., 2010a; WALLBERG et al., 2003), and fractionation by acid precipitation (GARCÍA et al., 2009; SANTOS et al., 2014; WADA et al., 1962; WANG; CHEN, 2013). Acid precipitation is a common method that extracts lignin from black liquor, decreasing the pH gradually by the addition of a strong acid (MUSSATTO et al., 2007; SUN; TOMKINSON, 2001; TOLEDANO et al., 2010b). However, most acid precipitation is related to a selective pH, in which the precipitation is made by a specific pH with no sequential fractionation. More recently, a sequential precipitation at low pH (5, 4 and 2) from steam-exploded stalk was studied and, interestingly, the procedure was considered as a fractionation and purification method at the same time (WANG; CHEN, 2013).

This work proposes a distinct fractionation approach, in which pH (9 to 3 pH unit) is modified sequentially from the same single sample. This allows the precipitation of a more purified fraction belonging to a specific pH, thus simplifying the process. Hardwood and softwood black liquor from the kraft process were submitted to sequential acid precipitation. The lignin fractions were physicochemically characterized and had their thermal properties analyzed. A comparison of the softwood and hardwood lignin fractions was also depicted, in order to obtain a better understanding for eucalyptus and pine kraft lignins in the different pH extracts.

III.2 MATERIALS AND METHODS

III.2.1 Materials

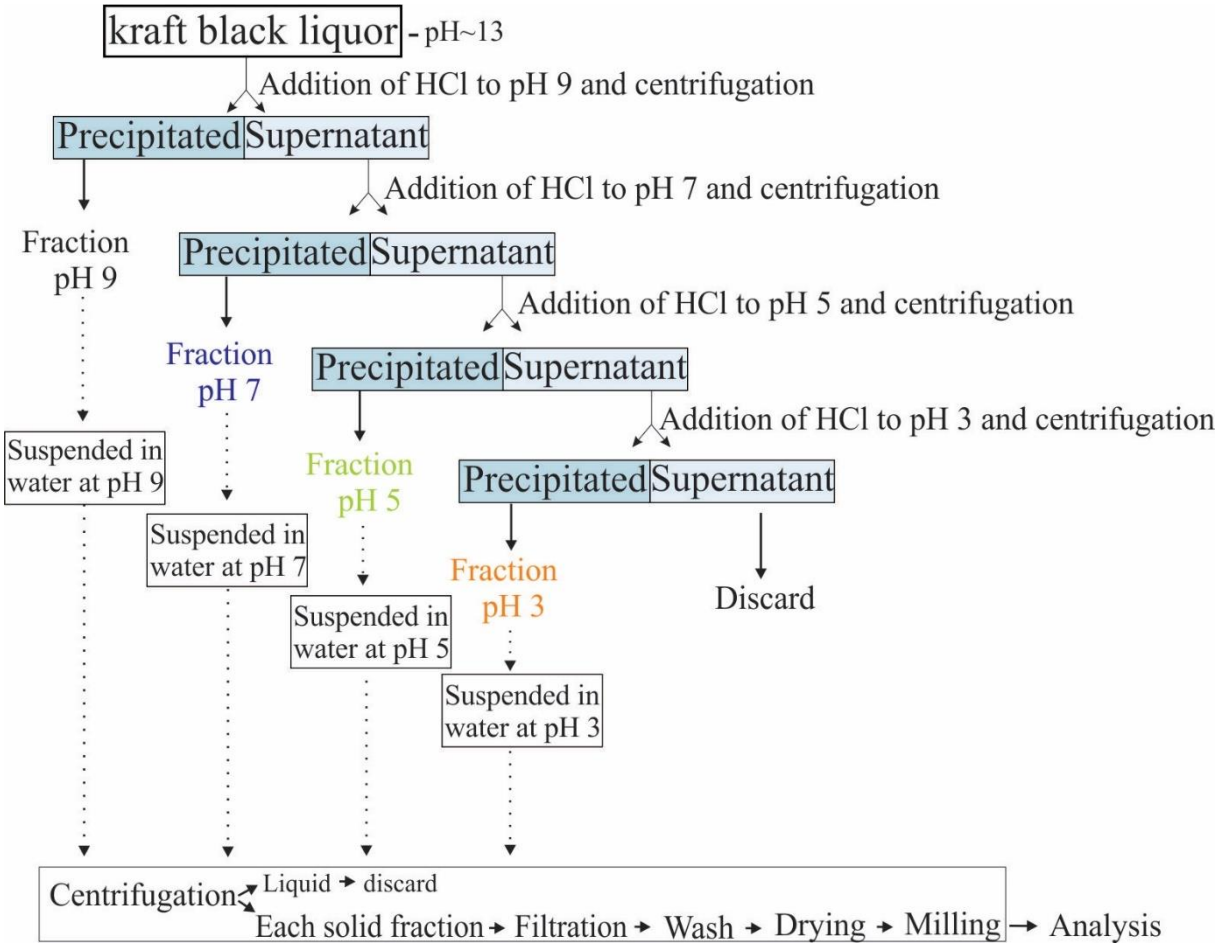
Eucalyptus sp. (hardwood) kraft black liquor was provided by Suzano Pulp and Paper Industry (São Paulo, SP, Brazil) and *Pinus* sp. (softwood) kraft black liquor was given by Cocelpa Pulp and Paper Industry (Curitiba, PR, Brazil).

III.2.2 Lignin precipitation

Drops of hydrochloric acid (started 12M and followed 1M) were added in a single sample of black liquor (~150 mL and pH ~13), until achieve pH 9, followed by centrifugation at 3500 rpm for 15 min. The precipitated fraction was reserved. The supernatant was acidified until pH 7 adding dropwise of HCl (12M and 1M) under agitation. Thereafter the same method was followed to obtain fractions at pH 5 and 3 sequentially (FIGURE III.1), in order to fractionate the lignin component.

Each reserved solid fraction was suspended in aqueous solution at the same pH of the fraction – pH's 9, 7, 5, and 3 - to avoid substances belonging to the other pHs and centrifuged again. The solution was prepared with water and controlling the pH by adding solutions of HCl (0.1M) and NaOH (0.1M). Then, each precipitated fraction was filtered (the liquid was discarded), washed with acidified water (*ca.* pH 2), oven-dried (50ºC) and milled in a mortar. The whole procedure was repeated in three independent replicates in order to test its repeatability.

FIGURE III.1- SCHEME OF SEQUENTIAL LIGNIN PRECIPITATION BY ACIDIFICATION



SOURCE: The author (2015).

III.2.3 Mass yield, ash content, elemental analysis and molar mass determinations

The yield of lignin was gravimetrically determined in the three replicates and each analytical measurement was presented as the average of three independent determinations on each isolated lignin sample after all process. The ash content was determined according to NREL standard (2008), using 10 mg of an oven-dried lignin fraction at 100ºC. Carbon, hydrogen, nitrogen, sulfur and oxygen (by mass difference) contents were determined in a CHNS Elemental Analyzer from Vario Macro Cube using 2.5 mg of the oven-dried fractionated lignin at 50ºC.

Lignin samples were acetylated before analysis by gel permeation chromatography (GPC) in order to render them completely soluble in THF (SCHORR et al., 2014): 50 mg lignin was mixed with pyridine:acetic anhydride under a nitrogen atmosphere (1:1 v/v, 1 mL, 24h) , the reaction medium was diluted with

dichoromethane:methanol (9:1 v/v, 25 mL, rest for 30 min). The solution was cleaned up with HCl (2M, 25 mL, 1x) and H_2O (25 mL, 2x), the residual water of the organic phase was removed by passing the solution through a column packed with Na_2SO_4 (*ca.* 3 g), and the solvent mixture was dried by rotary evaporation. The acetylated lignin was dissolved in dichoromethane (*ca.* 1 mL) and transferred to a glass vial (2 mL), and then dried in a gentle flux of nitrogen and an oven at 50 °C overnight. Gel permeation chromatography (GPC) was used to determine the lignin number-average (Mn) and mass-average (Mw) molar mass as well as the sample polydispersity (Mw/Mn). GPC analyses were performed in a Waters 1515 HPLC equipped with a Waters 2707 autosampler and a MIL-S800i-v2 interface. Two Supelco TSK-HXL columns (G2000 and G1000) and a G1000Hxl-G4000Hxl guard column were used at 40 °C under an isocratic THF flow rate of 0.8 mL min^{-1}. Detection was carried out by differential refractometry (Waters 2414) and UV spectrophotometry at 254 nm (Waters 2487). The injection volume was 20 µL and samples were prepared in THF at 5 µg/mL. Calibration was made using polystyrene standards (436-50000 Da).

III.2.4 Thermal analysis

Thermal properties of the different lignin fractions were studied by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). TGA was carried out in a Shimadzu DTG-60H thermobalance under a nitrogen atmosphere at 20 mL min^{-1}. The weight loss (TG) and the mass loss rate (DTG) were determined. About 5 mg of the sample was heated from 25ºC up to 600ºC at 10ºC min^{-1}. DSC was carried out in a Shimadzu DSC-60A under a nitrogen atmosphere at 20 mL min^{-1}. In this case, samples (~5 mg) were heated at 10ºC min^{-1} from 40 to 180ºC.

III.2.5 Infrared and ^1H NMR spectroscopy

Fourier transform infrared spectroscopy (FTIR) was performed in a Bruker Tensor 37 spectrometer, in the direct transmittance mode. FTIR spectra were recorded from KBr discs containing 1 wt% of lignin in the range of 4000-400 cm^{-1} with a 4 cm^{-1} resolution and 32 scans.

^{1}H NMR spectra of the acetylated lignin fractions were obtained using a Bruker DPX200 operating at 4.7 T at a frequency of 200 MHz. Samples were prepared in deuterated chloroform containing 0.1% TMS.

III.3 RESULTS AND DISCUSSION

III.3.1 Mass yield, ash content, elemental analysis and molar mass determinations

For the hardwood black liquor, the lignin yield decreased gradually as a function of the pH reduction (TABLE III.1). The greatest recovery was obtained at the highest pH value, yielding a lignin fraction with the highest average molar mass (TABLE III.2).

TABLE III.1- MASS YIELD, ASH CONTENT AND ELEMENTAL ANALYSIS OF KRAFT LIGNINS OBTAINED AFTER PRECIPITATION AT DIFFERENT pH

Lignin origin	Content (%)		pH 9	pH 7	pH 5	pH 3
Hardwood	Yield		50 ± 0.1	38 ± 2.3	7.5 ± 2.6	5.1 ± 0.9
	Ash		13.79	14.23	6.99	5.97
	Elemental analysis[a]	C	53.2	53.7	56.9	57.7
		H	4.9	4.8	5.3	5.4
		O	38.7	38.4	34.4	32.6
		S	2.5	2.6	2.9	3.4
Softwood	Yield		49.9 ± 0.5	37.2 ± 6.5	11.0 ± 7	0.8 ± 0.4
	Ash		11.1 ± 3.7	12.2 ± 2.9	13.3 ± 1.8	nd[b]
	Elemental analysis[a]	C	54.1	52.0	52.3	61.3
		H	5.0	4.9	4.8	5.5
		O	37.9	38.9	38.6	28.5
		S	2.4	3.4	3.51	3.8

[a]: Nitrogen content was not considered (~1%), [b]: not detected

Similar behavior was observed for the lignin ash content, which decreased gradually with the pH. The ash in kraft lignin is probably related to its sodium content (SANTOS et al., 2014), which derives from the chemistry of the cooking process. Considering that sodium is the counter ion of soluble lignin, and that during the sequential pH precipitation (mainly for hardwood) richer ash content was detected in

the higher pH, this suggests that a partial protonation of lignin occurred with more ash content. As the pH decreased, a more fully protonated lignin was precipitated (NORGREN et al., 2001).

Interestingly, the molar mass of acetylated hardwood lignins also decreased as a function of the pH reduction (FIGURE III.2a). Two distinct peaks were observed in the normalized GPC chromatograms, the first one at a retention time (Rt) of 13 min. and the latter at Rt of 14 min. The second peak (lower molar mass) was more abundant in all lignin fractions when compared to the first one, which decreased in intensity from pH 9 to pH 3. This indicates that lignins with a higher molar mass were obtained at higher pH values. This was reinforced at a higher elution time (Rt up to 15 min), in which the amount of low molar mass lignins increased their abundance with decreasing pH values (TABLE III.2 and FIGURE III.2a).

On the contrary, for the softwood kraft lignin, a fluctuation in the average molar mass, C, H, and O, and ash content (TABLES III.1 and III.2) were observed in the first three pH fractions (pH 9, 7 and 5). The yields for softwood lignins decreased with pH, similarly to what was observed for hardwood lignins. Nevertheless, both softwood and hardwood acetylated kraft lignins showed different normalized GPC elution profiles (FIGURE III.2). In fact, softwood lignin with higher molar mass prevailed in all GPC profiles (TABLE III.2).

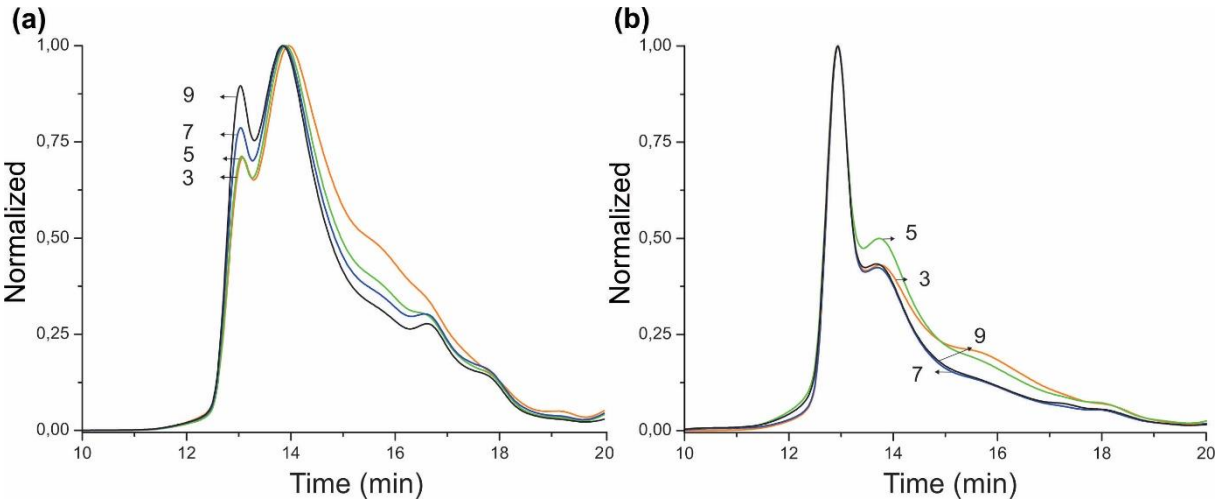
TABLE III.2- MASS-AVERAGE (Mw) AND NUMBER-AVERAGE (Mn) MOLAR MASS AND POLYDISPERSITY (Mw/Mn) OF BOTH HARDWOOD AND SOFTWOOD KRAFT LIGNINS

Lignin from		Lignin fractions			
		pH 9	pH 7	pH 5	pH 3
Hardwood	M_w	5316	4794	4352	3890
	M_n	621	413	442	276
	M_w/M_n	8.56	11.6	9.84	14.08
Softwood	M_w	13895	12443	12643	10301
	M_n	2543	2444	2106	1874
	M_w/M_n	5.46	5.09	6	5.5

Two main factors are involved in controlling lignin precipitation during acid addition in the kraft liquors: apparent lignin pKa and molecular mass (ZHU, 2013).

Before the addition of acid , the lignin was stable in the solution due to negative charges in phenolic and carboxyl groups that repel one another by means of electrostatic interactions (ROBERTS et al., 1996; WANG; CHEN, 2013). It is known that kraft lignin in the alkaline solution forms colloid structures (NORGREN et al., 2001), and that protonation and molecular mass acts concomitantly to lignin precipitation; thus, lignin with high molar mass and small apparent pKa tends to precipitate first due to lower solubility and weaker acid groups (NORGREN et al., 2001). The closely related ash content (*i.e*. sodium counter ion) and average molar mass with pH (TABLE III.1 and III.2) suggest that, despite higher apparent pKa, the solubility is governed by the high molar mass leading to coagulation and precipitation of lignin at higher pH, yielding a partial protonated product. This effect seems to be pronounced in the softwood lignin, for which no clear tendency was observed during precipitation at higher pH (9 to 5) (ash and M_w, TABLES III.1 and III.2). The broader GPC peak is indicative of a wide range of molar mass distribution of kraft lignin fractions (FIGURE III.2), which is formed by self-aggregation of colloidal structures that grow both in number and in size (NORGREN et al., 2001).

FIGURE III.2- MOLAR MASS DISTRIBUTION OF FRACTIONATED LIGNIN FROM HARDWOOD (a) AND SOFTWOOD (b) BLACK LIQUOR



SOURCE: The author (2015).

As highlighted, many high-value products derived from lignin require low ash content levels and high carbon content, such as in the case of carbon fibers (ROBERTS et al., 1996). At the pH 3 fraction, the ash content was the lowest for both hardwood and softwood lignin with high carbon content (> 57%, TABLE III.1), which is

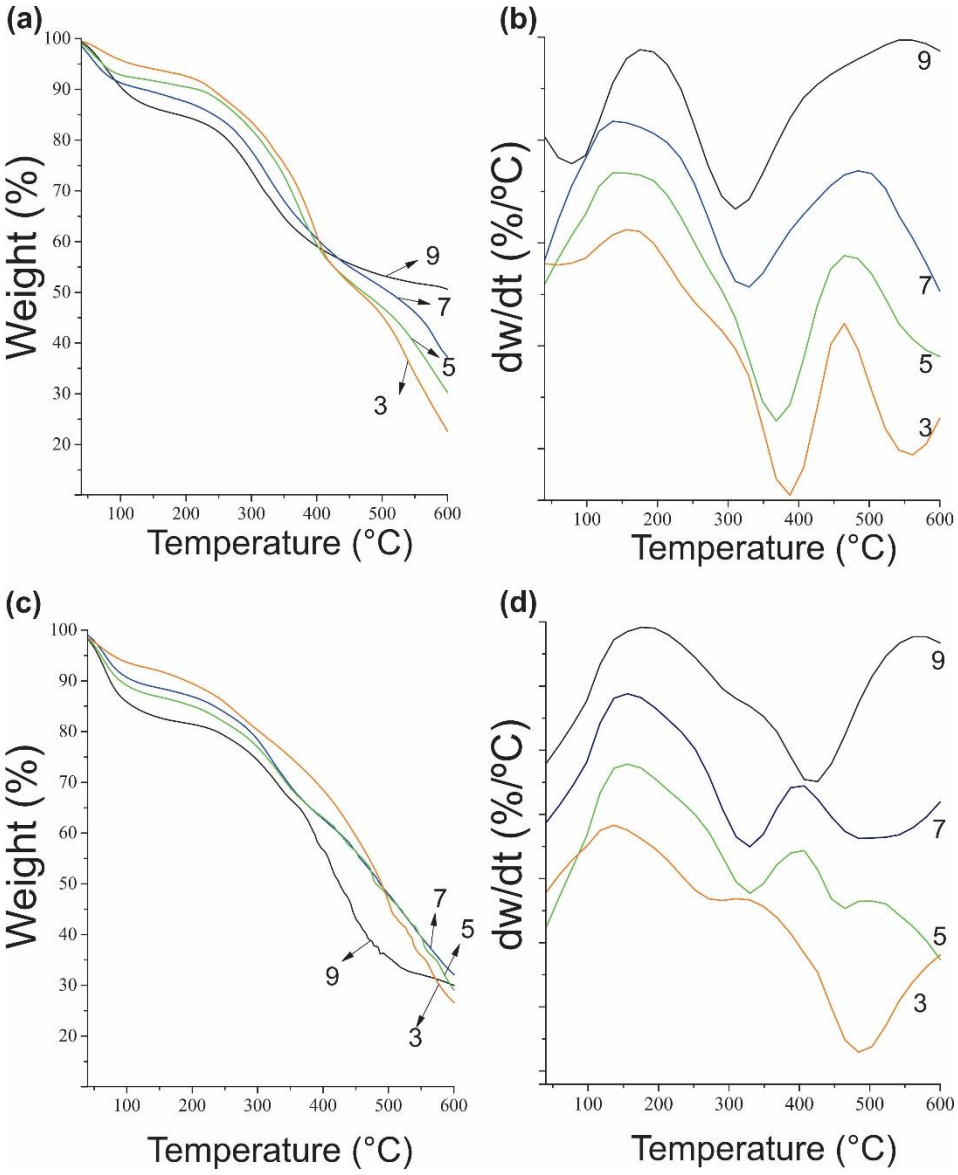
a promising product to explore for such application. However, the small yield obtained of this fraction remains a major disadvantage that needs to be economically evaluated (TABLE III.1).

The sulfur content, mainly in softwood kraft lignins, increased when precipitation was carried out at a lower pH. Dodd et al. (2014) and Helander et al. (2013) observed the same sulfur increment at lower pH lignin fractions. Kraft cooking severely degrade lignin into low molar mass oligomers with a high degree of sulfur substitution; some elementary sulfur is also released as a by-product and may contribute to sulfur content. However, the mechanism of this complex reaction is not fully understood (HELANDER et al., 2013). Compared with the membrane technique, acid precipitation consumes less energy and is considered easier to be used in a large scale (WANG; CHEN, 2013). On the basis of the GPC analyses (TABLE III.2 and FIGURE III.2), acid precipitation at different pHs can be an efficient method for fractionating hardwood and softwood kraft lignins into molecules with different relative molar masses. In this way, high molar mass lignins (high pH) could be used as dispersant or chelating agents, whereas the low molar mass lignins (low pH) would be a good matrix for adhesives (TOLEDANO et al., 2010b).

III.3.2 Thermal analysis

The first mass loss of all lignin fractions occurred at ~75 °C and this is related to the sample moisture content (FIGURE III.3). However, at higher temperatures, the thermal behavior of these fractions was slightly different. Kraft lignins obtained at high pH showed high desorption of water, suggesting the presence of a greater amount of hydrophilic groups in their structure. Toledano et al. (2010a) also found different water desorption behaviors at ~75 °C for lignin fractions by ultrafiltration.

FIGURE III.3- THERMOGRAMS (TG) AND DERIVATIVE THERMOGRAMS (DTG) OF FRACTIONS OBTAINED BY SEQUENTIAL PRECIPITATION OF HARDWOOD (a, b) AND SOFTWOOD (c, d) KRAFT LIGNINS



SOURCE: The author (2015).

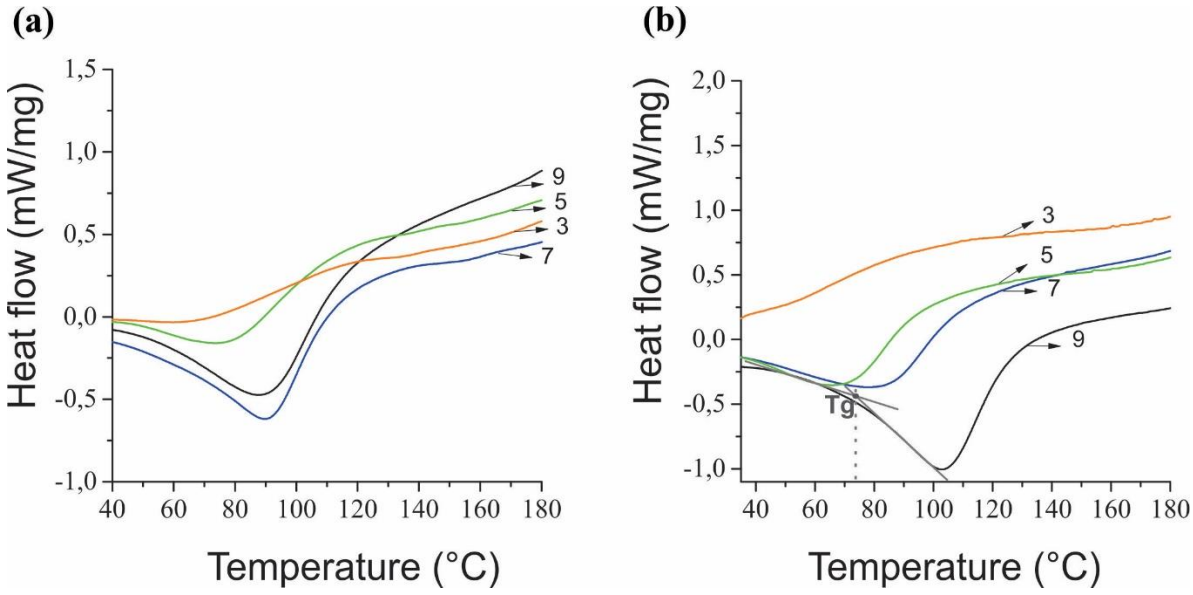
The second mass loss, with an onset around 235 °C, was similar for all lignin fractions. The mass loss around 235 °C was influenced by the remaining hemicellulose during the sequential extraction (BEALL, 1969 ; TOLEDANO; et al., 2010a; YOSHIDA et al., 1987). This degradation intermediate the decomposition between hemicellulose and lignin, suggesting the presence of a lignin-carbohydrate complexes (TOLEDANO et al., 2010b). According to Santos et al. (2014), hemicelluloses tend to precipitate at higher pH; thus, the tendency of a slight shoulder at ~235 °C in the higher pH fractions may be relating to a very small content of lignin-carbohydrate complexes (FIGURE

III.3b and III.3d). The low content of carbohydrates in the lignin fractions were further confirmed by HPLC (Supplementary data 2).

The next thermal degradation event was observed around 300-400 °C, particularly for hardwood kraft lignins, where the fractions obtained at pH 9 and 7 (higher average molar mass, TABLE III.2) displayed a slower degradation pattern. However, lignin degradation occurred in a rather broad temperature range (LAURICHESSE; AVÉROUS, 2014), ranging from around 300 °C, when aliphatic side chains start to break down, up to the cleavage of more stable carbon-carbon bonds, which arises at 370-400 °C (NASSAR; MACKAY, 1984).

The residual mass, related to non-volatile residues including ashes, increased with increasing molar mass (pH 9, 7 and 5, respectively) and this was consistent with the observed ash content in TABLE III.1. Such behavior has already been observed by other authors, working with kraft lignin fractionation by successive extraction with organic solvents (YOSHIDA et al., 1987; YUAN et al., 2009).

FIGURE III.4- DSC THERMOGRAMS OF FRACTIONS OBTAINED BY SEQUENTIAL PRECIPITATION OF HARDWOOD LIGNIN (a) AND SOFTWOOD LIGNIN (b)



SOURCE: The author (2015).

The glass transition temperatures (Tg) for all lignin fractions were obtained from DSC thermograms (FIGURE III.4). Hardwood kraft lignins showed Tg values decreasing from pH 9 and 7 (~65 °C) to pH 3 (~50 °C). For softwood kraft lignins, the highest pH showed a Tg value around ~75 °C and this gradually decreased with a decrease in the precipitation pH. In general, it is known that Tg decreases with

decreasing molar mass (HATAKEYAMA et al., 1975; LAURICHESSE; AVÉROUS, 2014) and this is in agreement with the observed decrease in the corresponding average molar mass (TABLE III.2). Tg variations with kraft lignin fractionation were also observed by Yoshida et al. (1987), in a successive extraction with organic solvents.

III.3.3 Infrared and ^{1}H NMR spectroscopy

FTIR was used for the molecular characterization of the main functional groups present in the precipitated kraft lignin fractions. In general (KIM; DALE, 2004; LI; MCDONALD, 2014, TOLEDANO et al. 2010b), the spectra revealed the most typical vibration modes for lignins, such as O-H axial deformation of associated hydroxyl groups (centered at 3450 cm^{-1}), the C-H axial deformation of CH_2 and CH_3 in aliphatic side chains (2932-2842 cm^{-1}), and the various skeletal vibrations of aromatic rings (1607-1515 cm^{-1}) (FIGURE III.5). TABLE III.3 shows the assignment of the most relevant information of the lignin FTIR spectra.

TABLE III.3- FTIR ASSIGNMENTS CORRESPONDENCE (FAIX, 1991; LI; MCDONALD, 2014; SILVERSTEIN et al., 2005)

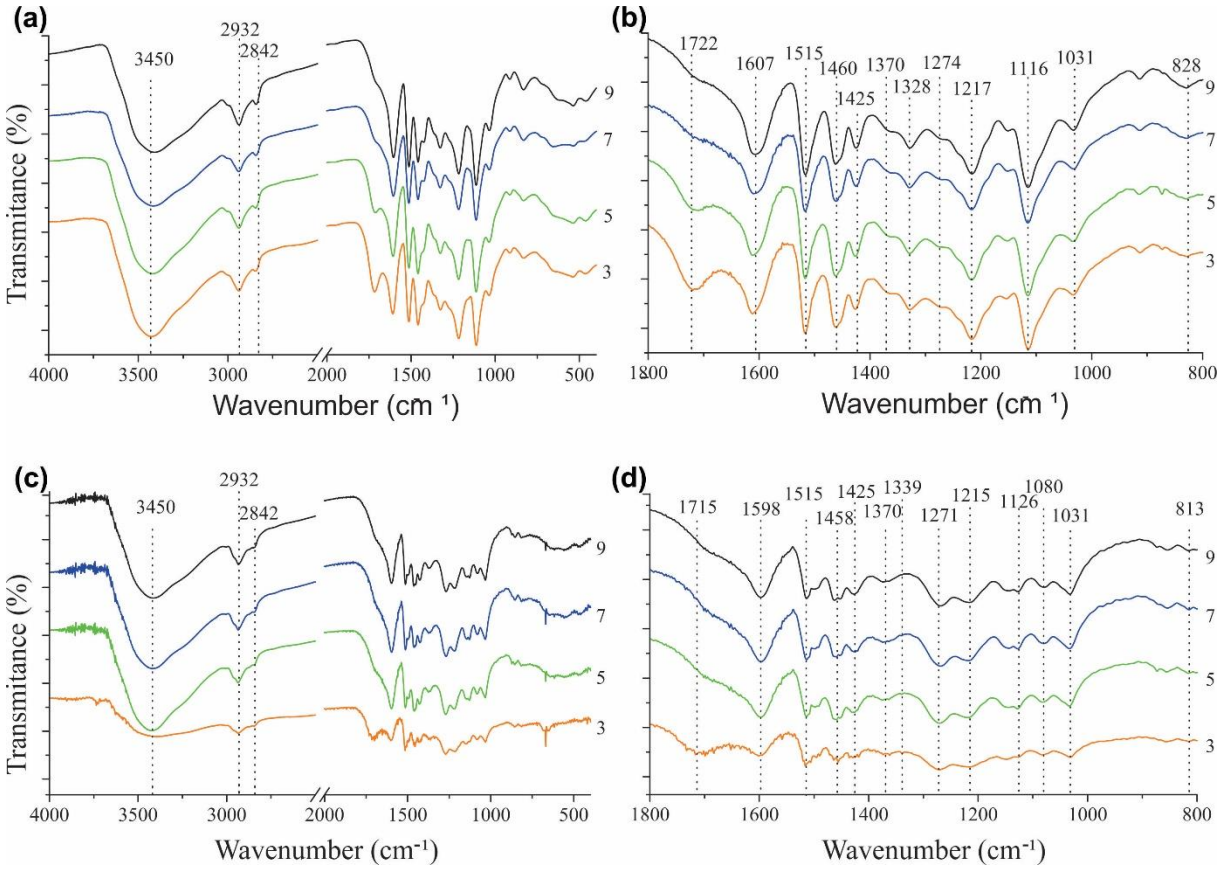
Band (cm^{-1})		Assignments
hardwood	softwood	
3450	3450	O-H stretching
2932	2932	C-H asymmetric stretching
2842	2842	C-H symmetric stretching
1722	1715	C=O stretching
1607	1598	Aromatic skeletal vibrations
1515	1515	Aromatic skeletal vibrations
1460	1458	Asymmetric bending deformation of methyl and methylene groups
1425	1425	C-H in-plane deformation with aromatic ring stretching
1370	1370	Symmetric bending deformation of methyl group
1328	1339	C-O of syringyl ring
1274	1271	C-O of guaiacyl ring
1217	1215	C-C plus C-O stretch
1116	1126	C-O deformation in ester bond
-	1080	C-O deformation in secondary alcohols and aliphatic ethers
1031	1031	Aromatic C-H in-plane deformation (G>S) plus C-O deformation in primary alcohols
828	813	C-H out-of-plane in position 2,5 and 6 of G units

The most visible change in the lignin structure is related to the axial deformation of conjugated carbonyl groups at 1722-1715 cm^{-1} (FIGURE III.5 b and d), which increased gradually from pH 5 to 3 for hardwood and at pH 3 for softwood kraft lignins, even though in lesser proportions for the latter. This shows that lignin fractions obtained at lower pH are more oxidized. This result supports the GPC analysis discussed above, since intense lignin degradation may result in oxidized molecules with lower average molar mass at low pH precipitation. The precipitated lignins from softwood kraft liquor showed similar FTIR spectra, indicating more stable molecules with higher molar mass. The basic unit in the softwood lignin is related to the guaiacyl groups, which leads to a more stable lignin due to chemical bound at carbon C5 in the guaiacyl monomers (TABLE III.2).

On the contrary, the greater amount of syringyl groups in hardwood kraft lignins explains why these fractions had a lower molar mass (TABLE III.2) and were more severely degraded than softwood kraft lignins. Syringyl lignin units are less condensed

and present a methoxy group at C5, which increase their chemical reactivity and make hardwood lignins more susceptible to alkaline attack, becoming easier to remove and more degraded during kraft pulping process (DEL RÍO et al., 2005). Therefore, a less oxidized product is pronounced for softwood kraft lignin, probably due to its chemical structure based on guaiacyl units (TABLE III.2).

FIGURE III.5- FTIR SPECTRA OF LIGNIN FRACTIONS OBTAINED BY SEQUENTIAL PH PRECIPITATION OF HARDWOOD (a, b) AND SOFTWOOD (c, d)



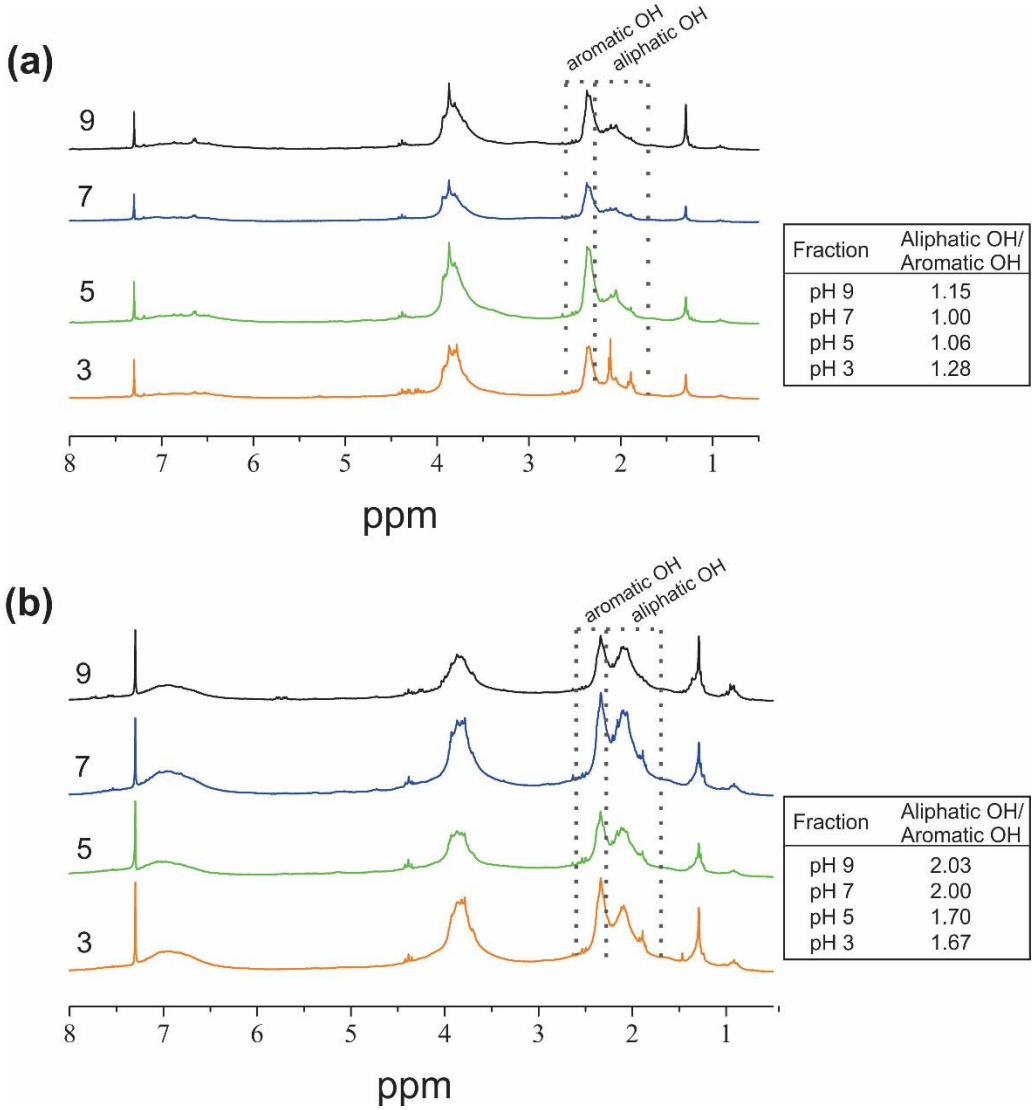
SOURCE: The author (2015).

The lignin-carbohydrate complexes can be evaluated through FTIR at 1030–620 cm^{-1} (GARCÍA et al., 2009, TOLEDANO et al., 2010b), at 1735 and 1043 cm^{-1} (YOU et al., 2015). Thus, the most visible signals related to hemicelluloses in the lignin fractions, were a slight vibration at 1031, 875, 854 and 813 cm^{-1} for softwood lignin at higher pH fractions, suggesting a small content of hemicellulose, as observed by TGA (FIGURE III.3), and confirmed by HPLC (Supplementary data 2). The highest molar mass of softwood (TABLE III.2) gives a more stable, condensed and preserved

structure (TABLE III.2, FIGURE III.5 and FIGURE III.6), explaining its greater amount of hemicellulose in relation to the hardwood lignin.

The analytical technique ¹H NMR was used as complementary characterization of the main functional groups present in the precipitated lignin fractions (FIGURE III.6). The main shifts evaluated are related to aromatic acetate (2.28-2.60 ppm) and aliphatic acetate groups (1.7-2.28 ppm) (LUNDQUIST, 1992).

FIGURE III.6- ¹H NMR SPECTRA OF ACETYLATED LIGNIN FRACTIONS FOR HARDWOOD (a) AND SOFTWOOD (b)



SOURCE: The author (2015).

It is known that during kraft processes the preferential group attacked is the aliphatic substituted in the macromolecular structure of lignin (GIERER, 1985). In the OH aliphatic/OH aromatic ratio (FIGURE III.6), the softwood lignin presented higher

ratio in relation to hardwood, which reflects better preserved structures. This result corroborates with the axial deformation of conjugated carbonyl groups in FTIR analysis (FIGURE III.5).

III.4 CONCLUSIONS

We presented a method to precipitate lignin decreasing sequentially the pH. The new method allows the fractionation of lignin from black liquor according to its molar mass. The methodology is better than others not sequential processes due the purification of the fractions, being the process able to be scaled up for industrial applications.

The lignin fractions precipitated at corresponding pH will be thoroughly characterized in order to be indicated for specific applications. As the fractionation process seemed to work better for hardwood over softwood black liquor, the next chapters will deeply investigate hardwood lignin fractions.

CHAPTER IV: ANTIOXIDANT CAPACITY AND BIOACTIVITY OF KRAFT LIGNIN FRACTIONS

IV.1 INTRODUCTION

Antioxidant compounds are desirable and valuable additives in many fields such as food, medicine, building and fuel in all production stages (processing, treatment, packaging, transportation, or storage) to slow or prevent oxidative degradation of materials. The lack of antioxidant activity can bring one of the biggest problems due the quality and/or shelf life reduction of products.

It is well known that mostly of the commercial antioxidants are from fossil resources. However, the substitution of fossil fuel stocks has gained attention towards to the use of natural antioxidants (KIRSCHWENG et al., 2017). Either because some results of traditional phenolic antioxidants showed health and environmental hazard (BROCCA et al., 2002) or by the idea of use an environment friendly and safe additive (CALEJA et al., 2017). In any case, fortunately, nature provides lignin that could be an excellent choice as antioxidant.

Lignin is a natural renewable phenolic polymer built up of methoxy phenylpropane structures, which depending on the methoxylation degree is called *p*-hydroxybenzyl, guaiacyl or syringil (CHAKAR; RAGAUSKAS, 2004). In plant, lignin protects against biochemical stress, and is responsible for rigidity, bringing strength and avoiding water cell elements to collapse (GRABBER, 2005). Lignin structure also contains different functional groups (carboxyls, carbonyls, phenolic and aliphatic hydroxyls) which become of great interest as natural polyphenol and has been quite exploited regarding to its antioxidant activity. Phenolic compounds present redox properties allowing them to inhibit an oxidation process (GARCÍA et al., 2017). Furthermore, such chemical structure makes lignin very attractive as bioactive agent. According to Sunthornvarabhas et al. (2017) antimicrobial activities from natural extracts present reduced toxicity compared to inorganic antimicrobial agents; therefore becoming a major scientific research topic.

Besides all these natural characteristics, lignin is the most abundant source of aromatic compounds in nature and can be largely recovered as byproduct during pulp and paper production. According to Roopan (2017), although there is a wide number of reports related to lignin application, few are related to biological activities. In this

study, four lignin fractions, obtained in a previous purification process made by a sequential acid precipitation (LOURENÇON et al., 2015), were analyzed regarding to its chemical composition by wet chemistry, ^{31}P NMR, its antioxidant capacity and distinct bioactivities. Two plant/crops phytopathogenic fungi and two pathogenic bacteria (gram-positive and gram-negative) were evaluated in the presence of lignin fractions. The characterizations were chosen in order to make the bases for potential technical applications and deeper scientific investigation as bioactive compounds, putting in perspective a new scenario for hardwood kraft lignin.

IV.2 MATERIAL AND METHODS

IV.2.1 Obtainment of the lignin fractions

The lignin used in this study refers to fractions precipitated from eucalypt kraft black liquor provided by Suzano Pulp and Paper Industry. The fractionation method is described in the Chapter III. Briefly, diluted hydrochloric acid was slowly added to the black liquor until reach pH 9, then it was centrifuged resulting in a supernatant and a precipitate. The supernatant was acidified and centrifuged again to get next supernatant and precipitate parts. This procedure was used to get samples at pH 7, 5 and 3. The precipitated material in each of these pHs were filtered off, washed with acidified water (*ca.* pH 2), oven-dried (50ºC) and milled in a mortar. The yield (%) recovered in each fraction was 50 ± 0.1 (fraction 9), 38 ± 2.3 (fraction 7), 7.5 ± 2.6 (fraction 5) and 5.1 ± 0.9 (fraction 3).

IV.2.2 Physicochemical characterization of lignin fractions

To determine the carbohydrate and lignin composition, the samples were hydrolyzed with sulphuric acid and the resulting monosaccharides were determined by HPAEC with pulse amperometric detection (Dionex ICS 3000A equipped with CarboPac PA1 column) according to NREL standard (2008) and Hausalo (1995). The polysaccharide content in the samples was calculated from the corresponding monosaccharides using an anhydrous correction of 0.88 for pentoses and 0.9 for hexoses. Klason lignin content *i.e.* the insoluble residue from the hydrolysis was

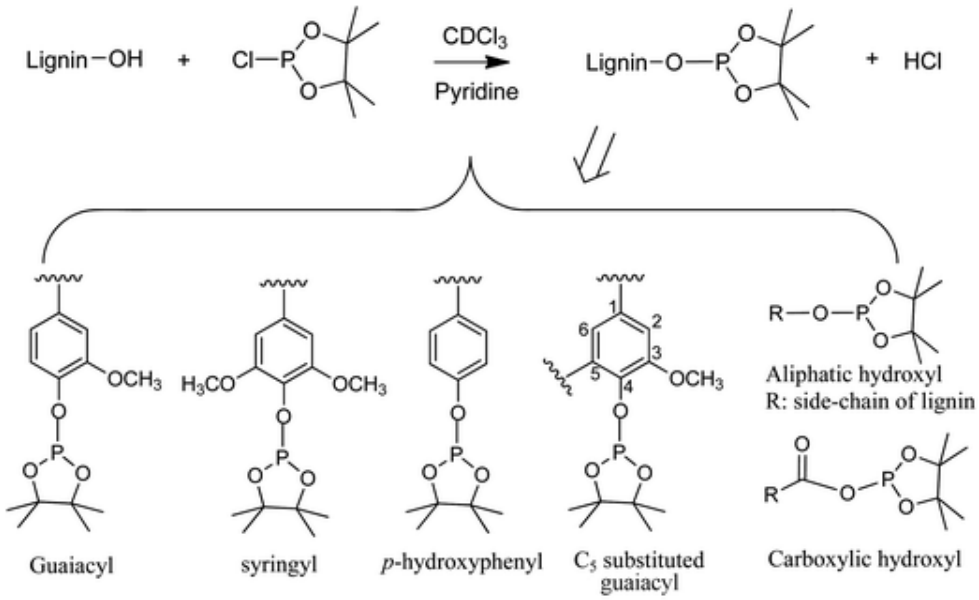
determined gravimetrically. Acid soluble lignin in the hydrolysate was detected at 215 and 280 nm using Equation IV.1, as described by GOLDSCHMID (1971).

$$C = 4.53 \times \frac{(A_{280} - A_{215})}{300} \qquad \text{Equation IV.1}$$

Where C: soluble lignin (g/L); A_{280}: absorbance at 280 nm; A_{215}: absorbance at 215 nm

The number of hydroxyl groups were determined with ^{31}P NMR spectroscopy by using the procedure by Granata; Argyropoulos (1995) and using a Bruker 500 MHz (Billerica, MA, USA) spectrometer. 1024 scans with pulse delay of 5 s, 90° pulse, line broadening of 2 and default baseline correction were used in spectral collection. The quantification limits applied were 150–146 ppm for aliphatic OH groups, 144.2-140.3 ppm for 3 and 5-subsituted phenolic OH groups, 140.3-138.5 ppm for guaiacylic OH groups, 138.5–137 ppm for *p*-hydroxyphenyl groups and 135.7–134 ppm for carboxylic acid groups (BALAKSHIN; CAPANEMA, 2015). The hydroxyl groups in the lignin samples were derivatized with phosphorus-containing reagent (FIGURE IV.1). After total dissolution of lignin in N,N-dimethylformamide, pyridine and internal standard solution (*endo*- N-Hydroxy-5-norbornene-2,3-dicarboximide in pyridine/$CDCl_3$) and $Cr(acac)_3$ in pyridine/$CDCl_3$ were added. Then phosphitylation reagent (2–chloro–4,4,5,5–tetramethyl–1,3,2–dioxaphospholane) followed by $CDCl_3$ were added.

FIGURE IV.1-PHOSPHITYLATION OF HYDROXYL GROUPS IN LIGNIN STRUCTURAL UNITS WITH 2–CHLORO–4,4,5,5–TETRAMETHYL–1,3,2–DIOXAPHOSPHOLANE



SOURCE: (PU et al., 2011).

IV.2.3 Antioxidant and biologic activity of lignin fractions

The Folin-Ciocalteu method was used to determine total phenol content in the lignin fractions. To this purpose, 0.5 mL of lignin fraction (0.15 mg/ml) dissolved in dioxane/water (90:10, v/v) was mixed with Folin-Ciocalteu reactive (2.5 mL) aqueous solution (1:10, v/v) and 2 mL of sodium carbonate (7.5% aqueous solution). The mixture was kept for 2 hours before measuring the absorbance at 760 nm in a spectrophotometer. The total phenol content was determined from the calibration curve of gallic acid standard solution (1- 10 mg/L) and expressed as mg of gallic acid equivalent (GAE)/g of lignin (on dry basis).

The antioxidant activity (AA) of lignin was evaluated as the capacity of the lignin to reduce the free-radical DPPH (2,2-diphenyl-1-picrylhydrazyl) (BRAND-WILLIAMS et al., 1995) using a Shimadzu UV/1800 spectrophotometer. Briefly, 0.1 ml of lignin sample (100, 250 and 500 mg/L) was dissolved in dioxane/water (90:10, v/v) and mixed with 3.9 mL of 6×10^{-5} mol/L DPPH in methanol. A control sample (0.1 ml of methanol and 3.9 ml of 6×10^{-5} mol/L DPPH) was prepared. The absorbances of DPPH (control sample) and lignin samples were measured at 515 nm after absorbances were stable (between 3 – 5 hours, increasing as the concentration increased). The AA was calculated according to Equation IV.2 for the three concentrations of lignin. The higher

the AA, the more efficient the antioxidant. The results were also given as the minimum amount needed to reduce 50% of the radical (EC_{50}). The lower the EC_{50} higher the antioxidant power.

$$ARP, \% = \frac{(A_{control} - A_{sample})}{A_{control}} * 100 \qquad \text{Equation IV.2}$$

where A_{sample} was the absorbance of sample and $A_{control}$ was the absorbance of the control.

The phytopathogenic fungi *Roselinia bunodes*, *Fusarium proliferatum* and *Fusarium oxysporum* were used to test the antifungal activity of the fractions. First, each fraction had the pH adjusted to the same pH as the substract (*ca.* 5.7). Then, the fractions were mixed to the potato dextrose agar (PDA) at three different concentrations 0.1, 0.5 and 1 wt%. All tests had a control sample (without lignin) prepared with the same conditions. The prepared PDA and PDA controls were distributed in Petri dishes (in triplicates). The fungi were inoculated from the same PDA, in the same day and close to the tests. Then, they were distributed in each dish containing 5mm as diameter. The evaluation was made measuring daily the fungus growing (mm) to the maximum allowed to the Petri dish. The results were expressed in % of fungi growth comparing to the control samples. The elected fungi are responsible for many diseases in many economically important crops and trees (CONG et al., 2017; HOOPEN; KRAUSS, 2006).

Antibacterial activity of the lignin samples was evaluated against two bacteria. *Escherichia coli* (gram-negative) and *Staphylococcus aureus* (gram-positive) are responsible of major pathogens that result in hospitalization. The lignin fractions were dissolved in Dimethyl sulfoxide (DMSO). The experiment was adapted from *minimum inhibition concentration* (MIC). Measurements were made in microplates at 600 nm using a Spectrophotometry TECNAL, model TEC-BIO-flex and software Softmax Pro6. In this methodology, each of the 4 fractions of lignin were tested in 7 concentrations (0, 0.25, 0.50, 1, 2, 3 and 4 µg/µl) and 12 replications. Both bacteria, previously maintained at -80ºC, were inoculated onto the surface Müeller-Hinton Agar (MHA) in a Petri dish and maintained at 30ºC for 24h. Then, a Drigalski handle of each bacterium was added to 15ml MHA media and incubated at 28 °C and 180 rpm in an Orbital shaker ThermoScientific, model MAXQ6000, for approximately 17 hours (time to

collect the bacterium peak growth). After incubation, the Optical density (OD) was read at 600 nm and according to OD a calculation was made to achieve 0.15 µg/µl as bacterium concentration and final volume as 100 µl. In each volume, the different lignin concentrations were added, and the total volume completed with MHA. A control microplate was prepared without the presence of bacteria and the absorbances (ABS) were subtracted from the ABS containing bacteria; thus, lignin ABS was isolated and only the bacteria growth could be, in fact, evaluated. Each concentration of lignin was calculated against 0% of lignin and the results were expressed in % of growth inhibition.

IV.3 RESULTS AND DISCUSSION

IV.3.1 Physicochemical characterization of lignin fractions

Lignin samples were obtained in a fractionation process from a single hardwood kraft black liquor sample. The resulted fractions presented different composition contents. As can be seen at TABLE IV.1 and Supplementary data 2, carbohydrate amounts were very low and similar among the fractions. Regarding the total lignin content, fractions 5 and 3 presented higher purity, 94.1 and 94.9%, respectively, while fractions 9 and 7 differentiate from the others by the lower amount of total lignin and higher inorganic compounds. This is probably related to the incomplete protonation of the acidic functionalities in lignin at those pH levels. In addition, some inorganic salt may have remained in the samples in the lignin isolation process. Lower purity lignin after acidification process has been previously associated to salt content in its composition (SANTOS et al., 2013).

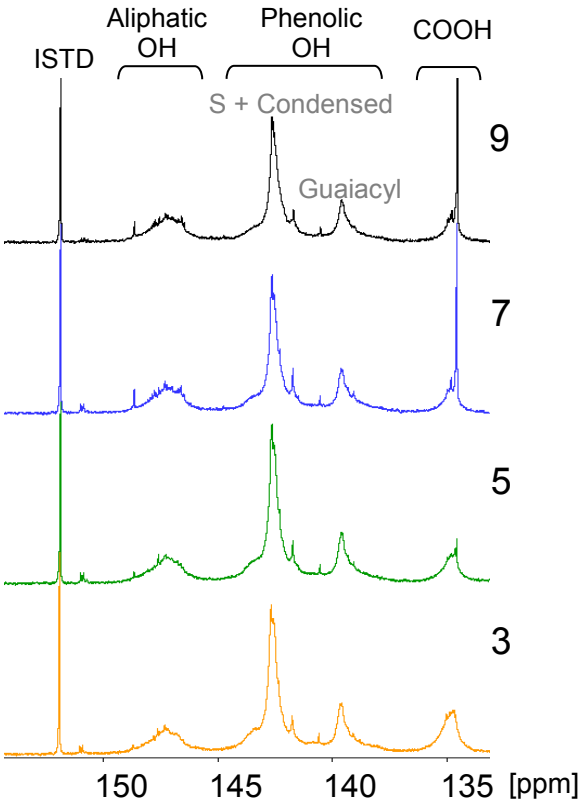
TABLE IV.1- COMPONENT COMPOSITION OF LIGNIN FRACTIONS

	Fraction			
	9	7	5	3
Klason Lignin (%)	70.4 ± 0.4	73.0 ± 0.9	83.5 ± 0.8	78.3 ± 0.2
Soluble Lignin (%)	9.7 ± 0.1	9.2 ± 0.3	10.6 ± 0.5	16.6 ± 0.7
Polysaccharides (%)	1.9 ± 0.0	2.0 ± 0.03	2.1 ± 0.02	2.5 ± 0.01
Ash (%)*	13.79	14.23	6.99	5.97
Mass Average (Mw)*	5316	4794	4352	3890

*From Chapter III.

The NMR spectral shifts (FIGURE IV.2) revealed high contents of syringyl (S) groups in all lignin fractions structure, which was expected since these lignins are from hardwood type (GELLERSTEDT; HENRIKSSON, 2008).

FIGURE IV.2- ^{31}P NMR SPECTRA OF LIGNIN FRACTIONS



SOURCE: The Author (2017).

From the ^{31}P NMR spectra, the number of hydroxyl groups in aliphatic, phenolic and carboxylic acid moieties were determined (TABLE IV.2) after derivatizing the hydroxyl groups in the lignin samples with phosphorus-containing reagent. From this analysis we can thus also evaluate the functional groups among lignin fractions (BALAKSHIN; CAPANEMA, 2015). As can be observed, phenolic S, G and H contents are increasing gradually in the fractions obtained at lower pH (5 and 3), which resulted in higher overall content of phenolic hydroxyl groups in same proportion. Large molecules are less degraded, contain less phenolic hydroxyl groups and thus aggregate and precipitate first. Consequently, smaller structures (as fraction 3, TABLE IV.1) require more acidity to protonate. This dependency has been reported earlier (ZHU et al., 2015), and the theory was now confirmed by ^{31}P NMR results.

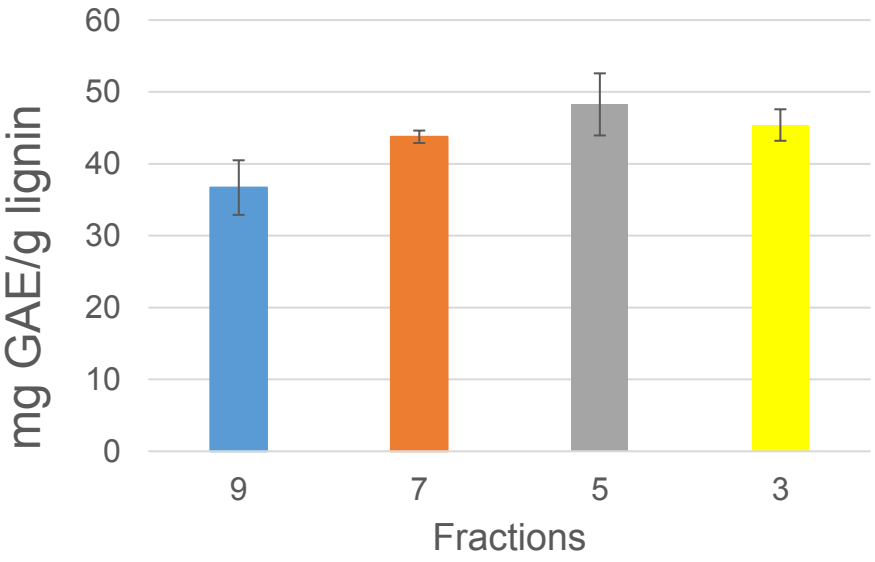
TABLE IV.2- NUMBER OF ALIPHATIC AND PHENOLIC HYDROXYL GROUPS AND CARBOXYLIC ACIDS IN LIGNIN FRACTIONS

Sample ID	Total Aliphatic OH	Total Phenolic OH	S + G_{cond}	G	H	COOH	Total OH
	mmol.g^{-1}						
9	0.99	2.94	2.20	0.65	0.09	0.63	4.56
7	0.98	3.19	2.39	0.71	0.10	0.59	4.76
5	0.92	3.79	2.87	0.82	0.12	0.58	5.30
3	0.83	3.83	2.86	0.83	0.14	0.89	5.54

$S+G_{cond}$= 5-substituted phenolic hydroxyls (S-units and 5-condensed G units); G= free phenols in guaiacylic structures: H = free phenols in para-hydroxyphenyl structures and COOH = carboxylic acids.

Despite the detailed revealed hydroxyl groups of lignins by ^{31}P NMR, the total phenolic content can also be expressed as gallic acid equivalent (GAE) (FIGURE IV.3). The quantification of phenolics as GAE is widely used as a correlation with antioxidant capacity of the investigated material (AADIL et al., 2014; GARCÍA et al., 2017). Here it confirmed high contents of total phenolic groups, mainly in the lower fractions.

FIGURE IV.3- TOTAL PHENOLICS AS GALLIC ACID EQUIVALENT

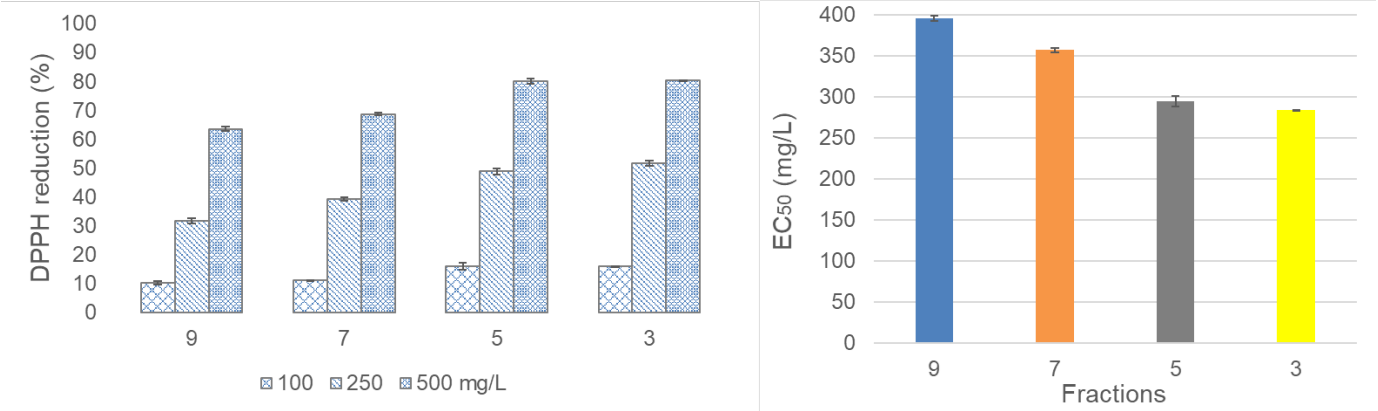


SOURCE: The Author (2016).

IV.3.2 Antioxidant activity (AA) of lignin fractions

In fact, those fractions which presented higher phenolic content (5 and 3), showed higher AA (FIGURE IV.4). Suggesting the positive correlation between phenolic content and antioxidant capacity. Nonetheless, factors as extraction method and other functional groups present in lignin structure, as methoxyl and carboxyl, can also act as electron donors and neutralize the free radical (AADIL et al., 2014). Fractions 5 and 3, besides present higher free phenolic hydroxyl groups, presented lower molar masses (TABLE IV.1), which have been suggested as a factor that increases radical scavenging activity (DIZHBITE et al., 2004).

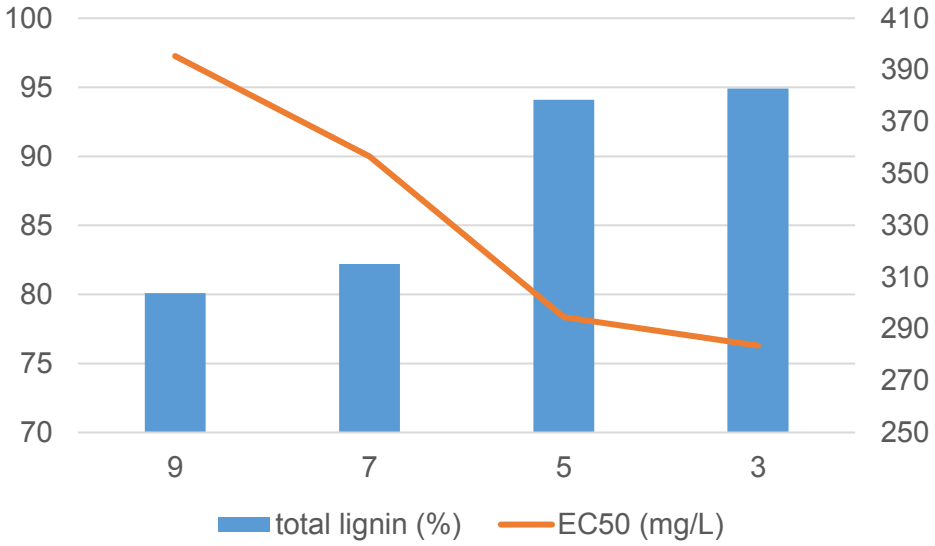
FIGURE IV.4- ANTIOXIDANT CAPACITY OF LIGNIN FRACTIONS; DPPH REDUCTION AT THREE DIFFERENT CONCENTRATIONS (LEFT) AND EC_{50} VALUES (RIGHT)



SOURCE: The Author (2016).

An increase over the radical reduction can be observed as function of concentration (FIGURE IV.4). All lignin fractions showed a satisfactory scavenging activity (>30%) from 250 mg/L. At higher concentration (500 mg/L) all fractions presented AA over 50%. In this case, 5 and 3 kraft lignin fractions presented scavenging activity over 80% (lowest EC_{50} values), quite superior to 30% found by García et al. (2010) in a study with three other types of lignin from *Miscanthus sinensis*, using this same concentration.

FIGURE IV. 5- LIGNIN CONTENT vs. EC_{50} OF LIGNIN FRACTIONS



SOURCE: The Author (2016).

Lignin fractions differences in AA, can be strongly related to their composition (heterogeneity and purity) (DIZHBITE et al., 2004). FIGURE IV.5 shows the EC_{50} of lignin fractions decreasing as total lignin content increased. Thus, the presence of non-lignin components as inorganics in those fractions 9 and 7 (TABLE IV.1) reduced proportion of lignin and consequently phenolic and other functional groups, reducing their AA. Although they still present great antioxidant power.

IV.3.3 Bioactivity of lignin fractions

When lignin was added to the PDA media, it was possible to observe some interferences in the fungi growth comparing to a control sample (without lignin addition). The two *Fusarium* (FIGURE IV.6 a and b) evaluated were not affected by 0.1 wt% of lignin increment. In both cases from 0.5 wt% of lignin addition was possible to observe a slight decrease in these fungi growth.

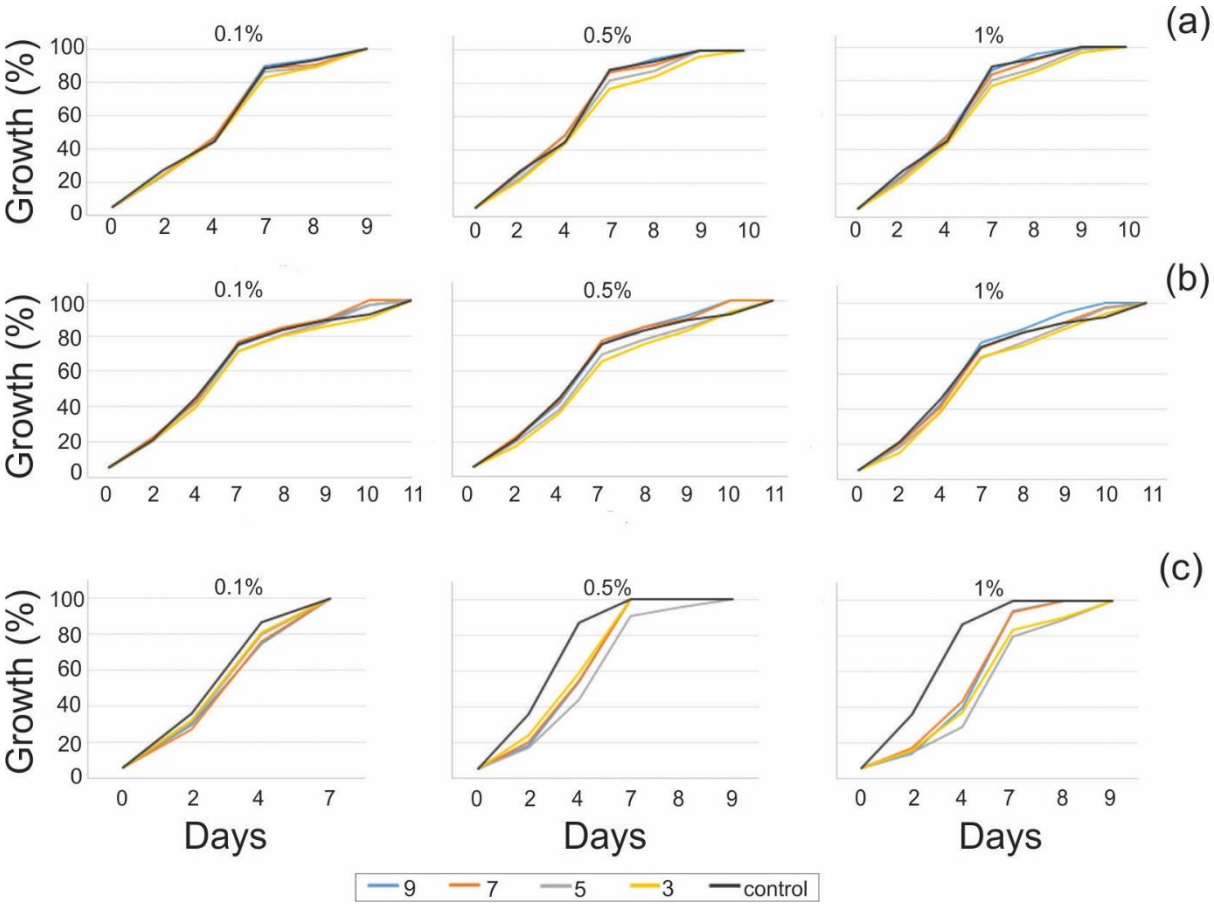
The *Rosellinia bunodes* fungus had a clear inhibition on its growth when lignin was incremented into the agar media (FIGURE IV.6 c). Such observation become more evident with higher increments of lignin. In the case of 1 wt%, after 4 days it can be observed that the control sample has grown 60% more than fungus in the presence of lignin (fraction 5).

Fractions 5 and 3 indeed presented the higher inhibitions (evidenced against *R. bunodes*) to these phytopathogenic fungi. These fractions are potential natural

antifungal to be further tested (*in vivo*). This fungus is responsible for losses in many economically important crops and trees like avocado (*Persea americana*), plantain (*Musa* AAB), coffee, cocoa, lime (*Citrus aurantifolia*), nutmeg (*Myristica fragrans*) (HOOPEN; KRAUSS, 2006).

Fractions 5 and 3, as already mentioned, present higher purity in terms of lignin content (TABLE IV.1) as well as higher total phenolic content (FIGURE IV.3, TABLE IV.2). These phenolic compounds seemed to present certain toxicity to fungi behaving as a barrier to its growth (mainly to *R. bunodes*). However, due molecular complexity of lignin it is difficult to assign this activity to specific structural components.

FIGURE IV.6- GROWTH EVALUATION OF THE FUNGI (a) *Fusarium proliferatum* (b) *Fusarium oxysporum* (c) *Rosellinia bunodes* SUBMITTED TO THREE DIFFERENT CONCENTRATIONS OF LIGNIN ADDITION COMPARED TO CONTROL SAMPLE (NO LIGNIN ADDITION)



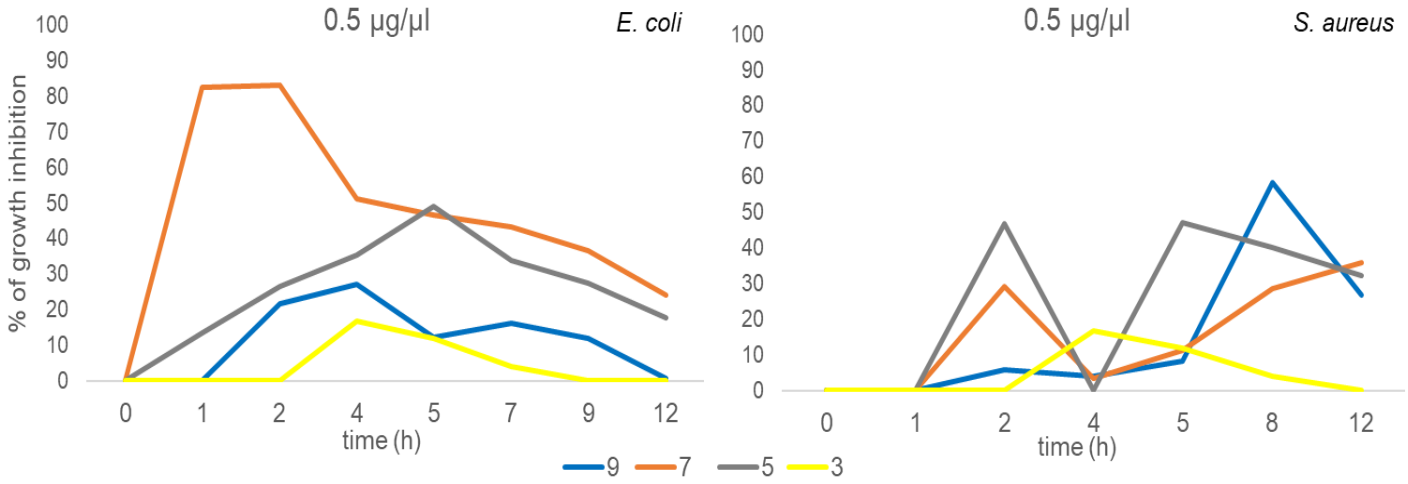
SOURCE: The Author (2016).

Each fraction of lignin was evaluated related to its capacity to inhibit pathogenic bacteria growth as function of time, in five different concentrations of lignin. In general,

all fractions inhibited both tested bacteria to growth even at lower concentrations of lignin (Supplementary data 1).

Differently from antioxidant capacity and antifungal activity - fractions 9 and 7 presented comparable inhibition capacity against both bacteria evaluated. FIGURE IV.7 shows % of bacteria growth inhibition at 0.5 µg/µl, which was the second lowest lignin increment tested (equivalent to 2% of lignin addition). Fraction 7 promoted the greatest inhibition activity against *E. coli*. Even at low evaluated concentration of lignin (0.5 µg/µl), this fraction promoted the highest growth reduction – *ca.* 80% in the first 2 hours - and maintained *ca.* 40% of inhibition over 12 hours. When higher concentrations of fraction 7 were tested, bacterial development was impressively reduced over 12 hours (Supplementary data 1).

FIGURE IV.7- GROWTH INHIBITION OF *E. coli* AND *S. aureus* BACTERIA BY ADDITION OF LIGNIN FRACTIONS AT 0.5 µg/µl OF CONCENTRATION



SOURCE: The Author (2016).

In the evaluation of *S. aureus*, at higher concentrations, an inhibition promoted by fractions 9 and 7 were more evident (Supplementary data 1). However, when lower concentration is evaluated, as in FIGURE IV.7, it can be seen similar effect among lignin fractions, with no trends.

All lignin fractions presented great results against gram-negative *E. coli* than gram-positive *S. aureus*. Higher growth reduction over gram-negative using different extracts have been reported in the literature (PRIYADARSHINI et al., 2013; SHANKAR et al., 2017). The higher inhibition obtained against gram-negative can be associated

to the bacteria cell wall structure. Gram-positive bacteria have thick peptidoglycan layer cross linked by short peptides making extracts difficult to penetrate (SHRIVASTAVA et al., 2007).

Some microbial effects have been reported in the literature but mostly have incremented an inorganic compound as silver nanoparticles to reach this effect (KLAPISZEWSKI et al., 2015; SHANKAR et al., 2017; YANG et al., 2016). However, this type of element can bring damage to the environment (KLAPISZEWSKI et al., 2015). Herein, antibacterial activity was resulted singly from lignin, only purified in a simple fractionation process. Therefore, the obtained results become of great interest to develop new biobased materials.

IV.4 CONCLUSION

All hardwood kraft lignin fractions showed a satisfactory antioxidant activity (AA). The great AA of this renewable and largely available resource, opens a new perspective to their potential use in pharmaceutical industry, food additive, polymers, agrochemicals, etc. Fractions 5 and 3 presented AA over 80% and higher inhibitions against phytopathogenic fungi (evidenced against *R. bunodes*). Higher antioxidant and antifungal activities of these fractions were associated to their higher content of phenolic compounds and to the lower molar mass. Antibacterial results, tested with *E. coli* and *S. aureus*, were very positive even at lower concentrations of lignin. The fractions showed comparable inhibition capacity against both bacteria evaluated.

CHAPTER V: HARDWOOD KRAFT LIGNIN FRACTIONS AS PHENOL SUBSTITUTE IN PHENOL FORMALDEHYDE RESINS

V.1 INTRODUCTION

Phenol-formaldehyde (PF) resins are thermosetting polymers resulted from the condensation of petroleum-based phenol with formaldehyde. These phenolic resins have been used in many industrial applications such as automotive, computing, aerospace, and building (GARDZIELLA et al., 2000), and widely used in the manufacturing of engineered wood products as in plywood, particleboard, laminates veneer lumber, and oriented strand board (STARK et al., 2010).

The price and availability of phenol depend heavily on petroleum cost, becoming one of the biggest disadvantages of PF adhesives. Furthermore, the growing socio-environmental-economical concerns about scarcity of fossil feedstock encourages the searching for petroleum replacement.

In this timely topic, some researches are highly focused on production of phenolic resins using a natural aromatic material, lignin, to replace phenol (MANSOURI; SALVADÓ, 2006; KALAMI et al., 2017; LORENTE et al., 2017). Lignin is the most abundant source of aromatic compounds in nature and can be largely recovered as byproduct during pulp and paper production. Kraft process is the main method applied for pulping, and according to ICIS (2017) the production of this technical lignin is estimated to reach 1.7m tonnes in 2025. Besides, this lignin has been considered a realistic prospective to produce high value-added products (SCHORR et al., 2014), highlighted by the fact that it forms a large proportion of the non-food biomass (DOHERTY et al., 2011).

The replacement of a fossil resource by a natural polymer would be, by itself, enough to justify all efforts on this area; moreover, the prices of technical lignins are estimated to be 600-800 € per ton while the price of phenol was 1350-1550 € per ton in 2014 (ICIS, 2017), making the lower price another driving force in this scenario. However, in spite of all evidenced benefits and researches in the area, still existing barriers to make this an industrial reality.

In lignin structure, phenolic hydroxyl groups with unsubstituted C3 and/or C5 positions and relatively low and narrow apparent molar mass are some of the desirable properties for LPF resin synthesis. These characteristics are dependent on the source

of wood used to isolate lignin. Softwood (*e.g.* pine) and hardwood (*e.g.* eucalypt) are the main raw materials used during kraft process while grasses are used in soda and organossolv processes. Their lignins present distinctions since softwood are composed exclusively by guaiacyl (G) and traces of *p*-hydroxyphenyl (H) while hardwood present syringyl (S), less amounts of G and traces of H. Lignins from grasses present HGS lignin type, with similar levels of S and G and higher amounts of H (LIN; DENCE, 1992). Thus, most of the studies have focused on the use of softwood or grasses in LPF resins, since G and H lignin present free C3 and/or C5 positions, able to react during resin synthesis. However, considering the statistics, Brazil is the second largest pulp producer in 2016 and the raw material for pulp and paper is from eucalypt (IBÁ, 2017), which means that all efforts to find value-added applications for this type of lignin are desirable.

Technical lignins present high molar mass and non-uniform chemical structures, which affect further solubility and reactivity (VISHTAL; KRASLAWSKI, 2011). Besides, the presence of impurities in the recovered lignin can restrict its utilization as high value-added products. To overcome such heterogeneity, fragmentation and/or purification processes have been used (GARCÍA et al., 2009; JÄÄSKELÄINEN et al., 2017; LI; MCDONALD, 2014; SANTOS et al., 2014; TOLEDANO et al., 2010b). To succeed over lignin heterogeneity and making efforts to use this largest available technical lignin, we previously developed a distinct simple purification approach, in which hardwood kraft black liquor was fractionated by sequential acid precipitation (LOURENÇON et al., 2015). Herein, this paper examines the compositions, structural characteristics, and chemical reactivity of these lignin fractions and their performance in LPF resins substituting phenol in 50 wt% by lignin. With this attempt, we hope this work can bring clarification about lignin characteristics needed for replacing phenol in PF adhesives, which goes beyond the frequency of reactive sites.

V.2 MATERIAL AND METHODS

V.2.1 Lignin fractions

The obtainment and physicochemical characterization of the lignin fractions used here are the same described in the chapter IV. Herein, besides the hardwood lignin fractions, a softwood kraft lignin was donated by Technical Research Centre of Finland (VTT) and was used for comparison.

V.2.2 Hydroxymethylation and Resin synthesis

Before resin synthesis a pre-polymerization (hydroxymethylation) experiment was made in order to understand lignin reactivity and the maximum consumption of formaldehyde. Lignin was mixed with NaOH (1:0.65) and formaldehyde was added according to reactive sites (mmol/g) calculated by ^{31}PNMR. Water was added to achieve 10% of dry matter. The samples were heated for 4-6 hours at 60ºC.

According to maximum formaldehyde consumption (mmol/g) the lignin-phenol formaldehyde (LPF) resin recipe was built up, using lignin as 50 wt% replacement of phenol. The dry matter of the resin was 50%. Lignin, NaOH (0.65 equivalent based on total acidic functionalities) and water were mixed at 60ºC overnight, to ensure total dissolution. Then, formaldehyde was added according to maximum consumption, measured by the hydroxymethylation experiment as described above, and mixed at 60ºC for 1h30 to make sure that lignin reacted with formaldehyde. Phenol, NaOH (0.65 equivalent) and formaldehyde (2:1 molar ratio formaldehyde to phenol, 0.67 equivalent) were added. The mixture was maintained at 60ºC for 30 min to ensure hydroxymethylation. The temperature was increased achieving 73ºC (measured directly from reaction mixture). The viscosity was monitored by a viscosimeter targeting 3,5-4,5 Poises (P). When *ca.* 4P was achieved, the reaction was stopped, and the resin immediately cooled in an ice bath. A reference sample without lignin increment - phenol formaldehyde resin- was made following the same above-mentioned steps.

V.2.3 Evaluation of hydroxylated fractions and LPF Resins

Physical properties of the resins

The pH values of the LPF resins were measured at 25 ºC using a digital pHmeter. Gel times were measured after resins preparation. Starting at room temperature, about 0.5 g of each resin was placed in a glass tube and immersed in oil bath at 100 ± 2 ºC, the time count started instantly and then, with constant stirring using a glass rod, the gel point was considered when no further stirring was possible.

Size exclusion chromatography (SEC)

The molar mass measurements of lignin fractions and resins were performed with size exclusion chromatography using alkaline eluent (0.1M NaOH). For the molar mass measurements, the samples were diluted with 0.1M NaOH for the measurement concentration. In all cases the samples were filtered (0.45 μm) before the measurement. The SEC measurements were performed in 0.1 M NaOH eluent (pH 13, 0.5 ml/min, T= 25 ºC) using PSS MCX 1000 & 100000 Å columns with a pre-column. The elution curves were detected using Waters 2998 Photodiode Array detector at 280 nm. The molar mass distributions (MMD) were calculated against polystyrene sulphonate (8 x PSS, 3420-148500 g/mol) standards, using Waters Empower 3 software.
Mn = number average molar mass = $\Sigma M \cdot n / \Sigma n$; Mw = weight average molar mass = $\Sigma M \cdot w / \Sigma w$; PD = polydispersity (Mw/Mn), the higher the PD the wider the distribution.

Free-formaldehyde and Free-phenol by Gas Chromatography-Mass Spectroscopy (GC/MS)

Free formaldehyde was analyzed as oxime by using (Agilent 7697A) Headspace Sampler coupled with Agilent 7890B gas chromatography, and the compounds were detected using Micro Electron Capture Detector (HS-GC-ECD). For calibration, a stock solution containing formaldehyde in water was prepared in the concentration of 1000 ppb. The calibration range was 5-40 ppb and calibration solution dilutions were done

into water. An aqueous solution of the derivatization agent O-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine (PFBOA) (Sigma-Aldrich) was prepared at a concentration of 6 g/L. 100 µl of PFBOA solution (6 g/L) and 10 mL of diluted sample was placed in a 20 mL glass vial and sealed with a crimp cap (Agilent), and run using HS-GC-ECD. For activated lignin and resin samples, the free aldehyde measurements were performed by HS-GC-ECD using HP-5 capillary column, 50 m x 0.32 mm x1.05 µm (J&W Scientific, Folsom, CA). Helium was the carrier gas at a flow rate of 1.0 mL/min and for ECD nitrogen make-up gas was applied at a flow rate of 30 mL/min. From the % of free-formaldehyde measured by GC/MS it was possible to calculate the % of formaldehyde consumed for the known lignin (g) and formaldehyde amount (mmol) need for making the resins.

Free-phenol was analyzed from small amounts (10 mg) of the received resins. The samples were directly trimethylsilylated in pyridine (0.2 ml) with a 0.4-ml mixture (3:1) of N,O-bis(trimethylsilyl)trifluoroacetamide and trimethylchlorosilane, after addition of 3-methoxyphenol as the internal standard. The trimethylsilylated samples were then analysed by GC/MS, using an Agilent 6890 series GC system, equipped with an Agilent 5973 mass selective detector and a DB-5 MS capillary column (30 m x 0.25 mm, film thickness 0.25 µm). The applied temperature programme was 1 min at 70 °C, 10 °C/min to 300 °C, and 9 min at 300 °C. The split ratio of 50:1 was used in the injection. The phenol concentrations were calculated from the peak areas, with the help of a calibration curve (for phenol concentrations from 20 to 60 g/L). The analyses were performed in triplicates.

Automated Bonding Evaluation System (ABES)

The bonding strength of five replicates of each LPF resin (50% replacement) were tested with ABES equipment. Matched conditioned silver birch (*Betula pendula*) veneer sheets (20 x 150 x 0.8 mm) were used. With a micropipette (HandyStep electronic, BRAND GMBH + CO KG, Wertheim, Germany) each resin was applied to an area of 4 x 20 mm² at one end of the veneer specimens to give a resin spread rate of *ca.* 237 g/m². Shear strength was measured after a preliminary test with three different temperatures, based on which 150ºC was settled as the best temperature to evaluate the resins. Various pressing times (45, 90, 180, 300, 480 s) were tested. The press pressure was 2.0 MPa. All resins were tested one day after their preparation.

The quantitative results from 180 s of press time were evaluated by analysis of variance (ANOVA) using ActionStat excel supplement. Differences among averages were compared and segregated by Tukey's test ($p < 0.05$).

Thermal behavior evaluation of the resins

Thermal properties of the resins were studied by differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). TGA was carried out in a Netzsch STA 449F1 under a nitrogen atmosphere at 20 mL min^{-1}. The weight loss (TG) and the mass loss rate (DrTG) were determined. About 5 mg of the sample was heated from 35ºC up to 800 ºC at 10ºC min^{-1}.

2D HSQC NMR

The relative amounts of different structural units were characterized by volume integration of the signals representative to those structures observed in a two-dimensional heteronuclear single quantum coherence (HSQC) NMR spectrum. 2D HSQC NMR spectra were recorded with a Bruker AVANCE III 600 NMR spectrometer with magnetic flux density of 14.1 T, and equipped with a cryogenically cooled 5 mm TCI probe head with inverse geometry (*i.e.*, optimized for proton signal detection). A sensitivity enhanced pulse program (hsqcetgpsisp2.2) that utilizes adiabatic pulses on carbon channel was used in acquisition. All samples were first freeze-dried, after which they were dissolved in D_2O (Sigma Aldrich, 99.96% deuterated), with a concentration of 80 mg in the 600 µl sample volume. Recorded spectral widths were 14 ppm (180 ppm) for proton (carbon), spanning a range from -2 to 12 ppm (0-180 ppm for carbon). The average value for one-bond J-coupling between protons and carbons was set as 145 Hz. The number of scans was 128 for each 256 increments in the indirect detected dimension, delay between successive scans was 1 s, and acquisition time was 120 ms. Prior Fourier transformation the data in directly detected dimension was zero-filled to 2048 real data points, for 13C dimension the data was zero-filled to 1024 points. Gaussian window functions were used for both dimensions. All data was processed using Bruker BioSpin's TopSpin 3.5 software. The integrals were normalized to the

integral of methoxyl signal, as the methoxyl group has been considered to be the most stable structure in the present reaction conditions (YELLE; RALPH, 2016).

V.3 RESULTS AND DISCUSSION

V.3.1 Lignin fractions

These variances in the component composition among the fractions (Chapter IV, TABLE IV.1) make them very attractive to be evaluate in PF resins, since they come from same source, however presenting different characteristics. Thus, the understanding of phenolic resin synthesis can be better understood.

From 31P NMR, described in the Chapter IV, it was possible to observed that with the increasing G and H content at fractions 5 and 3, the number of reactive sites increased as consequence (TABLE V.1). Similar estimated reactive sites from different lignocellulosics have been reported in the literature as from corncob (1.72 mmol/g) (YANG, et al., 2015), poplar wood (0.91-1.39 mmol/g) (YANG et al., 2014), from distinct hardwood lignins (0.65-1.37 mmol/g) and softwood kraft lignin (2.36 mmol/g) (BALAKSHIN; CAPANEMA, 2016).

TABLE V.1 - NUMBER OF ALIPHATIC AND PHENOLIC HYDROXYL GROUPS AND CARBOXYLIC ACIDS IN LIGNIN FRACTIONS AND EXPERIMENTALLY DETERMINED MAXIMUM FORMALDEHYDE CONSUMPTION. ALL UNITS ARE ($mmol.g^{-1}$) WITHOUT CORRECTION FOR LIGNIN IMPURITIES

Sample ID	Total Aliphatic OH	Total Phenolic OH	S + G_{cond}	G	H	COOH	Total OH	Reactive sites[a]	Maximum CH_2O consumption
9	0.99	2.94	2.20	0.65	0.09	0.63	4.56	0.83	0.83
7	0.98	3.19	2.39	0.71	0.10	0.59	4.76	0.91	0.89
5	0.92	3.79	2.87	0.82	0.12	0.58	5.30	1.04	0.99
3	0.83	3.83	2.86	0.83	0.14	0.89	5.54	1.11	0.87
softwood	1.88	4.15	1.83	2.05	0.26	0.50	6.53	2.98	0.76

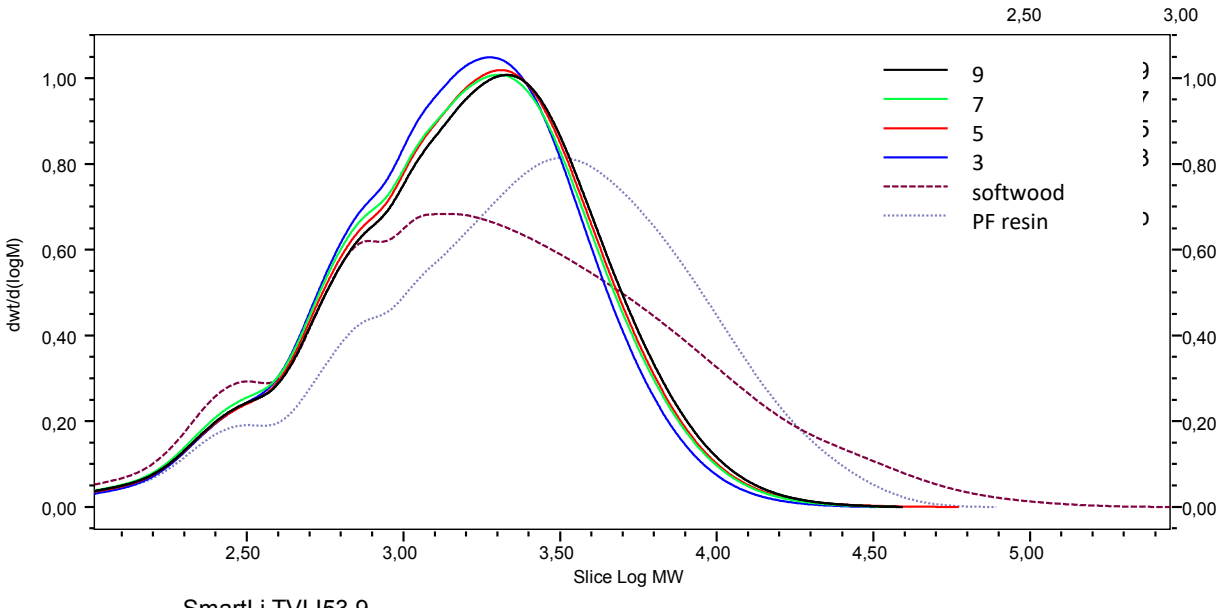
$S+G_{cond}$= 5-substituted phenolic hydroxyls (S-units and 5-condensed G units); G= free phenols in guaiacylic structures: H = free phenols in para-hydroxyphenyl structures and COOH = carboxylic acids.
[a]= G*1 + H*2

V.3.2 Evaluation of hydroxylated fractions and LPF resins

The LPF resins were prepared by replacing phenol by lignin in 50% of substitution. Viscosity of the resins was a stipulated parameter to stop the resin synthesis, then all resins were cooled when achieved about 4 poises. All physical properties measured for LPF resins were very similar to PF resin. The pH's were all about 11-12 and gel time 31-43 min. Percental free-formaldehyde measured was very low for PF resin (<0.05%) and all synthetized LPF resins (≤0.23%). This is a great result for such LPF resins since other studies reported high percental of free-formaldehyde when phenol was substituted by lignin (GHORBANI et al., 2016; KALAMI et al., 2017; LEE et al., 2011). The analyses indicated that in each case the free-phenol concentrations were in the range of 0.5-1.5 g/L, which was also considered satisfactory, mainly if it is compared to other studies using lignin to substitute phenol (PANG et al., 2017; ZHANG et al., 2013).

The molar mass distributions of LPF and PF resins were obtained by size exclusion chromatography (SEC). FIGURE V.1 shows rather similar distribution for all hardwood lignin fractions. On the other hand, these fractions showed pronounced differences, when compared to softwood lignin and PF resins. Hardwood lignin resins present clearly narrower molar mass distributions compared to softwood lignin, which yielded a resin with a significant fraction of high molar mass.

FIGURE V.1- MOLAR MASS DISTRIBUTION OF LPF RESINS AND PF RESIN



SOURCE: The Author (2017).

The calculated molar mass values of the resins, as well as starting lignins, are given in TABLE V.2. An increase in Mw of the resins when lignin was incremented is evident for hardwood samples (*ca.* 35%), while for softwood the average Mw barely increased suggesting low polymerization of resin containing softwood lignin.

There are some controversies in the literature about molar mass effect in resin synthesis. On one hand, it is believed that due the larger number of phenylpropane units per fragment in higher molar mass lignin, higher are the chances to contribute to polymerization, compared to monomeric and dimeric fractions (TEJADO et al., 2007). However, it seems that depending on how much higher the macromolecule is, it can affect negatively, as described above. On the other hand, higher portions of low molar mass molecules, characterized by cleaved main internal linkages, are more suitable to condensate with phenol formaldehyde since the cleavage introduces more active sites (PANG et al., 2017). Fraction 3 exemplifies that lower Mw fractions (TABLE V.2) can possess more theoretical reactive sites (TABLE V.1).

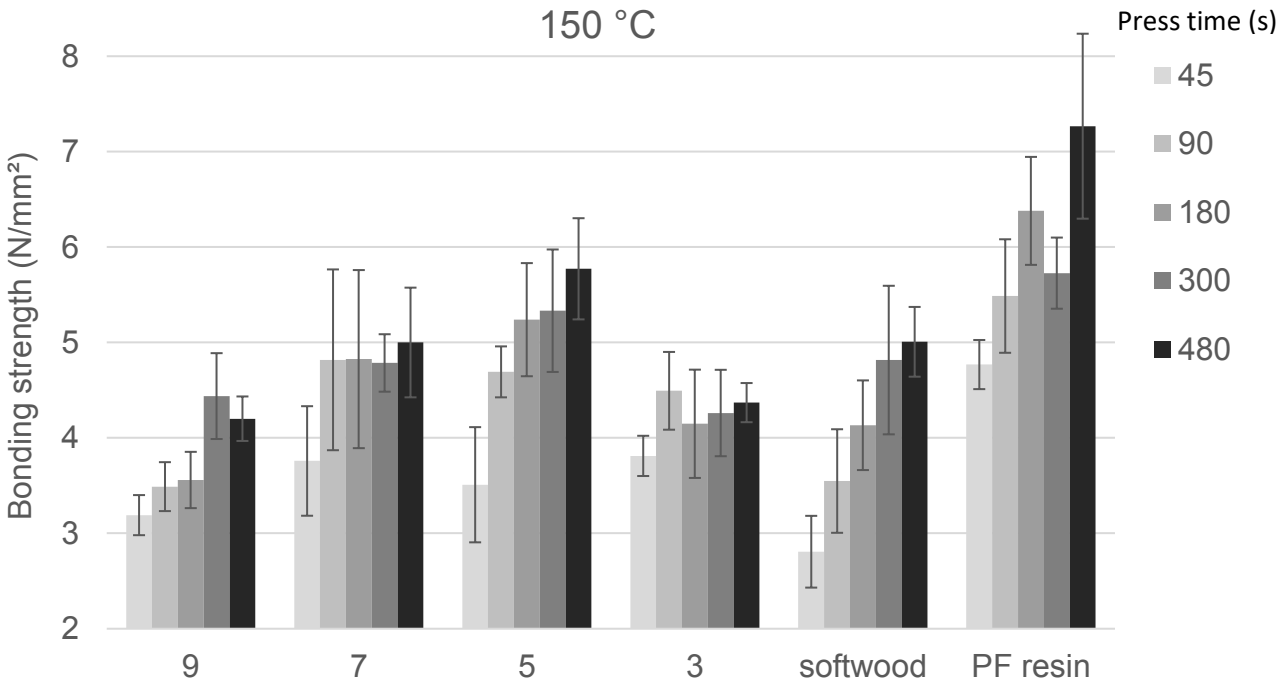
TABLE V. 2- MASS AVERAGE (Mw) AND NUMBER AVERAGE (Mn) MOLAR MASS AND POLYDISPERSITIES (PDI) OF RESINS MADE WITH DIFFERENT LIGNIN AND WITHOUT LIGNIN

	lignins			Resin (50% replacement)		
	Mw	Mn	PDI	Mw	Mn	PDI
9	1.867	973	1.9	2.369	992	2.4
7	1.839	964	1.9	2.247	953	2.4
5	1.847	984	1.9	2.287	982	2.3
3	1.731	957	1.8	2.103	960	2.2
softwood	5.125	2.009	2.6	5.179	970	5.3
PF resin				4.782	1.299	3.7

Theoretical reactive sites were first calculated by ^{31}PNMR to proceed with hydroxymethylation (without presence of phenol). Then, from hydroxymethylation evaluation the formaldehyde consumption has been calculated and compared with theoretical consumption. As can be observed in TABLE V.1, the formaldehyde consumptions determined for all the lignins were in the same range. Furthermore, for hardwood fractions the theoretical reactive sites were very close to the real formaldehyde consumption, while for softwood the real consumption was almost four times lower than the theoretical calculation. In spite of the fact that softwood lignin presents higher theoretical number of sites to react with formaldehyde, they seem not

be fully available. This steric barrier may be due to the significantly higher molar mass of softwood lignin. Thus, even if hardwood lignin only presents few reactive sites, they mostly react with the added formaldehyde. Balakshin and Capanema (2016) observed recently that the amount of reactive sites is not the only important factor in lignin-PF blends, suggesting that other characteristics as steric factors and flexibility of lignin macromolecule could play a more important role. Such lignin structure-performance correlations presented in the literature are insufficient, making very valuable any efforts to develop new models for a broader and deeper understanding of lignin in phenolic resins.

FIGURE V.2- BONDING STRENGTH OF RESINS WITH FIVE DIFFERENT PRESS TIMES



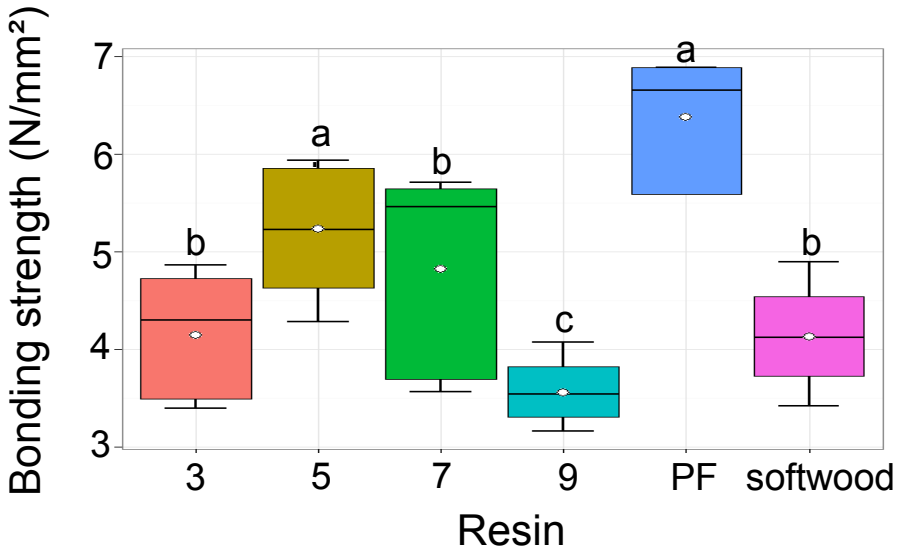
SOURCE: The Author (2017).

In fact, when the bonding strength of resins was evaluated by ABES, hardwood lignin performed with no disadvantage when compared to softwood lignin and even to PF resin. FIGURE V.2 shows each synthetized resin performing at 150ºC in five different press times.

For most of the resins the bonding strength increased as increased press time. To a better comparison between the resins, 180 seconds of press time has been

chosen as a fair condition to be deeper evaluated (FIGURE V.3). Herein, the bonding strength averages are represented by the boxes with their error bars. The average values followed by same letter means that they belong to the same statistical group, at level of 5% by the Tukey test. The increment of 50% of lignin in the phenolic resins led to a reduced bonding performance in the LPF resins when compared to PF without lignin. This is not the case of the hardwood fraction '5'. This resin was considered in the same statistical group as PF resin. Based on these results, it can be inferred that a lower (<50%) replacement of phenol by lignin could represent even a better performance of LPF resins.

FIGURE V.3- BONDING STRENGTH OF RESINS AT 180 s OF PRESS TIME



Note: Average values followed by the same letters are not statistically different at level of 5% by the Tukey test

SOURCE: The Author (2016).

Thermal behavior of PF and LPF resins was evaluated by TG and their DrTG (FIGURE V.4). Post curing, thermal reforming, and ring stripping are the three major thermal events reported for PF resins (CHAOUCH et al., 2014; ZHANG et al., 2013). From the thermogram profiles, it is possible to observe that LPF resins degraded in a broad temperature range.

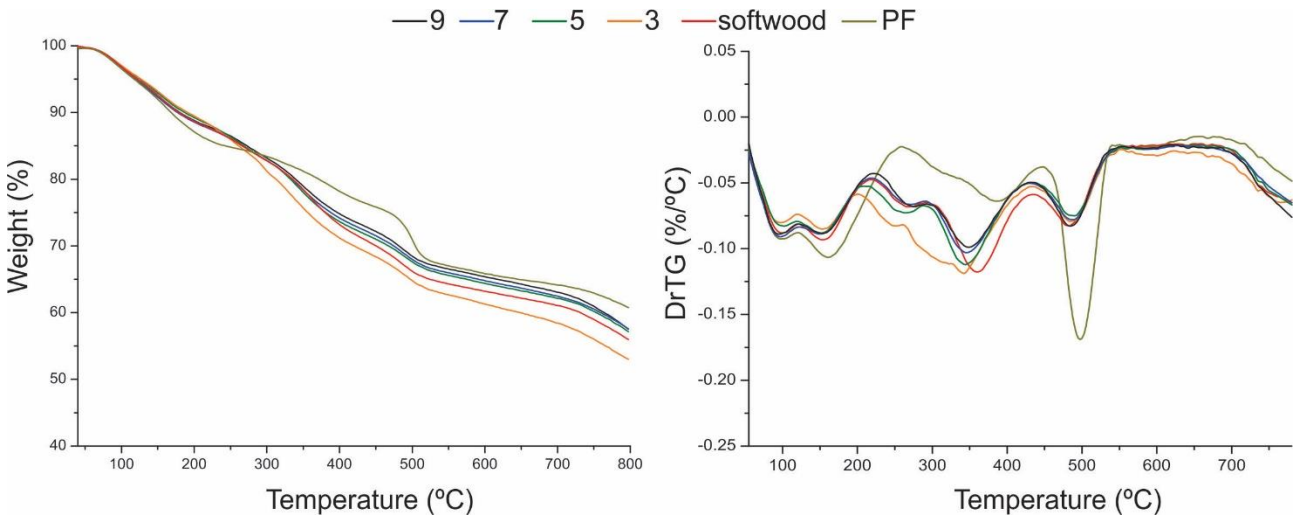
The initial weight loss in the temperature range of 90-300ºC for all resins included water, phenol and formaldehyde evaporation trigged by reaction of

hydroxymethyl groups with each other (KHAN et al., 2004). In this initial stage, LPF resins showed a higher thermal stability than PF resin.

The mass loss observed in the second event, at 390ºC for PF resin, might be due to the loss of water as consequence of the breakdown between phenolic-OH and methylene linkages (KHAN et al., 2004; ZHANG et al., 2013) as well as between two hydroxyl functional groups (LEE et al., 2011). This peak was shifted to lower temperature in the presence of lignin (350 °C). At this point, the higher molar mass softwood lignin (TABLE V.2) showed higher thermal stability among the LPF resins. The lower chemical activity of lignin compared to phenol and the relatively high substitution level of phenol by lignin, may lead to weak cross-linked networks, which may affect the thermal stability of the LPF resins (CHAOUCH et al., 2014). Besides, from *ca.* 300ºC, aliphatic side chains of lignin start to break down, up to cleavage of more stable carbon-carbon bonds, which arises at 370-400ºC (NASSAR; MACKAY, 1984).

The third thermal event at 500 ºC for PF resin was the most drastic thermo-degradation. This peak was also shifted to lower temperature when phenol was 50% substituted by lignin. This last event is associated to released methane and carbon monoxide formed by reaction of eliminated hydrogen and water with methylene, respectively (LEE et al., 2011). From 600 ºC the lower but continuous rate of weight loss is caused by dehydrogenation of aromatic structure in LPF resins (KHAN et al., 2004).

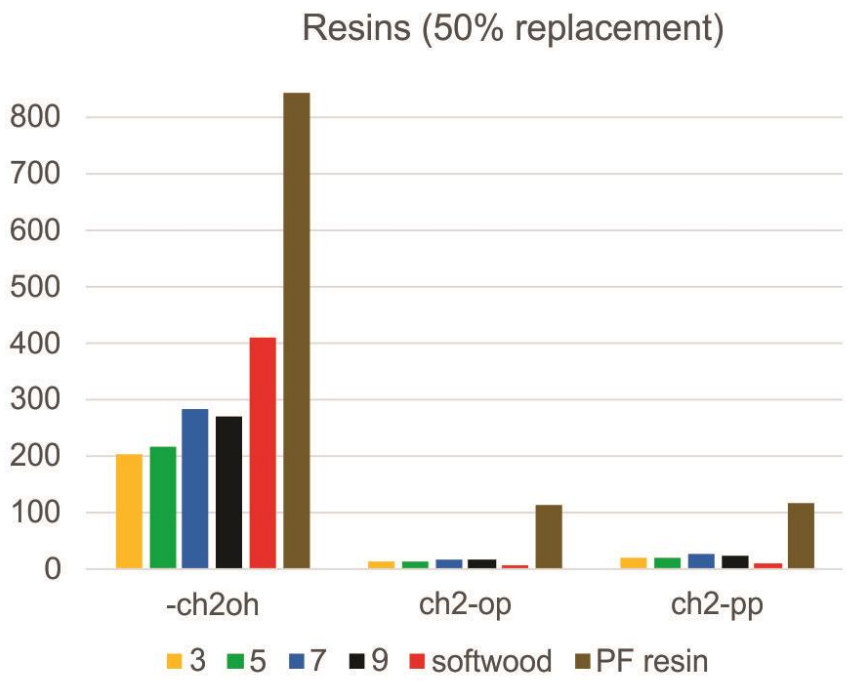
FIGURE V.4 - THERMOGRAMS (TG) AND DERIVATIVE THERMOGRAMS (DrTG) OF PF RESIN AND LPF RESINS 50% REPLACEMENT



SOURCE: The Author (2017).

The LPF resin from fraction 3 showed the highest thermal stability in the initial stage among all resins including PF without lignin. On the other hand, this fraction presented a wider and faster decomposition pattern from 250ºC and lower char content at 800ºC. Such thermal behavior can be associated to its lower molar mass (TABLE V.2) (LEE et al., 2011). From about 250 ºC, PF resin had a better thermal resistance than all LPF resins. The higher hydroxymethyl groups and methyl bridges (FIGURE V.5) confirmed from 2D NMR might be associated to such observation.

FIGURE V.5- PF RESIN AND LPF RESINS 50% OF REPLACEMENT EVALUATED BY 2D HSQC NMR. THE UNITS ARE RELATIVE TO THE METHOXYL CONTENT OF EACH SAMPLE



SOURCE: The Author (2017).

As can be seen at FIGURE V.5, the frequency of methylol (CH_2OH) substitution is slightly higher in the pH 7 and 9 samples compared to pH 3 and 5. The same order of reactivity is detected also for the formation of orto-para and para-para methylene bridges (CH_2-op and CH_2-pp). In the case of softwood lignin, only traces of methylene bridges were detected in comparison to the frequency of methylol substitution. PF resin had the highest substitution, as expected due to its higher reactivity. The easier formation of methylene bridges in hardwood resins may thus compensate for the lower degree of hydroxymethylation and explain their good performance as adhesives, observed in bonding strength tests (FIGURES V.4 and 5). The signals found by 2D HSQC NMR in hydroxymethylated and LPF resin from pH 3 can be seen in the Supplementary data 3.

V.4 CONCLUSIONS

In this work hardwood lignin fractions were tested as 50% of phenol substitute in PF resins. Softwood lignin was also tested in LPF resin and compared with hardwood samples. Hardwood fractions consumed formaldehyde close to the theoretical calculation (^{31}PNMR), while softwood lignin consumed almost four times less than the theoretical calculation. When the bonding strength of resins was evaluated by ABES, hardwood LPF resins performed similar to softwood LPF resin and even to PF resin. Besides, up to 200 ºC LPF resins showed a higher thermal stability than PF resin.

In spite of the fact that softwood lignin presents higher number of reactive sites, they seem not be fully available. The higher molar mass of softwood lignin and steric effects might decrease its polymerization, confirmed by lower formation of methylene bridges observed by 2D HSQC NMR. Thus, even that hardwood lignin only presents few reactive sites they react with mostly formaldehyde added and its lower molar mass allow further polymerization easily. Thus, the number of reactive sites is not the only important factor in the synthesis of lignin phenol formaldehyde resins. This subject is of great importance as it opens opportunities for further discussions related to all characteristics (steric factors, flexibility of lignin macromolecule, etc) that could play a more important role in LPF resins. Furthermore, our results showed new perspectives for application of hardwood kraft lignin, which so far was considered less valuable due the lower number of reactive sites.

FINAL CONSIDERATIONS

From the present study, it was possible to observe that after addition of NaCl and Na_2SO_4, hardwood and softwood kraft lignin were precipitated. The sulfate ion was more effective than chloride to precipitate lignin and a pronounced precipitation was observed for softwood lignin associated to its higher molar mass. As the anion Cl^- presented less influence during precipitation compared to SO_4^{-2}, hydrochloric acid (HCl) has been chosen to precipitate lignin in a sequential acid fractionation. From the fractionation method developed, it was possible to observe that different lignin fractions were obtained. Mainly regarding to molar mass which decreases as the pH decreases. Such fractionation process was considered more efficient to hardwood lignin than softwood lignin.

From that point, lignin fractions from hardwood were deeply evaluated. First, the study showed antioxidant activity of all fractions evaluated against the DPPH radical. The great AA of this renewable and largely available resource, opens a new perspective to their potential use in pharmaceutical industry, food additive, polymers, agrochemicals. Fractions 5 and 3 presented AA over 80% and higher inhibitions against phytopathogenic fungi (evidenced against *R. bunodes*). Higher antioxidant and antifungal activities of these fractions were associated to their higher content of phenolic compounds and to the lower molar mass. The characterizations were chosen in order to make the bases for potential technical applications and deeper scientific investigation as bioactive compounds.

When hardwood lignin fractions were tested as phenol substitute in lignin phenol formaldehyde (LPF) resins, it was possible to observe that this type of lignin performed similar to softwood LPF resin and even to phenol formaldehyde (PF) resin. Thus, it was concluded that the number of reactive sites is not the only important factor in the synthesis of LPF resins. This subject is of great importance as it opens opportunities for further discussions related to all characteristics (steric factors, flexibility of lignin macromolecule, etc) that could play a more important role in LPF resins. Furthermore, our results showed new perspectives for application of hardwood kraft lignin, which so far was considered less valuable due the lower number of reactive sites. Especially in Brazil, where hardwood (*Eucalyptus* genus) represents 88% of the used raw material during pulping process. Besides, this study presented a simple and cheap fractionation method and suggested potential applications of kraft lignin which still commercially little

exploited. Also, other developments and investigations may be carried out based on the presented results.

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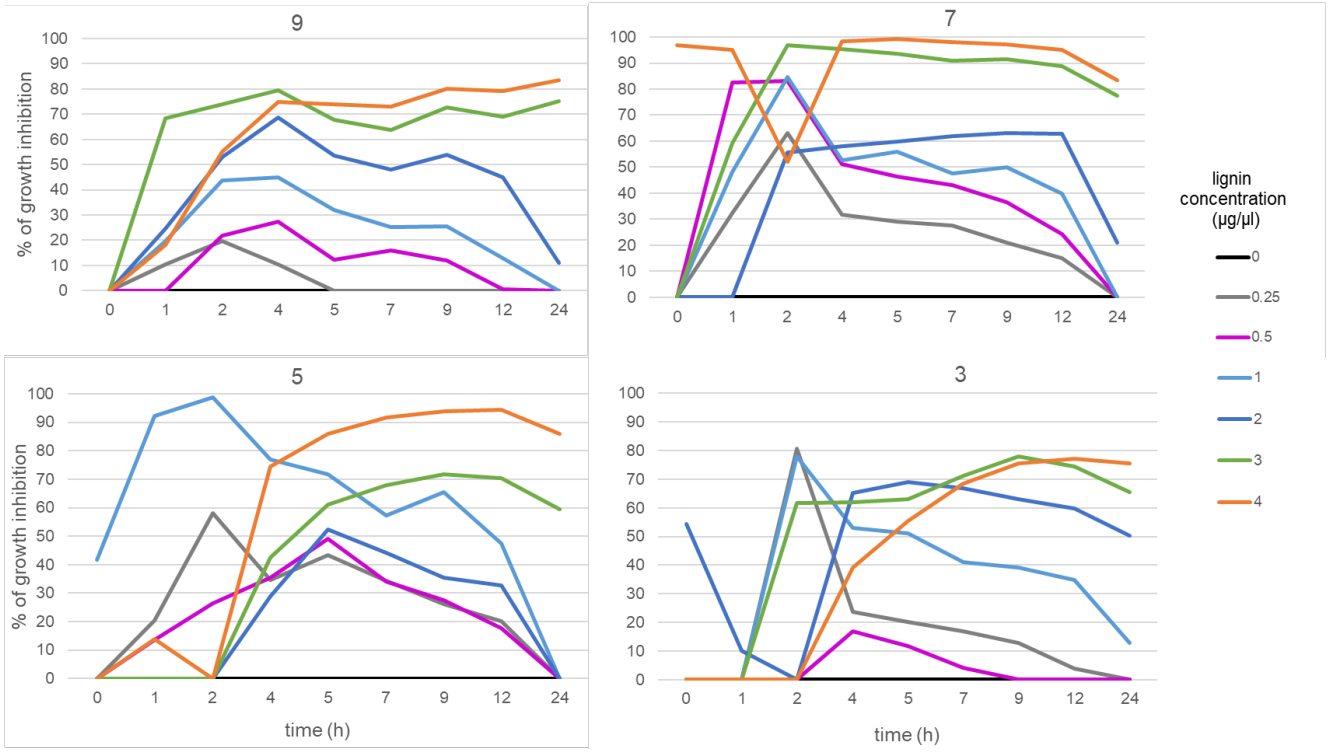
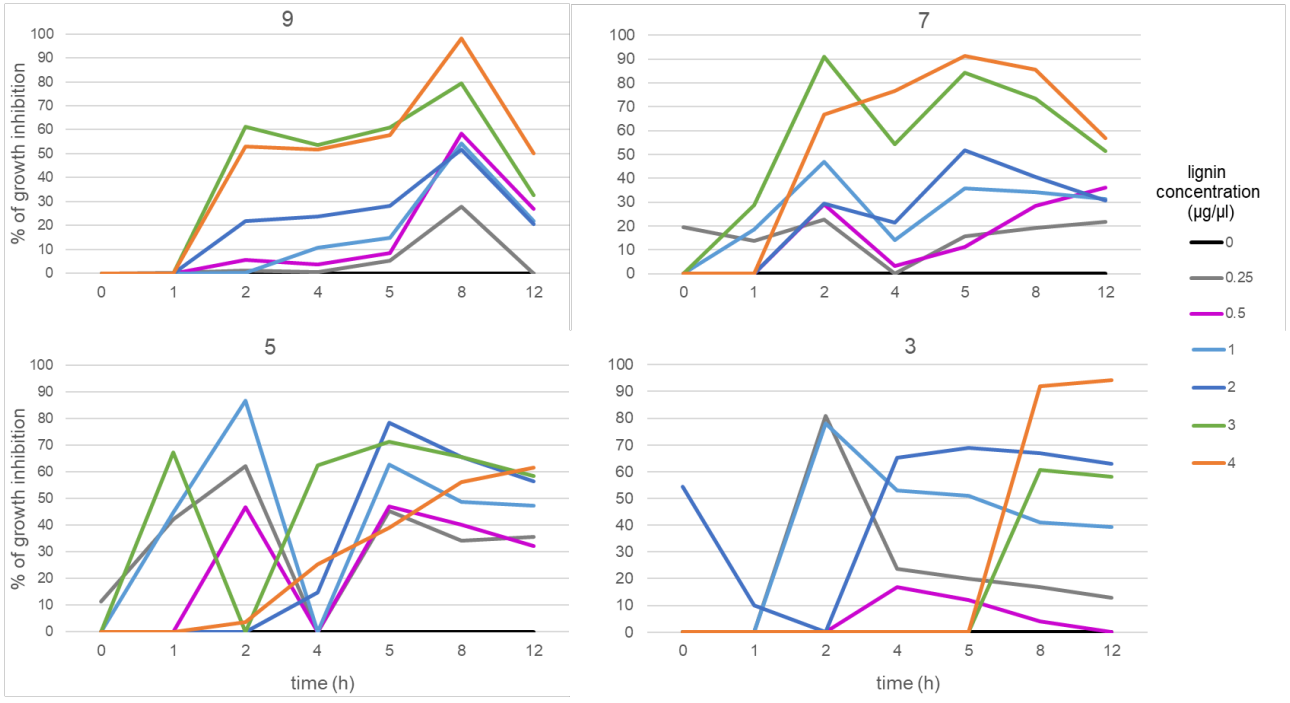
SUPPLEMENTARY DATA 1: GROWTH INHIBITION OF *E. coli* (A) AND *S. aureus* (B) BACTERA BY ADDITION OF LIGNIN FRACTIONS AT DIFFERENT CONCENTRATIONS

A)



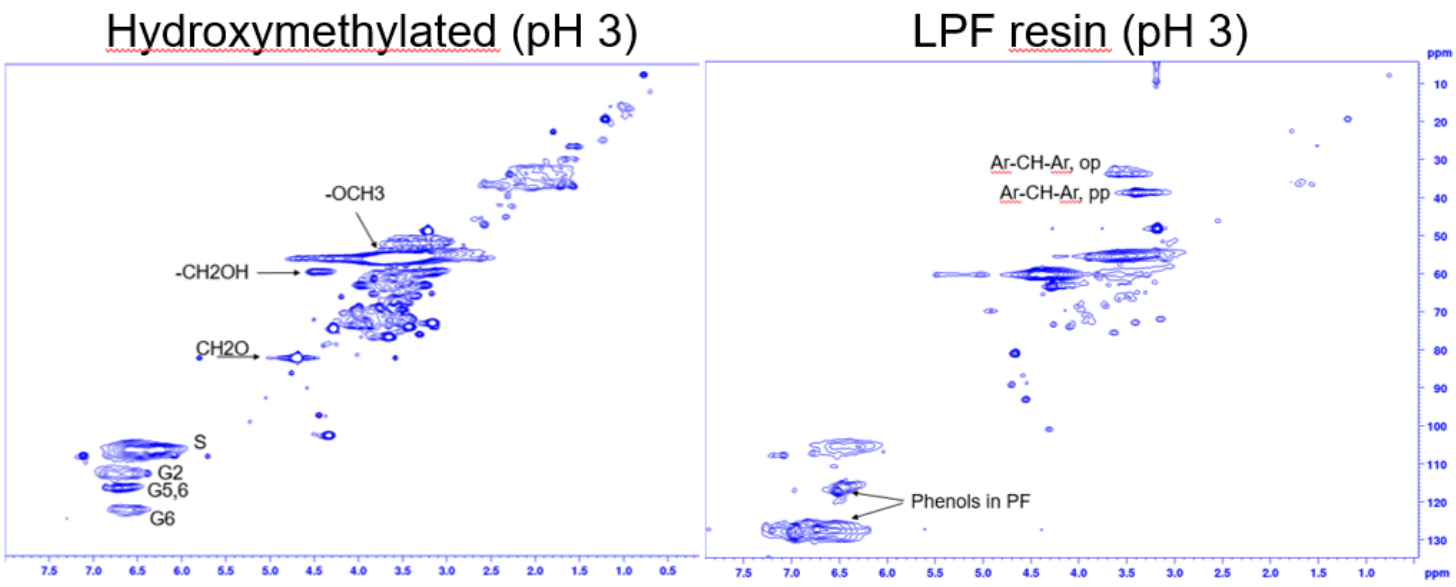
B)



SUPPLEMENTARY DATA 2: POLYSACCHARIDES IN EACH LIGNIN FRACTION

	Polysaccharides (%)					
Fraction	Glucose	Xylose	Arabinose	Galactose	Mannose	Rhamnose
9	<0,1	1,8	0,1	0,3	<0,1	<0,1
7	<0,1	1,9	0,1	0,3	<0,1	<0,1
5	<0,1	2,0	0,1	0,3	<0,1	<0,1
3	<0,1	2,5	0,1	0,3	<0,1	<0,1

SUPPLEMENTARY DATA 3: 2D HSQC SIGNALS FROM FRACTION 3



SOURCE: The Author (2017).

SUPPLEMENTARY DATA 4: RESUMO EXPANDIDO DA TESE

O setor de celulose e papel vem crescendo anualmente, de tal forma que em 2016 o Brasil alcançou o 2º lugar no ranking dos maiores produtores de celulose do mundo, de acordo com a Indústria Brasileira de Árvores (IBÁ, 2017).

Visto que nos processos de polpação (sulfito, kraft, organosolv ou soda) para obtenção da celulose a lignina é gerada como um resíduo, então, o aumento da produção de celulose significa crescimento direto da co-geração de lignina. Esse resíduo é comumente utilizado para produção de energia interna das indústrias. No entanto, a lignina apresenta uma estrutura química bastante interessante para substituir por exemplo, produtos de origem petroquímica como plásticos e resinas. Além disso, considerando o cenário atual do setor de celulose, a disponibilidade de matéria-prima pronta para ser explorada, é indiscutível.

O processo kraft é o principal método aplicado para polpação no mundo e o único no Brasil. Este processo é uma realidade prospectiva para produzir produtos de alto valor agregado a partir de biomassa, como químicos, fármacos, polímeros, adesivos, fibras de carbono e compósitos. Destacado ainda pelo fato de formar uma grande proporção de biomassa não alimentícia. Dessa forma, a aplicação da lignina com maior valor agregado que não seja sua queima torna-se de grande interesse.

A partir desse cenário, estudos indicam mudanças em um futuro próximo, em que a utilização eficiente de lignina será economicamente necessária, e os conceitos de biorrefinaria que consideram modificação e/ou incorporação de componentes na lignina, para obter produtos de valor agregado, se tornam uma alternativa interessante. Pesquisas relacionadas a química da lignina não são recentes, mas a possibilidade de escassez de matérias-primas atuais provenientes da exploração do petróleo, gerou espaço para o desenvolvimento de novos produtos e processos baseados em materiais aromáticos renováveis, como a lignina.

A lignina tem uma estrutura amorfa e complexa e suas propriedades variam muito dependendo da origem e de acordo com as reações ocorridas no processo de polpação. Além disso, a polidispersividade e a grande composição de grupos químicos podem ser problemáticos em sua utilização no biorrefino. Para contornar essa situação, o fracionamento deste material, tornou-se uma das maneiras de obter-se ligninas específicas. Seja utilizando enzimas, ultrafiltração, acidificação ou solventes seletivos. O objetivo do fracionamento é a obtenção de frações com massas molares

específicas, mais homogêneas, com propriedades definidas, de modo que possam ser então utilizadas em produtos de alto valor agregado. Um grande e promissor exemplo para a valorização da lignina é o crescente interesse na utilização de suas propriedades antioxidantes decorrente das unidades fenil-propanóicas e grupos funcionais (fenóis, carbonilas, carboxilas, hidroxilas alifáticas) capazes de inibir um processo de oxidação. Nesse sentido, a lignina vem sendo estudada na área dos bioplásticos, embalagens, cosméticos como bloqueador solar e em placas solares.

Tais grupos funcionais tornam a lignina também de grande interesse para a utilização na agricultura, na composição de herbicidas, fertilizantes, biocidas como também no desenvolvimento de compostos naturais bioativos de produtos para a saúde humana. Uma outra vertente dentre as alternativas mais promissoras para revalorização da lignina é sua aplicação na síntese de novos materiais poliméricos. A utilização da lignina para substituir o fenol em resinas fenol-formaldeído (FF), até então de origem petroquímica, é de grande interesse. O preço e disponibilidade do fenol dependendo totalmente do custo petróleo e a crescente preocupação sócio-econômica-ambiental com a escassez de produtos de origem fóssil alavancam a busca por substituintes do petróleo. Na estrutura da lignina as posições não substituídas C3 e/ou C5 são desejáveis para a síntese de resinas fenol-formaldeído. Essas estruturas são dependentes da fonte da lignina isolada. Assim, a maioria dos estudos focam no uso de lignina de coníferas e gramíneas por apresentarem as posições C3 e/ou C5 livre para síntese da resina. No entanto, no Brasil o gênero *Eucalyptus* representa 88% da matéria-prima utilizada no processo kraft, tornando de grande interesse pesquisas que visem a valorização desse tipo de lignina.

Nesse contexto, buscou-se como foco da pesquisa fracionar/purificar lignina de eucalipto (*hardwood*) e pinus (*softwood*) proveniente de licor negro do processo kraft, de maneira simples como acidificação, para obtenção de frações com diferentes e conhecidas propriedades. Primeiramente investigou-se o efeito dos ânions na precipitação da lignina, onde foi verificado que o íon cloreto teve menor influência durante a precipitação. O ácido clorídrico (HCl) foi então utilizado para uma precipitação ácida sequencial, em que os fatores pKa e tamanho molecular foram observados como principais fatores responsáveis pela precipitação. Foram obtidas frações com massas molares decrescendo conforme o pH decresceu. As frações provenientes da lignina de *hardwood* tiveram separação mais eficiente. Dessa forma, o segundo foco do trabalho teve como principal objetivo buscar e indicar potenciais de

aplicação para as frações de lignina de *hardwood* em duas grandes vertentes: testando suas atividades biológicas e testando a lignina como substituinte de fenol em resinas FF.

As frações de massa molar mais baixa e conteúdo maior de lignina total (menos impurezas) apresentaram maior capacidade antioxidante (podendo ser utilizadas em alimentos, embalagens, cosméticos) e antifúngica (potencial de aplicação como controle biológico) e todas as frações apresentaram atividade antibacteriana (podendo ser aplicada em rações, por exemplo). As resinas FF testadas com as frações de lignina kraft de *hardwood* apresentaram performance de cola similar e até superior a resina comparativa utilizada contendo lignina de *softwood*.

Com o trabalho foi provado que a lignina kraft de eucalipto tem, sim, potencial para ser utilizado em resinas FF. Tal resultado é de grande interesse e bastante vantajoso para o Brasil - considerando os altos índices de utilização de eucalipto como matéria-prima nas polpações kraft. Além disso, o estudo apresentou um método simples e barato de fracionamento e indicou potenciais de aplicação dessa lignina que ainda é pouco explorada e pouco utilizada comercialmente. A partir dos resultados apresentados, outros desdobramentos e investigações poderão ser realizados tendo como base a tese desenvolvida.