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FRANCISCO DE ASSIS AVELINO DE FIGUEREDO SOBRINHO

INSIGHTS ON THE STRUCTURE – PROPERTY RELATIONSHIP OF COCONUT SHELL LIGNINS OBTAINED FROM DIFFERENT MICROWAVE-ASSISTED ORGANOSOLV PROCESSES

FORTALEZA
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Thesis presented to the Chemistry Post-Graduation Program of Federak University of Ceará as a partial requirement to obtainment of the title of PhD in Chemistry. Concentrarion area: Chemistry.

Supervisor: Prof. Dr. Diego Lomonaco.
Co-supervisor: Prof. Dra. Selma Elaine Mazzetto.

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Aproved on: ___/___/______.

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Dedicated to my dear parents, brother and girlfriend. Thank you folks for the support, patience and mainly the love. Love you!

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To God for always surrounding me with nice people and for always guiding my steps.

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“The success is composed of 10 % of inspiration and 90 % of transpiration”.

(Thomas Edison)

RESUMO

A utilização de resíduos agroindustriais como matéria-prima na obtenção de produtos bio-baseados, como a lignina, tem sido amplamente explorada, a fim de substituir a utilização de compostos de origem petroquímica em diversos segmentos tecnológicos. No entanto, apesar da lignina ser bastante atrativa devido à sua estrutura fenólica, a complexidade de sua estrutura ainda representa um empecilho à sua valorização. O objetivo desse trabalho foi avaliar a influência da estrutura de ligninas do endocarpo do coco seco obtidas por diferentes processos de deslignificação organossolve assistido por micro-ondas (DOAM) nas suas atividades antioxidantes. As extrações de lignina a partir do endocarpo do coco seco foram realizadas utilizando-se soluções aquosas de ácido acético (90 % v/v) ou ácido fórmico (85 % m/m) aliado a um catalisador (ácidos de Lewis ou Brønsted; 2,0 % m/v ou v/v) a 110 ou 100 °C, respectivamente, durante 20 minutos. As ligninas acetosolv e formosolv foram completamente caracterizadas através de técnicas espectroscópicas, cromatográficas e termoanalíticas. Os dois processos empregados permitiram a obtenção de ligninas com diferentes rendimentos e purezas. Além disso, a utilização do processo acetosolv permitiu a obtenção de ligninas parcialmente acetiladas, enquanto que para o formosolv, tal modificação química não foi observada. A introdução de grupos acetil nas ligninas do endocarpo do coco seco resultou em uma diminuição de 20 % nas suas temperaturas de transição vítrea (T_g), obtendo-se ligninas com diferentes processabilidades. Entretanto, a maior disponibilidade de hidroxilas nas ligninas formosolv (cerca de 20 a 30 %), favoreceu a sua capacidade antioxidante, apresentando valores de IC_{50} menores do que as acetosolv. No entanto, ambas as ligninas acetosolv e formosolv apresentaram valores de IC_{50} cerca de dez vezes menores do que dois antioxidantes comerciais (BHT and Irganox 1010), ressaltando a sua potencialidade na substituição de derivados do petróleo. Conclui-se, portanto, que o processo DOAM pode ser considerado uma alternativa tecnológica para obtenção de ligninas de alta pureza, diferentes funcionalidades, processabilidades e capacidades antioxidante com potencial utilização em diferentes segmentos da indústria química e tecnológica.

Palavras-chave: Resíduo agroindustrial. Biopolímero. Micro-ondas. Organossolve. Elucidação estrutural.

ABSTRACT

Agroindustrial wastes have been extensively used as raw material for obtainment of bio-based products, such as lignin, in order to replace the petroleum-based compounds in several technological fields. Although lignin is very attractive due to its phenolic structure, its complex structure is still hinders its valorization. The aim of this work was evaluate the influence of the structural features of coconut shell lignins obtained by different microwave-assisted organosolv delignification (MWAOD) processes on their antioxidant activities. The lignin extractions were carried out using aqueous solutions of acetic or formic acid (90 % v/v and 85 % m/m, respectively) combined to an acid catalyst (Lewis or Brønsted; 2.0 % w/v or v/v) at 110 or 100 °C, respectively, during 20 minutes. Acetosolv and formosolv lignins were completely characterized through spectroscopic, chromatographic and thermal techniques. Both organosolv processes generated lignins with different yields and purities. Moreover, the utilization of acetosolv process favored the production of partial acetylated lignins, while such chemical modification was not observed when formosolv was used. The insertion of acetyl groups on acetosolv coconut shell lignins resulted in a decrease of 20 % in their glass transition temperature (T_g), yielding lignins with different processabilities. However, the higher amount of hydroxyls in formosolv lignins (approximately 20 – 30 %) favored their antioxidant capacities, presenting lower IC_{50} values than those of acetosolv. Nevertheless, both organosolv lignins had ten times lower IC_{50} values than two commercial antioxidants (BHT and Irganox 1010), which reinforces their potential on the replacement of petroleum-based derivatives. Therefore, it can be concluded that MWAOD process can be considered as a technological alternative for obtainment of lignins with high quality, different functionalities, processabilities and antioxidant capacities with potential for use in different fields of chemical and technological industries.

Keywords: Agrowaste. Biopolymer. Microwave. Organosolv. Structural elucidation

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS AND SYMBOLS

AA	Acetic acid
ACSL	Acetosolv coconut shell lignins
BDE	Bond dissociation enthalpy
BHA	Butyl hydroxyanisole
BHT	Butylated hydroxytoluene
Cl-TMDP	2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane
CS	Coconut shell
CSP	Coconut shell poder
CSALs	Coconut shell acetosolv lignins
CY	Char yield
FA	Formic acid
FAO	Food and agriculture organization
FCSL	Formosolv coconut shell lignins
G	Guaiacyl
GDP	Gross domestic product
H	*p*-hydroxyphenyl
HAT	Hydrogen abstract transfer
LCC	Lignin-carbohydrate complexes
LOI	Limiting oxygen index
MWAE	Microwave-assisted extraction
MWAOD	Microwave-assisted organosolv delignification
MWI	Microwave irradiation
PB	*p*-hydroxybenzoate
PDI	Polydispersity index
PLET	Proton decoupled electron transfer
RPIL	Relative proportion of interunit linkages
RPM	Relative proportion of monomers
S	Syringyl
TH	Traditional heating

SUMMARY

1 INTRODUCTION

In the recent years, the growing concern about the environment have made the scientific community explore alternative sources as raw material in detriment of the non-renewable fossil sources (ALI; BAHAITHAM; NAEBULHARAM, 2017). Among the alternative renewable sources, biomass becomes an interesting and promising solution to generation of greener energy and chemicals, especially for Brazil, which has an enormous area destined for agriculture, as well as the greatest biodiversity on Earth.

Along with this great biodiversity, climate variety provides to tropical regions the appropriate conditions for the development of the agribusiness, which generates large amounts of low-cost residues coming from the processing of crops, and represent a potential form of environmental pollution (MILLÁN; VARGAS; NZIHOU, 2017).

In Brazil, where the agricultural production accounts for 6.5 % of the Gross Domestic Product (GDP), the agrowastes are a concerning problem, since this activity is one of the most important to the country´s economy (MEDINA; SANTOS, 2017; SCHLEMPER; STÜRMER, 2014).

Among the main agrowastes generated in Brazil, coconut (*Cocos nucifera*) stands out since its economic relevance, where 285.000 hectares are destined to its cultivation, yielding approximately 2 billion coconut fruits per year (FARIAS et al., 2017). After its industrial processing, the majority of solid parts are discarded or burned in industrial furnaces, both consisting in its underutilization and in an environmental pollution. Except the coconut edible part, there are the mesocarp, approximately 25 % of the coconut weight, and the endocarp, or coconut shell (CS), representing 20% in mass of the coconut (BLEDZKI; MAMUN; VOLK, 2010; RAMBO; SCHMIDT; FERREIRA, 2015).

There are several works reporting the utilization of the coconut fibers as raw material for the generation of high value-added products, such as nanocellulose (AZEREDO; ROSA; MATTOSO, 2015; NASCIMENTO et al., 2014, 2016a; ROSA et al., 2010; UMA MAHESWARI et al., 2012). On the other hand, the most common uses of CS are production of char (CHEN; KUO, 2010), activated carbon (ARENA; LEE; CLIFT, 2016; PRAUCHNER; RODRÍGUEZ-REINOSO, 2012), fuels (ROUT et al., 2016; SIENGCHUM; ISENBERG; CHUANG, 2013) and filler in composites and concretes (BLEDZKI; MAMUN; VOLK, 2010; GUNASEKARAN; KUMAR; LAKSHMIPATHY, 2011; JAYAPRITHIKA; SEKAR, 2016; KEERTHIKA et al., 2016).

Although these applications highlight the elevated lignin content in CS, its use for noble applications such as lignin extraction is very limited and only started to be explored in the last years (LIYANAGE; PIERIS, 2015; ZAKARIA; IDRIS; ALIAS., 2017; AVELINO et al., 2018a, 2018b, 2018c, 2019). However, there is a long path to be explored and understood to promote the correct valorization of CS lignin, mainly that regarding to its structure.

Lignin is the second abundant natural polymer after cellulose and the major natural source of phenolic compounds. It has structural function in the cell walls, conferring rigidity and protection from hydrolytic attack of pathogenic microorganisms (BILAL et al., 2017). Chemically, lignin is a hydrophobic amorphous polymer, formed by phenylpropanoid units: *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S), randomly linked by ether (C-O-C) and carbon-carbon (C-C) bonds.

The chemical structure of lignin has been extensively researched by different analytical methods (LAURICHESSE; AVÉROUS, 2014). The existence of several functional groups in its structure offers different possibilities to chemical modifications, in order to produce environmentally friendly products with technological applications, replacing petroleum-based materials.

However, lignin chemical structure depends on the raw material origin, as also on the extraction and isolation methods, which will influence its reactivity and, consequently, its applications. Traditionally, lignin is a byproduct of the pulp and paper industry, where the processes require the utilization of acid or alkali medium to promote the delignification of the raw material at elevated temperatures under high pressure.

These harsh conditions are achieved by using high-pressure reactors or autoclaves, which are based on conductive heating from an external source for several hours. These long reaction times are undesirable due to high energy consumption, besides side reactions that can occur for lignin and the effects that pressure can cause in its structure (LI et al., 2012).

Recently, microwave-assisted extraction (MWAE) has been evaluated as an alternative method to promote the fragmentation of lignocellulosic biomass (HASSAN; WILLIAMS; JAISWAL, 2018; KOSTAS; BENEROSO; ROBINSON, 2017; LI et al., 2016, 2018b). This method uses the microwave irradiation to heat, which is based on dipole rotation and ionic conduction, promoting faster chemical reactions with higher yields than traditional heating (SANSEVERINO, 2002).

Therefore, this technique can be used as a sustainable methodology for lignin extraction, since it provides decrease in reaction times, as well as the utilization of low power ratings, reducing energy consumption and increasing efficiency.

Since in MWAE polar compounds must be used in order to absorb the radiation, the organosolv process is a suitable method for lignin extraction. In this process, organic solvents, such as alcohols and organic acids, are used to delignify lignocellulosic biomass, allowing their recovering and reutilization. Commonly, aqueous solutions of acetic acid, formic acid and ethanol combined with low concentrations of acid catalysts, such as H_2SO_4 and HCl, are used to separate lignin and carbohydrates. This process yields high purity and low molecular weight lignin than that obtained in industry, such as Kraft and sulfite, which have high sulfur and ash contents limiting their technological applications.

Despite the fact that Brønsted acids (HCl and H_2SO_4) catalyze lignin extraction in organosolv process, other works have used Lewis acids ($MgCl_2$, $FeCl_2$, $CuCl_2$, $FeCl_3$, $ZrCl_2$, $AlCl_3$) as catalysts, evaluating their effects in lignin structure (CONSTANT et al., 2015; HUANG et al., 2016; SCHWIDERSKI et al., 2014; WANG et al., 2016).

However, these works performed the lignin extraction at elevated temperatures and pressure, and it is necessary to consider the combined effects of these parameters. To the best of our knowledge, there are no reports on the use of microwave irradiation combined with Lewis acids as catalysts under mild conditions (atmospheric pressure) to promote delignification of biomass.

Therefore, the chapter 2 of this thesis reports the complete evaluation of microwave-assisted organosolv delignification (MWAOD) of coconut shell as a process to obtain high quality lignin under mild conditions. The influence of the type of catalyst (Brønsted or Lewis) and its concentration, as well as the reaction time were assessed by a deep structural characterization through wet chemical analysis, spectroscopic, chromatographic and thermal methods. Furthermore, the efficiency of MWAOD was compared to that of traditional heating by performing extractions under the same conditions used in the MWAOD experiments and characterizing the obtained lignins by the same analytical techniques.

Although acetic acid is more commonly used in organosolv process, formic acid can also be used, but it is less explored than the former, specifically in combination with microwave irradiation. During the organosolv pulping, side reactions can occur and the use of different solvents may influence on the lignin structure, conferring it specific functionalities. The chemistry involved in organosolv pulping using different solvents combined to microwave irradiation is still almost unexplored.

For this reason, the chapter 3 of this thesis reports the evaluation of microwave-assisted acetosolv and formosolv processes as delignification methods for coconut shell and the effects caused on the structure of the lignins by them through a complete structural characterization, in order to shed a light on the chemistry involved in each process. Moreover, their antioxidant activities were assessed in order to establish a structure-performance relationship, evaluating the main structural features that influence on this property and allowing to know how to take advantage of those features to employ them to a suitable application.

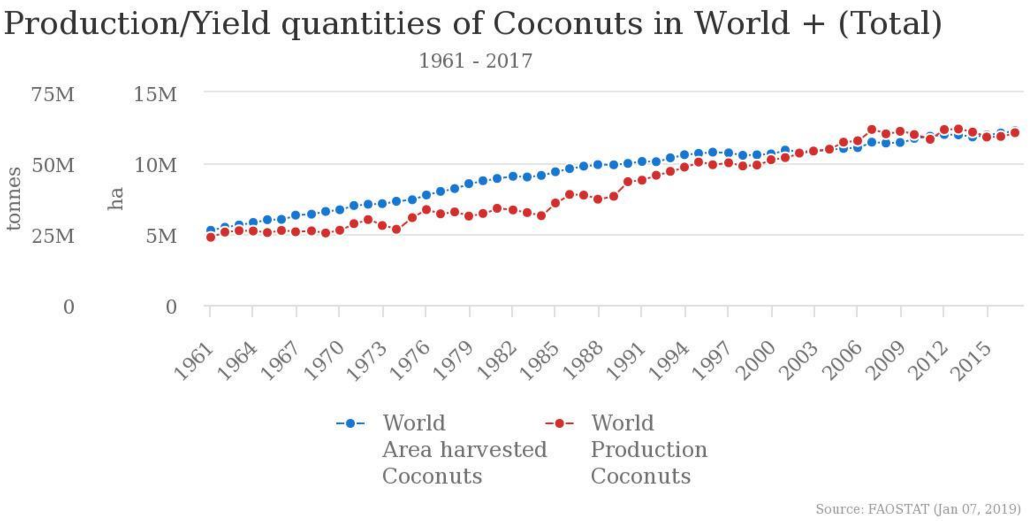
Therefore, the aim of this work was to promote the valorization of a cheap and available Brazilian agro-waste through the isolation of high purity and quality lignins from it using an eco-friendly process, yielding two types of organosolv coconut shell lignins. The potential application of those lignins as natural antioxidants was also evaluated through a complete understanding about their structural features, as well as their performance in relation to two commercial antioxidants, such as butylated hydroxytoluene (BHT) and Irganox 1010, in order to prospect alternatives to petroleum-based antioxidants currently used in different segments of chemical industry.

Chapter 1: State of the art

The culture of coconut (*Cocos nucifera*)

Coconut (*Cocos nucifera*) is a typical fruit cultivated in tropical countries that are located in coast zones. According to data from Food and Agriculture Organization (FAO), the production of coconut has increased significantly in the period of 1961 to 2017, almost reaching a value three times higher than the initial, as shown in **Figure 1**.

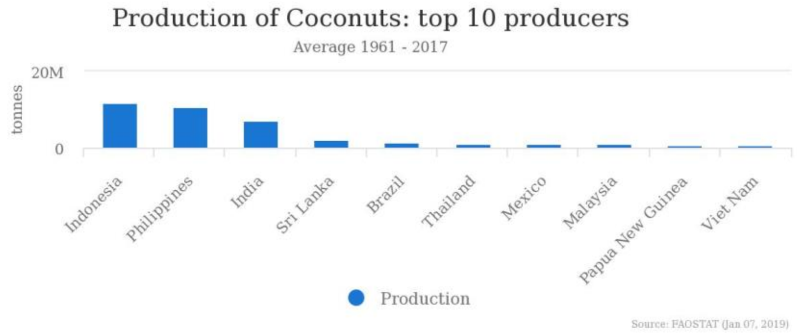
Figure 1 – Evolution of production and harvested area of coconut trees in the world from 1961 to 2017.



Source: (FAOSTAT, 2016)

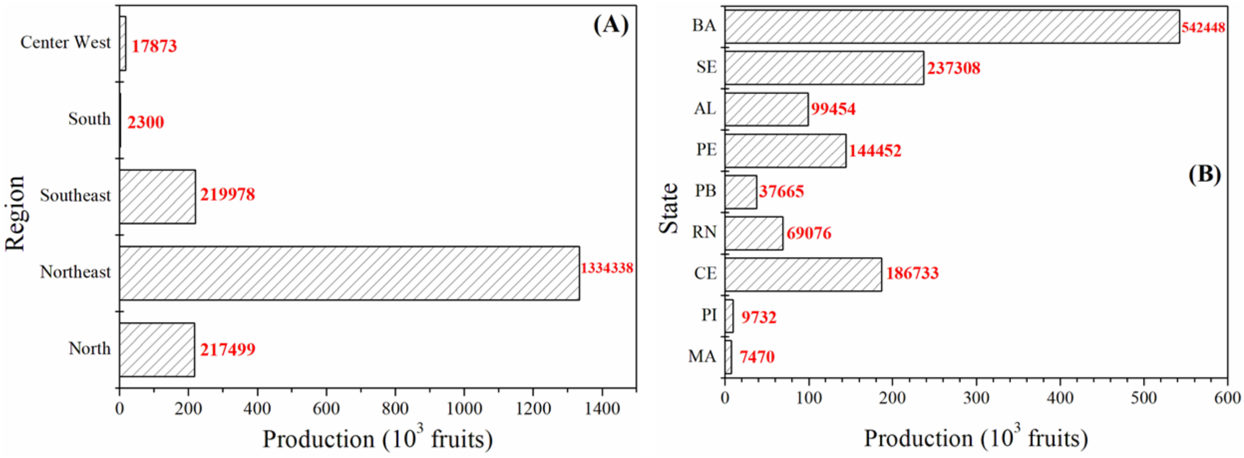
Moreover, according to FAO's statistics Brazil is one of the biggest coconut producers, occupying the fifth position on the worldwide producers ranking, **Figure 2**. According to Brazilian Institute of Geography and Statistics (IBGE), the Northeast of Brazil is the major producer of coconut in the country (**Figure 3A**) and among its states Ceará occupies the third position in the producers ranking (**Figure 3B**) (IBGE, 2017). The Northeast region is responsible for the high levels of production and consumption of coconut, either in its green form, in which the coconut water is consumed *in natura*, or in its dry form, in which the edible part is used for the production of coconut milk, coconut oil, grated coconut, etc (MARTINS; JUNIOR, 2014).

Figure 2 – The top 10 worldwide ranking of coconut producers.



Source: (FAOSTAT, 2016)

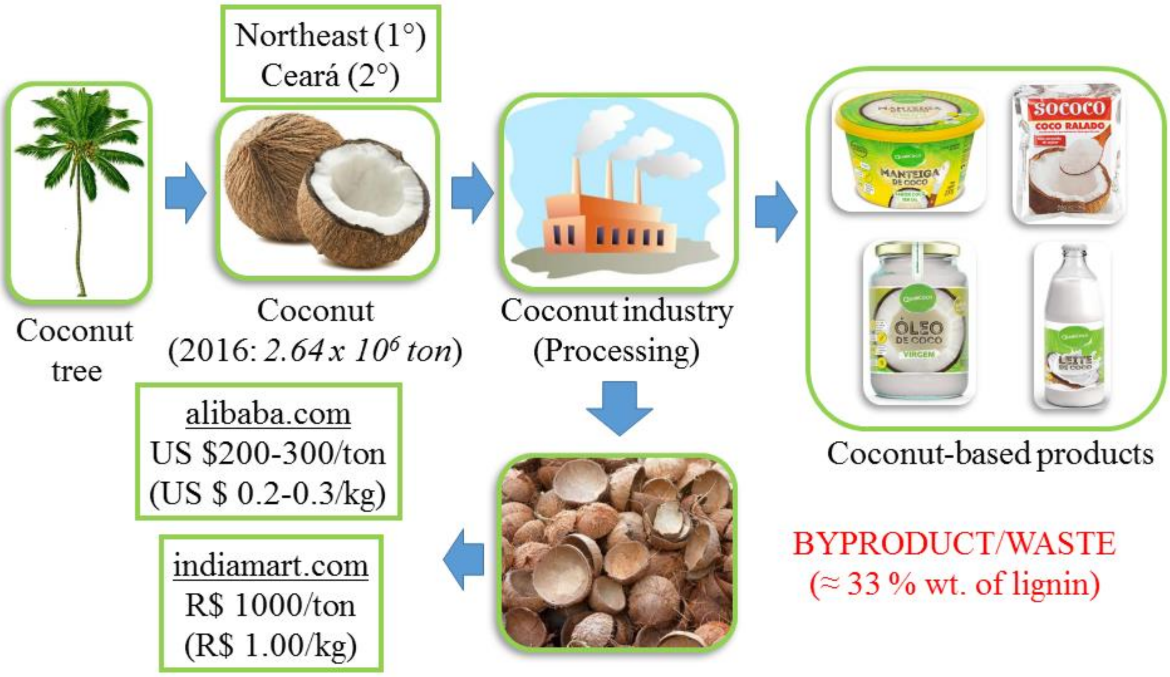
Figure 3 – The ranking of coconut producers considering (A) the Brazilian regions and (B) the states of Northeast region.



Source: (IBGE, 2017)

However, the increasing in the production and consumption of coconut, as well as its processing to obtain the industrialized products causes an increase in the generation of solid wastes, such the mesocarp (fibrous and outer part) and endocarp or shell (hard and inner part), **Figure 4**. At the most of the times, these residues are discarded in landfills, representing a serious environmental problem, since these materials can take at least 8 years to completely degrade (MARTINS; JUNIOR, 2014), **Figure 5**.

Figure 4 – Coconut processing chain and the generation of solid wastes as byproduct.



Source: The author.

Figure 5 – Solid wastes originated from coconut.

Source: http://wdn.com.ng/coconut-shells-can-produce-brake-pads-expert/, acessado em 15/04/2018.

These solid wastes are frequently used as fuels for heat generation in the industrial furnaces and boilers that is a destination that does not consume the large amount of residues generated in the coconut processing.

Besides this problem, the use of coconut waste as a source of energy represent an underutilization of this material, since according several works in the literature it has a rich biopolymer composition with high cellulose, hemicellulose and lignin contents (BLEDZKI; MAMUN; VOLK, 2010; CHEN; KUO, 2010; PRAUCHNER; RODRÍGUEZ-REINOSO, 2012). These are high added-value compounds with different applications in the chemical industry, which are wasted when submitted to combustion for energy generation.

There are significant amount of papers that report the valorization of coconut fibers, such as in the production of nanocellulose and lignin (AZEREDO; ROSA; MATTOSO, 2015; NASCIMENTO et al., 2014, 2016b; ROSA et al., 2010; UMA MAHESWARI et al., 2012).

Although the use of CS as feedstock for lignin extraction is still limited, there were some recent advances in this field (ZAKARIA et al., 2017; AVELINO et al., 2018b, 2019), however there are some issues about this matter that need to be solved. It is worth mentioning that previous studies already showed that CS has a high lignin content about 30 % wt., which shows its potential to be used as raw material for lignin extraction, although they used CS for another applications (BLEDZKI; MAMUN; VOLK, 2010; CHEN; KUO, 2010; PRAUCHNER; RODRÍGUEZ-REINOSO, 2012).

Therefore, since CS is a cheap and available solid waste with high incidence in the Northeast of Brazil, specifically in the State of Ceará (**Figure 4**) and combined to the fact that CS has a high lignin content in its composition and it is almost unexplored for lignin extraction, CS becomes a potential raw material for this application.

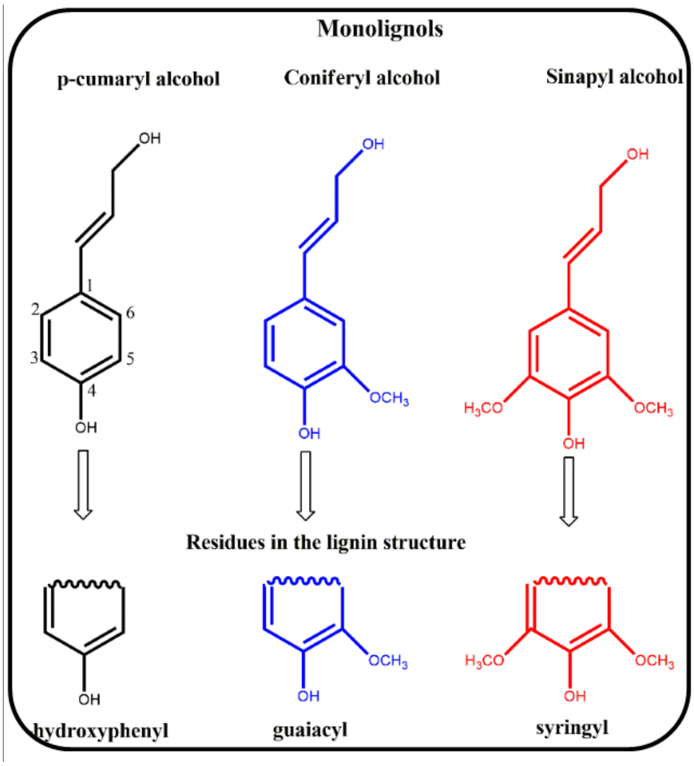
Lignin

Composition and chemical structure

Lignin is the second most abundant natural polymer after cellulose and also is the major source of aromatic compounds on Earth. It has structural function in the plants conferring rigidity to their wall cells and protecting cellulose from the hydrolytic attack of pathogenic microorganisms as well (BILAL et al., 2017).

In terms of chemical structure, lignin is a tridimensional amorphous polymer with hydrophobic character formed by phenylpropanoid units derived from three monolignols, such as *p*-coumaryl, coniferyl and sinapyl alcohols. The phenolic substructures originated from those three alcohols are so called *p*-hydroxyphenyl (H, from *p*-coumaryl alcohol), guaiacyl (G, from coniferyl alcohol) and syringyl (S, from sinapyl alcohol) and the difference between these units is the amount of methoxyl groups present in their structures, as shown in **Figure 6**.

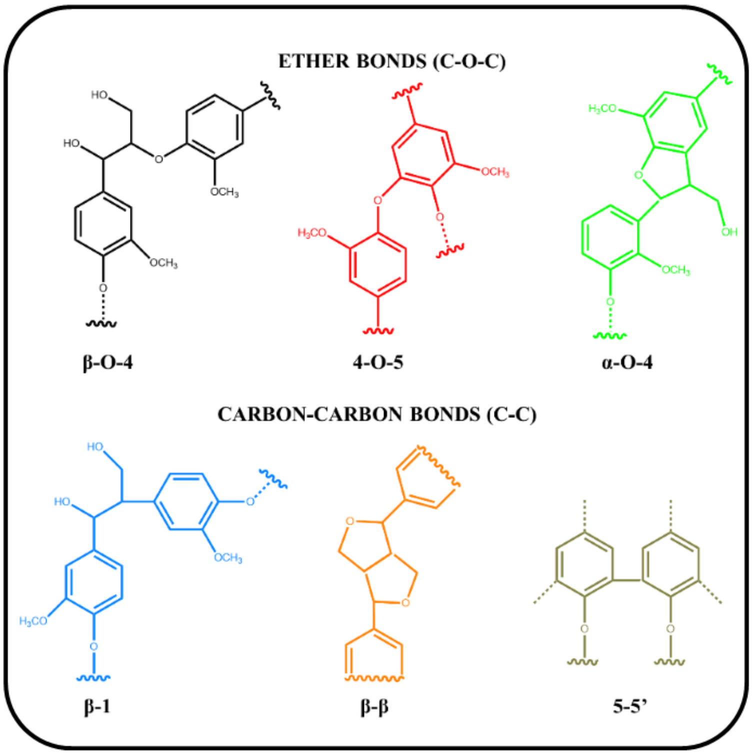
Figure 6 – The structures of the three units that form lignin macromolecule.



Source: The author.

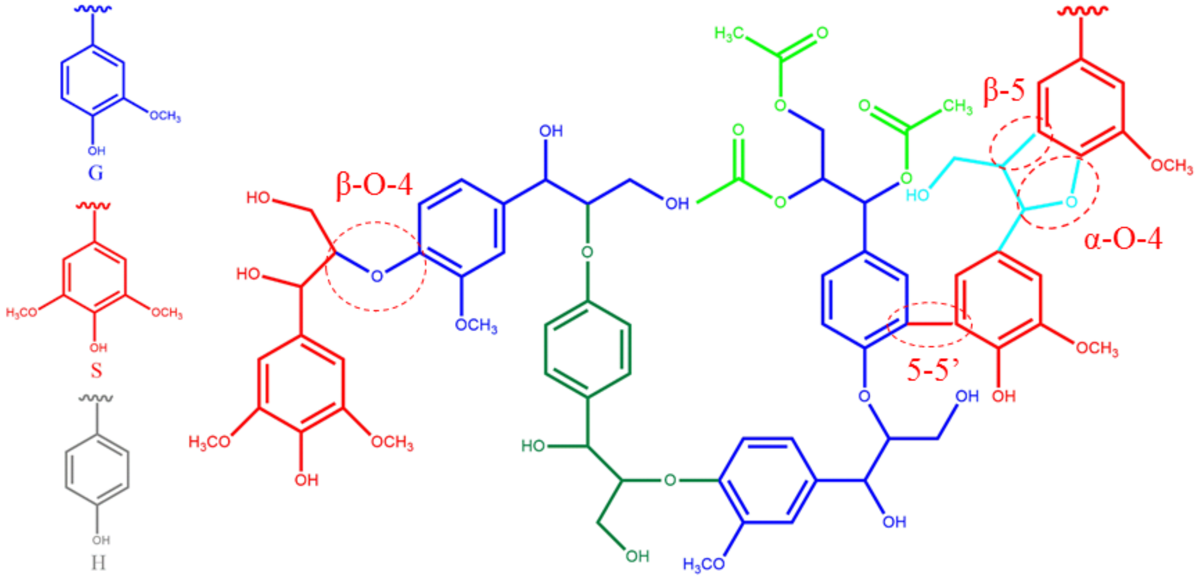
The three lignin's monomers are linked to each other in a random way through different types of bonds, such as C-C and C-O-C, being β-1, β-β, 5-5' and α-O-4, β-O-4 and 4-O-5 the main types of linkages, respectively, **Figure 7**. These substructures can be linked to each other as a result of a free radical polymerization between them, forming a complex, branched and tridimensional molecule, **Figure 8**, in which the main linkages are highlighted. The proportion of monolignols is strongly dependent of the plant species. In a general way, softwood lignin (pine, spruce) is formed exclusively by G units, whereas hardwood lignin (birch, poplar, eucalyptus) contains both G and S units. Lignin from herbaceous biomass is composed of all three monolignols, although the H-content is generally low (< 5%) (SCHUTYSER et al., 2018).

Figure 7 – Representation of the main types of ether and carbon-carbon bonds that link the lignin units.



Source: The author.

Figure 8 – Representation of the lignin structure.

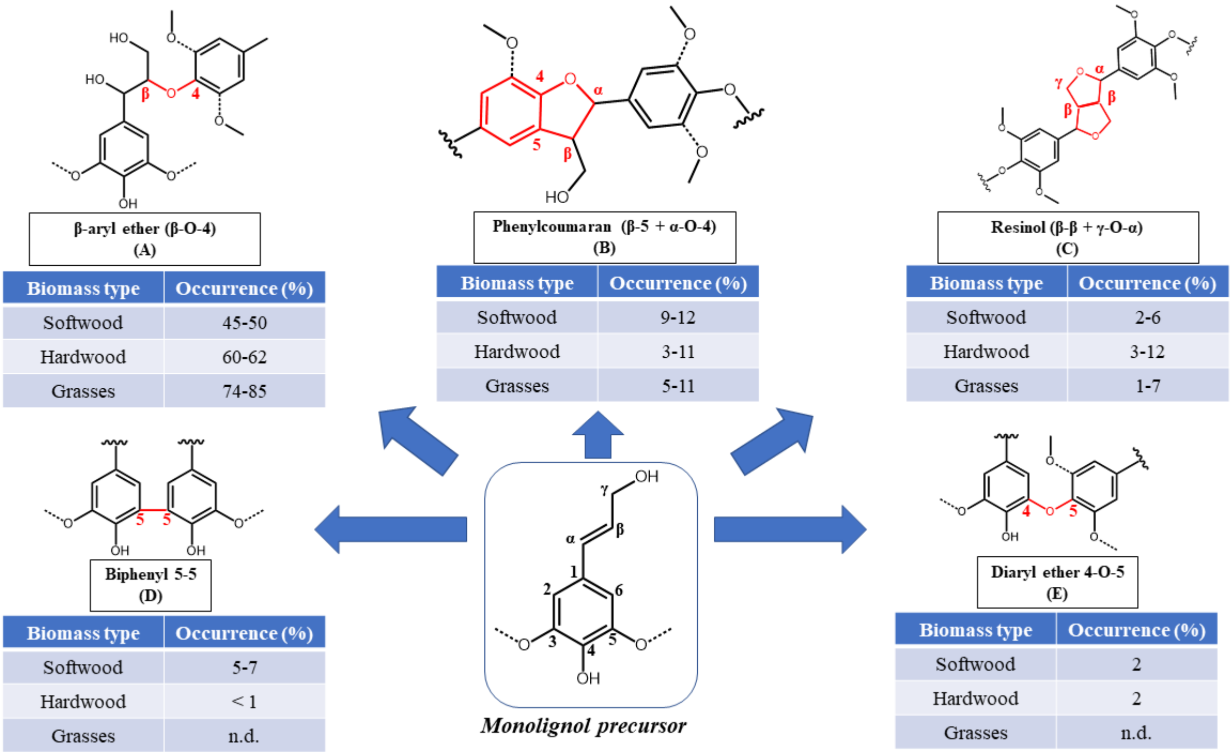


Source: The author.

To understand the complex chemical structure of lignin a detailed and careful characterization must be performed using different analytical techniques, such as wet, chromatographic and spectroscopic methods. However, among them the Nuclear magnetic resonance (NMR) is the one that provides the richest information about the lignin skeleton, such as the interunit linkages (the type of bonds existent between the monomers), the monolignol composition and the presence of other types of functional groups (acetyl, formyl, etc).

The modernization of NMR methods by the creation of the multidimensional experiments allowed the easier characterization of complex macromolecules, such as lignins. One of the most used experiments is the ^{1}H-^{13}C Heteronuclear single quantum coherence (^{1}H-^{13}C HSQC) that have replaced the traditional ^{1}H and ^{13}C experiments, since they suffered from lack of resolution which impacts on the distinguishment of subtle structural details, being widely used as a tool for elucidation of lignin structure (RINALDI et al., 2016). ^{1}H-^{13}C HSQC experiments have been employed in the determination and approximate estimation of the relative abundance of interunit linkages and monolignols that are present in native lignins, **Figure 9**. Furthermore, the use of ^{1}H-^{13}C HSQC experiments for characterization of technical lignins also allow the construction of insights about the effects of the extraction process and its operation conditions on the lignin structure.

Figure 9 – Scheme of the main interunit linkages and their abundance in native lignins according to the literature.



β-aryl ether (β-O-4) (A)

Biomass type	Occurrence (%)
Softwood	45-50
Hardwood	60-62
Grasses	74-85

Phenylcoumaran (β-5 + α-O-4) (B)

Biomass type	Occurrence (%)
Softwood	9-12
Hardwood	3-11
Grasses	5-11

Resinol (β-β + γ-O-α) (C)

Biomass type	Occurrence (%)
Softwood	2-6
Hardwood	3-12
Grasses	1-7

Biphenyl 5-5 (D)

Biomass type	Occurrence (%)
Softwood	5-7
Hardwood	< 1
Grasses	n.d.

Diaryl ether 4-O-5 (E)

Biomass type	Occurrence (%)
Softwood	2
Hardwood	2
Grasses	n.d.

Monolignol precursor

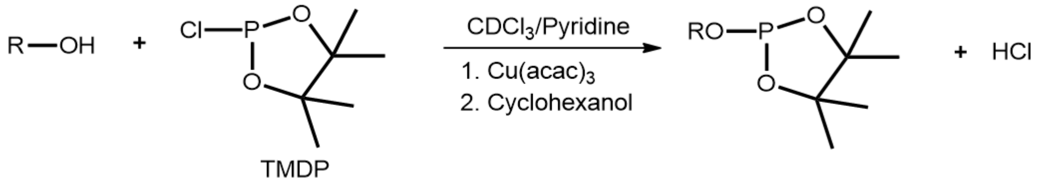
Source: The author adapted from Rinaldi et al. 2016.

However, although ^1H-^{13}C HSQC NMR is a versatile technique and tool for lignin characterization, it has a huge limitation that is the difficulty of performing quantitative analysis, being possible only to perform semi-quantitative determination of integral ratios, when the ^1H-^{13}C pairs are located in similar chemical environments, such as Cα-Hα signals for lignin phenylpropanoid chains or C_2-H_2/C_6-H_6 aromatic signals, since in these cases $^1J_{C\text{-}H}$ assumes similar values (RINALDI et al., 2016).

To overcome this problem to the use of HSQC NMR for quantification purposes, another NMR experiment has been used for obtainment of quantitative data on the nature of different types of hydroxyl groups in lignin, which is the ^{31}P NMR. It is a facile and sensitive method that allows the identification and quantification of hydroxyl groups present in the lignin structure, which makes possible the investigation of the interunit linkages and monolignol composition of native and technical lignins (LI et al., 2018a).

The analysis is based on the derivatization of the sample through the phosphitylation of labile proton present in lignin structure, such as aliphatic alcohols and phenols (OHs), as well as aliphatic and aromatic acids (COOHs). The derivatization of lignin is commonly carried out using 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (TMDP) as a phosphitylation agent in a solvent mixture of pyridine/$CDCl_3$ (1.6/1.0, v/v) according to Granata and Argyropoulos (1995), **Figure 10**.

Figure 10 – Phosphitylation reaction of lignin for subsequent ^{31}P NMR analysis.



Source: The author.

According to the pioneer methodology of phosphitylation of lignin samples for hydroxyl quantification by ^{31}P NMR (GRANATA; ARGYROPOULOS, 1995), besides the TMDP, a relaxation agent (chromium (III) acetylacetonate – $Cu(acac)_3$) and an internal standard (cholesterol) must be added to the medium prior the analysis. However, a recent study developed by Balakshin et al. (2015) showed that the use of cholesterol and cyclohexanol that are the most used internal standards for ^{31}P NMN analysis, cause an underestimation of hydroxyl groups up to 20 % (BALAKSHIN; CAPANEMA, 2015).

For this reason, the authors suggested the use of *endo*-*N*-hydroxy-5-norbornene-2,3-dicarboximide instead of the other ones. However, it was observed that the latter compound was not stable during the sample preparation mixture, whereas cholesterol was reported as stable under the applied conditions for at least 16 hours (BALAKSHIN; CAPANEMA, 2015). Therefore, it is more common the use of cholesterol and cyclohexanol as internal standards, due to their stability and also for their cost, since the *endo*-*N*-hydroxy-5-norbornene-2,3-dicarboximide is extremely expensive.

Besides the issues associated to the sample preparation, there are some other details about the ^{31}P NMR analysis of lignin that must be taken into account, such as the overlapping of S and G-condensed units, the presence of fatty acids, aldehyde and thiol groups in the lignin structure, which can cause an underestimation or overestimation of certain types of hydroxyl groups (CONSTANT et al., 2016). Thus, a meticulous interpretation of the results must be done to avoid the obtainment of misleading results.

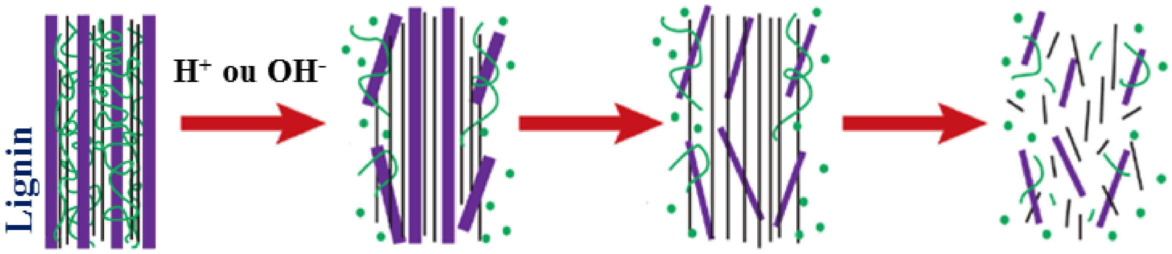
Despite the intrinsic limitations of ^{31}P NMR method, it is a versatile and powerful tool for structural elucidation and quantification of hydroxyl groups in lignin structure. Furthermore, ^{31}P NMR is also a robust technique for quantification purposes, which was proved by comparing the results obtained from ^{31}P and ^{13}C NMR and it was observed a good agreement between them (BALAKSHIN; CAPANEMA, 2015). It is worth mentioning that both methods have their pros and cons, but ^{31}P NMR has some advantages that make it very attractive, such as a low experiment duration, a fast sample derivatization and a beginner or intermediate operator to perform the analysis (BALAKSHIN; CAPANEMA, 2015).

Therefore, the combination of different spectroscopic techniques play a key role in the identification and quantification of the different functional groups present in the lignin structure as in the native as in the technical ones, such as methoxyls, aliphatic and phenolic hydroxyls, carboxyls, etc, providing possibilities to perform a wide range of chemical modifications. This allows the lignin to be used as a feedstock in several processes, mainly on those that involve the formation of aromatic products (LAURICHESSE; AVÉROUS, 2014).

Extraction methods

Generally, the lignin extraction from lignocellulosic biomass is carried out under severe conditions of temperature and pressure combined to the use of acid or base catalysts. During this process, lignin structure undergoes extensively cleavage, generating fragments with different molecular weight, **Figure 11**.

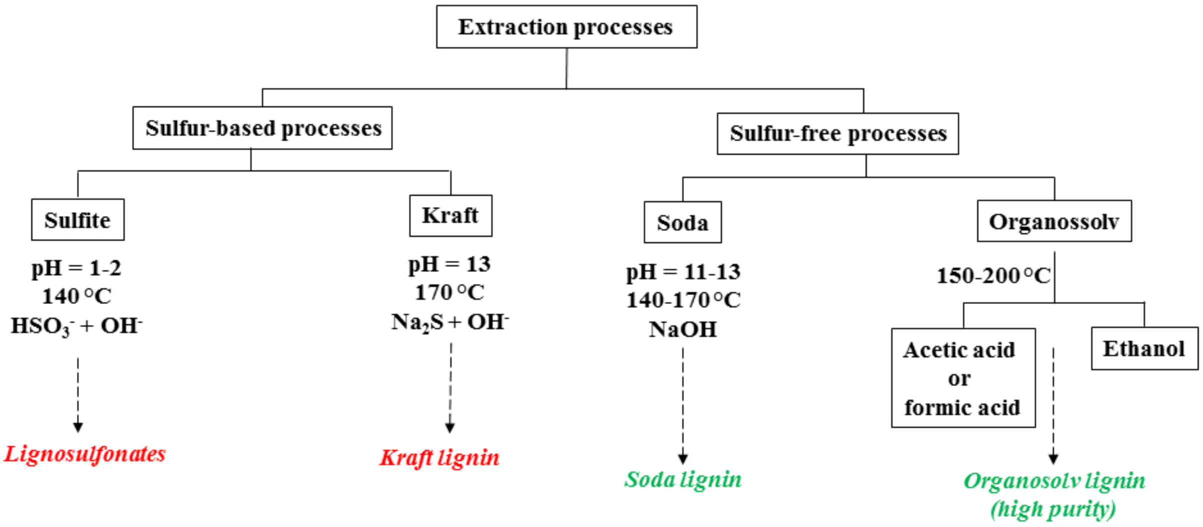
Figure 11 – Representation of the biomass fragmentation during the delignification process.



Source: The authors.

Basically, the processes used for delignification can be classified in two categories, such as the sulfur-based and the sulfur-free processes, **Figure 12**, yielding lignins with different structural features. Each process will be discussed in this section, in which the properties of each lignin will be highlighted as well.

Figure 12 – Flowchart of the main processes used for biomass delignification.



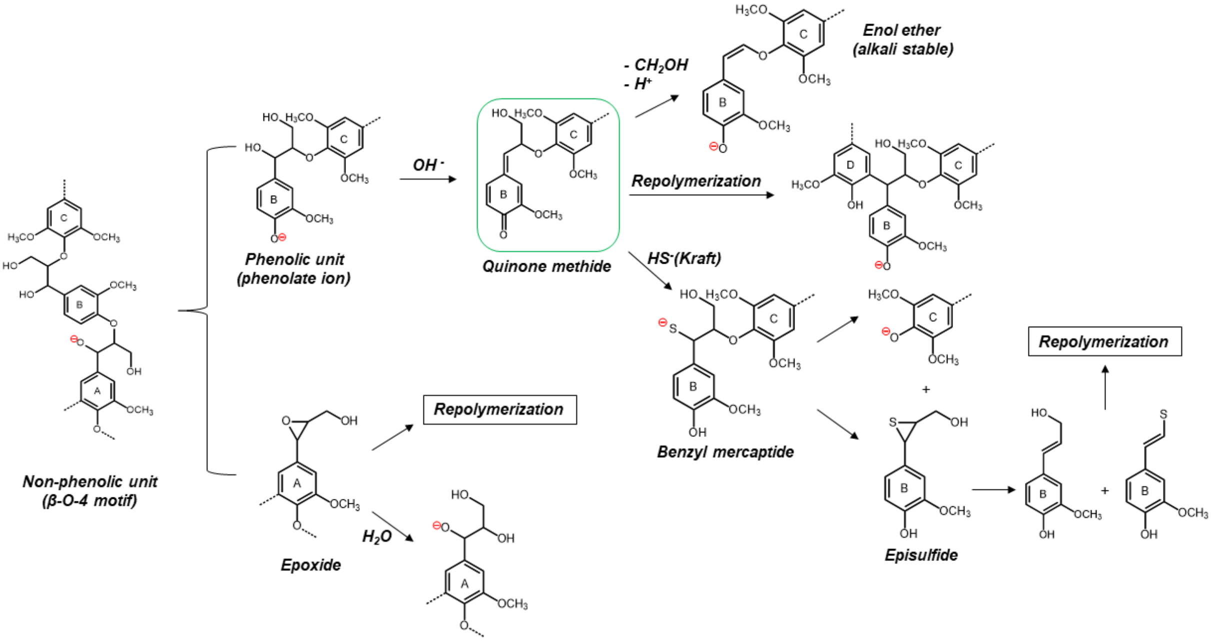
Source: The author.

Traditionally, there are three processes from which lignin is extracted in industrial scale: Kraft, sulfite and soda. However, lignin is produced mainly as a byproduct of paper and pulp industry that uses the Kraft process (MIAO; HAMAD, 2017). In this process, the feedstock is treated with “white liquor”, which is composed of a 1 mol L^{-1} NaOH and 0.25 – 0.70 mol L^{-1} Na_2S aqueous solution, at temperatures of 165 – 175 °C during 2 – 3 hours (CRESTINI et al., 2017; RINALDI et al., 2016).

The sulfite process is also commonly used in the pulp and paper industry and it is carried out by reacting lignin and a metal sulfite (usually, calcium, magnesium or sodium) and sulfur dioxide. The adjustment of the pulping medium allows the possibility of use of a wide range of pH varying from 2 to 12. The pulping is performed at a temperature range from 120 to 180 °C during 1 – 5 hours. On the other hand, the soda process is carried out by the digestion of the biomass in a 13 – 16 % wt. NaOH at 140 – 170 °C using anthraquinone as a catalyst (FIGUEIREDO et al., 2018).

The alkaline media favor the occurrence of cleavage of lignin-carbohydrate bonds (ester bonds in LCC), the fractionation of lignin molecule through the cleavage of ether bonds, mostly the β-O-4 motifs, the solubilization of the fragments through the deprotonation of phenolic groups and eventually the occurrence of repolymerization reactions (SCHUTYSER et al., 2018). However, the chemistry involved in such base-catalysed reactions is much more complex than the steps described previously, **Figure 13**.

Figure 13 – The main reactions involved in the alkaline lignin chemistry.



Source: The author adapted from Schutyser et al., 2018

Considering the β-O-4 motif as a starting point, since its abundance in lignin structure, there are two possible routes for the cleavage of β-O-4 linkages: the first leads to the epoxide formation, whereas the second leads to the formation of a phenolate ion and subsequently a quinone methide, which represents a key role in alkaline lignin chemistry, since it can undergoes nucleophilic attack due to its tendency to restore the aromaticity. During the Kraft process, the presence of a strong nucleophilic agent such as HS^- favors the formation of episulfide intermediates. These sulfur-based intermediates can undergo several types of reactions, yielding phenolic compounds, such as coniferyl alcohol that can suffers degradation and repolymerization (SCHUTYSER et al., 2018).

A parallel reaction is the reaction between the quinone methide with an *in situ* formed lignin nucleophile, leading to the occurrence of repolymerization reaction through the formation of a carbon-carbon bond. Alternatively, another possibility to restore the aromaticity of the quinone methide is the removal of the terminal γ-CH_2OH group *via* a retro-aldol type reaction. The occurrence of this pathway is more favorable when a strong nucleophile is absent, such as in the case of soda pulping. Therefore, the cleavage of β-O-4 linkages occurs in a less extension. The released formaldehyde can favors the repolymerization *via* formaldehyde-phenol type condensation (SCHUTYSER et al., 2018).

Although the Kraft and sulfite processes generate lignins with low β-O-4 content , a high amount of condensed structures resultant from the formation of new carbon-carbon bonds is also present in their structures. Furthermore, those lignins also have relative low purity, due to the high sulfur and ashes content, which decrease their reactivity and limit their industrial applications (SCHUTYSER et al., 2018). The use of soda process yields sulfur-free lignins, their structures also have considerable amount of condensed structures, since the absence of a strong nucleophilic agent favors the occurrence of repolymerization reactions.

Another factor that should be taken into account is the generation of extremely alkaline (Kraft process) or acid (sulfite process) effluents, which represent a potential source of environmental pollution, if they were discarded inappropriately. As an alternative to these problems, the organosolv process arises by using organic solvents (alcohols or acids) to promote the delignification of lignocellulosic biomass. This method yields high purity and reactivity, sulfur free lignins with low ashes content, besides the possibility of recuperation and reutilization of the organic solvents used in the lignin extraction.

Organosolv process

The organosolv process is based on the utilization of organic solvents to promote the cleavage of ether and ester bonds present in lignin structure. The most common solvents used in this process are acetic, formic acid and ethanol diluted in water and combined with low concentrations of an inorganic acid catalyst, usually sulfuric or hydrochloric acid. Compared with Kraft and sulfite processes, organosolv is extremely advantageous since it generates sulfur free lignins, which makes it an environmentally friendly process (FARIS et al., 2017).

Among the organic solvents, the most used in the organosolv process are the organic acids, mainly formic and acetic acids, and alcohols, mainly methanol, ethanol, propanol and butanol, besides ionic liquids (WANG et al., 2017). However, the organic acids provide a better separation between the lignin and carbohydrates, due to their contribution of hydronium ions, which the rate of this reaction (MANIET et al., 2017).

Furthermore, organosolv pulping is a promising technology since it promotes an efficient fractionation of lignocellulosic biomass into its three main components: the cellulosic pulp, the lignin precipitate and the hemicellulose-derived stream (SCHUTYSER et al., 2018). Therefore, organosolv process yields a high purity lignin that can be used as high value-added product for several technological applications.

Organosolv pulping can be carried out with or without the use of an acid catalyst (autocatalytic reaction) and in both cases, the lignin molecule undergoes acid-catalysed depolymerization and recondensation reactions. Commonly, inorganic diluted acids, such as sulfuric and hydrochloric acids are used as catalysts to increase the hydrolysis of hemicellulose and the porosity of biomass (IMMAN et al., 2014).

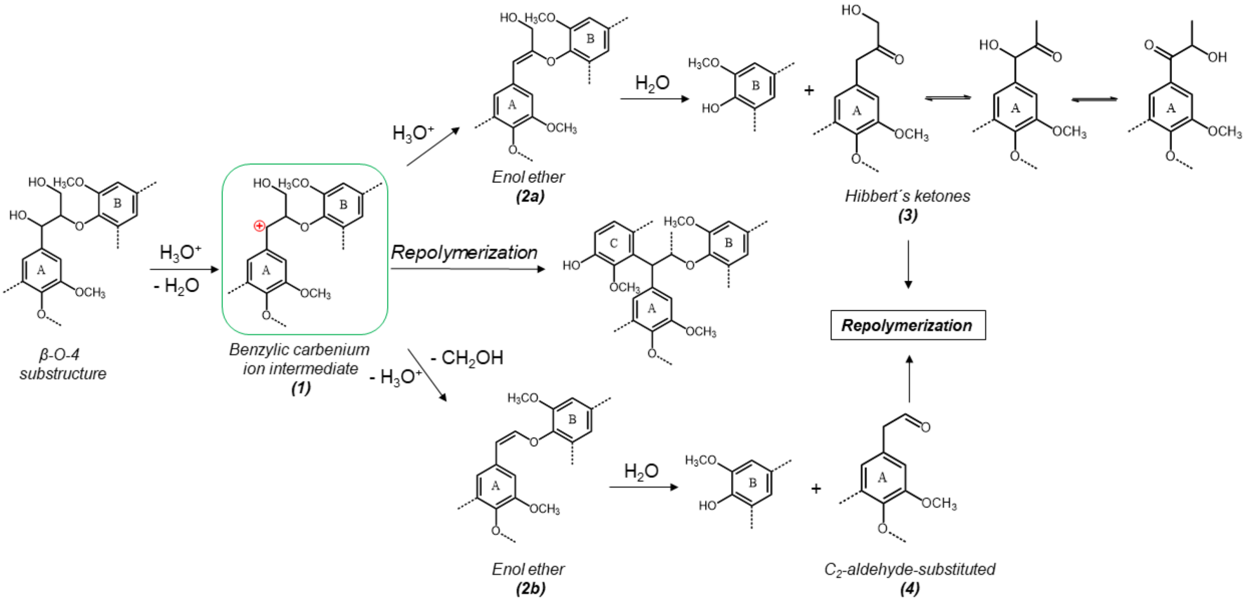
However, these acids have some disadvantages, such as danger of handling, equipment corrosion, recuperation problems and generation of effluents with high toxicity, such as organochloride compounds, which represent a concerning from the environmental point of view (SIRIPONG et al., 2016).

As an alternative to this problem, some Lewis acids have been explored as catalysts in order to evaluate their potential to replace the traditional Brønsted acids. Several Lewis acids have been used for this purpose, such as $FeCl_2$, $CuCl_2$, $FeCl_3$, $Ga(OTf)_3$, $ZrOCl_2$, $Sc(OTf)_3$ and $AlCl_3$ (CONSTANT et al., 2015; SCHWIDERSKI et al., 2014; WANG et al., 2016). As described by the authors, they provided comparable and sometimes better results than those obtained by using Brønsted acids, which shows their ability to catalyse the biomass deconstruction, besides to be safer and less hazardous, representing a potential class of compounds to replace the strong traditional acids currently used for it.

From the chemical point of view, the acid-catalysed chemistry involved in the delignification of lignocellulosic biomass using Brønsted or Lewis acids is similar and is based on the cleavage of ether bonds in the carbohydrate polymers, as well as the ester bonds existent in the lignin-carbohydrate complexes. This favors the solubilization of the lignin fragments by the organic solvent. The acidic medium also promotes the occurrence of depolymerization and repolymerization reactions in the lignin structure.

Considering the abundance of the β-O-4 motif, the most outstanding event in acid-catalysed lignin chemistry is the cleavage of β-O-4 bonds, as shown in **Figure 14**. The first step of the reaction is the formation of a benzylic carbenium ion (**1**) by the elimination of the OH-group in form of water from the α-position of phenylpropanoid chain. The carbenium ion can be transformed into two enol-ether species (**2a** and **2b**), with or without cleavage of the C_β-C_γ bond and the subsequently formation of formaldehyde. The applied acid will strongly influence on the prevailing pathway.

Figure 14 – The main reactions involved in the acid-catalysed lignin chemistry.



Source: The author adapted from Schutyser et. al. (2018).

Schutyser et al. (2018) claims that the use of H_2SO_4 favors the formation of intermediate **2b**, whereas the **2a** is the major specie when the catalyst is HCl or HBr. The subsequent hydrolysis of the acid-labile enol ethers (**2a** and **2b**) yields (i) C_2-aldehyde-substituted phenolics (**4**) and (ii) C_3-ketone-substituted phenolics (Hibbert's ketones, **3**). The latter compounds (**4**) in combination to the carbenium ions (1) and C_2-aldehyde-substituted phenolics (**3**) are involved in a series of complex repolymerization reactions that eventually lead to the formation of a condensed lignin structure.

Although organosolv pulping is a promising technology for biorefinery purposes, it has only been applied in laboratory and pilot plant scales. The increase in the demand of bio-based products (3 – 4 % growth rate per year) requires the scale up of biorefineries. However, the scale up of process for biorefinery proportion is still a challenge due to the several factors involved in the process. Since the experimental data acquired at lab and pilot plant scales may not necessarily have reproducibility when several tones biomass are used, the development of tools capable of design and simulate all the steps and factors involved in the process, such as accurate processing flow sheets, process modeling and simulation focusing on cost sensitivity analysis, life cycle assessment, forecasting and identifying risk factors, is extremely necessary to promote its scale up (CHANDEL et al., 2018).

According to previous studies related by Chandel et al. (2018), the main factor that influences in the success of biomass scale up is the correct integration of the biomass feedstock with process design, because each type of biomass requires a specific pretreatment to yield a specific bio-based compounds as well. Therefore, origin of biomass and its composition, pretreatment conditions and other factors exert great influence in scale up operations. Furthermore, technical details such as process integration, selection of products, clear-cut master plan, risk factors analysis, cost sensitivity analysis & regulatory norms and reproducible economic modelling play key role in a good management and operation for scaling up of biorefineries.

All those variables can influence on the operation and total costs for each process, as well as on the price of the final product, in this case the isolated lignin. Carvajal et al. (2016) performed a complete study about the economical aspects of four extraction process (soda, Kraft, organosolv and sulfite) for two biomasses (sugarcane bagasse – SCB and rice husk – RH) (CARVAJAL; GÓMEZ; CARDONA, 2016). The authors reported that surprisingly the organosolv pulping had the lower total cost value than the traditional industrial processes, **Table 1**. On the other hand, organosolv has the highest operating cost, which is responsible for 33.9 and 34.2 % of the total cost, for SCB and RH, respectively, **Table 1**.

This elevated cost is probably related to the use of organic solvents and the apparatus required to recover them, as well as to energy consumption, since the operation at middle and high pressures increases the energy expenditure. The main problem caused by these high costs related to operation is that the final product becomes more expensive as well, **Table 2**, in which shows that for both biomasses the organosolv process produces the highest price lignin.

The authors also attributed this behavior to the lower lignin yield provided by the organosolv process. However, it is worth mentioning that the lignin yield is strongly influenced by the operation conditions (temperature, reaction time, type and concentration of catalyst, pressure, etc) and that despite the technical and economical limitations the organosolv still yielding a high quality lignin and that these limitations can be surpassed or minimized using a process design, allowing the adjustment of the parameters involved on it.

Table 1 – Economical assessment (annualized costs) for lignin extraction processes without a preliminary pretreatment from sugarcane bagasse and rice husk.

Lignin extraction from sugarcane bagasse								
Item	**Soda**		**Kraft**		**Organosolv**		**Sulfite**	
	Million US$/year	Share (%)	Million US$/year	Share (%)	Million US$/year	Share (%)	Million US$/year	Share (%)
Raw material	31.02	88	37.68	68.1	11.82	41.8	34.73	64.6
Utilities	1.53	4.3	14.40	26.0	6.74	23.9	16.0	29.8
Operating	2.61	7.4	3.14	5.7	9.59	33.9	2.91	5.4
Capital depreciation	0.10	0.3	0.10	0.2	0.1	0.4	0.10	0.2
Total cost	35.26		55.33		28.25		53.74	
Lignin extraction from rice husk								
Item	**Soda**		**Kraft**		**Organosolv**		**Sulfite**	
	Million US$/year	Share (%)	Million US$/year	Share (%)	Million US$/year	Share (%)	Million US$/year	Share (%)
Raw material	30.99	88.3	37.12	69.3	11.12	42.0	34.41	65.9
Utilities	1.41	4.0	13.25	24.7	6.20	23.4	14.82	28.4
Operating	2.61	7.4	3.10	5.8	9.02	34.2	2.88	5.5
Capital depreciation	0.17	0.3	0.10	0.2	0.10	0.4	0.10	0.2
Total cost	35.11		53.56		26.44		52.21	

Source: Carvajal et al. (2016)

Table 2 – Assessment of the cost per kg of lignin for the extraction processes without a preliminary pretreatment from sugarcane bagasse (SCB) and rice husk (RH).

Process	**Soda**		**Kraft**		**Organosolv**		**Sulfite**	
Raw material	SCB	RH	SCB	RH	SCB	RH	SCB	RH
Production cost (USD/kg lignin)	5.1	3.5	6.3	4.3	16.0	10.2	7.8	5.3
NVP (millions USD)	22.4	72.8	50.0	114.8	- 494.6	- 440.9	24.5	81.5
Profitability index	1.13	1.40	1.24	1.51	0.16	0.20	1.13	1.41
Payout period (year)	3	1	2	1	-	-	3	1

Source: Carvajal et al. (2016)

Microwave irradiation

The majority of the studies in the literature about lignin extraction through organosolv method use hot plates or high pressure reactors as heating sources (AVELINO et al., 2018a; FERNÁNDEZ-RODRÍGUEZ et al., 2017; LØHRE; KLEINERT; BARTH, 2017; MANIET et al., 2017; PINHEIRO et al., 2017). These types of heating source use elevated temperatures and long reaction times, which imply high energy consumption due to the high energy dissipation. Moreover, there also are the significant costs associated to the maintenance of the high pressure reactors, as well as the risks to the security caused by the use of elevated pressures, **Figure 15**.

Figure 15 – The types of heating sources at lab and pilot plant scales.

Hot plate (atmospheric pressure)

High pressure reactor (lab scale)

High pressure reactor (pilot plant scale)

LONG REACTION TIMES

HIGH TEMPERATURES AND PRESSURES

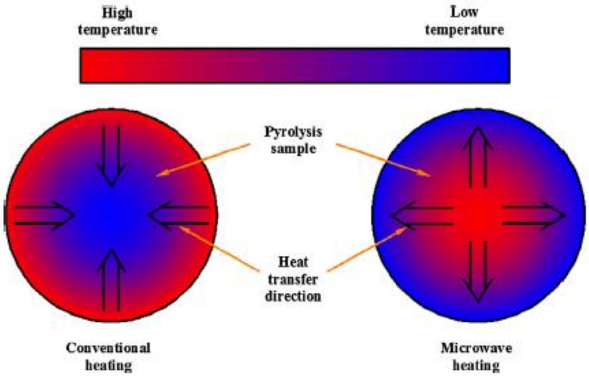
HIGH ENERGY CONSUMPTION

Source: The author.

As an alternative to the traditional heating sources there is the heat generated by the microwave irradiation (MWI), which is a type of electromagnetic radiation that has a frequency range from 300 to 300,000 MHz and is located between the infrared and radio waves regions in the electromagnetic spectrum. Since the MWI has an electric component, the presence of any material containing mobile electric charges, such as polar compounds or ions, will absorb this radiation causing an increase in the system's temperature. The microwave heating is based on the dipole's rotation and ionic conduction, which allows to carry out organic reactions in a few minutes with high yields (SANSEVERINO, 2002).

Therefore, the polar compounds present in the reaction medium will suffer some influence from the radiation's electric field causing changes in their orientation and producing heat by friction. This heat will be generated from inner to outer of the reaction mixture, while in traditional heating, such as oil baths, the heat will be produced from outer to inner to the medium through thermal conduction, as shown in **Figure 16**.

Figure 16 – Differences between the traditional and microwave heating.



Source: Miura, M., Kaga, H., Sakurai, A., Kakuchi, T., Takahashi, K., 2004. Rapid pyrolysis of wood block by microwave heating. J. Anal. Appl. Pyrol. 71 (1), 187–199.

Thus, this technique also permits the reduction of energy consumption due to the decreasing in the reaction times and the use of low power ratings as well. The possibility to perform several types of reactions in a matter of minutes is due to the changes in the dipoles orientation, which will produce heat by friction increasing their average kinetic energy and consequently the probability of effective collisions as well. Furthermore, the MWI also has selectivity and uniform volumetric heating performance, easy operation and low degradation or formation of side products when compared to traditional heating.

Therefore, MWI has been considered as an emerging technology for pretreatment of lignocellulosic biomass (HASSAN; WILLIAMS; JAISWAL, 2018; KOSTAS; BENEROSO;

ROBINSON, 2017; LI et al., 2016). The literature reports several MWI pretreatments as at atmospheric as at high pressure. At atmospheric pressure, there are the pretreatments carried out in a high boiling solvent (HBS), ionic liquids (ILs), alkali, salt and acid medium. On the other hand, at high pressure, the reactions can be performed using an alkali or acid medium (LI et al., 2016).

Among the several types of pretreatments using MWI, the microwave-assisted organosolv pretreatment has been showing a great potential to fractionate the lignocellulosic biomass into the cellulosic pulp, hemicellulose derivatives aqueous phase and lignin fraction. However, MWI combined with organosolv process also can be used for delignification purposes without any preliminary pretreatments, in order to yield a high quality lignin.

However, it is important to highlight that there are few works in the literature that use the microwave-assisted organosolv method for lignin extraction. This scenario shows there is a gap in this matter to be fulfill, since the amount of published works in this research filed in the last 7 years is very low, **Table 3**.

Table 3 – Compilation of papers that used microwave-assisted organosolv process for lignin extraction and the main extraction parameters used.

Agrowaste	**Solvent**	**Concentration (% v/v or wt.)**	**Fiber/solution ratio (g/mL)**	**Catalyst (% v/v or wt.)**	**Temperature (°C)**	**Time (min)**	**Lignin yield (%)**	**Pressure (bar)**	**Reference**
***Agave angustifólia* haw bagasse**	Ethanol	40 – 60	1:20	0.1 % HCl	150	60-120	58.8	10^{-3}	(HERNÁNDEZ et al., 2018)
***Agave angustifólia* haw bagasse**	Ethanol	40 – 60	1:20	0.1 % HCl	120	60-120	70.4	Atmospheric	(HERNÁNDEZ et al., 2018)
Bamboo	Ethanol	0 – 100	1:20	Formic acid (0-5)	110 – 190	20	2 – 32	Closed vessel	(LI et al., 2018b)
Coconut coir pith	Acetic/Formic acid	85/85 (7:3)	1:8	-	98	5-20	4.1	Atmospheric	(ASOKA PANAMGAMA; PERAMUNE, 2018)
Coconut shell	Acetic acid Formic acid	90 85	1:10 1:10	2.0 % $AlCl_3$, H_2SO_4, HCl	110 100	20 20	≈ 55 ≈ 35	Atmospheric Atmospheric	(AVELINO et al., 2018)
Eucalyptus sp.	Acetic/Formic acid	59.5/25.5	1:10	1.0 % H_2SO_4	108	150	15	Atmospheric	(ZHENG et al., 2018)

Ground agrowastes	Glycerol/Methanol	2:1	1:4	0.1 % H_2SO_4	120	9	17.6 – 23.0	Atmospheric	(XIE et al., 2015)
Ground agrowastes	Ethanol	92	1:20	0.32 M H_2SO_4	30	150	1.5 – 35.2	Closed vessel	(MANARA et al., 2014)
Ground agrowastes	Ethanol	81	1:10	0.5 M H_2SO_4	140	30	12.4 – 19.2	Closed vessel	(MONTEIL-RIVERA et al., 2013)
Triticale straw	Ethanol	27 – 95	1:20	0 – 1 M H_2SO_4	83 – 167	30	3.6 – 17.6	Closed vessel	(MONTEIL-RIVERA et al., 2012)
Bamboo	Acetic/Formic acid/water	3/5/2	1:20	6.0 % HCl	90 – 109	0 – 120	3.5 – 18.5	Atmospheric	(LI et al., 2012)
Birch	Formic acid	88	1:10	-	101	5 – 30	21.9 – 88.7	Atmospheric	(ZHOU et al., 2012)

Source: The author.

It is worth mentioning that there are several types of microwave reactors, such as the microwave penetration vessel (MWPV), microwave reflection tank (MWRT) and continuous MWI pretreatment reactor (CMWI). Furthermore, microwave reactors are a scalable technology allowing the lignin extraction in large amount, as described by Peng et al. (2014) who designed a pilot-scale CMWI reactor for biomass pretreatment with a maximum capacity of 5 kg/h (PENG et al., 2014). Tsubaki and Azuma (2011) also developed a CMWI reactor which had a maximum power of 4.9 kW, maximum temperature of 240 °C with treatment capacity of 7-20 L/h (TSUBAKI; AZUMA, 2011).

In addition, despite those reactors designed by some research groups previously commented, there is a japanese enterprise that manufactures microwave reactors for several scales and purposes, such as bath, bench, pilot and commercialization scale (MICROWAVE CHEMICAL CO., 2018), **Figure 17**, which shows that this technology is already available commercially and it could be used for biorefinery studies, specifically the microwave-assisted organosolv delignification.

Figure 17 – Scaling up of microwave reactor.

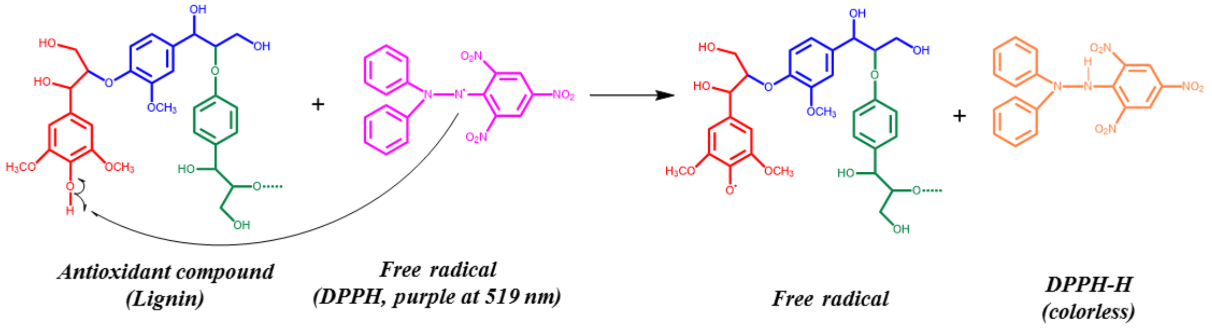
Source: Microwave Chemical Co., Ltd. (http://mwcc.jp/en/service_technology/platform03.html, accessed 01.13.2019).

Antioxidant activity

Antioxidants are compounds capable of scavenge free radicals preventing the oxidation of other molecules, **Figure 18**. They are commonly used in the food industry in order to avoid the oxidation of some food constituents, such as lipids, increase the shelf life of the products. Furthermore, antioxidants are also used in the polymers and fuels as additives, aiming their preservation from the heat and UV degradation.

In relation to the chemical structure, the phenolic antioxidant compounds have higher antioxidant capacities than the non-phenolic ones due to the high acidity of phenolic hydroxyls, which allow the neutralization of the free radical by hydrogen donation, as well as the stabilization of the phenoxy radical by resonance, **Figure 18**.

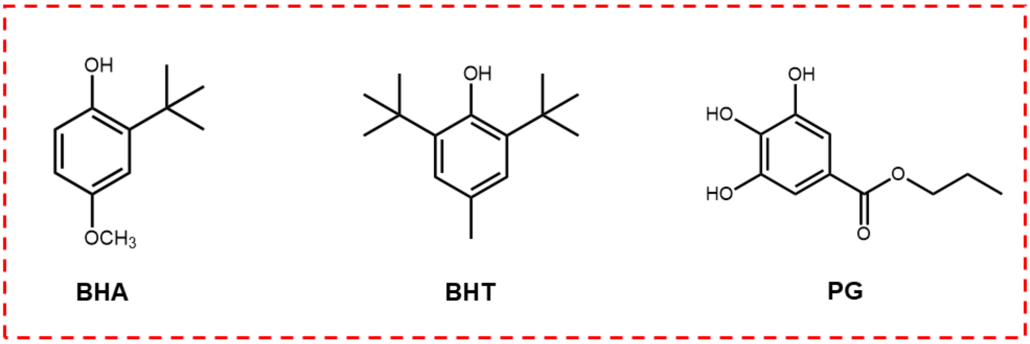
Figure 18 – Schematic representation of the mechanism of action of an antioxidant compound.



Source: The author.

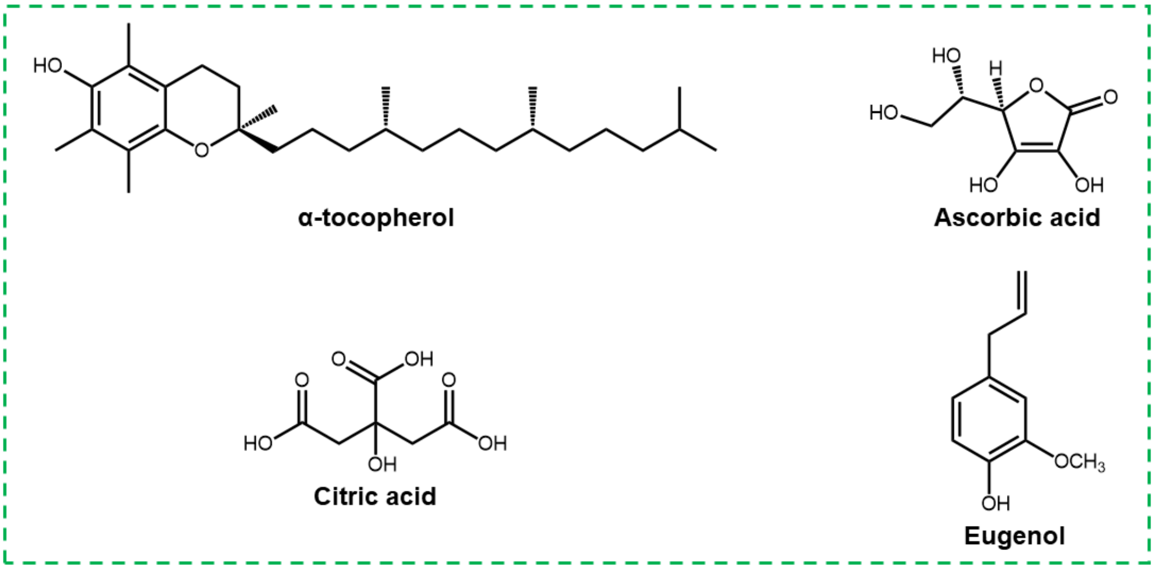
The antioxidants can be categorized into two classes such as the synthetic and the natural compounds. For a long time, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG) were the most used molecules in food to avoid its deterioration, **Figure 19**. However, these compounds have been reported as harmful being directly linked to stomach and bowel diseases, as well as food allergy (RIBEIRO et al., 2019). Therefore, the increasing concern of the consumers about the safety of such additives has made the food industry insert natural antioxidants in food composition, such as tocopherols (α-tocopherol), ascorbic and citric acids, carotenoids (β-carotene) and phenolic compounds (eugenol), **Figure 20**.

Figure 19 – The main synthetic antioxidant compounds used in food industry.



Source: The author.

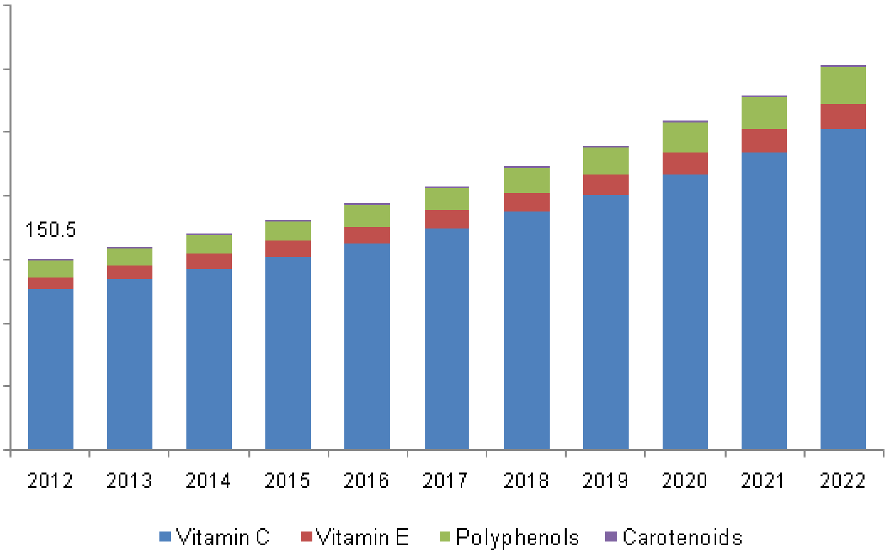
Figure 20 – Examples of some natural antioxidant compounds.



Source: The author.

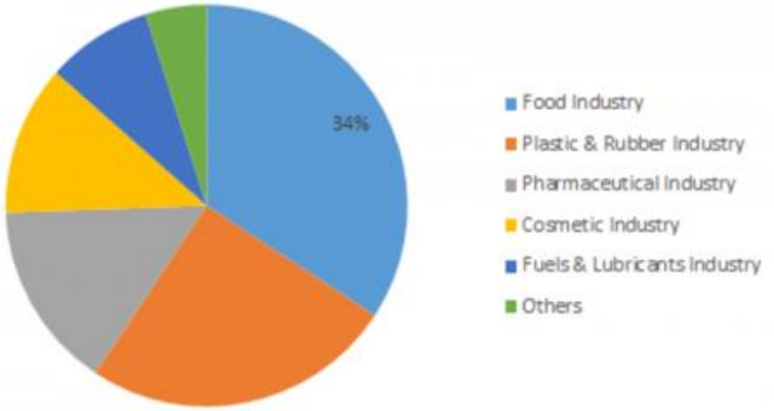
Among the natural antioxidants, the phenolic ones stand out due to their availability in the nature and also their high antioxidant capacity. Moreover, the polyphenols have dominated the natural antioxidants market, showing a continuous increase on their demand for different purposes, **Figure 21**. Furthermore, the use of the phenolic compounds in different segments of the chemical industry has increased and it is estimated that this market reach a USD 1830.05 million in revenues until 2023, Figure 21.

Figure 21 – Global natural antioxidants market, by category, 2012 – 2022 (kilo tons).



Source: https://www.grandviewresearch.com/industry-analysis/natural-antioxidants-market, accessed in 07/04/2019.

Figure 22 – Participation of the phenolic compounds as natural antioxidants in different technological fields.

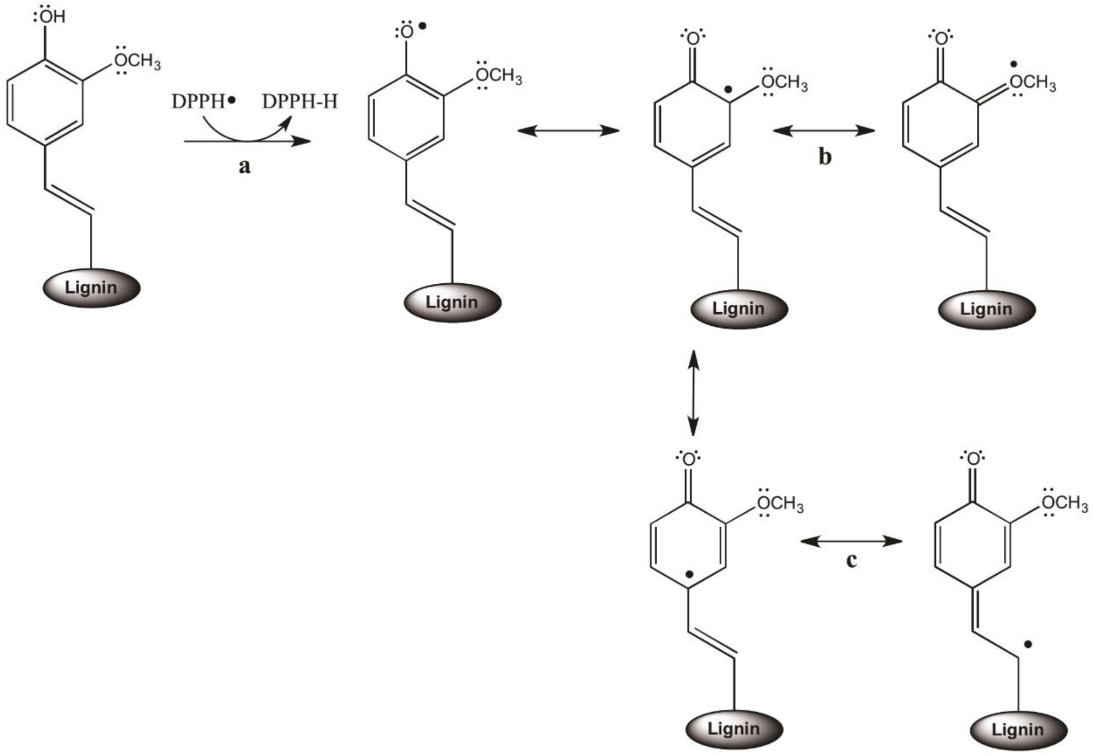


Source: https://www.reuters.com/brandfeatures/venture-capital/article?id=69322, accessed in 07/04/2019.

The importance of polyphenolic compounds to the chemical industry has made their consumption and production increase. The total amount of industrially extracted polyphenolic compounds reached 16,380 tons in 2015 and it is expected to reach 33,880 tons by 2024, which confirms their importance to some specific industries, such as functional beverages (44 %) and food (33 %) (OU et al., 2019).

As a natural polyphenolic molecule, lignin has a huge potential as an antioxidant to avoid the oxidation of biofuels, foods and polymeric composite materials. This is due to the presence of a considerable amount of phenolic hydroxyls and methoxyl groups in its structure, which are responsible for the neutralization of the free radicals, as well as the stabilization of the phenoxy radicals by resonance forming quinone structures. Furthermore, the presence of double bonds in the phenylpropanoid chain also helps to stabilize the phenoxy radicals, **Figure 23**.

Figure 23 – Proposed pathways involved in the neutralization of DPPH free radicals by lignin.



Source: (NOGUEIRA et al., 2018)

Basically, there are two main mechanisms involved in the radical scavenging: the hydrogen atom transfer (HAT), where radicals are neutralized by hydrogen atom donation of the antioxidant; and the single electron transfer (SET), in which the ability of an antioxidant to transfer one electron to reduce a compound plays a key role (ALZAGAMEEM et al., 2018), as shown schematically in **Equations 1** and **2**, respectively.

$$\text{HAT: } X\cdot + AH \rightarrow XH + A\cdot \quad (1)$$

$$\text{SET: } X\cdot + AH \rightarrow X^{-} + AH^{+} \quad (2)$$

where $X\cdot$ is the radical, AH is the antioxidant, XH is the protonated specie resultant from the radical scavenging, X^{-} is the anion and AH^{+} is the ionized form of the antioxidant.

It is common the occurrence of both mechanisms in parallel, resulting in complex reaction kinetics and numerous side reactions (ALZAGAMEEM et al., 2018). The effectiveness of an antioxidant depends on several factors, such as activation energy, rate constants, oxidation-reduction potential, antioxidant stability and solubility. In addition, the higher the bond dissociation energy (BDE) of AH the higher the activation energy and lower the antioxidant efficiency. On the other hand, the lower that BDE the higher the antioxidant efficiency (BREWER, 2011).

The capacity of an antioxidant to scavenge free radicals is quantitatively measured using several assays, such as the 2-2'-azino-bis(3-ethylbenzo thiazoline-6-sulphonic acid) (ABTS), the 2,2-diphenyl-1-picrylhydrazyl (DPPH), the Folin-Ciocalteu or Total phenolics (FC/TPC), etc. Each of those assays has specific features and peculiarities that are described in **Table 4**. These methods use UV-Vis spectrophotometry to monitor the progress of the kinetics of radical scavenging of free radicals, allowing the measurement of the antioxidant capacity, as well as the concentration of the antioxidant required to cause an inhibition of 50 % of the free radicals (IC_{50}).

Recent studies have been shown the potential of lignin as an antioxidant in both forms such as isolated and incorporated in polymeric matrices, showing its wide range of applicability (OLIVEIRA et al., 2017; AVELINO et al., 2019; NOGUEIRA et al., 2019). The common approach between those studies is the highlight of the structural features of lignin as a pivotal point on its antioxidant performance. Therefore, several approaches are proposed in order to improve the antioxidant capacity of lignins, such as organic and aqueous solvent fractionation, depolymerization, hydrogenolysis, etc.

Therefore, despite the approach chosen to modify the lignin structure, the studies has shown that a deep structural investigation is needed to provide reasonable insights about the structure – property relationship, allowing to design the modification process in order to obtain a lignin with suitable structural features and maximum performance.

A recent study of our group showed the huge potential of the acetosolv palm oil lignin fractions as natural antioxidants, since they presented very lower IC_{50} values than two commercial antioxidants, such as BHT and Irganox 1010, indicating that it is required a extremely low amount of those lignins to cause a similar inhibition value than that of BHT and Irganox 1010.

However, there is still a need for the perform of a cytotoxicity assay of lignins, in order to verify if the degradation products originated from them on the human body are toxic or non-toxic. This is extremely important when one is proposing an alternative for the synthetic antioxidants that are well known to cause some disorders to the health due to the release of toxic metabolites.

The understanding about the cytotoxicity of lignin will also influence the type of application that it will be used for, since if toxic compounds are released, it should not be used for food and cosmetic purposes, but it can be used for example, as additives in thermoplastics, epoxy resins and fuels.

Table 4 – The features of the main antioxidant assays.

Antioxidant assay	Mechanism	Advantages	Disadvantages	Reference
ABTS	HAT + SET	- simple, quick, wide pH-range, often used - soluble in aqueous and organic solvents - several wavelengths are available for photometric detection of the ABTS radical	- no exact time - reactivity is strongly varying for different samples - the bulky ABTS radical is not a good model for small, biologically more relevant radicals like OH·	(ALZAGAMEEM et al., 2018)
DPPH	HAT + SET	- simple, quick, often used, no special equipment needed - DPPH is commercially available	- no exact time - reactivity is strongly varying for different samples - DPPH radical may have a poor reactivity with antioxidants due to its stability and sterical hidrance	(MISHRA; OJHA; CHAUDHURY, 2012)
FC/TPC	HAT + SET	- simple, often used, does not require special equipment	- no exact time - reactivity is strongly varying for different samples - interferences with other reductive substances may influence the results	(BENAVIDES et al., 2013)

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Chapter 2 – Microwave-assisted extraction of coconut shell lignin by Brønsted and Lewis acids catalysts

Graphical abstract

RESUMO

O fracionamento de recursos renováveis em seus componentes tem sido explorado exaustivamente, a fim de que estes possam substituir a utilização de compostos do petróleo. Dentre eles, a obtenção de lignina de elevada qualidade para diversas aplicações ainda é um desafio, visto que sua estrutura é drasticamente afetada pelo método empregado em sua extração. O objetivo desse trabalho foi avaliar os efeitos causados pelo processo de deslignificação organossolve assistido por micro-ondas (DOAM) nas propriedades físico-químicas e estruturais da lignina do endocarpo do coco seco utilizando-se diferentes catalisadores. Um estudo exploratório da concentração do catalisador e do tempo de reação foi realizado para otimizar a condição de extração dos experimentos de DOAM. Obteve-se elevados rendimentos (≈ 55 %) ao utilizar-se ácido acético aquoso (90 % v/v) combinado ao uso de $AlCl_3$, H_2SO_4 e HCl 2,0 % m/v ou v/v como catalisadores a 110 °C durante 20 minutos. Extrações utilizando essas mesmas condições foram realizadas sob aquecimento convencional para comparar a eficiência da irradiação por micro-ondas em termos dos rendimentos e propriedades estruturais das ligninas. Sob as mesmas condições, os experimentos de DOAM geraram ligninas com elevados rendimentos e pureza (≈ 55 e 85 %, respectivamente), porém com menores massas moleculares (≈ 1792 g mol^{-1}). Em termos de composição monomérica, as ligninas obtidas por ambas as formas de aquecimento foram muito similares. Pode-se concluir que a DOAM é um procedimento eficiente e sustentável para a obtenção de lignina em questão de minutos, produzindo um produto com elevados rendimentos e pureza, permitindo sua utilização em diversas aplicações tecnológicas.

***Palavras-chave**: Resíduo agroindustrial; biopolímero; micro-ondas; organossolve; elucidação estrutural.*

ABSTRACT

The fractionation of renewable sources into their components has been extensively explored in order to replace the utilization of the petrochemical compounds. Among those, the obtainment of a high quality lignin for further applications stills a challenge, since its structure is severely affected by the extraction method. The aim of this work was to evaluate the physicochemical and structural effects on coconut shell lignin (CSL) caused by microwave-assisted organosolv delignification (MWAOD) under different acids catalysts. A screening of catalyst concentration and reaction time was employed to optimize the extraction condition for the MWAOD assays. Highest yields (≈ 55 %) were obtained using aqueous solution of acetic acid (90%) combined with 2.0 % of $AlCl_3$, H_2SO_4 or HCl under 110 °C for 20 minutes. Extractions using these catalysts were also performed under traditional heating in order to compare the efficacy of microwave irradiation in the yields and chemical structure of lignins. Under the same conditions, MWAOD produced lower molecular weight lignins (≈ 1792 g mol^{-1}) with higher yields and purities (≈ 55 e 85 %, respectively). In terms of monolignol composition, both lignins were quite similar. It can be concluded that microwave-assisted organosolv process is an efficient and eco-friendly procedure lignin extraction, in a matter of minutes, at high yields and purities, allowing their utilization for further technological applications.

***Keywords:** agrowaste; biopolymer; microwave; organosolv; structural elucidation*

Introduction

In the recent years, the growing concern about the environment have made the scientific community explore alternative sources as raw material in detriment of the non-renewable fossil sources (ALI; BAHAITHAM; NAEBULHARAM, 2017). Among the alternative renewable sources, biomass becomes an interesting and promising solution to generation of greener energy and chemicals, especially for Brazil, which has an enormous area destined for agriculture, as well as the greatest biodiversity on Earth.

Along with this great biodiversity, climate variety provides to tropical regions the appropriate conditions for the development of the agribusiness, which generates large amounts of low-cost residues coming from the processing of crops, and represent a potential form of environmental pollution (MILLÁN; VARGAS; NZIHOU, 2017). In Brazil, where the agricultural production accounts for 6.5 % of the Gross Domestic Product (GDP), the agrowaste is a concerning problem, since this activity is one of the most important to the country´s economy (MEDINA; SANTOS, 2017; SCHLEMPER; STÜRMER, 2014).

Among the main agrowastes generated in Brazil, coconut (*Cocos nucifera*) stands out since its economic relevance, where 285.000 hectares are destined to its cultivation, yielding approximately 2 billion coconut fruits per year (FARIAS et al., 2017). After its industrial processing, the majority of solid parts are discarded or burned in industrial furnaces, both consisting in its underutilization and in an environmental pollution. Except the coconut edible part, there are the mesocarp, approximately 25 % of the coconut weight, and the endocarp, or coconut shell (CS), representing 20% in mass of the coconut (BLEDZKI; MAMUN; VOLK, 2010; RAMBO; SCHMIDT; FERREIRA, 2015).

There are several works reporting the utilization of the coconut fibers as raw material for the generation of high value-added products, such as nanocellulose, while the utilization of CS is almost unexplored (AZEREDO; ROSA; MATTOSO, 2015; NASCIMENTO et al., 2014, 2016; ROSA et al., 2010; UMA MAHESWARI et al., 2012). Some papers report the use of CS in the production of char (CHEN; KUO, 2010), activated carbon (ARENA; LEE; CLIFT, 2016; PRAUCHNER; RODRÍGUEZ-REINOSO, 2012), fuels (ROUT et al., 2016; SIENGCHUM; ISENBERG; CHUANG, 2013) and filler in composites and concretes (BLEDZKI; MAMUN; VOLK, 2010; GUNASEKARAN; KUMAR; LAKSHMIPATHY, 2011; JAYAPRITHIKA; SEKAR, 2016; KEERTHIKA et al., 2016), applications that highlight its elevated lignin content, and turns it into a promising source for lignin extraction.

Lignin is the second abundant natural polymer after cellulose and the major natural source of phenolic compounds. It has structural function in the cell walls, conferring rigidity and protection from hydrolytic attack of pathogenic microorganisms (BILAL et al., 2017). Chemically, lignin is a hydrophobic amorphous polymer, formed by phenylpropanoid units: *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S), randomly linked.

The chemical structure of lignin has been extensively researched by different methods (LAURICHESSE; AVÉROUS, 2014). The existence of several functional groups in its structure offers different possibilities to chemical modifications, in order to produce environmentally friendly products with technological applications, replacing petroleum-based materials.

However, lignin chemical structure depends on the raw material origin, as also on the extraction and isolation methods, which will influence its reactivity and, consequently, its applications. Traditionally, lignin is a byproduct of the pulp and paper industry, where the processes require the utilization of acid or alkali medium to promote the delignification of the raw material at elevated temperatures under high pressure.

These harsh conditions are achieved by using high-pressure reactors or autoclaves, which are based on conductive heating from an external source for several hours. These long reaction times are undesirable due high energy consumption, besides side reactions that can occur for lignin and the effects that pressure can cause in its structure (LI et al., 2012).

Recently, microwave-assisted extraction (MWAE) has been evaluated as an alternative method to promote the fragmentation of lignocellulosic biomass (KURIAN et al., 2015; LI et al., 2012; MONTEIL-RIVERA et al., 2012; XIE et al., 2015; ZHOU et al., 2017, 2012). This method uses the microwave irradiation to heat, which is based on dipole rotation and ionic conduction, promoting faster chemical reactions with higher yields than traditional heating (SANSEVERINO, 2002). Therefore, this technique can be used as a sustainable methodology for lignin extraction, since it provides decrease in reaction times, as well as the utilization of low power ratings, reducing energy consumption and increasing efficiency.

Since in MWAE polar compounds must be used in order to absorb the radiation, the organosolv process is a suitable method for lignin extraction. In this process, organic solvents, such as alcohols and organic acids, are used to delignify lignocellulosic biomass, allowing their recovering and reutilization. Commonly, aqueous solutions of acetic acid and ethanol combined with low concentrations catalysts, such as H_2SO_4 and HCl, are used to separate lignin and carbohydrates.

This process yields high purity and low molecular weight lignin than that obtained in industry, like Kraft and sulfite, which have high sulfur and ash contents limiting their technological applications. Despite the fact that Brønsted acids (HCl and H_2SO_4) catalyze lignin extraction in organosolv process, other works have used Lewis acids ($MgCl_2$, $FeCl_2$, $CuCl_2$, $FeCl_3$, $ZrCl_2$, $AlCl_3$) as catalysts, evaluating their effects in lignin structure (CONSTANT et al., 2015; HUANG et al., 2016; SCHWIDERSKI et al., 2014; WANG et al., 2016).

However, these works performed the lignin extraction at elevated temperatures and pressure, and it is necessary to consider the combined effects of these parameters. To the best of our knowledge, there are no reports on the use of microwave irradiation combined with Lewis acids as catalysts under mild conditions (atmospheric pressure) to promote delignification of biomass.

Therefore, the aim of this study was evaluate the physicohemical and structural effects on coconut shell acetosolv lignins (CSALs) caused by microwave-assisted organosolv delignification (MWAOD) using different Lewis ($MgCl_2$, $MnCl_2$, $CoCl_2$, $ZnCl_2$ and $AlCl_3$) and Brønsted acids (CH_3COOH, H_2SO_4 and HCl), and compare the results with those obtained by traditional heating (TH).

Experimental

Materials

The following chemicals were used as received: glacial acetic acid (Synth), H_2SO_4 (98 %, Vetec), HCl (37 %, Synth), $MgCl_2$ (98 %, Sigma-Aldrich), $MnCl_2$ (98 %, Sigma-Aldrich), $CoCl_2$ (97 %, Sigma-Aldrich), $ZnCl_2$ (Cromoline), $AlCl_3$ (98 %, Sigma-Aldrich), DMSO-d_6 (99.96 %, Sigma-Aldrich), HPLC-grade tetrahydrofuran (Sigma-Aldrich), 1,4-dioxane (99 %, Sigma-Aldrich), chloroform-d (99.96 %, Sigma-Aldrich), pyridine (99 %, Sigma-Aldrich), chromium (III) acetylacetonate (99.99 %, Sigma-Aldrich), cyclohexanol (99 %, Sigma-Aldrich) and 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (95 %, Sigma-Aldrich).

Raw material preparation

The CSs were supplied by Ducoco Produtos Saudáveis (Itapipoca, Brazil). They were milled in a knife mill (Fritsch pulverisette 19) using sieves with pores diameter of 0.5 mm. The coconut shell powder (CSP) was characterized based on TAPPI T203 cm-99, T204 cm-97, T211 om-02, T421 om-01 and TAPPI T222 om-2 for determination of alpha-cellulose, extractives/fat, ash, moisture and lignin, respectively. The holocelullose and hemicellulose contents were determined by the procedure described by Yokohama *et al.* (YOKOYAMA; KADLA; CHANG, 2002). The composition of CSs was cellulose (25.1 ± 3.7 %), hemicellulose (20.9 ± 2.4 %), lignin (33.7 ± 1.8 %), extractives (10.3 ± 0.1 %), fat (0.6 ± 0.02 %) and moisture (3.6 ± 0.1 %).

Microwave-assisted extraction (MWAE)

The microwave-assisted extractions were performed in a Milestone (Sorisole, Italy) microwave reactor, model StartSYNTH, operating in an open-vessel configuration at a frequency of 2.45 GHz. The temperature was controlled by a contact-less infrared sensor, and the power applied was limited to a maximum of 500 W.

MWAE assays were divided in two stages. The first was a screening for the selection of ideal catalyst concentration and reaction time. In these experiments, CSP (10 g) was introduced in a round-bottom flask containing 100 mL of acetic acid/water solution (9/1, v/v) and the catalyst (H_2SO_4 or HCl) in different concentrations (0.5, 1,0 or 2.0 % v/v). The extractions were performed at 110 °C during defined reaction times (5, 10, 20 or 30 minutes), under atmospheric pressure. Extractions without any catalyst were carried out to evaluate the ability of acetic acid to catalyse lignin hydrolysis.

The second stage was the evaluation of the effects caused in lignin structure by using several Lewis acids as catalysts comparing them with the traditional Brønsted acids. In these experiments, the same amount of CSP was used and 2.0 % w/v $MgCl_2$, $MnCl_2$, $CoCl_2$, $ZnCl_2$ and $AlCl_3$ were added into the acetosolv solution. These experiments were carried out at 110 °C during 20 minutes under atmospheric pressure. For both stages, the temperature program used consisted of a fast heating step (3 minutes) followed by a plateau during which power varied in a narrow range (100 – 200 W) to maintain temperature at the target value.

At the end of the reactions, samples were cooled down for 10 minutes. The black liquor was isolated from the solid residue by filtration (pore diameter of 28 μm) and washed with acetic acid (60 °C). The resultant black liquor was concentrated and precipitated in deionized water at 60 °C, using a black liquor to water ratio of 1:10 (v/v) and allowing the mixture to rest at room temperature for 24 hours.

After that time, lignin was vacuum-filtered (pore diameter of 8 μm), washed with deionized water until pH 6.0 and dried at 105 °C. Lignin yield was calculated according to Equation 1:

$$\eta = \frac{m_{LigIsol}}{m_{LigCSP}} \tag{1}$$

where η is the lignin yield (%); $m_{LigIsol}$ is the mass of lignin isolated in the MWAOD process (g) while m_{LigCSP} is the mass of lignin in CSP (g) as determined by TAPPI standards (Section 1.2).

Lignin extractions using traditional heating (TH)

In order to compare the effects that the heating source would cause on the properties of lignins, the same extracting conditions used for MWAOD assays were applied for TH. The catalysts that produced the highest yields were chosen to be used in TH experiments. CSP (10 g) was introduced in a round-bottom flask with 100 mL of acetic acid/water solution (9/1, v/v) containing 2.0 % (w/v or v/v) $AlCl_3$, H_2SO_4 and HCl as catalysts. The reactions were performed in an oil-bath at 110 °C during 20 minutes under atmospheric pressure. The reaction time began to be counted when the solution temperature reached 110 °C. The treatments after the extractions were exactly the same used for those conducted using microwave irradiation. The TH-lignins were characterized by FTIR, ^{1}H-^{13}C HSQC, GPC and TGA techniques in order to assess differences in their structures in relation to those obtained by MWAOD.

Characterization of lignins

The purity of lignin samples was calculated by TAPPI T222 om-2 method with modifications (CORNERS, 2002). About 1.0 g of lignin was treated with 17 mL of sulfuric acid (72 wt %) at room temperature for 24 h. The solution was diluted with deionized water to 4 wt % sulfuric acid and refluxed for 4 h. After the end of the reaction, the system was cooled for 30 minutes at room temperature. The acid-insoluble residue was isolated by vacuum filtration and washed with deionized water until the pH of filtrates was equal to that of the deionized water. After these washings, the residues were dried at 105 °C for 24 h.

The acid-soluble lignin was determined using the first filtrate obtained in the acid-insoluble lignins. The filtrate was diluted with 4 wt % H_2SO_4 with 1:10 (v/v) ratio and the acid-soluble lignin was calculated from the UV absorbance at 215 and 280 nm. The sum of acid-insoluble and acid-soluble lignins was reported as the total lignin content (purity) of the samples.

Fourier transform infrared spectroscopy (FT-IR) was performed in a Perkin Elmer FT-IR/NIR FRONTIER, using an attenuated total reflectance (ATR) accessory with ZnSe crystal surface. Spectra were acquired between 4000 and 550 cm^{-1} with resolution of 4 cm^{-1} using the arithmetic average of four scans. The relative content of functional groups was calculated based on the methodology described by Bykov (2008) (BYKOV, 2008).

The heteronuclear single quantum coherence (HSQC) experiments were performed in a Bruker Avance DPX 300 operating at 300 and 75 MHz for ^{1}H and ^{13}C nuclei, respectively. Lignin (100 mg) was dissolved in 1.0 mL of DMSO-d_6, and solvent peak was used as internal reference (DMSO δ_H/δ_C 2.49/39.5).

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

Differential scanning calorimetry (DSC) analyses were performed in a Mettler-Toledo (Schwerzenbach, Switzerland) DSC 823e. Sample (10 mg) was placed in aluminum crucibles closed with a lid centrally punctured. Samples were heated from 25 to 90 °C, where temperature was kept constant for 10 minutes, then cooled from 90 to 0 °C, where temperature was kept constant for 3 minutes and heated from 0 to 250 °C, under a nitrogen atmosphere (50 mL min^{-1}) at a scanning rate of 20 °C min^{-1}.

Thermogravimetric (TGA) analyses were performed in a Mettler-Toledo (Schwerzenbach, Switzerland) TGA/SDTA851e. Sample (10 mg) was heated from 30 to 900 °C under nitrogen atmosphere (50 mL min^{-1}) at a scanning rate of 10 °C min^{-1}.

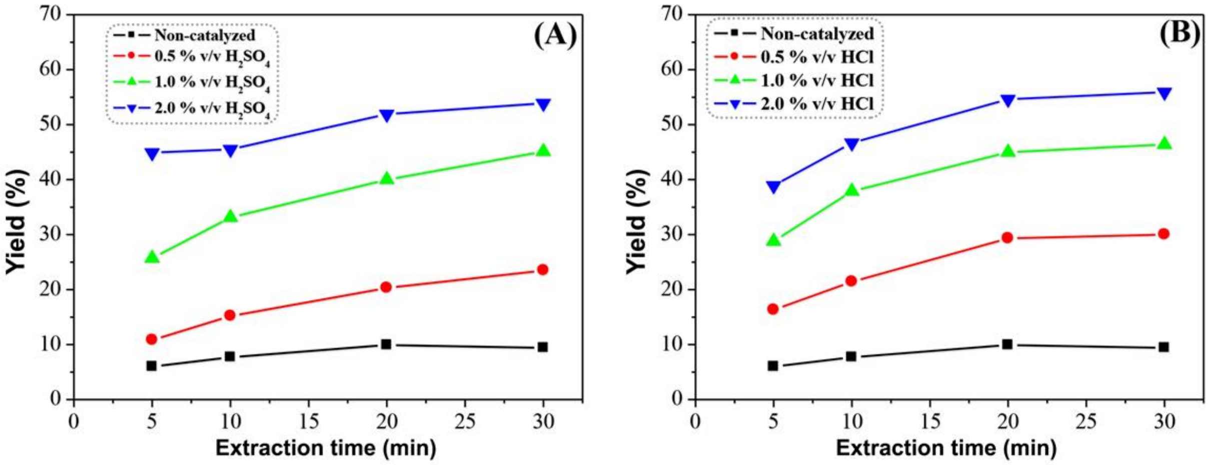
Gel permeation chromatography (GPC) analyses were performed in a Shimadzu LC-20AD (Kyoto, Japan) at 40 °C using a setup comprising two identical analytical GPC columns in series (Phenogel 5 Linear/Mixed columns, 7.38 mm x 300 mm, Phenomenex, Torrance, CA, USA), a flow rate of 1.0 mL min^{-1} and HPLC-grade THF as eluent. The samples were monitored by UV-Vis detector (Shimadzu SPD-M20A) at 280 nm. Lignin (2 mg) was dissolved in 2 mL HPLC-grade THF and filtered using 0.22 µm PTFE filter. Then, 100 µL of this solution was injected into the GPC system. Standard calibration was performed with polystyrene standards Phenomenex ALO-2761 (Phenomenex, Torrance, CA, USA, M_w range 362 – 2.52 x 10^6 g mol^{-1}).

Results and discussion

Microwave-assisted organosolv delignification (MWAOD)

Initially, the effect of three parameters was evaluated in the MWAOD experiments: the catalyst (without catalyst, H_2SO_4 and HCl), its concentration and the reaction time (**Figures 24 (A)** and **(B)**).

Figure 24 – Effect of catalysts, their concentrations and reaction time on lignin yield.



Source: The author.

Since the catalysts used were Brønsted acids, the most important feature that influenced on the lignin yield was their strength. This behavior can be seen by the increase on yield with the increase in strength of the acids, as shown in **Figures 24 (A)** and **(B)**.

The low yields obtained using CH_3COOH as catalyst shows its poor ability to produce H_3O^+ ions, which are responsible for the protonation of ether groups present in lignin, catalyzing their hydrolysis and increasing the yield.

Figures 24 (A) and **(B)** also show that the increase in the catalyst concentration combined with the increase in the reaction time enhanced the extraction yield. This behavior occurs due to the increase in the concentration of H_3O^+ ions, which enhances the amount of ether bonds that can be protonated and subsequently hydrolyzed. In addition, longer reaction times usually provide higher probabilities to occur effective collisions between the reactants, which increase the rate of reaction in this case represented by the lignin yield.

However, when catalysts' concentration was 2.0 % v/v there were no significant differences between yields using 20 and 30 minutes, as shown in Figures **24 (A)** and **(B)**. This can be attributed to kinetic factors, suggesting that from 20 minutes to higher reaction times the delignification process reaches a plateau and the amount of extracted lignin is practically constant. Therefore, condition of 2.0 % v/v of catalyst combined with 20 minutes of reaction was chose for the next steps of this work.

Another parameter investigated was the nature of catalyst. Based on the optimized conditions for the Bronsted acids, other five Lewis acids, with variable cation hardness, were employed in lignin extraction. The hardness of each cation was estimated by Pearson model (PARR; PEARSON, 1983; PEARSON, 1988).

The values of cation hardness, lignin yield, acid-insoluble lignin and purity of the lignins obtained by Lewis and Brønsted acids catalysts are shown in **Table 5**.

Table 5 – Influence of Brønsted and Lewis acids on the lignin yield, Klason and acid-soluble lignin and purity of lignins.

Catalyst	Cation hardness (eV)	Lignin yield (%)	Klason Lignin (%)	Acid-soluble lignin (%)	Purity (%)
CH_3COOH	6.4	9.20 ± 0.7	76.7	1.43	78.1
$CoCl_2$	8.2	14.8 ± 1.2	84.0	2.52	86.5
$MnCl_2$	9.3	14.6 ± 1.8	78.9	3.4	82.3
$ZnCl_2$	10.8	14.3 ± 0.1	82.8	1.58	84.4
$MgCl_2$	32.5	17.0 ± 1.7	78.0	2.92	80.9
$AlCl_3$	45.8	53.9 ± 2.4	80.0	3.40	83.4
H_2SO_4	∞	56.6 ± 5.2	83.5	2.26	85.8
HCl	∞	54.4 ± 3.3	81.9	3.82	85.8

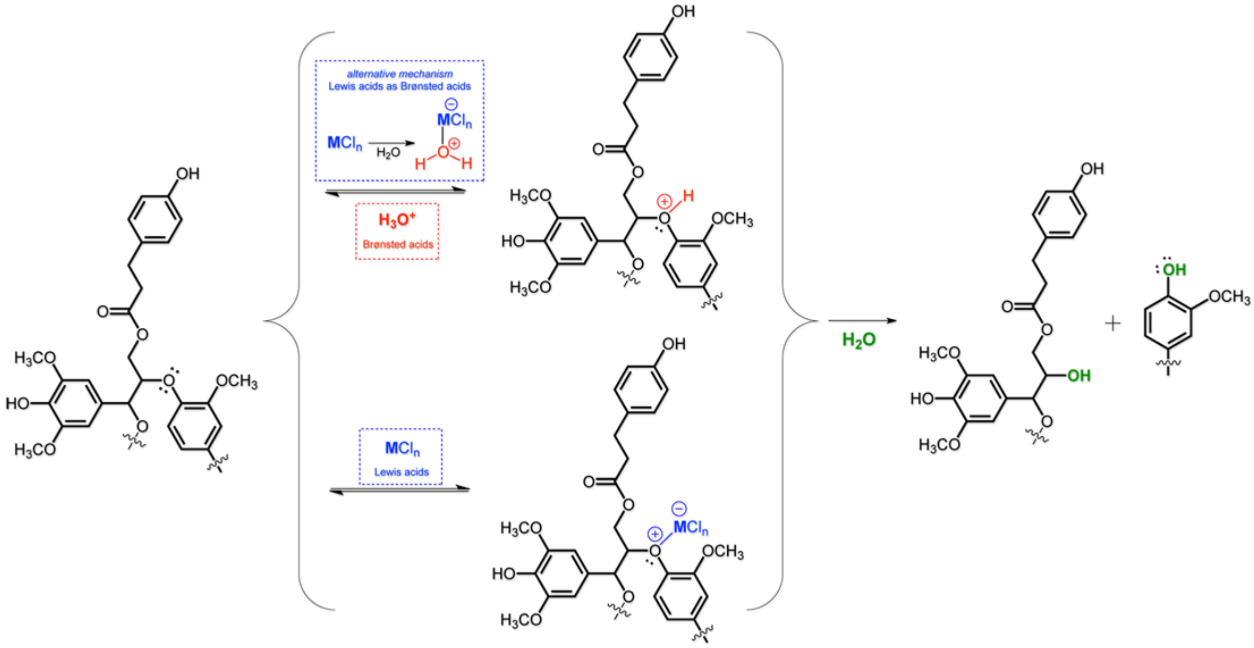
Source: The author.

In general, **Table 5** shows that the increase of cation hardness enhanced the extraction yield. This fact probably occurs because Lewis acids with higher cation hardness coordinate more efficiently with the oxygen atoms present in the ether groups, since the majority of bonds between the monomers in lignin structure are ether type, such as β-O-4 type. The greater the coordination power of the catalyst more electrophilic these sites will be, making them more susceptible to hydrolysis.

Comparing the results, the only Lewis acid that presented similar yield to Brønsted acids was $AlCl_3$, suggesting that they have similar acidic power despite the mechanism of the former be based on coordination and the latter based on protonation of oxygen atoms. Therefore, these results showed that the acidic power has a significant influence on yield, but not so much in the lignin purity.

Figure 25 shows the schematic representation of the action of the both types of acid catalysts during the lignin hydrolysis, since generally the literature does not show how they act during the extraction process. Thus, there were proposed pathways considering a fragment of lignin formed by H, G and S units linked by β-O-4 linkage, due to its abundance in lignin structure. Therefore, it is important to highlight that there are two possibilities of mechanism for the Lewis acids. The first would be based on the coordination of water molecules from the acetosolv solution, which will make their hydrogen more acidic to protonate the ether bonds (**Figure 25**). The second would be based on the direct coordination of ether bonds for the subsequent nucleophilic attack of water.

Figure 25 – Proposed mechanisms for both types of catalysts during lignin hydrolysis.



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It is worth mentioning that the major published papers about microwave-assisted delignification were performed under high pressure, which is a limiting factor since the amount of raw material used is too low (0.2 – 2 g) (LI et al., 2012; MONTEIL-RIVERA et al., 2012; XIE et al., 2015; ZHOU et al., 2017). This is unfavorable from an industrial perspective, since the scale-up of this process would be very difficult. Moreover, there are high-volume microwave reactors for pilot plant purposes (atmospheric pressure), which would simulate a scale up of certain process.

Some of the aforementioned works reported low lignin yield (10 – 25 %) even with the use of high temperatures and pressures, as reported by Monteil-Rivera *et al.* (2012), Li *et al.* (2012) and Xie *et al.* (2015).

Recently, Zhou *et al.* (2017) performed a delignification assays using softwood pellets and diluted sulfuric acid at 190 °C during 10 minutes, obtaining a lignin yield of 82.31 %

with purity of 80.64 %. Comparing this result with those presented in the present study, it is observed that we obtained lignins with comparable and higher purity under milder conditions and lower pressure (atmospheric).

Xie *et al.* (2015) performed delignification of different biomasses using a mixture of glycerol/methanol as solvent and sulfuric acid as catalyst at 120 °C during 30 minutes under high pressure and obtained low lignin yields (17.62 – 23.04 %) and low purity (75 – 77 %). Again, in this work under conditions much milder we report higher lignin yields and purities.

It is also necessary to consider that delignification experiments performed in reactors under traditional heating, combining high pressure with elevated temperatures and long reaction times, result in excessive energy consumption (AVELINO et al., 2018; FERNÁNDEZ-RODRÍGUEZ et al., 2017; LØHRE; KLEINERT; BARTH, 2017; MANIET et al., 2017; PINHEIRO et al., 2017).

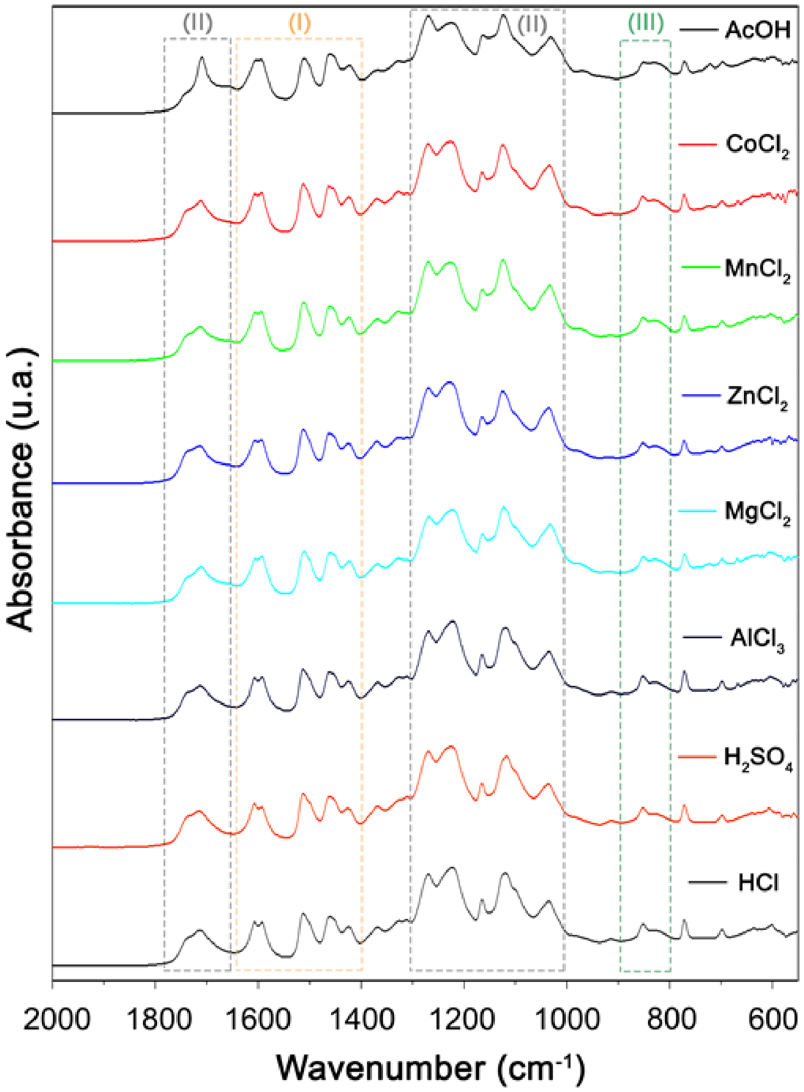
The results presented in this work show the energy efficiency of the microwave heating, producing lignins with higher yields and purities compared to those reported in the literature for MWAOD and TH experiments. It is worth mention that during the MWAOD the power applied was monitored during the whole experiments and the average value for all experiments was around 135 W, representing an extremely low energy consumption during the extractions, confirming that MWAOD represents a sustainable alternative to delignification of biomass under mild conditions.

Structural characterization

Fourier transform infrared spectroscopy (FTIR)

The main functional groups in the organosolv lignins, as well as any structural modification resultant from the extractions, were investigated by FTIR (**Figure 26**). The main bands in the fingerprint region were compared by the ratio between their areas and that of the band at 1510 cm^{-1} (**Table 6**).

Figure 26 – Fingerprint region of FTIR spectra of organosolv lignins.



Source: The author.

The peak assignments were conducted based on a previous report (FAIX, 1991). Figure 3 shows the fingerprint region of lignin spectra, where the characteristic bands of chemical bonds are found. Among these, some are related to the lignin backbone (region I), such as C=C stretching of the aromatic ring skeleton at 1593 and 1510 cm^{-1}; C-H deformations at 1459 cm^{-1} and aromatic skeletal vibrations coupled with C-H in plane deformation at 1420 cm^{-1}.

The absorption bands at 1708, 1268, 1163 and 1123 cm^{-1} were assigned to C=O stretchings (region II), which is commonly attributed to lignin-carbohydrate complexes (LCC). These bands can be used to estimate the efficiency of the delignification process, since their decrease indicates the cleavage of benzyl ester bonds (LIU et al., 2017). There were also residual carbohydrates in all organosolv lignins, although the intensities of these bands varied with the type of catalyst, which were evaluated by their relative contents (**Table 6**).

Table 6 – Relative content of the main functional groups in fingerprint region of CSALs.

Catalyst/Band (cm^{-1})	**1708**	**1593**	**1510**	**1459**	**1420**	**1330**	**1268**	**1228**	**1163**	**1123**	**1031**
CH_3COOH	1.51	1.44	1.00	0.97	0.67	0.84	2.04	2.28	0.83	2.66	2.10
$CoCl_2$	1.51	1.21	1.00	0.88	0.63	0.97	1.93	2.83	0.80	2.77	2.03
$MnCl_2$	1.36	1.19	1.00	0.85	0.58	0.77	1.78	2.69	0.66	2.87	2.12
$ZnCl_2$	1.69	1.17	1.00	0.84	0.59	0.69	1.75	2.78	0.69	2.75	2.10
$MgCl_2$	1.79	1.38	1.00	0.90	0.65	0.74	1.59	2.80	0.81	2.90	2.31
$AlCl_3$	1.44	1.28	1.00	0.89	0.55	0.91	1.56	2.90	0.67	2.77	1.93
H_2SO_4	1.46	1.22	1.00	0.83	0.62	0.63	1.73	2.98	0.71	2.85	1.73
HCl	1.56	1.26	1.00	0.86	0.64	1.08	1.82	2.93	0.84	2.97	1.70

Source: The author.

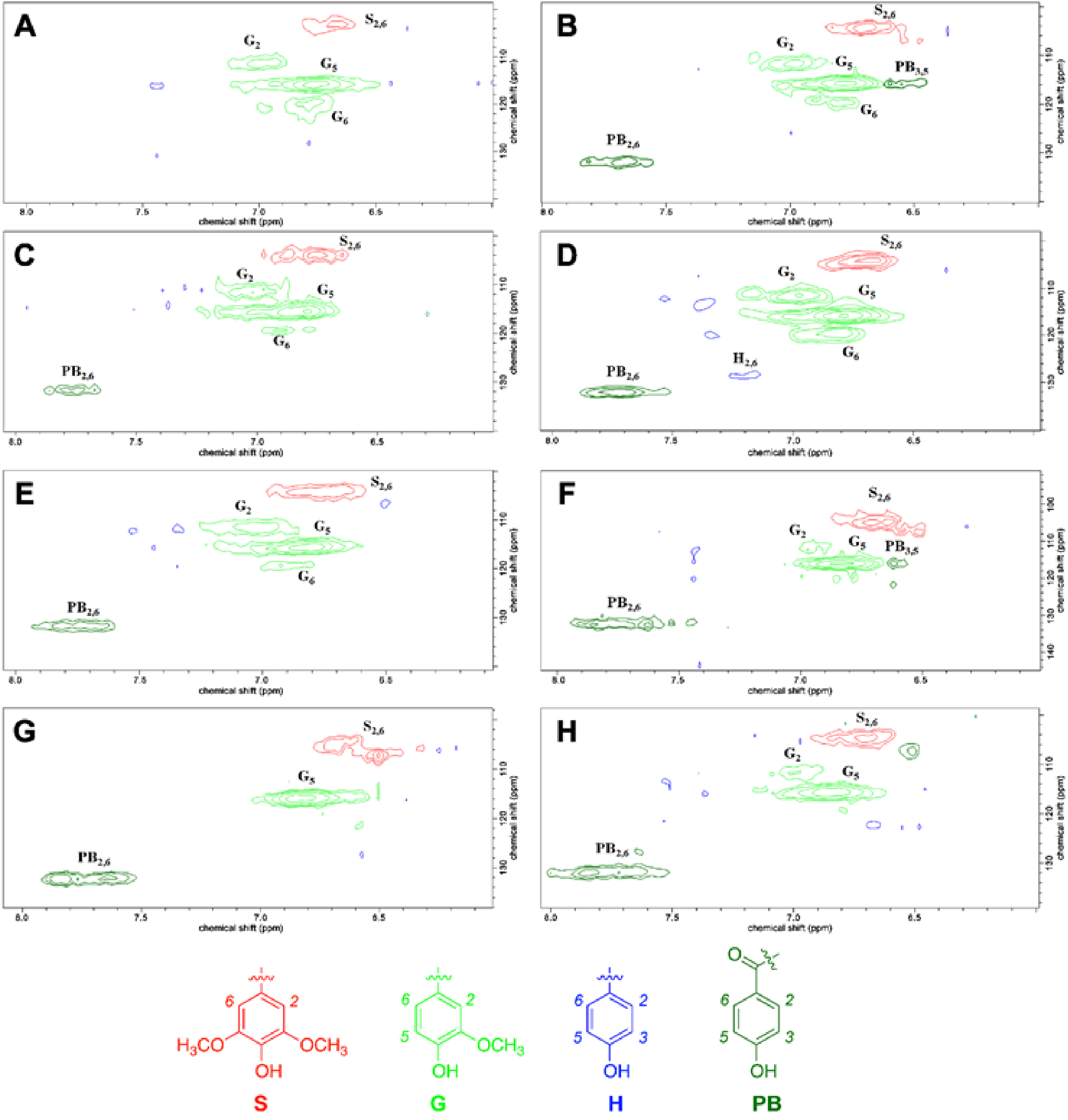
In **Figure 26**, the absorption bands at 1330 and 1123 cm^{-1} are characteristic of syringyl units (region II), while those at 1510, 1268 and 1031 cm^{-1} with relatively high intensity (Table S1) are related to guaiacyl units (regions I and II) (CONSTANT et al., 2016). The band at 853 cm^{-1} was assigned to C-H out-of-plane in positions 2, 5 and 6 of guaiacyl units (region III).

It is possible to identify in all lignin spectra the presence of a bands at 1163 cm^{-1}, assigned to C=O vibration of conjugated esters (region II), and at 831 cm^{-1}, assigned to the aromatic C-H out-of-plane vibrations in H units (region III), important spectral features of HGS lignins, although their intensities suggest that the amount of this monomer is very low.

^1H-^{13}C HSQC NMR

In order to obtain more detailed information on the structure of CSALs, samples were analyzed by HSQC NMR. **Figure 27** shows the aromatic regions (δ_C/δ_H 100-140 ppm/6.0-8.0 ppm) of CSALs, where the main cross-peaks were assigned according to Rencoret *et al.*(RENCORET et al., 2013) and are listed in **Table 7**.

Figure 27 – Aromatic regions in 2D-HSQC NMR spectra of CSALs obtained: (A) CH_3COOH; (B) $CoCl_2$; (C) $MnCl_2$; (D) $ZnCl_2$; (E) $MgCl_2$; (F) $AlCl_3$; (G) H_2SO_4 and (H) HCl. Main structures present in the CSALs: (S) syringyl; (G) guaiacyl; (H) *p*-hydroxyphenyl and (PB) *p*-hydroxybenzoates.



Source: The author.

Table 7 – Assignments of the lignin ^{1}H-^{13}C correlation signals in the HSQC NMR spectra of CSALs.

Label	δ_C/δ_H (ppm)	Assignment
$S_{2,6}$	6.67/104	C_2-H_2 and C_6-H_6 in S
G_2	6.98/111.6	C_2-H_2 in G
G_5	6.69/115.2	C_5-H_5 in G
G_6	6.78/119	C_6-H_6 in G
$H_{2,6}$	7.03/130.5	$C_{2,6}$-$H_{2,6}$ in H
$PB_{2,6}$	7.68/131.2	$C_{2,6}$-$H_{2,6}$ in PB
$PB_{3,5}$	6.61/114.3	$C_{3,5}$-$H_{3,5}$ in PB

Source: The author.

The obtained lignins presented similar composition, in which the monomeric units S, G, H and *p*-hydroxybenzoate (PB) were their main constituents.

Except for CH_3COOH, all the other catalysts generated lignins with significant amounts of PB units, originated from oxidized H units, which is in accordance with the results described by Constant *et al.* (CONSTANT et al., 2016).

Furthermore, the spectral features showed in the HSQC spectra corroborate with those in FTIR spectra. The latter showed the presence of bands attributed to C=O vibration of conjugated esters, considered a typical band for HGS lignins, probably due to the presence of PB units, which has an ester group conjugated with the aromatic ring, as shown in **Figure 26**. This was confirmed by the presence of cross-peaks related to $C_{2,6}$-$H_{2,6}$ (δ_C/δ_H 131.2/7.68) and $C_{3,5}$-$H_{3,5}$ (δ_C/δ_H 114.3/6.61) correlations for PB units in the HSQC spectra.

It is also evident that in all HSQC NMR spectra the cross-peaks related to G and S units had higher intensities, which is in accordance to the FTIR, since the bands at 1330 and 1123 cm^{-1} (S unit) and 1510, 1268 and 1031 cm^{-1} (G unit) stood out.

The relative proportion of monomers (RPM) was calculated as the ratio between the cross-peak area of the individual monomers and the sum of the cross-peaks areas of all monomers (**Table 8**).

Table 8 – Relative proportion of monomers for CSALs obtained by using different catalysts.

Lignin	% S	% G	% H	S/G ratio	H/G ratio
CH_3COOH	14.6	85.3	0	0.17	0
$CoCl_2$	18.5	68.6	12.9	0.27	0.19
$MnCl_2$	16.4	76.7	6.9	0.21	0.09
$ZnCl_2$	15.6	75.2	9.2	0.21	0.12
$MgCl_2$	19.9	67.9	12.1	0.29	0.18
$AlCl_3$	28.9	51.0	20.0	0.57	0.39
H_2SO_4	20.7	57.3	22.0	0.36	0.38
HCl	21.5	53.6	24.9	0.40	0.46

Source: The author.

Table 8 shows that the nature of the catalysts influenced in the RPM, suggesting that hardness and acidic power favored specific cleavages, as indicated by S/G and H/G ratios. This behavior can be seen in **Figure 27**, where the cross-peak related to $PB_{3,5}$ only appeared when $CoCl_2$ and $AlCl_3$ were used. This indicates a preferential cleavage of 5-5' and β-5 linkages, generating PB units with carbon 5 available to correlate with its respective hydrogen.

It is also suggested that CSALs samples that presented only cross-peak related to $PB_{2,6}$ had a different cleavage pattern than those which presented only $PB_{3,5}$. This means that the other catalysts may be acted in different type of linkages, such as the 5-O-4. This cleavage generates two fragments with hydroxyl groups in the carbons 5 and 4, which explains the absence of $PB_{3,5}$ units in those spectra.

In a general way, the relative proportion of guaiacyl units decreased in lignins obtained with $AlCl_3$, H_2SO_4 and HCl. These catalysts can possibly be favoring the occurrence of recondensation reactions, in which the position 5 of the G ring was involved.

^{31}P NMR

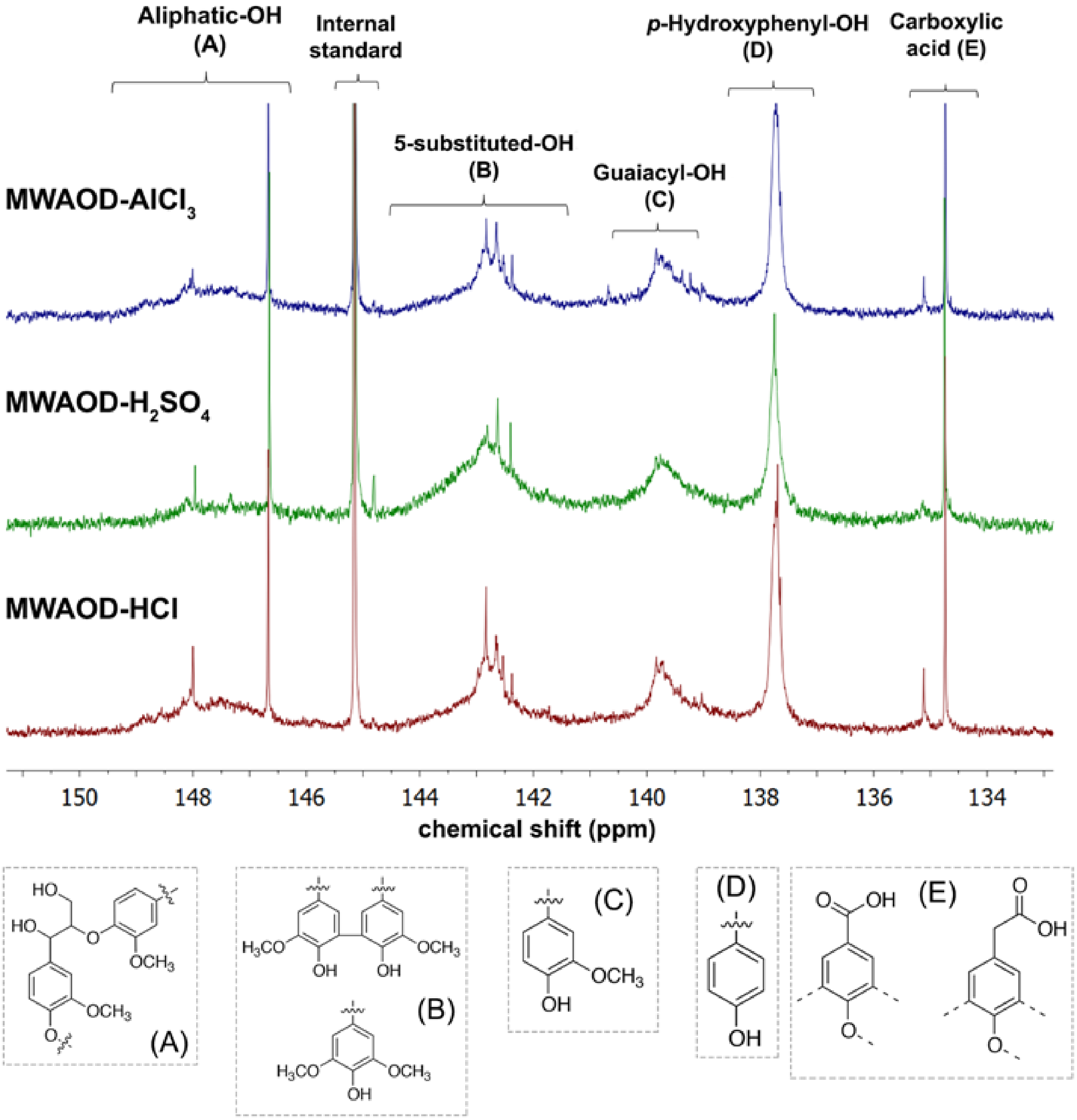
Quantitative ^{31}P NMR spectra were obtained to assess the amount of aliphatic, phenolic (5-substituted-OH, guaiacyl-OH and *p*-hydroxyphenyl-OH) and carboxylic acids hydroxyl groups present in MWAOD-lignins ($AlCl_3$, H_2SO_4 and HCl). The ^{31}P NMR spectra for these lignins and the identified substructures are shown in **Figure 28**. The data obtained from quantification are summarized in **Table 9**.

Table 9 – Quantification data of hydroxyl groups present in MWAOD lignins.

Index/Lignin	**$AlCl_3$**	**H_2SO_4**	**HCl**
OH aliphatic content (mmol g^{-1})	0.49	0.28	0.63
C_5-substituted-OH	0.65	0.84	0.80
Guaiacyl-OH	0.56	0.53	0.69
***p*-Hydroxyphenyl-OH**	0.51	0.39	0.60
OH phenolic content (mmol g^{-1})	1.72	1.76	2.09
COOH content (mmol g^{-1})	0.12	0.10	0.15
Total OH groups' content (mmol g^{-1})	2.33	2.14	2.87
$OH_{phen..}/OH_{total}$	0.74	0.82	0.73

Source: The author.

Figure 28 – ^{31}P NMR spectra of MWAOD lignins and their main substructures.



Source: The author.

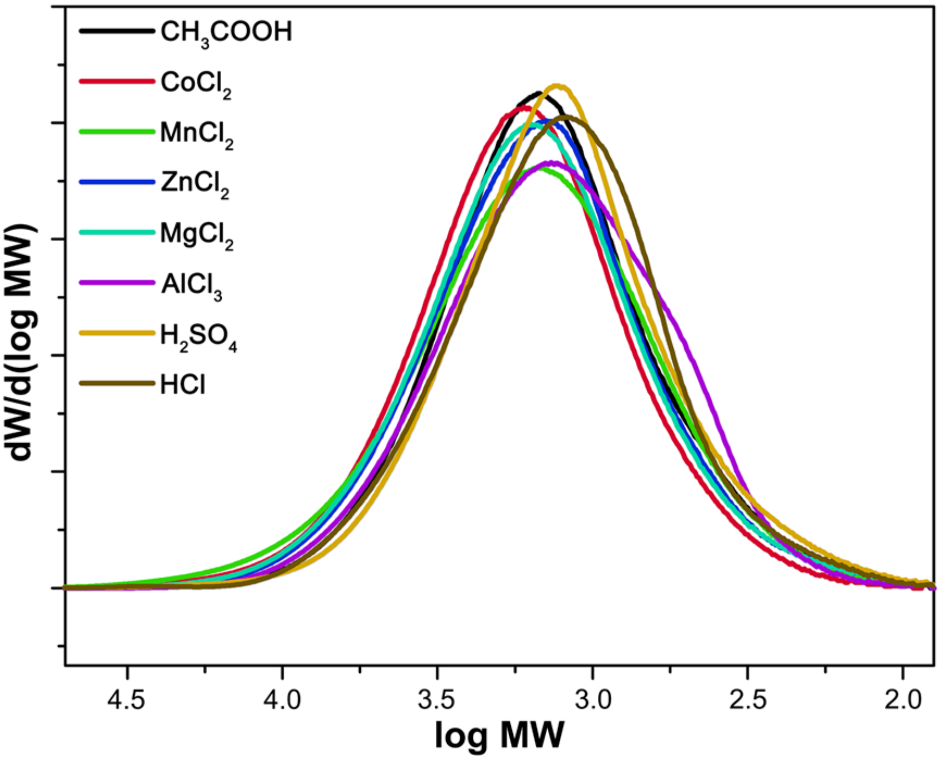
Figure 28 shows that the main hydroxyl groups are part of syringyl/G-condensed, guaiacyl and *p*-hydroxyphenyl units. These corroborates with HSQC spectra, in which were observed the presence of cross-peaks related to $S_{2,6}$, G_2, G_5, G_6, $H_{2,6}$, $PB_{3,5}$ and $PB_{2,6}$ substructures. In addition, the FTIR spectra of these lignins showed the presence of characteristic absorption bands related to S and G units, as also a characteristic band (1163 cm^{-1}), typical for HGS lignins.

The distribution of hydroxyl groups shown in **Table 9** are in good agreement with the literature (CONSTANT et al., 2016; PANG et al., 2017). Another observation from **Table 9** is the total OH phenolic content, which has its highest value for MWAOD-HCl lignin, reinforcing the hypothesis that this catalyst had the best performance in promoting cleavage of aryl alkyl ether bonds.

Molecular weight distribution

The molecular weight distributions curves of CSALs are shown in **Figure 29** and their weight-average (M_w) and number-average (M_n) molecular weights and polydispersity index (PDI) are given in **Table 10**.

Figure 29 – Molecular weight distribution curves of CSALs.



Source: The author.

Table 10 – Weight-average (M_w) and number-average (M_n) molecular weights and polydispersity (M_w/M_n) of CSALs.

Catalyst	M_w (g mol^{-1})	M_n (g mol^{-1})	PDI
CH_3COOH	1902	1027	1.85
$CoCl_2$	2328	1225	1.90
$MnCl_2$	2351	1053	2.23
$ZnCl_2$	2103	1056	1.99
$MgCl_2$	2169	1082	2.01
$AlCl_3$	1845	960	1.92
H_2SO_4	1753	906	1.93
HCl	1780	944	1.89

Source: The author.

The molecular weight distribution, M_w, M_n and PDI values of a lignin sample indicate the severity of the extraction process (combination of temperature and reaction time) used to the delignification of the biomass (CONSTANT et al., 2016). It is also possible to evaluate the effect of the catalyst on this process, since the temperature and reaction time was kept constant.

Table 10 shows that the M_w and M_n varied randomly by using different catalysts. In addition, the PDI values showed that CSALs had narrow molecular weight distributions, which means that these lignins were composed of fragments with low heterogeneity. In a general way, it was observed a trend in the M_w values, in which lignins with higher M_w values ($M_w > 1900$ g mol^{-1}) were obtained using less acidic catalysts (soft acids, from CH_3COOH to $MgCl_2$), while catalysts with high acidic power ($AlCl_3$, H_2SO_4 and HCl) generated lower molecular weight lignins ($M_w < 1900$ g mol^{-1}). All the M_w values are in good agreement with previous studies. (CONSTANT et al., 2016).

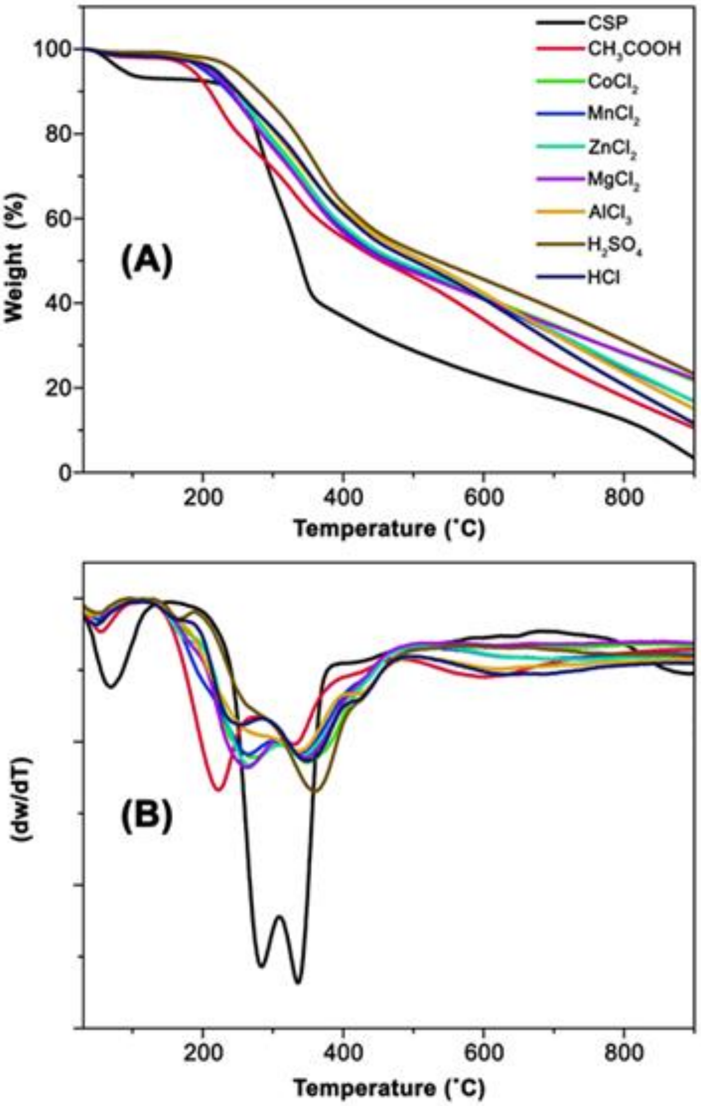
These results corroborates with those obtained by Constant *et al.* (CONSTANT et al., 2016), in which the authors studied the influence of several Lewis acid catalysts on the structural and chemical properties of lignins extracted from wheat straw. They reported that the use of harder Lewis acids, such as $Ga(OTf)_3$, $ZrOCl_2$ and $Sc(OTf)_3$ generated low molecular weight lignins and with lower amounts of β-aryl ether linkages.

The tendency observed in M_w values can be correlated to the RPM values obtained in HSQC experiments (**Table 8**), in which the increase in the cation hardness generated lignins with lower amounts of G unit. This can be due to the extensive cleavage of the 5-O-4 linkages, in which new phenolic hydroxyl groups were formed in the position 5 of G ring, decreasing the intensity of the cross-peak of $C_5 – H_5$ correlation and, consequently, the proportion of G unit present in those lignins.

Thermogravimetric analysis (TGA)

The thermal stability of lignin is a property that strongly depends on its molecular weight, its carbohydrates content and the identity of chemical bonds in its structure. Therefore, it is possible to establish a link between the thermal stability of lignin and its macromolecular structure. **Figure 30** shows the TGA and DTG curves for the CSP and CSALs. Onset and maximum degradation temperatures (T_{onset} and T_{max}), weight loss (Δw) and char yield values are summarized in **Table 11**.

Figure 30 – (A) TG and (B) DTG curves of CSP and CSALs.



Source: The author.

Table 11 – Thermal properties of CSP and CSALs obtained by TGA analysis.

Catalyst	T_{onset} (°C)	T_{max} (°C)	Δw (%)	Char yield (%)
CSP	252	334	29	3.45
CH_3COOH	168	223	22	10.6
$CoCl_2$	212	351	23	21.7
$MnCl_2$	204	265	22	16.8
$ZnCl_2$	211	267	23	16.7
$MgCl_2$	207	263	24	22.2
$AlCl_3$	214	340	17	15.1
H_2SO_4	235	360	11	23.3
HCl	205	348	15	11.7

Source: The author.

The weight loss in the range of 180-300 °C can probably be attributed to the degradation of extractives and carbohydrates in CSP and residual carbohydrates present in CSALs (SINGH et al., 2005). For this thermal event, were observed higher weight loss (Δw) for CSP than CSALs. This behavior was expected since Δw is proportional to carbohydrates content, which is higher in CSP.

Among CSALs samples, it was observed a dependence between purity of CSALs and T_{onset} values, which were also dependent on the catalyst used. This shows the great ability of the catalysts employed in cleaving the linkages between lignin and carbohydrates (LCC), yielding lignins with elevated purity and with higher thermal stabilities.

The weight loss in the range of 310-500 °C is mainly attributed to the cleavage of ether and carbon-carbon bonds, so called inter-unit linkages, between different lignin monomers (PANG et al., 2017). It was observed that more acidic catalysts ($AlCl_3$, H_2SO_4 and HCl) yielded lignins with higher T_{max}, indicating that these samples present significant amounts of carbon-carbon bonds, such as 5,5’, β-β and β-1, which could also justify their elevated T_{max} values.

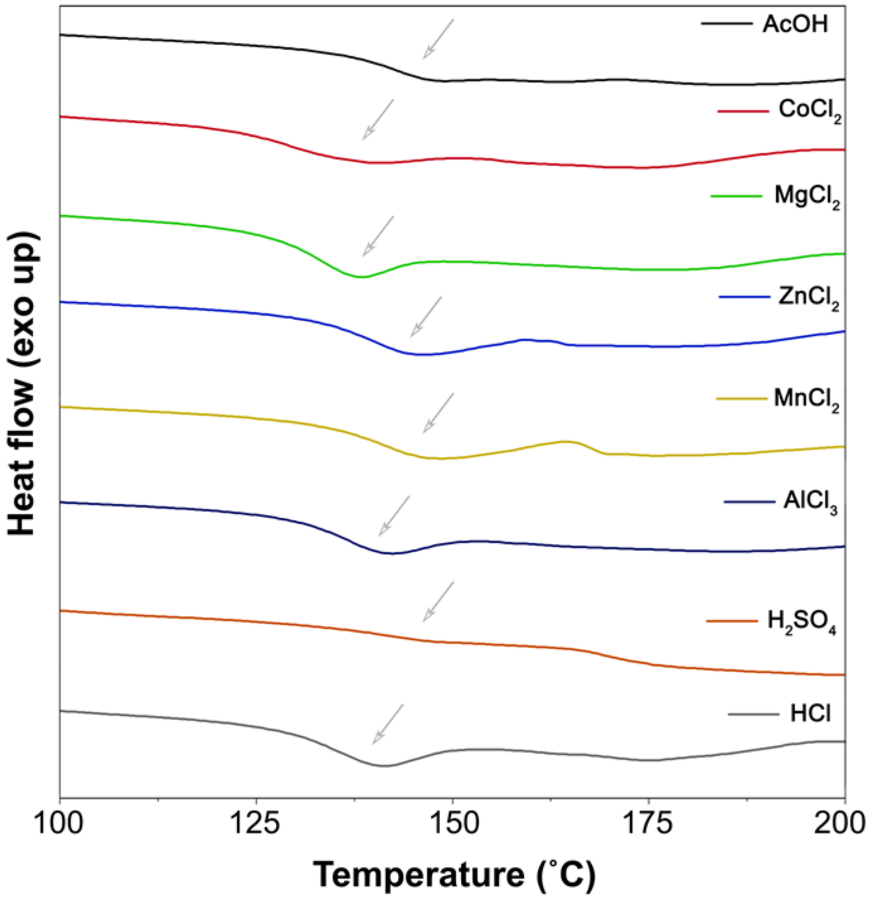
At temperatures higher than 500 °C there is the occurrence of condensation reactions between the aromatic rings of high molecular weight fragments, leading to the formation of a highly stable carbonaceous structures (COSTES et al., 2017), which justify the high char yield values shown in **Table 11**.

Differential Scanning Calorimetry (DSC)

DSC measurements were performed to determine the glass transition temperature (T_g) of CSALs, which is an important parameter that is directly involved in the processability of polymers determining their potential applications. The T_g of lignins is affected by several factors, such as their molecular weight, crosslinking degree and thermal history stored in the glassy state of the samples (LANGE et al., 2016). Considering the enormous polyphenolic structure of CSALs, there are some electronic interactions that can possibly occur, such as the classical hydrogen bonding, hydrogen bonding to aromatics and π-stacking between aromatic units (HU, 2002).

The thermal history of CSALs samples was eliminating by using an annealing temperature at 90 °C, making easier to detect and calculate the T_g of lignins. DSC curves shown in **Figure 31** show the second scan after the first cycle (annealing), where the T_g of CSALs are easily detected. The T_g values are summarized in **Table 12** (midpoint values).

Figure 31 – DSC curves indicating the T_g of CSALs obtained.



Source: The author.

Table 12 – T_g values (midpoint) obtained for CSALs by DSC analyses.

Catalyst	T_g (°C)
CH_3COOH	142
$CoCl_2$	131
$MnCl_2$	139
$ZnCl_2$	139
$MgCl_2$	131
$AlCl_3$	135
H_2SO_4	140
HCl	133

Source: The author.

Table 12 shows that the T_g values of CSALs varied in a narrow range from 131 to 142 °C, in which the higher T_g was obtained for lignin extracted without addition of catalyst, being the acetic acid the own catalyst. According to **Table 10**, CH_3COOH-lignin had a low M_w value, suggesting that it had high amount of phenolic and aliphatic hydroxyls, which favor the occurrence of strong intermolecular hydrogen bonding interactions that hinder the thermal mobility of CH_3COOH-lignin chains, resulting in its high T_g. In addition, according to the **Table 5** CH_3COOH-lignin had the lowest purity among the CSALs, which suggests that a significant amount of residual carbohydrates still on its structure. The hydroxyl groups present in these carbohydrates could contribute for the high T_g value observed for this lignin, because the amount of hydrogen bonding interaction between the polymeric chains would increase as well.

However, the addition of different types of catalyst to acetosolv solution yields lignins with lower T_g values than that of without catalyst. This is an evidence of the influence that the catalysts exerted on the structural properties of CSALs, yielding lignins with different values of T_g. The combined analysis of M_w values (**Table 10**) and T_g values (**Table 12**) show that there were not a linear correlation between these quantities, suggesting that the amount of hydroxyl groups had a significant influence on the T_g values, since the higher the M_w the higher the probability of the occurrence of steric effect, which hinders the formation of hydrogen bonding and intermolecular interactions, causing a decrease on the T_g values.

Comparison with TH-lignins

TH experiments were performed in order to compare the physicochemical and structural properties of TH-lignins with those obtained in MWAOD under the same extracting conditions, evaluating if microwave irradiation (MWI) favored the occurrence of selective reactions. In these comparisons, there were evaluated the lignin yield, purity, thermal stability, molecular mass distribution and monomeric constituents of TH-lignins, which are shown in **Table 13**.

Table 13 – Physicochemical and structural data obtained for TH-lignins.

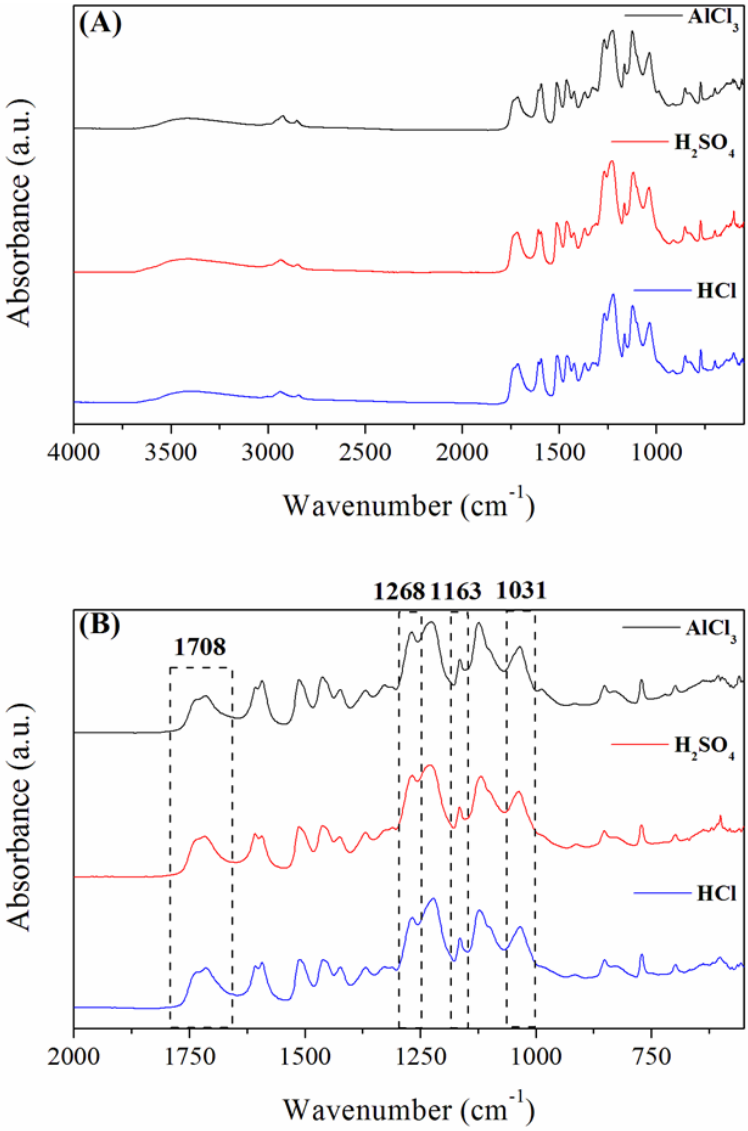
Catalyst	**Lignin yield (%)**	**Purity (%)**	**T_{onset} (°C)**	**T_{max} (°C)**	**Δw (%)**	**Char yield (%)**	**M_w (g mol^{-1})**	**M_n (g mol^{-1})**	***PDI***
$AlCl_3$	37	72.5	187	331	27.4	0	2380	1053	2.26
H_2SO_4	41	72.5	194	352	14.2	2.7	2709	1205	2.25
HCl	66	75.7	179	344	18.2	19	3018	1183	2.55

Source: The author.

Comparing the data of lignin yield shown in **Table 13** with those in **Table 5**, it is possible to observe that under the same extracting conditions, higher yields were obtained in MWAOD experiments when the catalysts were $AlCl_3$ and H_2SO_4. This shows that the MWAOD promoted a faster fractionation of the CSP due to the faster temperature increase induced by MWI. In addition, the high amount of lignin obtained in MWAOD was due to a more extensively cleavage of aryl alkyl ether bonds, such as α-O-4, β-O-4 and γ-O-4, yielding fragments with high solubility in the acetic acid, which was a result of the efficient heating generation that favored the rapid achievement of the activation energy of the hydrolysis reaction.

The FTIR spectra of TH-lignins presented a very similar pattern of bands to those of MWAOD-lignins (**Figure 26**), as shown in **Figure 32**. This shows that the heating source did not affect the type of functional groups present in both type of lignins. However, there are some spectral features in TH-lignins different of those in MWAOD-lignins, such as the relative content of some functional groups, as shown in **Table 14**.

Figure 32 – (A) Full and (B) fingerprint regions in FTIR spectra of TH-lignins with bands related to C=O stretching in carbohydrates highlighted.



Source: The author.

Table 14 – Relative content of the main functional groups in fingerprint region of TH-lignins.

Catalyst/Band (cm^{-1})	**1708**	**1593**	**1510**	**1459**	**1420**	**1268**	**1228**	**1163**	**1123**	**1031**
$AlCl_3$	1.65	1.38	1.00	1.01	0.68	1.99	3.42	0.95	3.47	2.49
H_2SO_4	1.68	1.28	1.00	0.87	0.60	1.74	3.23	0.78	3.11	2.40
HCl	1.87	1.33	1.00	0.84	0.64	1.61	3.18	0.94	2.90	2.32

*Absorption bands and relative contents highlighted in red are relative to C=O stretching in carbohydrates.
Source: The author.

Comparing the data in **Table 14** with those in **Table 6** is possible to observe that the TH-lignins had higher relative contents at 1708, 1268, 1163 and 1031 cm^{-1} than those of MWAOD-lignins, which suggest that the former had a higher carbohydrate content than the latter that is consistent with the lower values of purity of TH-lignins (**Table 13**), since these bands are related to C=O stretching present in carbohydrates. This behavior is a result of the efficient heating generation of MWI, which favored the more extensively cleavage of ester bonds in LCC yielding lignins with higher purities.

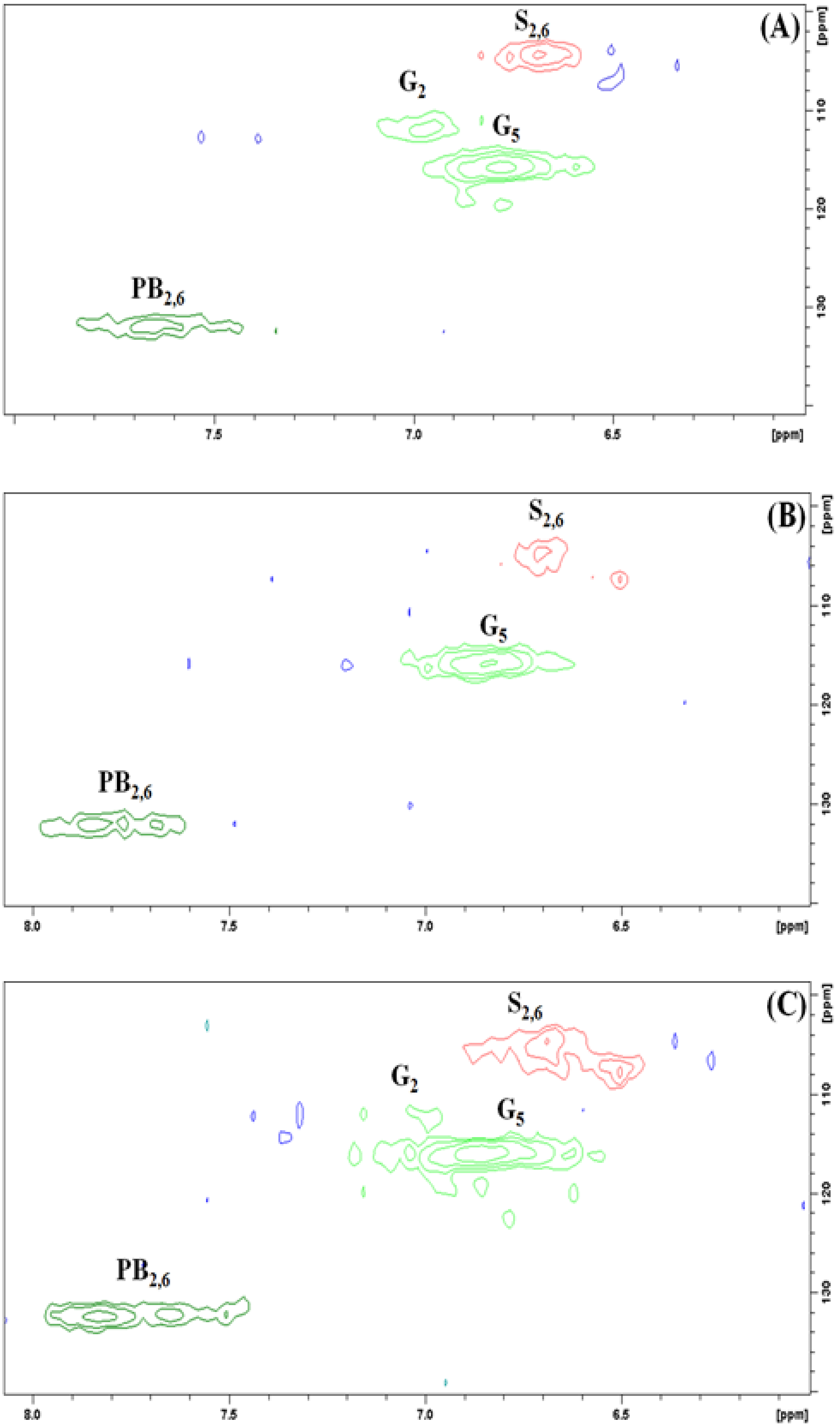
In terms of monomeric units composition, ^1H-^{13}C HSQC spectra of TH-lignins showed that the type of heating source did not cause a significant change in the lignins' structure, since there were observed the presence of the same monomers than those in ^1H-^{13}C HSQC spectra of MWAOD-lignins (**Figure 27**), as shown in **Figure 33**. However, the RPM values of TH-lignins were significant different than those of MWAOD-lignins, as shown in **Table 15**. Comparing these values with those presented in **Table 8**, it is possible to observe that for H_2SO_4 and HCl-TH-lignins there were a significant decrease in the relative amount of G units.

Table 15 – Relative proportion of monomers of TH-lignins.

Catalyst	**% S**	**% G**	**% H**	**S/G ratio**	**H/G ratio**
$AlCl_3$	16.1	65.9	18.0	0.24	0.27
H_2SO_4	15.0	54.3	30.7	0.28	0.57
HCl	26.2	49.4	24.4	0.53	0.49

Source: The author.

Figure 33 – ^{1}H-^{13}C HSQC spectra of (A) $AlCl_3$-; (B) H_2SO_4- and (C) HCl-TH-lignins.



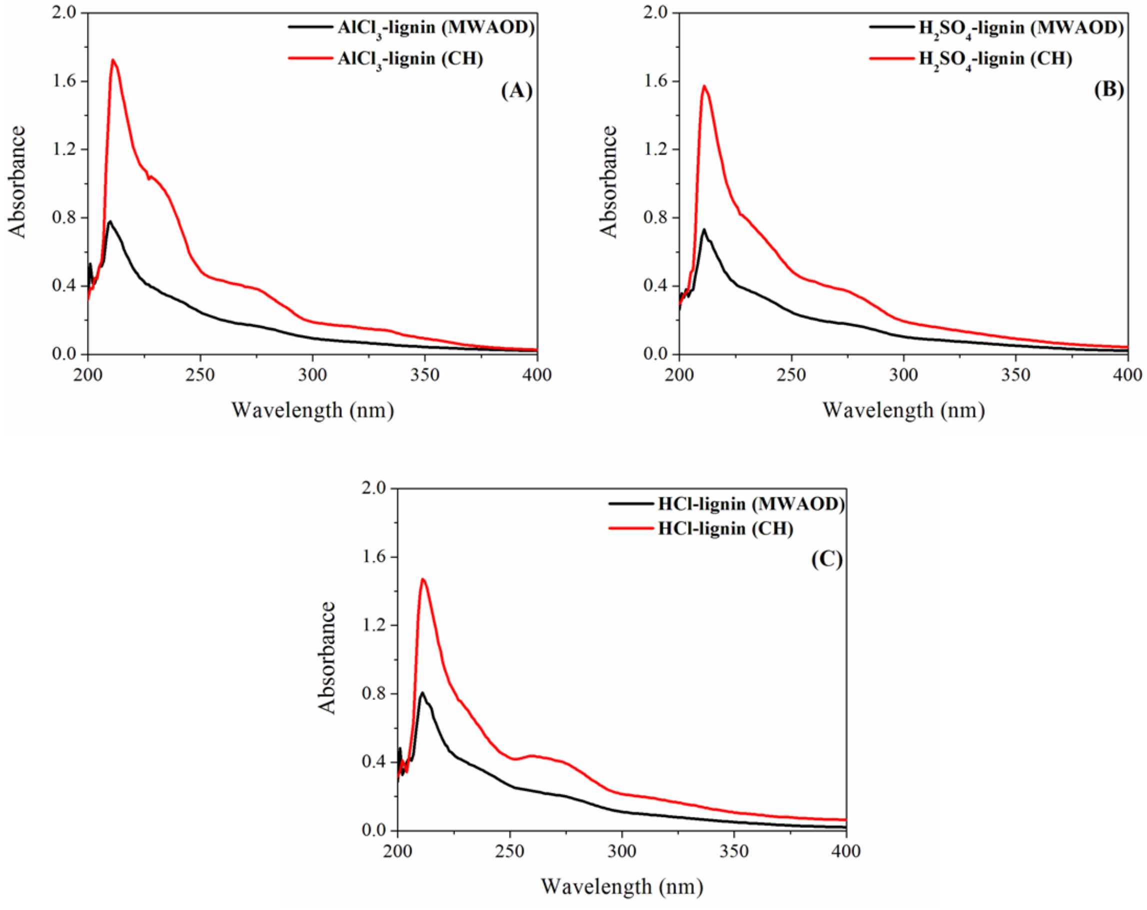
Source: The author.

This behavior suggests that the cleavage of ether bonds, such as 5-O-4 was less effective under TH, since the cross-peak related to G_5 had lower intensity, which means that the position 5 of G ring was substituted, probably by 5-O-4 or 5-5' linkages. This fact shows that the hydrolyses performed under TH were less effective than those in MWAOD, since in the former the slower heating transference led to the occurrence of less effective collisions between the species, which decreased the rate of hydrolysis reaction, as well as the extension of the cleavage of several types of bonds between the monomers.

Thus, these results show the higher energy efficiency of microwave irradiation that was capable to promote the cleavage of different types of bonds in different sites, due to the efficient heating transference that occurred in the bulk solution, allowing the generation of high amount of energy in the same period of time than in TH-experiments, yielding lignins with RPM values more distributed.

There were also observed some difference in the intensities of absorption bands at 210 and 300-400 nm in the UV-Vis spectra of MWAOD- and TH-lignins, as shown in **Figure 34**. The band in the range from 300 to 400 nm is attributed to carbonyl groups and/or double bonds conjugated with an aromatic ring (JABLONSKÝ et al., 2014), which can be an indicative of the presence of residual carbohydrates in the lignins. However, the double bonds present in the phenylpropanoid chains of lignins could also contribute to this increase in the absorption. Moreover, the increase of this band in $AlCl_3$-, H_2SO_4- and HCl-lignin for TH was not too significant to attribute to a higher carbohydrate content in these lignins.

Figure 34 – UV-Vis spectra of MWAOD- and CH-lignins obtained by using (A) $AlCl_3$; (B) H_2SO_4 and (C) HCl as catalysts.



Source: The author.

On the other hand, the band at 210 nm is usually used to detect residual carbohydrates in lignins, since other chromophores will absorb at higher wavelengths, as reported by Singh *et al*. (SINGH et al., 2005). This band is characteristic of carbonyl of ester groups, which are present in the LCC structure and is related to the $n \rightarrow \pi^*$ transitions. **Figure 34** shows that there was a significant increase in the intensity of this band for TH-lignins, which suggests that these lignins had higher residual carbohydrates content than that of MWAOD-lignins that is consistent with the spectral features observed in the FTIR spectra, as well as by their purities values. This behavior is due to the more efficient heating generated by MWI, which promoted a greater extensive cleavage of LCC yielding lignins with lower sugar content.

Comparing the data presented in **Table 13** with those in **Table 10**, is observed that the TH-lignins had higher values of M_w and M_n than that of MWAOD-lignins. These higher values can be attributed to the existence of higher amount of carbohydrates (LAN; LIU; SUN, 2011), which corroborate with the results observed in FTIR and UV-Vis spectra, as well as by their purities.

This behavior indicates that the microwave treatment promoted a more efficient cleavage between the lignin monomers through the hydrolysis of the ether and ester bonds, yielding lignins more fragmented, corroborating with their lower M_w and M_n values. In addition, the polydispersity values were also lower than those of TH-lignins, which indicates that the MWAOD-lignins were composed of much more homogeneous fragments suggesting that the MWI provided a better control of the molecular weight of the lignins.

The thermal data presented in **Table 13** show that there was a decrease in the thermal stability of CH-lignins in relation to that of MWAOD-lignins, as shown in **Table 11**. This can be seen by the lower T_{onset} and T_{max} values obtained for CH-lignins, which indicates the presence of high amount of residual carbohydrates in their structure. In addition, the values of weight loss related to the carbohydrates degradation were higher for TH-lignins than for MWAOD-lignins, corroborating with their purities. Therefore, it is clearly that the MWI favored the greater hydrolysis of linkages between lignin and carbohydrates, yielding lignins with lower sugar content and higher thermal stability.

Conclusions

The use of Lewis acids as catalysts in the microwave-assisted organosolv delignification (MWAOD) experiments showed that these compounds yielded lignins with structural and thermal properties comparable to those obtained by using traditional Brønsted acids. Among the Lewis acids, $AlCl_3$ generated a lignin with similar properties to those obtained by H_2SO_4 and HCl, which indicates this Lewis acid as an alternative catalyst.

Despite these interesting and promising results, further investigations are been conducted in order to assess the scaling-up of this protocol along with the optimization of reaction conditions for the utilization of other biomass residues as feedstock.

Thus, it can be concluded that microwave irradiation combined with the organosolv process promoted the delignification of coconut shell under mild conditions with higher efficiency and lower energy consumption than traditional heating, representing a great combination of green methods for extraction of lignin from biomass waste, with elevated yield, purity, and remarkable structural and thermal properties, making them suitable for further technological applications.

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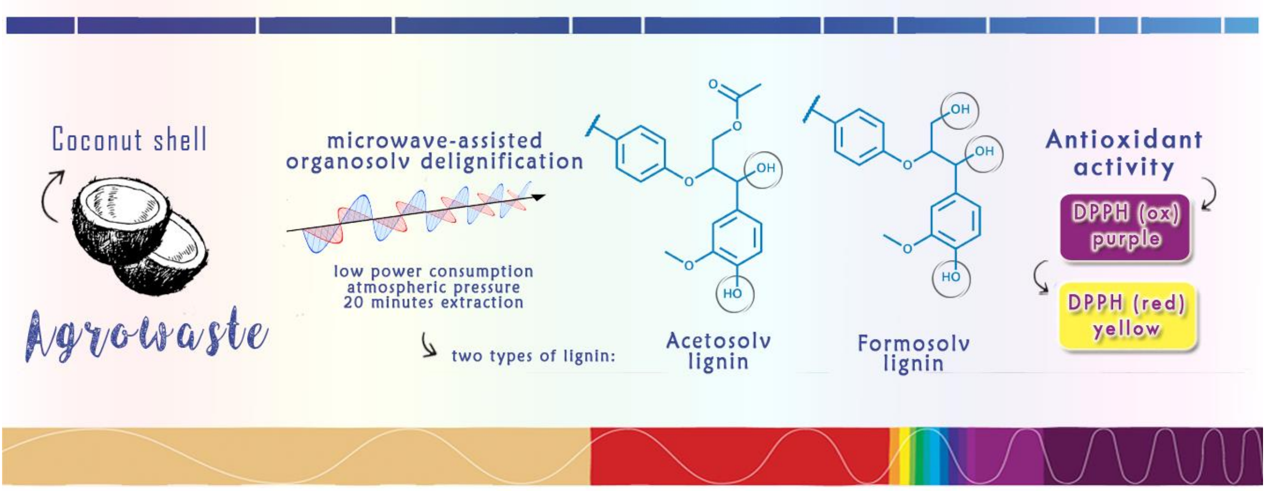
Chapter 3 - The effects of different organosolv processes on the structure of coconut shell lignins and their antioxidant capacities

Graphical abstract



RESUMO

Processos organossolve têm sido aplicados para a obtenção de ligninas de alta qualidade, permitindo a utilização de diferentes condições reacionais. O objetivo desse trabalho foi avaliar as diferenças entre ligninas obtidas do endocarpo do coco seco utilizando os processos acetosolv (LECA) e formosolv (LECF), além de avaliar as suas capacidades antioxidantes, comparando as suas performances com dois antioxidantes comerciais (BHT e Irganox 1010). LECA e LECF foram obtidas usando ácido acético ou fórmico co-catalisados por $AlCl_3$, H_2SO_4 e HCl. Caracterizações estruturais foram realizadas utilizando técnicas espectroscópicas, cromatográficas e termoanalíticas, enquanto que as suas atividades antioxidantes foram medidas através do método do DPPH. A principal diferença observada entre tais ligninas foi a acilação parcial das LECAs em um processo em uma única etapa (*one pot process*), fenômeno não observado para as LECFs. Essas características estruturais mostraram influência nos valores de temperatura de transição vítrea (T_g) e atividades antioxidante para ambas as classes de ligninas. As LECAs apresentaram menores valores de T_g (≈ 136 °C) e maiores valores de IC_{50} do que as LECFs (≈ 15 μg mL^{-1}), apesar de ambas as ligninas terem apresentado valores de IC_{50} cerca de dez vez menores (LECAs, 15 μg mL^{-1} e LECFs, 13,2 μg mL^{-1}) do que dois antioxidantes comerciais (BHT, 112 μg mL^{-1} e Irganox 1010, 127,9 μg mL^{-1}). Conclui-se, portanto, que o uso específico de processos organossolve tem grande potencial para a produção seletiva de ligninas com funcionalidades e processabilidades específicas, características valiosas para diferentes aplicações tecnológicas.

***Palavras-chave**: irradiação por micro-ondas; elucidação estrutural; DPPH; acetilação parcial*

ABSTRACT

Organosolv processes have been applied for obtainment of high quality lignin, allowing the utilization of different reaction conditions. Our aim was to evaluate the differences between lignins obtained from coconut shell under acetosolv (ACSL) and formosolv (FCSL) processes and assess their antioxidant activities, comparing them with those of two commercial antioxidants (BHT and Irganox 1010). ACSL and FCSL were obtained using acetic or formic acids co-catalyzed by $AlCl_3$, H_2SO_4 or HCl. Structural characterizations were performed using spectroscopic, chromatographic and thermo-analytical methods, while antioxidant capacities were evaluated by DPPH assay. The main difference observed was the partial acylation of ACSLs in an *one pot process*, not observed in FCSLs. These structural features influenced their glass transition temperatures and antioxidant capacities. ACSL presented lower T_g (≈136 °C) and higher IC_{50} values than FCSL ((≈ 15 μg mL^{-1}), but both lignins had ten times lower IC_{50} values (ACSLs, 15 μg mL^{-1} and FCSLs, 13.2 μg mL^{-1}) than commercial antioxidants (BHT, 112 μg mL^{-1} e Irganox 1010, 127.9 μg mL^{-1}). As conclusion, the use of specific organosolv processes have great potential for the selective production of lignins with specific functionalities and processability, very useful for further technological applications.

***Keywords**: microwave irradiation; structural elucidation; DPPH; partial acetylation*

Introduction

The crescent interest in the replacement of the petroleum-based products has led the scientific community to prospect alternative source of compounds. In this sense, lignocellulosic biomass represents a natural and renewable source of several chemicals that can be used as building blocks in different technological routes for developing bio-based products.

The lignocellulosic biomass has a rich chemical composition mainly by cellulose, hemicellulose and lignin. These compounds can be found in wood and non-wood plants and they can be extracted using suitable methods yielding their derivatives that can be used to produce high added-value products.

Due to the favorable weather conditions and the great availability of agricultural lands, Brazil has the agribusiness as one of the most important activity for its economy

(MEDINA; SANTOS, 2017). However, a collateral effect that arises from that activity is the expressive generation of agrowastes, as a result of the crops processing, which can be considered as a potential form of environmental pollution (MILLÁN; VARGAS; NZIHOU, 2017).

Among the several types of crops cultivated in Brazil, coconut has a great economical importance, due to its large production (DA SILVA et al., 2018). The side problem is the large amount of solid residues generated in the coconut processing chain, such as the coconut shell (CS), the hardest part of the coconut fruit, which represents approximately 20 % in mass of it (RAMBO; SCHMIDT; FERREIRA, 2015). As a byproduct of the process, CS forms big stacks in the coconut industries, where they are used as fuel in the boilers.

However, CS has a rich biopolymeric composition, presenting high lignin content (25 – 40 % wt.) (ARENA; LEE; CLIFT, 2016; BLEDZKI; MAMUN; VOLK, 2010; CHEN; KUO, 2010; PRAUCHNER; RODRÍGUEZ-REINOSO, 2012), which makes it a potential feedstock for lignin extraction for further valorization. Lignin has attracted the attention of scientists mainly because its availability in the nature and also for being the major source of phenolic compounds on Earth, representing a form to replace partial or totally the petroleum derivatives in different technological fields.

Lignin has a complex tridimensional structure formed by the random polymerization of three monolignols that constitute its monomeric components, such as guaiacyl (G), syringyl (S) and *p*-hydroxyphenyl (H). The type and amount of functional groups in lignin structure is strongly dependent on the biomass origin and the extraction method used for its fragmentation.

There are several types of extractions methods available for biomass delignification, but the most used is the Kraft process in the pulp and paper industry, yielding lignin as a byproduct. However, the presence of high amounts of sulfur and ashes in Kraft lignin structure limits its applications. Therefore, depending on the application the utilization of a high quality lignin is required.

An alternative method for the extraction of high quality lignin is the organosolv, which is based on the use of organic acids or alcohols as solvents, such as formic acid, acetic acid and ethanol, combined to acid catalysts, such as hydrochloric and sulfuric acids to promote the separation of lignin from hemicellulose. An important advantage of this method is the possibility of carry out the delignification under mild conditions. In addition, the use of low cost and toxicity solvents that can be recycled and reused makes organosolv an eco-friendly method.

Recently, our group used microwave-assisted organosolv delignification to extract lignin from coconut shell (AVELINO et al., 2018). There were obtained outstanding results in which high quality lignins were extracted with elevated yields under mild conditions (110 °C during 20 minutes under atmospheric pressure using 2.0 % $AlCl_3$, H_2SO_4 or HCl as catalysts). Through a deep structural characterization of those lignins, we found out that the use of acetic acid as solvent (acetosolv) promoted the partial acetylation of the aliphatic hydroxyls in an *one pot process*. This feature can be very interesting for several purposes, mainly for those that require a balance of polarity, such as the use of lignin as additives in thermoplastics (LI; SARKANEN, 2002, 2005).

On the other hand, for some other applications that require a great amount of hydroxyl groups, such as for antioxidant purposes, may be the partial acetylated lignin is not the most suitable one. In this case, in order to maximize the amount of hydroxyls in the lignin structure, the use of formic acid in the organosolv process (formosolv) would be more appropriate, in order to avoid the occurrence of acetylation, which probably would maximize its performance as a natural antioxidant compound.

However, since there are no studies correlating the structure of acetosolv and formosolv coconut shell lignins to their antioxidant capacities, a deep structural elucidation must be performed to make this correlation, allowing the choice of the most appropriated lignin for that purpose, in order to maximize its performance. This also favors the possibility of replacement of synthetic antioxidants, such as butyl hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl galate by lignin, especially for applications in food and chemical industry (REN et al., 2013), in which natural antioxidants are more desirable due to their purity, non-toxicity, high security and strong antioxidant capacity (HUANG; MENDIS; KIM, 2005).

Therefore, the aim of this work was to evaluate the features of formosolv process and the structural features of formosolv lignins by a full and deep characterization, comparing them with those of acetosolv lignins, in order to elucidate the effects of their structures on their antioxidant activities and establish a structure – property relationship to maximize their performance, also evaluating the potential of the organosolv coconut shell lignins as natural antioxidants in comparison with two commercial antioxidants, such as BHT and Irganox 1010.

Experimental

Materials

The following chemicals were used as received: glacial acetic acid (Synth), formic acid (85 % wt., Synth), H_2SO_4 (98 %, Vetec), HCl (37 %, Synth), DMSO (98 %, Synth), 4-hydroxybenzaldehyde (98 %, Sigma), $AlCl_3$ (98 %, Sigma), DMSO-d6 (99.96 %, Sigma), THF (HPLC-grade, Sigma), 2,2-diphenyl-1-picrylhydrazyl (Aldrich), 2,6-di-tert-butyl-4-methylphenol (≥99 %, Aldrich) and pentaerythritol-tetrakis (3,5-di-tert-butyl-4-hydroxyhydrocinnamate) (Irganox 1010, 98 %, Aldrich).

The CSs were gently supplied by Ducoco Produtos Saudáveis (Itapipoca, Brazil). The methodologies used for obtainment of coconut shell powder (CSP) and its lignocellulosic composition was previously reported (AVELINO et al., 2018).

Microwave-assisted organosolv delignification (MWAOD) experiments

MWAOD experiments were performed in a Milestone (Sorisole, Italy) microwave reactor, model StartSYNTH, according to the methodology described by our group (AVELINO et al., 2018). For the acetosolv experiments, CSP (10 g) was introduced in a round-bottom flask containing 100 mL of aqueous acetic acid solution (AA, 90 % v/v) and 2.0 % w/v or v/v of catalyst ($AlCl_3$, H_2SO_4 or HCl). The experiments were carried out at 110 °C during 20 minutes under atmospheric pressure. For the formosolv experiments, the same conditions were used except the temperature (100 °C) and the concentration of formic acid (FA, 85 % wt.) as solvent. The isolation and purification steps are described in our previous work (AVELINO et al., 2018) and they were used to yield the acetosolv and formosolv coconut shell lignins (ACSL and FCSL, respectively).

Characterization of the organosolv lignins

The purity of coconut shell lignins was determined by the sum of the acid-insoluble lignin (Klason lignin) and the acid-soluble lignin values. For determination of Klason lignin values of coconut shell lignins, a lignin sample (1.0 g) was mixed with 17 mL of H_2SO_4 (72 % wt.) at room temperature for 24 h. The solution was diluted with deionized water to 4 % wt. H_2SO_4 and refluxed for 4 h. After the end of the hydrolysis, the system was cooled for 30 minutes at room temperature. The acid-insoluble residue was isolated by vacuum filtration and washed with deionized water until the pH of the filtrate was equal to that of the deionized water.

After the washings, the residue was dried at 105 °C for 24 h. The experiment was performed in triplicate.

For determination of acid-soluble lignin, the filtrate was diluted with 4 % wt. H_2SO_4 with 1:10 (v/v) ratio and the acid-soluble lignin was calculated from the UV absorbance at 215 and 280 nm. The experiment was performed in triplicate.

Fourier transform infrared spectroscopy (FTIR) analyses were carried out using a Perkin Elmer spectrometer model FT-IR/NIR FRONTIER, using an attenuated total reflectance (ATR) accessory with zinc selenide (ZnS) crystal surface. The spectra were recorded between 4000 and 500 cm^{-1} with resolution of 4 cm^{-1} using the arithmetic average of four scans.

For ^1H-^{13}C HSQC experiments, lignin samples (30 mg) were solubilized in 500 μL of DMSO-d6. The central solvent cross-peak was used as internal reference (DMSO δ_H/δ_C 2.49/39.5). The ^1H and ^1H–^{13}C HSQC NMR spectra were recorded at 25 °C on a Bruker Avance DPX 300 spectrometer (operating at 300 MHz for ^1H nucleus), equipped with a 5-mm One Probe with z-gradient coils.

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

Gel permeation chromatography (GPC) analyses were performed in a Shimadzu LC-20AD (Kyoto, Japan) at 40 °C using a setup comprising two analytical GPC columns in series (Phenogel 5μ 50Å and Phenogel 5μ 10^3Å, 4.6 mm x 300 mm, Phenomenex, Torrance, CA, USA) and HPLC-grade THF as mobile phase. The samples were monitored by UV-Vis detector (Shimadzu SPD-M20A) at 280 nm. Lignin samples (2 mg) were dissolved in 2 mL HPLC-grade THF and then were filtered using a 0.22 µm PTFE filter. Thus, 20 µL of filtered solution was injected into GPC system at a flow rate of 0.35 mL min^{-1}. Standard calibration was performed with polystyrene standards PSS (M_w range 162 – 1.3 x 10^5 g mol^{-1}).

Differential scanning calorimetry (DSC) analyses were performed in a Mettler-Toledo (Schwerzenbach, Switzerland) DSC 823e. Sample (10 mg) was placed in aluminum crucibles closed with a lid centrally punctured. Samples were heated from 25 to 90 ^{o}C, where temperature was kept constant for 10 minutes, then cooled from 90 to 0 ^{o}C, where temperature was kept constant for 3 minutes and heated from 0 to 250 ^{o}C, under a nitrogen atmosphere (50 mL min^{-1}) at a scanning rate of 20 ^{o}C min^{-1}.

Thermogravimetric analyses (TGA) were carried out using a Mettler-Toledo TGA/SDTA851e analyzer. The measurements were performed from 30 to 900 ˚C, at a heating rate of 10 ˚C min^{-1} and under N_2 atmosphere (50 mL min^{-1}). The definition of the initial degradation temperature (T_{onset}) used in this work is the extrapolated onset temperature, which is basically the intersection of the tangents of the curve at the upper horizontal baseline and the steepest part of the curve.

The DPPH free radical scavenging assay were determined using a Cary 60 spectrophotometer (Agilent Technologies), as previously reported (NOGUEIRA et al., 2018). Initially, 1 mg mL^{-1} solution of ACSL or FCSL were prepared in DMSO. A stock solution of DPPH was prepared at a concentration of 10 mg mL^{-1} that was subsequently diluted to a working concentration of DPPH in DMSO of 0.04 mg mL^{-1}. Each different aliquot from the lignins' solution (6, 15, 30, 75 and 150 µL) was added to 1350 µL of working solution of DPPH in DMSO before the sample was brought to a final volume of 3000 µL with DMSO. After each addition, the sample was put in a dark place at room temperature for 30 minutes. In order to compare the antioxidant capacity of ACSL and FCSL there were performed assays with two commercial antioxidants, namely BHT and Irganox 1010. The same aforementioned procedure was used for these experiments. Measurements were carried out in the spectrum range from 300 – 600 nm with spectral resolution of 0.5 nm. The antioxidant activity is expressed in terms of IC_{50} values in µg mL^{-1}, which was determined using the linear regression of inhibition *versus* antioxidant concentration data, using $\lambda = 519$ nm as the standard wavelength for analysis. The analyses were performed in duplicate. The percentage of inhibition was calculated based on the **Equation 2**:

$$Inhibition\ (I, \%) = \frac{Abs_S - Abs_B}{Abs_B}\ .100 \quad (2)$$

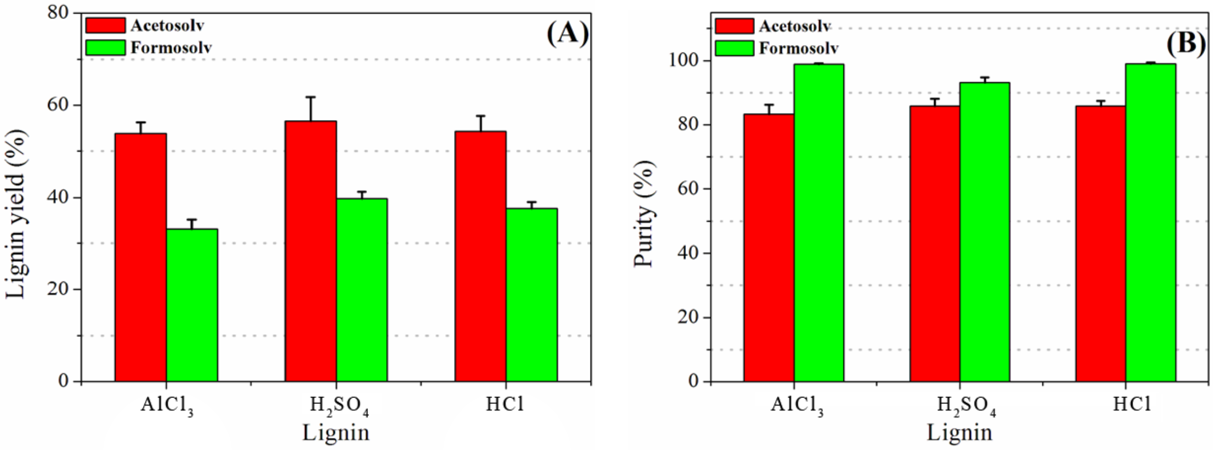
where Abs_S is the absorbance of DPPH at 519 nm in the presence of the antioxidant compound and Abs_B is the absorbance of DPPH at 519 nm without the addition of the antioxidant compound.

Results and discussion

General features of acetosolv and formosolv processes

Usually, the main parameters used to evaluate the efficiency of delignification process are the lignin yield and purity. In this sense, **Figures 35(A)** and **(B)** show the comparison of those parameters for the acetosolv and formosolv processes used to promote the delignification of CS.

Figure 35 – Comparison of (A) yields and (B) purities of coconut shell lignins obtained by acetosolv and formosolv processes.



Source: The author.

As can be seen in **Figure 35(A)**, the use of three different acid catalysts produced lignins with similar yields for both extraction processes, since they have comparable acid strength, although the catalysis mechanism is different, being based on coordination and protonation of ester and ether bonds for the Lewis acid ($AlCl_3$) and Brønsted acids (H_2SO_4 and HCl), respectively. However, it is also noticeable that the type of organosolv process had great influence on the lignin yield. As shown in **Figure 35(A)**, there was a significant decrease in the lignin yield approximately 20 % when the formosolv process was employed instead of acetosolv.

Since formic acid (pKa = 3.75) is ten times stronger than acetic acid (pKa = 4.76)

(GRAÇA et al., 2018), it is plausible to assume a more effective cleavage of bonds between lignin and hemicellulose under formosolv conditions. Nevertheless, this can cause the fragmentation of lignin in smaller fragments with high solubility in formic acid, impairing their precipitation during isolation step and, therefore, decreasing the yield.

In addition, the generation of a high amount of H_3O^+ by the formic acid will tend to increase the rate of hydrolysis reaction, since that would favor the heat production by ionic conduction, as result of the interaction of microwave irradiation with those species in the medium. This probably would favor the cleavage of different types of bonds in a greater extension, yielding low molecular weight fragments that will remain dissolved in formic acid, diminishing the lignin yield.

Although formosolv process generated lignins with lower yields than acetosolv, the former provided an isolation of a higher purity lignin, as shown in **Figure 35(B)**. The increase of approximately 10 % in the purity values of formosolv lignins can be caused by the higher acidic power of FA, which released a considerable amount of H_3O^+ that could catalyze the nucleophilic attack of water molecules to ester and ether bonds involved in lignin-carbohydrate complexes (LCC).

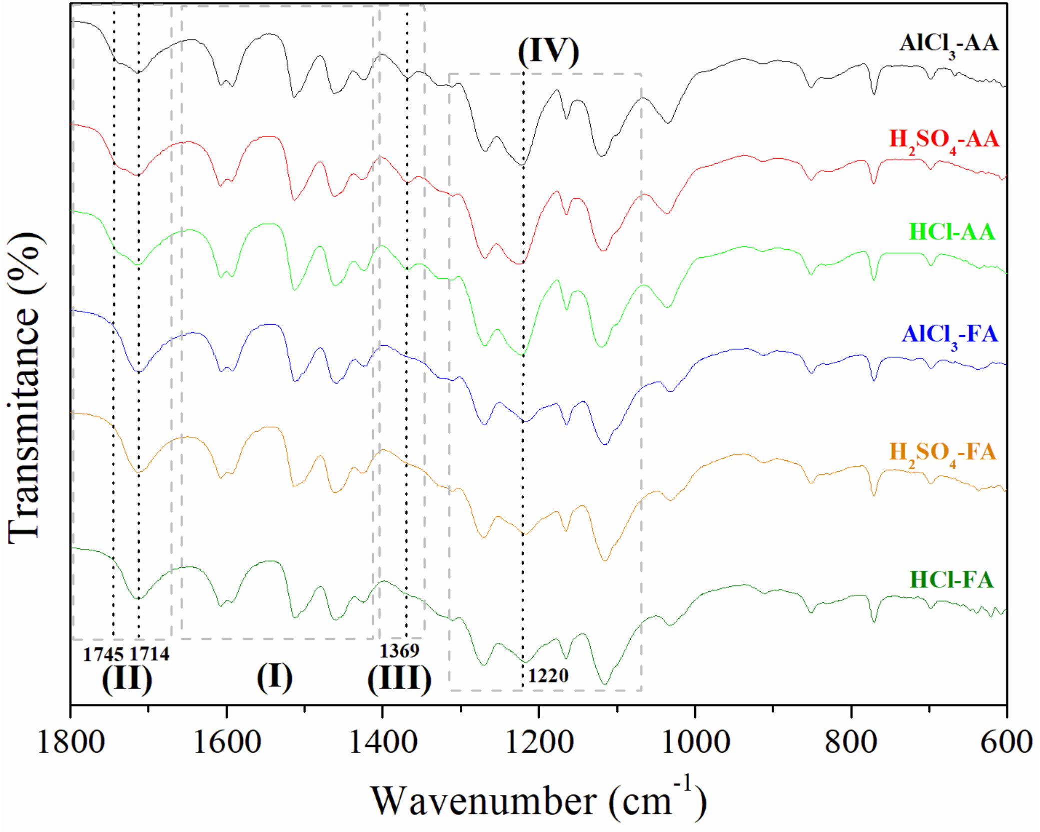
Therefore, the combination of lignin yields and purities values suggests that the formosolv process combined to microwave irradiation under mild conditions promoted a higher fragmentation of CS components than acetosolv, especially regarding the separation of lignin from hemicellulose.

Structural characterization of organosolv lignins

Fourier transform infrared spectroscopy (FTIR)

FTIR analysis provides valuable information about differences in lignin structure through the presence or absence of characteristic absorption bands related to the molecular vibration of specific functional groups. Therefore, **Figure 36** shows the fingerprint region of FTIR spectra of ACSLs and FCSLs, in which some spectral differences are highlighted. The main bands were assigned based on previous reports (ABDELKAFI et al., 2011; FAIX, 1991).

Figure 36 – FTIR spectra of organosolv coconut shell lignins.



Source: The author.

Figure 36 provides a general profile of the structure of ACSLs and FCSLs, in which is possible to observe that the use of three different acid catalysts in both processes generated lignins with very similar functional groups and only slight differences in the intensity of some absorption bands (AVELINO et al., 2018).

An important feature shown in **Figure 36** is the presence of bands comprised in region I, which are related to lignin backbone, such as those at 1593 and 1512 cm^{-1} (C=C stretching of aromatic ring); at 1459 cm^{-1} (C-H deformations) and at 1422 cm^{-1} (aromatic skeletal vibrations coupled with C-H in plane deformation). The presence of these bands in all organosolv lignins means that their aromatic cores were sustained even with the use of strong inorganic acids as catalysts and organic acids with different acidic power as solvents.

However, regions II and III revealed interesting spectral features of ACSLs and FCSLs that dictate remarkable differences between them. Region I comprises a common band for both organosolv lignins, which is that at 1714 cm^{-1} that combined to those in region IV (1269, 1163 and 1117 cm^{-1}) are related to C=O stretching of residual LCC that remained in lignin linked by ester linkages. The other band in region I appears as a shoulder at 1745 cm^{-1} only in ACSLs and it is also related to ester groups, specifically to acetyl groups.

Another interesting observation is the appearance of bands at 1369 and 1220 cm^{-1} (acetoxy methyl bending and C-O-C stretching, respectively), which are quite intense in ACSLs but very weak in the spectra of FCSLs. These bands can be related to the presence of an acetyl group, suggesting a partial acetylation of aliphatic hydroxyls during acetosolv process. In addition, it is important to consider that those bands can also be attributed to acetyl groups in LCC, since ACSLs have lower purity than FCSLs (**Figure 35**).

This finding opens a wide range of possibilities for lignin valorization, since the lignin structure has a great influence in its performance in the final material. Therefore, depending on the application it will be more suitable use a partial acetylated lignin, in which there is a balance of polarity in its structure or a non-acetylated lignin that has availability of considerable amount of hydroxyl groups.

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

A deep structural characterization through 2D NMR techniques provide important features about lignin's architecture, which includes the effects of the extraction method, catalyst identity and severity of the process used to promote the delignification. Therefore, three regions can be explored - aromatic (δ_C/δ_H 100 – 150/ 6.0 – 8.0), oxygenated aliphatic (δ_C/δ_H 50 – 90/ 3.0 – 5.5) and aliphatic (δ_C/δ_H 0 – 50/ 0 – 3.0) - from which informations about the monolignol composition (**Figure 37**), interunit linkages (**Figures 38 and 39**) and phenylpropanoid chains (**Figure 40**), can be extracted.

In addition, structural changes caused by different process and reaction conditions can be measured and compared through the calculation of the relative of proportion of interunit linkages (RPIL) and monomers (RPM) through the integration of the respective cross-peaks, as shown in **Figure 39**. The main cross-peaks were assigned according to previous reports (ABDELKAFI et al., 2011; RENCORET et al., 2013) and listed in **Tables 16 and 17**. The RPIL values are summarized in **Table 18**.

Table 16 – Assignment of the main cross-peaks of acetosolv coconut shell lignins shown in their ^{1}H-^{13}C HSQC NMR spectra.

Label	**δ_C/δ_H (ppm)**	**Assignment**
A'(CH_3COO)	20.5/1.93	C – H in acetate groups (CH_3COO-)
A'(CH_2COO)	2.17/34.4	C – H in ester groups present in LCC
-OMe	56.1/3.75	C – H in methoxyls
Aα/A'α	68.3/4.94	Cα – Hα in β – O – 4' structures (A) and γ-acetylated β – O – 4' substrucures (A')
Aγ	60.1/3.47	Cγ – Hγ in β – O – 4' substructures (A)
A'γ	64.9/4.22	Cγ – Hγ in acylated β – O – 4' substructures (A')
$S_{2,6}$	104.8/6.63	$C_{2,6}$ – $H_{2,6}$ in syringyl units (S)
G_2	111.3/6.97	C_2 – H_2 in guaiacyl units (G)
G_5	115.7/6.75	C_5 – H_5 in guaiacyl units (G)
G_6	119/6.78	C_6 – H_6 in guaiacyl units (G)
$PB_{3,5}$	114.3/6.61	$C_{3,5}$ – $H_{3,5}$ in *p*-hydroxybenzoate units (PB)
$PB_{2,6}$	131.2/7.68	$C_{2,6}$ – $H_{2,6}$ in *p*-hydroxybenzoate units (PB)

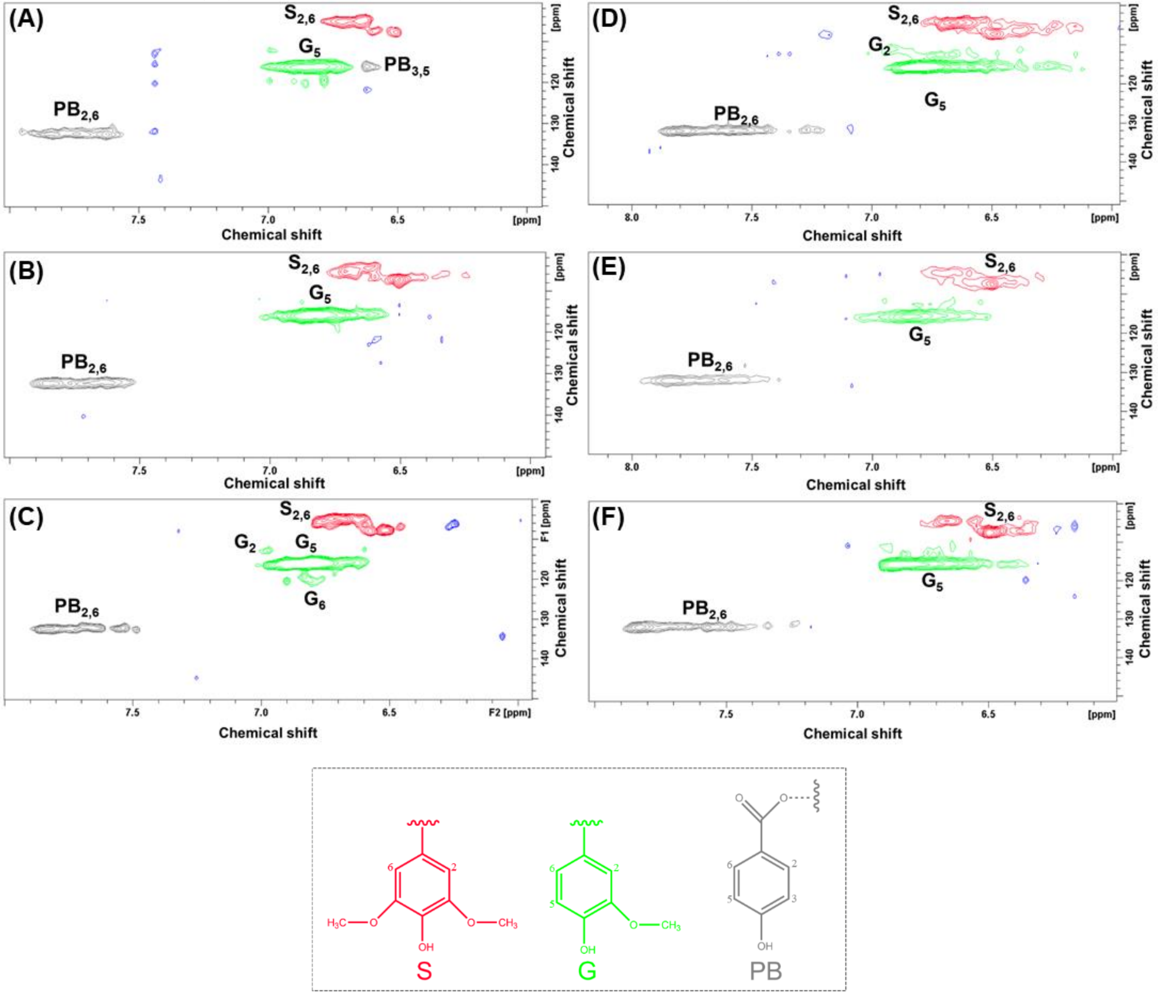
Source: The author.

Table 17 – Assignment of the main cross-peaks of formosolv coconut shell lignins shown in their ^{1}H-^{13}C HSQC NMR spectra.

Label	**δ_C/δ_H (ppm)**	**Assignment**
A'(CH_3COO)	-	C – H in acetate groups (CH_3COO-)
A'(CH_2COO)	2.21/34.7	C – H in ester groups (CH_2COO-) present in LCC
-OMe	56.2/3.65	C – H in methoxyls
Aα/A'α	68.4/5.04	Cα – Hα in β – O – 4' structures (A) and γ-acetylated β – O – 4' substrucures (A')
Aγ	60.5/3.62	Cγ – Hγ in β – O – 4' substructures (A)
A'γ	64.0/4.29	Cγ – Hγ in acylated β – O – 4' substructures (A')
$S_{2,6}$	105.8/6.58	$C_{2,6}$ – $H_{2,6}$ in syringyl units (S)
G_2	111.2/6.89	C_2 – H_2 in guaiacyl units (G)
G_5	115.4/6.78	C_5 – H_5 in guaiacyl units (G)
$PB_{2,6}$	131.2/7.62	$C_{2,6}$ – $H_{2,6}$ in *p*-hydroxybenzoate units (PB)

Source: The author.

Figure 37 – Aromatic region of ^{1}H–^{13}C HSQC spectra of **(A)** $AlCl_3$–ACSL; **(B)** H_2SO_4–ACSL; **(C)** HCl–ACSL; **(D)** $AlCl_3$–FCSL; **(E)** H_2SO_4–FCSL and **(F)** HCl–FCSL.



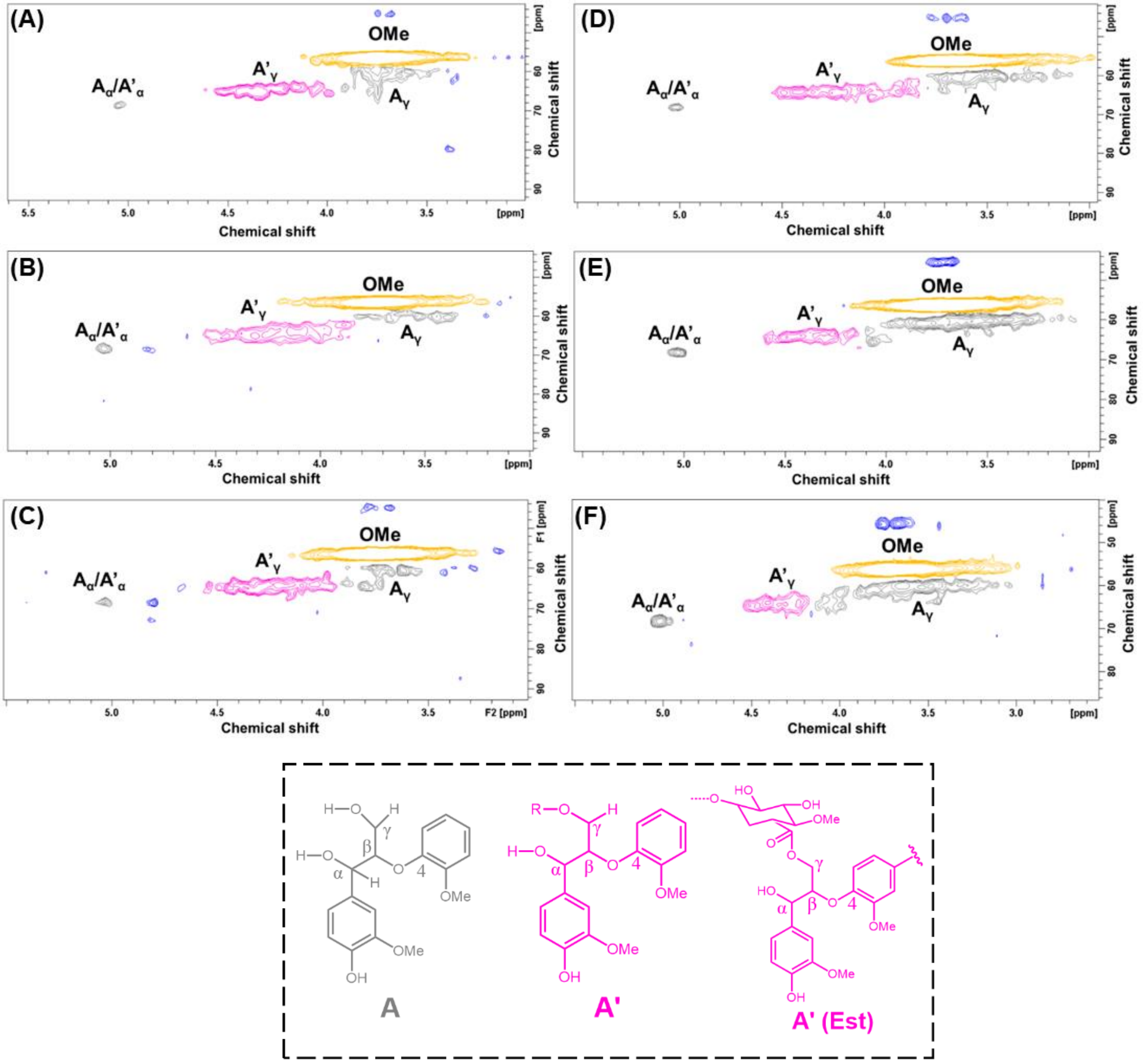
Source: The author.

Based on the spectra shown in **Figure 37**, it is noticeable that the ACSLs and FCSLs had similar monolignol composition, in which syringyl (S), guaiacyl (G) and *p*-hydroxybenzoate (PB) were the monomers composing those lignins, classifying them as HGS lignins.

Moreover, the RPM values presented in **Figure 38(A)** show that the relative amount of monomers were also quite similar for both processes and for all catalysts, which means that catalysts nature did not favor specific cleavages between the monomers, catalyzing the hydrolysis reaction of lignin molecule in comparable extensions.

Apparently, the use of organic acids with different acidicity also did not influence on the cleavage pattern of the monomers, as shown in **Figure 38(A)**. This observation suggests that the main cleaved linkages were the ether bonds, especially the β-O-4, the most abundant one, due to its low bond-dissociation energy (BDE), since carbon-carbon bonds would require more energy to be cleaved and, consequently, more severe conditions than those employed in MWAOD experiments.

Figure 38 – Oxygenated aliphatic region of ^{1}H–^{13}C HSQC spectra of **(A)** $AlCl_3$–ACSL; **(B)** H_2SO_4–ACSL; **(C)** HCl–ACSL; **(D)** $AlCl_3$–FCSL; **(E)** H_2SO_4–FCSL and **(F)** HCl–FCSL.

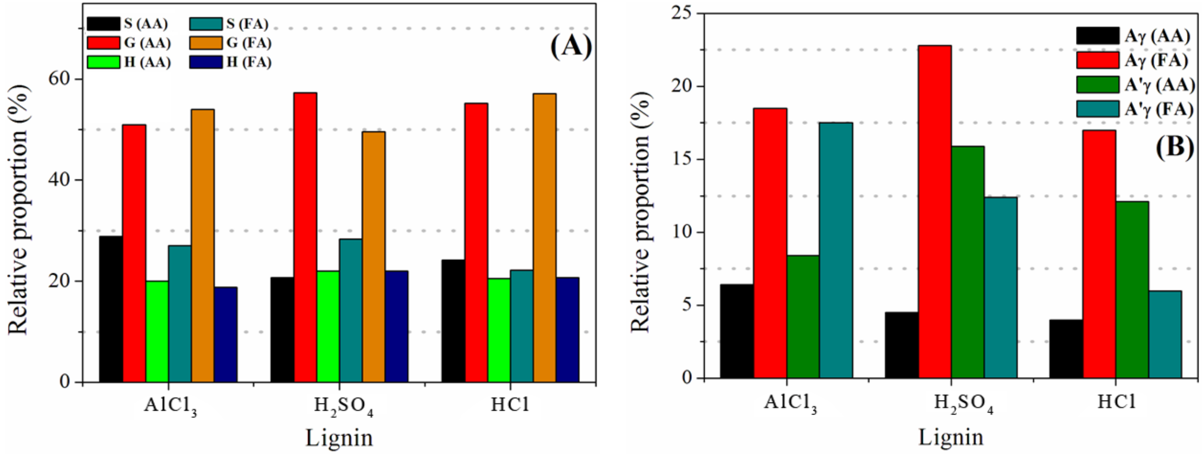


Source: The author.

Figure 38 shows that the oxygenated aliphatic regions of ACSLs and FCSLs are basically composed of A-type structures, in which the monomers are linked by β-O-4 alkyl-aryl ether bonds. The spectra show the presence of two substructures of A-type, the **A** and **A'**, where the latter can be overlaid by an **A' (Est)** substructures related to LCC (HUANG et al., 2018). The **A'** substructures of ACSLs have acetyl groups in α- or γ-positions of phenylpropanoid chains as result of the partial acetylation of aliphatic hydroxyls during the organosolv process, as observed by the FTIR analyses.

Comparing spectra in **Figure 38**, it can be seen that the cross-peaks related to **A'γ** have higher intensities for ACSLs than FCSL, while the cross-peaks related to **Aγ** have higher intensities for FCSLs than for ACSL, suggesting that the former have a higher amount of free hydroxyl groups. These observations can be better visualized in **Figure 39(B)**, where the RPIL values for ACSLs and FCSLs obtained by using different catalysts are shown.

Figure 39 – Relative proportion of (A) monomers and (B) interunit linkages of ACSLs and FCSLs obtained from their ^1H–^{13}C HSQC spectra.



Source: The author.

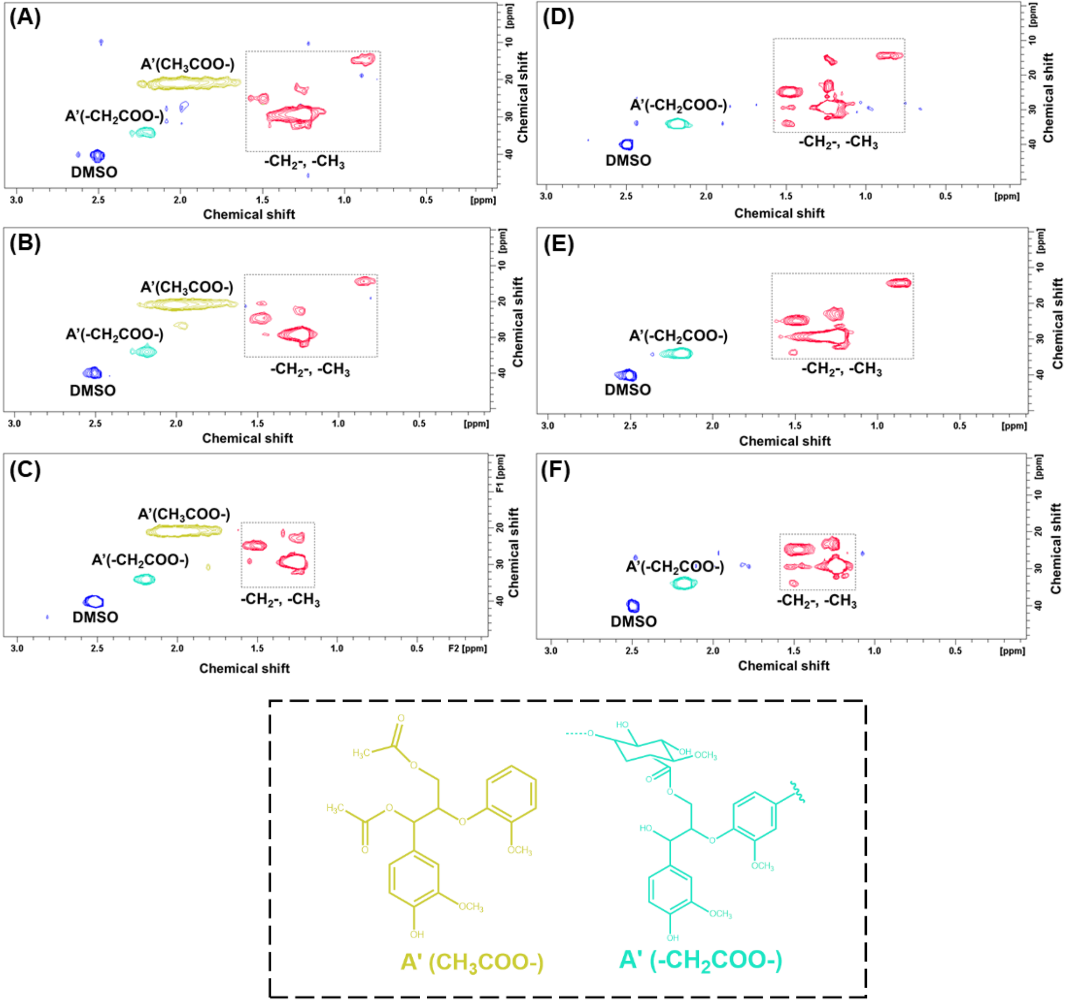
Table 18 – Relative proportion of interunit linkages (RPIL) of FCSLs and ACSLs.

Sample/RPIL (%)	**$-OCH_3$ (%)**	**Aγ (%)**	**A'γ (%)**	**Aα/A'α (%)**	**A'γ/Aγ**
$AlCl_3$-FCSL	63.1	18.5	17.5	0.9	0.95
H_2SO_4-FCSL	63.3	22.8	12.4	1.6	0.54
HCl-FCSL	74.9	16.9	6.0	2.2	0.35
$AlCl_3$-ACSL	85.0	6.4	8.4	0.3	1.3
H_2SO_4-ACSL	78.6	4.5	15.9	1.0	3.6
HCl-ACSL	83.4	4.0	12.1	0.4	3.0

Source: The author.

Interestingly, **Figures 38** and **39(B)** show that the $AlCl_3$-FCSL had a higher amount of **A'γ** than $AlCl_3$-ACSL. This could be caused by the contribution of the **A' (Est)** substructures present in LCC, since there is an overlapping between the **A'** and **A' (Est)** substructures.

Figure 40 – Aliphatic region of ^{1}H–^{13}C HSQC spectra of (A) $AlCl_3$–ACSL; (B) H_2SO_4–ACSL; (C) HCl–ACSL; (D) $AlCl_3$–FCSL; (E) H_2SO_4–FCSL and (F) HCl–FCSL.



Source: The author.

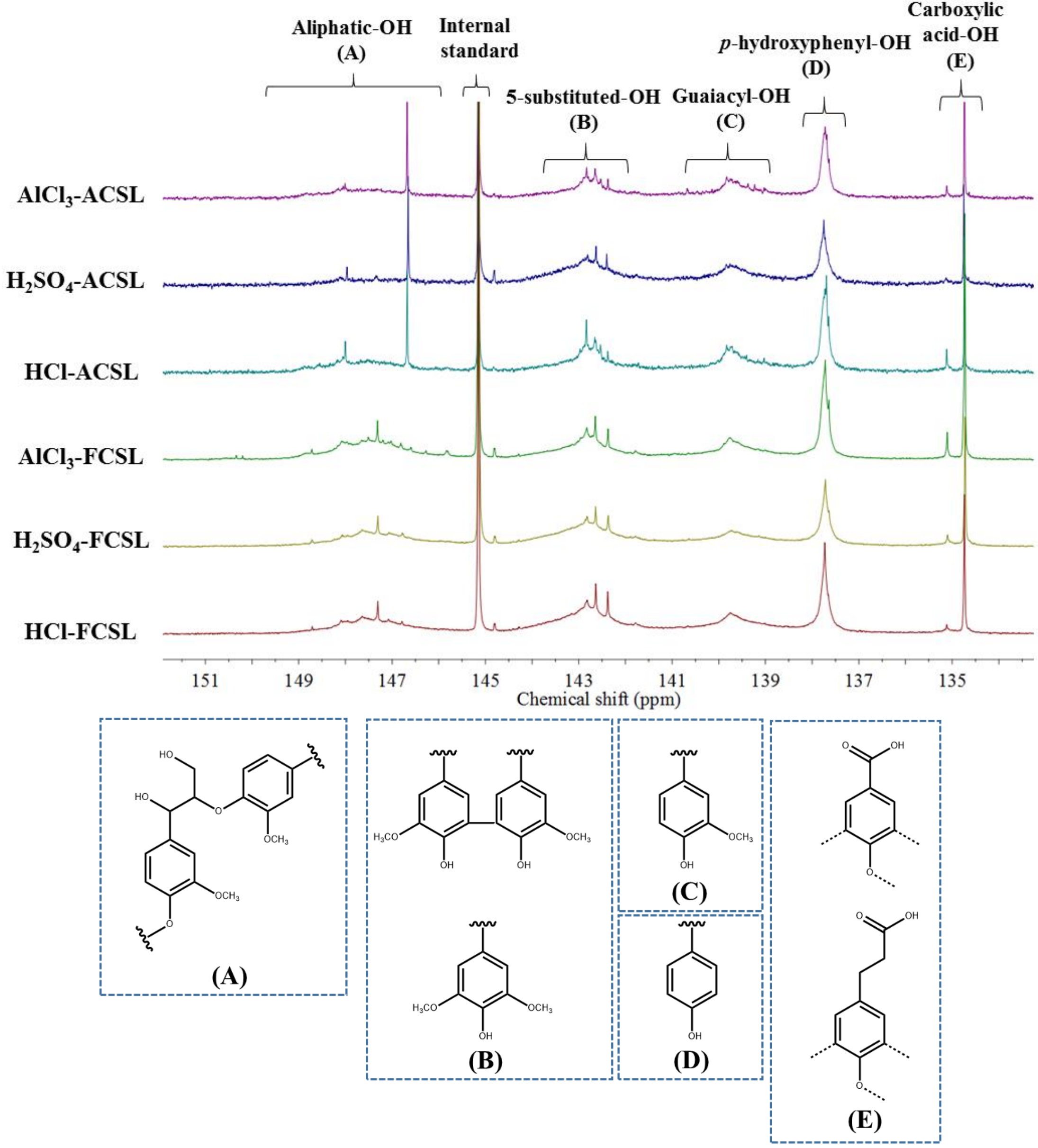
The aliphatic region of organosolv lignins provides valuable information about the occurrence of acetylation through the appearance of a strong and broad cross-peak in δ_C/δ_H 19 – 22.5/ 1.7 – 2.1 ppm, related to the aliphatic acetyl groups resultant from the acetosolv process, **Figure 40**. These cross-peaks are absent in the ^{1}H–^{13}C HSQC spectra of FCSLs, which combined to their FTIR spectra indicate that no acetylation was promoted when formic acid is used as solvent in MWAOD under mild conditions.

^{31}P NMR

Identification and quantification of different types of hydroxyl groups by ^{31}P NMR provide valuable information about the reactivity of the organosolv lignins. **Table 19** shows the data obtained from quantification of hydroxyl groups of ACSLs and FCSLs, while their ^{31}P

NMR spectra are shown in **Figure 41**.

Figure 41 – ^{31}P NMR spectra of ACSLs and FCSLs and their identified substructures.



Source: The author.

Table 19 – Quantification data of hydroxyl groups present in ACSLs and FCSLs.

Index/Lignin	**Acetosolv**			**Formosolv**		
Catalyst	**$AlCl_3$**	**H_2SO_4**	**HCl**	**$AlCl_3$**	**H_2SO_4**	**HCl**
OH aliphatic content (mmol g^{-1})	0.58	0.36	0.72	0.74	0.59	0.56
C_5-substituted-OH	0.71	0.95	0.88	0.63	0.80	0.81
Guaiacyl-OH	0.59	0.60	0.70	0.45	0.44	0.48
p-Hydroxyphenyl-OH	0.52	0.41	0.62	0.44	0.35	0.39
OH phenolic content (mmol g^{-1})	1.83	1.96	2.21	1.53	1.59	1.68

COOH content (mmol g^{-1})	0.14	0.12	0.18	0.23	0.19	0.17
Total OH groups' content (mmol g^{-1})	2.56	2.45	3.11	2.50	2.37	2.41
$OH_{phen..}/OH_{total}$	0.71	0.80	0.71	0.61	0.67	0.69

Source: The author.

Table 19 shows the slightly variation between monolignol composition of ACSLs and FCSLs, as pointed out by $^{1}H–^{13}C$ HSQC spectra and RPM values (**Figure 37** and **Figure 38**). It was observed a considerable decrease in the phenolic hydroxyls content in FCSLs, which can suggest the occurrence of repolymerization reactions during the pulping due to the higher acidic power of FA. In addition, FCSLs also present higher COOH contents suggesting that formosolv process is more oxidizing than acetosolv.

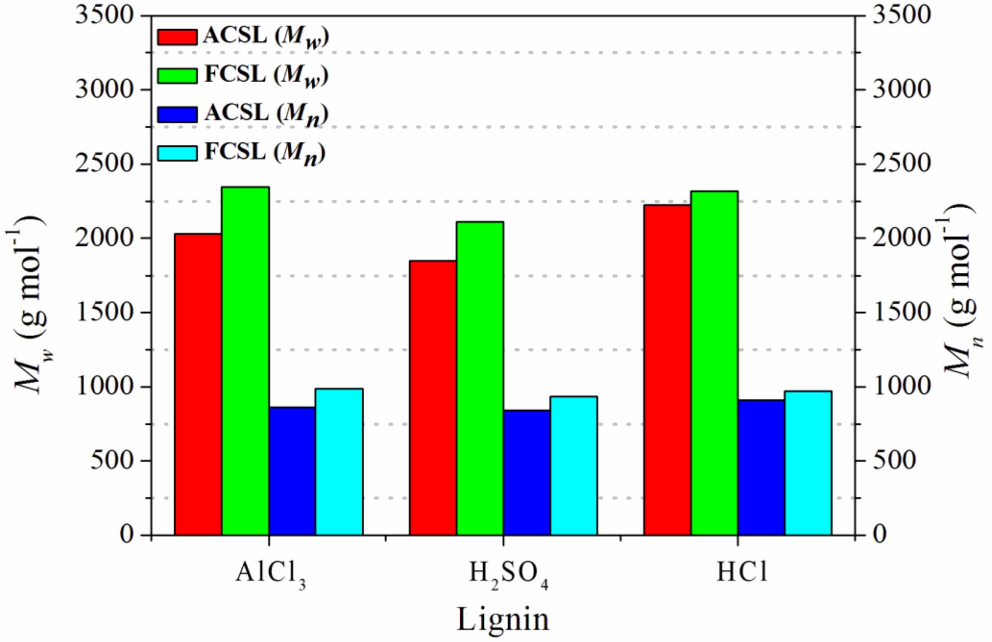
An interesting feature presented in **Table 19** is the difference between the aliphatic hydroxyls content of ACSLs and FCSLs. It is possible to observe that the aliphatic hydroxyls content of $AlCl_3$ and H_2SO_4-ACSLs decreased approximately 21.6 and 39 %, respectively, in relation to those of FCSLs, suggesting the occurrence of acetylation reaction during acetosolv process, as previously shown by $^{1}H–^{13}C$ HSQC spectra.

However, it is important to highlight that the residual carbohydrates in the lignins' structure can also undergo acetylation reaction. Since the ACSLs have lower purity than FCSLs by approximately 10 % (**Figure 35**), it is plausible to suggest that the carbohydrates present in ACSLs could be acetylated, contributing to the decrease in their aliphatic hydroxyls content.

Gel permeation chromatography (GPC)

The severity of the delignification process and its effects on the lignin structure can also be measured by its molecular weight distribution and the parameters that can be obtained from it, such as number-average (M_n) and weight-average (M_w) molecular weights and polydispersity index (PDI). **Figure 42** shows the M_w, M_n and PDI values of ACSLs and FCSLs and **Table 20** summarizes these parameters. The molecular weight distribution curves of the organosolv lignins are shown in **Figure 43**.

Figure 42 – Comparison between the M_w and M_n values of ACSLs and FCSLs.



Source: The author.

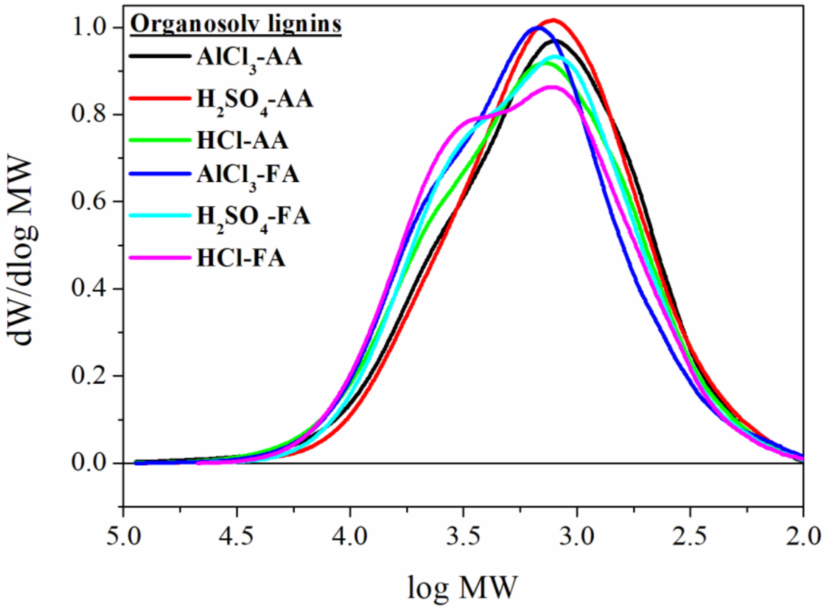
Table 20 – Molecular weight data of ACSLs and FCSLs obtained by GPC analysis.

Catalyst/Lignin	***Acetosolv***			***Formosolv***		
	M_w (g mol^{-1})	M_n (g mol^{-1})	PDI	M_w (g mol^{-1})	M_n (g mol^{-1})	PDI
$AlCl_3$	2032	861	2.36	2344	988	2.37
H_2SO_4	1849	841	2.20	2113	935	2.26
HCl	2224	908	2.45	2319	971	2.39

Source: The author.

The data presented in **Figure 42** and **Table 20** clearly show that the FCSLs had slightly higher M_w and M_n values than ACSLs, independent of the catalyst used in the extraction. The three catalysts are acidic enough to favor the cleavage of β-O-4 bonds in lignin structure. However, since FA is significantly stronger than AA, formosolv process probably creates an environment in which depolymerization and repolymerization reactions will compete (GALKIN; SAMEC, 2016; SCHUTYSER et al., 2018). Therefore, the results suggest that a higher acidity of the organosolv solution favors the occurrence of repolymerization reactions, which can also be seen by the displacement of the molecular weight distribution curves of FCSLs to higher log M_w values, as shown in **Figure 43**.

Figure 43 – Molecular weight distribution curves of ACSLs and FCSLs obtained by GPC analysis.



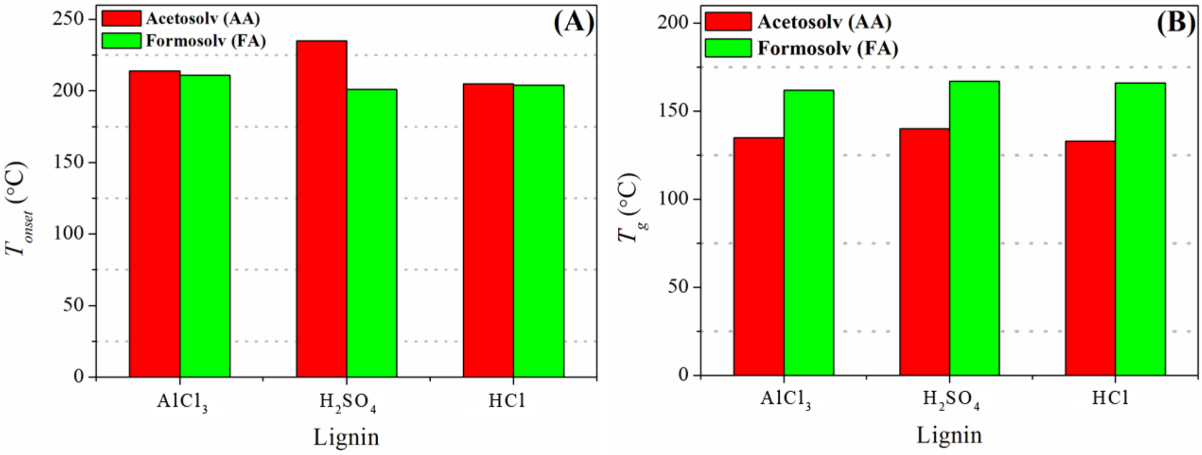
Source: The author.

Thermal properties

In order to establish their thermal stability and processability, ACSLs and FCSLs were evaluated by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC).

Figure 44(A) shows the onset temperature (T_{onset}) values for ACSLs and FCSLs obtained from their TGA curves (**Figure 45**), while **Figure 44(B)** shows the glass transition temperatures (T_g) for ACSLs and FCSLs obtained from their DSC curves (**Figure 46**). Other thermal data of those lignins, such as maximum degradation temperatures (T_{max}), char yield (CY) and limiting oxygen index (LOI) are summarized in **Table 21**.

Figure 44 – Comparison between (A) T_{onset} and (B) T_g values of ACSLs and FCSLs.



Source: The author.

Table 21 – Thermal data of ACSLs and FCSLs obtained from TGA and DSC analyses.

Lignin/Process	**Acetosolv**					**Formosolv**				
	T_{onset} (°C)	T_{max} (°C)	CY (%)	LOI* (%)	T_g (°C)	T_{onset} (°C)	T_{max} (°C)	CY (%)	LOI* (%)	T_g (°C)
$AlCl_3$	214	340	15.1	23.5	135	211	343	15.4	23.7	162
H_2SO_4	235	360	23.3	26.9	140	201	354	30.3	29.6	167
HCl	205	348	11.7	22.2	133	204	355	32.5	30.5	166

*Estimated based on van Krevelen equation (Equation 3) (VAN KREVELEN, 1975)

Figure 44(A) clearly shows that the T_{onset} of ACSLs and FCSLs had similar values. However, the char yields values described in **Table 21** were considerable higher for FCSLs than for ACSLs. This observation can be justified by the higher purity and higher M_w values (**GPC section**) of FCSLs, favoring the formation of a carbonaceous structure through the occurrence of condensation of aromatic rings at elevated temperatures. On the other hand, the higher residual carbohydrates content and the elevated degree of acetylation in ACSLs will decrease the amount of solid residues at the end of pyrolysis, since they undergo complete thermal degradation during the analysis.

The char yield values in **Table 21** can be used to estimate the potential of flame retardancy of lignins through the calculation of the LOI, which is the minimum O_2

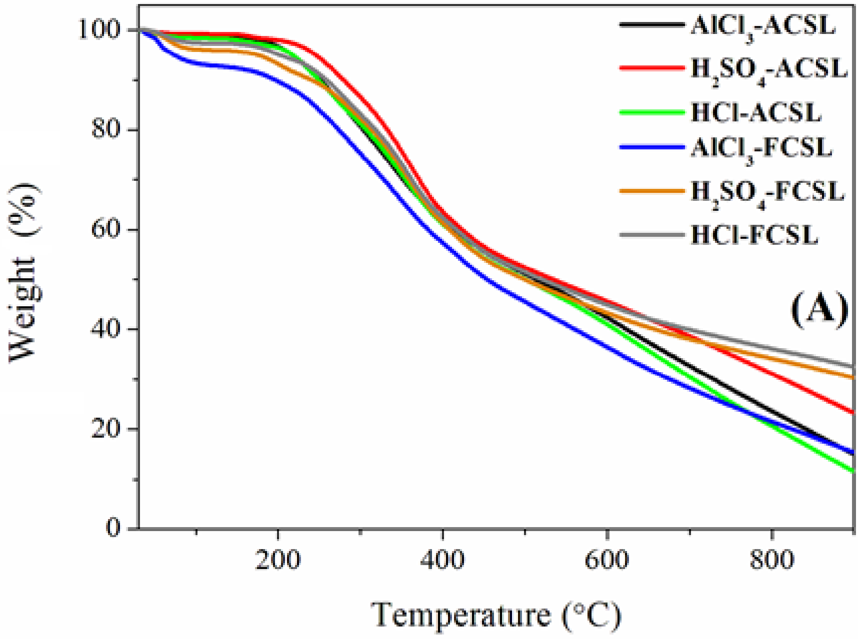
concentration required to promote the ignition of a material, causing its flammability. According to van Krevelen (1975), materials that have LOI values ≤ 26 are considered flammable and a simple method to estimate LOI using char yield values is based on van Krevelen equation (**Equation 3**) (VAN KREVELEN, 1975).

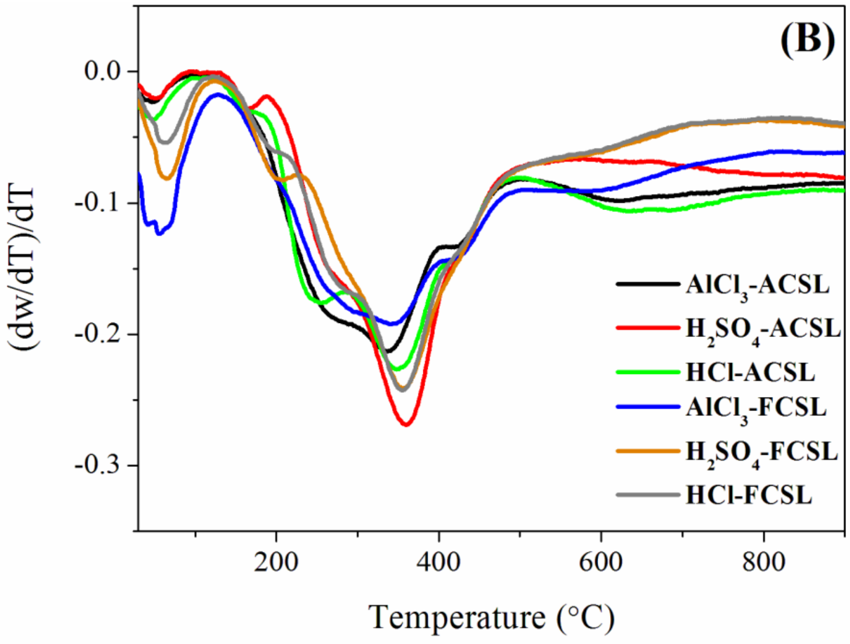
$$\mathrm{LOI} = 17.5 + 0.4\,(\mathrm{CY}) \qquad (3)$$

The LOI values presented in **Table 21** show that formosolv process generates lignins with LOI values > 28, which is the required value for polymers to be considered as self-extinguishing (MALLAKPOUR; ZADEHNAZARI, 2017). This is a valuable feature that shows the potential of FCSLs to be used as bio-based flame retardants, in order to replace the traditional halogen-, silane- and phosphorus-based materials commonly used in polymeric materials.

Another interesting feature presented in **Figure 44(B)**, is a significant difference between the T_g values of ACLs and FCSLs, in approximately 20 %. The higher T_g values of FCSLs can be attributed to a higher amount of aliphatic hydroxyl groups, while the acetylation of aliphatic hydroxyls in ACSLs increases the free volume in the molecules and reduces their T_g values. Therefore, acetosolv and formosolv processes can provide lignins with different processabilities, being a valuable tool for different technological applications.

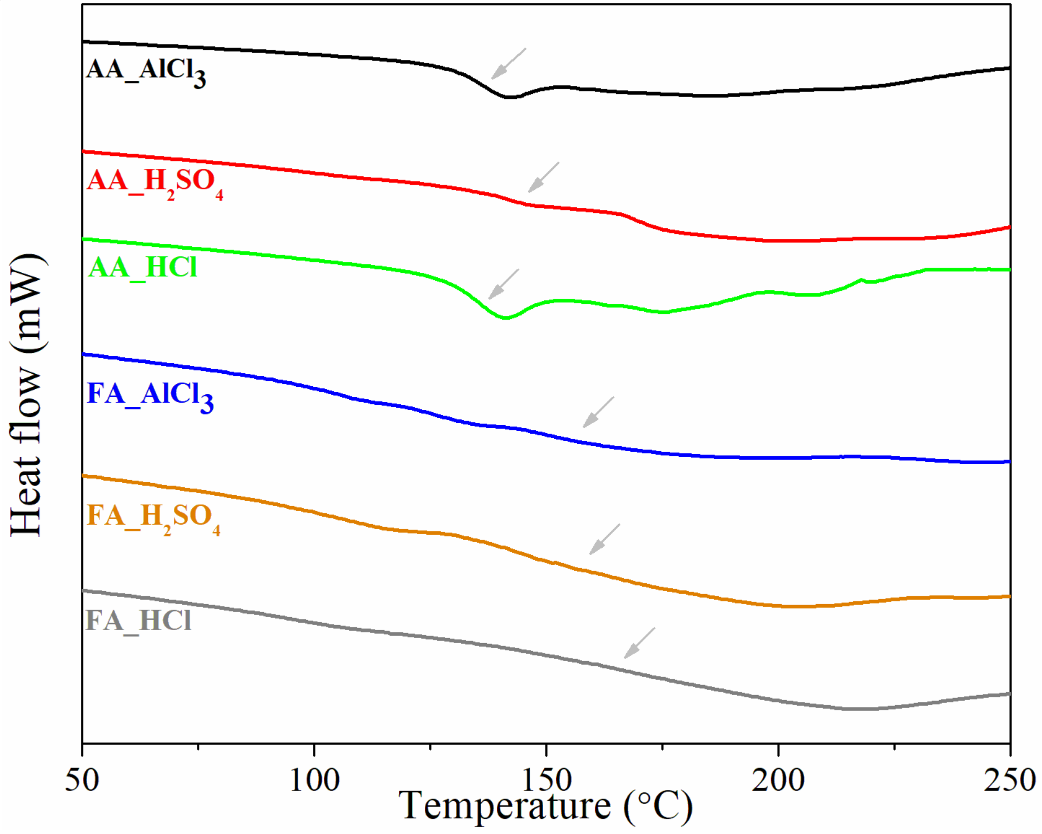
Figure 45 – (A) TGA and (B) DTG curves of ACSLs and FCSLs.





Source: The author.

Figure 46 – Comparison between DSC curves of ACSLs and FCSLs where the grey arrows highlight their glass transition temperatures (midpoint).

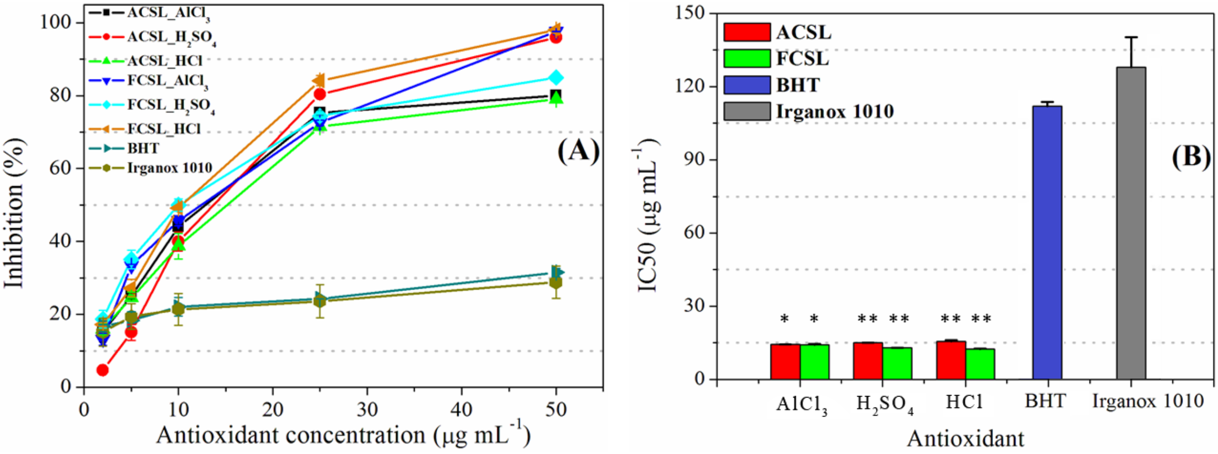


Source: The author.

Antioxidant activity of organosolv lignins

The antioxidant activity of ACSLs and FCSLs were assessed in order to establish a relationship between their structures and antioxidant capacities (AN et al., 2017). Therefore, their ability to scavenge DPPH radical was evaluated and compared to commercial antioxidants (BHT and Irganox 1010). The inhibition curves of ACSLs, FCSLs, BHT and Irganox are shown in **Figure 47(A)** and their IC_{50} values are shown in **Figure 47(B)**. **Table 22** summarizes the values of maximum inhibition, IC_{50} and slope of IC_{50} regression curves.

Figure 47 – (A) Inhibition curves and (B) IC_{50} values of ACSLs, FCSLs, BHT and Irganox 1010 obtained in the antioxidant activity assays.



Source: The author.

The main structural feature that influences on the antioxidant activity of lignins is the presence of phenolic groups due their ability to form stable phenoxyl radicals after donation of a hydrogen atom. The inhibition curves showed that ACSLs and FCSLs had elevated antioxidant activities, with FCSLs presenting better results (**Table 22)**. The IC_{50} values (**Figure 47(B))** also confirmed the improved performance of FCSLs, since their IC_{50} values were much lower than those of ACSLs, with 99.5 % of confidence (One Way ANOVA test).

Table 22 – Antioxidant capacity data of ACSLs, FCSLs, BHT and Irganox 1010.

Antioxidant/Parameter	**Max. inhibition (%)**	***IC_{50}*** **(μg mL^{-1})**	**Slope of *IC_{50}* curve**
ACSL – $AlCl_3$	80.1 ± 1.3	14.4 ± 0.06	2.56 ± 0.1
ACSL – H_2SO_4	96.0 ± 0.28	15.1 ± 0.01	3.26 ± 0.03
ACSL – HCl	79.0 ± 0.16	15.7 ± 0.48	2.38 ± 0.09
FCSL – $AlCl_3$	97.7 ± 0.05	14.3 ± 0.21	2.35 ± 0.07
FCSL – H_2SO_4	85.0 ± 0.25	12.9 ± 0.07	2.28 ± 0.05
FCSL – HCl	98.0 ± 0.75	12.4 ± 0.3	2.87 ± 0.03
BHT	31.5 ± 1.18	112.0 ± 1.7	0.29 ± 0.02
Irganox	28.8 ± 4.39	127.9 ± 12.3	0.23 ± 0.03

Source: The author.

The differences in the ability of FCSLs and ACSLs to scavenge free radicals are linked to their peculiar chemical structural. According to **Table 22**, FCSLs have lower phenolic hydroxyl than ACSLs, but similar total hydroxyls content, suggesting that there must be a combination of factors involved in the radical scavenging mechanism besides the phenolic hydroxyls.

An example of this is the carbohydrate content which can be associated to their purity (**Figure 35**), since residual carbohydrates can interact with phenolic hydroxyls through hydrogen bonds, decreasing the antioxidant capacity of lignins (DIZHBITE et al., 2004), as observed for ACSLs that have lower purities and antioxidant activities than those of FCSLs. The presence of *ortho*-substituted electron-donating groups, such as methoxyl groups favor the formation of phenoxy radicals by decreasing the bond dissociation enthalpy (BDE) of phenolic hydroxyl group (ZHAO et al., 2018), also helping in their stabilization by resonance.

However, according to **Table 18** (^{1}H-^{13}C HSQC section) apparently there was not a correlation between the relative proportion of methoxyl groups and the IC_{50} values, since ACSLs had higher amount of methoxyl groups and higher IC_{50} values. Interestingly, **Table 18** also shows that the relative proportions of Aα/A'α substructures for ACSLs are lower than those of FCSLs, suggesting the presence of *α*-carbonyl groups in that position resultant of its acetylation. The presence of these groups in the side chain of ACSLs can cause a significant decrease in their radical scavenging capacities (QIN et al., 2018).

Furthermore, it is worth mentioning that the aliphatic hydroxyl groups can also contribute to the antioxidant activity as a hydrogen donor [27, 30]. Therefore, this is also a considerable difference between ACSLs and FCSLs, since most of γ positions of ACSLs' side chain are acetylated (**Figures 39 and 40; Tables 18 and 19**), which hinder the occurrence of the neutralization of free radicals by hydrogen donation from those positions, reflecting in lower antioxidant capacities in comparison to those of FCSLs.

An important behavior is also observed in **Figures 47(A)** and **(B)** in which the maximum inhibition for organosolv lignins are three times higher than those of BHT and Irganox 1010, while the IC_{50} for those are ten times lower than that of commercial ones. A similar behavior was previously observed by our group for antioxidant activity assays using palm oil acetosolv lignins, BHT and Irganox 1010 in a different solvent (NOGUEIRA et al., 2018).

This difference in the IC_{50} values for ACSLs, FCSLs, BHT and Irganox 1010 can be caused by several factors, such as the solvent used in the assays and the radical scavenging method, which combined can interfere in the mechanism involved in the inhibition of free radicals by the phenolic hydroxyl groups, since they can inhibit them through hydrogen abstraction transfer (HAT), proton decoupled electron transfer (PCET) and sequential proton loss electron transfer (SPLET) (ZHAO et al., 2018).

It is important highlight that the structures of ACSLs, FCSLs, BHT and Irganox 1010 have considerable differences that can also influence in their abilities to scavenge free radicals. One of them is the higher amount of phenolic hydroxyls per mass unit in the organosolv lignins, which favors the neutralization of DPPH radicals. In addition, the values of the slopes of IC_{50} curves shown in **Table 22** suggest that the reaction between DPPH radicals, BHT and Irganox 1010 is very slow, since their slope values are approximately 10 times lower than those of ACSLs and FCSLs. This also reinforces the hypothesis of the influence of the concentration of phenolic hydroxyls in the organosolv lignins, since the kinetic of the inhibition is strongly affected by their concentration.

Therefore, the results obtained in the antioxidant assay showed a great potential of both acetosolv and formosolv coconut shell lignins to be used as natural antioxidants due to their very lower IC_{50} values than those observed for BHT and Irganox 1010. This indicates that the organosolv coconut shell lignins had a much better performance than those two commercial antioxidants at extremely lower concentrations. However, it is necessary to evaluate the cytotoxicity of those lignins, in order to assess the compounds released when they are exposed to human body cells, which can also be very useful to dictate the suitable application for them, such as use in food and cosmetics or as additives in thermoplastics and epoxy resins.

Conclusions

The simple use of different solvents in the organosolv process was capable to generate lignins with selective features. Partial acetylation of hydroxyls in an *one pot* process during the microwave-assisted delignification was the main difference between acetosolv and formosolv processes. These features have shown great influence on glass transition temperatures and antioxidant capacities. Acetosolv lignins presented lower T_g and higher IC_{50} than formosolv lignins. Both showed higher antioxidant capacities than commercial antioxidants, suggesting their potential as alternatives for these compounds. The results show great potential of organosolv process for the selective production of lignins with specific functionalities and processability, very useful for further technological applications.

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FINAL CONSIDERATIONS

This work demonstrated that microwave-assisted organosolv delignification (MWAOD) is a promising technology for obtainment of high quality lignin with elevated yields in a matter of minutes under mild conditions (atmospheric pressure) from an agroindustrial waste. MWAOD was also capable of yield lignins with similar monolignol composition that those obtained from traditional heating (TH), although the former had lower residual carbohydrates content. Furthermore, the combination of type of acid catalyst (Brønsted or Lewis) and its concentration and reaction time have shown to exert a great influence on lignin yield and purity, as well as on the structural features.

Therefore, the higher the acidic power of the catalyst and its concentration the higher the lignin yield and purity, suggesting a better fragmentation of lignocellulosic biomass into its components. In addition, the use of $AlCl_3$ as catalyst allowed the obtainment of a lignin with similar structural features than those obtained using H_2SO_4 and HCl, suggesting that the former could be considered as an alternative catalyst for lignin extraction.

The use of different organic solvents (acetic or formic acid) in the MWAOD also allowed the generation of coconut shell lignins with different structural features. Changing the solvent employed in the process but keeping its severity (mild conditions) allowed to yield partial acetylated lignins in an *one-pot* process (acetosolv), while this chemical modification was not observed in the formosolv process, yielding lignins with higher amount of hydroxyls.

These different functionalities had significant influence on certain structural features of coconut shell lignins, such as their processabilities through the decrease of the glass transition temperatures (T_g) of ACSLs approximately 20 %. This feature can be very useful for applications in polymer chemistry. In addition, the different amount of hydroxyl groups of ACSLs and FCSLs also influenced on their antioxidant capacities, in which FCSLs presented lower IC_{50} values than the ACSLs. However, both organosolv coconut shell lignins had ten times lower IC_{50} values than two commercial antioxidants (BHT and Irganox 1010), showing their potential to replace the petroleum-based antioxidants in different technological segments.

Finally, it can be concluded that MWAOD can be considered a promising technology for extraction of high quality lignins from agroindustrial wastes with different functionalities and structural features that allow their utilization in several technological applications, being their performance directly linked to their structures.

PERSPECTIVES

In order to explore deeper the potential of the organosolv coconut shell lignins as natural antioxidants and confirm them as alternative to replace the petroleum-based antioxidants, such as BHT and Irganox 1010, the evaluation of the cytotoxicity of those lignins are required, also comparing them with that of BHT and Irganox 1010. In addition, it is also interesting to verify if the different functionalities present in the ACSLs and FCSLs exert any influence on their cytotoxicity, which could be very useful to select the most suitable type of application to each one. Finally, since the Kraft lignin is the one that controls the Brazilian lignin market at least in the current moment, it would be interesting to perform the antioxidant and cytotoxicity assays for it, in order to compare its performance with that of the organosolv coconut shell lignins. This would be very useful to justify the use of a sulfur-free extraction process for obtainment of a high quality lignin, also helping the establishment of this process as an alternative and feasible process to the pulp and paper industry, as manner to consolidate it in this chemical industry for lignin extraction for specific purposes.

PUBLICATIONS

1st paper related to PhD thesis

Journal of Cleaner Production 189 (2018) 785–796

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Microwave-assisted organosolv extraction of coconut shell lignin by Brønsted and Lewis acids catalysts

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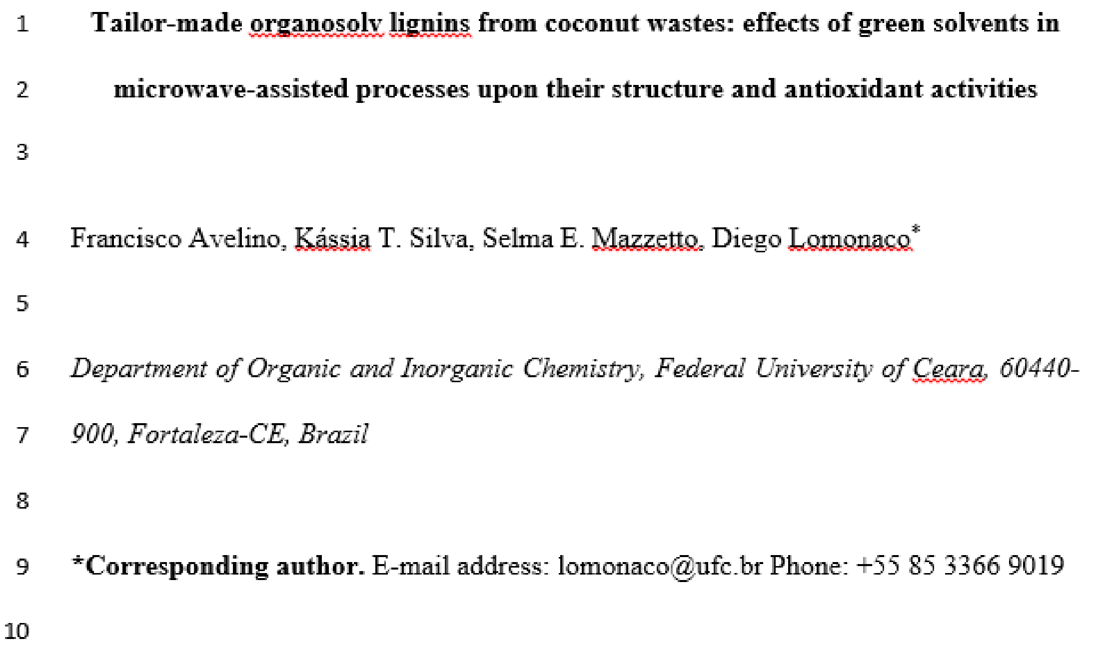
2nd paper related to PhD thesis (Submitted to Bioresource Technology Reports)



Tailor-made organosolv lignins from coconut wastes: effects of green solvents in microwave-assisted processes upon their structure and antioxidant activities

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Papers related to extra projects

International Journal of Biological Macromolecules 122 (2019) 1163–1172

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International Journal of Biological Macromolecules

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Organic solvent fractionation of acetosolv palm oil lignin: The role of its structure on the antioxidant activity

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Poly(methyl methacrylate) films reinforced with coconut shell lignin fractions to enhance their UV-blocking, antioxidant and thermo-mechanical properties

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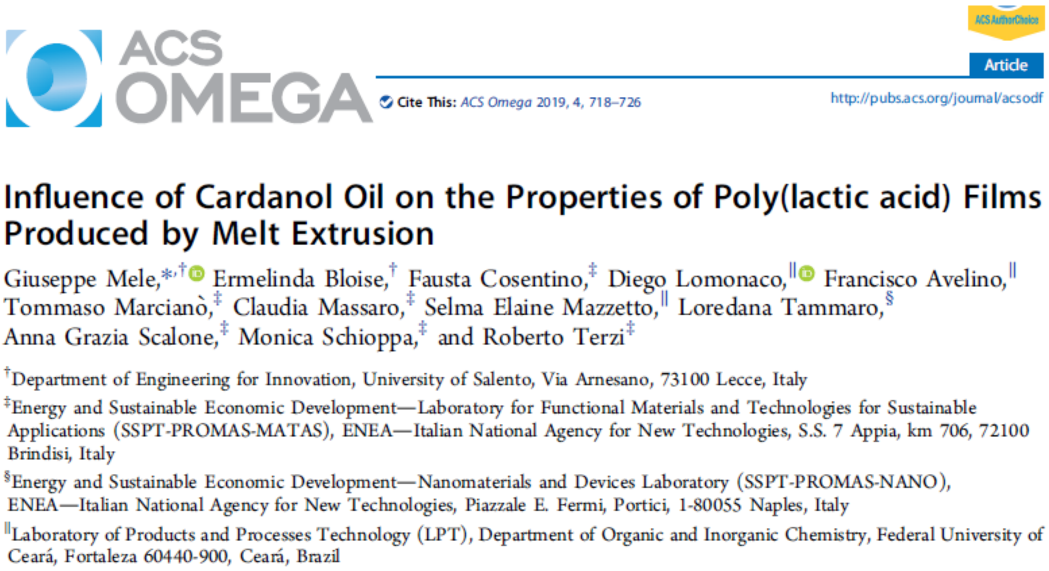

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Influence of Cardanol Oil on the Properties of Poly(lactic acid) Films Produced by Melt Extrusion

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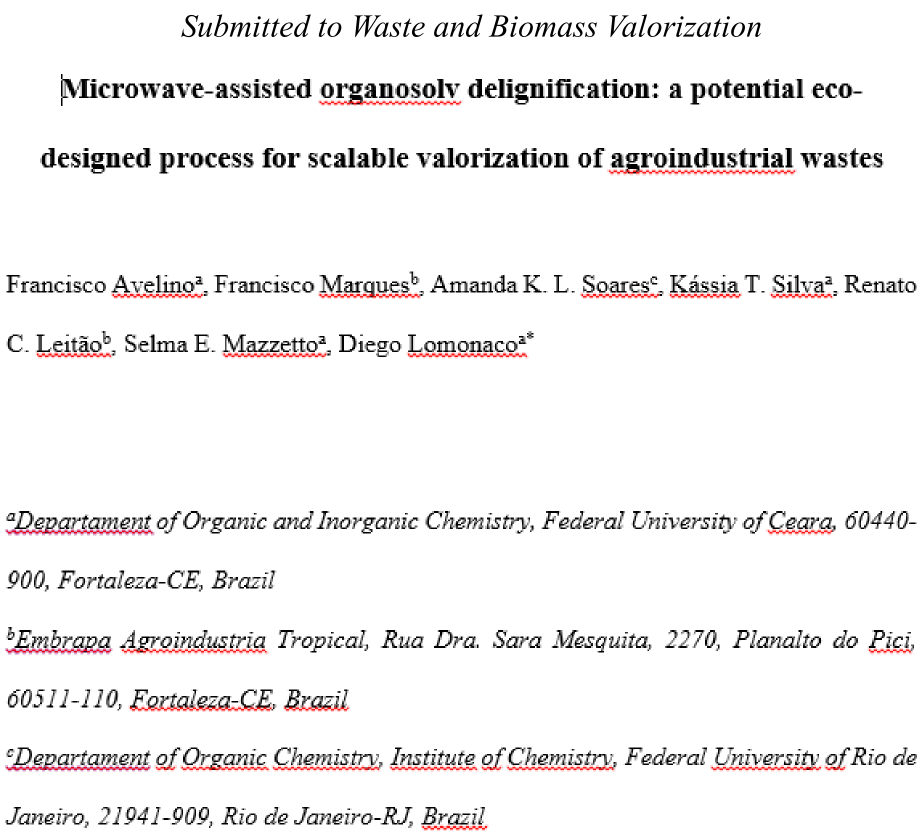

Submitted to Waste and Biomass Valorization

Microwave-assisted organosolv delignification: a potential eco-designed process for scalable valorization of agroindustrial wastes

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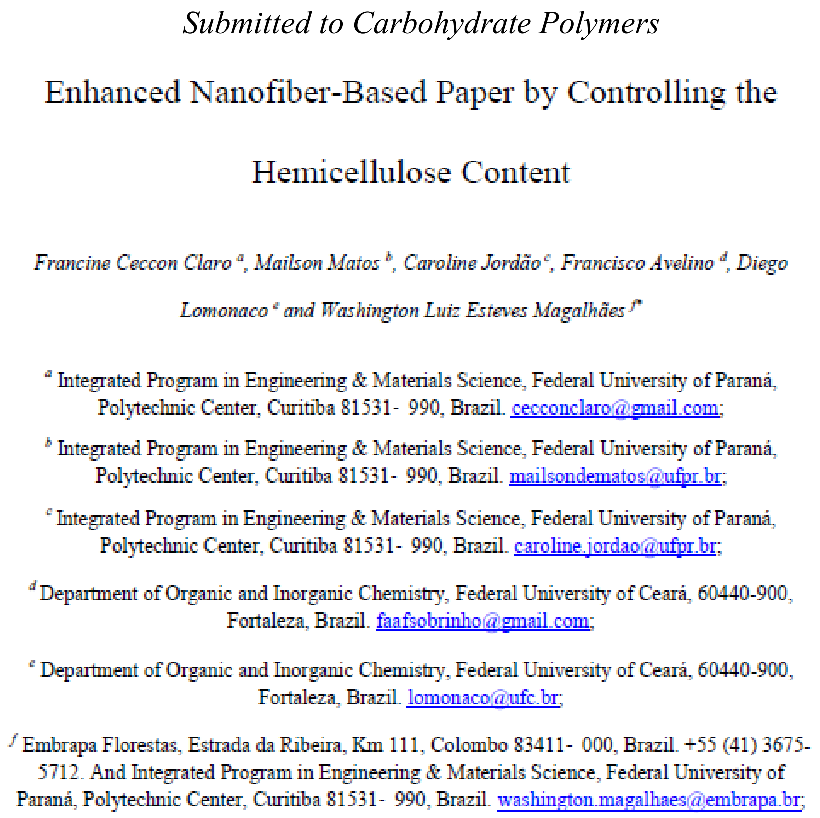

Submitted to Carbohydrate Polymers

Enhanced Nanofiber-Based Paper by Controlling the Hemicellulose Content

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