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NEW PRODUCTS FROM LIGNIN

Doctoral thesis presented by

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I. Preface

The present doctoral thesis was conducted in the Chemical Engineering department at the University of the Basque Country (Donostia-San Sebastian, Spain) during 2013-2017. Significant contribution was also accomplished at The Department of Fibre and Polymer Technology in The Royal Institute of Technology (Sweden) during a stay of three months in 2014 and one and half months in 2016.

Firstly, I would like to thank my supervisor, Dr. Jalel Labidi, for giving me the huge opportunity to be part of his prestigious research group to carry out my doctoral studies. In addition, I would also like to thank to Dr. Rodrigo Llano-Ponte, with which I took my first steps in the research world and who encouraged me to follow this path. Thank you very much!

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Moreover, I would like to express my gratitude to University of the Basque Country for providing me a financial support, which allowed me to work full time on the thesis during this period.

I must thank the working group of Biorefinery Process, to who started being my colleagues and ended up being good friends. Thanks to Itziar for helping me, especially in the beginning of this period. Thanks also to all those who have contributed in some way on my work during this period. I can´t forgot the colleagues of other international institutions from Brazil, Tunisia and Lithuania that has visited us, thank you for giving the opportunity to collaborate together.

Finally, thanks to my family, boyfriend and friends. Without you, nothing would have been the same.

II. Summary

Lignin is the only high-molecular-weight natural polymer with an aromatic structure present in plant cell wall of lignocellulosic biomass as structural component. As its chemical structure is highly dependent on the different production streams as well as the source from which it comes, a great variability of lignin types can be found. The high degree of chemical variability makes difficult the development of new and valuable uses for lignins, but its large availability, low price and properties that lignin offer, make that valorization strategies are urgently needed. Therefore, it is required a deeper knowledge of lignin in order to overcome its drawbacks and exploit its advantages.

In this context, this work has been structures in six chapters, described briefly in the following paragraphs.

The dissertation begins by introducing a theoretical background about lignin, one of the main structural component of the lignocellulosic materials. General chemical structure of lignin, the main industrial lignin sources and the most common properties of lignin polymer are reported. Moreover, the current valorization routes of lignin are summarized. Short outline about the synthesis of poly(lactic acid), its advantages and limitations, the main actual applications and main factors that influence on its degradation are presented.

The second chapter assess the influence of lignin from different sources as poly(lactic acid) additive on thermal and mechanical properties. Moreover, chemical modification by esterification using acetylation treatment is carried out in order to study if there is some improvement respect to unmodified lignin when is added to poly(lactic acid).

Afterwards, in the third chapter, chemical composition and structure of isolated lignins from hardwood and softwood by organosolv and kraft processes

are studied. The main objective of this part is the evaluation of isolated lignins for innovative applications in polymeric, pharmaceutical and cosmetic industries.

The fourth and fifth chapters are highly connected. Firstly, chemical modification by esterification of isolated organosolv lignins is investigated in order to increase their range of applications. In addition, in the fifth chapter the development of novel applications for synthesized lignin-esters are studied.

Finally, the conclusions of the work are summarized in the last chapter together with the suggestions for future works and publications during this period.

The experimental techniques and methods used are described in the in the appendix I and appendix II.

III. Research objectives and methodology

The main goal of this thesis is to contribute to the development of novel applications for lignin to convert it into a valuable product. Within the scope of the present thesis, two routes were explored for the valorization of lignin: the evaluation of potential properties and uses of unmodified lignin and the study of the chemical modification of lignin to be used in several applications. The general objectives were:

- To extract lignin from different sources.
- To analyze chemical and structural characteristics of different lignin samples.
- To evaluate thermal and mechanical properties of PLA containing lignin as an additive.
- To study the chemical modification of lignin in order to increase the range of applications.
- To determine functional properties of different lignins: antioxidant and antimicrobial capacity, potential as an energy source and the UV protection properties.
- To develop innovative applications for chemically modified lignins.

In order to achieve the proposed objectives, the following methodology was applied:

In the second chapter, environmental friendly material was elaborated by extrusion process. For that, poly(lactic acid) was used as matrix and three types lignins were used as an additive in different amounts. In order to obtain homogenous blends, lignin samples were acetylated before mixing with PLA by extrusion technique. The chemical composition, structure, molecular weight, and thermal properties of lignin samples were analyzed using FTIR, GPC, DSC and TGA, respectively. To verify the acetylation of lignins, several techniques and procedures were used, such as FTIR, GPC, DSC and TGA as well as the quantification of phenolic content before and after acetylation treatment. Regarding the extruded blends, visual appearance and morphology at microscopic scale was analyzed. AFM was also used to analyze the size of the acetylated lignin particles inside PLA. Finally, thermal behavior of blends were evaluated using TGA technique.

The third chapter was focused on the study of the most relevant structural and thermal differences between industrially available kraft lignins and lab-scale extracted organosolv lignins, both types from Eucalyptus and Spruce wood. In this chapter, chemical composition, chemical structure and thermal properties of four lignin samples were evaluated using Py-GC/MS, elemental analysis, FTIR, ^{31}P NMR, ^{13}C NMR, GPC, DSC and TGA. Moreover, some functional properties of lignins were assessed in order to provide new perspectives for innovative applications in polymeric, pharmaceutical and cosmetic industries. Antioxidant capacity of lignins was determined by DPPH and ABTS methods. In addition, antifungal activity against *Aspergillus Niger* as well as antibacterial activity against different food and human pathogenic microorganisms were analyzed. Furthermore, evaluation of lignins as an energy source was carried out by TGA. Finally, the potential of lignins as a natural additive for sunscreen was evaluated using an *in vitro* SPF method and analyzing the UV radiation transmittance of elaborated creams where lignin was incorporated in different amounts.

In the fourth chapter, the chemical modification of previously extracted organosolv lignins was described. For the confirmation of the chemical modification, chemical structure by FTIR, molecular properties by GPC, and thermal properties by DSC and TGA were studied. Moreover, the quantification of phenolic content before and after chemical modification, new solubility properties of the synthesized lignin-esters and the hydrophobicity were determined.

The fifth chapter was focused on the development of novel applications for previously synthesized lignin-esters. The first part of the chapter deal with the use of lignin-esters as an additive in PLA films. After elaboration of films by solvent casting, the appearance and optical properties were studied. Moreover, UV-protection ability of lignin-esters was analyzed by the use of the UV spectrophotometer. In addition, thermal stability of films was evaluate by TGA and finally the influence on lignin-esters addition on mechanical properties of PLA films was determined. The potential of lignin-esters as antifungal product was also assessed. The second part of the chapter was aimed to use lignin-ester as hydrophobic agent for wood products. Two types of treatments were applied to wood veneers. The first one was application of lignin-esters using press molding, while the second one was impregnation of modified lignins by immersion of the wood samples. Physical properties such as moisture, roughness and optical properties were analyzed. Moreover, surface properties using a dynamic contact angle before and after accelerated weathering were evaluated.

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Introduction

1.1. Lignocellulosic biomass

Nowadays, non-renewable sources as fossils fuels are the origin of the most common product that the society used today (fuels, asphalt, chemicals, plastics, paintings and varnishes, lubricants, fertilizers, detergent…). However, with constant changes in the price of oil and the future exhaustion of fossil fuels sources, in addition to their frightening environmental effects, such as global warming, our society has inevitably started to demand alternative sources of energy and chemicals[1,2]. Moreover, the European Union has already approved laws for the reduction of environmentally abusive materials and commenced to make greater efforts in finding sustainable and green products based on natural resources.

In this context, lignocellulosic biomass, the most abundant and bio-renewable biomass on Earth, represents the largest energy and organic matter source of the biosphere[3]. Many studies have proved its high potential for the sustainable conversion into energy, biofuel, value-added chemicals and new materials[4,5]. Furthermore, the biodegradability of their components and its non-human food application make it interesting feedstock.

Lignocellulosic materials include agricultural wastes, forestry residues, grasses and woody materials and are chemically composed of two types of components; structural and non-structural[5]. Non-structural components like water, inorganic material, extractives, and proteins, represent around 5-10% of the final composition but are not part of the cell wall. Three structural polymers form the cell wall of the lignocellulosic biomass: cellulose, hemicellulose, and lignin. Figure 1.1 shows a schematic representation of the chemical composition of wood compounds.

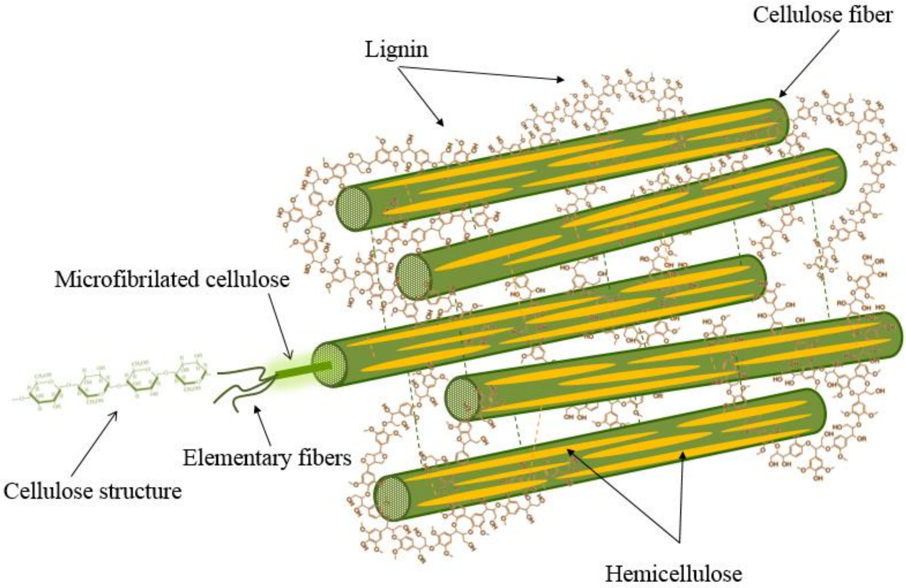


Figure 1.1. Representation of the structural compounds of plant cell wall.

The chemical composition of the lignocellulosic material is principally related to the botanical source, geographical location of the plant, growth conditions, plant tissue and age. Table 1.1 summarizes the types of lignocellulosic biomass and their general chemical composition.

Table 1.1. Types of lignocellulosic biomass and their chemical composition[4–6].

	Cellulose (%)	**Hemicellulose (%)**	**Lignin (%)**
Hardwood	40-55	15-35	15-25
Softwood	40-50	10-30	25-35
Agricultural wastes	25-40	15-35	5-20
Grasses	25-40	25-50	10-30

As can be observed, cellulose is the main components of lignocellulosic materials and is the main responsible of the plant mechanical strength. Approximately 40-55% of the dry substance in most wood species is cellulose. It is a linear polysaccharide based on D-glucose units linked by β-(1-4) glycosidic bonds resulting in a partially crystalline polymeric chain. Cellulose in lignocellulosic biomass is usually organized into microfibrils, each of which is formed by elementary fibrils. Figure 1.2 shows a schematic view of the cellulose structure. The intramolecular hydrogen bonds are responsible for the rigidity of cellulosic chain, while intermolecular hydrogen bonds originate its supramolecular structure forming cellulose microfibrils. The cellulose fibers are locked into the wood in a matrix composed of lignin and hemicellulose.

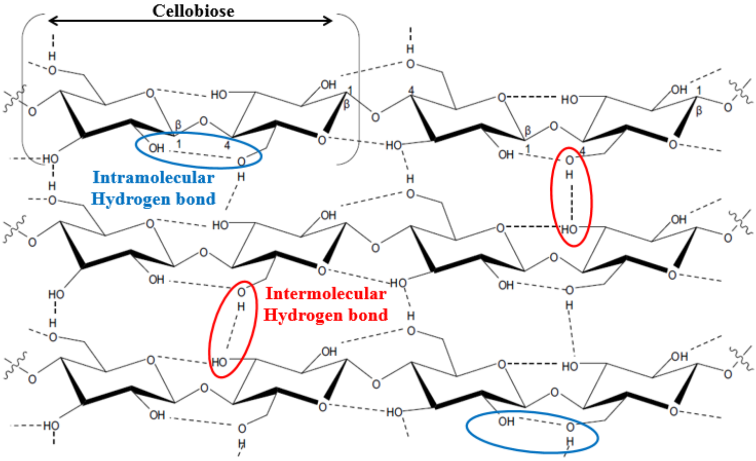
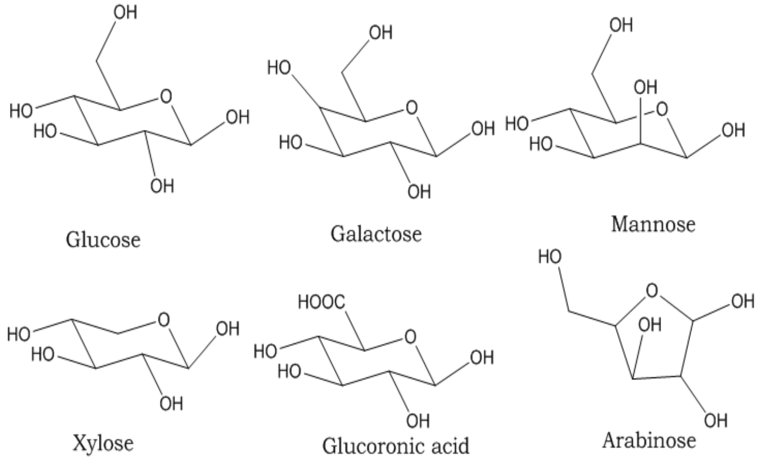


Figure 1.2. Representation of the cellulose structure.

Hemicelluloses, on the other hand, are a group of polysaccharides constituting the second most abundant polysaccharide in nature. The hemicelluloses have branched and amorphous structure, which is composed of different 5 and 6 carbon monosaccharide units: pentoses (xylose, arabinose), hexoses (mannose, glucose, galactose) and acetylated sugars (Figure 1.3). In contrast to cellulose, hemicellulose differs in composition depending on the lignocellulosic source; hardwood hemicellulose contains mostly xylans, whereas softwood hemicellulose contains mostly glucomannans. Moreover, they have a much lower degree of polymerization compared to that of cellulose. The amount of hemicellulose of the dry weight of wood is usually between 20 and 30%.


Glucose

Galactose

Mannose

Xylose

Glucoronic acid

Arabinose

Figure 1.3. The main monomeric sugars of hemicellulose polymer.

In addition to hemicelluloses, lignin accounts for 15-30 wt% of woody biomass and is also available in smaller amount agricultural residues such as straw, grass and bagasse[7]. It is the most complex aromatic polymer with a three-dimensional structure.

The main physical and biological functions of lignin are conferring structural support to the plant, glue different cells together in woody tissues, participate in the internal transport of water and nutrients[2] and provide protection against chemical and biological attack preventing the penetration of destructive enzymes and microorganisms through the cell wall and insects attack[8–10]. Moreover, lignin acts in the plants as antioxidant, stabilizer to UV radiation action and hydrophobic agent[11].

The following section will describe in depth the chemical structure and general properties of lignin polymer will be mentioned.

1.2. Lignin

1.2.1. Lignin structure and chemistry

Lignin belongs to a class of high-molecular-weight natural compounds and is present in almost all plants being the most abundant aromatic resource on the Earth. Commonly, the investigation of lignin structure has mainly been centered on the knowledge of the chemical composition of different lignin types from various non-wood/wood species, determination of structural units, linkages between units and the determination of specific functional groups.

Lignin can be described as complex three-dimensional and amorphous molecule derived from the polymerization of three phenylpropane units that come from three aromatic alcohols: *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol. The phenolic structural elements that originate from these monolignols are called *p*-hydroxyphenyl (H, from coumaryl alcohol), guaiacyl (G, from coniferyl alcohol) and syringyl (S, from sinapyl alcohol)[8,12].

Figure 1.4 shows chemical structure of the three primary elements of lignin molecule and schematic representation of the general chemical composition of different types of lignocellulosic biomass. As can be observed, the difference between the three lignin precursors is the number of methoxyl groups attached to the aromatic ring[10].

Regarding the chemical composition and structure of lignin polymer, there is no one unique, well-defined lignin molecule. The structure of lignin, and hence its properties, is dependent on its origin, external conditions during growth (season, climate) and the fractionation technology applied to isolate it[13,14].

Moreover, different amount of lignin can be found rely on the source. Softwoods usually have more lignin content than hardwoods, with values of 25-35 wt% and 15-25% respectively[15,16]. However, grasses typically contain up to

10% of lignin and annual plants present generally lower lignin content like flax (around 3 wt%)[17].

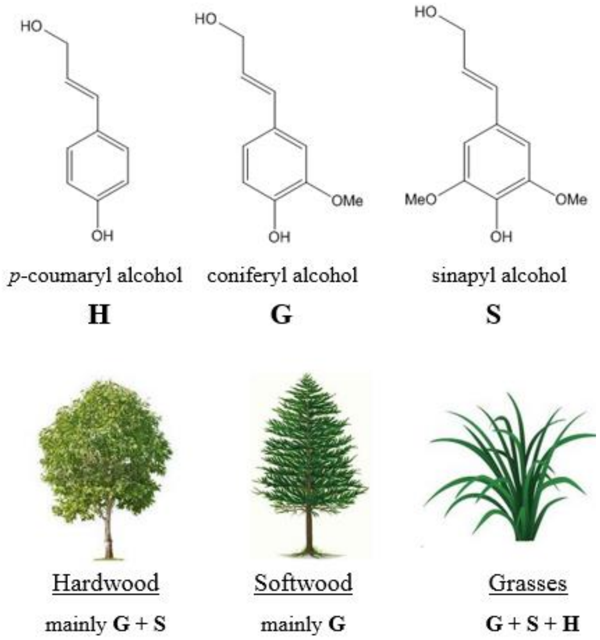
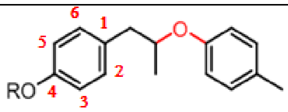
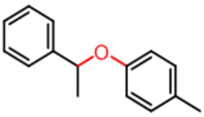
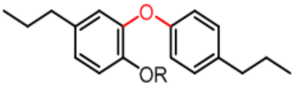
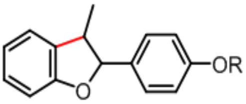
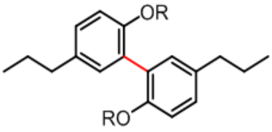
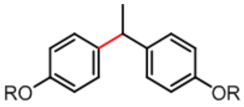
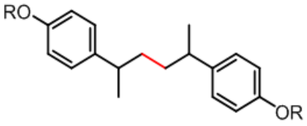


Figure 1.4. The molecular structure of the three monolignol building blocks of lignin and schematic representation of the chemical composition of lignocellulosic biomass.

As regards to the source, softwood lignins are composed mainly by G-units. However, hardwood lignins have more complicated structure because it comes from the polymerization of coniferyl and sinapyl alcohols with proportions from approximately equal amounts to three times higher levels of sinapyl alcohol[16]. The S/G ratio of hardwood lignins is usually interesting to determine for further applications. Although small amounts of *p*-hydroxyphenyl units (H) are found in both softwood and hardwood lignins, substantially more are found in non-woody species such as grasses, in which lignin molecule is a copolymer of all three lignin precursors being always *p*-coumaryl alcohol which is presented in a higher content[18,19].

These structural elements are linked to each other during biosynthesis through C-C (condensed bonds) and C-O-C (ether) bonds to form the polymer. The most common linkage in the lignin molecule is β-O-4 ether linkage (45-60%), followed by other types of ether and C-C linkages such as α-O-4, β-5, 5-5´, 4-O-5, β-1, and β-β´[20,21]. The most common bonds are depicted in Table 1.2 together with their approximate proportions in softwoods and hardwoods. The carbons in the aromatic ring are numbered from 1 to 6 starting with the carbon attached to the propyl chain. The aliphatic carbons of the propyl group are named with α, β and γ starting from the carbon next to the aromatic ring. A β-O-4´ bond is thus an ether linkage between a para-position in the aromatic ring and the central carbon in a propyl group, and a 5-5´ linkage represents a covalent linkage directly between the 5-carbons in two aromatic rings.

Table 1.2. Most common linkages connecting the phenylpropane units of lignin.

Linkage-type		**Softwood Lignin (%)**	**Hardwood Lignin (%)**
β-O-4		45-50	60
α-O-4		2-8	7
4-O-5		4-7	6-9
β-5		9-12	3-11
5-5´		20-25	3-9
β-1		7-10	1-7
β-β´		2-6	3-12

Lignin structure molecule contains a variety of functional groups which really affect the reactivity[10,21–23]. According to the current understanding of lignin, the most common functional groups are aromatic methoxyl groups, aliphatic and phenolic hydroxyl groups and small amounts of carbonyl groups and carboxyl groups[24]. The proportion of these groups depends on the origin of the lignin and its separation process.

Only a few phenolic hydroxyls (10-13 %) are free; most of the groups are occupied in inter-unit linkages[16]. The abundance and frequency of those functional groups have a great importance on the lignin physicochemical behavior. Moreover, almost all lignins have linkages to carbohydrates fragments through arabinose, xylose and galactose units, which are usually unavoidable in isolated lignins because of the presence of lignin-carbohydrate complex (LCC)[11].

Despite intensive studies and a great number of analytical data today available about the composition of lignin and the content of functional groups, this information cannot be summarized in a simple way because of large individual variations among the wood species. Nevertheless, several structures have been proposed as shown Figure 1.5 and 1.6.

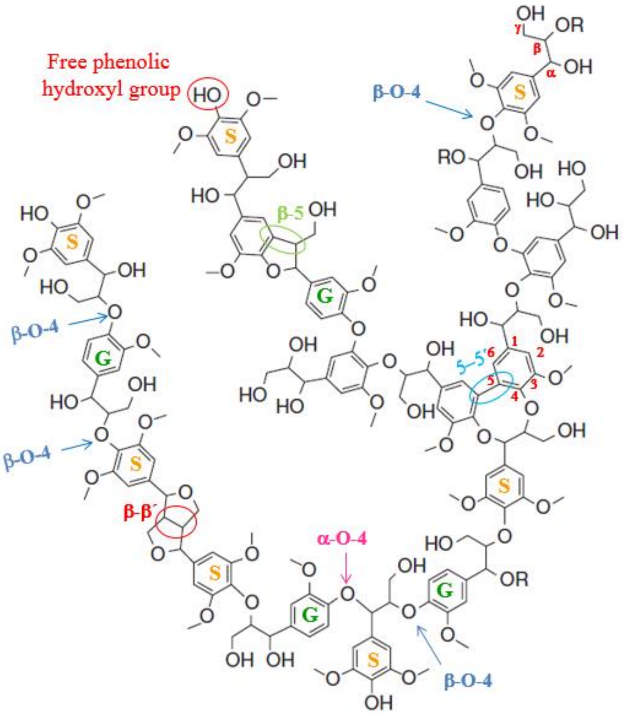


Figure 1.5. Hardwood lignin adapted from Macfarlane et al, 2014[10].

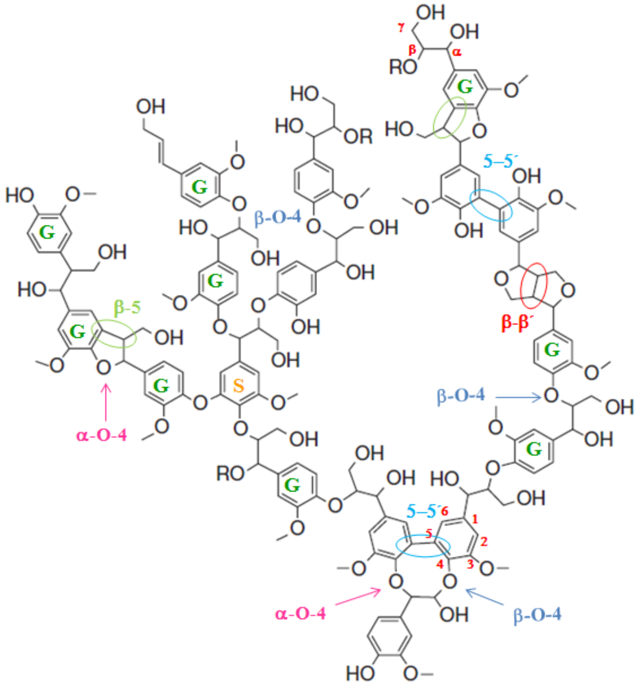


Figure 1.6. Softwood lignin adapted from Macfarlane et al, 2014[10].

The diversity of plant sources and processing methods results in a broad range of lignins. Consequently, the characteristics of each type of lignin are different and hence their physicochemical properties.

1.2.2. Lignin general properties

Chemical and structural characteristics of lignin polymer, such as molecular weight, composition and functional groups have a strong influence on the final properties of lignin and consequently determine its potential applications and the suitability for the manufacture of lignin-based products.

Lignin is an amorphous polymer and is presented as a brown thin powder in the solid state. The degree of polymerization in nature is difficult to estimate since lignin is consistently fragmented during the extraction process. However, higher molecular weight is usually found for softwood lignins than hardwood lignins[15]. Moreover, high polydispersity is also a usual characteristic, especially when compared to other biopolymers like cellulose.

It is a rigid and brittle polymer and presents poor film-forming ability. These characteristics are caused by its natural condensed structure and strong intermolecular hydrogen bonding interactions, which restrict the thermal mobility of lignin structure. Therefore, lignin polymer habitually presents high glass transition temperature (Tg)[25]. The glass transition temperature (Tg) is a characteristic thermal transition of amorphous polymers, and it is defined as temperature region where polymer chains are able to start moving.

Although, many researchers have established that Tg of lignin usually is found in the range of 90-180 °C[26–28], it is often difficult to determine due to its complex and heterogeneous structure and the broad molecular weight distributions caused mainly by isolation procedure. Generally, Tg depends on the molecular weight of the polymer and it shifts to higher temperatures with increasing average molar mass[12,21,29].

Other structural characteristics, such as degree of condensation (number of C-C linkages), as well as the content of both phenolic hydroxyl and methoxyl groups (syringyl content), have also a strong influence on the Tg value[11,30].

Regarding the isolation procedure, kraft lignins usually have large amounts of condensed structures that were formed in the final stage of the pulping process[8,12,21,24]. Moreover, as far as the source is concerned, the quantity of condensed structures normally is higher in softwood lignin than in hardwood lignin due to its composition, mainly made up of guaiacyl units (G).

As was previously mentioned, guaiacyl units (G) present a vacant in C_5 of the aromatic ring. This free position in the aromatic ring allows the formation of 5-5´and β-5 linkages that are related to the condensation degree of lignin structure[31]. Also, the amount of sorbed water and the presence of polysaccharides may affect the value of this thermal transition[21].

Another important property of lignin is its high thermal degradation temperature. The first decomposition step is associated with dehydration from the hydroxyl group located in the benzyl group and usually starts 150-200 ºC. Then, around 300 ºC, aliphatic side chains start splitting off from the aromatic ring while carbon-carbon cleavage between lignin units take place at 370-400 ºC. Finally, the breaking of the backbone occurs at higher temperatures (500-700ºC)[29] leading around 30-50% of char.

In addition, as a polyphenolic compound with diverse functional groups, lignin shows interesting functional properties, such as antioxidant and antimicrobial properties[32]. The free radical scavenging ability of phenolic groups gives lignin an excellent antioxidant property[33]. However, the antimicrobial properties are widely influenced by the concentration of lignin in cultivation medium and the type of microorganisms[30]. In addition to these properties, lignin has many other properties such as a dispersant and blending properties and biodegradability.

1.3. Sources of lignin

Lignin is usually classified according to the isolation procedure. Although the composition of lignin depends mainly on the botanical source, the fractionation process of biomass is also closely related to the final properties of lignin polymer, especially, chemical structure and purity[12,21].

Nowadays, the forestry industry produces huge quantities of lignin as a by-product of its pulping operations[6,8]. Kraft and Sulfite are the main processes employed in the pulp and paper industry whose main objective is to remove the lignin to obtain a cellulose pulp as pure as possible for the manufacture of paper and related products. Besides, for a long time, lignin has been considered as a waste from this industry and it has been used as fuel for the production of energy, having low economical usage[34]. Although today the high potential of lignin polymer for different applications is well known, very limited amount is actually isolated and commercially available.

Furthermore, sulfur-free lignins are an emerging class of lignin product. Having no sulfur, these lignins resemble more closely the structure of native lignin[35]. Sulfur-free lignins come from principally soda pulping and as a by-product of 2nd generation bioethanol production like organosolv process[6,7,36,37].

It is essential for researchers to understand how lignin processing changes the chemistry and properties of the material. The following section will summarize mentioned fractionation processing methods and the main characteristic of each type of available lignin.

1.3.1. Sulfite process

The origin of the sulfite process was found in 1866 when B. Tilghman patented a wood pulping method using aqueous solutions of calcium hydrogen sulfite and sulfur dioxide. However, around one decade later, C.D. Ekman was who started its production in Sweden, who is the principal initiator of the sulfite pulp industry[38]. Since then until the 1950s, when kraft process took over the pulp and paper industry, most of the pulp in the world was based on the sulfite process.

This method is based on a cooking with an aqueous sulfur dioxide (SO_2) and a base-calcium, sodium, magnesium or ammonium. During cooking, high amounts of sulfur are incorporated into the lignin structure. So, lignosulfonates contain a considerable amount of sulfur in the form of sulfonate groups (SO_3) present on the aliphatic side chains (Figure 1.7)[12]. Due to their sulfonate content, lignosulfonates are very soluble in water and are insoluble in organic solvents[34]. Usually, they present higher average molar mass than kraft lignin and high ash content and still contain a significant amount of carbohydrates[39]. Lignosulfonates cannot be precipitated through a change of pH, instead, they are isolated through complete evaporation of water[39].

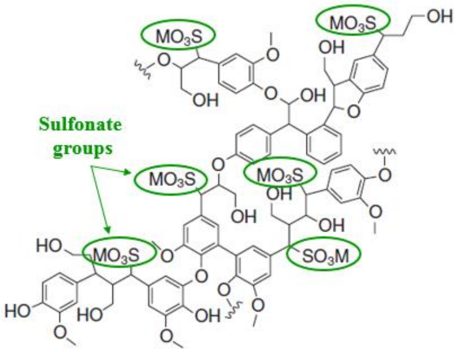


Figure 1.7. Spruce Lignosulfonate adapted from Macfarlane et al, 2014[10].

Lignosulfonates are commercially available from hardwood and softwoods and are the most exploited for several industrial applications. The largest current application of lignosulfonates is in the building sector as concrete additive[24,34] for providing plasticity and flowability to concrete with the less use of water. There are also widely used on a large scale as a binder in animal feed pellets[10].

Moreover, the high density of functional groups endows lignosulfonates with unique colloidal properties, accounting for their use as stabilizers, dispersing agents, emulsifiers, surfactants, and adhesives[39]. Many more specialty markets for lignosulfonates have been established such as its use in dust control, oil well drilling, road building, and pesticides used for agriculture applications and as a cleaning and decontaminating agent in water and soils[7,10,24].

1.3.2. Kraft process

The kraft-pulping process is actually the most traditional method employed in pulp and paper industry and hence produces the largest amount of lignin (85% of world production)[40].

Since the 1950s, the production of the kraft pulps increased much more than sulfite pulps because of a simpler and more economic recovery of chemicals and better pulp properties in relation to market needs[41]. Today, approximately 55 million tons of kraft lignin are generated from this process but only 75.000 tonnes is being isolated via the Lignoboost process for different uses.

This process consists of the treatment of wood chips with a mixture of sodium hydroxide (NaOH) and sodium sulfide (Na_2S) at elevated temperature (150-170 ^oC). During the process, the lignin molecule is fragmentized by attacking the ether bonds, decreasing the molecular weight and increasing the number of phenolic hydroxyl groups, which are susceptible to ionization in alkaline conditions by extraction of the phenolic proton. Thus, small water/alkali-soluble fragments of lignin are solubilized resulting in so-called “black liquor”[8]. The black liquor is concentrated by evaporation and is used as fuel in the recovery boilers[39].

At presents, in order to enhance the productivity, the extraction come part of the kraft lignin. The extraction is already being performed in Sweden on an industrial scale; it has been developed under the name "LignoBoost" process[39].

Lignin extracted from the pulping liquor in this process has so far only been used as an external fuel to increase the production capacity, but other applications of kraft lignins are possible. The most frequent method of extracting lignin from black liquor is by precipitation. The liquor is commonly acidified by mineral acids like sulfuric acid. Upon lowering the pH, a substantial portion of lignin is precipitated and may be recovered by filtering and washing.

As a result, kraft lignins usually contain between 1-2% of sulfur, some of it organically bound as aliphatic thiol groups (R-SH) and some as elemental sulfur and thus constitute important impurities that can have consequences for further valorization (Figure 1.8)[39,42]. It is the main cause of its particular odor. Moreover, this type of lignin contains high condensed structures due to carbon-carbon bonds during the last stage of the pulping[22].

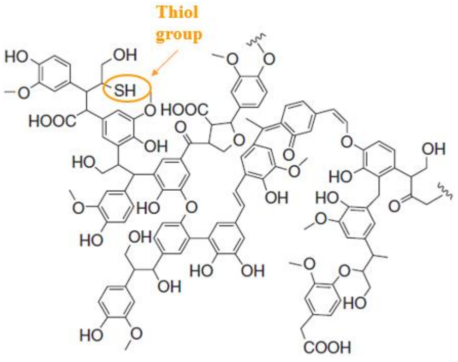


Figure 1.8. Kraft Pine lignin adapted from adapted from Macfarlane et al, 2014[10].

Although kraft pulping of wood is potentially the most important source of industrial lignins, it does not offer large amounts of lignins to the market, since the use of lignin as a by-product is highly integrated into the process optimizing the cycle of chemical energy. MeadWestvaco is the only company extracting kraft lignin at the moment. It is estimated that 70-75% of MeadWestvaco's total recovered kraft lignin is sulfonated in order to be sold for the applications typical of lignosulfonates, while the rest is sold unmodified[39]. Unmodified kraft lignin has a few applications, principally as an antioxidant, UV adsorbent and raw material for the production of low molecular weight chemicals.

1.3.3. Alkaline process

The alkaline process was the first chemical pulping method and was patented in 1845. However, nowadays is mainly employed for annual plants and agricultural residues because it is most effective with low lignin content biomass[7,42].

In this process, biomass reacts with sodium hydroxide under high pressure and high temperature[8]. The lignin recovered from this process is usually known as “soda lignin” and it has good potential for use in high-value product because of the absence of sulfur in its structure[21,22]. Moreover, since available industrial soda lignin is mostly obtained from non-woody plants, in addition to guaiacyl and syringyl moieties non-woods have significant amounts of *p*-hydroxyl groups.

Because of the absence of sulfur in its structure and composition, soda lignin has potential applications that are not available to kraft and sulfite lignins. Products from such lignins have been proposed for animal health and nutrition. Moreover, soda lignin is currently used as partial replacement of phenol in the manufacture of polyphenol (PF) resins[34].

1.3.4. Organosolv process

Organosolv pulping is considered more efficient and environmentally acceptable wood-conversion technology compared to kraft or sulfite, which cause serious air and water pollution. The ethanol organosolv process is among the pretreatment strategies currently being most intensively studied for the conversion of lignocellulosic biomass feedstock to biofuels[39].

The best known organosolv process is the Alcell® process based on ethanol/water pulping and it was developed in the early 1970s. Later, it was modified by Lignol Company in Canada and nowadays, this company is one of the most promising platforms in the conversion of waste wood materials, such as hardwoods, softwoods and agricultural into biofuels and biochemicals[7,12].

The cleavage of β-ether linkages seems to be the most important reaction in the lignin breakdown during organosolv pulping. The easier organosolv delignification of hardwoods compared to softwoods is primarily a result of differences in β-ether abundance. Moreover, when the organosolv processes are conducted at high temperatures (185-210 °C), there is no need for an acid addition, however, the delignification rate is substantially increased when acid catalysts are added, especially for softwoods[39].

The use of organosolv process promises several advantages. Firstly, lignin is isolated from lignocellulosic biomass using organic solvents, mainly, solvents with low boiling points, which can be easily recovered by distillation, leading to less water pollution[10]. Generally, the most usual solvents are methanol and ethanol (usually mixed with water), and organic acids such as formic and acetic acid[42]. Secondly, the absence of sulfur during pulping prevents the release of organic sulfur compounds and dust particles, contributing to lower emissions. Finally, in contrast to lignin produced by other technical processes, organosolv lignin is a sulfur-free product of high purity suitable for the development of a variety of products[22]. In addition, it has high solubility in organic solvents and is practically insoluble in water.

Although it is still under exploration, high-quality organosolv lignins could be used for the development of various polymeric materials such as phenolic powder resins, polyurethane foams and epoxy resins. Moreover, this type of lignin has attractive potential to be incorporated as filler or additive in new composite materials, especially with respect to the properties modification of biodegradable polymers. In this field, lignin can be used without modification or by preparing of their derivatives by chemicals modification of their structure. Other organosolv lignin applications include antioxidants, a precursor for chemicals such as vanillin, as well as a multitude of phenol-derivatives and its suitability for carbon fiber production[39].

1.4. Lignin valorization routes

The real difficulty in developing new and valuable uses for lignins in the future resides in their high degree of variability due to the different production streams as well as the utilization of different lignocellulosic materials. The chemical structure (functional groups, molecular weight…) is a cornerstone of many applications[43]. Nevertheless, a feedstock of such abundance clearly can not be ignored and sound valorization strategies are urgently needed. However, to become applicable on an industrial scale, lignins should have structure uniformity; in other words, have a defined quality as well as an acceptable price.

At present, lignin is already used for some industrial applications, but they are primarily low value. The vast majority of lignin is still combusted for the production of energy in order to use it in pulp and paper process. Moreover, there is also a large-medium market in which technical lignins are directly exploited as additives for concrete admixtures, dust control, feed and food additives, dispersants or binders.

However, recently, lignin has revealed itself as a high potential for numerous applications and therefore the development of conversion technologies could provide greater added value to lignin. Currently, for the lignin valorization, the study is centered on three main routes; fragmentation technologies, development of new strategies to incorporate it in the polymeric industry and use it as an energy source.

1.4.1. Lignin fragmentation processes

Figure 1.9 summarizes the current fragmentation processes which are being studied with the aim to reach an effective valorization of lignin into useful compounds like chemicals and biofuels. The main fragmentation technologies can be divided into two different groups such as thermochemical processes and biological processes. The efficiency of degradation and the products from these treatments would yield at least 10 times more value as compared to burning it for energy production[44]. Below, the most important fractionation processes are briefly described.

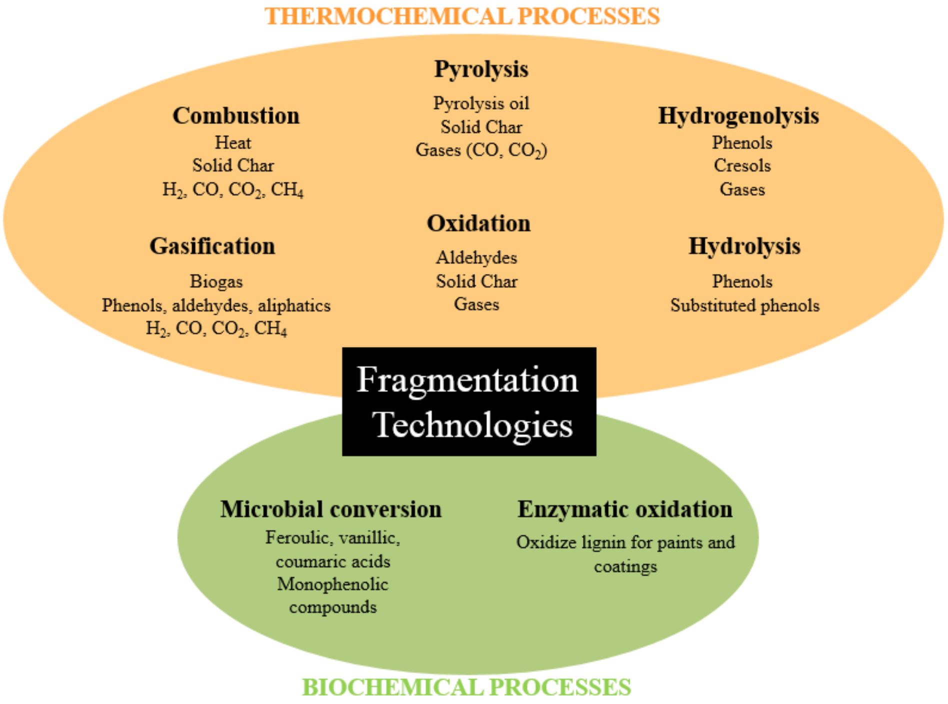


Figure 1.9. Thermochemical and biochemical methods of lignin depolymerization.

Pyrolysis is the most studied method for lignin depolymerization. It is a complex process that involves thermal treatment of lignin in the absence of oxygen at temperatures between 300-600 ºC. This degradation generates liquids (pyrolysis oil), monophenols (phenol, guaiacol, syringol and catechol), solid char and gaseous fractions (CO, CO_2, CH_4, etc.), in various proportions, depending on reactions parameters[12,45].

However, oxidation process of lignin involves heating the lignin in the presence of oxidants. Usually used oxidants are nitrobenzene, metallic oxides, air, and oxygen. These protect aromatic rings and form various aromatic compounds including aldehydes (vanillin and syringic) and acids (vanillic and syringic acids). The yield of products, in this case, aldehydes and acids, depends on the oxidant.

Lignin oxidation can provide several polyfunctional monomeric compounds and fine chemical products for chemical industry[46].

Gasification converts lignin into a gas called syngas consisting of CO_2, CO, CH_4, and H_2. It uses much higher temperatures than pyrolysis, between 700 °C and 1000 °C. Combustion occurs in the presence of oxygen at extremely high temperatures (around 800 °C to 1000 °C) and can be used to produce heat, electricity, gases and solid char.

Hydrogenolysis is probably the most promising chemical method for producing phenols from lignin. Compared to pyrolysis, hydrogenolysis leads to higher net conversion, higher yields of monophenols, and less char formation[45]. In addition, is fairly lower than those applied in pyrolysis processes. Another chemical fractionation technology is the hydrolysis, which is based on the depolymerization of lignin, by breaking the main C-O bonds present in its structure using acid or basic as a catalyst. The high pressures and temperatures produce low molecular weight compounds, being the most important a group of phenolic compounds, such as vanillin, cresols, catechols, guayacol.

On the other hand, the applicability of lignin breakdown by microbial degradation, which is a selective way of lignin depolymerization, is still at the laboratory level because of its high cost and long reaction time as compared to thermochemical fragmentation routes[24].

1.4.2. Incorporation of lignin in the polymer industry

Lignin as polymer offers a number of advantages suggesting a bright future for lignin-based products, such as high carbon content, compatibility with diverse industrial chemicals, good stability due to the presence of aromatic rings, small particle size and several functional groups, which make it suitable for chemical transformations. Moreover, it is produced in high volumes, making it an excellent candidate product to use in the polymer industry[47]. The preparation of novel materials from lignin or lignin-derived products would improve the economics of polymer materials as well as possibly address the problem of its waste disposal. The plastic industry might be one of the areas in which lignin could be used as an additive or raw material for the production of new plastics[6].

Therefore, numerous studies have been performed on the development of new applications of lignin in this field. Most of them have been focused on three different directions as is shown in the Figure 1.10.

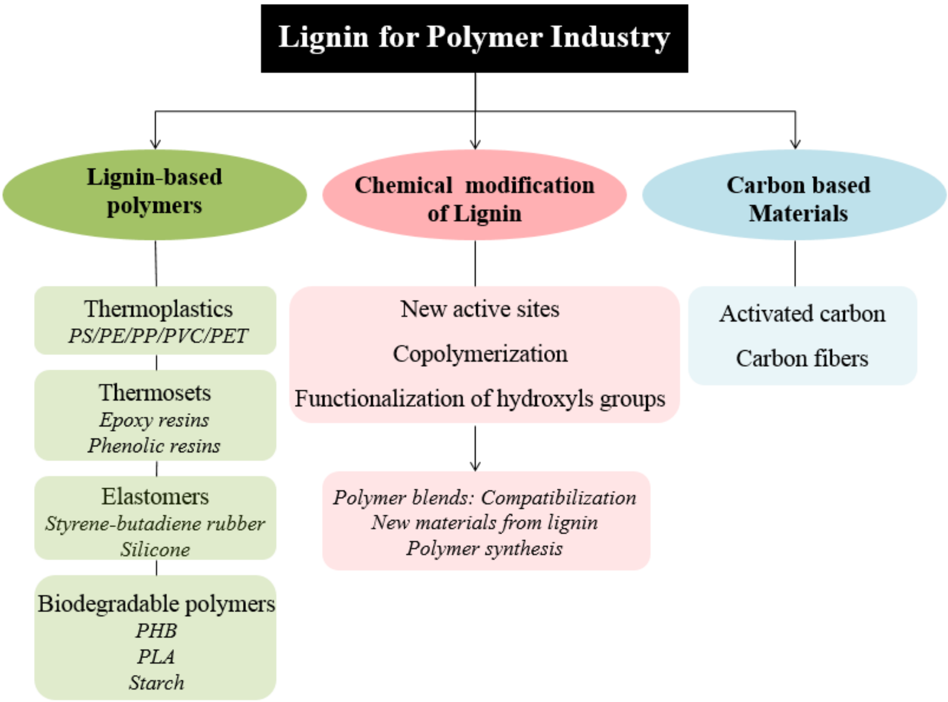


Figure 1.10. Research directions for the incorporation of lignin into polymer industry.

The first one consists of the study of the feasibility of using lignin as a component in polymer matrices to prepare more environmentally friendly and low-cost polymer composites. The second is based on the chemical modification of lignin structure mainly to increase the range of its application. In addition, the last one pursues the elaboration of carbon-based materials from lignin.

1.4.2.1. Lignin-based polymers

The use of lignin as a component in polymer matrices is an important branch of research to find new advances for its valorization in the plastic industry. Currently, lignin is considered an extremely interesting compound owing to its abundance, low cost and biodegradability[6]. Thus, the use of lignin as an additive in this area could contribute, largely, in the elaboration of partially or totally biodegradable polymer composites with new or improved properties. This section is centered on the achievements in the use of lignin in plastics.

Moreover, the use of additives in the polymer industry is a well-known strategy frequently used to obtain desirable properties of the polymer. The most common are reinforcements, fillers, plasticizers, thermal and UV stabilizers, antioxidants, flame-retardants, color pigments and lubricants. In some cases, compatibility between the polymer matrix and the incorporated additive is required, but in other cases, it is better that both polymer and additive form separate phases.

Nowadays, synthetic polymers are still used for most of the applications. Table 1.3 summarize the most common synthetic polymers used by the industry as well as their main applications. However, the demand for biobased polymers derived from natural sources has recently increased, and therefore, biopolymers are also being used as matrix material for some applications, especially for medical and packaging uses. These biobased polymers can be obtained from agro-resources such as polysaccharides (starch, pectin, chitin, chitosan) and proteins (casein, gelatin, collagen, soy, gluten). Moreover, some microorganisms produce biopolyesters like poly(hydroxybutyrate) (PHB) and poly(hydroxybutyrate co-hydroxyvalerate) (PHBV). In addition, polylactic acid (PLA), the most promising biopolyester developed by biotechnology using conventional synthesis but from monomers derived from bioresources.

Table 1.3. The most common commercial plastics used by polymer industry and their main applications.

Thermoplastics	**Application**
Polypropylene (PP)	Food packaging, reusable plastic containers, pipes, automotive parts, electrical appliances, insulation for electrical cables
Polyethylene (PE)	Food packaging, toys, reusable bags, bottles, trays and containers, house ware, moving machine parts (bearings, gears)
Poly(ethyleneterephtalate) (PET)	Bottles for water, soft drinks, juices, cleaners
Polystyrene (PS)	Eyeglasses frames, plastic cups, packaging, building insulation, CD and DVD cases
Polyvinyl chloride (PVC)	Construction industry, window frames, profiles, floor and wall covering, pipes, cable insulation, garden hoses
Polyamide (PA)	Electronic components, automotive parts, textile
Poly(methylmethacrylate) (PMMA)	Substitute for glass in aquariums, motorcycle helmet visors and aircraft windows. Automotive parts. Acrylic paints
Polytetrafluoroethylene (PTFE)	Coating for non-stick cookware, containers and pipes that come in contact with reactive chemicals
Acrylonitrile-Butadiene-Styrene (ABS)	Appliances, automotive parts, pipe, telephone components, shower heads, door handles, faucet handles
Thermosets	
Polyurethane	Manufacture of paintings, insulating, mattresses
Phenolic resins	Tool handles, billiard balls, insulation
Epoxy resins	Adhesives, coatings, manufacture of insulating materials, aircraft components, pipes, pressure vessels
Polyesters resins	Fibre-reinforced composites, boats, plastic agglomerates
Elastomers	
Natural rubber (NR)*	Latex products, footwear, adhesives, coatings
Styrene-butadiene rubber (SBR)	Wheels or tires of vehicles, gloves
Neoprene	Manufacture of wetsuits
Silicone	Pacifiers, medical prosthesis, lubricants, molds

**harvested mainly in the form of the latex from Hevea brasiliensis*

In this contents, lignin can be blended with synthetic or biobased polymers with the aim to provide some new property[46]. Several works have demonstrated in the last three decades the efficiency the use of lignin as a reactive component in plastics[6]. Although resulting properties have high dependence on the nature of polymer and functionality of lignin, in general, lignin could be used to fulfill the following functions:

- Antioxidant to protect the materials against deterioration by the oxidation caused by UV[9,48–51].
- Thermal stabilizer to prevent the degradation of polymers during the processing at high temperatures[13,48,52,53].
- Flame retardant[54].
- Nucleating agent[48,55].
- Reinforcement.
- Hydrophobic agent[56].

Since the phenolic OH groups of lignin are capable of eliminating free radicals, a considerable number of attempts were made to use it as a stabilizer and to protect the matrix polymer against oxidation. The ultraviolet exposure on some polymers surfaces can deteriorate the appearance and properties of the polymers, causing color change and the loss of physical properties. Many polymers like polyolefins (PE and PP), polystyrene, PVC, ABS, polyesters, and polyurethanes are so sensitive polymers to weathering by UV irradiation. Therefore, in the last years, the use of lignin as UV stabilizer has been extensively studied in some synthetic polymers[22].

Pucciariello et al., 2004[49] reported their study about the incorporation of straw lignin up to 20% in different commercial polymers (PE and PS) prepared by extrusion. They observed that the addition of lignin to polyethylene and polystyrene did not hinder their processability and further, demonstrated that the photodegradation behavior of lignin-containing blends was much lower than unblended polymer samples. Moreover, Mishra et al., 2007[9] prepared PVC-lignin films by solvent casting in order to explore the UV stability of elaborated films and the results suggested that the lignin acted as UV absorber in PVC-lignin films.

However, the study of Pouteau et al., 2003[51], where 15 lignin samples from different isolation processes were blended with polypropylene (PP), concluded that the compatibility between components greatly affects the stabilization properties of lignin in polymers. Hence, as happen for classical additives, a good compatibility between the polymer matrix and lignin is an essential requirement for a suitable response of lignin against oxidation induced by UV. Furthermore, the study revealed that the antioxidant activity of lignins varies with their genetic origin, isolation method and molecular characteristics. Therefore, for apolar systems like polyolefins, low molecular weight and low total OH content tend to improve the compatibility and consequently the antioxidant activity. In addition, the polydispersity of lignins, which is a typical feature of this biopolymer, seems to be a rather negative factor because high molecular weight chains limiting the solubility of active low molecular species. Other authors later confirmed these results, thus demonstrating that low molecular weight and narrow polydispersity of lignin derived from beech wood was suitable for the stabilization of both virgin and recycled polypropylene[52].

Degradation by thermo-oxidation is another important drawback of some polymers and therefore the use of thermal stabilizers are usually required during processing. For that reason, some authors have investigated the use of lignin as a thermo-oxidation stabilizer in different polymer matrices[48,52]. The results of the thermo-oxidative experiments of isotactic polypropylene blended with lignin showed that the presence of relatively small amounts of lignin (5-10%) was able to increase the thermal degradation temperature[48]. Moreover, Gregorová et al., 2006[52] who also analyzed the effect of lignin incorporation in natural rubber from the viewpoint of their thermo-oxidative aging, found that lignin exerts a stabilizing effect in the natural rubber. Lignin effect was comparable with that of conventional synthetic stabilizer usually used in this type of polymer such as N-phenyl-N-isopropyl-p-phenylene diamine (IPPD). Moreover, they noticed that the activity of commercial rubber stabilizer IPPD was enhanced by the addition of lignin.

Furthermore, due to the abundance of aromatic structures in lignin, it is able to generate a high amount of char residue (40-50%) upon heating at an elevated temperature in an inert atmosphere. This peculiarity is a basic aspect of flame retardant additives because this char is able to provide a barrier to both heat and oxygen diffusion, consequently, it can reduce the combustion heat and heat release rate of polymeric materials. Regarding environmental concerns, lignin as flame retardant additive could be useful for substitution of toxic halogen-flame retardants[57]. Thermal properties and flame retardancy of ABS-lignin blends elaborated by hot-pressing were investigated by Song et al., 2011[54]. The work demonstrated that the heat release rate decreased with increasing lignin loading determining that lignin incorporation can significantly improve the flame retardancy of ABS.

Furthermore, lignin particles can also act as nucleating agent in the composites increasing the crystallization rate. Canetti and Bertini., 2007[55] observed that the incorporation of lignin by extrusion in the poly(ethylene terephthalate) has an important influence on the melting behavior of composites promoting the crystallization and resulting in a faster crystalline reorganization than that of the pure PET. Similar behavior in PP-Lignin blends[48].

However, the introduction of lignin in polymers often have a big influence on the polymer mechanical properties. Usually, poor compatibility between the polymer matrix and lignin is observed and thus has a negative effect on mechanical properties because of poor stress transfer between the phases[6]. However, the compatibility between the phases can be improved in the presence of a compatibilizer or through chemical modification of lignin[22].

As can be observed, this section has centered on the literature based on lignin incorporation in synthetic polymeric matrices. However, in the last years, more efforts have been dedicated to the incorporation of lignin into natural polymers to form 100% bio-based materials. Several authors have added lignin to natural biopolymers such as starch (2), protein, polyhydroxyalkanoates composites PHB), to observe how it affects the thermal and mechanical properties as well as their interaction with moisture (lignin polymers).

1.4.2.2. Chemical modification of lignin

The chemical modification of lignin structure would open new perspectives for increasing its range of applications in the polymer industry. Lignin polymer, due to its characteristical chemical structure, is an excellent prospect to be chemically modified with several chemical compounds.

Different types of chemical modifications have been proposed in order to increase its reactivity and its solubility in organic solvents, use it in polymer synthesis or improve the compatibility with blended polymers[12,15,22,58].

The synthesis of new chemical active sites in lignin consists in increasing the reactivity of hydroxyl groups or changing the nature of chemical active locations in the molecule. The most relevant are halogenation, alkylation or dealkylation and hydroxyalkylation[2,8,12,15]. These modifications are mainly focused on the elaboration of prepolymers based on lignin for the synthesis of thermosets and thermoplastics[2]. Moreover, lignin can play an important role in lignin-based epoxy and lignin-based phenol formaldehyde resins preparation through different modification reactions[8,21].

Another important chemical modification is the elaboration of lignin graft copolymers. The main objective of this modification is attaching polymer chains to the lignin hydroxyl groups, obtaining a copolymer with a lignin core[22]. Not only is an effective way for the synthesis of polymers based on lignin, but also is widely used to overcome the incompatibility of lignin with commercial synthetic polymers[2,21,22].

However, among all of the reactions involving hydroxyl groups of lignin, esterification is probably the easiest way to modify lignin[12]. Indeed, acetylation is a routine method that is commonly used to enhance the solubility of lignin samples for molecular weight assessment and structural analysis. In general, acid anhydrides and chloride acids in the presence of acid (sulfuric acid or zinc chloride) or basic (pyridine) catalyst are used to modify lignin[2].

The main characteristics such as thermal properties, solubility in organic solvents, molecular weight, and physical appearance of lignin are greatly affected by the esterification. Moreover, the length of the attached aliphatic chain has a strong influence on the final properties.

Lewis et al., 1943[59] was the first author who published a study about the esterification of lignin with fatty acids and showed that esterified lignin gained new and very interesting properties like changes in solubility and thermal behavior. Later, Glasser and Jain, 1993[60] synthesized lignin acetate (LA), lignin butyrate (LB), lignin hexanoate (LH), and lignin laureate (LL) and revealed a consistent decline in Tg with increasing ester substituent size.

Although during the last decade several authors have studied the esterification of lignin with different compounds, not many have focused their study on finding a specific application for the synthesized lignin-ester[27,61]. However, more recently, researchers have centered on developing novel applications for synthesized lignin-ester derivatives. Sailaja and Deepthi., 2010[62], used phthalic anhydride to modify commercial lignin in order to improve the compatibility with PE. Moreover, Luong., 2012[63], isolated lignin from black liquor to synthesize a copolyester through polyesterification between lignin and sebacoyl chloride for the utilization of lignin for eco-friendly material development. Later, other authors demonstrated that the modified lignin with fatty acids can be used for coating applications[64,65].

Therefore, it has been proved that chemical modification of lignin approaches are used in the polymeric industry.

1.4.2.3. Carbon-based materials

Lignin consists of about 50-60 % of carbon, being able to be directly used as a precursor for carbon materials. The most interesting applications are activated carbons and carbon fibers.

Activated carbons are porous carbon materials, with high specific surface area, generally in the range of 500-1500 m^2g^{-1} for commercial products[22]. This type of material is able to remove organic and inorganic pollutants from either liquid or gaseous phases[10].

At present, two techniques exist for the conversion of lignin into activated carbon. The first one is physical activation where lignin is first carbonized by pyrolysis in an inert atmosphere at 600-900 ºC and then activated in an oxidizing atmosphere at 600-1200 ºC, resulting in the porous structure. In addition, the other one is chemical activation, which consists in impregnating lignin with chemicals and heat at 450-900 ºC. In this process, activation and carbonization proceed simultaneously to yield the final porous material.

Moreover, many efforts have been carried out in order to use lignin as a precursor for the development of carbon fibers. Carbon fibers are used as valuable reinforcement for the elaboration of composite materials especially used for sports equipment, marine products and automotive industry. Actually, carbon fibers are manufactured from polyacrylonitrile (PAN), which besides coming from no renewable sources is an expensive polymer. Therefore, lignin, as a polyaromatic macromolecule, could be an ideal sustainable biopolymer precursor for the production of carbon fibers. However, the development of high-quality structural lignin carbon fibers is being quite difficult. Still, lignin carbon fibers do not show required engineering properties (tensile strengths above 1 GPa and elastic modulus over 100 GPa)[22].

Nevertheless, the great cost reduction associated with the use of lignin still encourage further developments. Moreover, the properties of lignin-based carbon fibers depend largely upon the source of lignin[10].

1.4.3. Power/Fuel

Although biomass has been the main feedstock for energy production over the years, in Spain, as in most of the developed countries, the majority of consumption based on biomass was replaced, first by coal at the end of the 19th century and then by petroleum fuels during the 20th century.

However, currently, there is renewed interest in the use of renewable resources such as biomass for energy since it presents some advantages by providing a clean, renewable, and sustainable source of energy. Moreover, the use of biomass as renewable energy avoids the high dependence on external supply of fossil fuels[66]. The energy from biomass can be used for heating and hot water production in the domestic sector, heat for industrial processes and electricity generation.

In particular, in the European Union, but especially in the Nordic countries, Germany and Austria, the use of solid fuels for power generation and for residential heating appliances such as boilers and furnaces is well established. The installation of biomass boilers has significantly grown since 2004. Although different solid fuels can be used for energy production (Figure 1.11), wood pellets have experienced a large increase during the last decade. Wood pellets are typically prepared from wood residues from the secondary wood processing of softwoods or hardwoods.

Figure 1.11. Photos of different types of raw materials for biomass boilers.

One area of inquiry receiving attention is the manufacturing of wood pellets to produce high quality and high fuel-value pellets. Therefore, the incorporation of functional additives could a positive progress on the manufacture of wood pellets. For instance, Chen and Workman., 1990[67], combined wood and coal increasing the heating value of wood pellets because of the higher heating value of coal. However, nowadays the interest is focused on utilizing bio-additives like lignin in order to produce sustainable and environmentally friendly pellets from nontoxic and cheap raw materials.

Table 1.4 present ash content, volatile matter, fixed carbon and HHV (MJ/Kg) of the most used solid fuels and lignin. As can be observed, lignins, in general, possess less volatiles and higher fixed carbon content than commercial fuels, and thus it has a higher heating value than biomass.

Table 1.4. Ash content, volatile matter (VM), fixed carbon (FC) and HHV (MJ/Kg) of usual solid fuels, coal, and lignin[66,68–70].

Commercial Fuels	**Ash (%)**	**VM (%)**	**FC (%)**	**HHV (MJ/Kg)**
Almond Shell	2.2	82.0	15.8	18.3
Olive Stone	3.2	75.0	21.8	18.2
Briquette	7.4	74.9	17.7	17.2
Wood chips	1.5	68.6	29.9	15.2
Wood pellets	1.3	82.0	17.1	18.2
Coal	10-40	20-30	35-50	15-27
Lignin	2-4	60-70	25-40	25-27

Stevens and Gardner, 2010[71], explored the enhancement of wood pellet-fuel value by the addition of lignin and black liquor from kraft pulping (2-10%) and found that the lignin increased fuel value as a function of loading level. However, unexpectedly, the black liquor pellets decreased fuel value with increased loading levels. The moisture content and the incombustible inorganics of the black were the main factors that reduced the heating value of pellets. Moreover, lignin-wood pellets were well formed, dry to the touch, and had a shiny exterior finish.

Abedi and Dalai, 2017[72], studied the effect of bio-additives such as lignin and demonstrated that the lignin addition had a positive impact of the density and mechanical strength of the pellets as well as improvement of heating value.

Although some works have demonstrated that lignin could be exploited as an additive to wood pellets to enhance pellet fuel value, lignin composition, as moisture and ash content are parameters that influence negatively on the final properties of pellets.

1.5. Poly(lactic acid)

Although the development of polymeric materials has had a vital importance in the society and has contributed to facilitate our way of life since its creation in the 19th century, their use creates more and more environmentally damaging effects because of their non-renewable nature and ultimate disposal.

Nowadays most of the commercially available plastic materials are derived from petroleum. The world production of polymers has increased considerably since 1950 and is expected to keep growing in the following years. As can be observed in Figure 1.12, China is the largest producer of plastic materials, followed by Europe and North America. In 2015, world production of plastic materials was around 269 million tones[73].

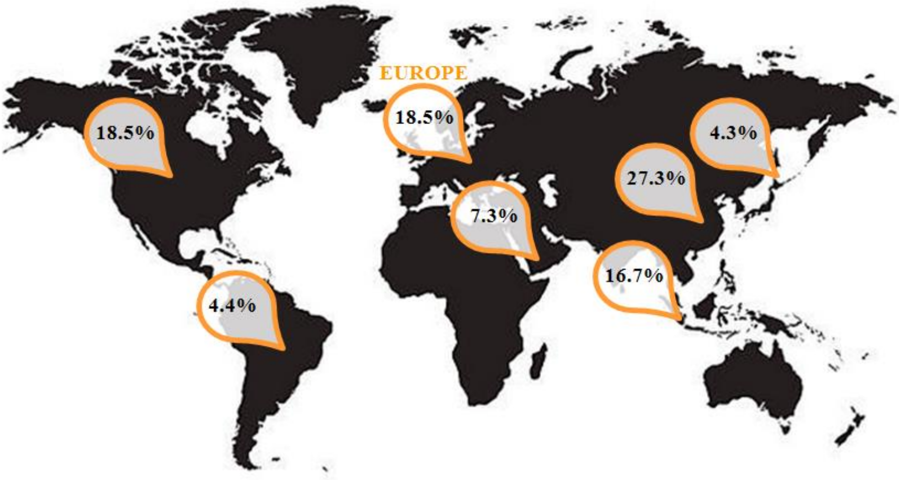


Figure 1.12. Distribution of global plastic materials production in 2015 adapted from PlasticEurope[73].

Regarding plastic materials demand in Europe, the 70% of the demand is concentrated in six countries, being Spain the fourth one. The packaging sector is the most plastic consumer segment assuming almost 40% of the total demand. The packaging sector is followed by the construction (19.7%), the automotive industry (8.9%), electrical and electronic devices (5.8%). Besides, others fields with around 22.4% of the total demand, include small segments such as sports, health and safety, leisure, agriculture, industry mechanics, and the furniture[73].

The most common polymers used in the packaging sector are represented in the Figure 1.13. Among the different uses of such polymers, we can highlight that PET is commonly used for water, soft drinks and juices bottles; PP for food packaging, sweet and snack wrappers, and microwave-proof containers and PE for milk bottles, shampoo bottles, reusable bags, and food packaging films.

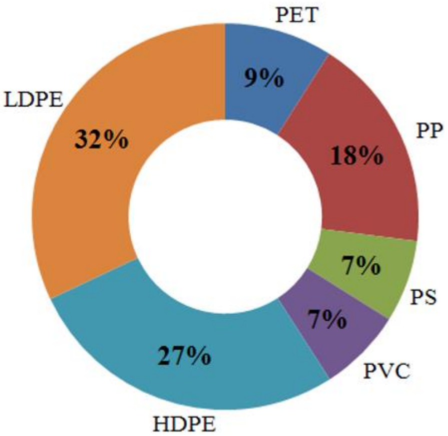


Figure 1.13. The most common polymers used in the packaging sector adapted from PlasticEurope[73].

All these products usually have a short service-life time, and after use, they are immediately discarded, causing a very alarming effect on the environment. Moreover, although in most of the developed countries a waste management policy has already been established, people have not yet become aware of how serious is the problem. Therefore, in addition to a continued raise in the awareness of the recycling and the consumption reduction of plastic products, especially those of short use, it is necessary to develop eco-friendly products with comparable properties to synthetic polymers and at an equivalent cost.

Biopolymers can be an environmentally sustainable alternative to current materials based exclusively on petroleum feedstock. One of the most promising biodegradable polymers is poly(lactic acid) (PLA) which has been extensively studied over last decades and now is playing an important role in replacing fossil-based polymers for certain applications[74]. It is an aliphatic thermoplastic polyester derived from renewable sources like corn, wheat, and other starch-rich products[75].

1.5.1. Synthesis and properties

Lactic acid (LA) is the monomeric precursor of PLA and is produced by chemical synthesis or by fermentation. Actually, bacterial fermentation of carbohydrates extracted from renewable sources is the preferred industrial technique for the production of lactic acid due to being more specific and friendly than chemical synthesis. Lactic acid exists as two stereoisomers, L-LA, and D-LA[58,76].

The synthesis of PLA starts from the production of lactic acid and ends with its polymerization. Figure 1.14 shows the three main methods available to produce high molecular weight (M_w) PLA from lactic acid: (1) polymerization through lactide formation, (2) direct condensation polymerization and (3) direct polycondensation in an azeotropic solution[74]. Variable molecular weights can be achieved depending on the polymerization technique, but only high molecular weight PLA is used by textile, plasticulture, and packaging industries.

The production of high molecular weight PLA by ring opening of lactide is the most employed process to produce PLA by the industry. In this process, as the first step, the water is removed in a continuous condensation reaction of aqueous LA to produce low molecular weight PLA. Then, the prepolymer is catalytically converted to lactide and then purified. Production of cyclic lactide results in three potential forms: D,D-lactide (D-lactide), L,L-lactide (L-lactide) and L,D- or D,L-lactide (*meso*-lactide). The polymerization process produces different PLA grades with finely controlled physical and chemical properties[77,78].

Direct condensation polymerization, although less used, also produces PLA at industrial scale. In this process, lactic acid is polymerized to yield a low molecular weight PLA, and then coupling agents are employed to increase the chain length. The azeotropic condensation polymerization is a method used to obtain high chain lengths without the use of chain extenders. This polymerization gives considerable catalyst residues because of its high concentration needed to reach an adequate reaction rate. This can cause many drawbacks during processing, such as degradation and hydrolysis[74,78].

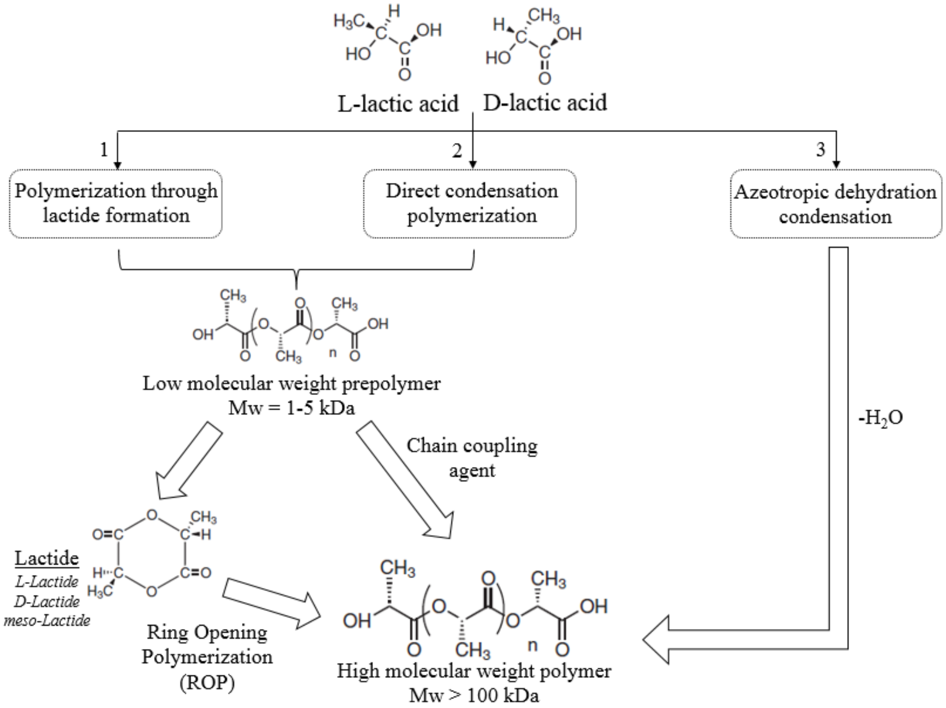


Figure 1.14. Synthesis methods for obtaining high molecular weight PLA adapted from Murariu and Dubois, 2016[76].

Poly(lactic acid) is one of the few polymers which exists in three stereochemical forms depending on the stereoisomers distribution within the polymer chains: poly(L-lactide) (PLLA), poly(D-lactide) (PDLA) and poly(DL-lactide) (PDLLA) (*meso*-lactide).

The stereochemical composition of the polymer has a significant effect upon polymer properties[79]. PLA can be produced as totally amorphous or with up to 40% crystallinity[80]. PLA containing more than 90% of L-lactic acid is mainly semicrystalline while having higher percentage of D-isomer is amorphous[76]. The crystallinity of PLA has a strong influence on the thermal, mechanical, barrier and biodegradable properties of the polymer[78]. Glass transition temperature and melting point decrease at higher D-isomer content. Moreover, the higher the crystallinity percentage is, the lower is the biodegradation rate[79]. In general, for the selection of a PLA matrix it is essential to take into account the requirements of the application. Furthermore, this polymer has relatively hydrophobic surface compared with other bio-polyesters and the best solvent able to solubilize is chloroform[78,81].

In comparison to other biopolymers, the production of PLA has numerous advantages. PLA is **eco-friendly** for coming from renewable sources in addition to being biodegradable, recyclable, and compostable. It also offers a significantly **reduced carbon footprint** versus traditional oil-based plastics[79]. Furthermore, PLA requires 25-55% less energy to produce than petroleum-based polymers.

It presents **high rigidity** and **high transparency** as well as good gas, aroma, and oil barrier. Its **high surface energy** makes it easy to print. Moreover, it is a **biocompatible** polymer. This is the most attractive aspect in biomedical applications, as it does not produce toxic effects in the human body.

From the point of view of the industry, its **ease processing by the conventional techniques** such as injection molding, film extrusion, blow molding, thermoforming, fiber spinning, and film forming, is one of the main benefit[75,81].

However, PLA has some shortcomings as well, which limit its use in certain applications. The main limitations of PLA include **poor toughness** and ductility as well as low heat and hydrolysis resistance[81]. PLA is a **brittle polymer** with less than 10% elongation at break[76]. Therefore, for some uses, plastic deformation is required. Moreover, PLA is susceptible to **heat deformation**[75]. The relatively low Tg of PLA (around 60 ºC) is considered an important drawback, especially to be used in hot-packaging applications such as microwaveable containers or single-use disposable drinking cups.

Regarding **poor water resistance** of PLA, it can interact with water molecules from the packed food and can be hydrolyzed during the storage time, reducing the performance of packaging and therefore compromising the quality and the safety of food[82]. **Low crystallization ability and degree**, also limits its industrial implementation in different applications, particularly in durable applications like automotive and electronics[75].

Therefore, to overcome these limitations and extend its range of applications, PLA requires some improvements of its properties and it could be solved by blending with other polymers, formulating new stereo-complexes, or using additives like reinforcing fibers, fillers, nucleating agents, stabilizers or plasticizers[74,80].

As regards the production of PLA, Cargill Dow Polymers LLC is the major producer of PLA with a capacity of 140 kT per year. This company commercializes the PLA under the trade name NatureWorks® and Ingeo®. During the last 15 years, this company has focused on the development of end-products based on PLA. For that, new compositions and copolymers with additives like nucleants, inhibitors or color pigments, have been developed. The following figure summarized where the market of this company is at present.

Figure 1.15. Products made with different formulations of Nature Works and Ingeo.

In addition, some new producers also emerged such as Galactic (Belgium), Toyota (Japan) and Corbion Purac. The last one is also an important

PLA producer, which operates 10 production plants, in the USA, the Netherlands, Spain, Brazil, and Thailand. Corbion Purac has developed a range of high heat PLA blends opening a multitude of possibilities for applications that require improved heat resistance, such as coffee cups or products for automotive or electronics (Figure 1.16)[83].

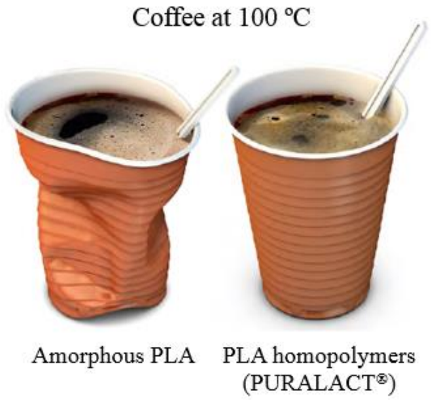


Figure 1.16. Difference between the behavior of amorphous PLA cup and PLA cup with improved heat stability (PRALACT®).

1.5.2. Applications

Initially, the use of PLA was limited to medical applications because of the polymer biocompatibility, high cost, low availability and limited molecular weight. However, the development of new techniques for the economical production of high molecular weight PLA, which can be processed by conventional processing techniques, have decisively contributed to its larger utilization, opening a new market for sectors such as packaging, textiles, automotive and electrical and electronic industry[76].

At present, in the biomedical sector, PLA-based products are still used for medical implants, surgical sutures, and medical devices because of its bioresorbability and biocompatibility properties with the human body. One of the main advantages in this area, especially in the case of medical implants, is that PLA can be degraded within the body, avoiding the removal of the implant.

Moreover, due to its hydrolytic degradability and low toxicity also shows important applications in drug delivery systems[74,77,78].

Apart from its use in the medical field, today, the most important market in volume for PLA is the packaging sector and it will probably remain the main use of PLA in the near future (Figure 1.17). PLA has had to solve numerous challenges for being used in commercial packaging applications due to its limited mechanical performance[74]. However, during the last decade, the use of PLA as a packaging material has increased all across Europe, Japan, and the United States, mainly in the area of fresh products, where PLA is being used as a food packaging for short shelf-life products, such as fruit and vegetables. Actually, package applications include containers, drinking cups, sundae and salad cups, wrappings for sweets, lamination films, blister packages, and water bottles[84].

One of the first companies to use PLA as a packaging material was Danone (France) in yogurt cups for the German market. Moreover, some food retailers are currently using PLA based packaging in some of their products such as Wal-Mart (USA), SPAR (Austria) and Auchan (France)[74].

In addition to packaging uses, PLA is increasingly being used in non-food related applications including agriculture, textiles, and 3D printing. However, the last trends show that electronics and automobile applications are expected to have a higher importance in the following years. Consequently, new PLA-based products (composites, nanocomposites, tailored formulations, etc.) with improved performances are under development in order to satisfy the specific application requirements.

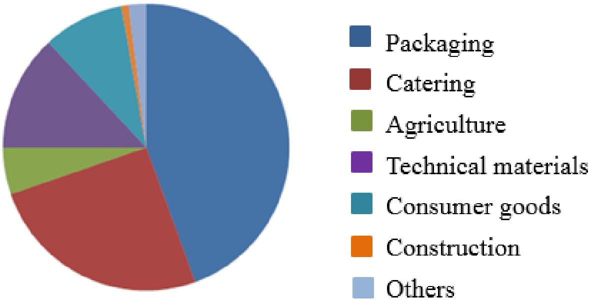


Figure 1.17. Global PLA application market in 2013 adapted from Karamanlioglu et al., 2017 [83].

1.5.3. Degradation mechanisms

PLA products will be exposed to different environments during their service life. Biotic and abiotic factors can promote irreversible changes of the polymers leading to the loss of various properties due to the chain scission[74].

The environmental degradation process of PLA is affected by its material properties such as molecular weight, optical purity, crystallinity, Tg, and Tm and by environmental factors such as humidity, temperature and catalytic species (pH and the presence of enzymes or microorganisms)[83]. However, depending on the application, degradation of PLA can be an advantage or a disadvantage and therefore, a good balance between its durability and biodegradability is needed. For example, on applications as packages or medical settings, is a very important advantage to be biodegradable. However, in products for durable uses, it can be a significant weakness. Figure 1.18 classify the main degradation processes that can suffer PLA polymer[74].

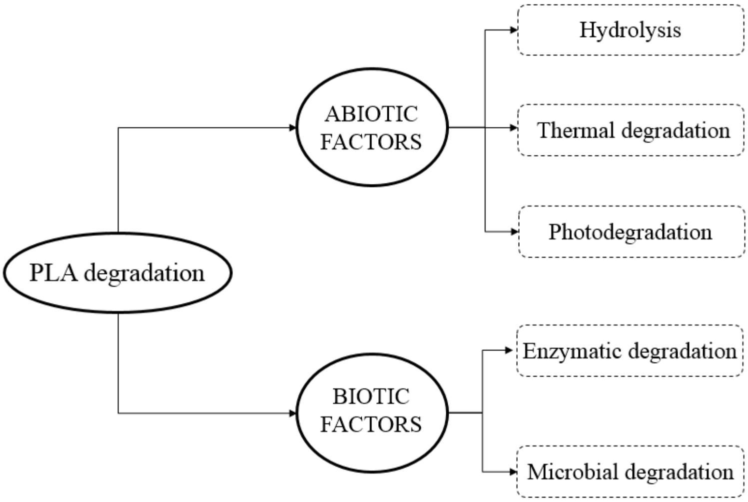


Figure 1.18. Main degradation factors of PLA.

Hydrolytic degradation takes place when PLA is exposed to moisture. The initial stage of the hydrolysis is located in the amorphous regions since the amorphous parts of the polyesters undergo hydrolysis before their crystalline regions due to a higher rate of water uptake[83,85]. Generally, ester groups of the polymer chain are randomly cleaved, resulting in a decrease of molecular weight and the release of soluble oligomers and monomers. Besides, the products of the hydrolysis self-catalyze the reaction[78].

Depending on the application, hydrolytic degradation is a phenomenon that can be both desirable (during the composting stage, for medical, plasticulture or packaging) and undesirable such as during processing or storage.

There are other factors like pH of the medium and the temperature, which promote the hydrolysis rate of PLA-based materials. In strong acidic and basic media, polymer chains are more easily degraded[74]. The temperature also plays a crucial role in the hydrolysis of PLA increasing the rate of degradation of PLA with temperature[83].

Moreover, PLA is susceptible to thermal degradation during processing due to being a hygroscopic material and very sensitive to high relative humidity

(RH) and temperature. Before processing, PLA should be dried to a water content less than 100 ppm (0.01%, w/w) to avoid hydrolysis and Mw reduction[74,78].

For outdoor applications such as plasticulture, packaging containers, or films, PLA is exposed to sunlight during its lifetime. UV radiation induces PLA plastic degradation. The carbonyl group present in the PLA chemical structure absorbs UV radiation at about 280 nm via n-π* electron transition, thus increasing the susceptibility of PLA to photodegradation. Several photodegradation mechanisms have been proposed for PLA such as Norrish II mechanism and photolysis of the ester group[74,86]. The amorphous regions are more susceptible to photodegrade than crystalline regions. Moreover, Janorkar et al., 2006[87], who studied the effect of wavelength on PLA photodegradation at a range of 232-500 nm, concluded that photodegradation mainly occurs between 200 and 300 nm. Photodegradation also is affected by temperature and humidity.

As regards to biotic degradation, it generally starts after exposure of the polymer to abiotic factors such as moisture and temperature, which lead to a reduction in molecular weight by chain scissions of the ester groups. Then, fragmented oligomers can be metabolized by cells in the case of medical applications, or be attacked by microorganisms for biodegradation in soil[78,80].

2

Lignin as additive in poly(lactic acid): Elaboration of PLA-lignin blends by extrusion process

2.1. Motivation

The overall aim of the present chapter was to find routes for lignin valorization in order to be used by the polymer industry. The incorporation of lignins as an additive for polymeric matrices could provide to lignin a new and interesting added-value.

This chapter is focused on the addition of three different lignins into poly(lactic acid) (PLA) matrix. The use of PLA as matrix was motivated for being one of the most extensively used biopolymers for the industry, especially for packaging applications. However, PLA has some limitations, such as its low thermal resistance as well as its high fragility and low ductility, which could be overcome using lignin as an additive.

Therefore, it was very exciting to study the combination of lignin and PLA obtaining a fully biobased material. With this aim, the study was focused on the following research questions, which were the origin of this work:

- Could lignin be used as additive for PLA and to elaborate blends using extrusion technique?
- How could affect the introduction of lignin in thermal and mechanical properties of PLA?
- As often lignin shows poor compatibility with polymers, could the chemical modification of lignin structure be a suitable alternative to improve the compatibility between lignin and PLA?
- How does acetylated lignin influence on the morphology, thermal and mechanical properties of PLA?

Owing to a high degree of variability of lignins, three lignins were studied. The first one was a commercial alkaline lignin, the second one comes from industrial black liquor (kraft process), and the last one was isolated from almond shells at lab-scale by an organosolv process. Almond shells were chosen as the raw material for lignin extraction because of their significant lignin content, as well as being an important agricultural waste in Spain, since the 63% of the total production of almond in the European Union comes from Spain. Therefore, in this research two types of blends were prepared by extrusion. Not only unmodified lignin was added to PLA in different amounts, but also blends of PLA and acetylated lignin were elaborated.

2.2. Objectives

The main purpose of this chapter was the evaluation of different types of lignin as a renewable additive for poly(lactic acid) (PLA). The study performed can be divided into four interesting points:

- Extraction and characterization of lignins.
- Functionalization of hydroxyls groups by esterification using acetylation treatment.
- Elaboration of PLA-lignin and PLA-acetylated lignin blends by an extrusion process.
- Evaluation of the thermal, mechanical and morphological properties of new materials.

2.3. Experimental procedure

2.3.1. Materials

Three types of lignin were used for composites elaboration with poly(lactic acid) (PLA). Commercial alkali lignin (CL), kraft lignin (KL) and lignin isolated from almond shells by an organosolv process (OL). Sigma Aldrich® supplied commercial alkali lignin. Papelera Guipuzcoana de Zicuñaga, S.A. (Hernani, Spain), kindly supplied the black liquor for kraft lignin obtention. The black liquor was characterized according to the procedures described in the Appendix I.2. The following values were obtained: density: 1.054 g/cm^3, pH 12.9, total dry solids (%): 15.8, lignin content: 67.4 g/L, content of organic material (%): 5.6 and content of inorganic material (%): 10.2.

Almond shells used in this study for lignin extraction by organosolv process were provided by “Eloy Castillo Fernandez” company which is involved in the production of almond in Arnedo (La Rioja). This company uses two varieties of almond called *Largueta* and *Marcona* produced by almond-tree *Prunus amygdalus*. The chemical composition of the almond shells was carried out according to TAPPI standards (Appendix I.1). The chemical composition of almond shells was: moisture content: 10.8±0.2, ashes: 0.81±0.09, extractives: 3.11±0.32, lignin: 31.3±0.30, cellulose: 18.2±0.20 and hemicellulose: 35.9±1.23.

PLA (NatureWorks PLA Polymer3051D) was from NatureWorks LCC (glass transition temperature: 55-65 ºC, melt temperature: 150-165 ºC, Mw: 169000 g/mol).

2.3.2. Lignin extraction processes

Kraft lignin was extracted by precipitation with H_2SO_4 (pH around 2). The precipitated lignin was filtered through a 0.45 μm nylon filter to recover lignin. After filtration, the precipitate was washed with acidified water (pH=2) and then was dried at 50 ºC.

Almond shells were previously milled to obtain particles between 0.25-0.40 mm. Then, the raw material was treated with a mixture of ethanol-water (70/30 wt%). The treatment was carried out at 180 ºC for 90 min in a 4 L pressure stainless steel batch reactor with constant stirring (EL0723 Iberfluid) with an electronic control unit for pressure and temperature control. The solid to liquid ratio was 1:6 (w/w). The liquid fraction (where lignin was dissolved) was separated from the solid fraction by filtration. Dissolved lignin was precipitated with two acidified volumes of an aqueous solution (pH around 2). The precipitated lignin was filtered through a 0.45 μm nylon filter to recover lignin, which was then washed and dried at 50 ºC.

2.3.3. Functionalization of hydroxyl groups: Acetylation

Previously obtained three lignin samples, CL, KL, and OL were acetylated. Acetylation of different lignins was carried out using the following experimental conditions. Dry lignin samples (1.00 g) were dispersed in formamide (25 mL) about 3 h, until complete solubilization. Pyridine (40 mL) was added, followed by acetic acid anhydride (6.6 mL). After stirring at room temperature for 3 h, another portion of acetic anhydride (6.6 mL) was added, followed by the same amount after another 3 h. After 30 h, the viscous dark solution was poured (under vigorous stirring) into 1.3 L of 2% ice-cold hydrochloric acid. The precipitated lignin was filtered with a 0.45 μm nylon filter and washed with 0.5 L distilled water and 0.5 L diethyl-ether. The samples were dried at 50 ºC overnight. Figure 2.1 represents the chemical modification of lignin by acetylation.

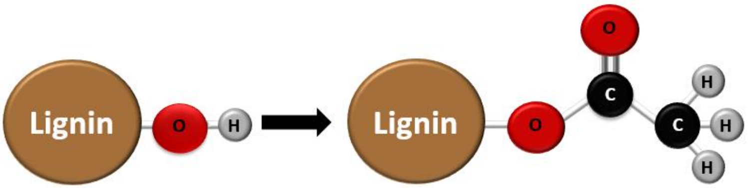


Figure 2.1. General scheme of lignin acetylation.

2.3.4. Processing of blends by extrusion

For PLA blends elaboration, Thermo Haake Minilab Rheomex CTW5 model with double screw (109.5 mm) extruder was used. The blends were made using PLA as a matrix with different percentages of original and acetylated lignin samples (0.5, 1, 5, 10, 20%). Process conditions for PLA mixed with commercial and organosolv original and acetylated lignins were 165 ºC, at rate 25 min^{-1} and recirculation time was 10 min. However, for PLA-KL composites, process conditions were 200 ºC, at rate 75 min^{-1} and recirculation time was 10 min, optimum conditions previously determined for successful mixing of the materials.

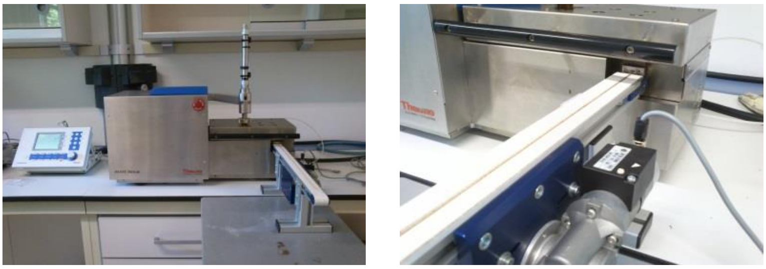


Figure 2.2. Equipment of PLA-lignin extrusion.

2.3.5. Characterization methods

Different techniques and procedures described in **Appendix I** and **Appendix II** were used to characterize lignin samples as well as elaborated PLA-lignin blends. The following table summarizes performed characterization:

Table 2.1. Characterization methods used for the analysis of original and acetylated lignins and PLA-lignin blends.

Analysis	Technique or Procedure	Appendix	Section
	Lignin		
Chemical composition			
Klason Lignin (IL)	Acid-insoluble lignin	**I**	3.1
Carbohydrate content (%)	HPLC	**I**	3.2
Ash content (%)	TGA	**II**	3.1
Chemical structure	FTIR	**II**	1.1
Total phenolic content	Folin-Ciocalteu (UV)	**I**	4.1
Molecular weight properties	GPC	**II**	2.1
Grass transition temperature	DSC	**II**	3.2
Thermal stability	TGA	**II**	3.1
	PLA-lignin blends		
Morphology	OM	**II**	4.1
	AFM	**II**	4.2
Thermal behavior	DSC	**II**	3.2
	TGA	**II**	3.1
Mechanical properties	MTS	**II**	6.1

2.4. Results and discussion

2.4.1. Chemical composition of lignins

Results of acidic insoluble lignin, soluble lignin, ashes and sugars content are presented in Table 2.2. Organosolv lignin was which showed the higher Klason lignin content, followed by alkaline and kraft lignin. Considering that polysaccharides are the most common contamination of lignin, it can be said that all lignin presented low sugars content (1-4%). Among the sugars present in lignins, xylose was the major hemicellulosic sugar in all lignins samples.

However, the lower ash content was unexpectedly found in Kraft lignin with only 0.3%. Generally, these types of lignins have a lot of salts due to their origin. Probably, in this case, the washing step after precipitation of lignin had a positive effect on it. Moreover, the high inorganic matter (20%) of commercial alkali lignin was surprising.

Table 2.2. Chemical composition of the different lignins in dry basis (wt.%)

	OL	**CL**	**KL**
Klason lignin (%)	81.4	76.1	69.7
ASL (%)	8.2	1.7	9.0
Ashes (%)	4.15	19.5	0.3
Carbohydrates (%)	3.8	1.2	3.4
Xylose (%)	3.6	1.0	3.1
Arabinose (%)	nd	0.2	nd
Glucose (%)	0.2	nd	0.3

nd: not detected

2.4.2. Chemical structure of lignins

Fourier transform infrared technique, gel permeation chromatography and the total phenolic content calculation were used in order to confirm the chemical modification of lignin samples.

FTIR spectra of lignin samples before and after acetylation are shown in Figure 2.3. Although similar spectra were observed for commercial alkaline (CL) and extracted lignins from kraft liquor (KL) and organosolv process (OL), only OL and KL showed a small peak at 1320 cm^{-1} which is related to the presence of syringyl units[88]. The presence of guaiacyl units (G) is associated with a peak at 1260 cm^{-1} [61,88]. Therefore, this indicates the existence of syringyl units (S) and guaiacyl units (G) in OL and KL structures, while guaiacyl units (G) are the main structures that compose CL, demonstrating that commercial alkaline lignin could come from softwood sources. Moreover, signals between 1400 and 1700 cm^{-1} were attributed to the aromatic skeletal vibrations. Characteristic peaks at 1595 cm^{-1} and 1510 cm^{-1} corresponds to C=C of aromatic skeletal vibrations[88].

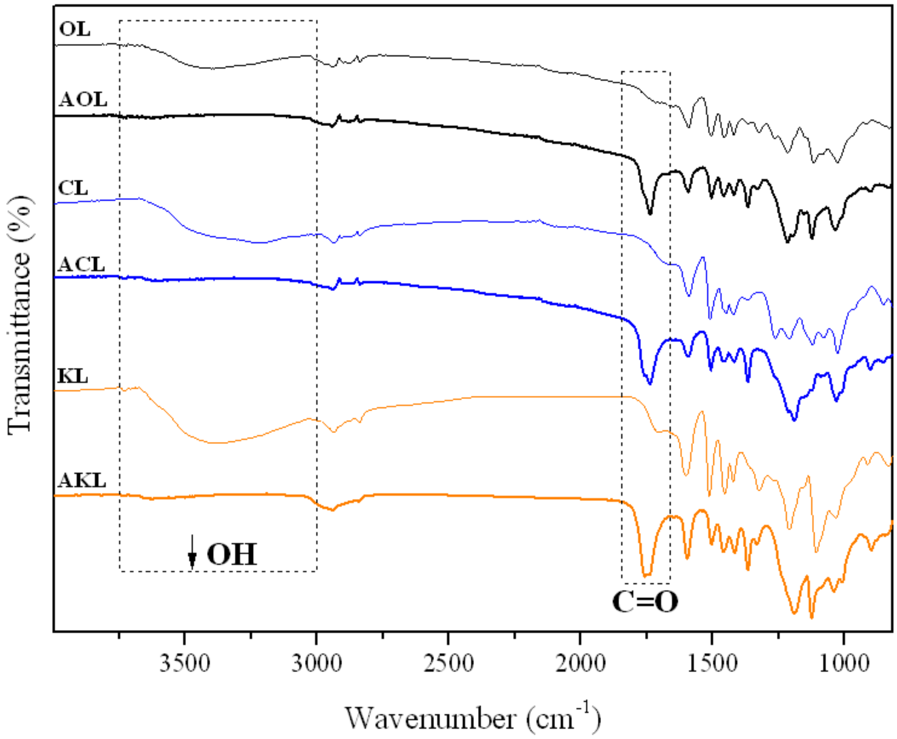


Figure 2.3. FTIR spectra of lignins before and after acetylation.

After chemical modification of lignins, spectra of acetylated samples (AOL, ACL, and AKL) showed that the signal around 3400 cm^{-1}, which is associated with the presence of O-H stretching vibrations in aromatic and aliphatic hydroxyl groups, was completely reduced[89]. Additionally, an intense peak appeared around 1735 cm^{-1} due to stretching of carbonyl groups (C=O) formed during acetylation, indicating that the acetylation process was successful[27,61,90].

The characteristic bands of acetic anhydride (≈ 1823, 1121, 995 and 896 cm^{-1}) were not observed in acetylated lignins spectra, demonstrating that acetylated samples did not contain traces of unreacted acetic anhydride[27].

Regarding the weight average of lignin samples (Table 2.3), it should be said that lignin structure and the weight average mainly depend on the feedstock and extraction process[90,91]. Kraft lignins do not have high molecular weights because of they come from industrial aggressive processes[6]. Moreover, it has been also reported that the carbohydrates chains linked to lignin can increase the hydrodynamic volume of lignin and therefore increase the apparent molecular weight of the lignin when it was measured using GPC[92].

According to the literature, weight average should increase when lignin is modified with ester groups. However, in this case, the weight average slightly decreased for AOL and ACL, but not for AKL which presented an increase.

Moreover, although the difference in molecular weight average was not very significant, unmodified lignins showed greater heterogeneity than acetylated samples because of the elimination of small fractions during acetylation process. It can be said that the acetylation reaction generates less polydisperse lignin.

Table 2.3. Weight average (Mw), number average (Mn) and polydispersity index (Mw/Mn) of unmodified and acetylated lignins.

	OL	**AOL**	**CL**	**ACL**	**KL**	**AKL**
Mn	1537	1536	1129	1598	579	787
Mw	8074	7928	10324	9295	2013	2276
IP	5.3	5.2	9.1	5.8	3.5	2.6

The phenolic content of original and acetylated lignin was calculated according to Folin-Ciocalteu procedure[6]. Although more techniques are available for determination of phenolics, in this chapter UV technique using a well-known procedure was chosen for it. No correlation of phenolic content of each lignin could be made due to the different origin and isolation process suffered.

However, as expected KL showed the higher phenolic hydroxyls group content because of the kraft process generates lignins with a high content of phenolic hydroxyl groups due to the cleavage of β-aryl and α-aryl ether linkages of lignin during the last stage of the process[8].

Table 2.4. Total phenolic content of original and acetylated lignins.

	GAE (%)	OH % (w/w)	mmol OH/g lignin	DS (%)
OL	27.2	10.8	0.640	
AOL	16.5	6.60	0.388	71.6
CL	32.3	12.9	0.760	
ACL	18.4	7.35	0.430	43.1
KL	57.9	23.2	1.361	
AKL	4.00	1.61	0.095	93.1

Table 2.4 shows the total phenolic content of lignin samples before and after acetylation and the degree of substitution of phenolic hydroxyls. Although also aliphatic hydroxyls groups participate in the chemical modification reaction, the degree of substitution was calculated taking account only phenolic hydroxyls groups. The lower degree of substitution achieved for commercial lignin was probably due to the high ash content, which affected to the modification. Moreover, a higher degree of modification was observed for lower molecular weights.

2.4.3. Thermal properties of lignins

The DSC curves of lignin before and after chemical modification are presented in Figure 2.4. DSC technique is the most accepted method to define glass transition temperature (Tg) of lignin polymer.

Normally, the Tg values of various non-derivatized lignins range from 90 ºC to 180 ºC[26]. However, glass transition temperature of lignin is often difficult to detect due to the complex structure and heterogeneity of this polymer. Therefore, it is sometimes possible to detect the range of the change in the curve[27]. Unmodified lignin has a relatively high glass transition temperature because of the condensed rigid phenolic moieties and strong intermolecular hydrogen bonding interactions, which restrict the thermal mobility of lignin molecules resulting in a high Tg[93].

After acetylation treatment, a clear reduction of the glass transition temperature was observed in all cases. During acetylation process, hydroxyl groups were replaced by ester substituent ($-COOCH_3$). Thus, reduce the number of hydrogen bonding and lead an increase free volume in the molecule and thus mobility of the chains[94].

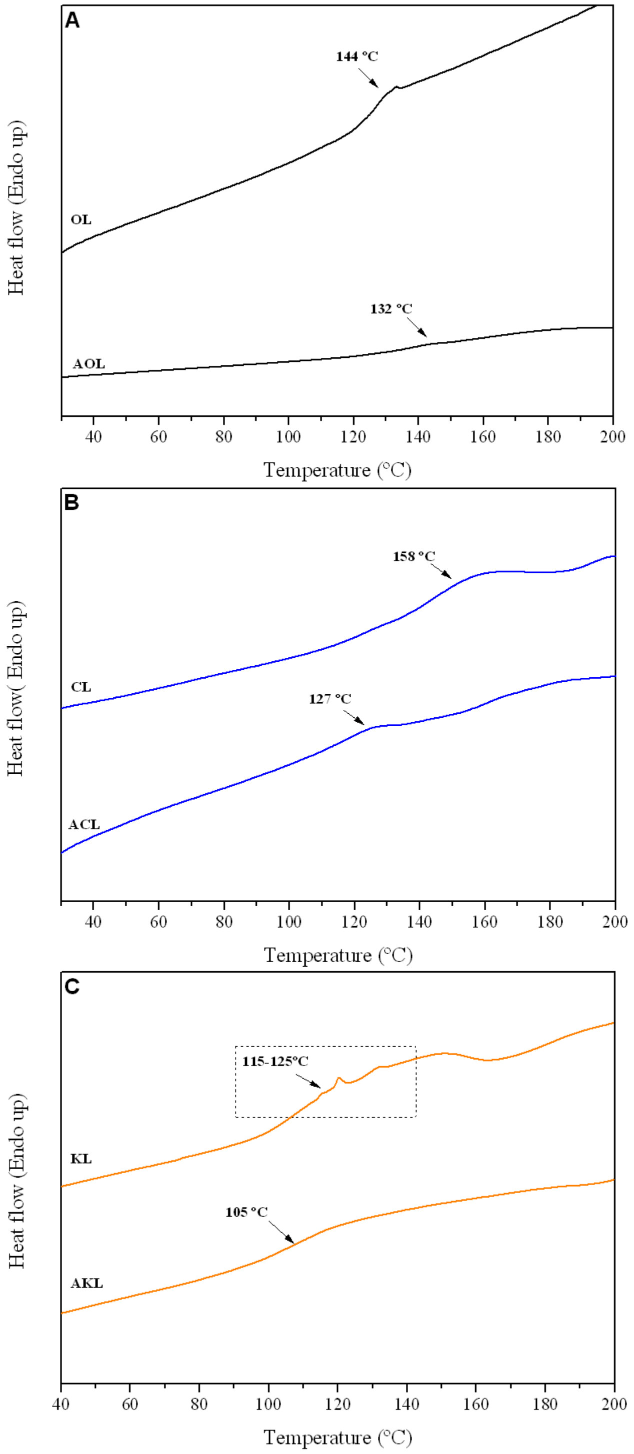


Figure 2.4. DSC of (A) OL and AOL, (B) CL and ACL and (C) KL and AKL.

For the use of lignin as an additive in the polymer industry, is important to determine their thermal degradation profile since thermoplastic polymers are usually processed at elevated temperatures and consequently lignin should be stable at processing temperatures employed[49,61].

TGA and DTG curves obtained for unmodified and acetylated lignins under nitrogen atmosphere are presented in Figure 2.5, 2.6 and 2.7, with detailed data listed in Table 2.5. Significant differences were observed between original and acetylated lignins, evidencing changes in the molecular structure of lignin samples after chemical modification.

All unmodified lignins presented a small weight loss (1-8%) below 100 ºC due to gradual evaporation of moisture. KL showed the higher moisture content, probably due to its high content of phenolic hydroxyls groups that makes it have higher water affinity. However, acetylated lignins did not show any weight loss below 100 ºC because, after acetylation, lignins became more hydrophobic.

Furthermore, as is showed in Table 2.5., the initial degradation temperature ($T_{10\%}$) of acetylated lignin samples is significantly higher than unmodified lignins, especially in the case of OL and KL, starting to decompose 80 ºC and 100 ºC later, respectively. Moreover, the main degradation step, which occurred in the temperature range between 300 and 500 ºC, also showed an improvement respect to unmodified lignins. In addition, except for CL, the weight loss at maximum degradation rate was lower for acetylated lignins, indicating that besides degrading later, also do it slowly.

Overall, it was found a lower amount of char residue at 800 ºC for acetylated lignins except in the case of OL. Similar results were reported by other authors[61] indicating that acetylation was a suitable route to enhance the thermal stability of lignins and make it more compatible with the actual processing techniques in the polymer industry.

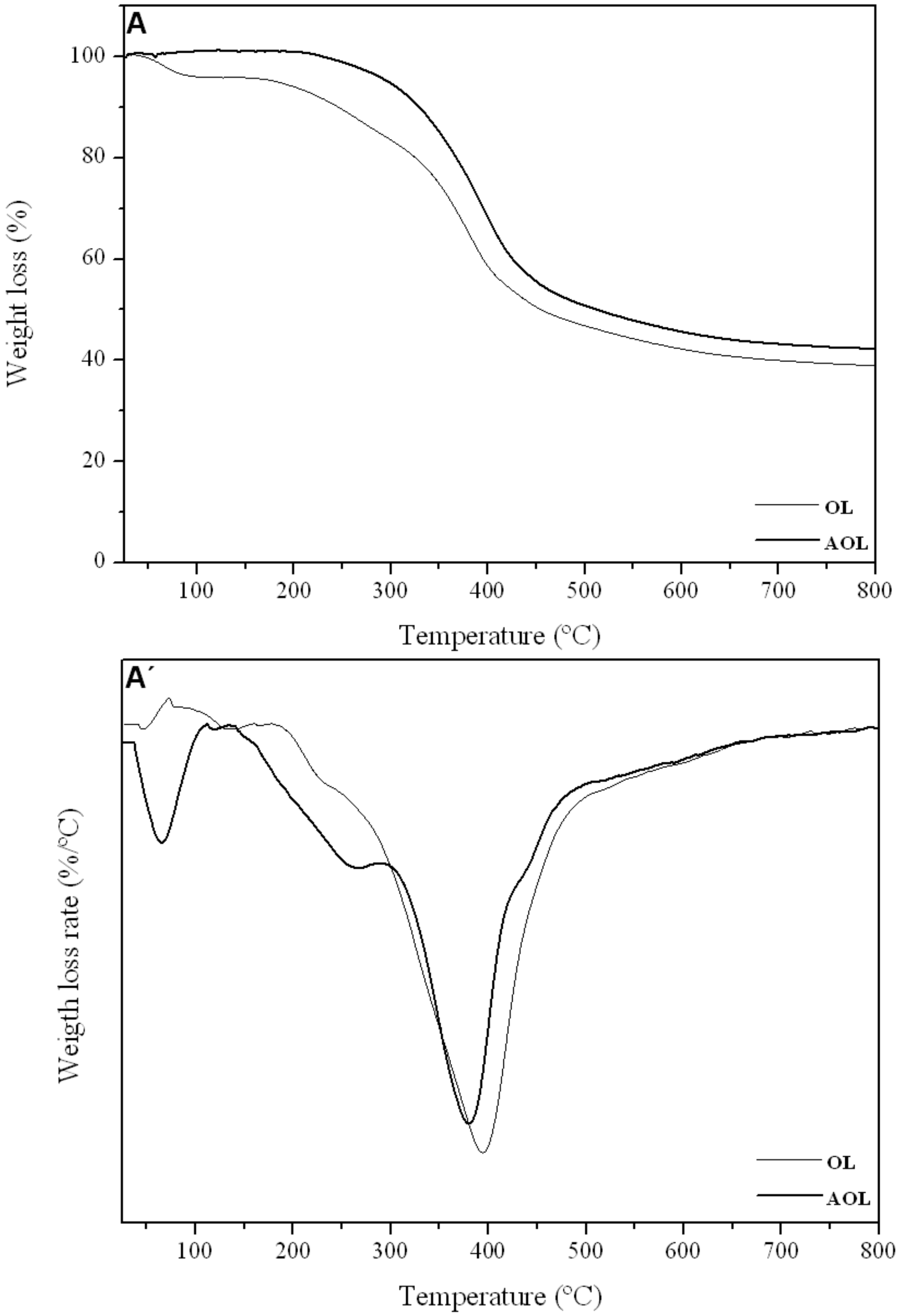


Figure 2.5. (A) TG and (A´) DTG curves of OL and AOL.

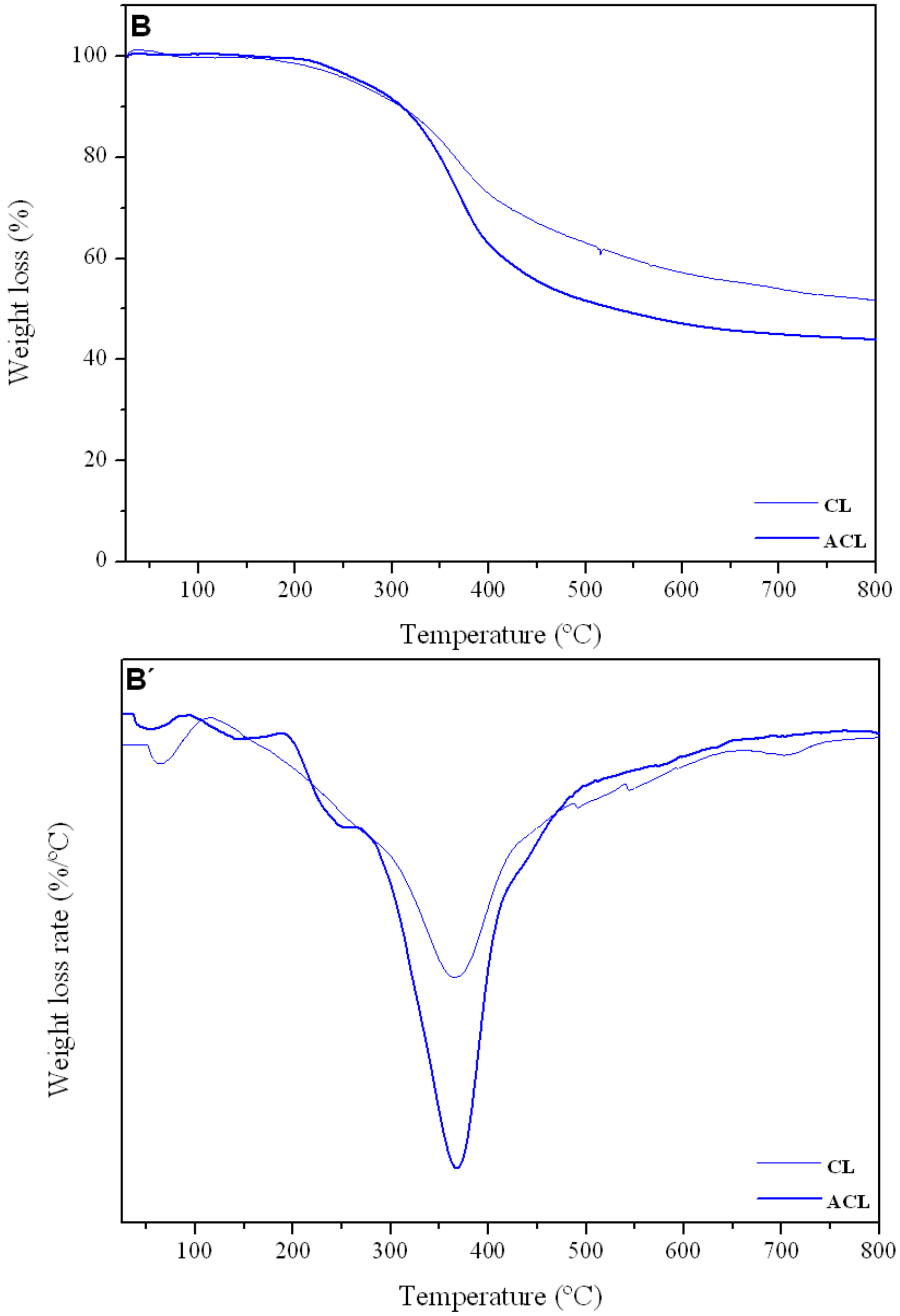


Figure 2.6. (B) TG and (B´) DTG curves of CL and ACL.

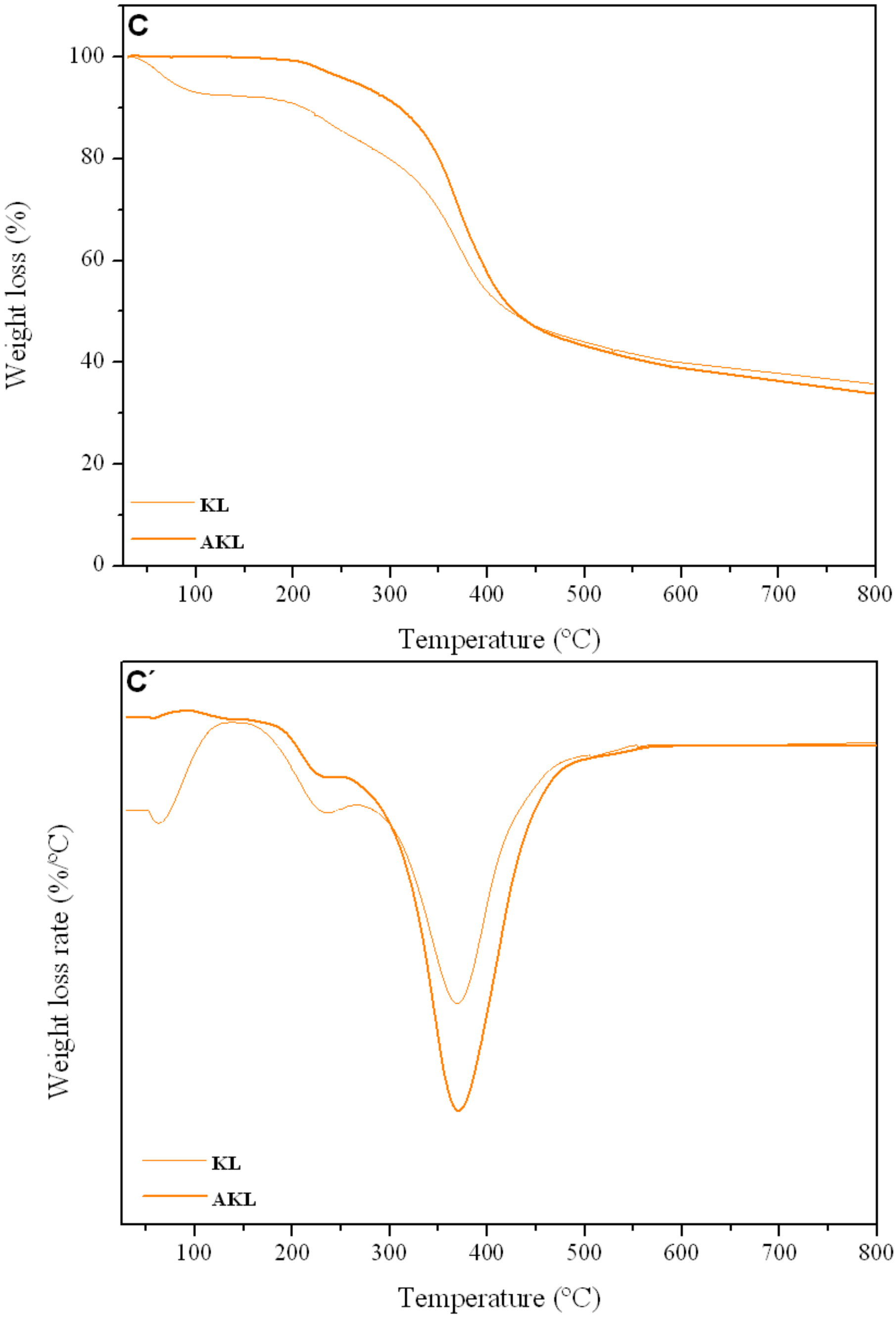


Figure 2.7. (C) TG and (C´) DTG curves of KL and AKL.

Table 2.5. Detailed data obtained from TGA measurements of unmodified and acetylated lignins.

Sample	**$T_{10\%}$ (ºC)**	**$T_{50\%}$ (ºC)**	**T_{max} (ºC)**	**wt (%)**	**Char Residue at 800 ºC (%)**
OL	245.6	454.2	380.2	34.8	38.8
AOL	329.8	513.2	395.5	26.7	42.3
CL	309.4	---	365.1	20.2	51.8
ACL	311.0	530.8	367.9	26.9	44.2
KL	211.7	423.5	369.3	36.4	35.9
AKL	310.7	428.1	372.8	30.5	33.9

2.4.4. PLA-lignin blends appearance and morphology

The appearance of the elaborated blends was visually analyzed. Figure 2.8 shows the elaborated blends with original and acetylated lignins. As can be observed, PLA-OL and PLA-CL blends present poor compatibility between PLA matrix and lignin. In both cases, is possible to appreciate the presence of aggregates. However, in the case of PLA-KL blends, better compatibility was found, without the existence of visually appreciable lignin particles. As was reported before by Pouteau et al., 2003[51], low molecular weight tend to improve the compatibility, therefore the low molecular weight of KL could have had a positive effect on the compatibility with PLA.

Furthermore, the acetylation of lignin clearly improves the compatibility between both components. PLA-acetylated lignin blends seem homogeneous at a macroscopic scale without visible particles. Moreover, it is remarkable, the change of color from clear brown to dark brown when lignin content increased.

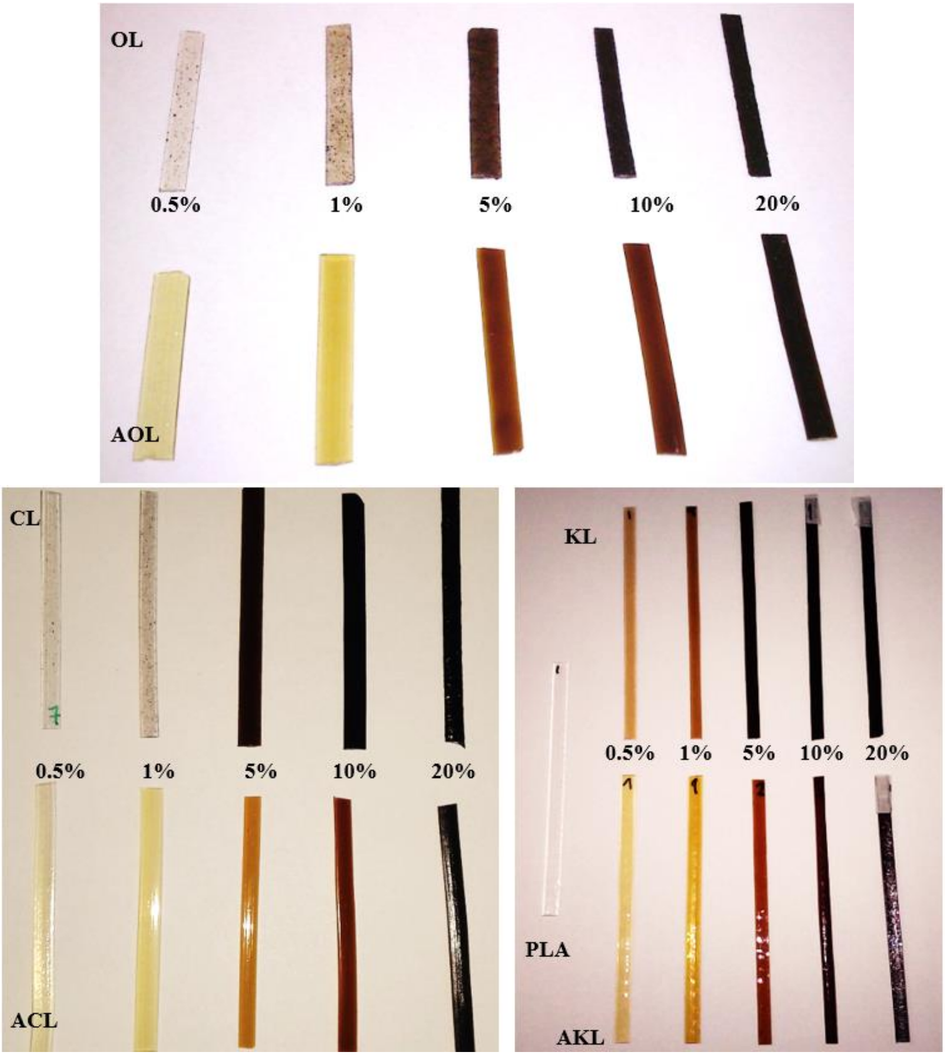


Figure 2.8. PLA-lignin and PLA-acetylated lignin blends prepared by extrusion.

In addition to visual analysis, optical microscope was used to analyze the morphology oh the extruded bends. Figure 2.9 shows micrographs of blends with 5% lignin (original and acetylated). As can be observed, blends with organosolv and commercial unmodified lignin presented aggregates due to incompatibility between PLA and lignin. However, as expected, PLA-KL blend revealed more homogeneous morphology, where kraft lignin particles were uniformly distributed along the PLA matrix. Moreover, it was evident the smaller size of kraft lignin particles (2-8 μm) compared to organosolv and commercial lignin particles (40-100 μm).

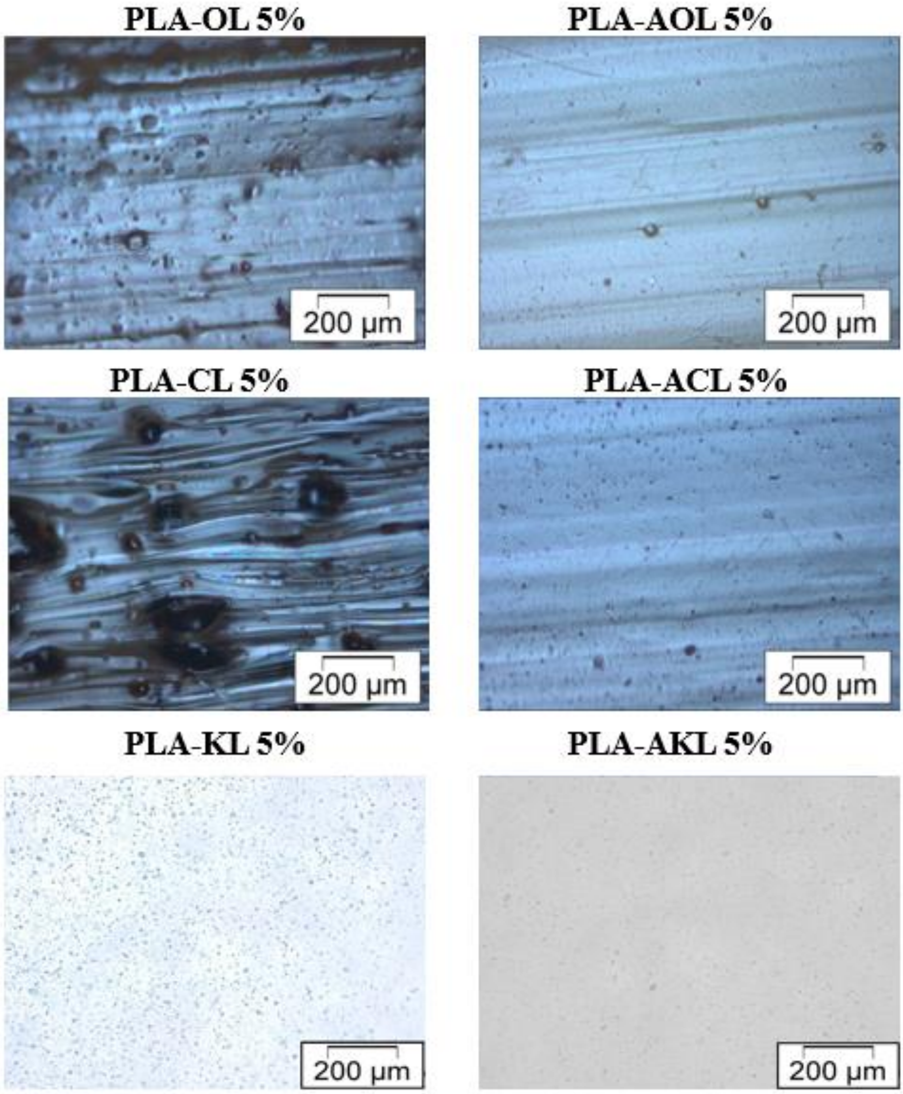


Figure 2.9. Micrographs of PLA-lignin and PLA-acetylated lignin blends with 5% of lignin prepared by extrusion.

Moreover, a clear difference in the morphology of blends with acetylated lignins was observed. Although blends with acetylated lignins also revealed the existence of lignin particles, less and significantly smaller particles than in blends with unmodified lignins were noticed. These results demonstrated that acetylated lignins presented greater compatibility with PLA matrix. Other authors also obtained an important compatibility improvement between polymer matrix and lignin after acetylation[95,96].

In order to check the size of lignin particles in PLA after acetylation, Atomic Force Microscopy was used. The results revealed the existence of spherical acetylated lignin nanoparticles (around 100 nm) homogeneously distributed in the PLA matrix. Figure 2.10 shows the image obtained from PLA-ACL 5%.

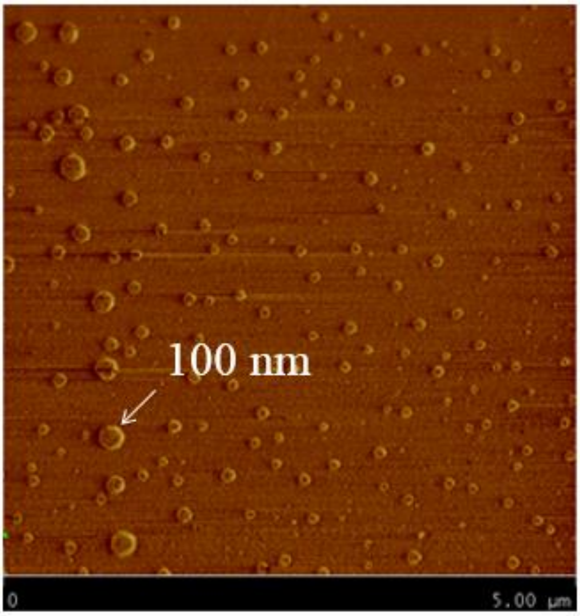


Figure 2.10. AFM non-contact mode height image of PLA-ACL 5%. The data scale is 5 µm x 5 µm.

2.4.5. Thermal properties of PLA-lignin blends

The effect of lignin's presence and content on the stability of the blends was studied by means of thermogravimetric experiments carried out inert atmosphere. TG and DTG curves of PLA-lignin and PLA-acetylated lignin with 0.5% and 5% are presented in Figures 2.11, 2.12 and 2.13. Moreover, Table 2.6 summarizes experimental data including initial degradation temperature ($T_{5\%}$), the temperature of 50% weight loss ($T_{50\%}$), maximum degradation temperature (T_{max}) and the char residue.

Although both PLA and PLA blends degraded in a single step, significant differences in thermal stability can be appreciated with the addition of unmodified and acetylated lignins.

In general, the initial thermal degradation temperature and the maximum degradation temperature of PLA increased with the addition of both unmodified and acetylated lignins. Moreover, higher thermal stability was observed for higher lignin content. However, no significant differences between the addition of unmodified and acetylated lignins were found. Char residue greatly varies depending on added lignin, but in general, can be said that the higher lignin content, the higher char residue is. Similar results were found by other authors[48,96,97].

Table 2.6. Thermogravimetric parameters of PLA blends.

Sample	**$T_{5\%}$ (ºC)**	**$T_{50\%}$ (ºC)**	**T_{max} (ºC)**	**wt %**	**Char Residue at 600 ºC (%)**
PLA	269.9	310.0	314.0	60.9	6.5
PLA-OL 0.5%	277.3	322.5	322.5	49.7	4.3
PLA-AOL 0.5%	282.0	326.0	326.1	50.5	4.4
PLA-OL 5%	339.8	363.0	363.2	50.6	10.2
PLA-AOL 5%	325.7	360.0	360.8	50.5	7.8
PLA-CL 0.5%	312.2	340.7	340.3	48.6	3.8
PLA-ACL 0.5%	313.7	341.3	340.3	47.7	4.3
PLA-CL 5%	341.9	365.4	365.3	49.2	8.7
PLA-ACL 5%	329.4	357.5	357.5	49.9	9.9
PLA-KL 0.5%	340.4	361.7	361.7	49.7	0.5
PLA-AKL 0.5%	328.6	360.4	360.6	50.4	0.2
PLA-KL 5%	336.3	364.1	364.2	50.5	2.1
PLA-AKL 5%	333.3	362.5	362.5	50.5	1.4

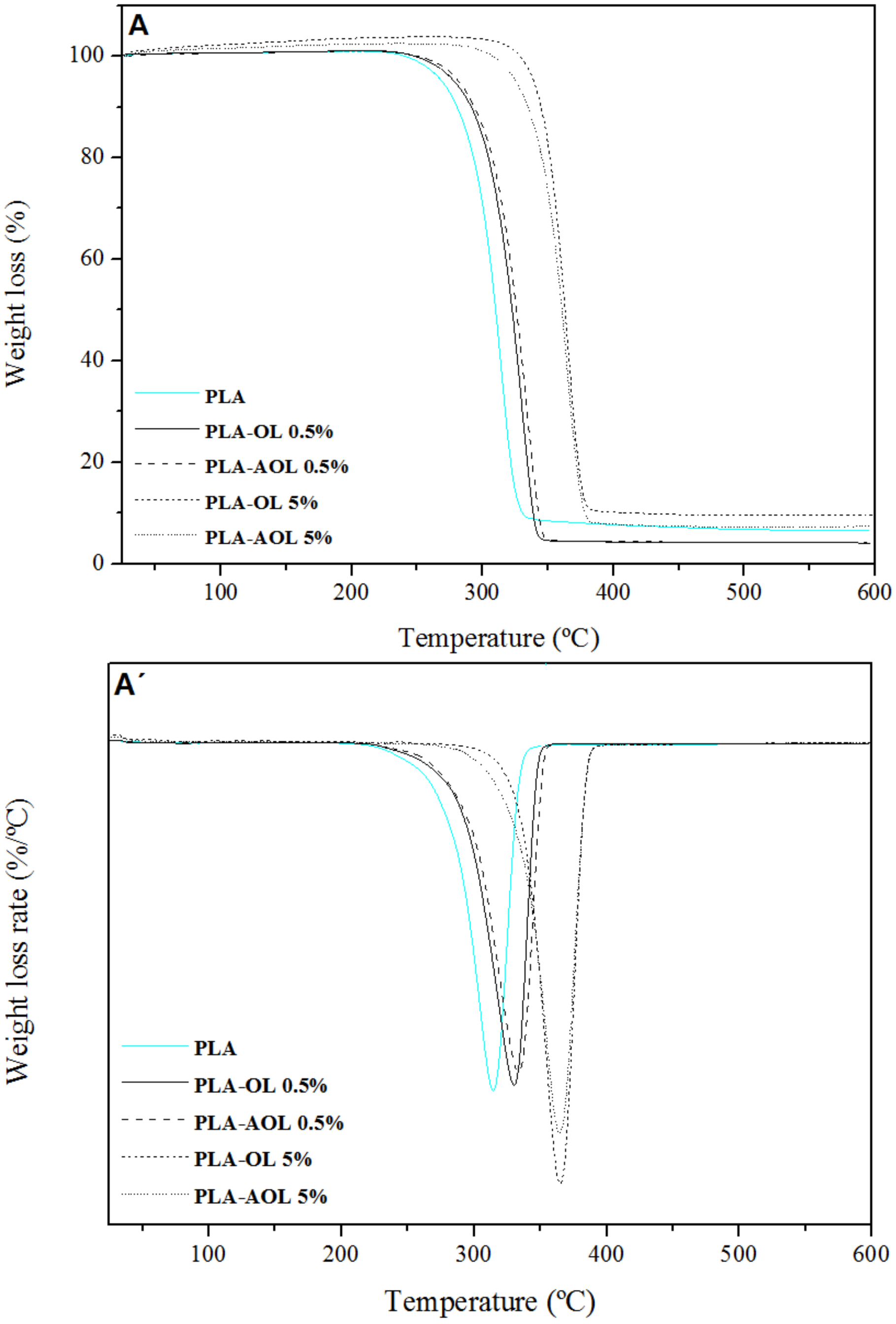


Figure 2.11. (A) TG and (A´) DTG curves under nitrogen atmosphere of PLA-organosolv lignin and PLA-organosolv acetylated lignin composites at 0.5% and 5%.

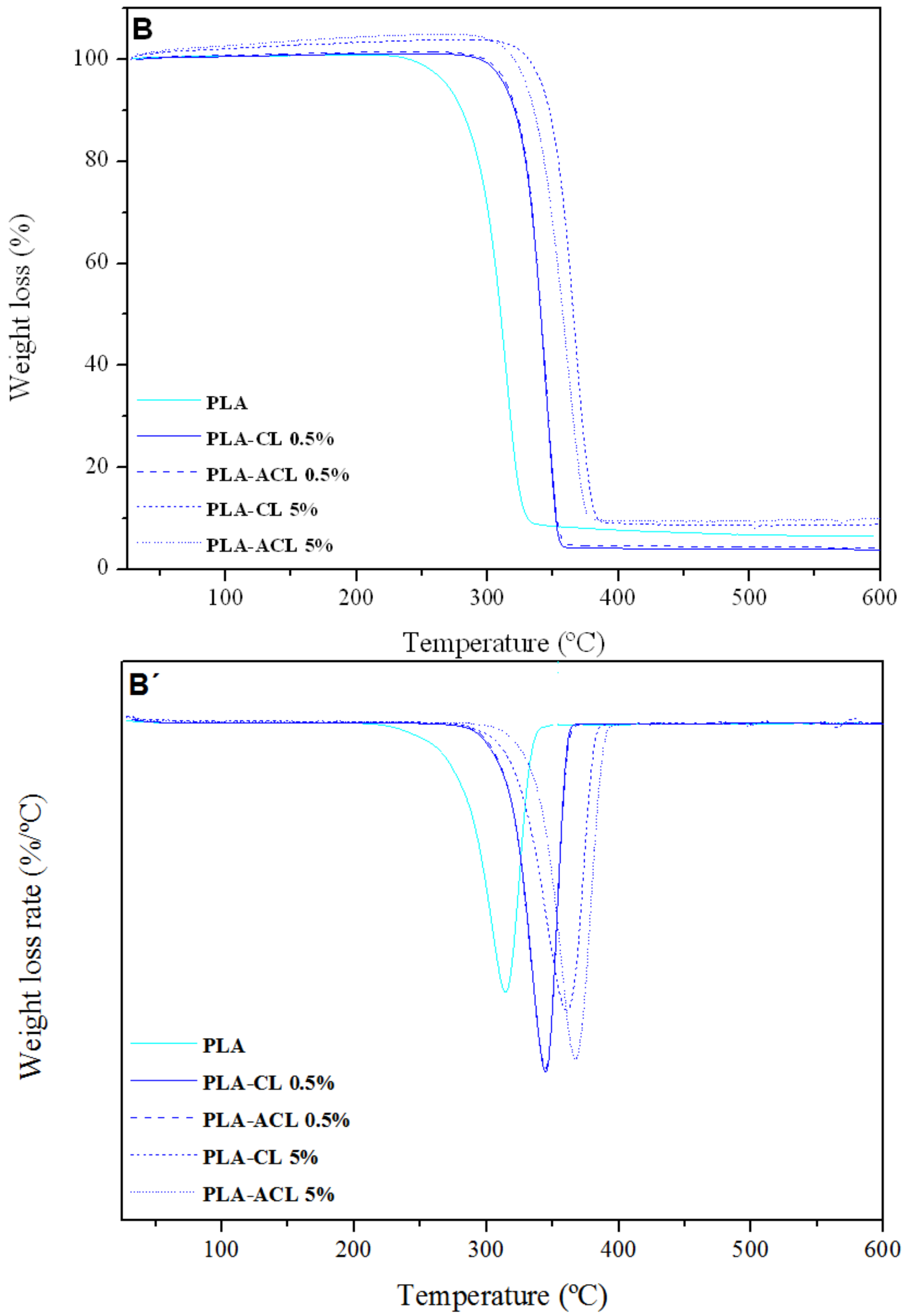


Figure 2.12. (B) TG and (B´) DTG curves of PLA-commercial lignin and PLA-commercial acetylated lignin composites at 0.5% and 5%.

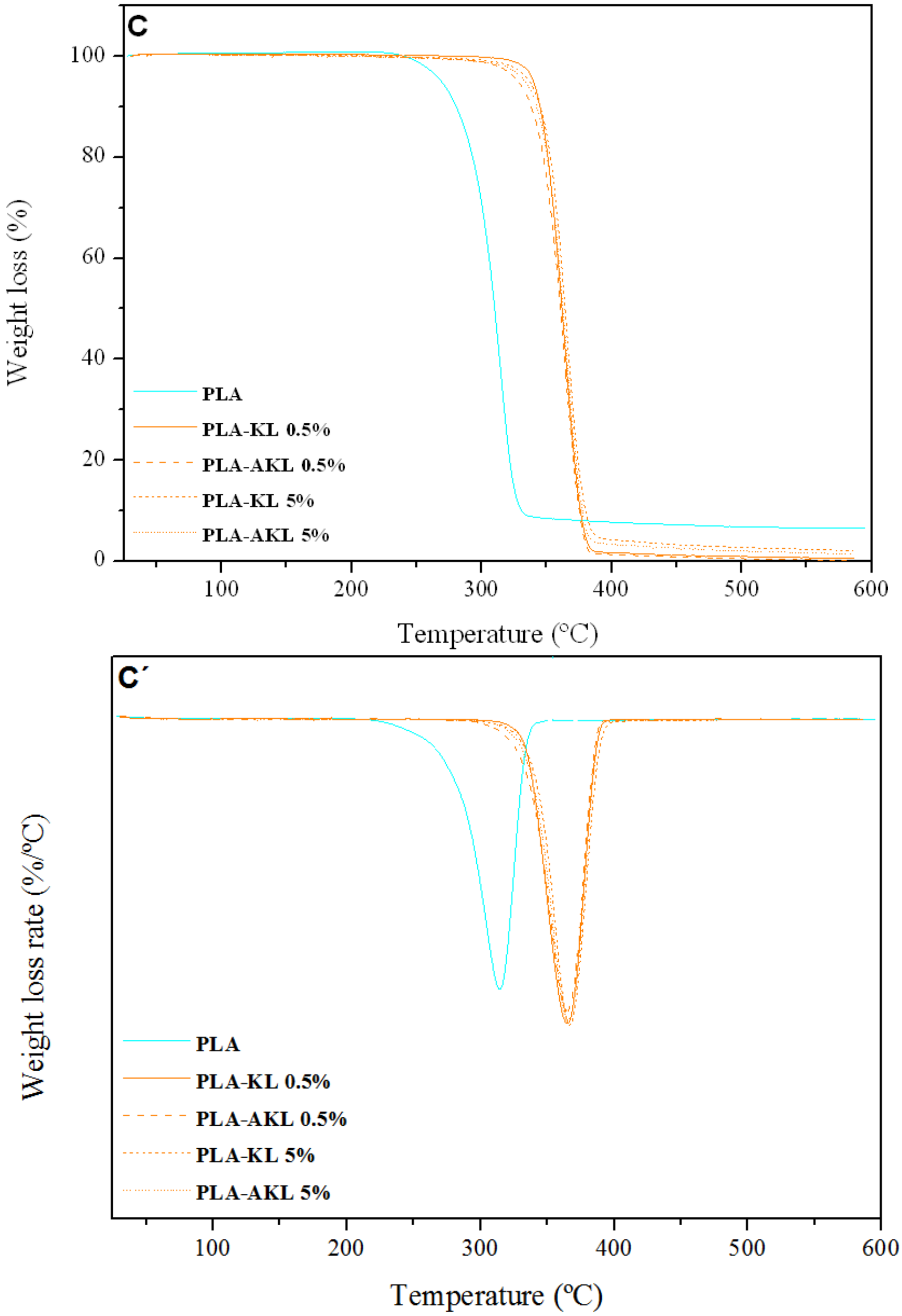


Figure 2.13. (C) TG and (C´) DTG curves of PLA-kraft lignin and PLA-kraft acetylated lignin composites at 0.5% and 5%.

2.4.6. Mechanical properties of PLA-lignin blends

Tensile tests have been performed to study the effect of three lignins on mechanical properties of PLA. The Young's modulus, elongation at break and maximum tensile strength of the different materials are shown in Figures 2.14, 2.15, 2.16.

The mechanical behavior of PLA was affected by the incorporation both original and acetylated lignins. Notable differences could be appreciated between the addition of original and acetylated lignins in all cases, proving that the chemical modification of lignin was beneficial and improve properties of the blends. Moreover, overall, the similar trend was observed by the addition of three different types of lignins.

The elongation at break was positively affected by the addition of low percentages (0.5 and 1%) of unmodified lignins, especially for OL and CL. However, the incorporation of more than 5% of unmodified lignin decreased the elastic deformation with increasing lignin content, mainly in the case of KL, which become very brittle material.

Moreover, low molecular weight esters usually can act as plasticizer[98]. Lignin can be considered a low molecular weight compound (2000-10000KDa) when there are compared to PLA (169000KDa). Other authors who prepared blends using cellulose triacetate as matrix and unmodified and acetylated lignin as filler found similar results; acetylated lignin also provided an improvement respect to unmodified lignin[99]. Other authors[56] also demonstrated a slight increase in the deformation of films formed with starch and lignin.

Although from 5% onwards also was reduced the deformation of blends, all blends with acetylated lignin, showed an improvement respect to blends with unmodified lignin, particularly, at high percentages.

The rigidity and the resistance of the PLA were reduced by unmodified lignins, especially at high contents. However, the Young Modulus and the maximum strength maintained similar to PLA with the addition of acetylated lignins up to 5%. Hence, the extent of decrease is much smaller for blends containing acetylated lignins. Blends with acetylated lignins presented a less rough surface due to the absence of agglomerations resulting in a better compatibility between PLA and acetylated lignins than PLA with unmodified lignins (see Figure 2.9).

The tensile strength of heterogeneous materials depends on the strength of interfacial adhesion, which is determined by the contact surface of the phases, where particle size in very important[6]. Therefore, the smaller size of the acetylated lignin increases the contact surface between both components. Moreover, after acetylation, due to the replacement of hydroxyls by ester substituents, weaker interactions among lignin molecules result. This is also confirmed by the reduction of glass transition temperature after acetylation treatment. Consequently, the combination of these two factors clearly has contributed to the improvement of blends after acetylation.

Another study in which there was elaborated a PLA/unmodified commercial lignin composite by similar methods, also observed a decrease in the stress at concentrations of 5% and 10%[100].

The obtained results can conclude that the incorporation of more than 5% of both lignins (acetylated and unmodified) worsen the elastic deformation as well as the rigidity and resistance of PLA. It can be due to the higher amount of glassy material (lignin) present in the blend, thus making it more brittle.

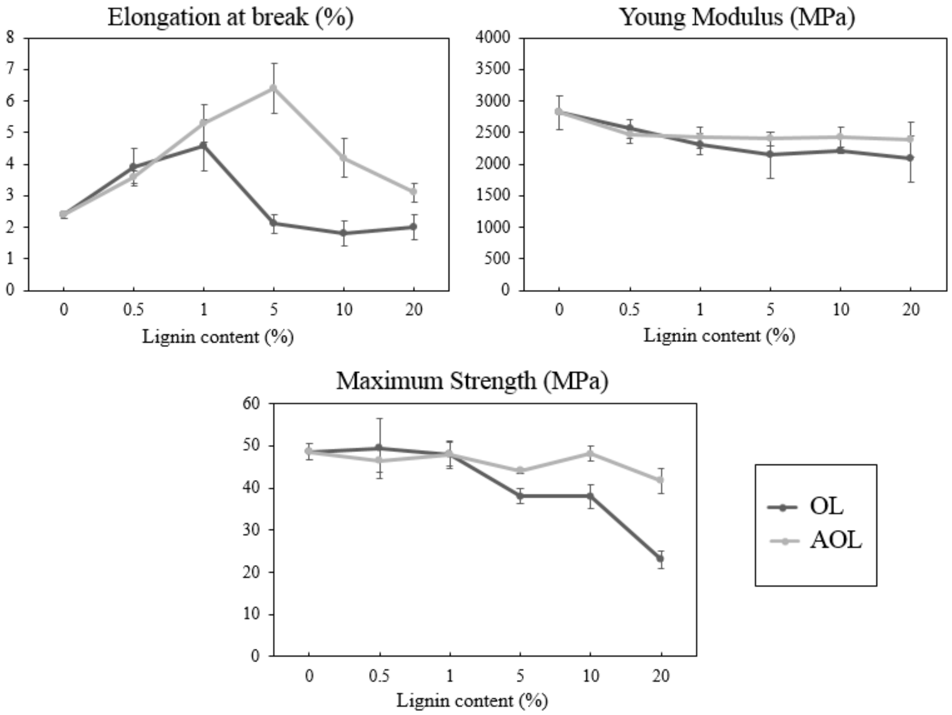


Figure 2.14. Mechanical properties of PLA-OL and PLA-AOL blends.

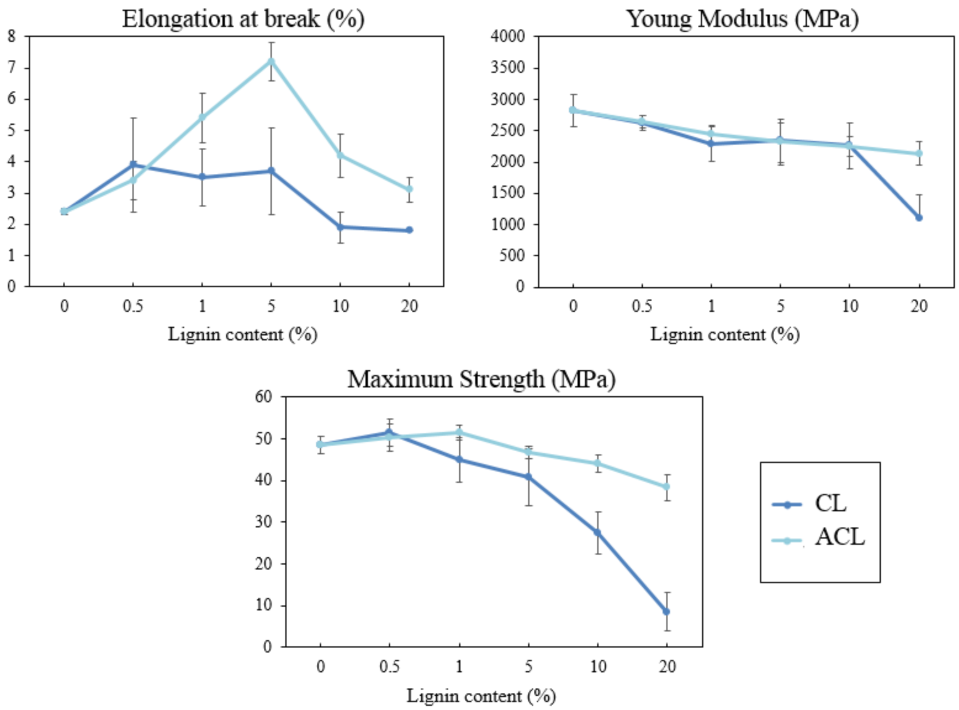


Figure 2.15. Mechanical properties of PLA-CL and PLA-ACL blends**.**

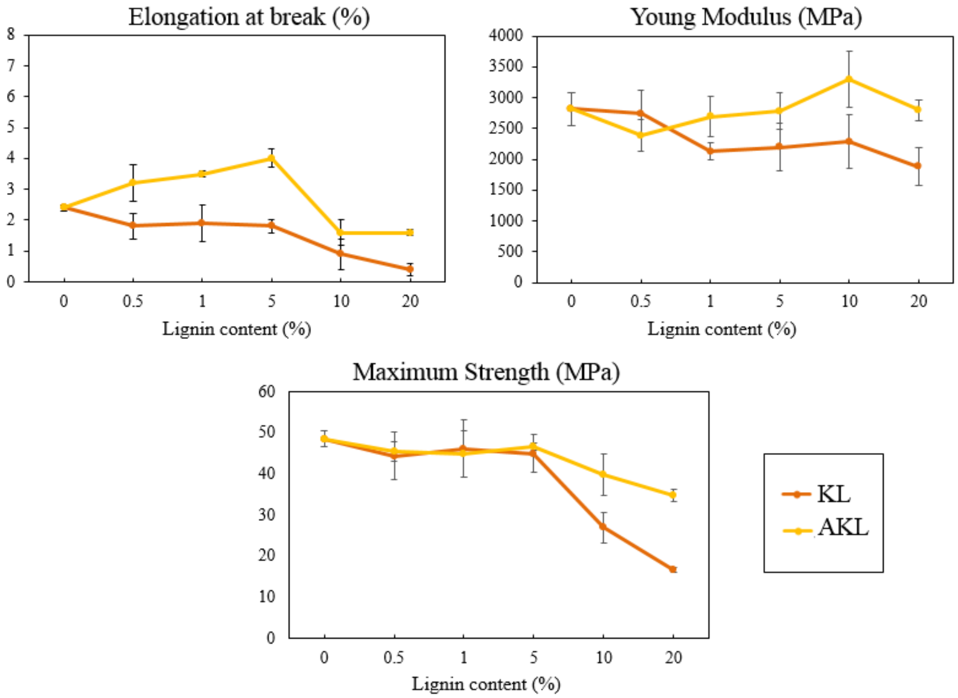


Figure 2.16. Mechanical properties of PLA-KL and PLA-AKL blends.

2.5. Conclusions

Lignin can be added to polymeric systems for the elaboration of blends using extrusion technique. Nevertheless, the addition of lignin into PLA had a significant effect on the PLA optical, mechanical and thermal properties.

The incorporation of unmodified lignins to PLA, in general, revealed poor compatibility with the presence of aggregates. However, the low molecular weight of lignin promoted the compatibility.

Acetylation was a suitable route to enhance the thermal stability of lignins and make them more compatible with the existing processing techniques in the polymer industry. Moreover, acetylation of lignins resulted in an easy treatment for improvement of the compatibility with PLA matrix. It had a positive effect on the mechanical properties. However, no more than 5% of lignin addition was recommendable. In addition, both unmodified and acetylated lignins provided higher thermal stability to PLA as an additive.

3

Lignin extraction and characterization of structure: Evaluation of functional properties

3.1. Motivation

The natural properties of lignin polymer, in addition to the growing commercial availability from the pulp and paper industry and the emergence of new biorefineries, make it a very attractive compound to be deeply studied.

Although the main functions of lignin in plants are well known, it was very interesting to determine if lignin maintains these properties once it has been isolated from the biomass. This chapter was focused on the study of industrial kraft lignins and lab-scale extracted organosolv lignins. The following research questions were the beginning of this chapter, aiming to contribute and motivate lignin valorization, thus improving the economic profit of the biomass fractionation industry.

- What are the main structural differences between industrial kraft lignins and lignin extracted at lab-scale by the organosolv process?
- What is really the potential of lignin to replace common synthetic antioxidants?
- Although lignin provides protection against chemical and biological attack in plants, does it present any inhibitory effect against microorganisms such as bacteria and fungus when it has been isolated?
- Can lignin be used as an additive for solid fuels?
- Is lignin able to act as natural sun blocker for applications as sunscreen additive to prevent the negative effects of sun exposure in the human skin?

In order to solve these questions, it was decided to study which were the most relevant differences between industrially available kraft lignins and lab-scale extracted organosolv lignins and how the chemical structure can be related to their properties. For that, wood from Eucalyptus and Spruce were selected as raw material for organosolv extraction. Both are widely used as feedstock for pulp and paper manufacture because of their high cellulose content and fast growth. Pulp industry of Latin America and Southern Europe are Eucalyptus consumers, while Spruce is more used in Nordic countries. Hence, this work offers a comparative study between organosolv lignins from Spruce and Eucalyptus and kraft lignins obtained from the industry.

3.2. Objectives

The main objectives of this chapter were the study of the chemical composition and structure of lignins from organosolv and kraft processes, as well as the evaluation of some functional properties. This work can be divided into 5 sections:

- Extraction of lignin: chemical, structural and thermal characterization.
- Analysis of the antioxidant capacity.
- Determination of antimicrobial properties.
- Evaluation of lignins as an energy source.
- Evaluation of lignin as sunscreen additive.

3.3. Experimental procedure

3.3.1. Materials

Spruce (softwood) and Eucalyptus (hardwood) were selected as raw materials for lignin extraction by the organosolv process. Eucalyptus kraft lignin (KE) was kindly provided by the Pulp and Paper Laboratory, Department of Forestry Engineering, Federal University of Viçosa, Brazil, and was used without any additional pretreatments. Softwood kraft lignin (KS) was Lignoboost lignin, Innventia AB, Stockholm, Sweden[101].

3.3.2. Lignin extraction processes

Firstly, Spruce and Eucalyptus woods were milled to obtain particles between 0.25-0.40 cm. Having taken into account the large experience of the groups on organosolv processes, it was chosen for the extraction of lignin from wood.

In case of Spruce, a mixture of ethanol-water (50/50 w/w) and sulfuric acid (H_2SO_4) as a catalyst (1.2% w/w) was used[102]. The treatment was carried out at 180 ºC for 60 min in a 4 L pressure (20 bar) stainless steel batch reactor with constant stirring (EL0723 Iberfluid) and with an electronic control unit for pressure and temperature control. The solid to liquid ratio was 1:7 (w/w). Dissolved spruce organosolv lignin (OS) was isolated by precipitation with four volumes of cold water.

For Eucalyptus wood, conditions reported by Dos Santos et al., 2014[103] were used. A mixture of ethanol-water (60/40 w/w) was used without a catalyst. The treatment was carried out at 180 ºC for 90 min in the same reactor. The solid to liquid ratio was 1:10 (w/w). Dissolved eucalyptus organosolv lignin (OE) was isolated by precipitation with acidified water at a pH of around 2. Precipitated lignins were recovered by filtration and then dried at 50 ºC.

3.3.3. Characterization methods

Isolated lignin samples were characterized by different procedures and techniques described in **Appendix I** and **Appendix II**, which are summarized in Table 3.1.

Table 3.1. Characterizations methods used for the analysis of lignins.

Analysis	**Technique or Procedure**	**Appendix**	**Section**
Chemical composition			
Klason Lignin (IL)	Acid-insoluble lignin	**I**	3.1
Carbohydrate content (%)	Py-GC/MS	**II**	2.3
Ash content (%)	TGA	**II**	3.1
Elemental analysis	Elemental analyzer	**II**	7.2
Chemical structure	FTIR	**II**	1.1
	^{31}P NMR	**II**	1.3
	^{13}C NMR	**II**	1.4
Molecular weight properties	GPC	**II**	2.1
Grass transition temperature	DSC	**II**	3.2
Thermal stability	TGA	**II**	3.1
Total phenolic content	Folin-Ciocalteu (UV)	**I**	4.1
Antioxidant capacity	DPPH (UV)	**I**	4.2
	ABTS (UV)	**I**	4.3
Antifungal capacity	Cellometer® Mini	**I**	5.1
Antibacterial properties	Length of inhibition	**I**	5.2
Proximate analysis	TGA	**II**	3.1
Sun protection factor	SPF (UV)	**I**	6.1

3.4. Results and discussion

3.4.1. Chemical composition and molecular weight

Results of the chemical composition analysis, together with molecular weight properties, are shown in Table 3.2. Carbon, hydrogen, and oxygen are the main components of lignin.

The purity of lignins expressed as Klason Lignin (%) showed high purity levels especially for OS (>90%) followed by KS and OE (>80%). However, KE presented low purity due to its very high ash content (>20%). Carbohydrates are usually the most known impurities in lignins; organosolv lignins presented higher sugar content than kraft lignins being OS the one with most carbohydrate content. For both kraft lignins, carbohydrate values were similar and corresponded to approximately 3% of all structures that were identified in the pyrolytic chromatogram.

Ashes, another type of impurity in lignins, were found in normal values, ranging between 2-4% for all lignins except for KE, which presented very high ash content (22.4%). Additionally, another interesting compound to control is sulfur content. It can be found free or covalently bonded to lignin structure. As expected, kraft lignins had sulfur-containing compounds because of their origin from pulp and paper production process. Besides, kraft lignins were also which showed the highest sulfur-free content in their composition.

With respect to molecular weight properties, it can be quite different depending on the feedstock and extraction process used[99]. As a result of different extraction conditions for each sample, no correlation could be made between the origin, isolation process and molecular weight characteristics of lignin. Typically, for organosolv lignins, lower molecular weights are reported for hardwood lignins than softwood lignins[43,102,104]. In this study, however, OE presented a higher molecular weight than OS probably due to the catalyst used in the extraction process.

The highest average molecular weight value was observed in KS lignin, while KE had the lowest Mw. GPC results showed that KE and OS lignins both had lower molecular weights and lower polydispersity. In general, all of the studied lignins presented high polydispersity, similar to results previously obtained by Baumberger et al., 2007[105].

Regarding the monomeric composition of lignin polymer, Figure 3.1 shows a schematic representation of the chemical composition. As was expected, no syringyl units were detected in spruce lignins (OS and KS) while eucalyptus lignins (OE and KE) are composed by *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) units in different proportions.

Table 3.2. Chemical composition and molecular weight properties of lignins.

	OS	**KS**	**OE**	**KE**
Klason Lignin (%)	94.3	88.5	83.7	58.6
Ash (%)	3.2	2.5	3.6	22.4
Carbohydrates (%)[a]	9.7	2.7	4.1	2.9
C (%)	68.8	63.7	61.4	49.8
H (%)	6.3	6.1	6.0	5.0
N (%)	0.08	0.10	0.13	0.11
S (%)	0.20	1.45	0.06	0.78
H/G/S [a]	23.1/76.9/nd	17.8/81.9/nd	6.1/31.0/62.9	12.4/23.4/59.2
S-containing (%)[a]	nd	0.26	nd	0.87
Mn [b]	1065	1540	1567	1059
Mw [b]	3081	7195	5079	2653
IP [b]	2.9	4.7	3.2	2.5

[a] Determined by Py-GM/MS.

[b] Determined by Gel Permeation Chromatography.

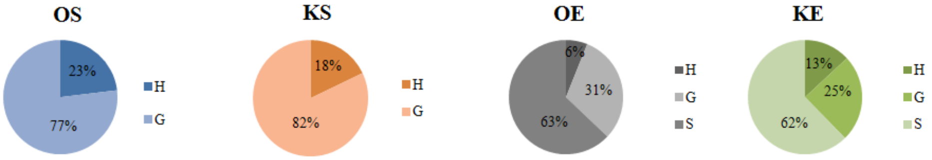


Figure 3.1. Composition of lignins according to H/G/S ratio.

3.4.2. Structural analysis

FTIR spectra of lignins are shown in Figure 3.2. All lignin samples showed a wide absorption band at 3400 cm^{-1}, which indicated the presence of O-H stretching vibrations in aromatic and aliphatic hydroxyls groups[106]. Bands around 2930 and 2840 cm^{-1} can be assigned to C-H stretching in $-CH_2-$ and $-CH_3$ groups[27]. Kraft lignin samples presented a peak at 1740 cm^{-1}, corresponding to stretching of carbonyl groups (C=O) in an ester or carboxylic acid group[99]. On the other hand, the small band al 1710 cm^{-1} indicated the presence of non-conjugated carboxylic acids in the lignins[27] and was only visible for organosolv lignins. A certain number of carboxylic groups were also quantified using the ^{31}P NMR method (Table 3.3), with the highest quantities being observed for the kraft lignins (KS and KE).

Signals between 1400 and 1700 cm^{-1} were attributed to the aromatic skeletal vibrations. The peaks at 1595 and 1510 cm^{-1} were due to C=C of aromatic skeletal vibrations. The bands found at 1460 and 1420 cm^{-1} were attributed to the C-H deformation in -CH_2-and -CH_3 groups and C-H aromatic ring vibrations, respectively. The band at 1365 cm^{-1} corresponded to aliphatic C-H stretching in -CH_3 groups. The band at 1325 cm^{-1} can be attributed to the presence of syringyl units (C-O stretch), but it could only be found in eucalyptus lignins. However, the peak at 1265 cm^{-1} assigned to guaiacyl (C-O stretch) ring appeared in all samples, although it had greater intensity in spruce lignin samples. Some characteristic bands associated with syringyl and guaiacyl units in lignin were detected at 1220, 1110 and 1030 cm^{-1}, corresponding to C-C, C-O and C=O stretching (G), aromatic C-H in-plane deformation (S) and aromatic C-H in-plane deformation (G > S)[107–109]. In addition, it could be observed that the band at 1110 cm^{-1} was very intense in eucalyptus lignins, but practically negligible in spruce lignins. However, the band at 1025 cm^{-1} that corresponds to G units was similar in all lignin samples. Meanwhile, the signal of aromatic C-H out-of-plane deformation in G and S units appeared at around 840-820 cm^{-1}.

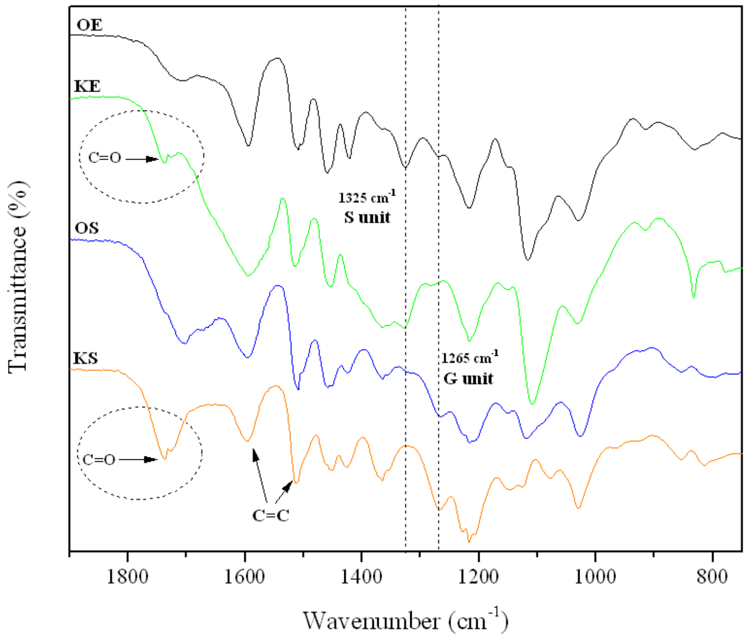


Figure 3.2. FTIR spectra of lignins.

Moreover, the functional groups of the lignin, such as hydroxyl and carboxylic groups, can easily be quantified by ^{31}P NMR[110], thereby providing the total content of potential active sites in the lignin polymer structures. For the elaboration of new materials based on lignin polymer, knowledge of the chemical structure of lignins is very important. Identifying the different functional groups in lignin, such as aliphatic and phenolic hydroxyl groups, is essential for the chemical modification of lignin.

Table 3.3 shows the contents of functional groups presented in lignin samples. Signals of aliphatic and total phenolic hydroxyl group appeared at 149-146 ppm and 144-137.5 ppm, respectively. The peaks at 139.5 ppm and 142.5 ppm were assigned to guaiacyl and syringyl units, respectively. Condensed guaiacyl OH groups were found at144-141 ppm and, at 135 ppm, the signal was attributed to carboxyl groups[65,111]. C_5 substituted condensed phenolic OH (β-5, 5-5´, 4-O-5 linkages) were integrated into the region between 144.7-142.8, 141.7-140.2 and142.8-141.7, respectively[112].

Table 3.3. Contents of functional groups (mmol/g) determined by ^{31}P NMR.

Functional groups (mmol/g)	**OS**	**KS**	**OE**	**KE**
Aliphatic OH	0.75	1.75	1.58	1.24
Phenolic OH	2.99	4.04	2.12	2.73
S[a] OH	nd	nd	0.69	1.19
G[a] OH	1.79	2.56	0.80	0.76
Condensed G[b]	1.20	1.48	0.63	0.78
β-5	0.38	0.66	0.49	0.46
5-5´	0.59	0.91	0.36	0.34
4-O-5	0.35	0.47	0.66	1.13
Total phenolic OH	1,20	1.48	0.63	0.78
Total OH	3.74	5.79	3.70	3.94
COOH	0.23	0.40	0.11	0.88

[a] G: guaiacyl, S: syringyl

[b] Area between 144 and 140.5 ppm minus the area of Syringyl phenolic[24].

In both eucalyptus lignins (OE and KE) spectra appeared a peak at 142.5 ppm, which corresponded to syringyl units that were not visible in spruce lignins (Figure 3.3). As was previously mentioned, lignins from industrial liquors usually have large amounts of condensed structures that formed in the final stage of the pulping process[8,12,21,24]. The ammount of condensed structures was higher in spruce lignins, with more condensed structures observed for kraft than for organosolv lignins. Regarding the analyzed linkages related to condensed structures, a higher content of β-5 and 5-5´in both softwood lignins were detected. In softwood lignins, it is usual to find a higher content of β-5 and 5-5´, which contributes to the higher degree of condensation in the lignin molecule[31]. While β-5 linkages can be present in native lignins, the formation of 5-5´ linkages takes place during the pulping stage, especially for softwood lignins that consist mostly of guaiacyl-type units with a vacant C_5 position in the aromatic ring. These results agree with the general percentages of linkages present in different types of wood, as reported by other authors[2,10,41].

The content of phenolic OH groups was also higher for kraft lignins than for organosolv lignins. The kraft process generates lignins with a high content of phenolic hydroxyl groups due to the cleavage of α-aryl and β-aryl ether linkages of lignin[8]. Spruce lignin had a higher content of phenolic OH groups than the eucalyptus lignin samples, with an especially high value for KS lignin[8,24]. The lower content of phenolic OH in eucalyptus could be associated with those units present in hardwoods that are preferentially linked through phenolic hydroxyls to form ether bonds such as β-O-4 and 4-O-5[113]. The ^{31}P NMR analysis showed that eucalyptus lignins have a higher content of 4-O-5 bonds, especially KE lignin. As found by other authors and demonstrated in our work also organosolv pulping generated lignins with lower amounts of carboxylic groups[88].

Quantitative ^{13}C NMR is another analytical method that can provide useful information on the structure of lignin. ^{13}C NMR analysis of acetylated lignin samples was carried out in order to determine, for each lignin sample, the distribution of functional groups per one C9 unit. The assignment of signals was based on information found in the literature regarding other acetylated lignin samples[31,114–117]. All integrations were performed assuming the integration of the aromatic region (160-100 ppm) as 6 carbon atoms. The obtained results are set out in Table 3.4.

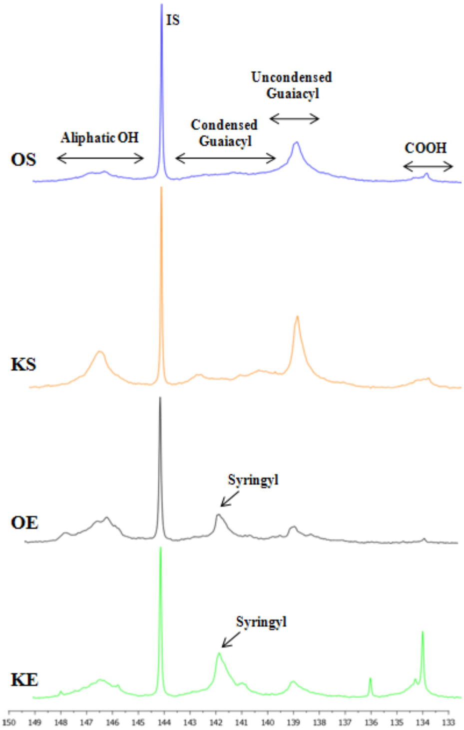


Figure 3.3. ^{13}C NMR spectra of lignins.

The content of phenolic and aliphatic hydroxyls groups determined after acetylation of the lignin samples showed the same trend as that evidenced by ^{31}P NMR. Each eucalyptus lignin had a different proportion of guaiacyl and syringyl units, while no syringyl units were detected in spruce lignins. The S/G ratios for both eucalyptus lignins were quite similar, 1.5 for OE and 1.6 for KE and are also in line with the results obtained from Py-GC/MS (Table 3.2). It is important to know the G and S content in lignins, as these units play a vital role for the use of lignins in the polymer industry[28]. Lignins with high guaiacyl content present a more condensable structure. However, for chemical modification, the lignin reactivity basically depends on the S/G ratio or the existence of uncondensed phenolic OH groups[18]. Low S/G ratio is more favorable for the chemical modifications. The reason for this is that the guaiacyl units are more reactive than syringyl units, as S units have the C_3 and C_5 substituted while G units have the C_5 position free.

Table 3.4. ^{13}C NMR of acetylated lignin samples.

Assignment	**(ppm)**	**Functional group per aromatic ring**			
		OS	**KS**	**OE**	**KE**
Aromatic C-O	160-140	1.74	1.62	1.84	2.04
Aromatic C-C	140-123	2.41	2.79	2.16	2.12
Aromatic C-H	123-100	1.88	1.60	2.00	1.84
Aliphatic C-O	90-58	1.31	0.32	2.14	1.24
Methoxyl OCH_3	57-54	0.70	0.85	1.19	1.14
CH3 acetyl	20	0.93	1.31	1.25	0.84
Primary Oac	170	0.15	0.33	0.33	0.23
Secondary Oac	169.4	0.02	0.70	0.33	0.18
Phenolic OH	168.1	0.63	0.77	0.46	0.50
S/G[a/c]		nd	nd	1.50	1.60
Degree of condensation[b]	123-100	1.12	1.40	1.00	1.16

[a] Based on the assumption that softwood lignin is made up of only G units and hardwood of S and G units.

[b] Calculated from $3.00\text{-}I_{123-106}$[31].

[c] S (104 ppm) and G (111 ppm).

The oxygenated aromatic C-O (140-160 ppm) and condensed aromatic regions C-C (123-140 ppm) have some degree of overlap, which makes the calculation of the degree of condensation less precise[118] (. The protonated aromatic region, on the other hand, does not suffer from overlap and, as a result, the degree of condensation can easily be estimated. Three minus the value of integration for this region will yield an estimate of the degree of condensation based on the assumption that completely uncondensed lignin will have protons at the C_2, C_5, and C_6 positions[31]. So, the content of aromatic C-H in the lignins could be closely related to the degree of condensation (DC). Due to the conditions during the isolation process, kraft lignins showed a higher degree of condensation than organosolv lignins. Moreover, softwood lignins presented a higher DC than hardwood lignins due to their structural features as being based almost entirely on guaiacyl units. In addition, both OE and KE had a higher content of methoxyl groups than softwood lignins due to the presence of syringyl units in their structures. Aromatic C-O content could be related to the ether bonds, so the lower content of aromatic C-O in softwood lignins may have been due to the lower content of ether bonds in their structures.

3.4.3. Thermal properties

The DSC curves of all lignins are reported in Figure 3.4. The glass transition temperature (Tg) is important to know when considering the use of lignins in polymer applications, but it is often difficult to determine. As was mentioned in the introduction, this difficulty is caused by the heterogeneity of the lignin chemistry, as well as the broad molecular weight distributions.

The results indicated that eucalyptus lignins presented lower Tg than spruce lignins, despite, for example, the higher molecular weight for organosolv eucalyptus than for spruce lignin (see Table 3.2). In general, hardwood lignins usually present lower Tg than softwood lignins, regardless of the extraction process used[21]. This may be because softwoods usually have a more condensed structure and higher phenolic hydroxyls group content than hardwoods, which restricts the mobility of the molecule by strong intermolecular hydrogen bonding interactions[93].

In the present study, as showed Table 3.3, spruce lignins (OS and KS) exhibited a higher content of condensed guaiacyl and phenolic OH groups than eucalyptus lignins (OE and KE). In addition, as seen from the ^{13}C NMR results, the degree of condensation of spruce lignins is higher than that for eucalyptus lignins, which resulted in their high Tg. Within the same species, on the other hand, Tg values correlated with molecular weights: OE presented higher Tg than KE, and KS presented higher Tg than OS.

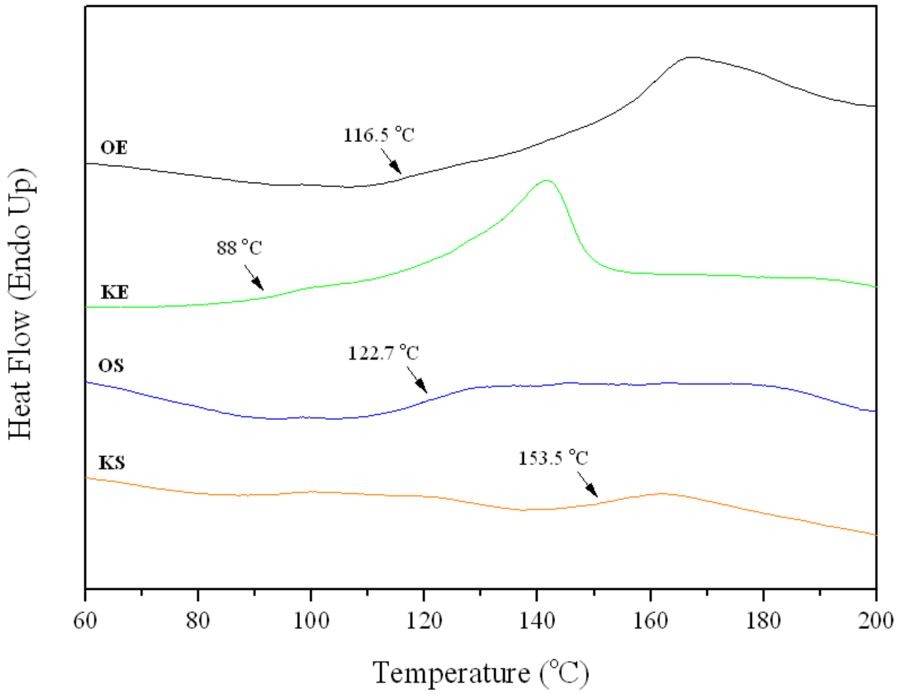


Figure 3.4. DSC of lignin samples.

Thermal degradation in polymers is another important parameter to know for thermal processing and to establish service life conditions. The lignin molecule has great potential for use it in the polymer industry. It can be used as a macromonomer or simply as an additive in other natural and synthetic polymers[21]. The thermal properties of lignin, therefore, are important when considering the impact of lignin addition to the polymeric system. In general, high molecular weight, high purity, and high thermal stability are required properties in the polymer field.

The thermogravimetric (TG) and first derivative (DTG) curves of lignins under nitrogen atmosphere are presented in Figure 3.5. All lignin samples exhibited various distinct weight loss stages. The first small weight loss (2-5%) below 100 ºC was assigned to the gradual evaporation of moisture.

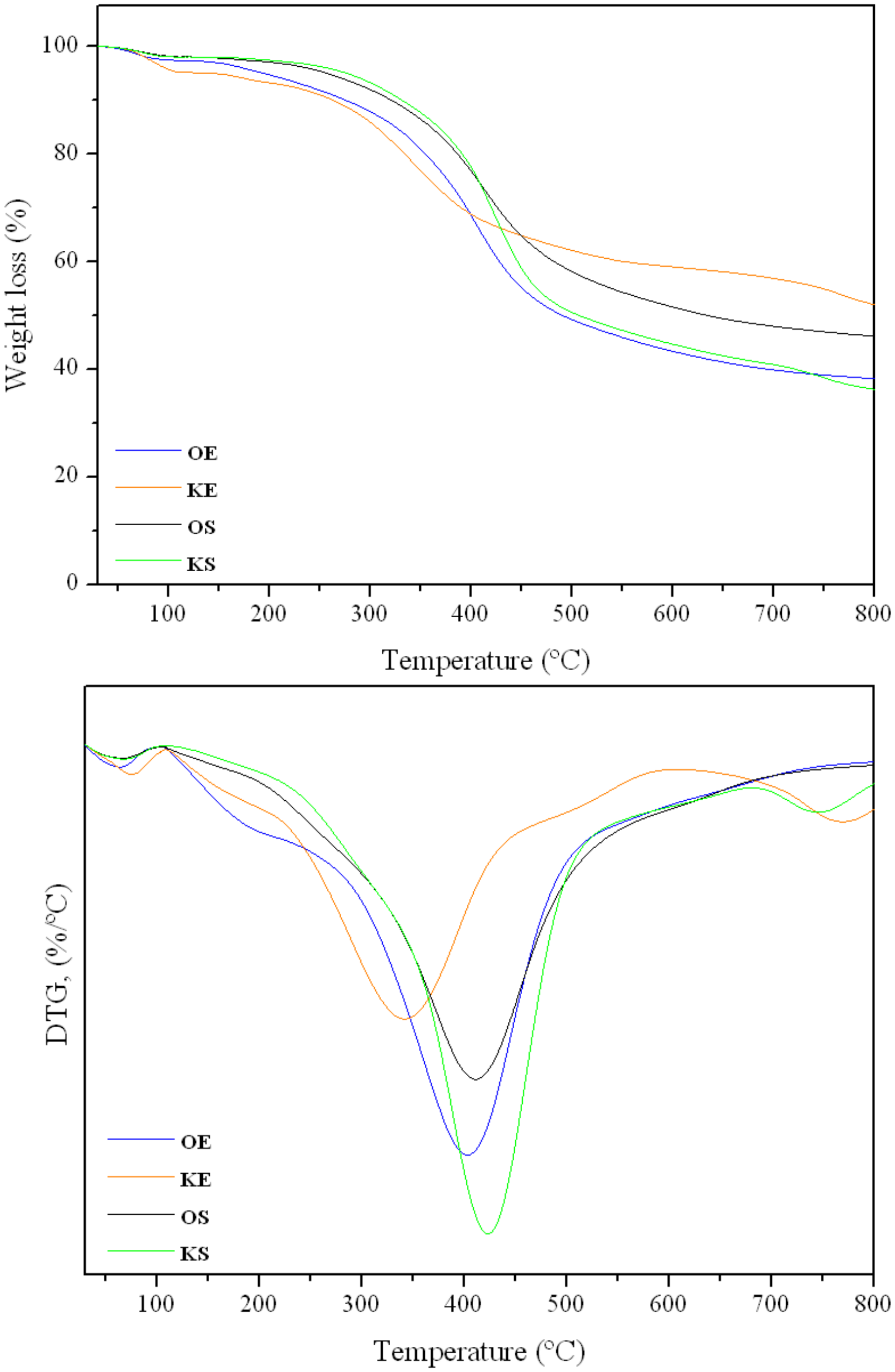


Figure 3.5. TG and DTG curves of lignin samples.

The main weight loss stage in all lignins occurs in the temperature range between 300 and 600 ºC[58] and is centered around 337-390 ºC. The main stage is associated with the fragmentation of inter-unit linkage[119], such as cleavage of typical ether linkages among the aromatic units. After that, cracking of aliphatic side-chains and cleavage of functional groups occurs[12,88,120]. The thermal stability of the samples was assessed at the onset temperature (determined as 5% weight loss based on a dry basis) of the main stage, and these parameters, together with the weight loss and maximum degradation temperature, are summarized in Table 3.5.

Table 3.5. Thermogravimetric parameters of different lignin samples.

Samples	**$T_{5\%}$ (°C)**	**$T_{50\%}$ (°C)**	**T_{max}(°C)**	**wt (%)**	**Residue at 800 ºC (%)**
OS	243.2	663.5	382.2	43.8	46.9
KS	262.0	507.9	390.7	52.8	38.8
OE	208.8	491.2	366.4	49.2	38.8
KE	239.5	>800ºC	338.9	33.5	46.9

Lignins from eucalyptus (OE and KE) presented lower thermal stability than spruce lignins (OS and KS). In general, it is common that hardwood lignins present lower thermal stability than softwood lignins[120]. The thermal stability of lignins depends, on the one hand, on the structure (functional groups and linkages) and, on the other hand, on their molecular weight and, therefore, on the origin and the applied extraction processes that determine the chemical composition of each sample.

The thermal stability of the samples showed a good correlation with the degree of condensation, with the highest values being those for the spruce lignins. As result, the KS exhibited the higher thermal resistivity. In addition, the spruce samples also showed higher Tmax than eucalyptus samples when subjected to the same extractive process. These results corresponded with those obtained by DSC and NMR techniques. Spruce lignins showed higher onset degradation temperatures and higher Tg than eucalyptus lignins, particularly KS, which also presented the highest content of OH groups and highest DC.

However, considering the influence of the isolation process on the same species, the Tmax did not show the same trend as the thermal stability, and higher values were exhibited for the OE than for the KE, as was evidenced in the Mw and Tg.

3.4.4. Antioxidant capacity and total phenolic content

ABTS and DPPH are simple indirect methods used for the evaluation of the antioxidant potential of lignins. In both methods, the radicals are reduced by the antioxidant, in this case, lignin, and the color change is measured by spectrophotometric technique[121]. In order to evaluate the inhibitory effect of lignin samples, BHT and Trolox were used as positive controls and the curves are shown in Figure 3.6. Moreover, Table 3.6 presents Efficient Concentration (IC_{50}) and phenolic content of the lignin samples.

As can be observed, the results of antioxidant activity of lignins did not show the same trend in ABTS and DPPH methods. These differences can be explained by the reaction mechanisms, in which ABTS radical reaction takes place at much faster rate due to electron transfer compared to DPPH[122]. Moreover, Wang et al., 1998[123] found that some compounds which showed ABTS scavenging activity did not have DPPH scavenging activity. Statistical analysis of antioxidant results (Table 3.6) showed that lignins OS, KS, and OE did not have statistical differences (Tukey and Bonferroni $p<0.001$) for IC_{50} value in ABTS method, indicating that IC_{50} value of KE lignin was significantly far from the other samples. In contrast, the IC_{50} value in DPPH method was statistically different for all lignin samples (Tukey and Bonferroni $p<0.5$). For this reason, it was considered that DPPH method could be more accurate than ABTS for the analysis of the antioxidant activity of lignins and the discussion was based on DPPH results.

According to previous studies, the phenolic groups have a high influence on the antioxidant activity of lignins[124,125]. However, the radical power of phenolics not only depends on the formation of the phenoxyl radical but also on its stability. Therefore, substituents like methoxyl groups and conjugated double bonds can stabilize phenoxyl radicals by resonance providing a positive effect on the antioxidant behavior of lignins[126,127]. In addition, having low molecular weight and narrow polydispersity is also beneficial for antioxidant properties[128]. Nevertheless, a conjugated carbonyl group has a negative effect on antioxidant activity[129], as well as impurities such as a high carbohydrate content, since the formation of hydrogen bonding with lignin block the formation of free radical[130].

In this study, the phenolic content of lignins did not present a correlation between the origin and isolation process presenting similar total phenolic content for OS, KS and OE with values between 33-35 mg GAE/g lignin. However, KE had a lower content of phenolic probably due to its low purity and high content of ashes. Moreover, the phenolic content determined by Folin-Ciocalteu did not show the same trend as the amount of phenolics determined by NMR techniques. However, being UV spectroscopy the technique used for the determination of the antioxidant capacity of lignins, it was decided to compare the results of phenolic content determined by Folin-Ciocalteu with the results of the antioxidant behavior of lignins. Moreover, in most of the works, which studied antioxidant properties of lignins, the results were compared to phenolics obtained by UV spectroscopy[125,131].

DPPH results showed a clear relation between lignin concentration and antioxidant activity, increasing the inhibitory effect at higher concentrations for all samples. The antioxidant activity of lignin samples decreased in the order OE > OS > KS > KE. Further, it was observed that antioxidant activity of OS, KS, and OE was higher than BHT control whereas it was lower than Trolox. However, KE presented lower antioxidant capacity than BHT as shows Table 3.6. Similar results are found by other authors[129,132,133]. Although OS, KS, and OE showed similar phenolic content, the stronger DPPH-scavenging capacity of OE was mainly due to the highest methoxyl content and lowest carboxylic acid content. On the other hand, the lower radical scavenging activity of KE could be partly attributed to the presence of very high content of carboxylic acids compared to other lignin samples as well as its low phenolic content together with its low purity.

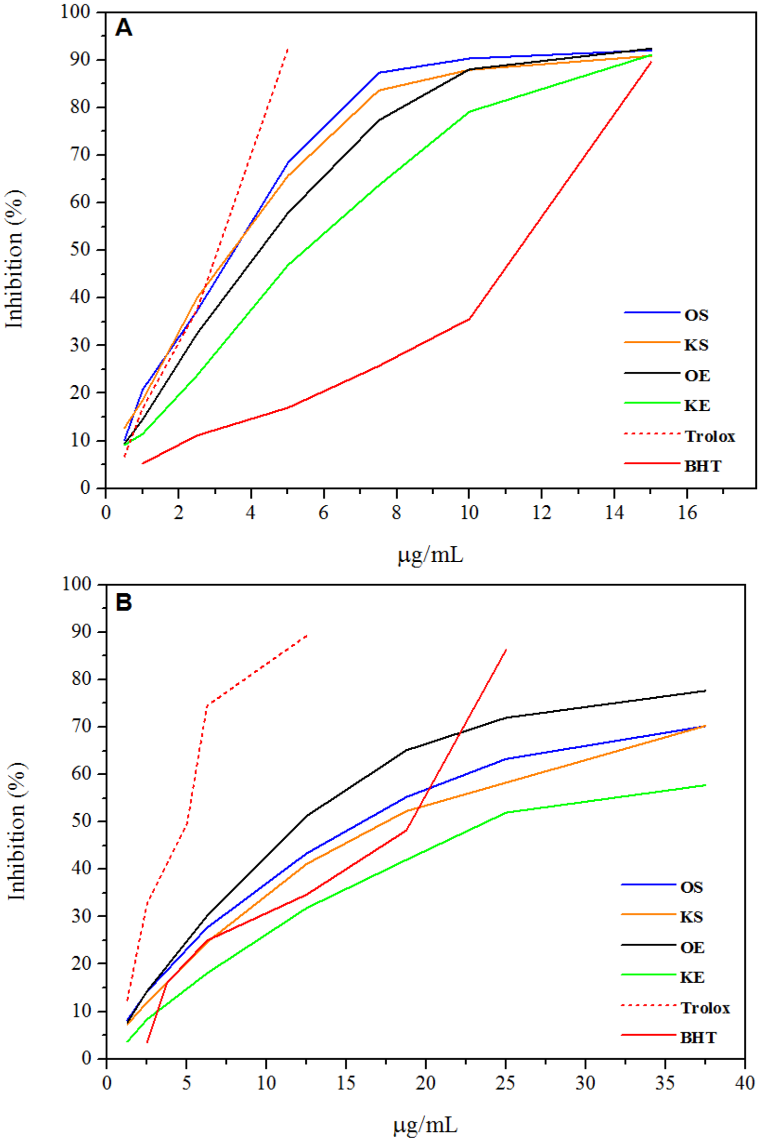


Figure 3.6. Antioxidant activity of lignin against (A) ABTS and (B) DPPH and their comparison with BHT and Trolox used as positive controls.

Concluding, the high antioxidant activity of the lignin samples, principally OS, KS, and OE, suggested that they could act as potential antioxidant using for food additive instead of using BHT. Table 3.7 shows the most common commercial antioxidants used in UE, together with their application field and some examples. In addition, recent publications have demonstrated the non-toxicity of lignin molecule for human tissues[128]. Therefore, new opportunities to lignin compound in the cosmetic industry would be interesting to develop.

Table 3.6. Phenolic content and efficient concentration (IC$_{50}$) of lignin samples.

	GAE	**OH (wt%)**	**ABTS***	**DPPH****
			IC$_{50}$	**IC$_{50}$**
OS	35.2	14.1	3.51 ± 0.1	15.85 ± 0.3**
KS	33.5	13.4	3.47 ± 0.4	16.63 ± 0.5**
OE	34.7	13.9	4.22 ± 0.3	12.85 ± 0.9**
KE	22.8	9.1	5.46 ± 0.2*	22.75 ± 0.3**
BHT	---	---	10.8 ± 0.8	19.01 ± 0.2
Trolox	---	---	2.80 ± 0.0	5.00 ± 0.1

Analysis of variance ANOVA: $p>0.001$; $p>0.5$ (Tukey and Bonferroni corrections:*= $p>0.001$ and **= $p>0.5$)

Table 3.7. The most common commercial antioxidants used in UE[134,135].

Name	**Application Industry**	**Examples**	**Negative effects**
L-ascorbic acid (E-300)	Food Cosmetic Pharmaceutical	Medicine (Pharmaton complex, Bayer pills)	none at usual doses
Butylated hydroxyanisole (BHA) (E-320) Butylated hydroxytoluene (BHT) (E-321)	Food Cosmetic Pharmaceutical	Tinned food, snacks, biscuits, sauces, drinks Make-up, face and body lotions Deodorant, ointments	toxic to skin, immune system, liver interferes with hormonal functions favors the growth of tumors

3.4.5. Antimicrobial properties

For the determination of antifungal activity, *Aspergillus Niger* was inoculated at different concentrations of lignin samples in order to know low, moderate and optimal potential inhibition effect of each lignin on cellulose pellets. Figure 3.7 shows the results of this analysis where three efficiency ranges were included in order to establish a clear difference in fungal inhibition power of lignins. Inhibition values higher than 75% were taken as optimal inhibition, values between 50-75% as moderate inhibition and values lower than 50% as low inhibition.

In general, both kraft lignins (KS and KE) presented higher inhibition potential than organosolv lignins (OS and OE). KS act as an antifungal product especially at low concentrations (up to 5%) while moderate inhibition potential was found at high concentrations. However, KE presented a quite stable inhibition power at studied concentrations with inhibition values between 73-87%. Organosolv lignins showed in general moderate inhibition effect. In addition, as can be observed, at high concentrations the inhibition effect decreased for all lignin samples, particularly in KS and OE (r=-0.749 and r=-0.482, respectively).

Only a few works have demonstrated that antifungal activity of lignin molecule depends mainly on the lignin origin and extraction method[136–138]. Moreover, their composition and chemical structure have a vital importance like Zemek et al., 1979[139] proved when studied different model compounds with guaiacyl and syringyl structure against some microorganism. Therefore, the higher inhibition power of kraft lignins could be due to their low carbohydrate content (2.5-3%) compared to organosolv lignins (4-10%), as well as the presence of sulfur and sulfur-containing derivatives as shown Table 3.2. Moreover, the high ash content (~23%) of KE could have an important influence on the antifungal inhibition effect since inorganic compounds usually are not desirable for fungus[140].

To sum up, regarding the antifungal study the authors conclude that kraft lignins could be more suitable than organosolv lignins for application as an antifungal product.

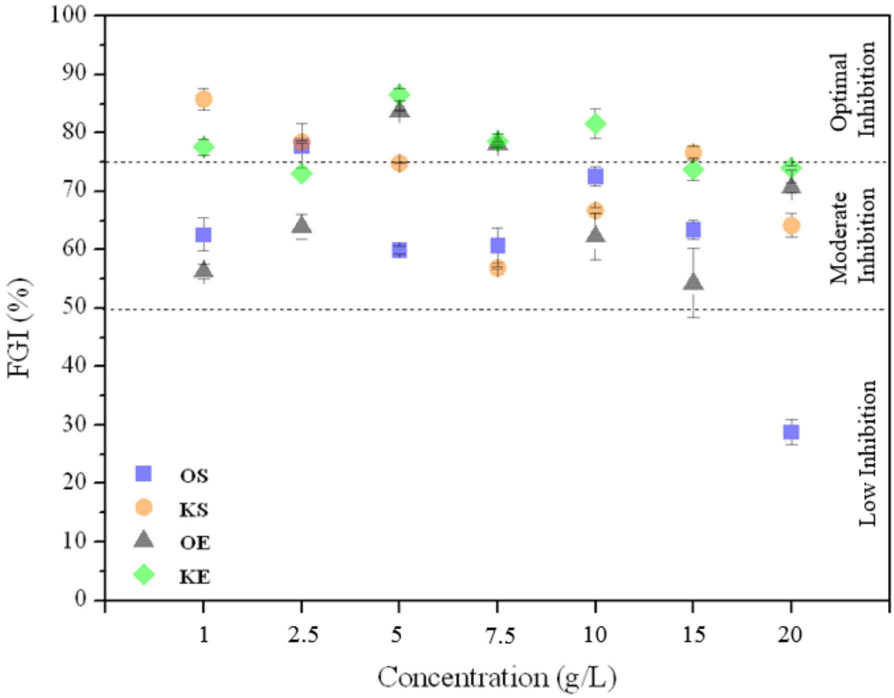


Figure 3.7. Fungal Growth Inhibition (%) of lignins.

Figure 3.8 shows the antibacterial activity of lignin samples against food and human pathogenic microorganisms. All the tested lignin samples exhibited greatly antibacterial affectivity against the studied bacteria.

KS lignin displayed greater antimicrobial activity against *E. coli* ATCC 25922 (17.72±1.16 mm), *P. microbilis* ATCC 14153 (19.12±1.22 mm), *P. vulgaris* ATCC13315 (24.68±1.31mm), *P. aeruginosa* ATCC 27853 (21.89±1.26 mm), *E. aerogenes ATCC13048* (18.97±1.08 mm), *S. aureus* ATCC 25923 (19.18±0.98 mm) and *B. thuringiensis* (24.76±1.19 mm) than tested KE lignin. In contrast, lignin of KE had higher antimicrobial property against microorganism *S. typhmurium* SL 1344 (33.36±1.42 mm) and *S. mutans* ATCC 25175 (17.03±1.05 mm) than KS lignin. On the other hand, OS lignin showed greater antimicrobial activity against *E. coli* ATCC 25922 (13.32±1.01 mm), *P. microbilis* ATCC 14153 (18.15±1.04 mm), *P. vulgaris* ATCC13315 (17.91±0.96 mm), *E. aerogenes* ATCC13048 (16.45±0.98 mm) and *B. thuringiensis* (18.36±1.09 mm) than tested OE lignin sample. In addition, OE lignin exhibited higher activity against *S. typhmurium* SL 1344 (28.92±1.36 mm), *P. aeruginosa* ATCC 27853 (16.10±1.06 mm), *S. aureus* ATCC 25923 (17.88±0.97 mm) and *S. mutans* ATCC 25175 (15.66±0.92 mm) than OS lignin form.

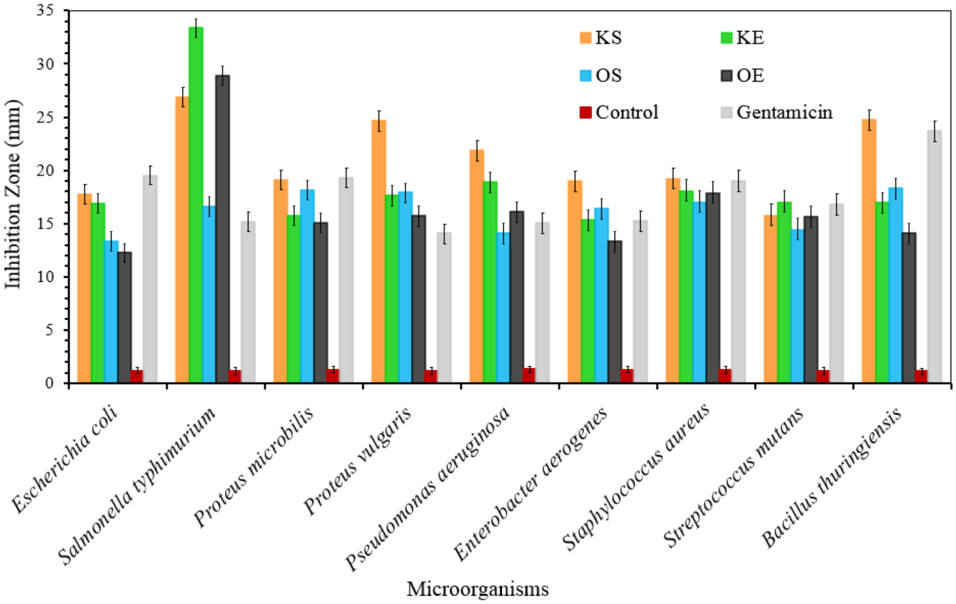


Figure 3.8. Antimicrobial activities of lignin samples expressed as inhibition zone diameter (mm).

Thus, it was obtained that almost similar bacteria culture showed similar sensitivity against Spruce lignins (KS and OS). In general, the antimicrobial activity of kraft lignins (KS and KE) had much higher than organosolv lignins (OS and OE). In all the tests, control disc with only solvent (DMSO) displayed slightly low (almost 1.2±0.06 mm) inhibition zone.

Interestingly, the antimicrobial activity of the kraft lignins (KS and KE) was recorded higher than commonly used antibiotic (gentamicin) against almost all tested microorganism. This high antimicrobial activity of the lignin was thanks to its rich antioxidant and polyphenolic nature[32,127,141]. Therefore, lignin obtained in our study could be used effectively as an alternative natural antimicrobial additive or agent in food, textile or chemical industry against pathogenic microorganisms.

3.4.6. Lignin as energy source

Proximate analysis was used to obtain information about moisture, ash content, volatile matter and fixed carbon in the samples[66]. The TGA is an appropriate technique for carrying out this assay[142]. The results of proximate analysis and HHV(MJ/Kg) are shown in Table 3.8 and have been calculated for dry lignin. The moisture content in all samples was 2-5%, which is similar to results for other lignins, but less than found in the literature for woods, annual plants, and agricultural residues[66,142–144].

Thermal conversion processes require low moisture levels because moisture greatly affects fuel combustion behavior, plant design, and transport costs[144]. The ash content also has a significant influence on the thermal conversion of lignin. This inorganic waste can affect transportation and processing costs and may generate harmful emissions and slag deposits that create greater thermal resistance to heat transfer and lead to corrosion[66,143]. In general, all lignins presented a normal value for ash content of 2-4%, when compared to other lignins and some species of wood and plants[144–146], but KE showed too high ash content (23%) due to the high content of salts formed during the extraction and precipitation processes.

In the present study, OE and KS presented normal values for volatiles of around 63% and for fixed carbon of 33%, while OS lignin showed lower values for volatile matter and high fixed carbon content. KE displayed very low values for volatiles and for carbon content, due to the high content of impurities in the studied lignin sample[68,147].

The heating values obtained varied between 18 and 24 MJ/Kg and were similar to those calculated by other authors[68]. These values are, in general, higher than those for the lignocellulosic biomass, which confirms lignins´ potential as an energy source[66,68,148]. It also indicates that volatiles and carbon content may have an important influence on the HHV. Lignins with higher volatiles and fixed carbon content are the most appropriate for pyrolysis and combustion processes due to their high HHV.

On the other hand, KE lignin presented the lowest HHV due to its low content of fixed carbon and volatiles and high ash content. For this reason, KE lignin is not suitable for these applications. In addition, this lignin could cause serious problems in boilers, as well as harmful emissions. Additional purification steps are necessary. Regarding the other lignins, although both sulfur and ash contents are above those recommended by European standards for pellets elaboration[149], they are not so high when compared with the other studied raw materials[144].

Table 3.8. Proximate analysis (% dry basis).

Sample	**Ash (%)**	**Volatiles (%)**	**Fixed carbon (%)**	**Organic matter (%)**	**HHV (MJ/Kg)**	
					Garcia et al., 2014[66]	Cordero et al., 2011[150]
OS	3.2 ± 0.3	55.0 ± 0.2	41.7 ± 0.0	96.7 ± 0.3	24.0	24.2
KS	2.5 ± 0.0	63.9 ± 2.0	33.5 ± 2.5	97.4 ± 0.0	23.0	22.8
OE	3.6 ± 1.9	63.4 ± 1.7	32.9 ± 0.0	96.3 ± 1.9	22.7	22.5
KE	22.4 ± 0.3	49.1 ± 0.0	27.2 ± 0.0	76.3 ± 0.1	18.3	18.0

3.4.7. Lignin as sunscreen additive

The *in vitro* SPF method is a useful quantitative test to check the effectiveness of the sunscreen product as a supplement of the *in vivo* measurements.

In this research, lignin samples were blended with a standard commercial cream (Cream-D) at 1% and 5% and their SPF values were determined using Mansur equation[51,136]. The SPF results are shown in Table 3.9.

Table 3.9. SPF Values of the pure cream blended with organosolv and kraft lignins from Spruce and Eucalyptus.

	Sun protection factor (SPF)	
Lignin	**1%**	**5%**
OS	11.3 ± 0.7	18.5 ± 1.2
KS	10.6 ± 0.7	12.2 ± 0.5
OE	11.2 ± 1.1	14.6 ± 0.5
KE	12.0 ± 0.8	19.7 ± 0.1

As expected, a great potential of lignins as sunscreen product was demonstrated. When lignin was added into the pure cream (cream-D), SPF values were increased. With the addition of only 1% to pure cream, SPF values of 10-12 were observed. Moreover, at 5% of lignin higher SPF values were noticed, especially for OS and KE following by OE, while KS remained almost similar.

Figure 3.9 showed that pure cream (Cream-D) had no absorbance in UVA and UVB areas; however, cream with SPF 50 (cream-D50) exhibited a transmittance of 0% demonstrating its high SPF. Higher lignin content decreased significantly the transmittance of elaborated creams.

The excellent UV-radiation absorber property of lignin molecule is mainly due to its chemical structure with chromophores such as carbon-carbon double bonds, carbonyl groups, and aromatic systems which affect on sunblock action[151–153]. In addition, auxochromes (hydroxyls groups and ethers) are important[154]. Apart from the structure and functional groups, Gutiérrez-Hernández et al., 2016[155] proved that the small size of lignin has a great positive effect on UV absorption properties of lignin. Moreover, Qian et al., 2016[151] found that lignin with more methoxyl groups and carbonyl groups has better sunscreen performance as well as the sunscreens of lignin improved after ultraviolet exposure due to auxochromes. In case of KE lignin, the highest sun protection factor found could be related to the highest content of methoxyl and carbonyl groups as well as the highest content of ether linkages in its structure. However, its high inorganic ash content, make it not suitable for sunscreen lotions. On the other hand, a high degree of condensation in lignins often causes a negative effect as sun blocker product[155]. Therefore, KS presented the lower SPF due to the highest content of condensed structures, in addition to having the lowest amount of ether bonds compared to the other lignin samples.

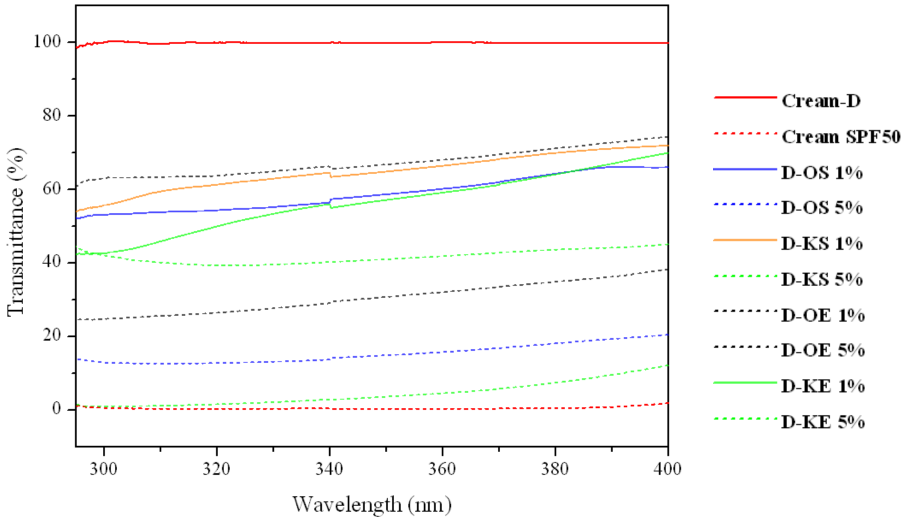


Figure 3.9. UV transmittance of creams containing 1% and 5% of organosolv and kraft lignins.

Concerning the creams visual appearance, the different solubility of lignins was appreciated. Kraft lignins presented better solubility than organosolv lignins; moreover, the origin had some influence, being more soluble hardwood lignins. An interesting relation between SPF values of creams and L* parameter of lignins was found, in which higher SPF values were exhibited in darker lignins, being the trend more noticeable in creams with 5% of lignin as can be observed in Figure 3.10. Consequently, the lightness of lignin had a big influence on sunscreen property of lignin samples. Due to lignin color, the formulated lignin sunscreen lotions exhibited a light brown to a brown color as its content increased providing a tanned look to the skin with their application besides being protected against solar UV radiations. Therefore, lignin would be an ideal candidate for using as natural active sunscreen against UV radiation.

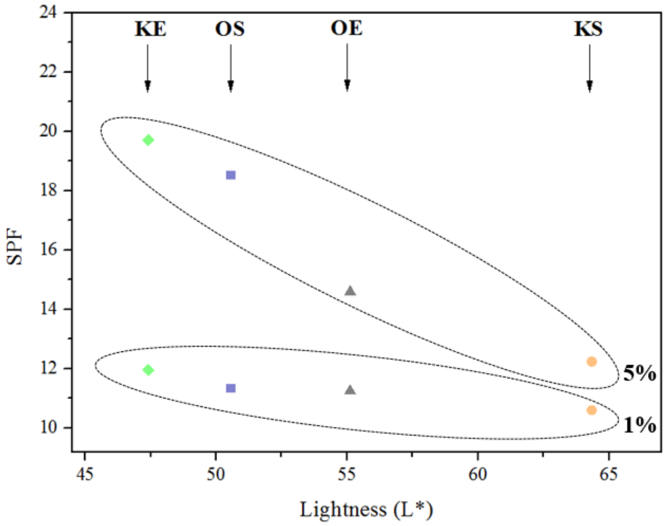


Figure 3.10. SPF *vs* L* parameter of lignins at [1%] and [5%] in standard commercial cream.

3.5. Conclusions

The results of the extensive study of chemical structure and functional properties of lignin samples obtaining from industry and at laboratory scale revealed that lignin could replace some products used today.

As regards the chemical structure, spruce lignins (OS and KS) presented more condensed structures and higher content of phenolic hydroxyls groups than eucalyptus lignins (OE and KE). Moreover, considering the influence of the isolation process on the same species, kraft lignins were also, which presented more condensed structures and higher content of phenolic hydroxyls groups.

Concerning thermal properties of lignins, higher molecular weight generally resulted in higher Tg values for lignins of the same origin. Additionally, higher thermal stability was found also for spruce lignins.

The results of antioxidant properties of lignins make them suitable for uses in cosmetic and topical formulations showing similar values to those found for commercial antioxidants. The remarkable antimicrobial capacity of lignins against *A. Niger* and diverse foodborne and human pathogenic microorganisms open new perspectives for food and pharmaceutical industry.

Lignin samples of high purity demonstrated appropriate HHV at 22-24 MJ/Kg, irrespective of their origin and the isolation process. KE lignin, which showed high ash content, would require additional purification to increase its HHV prior to use in biofuel applications.

High sun protection factor (SPF 20) of lignins suggested their potential as a natural additive for sunscreen avoiding the use of typical harmful commercial chemical and physical sunscreen products.

Therefore, the development of innovative applications using it as a commodity for several industries would improve the economic profit of the biomass industry.

4

Chemical modification of organosolv lignins by esterification with dodecanoyl chloride

4.1. Motivation

The motivation of this work was to increase the range of applications of organosolv lignins in the polymer industry. Owing to the limitations of lignin polymer, the chemical modification of hydroxyl groups presents in its chemical structure was considered the best alternative to get lignin with new and interesting properties, and thus opening new perspectives for several applications.

Moreover, both the results obtained after acetylation of lignins and the works made by other researchers demonstrated that chemically modified lignins can offer some advantages respect to unmodified lignins. Therefore, once again, esterification modification was chosen to functionalize the hydroxyls groups of lignins, as it is one of the easiest chemical reactions to perform considering the reaction parameters and used reactants.

In this case, in order to synthesize lignin-ester derivatives with low glass transition temperature and knowing that the reduction of Tg is higher when increasing the length of the attached chain, it was decided the use dodecanoyl chloride as a reactant. Dodecanoyl chloride is a 12 carbon aliphatic fatty acid in which the hydroxyl group of the terminal carboxylic group was substituted by halogen atom (Cl), making it much more reactive. With this aim, the work focused on the following research questions:

- Can lignin be chemically modified by esterification with dodecanoyl chloride using soft reaction conditions such as short time and without temperature?
- Which are the new properties of the synthesized lignin-ester derivatives?
- Will the reduction of the glass transition point and increment of lignins thermoplasticity be achieved with the used conditions?
- Will be able to use synthesized lignin-esters derivatives for further applications?

With the aim of answering these questions, previously extracted organosolv lignins were chemically modified in order to obtain a new product based on lignin but with higher capacity to be used for different applications.

4.2. Objectives

The main purpose of this chapter was the chemical modification of previously extracted organosolv lignin samples to obtain new lignin derivatives with innovative properties for further applications. The objectives were divided into different points:

- Functionalization of organosolv lignin with dodecanoyl chloride.
- Confirm the chemical modification by different techniques.
- Evaluation of the thermal properties of new lignin-ester derivatives.
- Analysis of the antioxidant capacity.

4.3. Experimental procedure

4.3.1. Materials

In this chapter, previously organosolv characterized lignins (OS and OE) were used for chemical modification.

4.3.2. Functionalization of hydroxyl groups: Esterification

The modification process took place in a two-necked flask where 0.5 g of lignin was dissolved in 15 mL of N,N-dimethylformamide (DMF) with a magnetic stirrer. Pyridine (2.75 mL) was used as a catalyst and dodecanoyl chloride was added (0.9 mL). Triethylamine (0.75 mL) was added in order to trap the hydrogen chloride formed during the reaction. The reaction was conducted at 20 °C for 2 h. After that, the solution was poured into 650 mL of 2% ice-cold hydrochloric acid.

The precipitate was filtered and washed with excess distilled water and ethanol to remove unreacted fatty acids. The samples were then dried in vacuum at 35 °C overnight. Figure 4.1 shows the general scheme of esterified lignins synthesis. The modified lignins were called OS_{12C} and OE_{12C} for Spruce and Eucalyptus lignins, respectively.

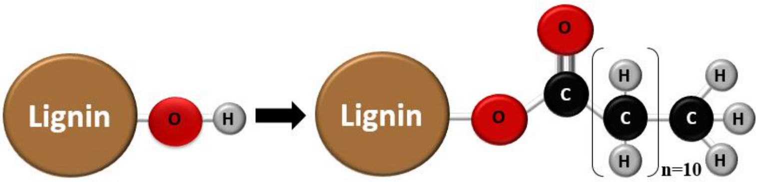


Figure 4.1. General scheme of lignin esterification.

4.3.3. Characterization methods

The synthesized lignin-ester derivatives were characterized according to the procedures and techniques described in Appendix I and II, which are summarized in Table 4.1.

Table 4.1. Characterizations methods used for the analysis of chemically modified lignins.

Analysis	Technique or Procedure	Appendix	Section
Chemical structure	FTIR	**II**	1.1
Phenolic content	Folin-Ciocalteu (UV)	**I**	4.1
Hydrophobicity	Contact angle	**II**	5.2
Molecular weight properties	GPC	**II**	2.1
Grass transition temperature	DSC	**II**	3.2
Thermal stability	TGA	**II**	3.1
Antioxidant capacity	DPPH (UV)	**I**	4.3

4.4. Results and discussion

4.4.1. Confirmation of the esterification reaction

The chemical modification was confirmed by different techniques such as FTIR, GPC and contact angle. Moreover, the reduction of phenolic hydroxyl groups was calculated.

FTIR spectra (Figure 4.2) of lignin-ester derivatives revealed that the esterification process was successful under studied conditions. This modification is highlighted for its short period of time without using temperature. Esterified lignins showed that the signal around 3400 cm^{-1}, which corresponds to O-H stretching vibrations in aromatic and aliphatic hydroxyl groups, was reduced and the appearance of two new peaks at 1740 $cm^{-1}$and 1760 cm^{-1} are clearly seen. They are assigned to aliphatic and aromatic ester bonds, respectively.

The intense increase of peaks at 2930 cm^{-1} and 2840 cm^{-1} are attributed to long chain alkyl groups (aliphatic carbon) which are present in fatty acid chloride[156,157]. Moreover, the absence of characteristic bands of dodecanoyl chloride (1800 cm^{-1} which is related to COCl) and dodecanoyl acid at 1700 cm^{-1} (COOH), showed that the modified lignins do not contain traces of free fatty acid. Therefore, the used washing method was suitable for removing impurities from the final product.

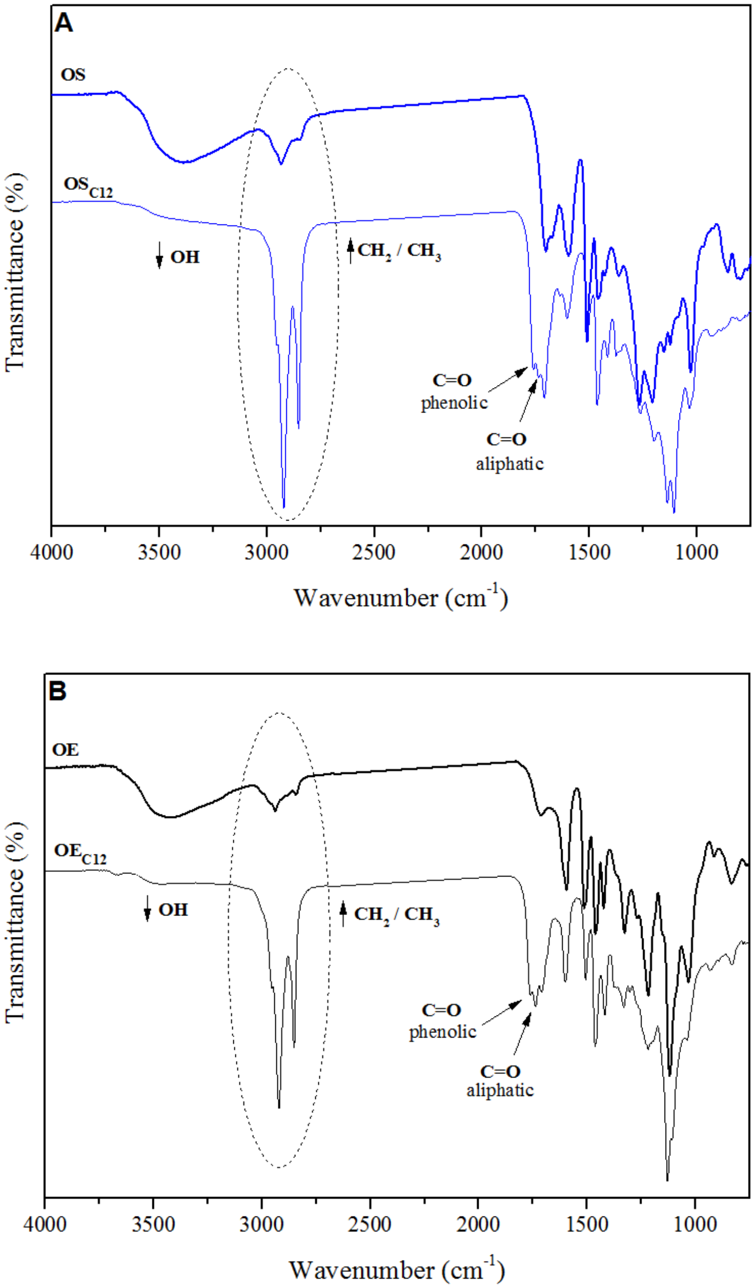


Figure 4.2. FTIR spectra of (A) isolated and chemically modified Spruce lignin and (B) isolated and chemically modified Eucalyptus lignin.

Moreover, chemically modified lignins presented some different properties compared to original lignins such as molecular weight, hydrophobicity as well as its physical appearance. Figure 4.3 shows the schematic representation of the visual appearance before and after modification. As can be observed, original lignins were a brown thin powder while lignin-esters become a soft and sticky dark resin.

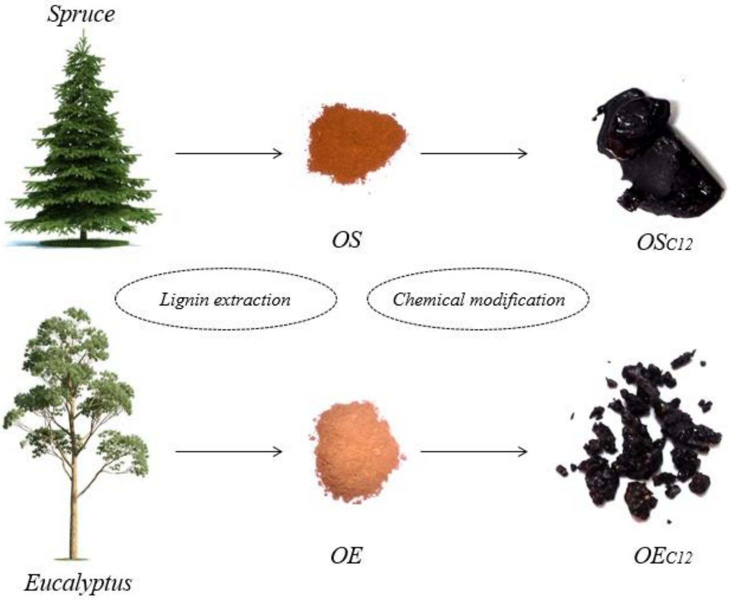


Figure 4.3. Schematic representation of the visual appearance before and after modification.

Table 4.2 displays the changes in molecular weight properties of chemically modified lignins. A significant increase in Mw can be observed after esterification with dodecanoyl chloride, as it was expected. This demonstrated that fatty acid has been successfully grafted to the lignins. It is known that the weight average should increase when lignin is modified with ester groups; also it has been reported that the molecular weight increase with the increase in the length of the ester substituent[60,64,90].

Regarding the yield of lignin after precipitation in acidified cold water, it was recovered more than twice introduced lignin in the reaction in both cases, indicating once again that dodecanoyl chloride has been successfully linked to organosolv lignins at the used conditions.

Table 4.2. Weight average (Mw), number average (Mn), polydispersity index (Mw/Mn) of isolated and esterified lignins.

	Mn	**Mw**	**IP**
OS	1065	3081	2.9
OS_{C12}	2774	10300	3.7
OE	1567	5079	3.2
OE_{C12}	3192	25837	8.1

The phenolic content of original and synthesized lignin-esters was calculated according to Folin-Ciocalteu procedure. A great reduction in phenolic hydroxyls was observed for both lignins, showing a higher degree of reduction organosolv spruce lignin. As in Chapter 2, a higher degree of modification was achieved for lignins with lower molecular weights.

Table 4.3. Total phenolic content of synthesized lignin-esters.

Sample	**GAE**	**OH %(w/w)**	**mmol OH/g lignin**	**DS (%)**
OS_{C12}	9.2	3.7	0.216	71.8
OE_{C12}	14.5	5.8	0.342	55.4

Finally, the contact angle with water measurements of isolated and esterified lignins were performed in order to confirm the change in their hydrophobicity. After chemical modification, hydroxyl groups present in lignins were substituted with non-polar groups [$-CO(CH_2)_{10}CH_3$] increasing the hydrophobicity. Figure 4.4 exhibits that esterified lignins presented higher hydrophobicity than isolated lignins and greater stability of contact angle values through time.

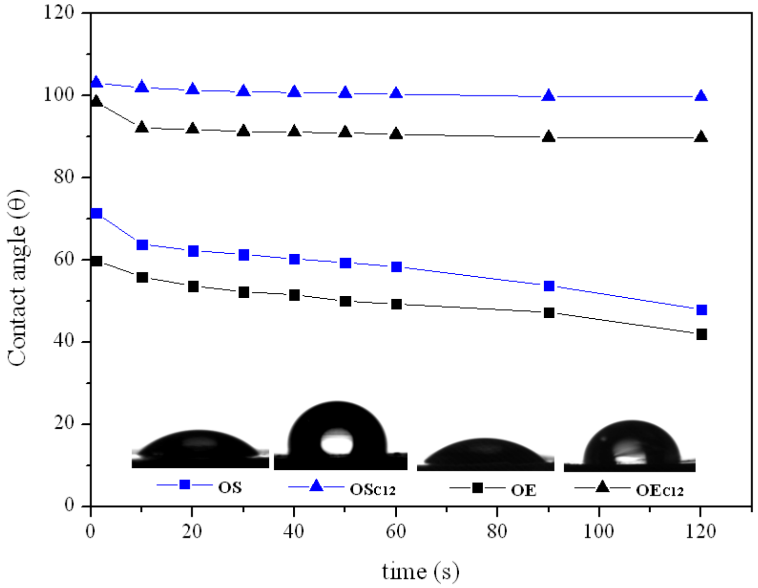


Figure 4.4. Dynamic contact angle of isolated and esterified lignins and with images of the contact angle of original and modified lignins after 120s.

4.4.2. Solubility

Owing to the change in lignins´ polarity after esterification, their solubility in different solvents were analyzed and the results are summarized in Table 4.4. As can be observed, esterified lignins become fully soluble in diethyl ether (nonpolar), chloroform, acetone and dimethylformamide and dimethyl sulfoxide. However, synthesized lignin-esters were totally insoluble in polar protic solvents such as ethanol and water.

Table 4.4. Solubility of lignin-esters.

Sample	**Diethyl ether**	**Chloroform**	**Acetone**	**DMSO**	**DMF**	**Ethanol**	**Water**
OS$_{C12}$	S	S	S	S	S	I	I
OE$_{C12}$	S	S	S	S	S	I	I

S: soluble; I: insoluble.

4.4.3. Thermal properties of lignin-ester derivatives

Regarding the thermal properties of lignin samples, glass transition temperature (Tg) parameter provides important information to know the suitability of using lignin in polymer applications and to process it by current industrial processing techniques like hot pressing.

DSC diagrams of different lignin samples are shown in Figure 4.5. The glass transition temperature (Tg) of lignin samples was very difficult to detect in the obtained thermograms. Therefore, based on the theory of glass transition temperature which defines it as the midpoint of the linear variation of the polymer heat capacity, and taking into account this inflection point is a maximum of the first derivative curve, it was possible to obtain a clearer information to identify this parameter.

Both lignins had a relatively high glass transition temperature (115-125 ºC) due to strong intermolecular hydrogen bonding interactions which restrict the thermal mobility of original lignin molecules and result in high Tg[93]. However, lignin derivatives presented an interesting change in its thermal behavior with a great reduction of glass transition temperature (Tg) with values of 15.8 °C for OS_{C12} and 17.4 °C for OE_{C12}[94]. This phenomenon occurs because the replacement of hydroxyls groups by ester substituent produced the reduction of the number of hydrogen bonding in lignin molecule and implied an increase free volume in the molecule and thus the mobility of the chains. Therefore, the use of esterification to elaborate lignin derivative products by attaching long aliphatic chains proved that it is a suitable method to obtain lignin derivatives with suitable glass transitions temperatures to use them in current industrial techniques.

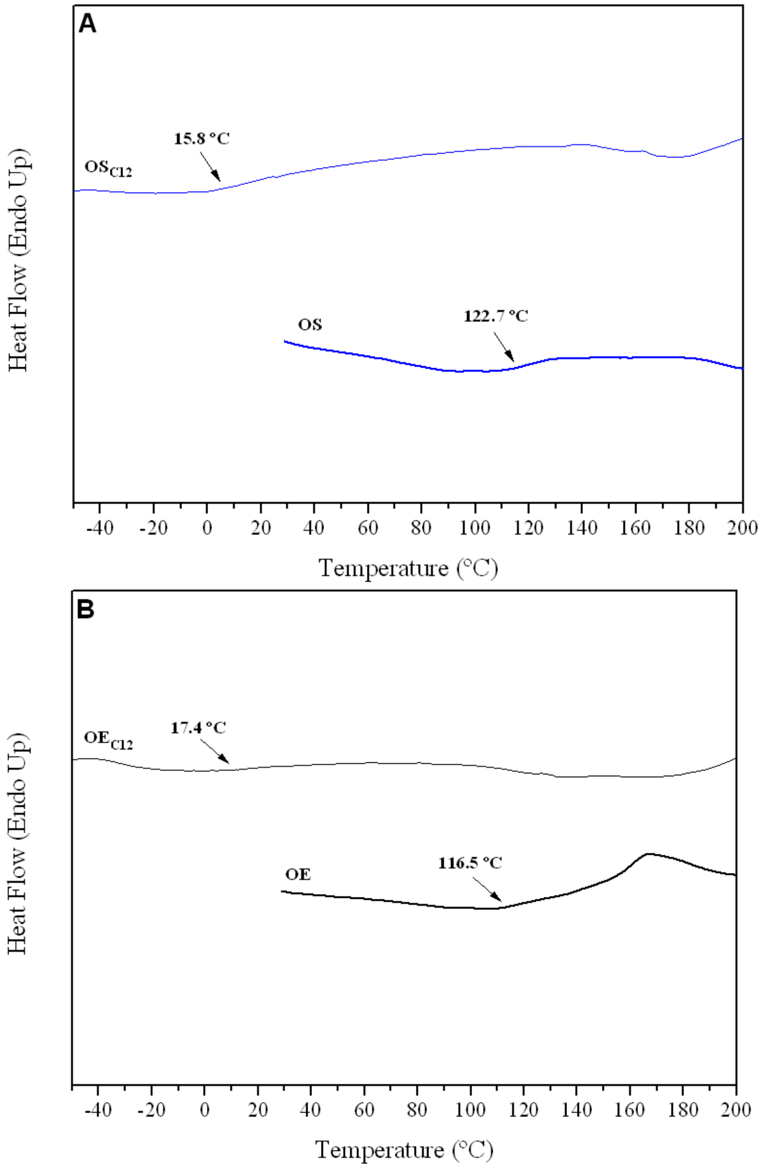


Figure 4.5. DSC curves of original and synthesized lignin-ester derivatives, (A) OS and OS_{C12} and (B) OE and OE_{C12}.

Moreover, the thermal degradation in polymers is also an important parameter to determine during thermal processing and to establish the service life conditions[61]. The thermogravimetric (TGA) curves obtained for original and modified lignins under nitrogen atmosphere are presented in Figure 4.6.

Lignins had a small weight loss (0-3%) below 100 °C due to the gradual evaporation of moisture, however, modified lignins did not show any weight loss due to moisture confirming that the esterified lignins were more hydrophobic than originals ones.

Thermal stability of the samples was assessed as the onset temperature (referred to 5% weight loss) and maximum degradation temperature together with the lost weight in this range and the obtained results are summarized in Table 4.5. The thermograms of original and modified lignins were indicative of the alterations in the chemical structure and thermal stability.

Original lignins only presented a wide single degradation step. This main weight loss stage occurs in the temperature range between 200 and 600 °C[88] and in this case, are centered around 385 °C for OS and 353 °C for OE. The main stage is associated with the fragmentation of inter-units linkage[158] like cleavage of typical ether linkages among the aromatic units. Then, cracking of aliphatic side chains and cleavage of functional groups occurs[12,21,105].

However, lignin-ester derivatives presented less thermal stability than originals starting to decompose ($T_{5\%}$) before and moreover, two degradation steps can be observed. The first one can be observed between 220 and 240 °C and the second between 370 and 395 °C.

The first weight loss is associated with the cleavage of the C=O bond present in the ester linkage and the consequent loss of the introduced long aliphatic chain. This first degradation step was a little more pronounced for OS_{C12} since it was which presented a higher degree of modification and therefore higher content of esters groups. In other studies, where other biomass components like cellulose were modified with the same reactive (dodecanoyl chloride), it were also observed two degradation steps[159–161]. The first one could be observed at similar temperatures to this study. Modified lignins also exhibited higher weight loss between 200 and 600 °C than original lignins, 70-75%, and 50%, respectively.

This is due to the lower content of phenolic OH groups in their structure of modifies lignins. On the other hand, the amount of char residue is lower for modified lignins.

Similar initial degradation temperatures (200-215 ºC) were found in other work where sebacoyl chloride was used to modify two types of lignins[63].

Synthesized lignin-ester derivatives presented acceptable values of initial degradation temperature (>200 ºC), being a good candidate to use them as an additive for polymers.

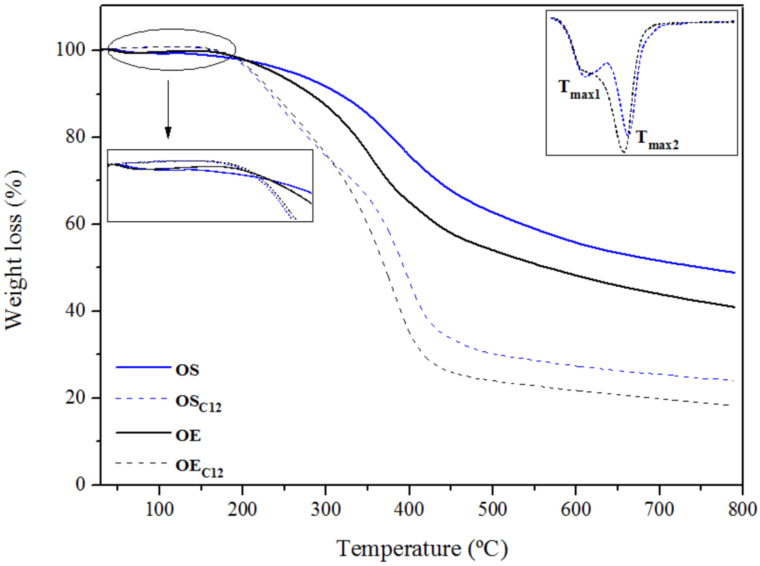


Figure 4.6. TG and DTG curves of OS_{C12} and OE_{C12}.

Table 4.5. Detailed data obtained from TGA measurements of unmodified and synthesized lignin-esters.

	$T_{5\%}$	$T_{50\%}$	T_{max1}	$wt_1\%$	T_{max2}	$wt_2\%$	Residue at 800 ºC (%)
OS	257.8	755.4			385.8	41.8	48.7
OS_{C12}	209.8	394.3	237.6	27.8	393.4	48.5	23.7
OE	261.6	561.7			353.4	49.5	40.8
OE_{C12}	213.2	371.3	222.2	23.9	376.2	57.6	18.5

4.4.4. Antioxidant capacity

While literature about the antioxidant capacity of lignin is vast, no works about the antioxidant capacity of lignin derivatives were found. As expected, due to the reduction of phenolic hydroxyls groups during esterification, lignin-ester derivatives did not show antioxidant behavior. It is well known that antioxidant capacity is highly related to phenolics, and as was reported in Table 4.3, a clear reduction of phenolic content was found after esterification with dodecanoyl chloride. Moreover, OS_{C12} was the lignin with a higher decrease on phenolic hydroxyl groups, and it reflected also in this analysis since it is necessarily higher amounts of lignin-ester to reduce 50% of the DPPH radical.

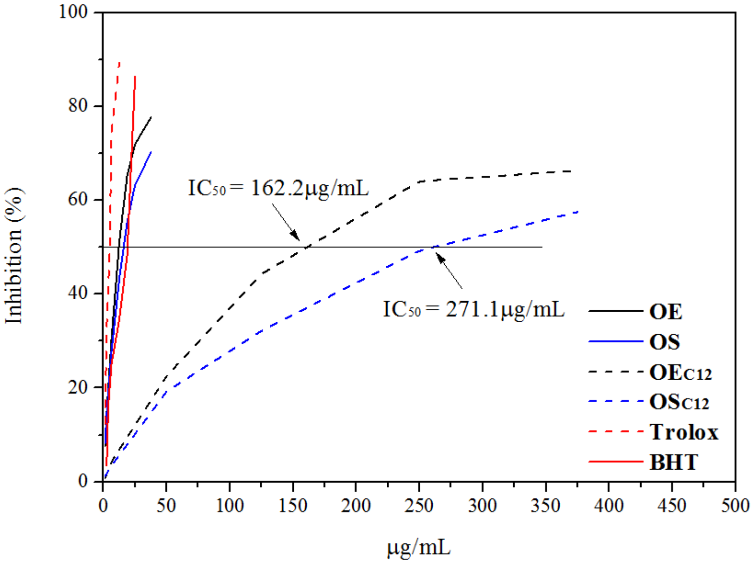


Figure 4.7. Antioxidant activity of esterified lignin against DPPH and their comparison with originals lignins, BHT and Trolox used as positive controls.

4.5. Conclusions

The developed strategy to attach fatty acid to softwood and hardwood organosolv lignins was suitable to prepare lignin-esters derivatives. Besides, the used techniques were appropriate to confirm the chemical modification of lignins.

A significant change in the lignin properties was achieved after chemical modification. Attaching long aliphatic chains resulted in an adequate method to obtain lignin-ester derivatives with higher hydrophobicity and suitable glass transitions temperatures allowing their use by conventional processing industrial techniques. Moreover, the initial degradation temperature was above 200 ºC, being a suitable product for uses as an additive in some polymeric materials, which are processed at temperatures below. However, lignin-ester derivatives did not show good antioxidant capacity.

5

Applications of synthesized lignin-ester derivatives

5.1. Motivation

As was described in the previous chapter, new lignin-ester derivatives showed an important change on their thermal properties, evidencing that esterification with chosen fatty acid was a potential route to lower the glass transition temperature and increase the thermoplasticity of lignin. Moreover, lignin-esters presented higher hydrophobicity and higher ability to be solubilized in organic solvents. Therefore, having determined the new resulting properties of esterified hardwood and softwood organosolv lignins with a long aliphatic chain, novel applications came to our minds.

- Could lignin-ester derivatives be used in PLA films as an additive to provide new properties? How would affect the addition of lignin-esters on thermal and mechanical properties of PLA films? Could lignin-ester offer some interesting property to PLA films?

- Might lignin-ester be able to use as a possible protective agent for wood products? How would influence the treatment of wood products on their appearance and surface properties?

Thus, in order to know the possibility of the use of lignin-esters as additive in PLA films and as hydrophobic agent for wood products, different experiments were carried out.

5.2. Objectives

The main objective of this work was the study of synthesized lignin-ester derivatives as an additive for PLA films and as hydrophobic agent for wood treatments. Thus, the research was divided into two different sections:

➤ *Lignin esters as PLA films additive*

- Elaboration of PLA films with different contents of lignin-ester derivatives using solvent casting method.
- Analyze their influence on optical, thermal and mechanical properties.
- Determine if lignin-esters have a positive effect on the transmittance of the light of PLA films.
- Study the antifungal behavior of PLA-lignin esters films against fungal food contaminants (*Aspergillus Niger*).

➤ *Lignin-esters as hydrophobic agent for wood products*

- Study the application of lignin-esters on wood products by two different methods (Coating and impregnation).
- Investigate the influence of the applied treatments on the appearance of wood veneers.
- Analyze the surface properties of treated wood veneers.
- Determine the resistance of the applied lignin-esters to artificial aging.
- Evaluate the antifungal behavior of treated wood veneers against wood fungus.

5.3. Experimental procedure

The utilization of previously synthesized lignin-ester derivatives has been focused on two different applications. The first one was the incorporation of new lignin products into PLA films, and the second one was for wood treatments. The following diagram summarized the experiments carried out in this chapter.

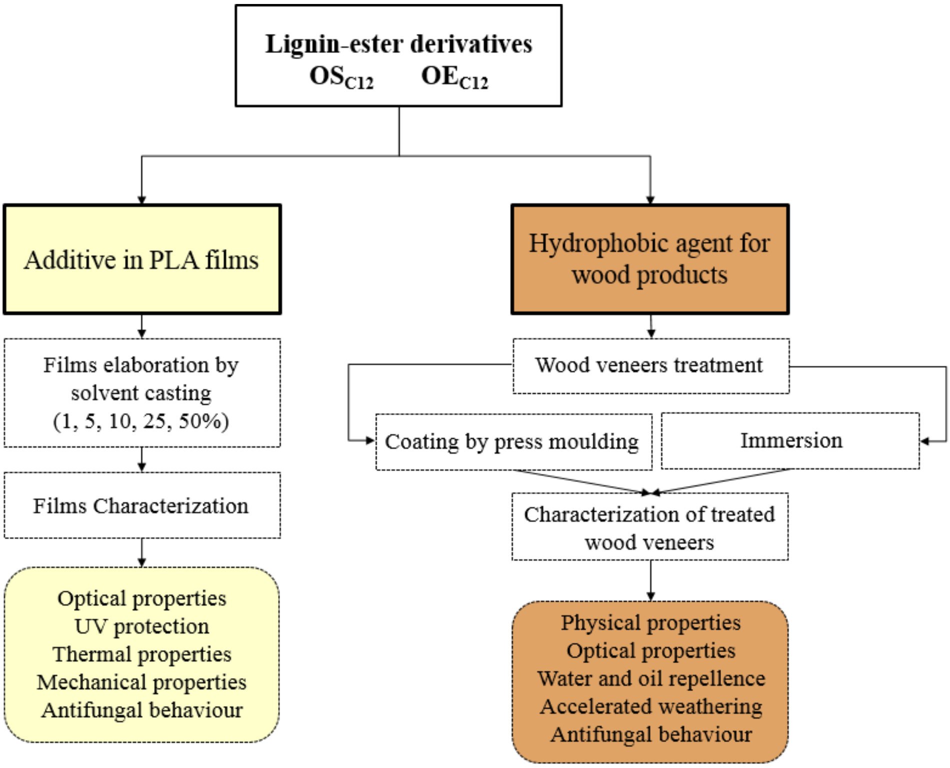


Figure 5.1. Diagram of the two applications for synthesized lignin-ester derivatives.

5.3.1. Additive in poly(lactic acid) films

5.3.1.1. Films elaboration by solvent casting

The films were elaborated by poly(lactic acid) (PLA) as matrix and different percentages of esterified lignins (1, 5, 10, 25, 50%) as an additive. The PLA was the same that was used in Chapter 2 and the esterified lignins (OS_{C12} and OE_{C12}) were previously described in Chapter 4.

Films have been prepared by solvent casting using chloroform (15 mL) as a solvent for its ability to dissolve PLA. When PLA was totally solubilized, the required amount of esterified lignin was added. The solution was then cast onto a 10 cm diameter glass Petri dish and then allowed to dry for about 24 h at RT.

The thickness of resultant films was a 60-100 µm. The total amount of dry substance (PLA and lignin-ester derivative) in each film was kept constant at 0.5g.

5.3.1.2. Films characterization methods

Elaborated films were characterized by different procedures and techniques described in **Appendix I** and **Appendix** II.

Table 5.1. Characterization methods used for the analysis of elaborated films.

Analysis	**Technique or Procedure**	**Appendix**	**Section**
Optical properties	Colorimeter	**II**	4.3
UV protection	UV	**I**	6.2
Thermal stability	TGA	**II**	3.1
Mechanical properties	MTS	**II**	6.1
Antifungal properties	Antifungal test	**I**	5.1

5.3.2. Hydrophobic agent for wood products

5.3.2.1. Wood veneers treatments

Samples prepared for finishing with lignin derivative products were sanded using silicon carbide (SiC) sandpaper with 280-grit (Beech) and 180-grit (Poplar). These wood veneers samples were treated with esterified lignins (OS_{C12} and OE_{C12}) using two different methods; the first method was coating by press molding at two different conditions (90 ºC/100 bar and 100 ºC/200 bar). The final experimental design was set at 2 min and a dosage of 0.002 g of product per cm^2. The treatment time assigned was the most suitable time where the product was coated to the substrate without visual defects as well as the quantity of product.

The second method was impregnation by immersion of veneers at room temperature in a prepared solution of acetone and esterified lignins (0.5 wt%) at different times (2, 4, 8, 12, 24, 48 and 72 h). Acetone was used as solvent because of its capacity of solubilize lignin-esters as well as allowing the immersion without using additional pressure. Prior to immersion wood veneers were dried at 105 ºC in an oven during 2 days and then samples were put under vacuum for 1 h.

5.3.2.2. Accelerated weathering

Wood veneers were artificially aged based on a modified ASTMD2559 technique[162]. To complete an aging cycle, samples were dried, weighed, placed in a sealed container and submerged in water (approx. 27 ºC), at 20 kPa for 15 h; then placed in a convection oven at 75 ºC for 9 h. Once removed from the oven, each specimen was checked for delamination or cracks and the cycle was repeated five times. Afterwards, samples were weighed and stored at 25 ºC and 50% relative humidity until they were tested to know the changes in water and oil repellency and color.

5.3.2.3. Characterization methods of treated wood veneers

Table 5.2. Characterization methods used for the analysis of treated wood veneers.

Analysis	**Technique or Procedure**	**Appendix**	**Section**
Moisture (%)	Denver	II	7.1
Roughness (mm)	Profilometer	II	5.1
Optical properties	Colorimeter	II	4.3
Surface properties			
Water/Oil repellence	Contact angle	II	5.2
Surface energy	Contact angle	II	5.3
Accelerated weathering			
Optical properties	Colorimeter	II	4.3
Water/Oil repellence	Contact angle	II	5.2
Antifungal capacity	Antifungal test	I	5.1

5.4. Results and discussion

5.4.1. Additive in poly(lactic acid) films

5.4.1.1. Appearance and optical properties of films

The optical characterization of PLA film was taken as control and it presented color values of lightness L* (92.99), red/green hue a* (0.45), yellow/blue hue b* (0.74), chroma C*(0.88) and hue angle (55.94).

The color change of films containing esterified lignins as an additive was very noticeable as can be seen in Figure 5.2. Films were acquiring from light to dark brownish tone with the content of lignin increased. Table 5.3 displays the color change parameters.

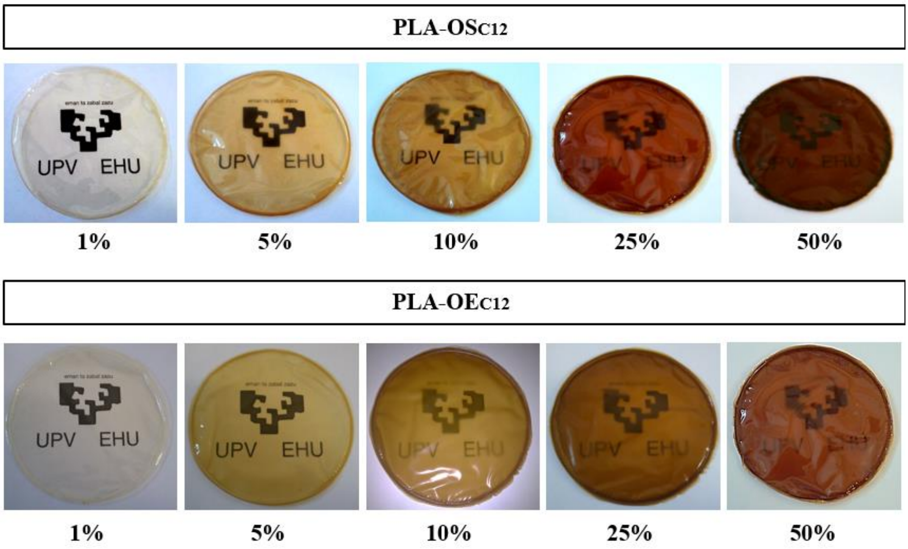


Figure 5.2. PLA-lignin ester derivatives films.

The total color difference (ΔE) of films was increasing with lignin content to values of 32 for OS$_{C12}$ and 29 for OE$_{C12}$. Moreover, the lightness also showed a great dependence on lignin content, decreasing when lignin content increased. OS$_{C12}$ lignin showed higher color change and reduction of lightness than OE$_{C12}$.

On the other hand, although the green-red (Δa) and yellow-blue (Δb) color coordinates of films with lignin significantly changed, hue values (H) demonstrated that the films presented similar tone with values between 55-60°. However, up to 10% of lignin content, the saturation of the color was increasing but higher than 25% of lignin addition tend to reduce the Chroma again due to films became darker.

Table 5.3. Color changes of PLA-lignin-ester derivatives.

% Lignin in PLA		**ΔL**	**Δa**	**Δb**	**ΔC**	**ΔH**	**ΔE**
OS$_{C12}$	**1**	-4.11	1.52	7.11	7.22	0.87	8.35
	5	-12.05	6.17	15.34	16.52	0.72	20.46
	10	-21.40	9.14	9.94	13.49	0.56	25.30
	25	-28.94	6.48	1.12	6.09	2.48	29.67
	50	-31.70	1.07	4.92	3.57	3.55	32.10
OE$_{C12}$	**1**	-1.11	0.77	4.17	4.19	0.69	4.39
	5	-7.48	3.95	12.44	13.02	0.88	15.04
	10	-11.68	5.63	14.74	15.76	0.77	19.63
	25	-22.37	9.43	9.58	13.42	0.67	26.09
	50	-27.95	7.60	1.36	7.47	1.96	28.99

5.4.1.2. UV protection analysis of films

PLA is actually the most extensively used bioplastic in packaging sector and one of the essential property of the packaging materials is the UV-vis protection capability for the safety of inside goods. Therefore, nowadays, synthetic chemicals such as titanium dioxide, zinc oxide, and others organic compounds are often used as active ingredients in sunscreens for UV absorption[163,164].

However, the use of lignin, natural broad-spectrum sun blocker, could be an interesting alternative to synthetic sunscreen additives for PLA materials maintaining thus the biobased content of the final product.

Although lignin is considered as UV protective product, its sunscreen effect can disappear when forms agglomerated because of poor compatibility with the polymer matrix[163]. Therefore, it was interesting to study the UV-vis protection of PLA and PLA-esterified lignin blends, since lignin-esters present wood compatibility with the matrix.

The UV-vis spectra of PLA and PLA containing esterified lignins was demonstrated in Figure 5.3. Moreover, the percentage of light transmitted in both UV and visible light regions was investigated since plastic materials tend to undergo photochemical degradation when exposed to UV radiation (200-400 nm) but food products exhibit high sensitivity towards the visible light (400-700 nm)[164]. The detailed transmittance results in UV and visible light regions are provided in Table 5.4.

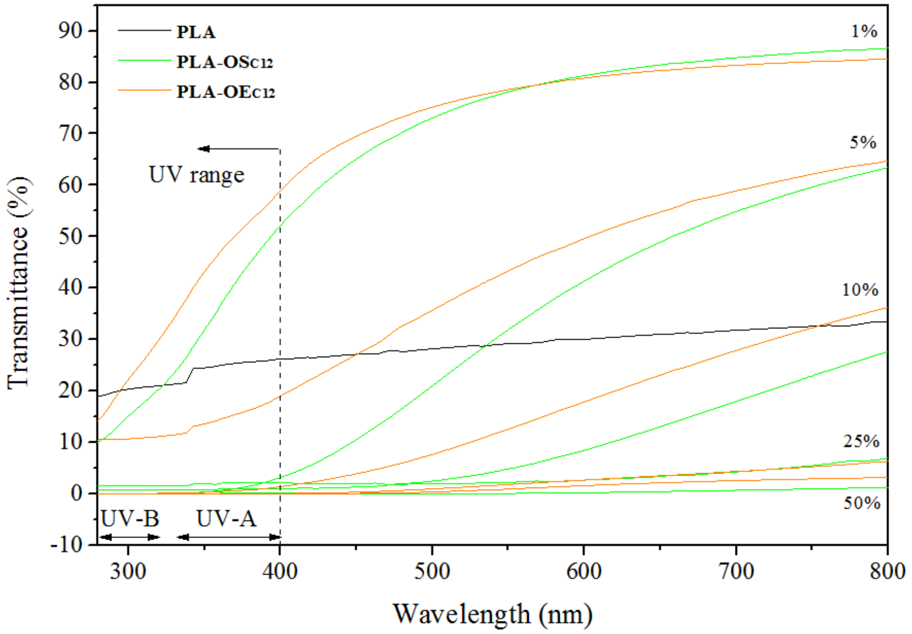


Figure 5.3. Typical UV-visible transmittance curves of PLA composites.

Although PLA usually present high transparency with more than 90% of light transmittance at 600 nm[163,165,166], in this work, PLA film present around 30% of transparency.

This could be associated with the elaboration step carried out by solvent casting, where the slow evaporation of chloroform might have caused this phenomenon. Probably, PLA chains arranged to form a crystalline structure producing an opaque PLA film. Images of PLA film and PLA-OE$_{C12}$ blends are presented in Figure 5.4. There is possible to appreciate that PLA-OE$_{C12}$ blends at 1% and 5% were transparent while PLA film is opaque.

Table 5.4. Percentage of light transmission (T%) values for PLA and PLA-RS films.

Film	**T (%) at 315 nm (UV-A)**	**T (%) at 400 nm (UV-B)**	**T (%) at 700 nm (Visible region)**
PLA	20.8	26.2	31.9
PLA-OS$_{C12}$ 1%	19.5	52.1	84.7
PLA-OS$_{C12}$ 5%	11.1	3.3	55.0
PLA-OS$_{C12}$ 10%	0.0	1.0	27.8
PLA-OS$_{C12}$ 25%	0.0	0.0	4.4
PLA-OS$_{C12}$ 50%	0.0	0.0	0.7
PLA-OE$_{C12}$ 1%	27.8	54.5	83.4
PLA-OE$_{C12}$ 5%	0.1	19.0	59.0
PLA-OE$_{C12}$ 10%	0.0	1.4	18.1
PLA-OE$_{C12}$ 25%	0.0	0.0	4.4
PLA-OE$_{C12}$ 50%	0.0	0.0	2.8

Figure 5.4. Image of PLA film, PLA-OE$_{C12}$ 1% and PLA-OE$_{C12}$ 5%.

Regarding the transmittance of PLA-esterified lignin blends, the results showed that the addition of 1% of OE$_{C12}$ and OS$_{C12}$ did not exhibit a light blocker capacity. However, 5% of lignin presented transparency and the transmittance decreased rapidly in the UV range (< 400 nm), especially with the addition of OS$_{C12}$.

For higher lignin-ester content, the transmittance values tend to decrease greatly both in UV and visible regions. The maximum reduction of transmittance was achieved for 25% and 50% contents with almost 100% of light absorbance in both light regions.

The results demonstrated that the introduction of 5% of esterified lignins in PLA could be used for packaging materials that require transparency and UV-protection capability. Moreover, higher esterified lignin content in PLA prevented the passage of visible light through PLA-esterified lignin blends, offering high protection for light-sensitive packed materials.

5.4.1.3. Thermal properties of films

The thermogravimetric (TGA) curves obtained for films with 1% and 25% of esterified organosolv lignins under nitrogen atmosphere are presented in Figure 5.5. In addition, the initial degradation temperature ($T_{5\%}$), the temperature of 50% weight loss ($T_{50\%}$), the maximum weight loss temperature (T_{max}) and char residue of the films are summarized in Table 5.5. The solvent casted PLA and PLA-esterified lignin films presented a small weight loss (2-3%) between 80 and 150°C because of the removal of the residual solvent in the films. Other authors who prepared films of PLA and unmodified lignin by solvent casting, also observed a small weight loss due to residual chloroform in the film.

The addition of lignins decreases initial degradation temperature of PLA when the chemically modified lignin content increases in the PLA-esterified lignin films. However, all studied composites showed higher maximum decomposition temperature than PLA and it increased at high percentages of esterified lignin.

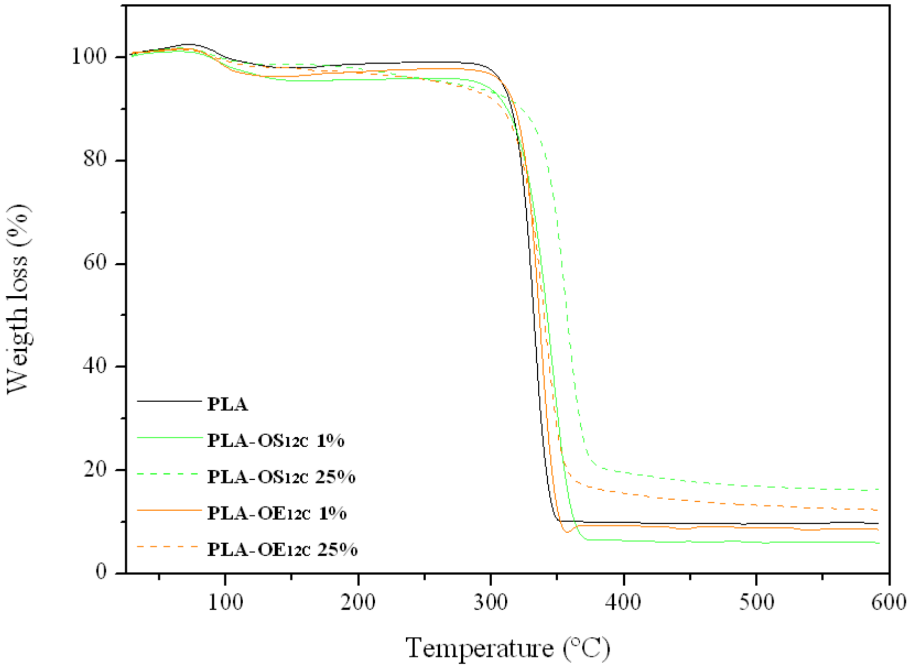


Figure 5.5. TG curves of elaborated films at 1% and 25% of esterified organosolv lignins.

Table 5.5. Thermogravimetric parameters of PLA-lignin ester derivatives films.

Sample	**$T_{5\%}$ (°C)**	**$T_{50\%}$ (°C)**	**T_{max} (°C)**	**wt %**	**Residue at 600°C**
PLA	309.7	331.9	331.6	48.8	9.7
PLA-OS$_{C12}$ 1%	292.2	342.0	346.1	59.4	5.9
PLA-OS$_{C12}$ 25%	270.7	356.9	357.1	49.3	16.1
PLA-OE$_{C12}$ 1%	309.8	336.0	336.3	48.8	8.4
PLA-OE$_{C12}$ 25%	266.7	33908	339.1	48.1	12.5

5.4.1.4. Mechanical properties of films

Tensile tests have been performed to study the influence of lignin-ester derivatives on the mechanical properties of PLA films. The Young's modulus, elongation at break, stress at break and stress at yield of the different films are shown in Figure 5.6.

The results demonstrated that neat PLA film show very low elongation at break (about 3%) and high elastic modulus (E = 2 GPa), indicating a brittle behavior. A clear difference was observed with the addition of esterified organosolv lignins into PLA matrix and both types of modified lignins showed similar trend on mechanical properties.

The addition of esterified lignins (1-50%) into the PLA matrix showed a progressive reduction in the elastic modulus with an increase of esterified lignin content, evidencing the reduction of the rigidity of the films.

Moreover, the elongation at break of the PLA-lignin-ester derivative films strongly increased with the addition of esterified lignins up to 25%, showing values up to 28% and 15% of deformation for OS_{C12} and OE_{C12}, respectively. However, more than 25% of esterified lignins became films brittle, especially in the case of OE_{C12}, probably due to the saturation of PLA and the lack of cohesion between lignin and PLA[167].

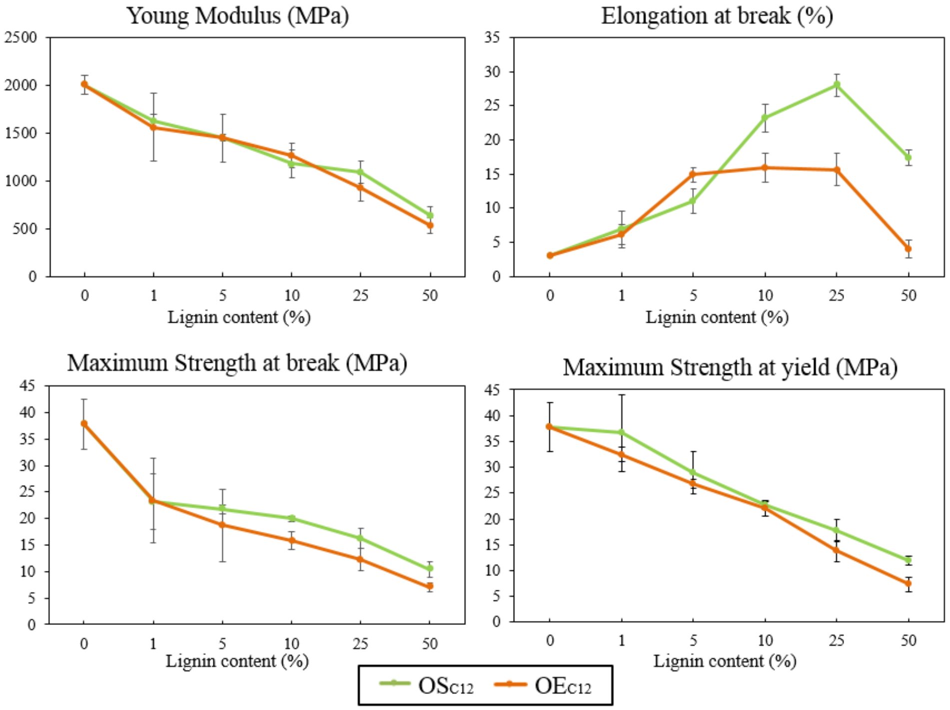


Figure 5.6. Mechanical properties of composites, PLA-OS_{C12} films, and PLA-OE_{C12} films.

It was remarkable the important enhancement of toughness and ductility of PLA since PLA is a very brittle and rigid material with high young modulus and low capacity to deform. However, the incorporation of both esterified lignins provides PLA plastic deformation. Figure 5.7 shows the sample before and after being mechanically tested, and as can be observed a clear plastic deformation of PLA with esterified lignin as an additive was achieved. The greater ductility or ability to deform plastically provided by OS$_{C12}$ probably was due to it had lower molecular weight than OE$_{C12}$[168].

On the other hand, films endured less stress at break and stress at yield than original PLA and decreased with increased lignin content. Other studies also showed that the addition of plasticizers decreased Young modulus and stress at break of final materials[167,169]. It was not possible the comparison of mechanical results with other authors because it was not found similar work in literature.

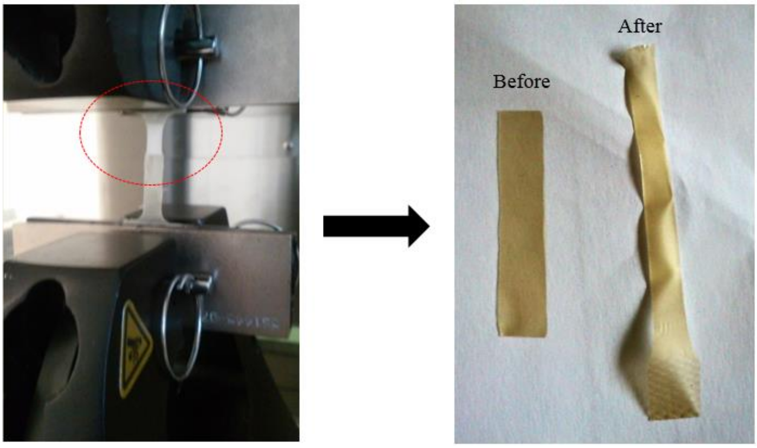


Figure 5.7. PLA-esterified lignin before and after mechanical testing.

5.4.1.5. Antifungal activity of films against *Aspergillus Niger*

The main purpose of the antifungal tests towards *A. Niger* was to assess the potential use of the PLA-esterified lignin films as fungal inhibitors for packaging applications. *A. Niger* was selected because it is well known as a widespread contaminant of fruits, vegetables, and food.

Figure 5.8 displays the increment of fungal inhibition of PLA-esterified lignin films respect to PLA films, which was taken as control. The results after seven days of *A. Niger* incubation at 25 °C, revealed that the addition of low concentration (1% and 5%) of lignin-ester derivatives did not cause any effect on the fungal inhibition of *A. Niger* on films. However, the fungal inhibition increased with the incorporation of 10%, 25% and 50% of esterified lignins into PLA.

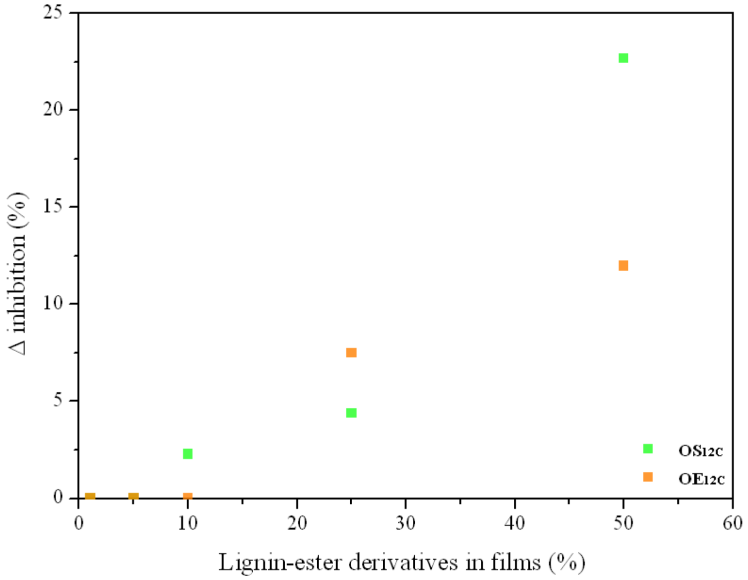


Figure 5.8. Increment of Fungal Growth Inhibition (%) of PLA-lignin-ester derivative films respect to PLA films.

5.4.2. Hydrophobic agent for wood products

5.4.2.1. Physical properties of treated wood veneers

The applied systems used for wood veneers showed certain differences. The press molding technique did not generate a film on wood veneers, but the esterified lignins were homogeneously extended over woods surfaces (Figure 5.9).

Despite the fact that the Tg of both products was below 18 ºC, there was necessary to provide additional energy in the form of heat to form homogeneous treatment. In order to obtain the most uniform product, the temperature was gradually increased from room temperature up to 100 ºC, checking that the initial product was melted-merged with the substrate to finally solidify on the cooling stage.

Beech-OSC12
90 ºC
100 Ba

Poplar-OSC12
100 ºC
200 Ba

Figure 5.9. Wood veneers treated by press molding of esterified lignins.

On the other hand, the impregnation method of veneers-esterified lignin products was carried out at different immersion times in order to obtain the adequate set-time where the maximum quantity of product was retained. Figure 5.10 shows the appearance of beech veneer before and after 72 h of immersion.

Figure 5.10. Difference on water wettability between beech veneer before and after 72h of immersion.

The product retention was calculated by weighting the veneers before impregnation and after impregnation under the same conditions (dried at 105 ºC).

The immersion method did not greatly affect the gain weight (0.2-1.4%) of the treated wood samples. Higher retention of the product at the higher time was observed, being almost constant from 24 h to 72 h (Table 5.6).

Although poplar wood is less dense and more porous than beech, in this case, beech veneers retained a higher amount of product with both modified lignins. These results probably are related to the pretreatments suffered, the humidity and thickness of veneers.

Table 5.6. Percentage of weight gained due to the product retention for impregnated wood samples.

Sample	**Immersion time (h)**	**Retention (%)**	
		Beech	**Poplar**
OS$_{C12}$	2	0.38 ± 0.11	0.26 ± 0.05
	4	0.39 ± 0.09	0.29 ± 0.07
	8	0.77 ± 0.12	0.44 ± 0.11
	12	1.02 ± 0.22	0.54 ± 0.09
	24	1.22 ± 0.22	0.88 ± 0.18
	48	1.33 ± 0.28	0.97 ± 0.38
	72	1.40 ± 0.19	1.01 ± 0.21
OE$_{C12}$	2	0.48 ± 0.10	0.27 ± 0.10
	4	0.54 ± 0.29	0.42 ± 0.08
	8	0.57 ± 0.10	0.66 ± 0.20
	12	0.65 ± 0.13	0.73 ± 0.07
	24	0.82 ± 0.22	0.74 ± 0.10
	48	0.85 ± 0.15	0.81 ± 0.15
	72	1.05 ± 0.27	0.96 ± 0.14

Table 5.7 shows the differences in the moisture content of veneers control and treated veneers. As it can be observed, after treatments the moisture content substantially decreased in all treated veneers, probably due to saturation of veneer pores after the application of the product avoiding thus moisture absorption. Less moisture content was noticed for coated wood veneers than for immersed veneers.

Table 5.7. Moisture content of control veneers and treated veneers.

Sample	Product	Treatment	Moisture content[a] (105ºC)
Beech	Control	Control	6.47 ± 0.58
Poplar	Control	Control	7.29 ± 0.45
Beech	OS_{12C}	Coating[b]	2.44 ± 0.18
Beech	OS_{12C}	Immersion[c]	2.82 ± 0.34
Beech	OE_{12C}	Coating[b]	2.19 ± 0.21
Beech	OE_{12C}	Immersion[c]	2.88 ± 0.48
Poplar	OS_{12C}	Coating[b]	2.46 ± 0.22
Poplar	OS_{12C}	Immersion[c]	4.56 ± 0.86
Poplar	OE_{12C}	Coating[b]	2.30 ± 0.41
Poplar	OE_{12C}	Immersion[c]	3.16 ± 0.61

[a] Measured with Denver instrument IR60Q.
[b] Coated at 90 ºC/100 bar.
[c] Immersed samples at 72h.

Moreover, Table 5.8 shows the roughness average of veneer samples. The average roughness (*Ra*) of veneers before treatments was influenced by the sanded-pretreatment, and in general, was lower than treated veneers; however, the control veneers presented significant differences between species (about 41%). After treatments, all veneers increased the roughness value, nevertheless the immersed samples presented similar values to that found in control (difference <12%). On the other hand, the roughness of coated samples was rather higher than in control with a difference above 65%. The layer formed due to the coating influenced the roughness, but in general, the values were acceptable and the visual assessment did not show remarkable defects.

Table 5.8. Roughness average of veneers samples.

Sample	Treatment	Roughness *Ra* (um)[a]	
		Beech	Poplar
Control	-----	19.89 ± 2.82	11.65 ± 4.62
OS_{C12}	Coating[b]	32.78 ± 9.41	28.10 ± 6.54
	Immersion[c]	22.14 ± 3.32	12.49 ± 5.63
OE_{C12}	Coating[b]	36.77 ± 11.32	24.17 ± 6.97
	Immersion[c]	21.24 ± 2.14	13.27 ± 3.79

[a] *Ra* measured across the grain orientation.
[b] Coated at 90 ºC/100 bar.
[c] 72h of immersion treated wood veneers.

5.4.2.2. Optical properties

The optical characterization of untreated beech wood veneers presented color values in the meaning of lightness L* (65.51), red/green hue a* (11.44), yellow/blue hue b* (24.73), chroma C*(27.26) and hue angle (65.08). For untreated poplar veneers, the results were lightness L* (84.28), red/green hue a* (3.35), yellow/blue hue b* (24.47), chroma value C* (22.72) and hue angle (81.51).

The color change in both kinds of wood after each treatment was visually appreciable, especially in the case of coated woods (Figure 5.11). The total color difference (ΔE) of coated beech wood varies between 30 and 40 while poplar woods presented higher values between 50 and 60. In general, the lightness (L*) decreased around 50% in coated beech and 60% in coated poplar. a*, b* C* and h parameters varied respect to the original woods in all cases, decreasing all these parameters in the case of coated beech but increasing the red-green coordinate (a*) in coated poplar, with both types of lignin-ester derivate.

On the other hand, impregnated woods presented less color change (ΔE) than coated woods with values between 2 and 12 for impregnated beech and 3-22 for impregnated poplar. The general trend was that OS_{C12} lignin provided greater color changes on woods than OE_{C12} as it can be observed in Figure 5.8 where samples treated with OS_{C12} presented darker color. The lightness (L*) decreased around 5-15% for impregnated beech and 5-20% for impregnated poplar.

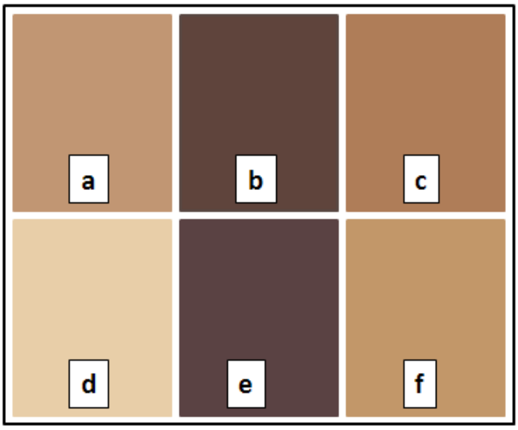


Figure 5.11. RGB color space calculated for wood samples (a) untreated beech, (b) beech coated with OE_{C12} at 90ºC/100Ba, (c) impregnated beech d with OS_{C12} (72 h), (d) untreated poplar, (e) poplar coated with OS_{C12} at 90ºC/100Ba and (f) impregnated poplar with OS_{C12} (72 h).

5.4.2.3. Surface properties

The contact angle of liquids on solid surfaces provides direct information about the wetting behavior of the surface. Kalnins et al.,1988[170] suggested the usage of a video-type technique to measure the dynamic contact angle of liquids on wood over time. The dynamic contact angle with water (WCA) and ethylene glycol (OCA) of coated and impregnated wooden surfaces are shown in Figures 5.12-5.17.

The analyses of contact angle exhibited important differences between treated and original woods veneers. The original wooden veneers presented hydrophilic and oleophilic character[171]. However, wetting properties of treated wood displayed a great improvement against water and oil. Coated wood veneers presented values between 90-120º for water and 70-100º for ethylene glycol. In the case of impregnated wood veneers, the contact angles were higher and more stable than for coated woods and remained practically unchanged during the test period with 120-140º for water and between 110-130º for oil.

Although the general tendency of the oil repellency of wood veneers with press molding treatment was the stability over time, higher values could be observed for coated beech veneers (90-110º) than for coated poplar veneer (80-100º). With respect to the results with poplar veneers, it was found that it surface was slightly apolar initially (without treatment) with WCA higher than beech veneers and with a total free surface energy lower than that found in press moulding treatments and specifically with lower disperse shares (leading to a better behaviour with ethylene glycol). It seems that the pretreatment of poplar veneers (180-grit sanded) was crucial to activating the free surface energy, showing an equitable distribution of shares; while in the treated poplar veneers the free surface energy was practically dispersive with lower polar shares.

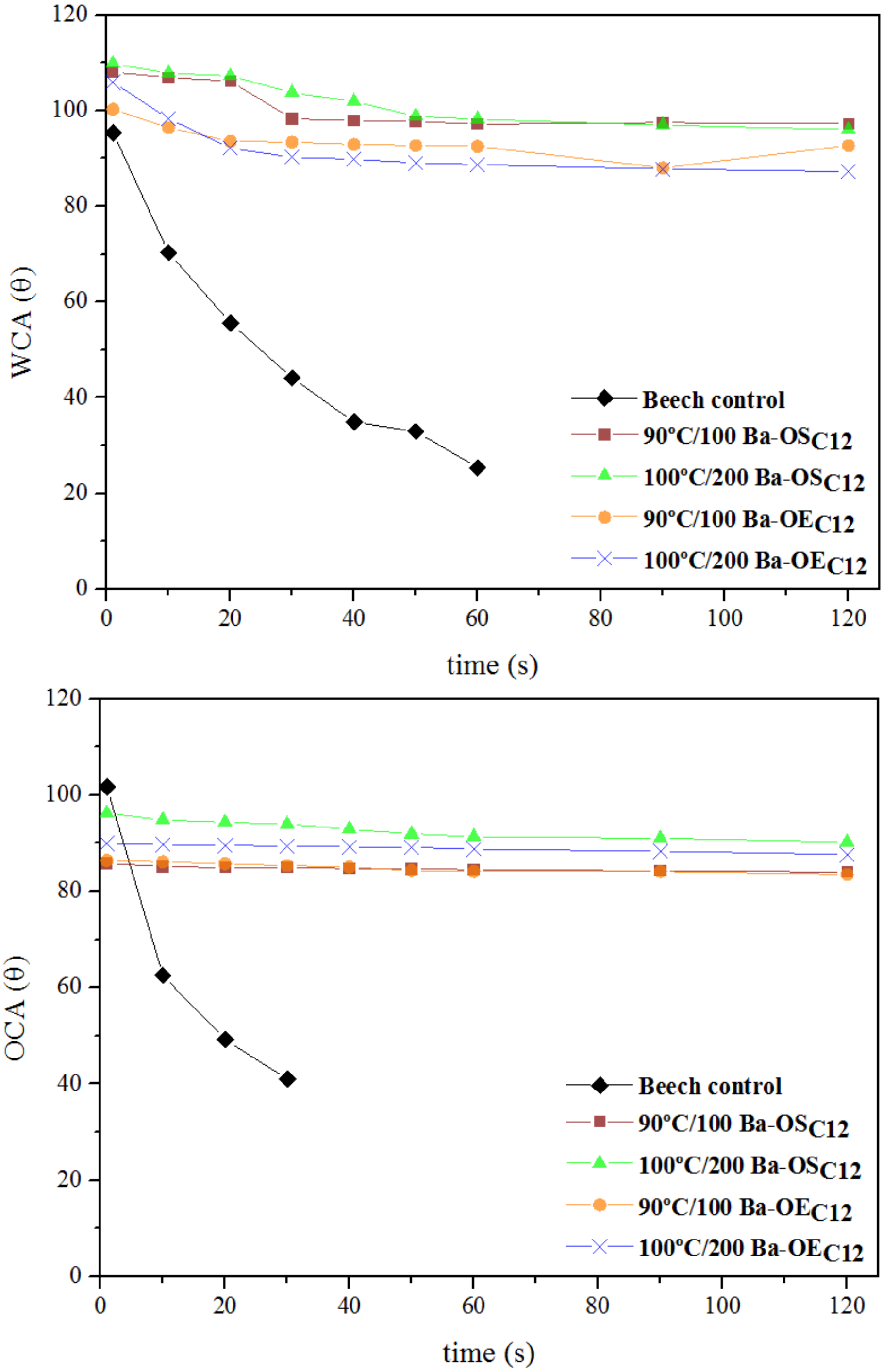


Figure 5.12. Water and ethylenglicol dynamic contact angle of coated beech.

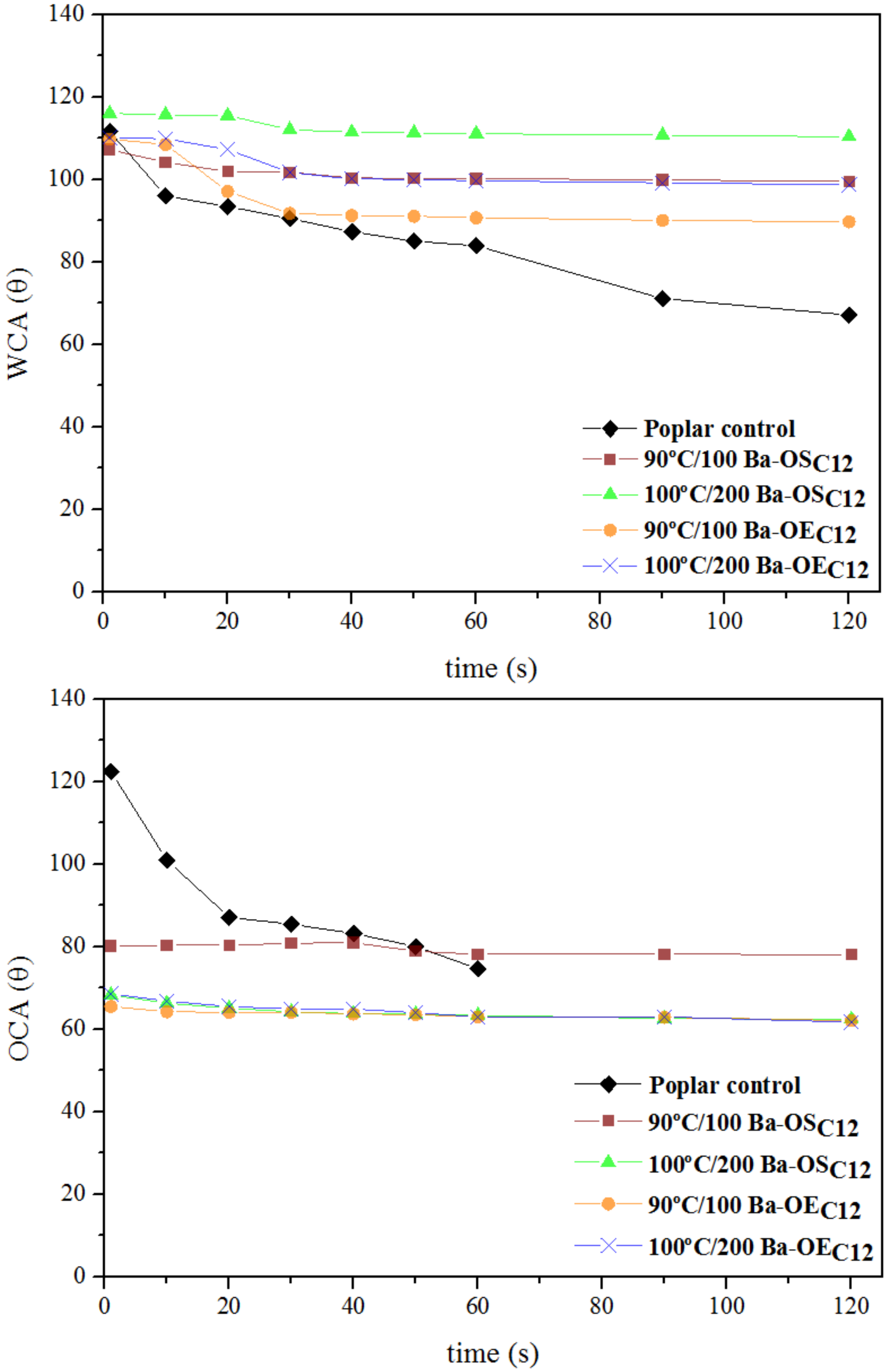


Figure 5.13. Water and ethylenglicol dynamic contact angle of coated poplar.

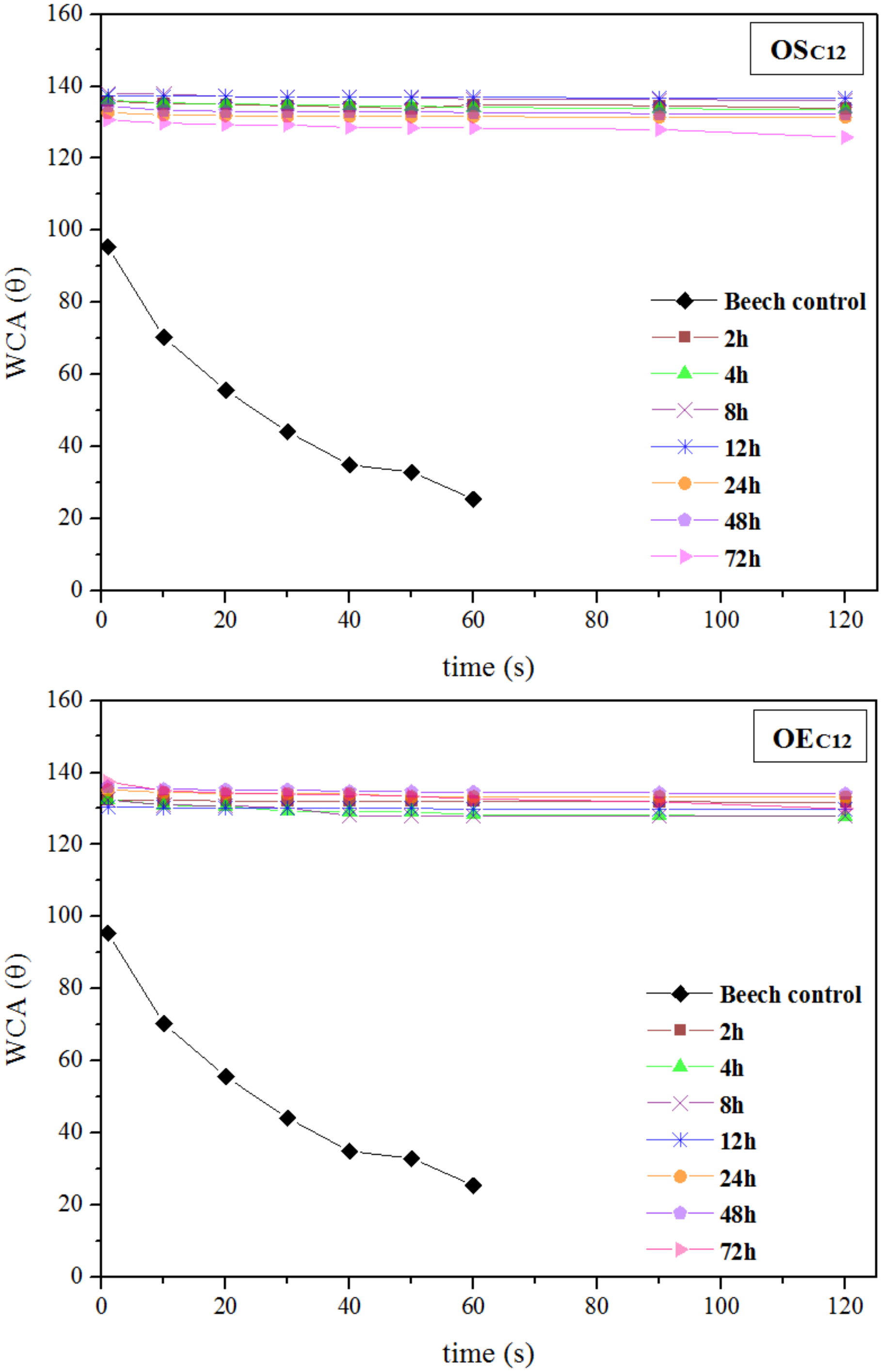


Figure 5.14. Water dynamic contact angle of impregnated beech with OS_{C12} and OE_{C12}.

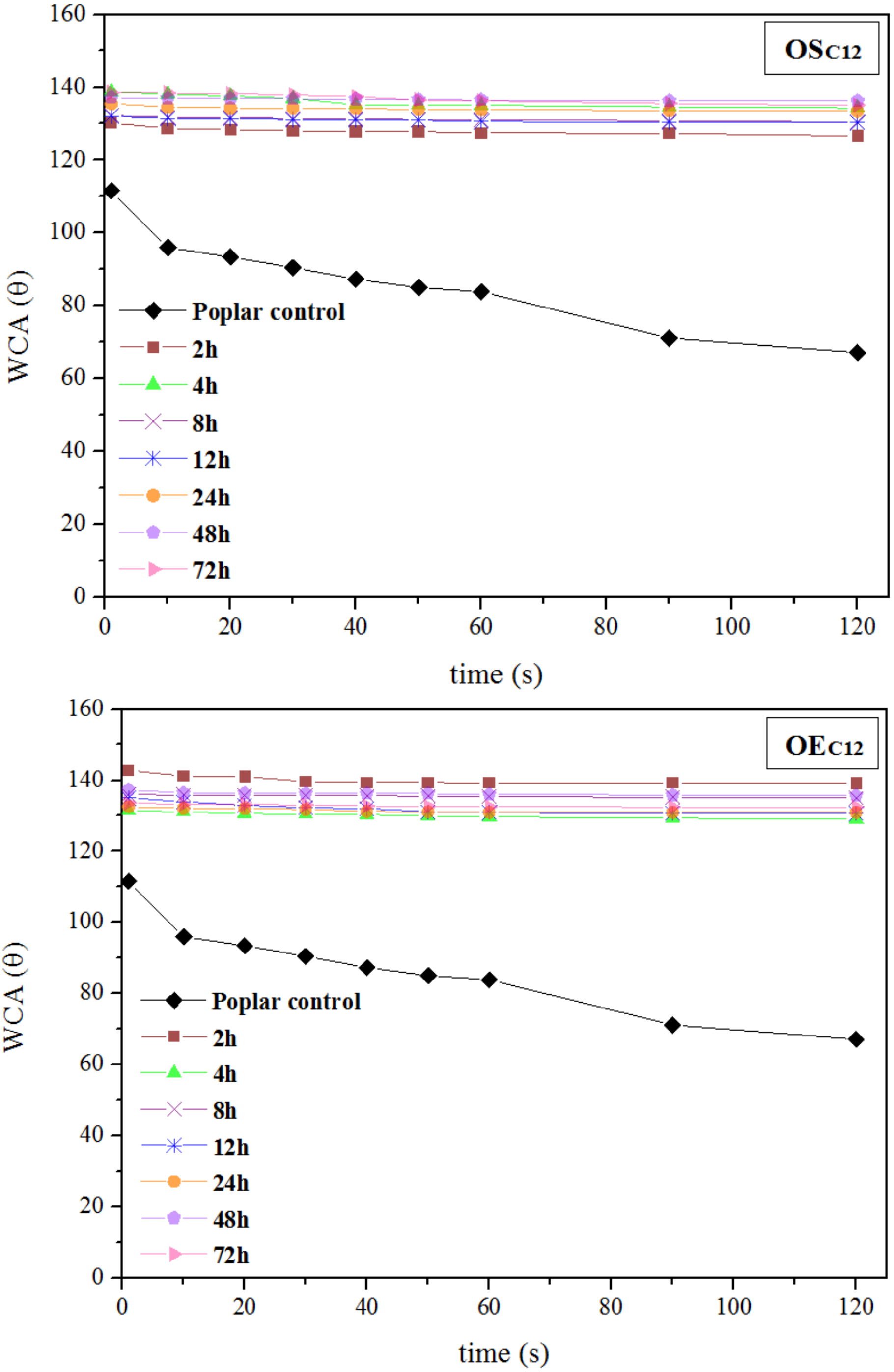
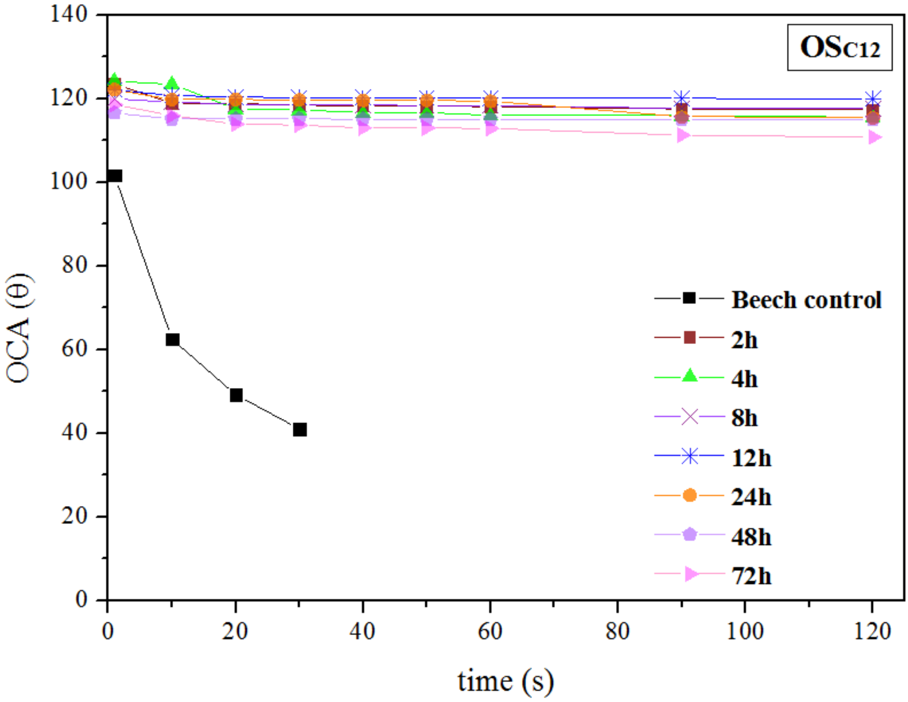


Figure 5.15. Water dynamic contact angle of impregnated poplar with OS_{C12} and OE_{C12}.



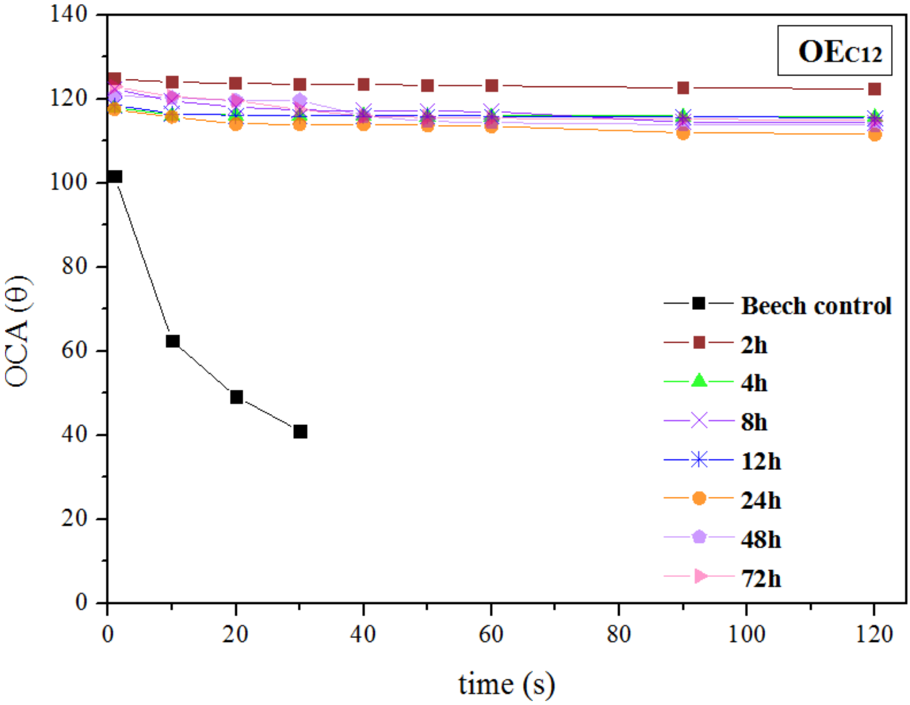


Figure 5.16. Ethylenglicol dynamic contact angle of impregnated beech with OS$_{C12}$ and OE$_{C12}$.

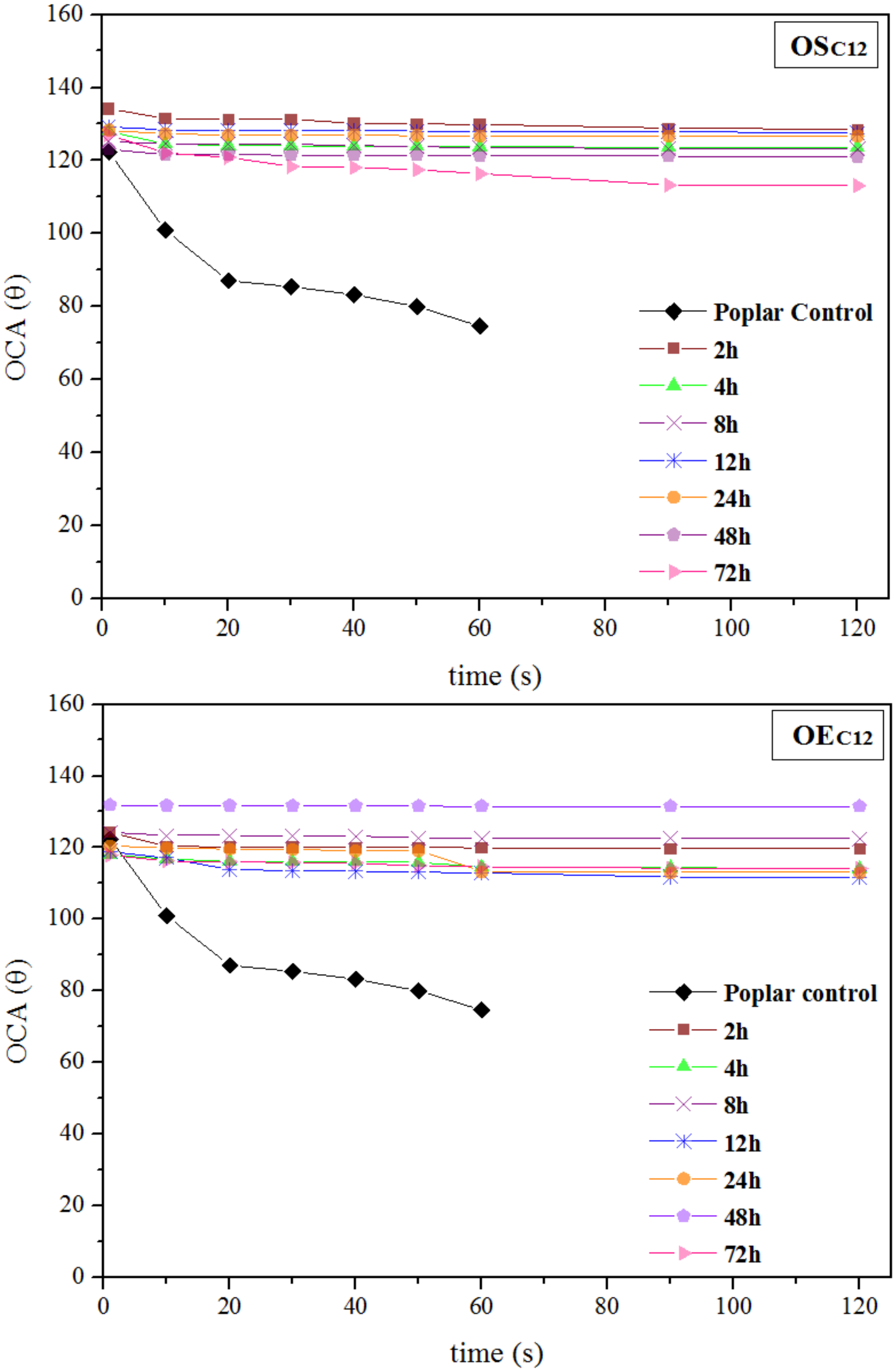


Figure 5.17. Ethylenglicol dynamic contact angle of impregnated poplar with OS_{C12} and OE_{C12}.

The study of the contact angles of three different liquids and the free surface energy of the veneers treated and control was studied, in order to complete the study of liquid-substrate interactions (Table 5.8). The hydrophobic character of synthesized lignin derivate provides the high contact angle values which are stable over time. Although used lignins come from a different origin (hardwood and softwood) and have different chemical structure; there were no significant differences between them regarding their hydrophobic behavior, thus proving that esterified lignin is an appropriate agent to increase the wood durability.

However, there can be observed few differences depending on the selected application methods, being more hydrophobic and oleophobic when impregnation method was used. Nowadays, the modification and hydrophobization of wooden surfaces by using silanes are being studied and several results are already presented in the literature with lower values than presented values[172,173]. Other authors obtained values below 90° using commercial coatings[174,175].

Table 5.8. Contact angles of three liquids with different polarity and free surface energy of veneers.

	Test[a]	**Control**	**OS_{C12}**		**OE_{C12}**	
			Coating[b]	**Immersion[c]**	**Coating[b]**	**Immersion[c]**
Beech	WCA	24.5 ± 3.7	96.9 ± 1.4	132.5 ± 3.2	88.7 ± 3.2	133.2 ± 2.7
	OCA	40.0 ± 18.3	91.4 ± 4.1	120.0 ± 2.7	86.5 ± 4.1	117.5 ± 5.1
	DCA	3.5 ± 2.1	56.7 ± 4.4	7.0 ± 5.9	55.5 ± 1.1	7.6 ± 3.7
	γ^p	24.6	0.5	9.3	2.3	9.6
	γ^d	36.7	26.1	40.1	24.2	40.9
	γ^T	61.3	26.6	49.4	26.5	50.4
Poplar	WCA	67.3 ± 5.3	111.3 ± 8.6	131.7 ± 4.7	99.6 ± 1.0	131.1 ± 3.8
	OCA	80.4 ± 8.1	66.3 ± 3.8	123.5 ± 6.7	62.9 ± 3.7	121.0 ± 4.4
	DCA	62.6 ± 6.0	40.5 ± 2.6	62.6 ± 4.6	42.0 ± 1.0	4.7 ± 1.0
	γ^p	12.9	0.69	3.0	0.04	8.8
	γ^d	18.3	42.9	21.4	39.1	39.7
	γ^T	31.3	43.6	24.5	39.1	48.5

[a] Values derived of average contact angle at time 60 s.

[b] Average contact angle of both press moulding treatments at time 60 s.

[c] Average contact at time 60 s.

5.4.2.4. Accelerated weathering

Following the initial successful results, it was necessary to analyze their effectiveness through material service life. For this purpose, an accelerated aging test on treated wood veneers was performed to know how could affect it on the wetting properties and also compare with aged control samples. In order to know the changes during the aging cycles, the weight loss of samples was measured. Although a clear trend was not found, controls and treated wood samples showed comparable weight loss with values between 1 and 3%.

Another way to follow the changes due to aging was the evaluation of color by the means of ΔE. In the case of coated samples, it is remarkable the stability of the samples treated at lower conditions (90 ºC/100 bar) for both esterified lignins recording values below controls (ΔE = 2-6) and being OS_{C12} lignin at process conditions of 90 ºC and 100 bar the most stable treatment. Regarding impregnated wood samples, all presented similar color changes compared with controls samples. However, the impregnated poplar samples were slightly more stable.

Finally, the dynamic contact angle was measured. As expected, aged controls presented higher affinity for water (Figure 5.18). In the case of ethylene glycol, it was not possible to measure the trend over time after aging due to its high affinity. On the other hand, aged coated surfaces exhibited a great improvement against oil and water compared to the aged controls (Figure 5.19). In the case of hydrophobicity, all wood samples showed WCA > 60º after two minutes of the test. However, the effectiveness of the esterified lignins against ethylene glycol after aging was lower than the previous values presented in Section 5.4.2.3. For this reason, it was taken as reference time of 20 s which allows recording the contact angle in all samples. Thus, less stability with an important decrease of contact angle (OCA) after aging at that time was observed, being the results between 40 and 80% less for OS_{C12} coatings and about 20-30% lower for OE_{C12} coatings.

Concerning impregnated aged samples it was found a higher stability against water and oil than in coated aged veneers. In the case of WCA, the values were higher than 80º after two minutes of the test. On the other hand, greater differences were observed during dynamic contact angle with ethylene glycol on aged impregnated veneers. At the reference time (20 s) it was observed a reduction of OCA between 25 and 100% for beech-OS_{C12}, 25-70% for beech-OE_{C12}, 25-40% for poplar-OS_{C12} and 10-30% for poplar-OE_{C12}. The obtained WCA and OCA values after artificial aging were positive, proving the resistance of the synthesized hydrophobic agent and thus improving the wood products service life. These contact angle values are comparable with commercial products and with those found in the literature.

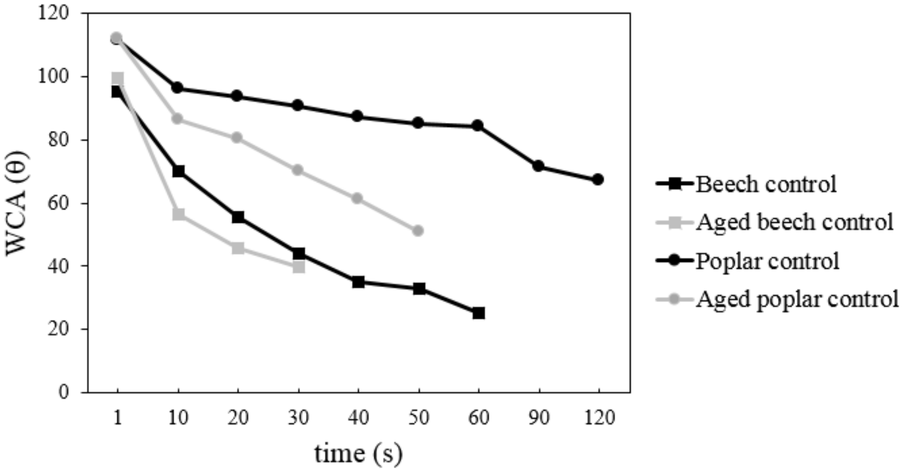


Figure 5.18. Differences on WCA between unaged controls and aged controls.

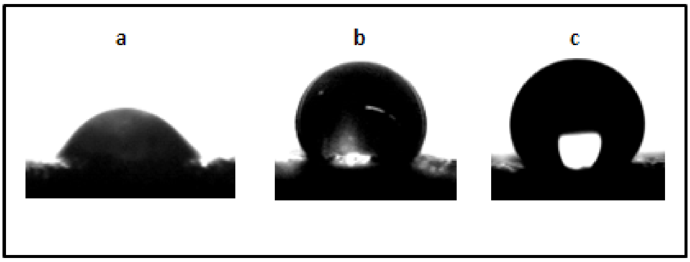


Figure 5.19. Wetting of a wood surfaces by liquid: (a) aged poplar at 30s with water, (b) impregnated aged poplar with OS_{12C} (2h) at 120s with water, (c) impregnated aged poplar with OE_{12C} (4h) at 120s with water.

The decay test of impregnated wood veneers after accelerated weathering was performed with white-rot fungus *Trametes versicolor*. The results of the experiment after 8 weeks of incubation at 25 ºC and at a relative humidity of 60 ± 5% indicated that the impregnation of beech and poplar wood veneers with both lignin-ester derivatives had a negative effect, showing very low antifungal capacity. Impregnated wood veneers mass loss values were between 70-90%, regardless the time of impregnation, while control samples (without impregnation) reached only 30-40% of mass loss while in contact with the white-rot fungus.

5.5. Conclusions

Synthesized lignin-ester derivatives were successfully used for proposed applications providing several improvements in both cases.

As PLA additive, a good compatibility with PLA matrix was observed, without the presence of aggregates. However, the color change on films containing lignin-esters was clearly appreciable, especially at high lignin contents. Moreover, the results suggested that lignin-ester derivatives could be an alternative to synthetic sunscreen additives since both esterified lignins demonstrated their capacity as UV light absorbing product. In addition, a significant influence on mechanical properties was observed by the incorporation of lignin-esters into PLA, having an important enhancement of the ductility of PLA, essential requirements for packaging uses.

On the other hand, esterified organosolv lignins were suitable for use as hydrophobic agents on wood products. The reduction of glass transition temperature after esterification process allows using them by industrial processing techniques such as press molding. Moreover, a stable and highly hydrophobic and oleophobic behavior of wood veneers was observed after treatments, being the immersion method, the one which provided the highest and more stable dynamic contact angle for water and oil. Besides, the color change on treated veneers was visually appreciable, especially in the case of coated woods. The dark shades generated by these products can be an advantage for undervalued woods by adding an aesthetic contribution. In addition, the efficiency of treatments over time was confirmed by the means of color analysis and dynamic contact angle, proving that lignin-esters improved the wood products service-life. However, treated wood veneers showed very poor antifungal capacity, making essential the incorporation of commercial antifungal additives for further applications in wood products.

6

General conclusions, future research and published works

6.1. General conclusions

The present thesis pretends to contribute to the revalorization of lignin in order to increase its economic value and exploit its numerous advantages. For this purpose, lignins from different sources were evaluated as feedstock for several applications. Moreover, chemical modification of lignin structure was considered as a simple strategy for the obtention lignin-based products with new and suitable properties for various uses. The esterification reactions used in this work resulted in an easy technique for lignin modification without using temperature.

The mixing of lignin with polymers, PLA in this case, can be performed by an extrusion process. However, a chemical modification of lignin structure was in general necessary for the obtention s blends with homogenous appearance. Regarding the compatibility of the elaborated blends in this work, a clear difference between them could be observed. Acetylation treatment resulted in a suitable route to get lignins more compatible with PLA. Moreover, the addition either of unmodified lignin or of acetylated lignins increased the thermal stability of PLA. Lignin incorporation affected to the mechanical properties of PLA, and although the addition of high content (more than 5%) presented a negative effect on PLA mechanical properties, acetylated lignin had always more positive influence than unmodified lignin on mechanical properties.

In addition, the extensive study about chemical structure and functional properties of lignin samples obtained from industry and at laboratory scale revealed that lignin has very high potential to be included in several industries for the development of innovative applications replacing some products used today.

Several differences were found concerning physicochemical properties of studied lignins. As regards the origin, spruce lignins presented more condensed structure being it the main cause of their high thermal stability.

The results of antioxidant properties and antimicrobial capacity of lignins against *A. Niger* and diverse foodborne and human pathogenic microorganisms open new perspectives for the utilization of lignin in cosmetic and topical formulations as well as in food and pharmaceutical industry. Moreover, lignins showed high potential to be used as natural sunscreen product. The HHV of lignins with high purity suggested a suitability for uses as an additive product in solid fuels; however, the sulfur and ash content of lignin were higher than recommended by European standards for pellets elaboration.

The attachment of fatty acids to softwood and hardwood organosolv lignins was suitable to prepare lignin-esters derivatives with higher hydrophobicity and low glass transitions temperatures, which can be used for several applications. Moreover, the increase of thermoplasticity of lignins after chemical modification makes possible their processing by press molding. Although lignin-ester derivatives showed lower thermal stability than original lignins, their initial degradation temperature was above 200ºC. Therefore, lignin-ester can be used in polymers processed below 200 ºC.

The use of synthesized lignin-ester derivatives for additive in PLA films as well as for hydrophobic agent in wood products revealed good results. As PLA additive, lignin-esters showed an appropriate UV light absorber capacity in addition to providing an important enhancement in the ductility of PLA.

Moreover, lignin-ester could be applied onto wood veneers by two different treatments such as coating using press molding and immersion. A stable and high hydrophobic and oleophobic behavior of wood veneers was observed after treatments, especially for woods treated by the immersion method. Moreover, the efficiency of treatments over time was confirmed, proving that lignin-esters improved the wood products service life. However, treated wood veneers showed very poor antifungal capacity, making essential the incorporation of commercial antifungal additive for further applications in wood products.

6.2. Future works

The following points are interesting studies yet to be carried out:

- Isolation of available lignin from industrial kraft black liquor using different methods in order to obtain different lignin samples with a great variety of chemical and structural characteristics from the same source.
- Study the functional properties and the applicability of each lignin samples regarding their chemical structure.
- Optimization of the chemical modification of kraft lignin samples using fatty acids with different chain lengths and different reaction conditions (concentration and temperature).
- Study the use of lignin and lignin derivatives as UV protection agents for packaging and cosmetic applications.

6.3. Published works

6.3.1. Publications in scientific journals

During this doctoral thesis, the following publications in peer-reviewed scientific journals related to the topic of the thesis have been written:

I. **Authors:** **Gordobil O**, Herrera Díaz R, Yahyaoui M, İlkc S, Kayac M, Labidi J
Title: Potential use of kraft and organosolv lignins as a natural additive for healthcare product
Journal: Biochemical Engineering Journal
Year: 2018
Impact factor:2.892 (Submitted)
JC and JR: --

II. **Authors:** **Gordobil O**, Herrera Díaz R, Llano-Ponte R, Labidi J
Title: Esterified organosolv lignin as hydrophobic agent for use on wood products
Journal: Progress in organic coating 103, 143-151

Year: 2017
Impact factor: 2.632 (2016)
JC and JR: Material Science, Coating and Films (3/19) Q

III. **Authors:** **Gordobil O**, Egüés I, Labidi J
Title: Modification of Eucalyptus and Spruce organosolv lignins with fatty acids to use as filler in PLA
Journal: Reactive Functional Polymers 104, 45-52
Year: 2016
Impact factor: 3.151 (2016)
JC and JR: Polymer Science (18/86) Q1

IV. **Authors:** **Gordobil O**, Moriana R, Zhang L, Labidi J, Sevatyanova O
Title: Assessment of technical lignins for uses in biofuels and biomaterials: Structure-related properties, proximate analysis and chemical modification
Journal: Industrial Crops and products 72, 46-53
Year: 2016
Impact factor: 3.181 (2016)
JC and JR: Agricultural engineering (3/14) Q1

V. **Authors:** **Gordobil O**, Delucis R, Egüés I, Labidi J
Title: Kraft lignin as filler in PLA to improve ductility and thermal properties
Journal: Industrial Crops and products 72, 46-53
Year: 2015
Impact factor: 3.449 (2015)
JC and JR: Agricultural engineering (2/14) Q1

VI. **Authors:** **Gordobil O**, Egüés I, Llano-Ponte R, Labidi J
Title: Physicochemical properties of PLA-Lignin blends
Journal: Polymer Degradation and Stability 108, 330-338
Year: 2014
Impact factor: 3.163 (2014)
JC and JR: Polymer Science (17/82) Q1

Moreover, other works and collaborations have also been published in peer-reviewed scientific journals:

I. **Authors:** Herrera Díaz R, **Gordobil O**, De Hoyos P, Llano-Ponte R, Labidi J
Title: Wood modification using fatty acids.
Journal: --
Year: 2018

Impact factor: Under Development
JC and JR: --

II. **Authors:** Yahyaoui M, **Gordobil O**, Herrera Díaz R, Abderrabba M, Labidi J
Title: New active food packaging based on PLA and plant extracts
Journal: Food Hydrocolloids
Year: 2018
Impact factor: 4.747 (Submitted)
JC and JR: --

III. **Authors:** Yahyaoui M, **Gordobil O**, Herrera Díaz R, Abderrabba M, Labidi J
Title: Evaluation of Mediterranean essential oils as additives in corn starch films for food packaging
Journal: Carbohydrate Polymers
Year: 2018
Impact factor: 4.811 (Submitted)
JC and JR: --

IV. **Authors:** Robles E, Fernández-Rodríguez J, Barbosa A.M., **Gordobil O**, Carreño N.L.V, Labidi J
Title: Production of cellulose nanoparticles from blue agave waste treated with environmentally friendly processes
Journal: Carbohydrate Polymer
Year: 2018
Impact factor: 4.881
JC and JR: Polymer Science (8/89) Q1

V. **Authors:** Podkoscielna B, **Gordobil O**, Riazanova A.V, Dobele G, Labidi J, Lindström M.E, Gunko V. M, Sevastyanova O
Title: Novel porous polymeric materials obtained from technical lignins or their corresponding methacrylates copolymerized with styrene and divinylbenzene
Journal: Chemistry Select 2, 2257-2264
Year: 2017
Impact factor: Not indexed
JC and JR: --

VI. **Authors:** Fernández-Rodríguez J, **Gordobil O**, Robles E, González-Alriols M, Labidi J
Title: Lignin valorization from side-streams produced during agricultural waste pulping and total chlorine free bleaching
Journal: Journal of cleaner production 142, 2609-2617
Year: 2017

Impact factor: 4.959 (2016)
JC and JR: Engineering (6/49) Q1

VII. **Authors: Gordobil O**, Robles E, Egüés I, Labidi J
Title: Lignin-ester derivatives as novel thermoplastic material
Journal: RSC Advances 6 (90), 86909-86917
Year: 2016
Impact factor: 3.108 (2016)
JC and JR: Chemistry Multidisciplinary (59/166) Q2

VIII. **Authors:** Yahyaoui M, **Gordobil O**, Herrera Díaz R, Abderrabba M, Labidi J
Title: Development of novel antimicrobial films based on poly(lactic acid) and essential oils
Journal: Reactive and Functional Polymers 109, 1-8
Year: 2016
Impact factor: 3.151 (2016)
JC and JR: Polymer Science (18/86) Q1

IX. **Authors:** Dávila I, **Gordobil O**, Labidi J, Gullón P.
Title: Assessment of suitability of vine shoots for hemicellulosic oligosaccharides production through aqueous processing
Journal: Bioresource Technology 211, 636-644
Year: 2016
Impact factor: 5.651 (2016)
JC and JR: Agricultural Engineering (1/14) Q1

X. **Authors: Gordobil O**, Egüés I, Urruzola I, Labidi J
Title: Xylan-Cellulose Films: Improvement of hydrophobicity, thermal and mechanical properties
Journal: Carbohydrate Polymers 112, 56-62
Year: 2014
Impact factor: 4.074 (2014)
JC and JR: Polymer Science (9/82) Q1

6.3.2. Contributions in scientific conferences

Authors	**Gordobil O**, Robles E, Herrera R, Egüés I, Labidi J
Title	Synthesis of new product based on esterified lignin and its different applications
Congress	10th World Conference of Chemical Engineering
Participation	Poster
Year	2017
Place	Barcelona, Spain

Authors	**Gordobil O**, Egüés I, Urruzola I, Labidi J
Title	Xylan-Cellulose Films: Improvement of hydrophobicity, thermal and mechanical properties
Congress	4th International Conference on Biobased Materials and Composites
Participation	Poster
Year	2017
Place	Nantes, France

Authors	Herrera R, **Gordobil O**, Labidi J
Title	Esterified lignin as hydrophobic agent for use on wood products
Congress	13th Pacific Rim Bio-Based Composite Symposium (BioComp2016)
Participation	Oral
Year	2016
Place	Concepcion, Chile

Authors	Podkościelna B, **Gordobil O**, Riazanova A, LabidI J, Sevastyanova O
Title	Lignin vinyl esters and their co-polymers with styrene, divinylbenzene and triethoxyvinylsilane
Congress	COST Action FP1306 "Valorization of lignocellulosic biomass side streams for sustainable production of chemicals, materials & fuels using low environmental impact technologies"
Participation	Oral
Year	2016
Place	Lisbon, Portugal

Authors	**Gordobil O**, Labidi J
Title	Elaboration of lignin-ester derivative with great ability to melt
Congress	14th International Symposium on Bioplastics, Biocomposites & Biorefining (ISBBB 2016)
Participation	Poster
Year	2016
Place	Guelph, Canada

Authors	**Gordobil O**, Podkościelna B, Zhang L, Labidi J, Lindström M.E, Gun'ko V.M, Sevastyanova O

Title	Novel porous polymeric materials obtained by copolymerization of lignin or lignin methacrylates with styrene and divinylbenzene: preparation and characterization
Congress	14th European Workshop on Lignocellulosic and Pulp (EWLP-2016)
Participation	Oral
Year	2016
Place	Grenoble, France

Authors	Sevastyanova O, **Gordobil O**, Podkoscielna B, Labidi J, Lindström M.E.
Title	Novel porous materials from lignin methacrylates copolymerized with styrene and divinylbenzene
Congress	251st ACS National Meeting & Exposition
Participation	Oral
Year	2016
Place	San Diego, California

Authors	Gullón P, **Gordobil O**, Dávila I, Egües I, Labidi J
Title	Synthesis of green hydrogels using lignin from organosolv process of almond shells
Congress	EPNOE 2015
Participation	Poster
Year	2015
Place	Varsovia, Poland

Authors	**Gordobil O**, Delucis R, Egüés I, Labidi J
Title	Kraft lignin as filler in PLA to improve ductility
Congress	5th International Conference on Biodegradable and Biobased Polymers (BIOPOL-2015)
Participation	Poster
Year	2015
Place	Donostia-San Sebastián, Spain

Authors	**Gordobil O**, Moriana R, Zhang L, Sevastyanova O, Labidi J
Title	Evaluation of softwood kraft and organosolv lignin as feedstock for biofuels and biomaterials
Congress	COST Action FP1105 “Understanding wood cell wall structure, biopolymer interaction and composition: Implications for current products and new materials”
Participation	Oral and Poster
Year	2015

Place	Donostia-San Sebastián, Spain

Authors	**Gordobil O**, Moriana R, Zhang L, Borges Gomes F.J, Labidi J, Colodette J.L., Lindström M.E., Sevastyanova O
Title	Assessing the potential of eucalyptus kraft and organosolv lignins as a resource for sustainable biomaterials or biofuels
Congress	7th International Colloquium on Eucalyptus Pulp
Participation	Oral
Year	2015
Place	Vitória, Espírito Santo, Brazil

Authors	**Gordobil O**, Labidi J
Title	Chemical modification of organosolv Spruce lignin with fatty acids
Congress	BiMate: Biopolymers and Engineering technology
Participation	Poster
Year	2015
Place	Slovenj Gradec, Slovenia

Authors	**Gordobil O**, Egüés I, Urruzola I, Labidi J
Title	Physicochemical properties of PLA-Lignin blends
Congress	13th European workshop on lignicellulosic and pulp
Participation	Poster
Year	2014
Place	Seville, Spain

Authors	**Gordobil O**, Egüés I, Llano-Ponte R, Labidi J
Title	Physicochemical properties of PLA-Lignin blends
Congress	International Conference on Biobased Materials and Composites, ICBMC'14
Participation	Poster
Year	2014
Place	Montreal, Canada

Authors	**Gordobil O**, Egüés I, Urruzola I, Labidi J
Title	Xylan-Cellulose Films
Congress	COST Action FP1105 "Understanding wood cell wall structure, biopolymer interaction and composition: Implications for current products and new materials"
Participation	Oral and Poster
Year	2014

Place	Coimbra, Portugal

Authors	Egüés I, **Gordobil O**, Llano-Ponte R, Labidi J
Title	Biofuel and biochemicals from Olive Tree pruning
Congress	4th International Conference on Biorefinery – towards Bioenergy (ICBB)
Participation	Oral and Poster
Year	2013
Place	Xiamen, China

Authors	Egüés I, **Gordobil O**, Eceiza A, Labidi J
Title	Effect of different hemicelluloses characteristics on film forming properties
Congress	4th International Conference on Biodegradable and Biobased Polymers (BIOPOL-2013)
Participation	Oral and Poster
Year	2013
Place	Roma, Italia

Authors	**Gordobil O**, Egüés I, Llano-Ponte R, Labidi J
Title	Physicochemical properties of PLA-Lignin blends
Congress	COST Action FP1105 “Understanding wood cell wall structure, biopolymer interaction and composition: Implications for current products and new materials”
Participation	Oral and Poster
Year	2013
Place	Trabzon, Turkey

Authors	Llano-Ponte R, **Gordobil O**, Robles E, Egües I, Labidi J
Title	Obta+C1:C80ining cellulose microfibrils from jute fibers and bleached by chemical and physical processes
Congress	Materiales Compuestos
Participation	Oral
Year	2013
Place	Algeciras (Spain)

Chapter 6

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Appendices

Appendix I. Procedures

1. Chemical composition of raw materials

1.1. Lignin

Procedure TAPPI T222 om-98. Acid-insoluble lignin in wood and pulp[176].

1.2. Extractives

Procedure TAPPI T204 om-97. Solvent extractives of wood and pulp[177].

1.3. Ashes

Procedure TAPPI T211 om-02. Ash in wood, pulp, paper and paperboard. combustion at 525 °C[178].

1.4. Holocellulose

Holocellulose comprises the water insoluble carbohydrates fraction of the lignocellulose, that is, the sum of hemicellulose and cellulose. The holocellulose content was determined according to the method proposed by Wise et al., 1946[179].

1.5. Cellulose

The procedure to obtain the wood cellulose is referred as the ‘’Rowell method’’ from the book: The Chemistry of Solid Wood, 1984, edited by Professor R. Rowell[180].

2. Characterization of the Kraft black liquor

2.1. pH

The pH of the liquid fraction was determined using a pH meter “CRISON basic 20”.

2.2. Density

The density was calculated gravimetrically by measuring the weight of known volume of a volumetric flask filled with the liquid fraction.

2.3. Total dry solids

The total solids content of liquor is the amount of material left as a residue upon drying at 105 ºC to constant weight. 5 ± 0.001 g of the liquid fraction (m) were weighed in a crucible free of moisture and tared (m_c). The filled crucible was placed in an oven at 105 ± 3 ºC for 24 hours. After cooling in desiccators, the crucible was weighed (m_f) until constant weight. The total dry solids (TDS) were determined as follows:

$$TDS\,(\%) = \left[\frac{(m_f - m_i)}{m}\right].100 \tag{1}$$

m_i = weight of the dried crucible (g)
m_f =weight of the crucible after the procedure (g)
m = weight of the liquid fraction (g)

The inorganic fraction (IM) of the liquid fraction can be determined after the combustion of the sample. The crucible assembly-solid residue obtained in the previous experiment (total dry solids) is combusted in the oven at 525 ºC for 3 hours. Subsequently, after cooling to room temperature in desiccators, the crucible is weighed assembly (m_f).

The inorganic matter (IM) is determined as follows:

$$IM\ (\%) = \left[\frac{(m_f - m_i)}{m}\right].100 \tag{2}$$

m_i = weight of dried crucible with solid dry residue (g)
m_f = final weight of the crucible after combustion (g)
m = weight of the liquid fraction (g)

The organic matter (OM) is determined as follows:

$$OM\ (\%) = TDS\ (\%) - \ IM\ (\%) \tag{3}$$

2.4. Lignin content

The method for determining lignin concentration in the liquid fraction varies depending on the pretreatment applied to the raw material for the lignin extraction. Nevertheless, the principle that governs all isolated procedures is the same, the insolubility of lignin in acid media. A known volume of liquid fraction (V) is treated with the corresponding lignin isolation procedure. Previously, the filters have to be dried over night at 50 ± 1 ºC and then tared (m_i). The liquid fraction is precipitated and left overnight depending on the isolation procedure. The precipitated liquid fraction is filtered and the filter is dried at 50 ± 1 ºC until the lignin gets dried. Once dried, samples were transferred to desiccators until they cool down. After cooling the samples, weigh (m_f) until weight of the sample is constant to ± 0.2 mg.

$$Lignin\ concentration\ (g/L) = \left[\frac{(m_f - mi)}{V/1000}\right] \tag{4}$$

V = liquid fraction volume (mL)
m_i = weight of the dried filter (g)
m_f = weight of the dried filter + lignin (g)

3. Characterization of lignin composition

3.1. Klason lignin (acid-insoluble lignin) and acid soluble lignin

3.75 mL of 72% of H_2SO_4 was added to 3.75 mg of lignin sample and was maintained for 1 h at 30 °C. Then, 36.25 mL of distilled water was added, and it was hydrolyzed at 100°C during 3 h. After cool down for 15 minutes, the solid residue was filtrated, oven dried and weighed as Klason lignin. The acid-insoluble content was determined as follows:

$$Acid - insoluble\ lignin = \frac{A}{W} \cdot 100 \tag{5}$$

A = weight of acid-insoluble lignin (g)
W = weight of the initial sample (g)

The filtrated liquid fraction was reserved for determination of acid soluble lignin fraction by spectrophotometry (UV absorption at 205 nm).

3.2. Monosaccharide content

The filtrated liquid fraction reserved from acid-insoluble lignin was used to inject into a high performance liquid chromatography equipment. (See Appendix II section 2.2.)

4. Phenolic content and antioxidant test

4.1. Total phenolic content

The total phenolic content of lignin samples was determined by the Folin-Ciocalteu spectrophotometric method using gallic acid as reference compound and dimethyl sulfoxide (DMSO) as solvent. As first step, a calibration curve using gallic acid as reference in DMSO was calculated with six different concentrations (100-1000 mg/L). Lignin samples were dissolved in DMSO (2 g/L). Na_2CO_3 at 200 g/L was prepared. For the analysis, 0.5 mL of lignin solution, 2.5 ml of Folin-Ciolcalteau reagent and 5 ml of Na_2CO_3 were added to 50 mL flask and covered with distilled water. The samples were kept in a thermostatic bath at 40 ºC for 30 min before spectrophotometric measurement of the absorbance at 750 nm (Jasco V-630 spectrophotometer). The blank was prepared in the same way but adding 0.5 mL of DMSO instead of sample. The total phenolic content of lignin samples was expressed as the gallic acid equivalent (% GAE) and hydroxyls content (wt%). All parameters were calculated on dry basis.

$$GAE = \frac{C_{GAE}}{C_{sample}[1-\frac{(H)}{100}]} \cdot 100 \tag{6}$$

$$OH\ (\%) = \frac{C_{GAE}}{170.12 \cdot 1000} \cdot 4 \cdot 17 \cdot \frac{1}{C_{sample}} \tag{7}$$

C_{GAE} = concentration of gallic acid obtained by the calibration curve
C_{sample} = concentration of the lignin sample in DMSO and
H = humidity content.

4.2. ABTS test

For ABTS assay, a radical solution (7 mM ABTS and 2.45 mM potassium persulfate) was prepared and left to stand in the dark at room temperature for 16 h before using. This solution was then diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm and equilibrated at 30 ºC. For the analysis, 2 mL of the diluted radical solution were mixed with 20 µL of the sample and the absorbance at 734 nm was read against ethanol in a Jasco V-630 spectrophotometer. The absorbance at 734 nm of all the samples was registered after 6 minutes. Commercial antioxidants (BHT and Trolox) were used as positive control. Each test was carried out in triplicate. The inhibition percentage of the ABTS radical was calculated according to the following formula:

$$Inhibition\ (\%) = \frac{A_0 - A_1}{A_0} \cdot 100 \qquad (8)$$

A_0 = absorbance of control

A_1 = absorbance of lignin sample.

The radical scavenging activity of the lignin was characterized using the term of “Efficient Concentration” or IC_{50}, which is the concentration required for 50% inhibition of the free radical.

4.3. DPPH test

For DPPH antioxidant test DMSO was also used to dissolve the lignin samples at different concentrations (0-2 mg/mL). The DPPH scavenging activity was evaluated using the method described by Brand-Williams et al, 1995[181] with some modifications. 0.1 mL of lignin solution was added to 3.9 mL DPPH (25mg/L in methanol). The absorbance was measured at 517 nm after 30 min of incubation at room temperature. Commercial antioxidants (BHT and Trolox) were used as positive control. Each test was carried out in triplicate. The inhibition percentage of the DPPH radical was calculated using the same formula described for ABTS antioxidant assay and it expressed as IC_{50}.

5. Biological assays

5.1. Antifungal test

For antifungal behaviour of lignins, lignin samples were diluted in DMSO at different concentrations (1, 2.5, 5, 7.5, 10, 15, and 20%) and then was evaluated the antifungal activity against *A. Niger* (Tiegh MB284309 CBS-KNAW, Holland) by impregnation and exposition to fungal strain in PDA. Cellulose pellets

(Ø=10mm) were soaked with 40 µL of each lignin solution and placed on Petri dishes with PDA (10 mL) and 0.4% Streptomycin. Each PDA dish was inoculated with fungal spores of *A. Niger*, and incubated for 7 days at 27 ± 1.5 °C. DMSO solvent was taken as control. Subsequently, the pellets were removed from Petri dishes and washed with sterile Ringer's solution (Sigma-Aldrich-96724). The solution was stained (Trypan blue) and homogenized to count the spores concentration on the pellets with a *Cellometer® Mini* (Nexcelom Bioscience LLC) automated cell counter by putting 20 µL of each spore solution inside counting chambers and using Cellometer® Mini software for the analysis. The fungal growth inhibition (FGI %) was calculated as concentration of spores (conidia) per milliliter according to the following equation:

$$FGI(\%) = \frac{Cg - Tg}{Cg} \cdot 100 \tag{9}$$

Cg =average concentration in the control sample.

Tg = average concentration in the pellets with lignin.

For assessment of the antifungal capacity of PLA-esterified lignin films, exposure of the films in an agar plate to the fungus (A. Niger) was carried out. After 7 days at 27 ± 1.5 °C of incubation, film samples were removed from Petri dishes and washed with sterile Ringer's solution. The solution was stained (Trypan blue) and homogenized to count the spores concentration on the pellets with a *Cellometer® Mini* (Nexcelom Bioscience LLC) automated cell counter by putting 20 µL of each spore solution inside counting chambers and using Cellometer® Mini software for the analysis. PLA films was taken as control. The equation 9 was also used for the determination of fungal growth inhibition (%). In this case, Cg is the average concentration in PLA film (control) and Tg the average concentration in PLA-esterified lignin films.

The white-rot fungus *Trametes versicolor* (L.) Lloyd (TR489) was used to asses the biological durability of treated wood veneers. The incubation periods were 8 weeks and 24 weeks at 23 ± 2 °C and at relative humidity of 60%±5 to allow the colonization by mycelium. After this period, colonized wood blocks were carefully removed from agar plates, taking away the surrounding mycelia of the samples. Finally, the samples were conditioned in a climatic chamber at temperature of 23 ± 2 °C and relative humidity of 50% ± 5 until they achieved a stable mass. The initial dry mass (mi) and the final dry mass after incubation (mf) were determined by oven drying the specimens at 103±1 °C. The mass loss (ML) due to fungal attack of each specimen was calculated as a percentage of the initial dry mass according to the following formula:

$$ML[\%] = \frac{m_i - m_f}{m_i} \times 100 \tag{10}$$

5.2. Antibacterial test

Bacteria Culture: Food borne and human pathogenic microorganisms (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Proteus microbilis* ATCC 14153, *Proteus vulgaris* ATCC13315, *Pseudomonas aeruginosa* ATCC 27853, *Enterobacter aerogenes* ATCC13048, *Bacillus thuringiensis*, *Salmonella enterica serotype typhmurium* SL 1344 and *Streptococcus mutans* ATCC 25175) were employed to evaluate the antibacterial properties of lignin samples. All bacteria strains were subcultured on (Luria Bertoni) LB agar culture at 37 °C for 24 h.

Disc Diffusion Assay: Disc-diffusion method (according to method of Clinical and Laboratory Standards Institute) was used for determination the antimicrobial properties of the lignin compounds against the food destroyed and human pathogen bacteria. For the disc diffusion assay, lignin samples (0.02 g/mL, w/v) were dissolved in DMSO. Microorganisms susceptibility was adjusted by 0.5 McFarland as a reference standard. The all prepared samples were sterilized under ultraviolet light for 5 min before test. Microorganism culture suspension (100 μL, 10^6 cells/mL) was swabbed onto a plate within Müller-Hinton agar. Filter discs (Ø 6 mm) were placed on the petri plate inoculated with microorganisms and then, 20 μL of the prepared lignin solution was loaded on the sterile discs.

Finally, bacteria cultures were incubated at 37 °C for 24 h. The disc containing gentamicin (10 μg/disc) and only solvent (DMSO) were used as positive and negative control, respectively. All experiments were repeated triplicate. The results were expressed as mean diameter of inhibition zone in mm ± standard deviation (mean ± SD).

6. UV protection test

6.1. Determination of sun protection factor (SPF)

Sun Protection Factor (SPF) of lignins was determined using *in vitro* screening method. It consists on measurement of UV radiation absorption through sunscreen product (25 mg) added to quartz plates (22x22 mm). Lignin-based sunscreen creams were prepared using DOVE body milk cream (Cream-D) and lignin at different concentrations (1% and 5%) by magnetic stirring under room temperature in dark for 48h. In addition, DELIAL SPF 50 was used as positive control. Taking into account that UVB is 1000 times more erythemogenic than UVA, SPF indicates the protection against UVB. The absorbance values of each sample were determined using Jasco V-630 spectrophotometer from 290-320 nm, at 5nm intervals by using the following formula. The values of EE and I are constants. They were determined by Sayre et al., 1979[182].

$$SPF = CFx \sum_{290}^{320} EE(\lambda)xI(\lambda)x\, Abs(\lambda) \quad (11)$$

CF: correction factor (10).

EE (λ): erythmogenic effect of radiation with wavelength λ.

Abs (λ): spectrophotometric absorbance values at wavelength λ.

Appendix II. Instrumental techniques

1. Spectroscopic techniques

1.1. Fourier transform infrared spectroscopy (FTIR)

Fourier Transform Infrared (FTIR) spectra were recorded on a Perkin Elmer spectrophotometer equipped with a Universal Attenuated Total Reflectance accessory (ATR) with internal reflection diamond crystal lens. The first step was to collect a background spectrum to subtract from the sample spectrum. Next, few milligrams of sample were analyzed using the module for eight scans in a range from 750 to 4000 cm^{-1} at a resolution 4 cm^{-1}. After scanning, the baseline was corrected and the bands of the FTIR profile were used to obtain the qualitative information regarding functional groups present on each sample. All spectra were smoothed and fitted to an automatic base line correction using OMNIC 8.6 software.

1.2. Ultraviolet spectrophotometer (UV)

Jasco V-630 spectrophotometer was used for the calculation of total phenolic content of lignin samples and the antioxidant capacity by DPPH and ABTS methods. The procedures of each method was described in Appendix I section 4.

The light transmittance of films was measured using a Shimadzu UV-3600 UV-VIS-NIR spectrophotometer. Spectra were recorded at room temperature in step of 1 nm, in the range of 280-700 nm.

1.3. Phosphorus-31 nuclear magnetic resonance spectroscopy (^{31}P NMR)

^{31}P NMR analysis with 90^{o} pulse angle, an inverse gated proton decoupling and a delay time of 10 seconds was used for the identification and quantification of hydroxyl and carboxyl groups. Prior to analysis, 20 mg sample of the respective lignin fraction was functionalized using 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane in a 1/1.6 mixture (V/V) of $CDCl_3$ and pyridine for 2 h at room temperature[110].

1.4. Carbon-13 nuclear magnetic resonance spectroscopy (^{13}CNMR)

For ^{13}C NMR analysis, 100 mg lignin was acetylated in 1 ml of anhydrous pyridine and 1 ml of acetic anhydride. After that, solution was poured into 20 ml of ethanol and the solvent was removed under reduced pressure (performed 3 times). The same procedure was performed with toluene and chloroform until pyridine was completely gone. For the quantitative ^{13}C NMR, the concentration of lignin was 20% in DMSO-d_6 as solvent. A 90^{o} pulse width, 1.4 s acquisition time and 1.7 s relaxation delay were used. Chromium (III) acetylacetone (0.01 M) was added to the lignin solution to provide a complete relaxation of all nuclei. A total of 200.000 scans were collected.

2. Chromatography Techniques

2.1. Gel-permeation chromatography (GPC)

25 mg of lignin was dissolved in 5 ml of N,N-dimethylformamide (DMF) and size exclusion chromatography analysis was used to evaluate the average molecular weight (Mw) and polydispersity (Mw/Mn) of the obtained lignin samples. N,N-Dimethylformamide eluent was used as GPC mobile phase, at a

flow rate of 0.7 mL/min and 35 ºC, using a Jasco Inc. chromatograph provided with an LC-NetII/ACD interface, a column oven CO-2065Plus and a RI-2031Plus intelligent refractive indexed. A guard column and two columns PolarGel-M (Varian Inc.) were employed. Calibration was made using polystyrene standards provided by Fluka, ranging from 250 to 70.000 g/mol.

2.2. High performance liquid chromatography (HPLC)

An aliquot of the liquid filtrate from acid-insoluble lignin analysis (Appendix I, section 3.2) was used to determine the sugar content of lignins. High Performance Liquid Chromatography [Jasco LC Net II/ADC with a ROA Organic Acid (00H-0138-K0) column (Phenomenex) equipped with a refractive index detector (RI-2031Plus) and a photodiode array detector (MD-2018Plus)]. 0.005M H_2SO_4 prepared with 100% deionized and degassed water was used as mobile phase (0.35 mL/min flow, 40 ºC, and injection volume 40 μL). High purity standards of D-(+)-glucose, D-(+)-xylose, and D-(-)-arabinose (provided by Fluka, with 99% of purity) were used for calibration. The sugars content is determined as follows:

$$Sugar\ content\ (\%) = \left[\frac{A \cdot B}{1000 \cdot C}\right] \cdot 100 \qquad (12)$$

A= obtained concentration by HPLC (ppm)
B= filtrated volume from acid-insoluble lignin test (L)
C= the initial lignin weight (g)

2.3. Py-GC/MS

Py-GC/MS, was performed on lignin samples using a Frontier Lab (Japan) Micro Double-shot Pyrolyser Py-2020iD, with pyrolysis temperature of 500 ºC and a heating rate of 600 ºC/s. It was directly coupled with the Shimadzu GC/MS-QP 2010 apparatus (Japan) with capillary column RTX-1701 (Restec, USA), 60m × 0.25 mm × 0.25 µm film, with injection temperature of 250 ºC with El of 70 eV, and MS scan range *m/z* 15-350 with gas helium at the flow rate of 1 mL/min and split ratio of 1:30. The sample size loaded was between 1.0-2.0 mg. The oven temperature program was 1 min isothermal at 60 ºC, then heated 6 ºC/min to 270 ºC, and finally held at 270 ºC for 10 min. The identification of the relevant peaks was performed based on GC/MS chromatogram using Library MS NIST 147.LI13. The relative area of the peaks was calculated using the Shimadzu software and for some corrected or integrated manually where it was needed on the basis of the GC/MS data. The relevant peaks were averaged between the double samples.

3. Thermal techniques

3.1. Thermogravimetric analysis (TGA)

Thermogravimetric equipment, Mettler Toledo TGA/SDTA 851 analyzer, was used to study the thermal degradation of isolated and chemically modified samples as well as blends and films of PLA and lignin. Samples about 5-10 mg were tested under nitrogen atmosphere at a heating rate of 10 ºC/min. Unmodified and modified lignin samples, from 25 ºC to 800 ºC. PLA blends and PLA films from 25 ºC to 600 ºC.

The ash content was measured in oxygen atmosphere at a heating rate of 10 ºC/min, from 25 ºC to 800 ºC.

In order to perform a proximate analysis of lignins, both nitrogen and oxygen atmospheres were used. This thermogravimetric method consisted of a heating ramp of 50 ºC/min from 25 ºC to 120 ºC (3 min), followed by a new 100 ºC/min heating ramp programmed to 950 ºC. Finally, the samples were cooled to 450 ºC and reheated to 800 ºC under oxygen atmosphere. The proximate analysis data (ash, volatile, organic matter and fixed carbon) were obtained through the direct measurement of weight changes in each experimental curve. The high heating value (MJ/Kg) was calculated according to equations found in literature[66,150].

3.2. Differential scanning calorimetric analysis (DSC)

The glass transition temperature of samples was determined by Mettler Toledo DSC 822 differential scanning calorimetry. Samples about 5-10 mg were tested under nitrogen atmosphere at a heating rate of 10 ºC /min. Sometimes due to the heterogeneity of the lignin samples other heating rates were checked (20 ºC /min, 3 ºC /min). The samples were first heated to 110 ºC to eliminate interferences due to moisture. Then, the samples were cooled to 25 ºC and reheated to 200 ºC. For chemical modified lignins were analyzed from -50 ºC to 200 ºC. In this case also the samples were heated until 110 ºC, then was cooled down to -50 ºC and reheated to 200 ºC. The obtained results are from the second heating thermogram.

4. Optical techniques

4.1. Optical microscope (OM)

Optical microscope (OM) Nikon Eclipse E600 working with the incidence of light in reflection was used to know the morphology of PLA-lignin blends and analyze compatibility between PLA matrix and lignin samples.

4.2. Atomic force microscopy (AFM)

Atomic force microscopy (AFM) images were performed in a Dimension 3100 NanoScope IV. The images were scanned in tapping mode under ambient conditions using a silicon nitride cantilevers having a tip nominal radiud of 10 nm at a frecuency if 1 Hz. For this purpose, samples were dissolved in chloroform and it was prepared by spin-coating (Spincoater P6700) at 2000 rpm for 120 s.

4.3. Colorimeter

The color and color changes were measured by a Konica Minolta CM-2600d device and expressed using the CIE-Lab color space coordinate system. Measurements were taken in triplicate for each sample. The overall color change (ΔE*) was also calculated from the L*, a*, and b* values according to the following equation:

$$\Delta E^{*} = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \qquad (12)$$

Where ΔE* is the total color difference between lightness (ΔL*), red-green axis (Δa*), and yellow-blue axis (Δb*). The evaluation criteria of overall color changes was as follow: 0.2<ΔE* (invisible difference); 0.2<ΔE*<2 (small difference); 2<ΔE*<3 (color change visible with high-quality filter); 3<ΔE*<6 (color change visible with medium-quality filter); 6<ΔE*<12 (high color changes); ΔE*>12 (different color).

In addition, the hue angle (h*) and chroma (C*) were computed on the base of CIE Lab parameters:

$$h^{*} = tan^{-1}({b^{*}}/{a^{*}}) \qquad (13)$$

$$C^{*} = \sqrt{a^{*2} + b^{*2}} \qquad (14)$$

5. **Surface characterization techniques**

5.1. Roughness

In order to follow the change in the surface veneer roughness, a profilometer (Mitutoyo Surftest SJ-301) was used to measure the average roughness Ra (ISO 428714) selected and calculated as an average of ten consecutive cut-off lengths for each pick-up travel length. The length of scanning line and cut-off were set to 15 mm and 2.5 mm, respectively, and the measurements were performed at 0.5 mm/s stylus speed.

5.2. Contact angle

Dynamic contact angle measurements were carried out with water using a Dataphysics Contact angle system OCA 20.

For the determination of changes in the hydrophilic character of lignin before and after chemical modification, water contact angle (WCA) was used by the placement of a droplet (5 mL) and at different times (1, 10, 20, 30, 40, 50, 60, 90, 120 s). The average value of five measurements per sample was calculated.

However, to examine water and oil repellence of treated wood veneers surfaces, water (WCA) and ethylene glycol (OCA) were used. Samples were stabilized at 25 ºC temperature and 50% relative humidity. The dynamic contact angle was determined by the placement of a droplet (3 µL) and at different times (0, 10, 20, 30, 40, 50, 60, 90, 120 s). The average value of five measurements per sample was calculated.

5.3. Surface Energy

Three different liquid with different dispersive and polar surface tensions were used to determine the surface free energy of the treated wood veneers. The following table are shown polar and dispersive components of employed solvents.

Table II.1. Values of polar and dispersive components of solvents.

Solvent	**γ_T (mJ/m2)**	**$\gamma_L{}^d$ (mJ/m2)**	**$\gamma_L{}^p$(mJ/m2)**
Water	78.2	21.8	51.0
Ethylene glycol	48.0	29.0	19.0
Diiodomethane	50.8	50.8	0

The free surface energy was calculated from the contributions of the liquid and the solid states, following the routine proposed by Owens, Wendt, Rabel and Kaelble[183,184]:

$$\gamma_L(1+\cos\theta) = 2\sqrt{\gamma_S^d \gamma_L^d} + 2\sqrt{\gamma_S^p \gamma_L^p} \tag{15}$$

Where γL, γL^p, γL^d are the surface free energy, polar and disperse shares of the testing liquids, γS^p, γS^d are the polar and disperse shares of the substrates free surface energy, respectively. It is possible to determine γS^p and γS^d by deriving the geometric mean from the equation and implementing values of γL^p, γL^d following the literature references:

$$\gamma_S^L = \gamma_S \ + \gamma_L \ 2\sqrt{\gamma_S^d \gamma_L^d} + \ 2\sqrt{\gamma_S^p \gamma_L^p} \tag{16}$$

Accordingly, the linear solution of transformed equation leads to the conclusion that γS^p corresponds to the slope of the linear function and γS^d to its intercept. The mathematical presentation of such relation is presented as follows:

$$y = a\mathrm{x} + b = \frac{1+\cos\theta}{2} \cdot \mathrm{x} + \frac{\gamma_L}{\sqrt{\gamma_L^d}} \tag{17}$$

Where:

$$\mathrm{x} = \sqrt{\frac{\gamma_L^p}{\gamma_L^d}} \ ; \ a = \sqrt{\gamma_S^P} \ ; \ b = \sqrt{\gamma_S^d} \tag{18}$$

It was assumed that measurement of wetting with three diverse liquids was sufficient to accurately determine the linear function. The work of cohesion (*W*$_{c1}$) can be determined according to the following equations:

$$W_{C1} = 2\gamma_{S1} \tag{19}$$

The interactions between unsaturated force fields of the wood-solid coating system were determined by applying valid relations in a common solid-liquid system by the free surface energy at the phase boundary of the wood-solid coating (γ_{S1S2}). The work of adhesion of the solid coating to wood (Wa) and work of cohesion (Wc_1=uncoated surface; Wc_2=coated surface) were determined according to the following equations:

$$\gamma_{S1S2} = \left(\sqrt{\gamma_{S1}^{d}} - \sqrt{\gamma_{S2}^{d}}\right)^2 + \left(\sqrt{\gamma_{S1}^{p}} - \sqrt{\gamma_{S2}^{p}}\right)^2 \tag{20}$$

$$W_a = 2\sqrt{\gamma_{S1}^{d}\gamma_{S2}^{d}} + 2\sqrt{\gamma_{S1}^{p}\gamma_{S2}^{p}} \tag{21}$$

$$W_{C1} = 2\gamma_{S1}\ ;\ W_{C2} = 2\gamma_{S2} \tag{22}$$

6. Mechanical testing techniques

6.1. Mechanical analysis

MTS Insight 10 equipment provided with pneumatic clamps (Advantage Pneumatic Grips) and with a loading cell of 250 N with a speed of 5 mm/min mechanically tested elaborated materials.

For extruded PLA-lignin and PLA-acetylated lignin blends, samples with 6 cm long were prepared with thicknesses between 0.25-0.8 mm, and width of 2.5-5 mm. The starting distance between the clamps was 25 mm.

The PLA-lignin-ester derivative films samples were prepared according to standard ASTM D-1708[185]. Thicknesses of samples range between 60-100 µm. Samples with 6 cm long were prepared, with a width of 4.75 mm. The values quoted are the average of eight measurements. The starting distance between the clamps was 22.9 mm.

7. Other techniques

7.1. Moisture

The moisture content of treated wood veneers was measured with a Denver instrument IR60Q.

7.2. Elemental analysis

Elemental analysis (C, H, N and S) was performed using 1-3 mg of lignin samples dried in a vacuum oven at 40 ºC for 48 h, using a Flash EA 1112, Thermo Finnigan, USA elemental analyzer.

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