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Microwave-assisted liquefaction of lignin to high value chemicals

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Riassunto

Negli ultimi anni si sta conducendo uno studio sempre più approfondito nel campo della bio-raffineria per migliorare il recupero di composti ad alto valore aggiunto dalla biomassa. Lo sviluppo di nuove tecnologie viene studiato per perseguire una diminuzione dell'utilizzo di prodotti da origine fossile, dato il loro negativo impatto ambientale e la loro decrescente disponibilità. Questo lavoro di tesi si sofferma sull'analisi della lignina, uno dei principali componenti della biomassa, da cui è possibile estrarre, dopo processi di pirolisi e solvolisi, composti fenolici ad alto valore aggiunto.

Lo studio si è focalizzato sull'analisi dei composti presenti in maggiore quantità nel bio-oil risultante: il guaiacolo, il creosolo ed il 4-etil-guaiacolo. La solvolisi della lignina è stata effettuata utilizzando il reattore a microonde mono modale Discover® ed impiegando come solventi il sulfolano, l'etanolo, l'eptano e l'acqua distillata. Questi stessi solventi sono stati poi riutilizzati come solventi per estrarre il bio-oil prodotto, sia dalla solvolisi che dalla pirolisi del campione. La lignina è stata poi pirolizzata nello stesso reattore, analizzando il processo al variare della potenza del reattore, a temperatura fissata, e del tempo di permanenza della reazione ed investigando l'influenza di catalizzatori acidi e basici. L'identificazione dei composti fenolici è stata possibile grazie al gas cromatografo con spettrometro di massa (GC-MS), mentre la quantificazione di resa e selettività è stata permessa mediante l'utilizzo del gas cromatografo con rivelatore a ionizzazione di fiamma (GC-FID). La curva di calibrazione per ogni composto principale è stata costruita a partire dai composti di riferimento per ricavare la concentrazione del composto chimico corrispondente nel bio-oil. Dopo aver constatato che la resa dei composti fenolici risultava molto bassa a seguito del trattamento a microonde, l'analisi si è focalizzata sulla pirolisi di tipo convenzionale. Il processo è stato condotto in un analizzatore termogravimetrico (TGA) accoppiato con uno spettrometro FT-IR. È stato considerato anche l'uso della lignina purificata. Con questa tecnica di riscaldamento la resa dei composti fenolici si rivela essere molto maggiore rispetto a quella ottenuta dopo la reazione nel reattore a microonde.

Abstract

In the last few years a major effort has been made at the bio refinery area to improve the recovery of useful chemical products from biomass treatment. The development of new techniques has been studied to decrease the excessive use of fossil chemicals because of their environmental concerns and the limited availability of fossil resources. The aim of this work regards the use of lignin, one of the main components of biomass, for the recovery of high value chemicals using pyrolysis and solvolysis treatment.

The study was mainly focused on the identification and quantification of the main components in the resulting bio-oil: guaiacol, creosol and 4-ethyl guaiacol. The lignin solvolysis was conducted with the microwave single-mode reactor Discover® and sulfolane, ethanol, heptane and distilled water as solvents reaction. These solvents were also used to extract the bio-oil produced from both pyrolysis and solvolysis processes. The lignin was pyrolyzed in the same reactor varying the microwave reactor power at constant temperature and the holding time inside the reactor and was also doped with acid and base catalysts. The identification of the valuable phenolic compounds was allowed by the Gas Chromatography with Mass Spectrometry (GC-MS), while yield and selectivity quantification was possible thanks to the Gas Chromatography with Flame Ionization Detector (GC-FID). From the reference run, a calibration curve was built for each interested chemical. This permitted to determine the concentration of the correspondent compound in the bio-oil.

The yields obtained from the microwave process resulted very low, so it was decided to focus on the pyrolysis using conventional heating. The process has been carried out in a thermogravimetric analyser (TGA) coupled with a FT-IR spectrometer. The use of purified lignin was also considered. With this heating method, the yield of the phenolic compounds reveals to be much higher than the one obtained after the microwave reaction.

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Introduction

In the past decades the conversion of biomass into useful energy and chemical products has emerged as a fundamental topic for the scientific research since the use of fossil fuels and derived products is leading to resource shortage and environmental concerns. The lignocellulosic biomass represents a big part of the bio-waste and is one of the more abundant material as a clean alternative to chemicals and fuels. It is referred mostly to plant or plant-based materials not used for food or feed and it is formed by three main components: cellulose, hemicellulose and lignin. Lignin constitutes 20%-30% of the lignocellulosic biomass and is a carbon-rich renewable biomass source so it is promising as a bioenergy and high value chemicals source. Its complex structure doesn't permit to determine the exact composition and proportion of the functional groups. The main units are phenylpropane type, namely coniferyl alcohol, sinapyl alcohol and *p*-coumaryl alcohol. However, the nature of the original source, the extraction process and the reaction conditions differentiate totally the chemical composition of this material. The lignin used in this work is a softwood type, obtained from the Kraft process. Valuable products, like phenolic compounds, could be obtained by thermochemical conversion of lignin, through pyrolysis or solvolysis. Those processes act on the breakage of the chemical bonds present in the lignin, producing radicalic reactions that lead to guaiacols or syringols formation. To perform this production, the microwave technology, which appears to be an energy-efficient, environmental-friendly and fast heating source, is often considered. Generally speaking, its advantages are related to the high selectivity and to the reaction control, with a more relevant energy efficiency compared to the conventional heating. This study aims to investigate the potentiality of the microwave heating in terms of yield and selectivity of valuable phenolic compounds produced from lignin after the solvolysis or pyrolysis processes. The reaction conditions were changed, varying the microwave power and the holding time inside the reactor and the presence of catalysts was examined. The results were then compared with the ones given by pyrolysis by conventional heating.

The thesis is structured in six chapters. Chapter 1 shows an overview of the biorefinery concern with a description of the different available methods to treat the biomass. The lignocellulosic biomass and its components are then presented, and a longer paragraph is reserved for the structure and the isolation methods of lignin. The second chapter regards the possible applications of the phenolic compounds extractable from lignin. The focus is given on guaiacol, creosol and 4-ethyl guaiacol, mainly utilised in the food industry as flavouring agents. Chapter 3 presents the state of art regarding the two main thermochemical techniques

used in this work to activate biomass. The physical principles about the microwave and the conventional heating are reported and a comparison between them is done, highlighting the inverted temperature gradients. A literature summary about the research already conducted for the microwave and the conventional lignin activation finds a place in Chapter 4. Both the pyrolysis and the solvolysis mechanisms are inserted to explain which probable products are expected from the lignin activation. Those reveal to be mainly phenolic compounds like guaiacols and creosols. In the same chapter it is reported also an overview about the existing technologies for the bio-oil components separation - extraction, distillation and chromatography. Chapter 5 illustrates the materials and the methods for the lignin characterisation, activation and bio-oil extraction and separation. The last chapter contains all the experimental results. In particular there are comparisons, initially between pyrolysis and solvolysis process using the microwave technology, and then between the two heating methods.

The experimental activity was carried out in the laboratory of the Green Chemistry Centre of Excellence at the University of York.

Chapter 1

Biorefinery overview

This chapter presents a general view of the literature about biorefinery and biomass. A specific section is focused on lignocellulosic biomass and its main constituent, lignin.

1.1 Biorefinery

In the last few years a major effort at biorefinery area has been made to develop new techniques for the recovery of useful chemical products through biomass treatment, since the excessive use of fossil fuels is leading to environmental concerns and limited availability of fossil resources. Alternative production chains, especially for the transport sector and chemicals production are needed [1]. Plant-based materials, such as biomass and its derivatives, are potentially a good replacement for a large fraction of fossil resources as feedstock for energy, materials and chemicals.

The use of renewable resources for energy supply is one of the most studied direction for biorefinery [1]. The major attempts in this area was done in the respect to combustion heat of bio-oil derived as a by-product in the process of materials/biogas production. But a substantial substitution of fossil resources to the renewable is still not achieved. The main problem for the use of bio-oil for energy supply is a high content of oxygen-containing compounds reducing the combustion heat capacity. The major attempts in this topic are currently directed on the bio-oil upgrading procedures [2]. This additional step of upgrading is essential to gain the same heat combustion properties to those one of fossil resources making this technology commercially non-profitable in the comparison to the relatively cheap fossil-based resources. In such a way the use of renewable resources for energy supply is restricted mainly on their combustion for additional heat supply.

The Executive Director of Bio-Based Industries Consortium Dr. Dirk Carrez pointed at the G2C2 annual conference (2018 Ghent, Belgium) that nowadays one of the most perspective direction of biorefinery is the production of chemicals. The prices for the chemicals produced from renewable and fossil resources are comparable. However, a real working technology is well-established only for cost-efficient bio-ethanol, while the rest of the areas, including such large industries of polymers, solvents and medicals, are still not covered.

The amount of solid waste per year, generated in the world, has been identified in 2012 as 1.3 billion tonnes, amounting to a footprint of 1.2 kilograms per person per day (Solid Waste Management). One of the major part of the waste is the bio-waste and in particular the so-called lignocellulosic waste. It is a real problem nowadays because of its inert nature and the complications conjugated with its activation.

Except for a small amount used in the paper or bio-refineries manufacture, most of the waste is not efficiently employed. Only in Italy indeed the generation of non-recycled lignocellulosic bio-waste reaches more than 4 million ton (Assocarta recycling statistic).

The main components of agricultural residues, namely lignocellulosic biomass, are cellulose, hemicellulose and lignin. Among these, cellulose and hemicellulose are used to obtain some industrial products while lignin is mainly used in the generation of energy. As a matter of fact, the definition of biorefinery, developed by IEA Bioenergy Task 42 is the following: "*Biorefinery is the sustainable processing of biomass into a spectrum of marketable products and energy*" and the main driver is the sustainability aspect (see Figure 1.1) [3].
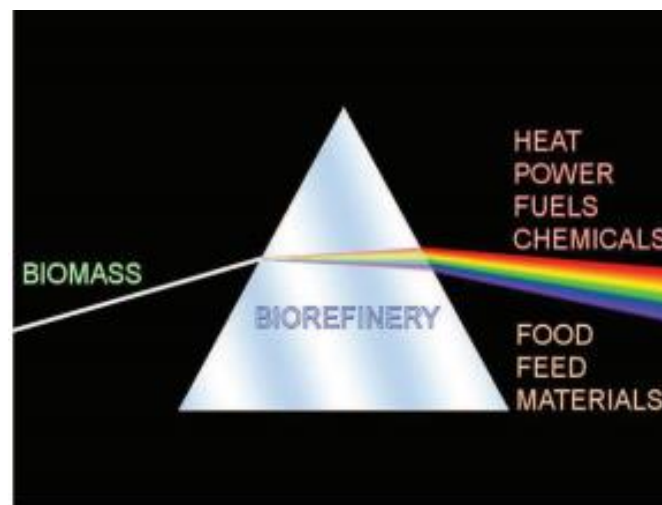
Figure 1.1. *High value products from the transformation of the biomass thanks to the biorefinery process.*

1.1.1 Biomass

Biomass is a renewable source of energy by burning wood and other organic matter and it has different value and characteristics depending on the chemical and physical properties of the large molecules from which is composed.

It represents approximately 14% of world final energy consumption [4], due to the fact that when the bonds between adjacent carbon, hydrogen and oxygen molecules are broken and these substances release their stored chemical energy.

There are several methods to treat the biomass. Combustion is the traditional energetic use, but other processes are: pyrolysis; gasification to produce a liquid fuel or a gas-like fuel such as methane, hydrogen and carbon monoxide; co-pyrolysis and co-gasification with coal.

The technological processes used to convert biomass can be divided in four main groups (Figure 1.2):

- Thermochemical processes – gasification and pyrolysis.

  Gasification is the reaction with carbon dioxide and steam at high temperature - >700 °C - to form syngas, a gas mixture of H_2, CO, CO_2 and CH_4 that can be used as stationary biofuel or like an intermediate for fuel or chemicals production. The pyrolysis process instead considers the heating of the biomass in absence of oxygen or by combustion with limited oxygen supply. The main products can be liquid pyrolytic oil (or bio-oil), solid charcoal and light gases like syngas. Flash pyrolysis tries to maximize the yield of the most desirable liquid bio-oil at different process conditions.

- Biochemical processes – fermentation and anaerobic digestion. Microorganism and enzymes are used in fermentation to convert a fermentable substrate, like hexoses and glucose, into recoverable products, for example ethanol.

  In the anaerobic digestion process the biodegradable organic matter is broken by bacteria in absence of oxygen over a temperature range from about 30 to 65°C to give as main product biogas (methane, carbon dioxide and other impurities).

- Mechanical processes. They are generally applied first to give to the biomass the right physics characteristics. In fact, the composition of biomass is not changed but there are only modifications in size reduction, shape and bulk density change (through cutting of commuting processes) or in feedstock components separation. After the separation of biomass substrate components, generally an extraction of valuable compounds is performed, and the products are concentrated.

- Chemical processes – hydrolysis and transesterification are the main one. This type of process carries a change in the chemical structure of the molecule by reaction performance. Hydrolysis is that process in which acids, alkalis or enzymes depolymerise polysaccharides and proteins into their component sugars or derivative chemicals. Transesterification instead uses vegetable oils that are converted to methyl or ethyl esters of fatty acids (biodiesel).
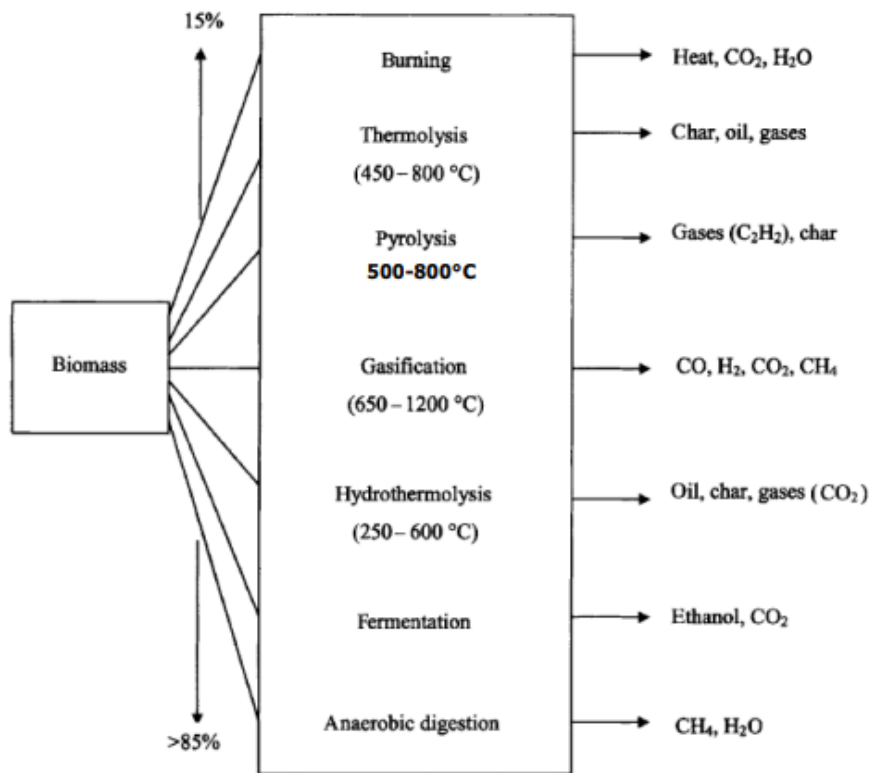
Figure 1.2. *Biomass main processes* [5].

There are two main kind of biofuels based on biomass:

- First generation biofuels refer to biofuels produced from raw materials in competition with food and feed industries and that could give rise to ethical, political and environmental issues;

- Second generation biofuels refer to raw materials based on waste, residues or non-food crop biomass (such as lignocellulosic material).

The main bio-based products obtained today are derivative of biomass conversion to basic products (starch, oil and cellulose) or chemicals for food industry (lactic acid and amino acid). The market of bio-based materials includes also adhesive, cleaning compounds, detergents, dielectric fluids, dyes, hydraulic fluids, inks, lubricants, packaging materials, paints and coatings, paper and box board, plastic fillers, polymers, solvents and sorbents [1].

1.2 Lignocellulosic biomass

Lignocellulosic biomass most refers to plants or plant-based materials that are not used for food or feed and is made up of three main components: cellulose, hemicellulose and lignin. The relative proportions of cellulose and lignin is the determining factor in identifying the suitability of the plant species in energy production [6]. In general, the biomass is synthesized via the photosynthetic process thanks to the conversion of carbon dioxide and water into sugars, that are successively treated by the plants. The major issue of this process is providing renewable carbon based raw materials.

1.2.1 Cellulose

Cellulose is a glucose polymer and its structure $[C_6H_{10}O_6]_n$ is illustrated in Figure 1.3. It presents a long chains of glucose molecules (C_6 sugars) that provide the strong molecular structure. The chains are constituted by (1,4)-D-glucopyranose units, linked 1-4 in the b-configuration [6]. This β(1-4) linkage confers the cellulose always the same unique structural features [7]. These ribbon-like glucan chains crystallize to form microfibrils that impart the characteristic flexible strength—similar to that of an equivalent thickness of steel—of cellulose [8]. Thanks to of its the configuration, different from the one of starch, cellulose (30-50% of total lignocellulosic dry matter) is much more difficult to hydrolyse and set free individual glucose monomers [1]. The average molecular weight is around 100,00 Da In nature, cellulose is the main structural component of the cell wall and responsible for many of its distinctive traits [7].
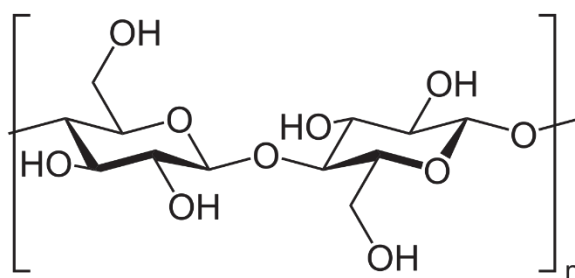
Figure 1.3. *Cellulose structure.*

1.2.2 Hemicellulose

The second main component of lignocellulosic biomass (about 20-40% of the total feedstock dry matter) is hemicellulose. Its structure (Figure 1.4) is not well identified. It is possible to recognize C_5 sugars and other different substituents arranged in different proportion [9]. The average molecular weight is lower than 30,000 Da (o g/gmol) [1]. In contrast to cellulose,

which is crystalline, strong and resistant to hydrolysis, hemicellulose is a relatively amorphous component and it's easily hydrolyzed with chemicals and/or heat.
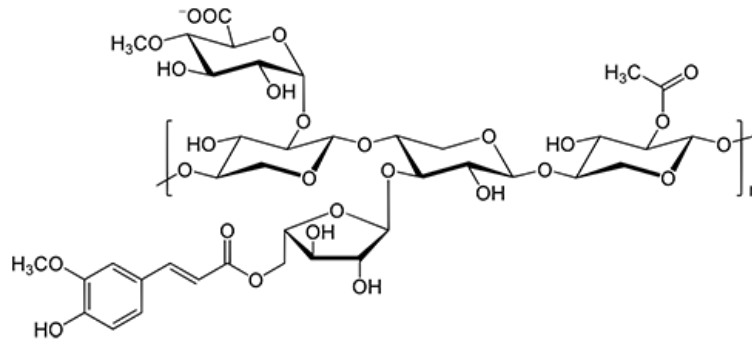
Figure 1.4. *Hemicellulose structure.*

1.2.3 Lignin

The third main component of lignocellulosic biomass (15-25% of total feedstock dry matter) is lignin. Its structure is based on cross-linked phenolic polymers (Figure 1.7) and it forms important structural materials, like the overall rigidity to plants and trees. However, like hemicellulose, it's not possible to identify exactly its structure since the substituted groups along the main chain can be every time very different and present in different proportion.

1.2.3.1 Lignin structure

The functional groups attached to the basic phenylpropanoid skeleton include hydroxyl phenolic, benzyl hydroxyl, carbonyl and methoxy groups. Figure 1.5 reports the main bonds between the structural units of lignin. Their content depends on the source and on the extraction process.
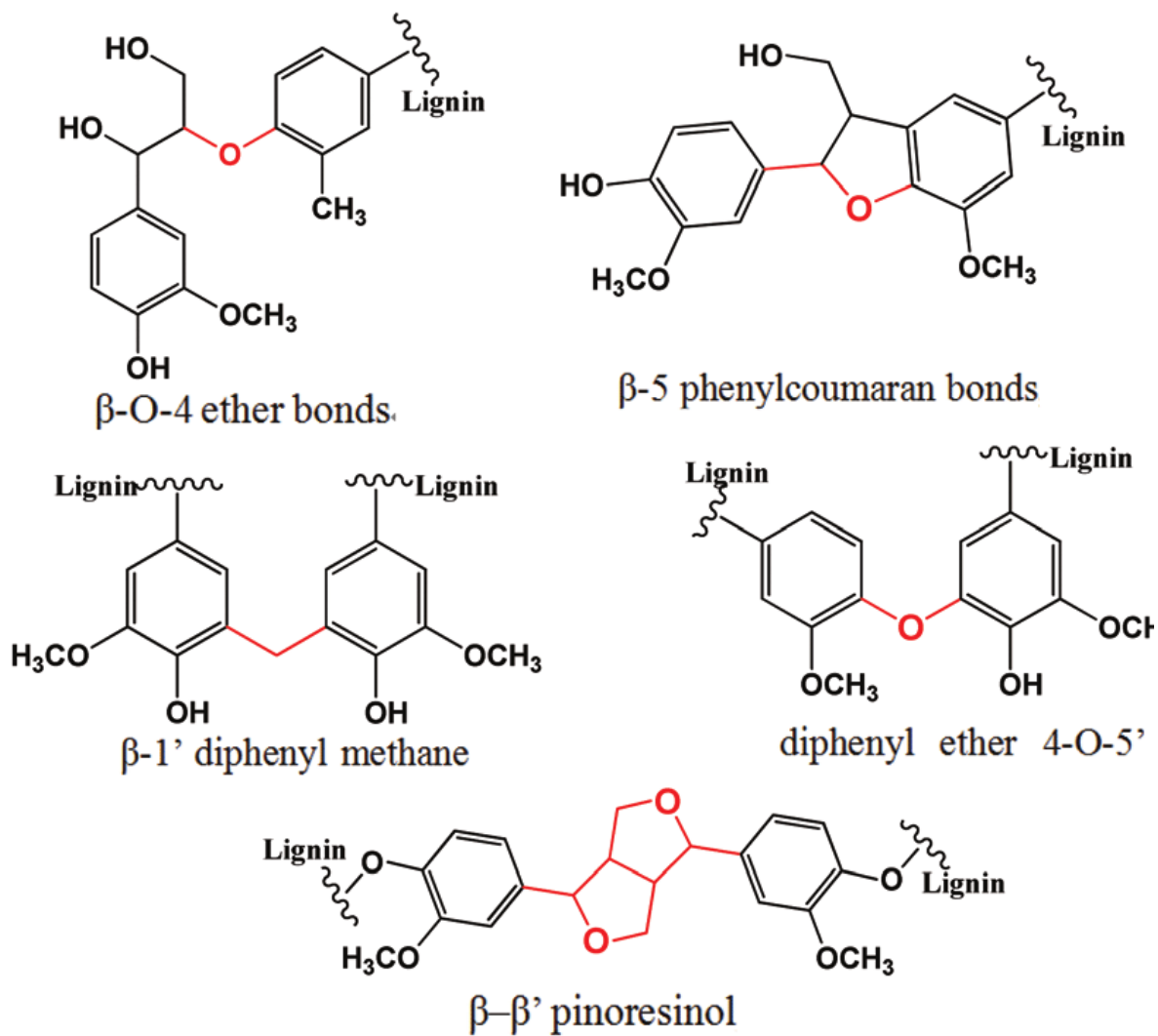
Figure 1.5. *Main bonds between the structural units of lignin* [10].

Lignin has three main identified structural components (Figure 1.6) – monolignol monomers - that are methoxylated to various degree:

- p-coumaryl alcohol;
- coniferyl alcohol;
- sinapyl alcohol.
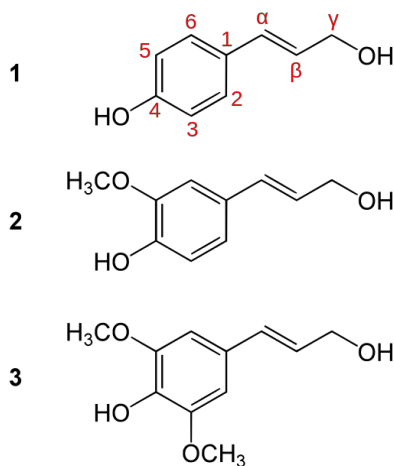
Figure 1.6. *Monolignol monomers precursors of lignin. (1) p-coumaryl alcohol; (2) coniferyl alcohol; (3) sinapyl alcohol.*

The main use of lignin is related to chemical extraction or energy generation purposes but cannot be used in fermentation process. In fact, two are the main reasons why lignin is so sought-after: its great availability and its enormous potential as a phenolic raw material.
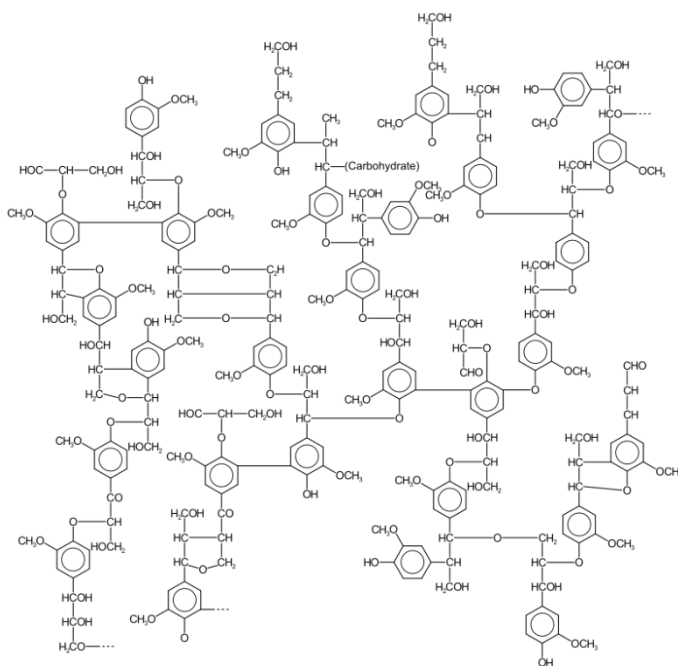
Figure 1.7. *Lignin structure.*

1.2.3.2 Lignin isolation methods

Several methods have been developed to isolate lignin from lignocellulosic biomass and each one has a different influence on the final characteristic of the lignin, in particular on the molecular weight, purity and functionality of the possible chemicals extractable.

The main processes are the following [11]:

- Kraft pulping. The lignin obtained through this process is mainly the byproduct of the paper industry. During the kraft pulping the sodium hydroxide converts the phenolic groups to quiononemethide group and then hydrogen sulphide is used to attack α-carbon atoms of ether linkage of lignin. The product is the abenzylthiolate anion with cleaving ether linkage. Next, benzyl thiolate anion releases β-phenolate anion to have free phenolic groups by sodium sulphide. These leads to quiononemethide. This mechanism is repeated again until a lower-molecular-weight fragments is obtained. The last stage implies the formation of lignin carbon-carbon bonds that involves condensation reaction giving a very robust lignin complex.

- Sulphite pulping – wood or biomass reaction with calcium/magnesium sulphite at high temperature (125-250°C) for 3-7 hours using an acid as catalyst to cleave α ether linkage and β ether linkages during the pulping process. The sulfonic acid is introduced to α-carbon of ether linkage. This permits an easy lignin dissolution and separation in aqueous solution. The resulting lignin contains lignosulfonic acid and lignosulfonate together with phenolic/aliphatic hydroxyl groups form. Due to the sulfuric high content it is used mainly as a fuel.

- Soda process. It can be carried out with wood and non-wood material, such as bagasse, sisal, kenaf, hemp and wheat straw. Under high temperature and high pressure, the biomass reacts with sodium hydroxide allowing the cleaving of the α ether bond and of the β-0-4 ether linkage if anthrahydoroquinone/anthraquinone catalyst is added. The absence of sulphur permits the soda lignin to be a precursor for raw materials of many applications.

- Organosolv lignin. In presence of acid or basic catalyst, like ethanol, methanol, formic acid and acetic acid, lignin is removed from wood using organic solvents. This kind of lignin is free from sulphur and the resulting molecular weight is lower than the lignin produced with the other methods. The main issue of this method is related to the cost of the organic solvents that could have also a non-negligible environmental impact.

- Steam explosion lignin. This process requires high pressure (200-2000 psi) and temperature (180-230°C) and a short processing time (1-20 min). Also, this kind of lignin has a low molecular weight and is easily soluble in organic solvents. The process can be the first step of the biofuel production prior to fermentation of polysaccharide.

- Dilute acid lignin. A pre-treatment of the biomass can be done diluting sulfuric acid at 0.5-1.4% at high temperature (165-195°C) for 3-12 min followed by a water-washing step. The acid conditions are relatively mild, and the solubility is quite high, but the conversion rate is quite low, and the resulting lignin can contain some sugar-derivative impurities.

Chapter 2

Applications of phenolic compounds

The extraction of phenolic compounds, after the lignin activation, is the main aim of this work. The depolymerization of lignin, indeed, could lead to the formation of these high value chemicals thanks to different thermal treatments. This Chapter describes the main applications related to the bio-oil recovered from the lignin pyrolysis and solvolysis and shows the structures of the main chemicals detected in this work.

2.1. Phenolic compounds from lignin pyrolysis

Since the various oxygen functional groups of lignin have different thermal stabilities, its thermal decomposition occurs in a broad temperature range and the scission of the bonds takes place at different temperature. At low temperature, low molecular weight compounds are produced while, at high temperature, char (30-50 wt%) and volatiles release are the consequence of the complete rearrangement of the backbone. It's possible the formation of products with increased stability thanks to the cleavage of the aryl-ether linkages which results in the production of reactive and free radicals [12]. The pyrolysis products can be obtained in the form of liquid (bio-oil), gaseous or solid. The pyrolysis bio-oil can be burn after mixture with other fuels or used to optimize the combustion system. Other applications, like the extraction of high value chemicals, depend on which fraction is used, since it is very rich in phenolic compounds [13], [14]. Simple phenols in particularly are very valuable chemicals, suitable for many applications in different industry fields.

2.1.1. Syringol and guaiacol compounds

Depending on the molecular weight, lignin can give the preference to syringol or guaiacol-type of compound. The softwood lignin returns mainly guaiacols, instead hardwood lignin gives both syringols and guaiacols. The main high value chemicals belonging to these categories are:
- 2-methoxy phenol (Guaiacol);
- 2-methoxy-4-methyl phenol (Creosol);
- 2-methoxy-4-ethyl phenol (4-Ethylguaiacol);
- 2,6-dimethoxy phenol (Syringol).

Since the lignin treated in this work is a softwood type, it is predictable to find, as pyrolysis products, an abundance of guaiacol, creosol and 4-ethyiguaiacol.

For these phenolics compounds, several production processes were developed in the past. They come mainly from non-sustainable resources so their extraction from lignin could be a very promising greener technique for many applications. The improving given by the lignin treatment can touch various production fields, like the food and the pharmaceutic ones. Anyway, the separation of pure compounds could be very difficult, so they are not much employed for commercial products yet. The bio-oil from pyrolytic lignin can find an application in the phenolic resins production, substituting petroleum-derived phenols. In this case a whole bio-oil is used. Its aldehydes can also partially replace the formaldehyde processed for the synthesis of phenol-formaldehyde (PF) resin.

The major challenge in this application lies on the relatively low reactivity in the phenolic resin synthesis since the reactive phenols are present in low amount in the bio-oil. The phenolic compounds represent the largest fraction, but they are less reactive than the pure phenol.

2.1.1.1. Guaiacol

The chemical present in major quantity in the softwood pyrolyzed lignin is guaiacol (or 2-methoxy phenol), which structure is reported in Figure 2.1.
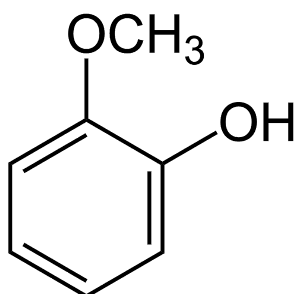
Figure 2.1. *Guaiacol structure.*

Its main application concerns the production of vanillin, about the 85% of the world supply of which is covered by guaiacol as intermediate [15]. Guaiacol is also an important platform chemical for the production of flavor ingredients [16], like in foods as roast coffee or smoked meat. It is used as an intermediate also in the pharmaceuticals manufacturing. It is employed as stabilizer and antioxidant for plastic and rubber or as a local anesthetic and antiseptic [6–10]. In addition, a recent research conducted by Barton et al. [22], exploited the production of *cis*-cis-muconic acid (MA) from guaiacol via metabolic engineering of *Amycolatopsis* sp. ATCC 39116. The MA obtained from guaiacol is recognized to be an important precursor for commercial plastics.

The commercial production of this compound includes first the formation of cumene via alkylation of benzene with propylene and then the partial oxidation to phenol. From the hydroxylation of phenol is possible to obtain the catechol that is methylated into guaiacol as the last step of the process [23], [24]. Since benzene and propylene are produced from petroleum, this production process is based on nonrenewable carbon sources.

2.1.1.2. Creosol

Creosol (or 2-methoxy-methyl phenol) is extracted directly from coal tar [25] and is employed as flavoring agent, especially for the alcohol industry. It's also patented for being active in anti-inflammatory disorders and is an important reagent in the bisphenols synthesis via stoichiometric condensation with short-chain aldehydes [25].
Figure 2.2 shows its structure.
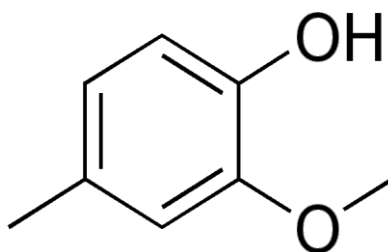
Figure 2.2. *Creosol structure.*

2.1.1.3. 4-Ethylguaiacol

The 4-ethylguaiacol or 2-methoxy-4-methyl phenol, which structure is in Figure 2.3, is extracted from coal tar like the creosol. It is relevant as smoke flavoring agent and displays oxidant activity. It is associated with off-flavors of wines since it can be formed by the yeast species of *Brettanomyces dekkera* [26].
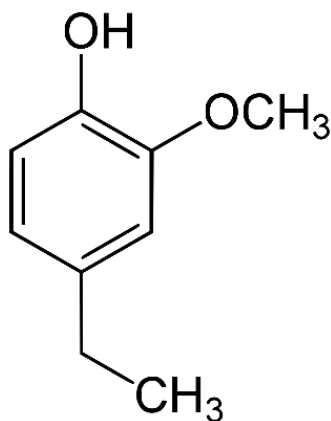
Figure 2.3. *4-Ethylguaiacol structure.*

As it will be possible to read in the Chapter 4, those compounds are the goal of production also in many solvolysis processes, depending on the reaction conditions and on the raw materials (lignin and solvent's nature). These phenolic compounds were detected in the bio-oil of the treated lignin.

Table 2.1. encases the boiling point values of the phenolic compounds just described. This property is important to understand if their separation from the bio-oil is possible through the distillation process.

Table 2.1. *Boiling point values of the main phenolic compounds detected in the bio-oil obtained from lignin.*

Phenolic compound	Boiling point [°C]
Guaiacol	205
Creosol	221
4-Ethylguaiacol	234

Chapter 3

Thermic methods of biomass processing

In this chapter it is presented the state of the art regarding the two-main thermochemical techniques used to process biomass. A comparison between microwave and conventional heating will be reported to underline the main advantages and disadvantages of the methods employed during the experimental procedure.

3.1. Conventional heating

Conventional heating follows three main mechanisms [27]:

- Convention. Energy is transported by the bulk motion of a fluid and in this kind of transport phenomena the density ρ of the fluid takes a relevant role;
- Conduction. Energy is transmitted through the material when there is a temperature gradient between adjacent region, without movement of the material.
- Irradiation. This energy transport doesn't require a material medium as instead conduction and convection do.

The rate of the heat flow per unit of area q, thanks to the conductive transport, is proportional to the temperature decrease over the distance y following the *Fourier's law of heat conduction* 3.1:

$$q = -k \cdot \partial T / \partial y \tag{3.1}$$

where the constant of proportionality k is the *thermal conductivity* of the material medium crossed by the energy flow. It represents the thermal resistance of this energy transport. The thermal conductivity can vary all the way from about 0.01 W/m • K for gases to about 1000 W/m • K for pure metals.

In addition to the thermal conductivity, equation 3.2 defined another important physical quantity, the *thermal diffusivity*:

$$\alpha = k / \rho \cdot \hat{C}_p \tag{3.2}$$

Here $\hat{C}_p$ is the heat capacity at constant pressure per unit of mass and ρ is the density of the medium. It's possible to notice that α has the same dimensions as the kinematic viscosity [lenght^2/time].

3.2. Microwave heating

Microwaves represent a kind of alternative energy source. The first studies about microwave interaction with matter were carried out by Von Hippel around the 50' but its application in the thermal activation of biomass has increased from mid-nineties.

3.2.1. Microwave irradiation

As it is possible to see in Figure 3.1, microwave range of wavelengths goes from 0.01 m to 1 m and the corresponding frequency range, according to equation (3.3) is settled between 0.3 and 300 GHz. However, MWs for household and industrial application are strictly regulated, due to the possible interferences with radar and telecommunication devices, and only some frequencies/wavelength are allowed (see Table 3.1).

$$f = c/\lambda \tag{3.3}$$

where f represents the frequency – i.e. number of oscillation if the magnetic or electric field in one second [sec^{-1}], λ the wavelengths [m] and c the speed of light equal to $3 \cdot 10^8$ [$m \cdot sec^{-1}$].

In general, most of the microwave reactors employed in chemistry work with a frequency of 2.45 GHz or 915 MHz. The microwave irradiation provides a too low energy to cleave chemical bonds, so it cannot induce properly chemical reaction by its direct absorption. It's possible to see in Table 3.2 the comparison between radiation types and bond energies that shows this behaviour.
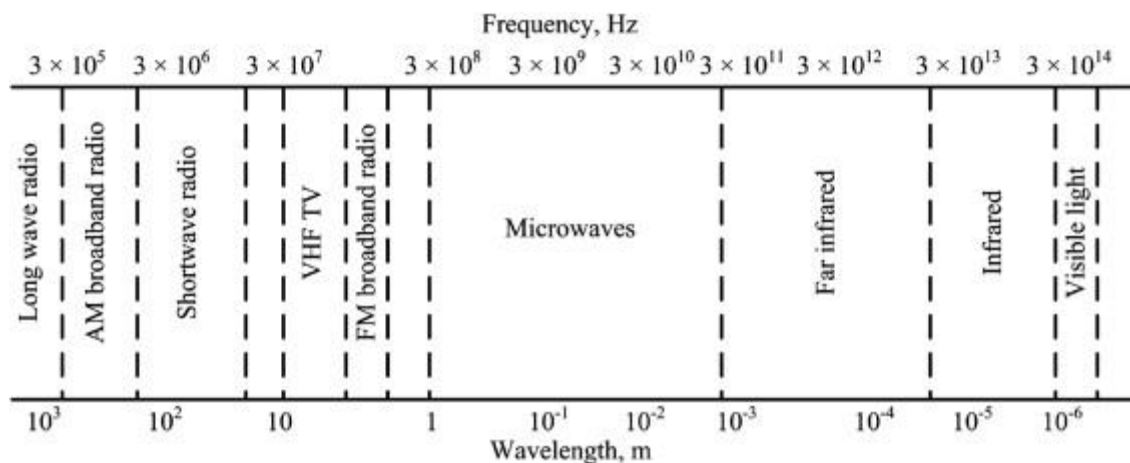
Figure 3.1. *Electromagnetic spectrum.*

Table 3.1. *According to international agreements, allowed ISM-frequencies (ISM – frequencies for industrial, scientific and medical use)*[28]

Frequency [MHz]	Wavelenght [cm]
433.92 ± 0.2%	69.14
915 ± 13 [a]	32.75
2450 ± 50	12.24
5800 ± 75	5.17
24125 ± 125	1.36
[a] Not allowed in Germany	

Table 3.2. *Energy of chemical bonds in comparison to different microwave energies*[28]

		Energy [eV]	Energy [Kj mol^{-1}]
1	CC single bond	3.61	347
2	CC double bond	6.35	613
3	CO single bond	3.74	361
4	CO double bond	7.71	744
5	CH bond	4.28	413
6	OH bond	4.80	463
7	Hydrogen bond	0.04-0.44	4-42
8	MW 0.3 GHz	$1.2 \cdot 10^{-6}$	0.00011
9	MW 2.45 GHz	$1.0 \cdot 10^{-5}$	0.00096
10	MW 30 GHz	$1.2 \cdot 10^{-3}$	0.11

Matter absorbs microwave energy through a mechanism called dielectric heating. Microwaves are electromagnetic waves composed by an electrical and a magnetic part (Figure 3.2). For the most cases is the electrical component that is important in the wave-material interaction, even in some fields, also the interaction of the magnetic field takes its own relevance.

The electrical component of the electromagnetic field causes heating by two main mechanisms: dipolar polarization and ion conduction.
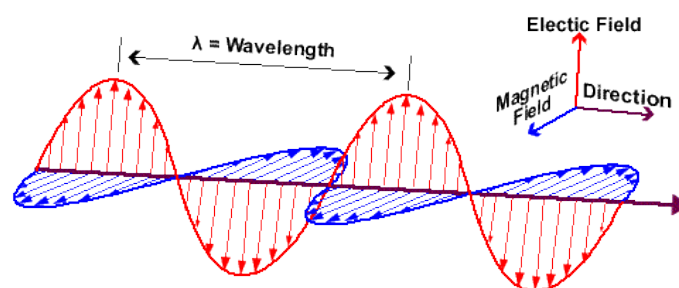
Figure 3.2. *Electric and magnetic fields in microwaves.*

The dipolar polarization is the interaction between the microwaves and a polar material. The heating mechanism becomes possible when the irradiated material has a dipole moment. In that case, its molecules are able to align themselves with the direction of the field by rotating completely or partly. Since the field oscillates, the molecules tend to follow its frequencies leading to phase shifts and dielectric losses that transform the electric energy into kinetic or thermal energy. In this part of the mechanism, besides the dielectric coefficient (permittivity), also the size (mass) of the excited molecules is important.

The possibility to heat is correlated to the ability of the molecules to align with the fields. If this process is too slow (high frequency irradiation) or too fast (low frequency irradiation), heating is not possible. Under high frequency field the dipoles don't have sufficient time to respond to the oscillation field, so they collide each other. Because of this phase lag, when they try to follow the field, the power is dissipated to generate heat. The second main heating mechanism is the ionic conduction. In this case, under the microwave effect, all the mobile charge carriers (electrons, ions, *etc.*) move back and forth through the material, creating an electric current. Due to the collisions of charged species with the close molecules or atoms an electric resistance is formed and heating is induced [29]. The microwave heating efficiency is highly depending on the dielectric properties of the sample.

3.2.2. Dielectric properties

The dielectric properties of the substance to be heated influence a lot the coupling of microwave energy in the material, i.e. the quantity of microwave that fails to penetrate the substance. The dielectric coefficient, ε_r, is the measure of this behaviour and is characteristic for each substance and its state. The ability to save electric energy is described by equation 3.4, in which ε_r is related to the capacity, C.

$$\varepsilon_r = C/C_0 \tag{3.4}$$

Follow equation 3.5 the dielectric factor can be divided in two different components, one real and one imaginary:

$$\varepsilon_r = \varepsilon_r' + i\varepsilon_r'' \tag{3.5}$$

Where ε_r'' is the dynamic dielectric factor and indicates the efficiency of the electromagnetic conversion into heat and ε_r' is the dielectric constant that describes the polarizability of the molecules in the electric field. The ability to convert the electromagnetic energy into heat – the dissipation factor tan δ - is given by the ratio between these two physical quantities (equation 3.6):

$$tan\delta = \varepsilon_r''/\varepsilon_r' \tag{3.6}$$

In accordance to the type of energy input (ion conduction or dipole rotation), the dissipation factor additionally depends on other factors like the ion concentration, the ion size, the dielectric constant, the microwave frequency, temperature and the viscosity of the reacting medium.

3.3. Conventional heating versus microwave heating

Between conventional (mostly carried out with an external heat source like an oil bath) and microwave heating there are some important differences.

In the conventional heating the heat is transferred by the three mechanisms described in section 3.1 from the surface toward the centre of the material. The efficiency of the heating system depends on the thermal conductivity of the material and on the convection currents and can be slow and inefficient; a contribution from irradiation mechanism can take place, depending on the temperature. In the microwave heating system, instead, is the dielectric heating that transfers electromagnetic heating to thermal energy so heat transfer is not limited by thermal conduction or convection [30]. The microwave can penetrate the material and the heat can be produced throughout the volume instead than coming from an external source.

This example of the inverted temperature gradient is well shown in Figure 3.3. The temperature of the reported sample is higher in the centre and lower near the walls on the case of microwave heating, unlike the conventional heating. This happens because the walls are usually made with materials transparent to MW, like borosilicate glass, quartz or teflon. The walls effects are so minimized, i.e. not hot vessel surface so the heating is more homogeneous and eliminates local overheating walls which can lead to side products. Microwave irradiations proceed so with higher purity and higher yields [31].
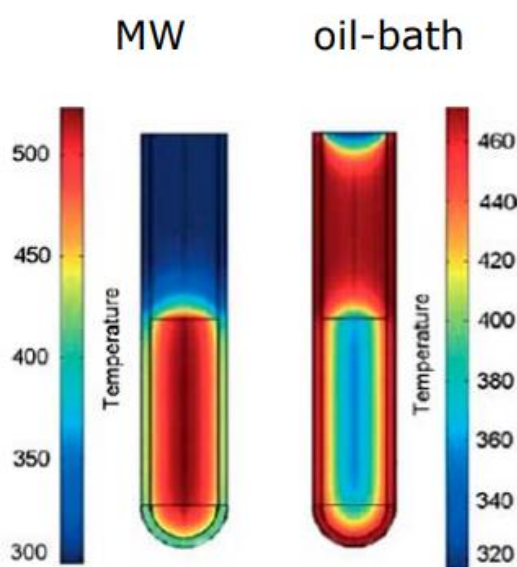
Figure 3.3. *Inverted temperature gradients in microwave versus oil-bath heating: difference in the temperature profiles after 1 min of microwave irradiation (left) and treatment in an oil-bath (right). Microwave irradiation raises the temperature of the whole volume simultaneously (bulk heating) whereas in the oil-heated tube the reaction mixture in contact with the vessel wall is heated first* [32].

The microwave heating leads to the observations of three main effects, not present in the conventional heating:

- Thermal effects. These effects concern the influence of a high reaction temperature which can be rapidly attained during the microwave irradiation of the material. In this case the reaction rate k described by the Arrhenius equation (eq. 3.7) is perturbed only in the temperature term and the activation energy, E_a, or the pre-exponential factor, A, are not affected.

$$k = Ae^{-E_a/RT} \tag{3.7}$$

- Specific effects. They are due to the proper nature of microwave irradiation heating mechanism in a microwave fields and shall be defined as accelerations of chemical transformations even if they are still thermal effects. Conventional heating cannot achieve them. One of them is the superheating effect of solvents at atmospheric pressure. As a matter of fact, when a liquid is heated by microwaves, the temperature increases rapidly to reach a steady state value that can be up to 40 K higher than the boiling point of the liquid. On the contrary under conventional heating this behaviour is not observed [30]. Also, the elimination of the walls effects is included in this classification. If a catalyst is used in the reaction its life time could be increased since it doesn't decompose due to the higher temperature. The mass heating moreover is more rapid, take the whole reaction mixture and could be very specific if, for example, are used a poorly MW absorbing solvent/reactant and a highly MW absorbing catalyst. The non-absorbent components are therefore not heated directly, but only by heat transfer from the heated component.

- Non-thermal effects. In this group are gathered all the chemical transformation accelerations that cannot be defined in terms of specific or thermal microwave effects.

  They are still a controversial matter and several theories and predictive models have been postulated but without a certainty. They are the result of the direct interaction of the electric field with specific molecules in the reaction medium. The presence of the electric fields probably leads to the orientation of dipolar molecules and hence change the pre-exponential factor A in the eq. 3.5 or the activation energy (entropy term). It's believed that the activation energy is decreased by the interaction with the electric field when a polar transition state is involved [32].

  Microwave effects are still subject of considerable current debate and a major effort on their research is needed to understand better the phenomena.

The temperature for the biomass activation is lower if the heating process is conducted with microwaves instead of radiation [33]. This phenomenon is maybe the most important for this work because permit us to carry the activation of lignin with a lower energy consume.

The only issue of the MW process is related to younger establishment of this technology that is still analysed.

Table 3.3. *Activation biomass temperature under MW heating and radiation heating.*

Biomass	MW activation temperature [°C]	Radiation activation temperature [°C]
Hemicellulose	160	280
Cellulose	180	320
Lignin	210	348
Wheat straw	160	341
Wood	164	371
Paper	200	420

3.4. Microwave reactors

The MW-assisted technology is possible thanks to the existence of laboratory-dedicated MW reactors that can convert the electromagnetic energy into heat in an efficient and reproducible way. The microwave reactor is composed by an oven in which is present a microwave source, a magnetron or a solid-state generator, with its power supply and controls. The electromagnetic energy is conveyed by a transmission line in a metallic cavity. The transmission line can consist in a waveguide for higher cavity or in a coaxial cable for lower power. The reactor is introduced inside the cavity. The material of the reactor vessel is transparent to the microwave energy to allow its transmission to the reactants or microwave-absorptive (susceptor) to supply heat by thermal conduction. Following there are other components needed for the impedance matching between generator and load.

The general features that characterize the commercial MW reactors are [34]:

- The presence of stirrer or rotative reactor vessel to guarantee a homogeneous electric field profile;
- A geometry design that considers the microwaves penetration depth;
- The temperature and pressure control system inside the reaction chamber to check the parameters of the reactions.
- A cost optimization and a sufficient availability of spare parts;
- Safety issue and microwave leakage intervention.

The reactors currently used can include also incorporated magnetic stirrer, fiber optic probes and IR sensors for a direct temperature and pressure control and a better microwave power regulation. In the market two main type of MW ovens are present: the multimode and the single-mode reactor. The main difference between those two systems lies in the number of

samples that can be irradiated and the uniformity of the electromagnetic field. The parallel synthesis is carried out in the multimode reactor since different reaction vessel can be heated simultaneously in rotors with multiple reactors, while in the single-mode system only one sample per time is run.

In Figure 3.4 is reported a general scheme of a microwave system in a closed applicator (single-mode).
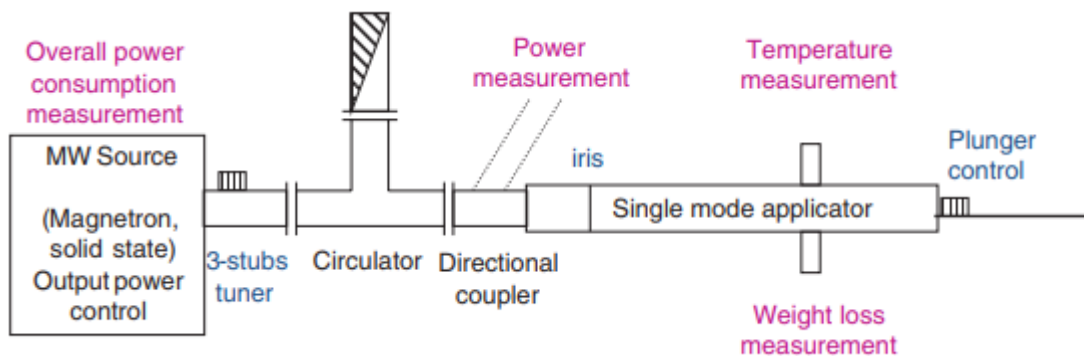
Figure 3.4. *Complete arrangement of a microwave system single-mode. The impedance matching is identified with the blue colour and the measurement parts is in purple.*

Chapter 4

Lignin activation

The chapter describes MW and conventional lignin activation techniques as an approach to obtaining valuable chemicals such as phenolic compounds. The last paragraph is focused on the available techniques for the separation of the chemicals contained in the resulting bio-oil.

4.1. Pyrolysis of lignin

4.1.1. Conventional Pyrolysis process

As it was seen in the first chapter, lignin has a complex composition and structure. This feature makes the thermal degradation composition and product yields strongly dependent on lignin nature, moisture content, reaction and degradation atmosphere, heat and mass processes. Even the physical properties and the quality of the pyrolysis products are affected so the challenge lies in finding the best lignin conditions treatment (including catalysts) and in economically separating valuable compounds for the market. Therefore, a characterisation before selecting the optimum method for upgrading or extracting the valuable products is needed [35].

Pyrolysis is one of the most promising thermochemical technologies [36] since it has more advantages than the standard incineration process, like a lower formation of harmful gases and a higher production of value-added chemicals [37]. Indeed, thanks to the high aromatic portions in lignin macromolecules, it's possible to obtain value-added phenolic compounds through depolymerisation [4–6].

There are two type of conventional pyrolysis processes depending on the heating rate: fast and slow pyrolysis. Windt et al. [41] indicated that fast pyrolysis has more advantages in terms of conversion due to the fact that longer time and lower temperature can favour the formation of coke and char.

4.1.1.1. Lignin decomposition during the Pyrolysis process

The decomposition mechanism during the pyrolysis lignin process is very complex [Figure 4.1], could occur in a wide range of temperature and depends on the nature of lignin and

reaction conditions. To control the production of the desire decomposition products, it is important to understand the thermochemical process behaviour. This behaviour is affected mainly by the lignin nature, the heating rate, the catalyst and the temperature [42].

At low temperature the weak bonds (hydrogen bonding and C-OH bonding) are the first to be broken and then, increasing the temperature, also the cleavage of stronger bonds occurs. During the first step of decomposition aldehydes, toluols, styrenes and guaiacyl hydroxyls are the main compounds, while the primary products of the further reactions are p-hydroxy phenol, cathecols and creosols [43].

Actually, the produced compounds depend on the nature of lignin – G or S type. Figure 4.2 shows the effect of temperature during the pyrolysis process on aromatic substitution pattern and side chain structure of the products from G-type lignin. 4-substituted guaiacols are predominant in the first stage of decomposition and then during the secondary stage they are transform in catechols and *o*-creosol along with phenol [44].

It is supposed that the first step of the decomposition includes the formation of free radicals after the breaking of the β-O-4 linkage. Some of them produce vanillin or 2-methoxy-methylphenols with the capture of the protons and others proceed with the lignin chain propagation, passing to other species for the next reactions. The depolymerisation reaction ends with the collision of two radicals to form a stable compound and the repolymerisation of the radicals at temperature higher than 350°C that leads to polyaromatic biochar [45]. Following Fergous et al. [46] in the products observed in the first step (120°C-300°C) there are also formic acid, formaldehyde, CO_2, CO, and water. Water is released after the cleavage of OH functional groups linked to β and γ carbons in aliphatic side chains while formaldehyde comes from the breaking of the β and γ carbons bonds in the alkyl side chains. The next bonds that are broken are the Aryl ether ones (α or β-O-4-bonds), while γ-carbons and methoxy groups are more resistant to the pyrolysis and become very reactive only after 400-450°C.

However, the complete pathway is still not very clearly understood due to the very complex structure of lignin that can give complex reactions products after unpredictable reactivity behaviour. For example, the recombination of the active radical intermediates can form oligomers and more condensed compounds, characterized by a higher molecular weight (solid chars).
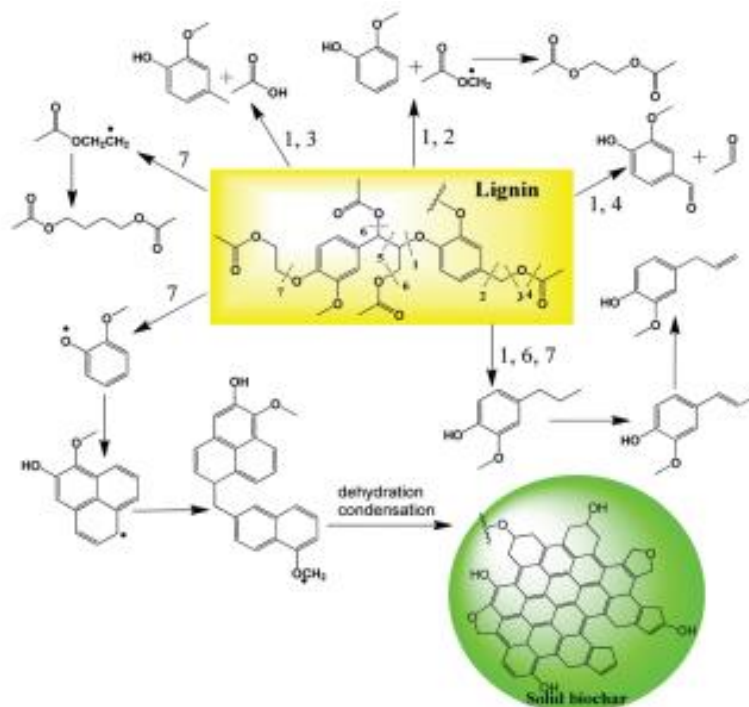
Figure 4.1. *Possible mechanism of lignin decomposition in the pyrolysis process [10].*
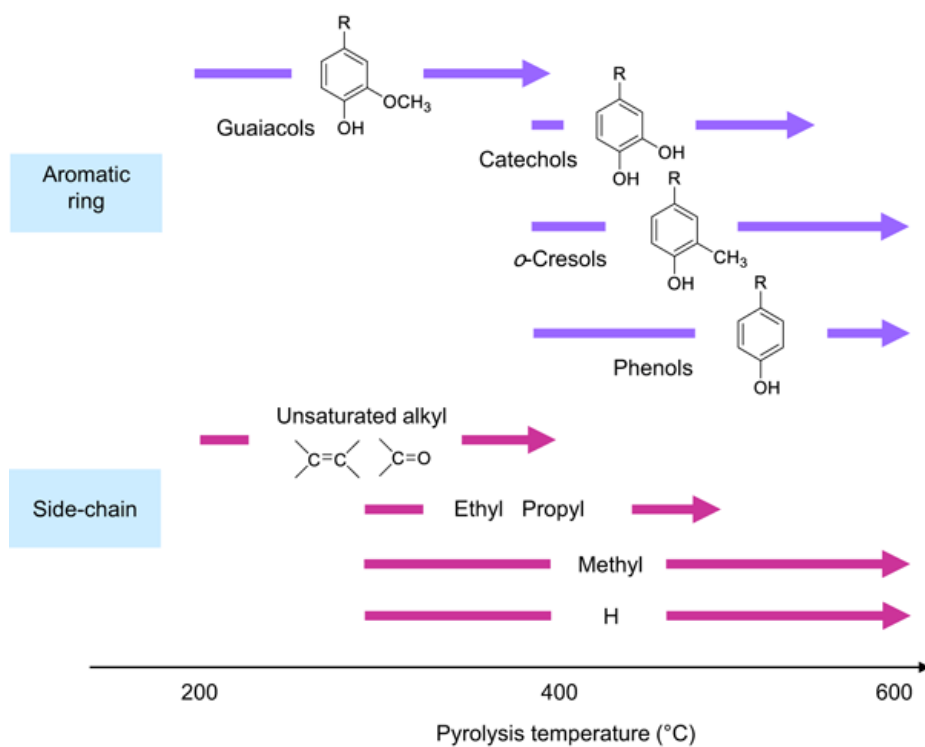
Figure 4.2. *Effect on aromatic substitution pattern and side-chain structure of temperature during the pyrolysis process. The products come from a lignin of G-type* [44].

4.1.1.2. Pyrolysis kinetics

In the literature works, the pyrolysis kinetics is studied to control the process and obtain the desired products and the reaction behavior related. The pyrolysis parameters have been studied by many researchers using the Arrhenius equation (3.7), seen in Chapter 3, that relates, for any reaction, the rate constant with the temperature.

The kinetic parameters are A, the frequency factor, and E_a, the activation energy. Both are constants for the single reaction.

Table 4.1 summarizes some kinetic parameters present in the literature. It is based on different reaction models (single-step, first-order for example) and on various lignin type, lignin isolation methods and computational methods of the parameters calculation [47].

Table 4.1. *Kinetic parameters of lignin pyrolysis, present in literature, according to lignin type, temperature range and pyrolysis method [48].*

Lignin type	E_a [kJ mol^{-1}]	A [min^{-1}]	T [°C]	Method
Kraft, SW	25.8	$4.7 \cdot 10^2$	160–680	microwave
MWL, HW	82.3	$2.03 \cdot 10^7$	327–1127	reactor
Kraft	36.7	39.3	227–727	
MWL	34–65	$10^{0.3}–10^{3\ a)}$		
Alcell	129–361	$6.2 \cdot 10^{11} – 9.3 \cdot 10^{22}$	272–532	TGA
kraft	80–158	$3.3 \cdot 10^7 – 1.84 \cdot 10^9$	234–503	TGA
SADF, SW	72.9–136.9	$6.8 \cdot 10^8 – 5.2 \cdot 10^9$	149–492	TGA-FTIR
SADF, HW	87.2–141.7	$1.3 \cdot 10^{10} – 7.9 \cdot 10^{13}$	154–527	TGA-FTIR
various	134–173	$2.4 \cdot 10^{10} – 9.4 \cdot 10^{12}$	105–900	TGA

4.1.2. Products of Lignin Pyrolysis

The pyrolysis products are present as gases, liquids and solids.

4.1.2.1. Solid products of Lignin Pyrolysis

Investigation of the solid products, the char, have been not done in many literature works so the information about them are limited. Ben and Ragauskas [48] reported that the structure of char is composed mainly by condensed aromatics and maintain up to 50% of starting biomass energy. Following the characterization done by Sharma *et al*. [15, 16] using SEM, FTIR and COMAS[13]C NMR the yield of the char decreases from 80% to 40% of lignin when the temperature increases from 523 K to 1023 K. The reason is related to the cleavages of aliphatic OH, carboxyl and methoxyl groups that, based on the results of FTIR and NMR, were improved at higher pyrolysis temperature. At those temperatures, values of both H/C

and O/C ratios decrease, representing the condensed aromatic structure. Hosoya et al. [51] considered GC-MS analysis to consider the importance in the char formation of methoxyl groups and *o*-quinone methide, a significant intermediate during the pyrolysis process.

4.1.2.2. Gas products of Lignin Pyrolysis

From the lignin pyrolysis it is possible to obtain gas products that have been the topic of many literature studies [12], [18–23]. The main found compounds are carbon monoxide and carbon dioxide that represent almost the 50% of the gas product. Methane is also present and shows, with the other two gasses, to increase in yield with the reactor temperature and to decrease at higher heating rate. The works of Ferdous et al. [12, 18] indicated that from the pyrolysis process, H_2 has also been produced (25 mol%). In this way the pyrolysis products can become valuable snice hydrogen and carbon monoxide are the major components of syngas which could be used for synthetic petroleum production.

4.1.2.3. Liquid products of Lignin Pyrolysis

Most of pyrolysis works employed GC-MS and FTIR techniques to study the composition of the liquid products obtained after the pyrolysis process of lignin.

Depending on the work conditions, different compounds have been detected. Jimenez et al. [58] used pyrolysis (Py)-GC-MS to show that softwood lignin leads to high yield of guaiacyl derivates, coniferaldehyde and coniferyl alcohol instead the major products from hardwood lignin are guaiacyl and syringyl derivates, syringaldehyde, coniferyl alcohol and sinapyl alcohol. The same method is used by Jiang et al. [59] in a wider range of temperature (673-1073 K). The phenolic compounds reach the maximum yield at 873 K and the major of them has an individual yield of less than 1 wt% of lignin on a dry-ash-free basis. Following the research of Lou et al [60] the composition of liquid products changes with the temperature. It indicates that the contents of methoxyl components – guaiacol, 4-methylguaiacol, 4-vinylguaiacol and syringol - is lower at higher process temperature, where prevails the contents of non-methoxyl contained compounds, such as creosols, ethyl-phenol and 2, 6-dimethyl-phenol. Many other compounds are reported in literature and they can be summarized and used to build simulation model to understand better the pyrolysis decomposition pathway [61].

If the compounds have a high molecular weight, they can be not detected by the GC because of the volatility limitation. FTIR analysis could be an alternative characterization method to avoid exclusion of part of the pyrolysis oil [62].

A study of hardwood and softwood lignin has been done by Liu et al. [63] with a TG-FTIR, a thermogravimetric analyzer coupled with a Fourier transform infrared spectrometry. Three reaction steps are identified: the evaporation of water; the formation of primary volatiles and the release of small molecular gases. The water is released at the beginning by evaporation

and is generated again at higher temperature (above 373 K) due to the lignin aliphatic groups dehydration. The major gaseous products in this case are phenols, alcohols, aldehydes, acids and CO, CO_2 and CH_4. A correlation between carbonyl absorption bands and oxygen and carbon content was found by Scholze et al. using FTIR [36].

4.1.3. Microwave-assisted Lignin Pyrolysis

The main products that could be obtained from lignin pyrolysis under microwave heating are reported in Table 4.2.

Table 4.2. *Literature about microwave-assisted pyrolysis of lignin.*

Lignin type	Reaction conditions	Major products	Yield	Ref
Kraft lignin	**900-1240K, 1.5-2.7 kW**	**Guaiacols**	**135-184 mg/g oil**	**[64]**
Alkali lignin	**700W, 309-591°C, 0.82-2.62 h^{-1}**	**Phenol**	**45 wt.%**	**[65]**
Softwood Kraft lignin	**1.5 kW, 800 s**	**Guaiacol** **4-methylguaiacol**	**14%** **13.58%**	**[66]**

Various studies were conducted using the microwave heating instead of the conventional one, to try an improvement of chemical reactions. Farag et al. [64] investigated the effects of the microwave absorber (20-40 wt%) and microwave irradiation power (1.5-2.7 kW) on the product distribution and crude liquid composition during the pyrolysis of Kraft lignin. The resulting products were mainly guaiacols in the oil phase, while phenols and catechols were produced in lower concentrations (74-108 mg/g and 31-50 mg/g respectively). The experimental design of Bu et al [65] included the microwave pyrolysis of lignin in presence of activated carbon (AC) as catalyst with the aim to produce renewable phenols and fuel. The influence of temperature and weight hourly space velocity (WHSV) was investigated in relation to the product yields.

The higher yield of phenol and phenolic compounds was observed at 550°C and 2.18 h^{-1}. In addition to phenols and guaiacols, also hydrocarbons and esters were found.

The pyrolysis of Kraft lignin was conducted by Fu et al. [66] with char as the microwave absorber and catalyst. The microwave power was changed from 1.5 kW to 2.7 kW producing non-condensable gas, heavy bio-oil, light bio-oil and solid charcoal. Due to the density difference, the bio-oil was obtained directly in two phases. Then, the extraction of phenols from the heavy pyrolysis oil was done with a switchable hydrophilicity solvent (SHS).

4.2. Lignin solvolysis

The solvolysis is a thermal-chemical process that permits the lignin conversion to high value products or fuels with the help of a solvent [6], [33], [67]. This process could guarantee simultaneously the depolymerization and the hydrogenation of the lignin.

A representation of the solvolysis mechanism can start with the same primary step of reaction described in the lignin pyrolysis mechanism. Compared to the pyrolysis, the difference lies in the fact that the reaction products are diluted and dissolved in the solvent instead of being exposed to a vapor/gas phase. It is still not sure if the solvent takes part in the primary process and different pathway are possible in relation to the solvent and reaction conditions used. The solvent could be water or any other organic/inorganic liquid, like alcohol or acid with a lower vapor pressure. Typical reaction conditions are 250-400°C and 100-250 bar.

It was demonstrated that the process under microwave heating can permit the reduction of the required activation energy, increasing the depolymerization reaction rate. It leads to high efficiency, high selectivity and low environmental pollution [68]. Indeed, following the studies of various researches, the application of the microwave, with or without catalyst, could enhance the depolymerization of lignin to high value compounds production. This leads to an easier reaction occur since the β-O-4 ether bonds and the C-C bonds in the lignin structure can be relatively easily broken [69].

The contents and selectivity of phenolic compounds was investigated by Dong et al. [70] varying the residence time and the reaction temperature during an acidic microwave-assisted solvolysis of black liquor lignin. The results show that yield of the liquid products decreases when the temperature and the reaction time increase. It is possible to consider that the depolymerization and the repolymerization reactions were both promoted as the temperature increases while during a longer residence time the first reaction is inhibited thanks to the simultaneously occurrence of the repolymerization one. Kim and Park [71] studied the MW-assisted solvolysis of sulfonated lignin in presence of KOH varying the temperature and obtaining guaiacol, vanillin, homovanillic acid, acetovanillone, phenol and syringol. It is showed that the products yield increases as the temperature increases and that the guaiacol yield is directly proportional to the concentration of KOH, despite of the other products that behaved in the opposite way.

In the past works low-boiling-point oxygen containing polar protic solvents were more frequently use, like water, methanol and ethanol. Hana et al. [72] liquefied alkali lignin in water obtaining the highest yield of guaiacol (11.23 wt%) at 553K and zero reaction time. Since the guaiacol can be hydrolysed into catechol when both the temperature and the reaction time increase [73], its yield decreases. If catechol becomes the main product, also in alcohol or water-alcohol mixture, the contents of guaiacol type compounds can be lower [74]. Peng at al. [72] tried to obtain selectively the production of guaiacol type compounds

via depolymerization of black liquor lignin in hydrocarbon liquids without a catalyst. They used non-polar, protic solvents to make the influence on the methoxy group attached into the aromatic ring less strong. The hydrocarbon liquids have also a high boiling point, so the reaction pressure is lower compared to the ones needed with low-boiling-point solvents. The results of this article confirm that the selectivity of guaiacol type compounds is enhanced in presence of non-polar solvents rather than polar solvents.

4.3. Catalyst in Lignin depolymerization

To improve the production of valuable compounds, the use of different type of catalyst, acid or base ones, was investigated in the depolymerization of lignin process.

In this work acid and basic catalyst were used to try to encourage the conversion of phenolic compounds so a summary of the related literature is here presented.

4.3.1. Acid catalyst

Acid catalysts are very common used in lignin depolymerization to phenolic compounds. The degradation of bamboo residues was, for example, carried out by Xie at al. [75] in a mixture of glycerol and methanol as solvent using sulfuric acid as the catalyst. The highest conversion yield was 96.7%. Other acid types were investigated in the literature. Mixtures with different proportion of ethanol and formic acid were used to catalyse the depolymerization of wheat straw lignin. Methoxyphenols were obtained with a yield of 2.9 wt.% adopting 10 wt.% of formic acid and 81 wt.% of ethanol.

4.3.2. Base catalyst

The production of phenolic compounds can be influenced a lot by base catalyst. The utilization of a strong base is a common strategy to enhance the lignin depolymerization and control the products formation. One of the most frequently homogeneous base catalyst is sodium hydroxide. At high temperature, 300°C, in 4 minutes of residence time and 250 bar pressure, is possible to produce simple aromatic compounds using 2wt% of this base in aqueous media from 5wt% of lignin. 11.5% of the staring materials was converted in the resulting oil (Figure 4.3), while the rest of the lignin remained unreacted [76].
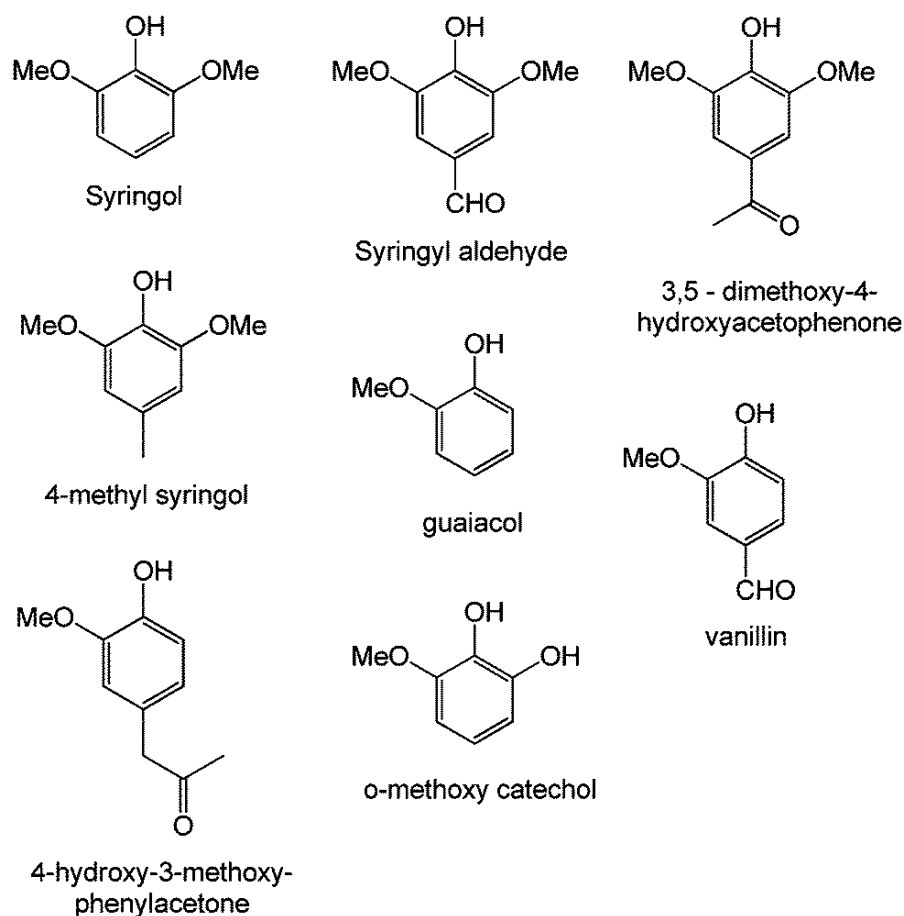
Figure 4.3. *Phenolic compounds obtained after NaOH-catalysed hydrolysis of lignin at 300°C and 250 bar [45].*

The contribution of base catalysis in the lignin depolymerization is related not only to the cleavage of lignin monomer linkages, but also to the breaking of other functional groups, like the methoxy one, connected to the aromatic rings.

In terms of reaction conditions, this study shows that high temperature doesn't help the depolymerization but encourages the molecules degradation and the formation of gas and coke.

The investigation of the effects of different base catalysts was done by Toledano et al. [77] working with KOH, NaOH, Ca(OH)$_2$, LiOH and K$_2$CO$_3$ as reaction catalysts and with organosolv olive tree pruning lignin as raw material. The yield of the oil and residual lignin was increased thanks to the base action and potassium catalyst gave the highest repolymerization degree.

4.5. Separation technologies for bio-oil components

The bio-oil resulting from the lignin depolymerization is a very complex phenolics mixture. Thus, a selective separation of the components into pure compounds is a big challenge [78] and often more expensive than the activation process of the biomass [79]. Very few industrial strategies for the valuable compounds were proposed in the literature and no processes are established, except the one for the vanillin production from lignosulfonates [80]. Often the separated products are not pure but the process yields a mixture of fractions that contains different chemicals with similar properties [81]. Anyway, the common methods are the solvent extraction, the chromatography column and the distillation.

4.5.1. Solvent extraction.

The solvent extraction is commonly performed with water, alcohols, alkaline solutions or other organic solvents specific to dissolve the desired components of the bio-oil. The process involves two phases, the raw solution and the solvent solution containing the products. Due to the large volume of solvent that may be needed, the industrial scale could be uneconomical. This technique can appear interesting if it's improved with special switchable hydrophilicity solvents (SHSs) or with supercritical CO_2. In literature some examples of separation of phenolic compounds from bio-oil using this technology were found. Amen-Chen et al. [82] managed to isolate phenol, cresols, guaiacol, 4-methylguaiacol, cathecol and syringol from wood tar converted to light oils. The separation was made with a five cross-current stage extraction using organic and alkali solvent. The highly alkaline conditions (pH of 12-13) showed to promote the process. A multi-step separation of monophenols and pyrolytic lignin was conducted by Wang et al. [83]. The chemicals were isolated from a water-insoluble phase composed by the bio-oil recovered from the Iauan sawdust pyrolysis. The chosen solvents were hydrochloric acid and sodium hydroxide combined with CH_2Cl_2, an organic solvent. From the extraction, filtration and distillation processes, three fractions were obtained. One is neutral, one contains the intermediate phenolic fraction and the last one the final phenolic fraction. After two filtration steps, it can be possible to get, as precipitates, a high-molecular weight pyrolytic lignin and a low-molecular weight pyrolytic lignin. In the intermediate phenolic fraction, the guaiacol was 48.3%. The high-molecular weight lignin had an abundance of polymers while the low-molecular weight lignin contained predominance of more reactive phenolic hydroxyl groups.

The use of new switchable polarity solvents (SHSs) was investigated recently due to their switch-ability from a liquid to on other one characterized by very different set of properties, upon application or removal normally by CO_2. They are completely miscible with water in presence of CO_2 but have a very little miscibility with water without it. This behavior is caused by a chemical reaction that gives a water-soluble bicarbonate salt of the protonates

SHS$_S$. Thanks to their alkalinity, SHS$_s$ are able to extract the phenolic compounds from the bio-oil without the need of a distillation step [84]. Fu et al. [66] used N,N-dimethylcyclohexylamine as switchability solvent and managed to concentrate the phenolic compounds in one fraction recovering 72% of the guaiacol and 70% of the 4-methylguaiacol present in the original bio-oil from softwood Kraft lignin microwave-assisted pyrolysis.

4.5.2. Chromatography column

The base principle of this process is the absorption in different way of the components into a stationary phase (silica gel or aluminum oxide) [85]. The interaction between the components and the stationary phase makes the components flow inside the column at different speeds, so they exit at different times. Since the processing rate is slow, this technique is more appropriated for high value chemicals.

4.5.3. Distillation

A distillation column acts on the difference between components volatilities, depending on the boiling point. Best condition (lower temperature) and reduced products decomposition can be achieved with steam distillation or vacuum distillation. In fact, operating at lower temperature, the products contain almost no water and few oxygenated compounds. Steam distillation performs the separation heating the bio-oil and so decreasing its viscosity. The bio-oil boils at lower temperature (below 100°C) permitting a reduced degradation of thermally sensitive chemicals. Also molecular distillation has been developed as a new method for bio-oil separation and it is commonly applied in petrochemical, fine chemical, medicine, pharmaceutical, and food-processing industries. It is particularly suitable for thermally unstable bio-oil separation since it is based in the various mean free paths of different molecules. The process conditions are lower temperature and shorter reaction times which imply higher separation efficiency and minimal losses because of thermal decomposition. The cost of equipment is very high.

Chapter 5

Materials and methods

In this chapter are described the materials and the methods used to investigate the activation of lignin with both conventional and MW assisted heating, the characterisation of the sample, and the quantification, extraction and separation of the phenolic compounds.

5.1. Materials

5.1.1. Lignin

The lignin pellets used in this work are softwood-type derived from the Kraft process. The characterisation was made with the methods described in paragraph §5.3 and the results are reported in the section §5.3.5.

5.1.2. Solvents

To investigate the behaviour of the lignin during MW solvolysis and select the best solvent for the phenolic compounds extraction, four different solvents were processed. Ethanol (Sigma-Aldrich – United Kingdom) and distilled water were chosen from the polar and protic solvent group, to compare also the phenol solubility in both; heptane (Fisher Scientific – United Kingdom) from the non-polar and aprotic solvent group and sulfolane (Sigma-Aldrich – France) from the polar and aprotic one. Table 5.1 contains some properties about those materials.

Table 5.1. *Solvents used for MW-assisted lignin solvolysis and phenolic compounds extraction.*

Solvent	Boilng point [°C]	Density at 25°C [g/mL]	Dielectric constant	Polar/Non polar	Protic/Aprotic	Molecular weight [g/mol]
Water	100	0.9970474	80.4 (20°C)	Polar	Protic	18.01528
Ethanol	78.37	0.7858	24.3 (25°)	Polar	Protic	46.07
Heptane	98.42	0.6795	1.9 (20°C)	Non polar	Aprotic	100.21
Sulfolane	285	1.261	42.22	Polar	Aprotic	120.17

5.1.2. Catalyst

Two acids and two bases were investigated as pyrolysis lignin catalysts, both using MW and conventional heating.

Table 5.2 presents the acids from the least strong to the strongest in dependence of the pKa value and Table 5.3 the bases following the same order criteria.

Table 5.2. *Acid catalyst and relative Ka value.*

ACID	pKa
Acetic acid	4.76
Sulfuric acid	-3.67

Table 5.3. *Base catalyst and relative Kb value.*

BASE	pKb
Ammonia	4.75
Sodium Hydroxide	-0.56

Acetic acid was purchased from Fluka (Germany), sulfuric acid and sodium hydroxide from Fisher Scientific (United Kingdom) and ammonia gas (anhydrous - 99.9%) from Aldrich.

5.1.3. Thin Layer Chromatography eluents

To find the eluent capable to separate the phenolic compounds in the bio-oil, several mixtures were proved. For this purpose, n-pentane, heptane, ammonia solution were purchased from Fisher Scientific (United Kingdom), benzene from Honeywell (United Kingdom), chloroform, ethyl acetate, butanol, dichloromethane and acetone from Sigma-Aldrich (France), 1-propanol from Acros Organic (Germany) and toluene and acetonitrile from Sigma-Aldrich (Israel). The eluents were suggested in the papers by Mark J. Kurth [86] and Fish W. et al. [87].

5.2. Methods for lignin activation

The lignin activation, with pyrolysis and solvolysis processes, was achieved using both microwave and conventional heating. The first method involves the microwave reaction and the latter one has been carried out in a thermogravimetric analyser.

5.2.1. The microwave reactor

The microwave reactor used to perform the experiments is the Discover® SP microwave synthesizer (Figure 5.1). The reactions can be carried out in both pressurized and open vessel and in flexible reaction vessel sizes for a wide range of organic and inorganic synthetic chemistry. It is a single-mode microwave cavity reactor, so it is possible to run only one sample per run.

The sample can be processed in glassware up to 80 mL with the pressurized feature or in 125 mL with the open vessel round bottom flask varying, if necessary, the speed of the magnetic stirring. To avoid over-pressurization an Activent® vent and a re-seal technology

permit the relieving of gaseous by-products (Fig 5.1*b*). It avoids vial failures and allows higher temperature achievement in a safe way. The data are reliable since is present a highly accurate infrared (IR) temperature control floor mounted and a cool-down regulated by compressed air. The temperature is measured below the sample (Fig 5.1*a*).
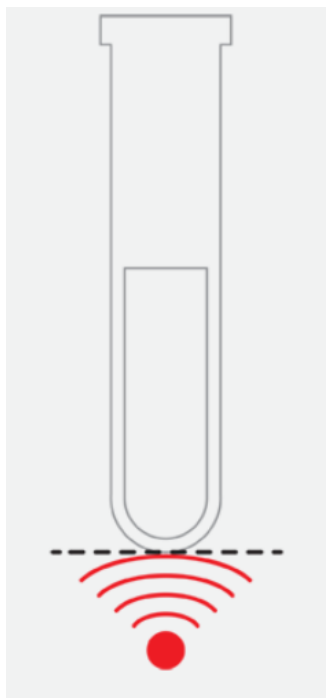
Figure 5.1*a*. *Temperature measurement.*
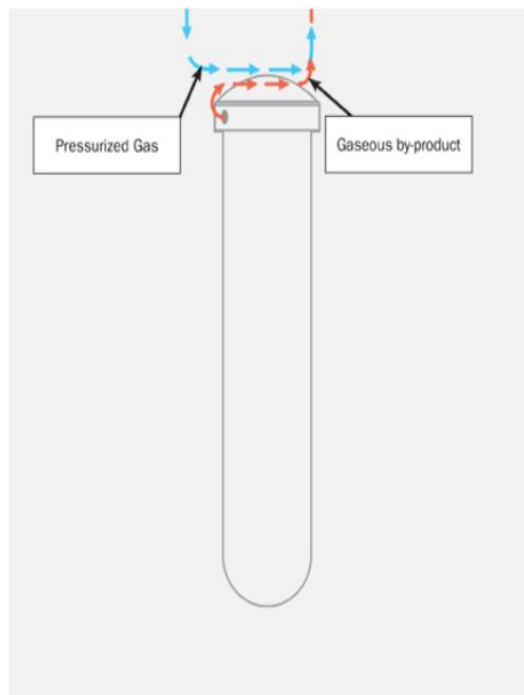
Figure 5.1*b*. *Pressure control.*

5.2.1.1. Reactor methods

Two methods were adopted in the microwave reactor to change the operative conditions for each experiment:

- The *Fixed Power method.* It permits to set the microwave power and change the reaction temperature. On the other way it is also used to investigate the influence of the microwave power on the lignin activation at a fixed temperature value;
- The *Dynamic Power method.* With this method is possible to follow the MW power behavior to reach a certain temperature or to maintain the reaction for the desired holding time.

The first method takes few minutes to reach the maximum temperature (280 °C) before cooling down the reactor. The second one instead, is slower in terms of heating rate since the power is not fixed but permits to keep the sample under the microwave radiation at the chosen temperature for the desire holding time before the cooling.

5.2.1.2. Solvolysis and Pyrolysis procedure

The solvolysis was conducted in the microwave reactor using the *Fixed Power method* (300 W) and no holding time after the highest temperature achievement. The solvents investigated were distilled water, heptane, ethanol and sulfolane. For each of them, one sample was run with 1 g of lignin in 1 ml of solvent. The standard procedure for the pyrolysis process included the run of 1 g of lignin without solvent. The reactor method was based on the desired parameter influence's knowledge. The power influence was investigated with the same *Fixed Power* method and the reaction time with the *Dynamic Power* one. 1 g of lignin, doped with acid or basic catalyst, was pyrolyzed with the first method at 300 W avoiding holding time after reaching the highest fixed temperature. The highest temperature value was 280°C, the pressure limit at 300 psia and the highest power allowed was 300 Watts. All the experiments were performed in closed vessel using 10 ml glass vials. The only exception was the samples of lignin doped with bases that were run in special 10 ml quartz vials to avoid breakages.

5.2.2. Thermogravimetric analysis (TGA)

The conventional pyrolysis of the lignin, with and without catalyst, was processed with Netzsch STA 409 (Fig. 5.2). This is a Simultaneous Thermal Analyser and is currently set up as a TGA. This equipment is composed by a thermobalance in a vertically-arranged instrument with top-loaded samples. Its design provides the protection of the digital balance, that measures the weight change simultaneously with heat flow as a function of time or temperature. The balance is placed on the bottom of the furnace and it is protected by an accurate flow of the purge and protective gases in a natural path to the top. It is also possible to couple FTIR and MS gas analysis system to the heated furnace outlet.

The temperature range is ambient to 1300°C and with the TGA sample carrier in place the range can reach 1600°C. The analysis can be run in flowing nitrogen or a mix of nitrogen and air. With special arrangement is also possible to run high oxygen concentrations experiments. The sample size can be up to 3 g in the TGA configuration.

Fig. 5.2. *Netszsch STA 409.*

The maximum temperature chosen for the experiments was 500°C with a heating speed of 50 K/min. The sample (less than 100 mg) is placed in the crucible inside the furnace and the vacuum is provoked before let the nitrogen passage. If the TG is connected to a FTIR technology, the gasses are detected by its detector. Other experiments were also run connecting a trap just after the furnace (Figure 5.3). In this case the volatile compounds go up inside the trap and condense due to the presence of the added liquid nitrogen in the trap cavity. Afterwards, to prepare the sample for the gas chromatography analysis the trap was washed with 20 ml of ethanol to dissolve the compounds.
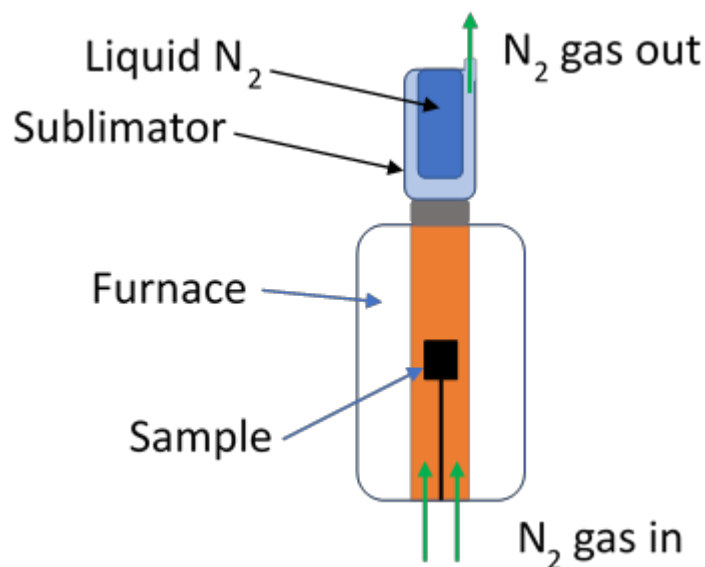
Figure 5.3 *Scheme of the TGA equipment. The sample is placed inside the furnace, where an inert atmosphere is guarantee by a nitrogen flow. On the top of the furnace the trap is located. This is composed by the sublimator, in which the volatiles are stopped, and the cavity for the liquid nitrogen storage. The nitrogen flow goes out from the trap and is breathed in the gas collector.*

5.2.3. Methods for lignin catalysis

The lignin (1 g) was catalyzed with the addition of several acids and bases, 2% by weight. First, the acid and base solution was prepared in distilled water. The grounded lignin was then added to the solution and a Rotor Evaporator was used to evaporate all the solvent. The evaporation time depends on the boiling point of the solvent and was usually around 1 hour per sample.

The doping with ammonia gas was obtained leaving 5 g of grounded lignin under the ammonia gas flow provided by a pressurized cylinder. The chosen residence times inside a two-necked flask was 1 hour.

5.3. Methods for lignin characterization

To investigate composition and the properties of the raw material four methods were used: TG-FTIR, 2D-NMR, GPC and CHN elemental analysis.

5.3.1. Fourier transform infrared spectroscopy (FT-IR)

The observation of the condensed phase decomposition is provided by a variable temperature chamber DRIFT (VT DRIFT) setup. The gases from the TGA are followed using the FTIR gas cell connected. This method is called TG-FTIR.

The real time FTIR measurement of the off-gasses from the thermogravimetric analysis is given by a Bruker Equinox 55 FT-IR which is coupled with the Netzsch STA 409 using a heated transfer line. This permits to know the composition of the volatile compounds coming from the furnace. Both TG and FTIR techniques are time resolved type since a series of spectra are taken at precise time intervals. Time and temperature can be correlated knowing the specific temperature program applied to the sample.

5.3.1.1. FT-IR mechanism

This technique is based on the absorption, by some molecules, of the infrared radiations (IR) that have wavelength between 0.78 and 100 μm. The IR radiation can provoke vibrations in the molecules bonds thanks to the resonance and the atoms vibration. This happens when the frequency of the radiation is equal or a multiple of the bonds natural one. There are two main type of bonds vibrations (Fig 5.4):

- Stretching: variation of the length bonds. It can be symmetric, if the shortening and the lengthening happen simultaneously, or asymmetric if one bond become longer and the other shorter;
- Bending: variation of the angle bonds. It can be in-plane or out-of-plane mode, depending on the plane of the angle vibration. The bending in the plane could be scissoring or rocking while the one out is called twisting or wagging.
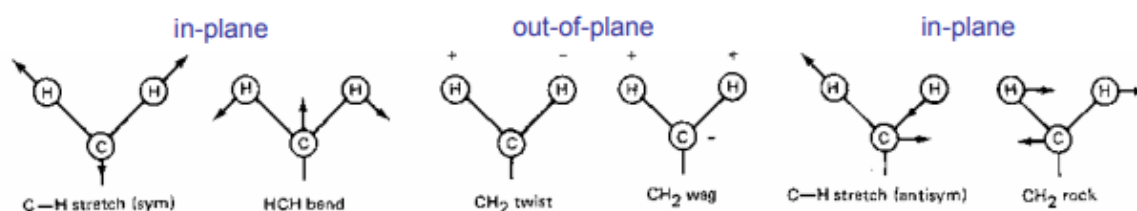
Figure 5.4. *Main type of bonds vibrations of the CH_2 group. The arrows show the direction of the motion and the + and – sign underline respectively the motion above and below the plane.*

The bonds vibrations of the sample molecules result in a change of the dipole moment after the IR selective absorption. Therefore, the sample molecules change their vibration energy levels from ground state to excited state. The energy gap determines the frequency of the absorption peak. This frequency depends on the molecule number of vibration freedom while the intensity of the absorption peaks is related to the variation of the dipole moment and the possibility of the transition of energy levels. The frequency can be calculated using an approximation of the Hooke law (Eq. 5.1):

$$\nu = 1/2\pi c \ \sqrt{(k/m)} \tag{5.1}$$

where ν is the wave lengths in cm^{-1}, c is the light speed, k is the bond strength constant [5 x 105 dine cm^{-1}] and m is the involved atom mass [$m_a m_b/(m_a+m_b)$]. The energy correlated to the radiation E is obtained from the equation 5.2:

$$E = h \cdot \nu \tag{5.2}$$

where h is the Planck constant (6.626·10-34 J·s) and ν is the radiation frequency previously calculated.

The most common absorption radiation of organic and inorganic compounds and ions is within the region 4000 ~ 400 cm^{-1}.

The information about the absorption are collected and analysed in form of a spectrum; the infrared absorption is capable to describe gas, liquid and solid samples.

Indeed, chemical bonds absorb varying intensities and at varying frequencies in different environments, so it is possible to know the functional groups and the chemical nature of the samples. Table 5.4 summarizes the characteristic wavenumber for the more common chemical bonds.

Table 5.4. *Peak wavenumber related to the more common chemical bonds [88].*

Peak wavenumber (without dislocation) (cm^{-1})	Peak wavenumber (with dislocation) (cm^{-1})	$\Delta v (cm^{-1})$	Bonds
3327	3332	5	OH stretching
2883	2882	-1	C-H symmetrical stretching
1724	1724	0	C=O stretching vibration
1623	1624	1	OH bending of absorbed water
1506	disappear	-	C=C aromatic symmetrical stretching
1423	1423	0	HCH and OCH in-plane bending vibration
1368, 1363	1367,1363	-1/0	In-the-plane CH bending
1325	1325	0	S ring stretching
1314	1313	-1	CH_2 rocking vibration at C6
1259	1261	1	G ring stretching
1245	1244	-1	C-C plus C-O plus C=O stretch; G condensed > G etherfied
1232	1231	-1	COH bending at C6
1204	1199	-5	C-O-C symmetric stretching, OH plane deformation
1152	1156	4	C-O-C asymmetrical stretching
1046	1043	-3	C-C, C-OH, C-H ring and side group vibrations
1020	1018	-2	C-C, C-OH, C-H ring and side group vibrations
994	996	2	C-C, C-OH, C-H ring and side group vibrations
895	894	-1	COC,CCO and CCH deformation and stretching
662	663	1	C-OH out-of-plane bending

5.3.1.2. Fourier-transform spectroscopy

The Fourier-transform spectroscopy, as opposed to the absorption ones, doesn't use a monochromator but a mechanical device called Michelson interferometer with movable mirrors.

In the scheme reported in Figure 5.5 is possible to identify:

- a *source*, that generates light across the spectrum of interest;
- the *sample*, that absorbs the light according to its chemical properties;
- a *detector*, that collects the radiation passing through the sample and compares its energy to that going through the reference.

The energy from the source is sent through an interferometer into the sample. The light passes through a beamsplitter (a semitransparent mirror) that divides the ray coming from the source and send it in two directions at right angles. One beam goes to the stationary

mirror and then comes back to the beamsplitter and the same happens to the other, but this is reflected by the moving mirror. The motion of the latter one varies the total path length versus the one taken by the stationary-mirror beam. This difference in the path lengths creates constructive and destructive interference when the two beams meet up again and recombine in the beamsplitter. This phenomenon generates an interferogram. The recombined beam passes through the sample and this absorbs all the different wavelengths characteristic of its spectrum, subtracting specific wavelengths from the interferogram. Since the position of the moving mirror and so the path lengths varying in time, also the interference between radiations and the sample trasmittance are doing that. The interferogram reports in this way the transmittance of the sample in function of time and using a superimposed laser beam it is possible to provide a reference for the equipment. Since it is difficult to interpret this kind of spectrum, the Fourier transform, a mathematical function, is applied to convert the variation of transmittance in time into the variation of transmittance in wavenumbers which are reciprocals of the wavelengths. The resulting spectrum is an intensity-vs.-frequency type and allows the interpretation of the functional groups present in the sample.
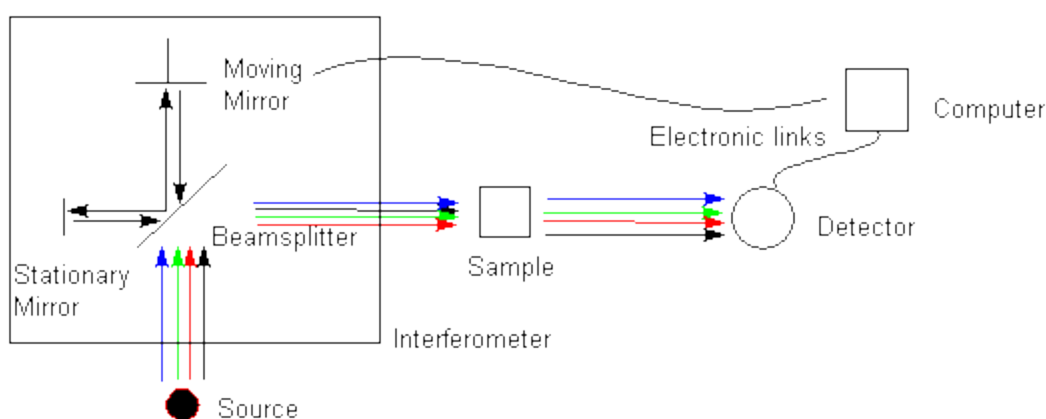
Figure 5.5. *Fuorier-transform spectrometer scheme.*

To study the sample surface, the Attenuated Total Reflection (ATR) is used in conjunction with the infrared spectroscopy. The ATR accessory measures the changes that occur in the IR beam when encounters the sample. The operation of this method is connected to the reflected light ability to penetrate in the sample surface for 1-5 μm. The machine used was UATR Accessory for Spectrum Two with Diamond/ZnSe (1 Reflection) as the high refractive index crystal. During the experiment the solid sample was placed directly in contact with the ATR crystal, through which the radiation is sent. The internal reflectance of the crystal creates an evanescent wave that covers beyond the crystal surface to the sample. The result is an IR spectrum. In the regions in which the sample absorbs energy, the

evanescent wave is attenuated. The beams are attenuated and return to the crystal before exiting the opposite end and meeting the detector in the IR spectrometer. This detector records the attenuated IR beams like interferogram signals that generate the IR spectrum. Figure 5.6. shows a scheme of the ATR-FTIR methodology:
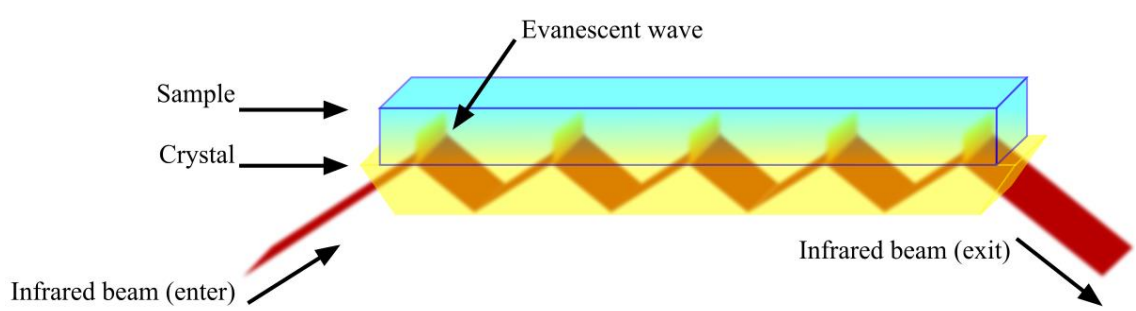
Figure 5.6. *Schematic representation of ATR-FTIR equipment. The infrared beam enters under multiple internal reflections in the crystal. The solid sample (light blue) is in contact with the crystal.*

The resulting spectrum can give information about the bonds and the number of molecules found in the sample.

5.3.2. Two-Dimensional Nuclear Magnetic Resonance (2D-NMR)

The 2D-NMR was operated to investigate the composition of the raw lignin. This technique permits an efficient in-situ analysis of lignin avoiding the isolation step. As it was reported in the previous chapters, lignin is synthesized mainly from three main alcohols: p-coumaryl, coniferyl and synapyl alcohols, that differ on their degree of methoxylation. Each of these monoligols gives a different unit of lignin type, H (p-hydroxyphenyl), G (guaiacyl) and S (syringyl) units respectively. It is known that softwood lignin contains almost only G-type lignin, while hardwood lignin has both G and S types and only minimally H type. A complex polymeric structure is then formed through a combinatorial fashion by free radical coupling mechanism generating a variety of structures and linkages.

The major structural features of lignin can be characterized by this methods that provides also information about the hemicellulose polysaccharides. In this work the Kraft lignin ($\sim$60 mg) were placed in NMR tubes with 800 μL DMSO-d_6. The samples were sealed and sonicated. The samples appeared to fully dissolve under these conditions. The two-dimensional (2D) $^{13}C–^{1}H$ heteronuclear single quantum coherence (HSQC) nuclear magnetic resonance (NMR) spectra were acquired at 398 K using a Bruker Avance-700 MHz instrument equipped with a 5 mm inverse gradient $^{1}H/^{13}C$ cryoprobe using the q_hsqcetgp pulse program (ns = 64, ds = 16, number of increments = 256, d1 =5.0 s) [89]. Chemical shifts were referenced to the central DMSO peak ($\delta C/\delta H$ 39.5/2.5 ppm). Assignment of the

HSQC (heteronuclear single quantum correlation) spectra is described by Rencoeret et al. [90] and Table 5.5 reports lignin ^{13}C-^{1}H correlation signals of *E. globulus wood.*

Table 5.5. *Assignments of lignin 13C-1H correlations in the HSQC spectra of E. globulus wood at different growth stages and their isolated MWLs.*

Labels	δ_C/δ_H	Assignment
	ppm	
C_β	53.5/3.46	C_β-H_β in phenylcoumaran substructures (C)
B_β	53.5/3.06	C_β-H_β in resinol substructures (B)
E_β	55.0/2.75	C_β-H_β in β-1′ substructures (E)
-OMe	55.6/3.73	C-H in methoxyls
A_γ	59.4/3.40 and 3.72	$C_\gamma H_\gamma$ in β-O-4′ substructures (A)
D_β	59.6/2.75	C_β-H_β in spirodienone substructures (D)
I_γ	61.3/4.09	C_γ-H_γ in cinnamyl (sinapyl/coniferyl) alcohol end groups (I)
C_γ	62.5/3.72	C_γ-H_γ in phenylcoumaran substructures (C)
B_γ	71.0/3.83 and 4.19	C_γ-H_γ in resinol substructures (B)
A_α	71.7/4.86	C_α-H_α in β-O-4′ substructures (A)
$D_{\beta'}$	79.3/4.11	$C_{\beta'}$-$H_{\beta'}$ in spirodienone substructures (D)
D_α	81.2/5.09	$C_\alpha H_\alpha$ in spirodienone substructures (D)
$A_{\beta(G)}$	83.5/4.28	C_β-H_β in β-O-4′ linked to a G unit (A)
F_β	83.8/5.23	C_β-H_β in oxidized (C_α=O) β-O-4′ substructures (F)
B_α	84.8/4.67	C_α-H_α in resinol substructures (B)
$D_{\alpha'}$	84.8/4.75	$C_{\alpha'} H_{\alpha'}$ in spirodienone substructures (D)
$A_{\beta(S)}$	85.8/4.11	C_β-H_β in β-O-4′ linked to a S unit (A)
C_α	86.8/5.46	C_α-H_α in phenylcoumaran substructures (C)
$S_{2,6}$	103.8/6.69	$C_{2,6}$-$H_{2,6}$ in etherified syringyl units (S)
$S'_{2,6}$	106.6/7.32 and 7.19	$C_{2,6}$-$H_{2,6}$ in oxidized (C_α=O) phenolic syringyl units (S')
G_2	110.9/6.99	C_2-H_2 in guaiacyl units (G)
$D_{2'}$	113.2/6.27	$C_{2'} H_{2'}$ in spirodienone substructures (D)
$H_{3,5}$	114.9/6.74	$C_{3,5}$-$H_{3,5}$ in p-hydroxyphenyl units (H)
G_5/G_6	114.9/6.72 and 6.94; 118.7/6.77	C_5-H_5 and C_6-H_6 in guaiacyl units (G)
$D_{6'}$	118.9/6.09	$C_{6'} H_{6'}$ in spirodienone substructures (D)
$H_{2,6}$	128.0/7.23	$C_{2,6}$-$H_{2,6}$ in p-hydroxyphenyl units (H)

5.3.2.1. NMR and 2D-NMR operating principles

This technology works on the orientation of the atomic nucleus in the presence of an external magnetic field (B_0) thanks to the nuclear spin I. The direction of the orientation can be in 2I+1 ways. In the most common NMR experiments (e.g. ^{1}H, ^{13}C, ^{19}F and ^{31}P) the value of the spin is ½ so there are only two possible states. These two states represent two energy levels separated by an energy gap ΔE. The high energy orientation is called β and is aligned against the field B_0, while the low energy orientation is aligned in the direction of the same field. The energy gap is defined as eq. 5.3:

$$\Delta E = h\gamma B_0/2\pi \tag{5.3}$$

where π is the magnetogyric ratio (a characteristic of a particular nucleus) and B_0 is the magnetic field strength. The two energy states can contain a certain number of nuclei following the Boltzmann distribution (Eq. 5.4):

$$Na/Nß = \exp(-\Delta E/kT) \tag{5.4}$$

and the frequency is given by the Larmor equation (Eq 5.5)

$$\nu = \gamma B_0/2\pi \tag{5.5}$$

The NMR aim is to obtain information about these active nuclei frequency. In fact, different nuclei react in different ways to an electromagnetic field, providing different signals. The main experiments are Carbon-13, Nitrogen-15 and Hydrogen-1. The possibility to distinguish the nuclei and relate the signals to the right position in the molecular structure, is given by the consideration that the real sample doesn't behave in an ideal way (absence of interaction forces). The real particle, in fact, is influenced by the external environment that induces all the protons to give different resonant signals at different frequency even if they have the same magnetic moments. The external electromagnetic field provokes in fact the motion of the electrons around a nucleus, generating a local magnetic field opposite to the stronger applied one. So, the actual resulting field becomes (Eq 5.6):

$$B_{local} = B_0(1 - \sigma) \tag{5.6}$$

where σ is called *shielding coefficient* and represents the modification of the applied field due to the non-ideal environment. In this way the frequency proves to be dependent on the shielding coefficient (Eq. 5.7). In particular it is reduced by the term $(1 - \sigma)$, since the induced field has an opposite sign compared to the applied one.

$$\nu = \gamma B_0(1 - \sigma)/2\pi \tag{5.7}$$

Thanks to this phenomenon the position of the absorption peaks is different relating to the particular chemical configuration of the atom. Observing the spectrum is then possible to make considerations about the electronic shield of the different nuclei that have generated the peaks.

To permit a universal comparison of the results, it was necessary to create a common standard. The data resulted from the NMR are in fact related to the reference standard ones of the Tetramethylsilane (TMS), that is very shielded and resonates at high frequency. The signal of the TMS is unique because of the symmetry of the molecule and so is taken as the zero in the spectrum axes. The other compounds are so referred to this, creating the *chemical shift*.

In addition, most compounds studied by ^{1}H NMR experiment absorb downfield of the TMS signal, avoiding interferences between the sample and the standard. To calculate the chemical shift regardless of the applied field, the *relative chemical shifting δ* is used [ppm]. Based on the chemical shift it is possible to discern, in the ^{1}H experiment for example, which function group belongs to each hydrogen and, depending on the signal type (single or multiple), if the hydrogen is surrounded by others H or is bounded with on other atom only.

The area under the spectrum peak is furthermore proportional to the absorbance intensity so gives important information about the number of active nuclei for the same signal. However, the peaks number is always higher that the effective nuclei number because of the *spin-spin coupling*.

While the conventional NMR spectra are plots of intensity vs. frequency; the 2D NMR spectra show intensity plotted as a function of two frequencies. The 2D NMR experiment employed in this work was HSQC. This method detects the correlations between two different nuclei types separated by one bond. The spectrum contains one peak per pair of coupled nuclei, whose two coordinates are the chemical shift of the two coupled atoms.

5.3.3. Gel permeation chromatography (GPC)

The instrument is composed by 3 columns, 4 detectors and a pump. 20 ul of sample were eluted with THF and injected with a flowrate of 1 ml/min. The temperature was set at 23°C. The delay volume was null and the acquisition interval 1 second.

Before the submission to the GPC the lignin was acetylated to assure the dissolution into the THF. The acetylation of 100 mg of grounded lignin was done with pyridine. The sample was afterwards sonicated to improve the dissolution, washed with ethanol three times and dried with nitrogen flow. 1 ml of THF was then added and the solution was filtrated to avoid solid particles.

5.3.3.1. GPC operating principles

The GPC technique has been used to provide the molecular weight of the lignin. This method permits the separation of the molecules of the sample depending on the size of the molecular chain. The sample solution is eluted in one or more columns containing a porous packing formed by small polymeric particles. If the sample particles have larger hydrodynamic diameter than pores one, they cross the column faster than the other particles. Indeed, the smaller the particles, the longer they remain inside the column pores, creating delay.

The GPC instrument is composed by the injection/pump system for the solvent, the separation column and the detector. The injection system and the pump need to guarantee, respectively, a constant solvent flow and a high pressure for the solvent elution. The more common measured parameter is the elution time.

The packing inside the column (or columns) is mainly made by high porosity rigid or semi-rigid spheres. The material is different if the solvent system is an aqueous or an organic liquid.

The efficiency of every column is connected to a strict molecular mass, so it is convenient to use more columns or a long one, with a high porosity distribution, to cover a larger range of molecular weights. The more important characteristic of the solvent is to dissolve completely the sample, avoiding secondary separation processes. Common solvents are:

- Tetrahydrofuran (THF). This is convenient if the polymers are soluble at ambient temperature;
- Dichlorobenzene or trichlorobenzene for polyolefins at 130°C and 150°C;
- 2-chlorophenol for polyamides and polyesters at 90°C.

The detector generally works on the difference of the refraction index between the pure solvent and the eluted solution. In this way it permits to build a chromatogram, describing the variation of the concentration as a function of the volume or the retention time.

A relation between the retention volume and the molecular weight is needed to obtain a molecular weight distribution. To provide that a calibration of the instrument is necessary. Samples with known molecular weight and small molecular weights distribution are ideals for that. The commercial standard ones are in polystyrene base with MW from 2.9 million to 3000 u.m.a..

5.3.4. Elemental analysis CHN

This analytical technique is used to know the elemental composition of a wide range of samples (organics, inorganics, oils and volatiles), thanks to a unique combustion technique. It is focused on the determination of carbon, hydrogen and nitrogen (CHN) and is carried out on an Exeter Analytical Inc. CE-440 analyzer. The high accuracy and precision of the instrument allows to reach consistently high-quality results and the experimental time takes typically less than 5 minutes.

5.3.4.1. CHN operating principles

The CE-440 measures the sample composition through the thermal conductivity detection, after combustion and reduction. The combustion products are also treated in the combustion tube with suitable reagents to complete the oxidation and remove undesirable byproducts. The oxides of nitrogen are converted in molecular nitrogen and the residual oxygen is removed. The final gasses are mixed to avoid heterogeneity and precise volume, temperature and pressure are set. This final mixture passes then into the conductivity detector. This is formed by three pairs of conductivity cells. The first one removes water from the gas with an absorption trap. Thanks to the reading of the water change it is possible to know the amount of hydrogen in the original sample. The second cell has a trap for the carbon dioxide removal and allows to determinate the carbon content. After the hydrogen and carbon subtraction, only helium and nitrogen are present. The remaining gas passes through the third thermal conductivity cell. The comparison between the output signal and a reference cell, through which pure helium flows, gives the nitrogen concentration. The analysis of the oxygen amount is then conducted by difference.

5.3.5. Results of lignin characterisation

In this section the results obtained from the previously described analyses are shown.

5.3.5.1. ATR-FTIR analysis

The ATR-FTIR analysis was conducted to identify better the raw lignin used in this work. The main asymmetric absorption bands are about 20 and they are typical for high molecular compounds with an irregular structure, like lignin [91]. The comparison between the typical bands for the common lignin structure (Table 5.4.) and the obtained spectrum (Fig. 5.7) allows the identification of the lignin composition in a qualitatively way.

Absorption bands caused by $C-H_n$ symmetrical stretching vibrations are located in the 2922-2844 cm^{-1} range. Up to 1706 cm^{-1}, there are no absorption bands while the region at lower wavenumbers is characterized by the presence of the carbonyl group stretching vibration. The peak at 1596 cm^{-1} is allocated to symmetric aryl stretching. The bands around the wavenumber 1510 cm^{-1} are assigned to the C=C aromatic symmetric stretching that can belong to the aromatic rings in the lignin structure. The absorption band at 1452 cm^{-1} can be related to H-C-H, O-C-H in-plane bending. The band at 1265 cm^{-1} regards G ring stretching (guaiacyl units) and the one at 1205 cm^{-1} represents C-O-C asymmetrical stretching and OH plane deformation. As regarding bands at 1126 cm^{-1} and 1031 cm^{-1}, the related bonds groups are C-C, C-OH and CH ring and side group vibrations.

Figure 5.7 shows the entire spectrum in which the main bands are underlined.
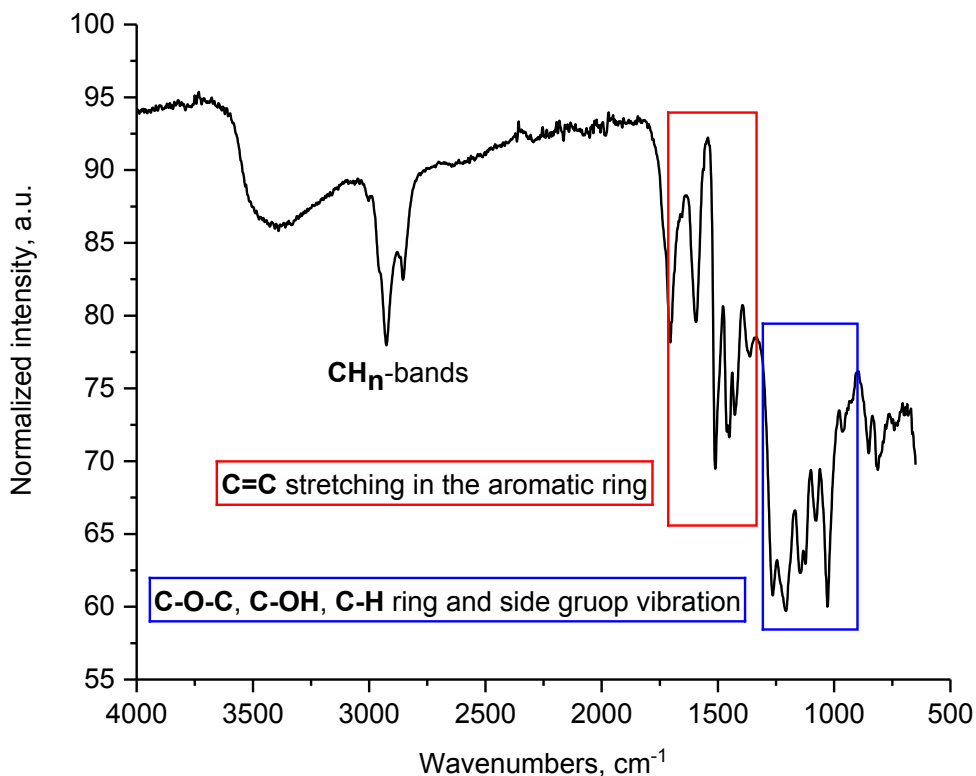
Figure 5.7. *ATR-FTIR spectrum of raw lignin (normalized intensity, a.u - wavenumbers, cm^{-1}). The main bands are highlighted.*

5.3.5.2. 2D-NMR analysis

The 2D-NMR analysis conducting with the HSQC, ^{13}C-^{1}H, experiment, allows to correlate the carbons of the lignin structure with their attached protons. The spectrum can be seen in Figure 5.8, here the x-axis is proton [ppm] and the y-axis is carbon [ppm]. In the following Fig. 5.9 and Fig 5.10 are shown the structures of the lignin unit types H (p-hydroxyphenyl) and G (guaiacyl) and of the main found bonds. Since the lignin is softwood-type it is expected the absence of the S (syringyl) unit (see § 5.3.2).

The characteristic lignin bonds are classified as:

- A (cyan) represents β-O-4 units;
- B (green) represents β-5 units;
- C (pink) represents β-β units.

The same colours were utilized to distinguish the bonds and the units in the 2D-NMR spectrum. The orange spots indicate therefore the double C=C bonds and the C-C bonds

(wax) and the yellow one the methoxyl group (OMe). The solvent used was the DMSO that is marked in the broad blue spot.
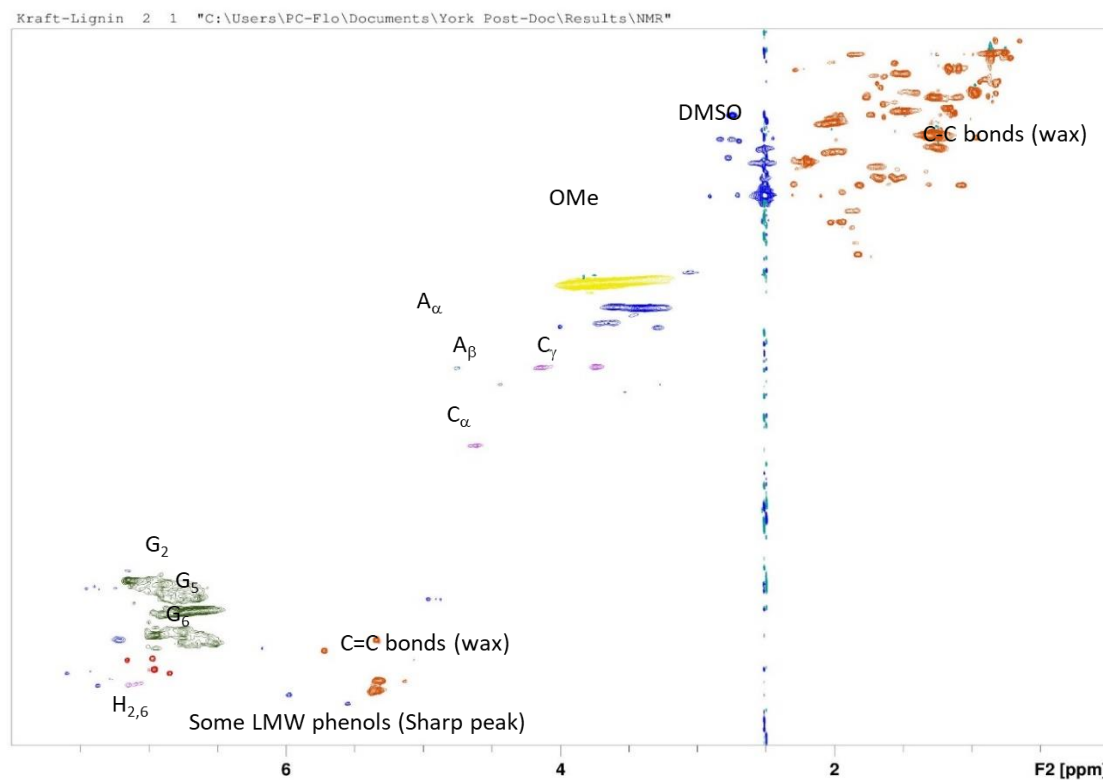
Figure 5.8. *2D-NMR spectrum of the raw lignin.*
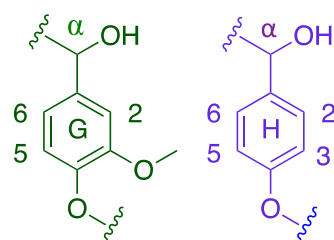
Figure 5.9. *G and H unit types that composed lignin.*
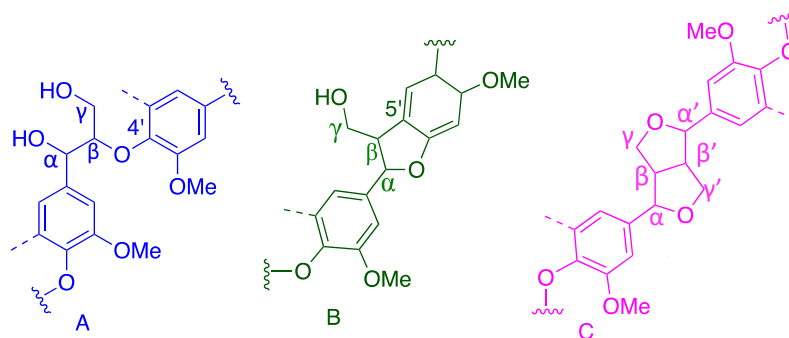
Figure 5.10. *Typical lignin bonds. A is related to β-O-4 bonds, B to β-5 bonds and C to β-β bonds.*

Three regions are noticed in the HSQC spectrum of the raw lignin, which are the aliphatic (δ_C/δ_H 10-50/0.5-2.5 ppm), side chain (δ_C/δ_H 50-95/2.5-6) and aromatic (δ_C/δ_H 95-130/5.5-8) ^{13}C-^1H correlations regions.

The more intense signals are related to the high amount of wax contained in the aliphatic region and some sharp and small peaks confirm the presence of low molecular weight phenols in the aromatic region (purple). The abundance of wax underlines the presence of oleates and fatty acid in the raw material.

The aromatic lignin region displays also all the C-H correlations found in the various G units (green) and it is evident that the main lignin side chain units are resolved, like the β-aryl ether (purple) unit A_α and A_β. However, A_γ is probably hidden and phenylcoumaran correlations (B) are too. The A units are anyway present at lower contour levels due probably to their lower abundance. From the other lignin unit, resinol (C), is possible to note only the C_γ and the C_α correlations.

5.3.5.3. GPC analysis

The molecular mass distribution can be seen in the following Figure 5.11. The chromatogram shows also the size separation. The molecular weights are present in the 100-100.000 range.
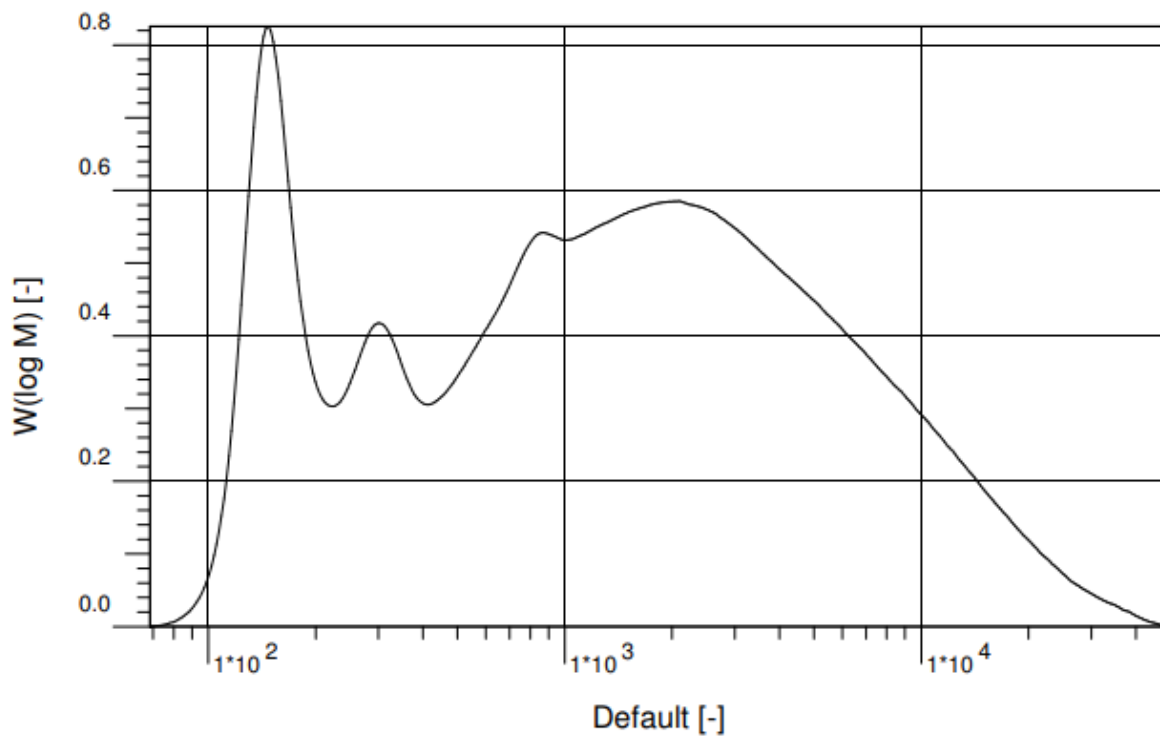
Figure 5.11. *Molecular weight distribution of the raw lignin sample.*

Since the lignin has no uniform chain lengths, the characterization of a specific molecular weight is precluded. Thus, it is needed to describe the sample in terms of average molecular weight. The most common ones are number molecular weight (M_n) and weight average molecular weight (M_w) (Eq 5.8 and 5.9). Eq 5.10 gives the polydispersity (D) that is a representation of the molecular weight distribution of the lignin.

$$M_n = \frac{\sum N_i M_i}{\sum N_i} \tag{5.8}$$

$$M_w = \frac{\sum N_i M_i^2}{\sum N_i M_i} \tag{5.9}$$

$$D = \frac{M_w}{M_n} \tag{5.10}$$

Where the index number *i* is the number of different molecular weight in the sample and N_i is the total number of moles related to the mass M_i.

In Table 5.6 the values of the two average and polydispersity are reported.

Table 5.6 *Values of molecular weight averages and polydispersity for the raw lignin sample.*

	Value	Uncert. [%]	Unit of measurement
M_n	$5.2633 \cdot 10^2$	15	g/mol
M_w	$3.2078 \cdot 10^3$	15	g/mol
D	6.0947	22	-

The molecular weights appear to be not very high. This depends also on the Kraft pulping process that generally causes the lignin fragmentation and depolymerization. During the process in fact the wood chips are mixed with an aqueous solution of sodium hydroxide and sodium sulphide that enhances the cleavage of select bonds.

In literature the molecular weights have been found within the range of 200 to 200.000 g/mol. However, the variation of those values can be very large since they are affected by wood type, analysis method and isolation procedure [92].

5.3.5.4. CHN analysis

As showed in Table 5.7 and in Table 5.8, the two samples submitted to the CHN analysis, reported an abundant amount of carbon (70%), oxygen (25%) and hydrogen (5%). These results follow the lignin structure found by the ATR-FTIR and the 2D-NMR experiments.

Table 5.7. *Elemental analysis data of raw lignin, sample 1.*

Element	%C	%H	%N	%O_2
Observed 1	67.71	6.66	0.20	25.42
Observed 2	67.71	6.74	0.19	25.36
Mean	67.709	6.701	0.199	-
Calc (theory)	70	5	-	25

Table 5.8. *Elemental analysis data of raw lignin, sample 2.*

Element	%C	%H	%N	%O_2
Observed 1	65.73	5.87	0.07	28.33
Observed 2	65.38	5.82	0.06	28.74
Mean	65.552	5.848	0.064	-
Calc (theory)	70	5	-	25

5.4. Methods for phenolic compounds extraction

5.4.1. Extraction after MW heating

After the microwave treatment the phenolic compounds were extracted from the pyrolyzed lignin or lignin-solvent system washing the sample with different solvents (distilled water, ethanol, heptane ad sulfolane). 5 ml of solvent were added to the pyrolyzed lignin and 4 ml of solvent to the solvolyzed one. Subsequently, the sample was inserted inside the centrifuge for 10 minutes at 2500 rpm to separate the liquid phase (the extraction solution) from the solid phase (the char). After a decantation step of several minutes (10-15 min) the extraction solution was filtered with RC 0.2μm Agilent filter (Figure 5.12) and then submitted to GC-MS and GC-FID.

Figure 5.12. *Filter used for sample preparation.*

5.4.2. Soxhlet extraction

The setup of the experiment includes the Soxhlet extractor, an electric heating mantle, the water condenser and the flash evaporator. The Soxhlet extractor scheme is reported in Figure 5.13.
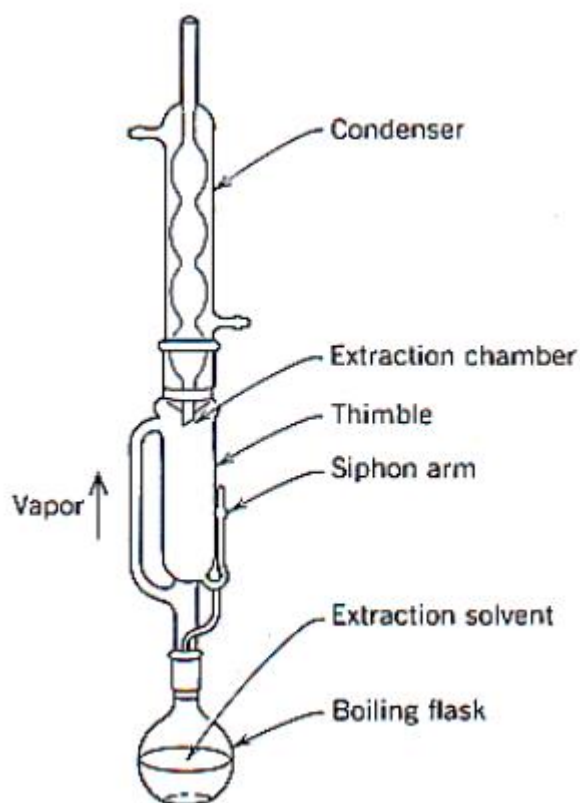
Figure 5.13. *Components of Soxhlet extractor.*

The extraction is a continuous solid/liquid extraction. The solid sample (milled lignin in this case) is allocated inside the thimble, made with a material that allows also the passage of the liquid and the contact between the two phases. The thimble is then placed in the Soxhlet extraction. In the boiling flask the extraction solvent is heated using a hot plate. When the liquid evaporates, its vapor rises up and is condensed by the water condenser on the top. The condensed solvent falls down inside the thimble and fills all the chamber until it is siphoned back down to the solvent flask. This operation takes place many times until the solvent is rich of the desired extracted compounds.

The extraction solvent was ethanol, capable to remove oleate compounds from the lignin as a purification step. The extraction took place for two days.

5.5. Methods for phenolic compounds quantification and identification

5.5.1. Identification of phenolic compounds using Gas chromatography mass spectrometry (GC-MS)

Every sample prepared in this work was analysed in the GC-MS to identify the constituent compounds. To gather the mass spectral data, a Perkin-Elmer Clarus 500 gas chromatograph coupled to a Clarus 560-S EI mass spectrometry (MS) was used. The column was a Rxi-5HT by Restek Restek with a diphenyldimethyl polysiloxane as the stationary phase. The internal diameter of the column is 0.25 mm thick, the height 30 m and the film thickness 0.25 m. A comparison of experimental data to NIST library data is made to identify the products. The software of the GC-MS produces a 'fit score' out of 1000 base on the match of the experimental to library data. This fit score represents how likely it is that the experimental data is from a pure sample of the library compound. The operating method was General 1. The GC-MS was autosampler.

5.5.1.1. GC-MS operating principles

This analysis method is very suitable for smaller and volatile molecules like benzenes, alcohol and aromatics, and simple molecules such as steroids, fatty acids and hormones. The samples could be run in liquid, gaseous or solid phase. The main abilities of the GC-MS are to separate very well complex mixtures, quantify analytes and determine trace levels of organic contamination. The sample is firstly volatized in the gas chromatograph that let also the chemical separation using a capillary column packed with a stationary (solid) phase. Then the compounds are forced by an inert carrier gas, generally argon, nitrogen or helium to flow in the column. The separated compounds elute from the column at different times, which is usually referred to as their retention times. After leaving the GC column, the components are ionized by the mass spectrometer with electron or chemical ionization sources. At this point the ionized molecules are accelerated through the instrument's mass analyzer (a quadrupole or an ion trap) and separated here based on their different mass-to-charge ratios *(m/z)*.

The ions are so detected and analyzed depending on the compound peak appearing as a function of their *m/z* ratios. The quantity of the chemicals can be calculated proportionally to their correspondent peak heights or area. If a sample is composed by different compounds, several peaks result from the analysis, forming a mass spectrum that gives the signal over the time. The identification of unknown compounds is therefore possible thanks to computer libraries of mass spectra for different chemicals. A general GC-MS system is illustrated in figure 5.14.
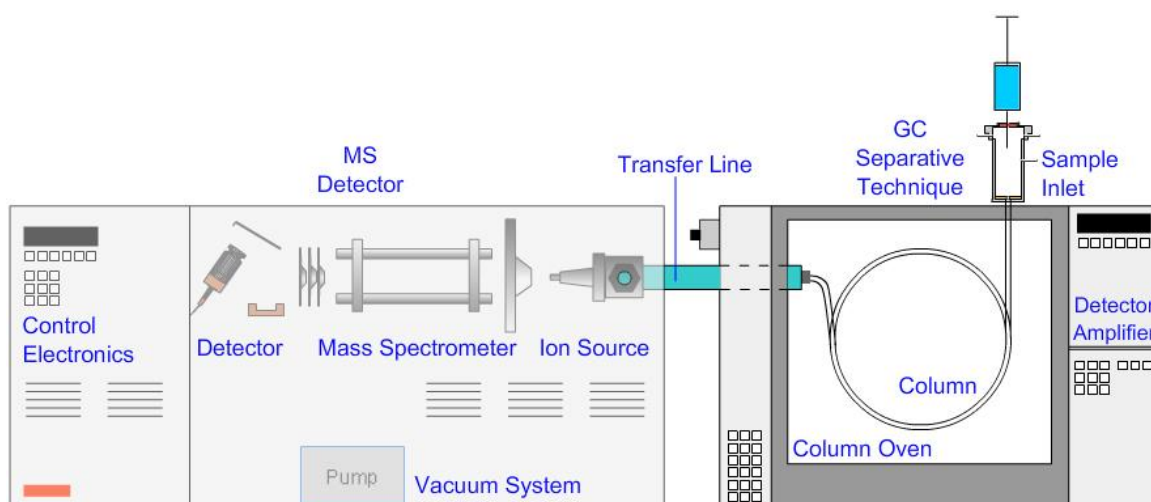
Figure 5.14. *General scheme of GC-MS.*

5.5.2. Quantification of phenolic compounds using Gas Chromatography with Flame Ionization Detector (GC-FID)

The phenolic compounds present in the extraction sample were quantified based on the data obtained from the GC-FID spectrum. This analysis was conducted with an Agilent 6890N GC-FID with a Restek Rxi-5HT fused silica column. The dimensions of the column are 30 m × 0.25 mm × 0.25 μm and the stationary phase was made with diphenyldimethyl polysiloxane. The temperature ramped in the column was from 50°C to 300°C at 30°C per minute with a hold for 5 minutes at 300°C. The column flow was 2.0 mL per minute helium and the detector held at 340°C. The operating method was General 1. The GC-FID was autosampler.

5.5.2.1. GC-FID Operating principles

After the evaporation of the sample in the GC injection point, the compounds are pushed by the carrier gas to move through the column. This column is generally tubular and packed with the stationary phase coated on the capillary walls. If the GC is coupled with a Flame Ionization Detector, after the column, the samples are burned in a hot, hydrogen-air flame. Indeed, FID is a destructive detector since the sample is pyrolyzed and is unaffected by noncombustible gases and water. The combustion produces carbon ions that are detected by electrodes as a current.

The current produced is proportional to the amount of the burned sample and is sensed by an electrometer and sent to an output device after the conversion to the digital form. The total efficiency of the process is slow since only 1 in 10^5 carbon ions produces an ion in the

flame, but the total amount of ions is directly proportional to the amount of carbon in the sample. Because the FID is mass sensitive, the signal amount is proportional to the mass of carbon in the sample so the compounds with more carbon give greater signals. This detector is one of the most sensitive, with only a limit in the picogram range and its response is linear over seven orders of magnitude. Figure 5.15 reports the general scheme of the GC-FID.
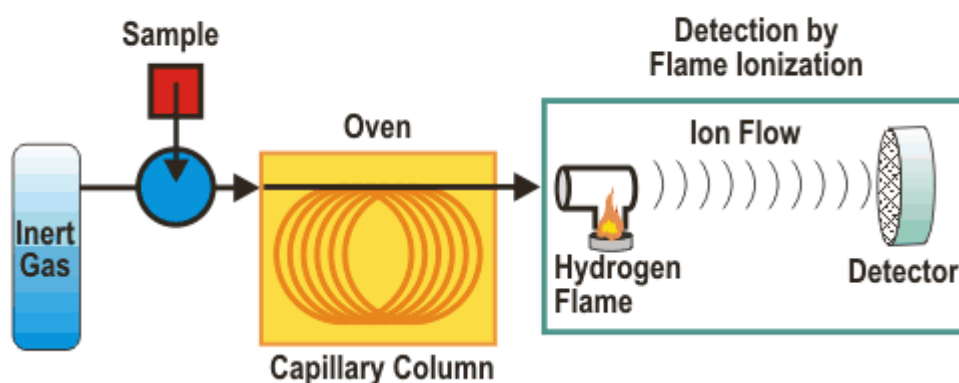
Figure 5.15. *General scheme of GC-FID.*

5.5.2.2. Yield and Selectivity quantification

The GC-FID report was used to calculate the yield of the phenolic compounds and to obtain the selectivity value.

After the identification of the three main phenolic compounds in the samples (Guaiacol, Creosol and 4-Ethyl Guaiacol) with the GC-MS, the references of them were run at different concentration, with the same method, in the GC-FID to build a calibration curve. For the guaiacol, 3-methoxyphenol was run as reference. The calibration graph reports the area of the spectrum in the y-axis and the concentration of the chemical in the x-axis (Figure 5.16-5.18). In the graphs the calibration straight line and the R-squared value are reported. Thanks to equations 5.8-5.10 it is possible to correlate the area under the curve in the GC-FID spectrum to the concentration of the same compound in the sample and obtain the yield by calculating the slope (cot α) of the calibration line:

$$\cot(\alpha) = C_{ref}/A_{ref} \tag{5.11}$$
$$C_{chemical} = A_{chemical} \cdot \cot(\alpha) \tag{5.12}$$
$$Yield = C_{chemical} \cdot V_s/m_{lig} \tag{5.13}$$

where C_{ref} and A_{ref} are respectively the concentration and the area of the reference compound, $C_{chemical}$ and $A_{chemical}$ the concentration and the area of the chemical in the sample, V_s is the volume of the solvent used to extract the phenolic compounds and m_{lig} is

the mass of the lignin sample before the run. The references were dissolved in 1 ml of solvent (acetone).

3-methoxyphenol

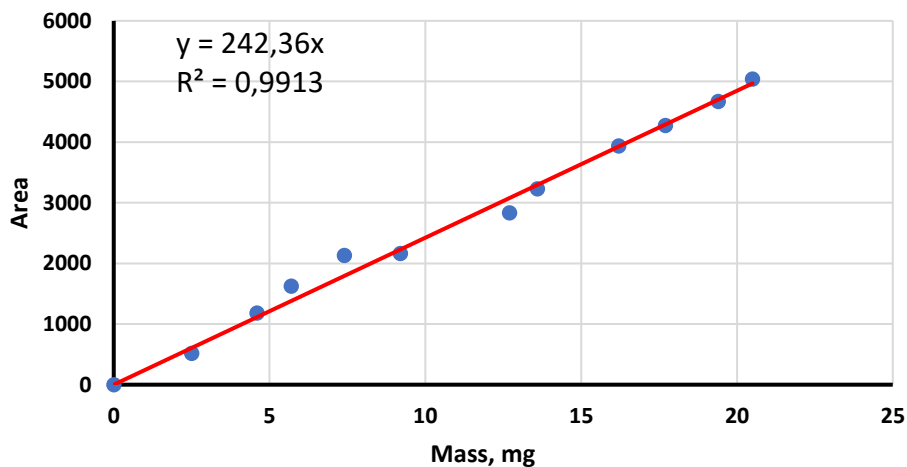
Figure 5.16. *Calibration curve for 3-methoxyphenol. The interpolation straight line and the R-squared value are reported.*
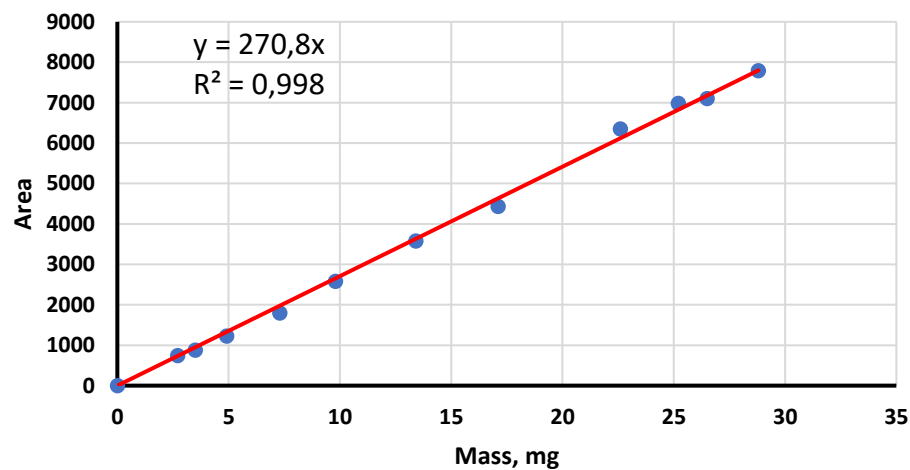
2-methoxy-4-methylphenol

Figure 5.17. *Calibration curve for 2-methoxy-4-methylphenol. The interpolation straight line and the R-squared value are reported.*

4-ethyl-2-methoxyphenol

$y = 272{,}42x$
$R^2 = 0{,}9858$
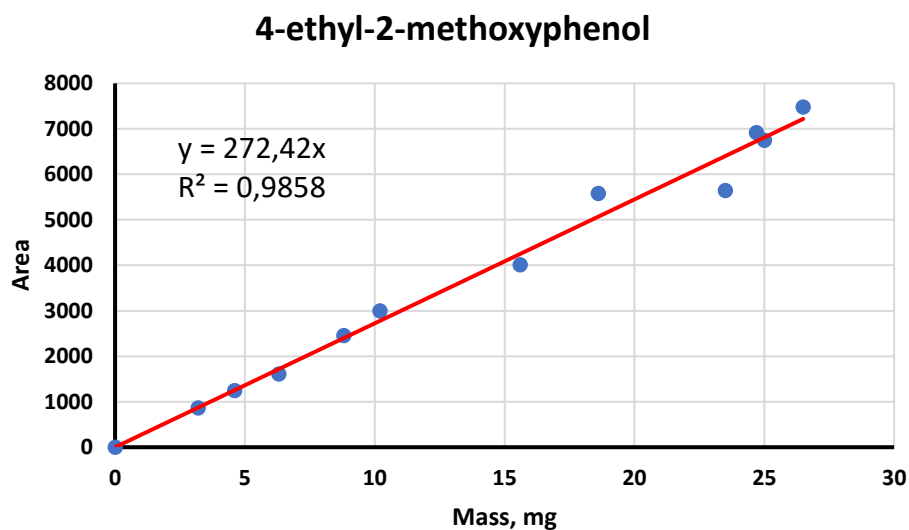
Figure 5.18. *Calibration curve for 4-ethyl-2-methoxyphenol. The interpolation straight line and the R-squared value are reported.*

The value of areas for each compound is issued by the GC-FID report as well as the selectivity value (% of compound amount in the total, excluding the solvent peak).

5.5.3. Thin layer chromatography (TLC) method for phenolic compounds separation

After the qualitative and quantitative analysis of the phenolic compounds, a separation system was proposed using the Thin Layer Chromatography technique (TLC).

This method is quite quick and inexpensive, and its principle is based on the separation of two or more compounds between two phases, one of which is moving and the other is stationary. In the TLC the phase system is solid-liquid. The stationary phase is normally a polar absorbent instead the moving phase is composed by one or a combination of solvents. The absorbent material of the stationary phase is generally coated on a glass slide or plastic sheet, creating a thin layer. After the experiment, it is possible to identify the number of the compounds and their identity and to setup the condition for the proper separation using a chromatography column.

The stationary phase used was a TLC silica gel plate and the moving phase, called eluent, a mixture of different solvents found after several tests. After finding the right eluent system, it is indeed possible to operate the separation of the compounds. The sample was added to one end of the sheet and dipped into the liquid phase for several minutes. Thanks to the capillary action, the solvent is drawn through the plate, so the molecules are shared between

the mobile and the stationary phase depending on their molecular structures and interactions with the plate. The partition coefficient, k, is the equilibrium constant for the molecules distribution and states the degree of separation of the sample components. The driving force of the chemicals separation lies in the equilibrium system that is related to the degree of adhesion to the plate. The equilibrium between the free and the absorbed states of molecules depends on:

- The polarity and size of molecules, determined by their structure;
- The polarity of the stationary plates;
- The polarity of the solvents.

In this way the equilibrium, and so the molecular repartition, can be influenced by varying the stationary and the mobile phase. The TLC plate in fact is usually very polar so the less polar chemicals will stay longer in the liquid phase despite of the more polar ones that will adhere more strongly to the stationary phase. In general, the more polar the functional group in the sample, the more slowly the molecules will move.

To identify the separated components on the TLC plates, it was necessary to develop a visualization method since the phenolic compounds are colourless. The UV lamp was used because the organic compounds contain a chromophore that can be visualized using a short or long wave UV lamp. Under the UV lamp the silica gel fluoresces while the organic compounds appear as dark spots after the UV light absorption. The dark spots were then recognized and circled with a pencil. A Potassium permanganate system was also employed to display the phenols. This stain is very suitable for functional groups sensitive to oxidation. To see in a better way the chemicals a gently heating was done following the TLC plate immersion into the stain. The spots appeared as bright yellow spots on a bright purple background and were circled with a pencil because the TLC could become brown upon standing for a prolonged time period. For the stain preparation the recipe includes the dissolution of 1.5 g of $KMnO_4$, 10 g of K_2CO_3 and 1.25 ml of 10% NaOH in 200 ml of water. The typical lifetime is 3 months.

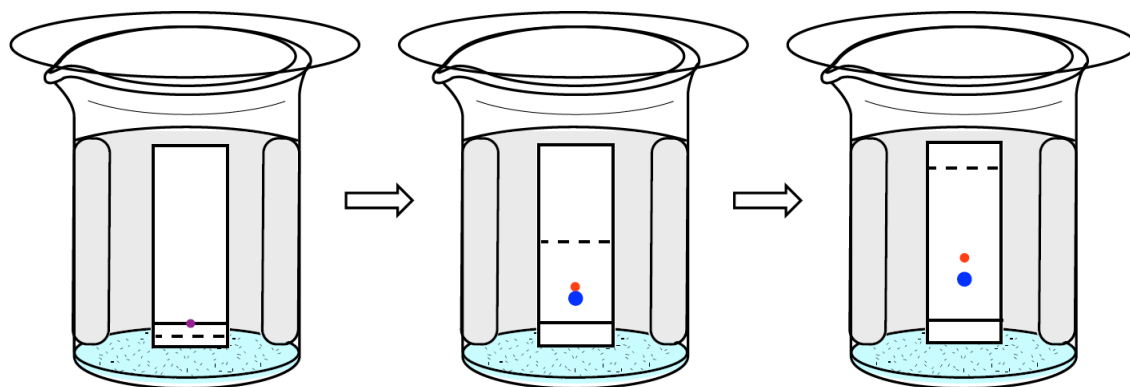Figure 5.19 shows the steps needed to develop the thin layer chromatography.

Figure 5.19. *TLC steps for chemicals separation.*

Chapter 6

Results of lignin activation

The main purpose of this work is the investigation of the potential microwave effects on the lignin activation to improve the extraction of valuable chemicals. For this reason, the main part of the chapter is related to the MW heating and the influence of various parameters in the MW-assisted lignin activation is discussed. The conventional heating effects are subsequently studied to highlight the difference, in phenolic compounds yield and selectivity, between the two heating methods. To follow, results about TLC are reported.

6.1. Microwave-assisted lignin activation

The activation of the raw lignin in the microwave reactor was done following two main reaction typologies: the solvolysis and the pyrolysis. After the extraction of the bio-oil from the MW treated lignin, the samples were submitted to the GC-MS for the identification of the products. Thanks to the library comparison, the phenolic compounds were found, as well as other chemicals like oleyl alcohol or phenanthrene derivatives, in all the experiments but in different amount.

6.1.1. Lignin pyrolysis

A typical resulting GC-MS spectrum (Fig. 6.1) shows the main chemicals obtained from the lignin activation by pyrolysis. It is related to the pyrolysis of 1 g of lignin, using the *Dynamic Power* mode, after the sample retention inside the reactor at 280 °C (the highest temperature value) for 5 minutes.

The main phenolic compounds are: 3-methoxy-phenol (guaiacol), 2-methoxy-methyl-phenol (creosol), 2-methoxy-ethyl-phenol (4-ethylguaiacol), 2-methoxy-4-propyl-phenol (4-propylguaiacol) and 2-methoxy-4-propylen-phenol (4-propylenguaiacol). To simplify the data calculation, the yield of only the first three compounds was calculated after every experiment.

As concern the yield and selectivity calculation, the same sample was then submitted to the GC-FID for a more precise quantification of the compounds concentration. The spectrum from the GC-FID shows similar peaks but slightly shifted, due to the different calibration system of the two chromatographers. The three main peaks are easily distinguishable from

the others as the ones referred to guaiacol, creosol and 4-ethylguaiacol (Figure 6.2) and they are repeated for each sample in the same time position of the GC-FID spectrum, as it happens with GC-MS results. The report from the GC-FID gives the peak area (pA) and the selectivity.

Figure 6.1. *GC-MS spectrum of the bio-oil extracted with ethanol after the lignin pyrolysis at Dynamic Power mode. The reaction was maintained for 5 minutes at 280°C. The main peaks are related to the correspondent chemicals and their chemical structure is showed.*
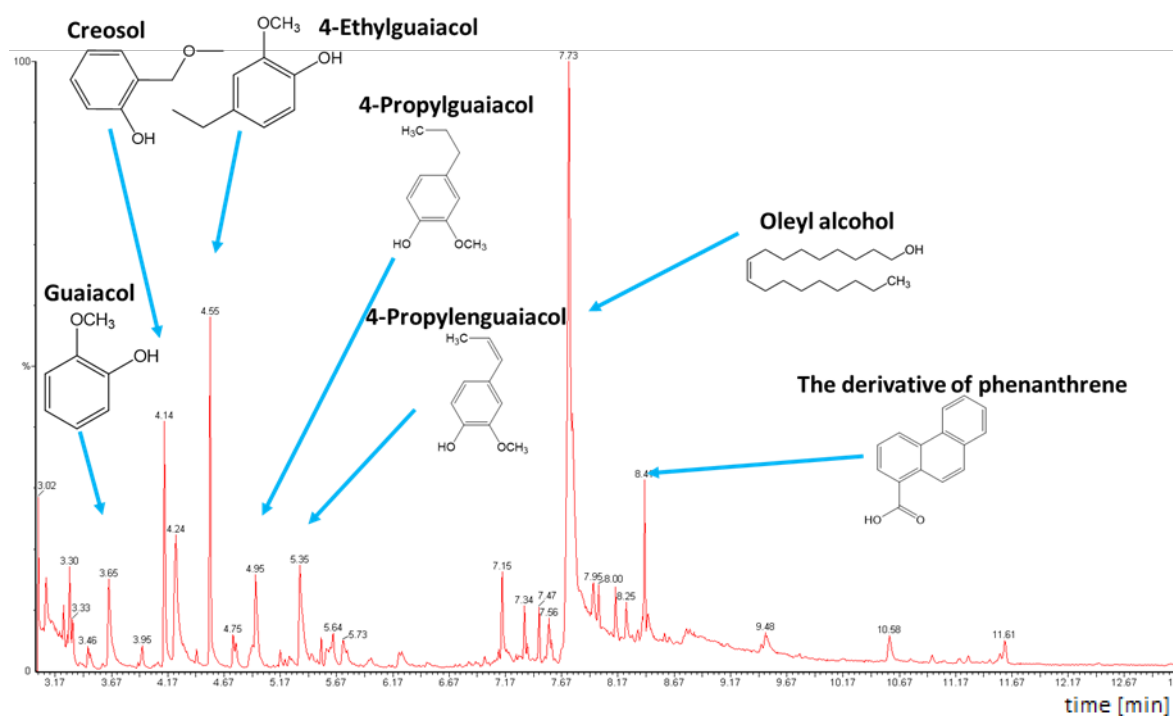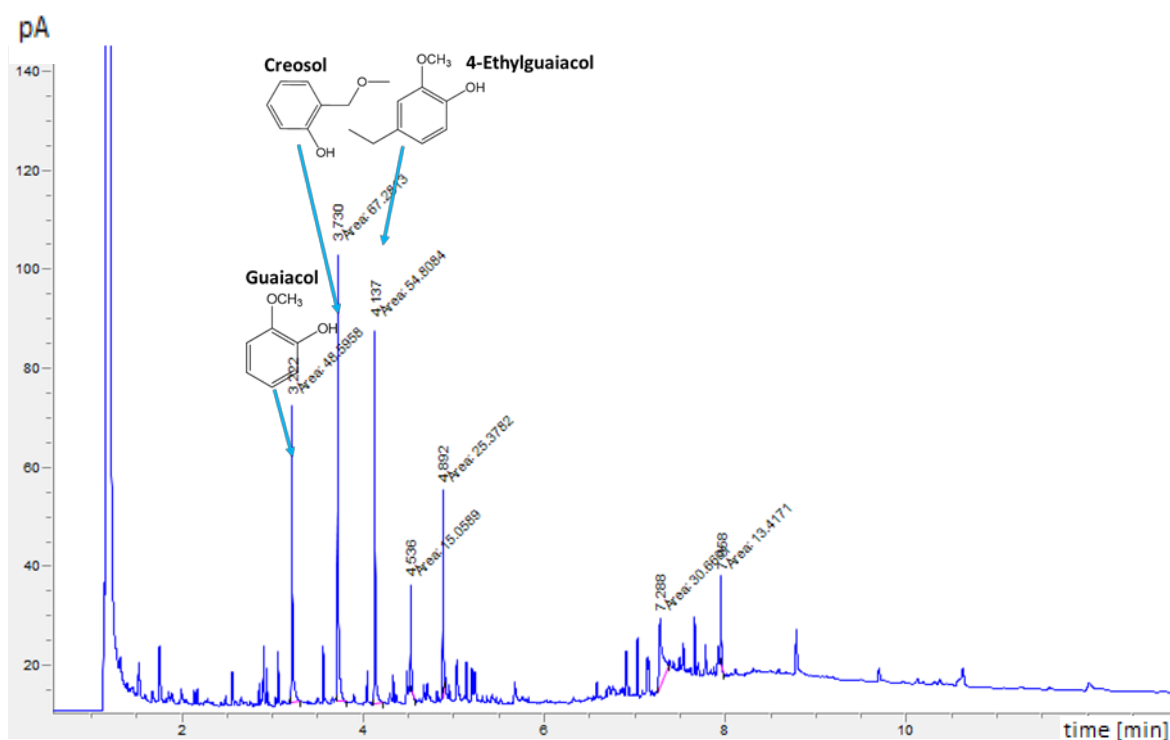
Figure 6.2. *GC-FID spectrum of the bio-oil extracted with ethanol after the lignin pyrolysis at Dynamic Power mode. The reaction was maintained for 5 minutes at 280°C. The peaks of the three main phenolic compounds, guaiacol, creosol and, 4-ethylguaiacol, are underlined.*

6.1.2. Lignin solvolysis

To decide which thermal treatment could have been the best in terms of chemicals extraction, a preliminary test was made comparing the solvolysis process with the pyrolysis one.

The solvolysis of lignin was carried out with water, heptane, ethanol and sulfolane. The yield of guaiacol, creosol and 4-ethylguaiacol is indicated in the graph of Figure 6.3 and their selectivity in Figure 6.4. In both figures it was chosen also to make a comparison between the various solvolysis processes and the pyrolysis of lignin at the same operating conditions (extraction with ethanol). The figures report a comparison between the four different solvents used (sulfolane, ethanol, heptane and water).

The yield of phenolic compounds after the solvolysis process demonstrates to be lower than the one achieved after the pyrolysis process. The pyrolysis process exhibits higher yield, so it was decided to persist with that for the lignin activation, although the value is nonetheless very low. The selectivity values in Figure 6.4. are higher during the water solvolysis than the other experiments. In particular guaiacol is obtained at 33% in the GC-FID spectrum.
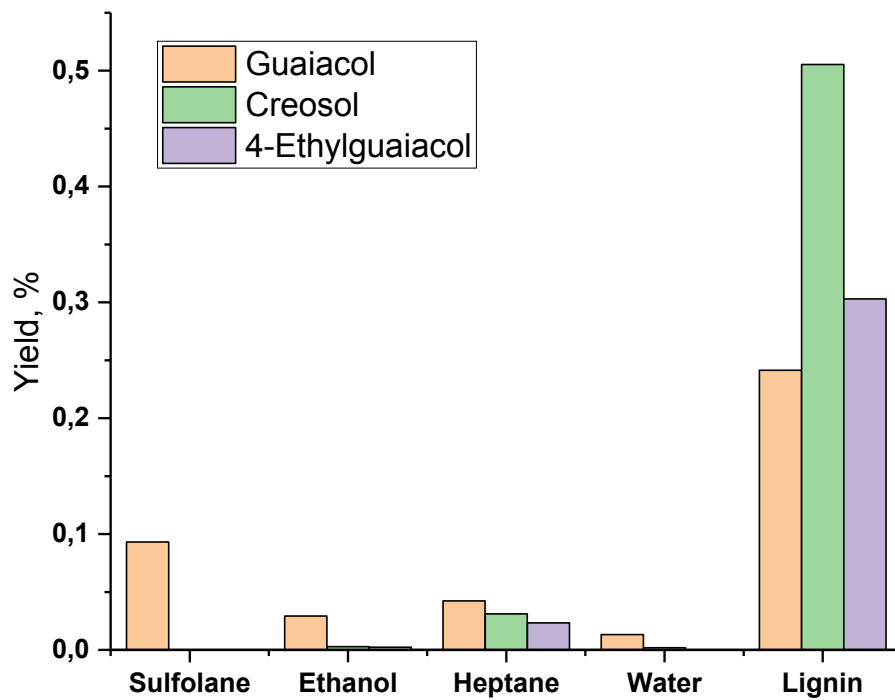
Figure 6.3. *Yield of guaiacol, creosol and 4-ethylguaiacol after the lignin solvolysis process with sulfolane, ethanol, heptane and water and after the extraction with the same solvent used in the MW run. These experiments are compared to the pyrolysis process.*
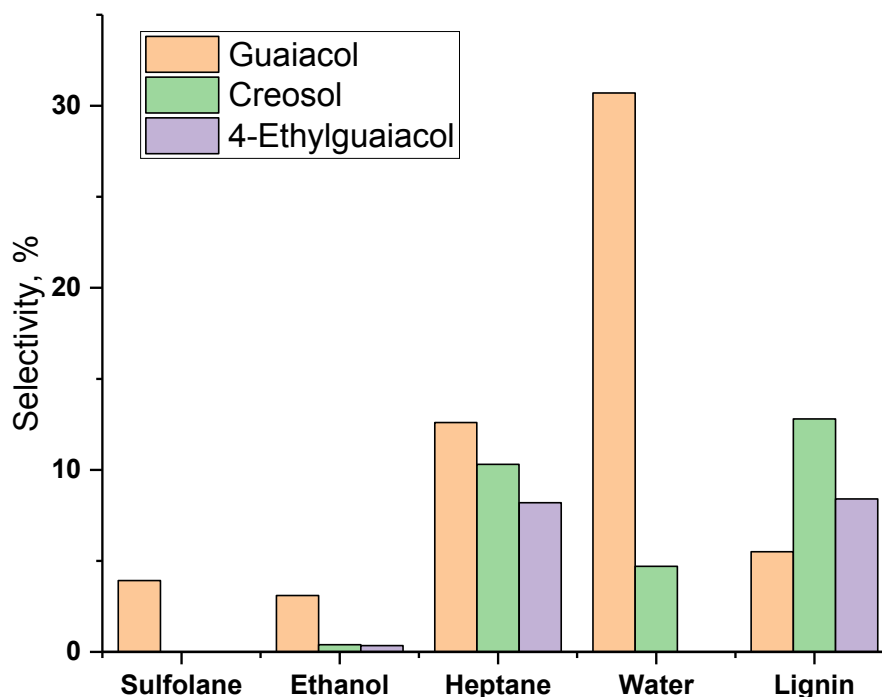
Figure 6.4. *Selectivity of guaiacol, creosol and 4-ethylguaiacol after the lignin solvolysis process with sulfolane, ethanol, heptane and water and after the extraction with the same solvent used in the MW run. These experiments are compared to the pyrolysis process.*

The solvolysis process gave also some operating problem when using ethanol and distilled water as process solvent. With these two solvents, in fact, the highest allowed pressure value (300 psia) was reached before the maximum temperature value (280°C). Running both the solvents themselves, it was noticed the same behavior. This means that the MW-solvent interaction is stronger than the MW-sample interaction, probably due to the high polarity of water and ethanol. The heating in this case happens via simple radiation heating and the MW solvolysis efficiency decreases.

6.1.3. MW power influence in lignin pyrolysis

Looking at the unsatisfactory solvolysis results, it was chosen to go on and try an improvement of the pyrolysis process. First it was changed the value of the microwave power to analyze its effects on phenolic compounds yield and selectivity. Four sample were run twice for each value of MW power – 200 W and 300 W - and the same four solvents, previously cited, were used for the extraction step.

In Figure 6.5 and in Figure 6.6 is reported the bio-oil yield of four runs of lignin pyrolysis at 200 W and 300 W respectively, using the *Fixed power* mode until the achievement of 280°C. The four samples, after collecting them from the microwave reactor, were washed with a different solvent each, sulfolane, ethanol, heptane and water, respectively. The phenolic compounds yield is still very low even if it exhibits a slight improvement increasing the microwave reactor power. The highest yield is recorded for creosol after the lignin pyrolysis at 300 W and the extraction with ethanol.

As it is possible to seen from Figure 6.7 and Figure 6.8, the selectivity isn't very influenced by the microwave power. The best result is given after the extraction step with water that is able to get creosol with a selectivity of almost 36%.

Figure 6.5. *Yield of guaiacol, creosol and 4-ethylguaiacol after the lignin pyrolysis process at 200 W MW power and after the solvent extraction. The reactor mode was Fixed Power and the highest value of temperature was 280°C.*
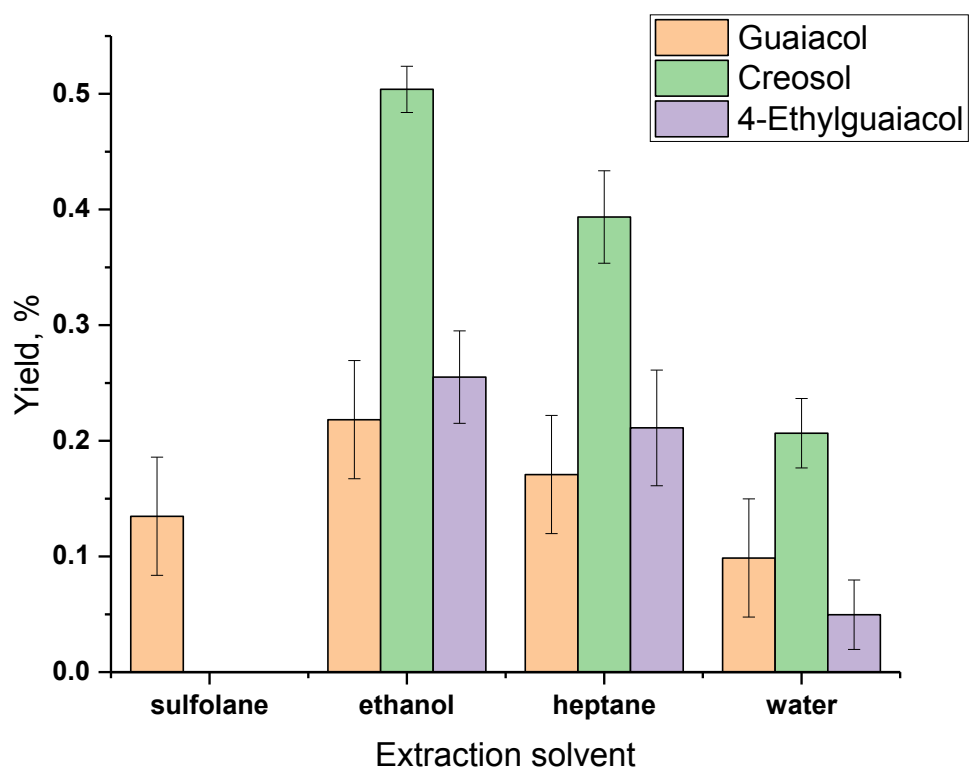
Figure 6.6. *Yield of guaiacol, creosol and 4-ethylguaiacol after the lignin pyrolysis process at 300 W MW power and after the solvent extraction. The reactor mode was Fixed Power and the highest value of temperature was 280°C.*

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Figure 6.7. *Selectivity of guaiacol, creosol and 4-ethylguaiacol after the lignin pyrolysis process at 200 W MW power and after the solvent extraction. The reactor mode was Fixed Power and the highest value of temperature was 280°C.*

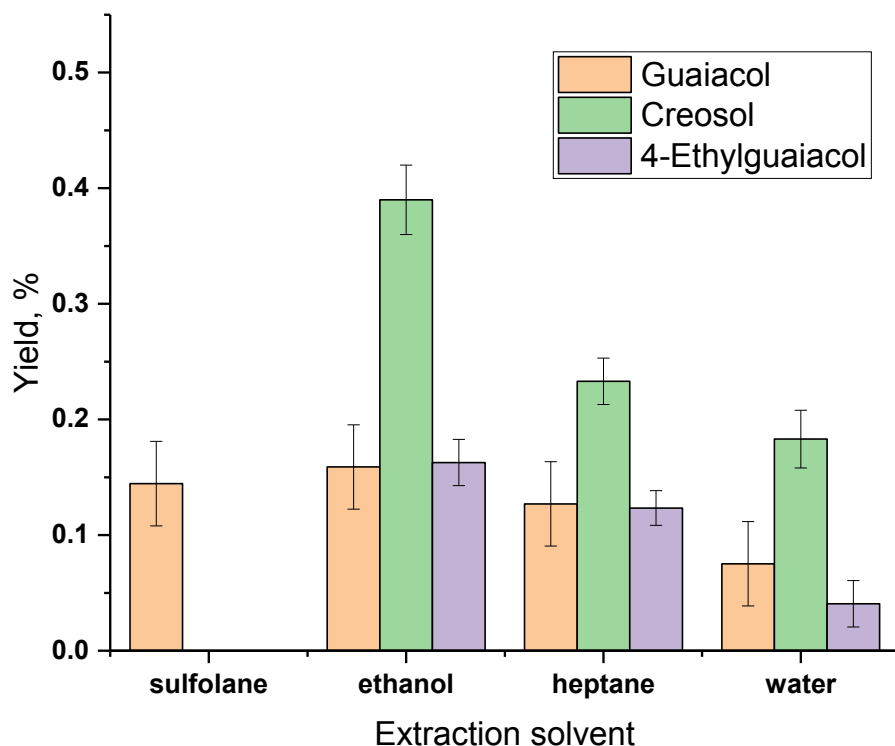**Figure 6.8.** *Selectivity of guaiacol, creosol and 4-ethylguaiacol after the lignin pyrolysis process at 300 W MW power and after the solvent extraction. The reactor mode was Fixed Power and the highest value of temperature was 280°C.*
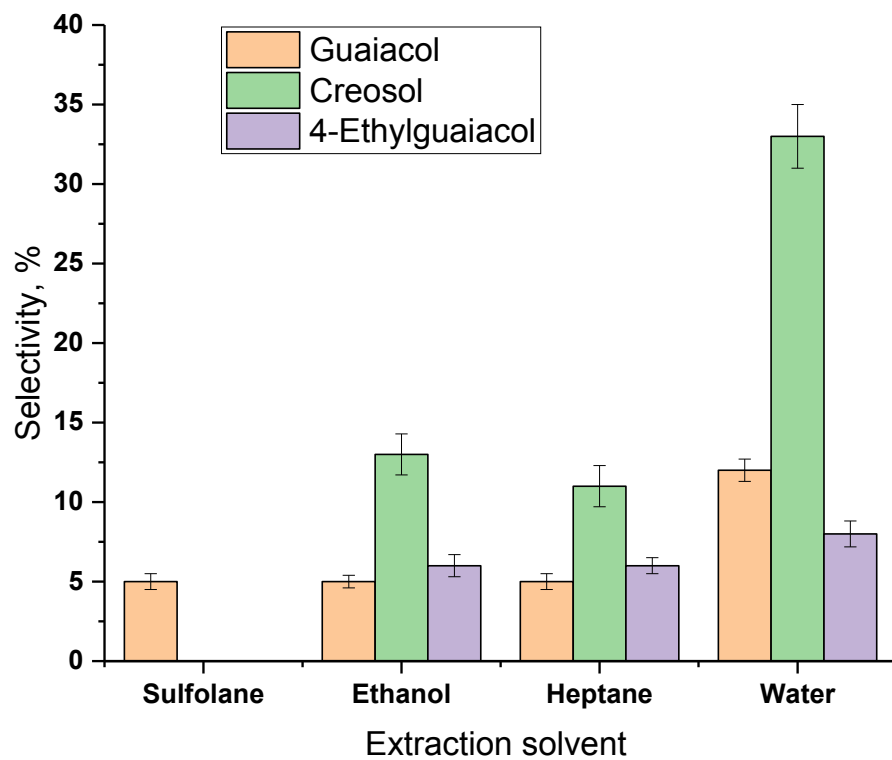
6.1.4. Hold time influence in lignin pyrolysis

Since the results from the previously experiments reveal to be not enough satisfactory for a future development, it was tried to extend the reaction time of the sample under the microwave heating. The idea was that the lignin activation, that theoretically started at 210°C (Table 3.3.), wasn't performed totally after reached the highest value 280°C in the Discover. A comparison was then made with a hold time of 1 s, the reaction was stopped as soon as it reaches 280°C, or 5 minutes. The runs were repeated twice. To permit the longer stay of the sample inside the reactor without overheating, the *Dynamic Power* mode adjusted the power depending on the actual reaction temperature. Graph in Figure 6.9 shows the % of yield of the sample hold for 1 s after 280°C and Figure 6.10 does the same for a hold time of 5 minutes. Results are still not satisfactory. A longer reaction time doesn't improve the lignin activation in terms of phenolic production. Presumably chemicals are produced only during the heating period. In generally the *Dynamic Power* mode is noticed to be worse than the *Fixed Power* one, so a constant power value is more efficient for the reaction success. The

sample washing with ethanol extracts more phenols than the other three solvents. Selectivity values are displayed in Figure 6.11 and 6.12 for hold time of 1 s and 5 min respectively. A lower residence time gives higher selectivity in case of ethanol extraction, probably due to secondary reactions which release other types of products. For guaiacol and 4-ethylguaiacol the selectivity is slightly higher, if extracted with water, with the *Dynamic Power* mode since the sample isn't heated for all the reaction with the maximum power. The best solvent in terms of selectivity values confirms to be water in almost all cases.

Figure 6.9. *Yield of guaiacol, creosol and 4-ethylguaiacol after the lignin pyrolysis process with 1 s hold time after the solvent extraction. The reactor mode was Dynamic Power and the highest value of temperature was 280°C.*
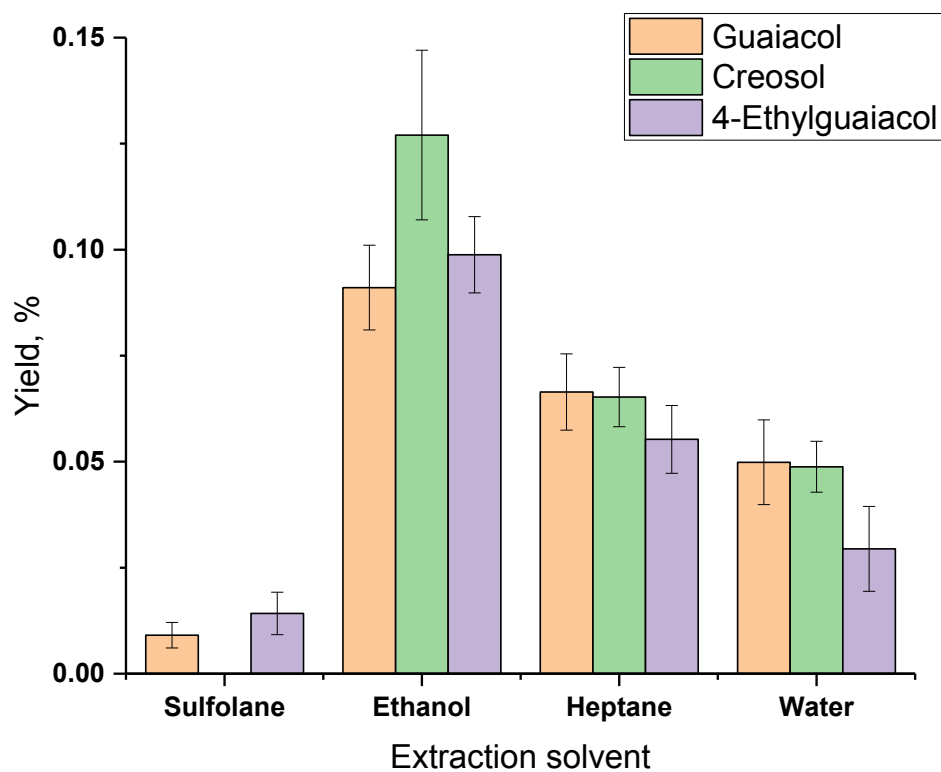
Figure 6.10. *Yield of guaiacol, creosol and 4-ethylguaiacol after the lignin pyrolysis process with 5 minutes hold time after the solvent extraction. The reactor mode was Dynamic Power and the highest value of temperature was 280°C.*
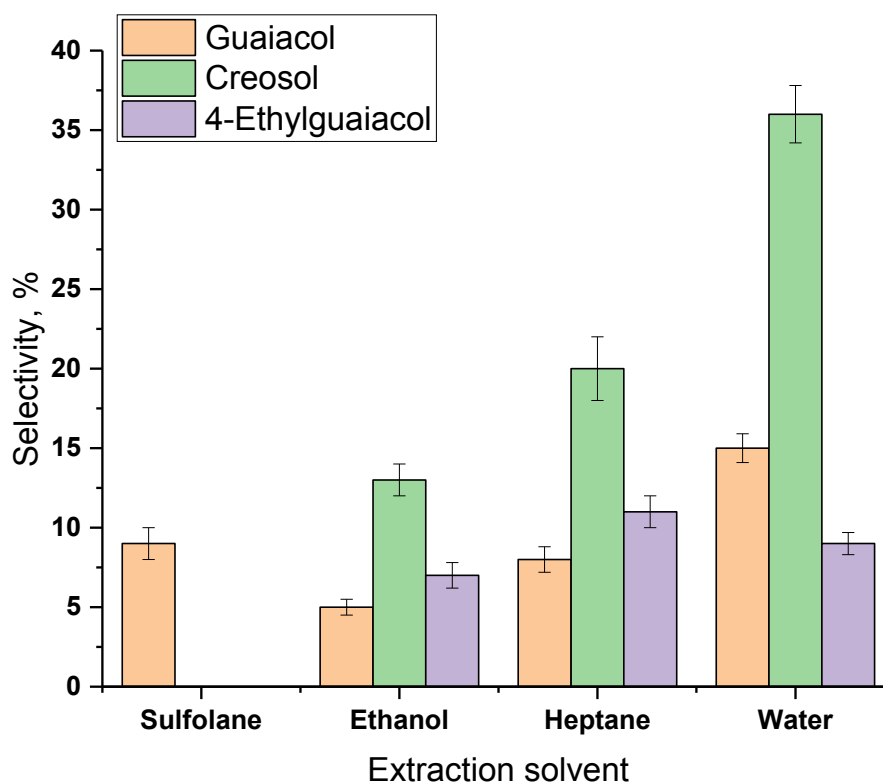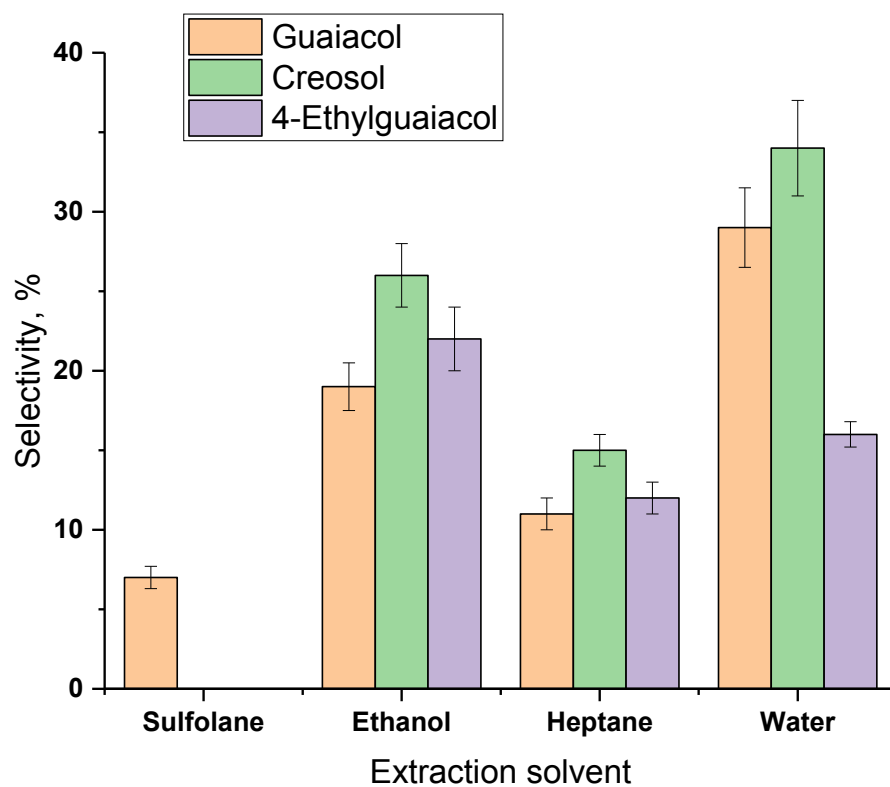
Figure 6.11. *Selectivity of guaiacol, creosol and 4-ethylguaiacol after the lignin pyrolysis process with 1 s hold time after the solvent extraction. The reactor mode was Dynamic Power and the highest value of temperature was 280°C.*
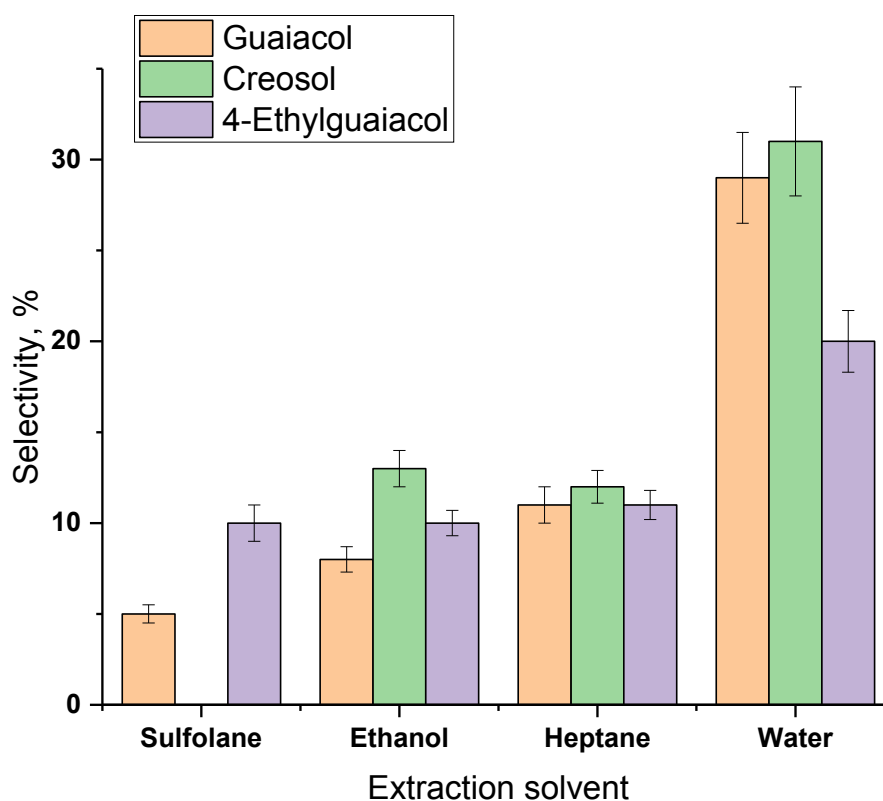
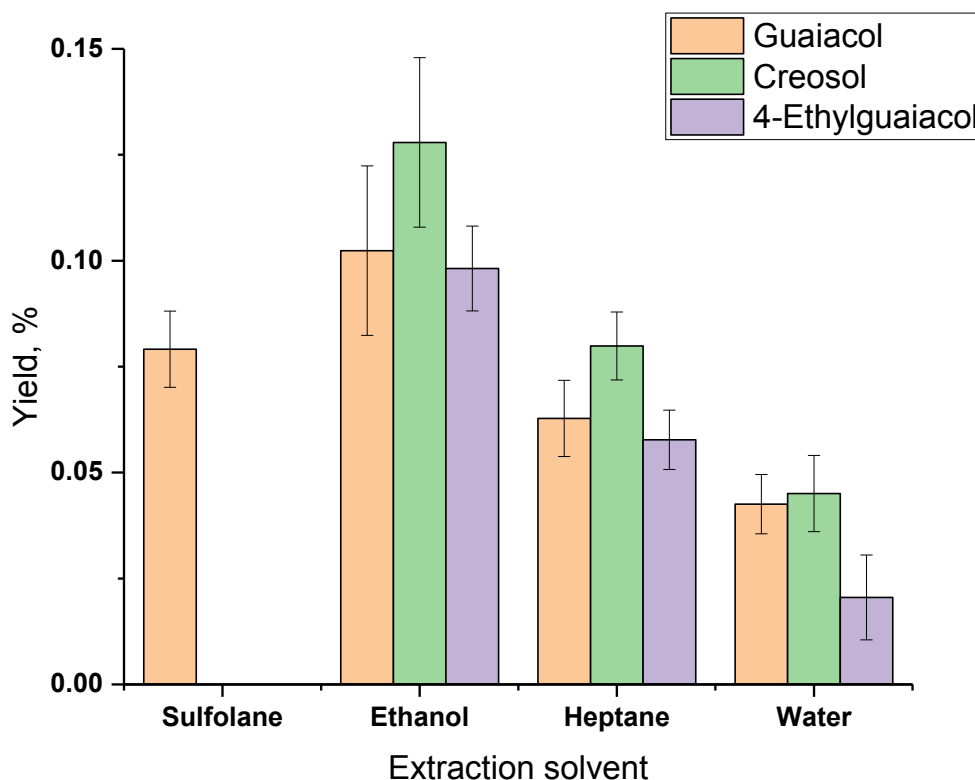**Figure 6.12.** *Selectivity of guaiacol, creosol and 4-ethylguaiacol after the lignin pyrolysis process with 5 min hold time after the solvent extraction. The reactor mode was Dynamic Power and the highest value of temperature was 280°C.*

6.1.5. Acid and base catalyst influence in lignin pyrolysis

In § 4.3, it was noticed that, in general, the addition of a proper catalyst could improve the lignin activation because the gas/liquid yield can be promoted by the addition of the catalyst during the microwave pyrolysis process. The last attempt with the microwave reactor was made doping the lignin with 2% by weight of two different acids – sulfuric and acetic acid – and two different bases – sodium and ammonium hydroxide. The runs were repeated twice. The reaction conditions were the best ones found until this moment in terms of yield: *Fixed Power* mode fixed at 300 W and highest allowed temperature always at 280°C. The extraction solvent was ethanol because it exhibited the best yields at 300 W. With the sodium hydroxide as catalyst, the phenols yield appears higher than the previously experiments, overcoming 1% of yield (Figure 6.13). Still, the results are not enough to proceed with the phenolic compounds separation since the yield remains very poor. A possible reason can be related to the polycondensation reactions that happen instead of the degradation to phenols. This is probably because of the too high microwave efficiency or temperature set. The lignin is activated but, instead of depolymerising in chemicals

as guaiacols phenol kinds, it condensates again to higher molecular weight compounds. These have a too high boiling point to be detected by the gas chromatograph. Selectivity is showed in Figure 6.14. Sodium hydroxide as catalyst promotes the formation of guaiacol with higher selectivity with respect to the other chemicals.
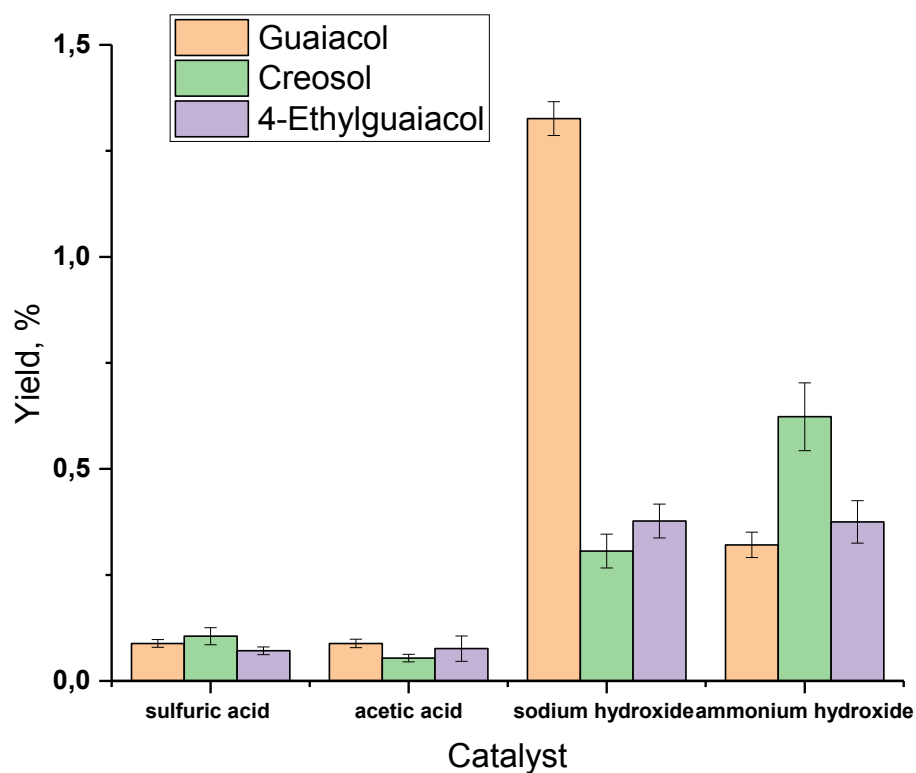
Figure 6.13. *Yield of guaiacol, creosol and 4-ethylguaiacol after the doped lignin pyrolysis. The reactor mode was Fixed Power at 300 W and the highest value of temperature was 280°C.The doping was 2% by weight of sulfuric acid, acetic acid, sodium hydroxide and ammonium hydroxide.*
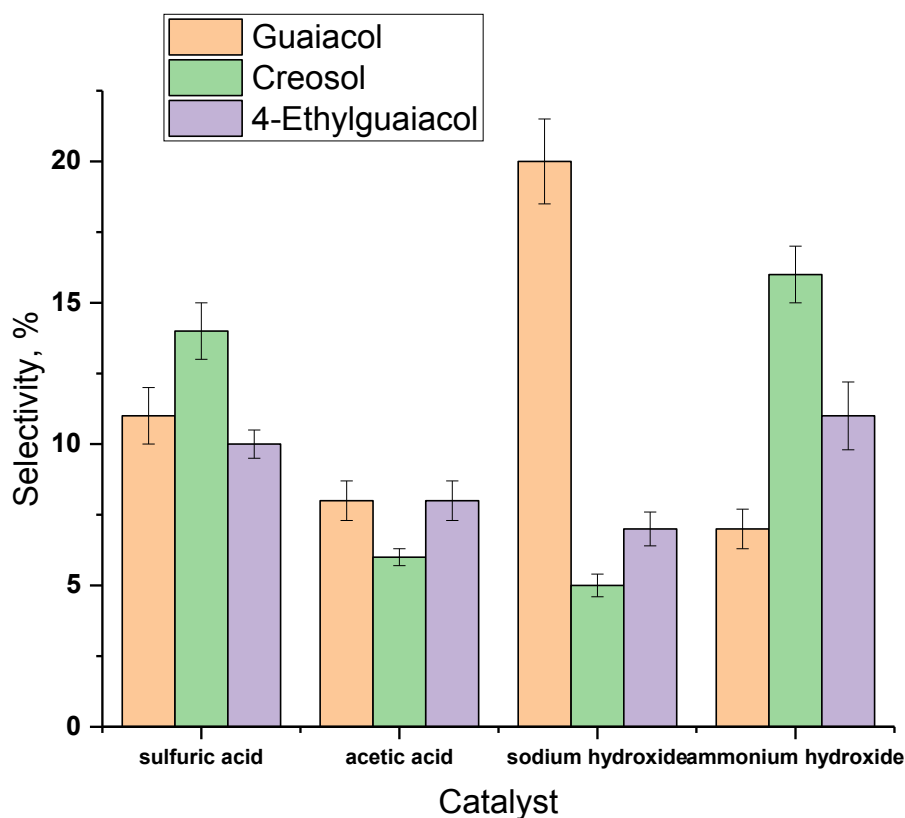
Figure 6.14. *Selectivity of guaiacol, creosol and 4-ethylguaiacol after the doped lignin pyrolysis. The reactor mode was Fixed Power at 300 W and the highest value of temperature was 280°C.The doping was 2% by weight of sulfuric acid, acetic acid, sodium hydroxide and ammonium hydroxide.*

6.2. Conventional lignin activation

Lignin pyrolysis has been also carried out using conventional heating by using thermogravimetric analyser on pristine lignin; in order to obtain higher yield and selectivity the use of purified lignin has also been considered.

The thermogravimetric analysis of raw lignin, at 50 K/min speed, from ambient temperature up to 500°C, gives a percentage mass loss around 55%. The beginning of raw lignin mass loss is detected at around 250°C. At this point the decomposition mechanism of lignin seen in §4.1.1.1 starts and the phenolics compounds are produced and other gases released. To reveal with more accuracy the sample weight variation, the differential TGA diagram was calculated. It is called differential thermogravimetry (dTG), it records the first derivative of the TGA curve respect to time and indicates the speed of the mass loss. When the sample loses weight during the pyrolysis, the thermogram TGA shows a step, while the dTG curve has a peak downwards.

Its lowest value corresponds to the inflection point of the TGA step profile. The two curves, TGA and dTG, are compared in Figure 6.15. A trap was located immediately on the furnace exit to capture the volatiles from the raw lignin pyrolysis for the GC-FID identification (Figure 6.16). The three main phenolics considered in the microwave pyrolysis were found also in this conventional heating process, correspondingly to the literature reported in § 4.1.1. The experiment was made again without the trap but directing the gas products to the FTIR detector.
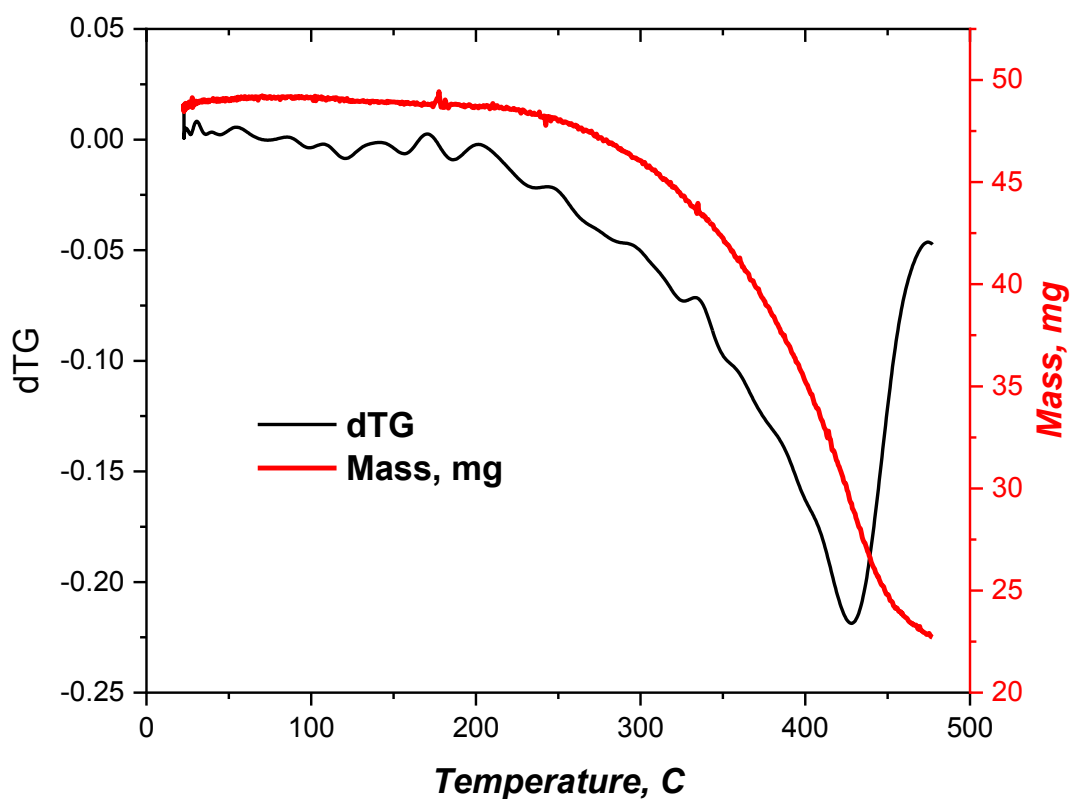
Figure 6.15. *TG mass loss [%] of raw lignin and dTG profile [°C] during the pyrolysis process related to temperature [°C]. The heating speed was 50 K/min and the temperature range from ambient to 500°C.*
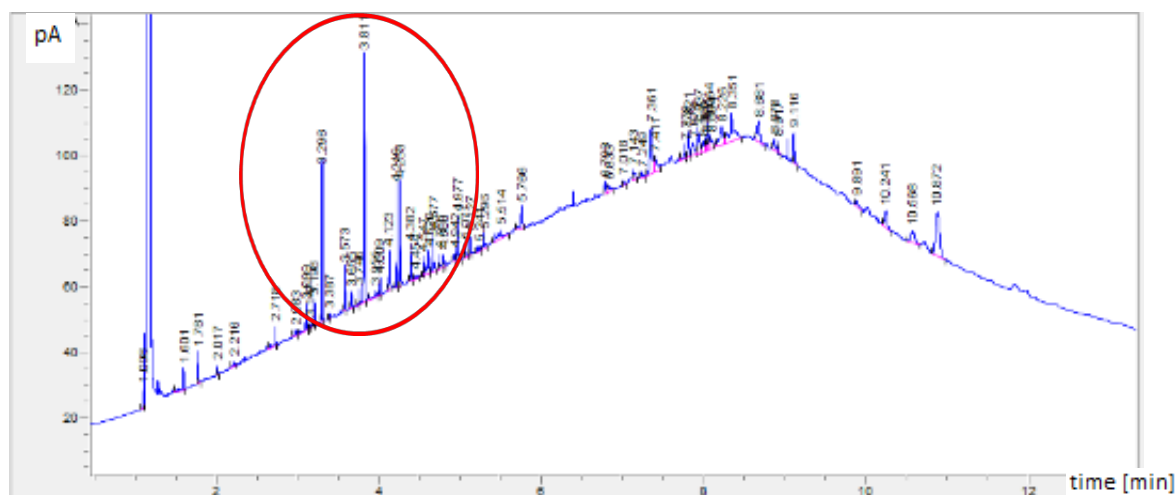
Figure 6.16. *GC-FID spectrum of the volatiles trapped after the thermogravimetric analysis. The trap was washed with ethanol. guaiacol, creosol and 4-ethylguaiacol peaks are underlined by the red circle.*

6.2.1. Lignin purification for pyrolysis trials

As it was possible to notice from the 2D NMR spectrum of the lignin (Figure 5.8), the raw material presents a large quantity of wax. It was so decided to perform a purification step using the Soxhlet extraction with ethanol. After the Soxhlet process, the solution containing the solvent and the extracted chemicals was concentrated using the rotor evaporator and then submitted to the GC-MS (Figure 6.17) and to the GC-FID (Figure 6.18) for the products identification and quantification.

The GC-MS library identifies mainly the presence of oleates compounds in the extracted solution. Their peak area in the GC-FID spectrum reveals also to be the biggest one. The remaining lignin was then dried overnight in the oven and pyrolyzed, like the raw one, to check if the yield of guaiacol derivatives increases respect to the microwave process. The lignin after the Soxhlet extraction contains more or less the same amount of phenolics, just a little bit of guaiacol was extracted, but is purified from oleates.
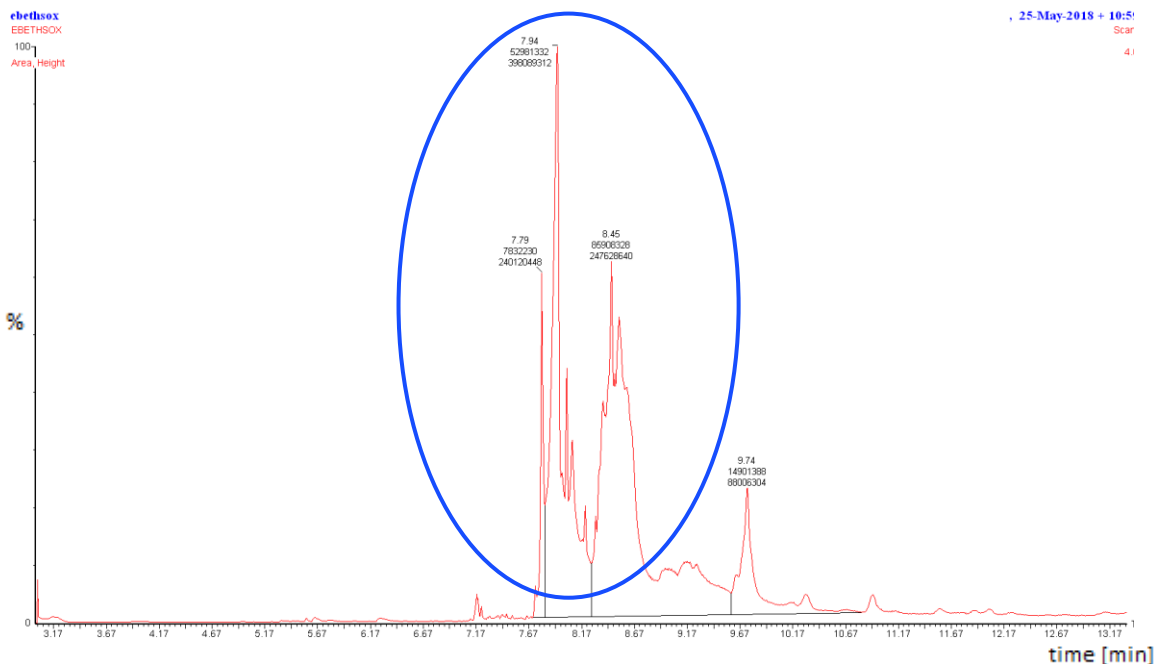
Figure 6.17. *GC-MS spectrum of the extracted solution in ethanol solvent system using the Soxhlet device. The blue circle surrounds the found oleates compounds.*
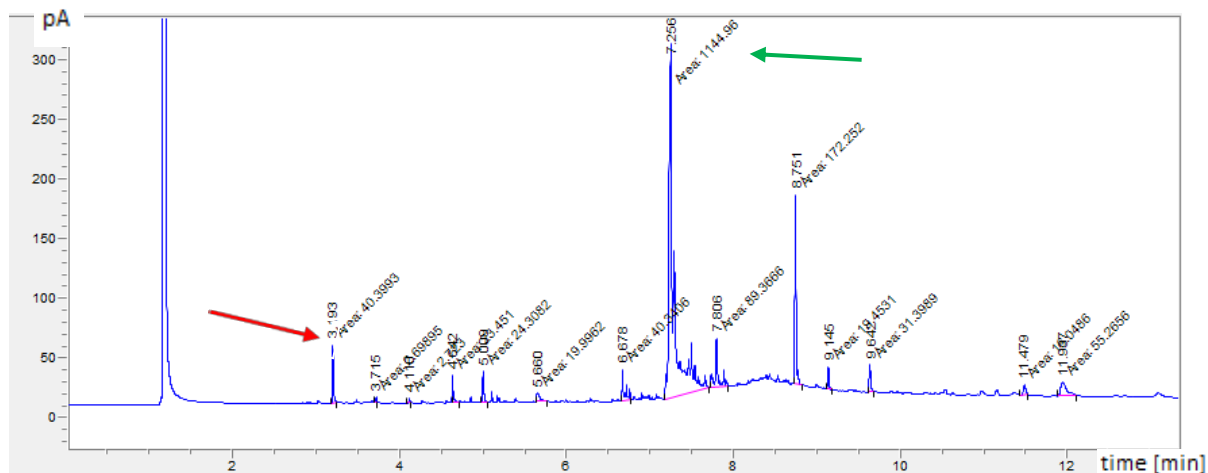
Figure 6.18. *GC-FID spectrum of the extracted solution in ethanol solvent system using the Soxhlet device. The area correspondent to the oleates compounds (green arrow) is the highest one excluding the solvent peak. The red arrow shows the guaiacol amount that is extracted by the ethanol.*

6.2.2. Conventional lignin pyrolysis on pristine and purified lignin

As said before, the TGA analysis was carried out twice for lignin both before and after extraction. To understand the spectra built by the FTIR device, the information from trap sample and literature were used [93]. After the chemicals identification, the reference

standards were run to obtain the calibration coefficients. They allow to understand how much chemicals are present in the volatiles. Table 6.1 compares the yield of different identified compounds derived from lignin both before and after the purification. [93]. After the chemicals identification, the reference standards were run to obtain the calibration coefficients. They allow to understand how much chemicals are present in the volatiles. Table 6.1 compares the yield of different identified compounds derived from lignin both before and after the purification.

Table 6.1. *Yield of identified compounds after the pyrolysis process of lignin before and after the Soxhlet purification with ethanol*

	Before purification	**After extraction**
	Yield mass loss, %	Yield mass loss, %
H_2O	13±0.7	13±0.8
CO_2	10±1	16±0.15
CO	7±0.4	8±0.6
Formaldehyde	5±0.2	2±0.1
4-ethyl-guaiacol	23±2	19±2
Creosol	10±0.5	7±0.8
Guaiacol	21±1.8	13±0.9
Hexanol	13±1.3	6±0.4
SUM	Around 100%	Around 85%
Mass loss	Around 55%	Around 50%

The lignin pyrolysis involves the formation of water, already confirms in literature, several combustion gases like CO and CO_2, and other chemicals as formaldehyde, hexanol and phenolics. Their yield results higher than the one calculated after the microwave process, the total phenolics yields is about the 55% of the total production yield.

Figures 6.19-6.26 allow to see in an easier way the difference between the chemicals released during pyrolysis for lignin before and after the purification. The major difference given by the purification step lies in the formaldehyde and hexanol content. They are presumably produced from the fatty acids pyrolysis. The phenols components are instead almost constant, just a little decrease of guaiacol yield is noticed after the purification step. The guaiacol is in fact partially extracted, as it was possible to seen from the GC-FID analysis of the extracted Soxhlet solution (Figure 6.18).
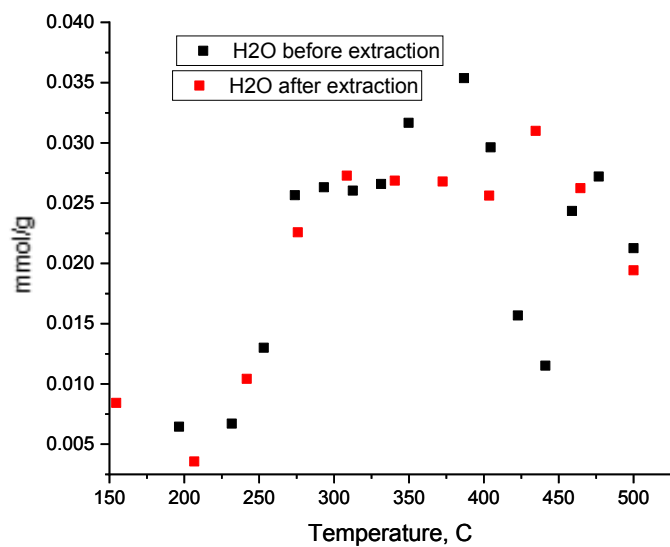
Figure 6.19. *Water profile [mmol/g] vs. temperature [°C] in pyrolyzed lignin before and after the Soxhlet purification step.*

Figure 6.20. *CO_2 profile [mmol/g] vs. temperature [°C] in pyrolyzed lignin before and after the Soxhlet purification step.*
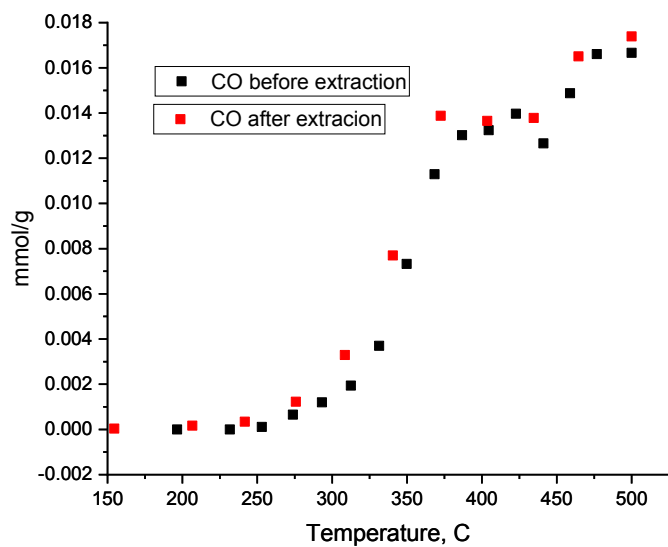
Figure 6.21. *CO profile [mmol/g] vs. temperature [°C] in pyrolyzed lignin before and after the Soxhlet purification step.*
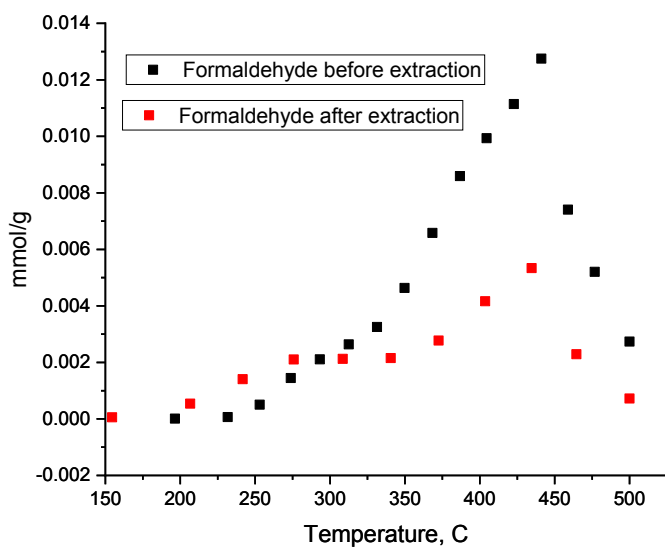
Figure 6.22. *Formaldehyde profile [mmol/g] vs. temperature [°C] in pyrolyzed lignin before and after the Soxhlet purification step.*
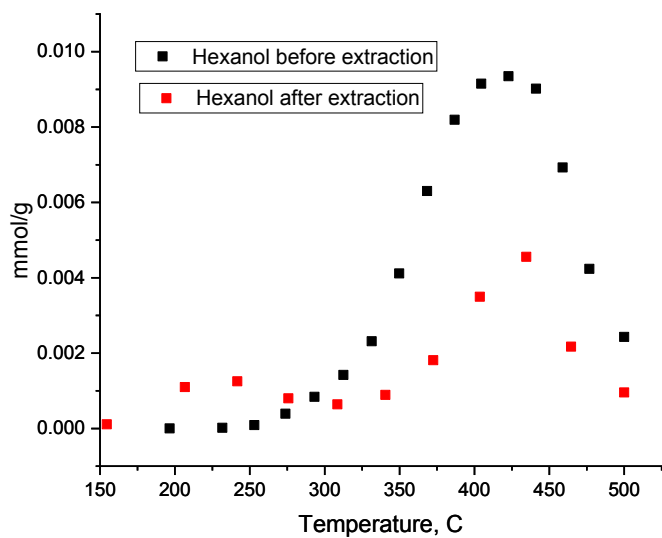
Figure 6.23. *Hexanol profile [mmol/g] vs. temperature [°C] in pyrolyzed lignin before and after the Soxhlet purification step.*
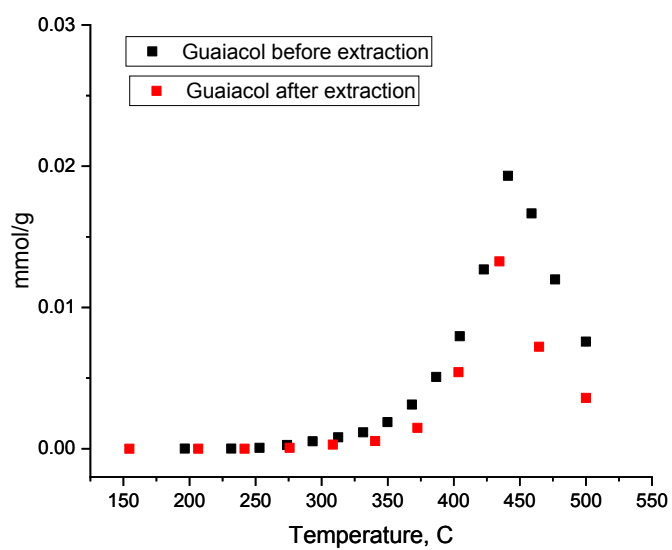
Figure 6.24. *Guaiacol profile [mmol/g] vs. temperature [°C] in pyrolyzed lignin before and after the Soxhlet purification step.*
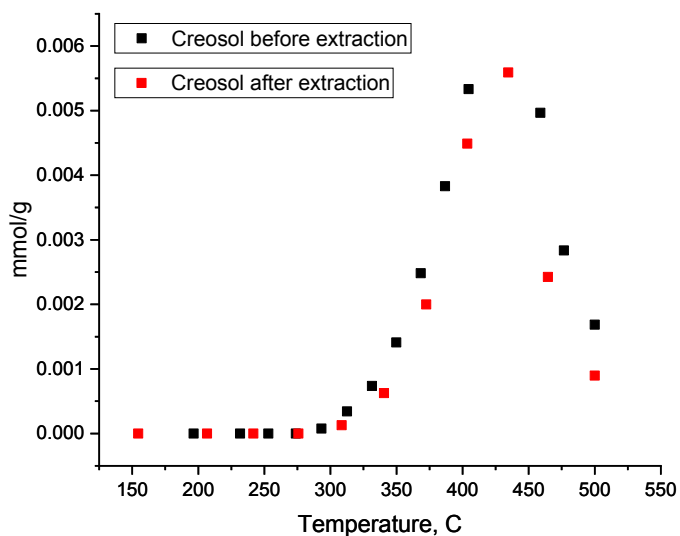
Figure 6.25. *Creosol profile [mmol/g] vs. temperature [°C] in pyrolyzed lignin before and after the Soxhlet purification step.*
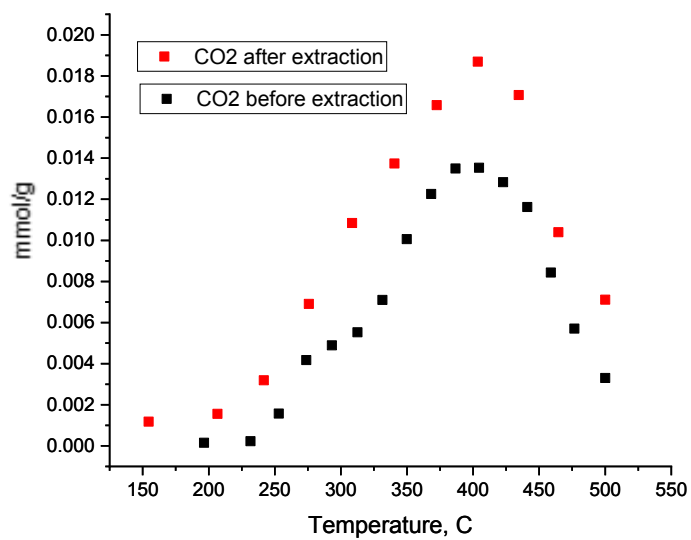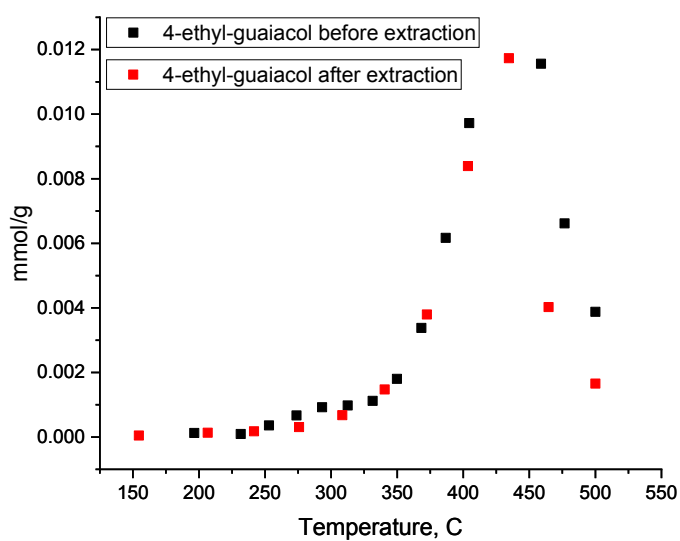

Figure 6.26. *4-ethylguaiacol profile [mmol/g] vs. temperature [°C] in pyrolyzed lignin before and after the Soxhlet purification step.*

6.3. TLC eluent system

Several eluent systems were investigated to guarantee the optimal separation of phenolics compounds in the samples. First the eluents were tested with the pure 3-methoxyphenol

(reference for guaiacol), creosol and 4-ethylguaiacol, and then also the sample was added on the TLC paper. The test mixtures were:

- heptane:ethyl acetate (6:1);
- toluene:acetone (95:5);
- heptane:ethyl acetate (9:1);
- chloroform:ethyl acetate:formic acid (5:4:1);
- ethyl acetate:DCE (3:2);
- ethyl acetate:DCM (1:1);
- ethyl acetate:formic acid:acetic acid:water (100:11:11:26);
- buthanol:acetic acid:water (35:5:12);
- acetonitrile (pentene saturated):ethyl acetate (4:1);
- ethanol:water (4:1);
- 1-propanol:ammonia solution (20:1);
- heptane:DCM (50:50);
- methanol:water (70:30);
- acetonitrile:water (70:30).

The best eluent found was the mixture benzene:methanol (95:5). This system permits to separate the three main phenolic references (Figure 6.27) and gives a good separation of these compounds inside the sample (Figure 6.28). Unlikely benzene is a dangerous material and not environmental friendly, so the research of a greener and safer eluent needs to be more elaborated. Since the resulting yields from the microwave experiments were too low, no further developments were carried out.

Figure 6.27. *TLC paper in benzene:methanol (95:5) eluent. The three separated references for guaiacol, creosol and 4-ethylguaiacol are circled with a pencil.*
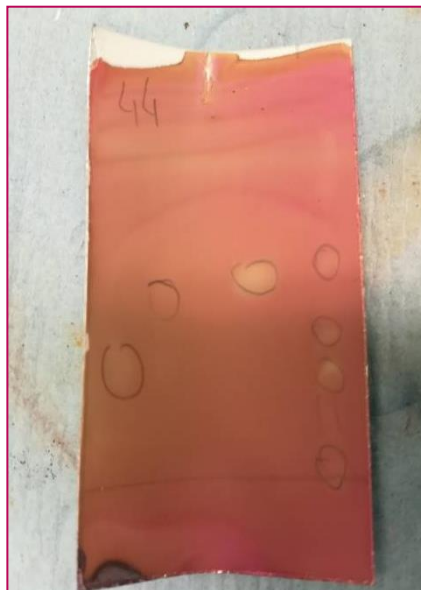
Figure 6.28. *TLC paper in benzene:methanol (95:5) eluent. A lignin sample is added at the very right part and shows a good separation of its components correspondent to the references of the phenolics.*

Conclusions

In this work it was studied the possibility to recovery high value chemicals after the thermal activation of lignin using microwave or conventional heating. The high value chemicals found were phenolic compounds as guaiacols and creosols, mostly used in the food industry like flavouring agents.

The lignin was first characterized to have information about the structure and the composition of the raw material used which derived from the Kraft process and proves to be a softwood type since the syringol units are not present in the structure. To activate the biomass, two types of thermochemical processes were conducted: solvolysis, in presence of four different solvents (sulfolane, ethanol, heptane and water), and pyrolysis. The same solvents were used to extract the bio-oil from the treated sample.

The identification of the compounds produced in the microwave activated process was made using the NIST library present in the GC-MS, while their quantification was based on a calibration curve built from GC-FID reports of reference chemicals.

The solvolysis process revealed to be not efficient at the reaction conditions tested, since the resulting yields of phenolic compounds were very low. After this preliminary test, the focus of the study was then directed to the pyrolysis process only, i.e. without solvent, using both microwave and conventional heating. To investigate the microwave effect in pyrolysis, it was used the single-mode reactor Discover®, varying the microwave power and the holding time of the reaction and doping the lignin with acid and base catalysts. The yields of phenolic compounds resulted to be very low as well, with only a little increment in the lignin doped with sodium hydroxide. However, the best operating condition for pyrolysis were found to be at the highest power value (300 W) using the *Fixed Power* reactor mode. Probably the cause of the very low yield is related to the polycondensation reactions, which happen during the microwave irradiation, and lead to higher molecular weight chemicals.

The results of conventional pyrolysis are different. Indeed, after identifying the phenolic compounds released and employing TG-FTIR technology to quantify the amount and the selectivity, it has been observed that the yields in this case are higher, so this technique seems to be preferable than the pyrolysis by using microwave. The use of purified lignin seems not to give important advantages since the major difference lies in the formaldehyde and hexanol content while the phenols components are instead almost constant, just a little decrease of guaiacol yield is noticed after the purification step.

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