University of Basilicata
Department of Science

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Department of Chemistry and Biology

Thesis Title

"Effect of thermo wood modification and polyoxometalates catalysts on yield and extractives composition"

PhD Course
Chemistry

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Abstract

The aims of this work were to investigate the impact of the heat treatment on the chemical composition (holocellulose, lignin and extractives) of different wood species, to characterise and quantify wood extractives and their composition by GC-MS analysis, to explore the effect of thermo vacuum process on the extractives content and composition, and to use molybdenum catalysts in order to increase the amount of recoverable extractives.

The results showed that there was an increase in the amount of lignin and extractives, and a decrease in the amount of holocellulose following thermo treatment, in the treatment temperature function.

Autoclave treatment of wood with water in the presence of some molybdenum catalysts can increase the amount of extracts, mostly in the presence of microcrystalline $H_3PMo_{12}O_{40}$, but it reduces solubility. Soxhlet extraction of wood with ethanol/toluene mixture in the presence of $H_3PMo_{12}O_{40}$ increased the amount of extractives and their solubility in chloroform, while in the presence of MoO_3 only the solubility of extractives increased.

GC-MS analysis of insoluble fraction showed the presence of myo-inositol and some simple carbohydrates, mainly ribose, xylose and glucose. GC-MS analysis of soluble fraction showed the presence of long-chain acids and fatty acid esters of 10-20 carbon atoms, mainly decanoic acid, hexadecanoic acid, and octadecanoic acid, which can be a source of fatty acids for biodiesel production.

Following the heat treatment and the use of molybdenum catalysts, the increased amount of extractives allowed to obtain valuable amounts of biological and pharmaceutical active compounds.

Furthermore, a new system for the complete separation of triglycerides and fatty acids of wood extractives has been developed: two-dimensional TLC through esterification with $NaOCH_3$ and the use of $AgNO_3$-TLC.

Preface

The work presented in this thesis was performed at the University of Basilicata, Italy, in collaboration with the University of Salerno, Italy, from November 2015 to October 2018. The work was supervised by Prof. Maurizio D'Auria, and was cosupervised by Dr. Luigi Todaro.

A part of this research was carried on at the University of BOKU, Wien, during the Erasmus project with supervisor Prof. Thomas Rosenau.

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others it is duly acknowledged in the text.

Publications

Poster and oral presentations:

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- M. D'Auria, I. Faraone, P. Prasad, **M. Mecca**, L. Milella, D. Russo, C. Sinisgalli. Metabolite profile and biological activity of essential oils from Saussurea costus roots evaluated with GC-MS, *High-throughput MS-based proteomics and metabolomics: from cells to clinic*, Novara, 2018, 23.
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III. **M. Mecca**, M. D'Auria, L. Todaro. Effect of heat treatment on wood chemical composition, extraction yield and quality of the extractives of some wood species by the use of molybdenum catalysts. *Wood Science and Technology*, 2018, 53(1): 119-133.

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VII. C. Benedetto, M. D'Auria, **M. Mecca**, P. Prasad, P. Singh, S. Singh, C. Sinisgalli, L. Milella. Chemical and biological evaluation of essential oil from Saussurea costus (falc.) Lipsch. from Garhwal Himalaya collected at different harvesting periods, *Natural Product Research*, 2018: 1-4.

VIII. L. Todaro, A. Rita, R. Pucciariello, **M. Mecca**, S. Hiziroglu. Influence of thermo-vacuum treatment on thermal degradation of various wood species, *European Journal of Wood and Wood Products*, 2018, 76(2): 541-547.

IX. **M. Mecca**, L. Todaro, M. D'Auria. Extractives from Cedar deodara and Alnus cordata in the presence of molybdenum catalysts, *Chemistry Select*, 2017, 2: 2536-2538.

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1. Introduction

1.1. Introduction

Historically, wood has been used for everything from needles to building churches. One might think that today's new technology and development of new materials would make wood obsolete. Despite these new competitors, wood is still used on a very large scale.

Wood is one of the sources for a lot human operations, like energy, building, paper and chemicals etc. Although wood continues to be used for many applications because of its many excellent material properties (such as a good strength to weight ratio, aesthetic appearance etc.), however, there are some obstacles when working with wood, and most of them are associated with its hydroscopic properties in combination with anisotropic swelling and shrinkage due to changes in moisture content (MC).

In addition to this problematic dimensional instability in response to altering atmospheric conditions, there are other moisture-related problems, such as biological degradation and susceptibility to biological attack. MC has major effects on the durability of wood, since fungi and bacteria are dependent on the presence of water for their survival. As long as the MC is kept under about 20%, there will not be a sufficient amount of water for either fungi or bacteria to grow. To physically protect wood from getting in contact with water after the lumber has been dried to below 20% MC is an environmentally friendly and efficient way of protecting it from biological degradation. This kind of protection is not always easy to accomplish, for instance when wood is used outdoors.

Impregnation of the wood with chemical substances toxic to fungi and bacteria is one of the most commonly used methods of preservation. One major drawback of this method is that it is toxic to many other organisms as well.

These preservatives do not prevent problems with dimensional instability. There are, however, methods to stabilize lumber chemically, but are very expensive, and the substances used can also, in some cases, be questionable in terms of their environmental effects.

During the last decades is increased the demand for sustainable material removing the use of toxic chemicals obtaining materials with a great potential and durability. For these reasons is born the necessity to modify the wood products. Wood modification it is necessary to produce a material that has the desired properties. The aim of a modification may be to bring about an improvement in decay resistance or dimensional stability, to reduce water sorption, to improve weathering performance and so on.

Some articles and patents on how to improve durability and dimensional stability of lumber by only using heat were presented during the first half of the previous century[1,2]. However, it took about 50 years before heat treatment was commercialized on a larger scale.

Contrary to traditional methods to wood modification, heat treatment of wood has been considered as an effective method to modify wood because it avoids use chemical and toxic substance and improve also significant changing. The hygroscopicity of wood is reduced, the dimension stability and durability are increased, but all of these positive effects are drawbacks to loss of strength proprieties.

Although many studies have been carried out on the improvement of overall properties of wood, there is a recent increasing interest in the characterization of the thermal degradation of treated wood products, which could be related to both increased demand for heat-treated wood as well as newly developed environmental management policies.

This research has drawn attention to the chemical change of wood after heat treatment, even using polyoxometalates as catalysts, having the potential to depolymerize lignin, but having never been used until now for this purpose.

1.2. Aims of the study

The aims of this work were:

- To investigate the impact of the heat treatment on the chemical composition of wood: holocellulose, lignin and extractives;
- To use water and ethanol/toluene as solvents to extract secondary metabolites from some wood species;
- To characterise and quantify wood extractives, and explore the effect of thermo vacuum process on their content and composition;
- Prove that the autoclave can be a competitive method compared to the Soxhlet;
- To use molybdenum catalysts to obtain wood degradation in order to increase the amount of recoverable extractives.

1.3. The Structure of Wood

Wood is a complex material and when considering the sorption properties of wood, it is necessary to take this complexity into account.

Wood is a natural material, and as such it exhibits great variability in its properties, it is cellular in structure, anisotropic and non-homogeneous. Wood is obtained from two broad categories of trees known commercially as softwoods and hardwoods. Softwoods are those woods that come from gymnosperms (spore-bearing plants with naked seeds), and hardwoods are woods that come from angiosperms (flowering plants with covered seeds). The wood of softwoods is more uniform than hardwoods which are easier to

distinguish from one another visually. Hardwoods and softwoods differ in terms of the types of trees from which they are obtained, but they also differ in terms of their component cells (Figure 1.1).

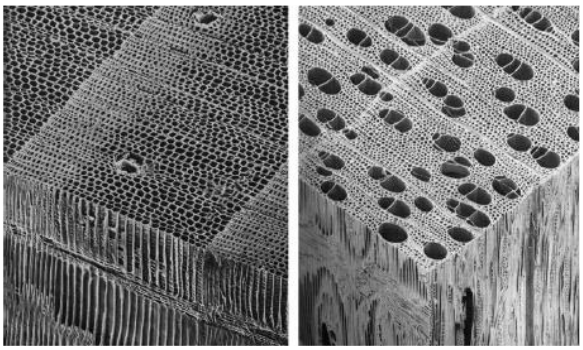

Figure 1.1. An example of the different structures of softwoods (Norway spruce) and hardwoods (birch)[3]

Due to their more complex structure, hardwoods are much more variable in their permeability and capillary behaviour[4]. The types of cells, their relative numbers and their arrangement are different, the fundamental difference being that hardwoods contain a type of cell called a vessel element which does not exist in softwoods. It should be noted that not all softwoods are soft and not all hardwoods are hard. Some softwoods produce wood that is harder and denser than wood produced by some hardwoods (e.g. balsa wood is a hardwood). There are more hardwood species which cover a greater ecological diversity including tropical and temperate regions. However softwoods such as coniferous species of the boreal forests cover a greater geographical area and represent a greater population of forest biomass.

1.3.1 Growth rings

For most species in temperate climates, where there are clear seasons, the difference between wood that is formed early in a growing season and that formed later is sufficient to produce well-defined annual growth rings. The age of a tree at the stump may often be determined by the counting of these rings.

Wood grown in a temperate climate nearly always produces one growth ring each year[4]. Typically, a growth ring consists of two distinct parts (i.e. earlywood and latewood). However, if the growth in diameter of the trunk is interrupted, by drought or defoliation by insects for example, more than one ring may be formed in the same season. In tropical climates where there is often no seasonal difference, growth rings are likely to be indistinct or absent.

1.3.2 Heartwood

Heartwood is wood that has become more resistant to decay as a result of deposition of chemical substances or extractives (a genetically programmed process). Once heartwood formation is complete, the heartwood is dead. Usually heartwood looks different; in that case it can be seen on a cross-section, usually following the growth rings in shape. Heartwood may be much darker than sapwood. It may be sharply distinct from the sapwood. It can be distinguished using an iodine test for starch, which is only present in the live parenchymatic tissue of sapwood.

1.3.3 Sapwood

Sapwood is the younger, outermost wood; its principal functions are to conduct water from the roots to the foliage. In sapwood, only the parenchymatic tissue and epithelial cells are alive and the other cells have a purely conduction or structural role. There is no definite relation between the annual rings of growth and the amount of sapwood, although sapwood width may correlate with the water demand from the foliage. Within the same species, the cross-sectional area of the sapwood is very roughly proportional to the size of the crown of the tree. As the tree gets larger, the sapwood must necessarily become thinner or increase materially in volume. Sapwood forms a greater proportion of the

upper portion of the trunk of a tree than near the base, because the age and the diameter of the upper sections are less.

1.4. Softwood and Hardwood

Wood consists of a lignified xylem that is composed of long slender cells mainly oriented with their longitudinal extension in the direction of the stem. The cells are hollow and interconnected to each other by pit openings. There are several different types of cells in the xylem to meet the different needs of the living tree, such as strength, transport and storage. The type and features of these cells differ not only between hardwoods and softwoods, but also between species, heartwood and sapwood, early wood and late wood.

1.4.1 Softwood

Softwood (the secondary xylem of gymnosperms) is composed of relatively few cell types: longitudinal tracheids, parenchyma, and epithelial cells. Softwoods are characterized by their simple anatomy, which consist mainly of longitudinal fibre tracheids (approximately 90-95 % by volume)[5]. Parenchyma cells, 5-10%, by volume and resin (epithelial) cells, 0.5-1.0% make up the remaining anatomy (Figure 1.1). The ray cells are radially arranged parenchyma cells and tracheids in softwoods that have storage function and frequently contain extractives (i.e. non-structural) material such as starch, fat, oils etc. The shape and size of rays vary significantly between species and are often used as a diagnostic feature in the microscopic identification of wood.

1.4.2 Hardwood

Hardwoods have a much more complex structure than softwoods. Hardwoods contain vessel elements (or pores), fibre tracheids, libriform fibres, and

parenchyma cells which are arranged longitudinally and horizontally (Figure 1.1). The wood consists of 36-70% fibres, 20-55% vessel elements, 6-20% ray cells, with approximately 2% parenchyma cells by volume[5]. Fibres are elongated cells with closed pointed ends and commonly have thick walls. Vessels are capillary tubes arranged vertically along the axis of the tree and are very important for conducting sap or minerals from the roots to the crown. Two structural features of vessel elements are the perforation plates and pits. Ring porous wood, such as oak and elm, contain large vessels in the early wood and narrow vessels in the latewood with an abrupt size change[6]. The parenchyma-cell content of hardwoods is, on the average, much greater than that of softwoods.

1.5. The chemical constituents of wood

In chemical terms, wood can be described like a three-dimensional biopolymer composed of lignin and holocellulose, the combination of cellulose (40-45%) and hemicellulose (15-25%), and this is the major carbohydrate portion of wood. The remaining compounds are extractives, e.g., fats, waxes and resin, which are not components of the actual cell wall (Table 1.1).

Component	Mass		Polymeric state	Molecular derivatives	Function	Degree of polymerization (unit)
	Softwood (%)	Hardwood (%)				
Cellulose	42±2	45±2	Crystalline, highly oriented, large linear molecule	glucose	'fibre'	~ 10^4
Hemicelluloses	27±2	30±5	Semi-crystalline, smaller molecule	galactose mannose xylose	'matrix'	~300
Lignin	28±3	20±4	Amorphous, large 3-D molecule	phenylpropane	'matrix'	none
Extractives	3±2	5±4	Principally compounds soluble in organic solvents	terpenes, polyphenols, stilbenoids	decay resistance and others	none

Table 1.1. Chemical composition of wood

1.5.1. Cellulose

About 40%-50% of the wood material consists of linear polymer chains of β-D-glucopyranose, i.e., cellulose. One cellulose chain consists of 7000–15,000 monomeric units that are linked together by glycosidic bonds (oxygen bridges)[5] (Figure 1.2).

A D-Glucose unit can exist in five different configurations wherein the β-D-glucopyranose is the most stable one, and open aldehyde is the most unstable. The units within the cellulose chain are fixated as β-D-glucopyranose form by the chain structure, while the last unit in the cellulose chain can exist as an open aldehyde and is therefore easily oxidized.

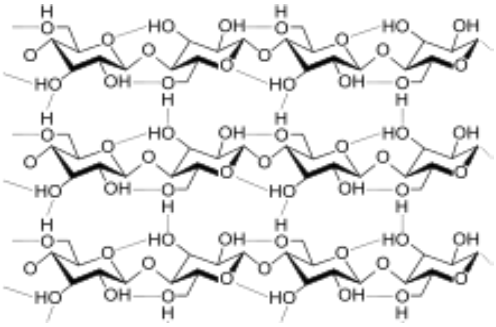

Figure 1.2. β-D-glucopyranose units of Cellulose chain

A bundle of cellulose chains held together by hydrogen bonding between their OH-groups is called a microfibril. The lack of side branches and the positions of the hydroxyl groups enable formation of strong crystalline regions within the microfibrils. The fiber structure, created by hydrogen bonds, provides important properties to cellulose, such as high tensile strength and insolubility in most solvent[7], and also contributes somewhat to the water adsorption of wood through its numerous hydroxyl groups, although many of these are located in the interior of the microfibril and as a consequence are inaccessible to water. The crystalline nature of the cellulose component of wood is relatively unreactive and thermally stable.

It is possible divide the cellulose in two categories, accessible and non accessible. This classification is important in moisture sorption, chemical modification, pulping, extraction and interactions with microorganisms[8].

1.5.2. Hemicelluloses

The Hemicellulose is a polysaccharide where the monomeric units are kept together by glycosidic bonds, but in contrast to cellulose, it is built up of highly branched chains of different types of sugar monomers, which vary with wood species. The monomer consists of six-carbon sugars (hexoses) including mannose, galactose and glucose, as well as, five-carbon sugars (pentoses) xylose and arabinose (Figure 1.3).

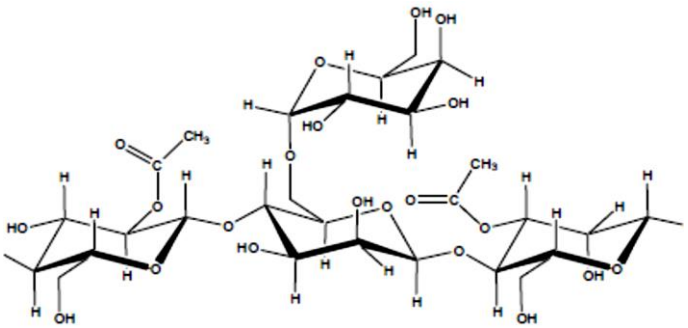

Figure 1.3. The molecular structure of hemicellulose

Typical hemicellulose polymers include glucomannan, galactoglucomannan, arabinogalactan, glucuronoxylan and glucuronarabinoxylan.

The presence of a high number of side branches contributes to the highly amorphous structure of hemicellulose. This means that its hydroxyl groups are easily accessible, react more readily, and are less thermally stable than cellulose or lignin. This is the reason most of the moisture in wood is bonded to the hemicellulose.

The total percentage of the hemicelluloses present in wood is larger in hardwoods compared to softwoods, with an higher level of acetylation.

Hemicellulose degradation results in wood becoming brittle and rigid, indicating the important role that they have in imparting viscoelastic properties to wood[9].

1.5.3. Lignin

Lignin is a complex amorphous high molecular weight polymer built from three different phenyl propane units (Figure 1.4), and has a low concentration of OH-groups compared to the polysaccharide components. The random coupling of the different units in lignin makes it the most complex polymer in wood. The two main bonding types found in lignin are: strong carbon-carbon and weaker ether bonds.

Lignin occurs between individual cells and within the cell walls. Between cells, it serves as a binding agent to hold the cells together. Lignin is a glassy material with a glass transition temperature around 140 °C, although this is reduced when water is present. About 25% of the total lignin in wood is to be found in the middle lamella, an intercellular layer composed of lignin and pectin, together with the primary cell wall. The bulk of the lignin (about 75%) is present within the secondary cell wall, having been deposited during cell senescence.

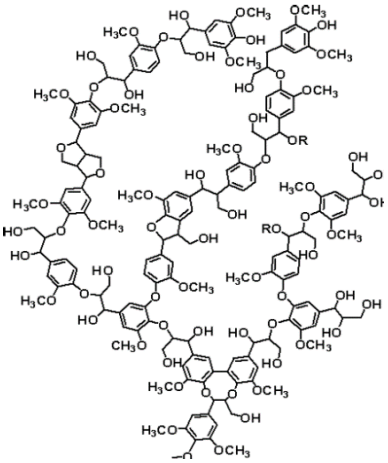

Figure 1.4. The molecular structure of lignin

The structure of lignin is different between hardwood and softwood. Hardwood lignins have a syringyl content varying from 20 % to 60 %, whereas softwood lignins have very low syringyl contents[10].

1.5.4. Extractives

Extractives are all substances that are extractable from wood without damaging the wood structure. The main groups of extractives are terpenes, fats/waxes, phenolic components, fats, steroids, resin acids, and many other minor organic compounds. These chemicals exist as monomers, dimers and oligomers. Most of the extractives in both softwoods and hardwoods are located in the heartwood rather than sapwood[11], and some are responsible for the colour, smell, and durability of the wood, in fact their main function is to protect the living tree as well as serving as backup nutrition.

Depending on the species, wood can contain levels of extractible material ranging from 0.5% to about 20%. Furthermore, various parts of the same tree, e.g. stem, branches, roots, bark and needles, differ markedly with respect to both their amount and composition of extractives[12].

The extractives comprise both inorganic and organic components.

Organic extractives of wood can be classified into two main classes i.e. lipophilic (fatty) and hydrophilic extractives (Table 1.2)[13].

Lipophilic	**Hydrophilic**
Resin acids	Phenols
Free fatty acids	Lignans
Triglycerides	Flavonoids
Steryl esters	Tannins
Free sterols	Sugars
Monoterpenes	Salts

Table 1.2. Lipophilic and hydrophilic extractives

In general, softwood has a higher extractive content than hardwoods.

Different types of extractives are necessary to maintain the diversified biological functions of the tree. For example, traces of certain metal ions are present as functional parts of the enzymes which are needed as catalysts for biosynthesis, fats constitute the energy source of the wood cells, whereas lower terpenoids, resin acids, and phenolic substances protect the wood against microbiological damage or insect attacks.

They are not only important for wood but they have also commercially use in polishes, lubricant, additives, fertilizer and so on[14] and gluing and finishing of wood[15]. In the last years, these types of compounds have found application in medicine and cosmetic fields.

1.6. Polyoxometalates

Polyoxometalates are large metal cluster anions formed mainly by transition metals and oxygen atoms that can adopt a variety of spatial structures. They have been known and used in the chemistry lab for nearly two hundred years, but only after the second half of the 20th century have we been able to fully perceive the richness of their chemistry, structure and activity.

Polyoxometalates, formerly and still occasionally known as Heteropoly- and Isopolyanions (or "acids"), comprise an immense and expanding class of oxyanions of the early transition metal elements, predominantly molybdenum(VI), tungsten(VI) and vanadium(V), and to a currently much lesser extent, pentavalent niobium and tantalum[16]. There are two major characteristics of these elements that distinguish them from other polyoxoanion formers such as chromium(VI), silicon and phosphorus. The first is the ability of the metal atom to increase its coordination by oxide from 4 to (commonly) 6 or 7, and the second, a consequence of the metal's vacant and accessible d-orbitals, the ability to engage in multiple bonding with

"terminal" oxygen atoms. The commonest environment of the metal atom in polyoxometalates (POMs) is quasi octahedral with one or two (mutually cis) unshared or terminal oxygens. In 1965, based upon the handful of POM structures that were known at that time, Lipscomb noted that no structures contained metal atoms with three terminal oxygens, and proposed that this would prove to be a general restriction[17]. Since that time, some POM structures have been reported to violate the so-called Lipscomb criterion but in most cases that can be examined closely, one of the three terminal oxygens (always in a *fac* configuration) has been found to be protonated or is a water ligand. The Lipscomb rule is of course a consequence of the strong trans influences of the M-$O_{terminal}$ multiple bonds. This leads, in the "octahedral" *fac*-MO_3 case, to long and therefore weak bonds attaching M to the remainder of the polyoxometalate structure.

One of the main reasons why polyoxometalates have not been considered in the past for the design of functional materials is precisely because their molecular nature makes them soluble in water and common organic solvents. Yet, they not only share structural and topological features with related transition metal oxides[18] but also resemble them concerning their redox, electron transfer or ion transport behaviour.

Polyoxometalates have been traditionally the subject of study of molecular inorganic chemistry. Yet, these polynuclear molecules, reminiscent of oxide clusters, present a wide range of structures and with them ideal frameworks for the deployment of a plethora of useful magnetic, electroionic, catalytic, bioactive and photochemical properties. A new trend towards the application of these remarkable species in materials science is beginning to develop.

On the one hand, there is their use as clusters with inherently useful properties on themselves, a line that has produced fundamental studies of their magnetic, electronic or photoelectrochemical properties and has shown these clusters as

models for quantum-sized oxides. On the other hand, the encapsulation or integration of polyoxometalates into organic, polymeric or inorganic matrices or substrates opens a whole new field within the area of hybrid materials for harnessing the multifunctional properties of these versatile species in a wide variety of applications, ranging from catalysis to energy storage to biomedicine.

Researches have also shown that polyoxometalates (heteropolyacids and their salts) and their transition-metal substituted derivatives have the ability of undergoing reversible one or multi-electron transfer while retaining their original structure, which has rendered these compounds attractive acid and redox catalysts in a variety of industrial catalytic applications[19].

Furthermore, while the use of oxidant catalysts in the lignin depolymerization is the object of a widespread research[20,21], the possible use of these catalysts to induce oxidative depolymerization of wood, increasing the amount of low molecular mass chemical compounds that can be obtained from wood, has not been studied until now.

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2. Thermo-treatment

One of the original reasons to heat-treat wood was to improve the properties of durability.

Some laboratory studies have shown durability properties comparable to (copper, chromium and arsenic) CCA impregnation[1], but a problem is that high durability requires high treatment temperatures, which means a great strength loss. In a review by Viitaniemi[2] it is concluded that the strength loss is from 0%-30% depending on treatment process.

After heat treatment, the wood acquires a darker colour. This colour change is often seen as a positive effect, especially in hardwoods. The colour change creates a potential for wood to reach new markets where more exclusive hardwoods are normally used. It has also been suggested that colour has the potential of predicting the quality of heat treatment by predicting various property changes such as chemical changes[3], strength loss[4] and mass loss due to thermal degradation[5].

One important reason for heat treatment is to obtain a more dimensionally stable material by reducing the equilibrium moisture content (EMC). The reduction of the EMC is about 0%-50%, depending on how the treatment is performed[6-8].

It is generally accepted that heat treatment also lowers the wettability of wood[9-11].

In the last years, wood heat treatment has increased greatly due to the declining of timber production, the introduction of restrictive regulations in the use of chemical products and the increasing request of sustainable material.

There are a lot of thermal commercial treatments, the technology used for this study is Thermovuoto®, a new system designed to combine a thermal treatment with a vacuum process.

It is an innovative process for the thermal modification of high temperature timber inside a vacuum autoclave, able to produce the modification of the wood structure through the multiple chemical and physical reactions of the substances constituting the same generated by the exposure of wood to temperatures at which the pyrolysis phenomenon begins, preventing its combustion anyway.
In order to perform the respective drying and heat treatment cycles, the trunks of the species identified were suitably sawed by means of a division with parallel cuts. It is the simplest procedure reducing waste to a minimum.

2.1. Thermovuoto® system

The technology used to carry out the heat treatment process at the Wood Technology Laboratory (UNIBAS) in Potenza is the press vacuum dryer, model PRESS M 1.6, mainly composed of the following elements (Figure 2.1):
a) a drying cell (1) of watertight stainless steel, having the shape of a parallelepiped and provided with a movable lid (2) composed essentially by an elastic silicone membrane resistant to high temperature (3) connected to a special support frame;

b) a wood heating circuit consisting of a series of plates heating elements (4) of aluminium in which a special pump circulates diathermic oil coming from an electric boiler, through inlet pipes (8) and outlets (9) which are stainless steel hoses, to allow the movement of the plates themselves;

c) a liquid ring vacuum pump with its hydraulic circuit;

d) an electric control unit (7) for the operation of the dryer, complete with microprocessor for the automatic conduct of wood drying;

e) a manual condensate discharge (10) of the drying cell.

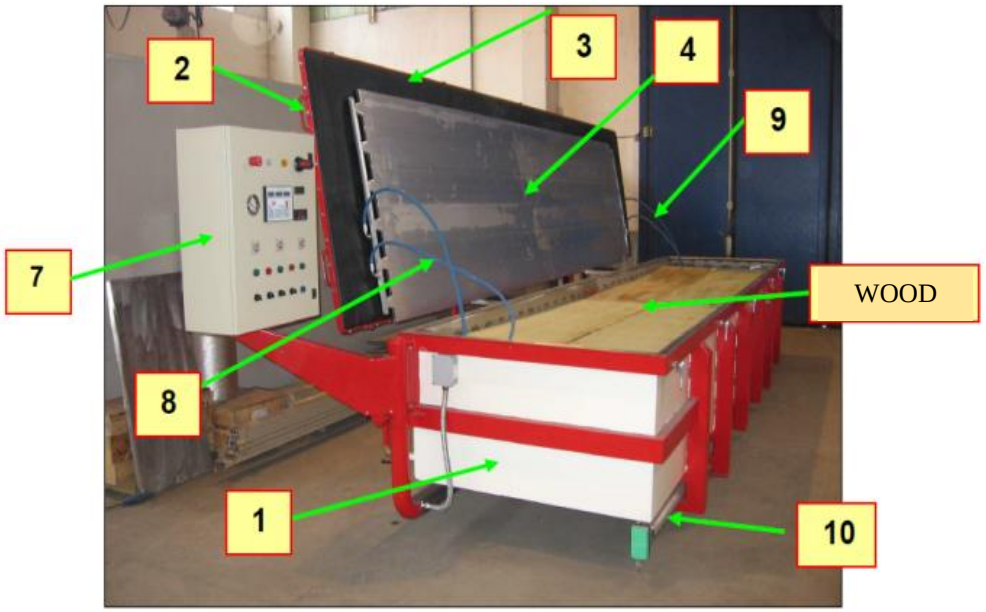

Figure 2.1. Press vacuum dryer model PRESS M 1.6

The thermo-treatment is applied to wood boards to 0% moisture content (MC). This value of MC is reached in vacuum condition in the same cylinder where the thermo-vacuum treatment is done. The temperature used during this drying phase don't have to pass the temperature (Tcr) above which occurs in the thermal modification inside the wood. This step is controlled by several sensors for the MC and depended from wood species.

For heating the indoor environment, the system uses diathermic oil. The heat is transferred by convection and the speed is proportional to the pressure reduction in the chamber. The pressure conditions are obtained by vacuum pump. The system is equipped with 9 sensors for measuring the temperature and 3 for measuring pressure. All probes are connected to the personal computer for monitoring and capturing process data. The figure below shows the layout of the plant (Figure 2.2).

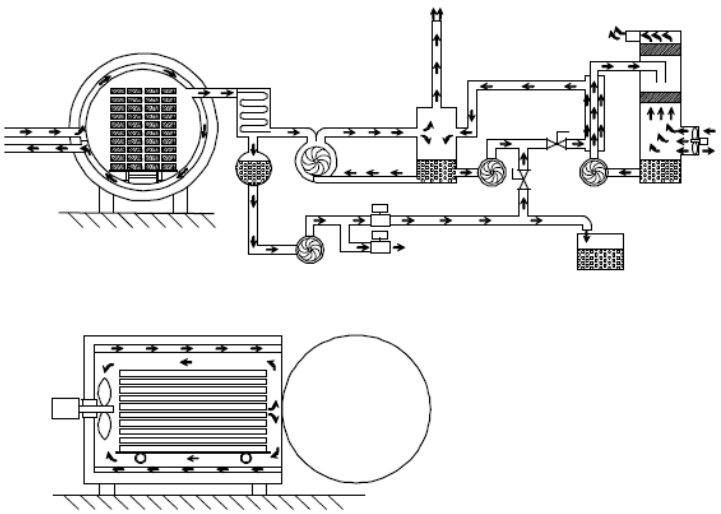

Figure 2.2. Thermovuoto® plant scheme

The operation of this technology begins with the manual loading of wooden boards to be dried inside the drying cell in alternating layers with the heating plates to form a pile that starts and ends with a plate. It is necessary to be careful to place the 3 wood moisture-measuring probes in three tables, chosen as a sample; subsequently, the cell is closed by positioning the deformable membrane cover.

Depending on the type of timber, its thickness and the desired final humidity, the operator must set the appropriate drying program using the appropriate manual timing control system, after which he can start the drying cycle that consists of mainly in three phases:

- Phase 1: wood preheating. During this phase, the plates heat up by contacting the wood, reaching the working temperature set by the treatment program;
- Phase 2: drying. In this phase, the vacuum pump allows extracting the air from the cell drying until the programmed working pressure is reached (variable between 150 and 400 mBar depending on the program made by the operator), while the heating system continues to supply heat to the wood through contact with the heating plates. The combined effect of vacuum and temperature stimulates the circulation of water from the heart to the surface

of the wood, from which it is removed in the form of steam, which, aspirated by the vacuum pump, is expelled into the external atmosphere, thus generating the drying of wood.

- Phase 3: cooling. During this phase the boiler is deactivated so that the plates do not transfer any more heat to the wood, which under the effect of evaporation of the residual water cools, while it continues to be subjected to the action of the vacuum and therefore of the pressure of the membrane of the lid.

After the drying phase, the boards are heat treated at different temperatures, depending on the different species. When the sensors inserted in the heart of the boards reach this value, the residence time between the plates is equal to 3 hours. The entire cycle has a duration of approximately 24 hours (Figure 2.3). The steps of the thermo-treatment are shown in the Figure 2.3.

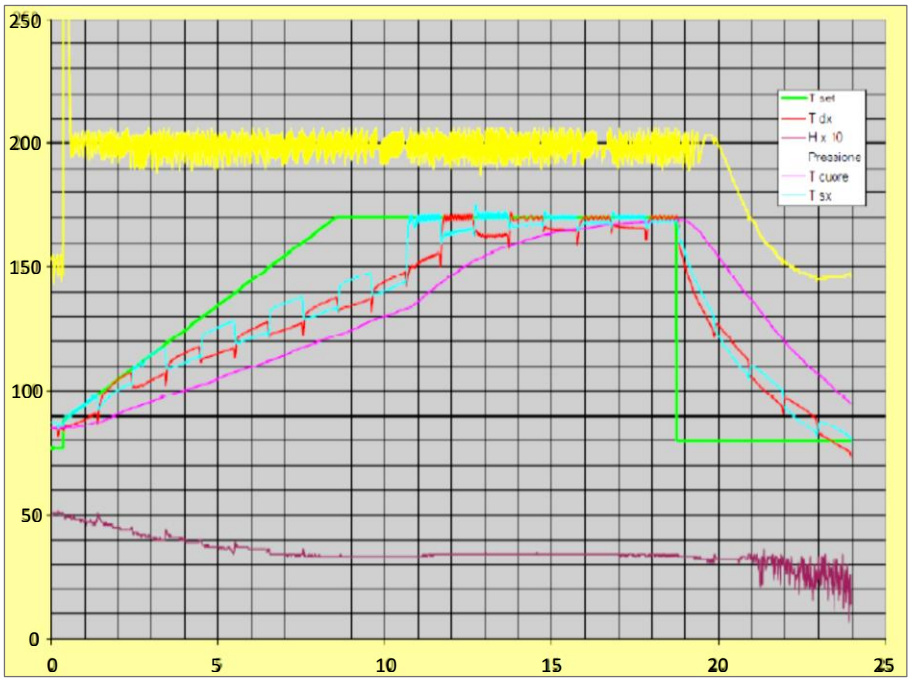

Figure 2.3. The heat treatment cycle (vacuum conditions for hours)

The wooden planks, which have become plastic due to the temperature and compression between two flat surfaces, are subjected to the phenomenon of

stretching the fibers and therefore become almost perfectly flat, with great advantage for the final quality of the dried wood.

2.2. General effects of the Thermo-Treatment

The temperature achieved in the heat treatment process is higher than those of conventional drying process, thus during heating not only removes water from wood but causes important chemical modifications of cell wall constituents and extractives of wood.

The chemical modification and degradation of component of wood occur by several reactions: dehydration, hydrolysis, oxidation, decarboxylation and so on[12]. Thus, a range of chemical reactions takes place simultaneously that are combinations of both endothermic and exothermic reactions, making determination of onset temperatures for different reactions nearly impossible. Analysis is complicated further by interactions between reactions in the different constituents. This means analysis performed on an isolated derivative of one of the components can be very different compared to what actually takes place inside wood.

Hemicellulose

The compound most sensitive to heat is also the most hydrophilic one, i.e., hemicellulose.

It is generally accepted that degradation of hemicellulose results in less accessible bonding sites for water (OH-groups). Hardwoods are less thermally stable than softwoods[13]. The lower stability of hardwood hemicellulose is thought to be mainly attributable to the higher presence of pentosans, which have a less favourable and thus a more reactive structure than hexosans.

The majority of the energy consumed by endothermic reactions below 150 °C is related to evaporation of cell wall bound water[13].

In order to get the improved properties in a reasonable time, heat treatment is normally performed at temperatures of at least 170 °C. According to Stamm (1964)[14] the dehydration is not the only explanation for lowered hygroscopicity but also formation of furfural polymers from degraded carbohydrates.

Degradation of hemicellulose also produces degradation products such as acetic acid, methanol and volatile heterocyclic compounds. The acetic acid is generated when the acetylated hydroxyl groups of the hemicellulose chains are split off.

Dimensional stability is dependent on the wood species and the anatomical direction considered, but in general after the thermo-treatment, the dimensional stability of wood boards increases thanks to reaction of cross-linking, destruction of several hydroxyl groups[15].

The transformation about hemicellulose, extractives compounds improves wood durability. Furthermore, the degradation of hemicellulose is the major factor for the loss of mechanical strength.

Cellulose

Crystalline cellulose starts to degrade at about 300 °C[16], which means that degradation of cellulose is not a big issue in heat treatment of wood. However, the degree of polymerization (DP) is already decreased at temperatures above 120 °C (100°C in isolated cellulose)[13]. Decrease in DP is due to chain scission (cleavage of the glucosidic bonding) that is accelerated by the presence of acids that are catalysing the reaction, and this implies the inaccessibility of hydroxyl groups to water molecules[17-19], which is one of the reasons that contribute to a decrease of equilibrium moisture content.

Sundqvist (2006)[20] analysed the molecule size of cellulose in heat treated wood by using intrinsic viscosity measurements and showed that heat treatment results in a considerable reduction in molecule size of cellulose. It

was also found that the reduction of intrinsic viscosity can be deferred by using buffer solutions at high pH during hydrothermal treatments at 180 °C.

Lignin

According to Sanderman and Augustin (1963)[21], lignin is the most thermally stable compound of wood when heating takes place in an inert atmosphere, but when oxygen is accessible, the oxidative decomposition of lignin can be substantial. However, even if lignin is considered the most stable component and does not undergo thermal degradation resulting in great mass loss, it undergoes modifications of its structure. Already at 130 °C, homolytic cleavage of the β-O-aryl ether linkage in lignin model compounds has been seen[22]. The formation of free radicals is believed to generate reactions creating condensation products and possibly also cross-linking between lignin and polysaccharides[23,24]. A decrease in methoxyl-group content might explain some of the improved dimensional stability found in heat-treated wood by increased cross-linking within the lignin complex[24].

Extractives

Recent studies have shown that fats and waxes migrate to the surface when pine sapwood is heat-treated at 100 °C-160 °C. In treatment above 180 °C, fats or waxes were no longer detectable. In treatments above 200 °C, resin acids were also undetectable[25].

According to Manninen (2002)[26], heat treatment lowers the emission of VOC by 8 times compared to air-drying in pine, but only 14 out of 41 identified compounds were found in both samples, which indicates that significant chemical changes had occurred.

Most of the wood extractives disappear upon heat treatment, especially the volatile compounds, but new extractives appear as degradation products of cell wall structural polymers[27,28].

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3. Wood Species

The main goal of this research was to explore and study the effects of thermo-vacuum treatment of *Cedrus deodara Roxb., Alnus cordata Desf., Populus nigra L., Larix decidua Mill., Paulownia tomentosa Steud., Castanea sativa Mill*. and *Quercus frainetto Ten.* woods on the content and composition of extractives.

The different structural and chemical compositions of hardwood and softwood have an important effect on wood modification.

The effect of treatment on low value softwood and hardwood species, such as the species covered by this study, is relatively little studied.

3.1 Cedrus deodara Roxb.

Cedrus deodara Roxb., *Pinaceae* family, is a species originating from the western Himalayan chains (Afghanistan, Pakistan, Kashmir, north-western India) (Figure 3.1, a). It was introduced in Europe for ornamental purposes in 1822 and is today widely used as an ornamental tree in large parks and gardens. It grows rapidly (up to 40-50 m tall and reaching more than 100 cm in diameter) from sea level to mountains. The sapwood is pink or greyish and differs from reddish/yellow heartwood. Its wood presents low hardness and high durability mainly in heartwood. The gum-resinous exudations are frequent. Its wood is used in naval construction, furniture, sculptures, temple, etc. From its aromatic characteristics wood oil is also extract and used as fragrance compounds natural product[1].

3.2 Alnus cordata Desf.

Alnus cordata Desf. belongs to the *Betulaceae* family and it is native to southern Italy where it is widespread, with particular reference to the Tyrrhenian regions (Figure 3.1, b).

It can grow very rapidly (up to 20 m tall and reaching 70-80 cm in diameter) in depressions at increased humidity and along water courses[2,3].

The bark is greyish/gray with lighter areas, but which tends to become dark with particularly dry climates.

It is widely used in reforestation throughout the Apennines and is also used to shade parks and roads, as it is a tree with rapid growth, high and compact foliage. It is mainly used for energy but also for furniture, paneling, plywood and paper pulp.

Thanks to the high concentration of tannins and other chemical compounds, alder wood is resistant and durable even under water. Thus, Italian alder wood, for its machinability and homogeneity, could be an interesting alternative for different industrial applications, especially for applications with improved durability.

3.3 Populus nigra L.

Populus nigra L. is a tree of the *Salicaceae* family that includes about thirty species commonly known as poplars (Figure 3.1, c). It is a geographically widespread tree species of which stands are found in large parts of Europe and Asia. The species is a typical pioneer species particularly in riverine areas. It is dioecious, heliophilous, and characterized by an efficient dissemination of both seeds and pollen, but also by a good ability to naturally undergo vegetative propagation. It is a species of both social and economic interest. In poplar-breeding programs, it plays a central role: 63% of the poplar cultivars

from the international register descend from this species, mainly as interspecific hybrids. In addition to wood production, it is used as a windbreak for the protection of agricultural land and for ornamental and landscaping purposes[4].

3.4 Larix decidua Mill.

Larix deciduas Mill., a deciduous conifer endemic to Europe belonging to the *Pinaceae* family, is an industrially important tree, characterized by a strong, water-resistant, and durable wood, but also flexible in thin strips (Figure 3.1, d). The heartwood is particularly weatherproof and, therefore, mainly used as construction timber. Due to its high resin content and difficult machinability, its use for furniture production is limited[5]. At present, larch sawdust is mainly used for the production of pellet fuels and contains phenolic compounds, such as lignans and flavonols (mainly dihydroquercetin and dihydrokaempferol)[6]. Barks and branches are waste material of the timber industry with important, but also partially underexplored, supplies of biologically-active compounds; this material can be considered a good source of antioxidants and phytoconstituents with possible use in cosmetic or nutraceutical products, furthermore, several conifer barks contain phytoconstituents, such as tannins, terpenes, and polyphenols[7].

3.5 Paulownia tomentosa Steud.

Paulownia tomentosa is a plant species belonging to the *Paulowniaceae* family, which includes 17 species of trees, all endemic to the South and East Asia (Figure 3.1, e). Paulownia wood, a light-colored hardwood, has been revered for centuries by Japanese craftsmen because of its workability and beauty. In the Japanese tradition, Paulownia was used to build kotos (Japanese

harps) because of the wood's superior acoustical quality, is now also used as timber and biomass, and in Chinese agroforestry systems because it grows fast, its wood is light but strong, its flowers are rich in nectar and its leaves make good fodder for farm animals. Paulownia wood also resists splitting and deformation in the drying process, and can retain nails and screws without splitting[8]. Fast developing programs for planting and growing Paulownia, with the help of financial support, are performed in Italy. Presently, they are the sources for the fast supply of high quality timber and energy.

3.6 Castanea sativa Mill.

Castanea sativa Mill., or sweet chestnut, is a plant species in the *Fagaceae* family, native to Europe and Asia Minor and a very long-lived tree, being able to reach 500 years of life (Figure 3.1, f). Castanea sativa heartwood is one of the most durable wood species in Europe. It reaches, in optimal conditions in the forest, 30-35 meters in height and considerable trunk diameters.

Due to its good durability, the wood is frequently used for utility poles and other outdoor applications, both in and above ground. In addition, this wood is used for manufacturing furniture, barrels and constructions, notably in southern Europe[9], and is sometimes used for the production of sulphate cellulose. One of the reasons for the good durability is the presence of extractives, predominantly tannins. Chestnut trees are a rich source of tannins, which were used in the past for the production of some medicines, leather production, as well as playing a part in the flavouring of various types of alcoholic beverages[10].

3.7 Quercus frainetto Ten.

Quercus is one of the most important woody genera due to its large number of species, including trees and shrubs in Turkey (Figure 3.1, g). Oak belongs to

the *Fagaceae* family, its botanical name is *Quercus frainetto Ten.*, and it is known by many common names across the globe such as Hungarian oak and Italian oak. It reaches 25 m in height after a very slow growth and the bark of the trunk is gray-brown.

These trees are also tolerant to different environmental and climatic conditions and contribute to erosion control. It is often used for firewood, and because of the rather high durability of its wood, Quercus frainetto wood sometimes has been used as construction material in civil engineering and mining. It is less suited for the manufacture of barrels and furniture[11].

Figure 3.1. Cedrus deodara Roxb.(a), Alnus cordata Desf.(b), Populus nigra L.(c), Larix decidua Mill.(d), Paulownia tomentosa Steud.(e), Castanea sativa Mill.(f) and Quercus frainetto Ten.(g) trees

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4. Materials and Methods

4.1. Sample Preparation and Heat Treatment

Paulownia tomentosa, Populus nigra and *Larix decidua* boards free of defect for each specie were supplied by a local manufactures located to Calitri (Avellino province, Campania Region, Italy).

Castanea sativa, Quercus frainetto, Alnus cordata and *Cedrus deodara* boards were obtained from a natural high forest located to Calvello municipality (Potenza province, Basilicata Region, Italy).

A total of eight sawn boards for each species with dimensions of 302 mm × 1850 mm × 15000 mm (thickness × width × length, respectively), having a moisture content of 20.0%, were used for the experiments.

Each wooden board was spitted into two parts in order to generate twin samples, corresponding to reference (no treated) and treated sample sets. Both wood drying and effective thermal treatment were performed in the same processor (autoclave) without removing samples.

The thermo-vacuum plant facility used for the test, following the description of Ferrari[1], was a semi-industrial prototype with an internal diameter of 1.7 m, modified to perform thermo-vacuum treatments at high temperature (up to 250 °C). The cylinder walls were heated by diathermic oil circulating between double layers of steel.

The samples were dried to a moisture content of 0.0% in the same cylinder prior to the thermo-vacuum treatments. The drying process of the samples having an initial moisture content of 20.0% was carried out at 100 °C and a pressure not exceeding 25,000 Pa, corresponding to a water boiling temperature of 65 °C[2].

In the next step, samples of the abovementioned species were exposed to the thermo-vacuum treatment. Each thermo-vacuum treatment consisted of a

heating phase that started at a temperature of 100 °C and reached a maximum of air temperature of 190 °C followed by a thermal treatment phase with a different constant air temperature for 2 h: 200 °C for *Cedrus deodara*, *Alnus cordata*, *Populus nigra*, and *Larix decidua*, 180 °C, 200 °C and 210 °C for *Paulownia tomentosa*, 170 °C for *Castanea sativa*, and 180 °C and 200 °C for *Quercus frainetto* (Figure 4.1).

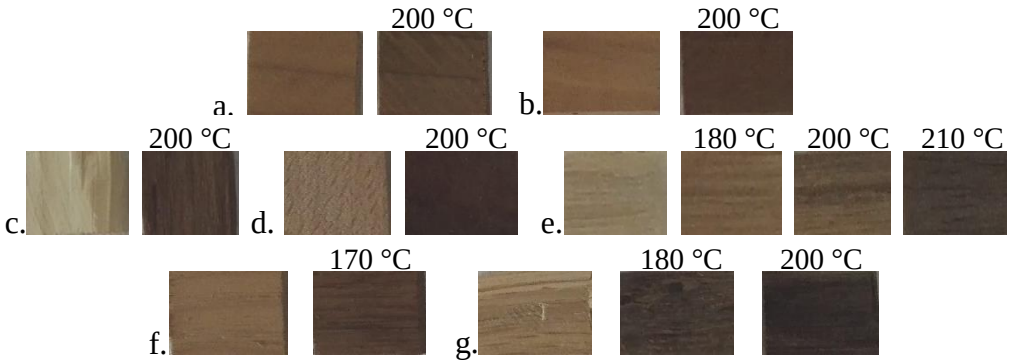

Figure 4.1. Cedrus deodara Roxb.(a), Alnus cordata Desf.(b), Populus nigra L.(c), Larix decidua Mill.(d), Paulownia tomentosa Steud.(e), Castanea sativa Mill.(f) and Quercus frainetto Ten.(g) untreated and thermotreated wood

The reason for the different treatment is that each type of wood is associated with one specific type of treatment, because of the different nature of hardwood and softwood[3].

Three-hour cooling phase decreased the temperature of the samples to 100 °C. The heating rate of the air was 12 °C per hour. The temperature variation of the diathermic oil, air, and wood samples was measured using thermocouples. Details regarding the ThermoVacuum process and its technical aspects are described in the literature[4-5].

4.2. Soxhlet Extraction

The samples of wood obtained in "Sample preparation and heat treatment" section were dried at 105 °C overnight. Then, it was ground through a 40 mesh

screen using a Wiley Mill (Thomas Scientific, Swedesboro, NJ, USA) in agreement with TAPPI T 204 cm-97 procedure[6]. This method can be used to determine the amount of solvent-soluble non-volatile material in wood.

The obtained material (1.0 g) was put into an extraction thimble, and it was put into a Soxhlet extraction apparatus (Figure 4.2). The extraction apparatus consisted of a 500-ml flask, Soxhlet tube, and 300-mm Allihn condenser. Samples were put in cellulose thimbles (33 × 80 mm) of medium porosity. The sample was extracted with 300 ml of 1:2 ethanol/toluene mixture (v/v) for 7 h. After this period, the solvent was then evaporated *in vacuo*, by using a rotary evaporator connected to a vacuum pump (Vacuubrand PC3001).

Extraction percentage was obtained by weighing the flask containing the residue and comparing the weight to that of the initial wood[7].

The obtained mixture was fractionated as follows: the mixture was treated with chloroform (20 mL) and filtered, the solvent was evaporated, and the residue was chromatographed using thin layer chromatography on silica gel in the presence of a fluorescent indicator and using a hexane/ethyl acetate (4:6) mixture. The different bands revealed by UV irradiation were scraped off and eluted with ethyl acetate. The solvent was evaporated, and the residue was analysed as described in Section 4.10. The chloroform insoluble fraction was treated as described in Section 4.9.

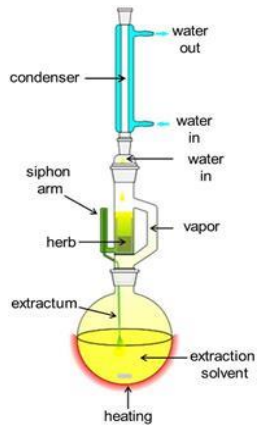

Figure 4.2. Soxhlet apparatus

4.3. Lignin Content

The remaining sawdust was transferred to a 50-ml beaker, a cold H_2SO_4 solution (72.0%) (15 ml) was added, and the mixture was frequently stirred for 2 h at room temperature according to TAPPI UM 250[8].

The mixture was then diluted to 3% (w/w) with 560 ml of distilled water, heated under reflux for 4 h, filtered, and washed with 500 ml of water. The residue was dried at 105 °C to a constant mass, and the lignin residue of each sample was finally determined.

The holocellulose content was determined by difference between the residue amount after extraction and the lignin content.

4.4. Autoclave Extraction

The Vapor Matic model 770 (Figure 4.3) is an autoclave with thermodynamic empty, suitable for cellular cultivation plots sterilization, glassware and hospital or laboratory refuses, that for this research was used to extract extractives of wood in a green way using water as solvent. The instrument has vertical charging of products, completely automatic sterilization cycle, thermoregulated and controlled by a microprocessor with high rejection HMOS logic, which permits to set up different times and temperatures. Temperature can goes from 100 °C to 134 °C equal to a 2 bar maximum pressure.

A sample of wood (10 g) was put into an airtight glass jar with 50 mL of distilled water and placed into the autoclave (Vapor Matic 770, Milan, Italy) for 20 min, at a temperature of 120 °C and a pressure of 1 bar for the extraction. Sample was filtered and frozen at a temperature of -28 °C.

Figure 4.3. Autoclave Vapor Matic model 770

Then, it was lyophilized to remove water[7]. The obtained mixture was fractionated as follows: the mixture was treated with chloroform (20 mL) and filtered, the solvent was evaporated, and the residue was chromatographed using thin layer chromatography on silica gel in the presence of a fluorescent indicator and using a hexane/ethyl acetate (4:6) mixture. The different bands revealed by UV irradiation were scraped off and eluted with ethyl acetate. The solvent was evaporated, and the residue was analysed as described in Section 4.10. The chloroform insoluble fraction was treated as described in Section 4.9.

4.5. Accelerated Solvent Extraction (ASE)

Extraction from wood chips was carried out using an Accelerated Solvent Extractor (Dionex ASE 350, Figure 4.4). Four grams of chips were weighted, dried over weekend and placed in the inox extraction cell in the oven of the instrument.

Extraction was carried out at the temperature of 60 °C using 1:2 ethanol/toluene mixture (v/v) as extraction solvent and of 120 °C using water as extraction solvent.

Figure 4.4. Dionex ASE model 350

After the injection of the solvent into the cell, a pressurised static extraction phase lasting 24 cycles of 30 min for ethanol/toluene and 6 cycles of 10 min for water was carried out (20 MPa), followed by a flow of fresh solvent. These settings produced the greatest yield of extractives and are similar to the optimal conditions used in other ASE methods[9,10]. They were successively evaporated in vacuo to remove ethanol/toluene mixture, and frozen at a temperature of -81 °C and lyophilized to remove water.

4.6. Synthesis of MoO_3 Supported on Silica Catalyst

The calculated amount of 5 g of aqueous ammonium heptamolybdate solution [81 wt% as MoO_3 (4.05 g)] was added into a suspension of 25 g of fumed silica (Aerosil 380) in distilled water (16.0% MoO_3/fumed silica). The solution was then evaporated to dryness at 100 °C in a rotary evaporator. The solid obtained was dried at 120 °C overnight and calcinated at 650 °C for 5 h in a muffle furnace, getting about 25 g of MoO_3/SiO_2[11]. XPS analysis of the catalyst showed a Mo $3d_{5/2}$/Si 2p ratio of 2.97, in agreement with the value of 3.06 reported in literature. The signal of Mo 3d was found at 233.3 eV, while that of Si 2p was detected at 103.8 eV. XPS spectra were acquired with a LH X1

Leybold (Germany) instrument using a dual achromatic Mg $K\alpha_{(1,2)}$ (1253.6 eV) and an Al $K\alpha_{(1,2)}$ (1486.6 eV) source operating at a constant power of 260 W. Wide and detailed spectra were collected using the fixed analyser transmission mode of operation with channel width of 1.0 or 0.1 eV, respectively, and pass energy of 50 eV. Under these conditions, the instrumental contribution to the line width is kept constant. The measured full width at half maximums of the Au $4f_{7/2}$ (84.0 eV) and Cu $2p_{3/2}$ (932.7 eV) signals used for calibration purposes are 1.3 and 1.6 eV, respectively.

4.7. Synthesis of $H_3PMo_{12}O_{40}$ Catalyst

The heteropolyoxometalate $H_3PMo_{12}O_{40}$ was prepared according to literature procedure[12]. 3.4 ml of H_3PO_4 (85.0% m/m) and 142 ml of $HClO_2$ 12 M were added successively to 210 ml solution of Na_2MoO_4 2.85 M. The warm solution took on a yellow colour, and the disodium salt $Na_2H[PMo_{12}O_{40}]$ has precipitated. After cooling to room temperature, the microcrystalline powder was filtered and air-dried. The salt was then recrystallized from a (20 ml/100 ml) Et_2O/H_2O mixture obtaining about 50.0 g of a greenish precipitate. $H_3PMo_{12}O_{40}$ was obtained from a solution of 50.0 g of $Na_2HPMo_{12}O_{40}$, just recrystallized, in 100 ml of H_2O, acidified by 25 ml of HCl 12 M, and extracted with 150 ml of Et_2O. When water was added, yellow crystals of $H_3PMo_{12}O_{40}$ precipitated. The solid so obtained was filtered and dried at 70-80 °C[13]. Fourier Transform-Infrared (FT-IR) spectrum of the catalyst showed peaks at ν_{max} 750, 830, 952, and 1064 cm^{-1}, in agreement with reported data[14]. Infrared spectra were obtained utilizing a Bruker Alpha FT-IR spectrophotometer (Bruker Photonics, Billerica, MA) configured for attenuated total reflectance (ATR) at ambient temperature (Figure 4.5).

The absorption at 750 and 830 cm^{-1} is due to Mo–O–Mo bending in Keggin structure, the absorption at 952 cm^{-1} is due to Mo=O stretching, while that at 1064 cm^{-1} is due to P–O stretching in Keggin structure.

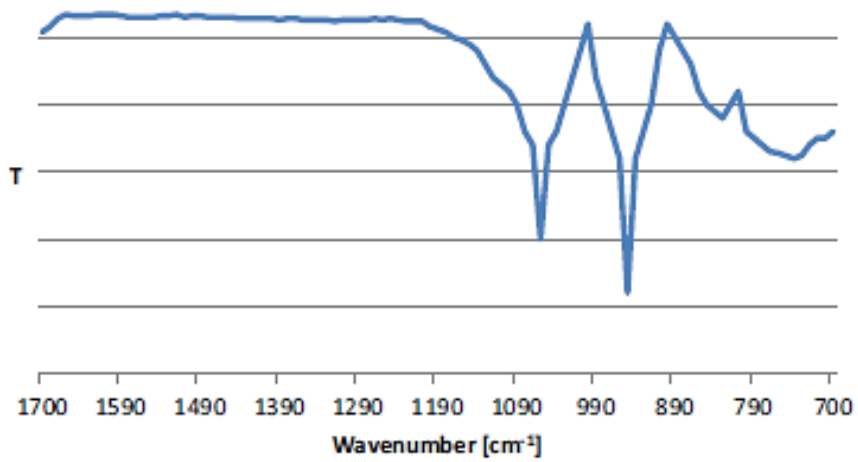

Figure 4.5. FT-IR of $H_3PMo_{12}O_{40}$

4.8. Synthesis of $H_3PMo_{12}O_{40}$ Nanoparticles

In a typical procedure 0.3 mmol of $H_3PMo_{12}O_{40}\cdot 13H_2O$ was dispersed in 50 mL hexane and the obtained mixture was stirred vigorously for 30 min at room temperature in order to forming a homogeneous dispersion. This dispersion was transferred into a teflon-lined stainless autoclave and filled 80% of its total volume. The autoclave was sealed and maintained at 150 °C for 12 h and then cooled to room temperature[14]. Finally, the produced powder was filtered and dried in vacuum. Particle size, morphology and size distribution of prepared nanoparticles was performed by SEM (scanning electron microscope) XL30 LaB_6 FEI image, and by this analysis we have seen that they are crystals of about 2 micrometers (Figure 4.6) and not nanoparticles as described in the procedure.

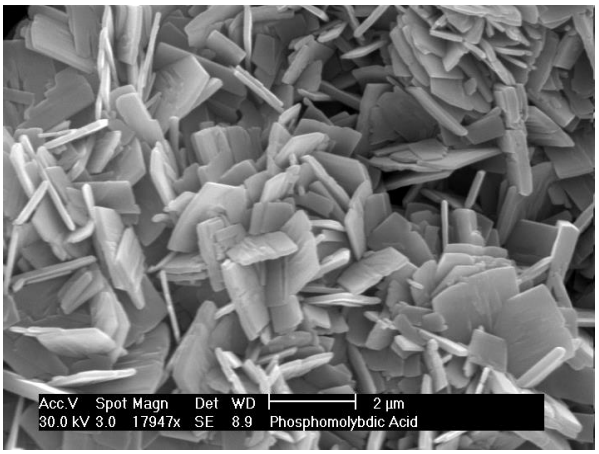

Figure 4.6. SEM image of $H_3PMo_{12}O_{40}$ Nanoparticles

4.9. Autoclave and Soxhlet Extraction in the Presence of Molybdenum catalyst

The sample (10.0 g) was put into an airtight glass jar with 50 ml of distilled water and 1.0 g of the catalysts MoO_3/SiO_2 or $H_3PMo_{12}O_{40}$, and was put into the Autoclave (Vapor Matic 770) for 20 min, at a temperature of 120 °C and a pressure of 1 bar. Then, the sample was filtered, frozen at a temperature of -28 °C, and was lyophilized to remove water[15]. While, for Soxhlet extraction, the sample (1.0 g) was put into an extraction thimble with 0.1 g of the catalysts MoO_3/SiO_2 or $H_3PMo_{12}O_{40}$, and it was put into a Soxhlet extraction apparatus. The sample was extracted with 300 ml of 1:2 ethanol/toluene mixture (v/v) for 7 h. After this period, the solvent was then evaporated *in vacuo*, by using a rotary evaporator connected to a vacuum pump (Vacuumbrand PC3001). Extraction percentage was obtained by weighing the flask containing the residue and comparing the weight to that of the initial wood.

4.10. Derivatization

To about 100 mg of the chloroform insoluble fraction of the extractives, 1 ml of pyridine and 1 ml of acetic anhydride were added, and the sample was

allowed to sit at room temperature for 48 h. Then, the solvent was exchanged with ethanol under reduced pressure followed by drying *in vacuo*[7,15]. The residue was chromatographed using thin layer chromatography on silica gel in the presence of a fluorescent indicator. The different bands revealed by UV irradiation were scraped off and eluted with ethyl acetate. The solvent was evaporated, and the residue was analyzed as described in Section 4.11.

4.11. Gas Chromatographic-Mass Spectrometric Analyses

Gas chromatography-mass spectroscopy (GC-MS) is one of the so-called hyphenated analytical techniques. As the name implies, it is actually two techniques that are combined to form a single method of analyzing mixtures of chemicals. Gas chromatography separates the components of a mixture and mass spectroscopy characterizes each of the components individually. By combining the two techniques, an analytical chemist can both qualitatively and quantitatively evaluate a solution containing a number of chemicals.

Gas chromatography (GC) was used to separate the compounds present in extractives while gas chromatography/mass spectroscopy (GC-MS) characterises the individual compounds based on their molecular structure.

Gas chromatography (GC) specifically gas-liquid chromatography is a highly developed technique, which involves a sample being vaporised while injected onto the head of the chromatographic column. The sample is transported through the column by the flow of an inert, gaseous mobile phase. The column itself contains a polymeric phase (stationary phase), which is coated onto the surface of an inert surface.

The mixture of compounds in the mobile phase interacts with the stationary phase. Each compound in the mixture interacts at a different rate. The lower the vapour pressure, i.e. the higher the boiling point, of any component of the analyte, the more time it spends dissolved in the stationary phase. Thus the

components emerge separately at different times[16]. Those that interact the fastest will exit (elute from) the column first. Those that interact slowest will exit the column last. By changing characteristics of the mobile phase and the stationary phase, different mixtures of chemicals can be separated. Further refinements to this separation process can be made by changing the temperature of the stationary phase or the pressure of the mobile phase. The GC method of separation used different length high temperature capillary columns with rapid temperature programming to separate compounds with a broad range of molecular weights in a short period of time. The components of a GC instrument are illustrated diagrammatically in Figure 4.7.

The carrier gas must be inert to both the sample and the solid phase. In this case the gaseous mobile phase carrier gas was helium at 0.8 ml/min. Liquid samples are injected into the column.

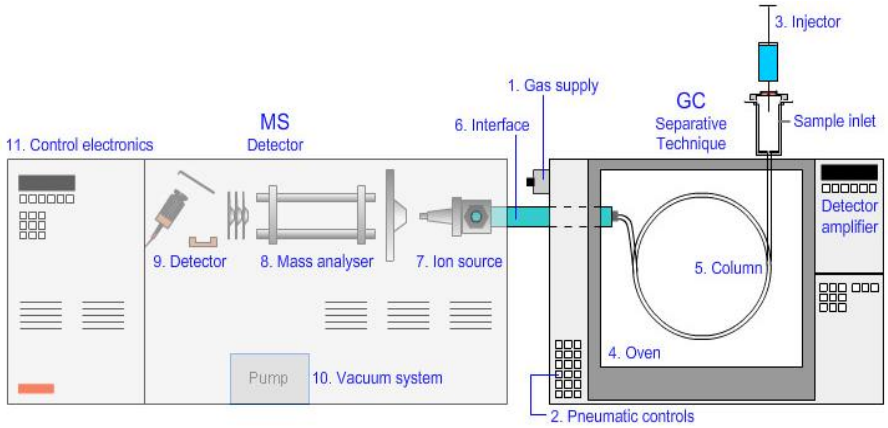

Figure 4.7. Schematic diagram of components of GC-MS instrument

The GC detector measures the concentration of the column effluent in a differential type analysis i.e. giving a zero signal for the carrier gas and a signal proportional to the concentration or mass of the eluted component. The type of detector used for this study was a quadrupole mass analyzer, HP 5973 MS selective detector (Agilent). The quadrupole consists of four parallel metal rods. Each opposing rod pair is connected together electrically, and a radio

frequency (RF) voltage with a offset voltage is applied between one pair of rods and the other. Ions must travel through the quadrupole rods. Only ions of a certain massto-charge ratio will reach the detector for a given ratio of voltages: other ions have unstable trajectories and will collide with the rods. This permits selection of an ion with a particular m/z or allows the operator to scan for a range of m/z-values by continuously varying the applied voltage[17].
In mass spectroscopy (MS), the sample (liquid, solid, solution, or vapour) enters the vacuum chamber through an inlet and is subjected to bombardment by an electron beam. The resultant ions are sorted in the mass analyzer according to their mass-to-charge ratios and then collected by a detector where the ion flux is converted to a proportional electrical current that is used to produce a mass spectrum[18].
GC-MS analyses used was an HP 6890 GC system equipped with a Phenomenex Zebron ZB-5 MS capillary column (30-m × 0.25-mm i.d. × 0.25 μm FT) (Agilent, Milan, Italy) with an HP helium as carrier gas.
As the compounds are separated, they elute from the column and enter a detector. The detector is capable of creating an electronic signal whenever the presence of a compound is detected; the greater the concentration in the sample, the bigger the signal. The signal is then processed by a computer, that generates a graph, called a chromatogram. Each of the peaks in the chromatogram represents the signal created when a compound elutes from the GC column into the detector. The time from when the injection is made (time zero) to when elution occurs is referred to as the retention time (RT). The x-axis shows the RT, and the y-axis shows the intensity (abundance) of the signal (Figure 4.8).

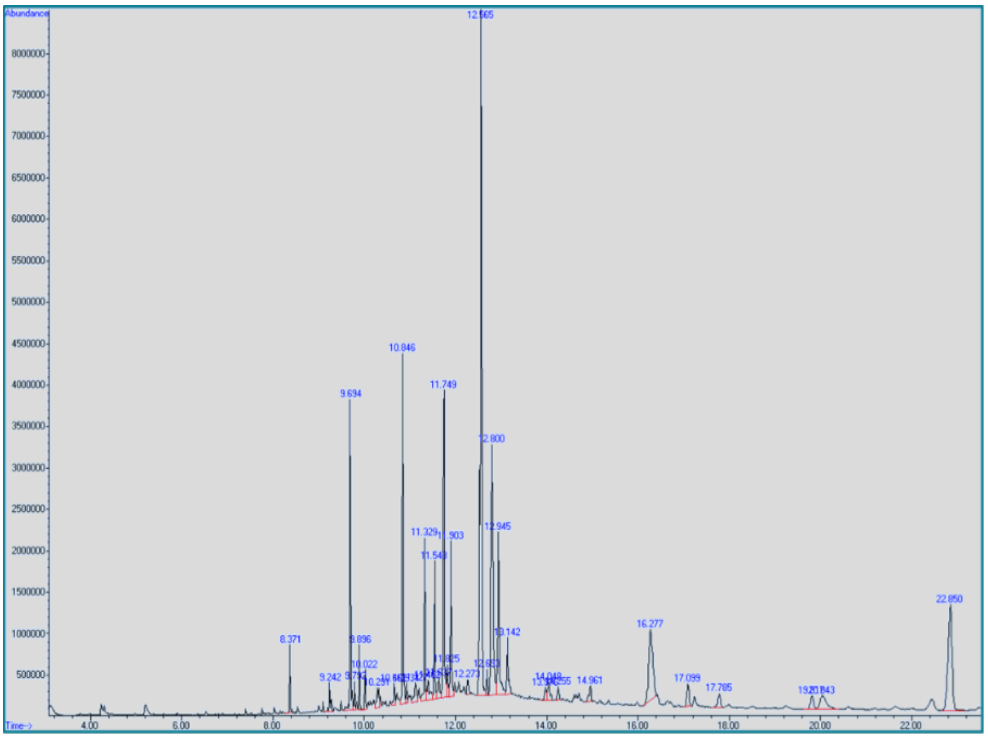

Figure 4.8. Chromatogram generated by a GC-MS

The conditions for the GC-MS analysis used are shown in Table 4.1.

CONDITIONS FOR GC-MS ANALYSIS	
Injector Port Temperature	250 °C
Initial Temperature	80 °C for 3 min
Ramp	20 °C /min to 250 °C hold for 20.00 min
Type of Column	HP-5MS, J&W Scientific
Column dimensions	L= 30 m, I.D= 250 µm and DF= 0.25 µm
Flow	0.8 mL/min
Average velocity	33 cm/sec

Table 4.1. Conditions used for GC-MS analysis

4.12. A new method: Two-dimensional Thin Layer Chromatography

The extractives obtained by Accelerated solvent extraction (ASE) techniques were chromatographed by using thin layer chromatography[19].

Because of the wide variety of fatty acids contained in wood, the characterization of its triglycerides (TGs) is a complex and difficult task. Before quantitative analysis, TGs must be grouped on the basis of some of their common characteristics (molecular weight, degree of unsaturation, etc.).

The TLC technique on silica gel with silver nitrate ($AgNO_3$-TLC) separates fat TGs and fatty acids according to the degree of unsaturation because of weak interactions between the π electrons of the double bonds and the silver ions[20](Figure 4.9).

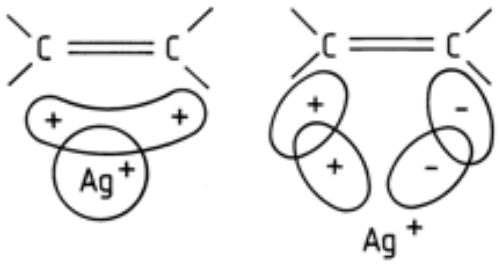

Figure 4.9. Model of interaction between a silver ion and an olefinic double bond

Other factors, such as geometric configuration and chain length, are also involved in TG separation on silica gel.

This separation, which is carried out prior to gas chromatography and/or mass spectrometry, has been used by Myher et al. (1988)[20] to identify and quantify over 100 TGs (the most significant from a quantitative standpoint) from a butter distillate.

One problem is the inherent difficulty of isolating all the individual components by silicic acid column or one-dimensional TLC.

Two-dimensional thin-layer chromatography (TLC) is a technique which has had limited application except in certain fields such as amino acids,

carbohydrates and phospholipids[21,22]. While many reported applications rely on the use of two different solvent systems acting on the same support system, combinations of normal, reversed and silver nitrate coated phases have also been reported.

Kaufman and co-workers[23] separated mixtures of lipids first by class of compound on silica gel, then coated the plate with dodecane or paraffin, and separated individual classes by carbon number in the second direction.

More recently, in a rather novel procedure, mercuric adducts were separated by degree of unsaturation in one direction, the adducts decomposed, the plate impregnated with paraffin, and partition chromatography carried out in the second direction.

Based on this background, in this research using two-dimensional TLC, we have developed a system that allows the complete separation of all known fatty acids and triglycerides components of wood extractives, and thus also allows an estimate of changes due to heat treatment: two-dimensional TLC through esterification with $NaOCH_3$ and the use of $AgNO_3$-TLC[24].

After the first development of the sample with hexane/ethyl acetate (4:6), the solvent residues were removed from the plate, and the bands were sprayed with $NaOCH_3$ (2 M); than the plate was plunged rapidly into a 10% (w/v) solution of $AgNO_3$ in water/ethanol (50:50), activated at 100 °C for 20 minutes, and developed in the second direction with chloroform/methanol (95:5).

This system allows the complete separation of all the known fatty acids and of the triglyceride components of the wood extractives[25] and thus also allows an estimate of changes due to heat treatment. A fingerprinting of the triglycerides of the extractives could be obtained.

4.13. References

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5. Results and Discussion

Heat treatment of wood changes its chemical composition by degrading cell wall compounds and extractives. The chemical changes due to heating depend on the duration and temperature of the treatment, the temperature being the main factor[1]. For low temperatures, the wood dries, beginning with the loss of free water and finishing with bound water, whereas at high temperatures the carbonization processes start with formation of CO_2 and other pyrolysis products.

5.1. Lignin and Holocellulose yield

The following table (Table 5.1) shows the results relative to the percentage of lignin and holocellulose of the wood species under study, both of untreated and thermotreated samples.

Wood species	Lignin	Holocellulose
Cedrus D.	29.0 %	62.4 %
Cedrus D. 200 °C	34.9 %	59.1 %
Alnus C.	25.1 %	71.7 %
Alnus C. 200 °C	38.8 %	57.5 %
Castanea S.	27.8 %	64.8 %
Castanea S. 170 °C	48.1%	36.2%
Q. Frainetto	39.6%	56.7%
Q. Frainetto 180 °C	45.1%	45.5%
Q. Frainetto 200 °C	40.4%	51.1%
Populus N.	37.7%	53.7%
Populus N. 200 °C	32.7%	61.6%
Paulownia T.	37.6%	54.5%
Paulownia T. 180 °C	31.9 %	60.8 %
Paulownia T. 200 °C	39.0%	45.7%
Paulownia T. 210 °C	39.5%	50.3%
Larix D.	45.2%	51.9%
Larix D. 200 °C	54.5%	42.1%

Table 5.1. Percentage (%) of holocellulose and lignin of the samples

Lignin is the most thermally stable compound of wood when heating takes place in an inert atmosphere[2], but when oxygen is accessible, the oxidative decomposition of lignin is substantial.

Already at 130 °C, homolytic cleavage of the β-O-aryl ether linkage in lignin model compounds has been seen and the formation of free radicals is believed to generate reactions creating condensation products and possibly also cross-linking between lignin and polysaccharides[3].

In fact, as you can see in Figure 5.1, the lignin content of the samples is relatively higher in the treated samples than in the untreated samples[4-6].

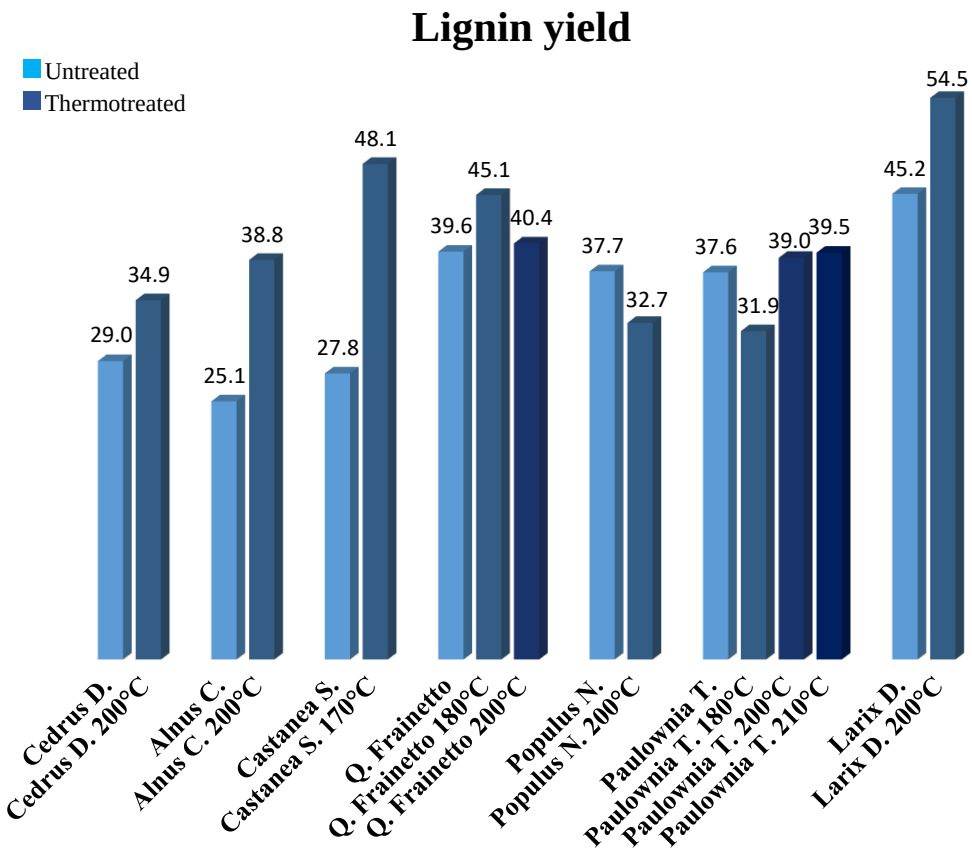

Figure 5.1. Lignin yield (%) of the samples

In particular, the content of lignin increased by 5.9% for *Cedrus d.*, by 13.7% for *Alnus c.*, by 20.3% for *Castanea s.*, by 5.5% and 0.8% for *Quercus f.* at 180

°C and 200 °C respectively, by 9.3% for *Larix d.*, and by 1.4% and 1.9% for *Paulownia t.* at 200 °C and 210 °C respectively, but the amount of lignin decreased, compared to those of untreated wood, by 5.0% for *Populus n.* and by 5.7% for *Paulownia t*. at 180 °C.

The change in lignin content is recognized to be strongly dependent on the temperature, treatment time, and on the characteristics of the wood species[7].

The thermal stability of lignin is therefore quite high and it is difficult to decompose. In fact, lignin has different structure with three types of benzene-propane units, which are heavily cross-linked and exhibit higher molecular weight than those of hemicellulose and cellulose[8,9].

It is generally accepted that the increase in lignin content in the thermo-treated wood is due to the loss of polysaccharide material that occurs during heating. However, as suggested by Esteves and Pereira (2009)[10], the increase in lignin content of the heat-treated wood cannot be considered pure lignin since polycondensation reactions occur in the cell wall of other wood components, resulting in polymerization and in further cross-linking, thus increasing the apparent content of lignin[9].

The cleavage of the ether linkages, especially β-O-4, leads to the formation of free phenolic hydroxyl groups and α- and β-carbonyl groups[11], which are responsible for cross-linking via formation of methylenic bridges. The methoxyl content decreases and the new reactive sites on the aromatic ring can lead to further condensation reactions[12].

Lignin can represent a feedstock for aromatic chemicals[13,14]. In particular, vanillin can be obtained through a lignin depolymerisation process[15,16], and vanillin, besides aroma application, can be the starting material for the production of new polymers, such as epoxy polymers, polycarbonates[17], and polyesters[18].

Another significant chemical compound that can be obtained from wood is ferulic acid. Ferulic acid can be used as precursor of vanillin, or for its biological properties, such as antioxidant, anti-diabetic, anticancer activities, as ultraviolet (UV) absorber in cosmetic applications, or in food preservatives[19]. Furthermore, the antioxidant activity of phenolic compounds extracted from different wood has been described[20].

As regards the holocellulose instead, while crystalline cellulose starts to degrade at about 300 °C[21], which means that degradation of cellulose is not a big issue in heat treatment of wood, hemicellulose is the compound most sensitive to heat is also the most hydrophilic one.

The histogram 5.2 shows the decrease of holocellulose in the thermo-treated samples compared to the untreated samples.

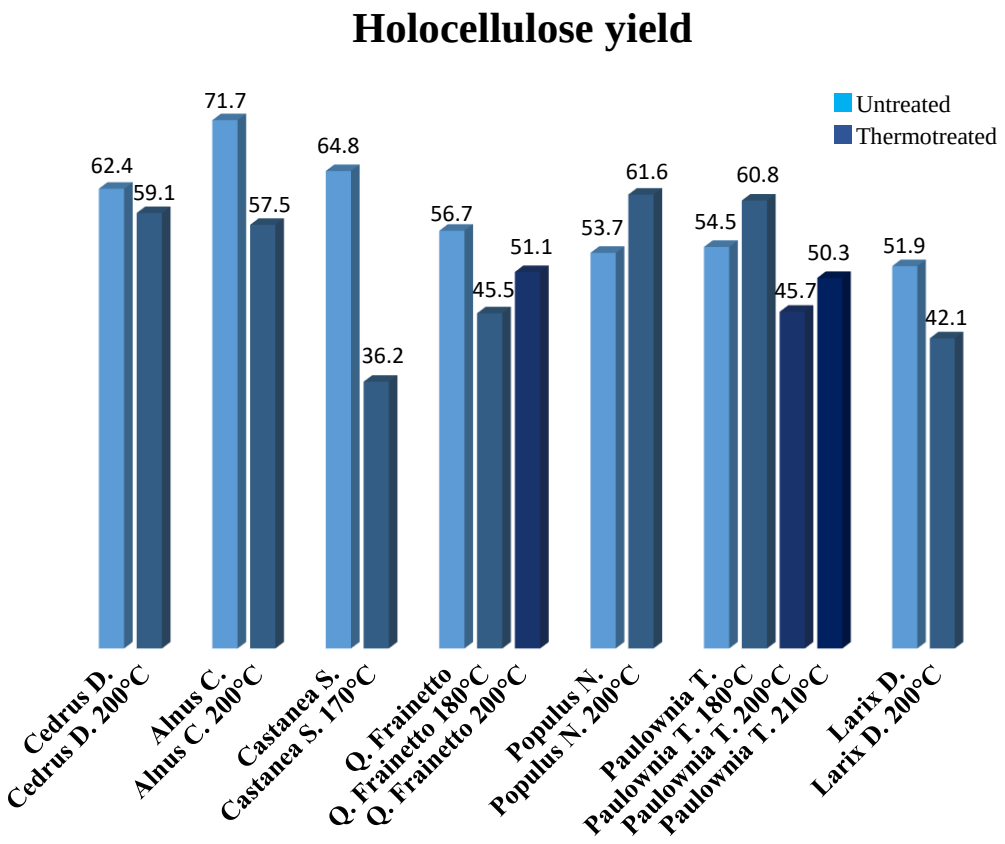

Figure 5.2. Holocellulose yield (%) of the samples

In particular, the content of holocellulose decreased by 3.3% for *Cedrus d.*, by 14.2% for *Alnus c.*, by 28.6% for *Castanea s.*, by 11.2% and 5.6% for *Quercus f.* at 180 °C and 200 °C respectively, by 9.8% for *Larix d.*, and by 8.8% and 4.2% for *Paulownia t.* at 200 °C and 210 °C respectively, but the amount of holocellulose increased, compared to those of untreated wood, by 7.9% for *Populus n.* and by 6.3% for *Paulownia t.* at 180 °C.

The increase in cellulose content of *Populus n.* and *Paulownia t.* after the thermal treatment is a new behaviour that can be explained only by assuming that in those species, the thermo-treatment induced a preferential lignin degradation with consequent decrease also in compounds deriving from lignin, such as the extractives.

It is a fact that the differences in the thermal decomposition of hemicellulose and cellulose are not well known. The higher activity of hemicellulose during thermal decomposition might be attributed to its chemical structure. Hemicellulose has a random amorphous structure and is easily hydrolysed[8,9]. In contrast, the cellulose molecule is a long polymer of glucose units, and its crystalline regions improve the thermal stability of wood[8,9].

The hemicelluloses therefore are the first structural compounds to be thermally affected, even at low temperatures. The degradation starts by deacetylation, and the released acetic acid acts as a depolymerisation catalyst that further increases polysaccharide decomposition. The content of carbohydrates decreases with the severity of the treatment and depends on wood species[22]..

5.2. Extractives yield

This section looks at the amount of extractives removed from the species by using hot water in an autoclave and a Soxhlet procedure in 1:2 ethanol/toluene mixture (v/v).

The extraction of the samples in the autoclave resulted in a reduced amount of extractives with respect to Soxhlet extraction in ethanol/toluene, as shown in Table 5.2, demonstrating that the content of the extractives also depends on the different solvents used.

Wood species	**Ethanol/Toluene**	**Water**
Cedrus D.	8.6%	2.8%
Cedrus D. 200 °C	6.0%	1.5%
Alnus C.	3.2%	0.8%
Alnus C. 200 °C	3.7%	1.6%
Castanea S.	7.4%	4.2%
Castanea S. 170 °C	15.7%	7.6%
Q. Frainetto	3.7%	5.3%
Q. Frainetto 180 °C	9.4%	4.8%
Q. Frainetto 200 °C	8.5%	3.3%
Populus N.	8.6%	0.5%
Populus N. 200 °C	5.7%	1.6%
Paulownia T.	7.9%	0.8%
Paulownia T. 180 °C	7.3%	1.0%
Paulownia T. 200 °C	15.3%	1.4%
Paulownia T. 210 °C	10.2%	0.9%
Larix D.	2.9%	1.4%
Larix D. 200 °C	3.4%	1.6%

Table 5.2. Extraction yield in percentage (%) for wood species

The ability of individual solvents were evaluated to determine which solvent extracts the most and whether the polarity index of the solvent has an impact on the amount and compounds extracted.

The difference of the solvents can be attributed to the different amounts of material extracted.

For all wood species, greater amounts of extracts were obtained by the ethanol/toluene blend[4-6].

Following the heat treatment, the content of extractives (by using ethanol/toluene) increased by 0.5% for *Alnus c.*, by 8.3% for *Castanea s.*, by 5.7% and 4.8% for *Quercus f.* at 180 °C and 200 °C respectively, by 0.5% for *Larix d.*, and by 7.4% and 2.3% for *Paulownia t.* at 200 °C and 210 °C, respectively. On the contrary, after the extraction by using Soxhlet apparatus, the amount of the extractives decreased by 2.6% for *Cedrus d.*, by 0.6% for *Paulownia t.* at 180 °C and by 2.9% for *Populus n.*, compared to those of untreated wood (Figure 5.3). That can be explained only by assuming that in those species, the thermo-treatment induced a preferential lignin degradation with consequent decrease also in compounds deriving from lignin, such as the extractives.

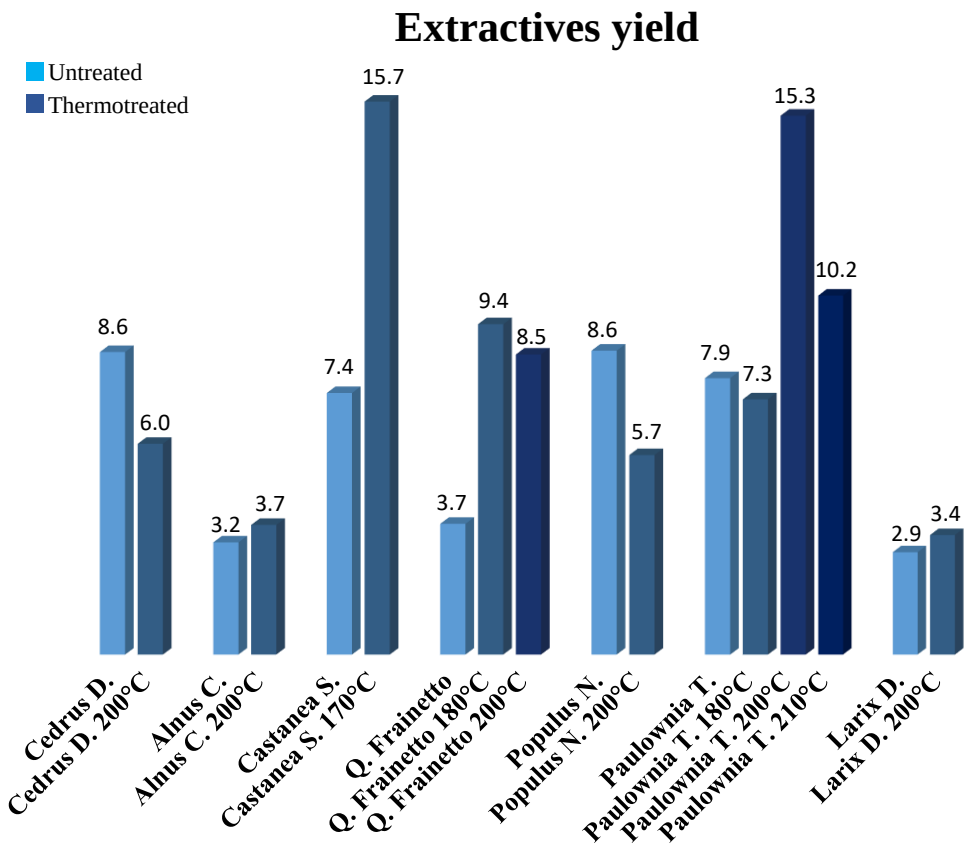

Figure 5.3. Amount of extractives (%) by Soxhlet extraction of samples

When the extraction was performed in an autoclave using water, the content of the extractives increased by 0.8% for *Alnus c.*, by 3.4% for *Castanea s.*, by 0.2% for *Larix d.*, by 1.1% for *Populus n.*, and by 0.2%, 0.6% and 0.1% for *Paulownia t.* at 180 °C, 200 °C and 210 °C respectively, but the amount of the extractives decreased by 0.5% and 2.0% for *Quercus f.* at 180 °C and 200 °C respectively, and by 1.3% for *Cedrus d.*, compared to those of untreated wood (Figure 5.4).

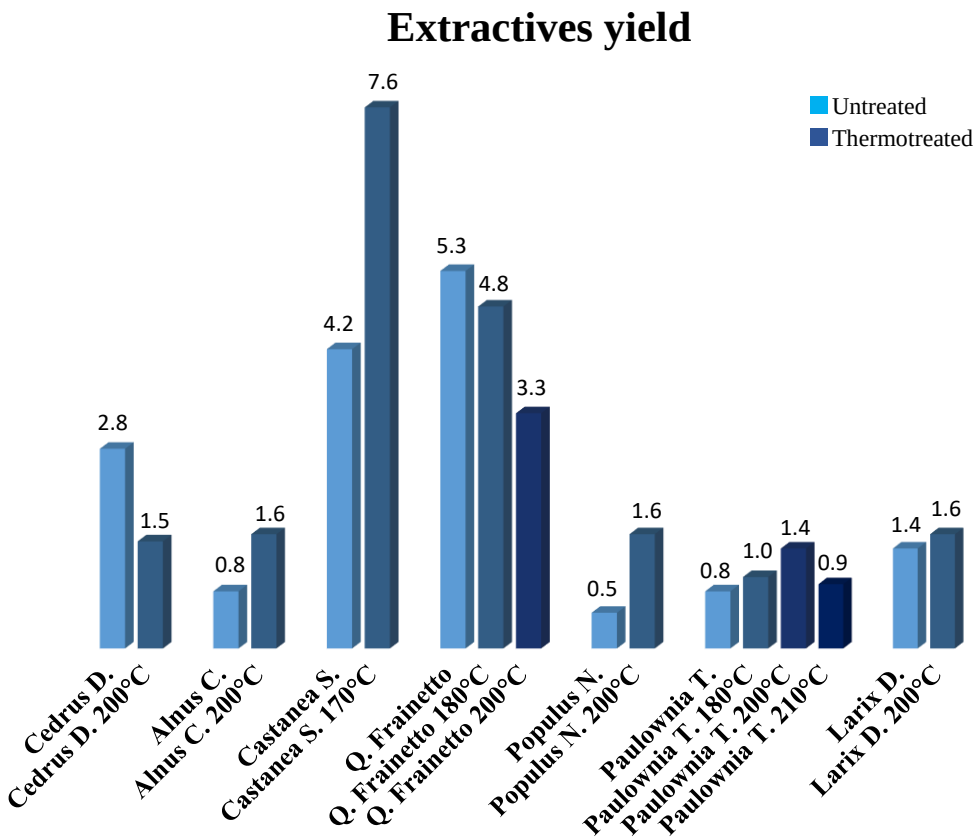

Figure 5.4.Amount of extractives (%) by Autoclave extraction of samples

The increase in extractives in the heat-treated samples, compared to the untreated ones, in the extraction with water in an autoclave is due to polysaccharide degradation.

Conversely, amounts of extracts obtained from *Cedrus d.* and *Quercus f.* wood by using water, were not higher for thermo-treated than for untreated wood. These data are in agreement with the reported greater cross-linking of thermo-treated wood compared to untreated wood[23]. During thermo-treatment, polycondensation reactions with other cell-wall components occur, resulting in further cross-linking. These reticulation reactions make it difficult to extract chemical compounds from wood.

As will be described below, the composition of water extractives mainly depends on the presence of pentoses and hexoses. It is reasonable that in thermo-treated species where the cellulose amount increases, the fraction that can be extracted in water increases[4-6].

From the results, it can also be concluded that the polarity of the solvents impacts on the yield of extractives. The polarity of the solvent determines what type of compounds it is able to dissolve. It was documented that the solvent plays a role in the material removed, in fact the polarity of the solvent has been suggested as the reason for the difference in the amount of extracts recovered[24].

Ethanol/toluene mixture removed a higher amount of extractives than water, which can be attributed to the fact that ethanol removes non-resinous compounds as well such as short polymers and lignin.

The last extraction method utilized was an Accelerated Solvent Extraction (ASE). ASE is a subcritical fluid application where organic solvents are heated above their boiling points but kept in the liquid state by pressurizing the system. Such an arrangement can greatly increase sample penetration and analyte desorption, often resulting in decreased preparation time and solvent usage.

Initial experiments set out to establish the optimal standard conditions to evaluate the extractives content of *Paulownia t.* untreated and *Paulownia t.* thermo-treated at 180 °C, 200 °C and 210 °C in ethanol/toluene and water.

The temperature of 60 °C for 24 cycles of 30 min using 1:2 ethanol/toluene mixture (v/v) as extraction solvent and of 120 °C for 6 cycles of 10 min using water as extraction solvent have been identified as the best extraction conditions.

These settings produced the greatest yield of extractives and are similar to the optimal conditions used in other ASE methods[25]. Longer extraction times were not found to increase this yield.

Table 5.3 presents the results from these two extraction conditions.

	Extractives (%)	
Wood species	**Ethanol/Toluene**	**Water**
Paulownia t.	2.5	4.4
Paulownia t. at 180 °C	3.1	5.1
Paulownia t. at 200 °C	3.5	5.3
Paulownia t. at 210 °C	3.1	3.0

Table 5.3. Extraction yield of *Paulownia t.* samples by using ASE method

In addition, these results confirmed the increase in extractives in the heat-treated samples, compared to the untreated ones, especially for *Paulownia t.* at 180 °C and 200 °C, while there was a decline in extractives yield for *Paulownia t.* at 210 °C, suggesting that at high temperatures the carbonization processes started with formation of CO_2 and other pyrolysis products.

The extraction of the samples in the ASE method showed, as a function of the different solvent used, an opposite trend with respect to the Soxhlet and autoclave methods previously used.

In fact, as can be seen in the Figure 5.5, by using ethanol/toluene (a) resulted in a reduced amount of extractives with respect to Soxhlet extraction (c) with the same solvent, while by using water (b) resulted in an increased amount of extractives with respect to autoclave extraction (d) with the same solvent. This demonstrated that the content of the extractives also depends on the different solvents and methods used.

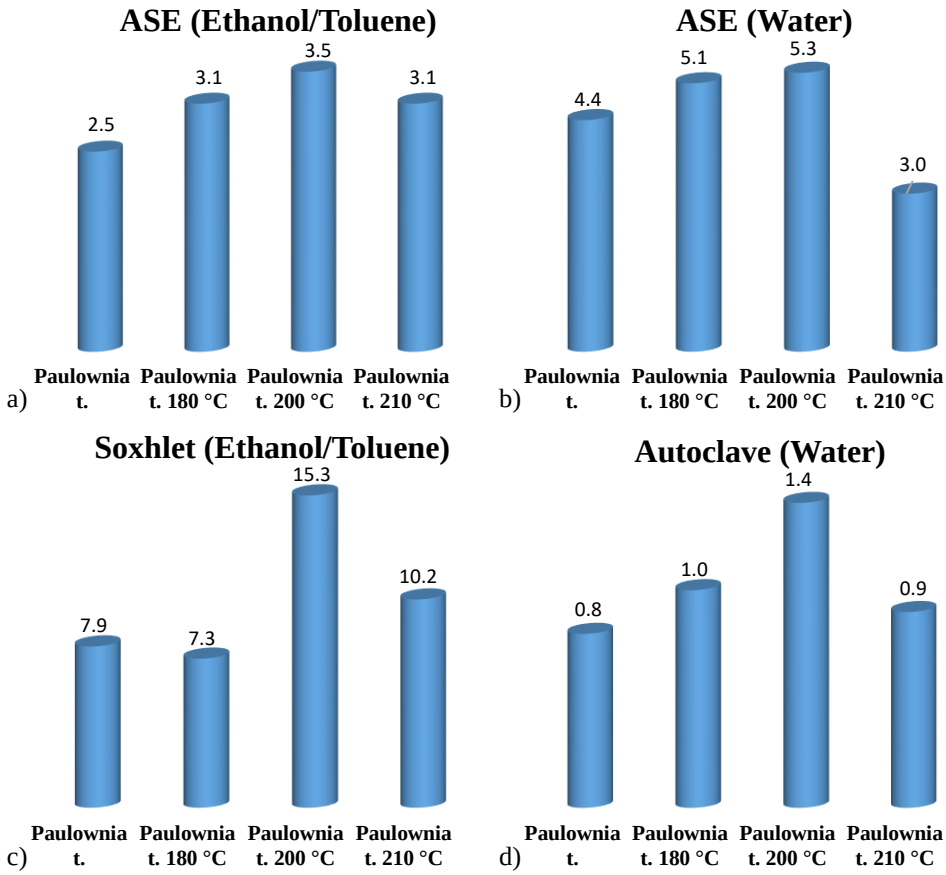

Figure 5.5. Amount of extractives (%) obtained by ASE (a,b), Soxhlet (c) and Autoclave (d) extraction of *Paulownia t.* samples

It is possible that the sample size in these experiments could have a greater impact on the extractives content determination, because 4.0 g of wood chips were used for ASE extraction, while 1.0 g of ground wood was used for Soxhlet extraction.

On the other hand, wood chips were used also for autoclave extraction, but ASE uses a sub-critical (i.e. superheated) solvent that has greater sample penetration capabilities than autoclave method, and can produce relatively larger extraction yields, by using the same solvent[26].

In terms of extraction time, the Soxhlet method requires a minimum of 7 h of solvent cycling, whereas ASE requires 10 min of static equilibration. While the Soxhlet procedure used 300 mL of ethanol/toluene per gram of sample, ASE utilized a total of 40 mL.

ASE was 30 times faster and used 85.0% less solvent than Soxhlet extraction. It can be performed with simple pumping and heating equipment, is compatible with numerous organic solvents of varying polarity, and can provide an efficient alternative to using a Soxhlet apparatus for extracting wood.

Moreover, the best extraction yield with ASE method was obtained using water as solvent, a great advantage and a key feature for green chemistry.

5.3. Extractives yield in the Presence of Molybdenum catalyst

The possible effect of oxidants of Mo(VI) to induce degradation of wood during the autoclave and Soxhlet extractions was tested. The effect of the presence of 10% of a polyoxometalate compound, such as $H_3PMo_{12}O_{40}$ and that of silica-supported MoO_3, was tested in particular for *Cedrus d.*, *Alnus c.* and *Castanea s.* thermo treated at 170 °C. The results are reported in Table 5.4.

Clearly, the presence of the Mo(VI) derivatives induced an extensive degradation of wood, a both lignin and cellulose oxidative depolymerization, thereby allowing an increased recovery of extractives.

It is noteworthy that when MoO_3/SiO_2 was used as additive, it increased the extractives soluble in chloroform[4-6].

	Ethanol/Toluene		Water	
Wood species	**Extractives (%)**	**$CHCl_3$ Soluble Fraction (%)**	**Extractives (%)**	**$CHCl_3$ Soluble Fraction (%)**
Cedrus d.	8.6	33.0	2.8	40.7
Cedrus d. with $H_3PMo_{12}O_{40}$	17.0	57.8	7.5	27.6
Cedrus d. with MoO_3/SiO_2	7.9	76.5	5.1	36.3
Alnus c.	3.2	/	0.8	29.5
Alnus c. with $H_3PMo_{12}O_{40}$	15.5	6.1	4.2	/
Alnus c. with MoO_3/SiO_2	3.0	37.6	4.4	/
Castanea s. 170 °C	15.7	3.2	7.6	2.7
Castanea s. 170 °C with $H_3PMo_{12}O_{40}$	19.1	12.4	15.8	1.2
Castanea s. 170 °C with MoO_3/SiO_2	14.8	15.5	8.8	0.3

Table 5.4. Amount of extractives and solubility of samples by Soxhlet and autoclave extraction in the presence of the Mo catalysts

In particular, in Soxhlet extraction by using ethanol/toluene, $H_3PMo_{12}O_{40}$ increased the amount of extractives by 8.4% for *Cedrus d.*, by 12.3% for *Alnus c.* and by 3.4% for *Castanea s.* at 170 °C, while on the other hand MoO_3/SiO_2 decreased the amount of extractives by 0.7% for *Cedrus d.*, by 0.2% for *Alnus c.* and by 0.9% for *Castanea s.* at 170 °C, compared to the control sample extracted without catalysts (Figure 5.6).

Furthermore, the obtained extractives were fractionated considering the capability of the components to be soluble in chloroform (dielectric constant 4.81, able to dissolve apolar and medium polar compounds).

In the case of the present samples, in Soxhlet extraction in the presence of $H_3PMo_{12}O_{40}$ the solubility of the extractives increased by 24.8% for *Cedrus d.*,

by 6.1% for *Alnus c*., and by 9.2% for *Castanea s*. at 170 °C, while it was increased by 43.5% for *Cedrus d*., by 37.6% for *Alnus c*., and by 12.3% for *Castanea s*. at 170 °C, when the extraction was performed in the presence of MoO_3/SiO_2, with respect to the control sample extracted without catalyst. (Figure 5.6).

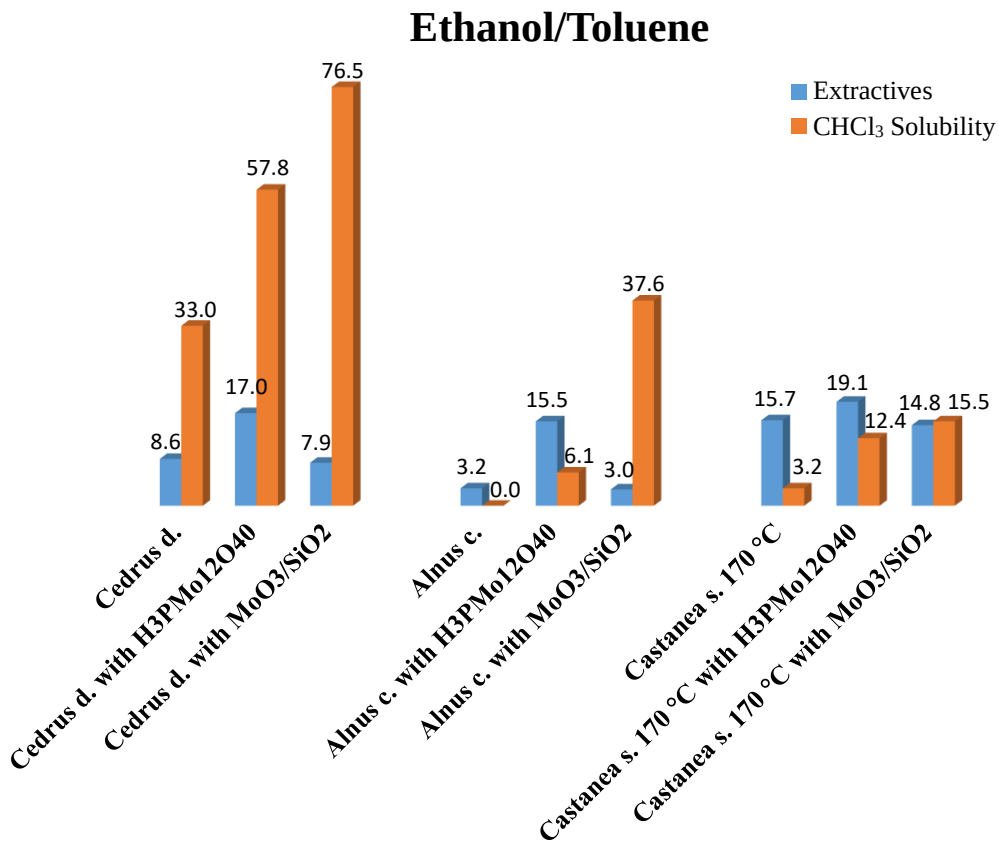

Figure 5.6. Amount of extractives and solubility by Soxhlet extraction in the presence of the Mo catalysts

In Autoclave extraction by using water, on the other hand, both catalysts increased the extraction yield and reduced the solubility in chloroform.

In fact, $H_3PMo_{12}O_{40}$ increased the amount of extractives by 4.7% for *Cedrus d.*, by 3.4% for *Alnus c.* and by 8.2% for *Castanea s.* at 170 °C, while MoO_3/SiO_2 increased the extractives yield by 2.3% for *Cedrus d.*, by 3.6% for

Alnus c. and by 1.2% for *Castanea s.* at 170 °C, compared to the control sample extracted without catalysts (Figure 5.7).

Furthermore, in Autoclave extraction in the presence of $H_3PMo_{12}O_{40}$ the solubility of the extractives decreased by 13.1% for *Cedrus d.*, by 29.5% for *Alnus c.*, and by 1.5% for *Castanea s.* at 170 °C, while it was reduced by 4.4% for *Cedrus d.*, by 29.5% for *Alnus c.*, and by 2.4% for *Castanea s.* at 170 °C, when the extraction was performed in the presence of MoO_3/SiO_2, with respect to the control sample extracted without catalyst (Figure 5.6).

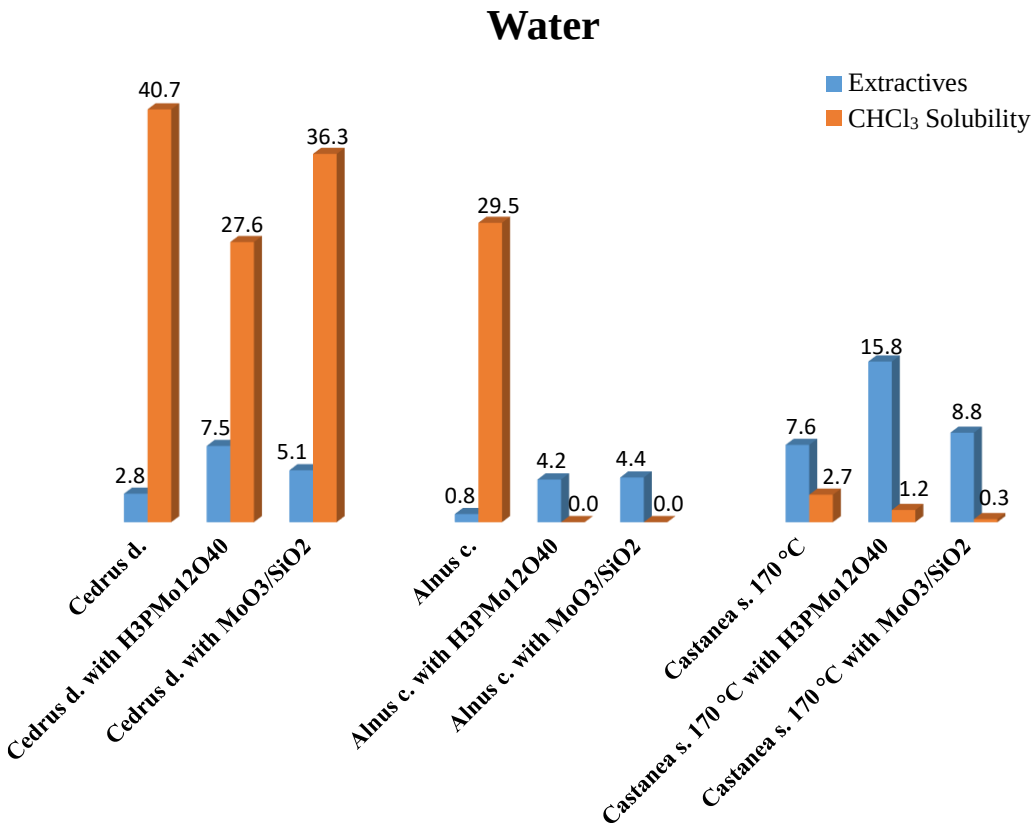

Figure 5.7. Amount of extractives and solubility by Autoclave extraction in the presence of the Mo catalysts

These results can be explained by considering the different effects of the oxidant on lignin and cellulose.

The polyoxometalated compound induced a selective degradation of lignin and cellulose inducing an increase in the amount of extractives both in Soxhlet and in autoclave extraction processes.

However, the autoclave treatment allows the selective extraction of polar components of the extracts, with the consequent reduction in the chloroform-soluble fraction.

When the Soxhlet extraction was performed in the presence of MoO_3, the oxidant was not able to oxidize wood, and the treatment induced only an oxidation process of the extractives that are reduced. The oxidation occurred mainly on the polar fraction of the extractives, and then, the solubility in chloroform increased, while the extraction yield decreased[4-6].

When the extraction was performed in the autoclave, both $H_3PMo_{12}O_{40}$ and MoO_3 catalysts were able to induce an oxidative depolymerization of wood with the consequent increase in the extractives amount. However, they reduced the solubility in chloroform considering that, in water, the extraction occurs mainly on polar compounds[4-6].

Furthermore, the wood samples of *Castanea s*. at 170 °C, *Quercus f*., *Paulownia t*. at 200 °C, and *Larix d*. at 200 °C, being the samples that showed a higher extraction yield (Table 5.2), were treated in autoclave by using water in the presence of 10.0% of 'nanostructured' $H_3PMo_{12}O_{40}$.

The synthesis of this nanostructured compound is described in literature[27]. The described methodology requires an autoclave treatment of the hexane suspension of the 12-molybdophosphoric acid.

Particle size, morphology and size distribution of prepared nanoparticles was performed by SEM (scanning electron microscope) XL30 LaB_6 FEI image, and by this analysis we have seen that nanostructured material was not obtained, but we obtained only crystals with micrometric dimension (Figure 5.8) and not nanoparticles as described in the procedure[16].

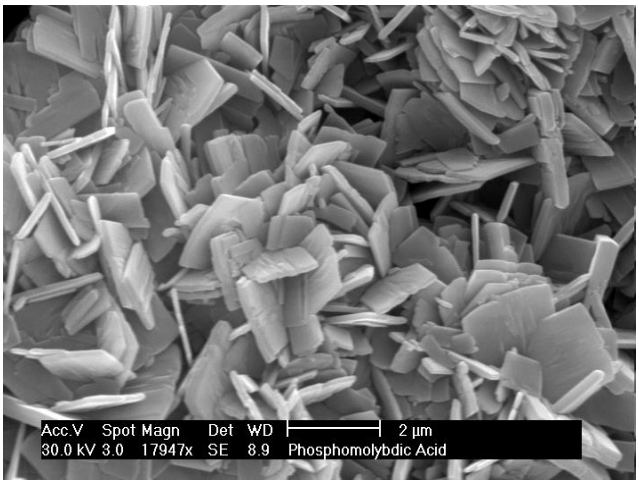

Figure 5.8. Environmental Scanning Electron Microscopy (ESEM) of crystals of $H_3PMo_{12}O_{40}$

However, the obtained crystals compound showed an interesting behavior. The results of the extraction in autoclave by using water in the presence of 10.0% of crystals of $H_3PMo_{12}O_{40}$ are reported in Table 5.5.

Wood species	**Extractives (%)**	**$CHCl_3$ Soluble Fraction (%)**
Castanea s. 170 °C	7.6	2.7
Castanea s. 170 °C with crystals	19.9	0.7
Quercus f.	5.3	0.7
Quercus f. with crystals	12.3	1.2
Paulownia t. 200 °C	1.4	8.5
Paulownia t. 200 °C with crystals	7.3	5.0
Larix d. 200 °C	1.6	5.1
Larix d. 200 °C with crystals	10.7	9.7

Table 5.5. Amount of extractives and solubility of samples by Autoclave extraction in the presence of crystals of $H_3PMo_{12}O_{40}$

As you can see from the histogram in Figure 5.9, in the case of the present samples, in Autoclave extraction in the presence of crystals of $H_3PMo_{12}O_{40}$ the extractives yield increased by 12.3% for *Castanea s.* at 170 °C, by 7.0% for *Quercus f.*, by 5.9% for *Paulownia t.* at 200 °C, and by 9.1% for *Larix d.* at 200 °C, with respect to the control sample extracted without catalyst. At the same time, the solubility of the extractives decreased by 2.0% for *Castanea s.* at 170 °C and by 3.5% for *Paulownia t.* at 200 °C, and it increased by 0.5% for *Quercus f.* and by 4.6% for *Larix d.* at 200 °C.

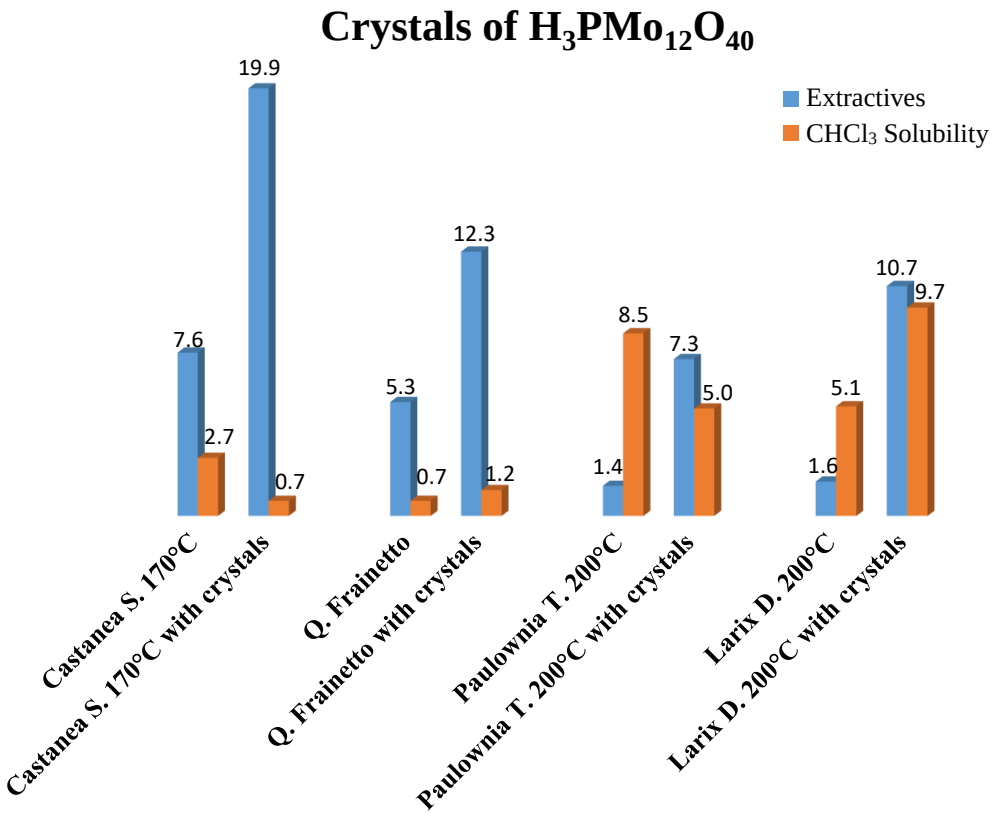

Figure 5.9. (%) Amount of extractives and solubility by Autoclave extraction in the presence of crystals of $H_3PMo_{12}O_{40}$

These results suggest that polyoxometalated compound induced a selective degradation of lignin and cellulose inducing an increase in the extractives yield in extraction processes. The use of a molybdenum polyoxometalated

compound depended on the evidences that molybdenum can act as catalyst in Fenton-like reactions[28].

In particular, the microcrystalline structure of phosphomolybdic acid considerably increases the yield of extractions even compared to simple phosphomolybdic acid. Indeed, in the case autoclave extraction of the *Castanea s.* at 170 °C samples, while simple $H_3PMo_{12}O_{40}$ increased the extractives yield by 8.2%, crystals of $H_3PMo_{12}O_{40}$ increased the amount of extractives by 12.3%.

However, microcrystalline molybdenum polyoxometalated compounds have not been used to this purpose until now[16].

5.4. Chemical characterization of the extractives by GC-MS analysis

All the bands, scraped off from semi-preparative TLC, were analyzed by GC-MS analysis, both of the soluble fraction in chloroform, and of the non-soluble fraction acetylated by treatment with pyridine and acetic anhydride.

Looking at the results of a chromatogram, Figure 5.10 from literature[29], it can be expected that fatty acids should be found at much shorter retention times followed by hydrocarbons, sterols and then sterol esters/triglycerides.

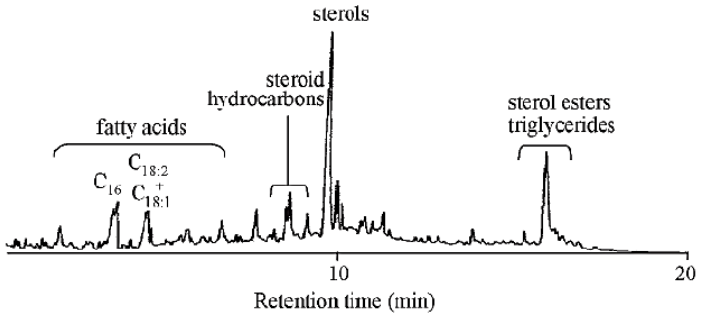

Figure 5.10. GC-FID chromatogram of lipophilic extractives

Cedrus deodara Roxb.

The main chemical components found in *Cedrus d.* wood extractives are reported in Table 5.6.

Compound	r.t.[min]
4-Hydroxy-3-(1,3-dihydroxy-2-propoxy(methyl))-1H-pyrazole-5-carboxamide	4.24
4-Oxopentanoic acid	5.27
Cyclooctanone	6.17
Vanillin	8.53
Longifolene	8.68
2,4-Bis(1,1-dimethylethyl)phenol	9.26
3,4-Dimethoxybenzenemethanol	9.57
8,9-Dihydroneoisolongifolene	10.04
Homovanillin	10.07
6,6-Dimethylhepta-2,4-diene	10.42
1,5-Diethenyl-2,3-dimethylcyclohexane	10.75
4-Acetyl-3-carene	11.02
Methyl b-ionone	11.05
Methyl hexadecanoate	11.54
7,9-Di-t-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	11.60
t-Butylhydroquinone	11.66
(E)-2-Isopropyl-5-methylphenyl 2-methylbut-2-enoate	12.05
5-Methyl-1-(2,6,6-trimethyl-2,4-cyclohexadien-1-yl)- 1,4-hexadien-3-one	12.11
1,13-Tetradecadiene	12.35
Methyl stearate	12.44
Cyclotetradecane	12.48
(Z)-8-dodecen-1-ol	12.49
1,15-Hexadecadiene	12.55
Octadecanoic acid	12.77
Ergosta-4,6-22-trien-3-ol	13.26
Butyl citrate	13.71
Homovanillinic acid	13.72
Butyl octadecanoate	14.79
Methyl docosanoate	16.66
Methyl pseudoecgonine	16.73
Octadecyl 3,5-bis(1,1-dimethylethyl)-4-hydroxybenzene propanoate	17.40
2-Hydroxy-1-(hydroxymethyl)ethyl hexadecanoate	17.76
Glicerol 1-palmitate	18.12
4-Methoxy-4',5'-methylenedioxobiphenyl-2-carboxylic Acid	19.18
1,2,3,4-tetrahydro-1-naphthalenol acetate	19.53
2-Hydroxy-1-(hydroxymethyl)ethyl octadecanote	20.47
β-Sitosterol	29.87

Table 5.6. Main chemical components of *Cedrus d.* wood extractives

In the case of autoclave extraction of *Cedrus d.* wood, the tin layer chromatography of the chloroform soluble fraction gave 2.5:6.5:1.0 mixture of three esters, 2-hydroxy-1-(hydroxymethyl)ethyl hexadecanoate (1), 2-hydroxy-1-(hydroxymethyl)ethyl octadecanoate (2), and 2,3-dihydroxypropyl

octadecanoate (3) (Figure 5.11). Compounds 1-3 were determined on the basis of their mass spectra. These compounds were obtained with an overall chemical yield of 49.7% (w/w)[5,6].

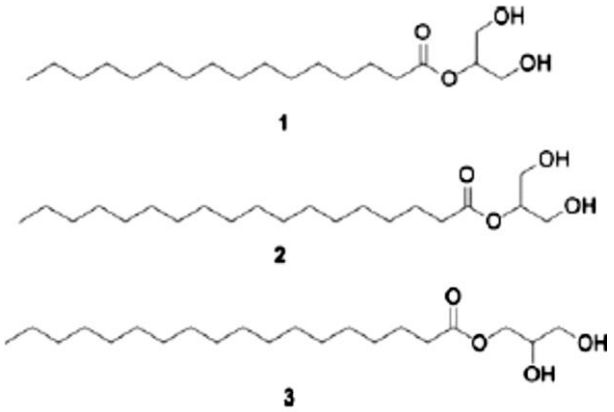

Figure 5.11. Esters determined in *Cedrus d.* extractives

The *Cedrus d*. chloroform-soluble fraction obtained by using autoclave, and chloroform-insoluble fractions obtained by both methods, on the other hand, were mainly formed by some simple carbohydrates.

The most abundant components were ribofuranose (4), ribopyranose (5), xylopyranose (6), and arabinose (7) (Figure 5.12).

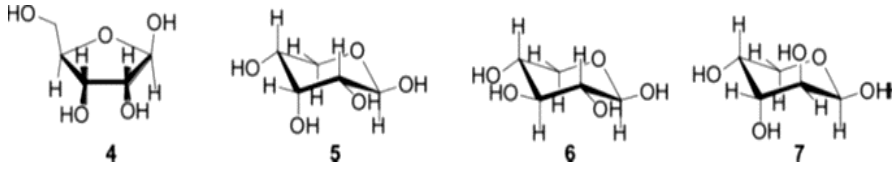

Figure 5.12. Carbohydrate compounds found in *Cedrus d*. extractives

Following the heat treatment of *Cedrus d*., the composition of the extractives was the same, especially long-chain compounds, but with a reduction in the yield of extractions with both Soxhlet and autoclave extraction methods.

When the autoclave extraction was performed in the presence of the $H_3PMo_{12}O_{40}$, in the chloroform-soluble fraction, only a 3.1:6.4:8.5 mixture of 1-3 was obtained. These compounds were obtained in 95.0% yield (w/w).

The same results were obtained when the autoclave extraction was performed in the presence of silica-supported MoO_3. In this case, the ester mixture was obtained in 93.0% yield (w/w).

It is evident that the presence of oxidant catalysts changes the composition of the extractives: the main components found were hexadecanoic and octadecanoic acid derivatives, clearly deriving from the oxidative fission of cellular membranes of the wood. In few cases some compounds can be originated from the waxes[5,6].

The chloroform-soluble fraction of cedar after autoclave treatment in the presence of both Mo polyoxometalate derivative and silica-supported MoO_3 gave excellent yields of a mixture of two fatty acids that can be used for the preparation of biodiesel.

It is noteworthy that fatty acids have been determined as a minor component in suberin.

Alnus cordata Desf.

Alnus c. wood showed a different behaviour. The extractives were not soluble in chloroform but they were analyzed by GC-MS only after derivatization. In this case, the main components of the extractives were D-arabinopyranose (6.3%), D-deoxyribopyranose (16.2%), D-glucopyranose (24.7%), and myo-inositol (3.4%), with an increase in yields following the heat treatment.

The same compounds were obtained by extraction in the presence of the catalysts. However, the catalysts have increased the solubility in chloroform (6.1%), so it was also possible to analyze the soluble fraction of the *Alnus c.*(Table 5.7)[6].

a.

Compound	r.t.[min]
4-(4-Hydroxyphenyl)-2-butanone	9.55
β-Curcumene	10.27
4-[(1E)-3-Hydroxy-1-propenyl]-2-Methoxyphenol	10.69
Decyl isobutyl phthalate	11.34
cis-9-Hexadecenoic acid	11.66
Hexadecanoic acid	11.75
3,5-Dimethoxy-4-hydroxycinnamaldehyde	12.01
cis-10-Heptadecenoic acid	12.20
Methyl (E)-9-octadecanoate	12.58
(Z,Z)-9,12-Octadecadienoic acid	12.85
Octadecanal	15.40
(E)-5-Eicosene	16.25
Bis(2-ethylhexyl) decanedioate	22.91

b.

Compound	r.t.[min]
1-Methyl-2-(phenylmethyl)benzene	9.48
2,2'-dimethylbiphenyl	9.52
Tetradecanoic acid	10.68
4-Hydroxy-3,5-dimethoxybenzoic acid	11.05
Decyl isobutyl phthalate	11.35
Stigmastan-3,5-diene	11.44
Methyl hexadecanoate	11.56
Hexadecanoic acid	11.74
Ethyl hexadecanoate	11.91
Methyl (Z,Z)-9,12-Octadecadienoate	12.84
Ethyl linoleate	12.94
Ethyl oleate	13.05
Ethyl octadecanoate	13.17
Ethyl eicosaonate	14.96
1,19-Eicosadiene	15.39
1-Nonadecene	16.25
(Z)-9-Octadecen-1-ol	16.77
Bis(2-ethylhexyl) decanedioate	22.88
β-Sitosterol	30.07
γ-Sitosterol	30.14

Table 5.7. Composition of the chloroform soluble fraction of *Alnus c.* extracted in presence of silica supported MoO_3 (a) and $H_3PMo_{12}O_{40}$ (b)

In this case, the extraction in the presence of MoO_3 and $H_3PMo_{12}O_{40}$ gave compounds whose origin could be the lignin[6].

Using the autoclave method with water, we were able to extract and identify a large number of compounds from thermo-treated *Alnus c.*, unlike the untreated sample. The results are shown in Table 5.8.

Alkyl compounds and higher amounts of carbonyl and phenolic compounds were obtained only from thermo-treated samples. These compounds could have resulted from the decomposition of volatile organic compounds (VOCs), terpenes, polyosides, and lignins[30].

Also fatty-acid derivatives (octadecanoic acid, 1-methylethyl dodecanoate, and glycerol 1,2-diacetate) were extracted from thermo-treated samples. Octadecanoic acid (12.7%) is a saturated fatty acid, which may have resulted from the hydrolysis or degradation of fatty acids during heat treatment.

Compound	r.t.[min]
Propanoic acid	4.85
Pentanoic acid, 4-oxo	5.42
2-Furancarboxylic acid	5.56
2-Pyrrolidinone	5.71
Catechol	6.95
Dianhydromannitol	7.05
5-Hydroxymethylfurfural	7.17
Hydroquinone	7.61
Benzaldehyde, 4-hydroxy	8.28
Vanillin	8.57
Apocynin	9.17
Propan-2-one, 1-(4-isopropoxy-3-methoxyphenyl)	9.45
3-Hydroxy-4-methoxybenzoic acid	9.65
Benzaldehyde, 4-hydroxy-3,5-dimethoxy	10.24
4-Hydroxy-2-methoxycinnamaldehyde	10.69
Desaspidinol	10.86
Methanone, (3,4-dimethoxyphenyl)-(5-oxotetrahydrofuran-3-yl)	12.83
Benzene, 1,1'-sulfonylbis[4-chloro]	13.86
Heneicosane	14.00
Tetracosane	14.99
Octacosane	16.25
Nonadecane	17.84

Table 5.8. Main chemical components of *Alnus c.* thermo-treated

Among the extractives from heat-treated *Alnus c.*, vanillin (2.8%) and methoxycinnamaldehyde (11.8%) are important extracts obtained (Table 5.8). Vanillin is commonly employed in the biological and biotechnological industries as a flavouring agent in foods and pharmaceuticals products and as a fragrance in perfumes. It has the potential to be a key intermediate for the synthesis of biopolymers[31].

Methoxycinnamaldehyde, is related to ferulic acid, an important precursor in the manufacture of aromatic compounds[32]. The aforementioned compounds were not found in untreated *Alnus c*. samples, reflecting the importance of thermo-treatment in generating mainly polar compounds[33].

Populus nigra L.

GC-MS analysis of the chloroform soluble fraction (31.2%) of *Populus n.* gave as main components 4-hydroxybenzoic acid (8.9%), 4-hydroxy-3,5-

dimethoxybenzaldehyde (1.4%), 4-(3-hydroxy1-propenyl)-2-methoxyphenol (14.0%), hexadecanoic acid (3.1%), methyl 9-octadecenoate (2.6%) and bis(2-ethylhexyl)decadioate (1.6%)[34]. The complete list of the identified compounds in the extractives is collected in Table 5.9.

Compound	**r.t.[min]**
Hexanoic acid	4.33
Phenol	4.63
2-Methoxyphenol	5.83
Benzoic acid	6.53
4-Methoxybenzaldehyde	7.41
2-Methoxy-4-vinylphenol	7.89
2,6-Dimethoxyphenol	8.16
1-Tetradecene	8.37
Vanillin	8.54
4-Hydroxybenzoic acid	9.32
Cetene	9.70
2,6-Dimethoxy-4-(2-propenyl)phenol	9.85
4-Hydroxy-3,5-dimethoxybenzaldehyde	10.23
(1E)-4-(3-Hydroxy-1-propenyl)-2-methoxyphenol	10.71
4-Hydroxy-3,5-dimethoxybenzoic acid	11.07
Methyl hexadecanoate	11.56
Hexadecanoic acid	11.73
Methyl (E)-9-octadecenoate	12.56
Methyl stearate	12.70
(Z,Z)-9,12-Octadecadienoic acid	12.78
Octadecanoic acid	12.93
1-Eicosanol	13.15
Dehydroabietic acid	15.87
Tetracosanol	16.45
Docosanoic acid	17.23
4,4′-Methylenebis(2,6-dimethoxy)phenol	20.85
Bis(2-ethylhexyl) decadioate	22.81

Table 5.9. Main chemical components of *Populus n.* soluble fraction

The fraction not soluble in chloroform of *Populus n.* was analyzed by GC-MS after derivatization and, in this case, the main components of the extractives were D-ribopyranose, D-galactopyranose, D-glucopyranose, myo-inositol, and γ-sitosterol.

Following the heat treatment, there was an increase in extractives yield by using water and a reduction by using ethanol/toluene, but most of all an

increase in compounds such as methyl stearate (6.1%) used as flavoring agents, octadecanoic acid (6.2%), and hexadecanoic acid (4.2%).

Larix decidua Mill.

While Soxhlet extraction of *Larix d.* extracted insoluble compounds in chloroform, especially D-galactopyranose, D-mannosan, D-galactose, D-glucose, D-glucopyranose and myo-inositol, by the extraction in autoclave with water, we could identify a greater number of compounds, shown in the Table 5.10.

Compound	**r.t.[min]**
Formamide, N,N-diethyl	3.97
Hexanoic acid	4.39
Phenol, 2-methoxy	5.83
Benzenemethanol,4-trimethyl	6.73
alpha-Terpineol	6.84
Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl	7.04
Cyclohexanemethanol, 4-hydroxy,4-trimethyl	7.83
2-Methoxy-4-vinylphenol	8.02
Phenol, 2,6-dimethoxy	8.17
Vanillin	8.57
Phenol, 2-methoxy-4-propyl	8.97
t-Butylhydroquinone	9.17
Homovanillic acid	10.25
2-Propenal, 3-(4-hydroxy-3-methoxyphenyl)	10.69
4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	10.76
Pentadecanoic acid, 14-methyl-, methyl ester	11.54
n-Hexadecanoic acid	11.73
Z-8-Methyl-9-tetradecen-1-ol formate	12.78
Octadecanoic acid	12.92
10,11-Dihydro-10-hydroxy-2,3-dimethoxydibenzoxepin	14.39
Pentadecane	14.96
Nonadecane, 9-methyl	16.19
4-Methoxy-4,5-methylenedioxybiphenyl-2-carboxylic acid	19.29
Heptadecane	19.80
Hentriacontane	22.46
Supraene	23.46
7,8-Diethylbenz[a]anthracene	24.04
Eicosane	25.81
Benzaldehyde, 2,4-dihydroxy-6-methyl	26.96

Table 5.10. Main chemical components of *Larix d.* soluble fraction

In this case too, in the heat-treated samples, the yield of many extractives increased, including 5-(hydroxymethyl)furan-2-carbaldehyde (12.7%) used as

flavoring agents, and 4-hydroxy-2-methoxycinnamaldehyde (5.2%), related to ferulic acid, an important precursor in the manufacture of aromatic compounds[32].

Larix d. thermotreated wood was also extracted in autoclave in the presence of crystals of $H_3PMo_{12}O_{40}$. The presence of an oxidant can induce both lignin and cellulose oxidative depolymerization, increasing the recovery of the extractives (9.1%), which showed the presence of large amounts of D-sesamin[16]. It will be described in section 5.5.

Paulownia tomentosa Steud.

The main compounds of chloroform soluble fraction of *Paulownia t.* are collected in the Table 5.11.

Compound	**r.t.[min]**
Benzaldehyde	4.29
5-Benzoylpentanoic acid	5.56
Vanillin	8.55
Octacosane	9.04
Triacontane	9.09
Pentadecane	9.15
Phenol, 2,4-bis(1,1-dimethylethyl)	9.24
1,3-Benzodioxole-5-carboxylic acid	9.28
Tetradecanoic acid	10.65
7-Hexadecene	11.36
Cyclotetradecane	11.37
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	11.63
n-Hexadecanoic acid	11.72
1,15-Hexadecadiene	12.35
9-Octadecen-1-ol	12.36
1,13-Tetradecadiene	12.42
Cetene	12.50
Methyl stearate	12.69
Oleic Acid	12.77
Octadecanoic acid	12.78
Alpha-Lapachone	12.92
Ferruginol	14.67
1-Pentadecene	16.28
Hexadecanoic acid	17.68
Octadecanoic acid, 2,3-dihydroxypropyl ester	20.66
13-Docosenamide	21.97
β-Sitosterol	30.17

Table 5.11. Main chemical components of *Paulownia t.* soluble fraction

The most abundant compounds were octadecanoic acid (8.8%), 9-octadecen-1-ol (4.4%), and alpha-Lapachone (5.9%).

Alpha-Lapachone is an "irreversible" inhibitor of topoisomerase II, it can inhibit initial non-covalent binding of topoisomerase II to DNA, can induce relegation of DNA breaks (even in pre-established ternary complexes) before dissociating the enzyme from DNA, and has also antineoplastic activity[35].

The fraction not soluble in chloroform of *Paulownia t.* was analyzed by GC-MS after derivatization and, in this case, the main components of the extractives were D-mannitol, D-glucopyranose, myo-inositol, and D-galactopyranose.

Following the heat treatment, there was an increase in extractives yield by using both extraction methods, but most of all an increase in compounds such as methyl stearate used as flavoring agents, and octadecanoic acid, hexadecanoic acid, nonadecanoic acid, and eicosanoic acid, clearly deriving from the oxidative fission of cellular membranes of the wood. This increase was observed mostly for *Paulownia t.* at 200 °C, with a drop for the sample thermo treated at 210 °C.

The most important feature for this species was that only in heat treated samples were found compounds with imported biological and pharmaceutical activities, described in section 5.5: apocynin, fenozan, sinapaldehyde, and asarinin[4].

When *Paulownia t.* thermo treated wood was extracted in autoclave in the presence of crystals of $H_3PMo_{12}O_{40}$, the chloroform soluble fraction of extractives revealed the presence of 4-hydroxy-3,5-dimethoxybenzaldehyde (known as Syringaldehyde), 3,5-dimethoxy-4-hydroxycimmanaldehyde (or Sinapaldehyde), and relevant amounts of long chain hydrocarbons, that can be used for the preparation of biodiesel[16].

Syringaldehyde has antioxidant, antioncogenic, antimicrobial, and antifungal activities, which make the compound important commercially[36].

Castanea sativa Mill.

While insoluble fractions in chloroform of *Castanea s*. revealed the presence only of D-ribopyranose, D-glucopyranose, D-mannopyranose, D-galactopyranose and myo-inositol, by both Soxhlet and autoclave extraction soluble fractions, we could identify a greater number of compounds, shown in the Table 5.12.

Compound	**r.t.[min]**
Phenol	4.47
Pentanoic acid, 4-oxo	5.34
2-Furancarboxylic acid	5.52
3-Furancarboxylic acid, methyl ester	5.74
Maltol	6.05
5-Hydroxymethylfurfural	7.15
3-Allyl-6-methoxyphenol	8.19
Phenol, 2,6-dimethoxy	8.27
Vanillin	8.54
alpha-Amino-3-hydroxy-4-methoxyacetophenone	9.21
Homovanillic acid	9.45
Benzoic acid, 4-hydroxy-3-methoxy	9.62
Phenol, 2,6-dimethoxy-4-(2-propenyl)	9.84
Benzaldehyde, 4-hydroxy-3,5-dimethoxy	10.22
4-Hydroxy-2-methoxycinnamaldehyde	10.67
4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	10.72
Benzoic acid, 4-hydroxy-3,5-dimethoxy	11.06
Benzoic acid, 4-hydroxy-3-methoxy, ethyl ester	11.17
n-Hexadecanoic acid	11.73
Mandelic acid, 3,4-dimethoxy-, methyl ester	11.92
Octadecanoic acid	12.92
9-Octadecenoic acid	14.96
Tetradecane	16.19
Eicosane	17.77
Heptacosane	19.80
Tetracosane	22.42
Octacosane	25.84

Table 5.12. Main chemical components of *Castanea s.* soluble fraction

The most abundant components were n-hexadecanoic acid (4.7%), octadecanoic acid (6.5%), and 9-octadecenoic acid (11.5%).

On the other hand, the chloroform soluble fraction of *Castanea s*. thermo treated contained mainly 5-(hydroxymethyl)furan-2-carbaldehyde (3.6%) used as flavoring agents, coniferyl alcohol (4.7%), and 4-hydroxy-3,5-dimethoxybenzaldehyde (or Syringaldehyde, 4.1%), that has antioxidant, antioncogenic, antimicrobial, and antifungal activities, which make this compound important commercially[36], and apocynin[4].

The amount of these compounds and of long chain hydrocarbons increased further by using $H_3PMo_{12}O_{40}$, MoO_3, and especially crystals of $H_3PMo_{12}O_{40}$.

In fact, the presence of 10.0% of crystals of $H_3PMo_{12}O_{40}$ increased the yields of methyl hexadecanoate, octadecanoic acid, hexadecanoic acid, and however, the main component of chloroform insoluble fraction was inositol (even up to 32.1%)[16], a biological active compound known as B7 vitamin, active in the control of insulin release and polycystic ovary syndrome in women[37].

Quercus frainetto Ten.

The chloroform soluble fraction obtained from the extracts of *Q. frainetto* by using water did not allow identification of a large number of components.

The insoluble fraction contained mainly D-ribopyranose, D-mannopyranose D-glucopyranose, myo-inositol, and mostly D-xylitol (11.5%).

On the contrary, Soxhlet soluble fraction of this species gave a lot of compounds, collected in Table 5.13.

The main compounds of this fraction were 9-octadecenoic acid methyl ester (8.1%), octadecanoic acid (5.6%), n-hexadecanoic acid (4.1%), and cetene (3.3%).

The amount of these compounds increased significantly by using Mo catalyst, in fact the GC-MS analysis of the chloroform soluble fraction of the extractives of *Q. frainetto* extracted in the presence of crystals of $H_3PMo_{12}O_{40}$ showed a greater amount of octadecanal and some long chain hydrocarbons[16].

Compound	r.t.[min]
1-Tridecene	6.75
Benzaldehyde, 4-methoxy	7.40
3-Allyl-6-methoxyphenol	8.21
Phenol, 2,6-dimethoxy	8.25
2-Tetradecene, (E)	8.37
5-Ethyl-5-methylnonadecane	8.43
Vanillin	8.54
Phenol, 2,4-bis(1,1-dimethylethyl)	9.24
Dodecanoic acid	9.49
Cetene	9.69
7-Hexadecene, (Z)	9.84
Dodecanoic acid, 1-methylethyl ester	9.89
Benzophenone	10.09
Tetradecanoic acid	10.65
1-Octadecene	10.85
Pentadecanoic acid	11.19
Nonadecane	11.40
Hexadecanoic acid, methyl ester	11.54
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	11.63
n-Hexadecanoic acid	11.74
Cycloeicosane	11.90
Heptadecanoic acid	12.28
9-Octadecenoic acid, methyl ester	12.56
Octadecanoic acid	12.92
E-15-Heptadecenal	13.13
1-Heneicosanol	14.05
Eicosanoic acid	14.59
5-Eicosene, (E)	16.28
Docosanoic acid	17.23
1-Heptacosanol	20.08
Tetracosanoic acid	21.58
Supraene	23.47
β-Sitosterol	29.87

Table 5.13. Main chemical components of *Q. frainetto* soluble fraction

Following the heat treatment, there was an increase in extractives yield by using both extraction methods, especially for *Q. Frainetto* thermo treated at 180 °C, but most of all new compound were detected: aspidinol, sinapaldehyde, syringaldehyde, and asarinin[4].

Moreover the main product obtained by insoluble fraction of thermo treated samples was monoacetyl derivative of gallic acid (10.2%)[38].

Obtaining a relevant amounts of gallic acid from wood wastes could be an interesting results considering that it shows a relevant number of biological and pharmacological activities: scavenger of free radicals, antioxidant, anticancer, inhibitor of squalene epoxidase, and agent able to interfere with signal pathways involving Ca^{2+} and oxygen free radicals[39,40].

5.5. New compounds detected in thermo-treated samples

Extractives are degraded or leave the wood at the same time that new extractable compounds emerge from wood degradation only in thermo-treated samples, resulting from the degradation of cell wall structural components, and they have important biological and pharmaceutical activities.

Potentially valuable bioactive compounds were extracted from *Cedrus d.* thermo-treated samples. For example, the terpene ar-Tumerone (8.2%, Figure 5.13), that is a compound found in curcumin, a perennial plant cultivated throughout tropical Asia, India, and China.

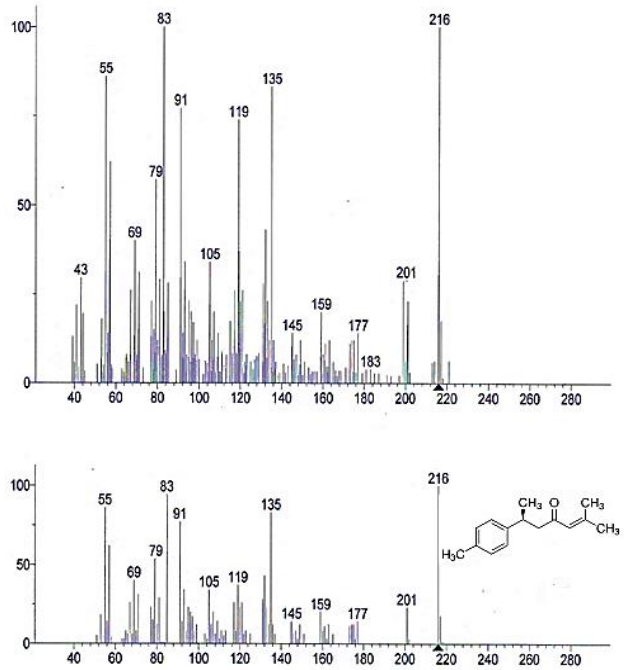

Figure 5.13. Ar-Tumerone GC-MS spectrum

This compound has been investigated in clinical trials for its potential therapeutic properties, including anticancer activity[41]. Ar-Tumerone acts through various mechanisms, including the induction of apoptosis, inhibition of angiogenesis, and modulation of tumor suppressor genes[42].

Q. Frainetto, *Populus n*. and *Paulownia t.* thermo treated samples revealed the presence of Sinapaldehyde (7.2-15.9%, Figure 5.14), an intermediate in the formation of lignin[4].

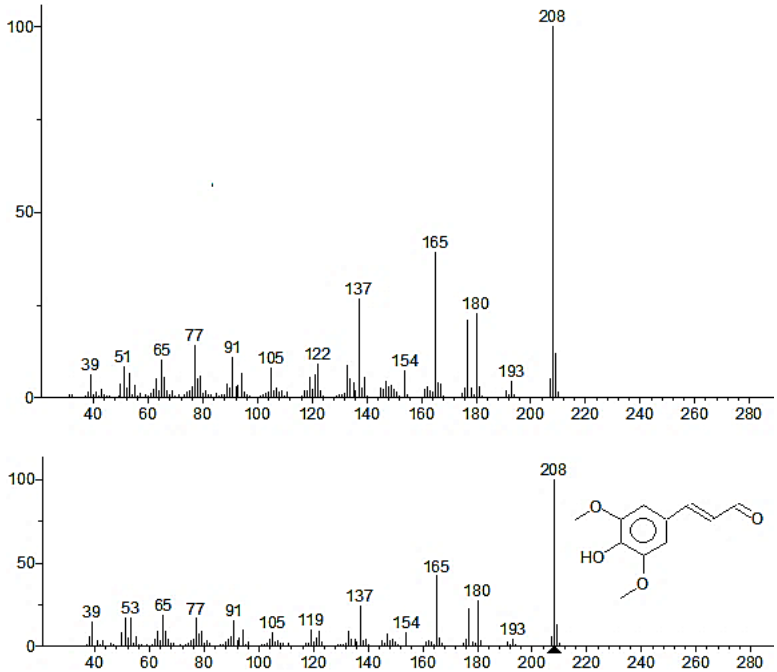

Figure 5.14. Sinapaldehyde GC-MS spectrum

Sinapaldehyde is used as a flavoring agent and it also is a cyclooxygenase (COX) inhibitor[43], that are responsible for formation of prostanoids, including thromboxane and prostaglandins such as prostacyclin, from arachidonic acid. Pharmaceutical inhibition of COX can provide relief from the symptoms of inflammation and pain.

Apocynin (1.6%, Figure 5.15), also known as acetovanillone, is a natural organic compound structurally related to vanillin, and it was detected in *Castanea s.*, *Populus n*., *Alnus c*. and *Paulownia t.* heat-treated woods[4].

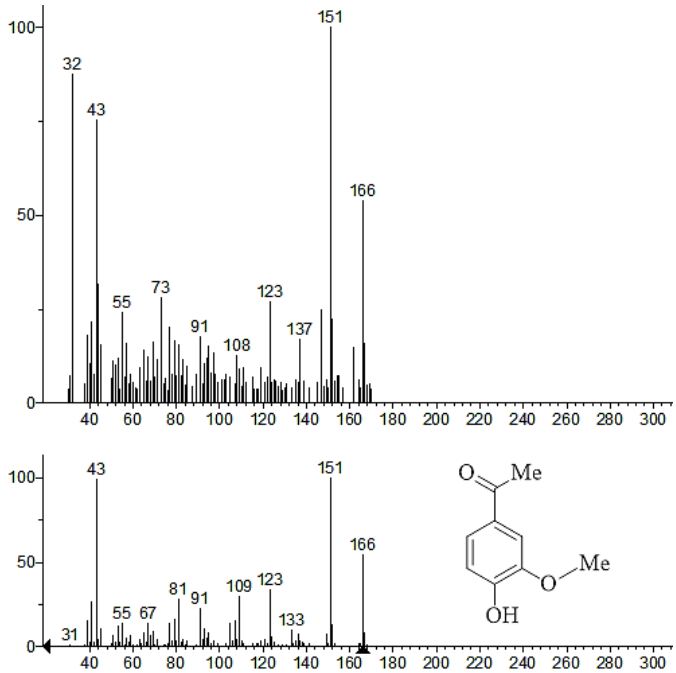

Figure 5.15. Apocynin GC-MS spectrum

Apocynin is an inhibitor of NADPH oxidase activity and thus is effective in preventing the production of the superoxide in human white blood cells or neutrophilic granulocytes. Due to the selectivity of its inhibition, apocynin can be widely used as an inhibitor of NADPH oxidase without interfering in other aspects of the immune system.

Apocynin has also anti-arthritic and anti-asthmatic properties[44,45].

Asarinin, also known as D-sesamin, was detected in *Paulownia t.*, *Larix d.*, and *Q. frainetto* thermotreated woods (6.9-17.1%, Figure5.16).

It is a non-competitive inhibitor of Δ5-desaturase without significantly influencing Δ6, Δ9, or Δ12-desaturase enzymes[46] and not influencing transcription of any enzyme; in otherwise healthy postmenopausal women given 50 g sesame seeds, there have been reductions in both eicosapentanoic acid (EPA) (12.0%) and arachidonic acid (8.0%)[47]. It appears to be able to inhibit the Δ5-desaturase enzyme which results in reduced circulating levels of EPA and arachidonic acids[16].

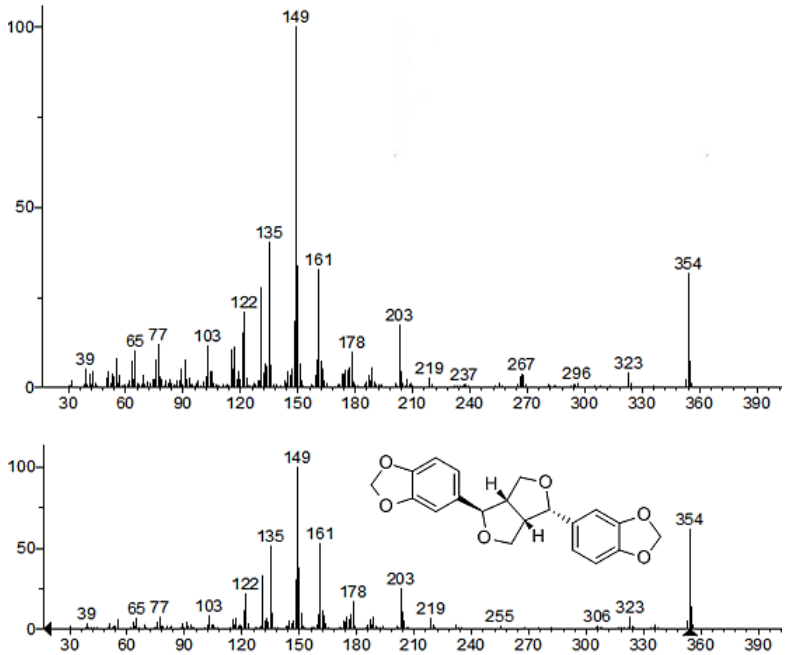

Figure 5.16. Asarinin GC-MS spectrum

Asarinin is also an antioxidants, vasodilators and antithrombotic agent[4].

Another important compound detected in *Paulownia t.* thermo treated samples was Fenozan (1.7%, Figure 5.17)[4].

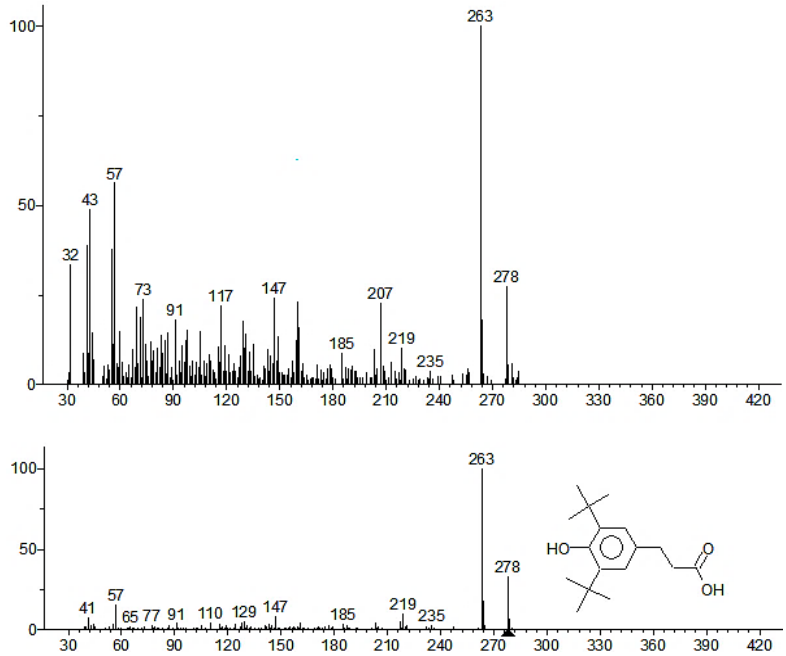

Figure 5.17. Fenozan GC-MS spectrum

Fenozan is a phenolic antioxidant, vasodilators, antithrombotic and antiepileptic agent. Moreover, according to recent research, it also inhibits the development of some spontaneous tumors in animals[48].

Finally, *Q. Frainetto* thermo treated samples revealed the presence of a compound with important biological activities: Aspidinol, a phloroglucinol derivate (5.4%, Figure 5.18)[4].

It is an anti-parasite[49] and antibacterial agent against *Staphylococcus aureus.* In fact, aspidinol achieved an antibacterial effect comparable to that of vancomycin, providing significant protection from mortality[50].

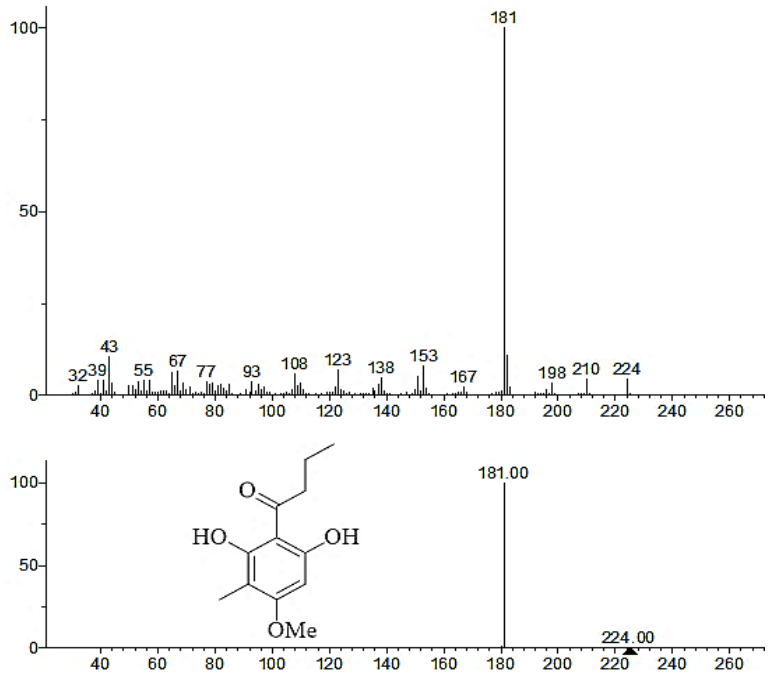

Figure 5.18. Aspidinol GC-MS spectrum

5.6. Two-dimensional Thin Layer Chromatography Images

The extractives of untreated and thermo-treated *Paulownia t.* wood obtained by Accelerated solvent extraction (ASE) techniques were chromatographed by using thin layer chromatography.

Because of the wide variety of fatty acids contained in wood, the characterization of its triglycerides (TGs) is a complex and difficult task.

One problem is the inherent difficulty of isolating all the individual components by silicic acid column or one-dimensional TLC (Figure 5.19, a).

The TLC technique on silica gel with silver nitrate ($AgNO_3$-TLC) separates fatty acids according to the degree of unsaturation because of weak interactions between the π electrons of the double bonds and the silver ions[51,52]. Other factors, such as geometric configuration and chain length, are also involved in TGs separation on silica gel.

On the other hand, Two-dimensional thin-layer chromatography (2-D TLC) is a technique which has had limited application, but the use of two different solvent systems acting on the same support system, combinations of normal, reversed and silver nitrate coated phases can extend its application.

The great limitation of this technique is that, since the wood extractives are made up of a large number of TGs, so it is not possible to obtain a good separation without a cleavage of the TGs in single fatty acids (Figure 5.19, b).

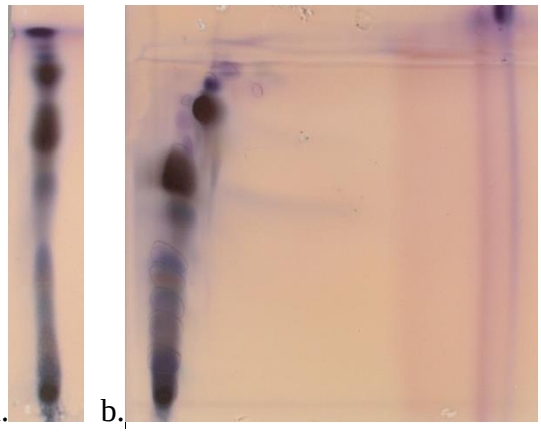

Figure 5.19. One-dimensional TLC (a) and Two-dimensional $AgNO_3$-TLC (b) of *Paulownia t.* extractives

To overcome this problem it was necessary to develop a new method that allowed the complete separation of all known fatty acids and triglycerides

components of wood extractives, and thus also allowed an estimate of changes due to heat treatment: two-dimensional TLC through esterification with $NaOCH_3$ and the use of $AgNO_3$-TLC.

After the first development of the sample with hexane/ethyl acetate (4:6), the solvent residues were removed from the plate, and the bands were sprayed with $NaOCH_3$ (2 M). Than the plate was plunged rapidly into a 10.0% (w/v) solution of $AgNO_3$ in water/ethanol, activated at 100 °C for 20 minutes, and developed in the second direction with chloroform/methanol (95:5) (Figure 5.20).

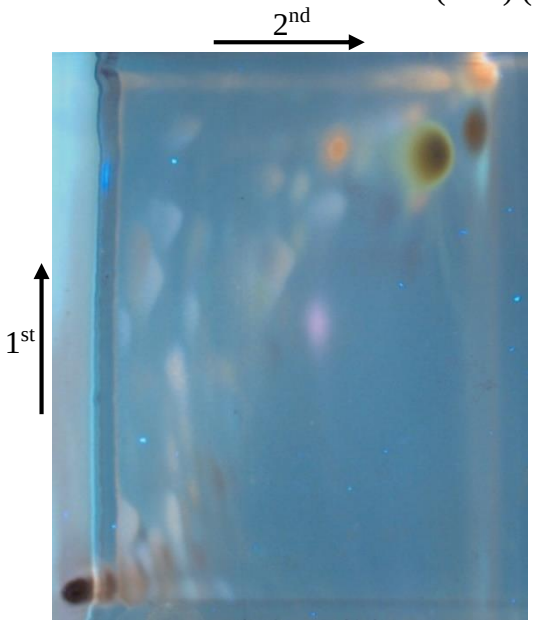

Figure 5.20. 2-D $AgNO_3$-TLC of *Paulownia t.* extractives by using esterification with $NaOCH_3$

This system allowed the complete separation of all the known fatty acids and of the triglyceride components of the wood extractives[53].

A fingerprinting of the triglycerides of the wood extractives could be obtained and this method thus also allowed an estimate of changes due to heat treatment. In fact from the fingerprints of fatty acids and TGs of *Paulownia t.* (a), *Paulownia t*. at 180 °C (b), *Paulownia t*. at 200 °C (c), *Paulownia t*. at 210 °C (d), it is possible to see how, following heat treatment, there was also a

variation in the qualitative and quantitative composition of fatty acids (Figure 5.21).

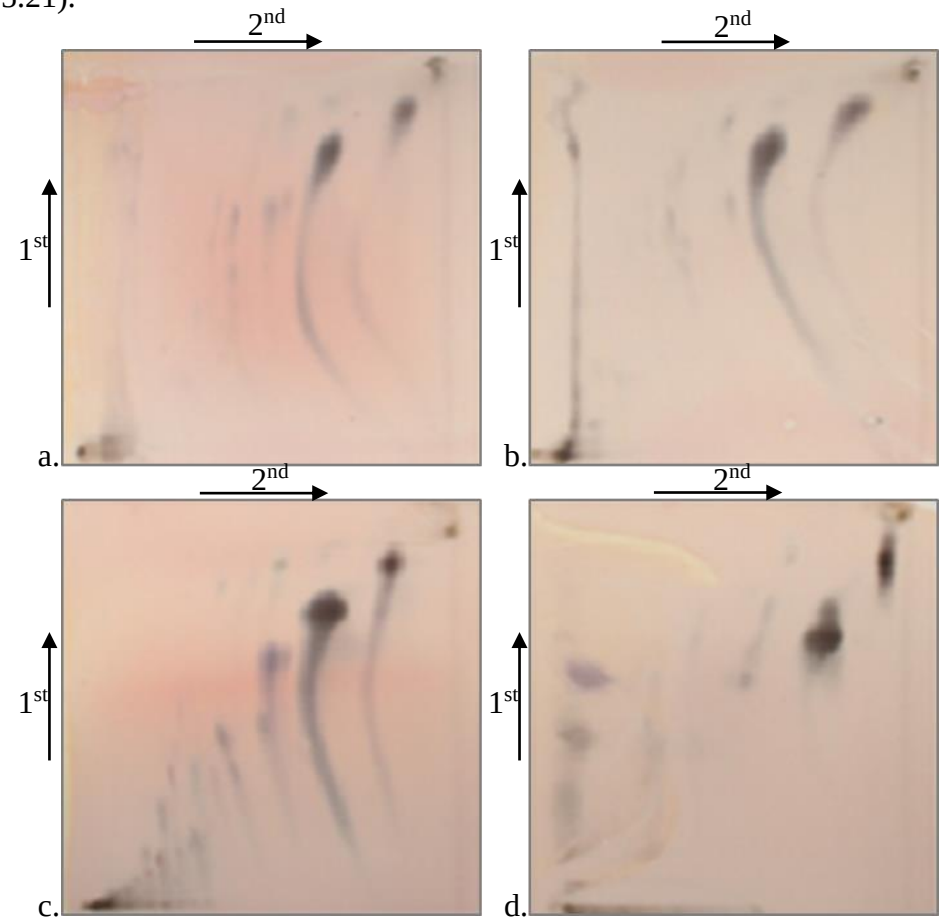

Figure 5.21. 2-D $AgNO_3$-TLC of *Paulownia t.* (a), *Paulownia t.* at 180 °C (b), *Paulownia t.* at 200 °C (c), *Paulownia t.* at 210 °C (d) extractives by using esterification with $NaOCH_3$

These data confirmed the results previously discussed, according to which the best condition was the thermo treatment of *Paulownia t.* at a temperature of 200 °C, in which we also saw an higher extraction yield, especially fatty acids, while at 210 °C the carbonization processes started with formation of CO_2 and other pyrolysis products.

Moreover, this method provides a useful rapid semi-quantitative chemist tool involved in triglyceride analysis, and, with different solvents, possibly also in other fields.

5.7. References

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6. Conclusions

In the experimental conditions, heat treatment changed the chemical composition of wood by degrading cell wall compounds and extractives. Hemicelluloses were the most affected compounds, whereas cellulose was more resistant to heat.

As a result, heat-treatment increased the yield of extractives and lignin, and reduced the amount of holocellulose, in the treatment temperature function. This trend was observed for all samples, except for heat-treated *Populus nigra* and *Cedrus deodara*, and *Paulownia tomentosa* thermally treated at 180 °C, in which the content of holocellulose increased and the content of lignin and extractives decreased. For these samples, cross-linking probably took place after polycondensation reactions between the chemical components of wood, making it difficult to obtain extractives.

In this research the possible effect of oxidants of Mo(VI) to induce degradation of wood during the autoclave and Soxhlet extraction was also tested particularly for *Cedrus deodara*, *Alnus cordata*, and thermo-treated *Castanea Sativa* wood. As general statement, the polyoxometalated compounds induced a selective degradation of lignin and cellulose, increasing the extractives amount both in Soxhlet and in autoclave extraction processes, with a greater activity on heat-treated wood.

About autoclave and Soxhlet treatment of the samples in the presence of molybdenum catalysts, in Soxhlet extraction both catalysts increased solubility, but only $H_3PMo_{12}O_{40}$ increased the extraction yields, while in autoclave both catalysts increased the yields of extractives, but they reduced solubility. Clearly, in these conditions, the presence of the oxidants induced a selective degradation of cellulose increasing the amount of chloroform insoluble carbohydrates. The amount of extractives considerably increased when the extraction was performed in the presence of microcrystalline

$H_3PMo_{12}O_{40}$, even compared to simple phosphomolybdic acid. However, molybdenum compounds had never been used for this purpose until now.

Moreover, the best extraction yield with ASE method was obtained using water as solvent, a great advantage and a key feature for green chemistry.

The organic solvent-soluble fraction was composed of 31-49% by long-chain acid and fatty acids esters, while the insoluble fractions of extractives can be a source of simple carbohydrates.

In conclusion, the heat treatment and the use of molybdenum catalysts increased fatty acid esters and long chain hydrocarbons, which have a possible use in biodiesel synthesis.

Furthermore, most of the extractives disappeared or degraded during the heat treatment, especially the most volatile ones. At the same time, hemicellulose and lignin underwent reactions that produce polar compounds that are easily extracted by polar solvents, allowing to obtain valuable amounts of biological active compounds: vanillin, α-lapachone, syringaldehyde, inositol, gallic acid apocynin, fenozan, sinapaldehyde, asarinin, aspidinol, and ar-tumerone.

Finally, a new system has been developed: two-dimensional TLC through esterification with $NaOCH_3$ and the use of $AgNO_3$-TLC.

This system allowed the complete separation of all the known fatty acids and of the triglyceride components of the wood extractives.

A fingerprinting of the triglycerides of the wood extractives could be obtained and this method thus also allowed an estimate of changes due to heat treatment.

It provides a useful rapid semi-quantitative chemist tool involved in triglyceride analysis, and, with different solvents, possibly also in other fields.

These results and methods could be widely used in the wood industry and particularly for waste treatment that can be used as a source of exploitable organic compounds in various fields, for an increasingly green chemistry.