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CHARACTERIZATION OF WOOD AGING BY MEANS OF VOLATILE ORGANIC COMPOUNDS (VOCS) ANALYSIS

Settore Scientifico Disciplinare: AGR/06

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1 INTRODUCTION

Objective and scientific approach

Wood is still today one of the most important raw materials used by human beings. The properties of recent wood have been therefore widely investigated and documented. On the other hand, very little is known about the aging process of wood or the properties of the aged material, although such information is fundamental for the proper conservation of wooden cultural heritage objects or for the worth re-use of aged wood. In addition, the research carried out on this topic often contain contradictious results, so there is not a clear standpoint for most of the properties of aged wood.

The current study aims to contribute to better address the knowledge about the aging processes of wood and its modifications, but, what is more, it includes the use of volatile organic compounds (VOCs) analysis to provide evidence of aging processes and to monitor degradation progress, in order to identify the causes of damage of wood-based-material or even wood artefacts.

To this aim, different experiments, both of spectroscopic characterization and emission analysis, on the chemical structure of aged wood were carried out, supplemented with investigations on recent wood and a thorough literature review.

In order to achieve the above mentioned goals, the characterization of wood aged in dry air was carried out by means of different analytical techniques. The following investigations were carried out:

- evaluation of the degradation of wood components by means of spectroscopic analysis, such as solid state nuclear magnetic resonance (NMR) spectroscopy;
- estimation of the crystallinity of cellulose and the cellulose/lignin ratio based on solid state NMR spectra;
- analysis of volatile organic compounds (VOCs) by means of mass spectroscopy.

The analyses were performed on specimens of different wooden species, and in particular on Norway spruce (*Picea abies* L. Karst.),

where recent and aged samples were analysed. In every case, recent wood was investigated alongside the aged samples to serve as a reference.

The following chapter gives a short overview of the anatomy, chemistry and variability of recent wood, followed by a definition of the aging phenomena and a thorough literature study regarding the properties of aged wood. Then, specific references concerning emissions of organic compounds from wood based-material introduce the issue of VOCs analysis for understanding the degradation of wood components. Chapter 3 introduces the investigated materials and the methods used in the study, while in Chapter 4 the results are presented and discussed, presented as different scientific papers. Finally, a summary of the main findings is given in Chapter 5.

2 STATE OF THE ART

In order to monitor and to determine the effect of aging on wood components, its influence in formation and subsequent emission of volatile compounds, an introduction to the anatomy and chemistry of recent wood is given in the first part of this section.

Subsequently, the second part focuses on the topic of aging. Influencing factors on the aging process are introduced and the literature considering aged wood is reviewed with an emphasis on sound wood aged in dry air.

Finally, third part concerns VOC emission issues (their source of emission, identification and monitoring).

2.1 ANATOMY AND CHEMISTRY OF RECENT WOOD

Wood is a complex biological structure, a composite of many chemistries and cell types, determined by its transport and structural functions in the living plant. Wood structure can be studied at several levels: macro, micro and molecular. However, all these three levels characteristics are intimately related and will be described in the following.

2.1.1 Macroscopic structure of wood

Observing a tree cross section from the innermost to the outermost part, several layers can be identified, as presented in Figure 1 (portrayal of a trunk cross section):

- Pith is the primary plant stem located in the middle of the trunk section.
- Heartwood is the inner part of the xylem consisting of dead cells with the function of long-storage of various extractives compounds, whose main task is the protection of wood.

- Sapwood is the outer part of the xylem in which transport processes take place.
- Vascular cambium is a thin layer composed by meristematic cells responsible for the secondary growth of the tree.
- Inner bark includes both primary and secondary phloem and provides the allocation of the sugars produced by photosynthesis to all living parts of the tree.
- Outer bark, the outermost part of the cross section, provides mechanical protection to the softer inner one and limits evaporative water loss.

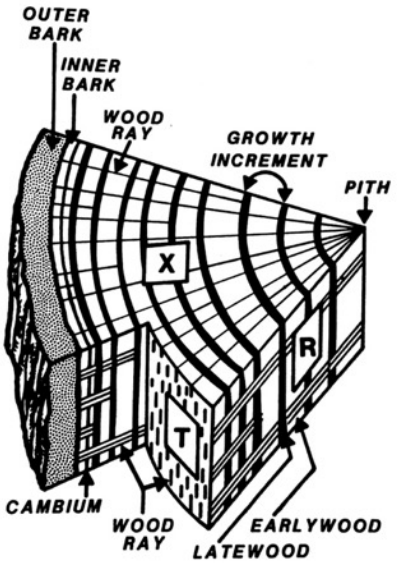


Figure 1: Representation of a tree cross section. X: cross-section or transverse surface; R: radial section; T: tangential section (adapted by Rowell "Chemistry of solid wood")

In temperate climates, the cambium's activity is periodical, which results in so called growth rings (also annual or year rings) that are visible even by the naked eye. However, the wood tissue is not uniform through the annual rings. Earlywood (EW) is produced in the beginning of the growing season and plays a major role in water transport. Accordingly, these cells have relatively big lumina and thin walls. Towards the end of the season, the cambium produces cells that have thick walls and smaller lumina and ensure mechanical support (latewood, LW). Changes from earlywood to latewood can be gradual

or abrupt, depending on the species (Parham & Gray 1984; Wiedenhoeft 2010).

2.1.2 Microscopic structure (wood tissue)

The arrangement of the wooden cells can be identified on the section cut in the three main planes used for the anatomical characterization of wood: the cross section, the tangential section and the radial section (Fig. 2) (Fengel & Wegener 1989).

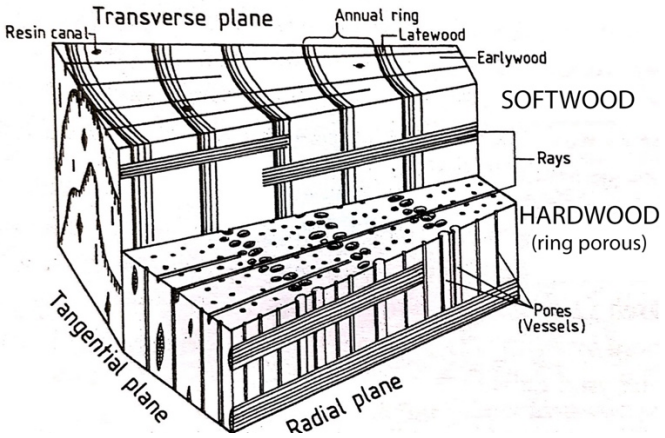
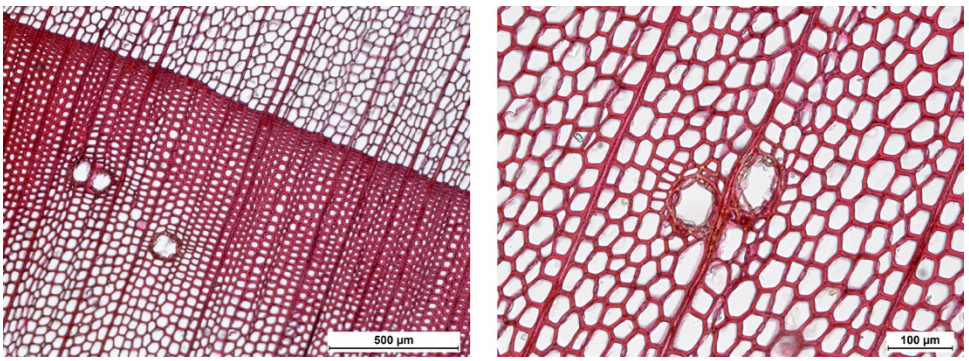


Figure 2: 3D representations of secondary xylem (adapted by Fengel & Wegener 1989)

Softwoods and hardwoods show remarkable differences in their tissues.

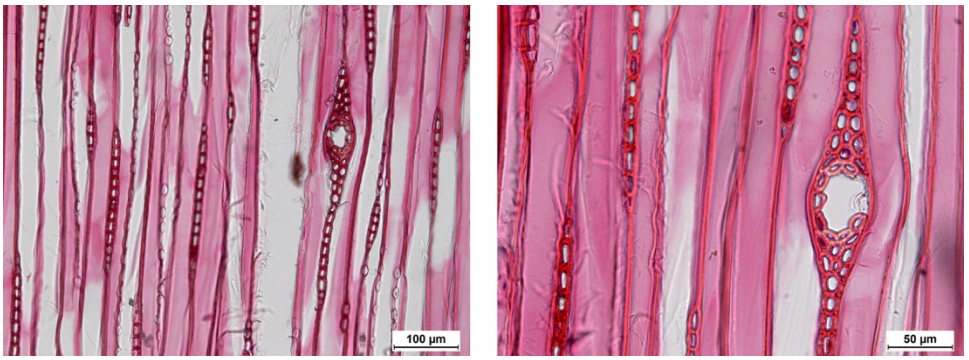
The cellular structure of softwoods is relatively simple (Fig. 3 a) and b), Fig. 4 a) and b), and Fig.5), made up of thin and long tube-like cells called tracheids (90-95% of the amount of the cells) which provide both water transport and mechanical support. Earlywood and latewood tracheids are aligned in the longitudinal direction of the stem. The former has thin walls and big lumen, while the latter have thick walls and smaller lumen. Tracheids radially arranged ensure storage and nutrient transport across the stem within the parenchyma cells, forming the rays. The amount of parenchyma cells aligned longitudinally is rather low (1-2%) and in some species they are even

completely missing. Species that have resin canals contain epithelial cells as well.



a) b)

Figure 3: Cellular structure of softwood in cross section: a) clear distinction between EW and LW in Picea abies and b) resin canals detail



a) b)

Figure 4: Cellular structure of softwood in tangential section: a) rays in tangential section and b) radial resin canal detail

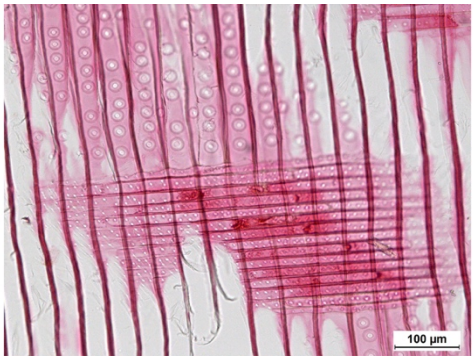
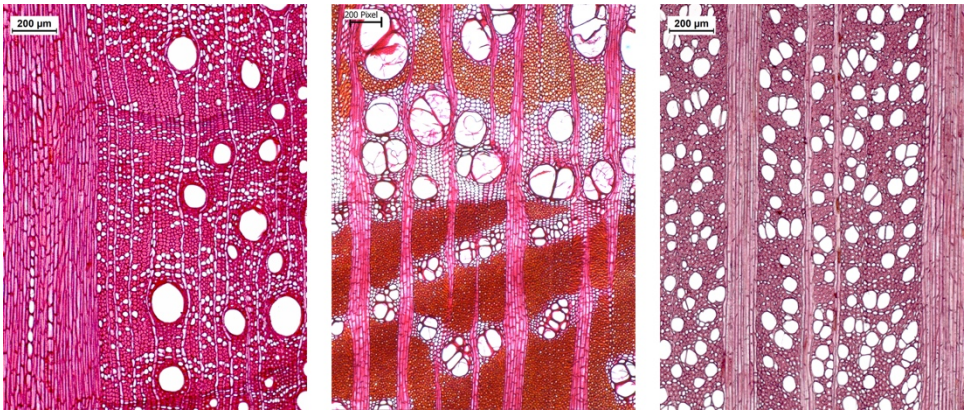


Figure 5: Cellular structure of softwood in radial section

Whereas hardwoods have different cell types for transport and supporting functions.

The supporting tissue consists of libriform fibres, a cell with an elongated shape with narrowed ends and mostly thick cell wall. Water transport along the stem takes place in the vessels, formed from multiple elements due to perforation of the cell walls at their ends. The diameter of the vessels ranges significantly, depending on the species and on their location within the annual ring. According to the arrangement of the vessels, hardwoods are categorized as ring-porous (Fig. 6 a) and b)) or diffuse porous (Fig. 6 c)). Axial parenchyma cells are more common in hardwoods than in softwoods and can be found either associated with the vessels (paratracheal parenchyma) (Figure 6 b)) or not (apotracheal parenchyma) (Fig. 6 a) and c)). Rays are composed of parenchyma cells as well.



a) b) c)

Figure 6: Cellular structure of hardwood: a) Quercus ilex; b) Robinia pseudoacacia; c) Platano sp.

2.1.3 Chemical components

The main chemical components of wood cell walls are cellulose, hemicelluloses and lignin, which are present in all wood species. The proportions and chemical composition of polyoses and lignin differ in softwood and hardwood, while cellulose is a quite uniform component of all woods (Pettersen 1984).

Cellulose is a linear high-molecular-weight homopolysaccharide that represents 40-45 wt% of the wood. It is built up of β-D-glucopyranose units linked together by β-(1-4)-glycosidic bonds (Fig. 7). The elementary unit of cellulose is cellobiose and the average degree of polymerization (DP) is 9000-10000 glucose units. The long straight chains of cellulose have got a strong tendency to create intra and inter molecular hydrogen bonds and van der Waals forces, letting the formation of a more complex structure: microfibrils, further arranged in a bigger structural element, called fibrils. A cellulose microfibril has crystalline and amorphous parts as well.

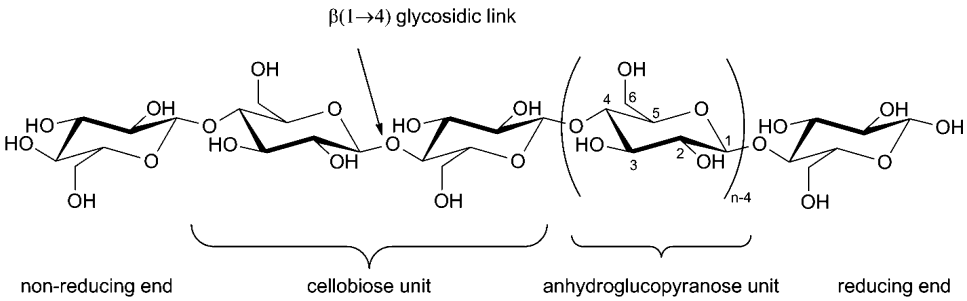


Figure 7: Molecular structure of cellulose chain

Hemicelluloses (polyoses) are heteropolymers in close association with cellulose in the cell wall. They include a wide variety of compounds composed of several types of sugar units (Fig. 8), arranged in branched chains, shorter than that of cellulose. Hemicelluloses are around 20-30 wt% but concerning their composition present vary greatly for softwood and hardwood and among different species.

Indeed, hardwoods contain more polyoses than softwoods and the sugar composition is different.

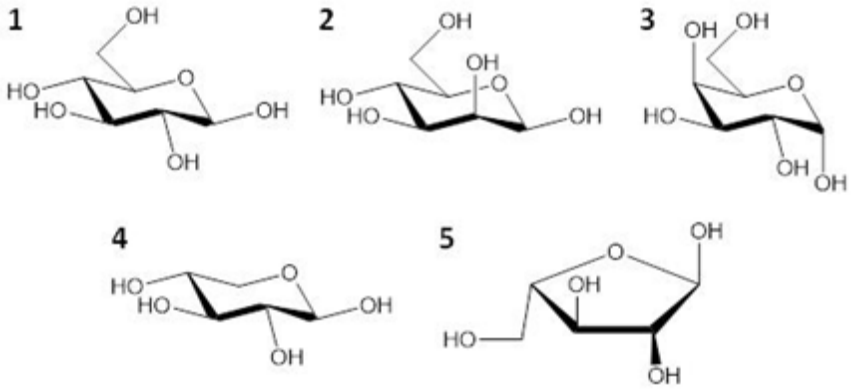


Figure 8: Structure of the principal components of the hemicelluloses (1 D-glucose, 2 D-mannose, 3 D-galactose, 4 D-xylose, 5 L-arabinose)

Softwood hemicelluloses: Galactoglucomannans (about 20%) are the main hemicelluloses in softwoods (Fig. 9). In addition, softwoods contain arabinoglucuronoxylan (5-10%), arabinogalactan and other polysaccharides.

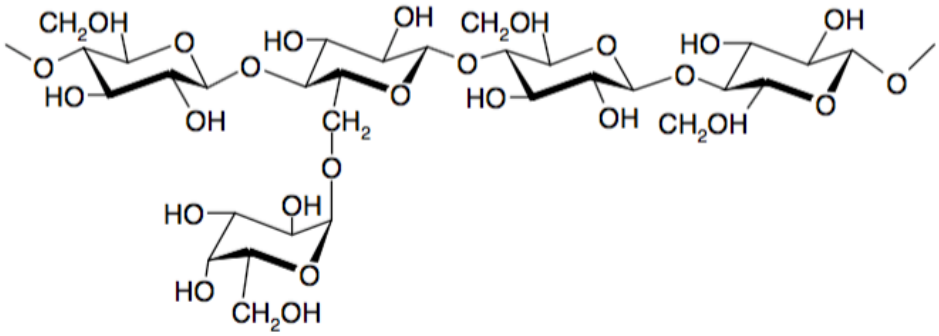


Figure 9: Principal chemical structure of galactoglucomannans

Hardwood hemicelluloses: Glucuronoxylans are the main hemicelluloses in hardwoods (Fig. 10). Depending on the hardwood species, xylan content varies between 15 and 30% of the dry wood. Besides xylan, hardwoods contain glucomannan (2-5%) and other polysaccharides.

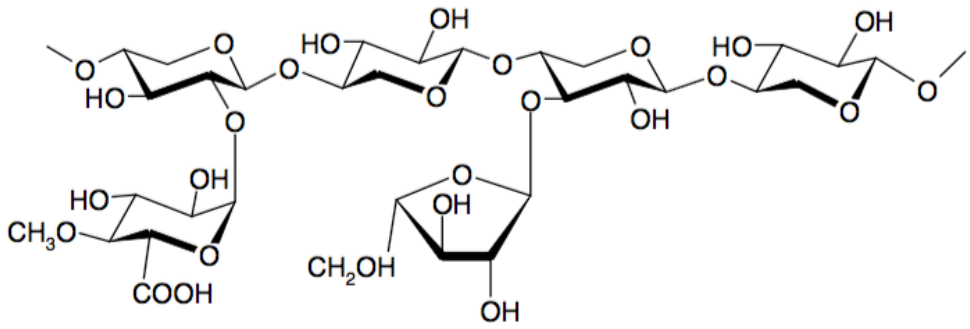


Figure 10: Principal chemical structure of arabinoglucuronoxylan

Lignin is the third macromolecular wood component. It consists of an aromatic, heterogeneous and amorphous system composed of phenylpropane units. The main components of lignin are: p-coumaryl alcohol, coniferyl alcohol (guaiacyl) and sinapyl alcohol (syringyl) (Fig. 11).

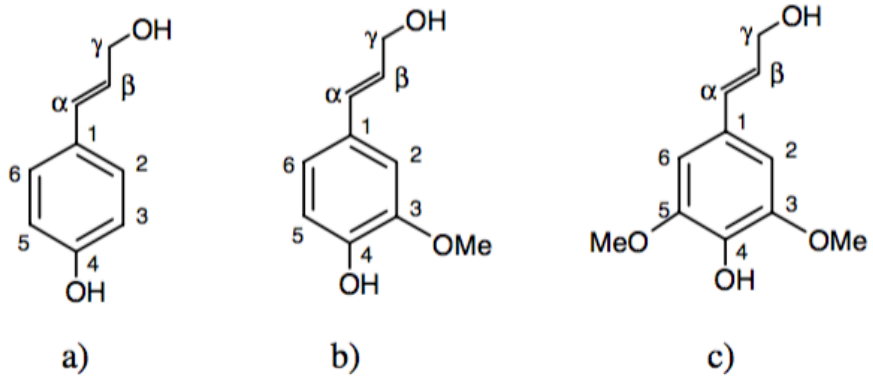


Figure 11: The monomeric units in lignin: a) trans-p-coumaryl alcohol, b) trans-coniferyl alcohol (guaiacyl units) and c) trans-sinapyl alcohol (syringyl unit)

Its amount in hardwoods and softwoods is about 20% and 30%, respectively. The three-dimensional structure of lignin is built up by C-O-C and C-C linkages. It is located in the compound middle lamella as well as in the secondary walls.

Lignin and hemicelluloses are intimately associated.

Beside these three main components, wood contains low-molecular-weight components as well: extractives (organic matter) and ash (inorganic matter).

Extractives include several chemical compounds: phenolic compounds, terpenes, fatty acids, alcohols, mono- and disaccharides. The quantity and quality of extractives shows variations depending on the species. Although they amount to only a few per-cent of the wood mass, they have remarkable influence on the colour, smell and durability of wood.

The ash content of wood is usually around 0.5%, consisting of various mineral substances (Ca, Mg and K).

2.1.4 Cell wall structure

The cell walls in wood are composed of multiple layers (Fig. 12). The concentric arrangement of this framework is caused by differences in the chemical composition and by orientations of the structural elements: cellulose fibres are structural components, while hemicelluloses and lignin constitute a blurring and encrusting matrix.

The cell wall consists of three main regions, from the outer part of the cell to the lumen (the void space): the middle lamella (ML), the primary cell wall (P) and the secondary wall (S) (Rowell 1984).

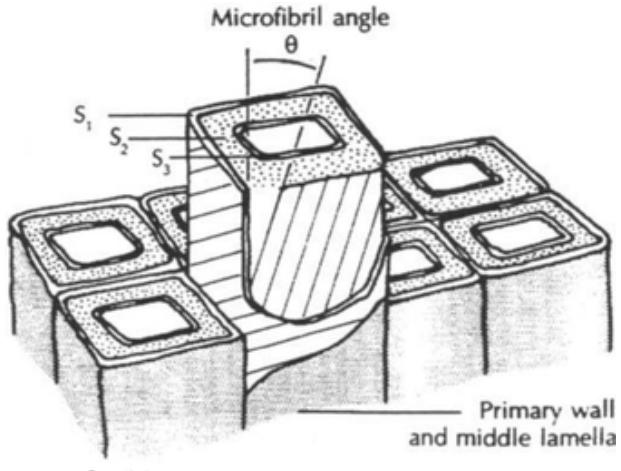


Figure 12: Scheme of the cell wall structure of normal wood fibers

The middle lamella is a layer free of cellulose which glues the cell together in order to form the tissue. In the adjacent primary wall, a limited amount of cellulose fibrils is arranged with irregular orientation in thin crossing layers. The subsequent wall is the

secondary cell wall which can be further divided into three layers (S1, S2, S3). In the S2 layer, the thickest and the most important part of the cell wall in a mechanical point of view, the cellulose microfibrils run parallel to each other and helical around the lumen with a particular angle to the cell axis (microfibril angle). S1 and S3 have microfibrils with helical alignment as well, but with a larger angle to the cell axis.

As already mentioned above, the chemical composition varies among the three layers. In the middle lamella and the primary wall there is the highest lignin content. The S2 layer has the highest proportion of cellulose, accompanied with a considerable amount of hemicelluloses. The hemicellulose content is the highest in the S3, while it contains only a little amount of lignin (Rowell 2005).

2.1.5 Variability of wood

During tree growth two different types of wood are produced (one after the other) by the cambium, respectively, juvenile and mature wood. Juvenile wood is always formed near the pith (for a number of years that may change with species), and is characterised, as compared to mature wood, by differences in both anatomical structure and physical-mechanical properties of wood. Often, this kind of differences concerns two main features:

-cell sizes (cell are shorter and smaller in tangential direction than ones in mature wood);

- microfibril angle (larger in rings closer to pith).

As consequence, these structural features determine an anomalous behaviour of juvenile wood especially deals with shrinkage and strength.

Moreover, in hardwoods the chemical composition shows little change from pith to bark and from base to top, while softwoods have wood cores with lower cellulose and higher lignin, but with little or no significant difference in hemicelluloses for either softwoods or hardwoods (Barnett & Jeronimidis 2003).

2.1.6 Hygroscopicity and wood moisture content (MC)

Wood is a capillary-porous material and hygroscopicity is an other important property of the material due to its ultrastructure, as well as the alignment of cellulose microfibrils in the cells. In fact, wood is a hygroscopic material because of the presence of -OH groups in the hydrophilic polymers of the cell wall (cellulose and hemicelluloses) which are able to fix water molecules forming hydrogen bounds. Hydroxyl groups are termed sorption sites, and the most sorption sites are found in the hemicelluloses followed by cellulose and lignin (Engelund et al. 2013).

Wood is able to adsorb and desorb water depending on the environmental surrounding conditions. Moreover, water in wood can be free located either in the lumen, or it can be bound within the cell wall. The state in which no free water is present in the lumina while the cell walls are saturated is called the fibre saturation point (FSP) and the moisture content stabilized at a certain climate is termed equilibrium moisture content (EMC). Moisture content (MC) is the ratio of mass of moisture to mass of dry wood substance.

Below the fibre saturation point, a change in water content results in shrinkage or swelling of the material and has an influence on its physical and mechanical properties as well. Most sorption sites are found in hemicelluloses and in the amorphous parts of the cellulose. By contrast, lignin is hydrophobic and limits the carbohydrates accessibility to water due to encrusting of them (Rowell 2005).

2.2 AGING OF WOOD

Aging of wood is a phenomenon of great complexity in the context of wood science. Wood is a natural polymer submitted to aging and, as such, a polymer aging is defined as "a change in polymer properties under influence of different environmental factors" (Bucur 2016). Wood structural degradation induced by aging is an irreversible process, an irreversible change of physical and mechanical properties of the material during longer storage or usage or due to variations of air humidity inducing wood moisture content fluctuations (Unger et al. 2001; Bucur 2016).

Despite the properties of recent wood having been widely studied, there is still a lack of knowledge concerning the aging process of wood or the properties of the aged material, crucial information for the proper conservation of wooden cultural heritage objects (Sonderegger et al. 2015).

Aging of wood begins with the cutting of the tree, when external influences come into play, and the subsequent changes of its components proceed depend on environmental factors in which wood material is. Humidity, temperature, solar irradiation, atmospheric content, biological factors, pollution, but also the kind of wood (softwood or hardwood), contribute significantly to wood degradation. Chemical components of wood structure, indeed, can be affected by oxidation, hydrolysis, dehydration, reduction and free radical reactions and, depending on the storage conditions over time, these reactions can be more or less aggressive for the structure of wood. In other words, the storage conditions determine the mechanisms and rates of degradation of wood.

The physical, chemical, morphological and structural characterizations by different physical and chemical methods are essential to understand several steps of the degradation processes that take place in wood material during its aging over years. Furthermore, this knowledge can contribute carrying out the most appropriate protection conditions.

A focus on naturally aged wood stored in dry air is presented. The biotic degradation of old wood, the effect of aging on the mechanical properties of the material or further the effect of aging on

archaeological or waterlogged wood are not themes dealt with this study.

2.2.1 Natural aging of wood

Since storage conditions determine what kind of chemical processes may occur, it is obvious that they have a significant effect on the aging process. In the case of wood, aerobic and anaerobic conditions have to be identified (Fengel 1991). Aerobic conditions are prevailing in wood buildings, architectural elements, sculptures, etc., whereas anaerobic conditions deal with wooden items buried in the ground and waterlogged such as foundation pillars, ships, etc.
In the case of wood stored in air, it has to be considered whether the objects are exposed to weathering or not. Wood exposed to direct sunlight undergoes chemical degradation caused by UV radiation. Moreover, wood exposed to weathering is also subjected to mechanical stresses due to fluctuations in temperature and humidity (Borgin, Faix, et al. 1975).
Studies related to dry aged wood are reviewed in section 2.2.2.

2.2.2 Changes in chemical composition and in the microstructure

As revealed and described in advance in section 2.1.3, cellulose, lignin and hemicelluloses are the main chemical components of wood. Their characteristic degradation processes as well as their sensitivity to degradation differ remarkably because of their different structures: for example, cellulose has a low solubility in most solvents and a strong enough resistance to hydrolysis because of its crystalline order system, i.e. its extremely ordered and high degree of intermolecular association within the fibrils (Fengel & Wegener 1989); by contrast hemicelluloses, due to their less ordered structure, have higher solubility and are easily hydrated and hydrolysable(Fengel & Wegener 1989; Fengel 1991); instead lignin is more resistant to hydrolysis, because of the presence in its 3D structure of ether and carbon-carbon bonds, but it is susceptible to oxidizing reagents, that could increase its degree of acid solubility (Fengel 1991).

Several studies concerning the chemical changes due to aging of wood are introduced in the following. The results reported in literature are not always concordant concerning lignin and holocelluloses amount, cellulose crystallinity degree, and their variation with age.

Very old dry wood is relatively rare and mainly comes from tombs in Egypt. Earlier researches have been conducted on samples collected by ancient and very old buildings or architectural items for construction purpose in Egypt (as already mentioned), Japan and India.

Borgin et al. (1975a, b) investigated samples of different wood species (*Pinus pinea, Juniperus phoenicea, Acacia nilotica, Pinus silvestris, Quercus robur*). The samples, varying in age from 900 to 4000 years and stored over time under different condition (dry air or in moist soil), were analysed by means of both electron microscopy (scanning and transmission electron microscopy: SEM and TEM) and polarized light microscopy and as well by analysis of their lignin content. By microscopical investigations (Borgin et al. 1975b), analysing the morphology of the cellulose, it was found to be intact. However, a reduction in birefringence observed indicated a reduction in the amount of crystalline cellulose. Also the amount of isolated lignin showed a decrease and it was assumed due to the oxidation of the macromolecules which degraded them in to smaller and soluble units (Borgin et al. 1975a).

Tomassetti et al. (1990) applied thermogravimetric analysis TG and DTG (differential TG) to the analysis of fresh and ancient spruce, fir and larch belonging to portals of ancient churches. For each species in the aged samples, an increase of the percentage lignin content was observed, mainly due to the decrease in cellulose, as a consequence of microbial degradation.

Erhardt et al. (1996) analysed samples of *Pinus sylvestris* used for construction purposes, aged 300-400 years. The type and amount of soluble saccharides were determined, including cellulose degradation products. While cellulose seemed to be essentially intact, some degradation of the hemicellulose (hydrolysis of xylan) and a decrease in the amount of crystalline material was observed.

Yonenobu & Tsuchikawa (2003), using near infrared spectroscopy (NIR), they examined changes due to aging in wood properties from modern timber and from an ancient wood building dated 7th century. Some modification in the amorphous region were observed, while no significant changes occurred in the semi-crystalline and crystalline regions.

Tsuchikawa et al. (2005), took dry-exposed archaeological wood sample from an old wooden temple in Japan (late 7th century). The degradation due to aging of the wood ultrastructure was investigated by FT-NIR with the aid of a deuterium exchange method and was compared with the state of a modern wood sample of the same species. From NIR spectra they observed a decrease of amorphous region, and partially semi-crystalline region, in cellulose, hemicellulose, and lignin by the aging degradation, whereas the crystalline region in cellulose was not affected by the aging. Hence the aging process of archaeological wood was clarified as a change in the state of order on a macromolecular structural level.

Luis García Esteban et al. (2006) compared the hygroscopicity of 205-year-old *Pinus sylvestris* L. with recently cut wood of the same species, in order to study the chemical variations and the crystallinity index of the cellulose using Fourier transform infrared spectroscopy (FT-IR) and X-ray diffraction (XRD). Despite the two infrared spectra were similar, preventing the attribution of the different hygroscopic behaviour to the chemical change in wood cell wall, a decrease in the cellulose crystallinity index was observed in old wood.

Popescu et al. (2005) described morphological and compositional changes in lime wood (*Tillia cordata* P. Mill.) from icons and iconostasis undergone to long-term degradation. By means of XRD and FT-IR, they provided evidence on the decrease of cellulose crystallinity and crystallite size. The disruption of the fibrils and changes in their dimensions was instead revealed by SEM analysis. Furthermore, Popescu et al. (2007) analysed aged lime wood also from painting supports (of different age, provenance, state of preservation and storage conditions) using analytical pyrolysis combined with gas

chromatography and mass spectrometry (Py-GC/MS) and electron spin resonance (ESR) spectroscopy. They observed the decrease of carbohydrates as result of the degradation of hemicelluloses (mostly due to the slow hydrolysis of O-acetyl groups of xylan). Concerning the most aged wood sample, an increase in the levoglucosan content upon pyrolysis was correlated to a potential increase in the more ordered part of cellulose during aging.

Nagyvary et al. (2006) have used solid state nuclear magnetic resonance (^{13}C CPMAS NMR) and infrared spectroscopy to analyse wood taken from antique instruments made by Stradivari and Guarneri. They compared ^{13}C CPMAS NMR spectra of maple wood samples, obtained as thin shavings during the repair of cracks in ancient violins, with samples of recent woods from Bosnia and central Europe. A decrease in intensity of some peaks in the spectra concern the signals of hemicelluloses and the main lignin signals was observed in samples from Guarneri violins, and in samples from Stradivari violins, respect spectra of modern maple wood. The differences between attenuated total reflection Fourier-transform infrared spectra (ATR FT-IR) are obvious at several peaks, particularly in the carbonyl (degradation of carbohydrates). The formation of quinones from lignin by oxidation was observed too.

Budrugeac & Emandi (2010) used thermogravimetric methods for investigation on fresh and old lime wood with the purpose of determine the conservative state of historical and cultural wooden objects. In fact, they compared TG, DTG and DSC (differential scanning calorimetry) curves for some new and old wood samples evaluating the percentage contents of the main components of wood, observing the thermal oxidizing steps of cellulose and lignin: the former decreased while the latter one increased as a result of natural aging.

Proietti et al. (2011) used ^{13}C CPMAS NMR spectroscopy to evaluate the state of conservation of an ancient wooden panel of an Egyptian sarcophagus. The ^{13}C CPMAS spectra of samples from the outer and the inner sides of the sarcophagus are compared with a seasoned yew wood sample used as control. They observed the absence

of acetyl groups of hemicelluloses signal in aged wood, clearly indicating the occurrence of hemicelluloses depletion. Evaluating the relative amount of carbohydrates and lignin and the state of conservation of lignin, both samples from the inner and the outer side of the sarcophagus showed a depletion of carbohydrates and of β-O-4 linkages inside lignin structure as well. Both these effects were definitely higher in the sample from the outer than in the sample from the inner side, due to a major degradation of this part.

Ganne-Chédeville et al. (2012) surveyed heartwood samples of spruce (*Picea abies* L.), used as structural elements dated 200 and 500 years old, by FTIR and UV resonance Raman (UVRR) spectroscopy. They observed IR spectra changes between the old and the recent wood due to the modifications of hemicelluloses (range of 1800-1500 cm^{-1}), and also a lower carbonyl/carbohydrate ratio and small differences in the UVRR spectra that could be caused by degradation products of lignin or unsaturated wood extractives.

These studies consider different wood species of various age and possibly submitted to different environmental conditions, furthermore different method were applied for the analysis. However, provided there is no microbial influence in wood chemical changes, to sum up, their results pointed out that the hemicelluloses are the mostly affected compounds due to degradation during aging of wood (Table 1). In fact, a decrease in hemicellulose amount (absolute and percentage one) has been reported increasing wood age. They are the most unstable components that can be depolymerized in oligosaccharides. Generally, data on chemical components obtained from old and fresh woods report that cellulose is the most stable component of wood, quantitatively unchangeable over time with age; by contrast the lignin decreases slightly due to oxidation during aging (significant effect on colour change of old wood)(Bucur 2016).

Table 1: summary of previous works regarding the chemical changes in wood during aging (+: increase; --: decrease; =: no change in proportion), (adapted by Kránitz et al. (2016))

	reference	species	age [years]	analytical method	lignin	holocellulose	cellulose	hemicelluloses	crystallinity	notes
Softwood	Borgin et al. (1975 a,b)	*Pinus pinea, Juniperus phoenicea, Pinus sylvestris*	900-4000	SEM, TEM, pol. LM Lignin content analysis	--*	+			--	*oxidation
	Tomassetti et al. (1990)	*Picea abies Abies alba Larix decidua*	300-800	TG, DTG	+		--*			*microbial degradation
	Erhardt et al. (1996)	*Pinus sylvestris*	300-400	Wet chemical analysis			=	--*	--	*hydrolysis of xylan
	Yonenobu & Tsuchikawa (2003)			FT-NIR	--		*	--	=	*decrease in the amorphous region
	Garcia Esteban et al. (2006)	*Pinus sylvestris*	250	FT-IR, XRD					--	
	Proietti et al. (2011)	*Taxus baccata*		^{13}C CPMAS NMR	--		--	--		
	Ganne-Chédeville et al. (2011)	*Picea abies*	200-500	FT-IR UVRR	*			**		*degradation **modification
Hardwood	Borgin et al. (1975 a,b)	*Acacia nilotica, Quercus robur*	900-4000		--*	+			--	*oxidation
	Popescu et al. (2005)	*Tillia cordata*	150-180	FT-IR, XRD, SEM					--	
	Nagyvary et al. (2006)	*Acer pseudo-platanus*		^{13}C CPMAS NMR, FT-IR ATR	--*			--		*oxidation
	Popescu et al. (2007)	*Tillia cordata*	150-180	Py-GC/MS ESR				--*	+	*hydrolysis of xylan
	Budrugeac et al. (2010)	*Tillia cordata*		TG, DTG, DSC	+		--			

A change in chemical composition of wood has a significant influence on its physical properties such as wood dimensional changes as a result of its hygroscopic behaviour (see below). In fact, considering that most problems with wooden cultural heritage objects occur due to swelling and shrinkage of wood, information about the hygroscopic behaviour of aged wood have to be well known in order to avoid irreversible damage.

The beliefs of craftsmen and instrument makers suggest that the dimensional stability of wood increases during aging. However, results of scientific investigations in this field are rather contradictory and no clear outcome can be determined (Bucur 2016).

2.2.3 Changes in the hygroscopic behaviour

Wood hygroscopicity can be modified by physical or chemical processes or by biological degradation, *e.g.* the exposure to high temperatures, acylation process and also fungi attack are able to reduce it, as consequence of a decrease in the hemicelluloses content (the most hygroscopic component). However, wood also modifies its hygroscopic behaviour naturally with the passage of time, related to the degree of crystallinity, following the reorientation of the molecules that most participate in the sorption process (hysteresis). The structure of wood, indeed, may be simplified into two basic components: fibrous cellulosic regions which provide most of the elastic stiffness, and are only slightly sensitive to moisture changes; and matrix regions (composed by hemicelluloses and lignin) which are more sensitive to moisture and contribute most to the swelling and creep of the material (Hunt & Gril 1996).

Below are reported a few references of works dealing with the hygroscopic behaviour, since this aspect has not been thoroughly studied in this thesis.

Erhardt et al. (1996) found no significant changes in the sorption behaviour of Scots pine specimens, aged 300-400 years.

Luis García Esteban et al. (2006) compared the sorption behaviour of a wood sample of Scots pine aged 205 years and a recent

sample. Sorption isotherms were taken at 35°C, and in their trial the aged sample showed higher EMC at every step of the sorption test than the recent reference sample. The difference may be a result of the lower crystallinity observed by the aged sample. However, based on higher hysteresis coefficients, the authors suppose that aged wood is more hygroscopically stable than recent wood.

Inagaki et al. (2008) determined sorption and desorption isotherms of Hinoki wood samples aged 1400 years. Beside the mass, NIR spectra were obtained at each humidity step. EMC values were shown to be lower for the aged wood than for recent wood. Spectral information suggests that the absorption mechanism of water into wood does not change considerably due to aging, although recent wood adsorbs molecules more strongly than aged wood.

Gereke et al. (2011) compared sorption hysteresis of new and old wood at room temperature. The response of wood to moisture variations, i.e. swelling and shrinkage, was tested in different steps of relative humidity simulating realistic conditions. Additionally, Young's and shear moduli were calculated from ultrasound velocity. The results demonstrate little if any difference between the two groups, indicating that conclusions drawn from the study of modern wood are also valid for historic wood in good condition.

Summarizing the results found in the literature, it can be supposed that the EMC decreases with aging, but the cause of the changes remains unknown in most cases. Degradation of hemicelluloses and an increase in crystallinity in the first few hundred years may have a contributing effect.

2.2.4 Accelerated/artificial aging

The study of the aging degradation on wood samples is not always easy, actually tests on naturally aged material are rather difficult to achieve, the amount of available material is limited and in most cases its history is unknown. Therefore, different artificial aging methods were developed to investigate the property changes of aged

wood samples. They are mostly designed to simulate the conditions of outdoor weathering or photodegradation processes indoors.

Colom et al. (2003) reported the detailed analysis of chemical modifications and structural changes in the cellulose and lignin of *Populus tremula* and *Buxus sempervirens*, as a result of photodegradation in a Xenon test chamber and the degradation was monitored by FT-IR. Lignin has been demonstrated to be the component most degradable in this kind of experiment.

Furthermore, thermal and thermo-hydrous treatments (usually aiming at property improvement of wood) can be considered as a kind of accelerated aging as well. However, the use of high temperatures modifies hygroscopicity which cause physical and chemical changes in the wood that are different from natural aging. In fact, in wood subjected to a high temperature the reduction of hygroscopicity is due to chemical transformations in the amorphous substances and it can not be explained by re-crystallisation of the cellulose. Moreover, heat-treated wood and aged wood are qualitatively different with respect to their hygroscopicity (and vibrational properties too). In conventional methods, wood is heated in the absence of moisture (oven-heating) or in saturated water vapor (steaming). However, it should be remembered that wood is aged in moderate humidity range (30-90% RH) and that the chemical changes in polysaccharides are much influenced by the humidity. Actually, the weight loss due to steaming is 10 times faster than that due to oven-heating, and the moisture sorption properties of oven-heated wood and steamed wood are qualitatively different. These must be reasons for qualitative difference between the aged wood and heat-treated wood (Obataya 2007).

Ganne-Chédeville et al. (2012) analysed spruce samples artificially aged by hydrothermal treatments by means of FT-IR ATR. They evaluated that esterified carbonyl structures in xylan decreased and free acid groups increased in artificial aging treated samples. Moreover, the formation of condensed structures has been attributed to changes in band intensities, such as C-H vibration in cellulose (1317 cm^{-1}).

Guo et al. (2015) studied the effects of an hygro-mechanical steam treatment on spruce wood in order to understand the chemical and physical changes of the secondary cell wall occurring under such conditions using imaging FT-IR microscopy. After this treatment, the cellular structure displayed significant deformations and the degradation of hemicellulose and lignin resulted in the decrease of hygroscopicity. This reduction in hygroscopicity of the wood after the combined treatment of compression and steam was associated to the probably reduction of accessible hydroxyl groups during treatment.

Therefore, the changes that wood undergoes during natural aging are not necessary similar to changes during artificial aging processes.

The other effect of the aging, as already mentioned above, have not been discussed in this research.

2.3 STUDY AND CHARACTERIZATION OF EMISSION FROM WOOD

The last part of the state of the art chapter deals with the fundamentals of emission analysis. The classes of compounds, some monitoring and sampling regulations, and a focus on the literature about emission from wood samples are presented below.

As wood aging is a subject that has been studied since 1950's, the issue of emission of organic compounds from raw material is a relatively recent situation that is gaining ever greater importance and more research is conducted on this topic.

2.3.1 Volatile Organic Compounds (VOCs) emission

Pollutants are in general distinguished in inorganic and organic compounds.

Organic substances are classified by World Health Organization (WHO 1997) according to their boiling points into very volatile organic compounds (VVOC; bp≤ 60°C), volatile organic compounds (VOC; bp = 60°C-290°C), semi-volatile organic compounds (SVOC; bp = 290°C-400°C) and particulate organic compounds or organic compounds associated with particulate matter (POM) (Table 2).

Table 2: the classification of organic substances of WHO

		boiling point
very volatile organic compounds	VVOC	<=60°C
volatile organic compounds	VOC	= 60-290°C
semi-volatile organic compounds	SVOC	= 290-400°C
organic compound associated with particulate matter	POM	>400°C

VOCs represent a large and chemically diverse group of carbon-based molecules, such as hydrocarbons and other organic molecules, with a high vapour pressure at room temperature. They are emitted into the atmosphere from anthropogenic and biogenic sources (plants, animals, microorganisms, production processes, and/or their products) (Jantunen et al. 1997; Guenther et al. 2000). These compounds may also be formed *in situ* in the atmosphere as products

of the transformation of other VOCs (Atkinson & Arey 2003). The major classes of emitted VOCs are alkanes, alkenes, aromatic hydrocarbons, and oxygenated compounds, with vegetative emissions typically being composed of alkenes (isoprene, monoterpenes, and sesquiterpenes) and oxygenated VOCs (including acetone and methanol) (Atkinson & Arey 2003).

2.3.2 Indoor air pollution: regulations, monitoring and sampling

Analysis of volatile organic compounds (VOCs) is a useful method for understanding the chemical processes involved in polymer degradation. A significant advantage of VOC analysis is its potential to be non-invasive, avoiding destructive sampling or even contact with an object.

Concentration of organic pollutants in indoor air normally range within a broad interval between few $\mu g/m^3$ to several hundred $\mu g/m^3$. Otherwise, in the museum environmental the detection of chemical contaminants even at a small scale is of high importance. Therefore, a precise identification of emission sources (e.g. different materials) and their emission profile (concentration *versus* time) are essential. As result, the study of the structural modification and transformation of different materials is possible as well.

2.3.3 Emission from wood

It was established in the last decades that the living vegetation of continents is the main source of a wide range of reactive volatile organic components (VOCs) of the atmosphere (Isidorov 1990; Fehsenfeld et al. 1992; Guenther et al. 1995).Much less attention was paid to the study of non-methane VOCs emitted by decomposing vegetable material (Isidorov et al. 2003).

Several studies concerning the emission of organic compounds from wood (raw wood or after specific treatments or processing procedure) are introduced in the following. Different analytical methods, characterized by their own detection limits, have been used, and different trials have been conducted in different conditions of

temperature, relative humidity and moisture content of samples. These studies mostly pointed out the emission of terpenes from softwoods and the emissions of carbonyl compound from hardwoods. In literature many methods have been proposed aim to identify these compounds and above all to reduce their emission.

Risholm-Sundman et al. (1998) monitored the emission of volatile organic compounds (VOC) from wood and wood products of nine different wood species in order to evaluate the impact on the indoor environment from different materials. The wood samples were felled between 0.5 to 1.5 year before the emission tests and most of them were dried to a moisture content of about 5%. All samples were planed to have a fresh surface just before the emission test started. The measurements were made with Field and Laboratory Emission Cell (FLEC) and with Head-Space (HS) analyses and the results from the different methods indicated that the main emissions are terpenes from softwood and acetic acid from hardwood.

Manninen et al. (2002) compared the emissions of VOCs from air-dried Scots pine wood and from heat-treated Scots pine wood by means of GC-MS analysis. Air-dried wood blocks released about 8 times more total VOCs than heat-treated (24h at 230°C) ones. Terpenes were clearly the main compound group in the air-dried wood samples, whereas aldehydes and carboxylic acids and their esters dominated in the heat-treated wood samples. Only 14 compounds out of 41 identified individual compounds were found in both wood samples indicating considerable changes in VOC emission profile during heat-treatment process. In fact, during heating of wood material, wood constituents start to degrade leading finally to remarkable chemical changes. The most volatile compounds are easily evaporated during the heating and drying process. Thermal degradation of cellulose is proceeding rapidly if the temperature is elevated above 200°C. Levoglucosan is the most important primary degradation product and finally furan derivatives are formed as end products. Concerning hemicellulose (especially xylans), their acetyl groups hydrolyse and acetic acid is formed as well.

Roffael (2006) studied the emission of formaldehyde from spruce, pine and beech samples, and summarized that undried and unprocessed woods also contain a small amount of releasable formaldehyde. The formaldehyde release of wood increases during its processing to lumber, particle- and fibre- boards due to drying, pressing and thermo-hydrolysis etc. Moreover, monoterpenes emission contributes to the emission of VOCs in two different ways: they are emitted directly from the wood, and on the other hand, they can contribute to the formation of degradation products such as formaldehyde by oxidation processes.

Hyttinen et al. (2010) compared VOC emissions from three untreated (air-dried) and heat-treated wood species (Norway spruce, Scots pine European aspen) during a four weeks test period in emission chamber test. Heat-treatment decreases and changes the composition of VOC emissions. The present study, which was conducted by using standardized emission testing, confirmed largely earlier findings on the emissions of heat-treated wood obtained by applying miscellaneous test methodology. TVOC emissions are significantly lower after the heat-treatment than before that. Especially emissions of terpenes from softwood decrease substantially during the heat-treatment. Heat-treatment increases the emission of furfural and decreases the emission of hexanal from all the tested wood species. Even though emissions of aldehydes and carboxylic acids, which have low odour threshold values, dominate emissions of heat-treated wood, their emission rates remained around the same level in softwood samples and in case of European aspen lower than the corresponding emissions from the air-dried wood samples.

Gibson & Watt (2010) examined using passive samplers a total of 14 different wood types for acetic and formic acid emission at room temperature (18–21 °C) 7 days after they were placed in a sealed environment at 54% RH. Then the experiment was repeated with extreme humidity levels to examine both ends of the humidity spectrum (approx. 100 or 5.8% RH) and at 45°C. It was observed that acetic acid was given off at higher concentration than formic acid for every wood type, much more from hardwoods than from softwoods. At

the extreme low end of RH, very little acetic acid was released by any wood indicating that retardation of acetyl hydrolysis of hemicelluloses was achieved. Besides, acetic acid concentrations increased by factors of 7–11 when the storage temperature of the wood increased from 20 °C to 45 °C.
Their simple experiment demonstrates the need to carefully select wooden materials used to construct museum furniture and the control of temperature appears to be an important factor when controlling acid emission.

Schumann et al. (2012) focused to detect formaldehyde (HCHO) as a very volatile compound (VVOC) among other volatile organic compounds in the low concentration ranges that are relevant for the production control in wood processing industries. They showed that a quick and easy derivatization of HCHO can be used for a reliable and straightforward identification with GC- FAIMS. Headspace samples of specimens made from wood-based panels were collected and pre-concentrated on a conventional solid phase microextraction fiber (SPME) and thermally desorbed in a split/splitless GC- injector. A standard gas-mixer generator based on the dynamical permeation principle was used to produce known concentrations of HCHO. The results are compared with gas chromatography-mass spectrometry (GC-MS).

Salem & Böhm (2013) presented an overview concerning the emission of formaldehyde from wood, which increases during its processing procedures of wood (i.e., particleboard and fiberboard). Formaldehyde is emitted from wood under very high heat and is not expected to be a significant source of the emissions from composite wood products during normal service. Formaldehyde is also detectable even if wood has never been heated as well as under more or less ambient conditions. The presence of formaldehyde in the emissions from wood that does not contain adhesive resin has been explained by thermal degradation of polysaccharides in the wood. The emission levels of formaldehyde depend on factors such as wood species, moisture content, outside temperature, and time of storage. Additionally, the pyrolysis of milled wood lignin at 450 °C yields

benzaldehyde, and the pyrolysis of spruce and pinewood at 450 °C generate formaldehyde, acetaldehyde, 2–propenal, butanal, and butanone, which can be attributed to the breakdown of the polysaccharide fraction of the wood.

Roffael et al. (2015) studied the influence of treating pine wood strands with hydrogen peroxide as an oxidising agent and sodium sulphite as a reducing agent on the emission of volatile organic compounds revealed that hydrogen peroxide increases the emission of aliphatic aldehydes (e.g. hexanal) and reduces the emission of monoterpene compounds (like a-pinene). Sodium sulphite as a reducing agent decimated the emission of monoterpene compounds without noticeably affecting the emission of volatile aldehydes.

Table 3: summary of previous works regarding the characterization of emission from wood and wood products

Reference	species	Analytical method	emission	note
Risholm-Sundman et al. (1998)	*Fraxinus excelsior, Fagus sylvatica, Acer saccharum, betula pubescens, Quercus robur, Prunus serotina, Pinus silvestris, Picea abies*	FLEC and HS	terpenes from softwood and acetic acid from hardwood	Samples dried to MC 5%. All samples had fresh surface just before the emission test started.
Manninen et al. (2002)	*Pinus sylvestris*	GC-MS	Terpenes in the air-dried wood samples; aldehydes and carboxylic acids and their esters in the heat-treated wood samples	Compare air-dried and heat-treated wood
Roffael (2006)	spruce, pine and beech samples	Emission chamber test (1 m^3, 30°C)	Formaldehyde and terpenes	
Hyttinen et al. (2010)	*Picea abies, Pinus sylvestris and Populus tremula*	emission chamber test	aldehydes and carboxylic acids	Heat-treatment decreases and changes the composition of VOC emissions
Gibson & Watt (2010)	14 different wood types	Passive samplers (palmes tubes)	acetic and formic acid	
Schumann et al. (2012)	Spruce and pine wod	GC-FAIMS SPME GC-MS	formaldehyde	
Salem & Böhm (2013)	Spruce and pine wod		formaldehyde, acetaldehyde, 2–propenal, butanal, and butanone	After pyrolysis
Roffael et al. (2015)	Pine wood	Emission chamber test (23 l chamber)	Aldehydes and monoterpenes	Compare hydrogen peroxide and sodium sulphite treatments on wood strands

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5.

3 MATERIAL AND METHODS

3.1 Wood samples and their preparation

The first phase of the research dealt with the qualitative characterization of VOCs emission from green state wood samples from different tree plants. Several samples, both of softwood and hardwood, have been prepared in order to study the emission from fresh wood cores (from both heartwood and sapwood unseparated).

The fieldwork was carried out in the spring of 2015 at Monte Morello (Florence, Italy). Trees of 13 different species were chosen among individuals belonging to the same class size (height between 15 and 20 m, diameter at breast height over 20 cm) (Table 4). From each tree, fresh wood core samples were extracted (Fig. 13). Three cores were collected on the same side of the tree trunk (S–SW–SE orientations) and at the same height (1.30 m from the ground) using a Pressler's increment borer (diameter = 5 mm) and a core extractor. Only cores without wounds or knots and including the pith were chosen for subsequent analysis. Residual bark for each sample was discarded. Afterward, the samples were sealed in a glass vial immediately upon extraction from the trunk and kept refrigerated (2–4 °C) until measurements.

Table 4: list of the 13 species analysed in the first work by means of PTR-TOF-MS

Species	Family	Group
Atlantic cedar (*Cedrus atlantica* Man.)	Pinaceae	Softwood
Austrian black pine (*Pinus nigra* Arn.)	Pinaceae	Softwood
Common cypress (*Cupressus sempervirens* L.)	Cupressaceae	Softwood
Bay laurel (*Laurus nobilis* L.)	Lauraceae	Hardwood
European walnut (*Juglans regia* L.)	Juglandaceae	Hardwood
Black poplar (*Populus nigra*)	Salicaceae	Hardwood
Common fig (*Ficus carica* L.)	Moraceae	Hardwood
Almond (*Prunus amygdalus* (Mill.) D.A.Webb)	Rosaceae	Hardwood
Wild cherry (*Prunus avium* L.)	Rosaceae	Hardwood
Black locust (*Robinia pseudoacacia* L.)	Fabaceae	Hardwood
Turkey oak (*Quercus cerris* L.)	Fagaceae	Hardwood
Downy oak (*Quercus pubescens* Willd.)	Fagaceae	Hardwood
Evergreen oak (*Quercus ilex* L.)	Fagaceae	Hardwood

Figure 13: wood cores

After the first analysis on fresh wood, the same wood specimens were subjected to several cycles of water desorption and adsorption, assuming that moisture variation might simulate a sort of accelerated aging process. The procedure is reported below in the next section (3.2.1).

Subsequently, several *Picea abies* solid samples (10cm x 2cm x 4mm) were prepared from a log, in order to compare emission from green state samples (immediately after tree felling, cut in Vallombrosa forest) and moisture conditioned samples (submitted to the same moisture cycles previously mentioned), both from heartwood and sapwood separately.

These specimens were used in preliminary trials (not reported in this thesis) concerning emission from Norway spruce wood, in order to define both the most suitable concentration time of the emission incubation for VOCs analysis and the masses scale of interest for this wood species, presented in this research. They were used also to set up the moisture cycles, explained below, using different salts, acted to simulate accelerating wood aging. Moreover, a set of these solid wood samples was submitted to extraction in alcohol, whereas an other set was submitted to UV irradiation. Both these methodologies were reported in the Paper I, where emissions from untreated fresh wood cores were compared with these treated samples.

After being stored in a climate chamber for 1 years, some set of these solid wood samples were powdered and were used as recent wood references (MOD1 and MOD2, see beyond in Paper II) for the study of the aged spruce wood, using both VOCs and spectroscopic analyses. Figure 14 outlines all the experimental attempt done on several solid specimens of *Picea abies*.

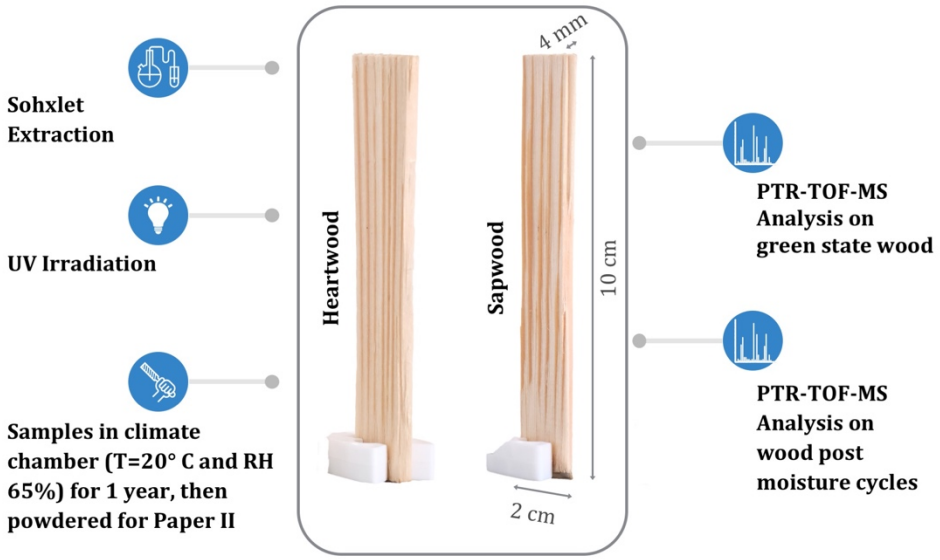


Figure 14: scheme of the different trials prepared on specimens of Picea abies

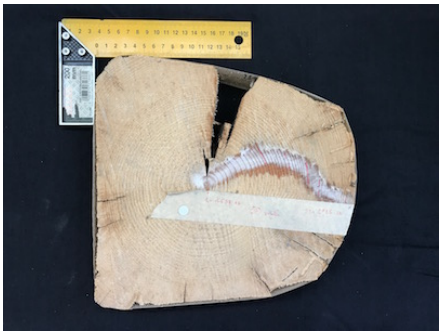
For the study concerning the aging of Norway spruce (Paper II), other powder samples from 2-years and 30-years old seasoned wedges were prepared and were used as recent wood references too (Fig. 15 and 16).

Figure 15: 2-years old seasoned wedges provided by CNR-IVALSA (Florence)

Figure 16: 30-years old seasoned wedge

Aged wood investigated in this study came from three different site (Table 5), and were provided by the professor Mauro Bernabei of the CNR-Ivalsa, in Trento, Italy. From these beam sections were prepared historical wood powder samples.

Table 5: list of historical samples

Name of the beam section		Origin	Period
	Corradini 1 **HIST_COR**	Casa Corradini, Cavalese, Val di Fiemme (Italy)	1731-1843
	Rosmini 3B **HIST_ROS**	Palazzo Rosmini, Rovereto (Italy)	1657-1715
	Vigo 8 **HIST_VIG**	Vigo di Fassa (Italy)	1312-1397

3.2 Methods

3.2.1 Moisture cycles

Wood samples were submitted to specific moisture cycles from fiber saturation point (FSP) to a moisture content (MC) of 10-12%, which were intended to simulate the natural aging of wood through cycles of moisture variation that typically occur in wood with the passing of time (Akahoshi & Obataya 2015).

The following drying-moistened conditioning procedure were followed:

- all the samples were placed in a sealed desiccator and dried with magnesium nitrate hexahydrate for analysis (EMSURE® ACS, Reag. Ph Eur. CAS 13446-18-9, EC Number 233-826-7, chemical formula $Mg(NO_3)_2 \bullet 6H_2O$) at T 20°C (obtaining a RH 54%-58%) until no weight loss was detected and a moisture content (MC) of 10-12% was achieved for the analysis of emissions;
- subsequently, the samples were moistened at T 20°C and RH 100% in the same desiccator until no weight loss was detected.

Then the cycles started again (Fig. 17).
The two steps were repeated several time.

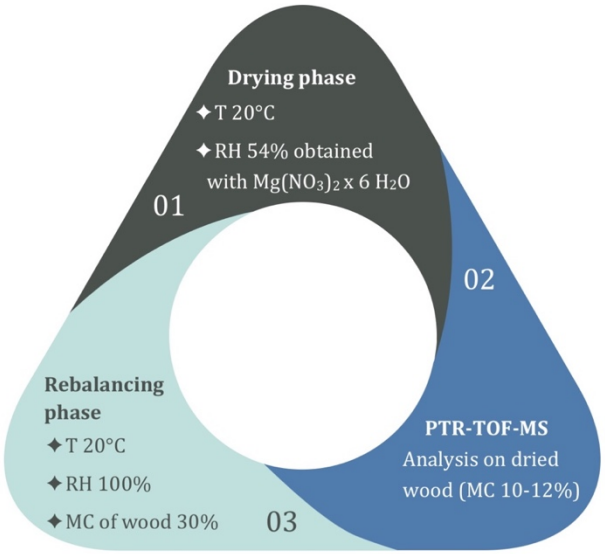


Figure 17: scheme of the moisture cycle

3.2.2 Proton- transfer reaction mass spectrometry (PTR-TOF-MS)

Mass spectrometry (MS) plays a critical major role in the analysis of VOCs. The significant feature of MS analysis of VOCs is detection limits on the order of parts-per-million to parts-per-trillion by volume (ppmv to pptv). Currently the application of combined chromatography and MS (GC/MS) techniques is the most widely used tool for both detecting and quantifying VOCs. GC/MS provides

sensitivities in the range of pptv, although a pre-concentration step is required to adsorb VOCs onto a suitable medium. The release of VOCs from the matrix occurs based on a partitioning effect that creates equilibrium between substances present in the matrix and the gas phase above the sample. In the most cases, standard methods for analysis specify the use of the adsorbent Tenax (Buchem B.V., The Netherlands), a porous polymer resin based on 2,6-diphenyl-*p*-phenylene oxide, specifically designed for the trapping of volatiles and semi-volatiles from air. However, the main disadvantage of GC/MS is the time required to achieve chromatographic separation of compounds (Maleknia 2012).

By contrast, another valuable technique for detecting and monitoring of VOCs (both with or not pre-concentration step) is the proton-transfer reaction/mass spectrometry (PTR/MS), which is ideal for the analysis of VOCs at low concentration (part per billion volume (ppbv) to pptv). Trace-level detection of atmospheric VOCs is facilitated by selective proton-transfer reactions with H_3O^+ (proton affinity of 166.5 Kcal/mol). Moreover, the incorporation of time-of-flight analyser (TOF-MS) with high resolving power improves the separation and identification of isobaric compounds.

Proton-transfer reactions of H_3O^+ (typical count rates of 10^6 counts/s) enable ionisation of trace levels of VOCs in air according to:

$$H_3O^+ + VOC \rightarrow VOCH^+ + H_2O$$

Concentration of VOCs are calculated by applying pseudo first-order kinetics (i.e. high ratio of $[H_3O^+]$ to trace levels of $[VOCH]^+$) according to:

$$[VOCH^+] = [H_3O^+]_0 \{1 - exp(-k[VOC]Dt\} \cong [H_3O^+]_0 \, k[VOC]Dt$$

where $[H_3O^+]_0$represents the concentration of primary reagent ions in the absence of VOCs, k corresponds to rate constants for proton-transfer reaction from H_3O^+ to VOCs and Dt is the time H_3O^+ ions require to traverse the drift tube.

3.2.3 Solid state NMR spectroscopy

The complex structure of wood sets conditions for measuring equipment. Nuclear magnetic resonance (NMR) spectroscopy is a proven tool in the analysis of wood and its components. Model compounds and isolated preparations of wood components are accurately characterised by several liquid state NMR techniques, while the structure of native wood has been less often characterised. Unfortunately, the necessary step of isolation or fractionation may cause significant modification in the structure of wood. An essential advantage of solid state NMR spectroscopy is that the samples can be studied in native state without component isolation or fractionation, and all chemical changes in the structure that may occur in chemical treatment are thereby avoided. Solid state NMR spectroscopy is a highly useful tool in the analysis of wood due to its non-destructive nature: the wood samples can be studied in native state (Maunu 2002).

As described, solid state ^{13}C NMR spectroscopy has been successfully applied in studies of wood and its components. The techniques of proton-carbon cross polarisation (CP), high- power proton decoupling and magic angle spinning (MAS) are combined in solid state CP/MAS NMR measurements. In solid state NMR spectroscopy, the advantage is that the samples can be studied in native state without fractionation or isolation of components and all chemical changes in the structure that might occur in chemical treatment are thereby avoided.

Cellulose, hemicelluloses and lignin, and to some extent extractives, give characteristic signals in the solid state NMR spectrum. Relatively sharp signals are assigned to ordered cellulose or hemicelluloses, while broader background signals are assigned to lignin and disordered hemicelluloses.

The ^{13}C CPMAS NMR spectrum of Norway spruce is shown in Fig. 18. The specific signal assignments for the spectrum of wood are presented in Table 6.

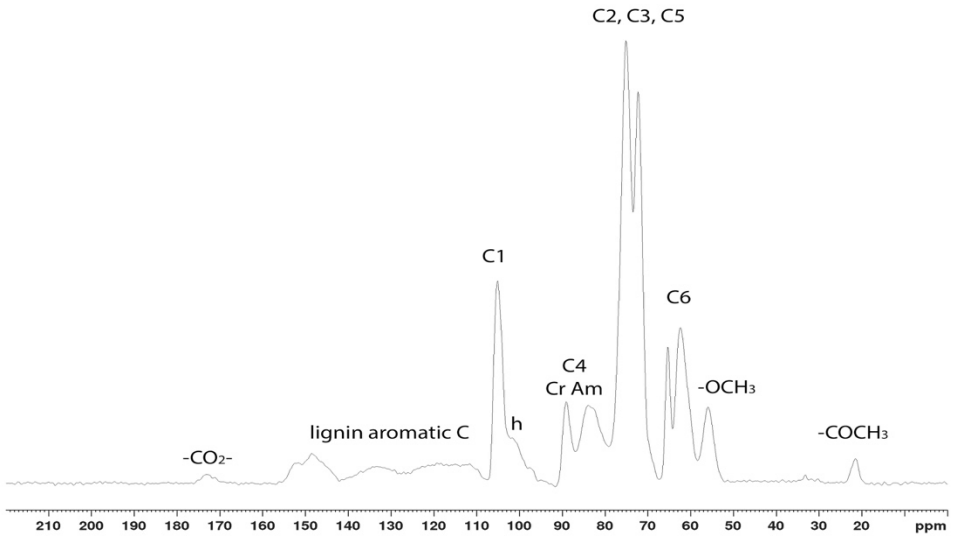


Figure 18: ^{13}C CP/MAS NMR spectrum of Norway spruce. Cr refers to crystalline cellulose, Am to amorphous cellulose and h to hemicelluloses

Table 6: Signal assignments for the ^{13}C CP/MAS NMR spectrum of wood

Chemical shifts (ppm)	Assignments
21.5	CH_3 in acetyl groups in hemicellulose
56	OCH_3 in lignin
62	C-6 of amorphous cellulose
65	C-6 of crystalline cellulose
72-75	C-2,3,5 carbon of cellulose and hemicellulose
84	C-4 of amorphous cellulose
89	C-4 of crystalline cellulose
101	C-1 of hemicellulose
105	C-1 of cellulose
112	G 2
116	G 5
120	G 6
133	S 1/4f, G 1f
137	S 1/4e, G 1e
146	G 4f
148	S 3/5f, G 3
153	S 3/5e, G 3
173	CO_2 in acetyl groups of hemicelluloses

G = guaiacyl, S = syringyl, e = etherified C-4, f = free phenolic C-4

3.2.4 Statistical evaluation

Multivariate data analysis techniques such as Principal component analysis (PCA) have been performed on spectroscopic data. PCA, indeed, offers a rapid means of identifying and classifying qualitatively great amounts of spectral data in a short time. PCA involves a mathematical procedure that transforms a number of variables into a smaller number of variables called principal components. The first principal component is the combination of variables that explains the greatest amount of variation within samples or sample groups. The second principal component defines the next largest amount of variation and is independent of the first principal component. There can be as many principal components as there are variables (Cordella 2012).

In addition, Partial least squares discriminant analysis (PLS-DA) approach was applied for the classification of the emission data matrix. PLS can be viewed as a multivariate regression framework where we want to predict the value of several target variables (Y1, Y2,,…) from the values of several input variables (X1, X2,…). When target variable Y is a categorical variable, classification with PLS is termed PLS-DA where the DA stands for discriminant analysis (Wold et al. 2001).

3.3 REFERENCES

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4 RESULTS AND DISCUSSION

In the followings, the researches concerning my studies dealt with emissions of organic compounds from different species of wood, chemical changes due to aging of wood and the case of study on the emission from stringed instrument are introduced.

Each paper is presented with its own bibliography and supplementary materials.

- PAPER I

The first work addresses the characterization of volatile organic compounds (VOCs) emitted by samples of 13 different wood species, belonging to both softwoods and hardwoods groups, regularly measured at different intervals of time, after the first measurement on green wood. The same wood specimens were subjected to several cycles of water desorption and adsorption. Proton Transfer Reaction-Time of Flight-Mass Spectrometry (PTR-TOF-MS) was used as a tool to characterize the emission of VOCs. Coupled with a multivariate class-modelling approach, this tool was able to discriminate between groups (softwood and hardwood) and in some cases between different species.

However, results showed that the discriminant capacity of VOCs emission to separate species and families rapidly decreases after the first cycles of moisture variation in wood. The green wood was characterized by a richness of volatile compounds, whereas, after only the first dry cycle, wood emitted a more restricted group of compounds. We hypothesized that most of these VOCs might have originated from structural changes and degradation processes that involve the main polymers (particularly hemicellulose) constituting the cell wall of wooden cells. The results obtained are in agreement with the physical and chemical modification processes that characterize wood aging.

[Sassoli M, Taiti C, Guidi Nissim W, Costa C, Mancuso S, Menesatti P, Fioravanti M (2017). Characterization of VOC emission profile of

different wood species during moisture cycles. iForest 10: 576-584. – doi: 10.3832/ifor2259-010]

- PAPER II

The aim of this second work was the study of wood aging process, comparing modern and historical powdered wood samples, and correlating structural changes, analysed by solid-state nuclear magnetic resonance (NMR), with the emission of volatile organic compounds (VOCs), carried out by means of Proton Transfer Reaction – Time of Flight – Mass Spectrometry (PTR-TOF-MS) technology.

The spectroscopic results showed only that the degradation rate of historical wood samples was higher for hemicelluloses than for cellulose and lignin, which appeared to be more stable under aerobic aging conditions. However, the volatomic approach was a useful tool to characterize VOCs profile of recent and aged wood samples. Coupled with a multivariate class-modelling approach, PTR-TOF-MS analysis was able to discriminate between the two different age groups (modern and historical wood), better than spectroscopic technique could do.

Moreover, the emission of specific compounds, such as acids (mainly acetic acid due to deacetylation of hemicelluloses), aldehydes, alcohols, from both modern and historical wood samples, provided that several modifications are acting in the structure of wood, immediately at the beginning of the aging (after tree falling/cutting), but these kind of structural changes could not be identified at these early stages of aging by means of spectroscopic techniques.

- PAPER III

In this case study, we tried to sample VOCs emissions from historical stringed instruments stored at the Museo del Violino, in Cremona, Italy. Emission profile of the stringed instruments can be compared qualitatively with the emission profile obtained by wood powdered samples.

4.1 PAPER I

CHARACTERIZATION OF VOC EMISSION PROFILE OF DIFFERENT WOOD SPECIES DURING MOISTURE CYCLES

1. Introduction

Volatile organic compounds (VOCs) represent a large and chemically diverse group of carbon-based molecules, such as hydrocarbons and other organic molecules, with a high vapour pressure at room temperature. They are emitted into the atmosphere from anthropogenic and biogenic sources (plants, animals, microorganisms, production processes, and/or their products) (Jantunen et al. 1997, Guenther et al. 2000). These compounds may also be formed *in situ* in the atmosphere as products of the transformation of other VOCs (Atkinson & Arey 2003). The major classes of emitted VOCs are alkanes, alkenes, aromatic hydrocarbons, and oxygenated compounds, with vegetative emissions typically being composed of alkenes (isoprene, monoterpenes, and sesquiterpenes) and oxygenated VOCs (including acetone and methanol) (Atkinson & Arey 2003). The classification proposal of the World Health Organization (WHO 1989) is the most widely used. The WHO classifies pollutants into very volatile organic compounds (VVOCs), volatile organic compounds (VOCs), semi-volatile compounds (SVOCs), and organic compounds associated with particulate matter (POM).

The emission from vegetation (biogenic emission) has been widely studied: conditions that have been explored include diurnal and seasonal variations (Grabmer et al. 2006, Karl et al. 2003) in response to temperature changes (Filella et al. 2007) and to different environmental conditions (Holzinger et al. 2000).

VOC emissions are also present in wood, and they are dependent upon many factors including species, age, and pH value; these emissions differ significantly between hardwoods and softwoods (Taiti et al. 2016, Roffael et al. 2015, Steckel et al. 2010, Roffael 2006). Specifically, Fengel and Wegener (1989) reported that softwood polyoses contain higher amounts of mannose and galactose than hardwoods, whereas hardwoods are richer in pentoses, characterized by higher amounts of

acetyl groups. VOCs from wood can originate either from compounds present in the native structure of wood, or through different chemical processes, such as oxidation and hydrolysis, that involve wood components (Roffael et al. 2015). In general, the most common VOCs emitted from wood are terpenes, aliphatic aldehydes, and organic acids (Schumann et al. 2012). Terpenes are the main constituent of the resin of softwoods, and aldehydes are formed by oxidative decay of fatty acids. Among the organic acids, acetic acid occurs due to cleavage of acetyl groups from hemicelluloses, whereas hexanoic acid originates from the decay of fatty acids. Furthermore, by increasing the time of storage and modifying the conditions of wood storage, the release of some VOCs declines dramatically over time, reaching very low emission levels (*e.g.* terpenes in softwoods and formaldehyde in both soft- and hardwoods are naturally occurring chemicals in wood (Roffael 2006)), whereas specific treatments could determine the transformation of the originals VOCs from wood, changing their release rates (Manninen et al. 2002, Hyttinen et al. 2010). In addition, during storage the composition of extractives changes: the content of extractives decreases and the content of free sugar, lipophilic fats, fatty and resin acids, and sterols substantially decreases (Salem & Böhm 2013).

Previous studies have already dealt with products that derived from the degradation of wood polysaccharide fraction, after specific treatments or processing procedures of wood, showing the formation of compounds such as: (1) formaldehyde, acetaldehyde, propenal, butanal, and butanone resulting from the breakdown of the polysaccharide fraction of the wood during pyrolysis (Salem & Böhm 2013); (2) acetic acid following acetyl group hydrolysis in hemicellulose (Risholm-Sundman et al. 1998); (3) pentanal, hexanal, and other aldehydes resulting from the oxidation of unsaturated fatty acids of triglycerides (Risholm-Sundman et al. 1998); (4) furfural due to hydrolysis of carbohydrates (Roffael et al. 2015); and (5) furan and furan derivatives as thermal degradation products of cellulose and other polysaccharides (Manninen et al. 2002, Fernández de Simón et al. 2009).

In the literature, no studies have been carried out on green wood (*i.e.* measured before the first drying cycle) and subsequently repeated on the same samples at regular intervals of time after wood moisture

cycles. Assuming that moisture cycles could have an effect in VOC formation and emission, having an active (hydrolysis) or passive (carrier) role in such phenomena described as wood aging (*i.e.* change in the chemical composition of constitutive polymers), the aims of the present study were:

i. assessing the VOCs emitted by the wood of different species in the green condition (*e.g.* immediately after tree felling or increment cores sampling) and over the time in response to several conditioning cycles (*e.g.* variation of moisture content of the sample);

ii. estimating the possibility to use VOC emission as a discriminant between different wood taxa after aging (*e.g.* after repetition of different cycles of moisture variation); and

iii. evaluating whether VOC compounds yield information about the processes of wood modification occurring during wood aging.

PTR-TOF-MS, used to characterize these emissions, is a useful analytical technique largely applied in order to provide an overview of the mass spectra of volatile compounds emitted by different materials (Vita et al. 2015, Mancuso et al. 2015, Soukoulis et al. 2013, Cappellin et al. 2013, Han et al. 2010). A detailed description of the PTR-TOF-MS tool is discussed by Blake et al. (2009).

2. Materials and methods

2.1 Study area and sampling design

Sampling procedures including site and species description have been thoroughly described in chapter 3 of this thesis. The list of the species studied is reported in Table 1.

Table 1: List of specimens studied

Code Species	*Species*	*Species Acronym*	*Family*	*Group*
1	Atlantic cedar (Cedrus atlantica Man.)	**CA**	Pinaceae	Softwood
2	Austrian black pine (Pinus nigra Arn.)	**PiN**	Pinaceae	Softwood
3	Common cypress (Cupressus sempervirens L.)	**CS**	Cupressaceae	Softwood
4	Bay laurel (Laurus nobilis L.)	**LN**	Lauraceae	Hardwood
5	European walnut (Juglans regia L.)	**JR**	Juglandaceae	Hardwood
6	Black poplar (Populus nigra)	**PoN**	Salicaceae	Hardwood
7	Common fig (Ficus carica L.)	**FC**	Moraceae	Hardwood
8	Almond (Prunus amygdalus (Mill.) D.A.Webb)	**PAmy**	Rosaceae	Hardwood
9	Wild cherry (Prunus avium L.)	**PAv**	Rosaceae	Hardwood
10	Black locust (Robinia pseudoacacia L.)	**RP**	Fabaceae	Hardwood
11	Turkey oak (Quercus cerris L.)	**QC**	Fagaceae	Hardwood
12	Downy oak (Quercus pubescens Willd.)	**QP**	Fagaceae	Hardwood
13	Evergreen oak (Quercus ilex L.)	**QI**	Fagaceae	Hardwood

The specimens, mainly constituted by increment cores, originated from the same plant and made up both heartwood and sapwood, were analysed in the green condition and after several cycles of moisture variation.

2.2 Moisture cycles

The first VOC analysis was performed by PTR-TOF-MS on green state increment cores (indicated as measurement time T1). Subsequently, samples were submitted to three moisture cycles from fiber saturation point (FSP) to a moisture content (MC) of 10-12%, which were intended to simulate the natural aging of wood through cycles of moisture variation that typically occur in wood during this process (Akahoshi & Obataya 2015). Emissions were regularly measured at different intervals of time on samples with moisture content of 10-12% (measurement times T2, T3, and T4).
The following drying-moistened conditioning schedules were followed:

- for T2 analysis (day 7): all the samples were placed in a sealed basin (volume: 72 l) for 7 days and dried with magnesium nitrate hexahydrate for analysis (EMSURE® ACS, Reag. Ph Eur. CAS 13446-18-9, EC Number 233-826-7, chemical formula $Mg(NO_3)_2 \bullet 6H_2O$) at T 20°C (obtaining a RH 54%-58%) until no weight loss was detected and a moisture content (MC) of 10-12% was achieved for the analysis of emissions;
- for T3 and T4 analysis: subsequently, the increment cores were moistened at T 20°C and RH 100% in the same basin for 7 days and submitted to another drying cycle with $Mg(NO_3)_2 \bullet 6H_2O$ for 7 additional days. At the end of the drying phase, samples were analysed by PTR-TOF-MS (T3, day 21). The same procedure was applied for T4 measurement (day 35) (Fig. 1).

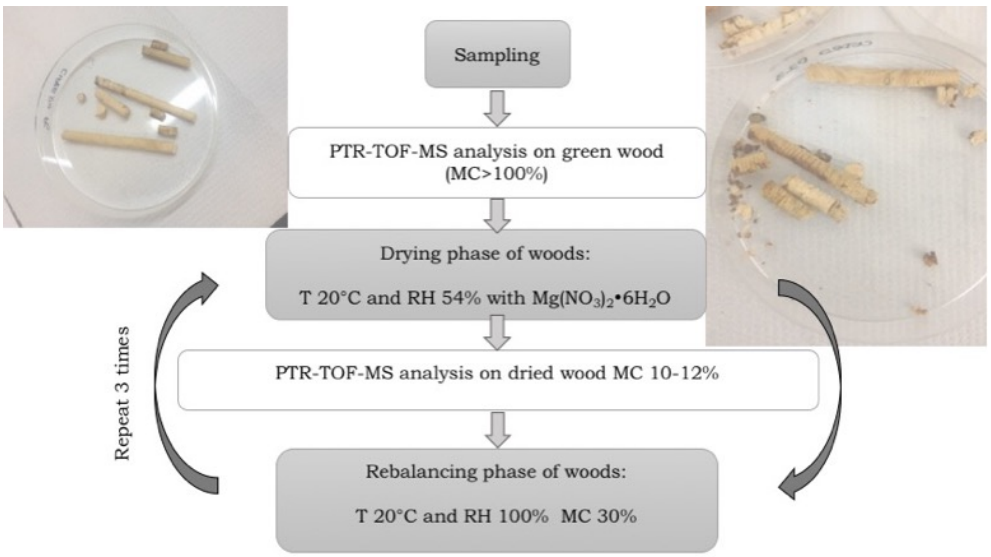

Figure 1: scheme of the experimental procedure.

2.3 Soxhlet extraction and UV treatment of core samples

In order to assess whether the emissions were due to, or influenced by, the presence of extractives in wood or to the microbiological activity (bacteria), a supplementary set of Norway spruce samples was prepared. One sub-sample was extracted in

alcohol, whereas a second sub-sample was subjected to UV radiation, maintaining for each test an untreated sample as control. Extractives were removed by means of Soxhlet extraction in ethanol (96%), according to standard prescription (TAPPI T 204 om-88), but without the grinding stage. The other specimen was exposed to UV treatment (Sankyo DenKi G20T10 lamp (20 Watt)) for 1 hour, in order to eliminate possible microbiological organisms.

At the end of these treatments, all the specimens, treated and control, were analysed by PTR-TOF-MS.

2.4 PTR-TOF-MS and VOCs analysis

The real-time detection of VOCs emitted by different wood cores was achieved using 8000 PTR-TOF system (Ionicon Analytik Innsbruck, Austria). The same samples were analysed again with the same equipment to monitor the VOCs' profiles in response to subsequent aging cycles. All VOCs emitted by the samples were assessed using a setup previously reported in Taiti et al. (2016). Before the analysis each jar was cleaned for 100 sec. and subsequently was hermetically sealed and incubated for 4 minutes at 20-25°C. Three replicates were prepared for each sample and a preliminary measurement on empty jars were run and used for background subtraction. The measurement order of samples was randomized. The inlet flow was set at 100 sccm. The headspace analysis was recorded in a range between m/z 20-210 for 60 s with an acquisition rate of one spectrum per second. All of the measurements were carried out under the following drift tube conditions: 600 V drift voltage, 60°C temperature, and pressure of 2.23 mbar, resulting in an E/N value (electric field strength/gas number density) of 130 Townsend (Td, 1 Td $= 10^{-17}$ cm^2/V s).

2.5 PTR-TOF-MS Data analysis

The raw data were acquired by the TOFDAQ Viewer software (Tofwerk AG, Thun, Switzerland). Data acquisition and quantification of peaks, expressed as normalized count for seconds (ncps), were corrected according to the duty cycle, and the signals were normalized

to the primary ion signal (cps→ncps) (Herbig et al. 2009). To allow a rapid identification of compounds with a high level of confidence, the internal calibration of mass spectral data was based on three points calibration using m/z=21.022 (H_3O^+), m/z=59.049 ($C_3H_7O^+$), and m/z=137.132 ($C_{10}H_{17}^+$); this was performed off-line (Lanza et al. 2015). The tentative identification of VOCs provided by the tool (high sensitivity and with a fast selective identification) was compared on models of fragmentation available in the literature and compared with published VOCs emitted by wood species (Tab. S1 in Supplementary material). Dead time correction and peak extraction were performed according to a procedure described elsewhere using a modified Gaussian peak shape (Cappellin et al. 2011). Subsequently, the peaks associated with the PTR-MS ion source including m/z = 32 (O_2^+) and m/z = 37 (water cluster ion) were eliminated.

2.6 Statistical analysis

The matrix composed by 80 VOCs x 156 wood samples (13 species; 4 measurement times T1, T2, T3, T4; 3 replicates each) was used to assess the potential of the method to discriminate: *i.* between softwoods and hardwoods (2 groups), *ii.* among families (9 groups), and *iii.* among plant species (13 groups). Three class-modelling approaches were applied to predict the aforementioned groups: *i.* the model M1 built on the first measurement dataset (T1), *ii.* the model M2 built on the fourth measurement dataset (T4), and *iii.* the model M3 that include all the measurement datasets (T1, T2, T3, T4). Matrices at each time were separately autoscaled by column.

After this procedure, the matrix was pre-processed using the normalization algorithm (which performed normalization of the rows; i.e., samples). A partial least squares discriminant analysis (PLSDA) approach was applied for the classification of the matrix to determine hardwoods and softwoods or the 13 species using the VOCs. The models were developed using a procedure written in the MATLAB 7.1 R14 environment. PLSDA (Sjöström et al. 1986, Sabatier et al. 2003, Infantino et al. 2015) is a PLS regression (SIMPLS algorithm) (de Jong 1993) in which the response variable is categorical, expressing the class membership of the statistical units. The objective of PLSDA is to

find a model, developed from a training set of observations of known class membership, that separates classes of objects on the basis of their X-variables. The percentages of correct classification were calculated for the calibration and validation phases, and then used for model selection. The PLSDA model selection was mainly based on the efficiencies and robustness parameters described above. For M1 and M2, T1 and T4 were respectively used as the calibration and validation sets, and the rest of the samples as the test set; for M3, the dataset was divided into a calibration/validation set composed by 66.67% of samples and an internal validation set represented by the remaining 33.3%. The partitioning of the models for M3, which include all the times, was conducted optimally by choosing the Euclidean distances based on the algorithm of (Kennard & Stone 1969) that selects objects without the *a priori* knowledge of a regression model. A summary of the relative importance of the X-variables for both Y and X model parts is given by Variable Importance in the Projection (VIP) (Taiti et al. 2015, Infantino et al. 2015). VIP scores estimate the importance of each variable in the PLS-based models. VIP scores were calculated according to Chong and Jun (2005). The explanatory variables with VIP scores values larger than 1 tend to be more important than others, although this does not imply that a variable with a low VIP score is not relevant for the model.

3. Results and discussion

Several mass peaks in the range of measured masses (m/z = 20-210) were collected from 13 different wood species at four different sampling times; 80 mass peaks were detected in the specimens from the first measurement (Taiti et al. 2016), but their number subsequently decreased after several aging cycles. In Table S1 in Supplementary material are reported the most significant putative molecules identified in the first analysis from wood in the green condition, including their measured m/z ratio, protonated molecular formula, chemical name, and related reference. The signals observed from green specimens varied in terms of the nature and intensity for each wood species.

According to our tentative identification, the main compounds detected were m/z = 33.033 methanol, m/z = 45.033 acetaldehyde, m/z = 47.049 ethanol, m/z = 59.049 acetone, m/z = 61.028 acetic acid, m/z = 69.036 furan, m/z = 81.069 monoterpene fragment, m/z = 93.070 toluene or p-cymene fragment, m/z = 153.127 terpenoid compound, m/z = 137.132 monoterpenes, and m/z = 205.195 sesquiterpenes. All these compounds were recorded in all the investigated species during the first measurement (Tab. S1 in Supplementary material). Their emission rates were successively monitored three times during the aging of specimens (Figs. 2 and 3).

All the VOCs emissions taken into account decreased in intensity starting from the second analysis (after the first drying of wood), and they subsequently disappeared after the second and third steps, excluding some VOCs that continued to be emitted in minimum quantities by some species. For instance, *Pinus nigra* and *Cupressus sempervirens* showed the highest signal intensity and the highest number of total peaks in softwood during first sampling time (Figs. 2 and 3, Tab. S1 in Supplementary material), but in the subsequent analysis, only their emission rates of acetaldehyde and acetone were slightly higher in comparison with all other VOC species. More generally, the softwoods group (*Cedrus, Pinus,* and *Cupressus*) continued to emit more methanol than the hardwoods group.

Fresh hardwood species released higher amounts of acetic acid, probably originating from the hydrolyses of acetyl groups in hemicellulose, than terpenes compounds (Figs. 2 and 3). Regarding specific profile emission from hardwoods during aging, only *Populus nigra* and *Ficus carica* showed ethanol emission even though in very low amounts, differentiating them from the other hardwood and softwood species here examined.

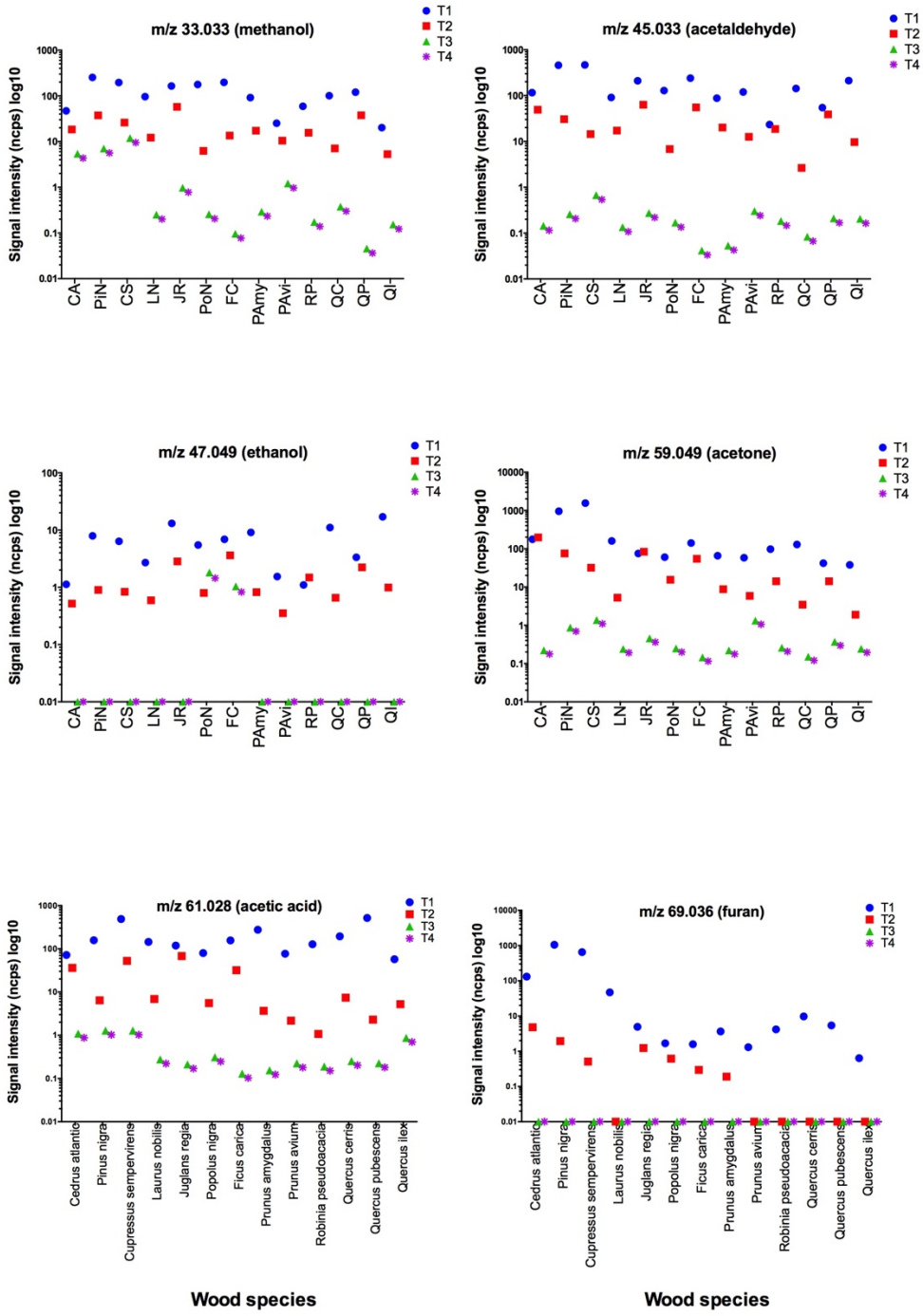

Figure 2: Evaluation of signal intensity of some compounds (reported by m/z ratio) detected in the 13 species, object of the study. Different symbols indicate a different time analysis (T1 for green state wood, whereas T2, T3 and T4 for samples after moisture cycles).

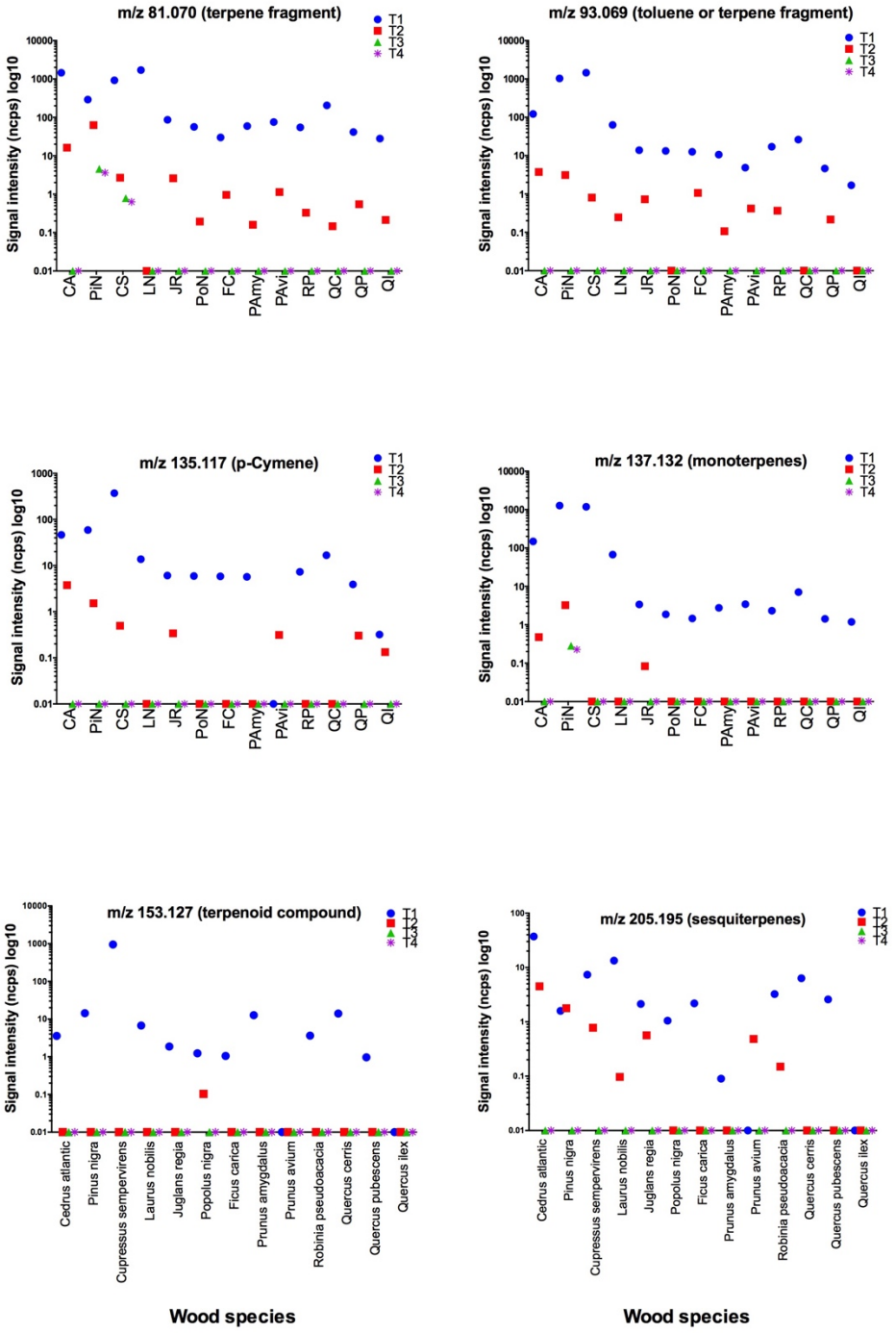

Figure 3: Evaluation of signal intensity of terpenes and terpenoid compounds detected in the 13 species analysed. Different symbols indicate a different time analysis (T1 for green state wood, whereas T2, T3 and T4 for samples after moisture cycles).

The emission intensity of compounds tentatively identified as terpenes, terpenoids, sesquiterpenes, and their fragments (m/z = 81.069; 93.070; 153.127; 137.132; 205.195) was higher in softwoods (*Cedrus, Pinus,* and *Cupressus*) in comparison with most hardwood species (Fig. 3), with terpene compounds being the essential part of the resin composition in many softwood species (Risholm-Sundman et al. 1998, Roffael et al. 2015, Baumann et al. 1999, Schumann et al. 2012). The only exception was represented by Bay laurel (*Laurus nobilis*) that showed similar peak intensities for the masses regarding terpenes, due to the richness of such compounds in this species (*e.g.* oxygenated monoterpenes and monoterpene hydrocarbons) (Flamini et al. 2007). However, given that terpenes are very volatile, their intensity decreased drastically by the second analysis, and, in the following cycles, terpene fragments and monoterpenes (m/z 81.070 and m/z 137.132, respectively) were recorded only in softwoods, especially in Black pine (*Pinus nigra)*.

The comparison of the emission profiles of extracted and UV-treated samples with reciprocal control samples showed no significant changes in the emitted compounds revealed. This observation seems to indicate that all the revealed emissions have to be attributed to modification processes occurring in wood and in its constitutive polymers, avoiding the hypothesis of possible interference in the results due to the presence of extractives or because of microbiological activity.

The statistical significance of the data sets measured at the different times (T1 to T4) was tested according to the models previously described (M1, M2, M3).

The performance indicators of the PLSDA models M1 and M2 tested on the other measurement times are reported in Table 2.

Table 2: Characteristics and principal results of the three PLSDA models. LVs = Latent Vectors; RMSEC = Root Mean Square Error of Calibration.

	M1			M2			M3		
	Hardwood/ softwood	**Families**	**Species**	**Hardwood/ softwood**	**Families**	**Species**	**Hardwood/ softwood**	**Families**	**Species**
N. Samples model	39	39	39	39	39	39	104	104	104
n. classes (y-block)	2	9	13	2	9	13	2	9	13
n. LVs	3	15	10	6	9	8	6	15	17
% Cumulated variance X-block	70.32	99.33	97.37	84.45	97.6	94.95	77.56	88.62	90.88
% Cumulated variance Y-block	68.15	85.1	71.15	65.59	51.13	50.55	32.24	48.67	45.6
Mean sensitivity. %	0.98	1	1	1	0.9	1	1	0.88	0.89
Mean specificity. %	0.93	1	1	0.98	0.95	0.94	1	0.93	0.93
Random probability. %	50	11.11	7.69	50	11.11	7.69	50	11.11	7.69
Mean classification error. %	0.05	0	0	0.01	0.08	0.03	0	0.1	0.1
Mean RMSEC	0.4	0.12	0.14	0.41	0.22	0.19	0.52	0.23	0.2
Mean % correct classification calibration/validation set	100	100	100	100	84.62	80.77	100	82.69	78.85
N. Samples test	117	117	117	117	117	117	52	52	52
Mean % correct classification test set	**T2: 84.615**	**T2: 10.256**	**T2: 17.949**	**T1: 66.667**	**T1: 7.6923**	**T1: 7.6923**	**100**	**67.31**	**73.08**
Mean % correct classification test set	**T3: 79.487**	**T3: 7.6923**	**T3: 25.641**	**T2: 84.615**	**T2: 20.513**	**T2: 20.513**			
Mean % correct classification test set	**T4: 79.487**	**T4: 7.6923**	**T4: 25.641**	**T3: 100**	**T3: 79.487**	**T3: 74.359**			

The M1 model adopted to determine hardwood *vs* softwood samples using 3 Latent Vectors showed 0.98 and 0.93 mean sensitivity and specificity values, respectively. The mean classification error was 0.05. The mean percentage of correct classification was determined to be equal to 100% for the calibration/validation set (at T1), whereas at T2, it decreased to 84.6%, and at T3 and T4 to 79.5%. The M1 model adopted to discriminate the 9 families used 15 Latent Vectors and showed 1 as the mean sensitivity and specificity values. The mean classification error was equal to 0. Even in this case, the mean percentage of correct classification was equal to 100% for the calibration/validation set (at T1), while at T2 it steeply decreased to 10.3%, and at T3 and T4 to 7.7%. The M1 model that was adopted to discriminate the 13 species used 10 Latent Vectors and showed 1 as the mean sensitivity and specificity values. The mean classification error was equal to 0.

The mean percentage of correct classifications in this case was determined be equal to 100% for the calibration/validation set (at T1), while at T2 it sharply decreased to 18.0%, and at T3 and T4 to 25.6%.

The results obtained applying the M1 model to the classification of the samples clearly showed the difference in the quality of the VOCs emitted at T1 (fresh wood) and in the subsequent steps (T2 to T4).

The M2 model adopted to determine hardwood *vs* softwood samples at T4 using 6 Latent Vectors showed mean sensitivity and specificity values of 1 and 0.98, respectively. The mean classification error was 0.01. The mean percentage of correct classification was determined to be equal to 100% for the calibration/validation set (at T4) and at T3, whereas at T2 it decreased to 84.6% and at T1 to 66.7%. The M2 model used 9 Latent Vectors to discriminate the 9 families and showed mean sensitivity and specificity values of 0.9 and 1, respectively. The mean classification error was equal to 0.08. The mean percentage of correct classification was found to be equal to 84.6% for the calibration/validation set (at T4), while at T3 it decreased to 79.5%, then at T2 it drastically decreased to 20.5%, and at T1 to 7.7%. Then, to discriminate the 13 species, M2 used 8 Latent Vectors and showed mean sensitivity and specificity values of 1 and 0.9, respectively. The mean classification error was equal to 0.03. The mean percentage of correct classification was determined to be equal to 80.8% for the

calibration/validation set (at T4), whereas at T3 it decreased to 74.4%, then at T2 it drastically decreased to 20.5%, and at T1 to 7.7%.

The performance indicators of the PLSDA model M3 built on 66.7% of the full dataset and tested on the remaining 33.3% are reported in Table 2. The M3 model adopted to determine hardwood *vs* softwood samples using 6 Latent Vectors showed mean sensitivity and specificity values of 1. The mean classification error was 0. The mean percentage of correct classification was found to be equal to 100% for both the calibration/validation and the test sets. The M3 model adopted to discriminate the 9 families used 15 Latent Vectors and showed mean sensitivity and specificity values of 0.88 and 0.93, respectively. The mean classification error was equal to 0.10. The mean percentage of correct classification was observed to be equal to 82.6% for the calibration/validation set and 67.3% for the test set. The M3 model adopted to discriminate the 13 species used 17 Latent Vectors and showed mean sensitivity and specificity values of 0.89 and 0.93, respectively. The mean classification error was equal to 0.10. The mean percentage of correct classification was found to be equal to 78.9% for the calibration/validation set and 73.1% for the test set.

Tables 3 and 4 show the classification test set results for the M3 families and species, respectively, reported as a confusion matrix. Each row of the square matrix represents the instances in the actual class (*i.e.* observed), while each column of the matrix represents the instances in the predicted class. Each entry, then, gives the number of instances of real classes that were classified as the predicted class. As a result, all correct classifications are on the main diagonal of the matrix; any value off that diagonal is an incorrect classification observation.

Table 3: Confusion matrix for M3 families test set.

	Pinaceae	*Cupressaceae*	*Lauraceae*	*Juglandaceae*	*Salicaceae*	*Moraceae*	*Rosaceae*	*Fabaceae*	*Fagaceae*	Total
Pinaceae	**6**	0	0	0	0	0	0	0	2	8
Cupressaceae	0	**3**	0	0	0	0	0	1	0	4
Lauraceae	0	0	**4**	0	0	0	0	0	0	4
Juglandaceae	0	0	0	**4**	0	0	0	0	0	4
Salicaceae	0	0	0	0	**2**	0	1	0	1	4
Moraceae	0	0	0	0	1	**2**	0	1	0	4
Rosaceae	0	0	0	0	0	2	**4**	0	2	8
Fabaceae	0	0	0	0	0	0	1	**1**	2	4
Fagaceae	0	0	1	0	0	1	1	0	**9**	12
Total	6	3	5	4	3	5	7	3	16	52

Table 4: Confusion matrix for the 13 M3 species test set.

	Cedrus atlantica	*Pinus nigra*	*Cupressus sempervirens*	*Laurus nobilis*	*Juglans regia*	*Populus nigra*	*Ficus carica*	*Prunus amygdalus*	*Prunus avium*	*Robinia pseudoacacia*	*Quercus cerris*	*Quercus pubescens*	*Quercus ilex*	*Total*
Cedrus atlantica	**4**	0	0	0	0	0	0	0	0	0	0	0	0	4
Pinus nigra	0	**4**	0	0	0	0	0	0	0	0	0	0	0	4
Cupressus sempervirens	0	0	**3**	0	0	0	0	0	0	1	0	0	0	4
Laurus nobilis	0	0	0	**4**	0	0	0	0	0	0	0	0	0	4
Juglans regia	0	0	0	0	**4**	0	0	0	0	0	0	0	0	4
Populus nigra	0	0	0	0	0	**2**	0	0	0	1	0	0	1	4
Ficus carica	0	0	0	0	0	1	**2**	0	0	1	0	0	0	4
Prunus amygdalus	0	0	0	0	0	0	0	**0**	0	1	3	0	0	4
Prunus avium	0	0	0	0	0	0	0	0	**4**	0	0	0	0	4
Robinia pseudoacacia	0	0	0	0	0	0	0	0	1	**2**	1	0	0	4
Quercus cerris	0	0	0	0	0	0	0	0	1	0	**3**	0	0	4
Quercus pubescens	0	0	0	0	0	0	0	0	0	2	0	**2**	0	4
Quercus ilex	0	0	0	0	0	0	0	0	0	0	0	0	**4**	4
Total	4	4	3	4	4	3	2	0	6	8	7	2	5	52

The accuracy of the statistical classification of the model is evaluated by two different measures, the producer's accuracy (PA) and the user's accuracy (UA), reported in Tables 5 and 6. The PA of a category indicates to what extent the reference samples of the category are correctly classified, whereas the UA of a category represents to what extent the other categories are less misclassified into the category in question. The two matrices return an accurate representation of statistical classification, and incorrect classifications based on the M3 model occur especially for the Fabaceae family (Producer Accuracy P.A. = 25% and User Accuracy U.A. = 33.3%) and regarding species for *Prunus amygadalus* (P.A.= 0%) (Tables 5 and 6).

Table 5: Producer accuracy and user accuracy for families

	P.A.(%)	U.A.(%)
Pinaceae	75	100
Cupressaceae	75	100
Lauraceae	100	80
Juglandaceae	100	100
Salicaceae	50	67
Moraceae	50	40
Rosaceae	50	57
Fabaceae	25	34
Fagaceae	75	56

Table 6: Producer accuracy and user accuracy for species

	P.A. (%)	U.A. (%)
Cedrus atlantica	100	100
Pinus nigra	100	100
Cupressus sempervirens	75	100
Laurus nobilis	100	100
Juglans regia	100	100
Populus nigra	50	67
Ficus carica	50	100
Prunus amygdalus	0	-
Prunus avium	100	67
Robinia pseudoacacia	50	25
Quercus cerris	75	43
Quercus pubescens	50	100
Quercus ilex	100	80

In Table 7, the protonated measurement masses presenting high VIP scores for taxonomic, families, or species discrimination based on the three different models are shown. In particular, is interesting to observe that the chemical species with higher significance for hardwoods and softwoods discrimination in the M1 model were m/z = 81.070 (TI: monoterpene fragment) and m/z = 205.195 (TI: sesquiterpenes), but in the other two models these masses were no longer suitable for taxonomic discrimination. On the other hand, compounds with higher VIP value that are able to discriminate families and species, both in M1 and M2 models and in some cases also in the M3 model, are m/z = 33.033 (TI: methanol), m/z = 43.050 (TI: alkyl fragment), m/z = 45.033 (TI: acetaldehyde), m/z = 47.049 (TI: ethanol), m/z = 61.028 (TI: acetic acid), and m/z = 123.116 (sesquiterpene fragment).

Table 7: Protonated masses having VIP scores higher than 2 (in italic) among the three PLSDA models

m/z	VIP scores								
	M1			M2			M3		
	Hard/softwood	Families	Species	Hard/softwood	Families	Species	Hard/softwood	Families	Species
27.022	0.969	0.855	0.918	1.776	***2.508***	***2.056***	0.745	1.712	1.703
33.033	0.582	***3.462***	***3.039***	***3.391***	***2.348***	***3.569***	1.172	1.933	1.947
39.020	1.238	0.803	1.129	1.682	***3.152***	***3.166***	0.835	1.928	***2.475***
41.038	1.288	0.757	0.722	1.911	***2.531***	***2.259***	1.253	1.524	1.521
43.050	0.953	***2.110***	***2.363***	***2.385***	***2.833***	***2.452***	1.313	1.515	***2.103***
45.033	1.057	***2.511***	***2.169***	1.528	***3.772***	***4.372***	0.799	1.927	***2.238***
47.049	1.011	***2.083***	***3.908***	1.657	***5.002***	***3.686***	1.081	***2.761***	***2.970***
49.011	0.492	***3.944***	***3.351***	0.000	0.000	0.000	0.582	***3.334***	***2.308***
53.040	1.266	0.932	1.308	1.773	***2.434***	1.883	1.338	1.612	1.803
55.055	1.290	0.839	1.142	1.491	***4.360***	***3.819***	1.219	1.996	***2.135***
59.049	1.144	0.782	0.624	1.751	***3.430***	***3.005***	1.072	***2.158***	***2.633***
61.028	0.453	***2.829***	***4.059***	***2.889***	***3.712***	***3.963***	0.809	***2.344***	***2.888***
69.069	1.290	0.774	0.989	1.865	***2.560***	1.978	1.295	1.406	1.638
75.043	0.642	***2.565***	***3.597***	0.000	0.000	0.000	0.614	1.976	***2.212***
77.038	1.281	0.819	1.152	1.549	***2.150***	***2.063***	1.196	1.473	1.636
79.054	1.359	1.050	0.874	***2.793***	1.743	1.822	1.542	1.592	1.571
81.070	***2.363***	***2.565***	***2.822***	1.997	***2.351***	1.877	1.649	1.990	1.883
89.059	0.880	1.201	1.563	0.000	0.000	0.000	0.766	***2.007***	1.558
91.054	0.923	1.399	1.380	1.857	***4.842***	***3.371***	1.399	***2.086***	***2.198***
95.086	1.192	0.792	0.786	***2.291***	***2.957***	1.626	1.770	1.543	1.358
107.085	0.413	***2.913***	***2.839***	0.000	0.000	0.000	0.758	1.302	***2.074***
123.116	1.133	***3.301***	***3.287***	***2.245***	***3.005***	***2.804***	1.088	***2.948***	***2.597***
137.137	1.270	0.734	0.892	1.791	***2.448***	***2.023***	1.387	1.389	1.862
153.126	0.929	0.869	0.912	0.000	0.000	0.000	0.784	***2.095***	1.767
169.090	0.944	***2.018***	1.299	0.000	0.000	0.000	0.655	0.709	1.134
189.165	***2.228***	***2.669***	***3.134***	0.000	0.000	0.000	1.054	1.141	1.609
203.180	***2.084***	1.856	***2.865***	0.000	0.000	0.000	1.185	1.145	1.303
205.195	***2.489***	***2.364***	***3.319***	0.000	0.000	0.000	1.224	1.404	1.791

The M1 model confirms that VOC emissions tend to decrease with time; in fact, green wood samples are characterized by a multiplicity of compounds not present or negligible in wood after a few moisture cycles. Softwoods and hardwoods are still discerned (> 79% for all sampling-analysis time), whereas the predictive capability for families, and especially for species, drastically decreases even as soon as T2. Even applying the M2 model, it is possible to discern between softwoods and hardwoods with a mean % value of at least 66.7% for T1; nevertheless, this model is able to identify families and species with a mean % value higher than 50% only at the T3 sampling time.

The M3 model maintains an accurate predictive capacity only because it also contains the data obtained with measurements carried out on fresh wood, in the absence of which the prediction capability of the models becomes very poor (as shown by Model 2).

Despite the usefulness of the three models in using VOCs as a possible tool for the discrimination of species, families, or groups, the statistical analysis clearly showed the significant difference between the measurements at T1 (green wood) and the measurements after a few cycles of moisture variation (T2 to T4).

In particular, the results of this study indicate that after the first cycles, both softwood and hardwood species tend to converge versus a common class of compounds, but a small residue of terpenes persists in the case of softwoods. It seems that green wood is still rich in compounds originated from physiological activity of the tree. Furthermore, it seems that the majority of these compounds are very volatile and, during moisture cycles, the residual compounds measured can be correlated to the processes of structural modification of the cell walls. This explanation finds confirmation in studies carried out on aged wood that show a lower content of hemicellulose and a significant reduction of its hygroscopicity (*e.g.* Obataya 2007). This analysis of VOCs indicates that this process starts when the first drying of wood occurs, and the tests on extractives and microbial-free wood allows us to exclude the influence of these two factors on the products emitted.

In this study, moisture cycles were used for simulating and accelerating a natural process that in wood normally occur gradually over time, with a speed that can change according to wood species and timber thickness. Further investigation will be required in order to

assess whether water and moisture changes inside wood have an active role in the alteration of polymers. However, despite the fact that heat treatment changes the composition of wood permanently and also modifies emissions of VOCs from the material, (*e.g.* it increases the emission of furfural that is the main thermal degradation product of hemicellulose), it is interesting to compare our results with those obtained by Manninen et al. (2002) and Hyttinen et al. (2010) on Norway spruce (*Picea abies),* Scot pine (*Pinus sylvestris*), and European aspen (*Populus tremula*). In their works, they demonstrated that the emissions of terpenes from softwoods and aldehydes from hardwoods decrease significantly after heat treatment. Assuming that the heat treatment produces an extreme and very intense aging of wood, this confirms that the trend showed by our results clearly attests to the loss of these compounds by different wood species during normal aging of wood.

4. Conclusion

The results of this study have shown that, as consequence of the moisture cycles, the spectra of the VOC emissions of wood are significantly changed. The emission rates of all revealed compounds decreased after only a few aging cycles, though we did not observe the increase of the emissions of some specific compounds or the presence of new compounds during the simulated aging.

The statistical analysis showed that the two models (M1 and M2) based on only one measurement dataset (T1 and T4, respectively) were not capable of discriminating groups (hardwoods *vs* softwoods, families and species) among aged wood samples, whereas the M3 model showed an effective ability to identify groups (softwoods *vs* hardwoods). This capacity depends on the fact that the M3 model also contains the data set acquired at T1 (green wood), combined with the other 3 datasets (T2, T3, T4).

During the moisture cycles in all the species studied, emission of VOCs has shown both a quantitative and a qualitative modification of the acquired spectra. Apparently, losing the compounds produced by the metabolic activity of the tree, the wood emits compounds possibly due to structural changes and degradation processes of the main

polymers constituting the cell wall; these polymers are common to all wood species excepting small percentage differences of chemical composition. These results clearly indicate that the analysis of VOCs might be applied to the identification of wood species only on very fresh wood, when the material still contains all the classes of compounds that are produced by the trees and that are characterized by strong volatility. As soon as the natural cycle of variation of moisture content (*i.e.* moisture desorption and adsorption) starts, the composition of the emissions changes, making very weak the discriminant capacity even at the highest hierarchical level of the taxa (*i.e.* families).

The results of this research do not clarify the role of water in the processes observed, and in particular, whether it can be considered as only a carrier of the VOCs or whether it has an active role in the hydrolysis of wood polymers (or probably on both of these processes). In this respect, further studies are needed. Specific treatments (Soxhlet extraction and UV treatment) applied to a separate and supplementary set of samples of Norway spruce (*Picea abies*) allowed us to exclude the potential effect of microbiological activity or extractives on the observed results.

Both the nature and the amount of the VOCs emitted by the wood at the different times assessed in the framework of this study seem to indicate that the native organization of the wooden structural polymers (i.e. cellulose, hemicellulose, lignin, and their reciprocal bonding) starts to be modified immediately after the first exposure to an external environment, initiating a process of aging, the practical importance of which is recognizable over a very long period, and that can affect the physical, mechanical, and acoustical properties of wood.

It is necessary to extend the analysis of VOCs by PTR-TOF-MS on increasingly aged samples and on naturally aged wood samples, in order to verify the presence of new signals and eventually match them to specific degradation processes of wood components.

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Table S1: Main compounds identified via PTR-TOF-MS during first measurement: Protonated masses (mass/charge = m/z), molecular formula, tentative identification, references of the investigated volatile compounds emitted from different wood species. The symbols indicate the presence (*) or the absence (-).

Measured m/z	Protonated formula	Tentative identification	References (PTR-MS #; Wood (plant and solid wood)*)	Code species number												
				1	2	3	4	5	6	7	8	9	10	11	12	13
27.022	C_2H_2—H^+	Acetylene	(Vita et al. 2015)#	*	*	*	*	*	*	*	*	*	*	*	*	*
33.033	CH_4O—H^+	Methanol	(Risholm-Sundman et al. 1998)*; (Maleknia et al. 2007)#*	*	*	*	*	*	*	*	*	*	*	*	*	*
39.020	C_3H_2—H^+	Isoprene fragment	(Maleknia et al. 2007)#*	*	*	*	*	*	*	*	*	*	*	*	*	*
41.038	C_3H_4—H^+	Alkyl fragment: propadiene	(Brilli et al. 2014)#*	*	*	*	*	*	*	*	*	*	*	*	*	*
43.050	C_3H_6—H^+	Alkyl fragment: propene	(Brilli et al. 2014) #*	*	*	*	*	*	*	*	*	*	*	*	*	*
45.033	C_2H_4O—H^+	Acetaldheyde	(Risholm-Sundman et al. 1998)*; (Filella et al. 2007)#*	*	*	*	*	*	*	*	*	*	*	*	*	*
47.012	CH_2O_2—H^+	Formic acid/Formates	(Sánchez Del Pulgar et al. 2014)#	*	*	*	*	*	*	*	*	*	*	*	*	*
47.049	C_2H_6O—H^+	Ethanol	(Maleknia et al. 2007)#*	*	*	*	*	*	*	*	*	*	*	*	*	*
49.011	CH_4S—H^+	Methanethiol	(Papurello et al. 2012)#; (Blake et al. 2009)#	*	*	*	*	-	-	*	-	-	*	*	*	-
53.040	C_4H_4—H^+	Alkyl fragment or cyclobutadiene	(Sánchez Del Pulgar et al. 2014)# or (Vita et al. 2015)#	*	*	*	*	*	*	*	*	*	*	*	*	*
55.055	C_4H_6—H^+	C4 aldehydes fragment	(Sánchez Del Pulgar et al. 2014)#	*	*	*	*	*	*	*	*	*	*	*	*	*
59.049	C_3H_6O—H^+	Acetone (2-propanone)	(Risholm-Sundman et al. 1998)*; (Maleknia et al. 2007)#*	*	*	*	*	*	*	*	*	*	*	*	*	*
61.028	$C_2H_4O_2$—H^+	Acetic acid	(Risholm-Sundman et al. 1998)*; (Maleknia et al. 2007)#*	*	*	*	*	*	*	*	*	*	*	*	*	*
69.036	C_4H_4O—H^+	Furan	(Maleknia et al. 2007)#*	*	*	*	*	*	*	*	*	*	*	*	*	*
69.069	C_5H_8—H^+	Isoprene (1,4-pentadiene)	(Maleknia et al. 2007)#*	*	*	*	*	*	*	*	*	*	*	*	*	*
75.043	$C_3H_6O_2$—H^+	Propanoic acid or Hydroxy-2-propanone	(Papurello et al. 2012)# or (Brilli et al. 2014) #*	*	*	*	*	*	*	*	*	*	*	*	*	*
77.038	C_6H_4—H^+	Alkyl fragment	(Goacher et al. 2010)#*	*	*	*	*	*	*	*	*	*	*	*	*	*
79.054	C_6H_6—H^+	Phenyl ion or benzene	(Maleknia et al. 2007)#* or (Brilli et al. 2014)#*	*	*	*	*	*	*	*	*	*	*	*	*	*
81.070	C_6H_8—H^+	Monoterpenes fragment	(Maleknia et al. 2007)#*; (Grabmer et al. 2006)#*	*	*	*	*	*	*	*	*	*	*	*	*	*
83.085	C_6H_{10}—H^+	C6 compounds: hexanal fragment or hexenol fragment	(Soukoulis et al. 2013)# or (Brilli et al. 2014)#*	*	*	*	*	*	*	*	*	*	*	*	*	*
89.059	$C_4H_8O_2$—H^+	Ethyl acetate or methyl-propanoate	(Yener et al. 2015)#	*	*	*	*	*	*	*	*	*	*	*	*	*

Measured m/z	Protonated formula	Tentative identification	References (PTR-MS #; Wood (plant and solid wood)*)	Code species number												
				1	2	3	4	5	6	7	8	9	10	11	12	13
91.054	C_7H_6—H^+	Xylene fragment	(Maleknia et al. 2007)#*	*	*	*	*	*	*	*	*	*	*	*	*	*
93.069	C_7H_8—H^+	p-Cymene fragment or toluene	(Maleknia et al. 2007)#* or (Holzinger et al. 2000)#*	*	*	*	*	*	*	*	*	*	*	*	*	*
95.086	C_7H_{10}—H^+	Monoterpene fragment	(Maleknia et al. 2007)#*	*	*	*	*	*	*	*	*	*	*	*	*	*
97.028	$C_5H_4O_2$—H^+	Furfural	(Brilli et al. 2014)#*; (Fernández de Simón et al. 2009)*	*	*	*	*	*	-	*	*	-	-	*	*	-
99.080	$C_6H_{10}O$—H^+	Hexanals	(Brilli et al. 2014)#*	*	*	*	*	*	-	-	-	-	*	-	*	-
101.059	$C_6H_{12}O$—H^+	Hexanal	(Risholm-Sundman et al. 1998)*; (Brilli et al. 2014)#*; (Roffael et al. 2015)*	*	*	*	*	*	*	*	*	-	-	*	*	*
105.069	C_8H_8—H^+	Olefin or styrene/ethylbenzene	(Brilli et al. 2014)#* or (Yener et al. 2015)#	*	*	*	*	*	*	*	*	*	*	*	*	*
107.049	C_7H_6O—H^+	Benzylaldehyde	(Yener et al. 2015)#; (Roffael et al. 2015)*	*	*	*	*	*	*	*	*	*	*	*	*	*
107.085	C_8H_{10}—H^+	Monoterpene fragment or p-xylene/ ethylbenzene	(Maleknia et al. 2007)#* or (Brilli et al. 2014)#*	*	*	*	*	*	*	*	*	*	*	*	*	*
123.116	$C_9H_{14}^+$—H^+	Sesquiterpene fragments	(Demarcke et al. 2009)#	*	*	*	*	*	*	*	*	*	*	*	*	*
135.117	$C_{10}H_{14}$—H^+	p-Cymene	(Maleknia et al. 2007)#*; (Courtois et al. 2009)*	*	*	*	*	*	*	*	*	-	*	*	*	*
137.137	$C_{10}H_{16}$—H^+	Monoterpenes	(Risholm-Sundman et al. 1998)*; (Maleknia et al. 2007)#*	*	*	*	*	*	*	*	*	*	*	*	*	*
153.126	$C_{10}H_{16}O$—H^+	Terpenoid-like compound/ ion of oxygen-containing terpenes	(Maleknia et al. 2007)#*; (Courtois et al. 2009)*	*	*	*	*	*	*	*	*	-	*	*	*	-
169.090	$C_9H_{12}O_3$—H^+	4-methylsiringol	(De Simón et al. 2009)*	*	*	*	-	*	-	-	-	-	-	-	-	-
189.165	$C_{14}H_{20}$—H^+	n.a.	n.a.	*	*	*	*	-	-	-	-	-	-	*	-	-
203.180	$C_{15}H_{22}$—H^+	n.a.	n.a.	*	*	*	*	-	-	-	-	-	-	*	*	-
205.195	$C_{15}H_{24}$—H^+	Sesquiterpenes	(Courtois et al. 2009)*	*	*	*	*	*	*	*	*	-	*	*	*	-

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4.2 PAPER II

VOCS ANALYSIS APPLIED TO THE CHARACTERIZATION OF AGING PHENOMENA IN WOOD

1. Introduction

Wood is an organic and complex material composed mainly of three biopolymers: cellulose, hemicelluloses and lignin, which are the main macromolecular cell components of all wooden species. Cellulose, the most abundant substance in the biosphere, is a polymer of glucose units. The chemical composition and conformation of cellulose chains, are responsible for their tendency to form aggregate of crystalline nature (Wertz et al. 2010). Hemicelluloses are relatively short-branched homo- and heteropolymers made up of pentose and hexose sugars and their uronic acid derivatives. Hemicelluloses constitute the alkali-soluble carbohydrate fraction of wood, and are chemically connected to both cellulose and lignin. Galactoglucomannans are the main hemicelluloses in softwoods. In addition, Softwoods contain arabinoglucuronoxylan, and other polysaccharides.

Lignin is a complex three-dimensional amorphous polymer synthesized by enzymatic polymerization of p-coumaryl, coniferyl and synapyl alcohols being the respective precursors of p-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) phenylpropanoid units linked by C–C and ether bonds. The softwoods lignin is a polymer consisting mostly of G units, with only small amount of H and S units (Fengel & Wegener 1989).

In addition to these polymers, wood also contains minor amounts of extractives (several organic compounds such as sugars, flavonoids, tannins, terpenes, fats or waxes) and inorganics (mineral substances, ashes). These low molecular-weight components are specific in kind and amount related within each wood species (Sjostrom 2013; Fengel & Wegener 1989).

In a tree at least two different type of wood are present, the juvenile wood (JW) (the wood produces by a young cambium, and located in the inner part of the cross section of the tree) and the mature one (MW). In terms of chemical compositions very few differences have been observed in hardwoods, while softwoods present JW with lower cellulose and higher lignin contents. Nor hardwoods or softwoods show significant differences between JW and MW in terms of hemicelluloses content (Pereira et al. 2003).

Because of the organic origin of wood, wooden products and wooden artefacts are potentially subject to biological, chemical, physical and mechanical alterations, that may take place along the time. The cumulative effects produced by these features, are called alteration or aging (with the first typically pertained to the biological origin), and they may produce an irreversible change in chemical, physical and mechanical properties of the material during its long storage or usage (Unger et al. 2001).

Nilsson & Rowell (2012) report a list of the major wood degradation pathways and the relevant chemical processes involved in, such as:

- chemical reactions (oxidation, hydrolysis, reduction, free radical formation);

- biological degradation by fungi, bacteria, insect, which cause enzymatic reactions oxidation, hydrolysis, reduction, free radical formation;
- thermal degradation (lighting, sun, fire);
- weather degradation (UV radiation, water, heat);
- mechanical degradation (stress, crack, fracture, abrasion, erosion, compression);
- water interactions (swelling, shrinking, freezing, cracking, erosion).

Many of these features may act at the same time, producing cumulative effects, and or facilitating the action of the other ones. In addition to biological alterations – produced by Fungi, Bacteria and Insects– both aerobic and anaerobic storage conditions may have a significant effects on aging process, determining different chemical transformations in wood structure (Fengel 1991), which start just after tree felling.

If wood is maintained in an aerobic environment, it is more stable when stored indoors, in dry air with other favourable conditions, such as low temperature and low UV radiation, and limited contact with water, or water vapour. In these conditions the aging on wood can be minimal and progressing very slowly. Whereas wood alteration and/or aging can be more severe and faster if wood is exposed in outdoors environments, under the effect of various organisms and agents (sunlight irradiation, erosion, weathering). Anaerobic aging conditions figure out typically when wood is submerged in water. In these particular environments alteration and aging are mostly due to Bacteria activity and to the chemical transformation of constitutive polymers (for a more extensive bibliography on aging of wood, see Kránitz et al. (2016)).

The assessment of both wood alteration and aging, has practical importance in the preservation of wooden products and wooden cultural heritage, where the characterization of material aging is considered one of the highest priority for its proper preservation (Hunt 2012; Sonderegger et al. 2015; Nilsson & Rowell 2012).

Several works dealing with aging of ancient wood samples (150- to- 4400- year-old), belonging to different wood species and coming from different environment, have been carried out by means of various techniques: polarized light microscopy and IR-spectroscopy (Borgin, Parameswaran, et al. 1975 a; Borgin, Faix, et al. 1975 b), thermogravimetry (TG), differential thermogravimetry (DTG) and differential scanning calorimetry (DSC) (Tomassetti et al. 1990; Campanella et al. 1991; Budrugeac & Emandi 2010), gas chromatography (GC) (Erhardt et al. 1996), FT-IR and x-Ray diffraction (Luis García Esteban et al. 2006), pyrolysis combined with gas chromatography and mass spectrometry (Py-GC/MS) and electron spin resonance (ESR) spectroscopy (Popescu et al. 2007), FTIR and UV resonance Raman (UVRR) spectroscopy (Ganne-Chédeville et al. 2012), and even solid-state nuclear magnetic resonance (NMR) to provide structural characterization of the wood components and in some cases to analyse the intrinsic crystallinity of cellulose, directly from solid wood samples in some cases (Maunu 2002; Andersson et al. 2004; Capitani et al. 2012).

The results of these studies pointed out that the hemicelluloses are the mostly unstable among the cell wall polymers, subjected to degradation during aging of wood, where a decrease in hemicellulose amount (absolute and percentage one) has been

reported with increasing wood age. However, concerning lignin and cellulose (amount and degree of crystallization) often the results reported in literature are not always concordant.
However, it could be assumed that chemical degradation, most of all depolymerisation of hemicelluloses, leads to a changing in wood physical and mechanical properties (colour, hygroscopic behaviour and still bending properties) (Cavalli et al. 2016; Sonderegger et al. 2015). As hemicelluloses are significantly degraded, the cell wall starts to shows a reduction in strength due to failure in the amorphous matrix: more strength is lost as more hemicellulose-lignin matrix is destroyed (Obataya 2007).

The main goal of this work was the study of aging process in wood, comparing modern and historical wood, and to correlate structural changes, analysed by means of spectroscopic method, with the emission of volatile organic compounds (VOCs). Analysis of VOCs is considered a useful method for understanding the chemical processes involved in polymer degradation (Curran & Strlic 2014), assuming that these compounds, or at least one part of them, can be the expression of the products released by the wood as consequence of the chemical alteration of the cell wall polymers.
After the assessment that on the specimens studied there were no contribution of biological attack, wood modification (aging), which sometimes took several hundred years to deteriorate the polymeric structure of wood, has been characterised through NMR spectroscopy. Carbon-13 Cross polarization/ Magic Angle Spinning Nuclear Magnetic Resonance (^{13}C CPMAS NMR) spectroscopy is a very powerful technique for studying structural changes in wood (Maunu 2002; Capitani et al. 2012). The position and the integral of resonances in the carbon spectrum give valuable information on the type of wood, namely hardwood or softwood, and on its state of degradation. The ^{13}C CPMAS spectrum acts as a fingerprint of the wood, and a qualitative analysis of such a spectrum is the first step in the investigation of structural changes occurring in ancient wood.
The VOCs analysis has represented an attempt to associate early structure modifications with emission of specific compounds, since the early beginning of the aging of the material (i.e. immediately after tree felling).
VOCs analysis were carried out by means of Proton Transfer Reaction – Time of Flight – Mass Spectrometry technology, this tool is one of a few analytical techniques that is suitable for online VOCs monitoring and is considered a very suited method for fingerprinting and monitoring VOCs emitted even in very small amounts in a very short time domain and without sample preparation (Taiti et al. 2017).

2. Materials and methods

2.1 Preparation of samples

Three discs of Norway Spruce (*Picea abies* Karst.) belonged to historical structural beams (previously dated by Dendrochronology and ^{14}C radiocarbon) were provided by CNR-IVALSA of Trento (Table 1). From each disc two samples were taken, one near the pith and one from the outermost parts (Fig.1), in order to verify possible differences in the aging of juvenile wood and mature wood.

Table 1: list of historical samples.

Name of the beam section		Origin	Period	Number of rings	Age of the sample
	Corradini 1 **HIS_COR**	Casa Corradini, Cavalese, Val di Fiemme (Italy)	1731-1843	113	174
	Rosmini 3B **HIS_ROS**	Palazzo Rosmini, Rovereto (Italy)	1657-1715	59	302
	Vigo 8 **HIS_VIG**	Vigo di Fassa (Italy)	1312-1397	86	620

The juvenile wood samples were taken from year rings 4-8, whereas the mature wood samples were taken from the last three growth rings of the beam.
In our sampling the juvenile wood is also the inner core of the wood beam, which was considered potentially not affected, or only partially, by environmental conditions during beam aging, whereas we considered the outer portion, constitute by mature wood, directly in contact with environmental degrading agents. Finally, historical powder samples were prepared by grinding a predetermined amount of dry wood for both spectroscopic analysis and VOCs analysis.

Figure 1: historical wood pieces used to obtain powder samples were taken both near the pith (juvenile= J) and from mature (M) wood from each sections.

The same procedure was applied to obtain modern powder samples from Norway Spruce pieces seasoned for 2 and 30 years in the mill of GESAAF Department of the University of Florence and in the CNR-IVALSA institute of Florence (Table 2).

Table 2: list of samples analysed by means of NMR and PTR-TOF-MS. j and m stay for juvenile and mature wood respectively, specifying the distance from pith of the samples. For historical samples j= inner, m=outer.

	Samples		NMR	PTR-TOF-MS
Modern wood	Modern reference cut **2 years** ago and seasoned in the mill of GESAAF department	**M1_j**		*
		M1_m	*	*
		M2_j		*
		M2_m		*
	Modern reference seasoned **30 years** in the mill of GESAAF department	**M3_j**	*	*
		M3_m	*	*
		M4_j		*
		M4_m		*
	Modern reference provided by CNR-IVALSA (**2 years** seasoned)	**M5_m**	*	
		M6_m	*	
		M7_m	*	
Historical wood	Corradini 1	**H1 _j**	*	*
	HIST_COR	**H1_m**	*	*
	Rosmini 3B	**H2 _j**	*	*
	HIST_ROS	**H2_m**	*	*
	Vigo 8	**H3_j**	*	*
	HIST_VIG	**H3_m**	*	*

These samples were used as a control during our analysis on aging of wood. Unfortunately, we did not have modern beam sections, but only wedges of logs or little pieces (Fig. 2); for this reason, the distinction between outer and inner portion was not applied for modern samples. They were identified as juvenile and mature in relation to their distance from the pith.

Figure 2: one of the modern wood edges used to obtain powder samples were taken both from juvenile (J) and from mature (M) wood. For modern samples both these two part has been considered as outer parts

2.2 Carbon-13 Cross polarization/ Magic Angle Spinning Nuclear Magnetic Resonance (^{13} C CP/MAS NMR) spectroscopy

^{13}C CP/MAS NMR measurements were done using a Bruker Avance AV Spectrometer operating at 701.22 MHz for the proton frequency and 175.305 MHz for the carbon, equipped with a 4 mm MAS BB/BB/1H Bruker Probe. The MAS spinning speed was 11 KHz regulated within +/- 2Hz by the MAS Control Unit. The Temperature was set at 290K and regulated by the BVT-3000 unit. The Contact Time during the Cross Polarization was 1ms, the relaxation delay was 2s, 1H decoupling at 96 KHz was applied during the acquisition time of ~ 30ms.
The integrals of all resonances were obtained by applying a deconvolution procedure, using TopSpin Software.

2.3 Analysis of wood volatiles by PTR-TOF-MS

The volatile compounds emitted by modern and historical powered wood samples were analysed with PTR-TOF-MS in its standard configuration, using H_3O^+ ions for the chemical ionization. The real-time detection of VOCs emitted by different wood samples was achieved using a PTR-TOF 8000 model (Ionicon Analytik GmbH, Austria). The abilities of the instrument, as well as production and ionization of H_3O^+ in the drift tube, are described elsewhere (Lindinger et al. 1998; Blake et al. 2009).
For the analysis, 1±0.15 g of powder wood (T 20°C and RH 65%) was used and subsequently each powder sample has been introduced in apposite glass jar (2/3L) provided with inlet and outlet Teflon pipes, which connect the glass jar to the PTR-TOF-MS system and to the zero-air generator, respectively. Before the analysis each jar was cleaned for 120 sec. and subsequently was hermetically sealed and incubated for 60 minutes at 20-25°C. Three replicates were prepared for each sample and a

preliminary measurement on empty jars were run and used for background subtraction. The measurement order of samples was randomized.

All the measurements were carried out under the following drift tube conditions: 600 V drift voltage, 60°C temperature, pressure of 2.23 mbar and resulting in an E/N value of 130 Townsend (Td, 1 Td = 10^{-17} cm^2/V s). In order to allow a direct comparison of the obtained spectra the E/N ratio was kept steady at 130 Td for all the measurements. The inlet flow was set at 100 sccm and the headspace analysis was recorded in a range between m/z 20-210 for 120 seconds using an acquisition rate of one spectrum per second.

All spectra were acquired, analyzed and filtered using a procedure previously reported by Taiti et al. (2017). Briefly, the raw data were acquired by the TOFDAQ Viewer software (Tofwerk AG, Thun, Switzerland) and subsequently, all signals were converted on the base of primary ion signal from cps to ppbv using a procedure described by (Lindinger et al. 1998). After mass peaks extraction, the putative identification of each peak detected was performed on the basis of high mass resolution and rapid identification of compounds with a high level of confidence provided by the tool (Lanza et al. 2015), which was compared (1) on models of fragmentation available in the literature (Maleknia et al. 2007; Demarcke et al. 2009; Kim et al. 2009; Aprea et al. 2015); (2) with published VOCs emitted by wood species (Risholm-Sundman et al. 1998; Courtois et al. 2009; Roffael et al. 2015).

At the end, a total of 42 compounds were detected with a minimum concentration of 1bbv and these data points were used to build the data matrix which was used for multivariate data analysis method.

2.4 Statistical analysis

A Principal component analysis (PCA) was applied on the dataset containing all the integral values derived from NMR spectra of the investigated samples to explore clustering of modern and historical wood, and inner and outer wood samples as well. PCA analysis was performed with RStudio software.

Furthermore, concerning VOCs analysis, the matrix composed of 42 mass peaks x 42 (*i.e.*, 14 samples, three replicates each - x-block) was used to discriminate between modern and historical wood samples, and among the four different sampling origin of the wood samples (modern juvenile and mature, and historical juvenile and mature) (y-blocks). A partial least squares discriminant analysis (PLSDA) (Sjöström et al. 1986; Sabatier et al. 2003; Infantino et al. 2015) approach was applied for the two y-blocks samples classification. The objective of PLSDA is to find a model, developed from a training set of observations of known class membership that separates classes (y-block) of objects on the basis of their X-variables. X-block matrix was preprocessed using an autoscaling algorithm (mean SD^{-1}) (Florack et al. 2016). The two models were developed using a procedure written in the MATLAB 7.1 R14 environment. The percentages of correct classification were calculated for the calibration and validation phases, and then used for model selection. This analysis expressed also the statistical parameters indicating the modelling efficiency indicated by sensitivity and specificity

parameters. The sensitivity is the percentage of the observations belonging to a category accepted by the class model. The specificity is the percentage of the observations belonging to a categories different from the modelled one, rejected by the class model. The PLSDA model selection was mainly based on the efficiencies and robustness parameters described above. The partitioning of the models was conducted optimally by choosing the Euclidean distances based on the algorithm of Kennard & Stone (1969) that selects objects without the *a priori* knowledge of a regression model. A summary of the relative importance of the X-variables for both Y and X model parts is given by Variable Importance in the Projection (VIP) (Taiti et al. 2015; Infantino et al. 2015). VIP scores estimate the importance of each variable in the PLS-based models. VIP scores were calculated according to Chong & Jun (2005).The explanatory variables with VIP scores values larger than 1 tend to be more important than others, although this does not imply that a variable with a low VIP score is not relevant for the model.

3. Results and discussion

The polymeric structure of both historical and modern Norway Spruce samples were analysed by means of ^{13}C CP/MAS NMR spectroscopy. In fact, this technique has been extensively applied to the structural studies of natural wood and the main characteristic of its spectrum is the broadening of most signals due to the unordered molecular structure of its components (lignin, hemicelluloses and partly cellulose) (Maunu 2002).

Figure 3 and 4 show ^{13}C CP/MAS NMR spectra with contact time of 1 ms for different samples (modern *vs* historical mature and modern *vs* historical juvenile samples).

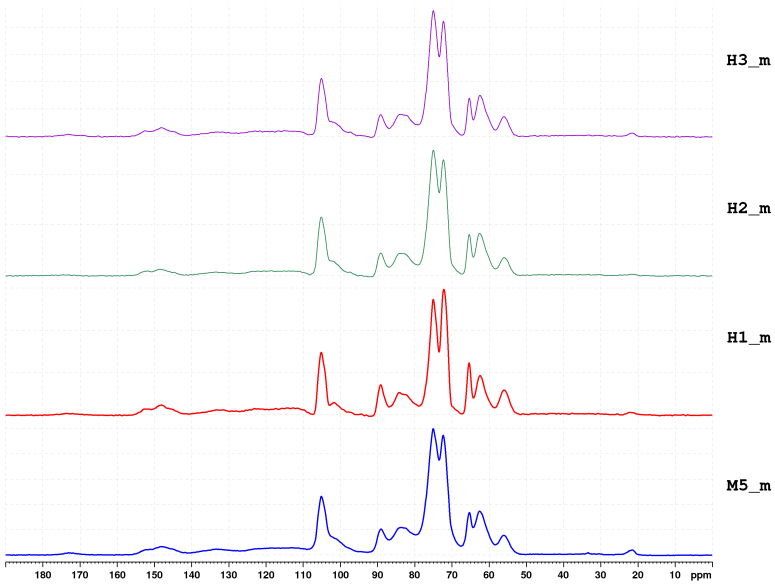

Figure 3: 13 C CP/MAS NMR spectra of 3 historical mature/outer samples and 1 modern sample as reference. Acquired with 1 ms CP contact time and normalized to the total cellulose peak at 105 ppm.

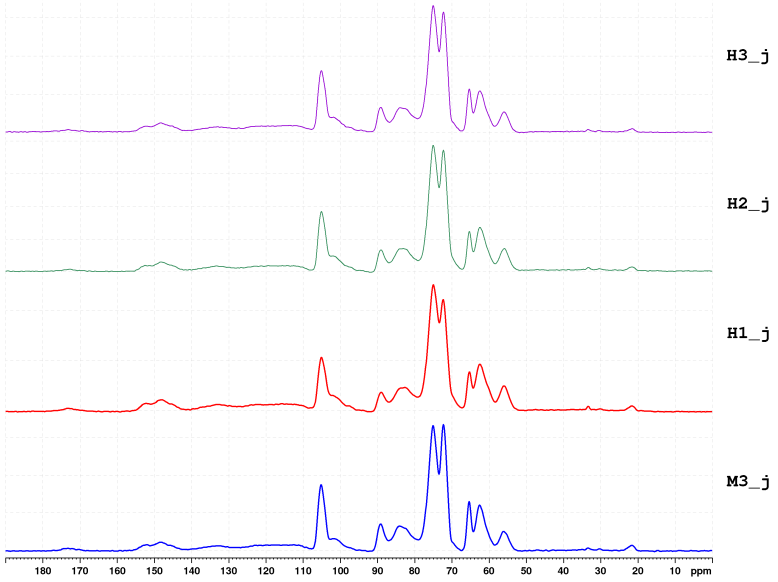

Figure 4: 13 C CP/MAS NMR spectra of 3 historical juvenile/inner samples and 1 modern sample as reference. Acquired with 1 ms CP contact time and normalized to the total cellulose peak at 105 ppm

The spectra present the typical resonances of wood components that produce highly overlapped signals. In the NMR spectra, two key regions can be distinguished: from 160 to 110 ppm characterised by broad and low intensity resonances typical of aromatic structures in lignin, and from 110 to 20 ppm, which includes sharp intense peaks due to carbohydrate polymers, overlapping each other due to their chemical similarity (Bardet et al. 2009). Table 3 shows the chemical shift assignments on the basis of several literature data (Santoni et al. 2015; Bardet et al. 2009; Capitani et al. 2012).

Table 3: Assignments of carbon-13 chemical shifts of CP/MAS NMR spectra of wood. Abbreviations: HC= hemicellulose; AC= amorphous cellulose; CC= crystalline cellulose; TC= total cellulose; S= syringyl (aromatic unit with two methoxyl groups); G= guayacyl (aromatic unit with only one methoxyl); e= etherified arylglycerol β-aryl ethers; ne= non-etherified arylglycerol β-aryl ethers.

Chemical shifts (ppm)	Resonance Assignments	Carbon Atom
21.5	Methyl carbon of acetyl groups in hemicellulose	$\underline{C}H_3$-COO-
56	Aryl methoxyl carbons of lignin	OCH_3
62	C-6 carbon of amorphous cellulose and hemicellulose	C-6 AC + HC
65	C-6 carbon of crystalline cellulose	C-6 CC
63-66	Background of C-6 carbon of hemicelluloses	C-6 HC
72	C-2,3,5 carbon of cellulose and hemicellulose	C-2,3,5 TC + HC
75	C-2,3,5 carbon of cellulose and hemicellulose	C-2,3,5 TC + HC
84	C-4 carbon of amorphous cellulose and hemicellulose	C-4 AC + HC
89	C-4 carbon of crystalline cellulose	C-4 CC
101	C-1 carbon of hemicellulose	C-1 HC
105	C-1 carbon of cellulose	C-1 TC
137	C-1,4 carbon of aromatic ring carbons of lignin	S1, S4 e + G1 e
148	carbon of lignin aromatic ring containing free phenolic groups attached	S3, S5 ne + G3 ne, G4 e
153	carbon of lignin aromatic ring containing methoxy groups	S3, S5 e
173	Carbonyl in acetoxy groups of hemicelluloses	CH_3-$\underline{C}$OO-, -$\underline{C}$OO-R

Only two signals can be undoubtedly attributed to hemicelluloses: the weak signal at 21.5 ppm due to the CH_3 carbon of the acetyl group in hemicelluloses and the signal at 172 ppm which arises from carbonyls in acetoxy groups of hemicelluloses and to acid groups possibly present in wood. The signal at 55.6 ppm is assigned to methoxyl groups of aromatic units of lignin. The region between 60 and 105 ppm is dominated by signals mostly assigned to cellulose, whereas the region between 110 and 160 ppm, as already mentioned, is specific to the aromatic carbons of lignin.

The NMR peak intensities were normalized with reference to the peak at 105 ppm (C-1, total cellulose "TC"). Noticeable variations were observed in specific intensity peaks of historical wood samples. It is worth noting that the resonance at 21.5 ppm (acetyl groups of hemicelluloses) which is clearly observed in modern *Picea abies*, is deeply decreased in the spectra of most of all historical samples obtained by ancient beams, clearly indicating the occurrence of hemicelluloses depletion. Excepting for juvenile/inner historical samples which present values similar to the modern wood. Historical mature/outer spruce showed an intensity decrease at hemicelluloses-related peaks and, in some cases, also at lignin-related peaks (21.5, 62, 65, 75, 137, 153 and 173 ppm). Figure 5 shows the comparison of the normalized intensities of the spectra of modern and historical spruce. The inner portion of historical wood showed higher intensities concerning lignin peaks, due probably to the different amount of lignin of the juvenile wood. In fact, softwoods have wood cores with lower cellulose

and higher lignin, but with little or no significant difference in hemicelluloses (Barnett & Jeronimidis 2003). Deacetylation of hemicelluloses was detected as an intensity decrease at 21.5 ppm (methyl group signal) and at 174 ppm (carboxyl group signal) too in historical mature/outer wood samples.

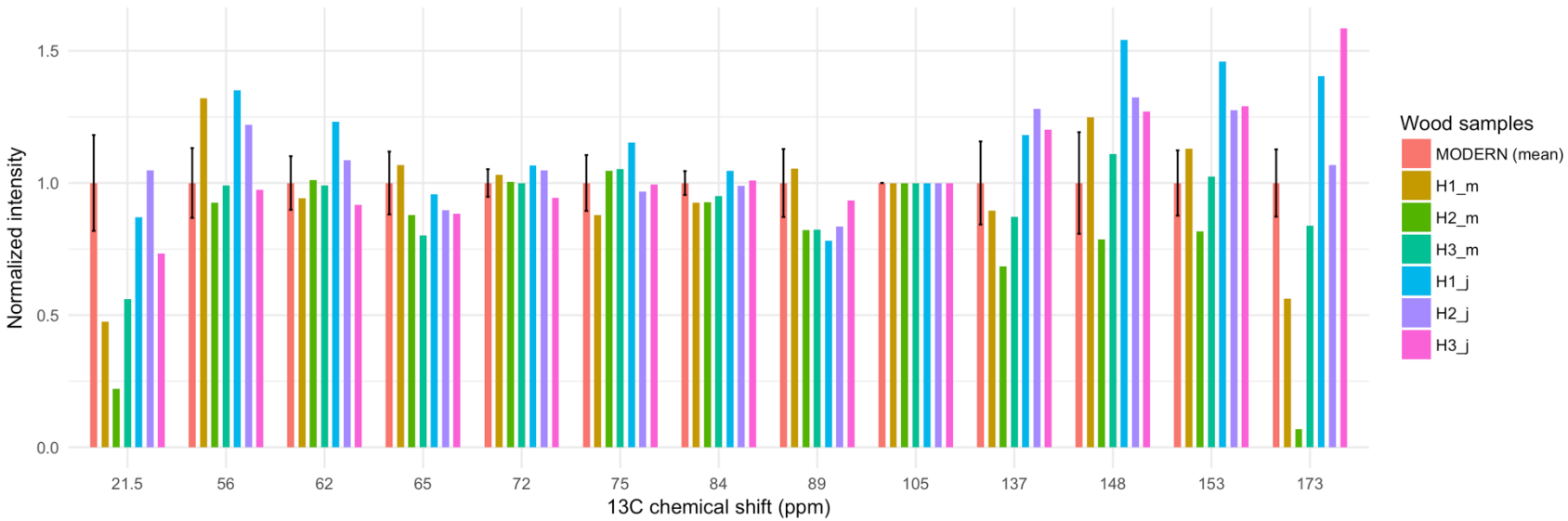

Figure 5: Comparison of ^{13}C peak intensities of the spectra of modern and historical spruce. Only the mean of 4 modern spruce samples is shown. Values of the historical samples were normalized with reference to the mean of the modern spruce.

Moreover, we estimated the relative degree of cellulose crystallinity by the intensity ratio of the the NMR peaks at 89 and 105 ppm (crystalline cellulose/ total cellulose) (Tai et al. 2017), which appeared to be stable in the tree historical samples (Fig. 6) respect to the modern ones, while the separation between different groups of samples is due to the relative hemicellulose level, established by the intensity of the peak at 21.5 ppm. It is worth noting that historical juvenile/inner samples were clustering together with the modern references.

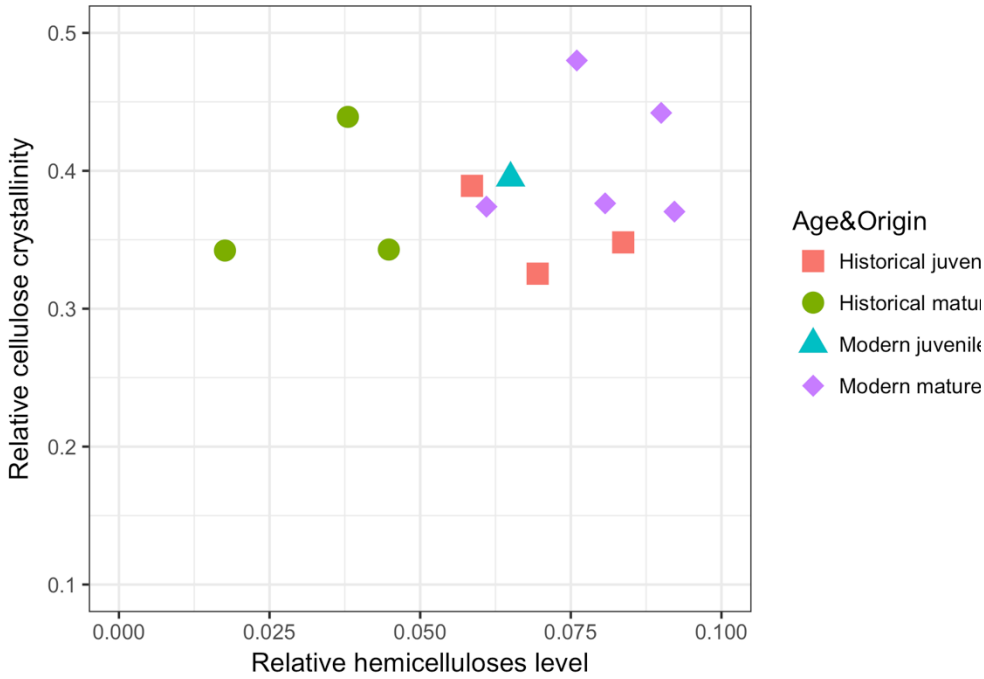

Figure 6: Relative cellulose crystallinity plotted versus hemicellulose level measured by ^{13}C CP/MAS NMR

According to the literature (Bardet et al. 2009), the relative amount of carbohydrates and lignin was evaluated from the integral of the signal at 105 ppm of the anomeric carbon of cellulose as a reference, and the integral at 56 ppm of the methoxyl groups of lignin. The ratio between the two signals is found to be 2.3 in modern wood, whereas it is 2.0 in the sample collected from the juvenile wood and 2.2 in the sample from the mature wood of the beam sections. So it was not possible do detect significant changes in carbohydrate relative amount between modern and historical wood.

The relative intensities of the two broad signals at 153 ppm and at 148 ppm, respectively assigned to C-3 and C-5 in syringyl involved in β-O-4 linkage structures of lignin and to the same carbons in non-etherified structures, were used to estimate the depletion of the β -O-4 linkages inside lignin polymers. For the modern and historical samples, it was not possible do detect significant change in these relative intensities. It clearly dues to the fact that these kind of linkages, the most frequent in lignin polymers, were not yet degraded during aging.

Principal component analysis (PCA) was performed on NMR spectra (peak intensities) collected from modern and historical samples. We observed that the PCA discrimination between all the modern and historical spectra did not provide sufficient differences for undoubtable discrimination of wood samples of different age (Fig. 7a). Clear clustering of wood samples, due both to the distance from the pith and to the provenience from the inner and outer part of the beam section, was notice between historical mature/outer and historical juvenile/inner samples (Fig. 7b). The samples were clearly separated along the first principal component (52.4% of the variance), which was primarily associated with the following peaks: 21.5, 84, 137, 148, 153, 173 ppm, strongly correlated with hemicelluloses changes and different amount of lignin in their composition. The second principal component further differentiated the historical mature samples based on peaks associated to cellulose.

However, we noticed that also the PCA discrimination between the modern samples did not provide a clear cluster (Fig. 7c), and some differences could be revealed in this group of samples due to their provenience, according to Santoni et al. (2015).

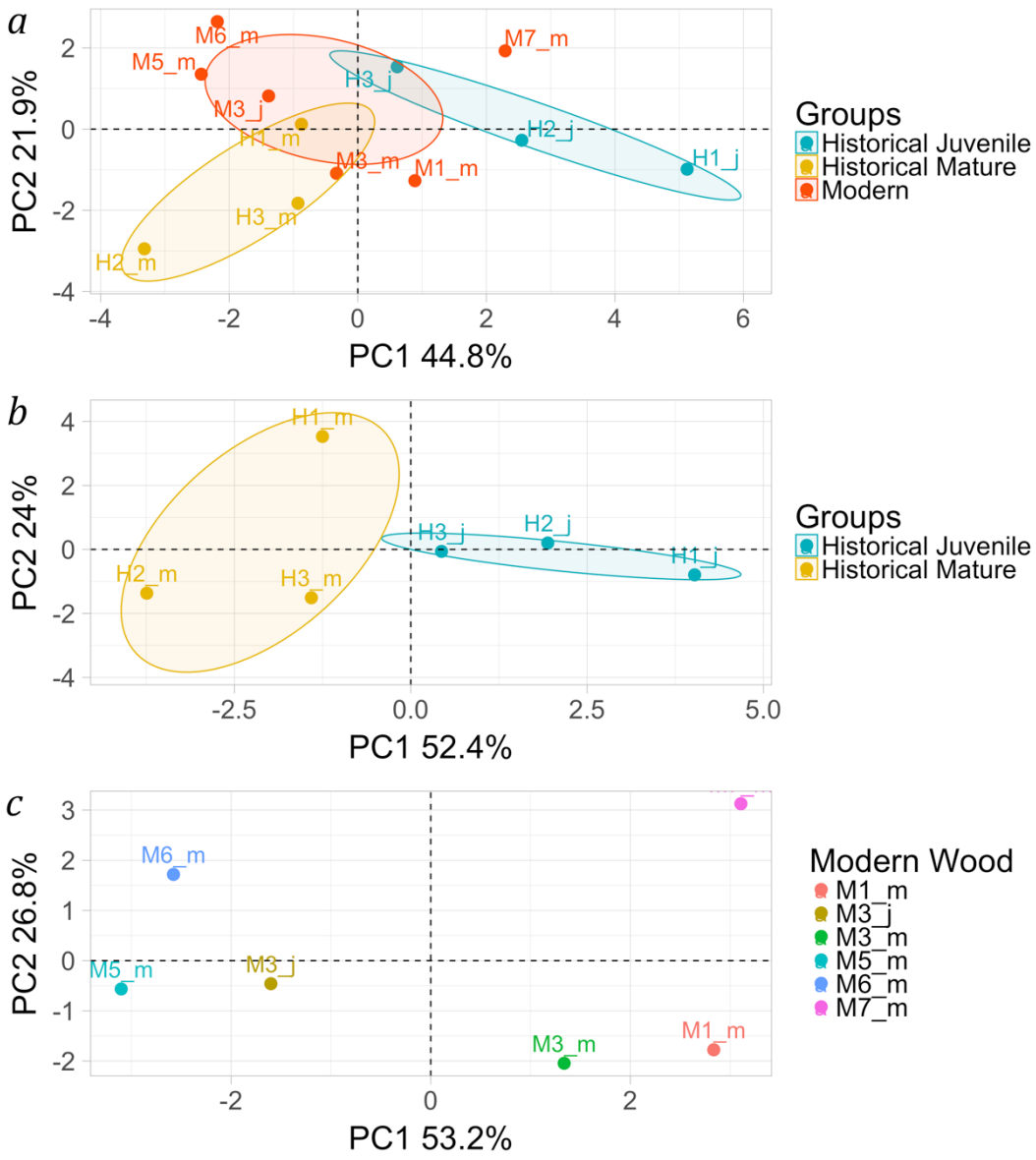

Figure 7: Principal component analysis of a) all the NMR spectra; b) historical samples and c) modern samples. Ellipses represents 95% Confidence intervals.

Dealing with VOC emissions, PTR-TOF-MS provided entire mass spectra from different wood samples with short response time and high mass resolution. In the current trial, several volatile compounds and more than 40 mass peaks in the range of measured masses (20-210 m/z) were detected from modern and historical wood specimens. The putative compounds identified for the two groups of samples, along with their mass/charge (m/z) ratio, molecular formula, chemical

classes, tentative identification and the related reference, are shown in Supplementary material (Table S1).

According to our tentative identification (TI), the main compounds detected and the most abundant volatile compounds in the headspace of modern and historical wood, both juvenile and mature, were m/z = 31.018 TI: formaldehyde, m/z = 33.033 TI: methanol, m/z = 45.033 TI: acetaldehyde, m/z = 59.049 TI: acetone, m/z = 61.028 TI: acetic acid, m/z = 69.069 TI: isoprene, m/z = 81.070 TI: monoterpenes fragment, m/z = 93.070 toluene or p-cymene fragment, and m/z = 97.064 furfural. All these compounds were recorded in all the specimens. However, many other compounds of different chemical class were emitted by the modern and historical wood samples, due to natural aging processes (Table S1, Fig. 8-9).

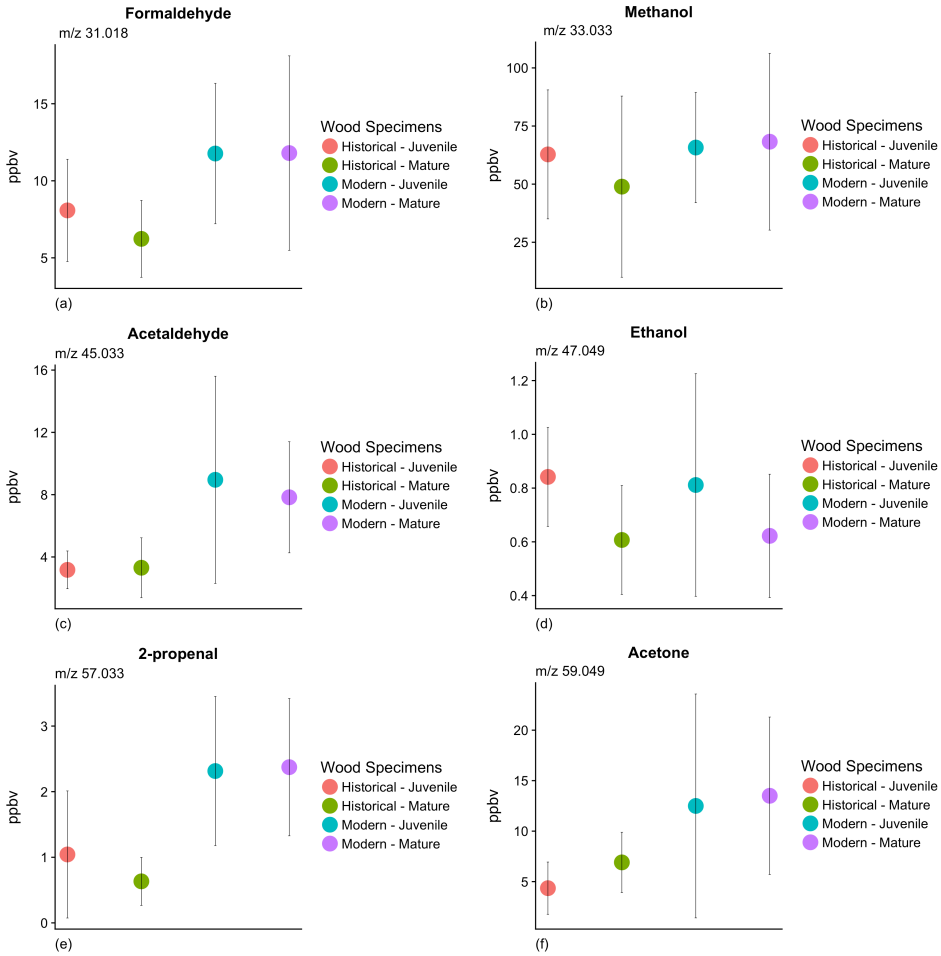

Figure 8: Emission rate of (a) formaldehyde, (b) methanol, (c) acetaldehyde, (d) ethanol, (e) 2-propenal, (f) acetone.

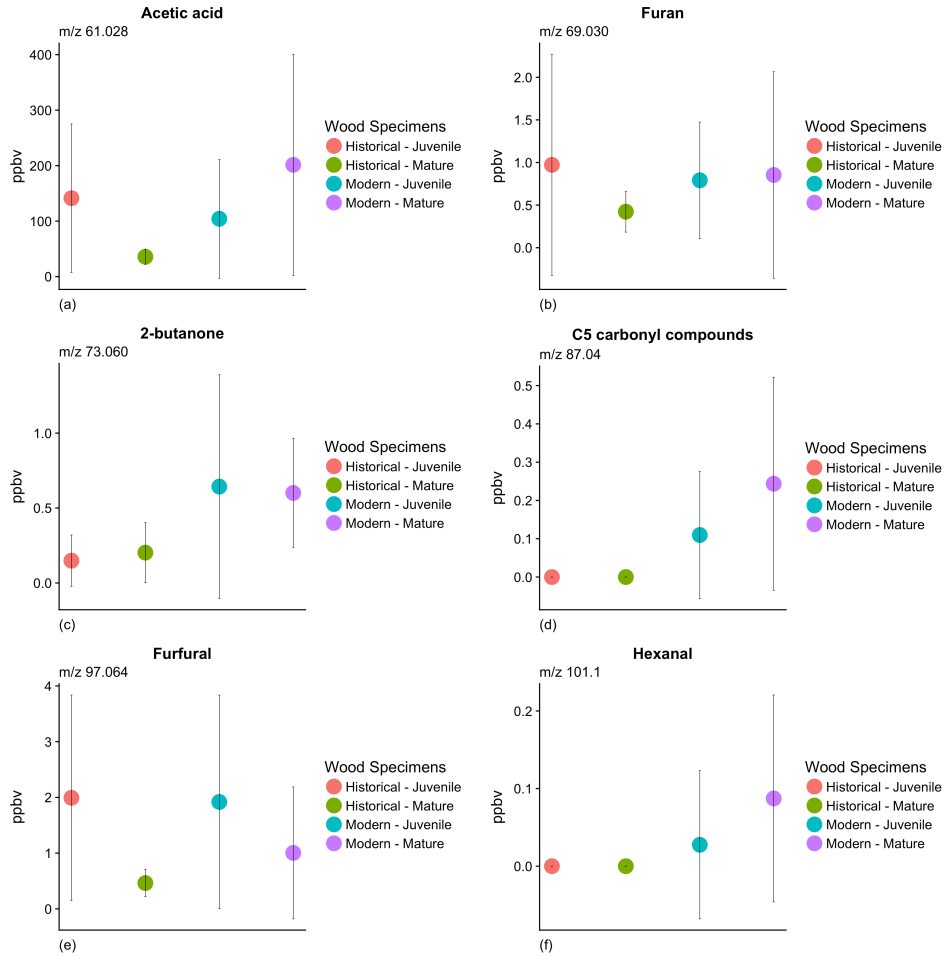

Figure 9: Emission rate of (a) acetic acid, (b) furan, (c) 2-butanone, (d) C5 carbonyl compounds, (e) furfural, (f) hexanal.

Terpenes and formaldehyde in softwoods are naturally occurring chemicals in wood (Roffael 2006), whereas, according to literature, specific treatments could determine the emissions of different VOCs from wood or changing their release rates over the time (Manninen et al. 2002; Hyttinen et al. 2010).

Previous studies have already dealt with products that derived from the degradation of wood polysaccharide fraction, after specific treatments or processing procedures of wood, showing the formation of compounds such as: (1) formaldehyde, acetaldehyde, 2-propenal, butanal, and butanone resulting from the breakdown of the polysaccharide fraction of the wood during pyrolysis (Salem & Böhm

2013); (2) acetic acid following acetyl group hydrolysis in hemicellulose (Risholm-Sundman et al. 1998); (3) acetone, butanal, pentanal, hexanal, heptanal, octanal and nonanal resulting from the oxidation of unsaturated fatty acids of triglycerides (Risholm-Sundman et al. 1998; Granström 2014); (4) furfural due to the conversion of carbohydrates (Roffael et al. 2015); and (5) furan and furan derivatives as thermal degradation products of cellulose and other polysaccharides (De Sińon et al. 2009; Manninen et al. 2002).

In our trial, comparing untreated modern powder wood samples (stored for few years in dry aerobic environment) and untreated historical ones (aged for hundreds years in the same condition), we similarly detected the emission of aldehydes and ketones, such as formaldehyde, acetaldehyde, 2-propenal, and butanone. Their emission could be due to the degradation of polysaccharides chains as autocatalytic process for the presence of reducing end group in the polysaccharides chains (Fig.10).

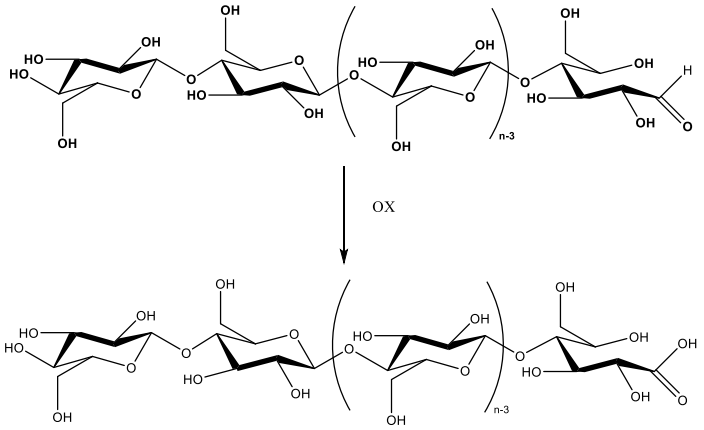

Figure 10: oxidation of reducing end group in polysaccharides chain

The oxidation of this reducing end group could activate reactions of degradation in the chain over longer time and with a more restrained effect than pyrolysis action produces. This process could take place in the galactoglucomannan chains of softwood hemicelluloses as well. The sensitivity to oxidation reactions, resulting in the formation of carboxyl groups (-COOH), is determined by the presence of reducing sugars with the free anomeric group in the terminal positions. The presence of reducing groups itself increases during degradation process due to the

advance of hydrolysis reaction, and thus it results in an autocatalytic process because it causes the increase of the carboxylic acid groups which catalyse the hydrolysis reaction. A further oxidative degradation process is due to the oxidation of primary alcohol groups to carboxylic acids which thus result in increased the acidity and a consequent hydrolysis ease (Fig. 11a). Finally, oxidation of secondary alcohol groups may result in the formation of carbonyl groups subject to further acid oxidation with ring opening only in extremely severe conditions (Fig. 11b).

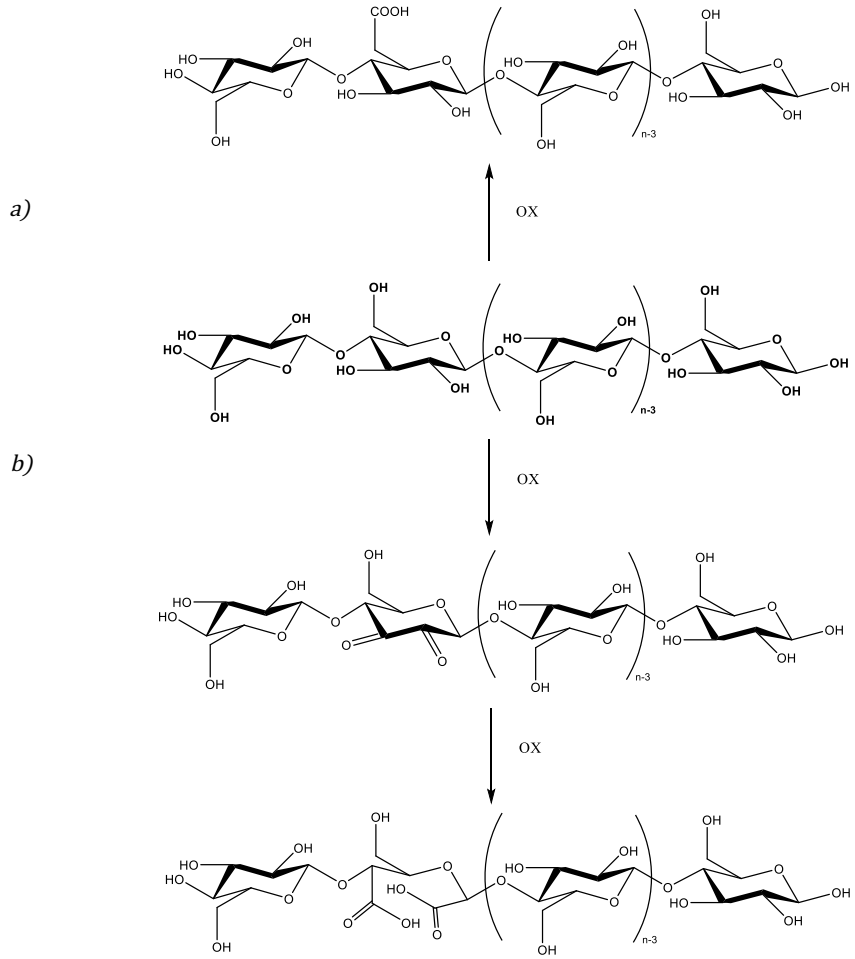

Figure 11: a) oxidation of primary alcohol groups; b) oxidation of secondary alcohol groups

However, the presence of certain types of compounds emitted as aldehydes with a C chain of atoms greater than 4 is difficult to correlate with this reactivity.

On the other hand, emission of acetone and other aldehydes such as pentanal and hexanal detected in our powdered samples, was in agreement with the oxidation of free unsaturated fatty acids of triglycerides, and this oxidative process is a complex self-catalysing free radical chain reaction (Granström 2014) (Fig. 12).

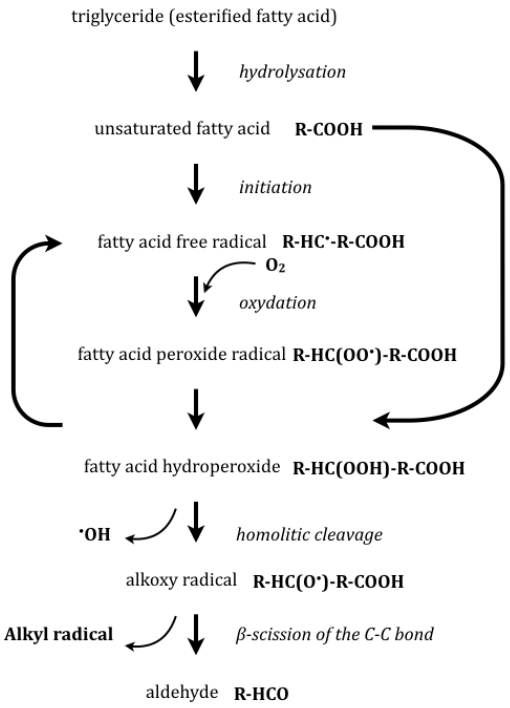

Figure 12: oxidation of free unsaturated fatty acids of triglycerides path (adapted by Granström (2014)

Acetic acid emission followed acetyl group hydrolysis in hemicellulose (Risholm-Sundman et al. 1998), in agreement with our NMR results too.

Moreover, according to Roffael et al. (2015), furfural emission possibly due to dehydration of carbohydrates (after dehydration of C5 hemicellulose fraction, such as pentosanes or xylans) (Fig. 13) was detected in our samples.

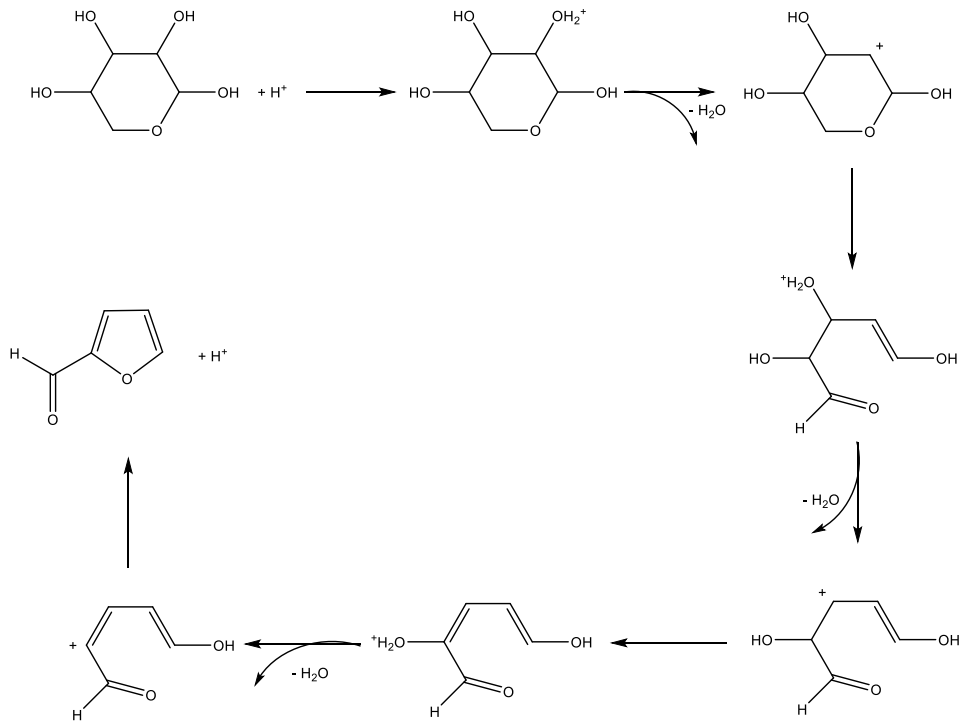

Figure 13: mechanism for the dehydration of pentose to furfural

Even furan and furan derivatives were detected in our samples. However, the emission of these compounds, indicated as thermal degradation products of cellulose and other polysaccharides (Manninen et al. 2002; De Siḿon et al. 2009) is difficult to correlate with the transformation of the same polymer structures at room temperature.

Concerning the emission of aromatic hydrocarbons, they could be originated by the transformation of lignin structure.

All these compounds seem to be emitted by the wood immediately at the beginning of its aging, even after only very few years of seasoned, and their releases last over the time. In fact, they were emitted by modern powder samples and by historical ones as well.

Subsequently, the PLSDA allowed identifying compounds which better contributed to discriminating between modern and historical samples. The performance indicator of the PLSDA model built to determine modern vs historical samples and the one built to determine the four different classes based on the sampling origin (modern-

juvenile, modern-mature, historical-juvenile and historical-mature) were summarized in Table 4.

Table 4: Characteristic and principal results of the two PLSDA models. LVs = Latent Vectors; RMSEC = Root Mean Square Error of Calibration

	PLSDA model modern *vs* historical wood	PLSDA model based on sampling origin
N. Samples Total	42	42
N. Samples model (75%)	32	32
n. classes (y-block)	2	4
n. LVs	5	11
% Cumulated variance X-block	87.05	96.91
% Cumulated variance Y-block	41.75	60.22
Mean sensitivity	1	1
Mean specificity	1	0.979
Random probability. %	50	25
Mean classification error. %	0	0.010
Mean RMSEC	0.537	0.315
Mean % correct classification calibration/validation set (75%)	100.0	100.0
Mean % correct classification test set (25%)	100.0	100.0

This model allowed a perfect discrimination (100% correct classification, sensitivity and specificity equal to 1) between modern and historical samples. Figure 14 shows the scatter plot of the scores of the samples based only on the first three PLSDA Latent Vectors (x-block 73.67%; y-block 32.96%) of the model used to determine modern *vs* historical samples. Modern samples were a more heterogeneous group and mainly separated from historical samples on the first axis.

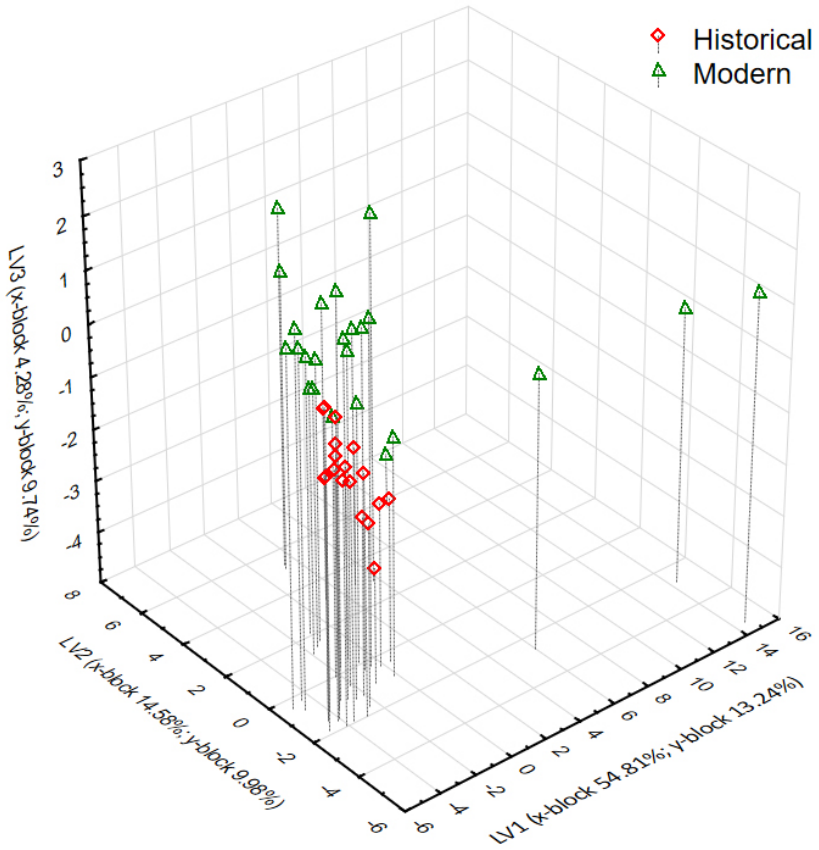

Figure 14: Scatter plots of the first 3 Latent Vectors scores of the PLSDA used to discriminate between modern and historical spruce samples.

Also the PLSDA model used to discriminate among the four different sampling origin of the wood samples (modern juvenile and mature, and historical juvenile and mature) showed a perfect classification of the samples into the 4 classes (100% correct classification, specificity equal to 1 and sensitivity equal to 0.98). Figure 15 shows how the 42 individuals (14 samples x 3 replicates each) were quite well separated using only the first three axes (x-block 81.34%; y-block 22.59%). Modern samples, juvenile and mostly mature, showed a larger variability than the other two classes of historical samples.

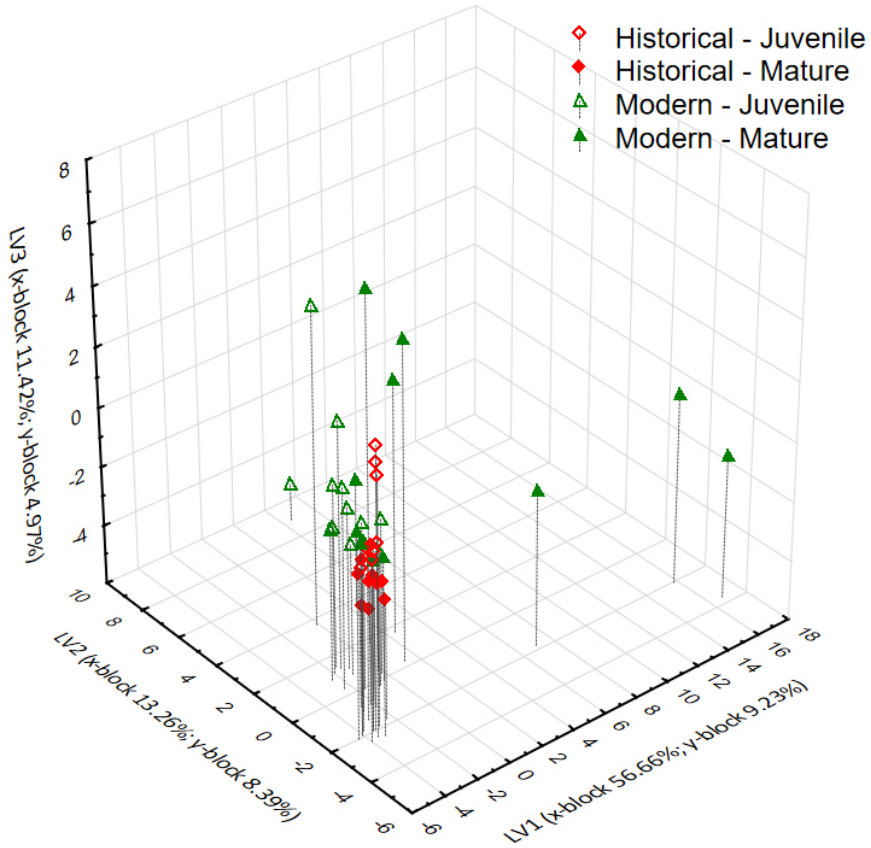

Figure 15: Scatter plots of the first 3 Latent Vectors scores of the PLSDA used to discriminate between the 4 classes based on the sampling origin.

For a more detailed characterization of the VOCs emitted by different woods, only the m/z presenting a high VIP score (higher than 1) for modern and historical wood discrimination and for the four sampling origin discrimination are presented in Table 5 and 6.

Table 5: Protonated masses having VIP scores greater than 1 between modern and historical wood and their tentative identification.

Protonated measured m/z	Tentative identification	VIP scores
		Historical vs Modern
31.018	Formaldehyde	**1.30**
41.038	Alkyl fragment: propadiene	**1.23**
45.033	Acetaldehyde	**1.48**
47.049	Ethanol	**1.49**
57.033	2-propenal (acrolein)	**1.94**
69.036	Furan	**1.19**
75.043	Propanoic acid or Hydroxy-2-propanone (acetol)	**2.06**
85.065	Pentenal/pentenone or cyclopentanone	**1.12**
87.043	C5 carbonyl compounds (pentanal / pentanone) or pentenol	**1.22**
89.059	Ethyl acetate or methyl-propanoate	**1.30**
101.059	Hexanal	**1.24**
103.075	Methylbutanoic acid	**1.49**
105.069	Styrene/ethylbenzene/ vinylbenzene	**1.13**
111.080	2-propyl furan or heptadienal	**1.38**

Table 6: Protonated masses having VIP scores greater than 1 (in bold) between the four classes based on sampling origin (Historical juvenile, Historical mature, Modern juvenile and Modern mature) and their tentative identification.

Protonated measured m/z	Tentative identification	VIP scores			
		Hist_j	Hist_m	Mod_j	Mod_m
31.018	Formaldehyde	0.947	0.945	**1.468**	0.765
33.033	Methanol	0.922	0.987	**1.074**	0.743
41.038	Alkyl fragment	0.933	**1.081**	0.967	0.859
43.054	Propylene	0.724	**1.052**	0.632	**1.167**
45.033	Acetaldehyde	**1.033**	**1.042**	**1.666**	0.635
47.049	Ethanol	**1.727**	**1.177**	**1.520**	**1.560**
57.033	2-propenal (acrolein)	**1.038**	**1.101**	**1.596**	0.824
57.070	Alkyl fragment	**1.055**	**1.286**	0.750	0.869
59.049	Acetone	0.720	0.835	**1.072**	0.659
61.028	Acetic acid	**1.053**	**1.439**	**1.162**	**1.233**
69.036	Furan	**1.723**	0.741	0.927	**1.601**
71.048	Methyl vinyl ketone (MVK) and methacrolein (MAC) or Butenal and butanoic acid fragment	0.689	**1.021**	0.729	0.871
73.065	2-butanone (methyl ethyl ketone MEK) and 2-methyl propanal	0.956	**1.415**	**1.319**	0.602
75.043	Propanoic acid or Hydroxy-2-propanone (acetol)	**1.097**	**1.599**	**1.698**	**1.146**
83.085	C6 compounds: hexanal fragment or hexenol fragment	0.855	**1.182**	0.931	**1.215**

Protonated measured m/z	Tentative identification	VIP scores			
		Hist_j	Hist_m	Mod_j	Mod_m
85.065	Pentenal/pentenone or cyclopentanone	0.897	**1.225**	**1.194**	0.698
87.043	C5 carbonyl compounds (pentanal / pentanone) or pentenol	**1.295**	**1.251**	0.875	**1.314**
89.059	Ethyl acetate or methyl-propanoate	0.980	**1.223**	0.970	0.987
97.064	Furfural	**2.142**	0.959	**1.333**	**2.010**
101.059	Hexanal	**1.680**	**1.379**	**1.630**	**1.513**
103.075	Methylbutanoic acid	**1.181**	**1.134**	0.981	**1.264**
105.069	Styrene/ethylbenzene/ vinylbenzene	**1.474**	**1.440**	**1.766**	**1.340**
111.080	2-propyl furan or heptadienal	**2.013**	**1.777**	**1.572**	**1.209**

Some of the most significant compounds for modern *vs* historical spruce samples discrimination were: m/z = 31.018 (TI: formaldehyde), m/z = 45.033 (TI: acetaldehyde), m/z = 47.049 (TI: ethanol), m/z = 57.033 (TI: 2-propenal), m/z = 75.043 (TI: propanoic acid or hydroxy-2-propanone), and m/z = 103.075 (TI: methylbutanoic acid) (Table 5). On the other hand, dealing with sampling origin discrimination (Table 6), some of the most significant compounds were: m/z = 31.018 (TI: formaldehyde), m/z = 33.033 (TI: methanol), m/z = 59.049 (TI: acetone) for modern juvenile samples; m/z = 43.054 (TI: propylene) and m/z = 61.028 (TI: acetic acid) for modern mature and historical mature wood; m/z = 47.049 (TI: ethanol) and m/z = 69.036 (TI: furan) for historical juvenile.

4. Conclusion

In the time domain studied (several centuries) the degradation rate, due to non-biological alteration, was higher for hemicelluloses than for the other two main wood polymers, cellulose and lignin, which appear to be very stable under these storage conditions.

The spectroscopic characterization by means of NMR is resulted very powerful in the assessment of the difference within the same sample (old timber beams): here the mature/outer historical wood, has systematically shown a higher degradation than the inner parts of the same sample. The aging process seems to start from the outer part of the samples, on the surfaces, and proceeds, with a speed that need to be quantified, to the inside of the beam sections, not yet affected.

However, NMR provided a weak discrimination between historical wood and modern wood, intended as whole groups.

The clusters obtained by means of PCA applied to spectroscopic results, allowed us to highlight the superimposition of two different effects: the aging of wood, which leads to a depletion of wood components and to changes in its structure, and that of initial quantitative composition of samples from different provenience. For this reason, in our trial, making chemical distinctions between historical spruce (aged for hundreds of years in aerobic condition) and modern one were not always so immediate and clear.

Regarding VOC analysis, the degradation of hemicelluloses, such as their deacetylation, contributes to the emission of acids (mainly acetic acid) that could remain in the wood structure and contributes to hydrolyse and depolymerise cellulose chains over the time.

A key conclusion is that the emission of acids, aldehydes, alcohols provided that several modifications are acting in the structure of wood, even at the very beginner stages of the aging. In fact, we detected emission of specific compounds both from modern samples aged 2 years and historical samples aged for hundreds of years. However, these kind of structural changes could not be identified at these stages of aging by means of spectroscopic techniques.

The volatomic approach was a useful tool to monitor and characterize VOCs profile during natural aging process. Moreover, statistical analysis on VOC analysis was able to discriminate between the two different age groups.

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Supplementary materials Paper II

Table S1: Compounds identified via PTR-TOF-MS: Protonated masses (mass/charge = m/z), molecular formula, tentative identification, references of the investigated volatile compounds, and their rate of emission (ppm) from modern and historical samples.

Measured m/z	Protonated formula	Chemical class	Tentative identification	References (PTR-MS #; Wood (plant and solid wood)*)	Modern wood		Historical wood	
					Juvenile	Mature	Juvenile	Mature
27.022	C_2H_2—H^+	Alkenes	Acetylene	(Vita et al. 2015)#	6.723±4.523	9.405±5.895	6.213±2.706	7.015±2.543
31.018	CH_2O—H^+	Aldehydes	Formaldehyde	(Roffael 2006)*	11.769±4.556	11.799±6.309	8.079±3.319	6.232±2.497
33.033	CH_4O—H^+	Alcohols	Methanol	(Risholm-Sundman et al. 1998)*;	65.74±23.734	68.253±38.022	62.795±27.744	48.925±38.965
41.038	C_3H_4—H^+		Alkyl fragment: propadiene	(Brilli, Gioli, Ciccioli, et al. 2014)#*	12.016±7.481	14.844±10.92	10.635±8.422	12.486±4.11
43.054	C_3H_6—H^+	Alkenes	Propylene	(Brilli, Gioli, Ciccioli, et al. 2014)#*	13.923±9.32	27.466±21.372	14.196±8.266	8.563±2.643
45.033	C_2H_4O—H^+	Aldehydes	Acetaldehyde	(Risholm-Sundman et al. 1998)*	8.955±6.653	7.832±3.567	3.171±1.214	3.307±1.922
47.049	C_2H_6O—H^+	Alcohols	Ethanol	(Maleknia et al. 2007)#*;	0.812±0.414	0.622±0.23	0.842±0.184	0.607±0.203
53.040	C_4H_4—H^+	Alkanes	Cyclobutadiene or alkyl fragment	(Vita et al. 2015)# or (Sánchez Del Pulgar et al. 2014)#	0.202±0.224	0.77±1.071	0.129±0.256	0.17±0.125
57.033	C_3H_4O—H^+	Aldehydes	2-propenal (acrolein)	(Brilli, Gioli, Ciccioli, et al. 2014)#*	2.314±1.137	2.373±1.046	1.043±0.969	0.633±0.366
57.070	C_4H_8—H^+	Alkenes and esters	Butene and hexyl acetate fragment	(Brilli, Gioli, Ciccioli, et al. 2014)#*	3.913±2.881	4.333±2.821	1.537±1.165	3.611±1.774
59.049	C_3H_6O—H^+	Ketones	Acetone (2-propanone)	(Risholm-Sundman et al. 1998)*; (Maleknia et al. 2007)#*	12.496±11.086	13.501±7.798	4.352±2.589	6.905±2.984
61.028	$C_2H_4O_2$—H^+	Esters and acids	Acetic acid	(Risholm-Sundman et al. 1998)*; (Maleknia et al. 2007)#*	104.25±107.133	201.428±199.226	141.329±133.908	35.823±13.819

Measured m/z	Protonated formula	Chemical class	Tentative identification	References (PTR-MS #; Wood (plant and solid wood)*)	Modern wood		Historical wood	
					Juvenile	Mature	Juvenile	Mature
69.036	C_4H_4O—H^+	Furans	Furan	(Brilli, Gioli, Ciccioli, et al. 2014)#*	0.791±0.683	0.854±1.212	0.972±1.297	0.423±0.239
69.069	C_5H_8—H^+	Terpene fragment	Isoprene (1,4-pentadiene)	(Filella et al. 2007)#*	2.964±2.058	4.254±4.362	2.384±1.245	2.684±0.753
71.049	C_4H_6O—H^+	Aldehydes/ ketones	Methyl vinyl ketone (MVK) and methacrolein (MAC) or Butenal and butanoic acid fragment	(Liu et al. 2013)#*or(Yener et al. 2016)#.	0.478±0.465	0.772±0.786	0.192±0.311	0.262±0.169
73.065	C_4H_8O—H^+	Aldehydes/ ketones	2-butanone (methyl ethyl ketone MEK) and 2-methyl propanal	(Brilli, Gioli, Zona, et al. 2014)#*; (Holzinger et al. 2000)#*;	0.643±0.747	0.601±0.365	0.149±0.171	0.203±0.201
75.044	$C_3H_6O_2$—H^+	Esters and acids	Propanoic acid or Hydroxy-2-propanone (acetol)	(Papurello et al. 2012)# or (Brilli, Gioli, Ciccioli, et al. 2014)#*	0.899±0.586	0.875±0.641	0.42±0.381	0±0
77.038	C_6H_4—H^+		Alkyl fragment	(Goacher et al. 2010)#*	1.284±1.942	10.469±12.977	1.237±0.773	1.556±1.158
79.054	C_6H_6—H^+	Aromatic hydrocarbons	Benzene	(Brilli, Gioli, Ciccioli, et al. 2014)#*	0.832±1.114	7.805±10.612	1.755±1.199	1.167±0.466
81.070	C_6H_8—H^+	Terpene fragment	Monoterpenes fragment	(Grabmer et al. 2006)#*	1.657±2.125	14.452±24.19	2.603±1.148	1.47±0.463
83.048	C_5H_6O—H^+	Furans	2-methyl furan	(Brilli, Gioli, Ciccioli, et al. 2014)#*	0.069±0.18	1.22±2.178	0±0	0±0
83.085	C_6H_{10}—H^+	Aldehydes/ alcohols	C6 compounds: hexanal fragment or hexenol fragment	(Soukoulis et al. 2013)# or (Brilli, Gioli, Ciccioli, et al. 2014)#*	1.678±1.068	1.885±2.001	0.936±0.564	1.826±1.285

Measured m/z	Protonated formula	Chemical class	Tentative identification	References (PTR-MS #; Wood (plant and solid wood)*)	Modern wood		Historical wood	
					Juvenile	Mature	Juvenile	Mature
85.065	C_5H_8O—H^+	Aldehydes/ ketones	Pentenal/pentenone or cyclopentanone	(Risholm-Sundman et al. 1998)* ; (Yener et al. 2016)# or (Brilli, Gioli, Ciccioli, et al. 2014)#*	0.796±1.012	0.662±0.612	0.178±0.278	0.492±0.353
87.043	$C_5H_{10}O$—H^+	Aldehydes/ketones or Alcohols	C5 carbonyl compounds (pentanal / pentanone) or pentenol	(Brilli, Gioli, Ciccioli, et al. 2014)#*or (Yener et al. 2016)#	0.11±0.166	0.244±0.278	0±0	0±0
89.059	$C_4H_8O_2$—H^+	Esters	Ethyl acetate or methyl-propanoate or 3-hydroxy-2-butanone	(Yener et al. 2015)# or (Schripp et al. 2014)# or (Aprea et al. 2015)#	0.326±0.379	0.466±0.49	0.237±0.356	0±0
91.054	C_7H_6—H^+	Aromatic hydrocarbons	Xylene fragment	(Maleknia et al. 2007)#*	1.595±2.139	6.668±7.178	1.597±0.872	2.337±0.889
93.069	C_7H_8—H^+	Terpene/aromatic hydrocarbons	p-Cymene fragment or toluene	(Maleknia et al. 2007)#* or (Holzinger et al. 2000)#*	2.326±2.381	16.024±19.013	2.84±0.675	2.813±0.696
95.086	C_7H_{10}—H^+	Terpenes	Monoterpene fragment	(Maleknia et al. 2007)#*	0.64±0.554	3.604±5.511	0.763±0.141	0.451±0.14
97.064	$C_5H_4O_2$—H^+	Aldehydes	Furfural	(Yener et al. 2016)#	1.919±1.917	1.004±1.184	1.993±1.845	0.464±0.245
97.101	C_7H_{12}—H^+		Alkyl fragment	(Aprea et al. 2015)#	0.381±0.259	0.539±0.61	0.225±0.21	0.268±0.15
99.080	$C_6H_{10}O$—H^+	Aldehydes/ ketones	Hexenals/ methylpentanone	(Brilli, Gioli, Ciccioli, et al. 2014)#*	0.23±0.259	0.252±0.203	0.042±0.094	0.15±0.152
101.059	$C_6H_{12}O$—H^+	Aldehydes	Hexanal	(Risholm-Sundman et al. 1998)*; (Brilli, Gioli, Ciccioli, et al. 2014)#*; (Roffael et al. 2015)*	0.028±0.095	0.087±0.133	0±0	0±0
103.075	$C_5H_{10}O_2$—H^+	Esters and acid	Methylbutanoic acid	(Yener et al. 2016)#	0.573±0.576	0.756±0.659	0.173±0.261	0.101±0.158

Measured m/z	Protonated formula	Chemical class	Tentative identification	References (PTR-MS #; Wood (plant and solid wood)*)	Modern wood		Historical wood	
					Juvenile	Mature	Juvenile	Mature
105.069	C_8H_8—H^+	Aromatic hydrocarbons	Styrene/ethylbenzene / vinylbenzene	(Brilli, Gioli, Ciccioli, et al. 2014)#*; (Yener et al. 2015)#	0.875±0.836	2.322±2.105	1.11±0.306	1.113±0.321
107.085	C_8H_{10}—H^+	Terpene/ aromatic hydrocarbons	Monoterpene fragment or p-xylene/ ethylbenzene	(Maleknia et al. 2007)#* or (Brilli, Gioli, Ciccioli, et al. 2014)#*	2.374±2.07	6.727±6.552	2.457±0.804	2.626±0.769
111.080	$C_7H_{10}O$—H^+	Furans/aldehydes	2-propyl furan or heptadienal	(Risholm-Sundman et al. 1998)* or (Yener et al. 2016)#	0.032±0.112	0.16±0.258	0.09±0.135	0±0
121.101	C_9H_{12}—H^+	Aromatic hydrocarbons/ terpenes	Methylethylbenzene or sesquiterpene fragments	(Yener et al. 2016) # or (Demarcke et al. 2009)#	1.251±0.734	2.705±2.71	0.9±0.226	1.036±0.346
123.116	C_9H_{14}—H^+	Terpenes	Sesquiterpene fragments	(Demarcke et al. 2009)#	0.211±0.25	2.171±3.268	0.549±0.126	0.293±0.164
137.137	$C_{10}H_{16}$—H^+	Terpenes	Monoterpenes	(Risholm-Sundman et al. 1998)*; (Maleknia et al. 2007)#*	0.184±0.349	2.358±4.075	0.505±0.133	0.081±0.112
149.135	$C_{11}H_{16}$—H^+	Terpenes	Sesquiterpene fragment	(Kim et al. 2009)#	0.022±0.077	0.901±1.471	0.134±0.133	0±0
153.126	$C_{10}H_{16}O$—H^+	Terpenes	Terpenoid-like compound/ ion of oxygen-containing terpenes	(Maleknia et al. 2007)#*; (Courtois et al. 2009)*	0.104±0.224	0.701±1.077	0.169±0.12	0±0
205.195	$C_{15}H_{24}$—H^+	Terpenes	Sesquiterpenes	(Courtois et al. 2009)*	0.024±0.083	1.925±3.085	0.241±0.192	0.078±0.106

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4.3 PAPER III

EMISSION FROM WOODEN CULTURAL HERITAGE ARTEFACTS. THE CASE STUDY OF EARLY STRINGED INSTRUMENTS.

1. Introduction

A first detailed insight into conservation problems copes with gaseous outdoor pollutants is delivered by (Saunders 2000) giving a comprehensive literature research about pollution in the London National Gallery during the early nineteenth century. The report refers to indoor air pollution due to exogenous pollutants and damages resulting from inappropriate climatic conditions both to protect artworks and for museum staff and visitors, a problem which became evident even in these early times.

In the last years the science applied to the Cultural Heritage has turned its attention to study the effects of air pollution present in the museums and in the showcases (confined indoor environments), emitted by artefacts themselves (Camuffo et al. 2001; Pavlogeorgatos 2003; Shiner 2007; Lee et al. 2011). Indeed, the monitoring of VOCs present in the confined spaces of showcases is fundamental in order to detect situations of risk and prevent damage and/or corrosion phenomena (Schieweck & Salthammer 2009). This type of pollution is unique, both for its formation and for its subsequent dynamic evolution. In particular, it is important to evaluate the close interaction that exists between the composition of air pollutants and materials exposed (inside of the showcases). The dynamic of movements and the air exchanges inside the museums must to be considered. Moreover, the airtight of the showcases protects from external pollutants but could promote an undesirable concentration of compounds emitted by materials displayed (Camuffo et al. 2000; Schieweck & Salthammer 2011).

Recently, several studies have been performed on the air sampling methods and on the analysis techniques for gaseous organic pollutants in order to select those suitable for museums indoor environments (Ryhl-Svendsen & Glastrup 2002; Ramalho et al. 2009; Fenech et al. 2010; Weston et al. 2012; Lattuati-Derieux et al. 2013).

Changes in temperature and humidity should be monitored as described in specific regulations in order to prevent the damage due to these physical agents. The control of the microclimatic parameters has been joined by the study of the effects of the chemical pollution, checking the concentrations of reactive inorganic and organic gases, observing also synergistic actions between pollutants and factors thermo-hygrometric (Fenech et al. 2010; Weston et al. 2012; Lattuati-Derieux et al. 2013).

The review of the above reported literature shows clearly that the interest in studying the VOCs emission in conservation, mainly deals with the effects of these compounds on the preservation of the objects. Almost no research has been carried out on the study of VOCs as an indicator of the on going aging/degradation process that can interest a specific Wooden Cultural Object, which, conversely is the main topics of the present work.

The study of the emission from art materials may address the preservative state of the material itself and give useful information about its history and its own aging processes. In fact, the analysis of historical objects is often difficult due to restricted sampling. The value of historical substance and its integrity is rarely outweighed by the information we gain through destructive sampling and subsequent chemical analysis. However, VOCs emitted from materials during degradation can be a valuable source of information.

At the same time, having full knowledge of the degradation phenomena that may affect the material itself and its polymeric components is fundamental.

The two previous works, presented in this thesis, have been conducted in order to study and understand green state, dried, modern and historical raw wood behaviour in terms of emission of VOCs.

The results of Paper I have shown that, as consequence of the natural cycle of variation of moisture content (meant as early stages of aging process of wood), the spectra of the VOC emissions of different species of wood are significantly changed between green state wood and dried wood. Losing the compounds produced by the metabolic activity of the tree, and specific for each species, the capacity of identify and discriminate different wood species decreases. Thus, we supposed that immediately after tree felling, or after any kind of sampling, or

more in general after any variation of the natural condition of the wood in the tree, the wood emits compounds possibly due to structural changes and degradation processes of the main polymers constituting the cell wall, common to all wood species.

The effect of aging on wood has been studied in Paper II, comparing modern and historical powdered spruce wood samples, and correlating structural changes, analysed by spectroscopic method, with the emission of VOCs. Once again, the emission of acids, aldehydes, alcohols provided that several modifications are acting in the structure of wood, from the early stages of aging. However, this type of structure transformation can be evidenced by spectroscopic techniques only when the degradation of the material is very advanced (not on modern wood). Spectral analysis allows non-invasive measurement, but it can hardly detect small chemical changes during aging for several hundreds years. In fact, it is possible to appreciate the degradation by means of spectroscopic techniques when the degradation itself exceed the range of natural variability that we find in fresh or modern wood.

We have also tried to specify different reaction paths that affect the wood components, especially polysaccharides components, which result in emissions of specific compounds of interest. These compounds resulted by oxidative and hydrolysis processes which could naturally occur in wood structure.

At this point of the research, the analysis of emission from wooden artefacts, such as stringed instruments, allowed us to study and verify the correspondence of the phenomena observed on wooden powders, with the emissions collected from real Wooden Objects. Early string instrument have represented the perfect case studies for different reasons:

-the reduced thickness of the artworks (violins, cellos) is in an order of magnitude (few millimetres) that allows to produce measurable effect in a relatively long term (few centuries);

- it is well known that with the time, acoustic properties of the instruments improve, with an increase in elasticity and reduction of the damping of wood

- the degradation phenomena, which we have hypothesized, affecting the entire artefact and, it could be the explanation of the variation in the acoustic properties of early wooden musical instruments, providing useful inputs for conservation strategies.

In this case study, it was our primary interest to relate the information on VOC emission, which were obtained using PTR-TOF-MS technique, with chemical information on the emitting material. VOCs can be produced in a variety of cellulose, hemicelluloses and lignin degradation reactions, as already mentioned. It might be expected that during natural aging, some components produced by the natural modification of constitutive polymers, are slowly emitted, releasing these accumulate products in the wood sound box of the instrument, and or in the showcase where it is stored.

In this case study, we tried to sample VOCs emissions from historical stringed instruments stored at the Museo del Violino, in Cremona.

2. Experimental section

In this trial, we used the sound box of the instruments, as the volume in which concentrate the emissions released by the structural wooden components (in early Italian violins mainly Norway spruce and Maple) from the wood instrument.

We decided to operate in this way in order to try to isolate emissions from barely wood material and try to avoid to collect emissions due to terpenic varnishes and resins from the external surfaces. Six wooden stringed instruments, had been analysed (Table 1).

Table 1: list of stringed instrument studied

Instruments	Author	Year
Violin	Antonio Stradivari (AS)	1679
Violin	Antonio Stradivari (AS)	1734
Violin	Giuseppe Guarneri "del Gesù" (GdG)	1734
Violin	Andrea Amati (AA)	1566 ca.
Violin	-	2012
Cello	-	1700 ca.

The f-holes of the instruments were specially sealed (by the Maestro Fausto Cacciatori, restorer of the Violin Museum) with a thin film of aluminium (Fig. 1). The wooden end button of the instruments, was replaced by a shaped Teflon cap provided with inlet and outlet brass tubes and teflon pipes (Fig. 2), which connect, respectively, the instrument to the PTR-TOF-MS system and to the zero-air generator, which generates zero air by removing carbon dioxide and moisture from the air of the volume where the emissions were incubated. Before the analysis each instruments were cleaned with the zero-air flow for 4 minutes and subsequently the Teflon pipes were hermetically sealed and wood emission were incubated for 60 minutes in a climatic box where the condition of the conservation showcases was reproduced (T 20°C and RH 55%) (Fig. 3). After 60 minutes of incubation, the instrument was removed from the climatic chamber and analysed (Fig. 4). All the measurements were carried out under the same condition previously reported in Paper II.

Figure 1: Stradivari violin with sealed f-holes

Figure 2: Teflon cap replaced the wooden end button

Figure 3: climatic box (T 20°C and RH 55%)

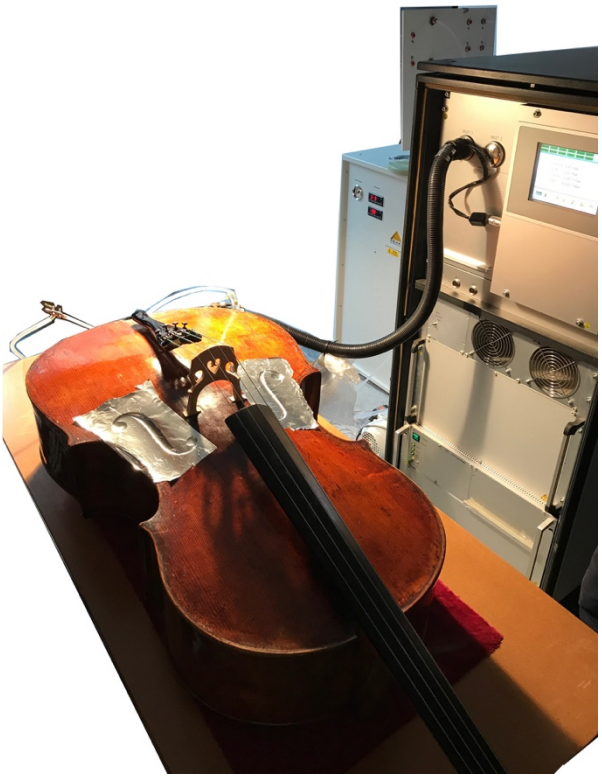

Figure 4: stringed instrument connected to the PTR-TOF-MS system and to the zero-air generator during the measurement

3. Preliminary results

Emission profile of the stringed instruments can be compared qualitatively with the emission profile obtained by wood powdered samples.

The compounds concentrated in the sound box of the different instruments were reported in Table 2 and compared with emission from the wooden powders.

m/z	Protonated formula	Tentative identification	Emission concentration (ppbv)							
			Modern wood (1±0.15 g powder sample)	Historical wood (1±0.15 g powder sample)	Violin (2012)	AS Violin (1734)	GdG Violin (1734)	AS Violin (1679)	AA Violin (1566 ca.)	Cello (1700 ca)
27.022	C_2H_2—H^+	Acetylene	9.405±5.895	7.015±2.543	35.04	36.35	46.01	34.52	33.13	34.53
31.018	CH_2O—H^+	**Formaldehyde**	**11.799±6.309**	**6.232±2.497**	**37.93**	**38.91**	**54.96**	**40.52**	**47.49**	**32.5**
33.033	CH_4O—H^+	**Methanol**	**68.253±38.022**	**48.925±38.965**	**160.9**	**106.9**	**136.5**	**108.3**	**127.7**	**133.9**
41.038	C_3H_4—H^+	Alkyl fragment: propadiene	14.844±10.92	12.486±4.11	39.78	34.81	44.61	31.88	36.9	39.9
43.054	C_3H_6—H^+	Propylene	27.466±21.372	8.563±2.643	38.84	15.46	30.51	20.34	20	27.11
45.033	C_2H_4O—H^+	**Acetaldehyde**	**7.832±3.567**	**3.307±1.922**	**40.28**	**25.95**	**27.68**	**19.19**	**22.09**	**21.32**
47.049	C_2H_6O—H^+	**Ethanol**	**0.622±0.23**	**0.607±0.203**	**1.833**	**1.206**	**1.608**	**1.604**	**1.331**	**1.638**
53.040	C_4H_4—H^+	Cyclobutadiene or alkyl fragment	0.77±1.071	0.17±0.125	1.528	1.074	1.439	1.021	1.311	1.112
57.033	C_3H_4O—H^+	**2-propenal (acrolein)**	**2.373±1.046**	**0.633±0.366**	**10.58**	**4.177**	**7.669**	**5.393**	**5.166**	**5.057**
57.070	C_4H_8—H^+	Butene and hexyl acetate fragment	4.333±2.821	3.611±1.774	8.23	7.439	10.5	7.678	7.455	10.14
59.049	C_3H_6O—H^+	**Acetone (2-propanone)**	**13.501±7.798**	**6.905±2.984**	**54.81**	**31.54**	**37.91**	**29.37**	**34.98**	**39.44**
61.028	$C_2H_4O_2$—H^+	**Acetic acid**	**201.428±199.226**	**35.823±13.819**	**330.9**	**15.08**	**199**	**109.2**	**109**	**167.8**
69.036	C_4H_4O—H^+	**Furan**	**0.854±1.212**	**0.423±0.239**	**1.374**	**1.341**	**1.773**	**1.437**	**1.573**	**1.596**
69.069	C_5H_8—H^+	**Isoprene (1,4-pentadiene)**	**4.254±4.362**	**2.684±0.753**	**5.559**	**4.975**	**4.709**	**3.655**	**4.335**	**4.325**
71.049	C_4H_6O—H^+	**Methyl vinyl ketone (MVK) and methacrolein (MAC) or Butenal and butanoic acid fragment**	**0.772±0.786**	**0.262±0.169**	**2.206**	**1.376**	**1.822**	**1.372**	**1.4**	**1.989**
73.065	C_4H_8O—H^+	**2-butanone (methyl ethyl ketone MEK) and 2-methyl propanal**	**0.601±0.365**	**0.203±0.201**	**4.296**	**2.08**	**2.774**	**2.365**	**2.646**	**3.557**
75.044	$C_3H_6O_2$—H^+	**Propanoic acid or Hydroxy-2-propanone (acetol)**	**0.875±0.641**	**0±0**	**12.85**	**1.575**	**9.821**	**7.464**	**5.812**	**20.18**
77.038	C_6H_4—H^+	Alkyl fragment	10.469±12.977	1.556±1.158	6.92	2.625	4.923	3.972	6.142	37.15
79.054	C_6H_6—H^+	Benzene	7.805±10.612	1.167±0.466	17.74	3.153	5.935	5.021	10.58	4.068
81.070	C_6H_8—H^+	Monoterpenes fragment	14.452±24.19	1.47±0.463	5.2	2.428	3.328	2.293	3.042	2.373
83.048	C_5H_6O—H^+	**2-methyl furan**	**1.22±2.178**	**0±0**	**1.06**	**0.7667**	**0.8349**	**0.728**	**0.7849**	**0.8579**
83.085	C_6H_{10}—H^+	**C6 compounds: hexanal fragment or hexenol fragment**	**1.885±2.001**	**1.826±1.285**	**2.965**	**2.351**	**2.294**	**1.841**	**2.129**	**2.319**
85.065	C_5H_8O—H^+	**Pentenal/pentenone or cyclopentanone**	**0.662±0.612**	**0.492±0.353**	**1.269**	**0.8338**	**0.8714**	**0.7515**	**0.847**	**1.465**
87.043	$C_5H_{10}O$—H^+	**C5 carbonyl compounds (pentanal / pentanone) or pentenol**	**0.244±0.278**	**0±0**	**1.823**	**0.9592**	**1.27**	**0.9492**	**1.052**	**1.272**

m/z	Protonated formula	Tentative identification	Emission concentration (ppbv)							
			Modern wood (1±0.15 g powder sample)	**Historical wood (1±0.15 g powder sample)**	**Mod Violin (2012)**	**AS Violin (1734)**	**GdG Violin (1734)**	**AS Violin (1679)**	**AA Violin (1566 ca.)**	**Cello (1700 ca)**
89.059	$C_4H_8O_2$—H^+	Ethyl acetate or methyl-propanoate or 3-Hydroxy-2-butanone	0.466±0.49	0±0	1.562	0.3147	0.4437	0.3707	0.4565	1.617
91.054	C_7H_6—H^+	Xylene fragment	6.668±7.178	2.337±0.889	24.555	3.0205	6.378	3.905	17.15	17.17
93.069	C_7H_8—H^+	p-Cymene fragment or toluene	16.024±19.013	2.813±0.696	6.049	1.103	1.731	1.204	3.177	32.29
95.086	C_7H_{10}—H^+	Monoterpene fragment	3.604±5.511	0.451±0.14	2.357	1.431	1.843	1.599	1.57	1.564
97.064	$C_5H_4O_2$—H^+	**Furfural**	1.004±1.184	0.464±0.245	**1.268**	**0.7453**	0.9835	0.8156	0.8385	1.143
97.101	C_7H_{12}—H^+	Alkyl fragment	0.539±0.61	0.268±0.15	1.033	0.8862	0.7909	0.8522	0.8392	1.127
99.080	$C_6H_{10}O$—H^+	**Hexenals/ methylpentanone**	**0.252±0.203**	**0.15±0.152**	**0.4225**	**0.2456**	**0.3156**	**0.2535**	**0.2741**	**0.4223**
101.059	$C_6H_{12}O$—H^+	**Hexanal**	**0.087±0.133**	**0±0**	**0.4722**	**0.3312**	**0.406**	**0.3565**	**0.3361**	**0.9441**
103.075	$C_5H_{10}O_2$—H^+	Methylbutanoic acid or Isovaleric acid/valeric acid	0.756±0.659	0.101±0.158	0.4094	0.2333	0.2897	0.2662	0.3188	1.334
105.069	C_8H_8—H^+	Styrene/ethylbenzene/ vinylbenzene	2.322±2.105	1.113±0.321	7.433	1.247	2.879	2.117	5.137	1.896
107.085	C_8H_{10}—H^+	Monoterpene fragment or p-xylene/ ethylbenzene	6.727±6.552	2.626±0.769	35.68	2.492	4.707	3.03	18.46	5.856
111.080	$C_7H_{10}O$—H^+	**2-propyl furan or heptadienal**	**0.16±0.258**	**0±0**	**0.5736**	**0.3588**	**0.3935**	**0.4021**	**0.433**	**0.4789**
121.101	C_9H_{12}—H^+	Methylethylbenzene or sesquiterpene fragments	2.705±2.71	1.036±0.346	3.893	0.6882	0.9836	0.7527	1.956	0.9868
123.116	C_9H_{14}—H^+	Sesquiterpene fragments	2.171±3.268	0.293±0.164	0.5176	0.3178	0.4796	0.4305	0.3625	0.4134
137.137	$C_{10}H_{16}$—H^+	Monoterpenes	2.358±4.075	0.081±0.112	0.5988	0.2793	0.3236	0.266	0.3349	0.3347
149.135	$C_{11}H_{16}$—H^+	Sesquiterpene fragment	0.901±1.471	0±0	0.2116	0.1201	0.1451	0.1612	0.1199	0.1629
153.126	$C_{10}H_{16}O$—H^+	Terpenoid-like compound/ ion of oxygen-containing terpenes	0.701±1.077	0±0	0.2722	0.1231	0.3818	0.4074	0.1461	0.1242
205.195	$C_{15}H_{24}$—H^+	Sesquiterpenes	1.925±3.085	0.078±0.106	0.0563	0.0656	0.0662	0.0680	0.0558	0.0815

We used the same masses scale of emission recorded during the trial on wood powders. However, we did not detect many differences in the amount of the several masses. There were no significant differences between the wooden powders (both modern and historical) and the instruments, and neither between modern violin and the historical instruments. It seems reasonable to suppose that these compounds were emitted very slowly by wood over the time, until the complete depletion of its components, which, due to their aging/degradation, determine the emission themselves.

Methanol and acetic acid were the main compounds in the VOC emission profile of wooden stringed instruments. The other abundant compounds were formaldehyde, acetaldehyde and acetone. Other masses, with their tentative identification as alcohols, aldehydes, ketones, carboxylic acid and aromatic hydrocarbons were found in lower quantities.

Below the emission rate of several masses are reported, comparing the six instruments analysed during this measurement campaign (Fig. 5).

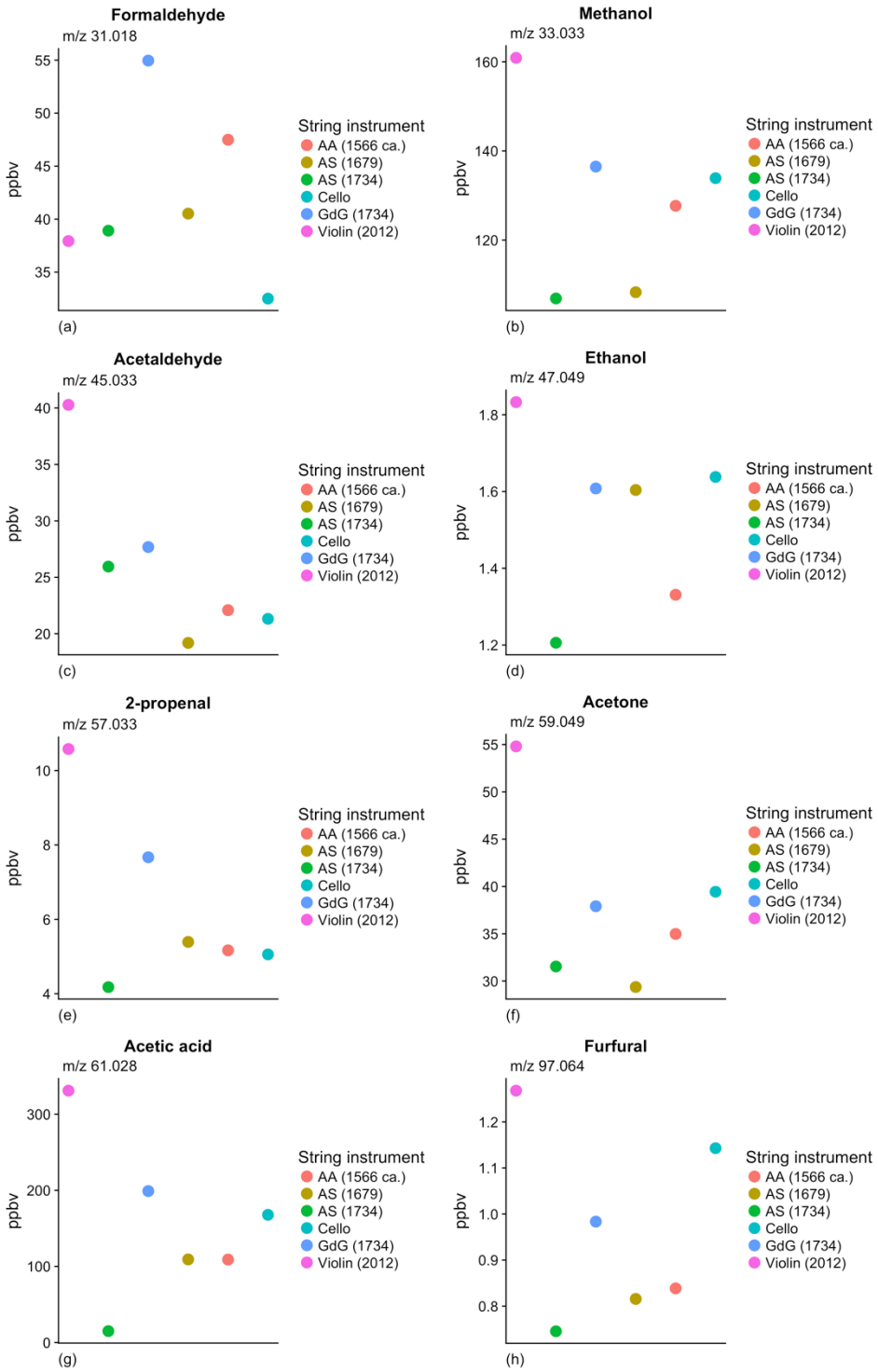

Figure 5: Emission rate of (a) formaldehyde, (b) methanol, (c) acetaldehyde, (d) ethanol, (e) 2-propenal, (f) acetone, (g) acetic acid, (h) furfural.

Based on the observations made in the Paper II, on the possible reactions that occur in wood during aging, emission of formaldehyde, acetaldehyde and 2-propenal could be caused by the oxidation of the reducing end group in the polysaccharides chains. Whereas, emission of acetone and other aldehydes with a C5 – C9 atom chain could be due to the oxidation of free unsaturated fatty acids of triglycerides. Moreover, during aging, acetyl groups of hemicelluloses hydrolyse and acetic acid, one of the most emitted compounds in the VOC emission from aging of wood, is formed. Finally, the dehydration of C5 hemicelluloses fraction could cause the emission of furfural.

4. Conclusion

Emission profile of stringed instruments are comparable to wood powder samples profile. This observation seems to indicate that the phenomena affecting the aging of the violins are related to the aging of wood components.

The useful approach of using emissions of VOCs from materials as source of information not only on material identity but also on its quality deserves further attention. Furthermore, the presence of VOCs, especially acetic acid and formaldehyde in museum environments can have corrosive effects on a variety of other materials.

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5 CONCLUSION

In this research work, the study of aged wood by means of NMR spectroscopic characterization, has pointed out that the hemicelluloses are the mostly affected compounds in aging phenomena. The results got confirm, according to literature, that hemicellulose can be subjected to hydrolysis, and depolymerisation to oligosaccharides during aging.

Concerning the relative degree of cellulose crystallinity, there was no clear evidence of its variation due to aging process. It appeared to be stable in historical wood samples respect to the modern ones analysed in this thesis. Furthermore, it was not possible do detect significant changes in carbohydrate relative amount and in lignin polymeric structures between modern and historical wood.

However, it was worth noting the presence of a systematic difference between historical juvenile-inner samples and historical mature-outer ones. These differences are strongly correlated with hemicelluloses depletion rate and different amount of lignin.

On the other hand, the variability recorded in modern wood samples (typically used as term of reference for assessing the modification undergone by old wood) is resulted to be greater than the effect produced by aging phenomena on historical samples (aged for hundreds of years in aerobic condition). For this reason, making comparisons between historical and modern samples (whose history and origin are unknown) may not be so immediate, and the evaluation of comparative results has to be prudently.

The analysis of volatile organic compounds (VOCs), has been applied for a better addressing the knowledge about both the aging processes of wood and its relevant modifications. Starting from the hypothesis that wood during aging is affected by structural modification which could determine the emission of different compounds, the study of VOCs emitted from wood were a considerable part of this research work.

In this respect, PTR-TOF-MS has shown to be a very useful technique to detect VOCs emitted by different kind of wood specimens

(increment cores, powder samples, wood artefacts, green state wood and dried wood too).

We observed that wood in green condition emits a blend of VOCs specific for softwood and hardwood, and for each species, in fact the material still contains all the classes of compounds that are produced by the trees. However, immediately after the first variation of moisture content of wood, the profile of the emissions changes and the capacity to discriminate between different species decreased. Apparently, losing the compounds produced by the metabolic activity of the tree, the wood continues to emit mostly compounds possibly derived by the structural modification that occur on the main polymers constituting the cell wall.

Detecting the emission of specific compounds, such as organic acids, aldehydes, ketones and alcohols, hypothesis of their possible pathway of formation during natural aging of the material has been proposed in the thesis. These emissions could be due to:

- the degradation of polysaccharides chains as autocatalytic process for the presence of reducing end group in the polysaccharides chains;
- the hydrolysis of the acetyl groups in hemicellulose;
- the oxidation of free unsaturated fatty acids of triglycerides;
- the conversion of carbohydrates.

The comparison between VOC emissions from recent (few years after tree felling) and historical (hundreds of year aged) wood samples has shown that historical and recent wood emitted qualitatively the same masses of compounds. The emission of acetic acid, formaldehyde, acetaldehyde, 2-propenal, butanone, acetone, furfural, pentanal and hexanal, resulted by hydrolysis and oxidative processes which could naturally occur in wood structure, have been detected since the early stages of aging. However, the different amount of emissions allowed the statistical PLSDA model to discriminate between modern and historical samples, better than PCA on NMR spectra data did.

Finally, the analysis of emission from wooden stringed instruments (both modern and historical) confirmed the observed behaviour on wood specimens, such as powdered wood samples. This observation seems to indicate that the characteristics attributed to older instruments (higher elasticity, lower damping, better sound quality) can be related to the aging of wood components.

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