New markers of natural and anthropogenic chemical alteration of archaeological lignin revealed by *in situ* pyrolysis/silylation-gas chromatography-mass spectrometry

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Abstract

Analytical pyrolysis coupled with gas chromatography and mass spectrometry with *in situ* silylation using hexamethyldisilazane (Py(HMDS)-GC/MS) was used to investigate the chemical alteration patterns of a set of archaeological waterlogged oak and silver fir wood. The samples were collected from five piles removed from stilt houses found in a Neolithic village (Bracciano lake, Rome, Italy) and from various parts of the roof of a Roman house (Herculaneum, Italy).

We discuss on how the molecular information provided by Py(HMDS)-GC/MS revealed the causes and effects of natural and anthropogenic alteration and degradation of lignin, and how the adoption of silylation reactions leads to the detection of very informative pyrolysis products. Very particular pyrolytic patterns were obtained for the archaeological samples investigated, which were mainly characterised by the presence of oxidised compounds such as vanillin, acetovanillone, vanillic acid, syringaldehyde, acetosyringone, and syringic acid.

We also report the first ever identification of the methyl esters of vanillic and syringic acids in their silylated form using this method. The results are consistent with heating processes undergone by archaeological wood due to natural or anthropogenic causes: the wooden roof from Herculaneum was naturally exposed to high temperatures during the eruption of Vesuvius, and the wood piles may have been artificially heated by people in the Neolithic Age to enhance the waterproof properties of wood.

Due to the importance of identifying lignin pyrolysis products, the identification and mass spectra of sixty lignin pyrolysis products in their silylated form are also presented.

Keywords: analytical pyrolysis, silylation, archaeological wood, lignin, methyl esters

1. Introduction

Archaeological wood findings are an invaluable source of information regarding technological skills, habits and traditions of people from past civilizations. Unfortunately, wood undergoes biological, chemical, and thermal degradation, which is why it is rarely found in archaeological findings [1,2]. Assessing the degradation state of archaeological wood and identifying the main threats is the starting point for understanding the best preservation strategy [3-6].

Several different analytical techniques are used to obtain information on the wood composition and to measure the extent of wood decay [7-11]. Given that Py-GC/MS is highly sensitive, requires only small sample (usually µg range), and has negligible sample preparation and a short analysis time (*ca*. 40 minutes), it has been successfully used to determine the state of degradation of archaeological wood [7,12-14]. In fact, these technical characteristics are often required in the cultural heritage field.

However, during pyrolysis, wood releases many compounds that are not volatile enough to be efficiently separated in a gas chromatographic column. In fact, both carbohydrates and lignin pyrolysis products contain a high number of alcoholic/acidic functionalities. A derivatising agent, which can be added *in situ* in the pyrolyser, is thus extremely useful in such cases [7]. The agent reacts with mobile hydrogen atoms, thus reducing the polarity of pyrolysis products and ensuring a better chromatographic performance and longer column lifetime [15]. Methylating and silylating agents are the most commonly used [7,16]. Hexamethyldisylazane (HMDS) has several advantages over tetramathylammonium hydroxide (THMA) for online derivatisation of wood pyrolysis products [17]. In fact, the methylation of phenolic groups turns them into methoxy groups. Pyrolysis products from guaiacyl and syringyl lignin differ in terms of a methoxy group on the aromatic ring, thus methylation makes lignin pyrolysis products difficult to identify, unless isotopically labelled reagents are used [18]. Furthermore, the trimethylsilyl group protects alcoholic functionalities, reducing the occurrence of radical oxidation secondary pyrolytic reactions, thus producing aldehydes and ketones [19].

Regardless of the technique adopted, the degradation of archaeological waterlogged wood seems to be mainly related to the partial loss or alteration of cellulose and hemicelluloses [3,20-24]. Less attention has been given to lignin, because of its higher stability with respect to carbohydrates [25]. However, lignin has been proven to undergo chemical changes in some archaeological woods, involving demethylation [14], oxidation [12,18,26] and depolymerisation [27].

In two previous works, we investigated the preservation state of various archaeological wood samples from Herculaneum (Naples, Italy) [12] and from “La Marmotta” archaeological site (Lake Bracciano, Rome, Italy) [27]. “La Marmotta” is a Neolithic village (*ca.* 5500 BC), found 8 m below the water level of lake Bracciano (Anguillara Sabazia, Rome, Italy). It is the most ancient Stone Age shore village in Western Europe. Excavations started in 1989 and various faunal, botanical, pottery, wooden and lithic remains have been discovered. In particular, 3000 wood piles were found embedded in mud in the lake. Their function was to support the houses of the village, and the large number found suggests that the village was a sizable settlement [28,29]. The house of the Telephus Relief is one of the most important Roman *domus* found in the archaeological site of Herculaneum (Naples, Italy) and was covered by a series of pyroclastic surges and flows from the eruption of Vesuvius in 79 AD. The house was originally built on the slope leading down to the marina and had a decorated wooden roof. The remains of the roof were found in the area of the ancient shoreline, since, during the volcano's eruption, the roof was swept off by the first mud flow, turned upside down and then smashed onto the beach. In 2009 the roof was found embedded in wet sand [30].

In Herculaneum, the results obtained by Py-GC/MS with *in situ* silylation using HMDS showed a great variability in the preservation state of the wood samples. In addition, the O/L coefficient was calculated for the first time: the ratio between the relative abundance of lignin pyrolysis products with carbonyl and carboxyl functionalities compared to the total relative abundance of lignin pyrolysis products highlighted that significant lignin oxidation had occurred in some samples from Herculaneum. In the "La Marmotta" archaeological site, evolved gas analysis coupled with mass spectrometry (EGA-MS) was applied for the first time in the analysis of archaeological wood. Again, the evaluation of specific *m/z* peaks attributed to lignin highlighted the unusual oxidation of lignin in the samples. Despite this, the cause of this particular oxidation was not revealed.

This work presents new data obtained by Py(HMDS)-GC/MS for the analyses of 25 archaeological wood samples collected from five piles found in "La Marmotta", and critically reviews the data obtained from the wood samples from Herculaneum [12].

2. Materials and methods

2.1. Samples

A set of five waterlogged oak (*Quercus* sp.) wood piles from "La Marmotta", a Neolithic village, was investigated. The piles were sampled from the external to the internal parts, following their annual growth rings in groups of five. There was a total of 25 archaeological samples (see Table 1). The numbers for the annual rings increase from the external to the internal part. To simplify the discussion of the results, the samples for each pile were labelled in alphabetical order from the external to the internal parts.

Four samples of waterlogged wood belonging to the species silver fir (*Abies alba* Mill.) were collected from various parts of a roof from a Roman *domus* found in the archaeological site of Herculaneum (Naples, Italy). The parts of the roof were numbered according to the inventory of the Archaeological Superintendence of Naples and Pompeii. Sample J3 was taken from the panel No. 40. Sample J9 from the fragment No. 197. Sample J15 from the frame No. 94. Sample J21 from the border No. 209.

Two samples of sound oak and silver fir wood were analysed and used as references to compare the results.

All the samples were oven dried for 24 h at 50 °C, and then homogenised and powdered using a ball mill made of zirconium oxide (Pulverisette 23, Fritsch GmbH, Germany) before analysis.

2.2. Instrumentation

Two different pyrolysers were used:

- 5150 CDS Pyroprobe 5000 Series (CDS Analytical, USA) filament (platinum coil) pyrolyser for the samples from Herculaneum and the reference sample of sound silver fir wood. The pyrolysis temperature was 550°C and was carried out for 20 s. The Py-GC interface was kept at 180 °C. Similar amounts (*ca.* 100 µg) of sample were inserted into the centre of the pyrolysis quartz tube with glass wool and 7 µL of HMDS, and then put into the filament coil. The GC injector was used with a split ratio of 1:10 and 280°C. Chromatographic conditions were as follows: initial temperature 60°C, 2 min isothermal, 15°C min^{-1} to 100°C, 3 min isothermal, 4°C min^{-1} to 200°C, 5 min isothermal, 15°C min^{-1} to 280°C, 5 min isothermal. Carrier gas: He (purity 99.995%), constant flow 1.0 mL min^{-1}.
- EGA/PY-3030D Multi-Shot micro-furnace pyrolyser (Frontier Lab, Japan) for the samples from "La Marmotta" and the reference sample of sound oak wood. The pyrolysis temperature was 550°C and interface temperature was 250°C. Similar amounts (ca. 50 µg) of sample and HMDS (5 µL) were put into a stainless steel cup and placed into the micro-furnace. The GC injector was used with a split ratio of 1:5 and 280°C. Chromatographic conditions were as follows: initial temperature 50°C, 1min isothermal, 10°C min^{-1} to 100°C, 2min isothermal, 4°C min^{-1} to 190°C, 1min isothermal, 30°C min^{-1} to 280°C, 30min isothermal. Carrier gas: He (purity 99.995%), constant flow 1.0 mL min^{-1}.

1,1,1,3,3,3-hexamethyldisilazane (HMDS, chemical purity 99.9%, Sigma Aldrich Inc., USA) was used as a silylating agent for the *in situ* derivatisation of pyrolysis products. The pyrolysers were both connected to a gas chromatograph 6890 Agilent (USA) equipped with a split/splitless injector, an HP-5MS fused silica capillary column (stationary phase 5% diphenyl and 95% dimethyl-polysiloxane, 30 m x 0.25 mm i.d., Hewlett Packard, USA) and with a deactivated silica pre-column (2 m x 0.32 mm i.d., Agilent J&W, USA). The GC was coupled with an Agilent 5973 Mass Selective Detector operating in electron impact mode (EI) at 70 eV. The MS transfer line temperature was 300°C. The MS ion source temperature was kept at 230°C and the MS quadrupole temperature at 150°C.

3. Results

The pyrolysis profiles obtained for the "La Marmotta" samples highlighted that holocellulose was almost absent, since lignin pyrolysis products were the most abundant peaks in the pyrograms.

This was in agreement with the results obtained by EGA-MS [27]. Several similarities were revealed in the pyrolytic profiles of samples from different piles, with the exception of pile 261. In particular, samples from the external part of the piles showed different relative abundances of the major lignin pyrolysis products compared to the samples from the internal part of piles. Figure 1 shows the pyrograms obtained by Py(HMDS)-GC/MS analysis of samples 2212-A (external part) and 2212-G (internal part). Sixty lignin pyrolysis products were identified in their silylated form (Table 2). The identification of these compounds was based on the literature [17,31-36] and interpretation of the mass spectra. The mass spectra of the sixty identified lignin pyrolysis products are reported in the Supplementary Material.

Guaiacol (#6), syringol (#11), 4-vinylguaiacol (#13), 4-methylsyringol (#16), and 4-vinylsyringol (#23) were among the most abundant pyrolysis products for sample 2212-A. These pyrolysis products have a shortened side chain with respect to lignin monomers coniferyl and sinapyl alcohols (# 47, 58), and their higher relative abundance compared to lignin monomers can be related to lignin depolymerisation [23]. Also vanillin (#19), acetovanillone (#24), syringaldehyde (#31) and acetosyringone. (#36) were detected with a high abundance in sample 2212-A. As these compounds have a carbonyl functionality at the benzylic position, they provide information on the oxidation reactions undergone by lignin [23,27]. For sample 2212-G, syringol (#11), 4-methylsyringol (#16) and 4-vinylsyringol (#23) showed the highest relative abundances, however *E*-4-isopropenylsyringol (#32) and *E*-sinapyl alcohol (#58) also had high relative abundances. These two latter pyrolysis products still conserve the side chain with three carbon atoms, typical of unaltered lignin monomers, thus indicating a better preservation of lignin in the core of the pile. In addition, carbonyl compounds had lower relative abundances compared to sample 2212-A, highlighting a lower extent of lignin oxidation.

Another important difference between these two samples was the presence of vanillic and syringic acid methyl esters (#27, 39) in sample 2212-A which were not present in sample 2212-G. Methyl esters of vanillic and syringic acids are reported in the literature as wood pyrolysis products and they always show a very low abundance (<1% of lignin pyrolysis products) in sound or archaeological wood [37-39]. However, they have never been reported as trimethylsilylated derivatives, thus their identification was based on the interpretation of mass spectra, as shown in Figure 2. The peaks at *m/z* 254 and 284 are attributed to the molecular ions ($M^{+\bullet}$) of vanillic acid methyl ester and syringic acid methyl ester, respectively. The fragmentation paths of these molecules lead to the formation of peaks at *m/z* 239 and 269 ($[M-15]^{+}$), respectively, due to the loss of a methyl radical ($CH_3^{\bullet}$). The peaks at *m/z* 224 and 254 ($[M-15-15]^{+\bullet}$) originate from the consecutive loss of two methyl radicals. Finally, the peaks at *m/z* 193 and 223 ($[M-15-15-31]^{+}$) are due to the further loss of a methoxy radical ($CH_3O^{\bullet}$). According to the literature [40], for the *ortho*-substituted trimethylsilyl ethers of phenols, the first methyl radical is lost from the trimethylsilyl group, and the second one from the *ortho* position.

Despite these important differences detected in the pyrolytic profiles, the calculation of the pyrolytic H/L ratio (holocellulose (H)/lignin (L)) was not able to differentiate between the samples taken from the same pile (Table 3). This parameter is reported as a good index of the preservation state of the wood, since it indicates the relative loss of one wood component (holocellulose or lignin) with respect to the other [13,23]. The sum of the percentage areas of chromatographic peaks of holocellulose (H) and lignin (L) pyrolysis products were calculated respectively, together with the ratio between these two values, thus obtaining the pyrolytic H/L ratio. The values calculated for the samples were extremely low, ranging from 0.1 to 0.6 and indicating that 80-90 % of holocellulose was depleted from these woods. Only samples B, C and D from pile 261 showed very high H/L ratios (*ca.* 5), highlighting the different type of degradation undergone by this pile. Carbohydrates were very well preserved in the core of this pile compared to the other piles analysed. However, no significant trends were obtained for all the piles. Just a slight increase in the H/L ratio was generally obtained for the innermost samples of the piles.

The pyrolytic profiles obtained for the samples from Herculaneum showed similar features. A previous work [12] included a discussion regarding the H/L ratio and the preservation state of these samples. PCA enabled us to determine a great variability in the degradation patterns of these samples. In particular, samples J3, J9, J15 and J21 were found to have an unusual degree of lignin oxidation and a high depletion of carbohydrates, similarly to the samples from “La Marmotta”. At that stage of the research, the database of the wood pyrolysis products was not complete. The present research enabled us to identify the presence of vanillic acid methyl ester (#27) in most of the samples from the Herculaneum site and was particularly abundant in samples J3, J9, J15 and J21. These samples all belonged to the wood species silver fir (*Abies alba Mill.*), a coniferous tree, thus only the guaiacyl component of lignin was present. Figure 3 shows the pyrograms obtained by Py(HMDS)-GC/MS analysis of samples J9 and J21.

All these observations indicate that the focus should be on the lignin pyrolysis products, in order to better investigate the alteration patterns of these wood samples.

During the pyrolysis of lignin, the formation of the original monomers (coniferyl and sinapyl alcohols) is the primary pyrolytic reaction, due to the predominant initial cleavage of the β-ether bonds between phenylpropane units. Reactions involving conversion/alteration of the side-chain and the methoxy groups on the aromatic ring are secondary reactions, which lead to the formation of guaiacyl and syringyl units with shorter side chains and different functionalities. The formation yields of the various pyrolysis products depend on the pyrolytic conditions and on the state of preservation of the material [41-44].

In order to provide information on the differences in the relative abundances of lignin pyrolysis products with a semi-quantitative approach, lignin pyrolysis products were divided into categories on the basis of their molecular structure and pyrolytic formation [23,45] (Table 2). The peak area of each lignin pyrolysis product was expressed as a percentage with respect to the sum of the peak areas of all lignin pyrolysis products. The sum of the percentage areas of the lignin pyrolysis products assigned to each category was then calculated. Table 3 reports the results of these calculations performed for the archaeological wood samples and the references of sound oak and silver fir woods , together with the H/L ratios.

Although lignin from oak has a guaiacyl-syringyl composition and lignin from silver fir contains only guaiacyl compounds, the relative abundances of the categories of lignin pyrolysis products for the sound oak and silver fir woods showed similar results. Monomers accounted for *ca.* 40-50 % of the total abundance of lignin pyrolysis products; short chain compounds were *ca.* 15 %; long chain compounds *ca.* 10 %; carbonyl *ca.* 5-10 %; acids *ca.* 1%, esters < 0.1 %; demethylated/demethoxylated compounds *ca.* 10-15 %. This was expected, since pyrolysis mechanisms involving lignin are generally the same for softwood and hardwood lignin [41,46].

The distribution of lignin pyrolysis products was very different for the archaeological samples and, to clarify the trends and differences in these distributions, Figures 4 and 5 present two histograms, which report the results for pile 2212 and for the Herculaneum samples respectively, together with the corresponding sound wood.

The relative abundance of monomers was generally much lower than for sound wood. An increase in short chain compounds was observed. The shortening of the side chain could be due to degradation (depolymerisation) of lignin in the burial environment [47] or the enhanced yield of secondary pyrolytic reactions for degraded wood compared to sound woods [23,41,46]. In both cases, the increase in short chain compounds and the decrease in monomers can be taken as an indication of the occurrence of reactions involving the alteration of the side chain of the phenylpropane units in lignin. An increase in carbonyl, acid and ester compounds was also observed in archaeological samples, which was related to lignin oxidation. Regarding the samples from "La Marmotta", lignin oxidation was particularly evident for the samples taken from the external part of the piles, in agreement with the observations obtained by EGA-MS [27]. Through the molecular detail achieved by Py(HMDS)-GC/MS, it was possible to specify that the oxidation of lignin was mainly due to aldehyde and ketone functionalities present in the lignin network. In fact, acids were detected with generally low relative abundances (5-10 %) compared to aldehydes and ketones (up to *ca.* 40 % in some samples), with the exception of pile 2020. In this case acids were detected with relative abundances of *ca.* 15-20 % for the outermost samples of the pile, highlighting the higher extent of lignin oxidation for this pile. In the samples from Herculaneum, carbonyl compounds were *ca.* 20-25%, similarly to the external samples from pile 2212. Comparable relative abundances of acids and esters were also detected for these samples, indicating a similar extent of lignin alteration and oxidation.

Vanillic and syringic acid methyl esters were found with a relative abundance of *ca.* 5-10 % for the external samples of the piles from "La Marmotta" (samples A and B) and for the Herculaneum samples. Negligible relative abundances of esters were found in the inner parts of the piles.

We therefore attempted to explain all the observations obtained for these samples, in particular the uncommon presence of vanillic and syringic acid methyl esters as major pyrolysis products. The first hypothesis involves the chemistry of the wood heating process. When wood is heated to around 200-300°C, several reactions and modifications occur, primarily the release of small molecules, such as water, formaldehyde, acetic acid, carbon dioxide, methanol and formic acid [48,49]. The relative yield of these products depends on the availability/unavailability of oxygen during the process [50,51]. These molecules can easily react in a gaseous phase with acid moieties leading to the formation of new compounds, above all methyl esters, due to the presence of methanol (Figure 6). There is at least one precedent for this in archaeological materials: the formation of methyl esters of diterpenoid acids, mainly methyl-dehydroabietate, has been attributed to the reaction occurring during intentional heating processes used to produce pitch and tar from resinous woody materials [52].

In addition, methyl esters of vanillic and syringic acids are markers of particular toasting processes involving wood barrels for wine production and have been detected both in the wood and in the wine with significant concentrations, along with vanillin, acetovanillone, syringaldehyde and acetosyringone [53-55]. In fact, during heating, oxidation can also occur in wood constituents, with a consequent increase in aldehyde, ketone and acid functionalities [53,56,57]. Wood is also an isolating material and when it is exposed to high temperatures, the surface can reach several hundred degrees, whereas the core (2-3 cm under the surface) remains at room temperature. Archaeological studies [58,59] have shown that wood used to be processed before being used, in order to change the mechanical, colour and acoustic properties and to increase the durability and the resistance to decay, also biological. In ancient Africa, natives hardened wooden spears by placing a sharpened straight wooden stick into glowing coals, then pounding the burned end with a rock, repeating this process many times until the end was sharp and hard. In Sweden more than 5200 years ago, the Saami bent the front tips of their wooden skis. Hieroglyphic Egyptian pictures (1440 BC) seem to show that poles and chariot yokes were made using heat-bent wood components. There are also examples of methods used to increase the durability of wood in the ground and water: the most common method was to burn the outer part of the wood in order to obtain an insulating layer of char, thus producing wood with higher waterproofing properties.

Today, most of these processes have been industrialised and are known as thermo-hydro-mechanical wood processing [58,59].

Thus, the results obtained for the archaeological wood samples from “La Marmotta” and Herculaneum pointed to the hypothesis that the surface of these samples had been exposed to uncontrolled heating. The eruption of Vesuvius could represent the cause of the heating for the Herculaneum samples. The wood ceiling was exposed to high temperatures before being transported by the mud flow in the sea water. As regards the wood piles from “La Marmotta”, artificial heating was most likely performed before placing the piles in water to hold up the stilt houses. In addition, since the heating involved only the external part of the pile, the trends observed from the external to the internal parts are also justified. It was thus also possible to hypothesise that the heat treatment had not been performed for pile 261. Only the outermost part of the pile underwent a depletion in carbohydrates, whereas in the inner part, some other degradation phenomena selectively involving lignin had occurred. It is currently known that uncontrolled heat treatments can easily affect wood, and, depending on the presence of oxygen and humidity, the hydrolysis of carbohydrates is enhanced [59]. In addition, wood can apparently be strengthened by uncontrolled heat treatment, however the chemical changes make wood more likely to undergo further decay [58]. In contrast, this pile presented an extremely good preservation of carbohydrates.

However, the extreme age of these wood samples (*ca.* 7000 years old for “La Marmotta” and *ca*. 2000 years old for Herculaneum) and the lack of knowledge regarding their “life” do not rule out other hypotheses for this unusual degradation. For instance, a reduction in the water level of the lake could have exposed this wood to air and sunlight for some periods. This could have caused a partial lignin oxidation, especially in the outer part of the piles. Still hypothesising an exposure to air, particular species of white rot fungi can degrade lignin, producing an effect referred to as “enzymatic combustion”, since the residual lignin is significantly oxidised [60,61]. In addition, some brown rot fungi can demethylate various methoxy groups present in lignin, with the consequent production of methanol [60,62]. Although there is no evidence of methyl ester formation in these processes, the combined action of white rot and brown rot fungi could have created an environment in which the formation of these compounds is possible.

4. Conclusions

Our results demonstrated that Py-GC/MS with *in situ* silylation using HMDS is not only a suitable tool to study the chemistry of the natural degradation of archaeological wood, but is also unique in revealing new molecular markers, such as the chemical signature of the anthropogenic alteration undergone by lignin. Vanillic and syringic acid methyl esters have never before been detected using Py-GC/MS with online derivatisation by TMAH. In fact, the methylating agent renders vanillic and syringic acids indistinguishable from vanillic and syringic acid methyl esters. This limitation of the Py(TMAH)-GC/MS method proves the better suitability of Py(HMDS)-GC/MS for the analysis of wood.

The calculation of the percentage relative abundances of the lignin pyrolysis products grouped into categories (monomers, short-chain, long-chain, carbonyl, acids, esters, demethylated/demethoxylated) highlighted molecular differences among the samples, indicating the importance of a correct and detailed interpretation of the data. In particular, the decrease in monomers and the corresponding increase in short chain compounds with respect to the reference sound wood was indicative of lignin depolymerisation. An increase in carbonyl, acid and ester compounds indicated the high extent of lignin oxidation. In addition, the detection of the methyl esters of vanillic and syringic acids with unusual high abundances, above all in the outermost samples of the piles from Neolithic stilt houses, led to hypothesise that a wood heating process was performed by people from the Neolithic Age before putting the piles in the water and using them to hold up the stilt houses. Similar results were obtained for various samples from the Herculaneum site. In this case the heating process was caused by the eruption of the Vesuvius volcano in 79 AD, which naturally exposed this wood to high temperatures. Thus, our data and results suggested that the archaeological wood from both sites share a common cause of alteration. Although other unknown causes of degradation cannot be ruled out, since these wood samples remained in their burial environment for millennia, the hypothesis of a surface heat process appears reasonable.

These results highlighted that a correct interpretation of the data obtained by Py-GC/MS can provide answers useful to archaeologists, conservators and art historians in order to reconstruct the history of such precious artefacts from past civilisations.

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References

[1] R.A. Blanchette, *International Biodeterioration & Biodegradation*, 46, (2000) 189.

[2] T. Nilsson and R. Rowell, *Journal of Cultural Heritage*, 13, (2012) S5.

[3] G. Giachi, F. Bettazzi, S. Chimichi and G. Staccioli, *Journal of Cultural Heritage*, 4, (2003) 75.

[4] Y. Fors and S. Magnus, *Chemical Society Reviews*, 35, (2006) 399

[5] J. Preston, A.D. Smith, E.J. Schofield, A.V. Chadwick, M.A. Jones and J.E.M. Watts, *PLoS One*, 9, (2014) 1.

[6] S. Braovac and H. Kutzke, *Journal of Cultural Heritage*, 13, (2012) S203.

[7] J.J. Łucejko, F. Modugno, E. Ribechini, D. Tamburini and M.P. Colombini, *Applied Spectroscopy Reviews*, 50, (2015) 584.

[8] C.N. Passialis, *Holzforschung*, 51, (1997) 111.

[9] M.-Y. Li, B.-S. Fang, Y. Zhao, T. Tong, X.-H. Hou and H. Tong, *Polymer Degradation and Stability*, 109, (2014) 71.

[10] M. Bardet, M.F. Foray and Q.-K. Trân, *Anal. Chem.*, 74 (17), (2002) 4386

[11] F. Modugno, E. Ribechini, M. Calderisi, G. Giachi and M.P. Colombini, *Microchemical Journal*, 88, (2008) 186.

[12] D. Tamburini, J.J. Łucejko, F. Modugno and M.P. Colombini, *International Biodeterioration & Biodegradation*, 86, Part B, (2014) 142.

[13] J.J. Łucejko, M. Zborowska, F. Modugno, M.P. Colombini and W. Pradzynski, *Analytica Chimica Acta*, 745, (2012) 70.

[14] P.F. van Bergen, I. Poole, T.M. Ogilvie, C. Caple and R.P. Evershed, *Rapid Communications in Mass Spectrometry*, 14, (2000) 71.

[15] I. Bonaduce and A. Andreotti, in M.P. Colombini and F. Modugno (Eds.), *Organic Mass Spectrometry in Art and Archeology*, Wiley, Chichester (UK), 2009, Chapter 11, p. 303.

[16] J.M. Challinor, *Journal of Analytical and Applied Pyrolysis*, 61, (2001) 3.

[17] D. Fabbri and G. Chiavari, *Analytica Chimica Acta*, 449, (2001) 271.

[18] M.P. Colombini, J.J. Łucejko, F. Modugno, M. Orlandi, E.-L. Tolppa and L. Zoia, *Talanta*, 80, (2009) 61.

[19] K.-i. Kuroda, *Journal of Analytical and Applied Pyrolysis*, 56, (2000) 79.

[20] D. Dedic, T. Iversen and M. Ek, *Studies in Conservation*, 58, (2013) 308.

[21] I. Bjurhager, H. Halonen, E.L. Lindfors, T. Iversen, G. Almkvist, E.K. Gamstedt and L.A. Berglund, *Biomacromolecules*, 13, (2012) 2521.

[22] M. Bardet, G. Gerbaud, M. Giffard, C. Doan, S. Hediger and L. Le Pape, *Progress in Nuclear Magnetic Resonance Spectroscopy*, 55, (2009) 199.

[23] D. Tamburini, J.J. Łucejko, M. Zborowska, F. Modugno, W. Prądzyński and M.P. Colombini, *Journal of Analytical and Applied Pyrolysis*, 115, (2015) 7.

[24] C.-M. Popescu, M.-C. Popescu and C. Vasile, *Carbohydrate Polymers*, 79, (2010) 362.

[25] J.I. Hedges, G.L. Cowie, J.R. Ertel, R. James Barbour and P.G. Hatcher, *Geochimica et Cosmochimica Acta*, 49, (1985) 701.

[26] M.P. Colombini, M. Orlandi, F. Modugno, E.-L. Tolppa, M. Sardelli, L. Zoia and C. Crestini, *Microchemical Journal*, 85, (2007) 164.

[27] D. Tamburini, J.J. Łucejko, E. Ribechini and M.P. Colombini, *Journal of Mass Spectrometry*, 50, (2015) 1103.

[28] M.A. Fugazzola Delpino, G. D'Eugenio and A. Pessina, *Bullettino di Paletnologia Italiana*, (1993) 181.

[29] M.A. Fugazzola Delpino, *Bullettino di Paletnologia Italiana*, 95, (2004) 1.

[30] D. Camardo and M. Notomista, *Rivista di Studi Pompeiani*, XXI, (2010) 154.

[31] D. Fabbri, G. Chiavari, S. Prati, I. Vassura and M. Vangelista, *Rapid Communications in Mass Spectrometry*, 16, (2002) 2349

[32] S.C. Moldoveanu, in D. Coleman and B.F. Price (Eds.), *Analytical Pyrolysis of Natural Organic Polymers*, Elsevier Science, Amsterdam, 1998, p. 217.

[33] S.C. Moldoveanu, in S.C. Moldoveanu (Ed.), *Pyrolysis of Organic Molecules with applications to health and environmental issues* Elsevier Science, Amsterdam, 2010, Chapter 16, p. 419.

[34] D. Scalarone, O. Chiantore and C. Riedo, *Journal of Analytical and Applied Pyrolysis*, 83, (2008) 157.

[35] A.D. Pouwels, G.B. Eijkel and J.J. Boon, *Journal of Analytical and Applied Pyrolysis*, 14, (1989) 237.

[36] J.J. Łucejko, *Waterlogged Archaeological Wood: Chemical study of wood degradation and evaluation of consolidation treatments*, VDM Verlag Dr Muller, Saarbrucken, Germany, 2010, p. 157.

[37] J. Ralph and R.D. Hatfield, *J. Agric. Food Chem.*, 39, (1991) 1426.

[38] E.R.E. van der Hage and J.J. Boon, *Journal of Chromatography A*, 736, (1996) 61.

[39] J.J. Łucejko, F. Modugno, E. Ribechini and J.C. del Río, *Analytica Chimica Acta*, 654, (2009) 26.

[40] D. Krauss, H.G. Mainx, B. Tauscher and P. Bischof, *Organic Mass Spectrometry*, 20, (1985) 614.

[41] T. Kotake, H. Kawamoto and S. Saka, *Journal of Analytical and Applied Pyrolysis*, 105, (2014) 309.

[42] T. Kotake, H. Kawamoto and S. Saka, *Journal of Analytical and Applied Pyrolysis*, 104, (2013) 573.

[43] H. Kawamoto, M. Ryoritani and S. Saka, *Journal of Analytical and Applied Pyrolysis*, 81, (2008) 88.

[44] J. Huang, C. Liu, D. Wu, H. Tong and L. Ren, *Journal of Analytical and Applied Pyrolysis*, 109, (2014) 98.

[45] S. Braovac, D. Tamburini, J.J. Łucejko, C. McQueen, H. Kutzke and M.P. Colombini, *Microchemical Journal*, 124, (2016) 368.

[46] T. Kotake, H. Kawamoto and S. Saka, *Journal of Analytical and Applied Pyrolysis*, 113, (2015) 57.

[47] Á.T. Martínez, M. Speranza, F.J. Ruiz-Dueñas, P. Ferreira, S. Camarero, F. Guillén, M.J. Martínez, A. Gutiérrez and J.C. del Río, *International Microbiology* 8, (2005) 195.

[48] M.J. Prins, K.J. Ptasinski and F.J.J.G. Janssen, *Journal of Analytical and Applied Pyrolysis*, 77, (2006) 35.

[49] T. Nocquet, C. Dupont, J.-M. Commandre, M. Grateau, S. Thiery and S. Salvador, *Energy*, 72, (2014) 180.

[50] P. Trop, B. Anicic and D. Goricanec, *Energy*, 77, (2014) 125.

[51] J.D. McDonald, B. Zielinska, E.M. Fujita, J.C. Sagebiel, J.C. Chow and J.G. Watson, *Environmental Science & Technology*, 34, (2000) 2080.

[52] M.P. Colombini, F. Modugno and E. Ribechini, *Journal of Mass Spectrometry*, 40, (2005) 675.

[53] B. Fernández de Simón, J. Martínez, M. Sanz, E. Cadahía, E. Esteruelas and A.M. Muñoz, *Food Chemistry*, 147, (2014) 346.

[54] L. Culleré, B. Fernández de Simón, E. Cadahía, V. Ferreira, P. Hernández-Orte and J. Cacho, *LWT - Food Science and Technology*, 53, (2013) 240.

[55] P. Hernández-Orte, E. Franco, C.G. Huerta, J.M. García, M. Cabellos, J. Suberviola, I. Orriols and J. Cacho, *Food Research International*, 57, (2014) 234.

[56] P. Navi and D. Sandberg, *Thermo-Hydro-Mechanical Wood Processing*, CRC Press, Boca Raton, USA, 2012, p.

[57] J. Zhang, H. Deng and L. Lin, *Molecules*, 14, (2009) 2747.

[58] D. Sandberg, P. Haller and P. Navi, *Wood Material Science & Engineering*, 8, (2012) 64.

[59] D. Sandberg and P. Navi, in, Växjö University, Sweden, 2007.

[60] T.K. Kirk and R.L. Farrell, *Annual Review Microbiology*, 41, (1987) 465.

[61] D. Geoffrey, in *Deterioration and Protection of Sustainable Biomaterials*, American Chemical Society, 2014, Chapter 2, p. 23.

[62] A. Hatakka, in M. Hofrichter and A. Steinbüchel (Eds.), *Lignin, Humic Substances and Coal*, Wiley VCH, Germany, 2005, p. 129.

Figure 1. Py(HMDS)-GC/MS profiles for samples **a)** 2212-A (external part) and **b)** 2212-G (internal part) from “La Marmotta”. Lignin pyrolysis products are indicated as numbers, and peak labelling refers to Table 2. H = holocellulose pyrolysis product.

Figure 2. Mass spectra of **a)** vanillic acid methyl ester, and **b)** syringic acid methyl ester.

Figure 3. Py(HMDS)-GC/MS profiles for samples **a)** J9 and **b)** J21 from Herculaneum. Lignin pyrolysis products are indicated, and peak labelling refers to Table 2.

Figure 4. Distribution of categories of lignin pyrolysis products expressed as percentages for samples from pile 2212 (A = external pile ; G =.internal pile) and for the reference oak sample.

Figure 5. Distribution of categories of lignin pyrolysis products expressed as percentages for samples J3, J9, J15 and J21 from Herculaneum and for the reference silver fir sample.

Figure 6. The formation of vanillic acid methyl ester during wood heating.