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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 woods. 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 lead 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.

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 small samples (usually μg range), negligible sample preparation and 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

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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 tetramethylammonium hydroxide (TMAH) 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, which produce 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:

- 5150CDS 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, 1 min isothermal, 10 °C min^{-1} to 100 °C, 2 min isothermal, 4 °C min^{-1} to 190 °C, 1 min isothermal, 30 °C min^{-1} to 280 °C, 30 min 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