Characterise Lignin in Archaeological Wood

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With comparison to cellulose and hemicelluloses, lignin is generally less prone to most degradation processes affecting archaeological artefacts in burial environments, especially waterlogged ones, which are the most favourable for wood preservation. Nevertheless, lignin also undergoes significant chemical changes. As wood from waterlogged environments is mainly composed of lignin, knowledge of its chemical structure and degradation pathways is fundamental for choosing preventive conservation conditions and for optimising consolidation methods and materials, which directly interact with the residual lignin. Analytical pyrolysis coupled with mass spectrometry, used in several complementary operational modes, can gather information regarding the chemical modifications and the state of preservation of lignin, especially concerning oxidation and depolymerisation phenomena. Several applications to the analysis of wood from archaeological artefacts affected by different conservation problems are presented to showcase the potential of analytical pyrolysis in various scenarios that can be encountered when investigating archaeological waterlogged wood.

Keywords: archaeological wood ; lignin ; analytical pyrolysis

1. Introduction

Archaeological wooden artefacts are relatively rare and represent an invaluable source of knowledge on the culture, technology, and everyday activities in ancient human societies, and thus have a high historical significance[1]. Wood is a complex heterogeneous organic material, susceptible to various type of degradation processes, and this makes the preservation of archaeological wood a fundamental challenge for archaeologists and conservators all over the world. The study of wood degradation can be approached in several ways: mechanical, physical, and structural degradation are often evaluated [1], but these aspects are ultimately all related to the chemical degradation of the lignocellulosic matrix constituting wood.

Around 95% of wood is composed by three biopolymers: cellulose, hemicelluloses (together defined as holocellulose), and lignin. The remaining part is made up of organic and inorganic extractives[2]. It is not common for wooden historical artefacts to survive natural degradation in the natural environment due to biodegradation [3], since fungi, bacteria, and insects attack and metabolise wood components [4][5]. Waterlogged conditions favour the preservation of wood, as low temperatures and limited oxygen availability inhibit or decelerate the action of microorganisms [6][7].

Assessing the state of degradation of wood in archaeological artefacts has recently acquired even greater interest due to the severe degradation phenomena observed in consolidated archaeological wooden artefacts exhibited in museum galleries. In fact, it is evident that the degradation processes continue after recovery from the burial environment [8][9][10][11][12][13][14]. A range of analytical approaches have been exploited to assess wood decay [15][16][17][18]. The chemical alteration of archaeological waterlogged wood is often evaluated in terms of the degradation of cellulose and hemicelluloses [19][20][21][22][23]. The loss of polysaccharides can be evaluated using the holocellulose over lignin ratio (H/L), which can be estimated by several techniques and that, when compared with the values obtained for reference sound wood, is considered a proxy for the extent of wood degradation in terms of loss of polysaccharides [24]. Less research has been dedicated to the degradation of lignin, due to its relative resilience in the burial environment [25]. Lignin is known to be attacked by white rot fungi [26][27], but these microorganisms are not active in waterlogged environments [4]. Nevertheless, chemical changes affecting lignin have been in some cases described in archaeological waterlogged wood, and they involve demethylation [28][33], oxidation [29][30][31][32], and depolymerisation [33]. Recent studies performed by Py-GC/MS have also shown some minor chemical changes in the structure of the lignin, induced by the burial environment, when fragments of oak wood were buried in the archaeological site for a relatively short time of 10 years [34]. Another recent study, also carried out by Py-GC/MS, reports on the oxidation of lignin observed in ancient papyri. An increase in the relative abundance of some diagnostic compounds with carbonyl and carboxyl functionalities was determined [35]. These phenomena are not effectively described by changes in the H/L ratio. The severe depletion of carbohydrates in waterlogged archaeological wood further prevents the use of the H/L ratio as a chemical parameter to differentiate the extent of wood degradation in different samples or objects, whereas assessing the degradation undergone by the residual

lignin has been demonstrated to be an effective discrimination method [36]. Furthermore, molecular information on lignin is particularly useful to select conservation treatments and consolidation materials: The interactions between the restoration/consolidation materials and the residual lignin can be crucial in determining the stability of the consolidated object.

2. Analytical techniques

Analytical techniques based on pyrolysis have proven particularly successful to investigate lignin in archaeological wood [37][38][39][40][41]. They provide information at a molecular level on complex polymers by studying their pyrolysis products, which are small volatile molecules produced by selective bond cleavage induced by thermal energy [42]. When coupled with gas chromatography (GC) and/or mass spectrometry (MS), thermo-analysis is highly sensitive, requires only small amounts of a sample (usually 10–50 µg), very little sample preparation, and quite short analysis time (from a few minutes to one hour). These technical features are highly desirable in the heritage science field [43].

Lignin pyrolysis products have been identified in pioneering studies [44][45][46], proving that hydroxyphenyl, guaiacyl, and syringyl components of lignin can be distinguished by this technique. Lignin can also be separated from the other wood components before analysis. However, the results obtained on lignin extracted from archaeological wood can be biased by alterations induced by the wet-chemical extraction steps [47]. It is thus preferable to pre-treat the sample as little as possible when dealing with archaeological degraded wood, and this requirement is fulfilled by analytical pyrolysis.

When analytical pyrolysis is directly coupled with MS (DE-MS, DT-MS, Py-MS), the time of analysis is very short (a few minutes) and the amount of sample is extremely small (a few grains of milled wood). However, the mass spectra obtained may be very complex as they are the combination of the mass spectra of all the thermal degradation products. Therefore, in some cases, the comparison, classification, and differentiation of the analysed samples are successfully achieved by multivariate analysis of the mass spectral data [48][49][50][51][52][53][54]. Such analysis can be very useful as a screening step, in order to choose the representative samples for more detailed studies.

Another relatively new approach, which does not involve chromatographic separation, is evolved gas analysis coupled with mass spectrometry (EGA-MS). Based on a thermal degradation occurring over a temperature gradient, this technique provides both thermal and compositional information on the analysed material as well as insights into the interactions between lignin and carbohydrates [55][56]. EGA-MS has been applied to treated and untreated archaeological wood and has enabled the chemical changes undergone by lignin to be clarified [57].

Recently, direct analysis in real time—mass spectrometry (DART-MS) has been proposed to evaluate the degradation state of archaeological wood. This technique is technically not a pyrolysis-based approach, as wood components are thermally desorbed at ambient pressure. Nevertheless, it shares the same principles of DE-MS, with the advantage that it can be easily coupled to high resolution MS. The use of chemometrics delineates a promising scenario for further application of this technique to archaeological wood analysis.

When chromatographic separation is introduced between pyrolysis and MS analysis (Py-GC/MS), more detailed information is obtained, as the pyrolysis products are separated and singularly identified. However, many lignin pyrolysis products are polar compounds, which are not volatile enough to be effectively separated in a gas chromatographic column. A derivatising agent to be added *in situ* is useful to improve the chromatographic separation of pyrolysis products bearing carboxylic or alcoholic functionalities [58]. Methylating and silylating agents are the most commonly used [59]. Hexamethyldisylazane (HMDS) is preferred over tetramethylammonium hydroxide (TMAH) in wood analysis [60], because silylation of phenol groups permits to differentiate them from methoxy groups even after the derivatisation. In addition, trimethylsilylation reduces the reactivity of alcoholic functionalities during pyrolysis, improving the GC-MS detection of the primary lignin pyrolysis products with alcoholic moieties in the side chain such as coniferyl alcohol and sinapyl alcohol [61].

Recent instrumental configurations have led to the development of double-shot and multi-shot Py-GC/MS. A single sample can be exposed to increasing pyrolysis temperatures (shots) and a chromatographic run follows each shot, thus allowing the separation of specific pyrolysis products into different chromatographic runs according to the pyrolysis temperature at which they are formed. Double-shot pyrolysis has been applied to PEG-treated waterlogged wood, enabling the wood and the consolidant to be investigated separately.

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