Characterisation of archaeological waterlogged wood by pyrolytic and mass spectrometric techniques

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Abstract

Two analytical approaches based on analytical pyrolysis and mass spectrometry were used for the chemical study of archaeological waterlogged wood: direct exposure-mass spectrometry (DE-MS) and pyrolysis-gas chromatography/mass spectrometry Py-GC/MS with in situ thermally assisted silylation of pyrolysis products using hexamethyldisilazane (HMDS). The wood remains derived from Polish early-Mediaeval settlement in Żyłe (Central Maritime Province, Poland). The data were compared to those relative to native sound wood of the same species alder (Alnus glutinosa Geartn.), oak (Quercus robur L.), beech (Fagus sylvatica L.). The potentials of the adopted mass spectrometric techniques were compared. Both of them achieved semi-quantitative results on the content of lignin and polysaccharides in degraded wood, on syringyl vs. guaiacyl ratio, and on the chemical structure of lignin, avoiding the long wet-chemical procedures that are commonly used in wood analysis, and allowing us to use a minimal sample size.

1. INTRODUCTION

Although wood is one of the most resistant organic materials, archaeological wooden objects are relatively rare, and are preserved for long periods of time only under particular conditions as wet environments, because normally in sediments they are subjected to attack by biological agents such as fungi, bacteria and insects. In waterlogged conditions, characterised by low temperatures and oxygen concentrations, fungi and insects are not active, and consequently wooden artefacts often survive in a surprisingly good state, maintaining their original shape and features even after centuries underwater. Nevertheless, it has been shown that some species of anaerobic bacteria can slowly attack waterlogged wood even under near anoxic conditions, mainly by eroding the cellulose and hemicelluloses as sources of nutrients [1-4]. This leads to long term degradation phenomena that seriously compromise the stability of the artefacts, especially during/after the recovery and drying.

Knowledge of the chemical transformation occurring underwater in wooden historical objects is at present extremely inadequate, yet it is extremely important for their recovery, conservation and exposition. Conservation treatments of waterlogged wooden artefacts such as shipwrecks are technically difficult, expensive and not reversible, requiring the introduction of polymeric impregnants in the material [1], and the many factors influencing their long term stability have not been fully assessed.

The aim of this study was to examine the chemical composition of archaeological waterlogged wood and the chemical transformation undergone in waterlogged environments, and to highlight the chemical differences between archaeological wood and sound wood of the same specie. This was done in order to investigate if these differences are relevant in the choice of conservation/restoration treatments.

The study of archaeological waterlogged wood was performed by means of two analytical techniques based on pyrolysis and mass spectrometry, namely direct exposure electron ionisation - mass spectrometry (DE-MS) and pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) using hexamethyldisilazane (HMDS) for the in-situ thermally assisted silylation of pyrolysis products. Exploratory multivariate analysis of DE-MS mass spectral data was performed by principal component analysis (PCA). DE-MS is a fast fingerprint technique that allows us to obtain an overall mass spectrum of organic materials, without any sample pre-treatment. In previous papers we successfully used DE-MS for the study of archaeological wood samples [2] and of lignin samples extracted from archaeological wood [2, 3]. A similar approach, direct temperature mass spectrometry (DTMS), was previously shown to be useful in studying the composition of different kinds of wood and lignin [9,10].
Although the macromolecular complexity of wood limits the possibilities of obtaining complete chemical information, the use of Py-GC/MS has proven to be a very useful tool for investigating wood, lignin, cellulose and hemicelluloses. In Py-GC/MS the macromolecules are depolymerized by heat, and the fragments produced are identified by mass spectrometry, after gas chromatographic separation. Polymer subunits undergo thermal degradation under pyrolysis, consequently the information obtained is strictly dependent on the adopted instrumental conditions: primarily the selected pyrolysis temperature and asset of the pyrolyser; secondly, the parameters of the transfer line/interface between the pyrolyser and the GC, where secondary reactions occur. (A low temperature will not produce sufficient fragmentation of the stronger bonds in the macromolecules, while on the other hand a high pyrolysis temperature will produce extensive fragmentation of the sample, yielding less informative low molecular weight fragments, and producing secondary reactions.) A possible method to increase the volatility of pyrolysis products for GC/MS analysis, thus improving the analytical response, accelerating their transfer to the GC column and limiting the possibility of thermal degradation, is to perform in-situ thermally assisted derivatisation of the pyrolysis products. In the literature, the application of in-situ derivatisation methods in the pyrolysis of wood appears to be limited: results obtained with methylating [4-6] and silylating [7, 8] reagents are described, while the majority of published applications do not use derivatisation.

Py-GC/MS has been applied in the study of cellulose and lignin, to investigate decay processes in degraded wood [9-14] and also in a few cases in the specific study of historical or archaeological wood samples [15-18]. The literature data show that highlighting chemical alterations in the residual lignin structure of waterlogged wood is not straightforward. A comparison of Py-GC/MS profiles of degraded wood with those obtained from sound wood of the same species enables differences in the variations of the relative amounts (in terms of chromatographic peak areas) of minor pyrolysis products of lignin [14] to be observed. A comparison of pyrolysis profiles with those in the literature is of limited use because of the variability of pyrolysis products depending on the instrumental conditions used.

The waterlogged wood samples examined in this study derive from the excavation from Polish early-Mediaeval settlement in Zółte (Central Maritime Province). The site is located on a small island on Lake Zaranskie, in Zółte in Drawsko Lakes region. The settlement are date to a period between the 9th century and the 12th century AD.[19-21], where the elements of: palisades, bridges and structures along the shore, have been found in relatively good conditions (Figure 1). Native woods of the same species were also analysed for comparison.

2. MATERIAL AND METHODS

2.1 SAMPLES

Eight samples of archaeological waterlogged wood (oak: 2.2Q, 2.5Q, 2.7Q and 2.8Q; beech: 2.4F, 2.9F; alder: 2.1O and 2.3O) from the excavation of the early-Mediaeval settlement in Zółte (Central Maritime Province, Poland) were provided by the Institute of Wood Technology, Agricultural University of Poznań, Poland. The settlement is date to a period between the 9th century and the 12th century AD. Native sound wood samples of the same species (Quercus roburs, Fagus sylvatica, Alnus glutinosa) were provided by the same Institute of Wood Technology in Poland. The wood was dried in an oven at 50°C for 48 h and ball-milled for DE-MS and Py-GC/MS analyses. The derivatization reagent for Py-GC/MS analysis was 1,1,1,3,3,3-hexamethyldisilazane (HDMS, 99.9%, Sigma-Aldrich Inc., USA).
2.2 DE-MS
Samples were analysed in duplicate, by direct deposition on the exposure probe filament of the DE-MS using a capillary. The instrumentation (Thermo Electron Corporation, USA) was made up of a Direct Probe Controller and a Direct Exposure Probe (rhenium filament, current programmed mode 0 mA to 1000 mA in 2 s then 1000 mA for 60 s), coupled with a Polaris Q ion trap external ionisation mass spectrometer (electron impact ionisation 70 eV). Source temperature: 230°C. M/z range: 50–350. Optimal conditions (final temperature and speed of heating) to obtain a convenient peak shape of the total ion current (TIC) curve as a function of time were achieved by programming the probe as follows: 0 mA for 20 s, from 0 to 1000 mA in 2 s and 60 s at 1000 mA [2]. A mass spectral fingerprint was obtained by averaging the mass spectra in the desired time range.

2.3 Py(HMDS)-GC/MS
Pyrolysis (HMDS)-gas chromatography/mass spectrometry was performed in a 5150 CDS Pyroprobe 5000 Series with a coil filament connected to a 6890 Agilent gas chromatograph (USA) coupled to a 5973 Agilent mass selective detector operating in electron impact mode (EI) at 70 eV. On the basis of a careful comparison of the literature, pyrolysis temperatures between 450 and 600°C were tested and 500°C was chosen. Pyrolysis was carried out for 20 µs using a platinum coil probe and quartz sample tubes. We adopted hexamethyldisilazane (HMDS) as in-situ derivatizing agent for hydroxyl and acidic moieties [22]. Constant amounts (40–60 µg) of lignin and hexamethyldisilazane (5 µl) solution (70% in acetone) were inserted in the center of the pyrolysis quartz tube and placed in the pyrolysis coil filament. Chromatographic separation was performed on an HP-5MS fused silica capillary column (stationary phase 5% diphenyl–95% dimethyl-polysiloxane, 30 m × 0.25 mm i.d., Hewlett Packard, USA) with a de-activated silica pre-column (2 m × 0.32 mm i.d., J. and W. Scientific, Agilent Technologies, USA). Chromatographic conditions were as follows: an initial temperature of 31°C, 8 min isothermal, 10°C min⁻¹ up to 240°C, 3 min isothermal, 20°C min⁻¹ up to 300°C, 30 min isothermal. Carrier gas: He (purity 99.995%), constant flow 1.0 ml min⁻¹. Each sample was analyzed in four replicates. Quantification was based on peak areas (total integral of identified compounds equals 100).

2.4 PCA Data Analysis
Unsupervised pattern recognition analysis of DE-MS mass spectral data corresponding to the mass range 50–350 m/z, was performed by principal component analysis (PCA, Nipals algorithm) [23] on the covariance matrix of centered data, after row normalisation of the full 50–1000 m/z spectra. The
region 50–350 m/z was selected because it contains all the pyrolysis fragments corresponding to lignin monomers and dimers. The software used was XLSTAT 2009.1.02 (Addinsoft, Paris, France).

3. RESULTS AND DISCUSSION

3.1 DE-MS

The mass spectra of sound and archaeological woods showed a high complexity, as expected for the complex mixture of products formed in the pyrolysis of wood. The presence of peaks deriving from guaiacyl (m/z 124, 137, 150, 152, 164, 178, 180) and syringyl (m/z 154, 167, 180, 182, 194, 196, 208, 210) structures was evident in the mass spectra of investigated samples. Figure 2 shows the mass spectra obtained for the archaeological sample 2.9F (*Fagus sylvatica*) and for the reference beech wood. Both spectra are characterised by the occurrence of peaks indicative of a guaiacyl-syringyl lignin. The guaiacyl-derived fragments are at m/z 124, corresponding to the molecular peak of guaiacol (2-methoxy-phenol), at m/z 137 (guaiacol + CH₂⁺), which could derive from several compounds formed in the pyrolysis of guaiacyl lignin including ethylguaiacol, propylguaiacol and coniferyl alcohol, and at m/z 151 (guaiacol + CH₂CH₂⁺). The syringyl-derived fragments can be seen at m/z 167 (syringol + CH₂⁺) and m/z 181 (syringol + CH₃CH₂⁺), while the peak at m/z 210 corresponds to the molecular ion of sinapyl alcohol [3]. The main difference observed between the native and archaeological wood is the presence in the former of peaks deriving from the pyrolysis of polysaccharides, (cellulose and hemicellulose) at m/z 55, 69, 73, 85, 97, 114 and 126, which are drastically reduced in the archaeological wood. The fragment at m/z 69 is formed in the pyrolysis of furans; m/z 114 can be attributed to xylans; m/z 57 and 73 are derived from levoglucosan.

![Figure 2 DE- mass spectra of: archaeological beech wood (2.9F) from the Žólte site, and reference sound beech wood.](image)

Due to the complexity of the mass spectra obtained in the DE-MS analysis of wood, PCA was used as a pattern recognition technique to quantitatively compare the mass spectra obtained and to highlight differences and similarities between the various samples. The mass spectral data corresponding to sound and archaeological woods (two replicated samples for each material) were submitted to PCA...
based on the covariance matrix after row normalisation of the data matrix (m/z from 50 to 350). The resulting score plot for the first two principal components is shown in Figure 3. The score plot, accounting for 87% of the total variance, highlights that the first principal component discriminates between sound wood (PC1 positive values) and archaeological wood (PC1 negative values). Examination of PC1 loadings shows that PC1 is positively correlated to the intensities of the peaks due to the fragmentation of polysaccharides (m/z 55, 69, 73, 85, 97, 114, 126). This means that PC1 differentiates the samples on the basis of polysaccharide content, and gives an indication of the degree of wood decay. All archaeological samples are located on the left side of the PC2/PC1 score plot, which highlights their lower content of cellulose with respect to native samples of the same species.

![Wood DE-MS mass spectra PC1/PC2 score plot](image)

Figure 3 PCA score plot of PC1 and PC2 of mass spectral data, accounting for 86.98% of total variance.

### 3.2 Py(HMDS)-GC/MS

The pyrograms of sound beech wood and of archaeological beech wood derived from Žólte site are reported in Figure 4. Wood pyrolysis produces a mixture of low molecular weight compounds derived from polysaccharides, which also lead to the formation of levoglucosan, and relatively simple phenols resulting from the cleavage of ether and C-C bonds of lignin [15,30,31]. The phenols produced retain their substitution patterns from the lignin polymer [24], thus it is possible to identify its components from the p-hydroxyphenylpropanoid (H), guaiacylpropanoid (G) and syringylpropanoid (S) lignin units.

From a qualitative point of view, archaeological wood samples produce silylated pyrolysis products analogous to those observed in sound wood. The most abundant pyrolysis products of lignin under adopted conditions are: 4-methylguaiacol, 4-vinylguaiacol, E-isoeugenol, and E-coniferyl alcohol for guaiacyl compounds; 4-methylsyringol, 4-vinylsyringol, E-propenylsyringol and E-sinapyl alcohol, for syringyl ones. Other significant peaks in the chromatogram are related to vanillin, acetovanillone, 4-propenylsyringol (41), syringaldehyde (46), acetosyringon (52), syringic acid (58), and E-sinapaldehyde. In addition, products from pyrolysis of carbohydrate were also recognized including: 4-hydroxy-5,6-dihydro-(2h)-pyran-2-one, 3-hydroxy-2-methyl-2-cyclopenten-1-one, 1,3-dihydroxypropan-2-one, 3-hydroxy-2-(hydroxymethyl)-2-cyclopenten-1-one, E-4,5-dihydroxy-2-cyclopenten-1-one, 1,2,4-trihydroxybenzene, levoglucosan, and other minor peaks holocellulose derivatives.

The pyrograms clearly reflect wood degradation in archaeological samples. Carbohydrate pyrolysis products represent on average 53% of the total area of identified compounds for sound beech wood.
and 20% for archaeological beech wood. Phenols from lignin are 40% of the total area in sound wood samples and 74% for waterlogged beech wood (2.9F). The values obtained for all the analysed samples are reported in Table 1.

The Carbohydrate/Lignin coefficient, defined as the ratio between relative abundances (in %) of holocellulose (cellulose and hemicelluloses) and lignin provides valuable and reliable information concerning the extent of degradation of archaeological wood [25]: the lower its value, the more degraded is the raw material. This coefficient, in the sound wood, has the values of 2.23 for oak, 1.35 for alder and 1.33 for beech, whereas in archaeological wood is consistently lower, and depends on how degraded is the wood in the sample.

![Py(HMDS)-GC/MS profiles of sound beech wood and archaeological beech wood (2.9F) from Zőlte site](image)

Table 1: Total amount of C, S and G compounds from the Py (HMDS)-GC/MS of archaeological and sound wood.

<table>
<thead>
<tr>
<th></th>
<th>Beech 2.4F</th>
<th>2.9F</th>
<th>Alder 2.1O</th>
<th>2.3O</th>
<th>Oak 2.2Q</th>
<th>2.5Q</th>
<th>2.6Q</th>
<th>2.7Q</th>
<th>2.8Q</th>
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<tbody>
<tr>
<td></td>
<td>Sound</td>
<td>Arch.</td>
<td>Sound</td>
<td>Arch.</td>
<td>Sound</td>
<td>Arch.</td>
<td>Sound</td>
<td>Arch.</td>
<td>Sound</td>
</tr>
<tr>
<td>Carbohydrate peaks</td>
<td>52.9</td>
<td>14.0</td>
<td>19.7</td>
<td>52.5</td>
<td>17.7</td>
<td>15.2</td>
<td>64.5</td>
<td>18.7</td>
<td>20.1</td>
</tr>
<tr>
<td>Lignin peaks</td>
<td>39.8</td>
<td>81.4</td>
<td>74.1</td>
<td>38.8</td>
<td>76.9</td>
<td>80.3</td>
<td>28.8</td>
<td>72.5</td>
<td>70.6</td>
</tr>
<tr>
<td>Carbohydrate/Lignin</td>
<td>1.3</td>
<td>0.2</td>
<td>0.3</td>
<td>1.4</td>
<td>0.2</td>
<td>0.2</td>
<td>2.2</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>G</td>
<td>14.9</td>
<td>22.7</td>
<td>22.9</td>
<td>17.8</td>
<td>31.4</td>
<td>31.3</td>
<td>11.3</td>
<td>25.3</td>
<td>28.4</td>
</tr>
<tr>
<td>S</td>
<td>24.8</td>
<td>56.9</td>
<td>49.9</td>
<td>21.2</td>
<td>45.8</td>
<td>49.0</td>
<td>17.8</td>
<td>47.4</td>
<td>42.2</td>
</tr>
<tr>
<td>Syringyl/Guaiacyl</td>
<td>1.7</td>
<td>2.5</td>
<td>2.8</td>
<td>1.2</td>
<td>1.5</td>
<td>1.6</td>
<td>1.6</td>
<td>1.9</td>
<td>1.5</td>
</tr>
</tbody>
</table>
The strongest changes due to the degradation processes (e.g.: low polysaccharides content) were observed for beech wood samples 2.4F and 2.9F; for both of investigated alder wood samples (2.1O and 2.3O) and for oak samples 2.2Q, 2.5Q and 2.6Q. The carbohydrates and lignin content in archaeological oak wood samples: 2.7Q and 2.8Q show that in these samples wood is better conserved.

Beech wood samples 2.4F and 2.9F show peculiar results in the acidic functionalities content, that are relatively higher with respect to other archaeological and sound wood samples. This behavior lets us suppose that these samples underwent an oxidative degradation process. The nature of this oxidative degradation process is still under investigation. The samples have been provided under wet conditions; therefore oxidative degradation process during the excavation is unlikely.

The syringyl/guaiacyl ratio (S/G) is also a significant chemical parameter in the characterisation of hardwood lignin. If the S/G ratio of archaeological wood is comparable to those of sound wood of the same specie, the lignin is well preserved; otherwise its structure can be supposed to be altered. Some of the archaeological samples show an increase in the S/G ratio in respect of sound wood of the same specie. This trend needs to be better investigated, and suggest depolymerisation of lignin with preferential loss of guaiacyl units, or preferential demethoxylation of guaiacyl units.

4. CONCLUSIONS

In the investigated waterlogged wood samples from Žőlte site we observed different level of degradation. The pyrograms clearly reflect wood degradation in archaeological samples, expressed by the ratio between polysaccharides and lignin pyrolysis products.

Our results highlight some advantages of pyrolysis-mass spectrometric techniques in the characterisation of archaeological wood: to use a minimal sample size and to perform the analysis in short time, thus avoiding the long wet-chemical procedures that are commonly used in wood analysis. DE-MS is a fast method for the screening, evaluation and comparison of archaeological wood samples in a few minutes. This enables us to have a rapid semi-quantitative indication of the syringyl/guaiacyl ratio and of the loss of polysaccharides as the effect of degradation in a waterlogged environment. Although directly combining pyrolysis with mass spectrometry does not offer the detailed chemical information achieved with Py-GC/MS, it has the advantage of achieving a mass spectral fingerprint of the samples within a few minutes. Moreover, the application of pattern analysis based on PCA enables the mass profiles of many samples to be compared quantitatively in an easily readable manner, highlighting similarities and differences among samples. The examination of the PC loadings enables the differences to be correlated to specific chemical features. This analytical approach is suitable for fast screening and we believe it has great potential when applied to large wooden artefacts, such as shipwrecks. In fact, it can be used to monitor the state of decay in various regions of the wood.

References