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Characterisation of archaeological waterlogged wood by pyrolytic and mass spectrometric techniques

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ABSTRACT

Two techniques based on analytical pyrolysis and mass spectrometry, direct exposure-MS (DE-MS) and pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS), were used to characterise waterlogged archaeological wood and to study degradation patterns of wood in aqueous environments. The two techniques were applied to samples from the excavation of the Site of the Ancient Ships of Pisa San Rossore in Pisa (Italy), and data were compared to those relative to native sound wood of the same species (pine, elm, beech). Both the methods result valuable in the analysis of ancient wood artefacts, avoiding the long wet-chemical procedures that are commonly used in wood analysis, and allowing us to use a minimal sample size. DE-MS achieves a global mass spectral fingerprint of lignin and polysaccharides pyrolysis compounds in few minutes, and the results have been interpreted with the support of principal component analysis (PCA) of mass spectra. Py-GC/MS permits detailed molecular analysis of pyrolysis compounds and highlights some chemical modifications of lignin in archaeological samples, as demethylation of both guaiacyl and syringyl lignin units. Both the techniques demonstrate consistent loss of polysaccharides in archaeological wood.

1. Introduction

The availability and unique physical properties of wood have made it the material of choice since ancient times for the fabrication of tools, furniture, shelters, ships and everyday or artistic artefacts. 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. This is sometimes observed in shipwrecks, which maintain 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 hemicellulose as sources of nutrients [1–4]. This leads to long term degradation phenomena that seriously compromise the stability of the artefacts, especially during the recovery and drying.

Knowledge of the chemical transformations occurring underwater in wooden historical objects is at present 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 [4], 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 by lignin 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 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). Exploratory 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 [5–7]. In a previous preliminary paper we successfully used DE-MS for the study of lignin samples extracted from archaeological wood [8]. A similar approach, direct temperature mass spectrometry (DTMS), was pre-

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viously shown to be useful in studying the composition of different kinds of wood and lignin [9,10]. Although directly combining pyrolysis with mass spectrometry does not offer the detailed chemical information achieved with Py-GC/MS, DE-MS 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. Furthermore, 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.

Although the macromolecular complexity of wood limits the possibilities of obtaining complete chemical information on its alteration, the use of Py-GC/MS has proven to be a very useful tool for investigating wood, lignin, cellulose and hemicellulose. In the literature there are many papers describing the Py-GC/MS analysis of complex macromolecules in wood [9,11–13], non-wood lignocellulosics materials [14] and isolated lignin [8,15], thus confirming the reliability and potentiality of this technique.

Py-GC/MS has been successfully employed to study the chemical alterations of wood components [9,16,17], during degradation induced by fungi [18] such as brown-rot [19] and white-rot [20,21], and chemical [22–24] and enzymatic treatments [25–27] for the pulp and paper industry. Py-GC/MS has also been used for investigating the degradation processes occurring in archaeological wood [28] and in lignin extracted from waterlogged wood [29]. Although quantitative data were not reported, Py-GC/MS analysis of samples from waterlogged oak wood (ca. 4000 BC) from the South coast of England highlighted the occurrence of demethylation of lignin units during degradation [28].

When using this technique, wood is pyrolyzed producing a mixture of low molecular weight compounds, including levoglucosan, derived from polysaccharides and relatively simple phenols resulting from the cleavage of ether and C–C bonds of lignin [15,30,31]. The phenols retain their substitution patterns from the lignin polymer [32], thus it is possible to identify its source as *p*-hydroxyphenylpropanoid (H), guaiacylpropanoid (G) and syringylpropanoid (S) lignin units.

The waterlogged wood samples examined in this study derived from the excavation of the Site of the Ancient Ships of Pisa San Rossore in Pisa (Italy), where more than 30 Roman shipwrecks have been found in relatively good conditions [33]. Native woods of the same species were also analysed for comparison. The two techniques were compared in order to verify the efficacy of the analytical protocol based on DE-MS and PCA analysis, whose applicability to extracted lignin was investigated in a previous study [8], and it is here applied for the first time to archaeological wood.

2. Material and methods

2.1. Samples

Eight samples of archaeological waterlogged wood (pine: F5, F18, F26 and C19, beech: G26, elm: D5, D21, D24) from the excavation of the Site of the Ancient Ships of Pisa (Italy) were provided by the Archaeological Superintendence of Tuscany. The archaeological artefacts from the site date to a period between the 4th century BC and the 2nd century AD.

Native sound wood samples of the same species (*Pinus pinaster, Fagus sylvatica, Ulmus minor*) were provided by IVALSA (Trees and Timber Institute) CNR (Florence).

The wood was dried in an oven at 50 °C for 48 h and ball-milled for before analyses.

Cellulose was Sigmacell® Type 101 by Sigma–Aldrich (Germany).

2.2. DE-MS

The instrumentation (Thermo Electron Corporation, USA) was made up of a Direct Probe Controller and a Direct Exposure Probe (rhenium filament), coupled with a Polaris Q ion trap external ionisation mass spectrometer (electron impact ionisation 70 eV). The source temperature was 230 °C. Samples were analysed by direct deposition on the exposure probe filament using a capillary. Each sample was analysed in duplicate.The m/z range 50–1000 was firstly scanned over for all the samples, and subsequently, once the presence of high molecular mass and/or polymeric components had been excluded, the analysis was repeated twice scanning the m/z range 50–500. Optimal conditions 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 [8]. A mass spectral fingerprint was obtained by averaging the mass spectra in the desired time range.

2.3. Py-GC/MS

Pyrolysis was performed with a Py-2020iD double-shot micro furnace pyrolyser (Frontier Laboratories Ltd., Fukushima, Japan) connected to an Agilent 6890 GC/MS system equipped with a DB-1701 fused-silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness, Agilent J&W, USA) and an Agilent 5973 Mass Detector (EI at 70 eV). Pyrolysis temperature was 500 °C. The GC oven temperature was programmed from 50 °C (1 min) to 100 °C at 30 °C min^{-1}, and then up to 290 °C (10 min) at 6 °C min^{-1}, using Helium as a carrier gas (1 ml min^{-1}). The compounds were identified by comparing their mass spectra with those reported in Wiley and NIST libraries (match factor ≥95%), and in the literature [34,35]. Identification was always supported by inspection of the mass spectra and evaluation of the ion-ratios. Peak areas of the lignin-degradation products were calculated, the summed areas normalised, and the data for two replicated analyses averaged and expressed as percentages of the total.

2.4. PCA data analysis

Exploratory 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) [36] on the covariance matrix of centered data, after row normalisation of the full 50–500 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 mixture of products formed in the pyrolysis of wood. The presence of peaks deriving from guaiacyl structures was evident in the mass spectra of both hardwoods and softwoods, while peaks deriving from syringyl units were only observed in the mass spectra of beech and elm (hardwoods) (Fig. 1).

Fig. 2 shows the mass spectra obtained for the archaeological sample G26 (*Fagus* spp., Fig. 2A) and for the reference beech wood

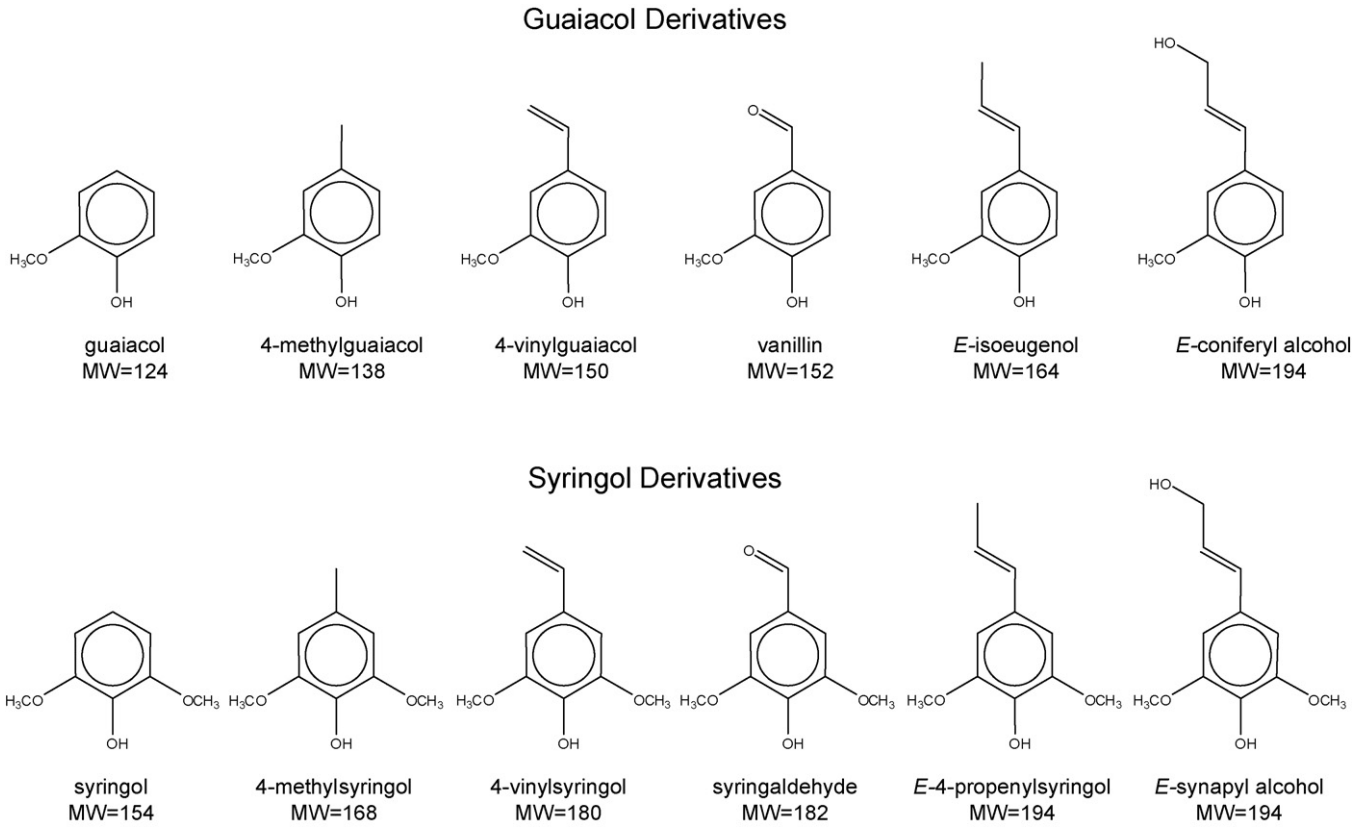

Fig. 1. Chemical structures of the main pyrolysis products of lignin.

(Fig. 2B). 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 + CH_2^+), 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 + $CH_2CH_2^+$). The syringyl-derived fragments can be seen at *m*/*z* 167 (syringol + CH_2^+) and *m*/*z* 181 (syringol + $CH_2CH_2^+$), while the peak at *m*/*z* 210 corresponds to the molecular ion of sinapyl alcohol [8]. The main difference observed between the native and archaeological wood is the presence in the former of intense 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.

As a comparison, Fig. 2C shows the mass spectra obtained in the analysis of a reference sample of pure cellulose. 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.

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 Fig. 3. The score plot, accounting for 78.61% of the total variance, highlights that the first principal component discriminates between softwoods (PC1 negative values) and hardwoods (PC1 positive values), while the second principal component discriminates between sound wood (PC2 positive values) and archaeological wood (PC2 negative values).

The loadings for PC1 and PC2, representing the coefficients or "weights" used to calculate the scores as linear combinations of the original variables, are showed in Fig. 4 in the form of reconstructed mass spectra.

PC1 loadings (Fig. 4A) show that PC1 is positively correlated to the intensities of the peaks due to the fragmentation of syringyl monomers, and negatively correlated to the intensities of the peaks due to the fragmentation of guaiacyl monomers. Thus, PC1 gives a rapid indication of the ratio between the amounts of syringyl and guaiacyl fragments, and on the type of wood, discriminating between softwoods and hardwoods. The softwood samples, sound pine and archaeological samples F26, F18, F5 and C19, score lowest on PC1 because they contain guaiacyl lignin only, and are well differentiated from the hardwood samples that contain both guaiacyl and syringyl monomers, and score higher on PC1. Sound elm wood and the archaeological elm samples D24, D21 and D5 score a PC1 intermediate position, while sound beech wood and the archaeological beech sample G26 are in the right hand area of the score plot. The ratio between the relative abundance of peak at *m*/*z* 167, the most abundant fragment from syringyl monomers, and the peak at *m*/*z* 137, deriving from guaiacyl monomers, can be considered as a rough indication of the S/G ratio for the wood samples compared. This index should be considered as relative and not absolute: it does not reflect the real value of the ratio between syringyl and guaiacyl monomers in the wood, but is useful for comparing samples analysed under the same conditions. In the elm wood samples the average ratio was 0.6, while in the beech wood sample the average ratio was 1.5.

Examination of PC2 loadings (Fig. 4B) shows that PC2 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 PC2 differentiates sound wood from archaeological wood on the basis of polysaccharide content, and gives an

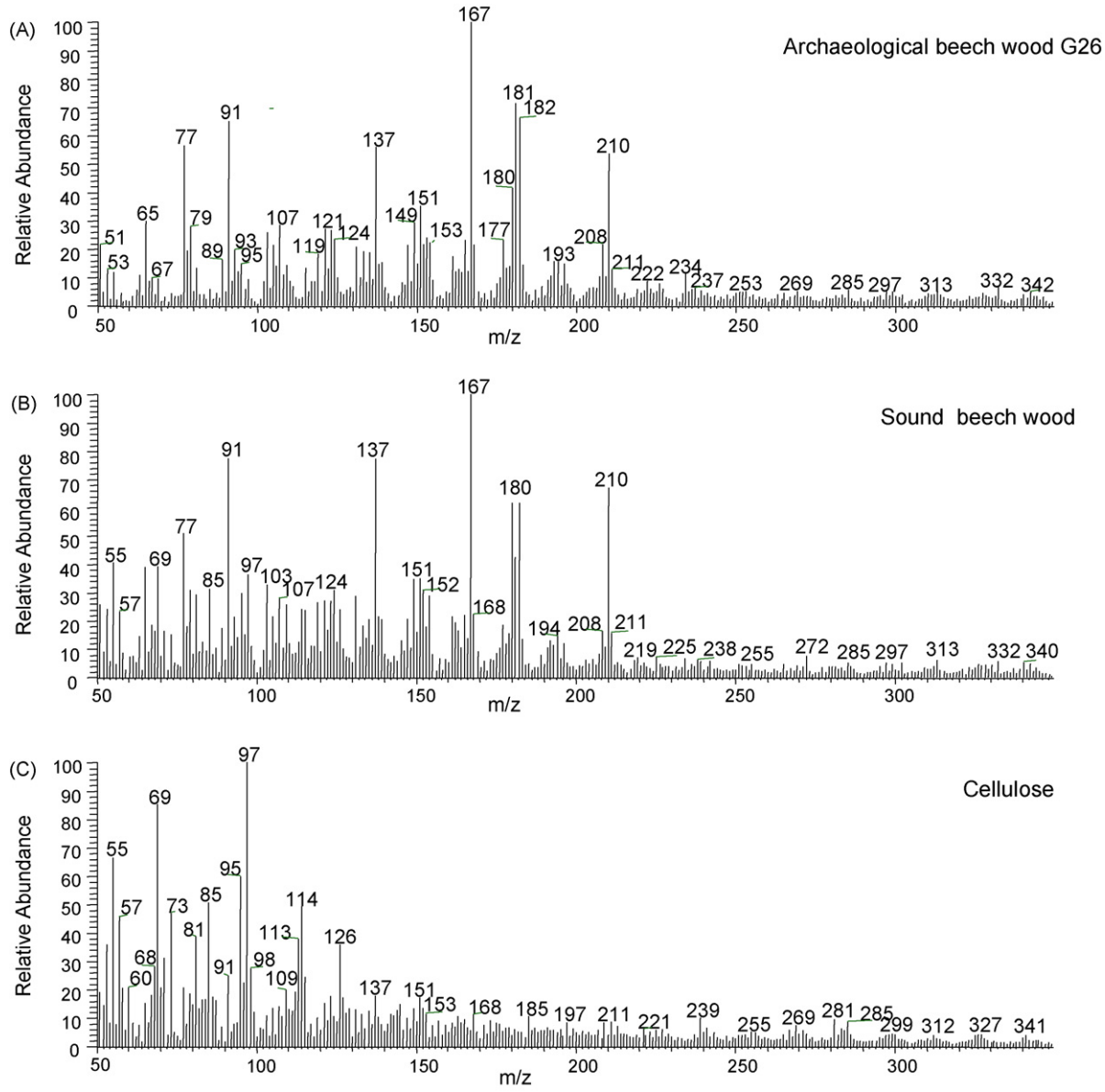

Fig. 2. DE- mass spectra of (A) archaeological beech wood (G26) from the Pisa San Rossore site, (B) reference sound beech wood and (C) pure cellulose.

indication of the degree of wood decay. All archaeological samples are located in the lower part of the PC2/PC1 score plot, which highlights their lower content of cellulose with respect to native samples of the same species. Elm archaeological samples show a higher heterogeneity in PC2 values, suggesting a different degree of polysaccharide decay among the examined samples.

3.2. Py-GC/MS

In order to get more detailed information on the chemical composition of all the wood constituents, i.e. polysaccharides as well as lignin, the samples of sound and archaeological wood were studied by analytical pyrolysis coupled to GC/MS. The first part of the pyrograms (3–7 min) is dominated by the pyrolysis products of polysaccharides, while lignin thermal cleavage leads to a complex mixture of phenolic products (7–27 min), with a guaiacyl structure in the case of pine, and with both guaiacyl and syringyl structures in the case of elm and beech.

Table 1 lists the identities and the relative abundances of the cellulose- and lignin-derived compounds detected with Py-GC/MS in the conditions adopted for the various samples of sound and archaeological wood of different species. The data relative to archaeological wood were obtained averaging the results for all the samples of the same specie: four for pine (F5, F18, F26, C19), one for beech (G26), and three for elm (D5, D21, D24). Each sample was analysed twice. The summed areas of the relevant peaks were normalised to 100%. Relative peak areas were calculated for the total amount of carbohydrate-derived compounds and for G and S type lignin products

From a qualitative point of view, archaeological wood samples of pine, beech and elm produce analogous pyrolysis products to those observed in sound wood. The differences between archaeological waterlogged wood and sound wood can be seen in the relative amounts of the products observed, as shown in Fig. 4A and B with the pyrolysis profiles of sound beech wood and the corresponding degraded archaeological wood from the Pisa San Rossore site.

The pyrogram of sound beech wood, in Fig. 5A, shows a large number of compounds derived from phenylpropanoid lignin units: mainly guaiacyl (G) and syringyl (S) moieties with different alkyl substituents. The major peaks are guaiacol (17), 4-vinylguaiacol

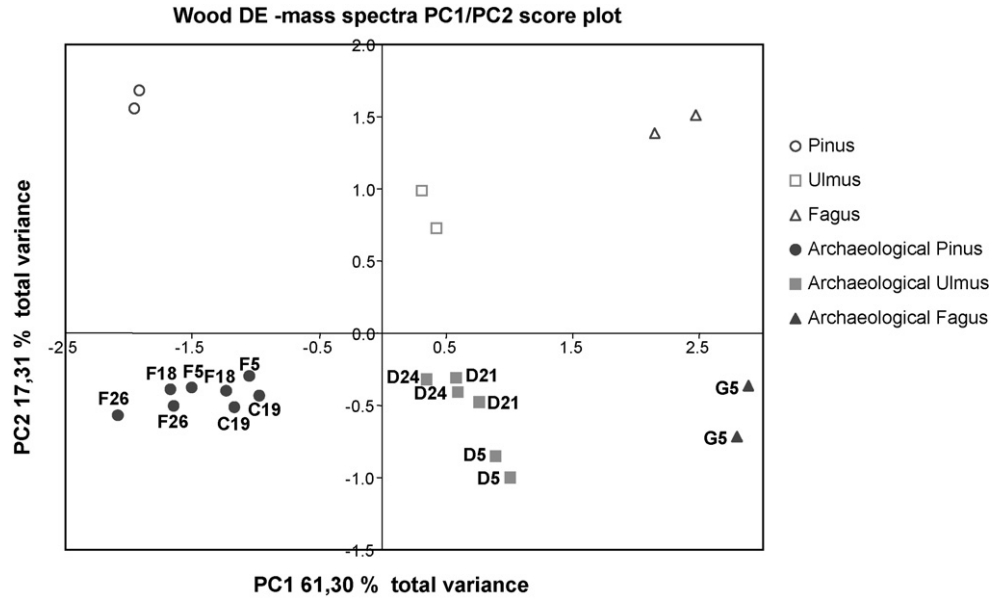

Fig. 3. PCA score plot of PC1 and PC2 of mass spectral data, accounting for 78.61% of total variance.

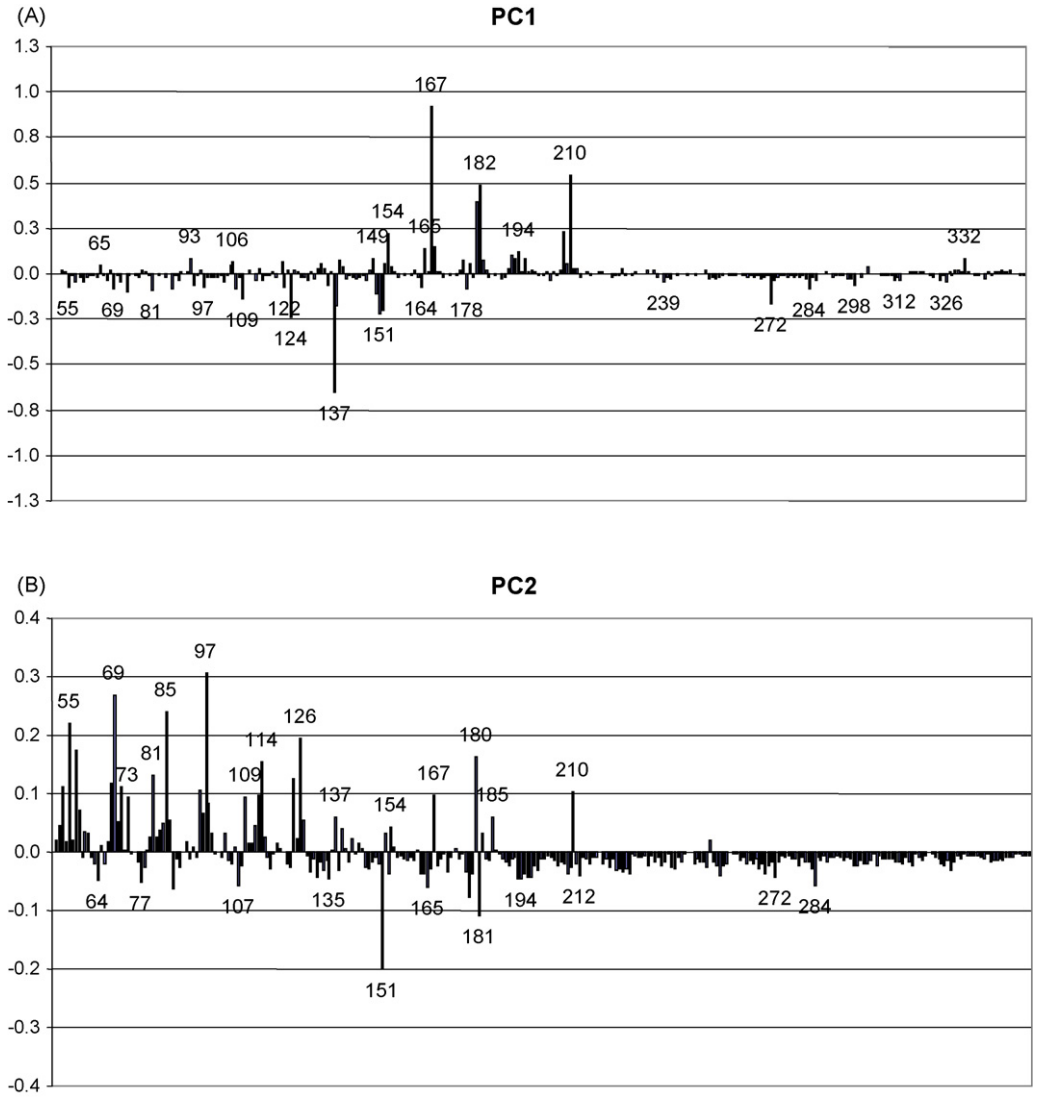

Fig. 4. Loading plots of (A) PC1 and (B) PC2 in the form of reconstructed mass spectra.

Table 1
Compounds identified by Py-GC/MS from the Pyrolysis fragments of archaeological and sound wood samples, and relative amounts. Peak intensities are expressed as relative percentages of the area respect to the total area of determined compounds, and are the average of all the samples analysed for each specie. C—carbohydrates, H—p-hydroxyphenyl, G—guaiacyl, S—syringyl; arch.—archaeological wood samples from San Rossore site (Pisa, Italy).

Peak no.	Ret. Time [min]	Compound	Main MS peaks (*m*/*z*)	MW	Origin	Elm		Beech		Pine	
						Sound	Arch.	Sound	Arch.	Sound	Arch.
1	3.98	Furfural	96, 67	96	C	3.2	1.4	3.3	0.5	4.2	1.8
2	4.37	2-Furanmethanol	98, 81, 69	98	C	0.6	0.1	0.8	0.1	0.5	0.8
3	4.40	2(5H) furanone-5-methyl	98, 70, 55	98	C	0.3	0.1	0.3	0.1	0.4	0.2
4	4.63	2-Methyl-2-cyclopenten-1-one	96, 67	96	C	0.1	–	0.1	–	0.4	0.1
5	4.74	2-Acethylfuran	110, 95	110	C	0.4	0.1	0.4	0.0	0.3	0.1
6	4.86	4H-pyran-4-one	96, 69	96	C	0.1	0.0	0.2	0.0	0.3	0.1
7	4.92	4-Cyclopenten-1,3-dione	96, 68	96	C	0.3	0.1	0.3	0.0	0.5	0.1
8	5.02	2,5-Furandione	98, 70, 54	98	C	–	–	–	–	0.3	0.0
9	5.26	2,3-Dihydro-5-methylfuran-2-one	98, 69, 55	98	C	3.2	0.2	2.7	0.1	2.2	0.7
10	5.30	3-Furanmethanol	98, 70	98	C	–	–	0.4	–	0.9	0.1
11	5.58	5-Methyl furancarboxyaldehyde	110, 95, 81	110	C	0.6	0.4	0.7	0.1	0.6	0.8
12	6.06	2(5H) Furanone	84, 55	84	C	2.4	0.3	2.0	0.1	4.5	0.6
13	6.23	2(3H) Furanone-5-methyl	110, 98, 83	110	C	0.7	0.2	0.7	0.1	0.5	0.3
14	6.31	4-Hydroxy-5,6-dihydro-(2H)-pyran-2-one	114, 58	114	C	0.2	0.1	0.6	0.1	1.7	0.8
15	6.67	2-Hydroxy-3-methyl-2-cyclopenten-1-one	112, 83, 69, 55	112	C	3.6	0.8	3.0	0.4	2.4	1.5
16	7.07	Phenol	96, 66	94	H	0.8	0.6	0.5	0.4	0.4	0.8
17	7.46	Guaiacol	124, 109	124	G	6.7	5.9	2.7	3.3	5.6	5.6
18	7.91	Cresol	108, 90	108		0.5	0.6	0.3	0.4	0.2	0.8
19	8.21	3-Hydroxy-2-methyl-(4H)-pyran-4-one	126, 97, 71	126	C	0.9	–	1.3	0.4	1.3	0.6
20	8.63	2,4-Dihydropyran-3-one	98, 69	98	C	0.2	–	0.3	-	0.5	0.1
21	9.27	4-Methylguaiacol	138, 123	138	G	3.3	6.8	2.3	3.5	5.0	10.7
22	10.87	4-Ethylguaiacol	152, 137	152	G	1.5	2.8	0.7	1.6	0.6	3.0
23	11.98	3-Methoxy-1,2-benzenediol	140, 125, 97	140		1.5	2.1	–	4.4	–	–
24	12.01	4-Vinylguaiacol	150, 137	150	G	9.0	6.8	5.6	3.3	9.4	8.3
25	12.47	Eugenol	164, 149	164	G	1.9	2.6	1.0	1.3	2.6	4.3
26	12.54	4-Propylguaiacol	166, 137	166	G	–	0.4	0.2	0.5	0.4	0.9
27	12.93	1,2-Benzenediol	110, 92	110		3.1	2.6	2.2	1.6	0.4	2.2
28	13.07	Syringol	154, 139	154	S	5.3	3.8	6.9	7.7	–	–
29	13.57	*Z*-Isoeugenol	164, 149	164	G	1.5	2.9	0.8	1.7	1.8	3.8
30	13.65	3-Methyl-1,2-benzenediol	124, 107	124		0.5	0.8	0.5	0.6	–	0.7
31	13.73	5-Methyl-3-methoxy-1,2-benzenediol	154, 139	154		0.6	1.1	1.2	2.9	–	–
32	14.43	4-Methyl-1,2-benzenediol	124, 107	124		1.1	2.0	0.7	1.0	–	3.6
33	14.59	*E*-Isoeugenol	164, 149	164	G	7.2	9.5	3.3	5.0	8.1	13.3
34	14.86	4-Methylsyringol	168, 153	168	S	1.9	3.5	4.1	6.9	–	–
35	15.03	Vanillin	152, 123	152	G	2.3	3.2	1.4	2.7	4.9	4.7
36	15.19	Propineguaiacol	162, 147	162	G	0.8	0.4	0.4	0.3	4.9	0.9
37	15.36	5-Ethyl-3-methoxy-1,2-benzenediol	168, 153	168		0.7	0.7	0.9	1.8	–	–
38	15.39	Propineguaiacol	162, 147	162	G	0.8	0.6	0.4	0.4	4.7	0.9
39	16.14	Homovanillin	166, 137, 122	166	G	0.6	–	0.8	0.2	2.2	2.0
40	16.27	4-Ethylsyringol	182, 167	182	S	0.7	1.5	1.6	3.5	–	–
41	16.43	Vanillic acid methyl ester	182, 151, 123	182	G	0.1	0.6	–	0.7	0.7	0.6
42	16.57	Acetovanillone	166, 151	166	G	1.4	4.0	0.9	2.4	2.1	3.1
43	16.79	3-Methoxy-5-vinyl-1,2-benzenediol	166, 151, 123	166		0.5	0.6	0.7	0.6	–	–
44	17.37	4-Vinylsyringol	180, 165	180	S	4.6	3.6	6.1	5.7	–	–
45	17.55	Guaiacylacetone	180, 137	180	G	1.1	1.3	0.5	0.7	1.1	1.7
46	17.71	4-Allylsyringol	194, 179	194	S	1.6	2.5	2.6	4.9	–	–
47	18.15	Propiovanillone	180, 151	180	G	0.3	1.3	0.5	1.0	0.8	1.8
48	18.51	Guaiacyl vinyl ketone	178, 151, 123	178	G	0.0	0.3	0.0	0.1	4.7	0.3
49	18.65	*Z*-4-Propenylsyringol	194-179	194	S	1.0	2.0	1.5	3.4	–	–
50	19.19	3-Methoxy-5-propenyl-1,2-benzenediol	180, 165	180		-	0.5	1.2	1.1	–	–
51	19.19	Propinesyringol	192, 177, 131	192	S	0.4	0.3	0.8	0.5	–	–
52	19.42	Propinesyringol	192, 177, 131	192	S	–	0.3	1.8	0.7	–	–
53	19.46	Levoglucosan	98, 73, 60, 57	162	C	4.5	2.8	8.5	–	15.4	8.8
54	19.74	*E*-4-Propenylsyringol	194, 179	194	S	3.9	5.8	5.4	8.9	–	–
55	19.82	Dihydroconiferyl alcohol	182, 137	182	G	1.6	1.9	0.6	0.2	1.7	4.2
56	20.25	Syringaldehyde	182, 181	182	S	1.0	1.7	3.2	3.2	–	–
57	20.30	*Z*-Coniferyl alcohol	180, 137	180	G	0.8	–	–	0.3	0.2	0.1
58	21.02	Homosyringaldehyde	196, 167	196	S	0.2	–	0.8	–	–	–
59	21.44	Acetosyringone	196, 181	196	S	0.6	1.9	1.5	3.0	–	–
60	21.74	*E*-coniferyl alcohol	180, 137	180	G	3.0	0.1	1.4	0.2	0.6	0.4
61	22.07	*E*-coniferaldehyde	178, 147	178	G	2.5	1.1	1.5	0.9	0.1	4.0
62	22.20	Syringylacetone	210, 167	210	S	0.6	0.7	1.0	1.5	–	–
63	22.71	Propiosyringone	210, 181	210	S	0.3	0.6	0.5	1.0	–	–
64	22.77	Syringyl vinyl ketone	208, 181	208	S	–	0.2	0.1	0.2	–	–
65	23.01	*E*-synapyl alcohol	210, 181, 167	210	S	–	–	0.5	–	–	–
66	24.24	Dihydrosinapyl alcohol	212, 168	212	S	0.7	0.6	1.0	0.5	–	–
67	26.24	*E*-Sinapaldehyde	208, 180	208	S	0.7	0.3	2.2	1.8	–	–

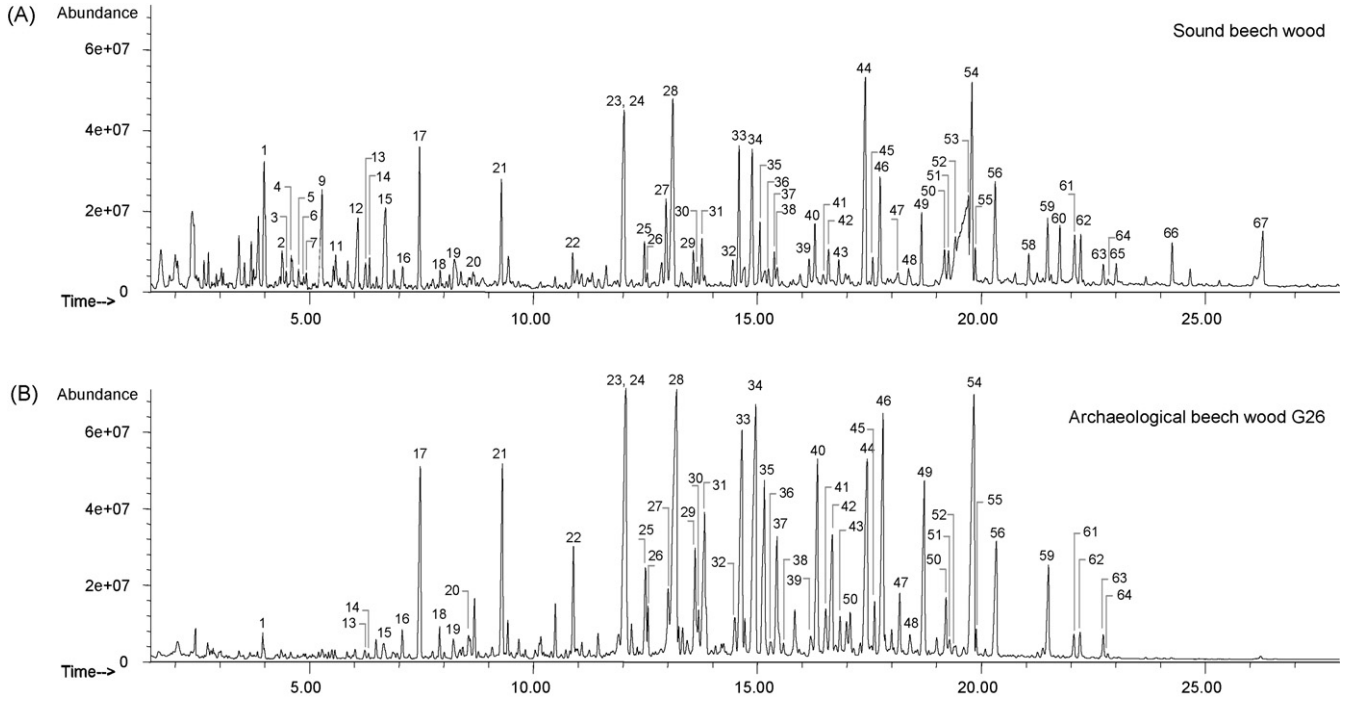

Fig. 5. Py-GC/MS profiles of (A) sound beech wood (*Fagus sylvatica*) and (B) archaeological beech wood from the Pisa San Rossore archaeological site.

(24), syringol (28), *E*-isoeugenol (33), 4-methylsyringol (34), 4-vinylsyringol (44), 4-allylsyringol (46), *E*-4-propenylsyringol (54) and syringaldehyde (56). Other prominent pyrolysis products identified in the beech wood pyrograms were vanillin (35), 4-ethylsyringol (40), *Z*-4-propenylsyringol (49), acetosyringone (59), *E*-coniferyl alcohol (60) and *E*-coniferaldehyde (61). In addition, products arising from the pyrolysis of carbohydrates were also recognized, including: furfural (1), 2,3-dihydro-5-methylfuran-2-one (9), 2(5H)-furanone (12), 2-hydroxy-3-methyl-2-cyclopenten-1-one (15) and levoglucosan (53).

The examination of the pyrograms of archaeological waterlogged wood samples, in Fig. 5B, shows that lignin was well preserved in archaeological wood, and, at the same time, clearly reflects the polysaccharide degradation, as already observed by DE-MS.

A comparison between the pyrograms of archaeological wood and sound wood of the same species shows that the archaeological samples of the three wood species showed a relatively low amount of polysaccharidic products, and of levoglucosan. The amounts of guaiacyl and syringyl units derived from lignin strongly increase, with respect to the polysaccharide derivates, in the pyrograms of archaeological wood, compared to the pyrograms of native wood. This provides evidence of a substantial degradation in the wood: due to the selectivity of the anaerobic degradation process during a prolonged period in an aqueous environment, the polysaccharide component had been preferentially removed, thus leaving a residue enriched in lignin. Table 2 shows the relative loss of polysaccharide pyrolysis products, in terms of peak areas, from sound to archaeological wood. The reduction in the relative intensities of the peaks deriving from carbohydrates is about 50% for pine, 70% for elm and 90% for beech.

The pyrolysis S/G ratio, a common parameter for the characterisation of hardwood lignin [37,38], was also calculated from the total ion chromatograms of sound and archaeological elm and beech wood, and are reported in Table 2. It is interesting to note that the values of the S/G ratios obtained by Py-GC/MS are comparable with those obtained by DE-MS and reported in the previous section. The S/G ratios observed for the pyrolysis products of archaeological wood are comparable to those of sound wood, which indicates a remarkable preservation of the lignin moiety.

The molecular profiles of the pyrolysis products of lignin were carefully inspected to highlight any alteration that could be attributed to the chemical decay of lignin. Table 1 highlights some differences in the pyrolysis patterns of lignin derivates, between the sound and archaeological wood samples of the same species.

In the archaeological pine wood, a net increase in 1,2-benzenediol (27) and 4-methyl-1,2-benzenediol (32) was observed. Catechols have been reported [28] to form with the degradation of guaiacyl rings, as products of the demethylation of the methoxy group. In the elm and beech archaeological wood, diols deriving from the demethylation of syringyl lignin units were observed: 3-methoxy-1,2-benzenediol (23), 5-methyl-3-methoxy-1,2-benzenediol (31), 5-ethyl-3-methoxy-1,2-benzenediol (37), and 3-methoxy-5-propenyl-1,2-benzenediol (50). The degradation pattern observed in archaeological waterlogged wood is very similar to that produced by brown-rot fungi, which selectively degrade carbohydrates and produce only small changes in lignin structure, mostly related to demethylation of methoxy groups.

Another interesting feature is the higher amounts of compounds with intact propyl side-chain (*E*-coniferaldehyde (61), sinapaldehyde (67), coniferyl alcohol (60), sinapyl alcohol (65)) in the pyrograms of sound beech and elm wood compared to the archaeological wood of the same species. Their decrease in the

Table 2
Total amounts of C, S and G compounds observed in the Py-GC/MS of archaeological and sound wood.

		Elm		Beech		Pine	
		Sound	Arch.	Sound	Arch.	Sound	Arch.
Polysaccharides	C	21.2	6.6	25.5	2.1	36.9	17.3
Lignin	S	23.2	29.3	41.5	53.4	–	–
	G	46.3	52.5	24.9	30.2	62.2	74.6
	S/G	0.5	0.6	1.7	1.8	–	–

archaeological wood indicates lignin side-chain degradation [37]. Moreover, in archaeological woods we observed an increase in the relative abundance of oxidized units such as acetovanillone (42), guaiacylacetone (45), propiovanillone (47), syringylacetone (62), and propiosyringone (63). A similar behaviour has been observed for the enzymatic degradation of wood [23].

4. Conclusions

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 fingerprint 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. The comparison of DE-MS results with those obtained by Py-GC/MS suggests that DE-MS can be used as a fast screening method to select subgroups of samples to be submitted to further analysis.

The results also confirm the value of Py-GC/MS as a tool for shedding light on the chemical modifications of wood macromolecules in archaeological objects. In particular, waterlogged wood from the site of Pisa San Rossore has been shown to undergo an extensive loss of polysaccharides. Pyrolysis also highlighted the partial demethylation of lignin units, both guaiacyl and syringyl monomers. In fact, small but not negligible amounts of catechols and methoxy catechols were identified among the various pyrolysis products of waterlogged wood samples. Further research on the structural and chemical modifications occurring in archaeological wood is needed in order to better understand these complex processes. In addition, the use of techniques that enable us to better evaluate the molecular weight and possible depolymerisation of lignin could contribute to a more complete picture of the chemical modifications in archaeological wood.

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