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Potential of pyrolysis-GC–MS molecular fingerprint as a proxy of Modern Age Iberian shipwreck wood preservation

Mohamed Traoré[a,*], Joeri Kaal[a,b], Antonio Martínez Cortizas[a]

[a] Ciencia do Sistema Terra, Departamento de Edafoloxía e Química Agrícola, Universidade de Santiago de Compostela, Campus Sur s/n, Santiago de Compostela 15782, Spain

[b] Instituto de Ciencias del Patrimonio (Incipit), Consejo Superior de Investigaciones Científicas (CSIC), San Roque 2, Santiago de Compostela 15704, Spain

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ABSTRACT

Even though pyrolysis in combination with gas chromatography and mass spectrometry (Py-GC–MS) is widely used for molecular characterization of wood, its abilities to determine the taxonomy (species), provenance and the nature and intensity of degradation of archaeological woods are hardly explored. We performed principal component analysis (PCA) on Py-GC–MS data of sound woods and shipwreck woods of *Pinus* sp. and *Quercus* sp., to identify the impact of diagenesis on pyrolysis fingerprints. It was found that the proportion of most polysaccharide products decreased significantly upon diagenesis with the exception of 3-hydroxy-2-methyl-2-cyclopenten-1-one, which remains relatively well preserved. Furthermore, the guaiacyl lignin products were generally well preserved with the exception of 4-propylguaiacol, the relative contribution of which decreased considerably. New indices are proposed to establish the preservation state of shipwreck wood (shipwreck wood preservation index; SWPI) on the basis of polysaccharides ($SWPI_{PS}$) and guaiacyl lignin ($SWPI_{LG}$) and syringyl lignin ($SWPI_{LS}$) fingerprints. Stepwise multiple linear regressions analyses applied on FTIR data of the same samples are indicative of the consistency of both techniques and the potential to identify changes in wood chemistry as a result of degradation. Other factors that influence wood composition, such as the differences between soft- and hardwood lignin and sap- and heartwood were also recognized.

1. Introduction

One of the main challenges in archaeological research of shipwrecks is the identification of the tree species used as timber and its growing location, i.e. provenance [1,2]. The microscopic and dendrologic analyses of shipwreck wood have limited capacities to solve these issues, because of the large anatomical variation between woods of different species, individuals of the same species or even different anatomical sections of the same tree [3,4]. Organic geochemical approaches are beginning to be of significant aid in the analysis of archaeological wood materials [5,6].

The composition of arboreal wood depends mainly on tree taxonomy and environmental factors of the growing location [7–10]. For example, lignin of softwood trees (gymnosperms) is composed of guaiacyl units whereas that of hardwood (angiosperms) contains both guaiacyl and syringyl moieties [11–13]. Latewood production in annual ring is one of the consequences observed due to the environmental factors [7,8]. After death, the nature and intensity of the degradation processes depends primarily on the storage conditions, which control the availability of oxygen and the composition of the wood-scavengers community [14,15]. In case of archaeological and historical woods, degradation can often be recognized on the molecular level, even if the morphological appearance remains unaltered [16,17]. The intensity of degradation depends heavily on storage conditions (e.g. open air storage in buildings, burial in sediments and soils, aerobic/anaerobic conditions, etc.) and time lapse. Numerous studies focused on understanding wood chemical changes (e.g. [18–20]), showing that archaeological woods are subjected to biological agents such as fungi, bacteria and insects. More specifically, under aerobic conditions fungi are usually the most important decomposers whereas under anaerobic conditions, bacteria are more significant [21–23]. In archaeological woods, cellulose and hemicellulose are typically more strongly affected than lignin compounds due to bacterial decomposition under anaerobic conditions [17,24–27].

Pyrolysis in combination with gas chromatography and mass-spectrometry (Py-GC–MS) is among the most frequently used techniques for wood studies due to its ability to provide details on the molecular structure of lignocellulose [18,19,28–33]. Py-GC–MS uses thermal degradation in an inert atmosphere (pyrolysis) in combination with chromatographic separation and spectrometric identification of

* Corresponding author.
E-mail addresses: mohamed.traore@usc.es (M. Traoré), joeri.kaal@usc.es (J. Kaal), antonio.martinez.cortizas@usc.es (A. Martínez Cortizas).

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pyrolysis products, in order to assess the molecular composition of intractable materials such as synthetic and natural organic polymers. It has been used to characterize lignin in grass, softwood and hardwood according to distinctive features of the phenylpropane units [12,34–37]. It can also be used to distinguish species of the same genus, at least in the case of sound wood, such as several white oak species [38]. Py-GC–MS also showed the similarity of the lignin structure in bark and xylem and chemical differences between sapwood and heartwood [39,40]. This implies that Py-GC–MS has a potential to identify the wood type (species), source (in terms of plant anatomy) and perhaps growing location of archaeological wood when morphological features inhibit identification at the species level [4]. However, there are few studies on the application of Py-GC–MS to archaeological woods. In order to be useful for species identification and determination of provenance, Py-GC–MS fingerprints need to be "corrected" for the molecular signature imposed by diagenesis. Py-GC–MS was successfully applied on several shipwreck woods to assess the impact of diagenesis on the molecular compositions [25,33,41]. Furthermore, wood specimens from angiosperms and gymnosperms from different waterlogged environments were characterized by Py-GC–MS and showed that lignin was less affected by degradation than polysaccharides [42,43]. Also, delignification of eucalypt wood by basidiomycetes was described by this technique [44]. Interestingly, some studies using Py-GC–MS showed that inorganic chemicals from the marine environment, or from wood preservation agents, could interfere during pyrolysis [45,46]. Therefore, chemicals used for preservation purposes should be considered because they may affect wood chemistry [47–49].

In the present study we applied Py-GC–MS to samples of both living trees and shipwreck wood of a softwood (*Pinus* sp.) and hardwood (*Quercus* sp.). The main objective was to provide better insights on the influence of degradation on archaeological shipwreck wood composition and designate proxies of wood decay. For that purpose, we applied principal component analysis (PCA) on semi-quantitative Py-GC–MS data. Stepwise multiple linear regressions were also applied on infrared spectroscopic data in a view to support the result obtained by Py-GC–MS.

2. Materials and methods

2.1. Description of samples

We used wood fragments from two shipwrecks sampled by nautical archaeologists of the ForSEAdiscovery project (http://forseadiscovery.eu/), in June 2015. The two Iberian shipwrecks were dated to the period between the 16th and the 18th century: the *Magdalena* wrecked near Viveiro, and an unnamed galleon in the bay of Ribadeo, both in the north of Spain. Four *Pinus* sp. wood fragments are from the *Magdalena* wreck (MAG02, MAG03, MAG15 and MAG16). A *Quercus* sp. sample was also collected from the *Magdalena* wreck (MAG10). Two other oak samples were collected from the shipwreck obtained in the bay of Ribadeo (RIB08 and RIB10). The wood fragments were oven-dried for two weeks at 30 °C and then their surfaces were clean-cut in order to visualize tree ring patterns. The samples were not subjected to chemical treatments.

Woods from living pine and oak were also studied. Four *Pinus nigra* cores collected in the south of Spain in the Cazorla mountain (L113C, L17C, L311A and L34D), two *Pinus nigra* cores in La Sagra mountains (LSA16, LSA19) and two *Pinus sylvestris* cores collected in the north of Spain in the Artikutza Park (PS01, PS02). The oak samples (*Quercus robur*) were collected in the north of Spain in the Artikutza Park (AZK-01) and near Oiartzun (OIR-P2-01 and OIR-P2-04). Wood cores were retrieved using an increment borer, at breast height. Those samples were also dried and their surfaces cleaned.

Analyses were done on consecutive positions from the outer (recent tree ring: sapwood of sound woods) to the inner part (older tree rings: heartwood of sound woods).

2.2. Pyrolysis-GC–MS measurement

Pyrolysis–GC–MS was performed on wood chips sampled from different tree rings from the sapwood and heartwood sections, for both shipwreck wood and sound wood. It is preferred to work with small fragments (< 1 mg) of wood in order to avoid losing sample material and to avoid analysis of rays instead of xylem. Samples were embedded in quartz wool in quartz tubes. Pyrolysis was performed using a CDS 5250 at 650 °C for 20 s (10 °C ms^{-1} heating rate). Pyrolysis products were swept online into a 6890 N gas chromatograph (Agilent Technologies) using He as the carrier gas (1 ml min^{-1}), and a HP-5MS column (length 30 m; internal diameter 0.25 mm; film thickness 0.25 mm). The GC temperature program was from 50 °C (initial temperature) to 325 °C (final temperature, 5 min dwell time), at a rate of 20 °C min^{-1}. The pyrolysis products were identified using an Agilent 5975 mass spectrometer operating in 70 eV electron impact (*m/z* 50–500). Compound identifications were based on the NIST '05 library as well as on mass spectral data and retention time comparisons reported in literature. Quantification of pyrolysis products was based on the peak area of dominant fragment ions. The relative proportions of each pyrolysis product were calculated as the percentage of the sum of all peak areas, i.e.% of total quantified peak area (TQPA).

2.3. Data analysis

We applied principal component analysis (PCA) to the whole dataset (PCA_{All}) in order to extract information related to chemical composition, using the relative proportions of the pyrolysis products as variables. We also applied individual PCA on datasets of *Pinus* sp. (PCA_{Pine}) and on *Quercus* sp. (PCA_{Oak}) to obtain specific information from each wood species. The PCA were performed on the correlation matrix. We applied One-way ANOVA to assess the significance ($P < 0.05$) of the observed differences. For the ANOVA test, the number of observations was 24 and 9 for pine and oak sound wood, respectively, and 16 and 9 for pine and oak archaeological woods, respectively. All the statistical analyses were performed using SPSS 20.

Additionally, all the samples were analysed by Fourier-transform infrared spectroscopy in attenuated total reflectance mode (FTIR-ATR). The instrument and method is described in detail in our previous study [50]. Stepwise multiple linear regression (MLR) analysis was applied to the FTIR data to fit the indices of preservation calculated from the Py-GC–MS data (see below).

3. Results and discussion

3.1. Pyrolysis products of sound and archaeological pine and oak wood

Examples of total ion chromatograms corresponding to pine and oak samples are shown in Fig. 1. The pyrolysis products with their ion fragments (*m/z*) that were used for the semi-quantification are listed in Appendix A. The samples produced predominantly derivatives of polysaccharides (cellulose and hemicellulose) and lignin (guaiacyl and syringyl lignins). For the pine samples, especially the heartwood, large proportions of diterpenoid resin products were recorded. Several smaller peaks for mono- and sesquiterpenoid resin compounds were also identified in pine samples. As expected, no peaks were identified for syringyl lignin in pine samples while in oak samples we identified several syringyl compounds.

3.2. Principal component analysis (PCA_{ALL}) with all samples: general composition of wood

The three first factors from the PCA_{ALL} explain 66% of the total variance. From the factor scores, it appears that the first factor ($PC1_{ALL}$) separates pine (softwood) and oak (hardwood) samples (Fig. 2a). The second factor ($PC2_{ALL}$) mainly separates heartwood and sapwood in

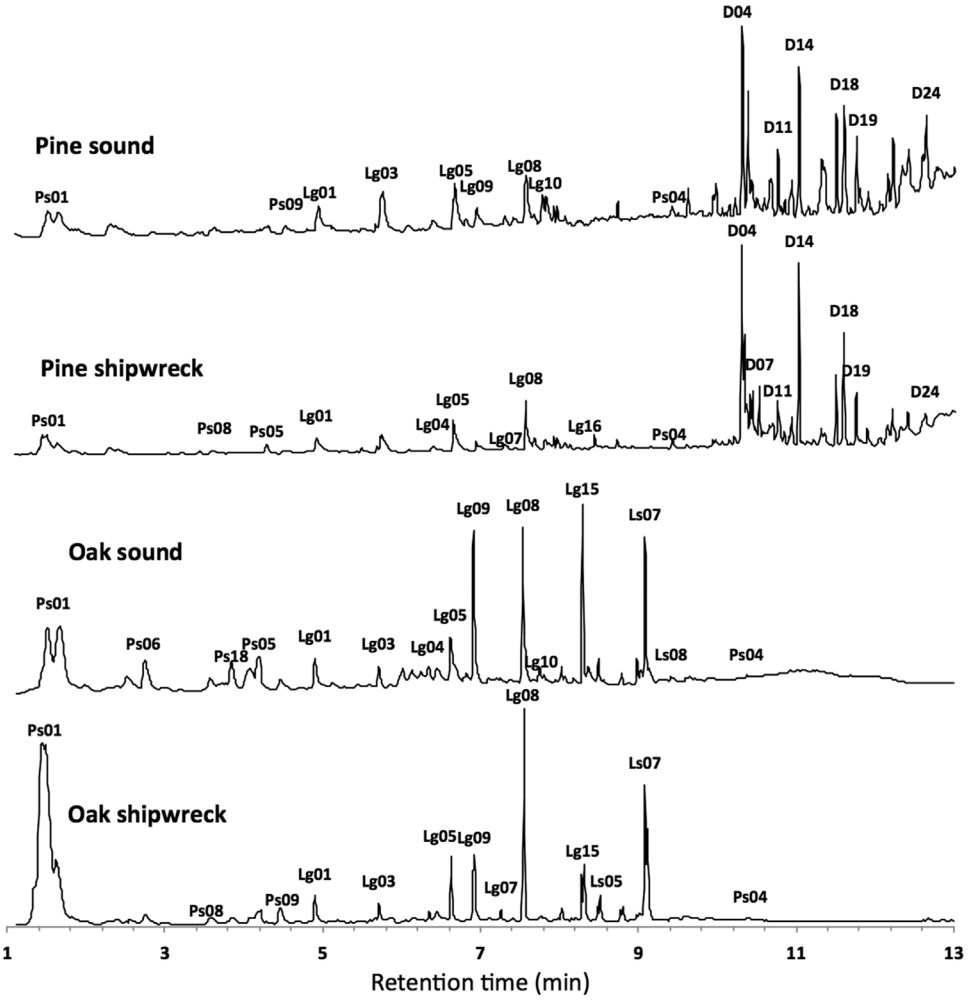

Fig. 1. Example total ion chromatograms of pine and oak wood (sound and shipwreck samples). Peak labels refer to the pyrolysis product list (Appendix A); Ps for polysaccharide products, Lg for guaiacyl lignin, Ls for syringyl lignins and D for diterpene products.

pine samples (Fig. 2b), and the third factor ($PC3_{ALL}$) separates sound woods and archaeological woods (Fig. 2c), as discussed in detail in the following sections.

3.2.1. PCA factor for softwood and hardwood distinction

The first factor ($PC1_{ALL}$ with 39% of the total variance) displayed two groups of samples (Fig. 2). Positive scores characterized the pine samples (sound and archaeological), while negative scores characterized the oak samples (sound and archaeological). Hence, the compounds with strong positive loadings such as many guaiacyl lignin products, several polysaccharides and all resin compounds (Table 1) are more abundant in pine samples, whereas compounds with negative loadings (essentially syringyl products and polysaccharide products) were associated with oak samples. $PC1_{ALL}$ highlights the differences between pine (softwood) and oak (hardwood) wood samples.

Lignin composition, and in particular the ratio of syringyl to guaiacyl products (S/G) is the most frequently used parameter to differentiate between softwood (guaiacyl lignin) and hardwood (guaiacyl as well as syringyl lignin) [12,51]. This differentiation is not affected by the possible diagenetic state of archaeological shipwreck woods [18], because at least some of the syringyl lignin is preserved. The S/G ratio is zero for all pine samples while it is 2.0 ± 0.5 for the sound oak samples in comparison with 1.7 ± 0.4 for archaeological oak wood. According to literature, an incipient selective decomposition of syringyl lignin may cause a decline in the S/G ratio of archaeological wood in comparison with sound wood [22,24,44,52]. In our study, the difference in the S/G ratio was not significant ($P > 0.05$).

Regarding the polysaccharides fingerprints, pine wood samples are characterized by positive loadings for 5-hydroxymethyl-2-dihydrofuraldehyde-3-one (Ps02), which was not found in the oak samples. On the other hand, negative loadings of polysaccharide products Ps16, Ps07, Ps12, Ps18, Ps17, Ps10, Ps15, Ps05, Ps11, Ps06, Ps14, and Ps13 show that these are relatively abundant in oak wood samples. These differences may be associated with the difference in hemicellulose structures: the hemicellulose of hardwood is mainly composed of xylan moieties whereas in softwood species it is mainly mannan hemicellulose [14,53].

Most resin products were identified in the pyrolyzates of pine samples but not of oak samples, which is reflected by the positive loadings of several mono- and diterpene resin products. Indeed, the resin compounds (mono- and diterpenes) are wood extractive compounds, which in general are particularly found in higher proportion in softwood [5,36,54–58].

3.2.2. PCA factor for sapwood and heartwood differentiation

$PC2_{ALL}$ explains 20% of total variance. The pine heartwood samples presented high positive scores, the pine sapwood samples presented high negative scores and the oak samples presented low positive scores (Fig. 2). Resin compounds with positive loadings (Table 1) are mainly associated with pine heartwood samples. This factor highlights the differences between pine sapwood and heartwood due to the higher abundance of resinous substances in the heartwood [39,59]. In general softwood resin canals are filled by mono- and diterpernoid resins. The latter are particularly abundant in heartwood [60].

Negative loadings were found for 2,3-dihydro-5-methylfuran-2-one (Ps08) and 4-2-propenyl-guaiacol (*trans*) (Lg08) (Table 1), suggesting that also the polysaccharide and lignin fingerprints are different for

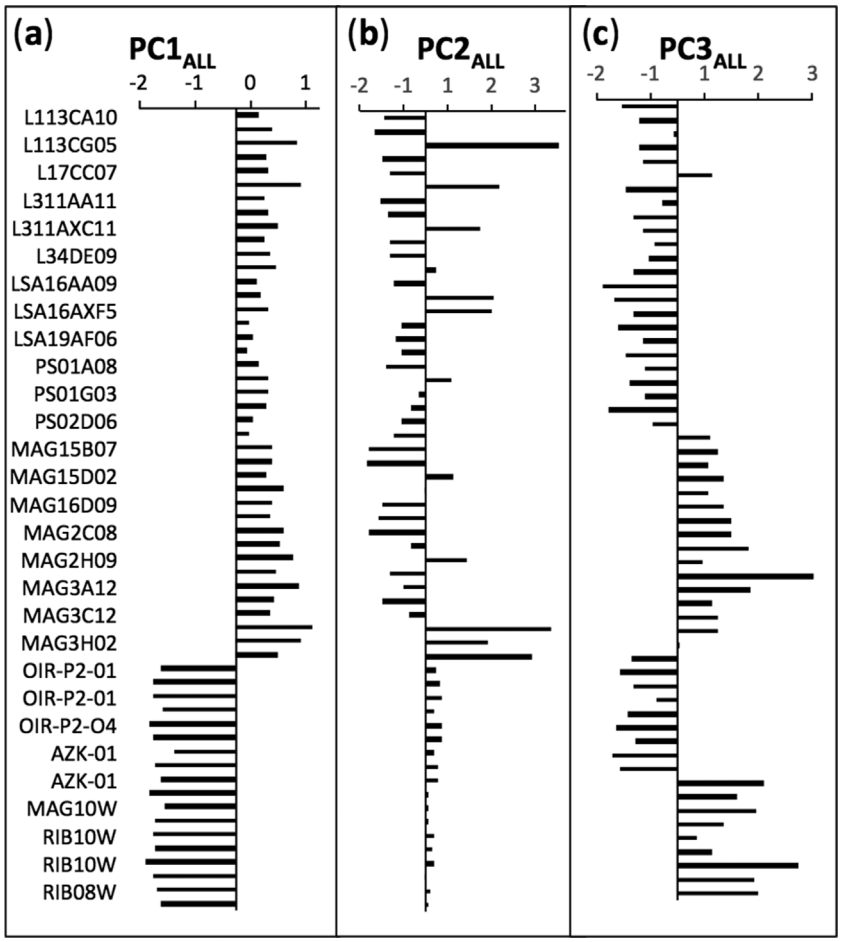

Fig. 2. Component scores of the three extracted factors from the PCA applied to all wood samples.

heart- and sapwood. They are in higher proportion in the sapwood than in the heartwood of sound woods ($P < 0.001$) for both pine and oak (Fig. 3). However, sapwood and heartwood overlap in archaeological samples for both species so that the two sections of the wood samples are not clearly separated ($P > 0.05$). Moreover, 2,3-dihydro-5-methylfuran-2-one (Ps08) is more abundant among the pyrolyzates of sapwood than of heartwood. Contrary to polysaccharides, the pyrolysis products of lignin in the heartwood samples are influenced by the higher resin content. The number of samples is insufficient to unambiguously conclude that there is a difference between polysaccharide and lignin composition between sap- and heartwood, but has been documented previously [39,51,59].

3.2.3. PCA for sound and archaeological wood differentiation

$PC3_{ALL}$ (7% of variance) discriminates between sound (negative scores) and archaeological (positive scores) woods (Fig. 2). The positive loadings of the 3-hydroxy-2-methyl-2-cyclopenten-1-one (Ps09) and 2/3-methylguaiacol (Lg02) (Table 1) associate these compounds with the shipwreck samples, while levoglucosan (Ps04) and 4-propylguaiacol (Lg10), with negative loadings (Table 1), are associated with sound wood samples.

In our study, 3-hydroxy-2-methyl-2-cyclopenten-1-one (Ps09) appeared useful to distinguish between sound and archaeological woods for both pine and oak. The proportion of this compound of the sum of all polysaccharide products (see Fig. 4a) in both types of wood is significantly higher ($P < 0.001$) in archaeological samples than in sound wood samples. Similar results have been found by Łucejko et al. [33] and Heigenmoser et al. [61] related to 3-hydroxy-2-methyl-2-cyclopenten-1-one (Ps09), which suggests that it originates from a polysaccharide precursor that is relatively well preserved during decay (see also [18]). 2/3-methylguaiacol (Lg02) also allows to differentiate between sound and archaeological wood, but a significant difference was only found for pine samples (Fig. 4b): the proportion of this compound of the sum of guaiacyl products in the pine woods is significantly higher ($P < 0.001$) in archaeological than in sound samples. 2/3-methylguaiacol (Lg02) has been found in lower abundance in recent wood than in archeological pinewood [33]. Hence, the relative proportion of Lg02 could be a proxy of archaeological wood preservation. There is a strong correlation between 3-hydroxy-2-methyl-2-cyclopenten-1-one (Ps09) and 2/3-methylguaiacol (Lg02) ($r = 0.76$ for pine samples; and $r = 0.67$ for oak samples).

Negative loadings for levoglucosan (Ps04) and 4-propylguaiacol

Table 1
List of compounds with loadings above 0.5 on factors 1, 2 and 3 (for compound labels, please refer to Appendix A).

Loadings	$PC1_{ALL}$	$PC2_{ALL}$	$PC3_{ALL}$
Positive	Lg06, M05, Lg16, M02, Ps02, Lg03, Lg14, Lg07, Lg09, Lg05, Lg04, D08, Lg15, Lg01, Lg12, Lg13, Lg11, D07	D17, D23, D14, D18, M04, D16, S01, D21, S02, D20, D22, D09, M07, M06, M01, S05, D24, D04, D13, D02, D05, D03, D19	Lg02, Ps09
Negative	Ls12, X02, Ls04, Ps16, X01, Ls10, X04, Ps07, Ps12, Ps18, Ls08, Ps17, Ls03, Ps10, Ps15, Ls05, Ls01, Ls09, Ps05, Ps11, Ls06, Ps06, Ps14, Ls07, Ls02, Ps13	Ps08, Lg08	Ps04, Lg10

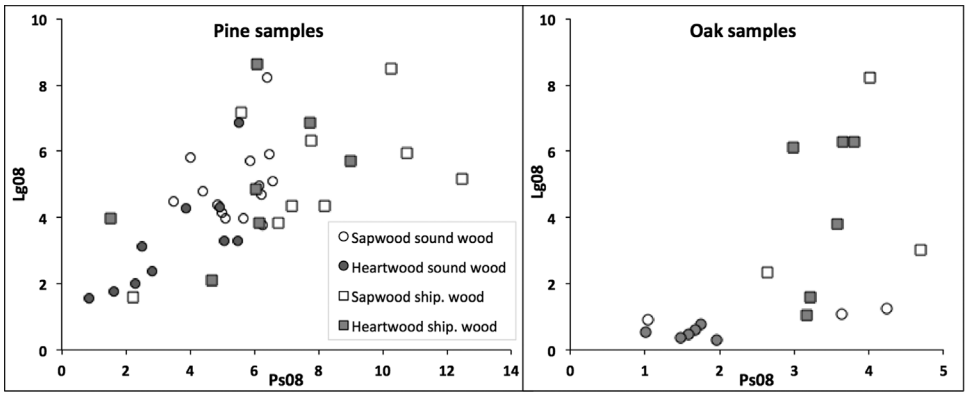

Fig. 3. Scatter plot of the relative proportions of Ps08 (2,3-dihydro-5-methylfuran-2-one) and Lg08 (4-2-propenyl-guaiacol (*trans*)) of sapwood and heartwood samples from pine and oak.

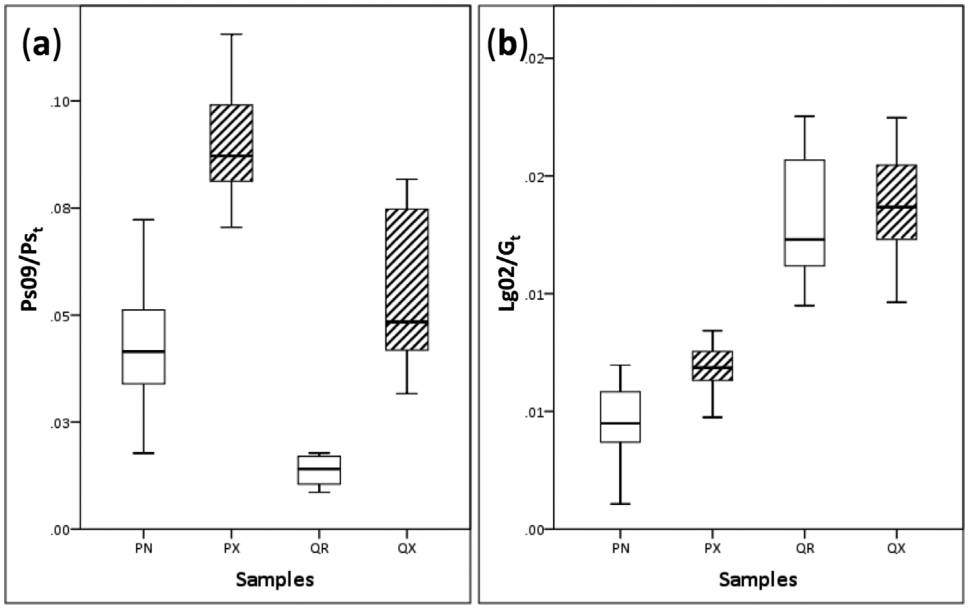

Fig. 4. Boxplot of polysaccharide ($Ps09/Ps_t$) and guaiacyl lignin ($Lg02/G_t$) ratios to differentiate between sound (pine: PN, oak: QR) and archaeological (pine: PX, oak: QX) samples.

(Lg10) suggest that they become depleted upon increased impact of degradation in archaeological samples. The polysaccharide ($Ps04/Ps_t$) and lignin ($Lg10/G_t$) ratios (Fig. 5) show that Ps04 and Lg10 are depleted relative to total polysaccharide and lignin products, respectively, as well. This is in agreement with literature regarding the relatively low abundance of polysaccharides, in particular, levoglucosan (Ps04) in archaeological wood [6,22,33,41]. Colombini et al. [62] showed that lignin compound Lg10 (4-propylguaiacol) has lower abundance in archaeological waterlogged samples (softwood and hardwood species). It is concluded that, $PC3_{ALL}$ represents the effects of diagenesis on both lignin and polysaccharides in archaeological woods. A prolonged storage of wood material underwater implies swelling of the secondary cell walls, which is favourable to the hydrolysis of carbohydrates [15,42]. According to previous studies, this degradation process happens simultaneously on the whole wood piece.- However, depending to the burial conditions, the mechanism and the rate of degradation can be different for individual pieces due to differences in anatomical structure, permeability and chemical composition [63]. Bacteria also play a significant role on the degradation of waterlogged woods. These microbial agents preferentially attack polysaccharides over lignin [64]. Furthermore, polysaccharide hydrolysis does not require molecular oxygen whereas oxidative enzymes are necessary for the degradation of lignin [16].

It appeared that the PCA on the basis of all samples shows some obvious differences between softwood and hardwood ($PC1_{ALL}$) and sapwood and heartwood ($PC2_{ALL}$), and that only 7% of the variance ($PC3_{ALL}$) is related to shipwreck *vs.* living wood. In order to better understand the changes in molecular structure of the wood in the shipwrecks, we performed additional PCA of the pine and oak samples separately (to eliminate the variation associated with $PC1_{ALL}$) and, in case of pine samples, we also recalculated the% TQPA data omitting the resin compounds and therewith the influence of differences between heartwood/sapwood ($PC2_{ALL}$). Furthermore, these resin compounds are severely overrepresented in the pyrolysis chromatograms and do not reflect pyrolytic fragments of the macromolecular backbone of wood (lignocellulose) but instead evaporation products of volatile/extractive constituents. The application of separate PCA for the pine and oak series is also desirable as the sample number of each group is different and as a kind of independent control for consistency in diagenetic effects on wood composition.

3.3. PCA of Py-GC–MS data

The PCA of the pine series reveals one dominant factor ($PC1_{PINE}$) that explains 43% of variance and that clearly differentiates between sound (negative scores) and shipwreck (positive scores) woods (Fig. 6a). For the PCA of the oak samples, a similar observation can be made, with $PC1_{OAK}$ explaining 43% of the variance and also differentiating between sound (negative scores) and shipwreck (positive scores) woods (Fig. 6b). The dominance of variation associated with living and/or dead woods is not a surprise, because we removed the variation associated with gymnosperms *vs.* angiosperms and that of heartwood *vs.* sapwood. More interestingly, the PC1 loadings of these two independent datasets are highly correlated ($r = 0.99$; $P < 0.001$),

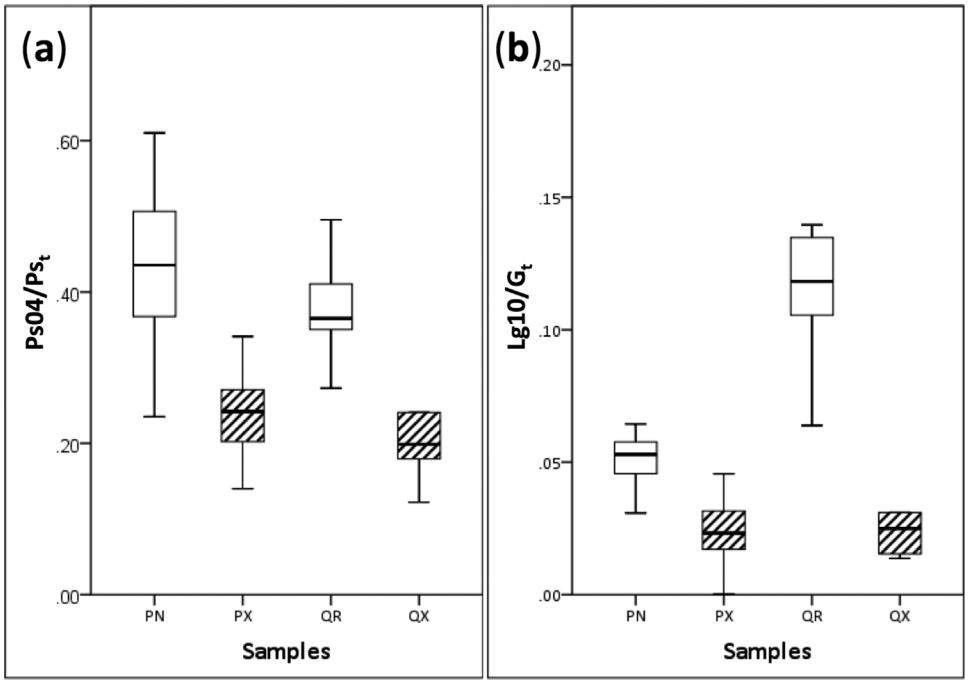

Fig. 5. Boxplot of polysaccharide ($Ps04/Ps_t$) and guaiacyl lignin ($Lg10/G_t$) ratios to differentiate between sound (pine: PN, oak: QR) and archaeological (pine: PX, oak: QX) samples.

suggesting that $PC1_{PINE}$ and $PC1_{OAK}$ reflect the same lignocellulose alteration process in these two different wood species. The loadings of the individual PCA allowed the identification of common diagenesis marker for pine and as well as for oak samples. The degradation process produced a relative increase (with positive loadings) of Lg01, Lg02, Lg04, Lg05, Lg07, Lg08, Lg09, Ps08 and Ps09; and a relative decrease (with negative loadings) of Lg10, Ps04 and Ps05. On the basis of the relative abundances of these pyrolysis products we can calculate an index of wood preservation, i.e. Shipwreck Wood Preservation Index (SWPI).

We propose two SWPIs that reflect the preservation state of polysaccharide ($SWPI_{PS}$) and guaiacyl lignin ($SWPI_{LG}$) in archaeological woods. They are strongly correlated for the pine samples ($R^2 = 0.74$; Fig. 7a) and for the oak samples ($R^2 = 0.73$; Fig. 7b):

$$SWPI_{PS} = \frac{Ps04 + Ps05}{Ps04 + Ps05 + Ps08 + Ps09} \quad (1)$$

$$SWPI_{LG} = \frac{Lg10}{Lg01 + Lg02 + Lg04 + Lg05 + Lg07 + Lg08 + Lg9 + Lg10} \quad (2)$$

$SWPI_{PS}$ (Eq. (1)) showed a significant difference between sound and archaeological woods for both species (Fig. 8a). For the pine shipwreck samples, $SWPI_{PS}$ is strongly correlated ($n = 16$, $R^2 = 0.87$; Fig. 9a) with one of the most commonly used ratios of polysaccharide degradation from Py-GC–MS fingerprints (levoglucosan/total carbohydrate markers: $Ps04/Ps_t$) [65,66], which provides support for $SWPI_{PS}$ and suggests that polysaccharide decomposition in shipwrecks proceeds according to similar reactions as decay in soils and sediments. The relation is much less explicit for archaeological oak samples ($n = 9$, $R^2 = 0.55$; Fig. 9a), but this may be associated with the smaller sample number.

$SWPI_{LG}$ (Eq. (2)) also showed a significant difference between sound and archaeological wood for both species (Fig. 8b), suggesting that lignin preservation in archaeological shipwrecks is reflected by the relative abundance of 4-propylguaiacol. Alternatively, the ratio of the propenyl guaiacols to the total guaiacyl lignin content (C_3G/G_t) has been used to assess the rate of degradation of guaiacyl lignin contents of wood [66–68]. In our study, this ratio showed no significant difference between sound and shipwreck pine wood and a no correlation was found between C_3G/G_t and $SWPI_{LG}$ ($R^2 = 0.11$; Fig. 9b). Even though for oak samples the C_3G/G_t ratios of shipwreck wood were significantly lower than for sound wood, and a significant correlation was found between C_3G/G_t and $SWPI_{LG}$ ($R^2 = 0.68$; Fig. 9b), our results suggest that $SWPI_{LG}$ could be more suitable for the assessment of shipwreck wood decay than C_3G/G_t.

For comparison, we calculated the SWPI of syringyl lignin ($SWPI_{LS}$) (Eq. (3)) for oak. The strong correlations (Fig. 10) between $SWPI_{LS}$ and $SWPI_{PS}$ and $SWPI_{LG}$ imply that the preservation state of syringyl lignin is also reflected by the relative abundance of the propyl-substituted syringol Ls09. The lower proportion of Ls09 in degraded wood is probably associated with alkyl side chain modification, which in term is related to disruption of the mainly β-O-4 bonds between lignin monomers and side-chain shortening [65,69]. Apart from 4-propylsyringol, 4-methylsyringol (Ls02) seemed to be preferentially depleted upon degradation but, after more detailed inspection, it appeared that Ls02 was affected by co-elution with traces of vanillic acid (also *m/z* 153 + 168), which is why only 4-propylsyringol is used for proxy calculation.

$$SWPI_{LS} = \frac{Ls09}{Ls01 + Ls03 + Ls04 + Ls05 + Ls06 + Ls07 + Ls09 + Ls10} \quad (3)$$

Finally, we calculated the ratios of two products that probably originate from demethylated syringyl units, i.e. 3-methoxycatechol (X01) and 5-methyl-3-methoxycatechol (X02) [70]. These compounds, and especially X01, were correlated with $SWPI_{LG}$ ($R^2 = 0.32$) and $SWPI_{LS}$ ($R^2 = 0.39$), but their proportions relative to total syringols were not. Thus, even though the pyrolysis fingerprints show that decay causes some demethylation of syringyl lignin, this reaction has a smaller effect on lignin composition than the propanoid side chain alterations reflected by $SWPI_{LG}$ and $SWPI_{LS}$.

3.4. Effects of degradation on infrared spectra of shipwreck wood

The FTIR spectra of the two wood types share 16 bands (Appendix B). Stepwise multiple linear regressions (MLR) were applied to the peak absorbances of these bands in order to identify those that fit the SWPIs. Appendix C shows the original indices calculated from the Py-GC–MS plotted against the indices predicted using FTIR.

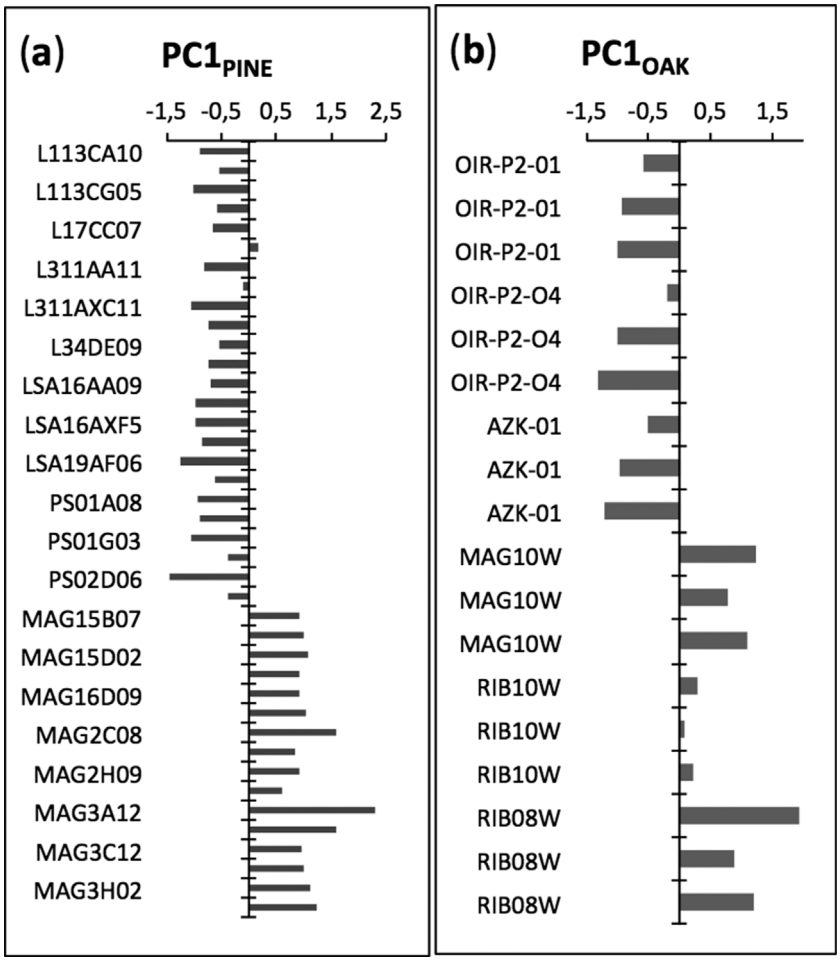

Fig. 6. Component scores of the first extracted factor from the individual PCA to pine ($PC1_{PINE}$) and oak ($PC1_{OAK}$) samples.

The statistical summaries of the stepwise MLR analyses are shown in Table 2. The bands selected by the regression model are related to molecular bond vibrations of polysaccharide and lignin structures. The bands at 895, 1155, 1315 and 1730 cm^{-1} are assigned to C═O, C—H, C—O—C and C—O deformation or stretching vibrations of different groups in carbohydrates [71,72]. The bands at 1225, 1420, 1510 and 1590 cm^{-1} are related to C═C, C—O, C—H, C—O stretching or bending vibrations of different groups primarily from lignin [71,72]. The MLR models explain between 62 and 81% of the variance in SWPIs (R^2 = 0.62–0.81; Table 2). The reasonably good fit of the MLR obtained from FTIR is indicative of the consistency of both techniques and their potential to identify changes in wood chemistry as a result of degradation. Infrared spectroscopy has been used previously in studies of archaeological wood to support results from other methods [6,73], because it can also provide information on the physical state of wood. For example, it has been shown that the preferential elimination of amorphous phases of polysaccharide and lignin compounds occurs at the structural level [74,75].

The proposed indices in this study could be useful to evaluate the state of preservation of wood materials retrieved from other shipwrecks of the same time period. In an upcoming work the SWPIs will be applied to a large number of Iberian shipwreck woods using Py-GC–MS and thereby, expectedly, isolate the signal of oak species and source area, in an attempt to improve provenance studies in the framework of the ForSEAdiscovery project.

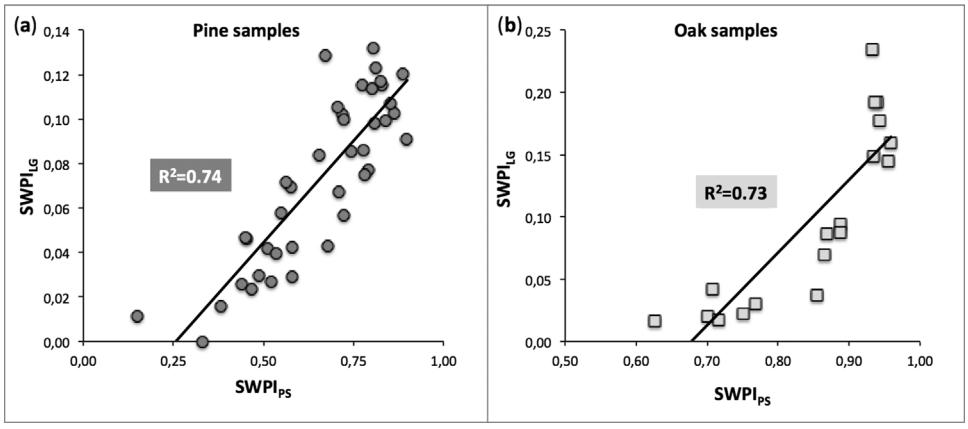

Fig. 7. Scatter plot of the wood preservation indices ($SWPI_{PS}$ and $SWPI_{LG}$) for pine (a) and oak (b) samples.

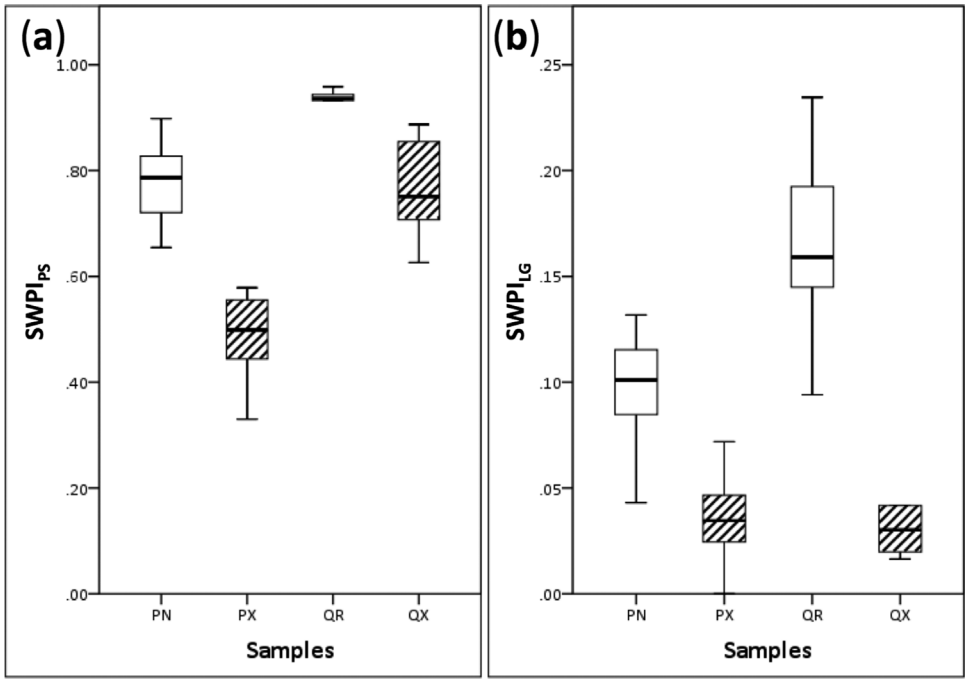

Fig. 8. Boxplot of polysaccharide ($SWPI_{PS}$) and lignin ($SWPI_{LG}$) indices for wood preservation for sound (pine: PN, oak: QR) and archaeological (pine: PX, oak: QX) samples.

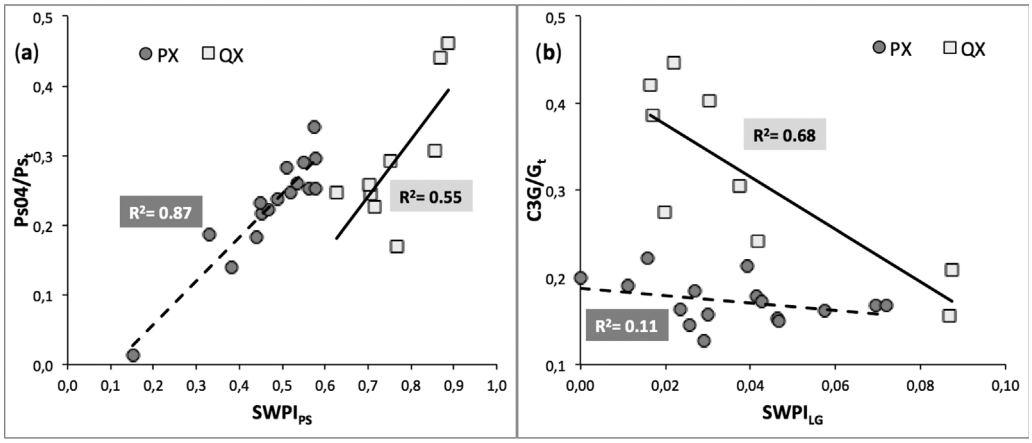

Fig. 9. Comparison of the shipwreck wood preservation indices ($SWPI_{PS}$ and $SWPI_{LG}$) and alternative ratios used in lignocellulose degradation studies (a: $Ps04/Ps_t$ and b: C_3G/G_t) for pine (PX) and oak (QX) shipwreck samples: $C_3G = Lg06 + Lg07 + Lg08$.

4. Conclusions

This study highlights the usefulness of principal component analysis on Py-GC–MS data to characterize different types of wood samples. Besides the difference in lignin structure related to the guaiacyl and syringyl units, our study shows differences between pine and oak samples on the basis of their polysaccharide fingerprints. The degradation processes detected for the archaeological samples seem to have a similar effect on pyrolysis fingerprints for pine and oak shipwreck wood samples. Diterpene resin compounds were identified in

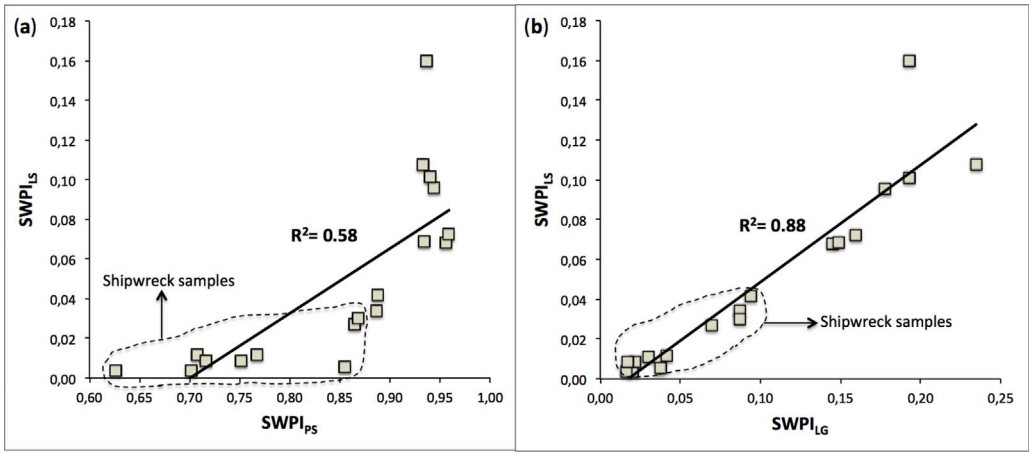

Fig. 10. Relationship between the polysaccharide and guaiacyl lignin based indices ($SWPI_{PS}$ and $SWPI_{LG}$) and syringyl lignin products based index ($SWPI_{LS}$) for oak samples.

Table 2
Statistical summaries of the stepwise multiple linear regressions analyses.

Wood species	Indices	Selected Bands (cm^{-1})			Model summaries			
		1st	2nd	3rd	R	R^2	SEP[a]	Pr (> F)
Pine	$SWPI_{PS}$	1730	1225	–	0.79	0.62	0.11	0.04
	$SWPI_{LS}$	1730	1510	895	0.79	0.63	0.02	0.03
Oak	$SWPI_{PS}$	1730	1420	–	0.82	0.67	0.06	0.04
	$SWPI_{LG}$	1420	1155	–	0.89	0.79	0.03	0.00
	$SWPI_{LS}$	1730	1315	1590	0.90	0.81	0.02	0.04

[a] Standard error of prediction.

pine archaeological samples and the higher proportion of resin in sapwood than in heartwood samples was not affected by decay. Moreover this study showed that 2,3-dihydro-5-methylfuran-2-one and 4-2-propenyl-guaiacol (*trans*) were more abundant in sapwood of living trees, but they are unable to differentiate between sapwood and heartwood in archaeological woods.

All the shipwreck samples show evidence of degradation of polysaccharides, expressed by a decline in the relative proportion of levoglucosan. However, our study shows that even if the polysaccharide contents are the most affected, 3-hydroxy-2-methyl-2-cyclopenten-1-one appears to be well-preserved in shipwreck wood after centuries of storage under marine conditions. To the contrary, the precursor of the pyrolysis product 4-propylguaiacol appears to be poorly preserved whereas guaiacyl lignins in general are usually among the well-preserved components in waterlogged wood. Pyrolysis products of polysaccharides and lignin that remain in archaeological wood enabled to propose indices to evaluate the degree of preservation of the studied shipwreck samples ($SWPI_{PS}$, $SWPI_{LG}$ and $SWPI_{LS}$).

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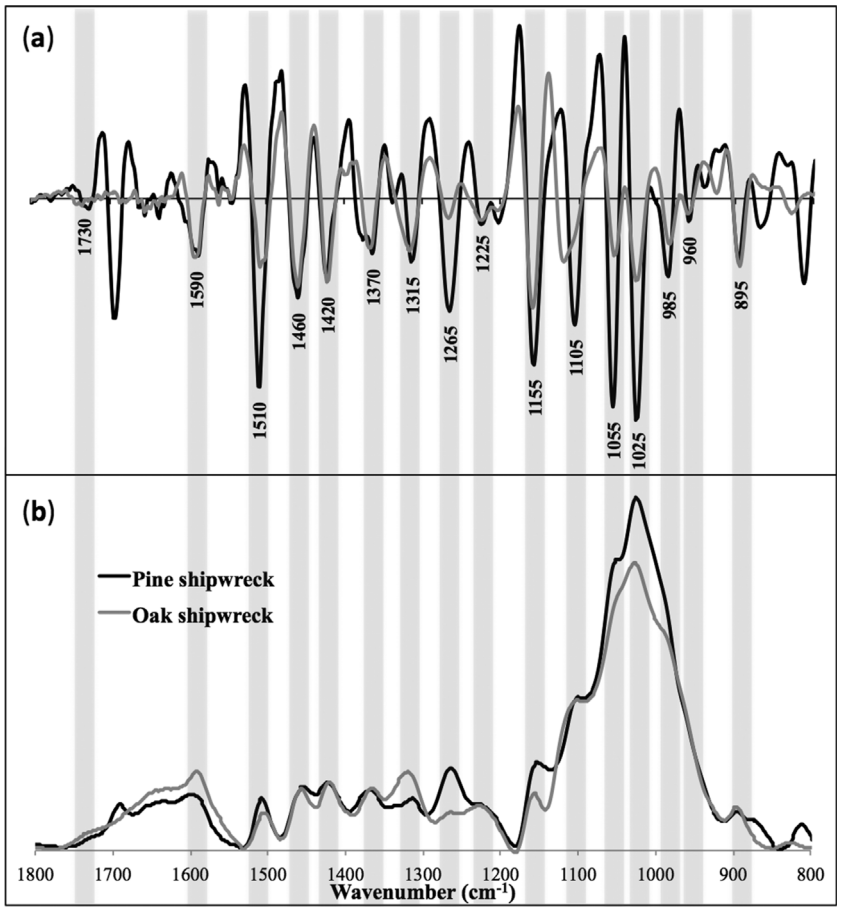
Appendix A. Pyrolysis products

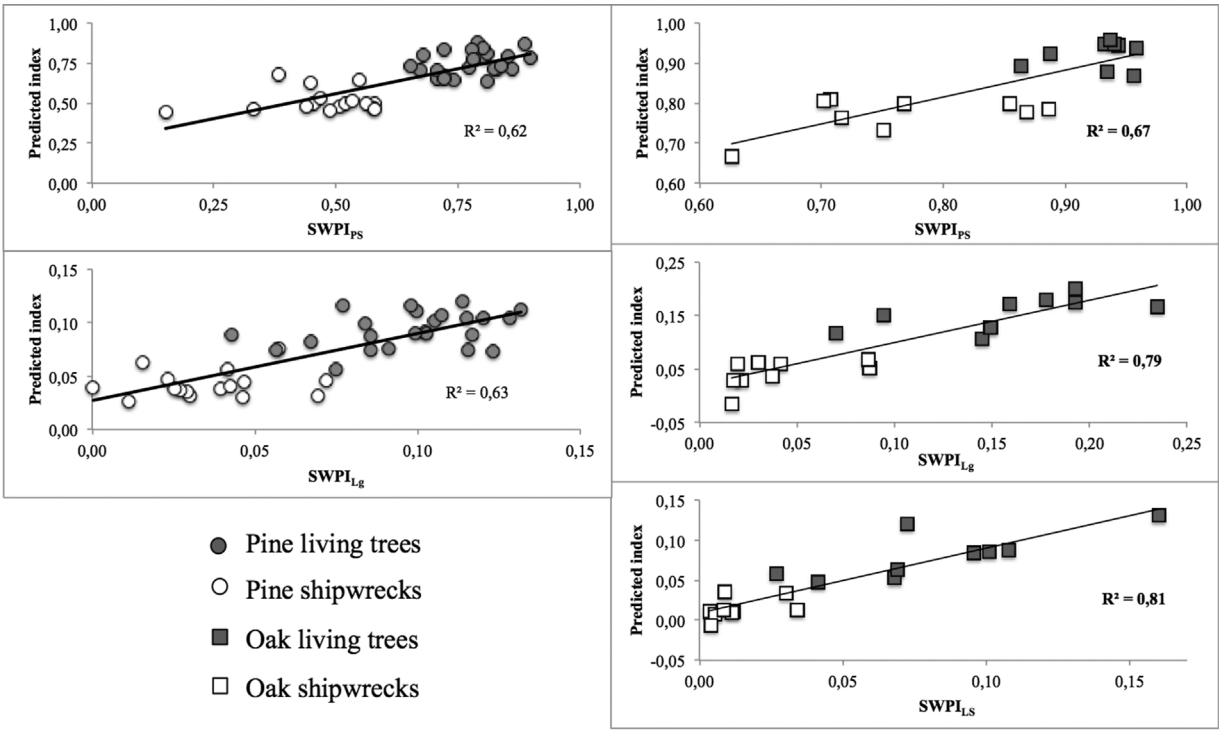
Code	Name	*m/z*	RT (Pine)	RT (Oak)	Group
Ps01	Acetic acid	60	1669	1694	Polysaccharide
Ps02	5-hydroxymethyl-2-dihydrofuraldehyde-3-one	57 + 69	5762	–	Polysaccharide
Ps03	1,4:3,6-dianhydro-alpha-D-glucopyranose	57 + 69	6042	5886	Polysaccharide
Ps04	Levoglucosan	60	9400	10,4	Polysaccharide
Ps05	4-hydroxy-5,6-dihydro-(2H)-pyran-2-one	58 + 114	4262	4185	Polysaccharide
Ps06	3/2-furaldehyde	95 + 96	2965	2758	Polysaccharide
Ps07	2-(hydroxymethyl) furan	55 + 98	–	3017	Polysaccharide
Ps08	2,3-dihydro-5-methylfuran-2-one	55 + 98	3639	3567	Polysaccharide
Ps09	3-hydroxy-2-methyl-2-cyclopenten-1-one	55 + 112	4527	4465	Polysaccharide
Ps10	Unidentified deoxyhexose	69 + 110	–	6151	Polysaccharide
Ps11	3-hydroxy-2-methyl-4H-pyran-4-one	68 + 142	–	6063	Polysaccharide
Ps12	Pyran compound	128	–	5648	Polysaccharide
Ps13	3,5-dihydroxy-2-methyl-(4H)-pyran-4-one	57 + 126	–	5284	Polysaccharide
Ps14	Furan	68	–	1512	Polysaccharide
Ps15	2-methylfuran	53 + 82	–	1673	Polysaccharide
Ps16	2,5-dimethylfuran	96	–	1979	Polysaccharide
Ps17	(2H)-furan-3-one	84 + 54	–	2514	Polysaccharide
Ps18	5-methyl-2-furaldehyde	110 + 109	–	3832	Polysaccharide
Lg01	Guaiacol	109 + 124	4957	4895	G-Lignin
Lg02	2/3-methylguaiacol	123 + 138	5627	5596	G-Lignin
Lg03	4-methylguaiacol	123 + 138	5752	5710	G-Lignin
Lg04	4-ethylguaiacol	137 + 152	6390	6353	G-Lignin
Lg05	4-vinylguaiacol	135 + 150	6696	6634	G-Lignin
Lg06	4-(1-propenyl)guaiacol	149 + 164	6934	7220	G-Lignin
Lg07	4-(2-propenyl)guaiacol (*cis*)	149 + 164	7287	7266	G-Lignin
Lg08	4-(2-propenyl)guaiacol (*trans*)	149 + 164	7552	7557	G-Lignin
Lg09	4-propylguaiacol	137 + 166	6981	6971	G-Lignin
Lg10	4-propylguaiacol	137 + 166	7754	7754	G-Lignin
Lg11	4-formylguaiacol (vanillin)	151 + 152	7401	7396	G-Lignin
Lg12	C_3H_3-guaiacol	147 + 162	7770	7770	G-Lignin
Lg13	C_3H_3-guaiacol	147 + 162	7827	7822	G-Lignin
Lg14	4-acetylguaiacol	151 + 166	8044	7983	G-Lignin
Lg15	4-propan-2-one guaiacol	137 + 180	8185	8206	G-Lignin
Lg16	Coniferyl alcohol	137 + 180	8444	–	G-Lignin
Ls01	Syringol	139 + 154	–	6919	S-Lignin
Ls02	4-methylsyringol	153 + 168	–	7666	S-Lignin
Ls03	4-ethylsyringol	167 + 182	–	8040	S-Lignin
Ls04	4-vinylsyrinngol	165 + 180	–	8315	S-Lignin
Ls05	4-(1-propenyl) syringol	194 + 179	–	8517	S-Lignin
Ls06	4-(2-propenyl) syringol (*cis*)	179 + 194	–	8792	S-Lignin

Ls07	4-(2-propenyl) syringol (*trans*)	179 + 194	–	9114	S-Lignin
Ls08	C_3H_3-syringol	172 + 192	–	9031	S-Lignin
Ls09	4-propylsyringol	167 + 196	–	9409	S-Lignin
Ls10	4-acetylsyringol	181 + 196	–	9461	S-Lignin
Ls11	4-(propan-2-one) syringol	167 + 210	–	9653	S-Lignin
M01	Alpha-pinene	91 + 93	3443	–	Monoterpene
M02	3-Carene	98 + 121	3567	–	Monoterpene
M03	m/p-cymene	119 + 134	4273	–	Monoterpene
M04	Limonene	67 + 68	4293	–	Monoterpene
M05	Unidentified monoterpene	69 + 112	4522	–	Monoterpene
M06	Isoborneol	95	5492	–	Monoterpene
M07	Alpha-terpineol	59 + 67	5679	–	Monoterpene
S01	Longifolene	79 + 161	7676	–	Sesquiterpene
S02	Caryophyllene	91 + 161	7,92	–	Sesquiterpene
S03	Bergamotene	91 + 119	7962	–	Sesquiterpene
S04	Calamenene	159 + 202	7983	–	Sesquiterpene
S05	Muurolene	105 + 161	8066	–	Sesquiterpene
S06	Probably (1,4,9-cadalatriene)	157 + 115	8118	–	Sesquiterpene
D01	Phenantrene, ethenyl dodecahydro-dimethyl methylene	91 + 145	10,042	–	Diterpene
D02	Phenantrene, ethenyl dodecahydro-dimethyl methylene	91 + 241	10,361	–	Diterpene
D03	Phenantrene, ethenyl dodecahydro-dimethyl methylene	91 + 239	10,203	–	Diterpene
D04	Ent-kaur-16-ene	105 + 229	10,297	–	Diterpene
D05	Unidentified diterpene	161 + 229	10,369	–	Diterpene
D06	Ent-kaur-16-ene	91 + 241	10,39	–	Diterpene
D07	Dehydroabietic acid	239 + 240	10,572	–	Diterpene
D08	Norabieta-tetraene	197 + 239	10,65	–	Diterpene
D09	Limonene dimer	67 + 68	10,681	–	Diterpene
D10	18-Norabietatriene (or dehydroabietine)	159 + 241	10,727	–	Diterpene
D11	Unidentified diterpene	91	10,743	–	Diterpene
D12	Unidentified diterpene	193 + 254	10,888	–	Diterpene
D13	Tetradehydroabietic acid	237 + 238	10,919	–	Diterpene
D14	Dehydroabietic acid	239 + 254	11,018	–	Diterpene
D15	Bisnorabieta-pentaene (or salvinane)	223 + 238	11,127	–	Diterpene
D16	Bisnorabieta-pentaene (or salvinane)	223 + 238	11,298	–	Diterpene
D17	Unidentified Labdane (or 19-hydroxy-miltiradien)	257 + 271	11,49	–	Diterpene
D18	Dehydroabietic acid	239 + 254	11,589	–	Diterpene
D19	Unidentified diterpene	152 + 240	11,807	–	Diterpene
D20	C_5 hydrophenanthrene compound	91 + 257	11,9	–	Diterpene
D21	Unidentified diterpene	109 + 257	12,133	–	Diterpene
D22	Dehydroabietic acid, methyl ester	239 + 240	12,196	–	Diterpene
D23	Abietate	241 + 256	12,393	–	Diterpene
D24	Methyl abietatetraeonate (or phenanthrene carboxylic acid)	237 + 312	12,616	–	Diterpene
X01	3-methoxycatechol	97 + 125 + 140	–	6462	Other
X02	5-methyl-3-methoxycatechol	139 + 154	–	7101	Other
X03	Triacetin	103 + 145	–	6815	Other
X04	Unidentified compound	189 + 204	–	10,017	Other

Appendix B. Averages of second derivative spectra (a) and FTIR spectra (b) for the fingerprint region (1800–800 cm^{-1})



Appendix C. Correlation plots indices calculated from the Py-GC–MS plotted against the indices predicted using FTIR



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