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CENTRO INTERNACIONAL DE ESTUDOS
DE DOUTORAMENTO E AVANZADOS
DA USC (CIEDUS)

TESE DE DOUTORAMENTO

POTENTIAL BIOMARKERS OF PROVENANCE OF THE WOOD FROM IBERIAN TYPOLOGY SHIPWRECKS (15TH TO 17TH CENTURIES)

Mohamed Traoré

ESCOLA DE DOUTORAMENTO INTERNACIONAL

PROGRAMA DE DOUTORAMENTO EN MEDIO AMBIENTE E RECURSOS NATURAIS

SANTIAGO DE COMPOSTELA

2018


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Potential biomarkers of provenance of the wood from iberian typology shipwrecks (15th to 17th centuries)

Antonio Martínez Cortizas, Catedrático de Universidade del Departamento de Edafoloxía e Química Agrícola da Facultade de Bioloxía da Universidade de Santiago de Compostela e **Joeri Kaal,** Doutor investigador invitado do Grupo de investigación Ciencia do Sistema Terra da Universidade de Santiago de Compostela

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This thesis is based on the following manuscripts:

Manuscript 1: Traoré, M., Kaal, J., Martínez Cortizas, A., 2016. Application of FTIR spectroscopy to the characterization of archeological wood. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 156, 63-70. **JCR IF (2016): 2.536, Q2 in Spectroscopy**

Manuscript 2: Traoré, M., Kaal, J., Martínez Cortizas, A., 2017. Potential of pyrolysis-GC–MS molecular fingerprint as a proxy of Modern Age Iberian shipwreck wood preservation. *Journal of Analytical and Applied Pyrolysis* 126, 1-13. **JCR IF (2016): 3.471, Q1 in Spectroscopy**

Manuscript 3: Traoré, M., Kaal, J., Martínez Cortizas, A., 2018. Differentiation between pine woods according to species and growing location using FTIR-ATR. *Wood Science and Technology* 52, 487-504. **JCR IF (2016): 1.509, Q1 in Materials Science, Paper & Wood**

Manuscript 4: Traoré, M., Kaal, J., Martínez Cortizas, A. A promising approach for provenance studies of wood from Iberian shipwrecks: FTIR and Py-GC-MS in combination with multivariate statistical modelling. Under review at *Journal of Archaeological Science.*


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This work has been carried out in the framework of the ForSEAdiscovery project (PITN-2013-GA607545) funded by the European Research Council through the Seventh Framework Programme under Grant Agreement Number 607545, coordinated by Dr. A. Crespo Solana (Centro de Ciencias Humanas y Sociales; Consejo Superior de Investigaciones Científicas).


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SUMMARY

Shipbuilding in the Iberian Peninsula was one of the animating elements of the European expansion overseas during the Age of Discovery (between 15th and 16th century). During this period, the most valued ships were those built in the Iberian Peninsula. On one hand, this reputation was due to the particular techniques employed by shipyards in this region and, on the other hand, it was due to the availability of high quality timber from forests located in the Atlantic coast of Iberia. In addition to the high demand of ships, the increase in social and economic development induced an augmentation of wood exploitation for the production of building material, furniture and as a fuel. The problematic of wood scarcity led to initiate several plans of action. Therefore, to cope with the shortage, wood was imported through a complex and extensive commercial network of timber trade, spanning across Western Europe, Scandinavia, the Baltic, etc. Furthermore, shipbuilding techniques were adapted to the emerging and worsening situation of wood shortage. Actually, the shortage of wood resources opens several questions about the use of local and imported timber for shipbuilding in the Iberian Peninsula. Nowadays, there is significant interest in this subject, and efforts to obtain a better understanding of its role in the European expansion are being made. A thorough revision of historical documents and archaeological evidence permitted to get much information about the use of wood resources including measures taken for sustainable use of Iberian forests. For instance, several laws and legislations were introduced, in order to improve the policies related to the management of forest resources, and also to deal with timber commercialisation networks that have been established between European states. Hitherto, there is no certainty that increased use of wood for shipbuilding was the main cause of the overexploitation of Iberian forests. In the ForSEAdiscovery project (http://forseadiscovery.eu/), attention has been paid to ancient documents related to shipbuilding ethics and timber supply, to get insights on the use of wood and the management of forest resources. In order to get further understanding about the type and the origin of wood collected from shipwrecks, with features proper to Iberian shipbuilding, attention was also paid to specific applications to study wood materials. For this purpose, this multidisciplinary project was composed of three main working groups or work packages. The first work package was dedicated to study the history of wood supply and the dynamics of the commercial networks in the first global era (AD 1500-1800). The aim of the second work package consisted in nautical archaeology campaigns and investigation of shipbuilding techniques of the Iberian Peninsula, focusing on the earlier phases of the European expansion in the 16th – 17th centuries.

The third work package involved the application of wood analyses, among others, dendrochronology, wood anatomy and wood chemical (organic and inorganic) composition. To investigate the provenance of wood fragments from different Iberian shipwrecks, various approaches related to wood physical and chemical characteristics were applied.

Wood is a complex lignocellulosic and biodegradable material, which is widely used and has applications in various fields, such as energy generation, paper production, or the construction industry. The complexity of this material is associated to the occurrence of various cell elements that play specific functions (mechanical, transport and storage) in woody plants. This complex polymeric material is composed of cellulose, hemicellulose, lignin and extractive compounds (organic and inorganic). The nature of wood largely refers to its structure and chemical composition, which are intimately related to taxonomy and to environmental factors of the growing location. Softwoods (gymnosperms) and hardwoods (angiosperms) are the two main groups of wood. They can be distinguished by the structure of wood cells and also by the chemical composition. Softwoods are characterised by the lack of pores (or vessels) and wood tissues of this group are mostly formed by tracheids. In hardwoods, vessel elements and parenchyma cells are the major constituents. Additionally, numerous chemical patterns related to polysaccharide, lignin and extractive contents show differences between softwoods and hardwoods. The best-known chemical difference is related to the molecular structure of lignin compounds. Lignin in gymnosperms is mainly composed of guaiacyl moieties, whereas lignin in angiosperms is composed of both guaiacyl and syringyl moieties. Wood chemical composition can also be used to explain dissimilarities between individual species of the same genus, as well as between trees grown at different locations. In the particular case of archaeological contexts, the degradation processes induce significant changes on the structure and the chemical composition of original wood by alterations of the chemical compounds, depending on the type of agents. Changes are directly related to the environmental conditions of the storage locations. Under moist and aerobic conditions, the enzymatic system developed by fungi attacks wood cells through oxidation and demethoxylation of lignin compounds. In water-saturated conditions (anaerobic environments), on the other hand, bacteria are the main agents that alter primarily carbohydrate polymers (cellulose and hemicellulose). Certainly, for chemical characterisation of shipwreck woods, it is necessary to pay attention to the environmental conditions of the storage location and the related degradation processes. Therefore, it seems fundamental the application of analytical techniques that allow getting such detail from wood chemical composition. Among many techniques, Fourier Transform Infrared Spectroscopy (FTIR) and Pyrolysis-Gas Chromatography-Mass Spectrometry (Py-GC-MS) are two techniques known to be relevant for studies in wood chemical characterisation. Due to the structure of data provided by them (FTIR and Py-GC-MS), the application of multivariate data analysis (MVDA) methods is usually required to extract synthetic information. In fact, development of a strategy of MVDA for data evaluation, especially for FTIR spectroscopy, has been one of the key aspects of this research.

The principal aim of this thesis is to explore the potential of the use of wood organic chemical contents for studies about species and provenance identification of underwater archaeological wood. It particularly focuses on identification of species and provenance for wood samples from shipwrecks recognised with the Iberian shipbuilding characteristics (hull design, position of floor timbers, etc.). To achieve this objective we carried out studies on two groups of wood samples. On one hand, we studied living trees of two species of pine (*Pinus nigra* and *Pinus sylvestris*) and four species of oak (*Quercus faginea*, *Quercus petraea*, *Quercus pyrenaica* and *Quercus robur*), from different forests pointed out by historical information as potential sources of timber production for shipbuilding. On the other hand, our studies focused on woods from four shipwrecks (Belinho, Magdalena, Ribadeo and Yarmouth) with Iberian features. Particular attention has been paid on the main wood organic polymers (cellulose, hemicellulose and lignin). The impact of the degradation processes undergone by the wood material during centuries of water-saturated conditions was also taken into account. The development of the research can be summarised in the following steps: 1) first, we explored the potential of FTIR and Py-GC-MS techniques to characterise the chemical structure and composition of archaeological woods; 2) then we identified relevant chemical parameters (structures, molecules, indices, etc.), including those related to wood degradation, for the appropriate evaluation of archaeological woods; 3) after that, the investigation was concentrated on wood chemicals associated to signals related to species and/or location; and 4) we applied the developed methodologies to identify the species and possible origin of the shipwreck woods. The outcomes within the framework of this research were reported in four scientific manuscripts. Three of the manuscripts have been published in peer-reviewed journals and the fourth has been submitted for publication and is being revised at the moment of the writing of this thesis, after a first round of revisions.

Manuscript 1 focuses on two archaeological woods studied by FTIR and Py-GC-MS: a pine (*Pinus* sp.) wood sample collected from a beam of an ancient building (Cathedral of Segovia) and an oak (*Quercus* sp.) wood fragment collected from a ship that wrecked in Ribadeo Bay (the colossal "Galleon of Ribadeo"). Principal Component Analysis (PCA) was applied to the transposed data matrix of the recorded spectra (samples as columns and spectral bands as rows). The obtained results showed differences between the two samples through the chemical structure of lignin compounds, with a mixture of guaiacyl and syringyl lignin in the oak (hardwood) wood, whereas, lignin in the pine (softwood) wood was only composed of guaiacyl moieties. There were also differences between the two samples according to the relative proportion of carbohydrates and lignin contents. Indeed, the relative proportion of these two components was related to storage conditions (i.e. the chemical alteration of the wood fragments). Highest relative proportions of lignin were recorded for the shipwreck wood, most probably due to the intense degradation of polysaccharides by the hydrolysis underwent during the long period it remained under anoxic condition at the sea bottom. Lower relative content of lignin characterised the chemical composition of the wood fragment from the beam. For this sample, intense alteration of lignin has been attributed to its oxidation under an air-dry condition. Additionally, the results of this paper suggest a gradual impact of the degradation processes, with a clear tendency of decreased intensity from the outer (exposed) to inner (preserved) sections of the wood. Lignin proportion within the beam wood was found to be lower in the recent (outer) than in the older (inner) tree rings. In most cases, the outer part of archaeological woods are subject to more intense alteration as they are the ones in greater contact with degradation agents, facilitating the accessibility to wood chemical contents. In parallel, the application of Py-GC-MS to the samples studied permitted to confirm the obtained conclusions and to show the consistency of the data. Also, this study demonstrated that FTIR and Py-GC-MS could be used as complementary techniques for wood chemistry investigations. Beyond any doubt, the application of PCA to the transposed data matrix of FTIR spectra appeared to be relevant to extract meaningful chemical information between and within wood samples, and this proved to be a crucial step towards the optimization of the use of FTIR data for wood analysis.

In **manuscript 2**, focus was placed on the assessment of the intensity of wood decay. This information was considered paramount to be able to recognise degradation patterns, and avoid the undesired use of signals related to decay in the models of wood species or provenance assessment. In this study, Py-GC-MS was applied to samples from four pine (*Pinus* spp.) wood fragments, an oak (*Quercus* sp.) fragment from the Magdalena shipwreck and two oak (*Quercus* spp.) fragments from the Ribadeo shipwreck. The application of PCA to the Py-GC-MS data permitted to get detailed information about the chemical composition of the studied archaeological woods. The observed degradation appeared to depend not only on the type of wood (softwood and hardwood), obviously expected, but also to a significant extent on the anatomical section of the wood (sapwood and heartwood) analysed. For most wood samples, polysaccharide contents appeared to be the most altered, which is attributed to the hydrolysis reactions underwent under anoxic conditions in seawater. However, not all of the polysaccharides altered with the same intensity. We found that 3-hydroxy-2-methyl-2-cyclopenten-1-one (*m/z*: 55+112) was well-preserved among the polysaccharide pyrolysis products, and differences of degradation trend were shown by levoglucosan (*m/z*: 60). In fact, separate PCA models for carbohydrate and lignin markers allowed the decay of polysaccharides and lignin to be examined, the results of which are far more relevant for wood analysis than the often-reported shift in relative contents of lignin and polysaccharides. For both types of wood, the lignin compounds were well-preserved except for the homovanillin (a guaiacyl product, *m/z*: 137+166) and homosyringaldehyde (a syringyl product, *m/z*: 167+196), which appeared to be the less preserved lignin compounds. Although wood extractive compounds can be soluble in water and may be expected to be released after centuries of immersion, they were actually quite well-preserved in the archaeological wood, in spite of the clear evidence of significant alteration of lignin and polysaccharides. Resin diterpene compounds were found in pine (*Pinus* spp.) shipwreck woods, whereas phenolic extractive compounds were detected in oak (*Quercus* spp.) shipwreck woods. These compounds were in general better preserved in the inner part than in the outer part of the wood. The application of PCA enabled to bring attention to potential polysaccharide and lignin compounds in order to formulate new proxies for the evaluation of the degradation state of shipwreck woods. Thereby, new indices (Shipwreck Wood Preservation Index: SWPI) were proposed on the basis of polysaccharides ($SWPI_{PS}$), guaiacyl lignin ($SWPI_{LG}$) and syringyl lignin ($SWPI_{LS}$) compounds, that are particularly susceptible to remain in decayed woods. These indices may be specifically applicable to shipwreck wood alteration assessment as they differ from similar proxies used in environmental studies of e.g. peat deposits. Stepwise multiple linear regressions analyses applied on FTIR data of the same samples permitted to point out the consistency of our findings. Also, in this study the two techniques (FTIR and Py-GC-MS) have been proved to be adequate to characterise the chemical composition of decayed woods.

Manuscript 3 represents an attempt to distinguish between pine samples according to species and growing location, through the combination of Fourier Transform Infrared Spectroscopy (FTIR) and Stepwise Discriminant Analysis. FTIR spectra were recorded from heartwood samples from six *Pinus nigra* trees sampled in two forests from southern Spain, and two *Pinus sylvestris* trees sampled in a forest in northern Spain. The ability of FTIR data to discriminate between wood species and growing location was explored by the application of various discriminant analysis tests on some selected FTIR bands (mainly lignin and polysaccharides bands selected from the 2nd derivative spectra). For the first time, the results showed that lignin, as well as polysaccharide compounds, could be used to differentiate between *Pinus nigra* and *Pinus sylvestris*. Absorption bands related to lignin compounds permitted to differentiate between the two pine species, wherea bands related to polysaccharides enabled differentiation between both species and growing location. Signals for aromatic structures in lignin were significantly more prominent in *Pinus nigra* than in *Pinus sylvestris*. This was attributed to the greater shade tolerance of *Pinus nigra*. A noticeable difference between the two pine species was also related to the strength of hydrogen bonds between xylan chains in *Pinus nigra* trees. Discrimination between growing locations was mainly regulated by the molecular structure of wood cellulose. We found a greater degree of crystallinity in the structure of cellulose in trees located at higher altitude. According to the results of this study, the combination of FTIR with discriminant analysis was suggested as a relevant tool for the identification of species and provenance of wood samples from different origins.

The investigation reported in **manuscript 4** further built on the approaches established in the above-mentioned works, i.e. the combination of FTIR and Py-GC-MS techniques with MVDA, but on the basis of much larger datasets. The main aim was now to identify species and possible provenance of shipwreck oak wood (*Quercus* spp.) fragments of four Iberian shipwrecks (Belinho, Magdalena, Ribadeo and Yarmouth) studied in the ForSEAdiscovery project. For this purpose, living tree cores were collected from four oak species native from the Iberian Peninsula (*Quercus faginea*, *Quercus petraea*, *Quercus pyrenaica* and *Quercus robur*). According to the objectives of the project, only oak species that can be used for accurate dendrochronological studies were selected (e.g. *Quercus ilex* was ignored). The PCA applied on the FTIR and Py-GC-MS data indicated that most archaeological woods have the expected composition of heartwood (sapwood virtually absent in shipwreck samples). In fact, heartwood is the most used wood part for building due to its mechanical and durable properties. Furthermore, from Py-GC-MS, the identification of diagenetic alteration appeared to be more problematic for sapwood. For these reasons, specifically to avoid using variability in wood composition related to differences in sapwood and heartwood and their differential diagenetic development, the analysis of the of wood cores from the living trees was also focused on heartwood. The application of Discriminant Analysis enabled to develop a model that separated living tree samples according to species. Thereby, the discriminant models were used to identify which species the shipwreck wood samples were more likely to originate from. A large number of samples indicated a signal similar to either *Q. petraea* or *Q. robur*, which are two analogous species and widely spread throughout Europe. Another part of the shipwreck samples was more similar to wood from *Q. faginea* and a smaller amount to wood from *Q. pyrenaica*. These two species (*Q. faginea*, *Q. pyrenaica*) show a more confined distribution in the Iberian Peninsula. Information of the geographical distribution of oak species (nowadays), in combination with historical information, indicated that neighbouring forests were the most likely source of timber used by shipyards. For instance, written information about the construction of the Magdalena frigate in the North-West of the Iberian Peninsula is consistent with the discriminant model, in the prediction of the probable provenance of the shipwreck wood. Our results also showed higher probability for the Iberian forest as the likely source for wood samples from the other shipwrecks (Belinho, Ribadeo and Yarmouth). However, these conclusions are, to a certain degree, constrained by the limitations imposed by the number of samples and the geographical area studied in the ForSEAdiscovery project. It is pertinent to analyse oak materials from other regions in Europe to elucidate whether local material was indeed used. Finally, the results in this manuscript showed that FTIR and Py-GC-MS techniques, if combined with appropriate MVDA techniques and avoiding molecular modifications induced by decay, could be considered as promising tools for provenance studies of archaeological woods. And, more unequivocally, this approach can be used to differentiate the species of living wood to high levels of probability.

To summarise, the major finding of the research carried out in the framework of this thesis is that the organic chemical composition of wood can be considered as a very important parameter in wood identification studies. Certainly, it is essential to apply adequate techniques and methodologies for this purpose. The application of FTIR and Py-GC-MS permitted to get detailed information on wood chemical composition, which is mainly determined by factors (taxonomy and growing location) that contribute to wood formation. It has also been shown that the data provided by these techniques on archaeological woods enables further understanding of chemical modification processes (early wood diagenesis). As shown by the results obtained within this research, the application of correct MVDA techniques and pre-selection of the dataset (use of 2nd derivative to identify bands, avoid signals affected by decay, focus on heartwood not sapwood, etc.) is also crucial to extract insightful information and obtain answers to the numerous questions about wood (living trees and archaeological) samples. Application of MVDA methods has proved to be valuable for FTIR and Py-GC-MS data analysis. The application of PCA permitted to extract factors that are directly related to wood chemical content. This statistical method has been shown to be useful to determine chemical changes between and within wood samples (living trees and archaeological). Furthermore, the application of discriminant analysis was found to have good potential for classification of wood samples according to taxonomy and growing location, which are the primary factors that control wood chemical composition. Actually, discriminant analysis helped to elucidate differences even between species that are believed to be very much alike.


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RESUMEN

La construcción naval en la Península Ibérica fue uno de los elementos que alentaron la expansión europea hacia el exterior durante la Era de los Descubrimientos (entre el siglo XV y el siglo XVI). Durante este período, los barcos construidos en la Península Ibérica se encontraban entre los más valorados. Por un lado, esta reputación se debió a las técnicas particulares empleadas por los astilleros en esta región y, por otro, se debió a la disponibilidad de madera de alta calidad de los bosques ubicados en la costa atlántica de Iberia. Además de la alta demanda de barcos, el incremento en el desarrollo social y económico provocó un aumento de la explotación de madera para la producción de materiales de construcción, mobiliario y como combustible. La problemática escasez de madera a la que esto conllevó, provocó que se pusieran en marcha diversos planes de acción. Por lo tanto, para hacer frente a la demanda, la madera se importó a través de una compleja y extensa red comercial, a través de Europa Occidental, Escandinavia, el Báltico, etc. Asimismo, las técnicas de construcción naval se adaptaron a la nueva situación y al empeoramiento en el suministro de madera. En realidad, la escasez de recursos forestales plantea preguntas sin resolver sobre el uso de madera local y la importada para la construcción naval en la Península Ibérica. Hoy en día, existe un interés significativo sobre este tema y se están haciendo muchos esfuerzos para obtener una mejor comprensión de su papel en la expansión europea. Una revisión exhaustiva de los documentos históricos y las evidencias arqueológicas ha permitido obtener mucha información sobre el uso de los recursos madereros, incluidas las medidas adoptadas para el uso sostenible de los bosques ibéricos. Entre ellas se encuentra, por ejemplo, la introducción de leyes cuyo objeto era mejorar las políticas relacionadas con la gestión de los recursos forestales y el control de las redes de comercialización de la madera que se habían establecido entre los estados europeos. Hasta ahora, no hay seguridad de que el uso creciente de madera para la construcción naval haya sido la causa principal de la sobreexplotación de los bosques ibéricos.

En el proyecto ForSEAdiscovery (http://forseadiscovery.eu/) se ha prestado atención a la documentación históricos relacionada con la ética de la construcción naval y el suministro de madera, para obtener información sobre el uso de la madera y la gestión de los recursos forestales. Con el fin de obtener una mayor comprensión sobre el tipo y el origen de la madera de los pecios estudiados, con características propias de la construcción naval ibérica, se prestó particular atención a metodologías adecuadas para el estudio de materiales de madera. Para este propósito, este proyecto multidisciplinar estaba compuesto de tres grupos de trabajo. El primero se dedicó estaba dedicado a la historia del suministro de madera y la dinámica de las redes comerciales en la primera

era global (siglos XV a XIX). El segundo grupo de trabajo se encargó de las campañas de arqueología náutica y la investigación sobre las técnicas de construcción naval en la Península Ibérica, centrándose en las fases primitivas de la expansión europea entre los siglos XVI-XVII. El tercer grupo de trabajo combinó aplicaciones analíticas y disciplinas para la caracterización de la madera; entre otras, la dendrocronología, la anatomía de la madera y la composición química (orgánica e inorgánica). Se aplicaron diversos enfoques relacionados con las características físicas y químicas de la madera, para investigar la procedencia de fragmentos de madera de diferentes naufragios ibéricos.

La madera es un complejo material lignocelulósico y biodegradable que se utiliza ampliamente y tiene aplicaciones en diversos campos, como la producción de energía, la producción de papel o la industria de la construcción. La complejidad de este material está asociada a la presencia de varios elementos celulares que desempeñan funciones específicas (mecánica, transporte y almacenamiento) en las plantas leñosas. Este complejo material polimérico está compuesto de celulosa, hemicelulosa, lignina y compuestos extraíbles (orgánicos e inorgánicos). La composición y propiedades de la madera están relacionadas en gran medida con su estructura y composición química, que están profundamente relacionadas con la taxonomía y los factores ambientales del lugar de crecimiento. Las maderas blandas (gimnospermas) y duras (angiospermas) son los dos grupos principales de madera. Estos dos grupos se pueden distinguir por la estructura de las células vegetales y también por la composición química. Las maderas blandas se caracterizan por la ausencia de poros (o vasos) y sus tejidos están formados principalmente por las traqueidas. En las maderas duras, los vasos y las células del parénquima son los principales componentes. Además, numerosos patrones químicos relacionados con polisacáridos, lignina y contenido en extraíbles permiten diferenciar entre maderas blandas y duras. La diferencia química más conocida está relacionada con la estructura molecular de los compuestos de lignina. La lignina en las gimnospermas está principalmente compuesta unidades de guaiacil, mientras que la lignina de angiospermas está compuesta tanto por guaiacil como siringil. Igualmente, la composición química de la madera se puede utilizar para explicar las diferencias entre especies de un mismo género, como entre árboles que crecieron en diferentes lugares. En los contextos arqueológicos, los procesos de degradación provocan cambios significativos en la estructura y composición química de la madera, en función del tipo de agente. Estos cambios están directamente relacionados con las condiciones ambientales de los yacimientos. En condiciones de humedad, pero en ambientes aeróbicos, el sistema enzimático desarrollado por los hongos ataca las células de la madera a través de la oxidación y la demetoxilación de los compuestos de lignina. En condiciones de saturación por agua (ambientes anaeróbicos), por otro lado, las bacterias son los principales agentes que alteran los polímeros de carbohidratos (celulosa y hemicelulosa). Sin duda, para la caracterización química de los barcos hundidos es necesario prestar atención a las condiciones ambientales de los yacimientos y los procesos de degradación asociados. Por lo tanto, parece fundamental la aplicación de técnicas analíticas que permitan obtener detalles sobre la composición química de la madera. Entre otras técnicas, la Espectroscopia de Infrarrojo por Transformada de Fourier (FTIR) y la pirolisis-cromatografía de gases-espectrometría de masas (Py-GC-MS) son dos de las consideradas como relevantes para los estudios de caracterización química de la madera. Debido a la estructura de los datos proporcionados por estas técnicas (FTIR y Py-GC-MS), generalmente se requiere la aplicación de métodos estadísticos multivariados (MVDA) para extraer información sintetizada. De hecho, la aplicación de MVDA para la evaluación de datos, especialmente para la espectroscopia infrarroja (FTIR), ha sido uno de los aspectos clave de esta investigación.

El objetivo principal de esta tesis es investigar el potencial de los compuestos orgánicos de la madera en los estudios sobre la identificación de especies y procedencias de la madera arqueológica subacuática. Se centra particularmente en la identificación de especies y procedencia para muestras de madera de pecios reconocidos con las características (diseño del casco, posición de las vigas del piso, etc.) de construcción naval ibérica. Para lograr este objetivo, llevamos a cabo estudios en dos grupos de muestras de madera. Por un lado, estudiamos árboles vivos de dos especies de pino (*Pinus nigra* y *Pinus sylvestris*) y cuatro especies de roble (*Quercus faginea*, *Quercus petraea*, *Quercus pyrenaica* y *Quercus robur*) de diferentes bosques, que la investigación histórica sugiere como fuentes potenciales de la producción de madera para la construcción naval. Por otra parte, nuestros estudios sobre madera arqueológica se centraron en muestras de cuatro pecios (Belinho, Magdalena, Ribadeo y Yarmouth) con características ibéricas. Se ha prestado especial atención a los principales polímeros orgánicos de madera (celulosa, hemicelulosa y lignina). También se tuvo en cuenta el impacto de los procesos de degradación sufridos por las maderas durante siglos bajo condiciones saturadas de agua. El desarrollo de la investigación puede resumirse en los siguientes pasos: 1) primero, exploramos el potencial de las técnicas FTIR y Py-GC-MS para caracterizar la estructura y la composición química de las maderas arqueológicas; 2) luego identificamos parámetros químicos relevantes (estructuras, moléculas, índices, etc.), incluidos los relacionados con la degradación de la madera, para la evaluación adecuada de maderas arqueológicas; 3) después de esto, la investigación se concentró en aquellos productos químicos de la madera asociados a señales relacionadas con especies y / o procedencia; y finalmente 4) aplicamos las metodologías desarrolladas para identificar las especies y el posible origen de las maderas de los pecios. Los resultados obtenidos en el marco de esta investigación se formalizaron en cuatro artículos científicos. Tres de ellos han sido revisados por pares y publicados en revistas indexadas y el cuarto se ha enviado para su publicación (en el momento de redacción de esta tesis se encuentra en fase de reelaboración tras una primera ronda de revisiones).

El **manuscrito 1** se centra en estudiar por FTIR y Py-GC-MS dos maderas arqueológicas: una muestra de pino (*Pinus* sp.) tomada en una viga de la Catedral de Segovia y un fragmento de roble (*Quercus* sp.) de un barco que naufragó en la Bahía de Ribadeo (el colosal "Galeón de Ribadeo"). Se aplicó análisis por componentes principales (PCA) a la matriz transpuesta (muestras en columnas y bandas espectrales en filas) de los datos de espectroscopia. Los resultados obtenidos mostraron diferencias en la estructura química de los compuestos de lignina entre las dos maderas: mezcla de guaiacil y siringil en la madera de roble (madera dura), mientras que la de pino (madera blanda) estaba compuesta solo por guaiacil. Además, también se encontraron diferencias en la proporción relativa de carbohidratos y contenido de lignina. De hecho, la proporción relativa de estos dos componentes se relacionó con las condiciones ambiental del yacimiento (es decir, la alteración química que sucede a los fragmentos de madera). Las proporciones relativas más altas de lignina se registraron en la madera del pecio, muy probablemente debido a la intensa alteración de los polisacáridos por la hidrólisis que sucede después de un largo período en condiciones anóxicas en el fondo del mar. La composición química del fragmento de la viga se caracterizaba por un bajo contenido relativo de lignina. Para esta muestra, la alteración intensa de la lignina se ha atribuido a su oxidación bajo condiciones secas al aire libre. Conjuntamente, los resultados de este trabajo sugieren un impacto gradual de los procesos de degradación, con una clara tendencia a la disminución de la intensidad de los mismos desde las secciones externas (expuestas) a las secciones internas (preservadas) de la madera. Se encontró que la proporción de lignina en la madera de la viga era más baja en los anillos mas recientes (las secciones externas) que en los más antiguos (las secciones internas). En la mayoría de los casos, la parte exterior de las maderas arqueológicas está sujeta a alteraciones más intensas ya que es la que está en mayor contacto con los agentes de degradación, lo que facilita la accesibilidad a los contenidos químicos de la madera. En paralelo, el análisis de las mismas muestras por Py-GC-MS permitió confirmar las conclusiones obtenidas y demostrar la consistencia de los datos. Además, este estudio demostró que FTIR y Py-GC-MS pueden emplearse como técnicas complementarias para investigaciones sobre la química de la madera. Más allá de cualquier duda, la aplicación de PCA a la matriz transpuesta de los datos de espectroscopia resultó ser muy adecuada para extraer información química explicativa entre muestras y dentro de las muestras de madera, y esto demostró ser un paso crucial para la optimización del uso de datos FTIR en los estudios de la madera.

El trabajo del **manuscrito 2** se centró en la estimación de la magnitud de la alteración de la madera. Es ésta una información esencial para poder identificar los patrones de degradación y evitar el uso no deseado de señales relacionadas con la descomposición en los modelos para la identificación de especies o de procedencia de la madera. En este estudio, el análisis de Py-GC-MS se aplicó a muestras de cuatro fragmentos de madera de pino (*Pinus* spp.) y un fragmento de roble (*Quercus* sp.) del pecio Magdalena, y dos fragmentos de roble (*Quercus* spp.) del pecio de Ribadeo. La aplicación de PCA a los datos de pirolisis permitió obtener información detallada sobre la composición química de estas maderas arqueológicas. La degradación observada parece no depender en exclusiva del tipo de madera (blanda o dura) sino también, en gran medida en la parte anatómica (albura o duramen) de la madera estudiada. Para la mayoría de las muestras de madera, el contenido de polisacáridos parece ser el más alterado, lo que se atribuye a la hidrólisis que sucede bajo condiciones anóxicas en los fondos marinos. Sin embargo, no todos los polisacáridos se alteraran con la misma intensidad. Encontramos que entre los productos de pirolisis el compuesto 3-hydroxy-2-methyl-2-cyclopenten-1-one (*m/z*: 55+112) estaba mejor conservado. Diferencias en la degradación se encontraron también para el levoglucosano (*m/z*: 60). De hecho, los modelos PCA separados para marcadores de carbohidratos y ligninas permitieron una mejor comprensión de la descomposición de ambos tipos de compuestos, resultados que demostraron ser mucho más relevantes para el análisis de la madera que la habitual relación del contenido total de lignina y polisacáridos. En ambos tipos de madera (blanda y dura) los compuestos de lignina estaban bien conservados, excepto el homovanillin (un guaiacil, *m/z*: 137+166) y el homosiringaldehido (un siringil, *m/z*: 167+196), que parecían ser los compuestos de lignina menos conservados. A pesar de la clara evidencia de una alteración significativa de las ligninas y los polisacáridos, los compuestos extraíbles estaban bastante bien conservados en la madera arqueológica, a pesar de que estos compuestos suelen ser solubles en agua y por lo tanto cabría esperar que se hubiese lavado tras de siglos de sumersión. En los pecios, las maderas de pino (*Pinus* spp.) contenían compuestos de resina diterpenos, mientras que las de roble (*Quercus* spp.) contenían compuestos fenólicos extraíbles. En general, estos compuestos se conservaron mejor en la parte interna que en la parte exterior de la madera. Además, el PCA permitió determinar que algunos compuestos de polisacáridos y lignina eran susceptibles de ser empleados como indicadores para la estimación del estado de alteración de las maderas de pecios, al preservarse bien en las maderas arqueológicas. De esta manera, se propusieron nuevos índices (Índice de Preservación de la Madera de Pecio: SWPI) sobre la base de compuestos de polisacáridos ($SWPI_{PS}$), de guaiacil-lignina ($SWPI_{LG}$) y siringil-lignina ($SWPI_{LS}$). Estos índices pueden ser aplicables para la evaluación de la alteración de maderas de pecios, ya que difieren de otros indicadores similares utilizados en los estudios ambientales, como los depósitos de turba por ejemplo. El análisis de los datos de FTIR de las mismas muestras, mediante regresión lineal múltiple por pasos, permitió demostrar la consistencia de nuestros resultados. Además, en este estudio, las dos técnicas (FTIR y Py-GC-MS) han demostrado ser adecuadas para caracterizar la composición química de las maderas arqueológicas.

El **Manuscrito 3** representa un diseño que combina la Espectroscopia de Infrarrojo por Transformada de Fourier (FTIR) con el Análisis Discriminante para distinguir entre varias muestras de pino según especie y procedencia. Los espectros de FTIR se registraron a partir de muestras de duramen de seis árboles de *Pinus nigra* muestreados en dos bosques del sur de España, y otros dos árboles de *Pinus sylvestris* muestreados en un bosque en el norte de España. La capacidad de los datos FTIR para distinguir entre muestras de maderas de distintas especies procedencia se exploró mediante varias pruebas de análisis discriminante, con bandas seleccionadas (especialmente bandas de lignina y polisacáridos seleccionadas a través del espectro de la segunda derivada). Por primera vez, los resultados mostraron que los compuestos de lignina, así como los polisacáridos, pueden emplearse para diferenciar entre *Pinus nigra* y *Pinus sylvestris*. Las bandas de absorción relacionadas con los compuestos de lignina permitieron diferenciar entre las dos especies de pinos, mientras que las bandas relacionadas con los polisacáridos permitieron la diferenciación entre ambas especies y su procedencia. Las señales de las estructuras aromáticas de la lignina fueron significativamente mas intensas en *Pinus nigra* que en *Pinus sylvestris*. Esto se atribuyó a la mayor tolerancia del *Pinus nigra* a la sombra. Otra diferencia notable entre las dos especies se relacionó con la fuerza de los enlaces de hidrógeno entre las cadenas de carbohidratos de las maderas de *Pinus nigra*. La discriminación de la procedencia se relacionó principalmente con la estructura molecular de la celulosa. Encontramos un mayor grado de cristalinidad en árboles ubicados a mayor altitud. De acuerdo con los resultados de este estudio, la combinación de técnica espectroscópica con análisis discriminante se sugirió como un método de trabajo relevante para la identificación de especies y procedencias de maderas de diferentes orígenes.

La investigación descrita en el **manuscrito 4** tiene el mismo enfoque que los trabajos descritos anteriormente, es decir, la combinación de FTIR y Py-GC-MS con el análisis estadístico multivariante, pero en este caso sobre una base de datos mucho más amplia que incluyen tanto árboles vivos como todas las muestras arqueológicas. El objetivo principal era identificar especies y posible procedencia de los fragmentos de madera de roble (*Quercus* spp.) de cuatro pecios ibéricos (Belinho, Magdalena, Ribadeo y Yarmouth), estudiados en el proyecto ForSEAdiscovery. Para este propósito, se sacaron testigos de madera de árboles vivos de cuatro especies de roble nativas de la Península Ibérica (*Quercus faginea*, *Quercus petraea*, *Quercus pyrenaica* y *Quercus robur*). De acuerdo con los objetivos del proyecto, el enfoque era sobre especies de roble que permiten estudios dendrocronológicos de precisión (por ejemplo, *Quercus ilex* se ignoró). El PCA aplicado a los datos de FTIR y Py-GC-MS indicó que la mayoría de las maderas arqueológicas tienen la composición esperada del duramen (albura prácticamente ausente en maderas de pecios). De hecho, el duramen es la parte de madera más utilizada para la construcción debido a sus propiedades mecánicas y su duración. Además, de Py-GC-MS, la identificación de la alteración diagenética parece ser más problemática para la albura. Por estas razones, los análisis sobre los testigos de madera de los árboles vivos se centró en el duramen, concretamente para evitar la variabilidad en la composición química de la madera relacionada con las diferencias entre albura y duramen, y también su proceso diagenético diferencial. El Análisis discriminante permitió desarrollar un modelo que es capaz de separar entre las muestras de árboles vivos según la especie. De este modo, los modelos discriminantes se usaron para identificar la especie mas probable de las maderas de pecios. Una gran parte de podrían atribuirse a *Q. petraea* o *Q. robur*, que son dos especies similares y con una amplia distribución en toda Europa. Otra parte de las maderas de pecios mostraron una mayor probabilidad de pertenecer a *Q. faginea* y una pequeña cantidad a *Q. pyrenaica*. Estas dos últimas especies (*Q. faginea*, *Q. pyrenaica*) tienen una distribución más restringida en la Península Ibérica. La información de la distribución geográfica de las especies de roble (hoy en día), en combinación con la información histórica, sugiere que los bosques cercanos fueron las fuentes más probables de madera utilizada por los astilleros. Por ejemplo, la información escrita sobre la construcción de la fragata Magdalena en el noroeste de la Península Ibérica es consistente con el modelo discriminante, en la predicción de la probable procedencia de la madera del pecio. Nuestros resultados también mostraron una mayor probabilidad para el bosque ibérico como fuente de las muestras de madera de otros pecios (Belinho, Ribadeo y Yarmouth).

Sin embargo, estas conclusiones están, hasta cierto punto, limitadas por el número de muestras y el área geográfica estudiada en el proyecto ForSEAdiscovery. Es relevante analizar materiales de roble de otras regiones de Europa para elucidar si realmente se utilizó únicamente material local para la construcción naval. Finalmente, los resultados en este manuscrito mostraron que las técnicas empleadas (FTIR y Py-GC-MS), si se combinan con técnicas apropiadas de MVDA podrían considerarse como una herramienta prometedora para los estudios de procedencia de maderas arqueológicas. Y, de manera más inequívoca, este enfoque se puede utilizar para diferenciar las especies de madera viva con una probabilidad muy alta.

En resumen, el principal resultado de la investigación llevada a cabo en el marco de esta tesis es que la composición química orgánica de la madera puede considerarse un parámetro muy importante en los estudios de identificación de la madera. Ciertamente, es esencial aplicar técnicas y metodologías adecuadas para este propósito. La aplicación de FTIR y Py-GC-MS permitió obtener información detallada sobre la composición química de la madera, que está definida principalmente por varios factores (taxonomía y ubicación de crecimiento) que influyen en su formación. También se ha demostrado que los datos proporcionados por estas técnicas en maderas arqueológicas permiten una mayor comprensión de los procesos de degradación química (diagénesis incipiente de la madera). Como muestran los resultados obtenidos en esta investigación, la aplicación de técnicas MVDA y la preselección del conjunto de datos (uso del espectro de la segunda derivada para identificar bandas, evitar señales afectadas por la descomposición, centrarse en el duramen y no en la albura, etc.) también es crucial para extraer información adecuada y obtener respuestas a las numerosas preguntas sobre muestras de madera (árboles vivos y arqueológicos). La aplicación de métodos MVDA ha demostrado ser ventajosa para el análisis de datos FTIR y Py-GC-MS. La aplicación de PCA permitió extraer factores que están directamente relacionados con el contenido químico de la madera. Este método estadístico ha demostrado ser útil para determinar los cambios químicos entre y dentro de las muestras de madera (árboles vivos y arqueológicos). Además, se encontró que la aplicación del análisis discriminante tiene un buen potencial para la clasificación de muestras de madera de acuerdo con la taxonomía y la procedencia. De hecho, el análisis discriminante permitió elucidar las diferencias incluso entre especies que se cree que son muy similares y difíciles de distinguir.


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1. GENERAL INTRODUCTION


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1.1. ForSEAdiscovery project

1.1.1. Context of the project

The Iberian Peninsula played an important role in the European economic, political, and social development during the European expansion period. The shipbuilding tradition in the peninsula was a key factor for sustaining the Iberian Golden Age, which was largely a product of its overseas expansion, during the 15th and the 16th centuries (Castro 2008). It has been recognised that the Iberian ships were among the most appreciated, as they were built with high-quality timber from the Atlantic coast of Iberia, and were also recognised by characteristics (shape, size, and performance) that depended on personal skills of craftsmen (Castro 2008, Bojakowski 2011). However, historical information shows that the Iberian ships shared several characteristics with other European and Mediterranean ship typologies (Loewen 1998, Castro 2008). During the expansion period, the soaring ship demand contributed to significant changes in shipbuilding technologies (based on ship capacity, type and amount of wood, etc.) and on the selection and use of wood resources (Creasman 2008, Reboredo and Pais 2014). As a result, commercial timber networks between European states became increasingly large and complex (Albion 1926, Crespo Solana 2015, Gallagher 2016, Reichert 2016, Kumar 2018). Besides being the main raw material for shipbuilding activity, wood was in high demand due to the increase in social and economic development of the Iberian Peninsula. Wood was increasingly used in building of houses, agricultural activities, for heating and was also used in food processing. The higher demand (for fuel, building materials, furniture, etc.) of this natural resource created a problematic shortage. But it is not yet clear from the historical studies, if shipbuilding was the main cause of overexploitation of forest resources. Nevertheless, the imperial states were actively concerned about the increasing pressure on its forests; consequently, new legislations were introduced for the regulation of the wood economy (Albion 1926, Wing 2012, Reboredo and Pais 2014, Wing 2015). Furthermore, new laws and regulations were introduced to face the shortage of timber. For instance, "*Fueros*" or municipal laws played a significant role to guarantee access to resources of the commons all over Spain (Loewen 2000). Likewise, changes in naval architecture permitted the use of wood from forests that were not specifically cultivated to produce timbers for shipbuilding (Loewen 2000).

In the ForSEAdiscovery project, attention was paid to ancient documents concerned with shipbuilding and timber supply in a view to obtain a better understanding of the use and management of Iberian forest resources (Crespo Solana 2015) during the early modern ages. This international project was a multidisciplinary network funded by the European Union through the Research Executive Agency (REA). Ancient manuscripts, archaeological evidence and specific applications (dendrochronology, wood anatomy and wood chemistry) to the study of wood are known to hold a potential to piece together information on past socio-environmental policies and practices related to shipbuilding and timber resources management (Jones 2004, Creasman 2008, Rich et al. 2017). Another important aspect in the project was its objective to provide an opportunity to young research fellows to gain transferable competences and specific research skills within the framework of a multidisciplinary research-training programme.

1.1.2. Structure and design of the project

Various research teams were organised in three main working groups or Work Packages (WP). The multidisciplinary approach of the project facilitated collaborations and interactions between researchers, which resulted in a general participation of fellows in research actions of diverse disciplines. The first work package (WP1) was dedicated to investigate the historical wood supply and the dynamics of the commercial networks. WP1 focussed on historical research in the Age of Discovery (between the 16th and 18th century) about shipbuilding timber supply and Iberian forest management. Research in this work package permitted to identify potential sites for Iberian shipyards and also to specify the main techniques used for shipbuilding. The research methodologies are based on exploitation of available historical materials about the issue (archival documentations, historiography and historical databases). Research conducted in national archives (Portugal, The Netherlands and Spain) contributed to advance investigations of documents such as treatises and shipbuilding contracts. The second work package (WP2) was dedicated to studies related to nautical archaeology and shipbuilding ethics. Attention was paid to the archaeological evidence of Iberian shipbuilding techniques as well. Furthermore, researchers in WP2 were in charge of research and maritime excavation of Iberian shipwrecks. The research methodologies in WP2 were based on historical archives and underwater sampling techniques in order to collect appropriate shipwreck wood materials required for various approaches with specific applications to the study of wood. Through various exploration campaigns, many shipwrecks with features that are characteristic of Iberian shipyards were excavated. Moreover, innovative techniques (3D digital approaches) were applied in order to reproduce the original ships from the wreckages. The third work package (WP3) was oriented to define the provenance of the collected shipwreck wood. Various approaches (dendrochronology, wood anatomy and wood chemical composition) were applied so as to get consistent and additional information about the wood materials (living trees from Iberian forests and wood from Iberian shipwrecks) used in the project and to identify the possible sources of timber. The investigations enabled to extend tree ring chronologies back in time using oak and pine samples (from historic buildings and living trees) from selected areas throughout the Peninsula. Studies on wood anatomical and chemical properties provided evidence about the studied wood species and growing locations. These specific applications of wood study enabled the development of novel processes for wood provenance studies, particularly relevant for archaeological wooden artefacts.

1.1.3. Assumptions and working hypothesis

The specific applications to study wood within the ForSEAdiscovery project consisted in investigating the provenance of wooden fragments from various Iberian shipwrecks. The parameters used for these approaches are related to the physical and chemical properties of wood. The chemical composition of wood depends on tree species and the growing location, as well the anatomical part of tree and tree age. The taxonomic and environmental factors play an important role in the growing process of living trees (Fengel and Wegener 1984, Poletto et al. 2012). But in the particular context of shipwreck wood, there are additional factors that induce disturbance in the original signal of wood. Hence, to study the origin of shipwreck wood, it is fundamental to pay particular attention to taxonomy and the growing location of trees, but also to the degradation factors that cause changes in wood composition. Within this framework, Fourier-transform infrared spectroscopy (FTIR) and pyrolysis coupled to gas chromatography and mass spectrometry (Py-GC-MS) have been applied to several living tree samples (collected from various forest of the Iberian Peninsula) and samples from various shipwrecks (of Iberian typology). These two techniques have been widely used for studies on lignocellulosic materials (Zhao et al. 2014, Łucejko et al. 2015, Traoré et al. 2016). They are known as easy to use techniques and provide detailed information on the structural and molecular composition of wood compounds. Furthermore, the chemical data were analysed with multivariate statistical methods to work out methodology of identification of chemical proxies for wood provenance.

1.2. Wood structure and chemical composition

Wood is an important natural material that is used for many applications, such as source of energy, paper production and it is an important material for building and furniture, etc. (Hillis 1987, Higuchi 1997). Wood is a complex polymeric composite consisting of predominantly lignocellulose. The complexity of wood structure is due to the existence of various cell elements that provide multiple functions in woody plants such as mechanical support, water and nutrient transport and the storage of biochemicals (Wiedenhoeft and Miller 2005, Zanne et al. 2006). Beside these multiple functions, the variety of wood structures is also associated to environmental conditions at temporal and geographic scale and also tree taxonomy (Fritts 1976, Creber and Chaloner 1984, Wodzicki 2001, Dobbertin 2005, Gratani 2014). The structure and chemical composition of wood vary greatly with tree taxonomy (age, genus, species, etc.), the part of tree (root, stem and branch) and the environmental conditions of the growing location (climate and soil properties). The structural and also chemical aspects of wood permit its classification into two main groups: softwoods (gymnosperms) and hardwoods (angiosperms) (Figure 1). The most obvious difference between these two groups is the lack of pores (or vessels) in the former whereas the most visible characteristics for the latter are related to the pores (Higuchi 1997, Hon and Shiraishi 2000). In gymnosperm trees, wood tissues are mostly formed by tracheids, whereas they are mostly formed by vessel elements and parenchyma in angiosperm trees (McCulloh et al. 2010).

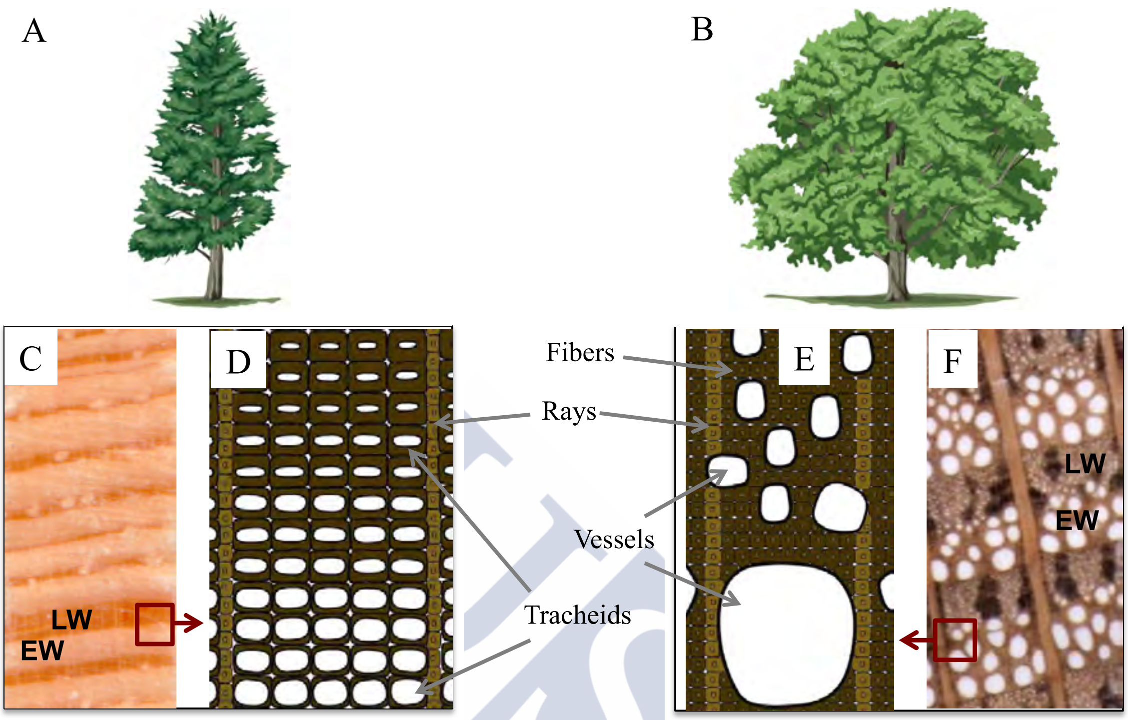

Figure 1: General form of a generic softwood tree (A) and hardwood tree (B). Hand-lens views of transverse section showing earlywood (EW) and latewood (LW) in *Pinus nigra* (C) and *Quercus robur* (F). Structural scheme of softwood (D) and hardwood (E).

1.2.1. Wood macroscopic structure

Wood is a composite of tissues produced by the vascular cambium. The conspicuous concentric number of cell layers at determined interval is known as growth increment or growth rings (Butterfield and Meylan 1980, Wiedenhoeft and Miller 2005). The growth rings, also called tree rings, are delimited by thick-walled cells called latewood, which are formed at the end of the growing season, and the thin ones named earlywood, formed at the beginning of the growth period. Notwithstanding the structural differences between the nonporous woods (softwoods) and the porous woods (hardwoods), the patterns of formation within a growth ring appear to be similar (Fengel and Wegener 1984, Back 2002, Ramage et al. 2017). This fundamental pattern consists of a gradual change of the inner diameter of wood cells. The size of the inner diameter is considerably smaller in latewood than in earlywood as well (Uggla et al. 2001, Wiedenhoeft and Miller 2005). In temperate latitudes the growth increment is commonly associated with yearly seasonal changes, in general wood cell formation ceases during winter contributing to an annual cycle of wood formation. On the other hand, growing seasons are more or less continuous throughout the year in the tropical zones whereby the differences between earlywood and latewood are more related to longer-term (years) than to seasonal changes (Tsoumis 1968, Fritts 1976, Creber and Chaloner 1984).

At a certain age, the oldest growth rings stop participating in the vital life processes of the tree, and their main function becomes mechanical support (Bertaud and Holmbom 2004, Metsä-Kortelainen 2011). In both softwood and hardwoods, the cross section of the stem of many wood species is not uniform in colour; the inner zone usually appears darker than the outer zone, referring to heartwood and sapwood, respectively. According to tree taxonomy, age and other external factors, the relative proportion of heartwood and sapwood may vary within a tree. Heartwood formation is progressively initiated from the oldest growth rings of the sapwood toward the outside; whereas, sapwood is formed next to the bark by a necessary layer of new growth rings that are involved in maintaining the required sap conduction (Holmgren et al. 1999, Back 2002, Balouet et al. 2009). By definition, the sapwood in a cross section is the physiological section that contains living cells and reserve materials (Hillis 1962, Unger et al. 2001). The heartwood represents the section with no living cells and where the reserve materials were eliminated or consumed to produce heartwood substances (Wiedenhoeft and Miller 2005). These substances are known collectively as extractives, which are formed by wood cells at the boundary between sap- and heartwood. Several characteristics and physical properties of the heartwood such as natural durability and resistance to decay processes can be related to extractive compounds (Brown et al. 1949, Hillis 1987, Taylor et al. 2002).

1.2.2. Wood microscopic structure

In addition to the differences aforementioned between softwoods and hardwoods, there are also significant differences regarding the cells they are composed of (Sjöström 1993, Ramage et al. 2017). Softwoods' cell arrangement appears to be more simple and homogenous, with only two main types of cells (tracheids and ray parenchyma cells). On the contrary, the cell arrangement of heartwood is more complex with a greater diversity of cell types (e.g. vessels, fibers, axial parenchyma and ray parenchyma), which vary in size and shape to a larger extent than softwood as well (Tsoumis 1968, Unger et al. 2001, Wiedenhoeft and Miller 2005). The middle lamella permits the bonding of neighbouring cell units. Additionally, the conducting function between two adjacent cell lumina is assured by pit membranes (Carlquist 2001, Rowell et al. 2005).

Tracheid cells are long tubes and narrow cells that mainly compose the axial (vertical) cell system of softwood. They constitute over 90% of the total volume of the wood, and provide both conductive and mechanical functions (Wang and De Groot 1996, Hon and Shiraishi 2000, Unger et al 2001). Tracheids with thin cell walls in earlywood are appropriate for the conducting function whereas the mechanical function is covered by the latewood tracheids which have thicker cell walls. In some softwood species, tracheids are associated in the radial (horizontal) system with some small parenchymatous ray cells (Back 2002, Wiedenhoeft and Miller 2005). These ray parenchyma cells survive in the sapwood for many years with their physiological functions, and represent important channels for the radial flow of nutrients (Wang and De Groot 1996). Parenchyma cells constitute less than 1% of the cells in the structure, they are not common for all the softwood species but are present in the axial system of some. Furthermore, some softwood species have additional structures called resin canals or resins ducts. These structures with particular surrounding parenchyma cells contribute to resin production and transport.

Hardwoods are mainly characterised by vessel elements and wood fibers that maintain conduction and mechanical properties, respectively (Rowell et al. 2005, Ramage et al. 2017). Vessel elements are long thick structures that are connected with one another by a perforation similar to the pit pairs of softwood tracheids, but more developed than the latter. The arrangement of vessel elements divides hardwoods in two main groups. Hardwoods are ring porous when the earlywood vessels are much larger than the latewood vessels, and hardwoods are diffuse porous when vessels distributed throughout the growth ring are of the same size (Fritts 1976, Carlquist 2001, González-González et al. 2014). Throughout the cross-section, the conducting function of the vessels becomes no more active in the heartwood part because of the plugging of vessel elements with tyloses. The fibers are also specific of cells present in the axial system of hardwoods (Unger et al. 2001). They are elongated cells with thicker walls than vessel elements and play an important support function of the trunk. The sizes of fibers vary according to the hardwood species. Contrary to softwood, the axial parenchyma cells are commonly present in the structure of hardwood. Axial parenchyma cells are relevant structures for species identification due to their particularity in each species (Butterfield and Meylan 1980, Wiedenhoeft and Miller 2005). Like in softwoods, ray parenchyma cells are present in the radial system of hardwoods. Even if there are various types of ray parenchyma, they generally play a significant role in nutrient transport (Higuchi 1997, Wiedenhoeft and Miller 2005, Morris 2016).

1.2.3. Wood chemistry

The chemical composition of wood (Figure 2) may be described as a complex composite of biopolymers that form an interconnected network: cellulose, hemicellulose and lignin (Maga 1992, Sjöström 1993, Unger et al. 2001). There are also lower molecular-weight components (extractives and inorganic compounds) that usually vary in amount and type according to tree species (Unger et al. 2001). Dried wood mainly consists of a combination of carbohydrate polymers (polysaccharides) and lignin, and is elementally composed of carbon (50%), hydrogen (6%), oxygen (44%) and trace amounts of inorganic compounds (Pettersen 1984, Rowell et al. 2005). Softwood and hardwood can be differentiated due to the variation in chemical composition. Numerous features are determined by particular chemical patterns due to wood polysaccharide, lignin and extractive contents (Di Blasi et al. 2001, Humar et al. 2008, Duca et al. 2016). Also, advanced studies about wood chemical composition can be used to differentiate between individual species of the same genus (Sandak et al. 2011, Carballo-Meilán et al. 2016). Variation between wood species can be related to chemical properties such as the correlation between the chemical composition, the influence of extractives content on wood degradation, and wood cellulose crystallinity (Colom et al. 2003, Popescu et al. 2011, Poletto et al. 2012).

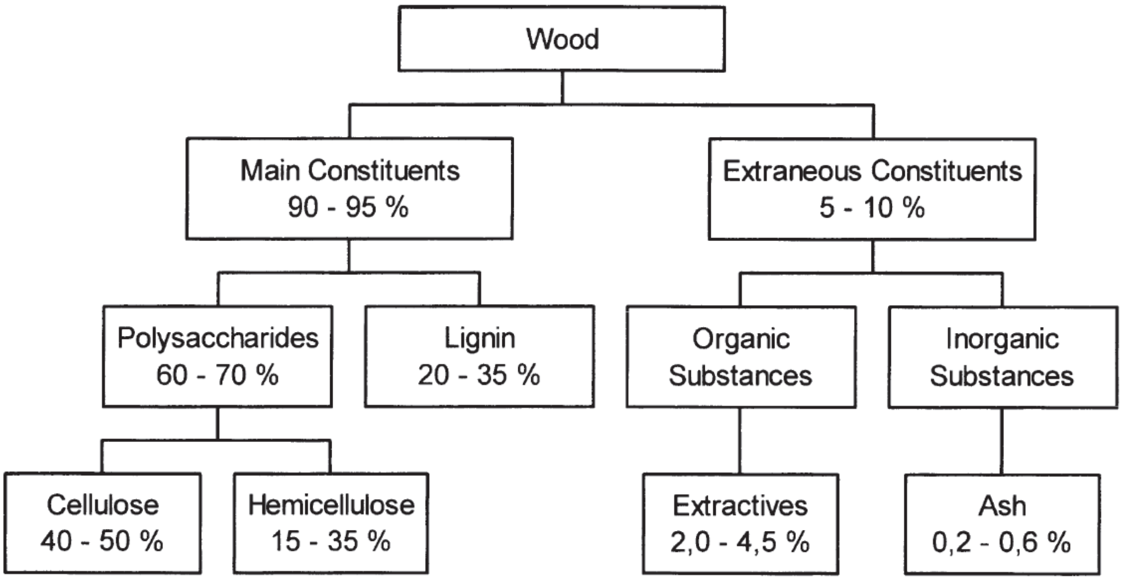

Figure 2: Scheme of the composition of wood (Unger et al. 2001)

Cellulose is the most abundant wood component, representing up to 50 % of the wood (dry weight) in both softwood and hardwood (Sjöström 1993, Klemm et al. 2005). This polysaccharide is a main structural component of the cell walls. It is a linear homopolysaccharide consisting of beta-glycosidically-bound glucose monomers formed by the reaction of glucose molecules and the elimination of a water molecule between their hydroxylic groups (Pouwels et al. 1989, Poletto et al. 2014). The cellulose polymer (Figure 3) comprises 7000-15000 glucose units and has a length of 3-8 µm (Unger et al. 2001). The abundant hydroxyl groups in cellulose play an important role in the chemical reactivity, physical behaviour and the supramolecular structure of the polymer. Additionally, the hydroxyl groups enable the formation of intra and intermolecular hydrogen bonds within and between neighbouring molecules (Tsoumis 1968, Rowell et al. 2005, Park et al. 2010). In bundles of cellulose molecules, there are highly ordered phases called "crystalline intertwined" with less ordered phases considered "amorphous" (Colom et al. 2003, Shen et al. 2013). The arrangement of the intra- and intermolecular hydrogen bonds between cellulose molecules is responsible for crystalline properties in the structure of the polymers (Park et al. 2010, Ciolacu et al. 2011). Intensely packed molecules build up the crystalline region of cellulose. Crystalline regions represent over 65% of wood derived cellulose, the remaining less dense packed portion refers to amorphous cellulose (Klemm et al. 2004, Rowell et al. 2005). The non-crystalline (amorphous) cellulose is relatively susceptible to degradation; it is the most accessible structure to degradation agents (Ciolacu et al. 2011, Lionetto et al. 2012, Poletto et al. 2012).

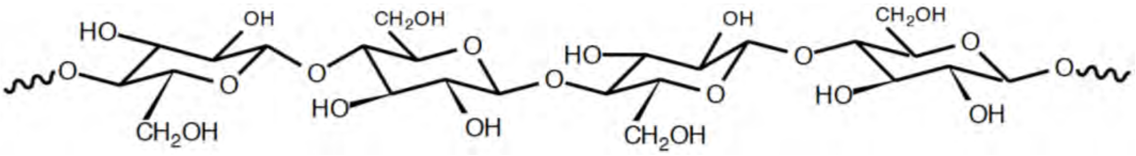

Figure 3. Cellulose, a linear polymer of 1,4-ß-D-glucopyranose units (modified from Barker and Owen 1999)

Hemicelluloses, or polyoses, are another types of polysaccharide that are usually located between cellulose fibers and bundles (Pettersen 1984, Sjöström 1993). Generally, hemicellulose (between 15 and 30% of wood biomass dry weight) consists of shorter molecules than cellulose (with 100-200 carbohydrate units). They also present important properties in wood cell walls due to their intimate association with cellulose, contributing to wood structural components in the cell walls (Timell 1967, Hillis 1987). In contrast to cellulose, hemicellulose (Figure 4) occurs as heteropolysaccharides formed by combination of two or more varieties of monomeric sugar units, hexoses (D-glucose, D-mannose, and D-galactose) or pentoses (D-xylose and L-arabinose). Small amounts of uronic acids are also present, of which 4-*O*-methyl-D-glucuronic acid residue dominates. The composition and structure of hemicellulose in softwoods differs from those in hardwoods. Glucomannan is the most important hemicellulose constituent in softwoods, whereas the dominant one in hardwood is glucuronoxylan (Timell 1967, Unger et al. 2001). Hemicellulose polymers are linked to each other and also to other wood polymers by predominantly hydrogen bonds. The chemical structure of hemicellulose makes it less resistant than cellulose due to shorter molecular chains and lower degree of crystallinity. Hence, when subjected to a degradation process, hemicellulose molecules appear to be more vulnerable than cellulose (Sundqvist 2002, Müller-Hagedorn et al. 2003, Lionetto et al. 2012).

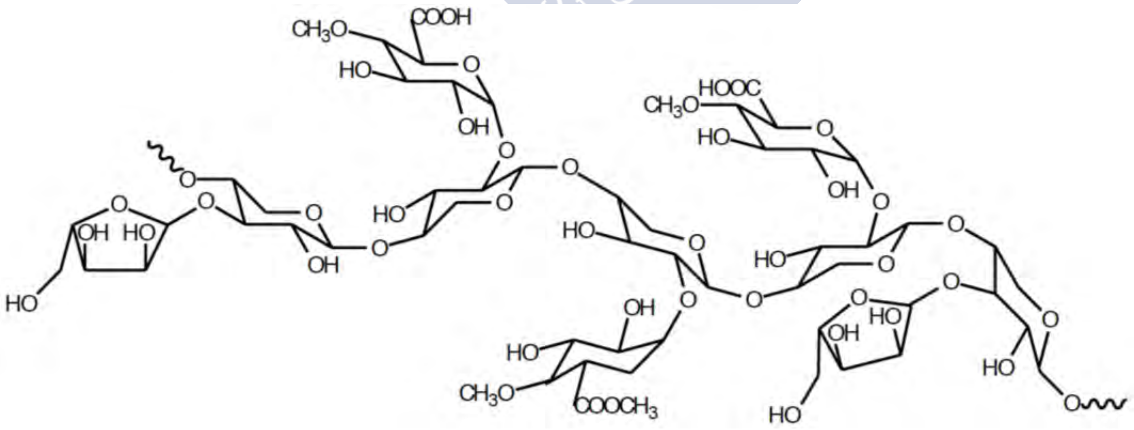

Figure 4: Hemicellulose, a branched-chain polymer composed of different sugars and side groups (residues). The figure shows a 1,4-ß-D-xylopyranose fragment with 4-O-methyl-D-glucopyranosyl uronic acid side chains (modified **from Barker and Owen 1999).**

After cellulose and hemicellulose, lignin is the most important polymeric substance in wood. It is a highly complex, heterogenous and amorphous aromatic polymer consisting of methoxylated phenylpropane structures (Tsoumis 1968, Fengel and Wegener 1984, Dence and Lin 1992, Mohan et al. 2006). The main function of this complex polymer is to contribute to the mechanical strength and liquid transport in wood cell walls (Whetten et al. 1998, Novaes et al. 2010, Dimmel 2010). The three main phenylpropanoid monomer precursors of lignin biosynthesis, also called monolignols are *p*-coumaryl, coniferyl and sinapyl alcohols (Figure 5). These phenylpropane units are linked in lignin by ether and carbon-carbon bonds. According to the three types of monolignols, lignin compounds are classified as guaiacyl (G), syringyl (S) and *p*-hydroxyphenyl (H) lignins, polymerised by guaiacyl propane, syringyl propane and hydroxyphenyl propane, respectively (Fengel and Wegener 1984, Boerjan et al. 2003). Lignin composition and contents vary greatly according to the type and part of a woody plant. Softwood lignin is mainly composed of guaiacyl lignin (produced by polymerisation of coniferyl alcohol) whereas hardwood lignin is composed of both guaiacyl and syringyl lignin (produced by copolymerisation of coniferyl and sinapyl alcohol) (Lin and Dence 1992, Rowell et al. 2005, Novaes et al. 2010). Furthermore, both softwoods and hardwoods contain lower amounts of *p*-hydroxyphenyl lignin (produced by polymerisation of coumaryl alcohol). Unlike carbohydrate polymers, lignin polymers are built up of many types of interunit linkages (about 20 types) that cross-link the phenyl propane units; the most prominent intermonomeric linkages are C-O-C (e.g. β-*O*-4 linkage) and C-C bonds (Kutscha and Gray 1970, Whetten et al. 1998). The interconnection of lignin with carbohydrates (especially hemicelluloses) is made with ester, ether and glycosidic bonds (Kutscha and Gray 1970).

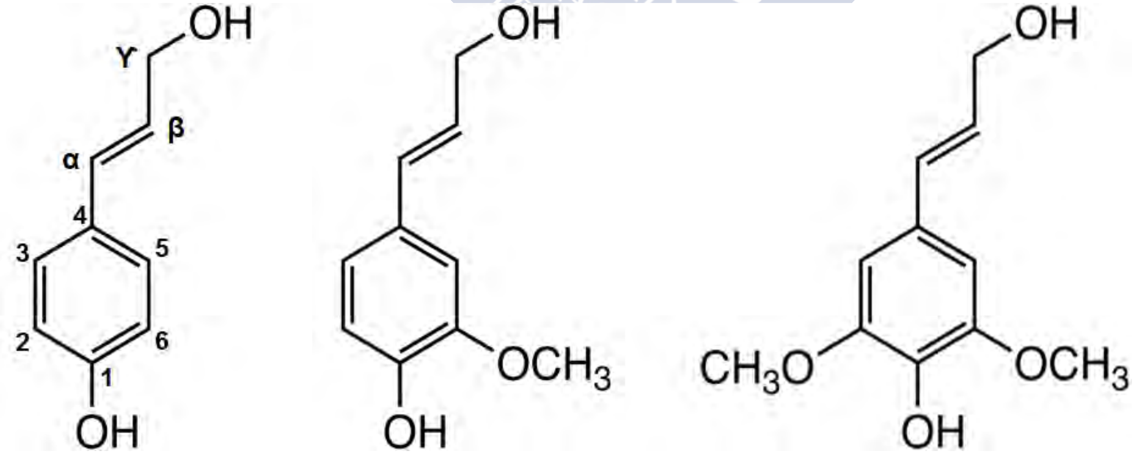

Figure.5. The three monomeric precursors of lignin: from left to right 4-coumaryl, coniferyl and sinapyl alcohols.

Extractive compounds comprise a small proportion of wood chemical contents that can be removed using neutral organic solvents or water (Sjöström 1993, Umezawa 2000). Contrary to carbohydrate and lignin compounds, extractive compounds do not contribute to cell wall structures. They play a considerable function being important as metabolic intermediates, as energy reserves and as defences against microbial and insect attack (secondary metabolites) (Hillis 1987, Higuchi 1997). Some of the extractives contribute to wood properties such as durability, colour and odour (Chen 2014). Extractive chemicals can be presented as monomers, dimers and polymers. Wood extractives comprise a variety of organic compounds consisting of polymers such as proteins or tannis and low molecular weight compounds such as fatty acids, fatty alcohols, phenols, terpenes, steroids, resin acids, etc. The amount of extractive compounds can vary according to the part of wood from a tree (stem, branches and roots); additionally, sapwood and heartwood extractives are usually significantly different in proportion and type (Taylor et al. 2002, Esteves et al. 2005, Miranda et al. 2017). Also, relatively higher concentrations of extractives are found in resin canals and ray parenchyma cells, whereas lower quantities are found in the middle lamellae and cell walls of tracheids and fibers. Extractable compounds from various wood species show characteristic (qualitative and qualitative) differences between wood samples according to species and growing location (Cadahía et al. 2001, Sanz et al. 2012, Zhang et al. 2015).

In addition to the above mentioned extractive compounds, there is another group of non-structural components of wood; inorganic compounds, or ash (Saka 2000, Rowell et al. 2005). These chemicals are also extraneous components but can be extracted only with specific solvents such as aqueous solutions or complexing agents (Fengel and Wegener 1984, Sjöström 1998). Many of the inorganic compounds are essential for wood growth, but others are not. The inorganic compounds consist of a large variety of elements and are usually present as carbonates, oxalates and sulphates, or bound to carboxylic groups in pectic materials (Rowell et al. 2005). The most abundant elements in wood ash are calcium (Ca), magnesium (Mg), and potassium (K) that make up 80% of the ash (Pettersen 1984, Demeyer et al. 2001, Rowell et al. 2005, Chen 2014). These chemical elements are taken up by trees through the roots, so that they widely vary in plants, both between and within species. Furthermore the growing environmental conditions (site and climate) and location of trees appears to be significant factors of variation for the inorganic content (Fengel and Wegener 1984, Saka 2000, Demeyer et al. 2001, Chen 2014).

1.2.4. Degradation of wood

Wood materials are subject to a wide variety of chemical modifications. These changes depend on the kind of wood (e.g. softwood or hardwood). There are also external factors associated to the environmental conditions that contribute to the deterioration of wood chemical composition (Lai 2000, Williams 2005). In general the factors that affect wood composition are: humidity, temperature, solar irradiation, chemicals and biological agents (Unger et al. 2001, Kránitz et al. 2016). For example, outdoors exposure of wood results in a slow degradation of the material described as weathering, which is initiated primarily by solar radiation. The changes that occur depend on the length of exposure and also on e.g. wood density. The weathering is initiated by discoloration of the wood surface, and also a modification of its texture (Feist and Hon 1984, Sandak et al. 2015, Liu et al. 2017). Thin layers of wood are more severely affected than thick layers. Discoloration is due to long irradiation exposure, which affects wood structural components (Williams 2005). Anderson and co-workers (1991) reported that lignin and hemicellulose compounds are primarily affected by natural weathering. Changes occurred in weathered wood surfaces contribute to enhance the effect of biological agents of wood decay. Weathering should not be confused with decay, which results from changes on wood in the presence of excess moisture by organisms (e.g. xylophagues, fungi or bacteria), which is largely an enzymatic breakdown (Feist and Hon 1984, Williams et al. 2001, Williams 2005, Schweingruber 2007). Under moist conditions in aerobic environment, fungal spores germinate and their hyphae cause wood deterioration by attacking wood cells (Blanchette et al. 1989, Blanchette 2000, Pandey and Pitman 2003). The enzymatic systems developed by fungi (brown-, white- and soft-rot) enable modifications in lignin by oxidation and by demethoxylation. Oxygen and the "right" temperature boost the deterioration of wood by fungi (Viitanen 1997, Brischke and Rapp 2008). Under water-saturated conditions in anaerobic environment, bacteria are the main biological agents of deterioration process on a long term (Blanchette et al. 1989, Eriksson et al. 1990, Blanchette 2000). Decay provoked by bacteria is common for wood submerged in seawater and freshwater, aboveground exposure, or mineral soil/sediment. These microorganisms are able to produce specific enzyme systems and also to recognise carbohydrate polymers (especially hemicelluloses). For that reason polysaccharides are usually considerably altered whereas modifications of lignin are less intense, even in more advanced stages of decay (Blanchette et al. 1989, Kim and Singh 2000, Schmidt 2006, Gelbrich et al. 2008).

1.2.5. Approaches for wood identification and provenance studies

Wood identification and provenancing are interdependent. They are highly demanded by industrial users, government agencies and museums, as well as in wood science disciplines such as botany, ecology, anthropology, forestry and so on (Tsoumis 1968, Wiedenhoeft and Miller 2005). The identification of wood anatomy consists of the analysis of characteristic cell patterns (macroscopic and microscopic features) (Brown et al. 1949, Wilson and White 1986). The most commonly applied approach is "identification by comparison", using field guides such as atlases of wood anatomy, for example (Wheeler and Baas 1998, Mazlum and Serdar 2017). Dichotomous keys have also been widely used for wood identification, but these present limitations, as they usually do not contain enough features of wood materials. Additionally, there are several computer-assisted packages that appear to be efficient for wood identification (Miller 1980, Pearson and Wheeler 1981). The adaptations of species to local environments is intimately connected to wood structure and composition, thus it influences the possibilities to obtain accurate identifications (Rana et al. 2008, Santoni et al. 2015, Traoré et al. 2018). Wood properties and characteristics depend significantly on the species and also the trees' growing environments. Furthermore, in the particular case of archaeological wooden artefacts, the storage environment is an influencing factor (Unger et al. 2001, Kránitz et al. 2016). In the last few decades, dendrochronological studies showed potential for archaeological wood identification and provenance studies (e.g. Nayling 2008, Domínguez-Delmás et al. 2013). However, shortages and divergences that often coincide with tree ring studies appear to be a significant limitation for this approach (Wilson et al. 2007, Büntgen et al. 2009, Haneca et al. 2009, Tegel et al. 2014). Wood anatomical features (phloem, ray, vessel etc.) have also been shown to be relevant for provenance studies (Martín et al. 2010, Esteban et al. 2012), but this approach is novel and more studies are needed for better elucidation for identification studies. Similarly, studies on strontium (Sr) isotopes showed that isotope ratios (^{87}Sr/^{86}Sr) could be of value to determine the provenance of ancient wooden material (English et al. 2001, Rich et al. 2015, 2016). However, it is worth mentioning that this technique presents limitations related to the complexity of wood associated to species diversity and local environmental factors, such as introduction of allochthonous Sr from seawater (Bridge 2012, Rich et al. 2016, Hajj et al. 2017). Alternatively, the structure and composition of wood chemical contents can be of value for wood identification and provenancing. Notwithstanding the complexity of wood chemical structure, several studies showed the potential of the combination of wood chemical characteristics with multivariate statistic for understanding recent and ancient wood with diverse taxonomy and origin (Colom and Carillo 2005, Colombini et al. 2007, Rana et al. 2008, Łucejko et al. 2012, Xu et al. 2013, Pizzo et al. 2015, Santoni et al. 2015, Traoré et al. 2017, 2018).

1.3. Methodology

1.3.1. Living tree and shipwreck samples

Two main reasons incited to be selective among the Iberian native tree species that are considered in the framework of the ForSEAdiscovery project. On one hand, based on historical information, oak and pine timber are indicated as the most used materials in Iberian shipbuilding (Goodman 2003, Holmgren et al. 1999, Wing 2015). On the other hand, the multidisciplinary aspect of the ForSEAdiscovery project motivated the particular attention on the potential of studies on tree ring series. However, not all the oak and pine species in the Peninsula are suitable for dendrochronological studies. There are species that have been stripped of the older trees, leaving no material for reference (Schweingruber 2012). Furthermore, the distinction of annual ring is difficult, or impossible for some species (Campelo et al. 2009), and the occurrence of intra-annual density fluctuations is frequent for others (Campelo et al. 2006, Bogino and Bravo 2008, Vieira et al. 2009, de Luis et al. 2011). Samples from four oak species (*Quercus faginea*, *Quercus petraea*, *Quercus pyrenaica* and *Quercus robur*) and two pine species (*Pinus nigra* and *Pinus sylvestris*) were collected throughout the Peninsula (**Figure 6**). The potential trees (with the largest number of rings) were targeted at the sampling sites. All the wood cores were retrieved at breast height using an increment borer. The cores were stored in paper tubes and then dried at 30 ºC for two weeks and stored in paper tubes again afterwards. Prior to any analysis, the surfaces were clean-cut on both sides to visualise tree rings.

We also analysed samples from four shipwrecks with Iberian typology, collected by the nautical archaeology team of the ForSEAdiscovery project. Two of the shipwrecks (Magdalena and Ribadeo) were located in the North of Galicia (Spain), one (Belinho) in Portugal and the last one (Yarmouth Roads) near the Isle of Wight in the English Channel (Figure 6). All shipwrecks exhibited characteristics related to techniques of Iberian typologies, although no detailed information on e.g. historical documents is available about the possible origin of the Ribadeo and Belinho wrecks. Investigations made on the Yarmouth wreck permitted to indicate features belonging to Iberian shipyards (Watson and Gale 1990, Plets et al. 2007). As for the Magdalena frigate, historical data showed that it was built and launched in July 1773 in the royal shipyard of Ferrol and had sunk in the Viveiro estuary in 1810 (Ministerio de Defensa 2011, Lema Mouzo 2014). Most of the wood samples presented anatomical features typical of oak species (*Quercus spp.*) and the remaining materials presented anatomical features of pine species (*Pinus* spp.). Besides, a pine (*Pinus* sp.) wood fragment collected from the roof structure of Segovia Cathedral was analysed. Timbers used to build this 16th century building are probably pine from the Guadarrama Mountains in central Spain (Domínguez-Delmás et al. 2017). No particular treatment was applied to the ancient wood samples; they were dried at 30 ºC for two weeks, similar to the living tree wood cores. Before any analysis the samples were cleaned by removing sediment and shipworm shells, and the surfaces were clean-cut to better visualise growth rings.

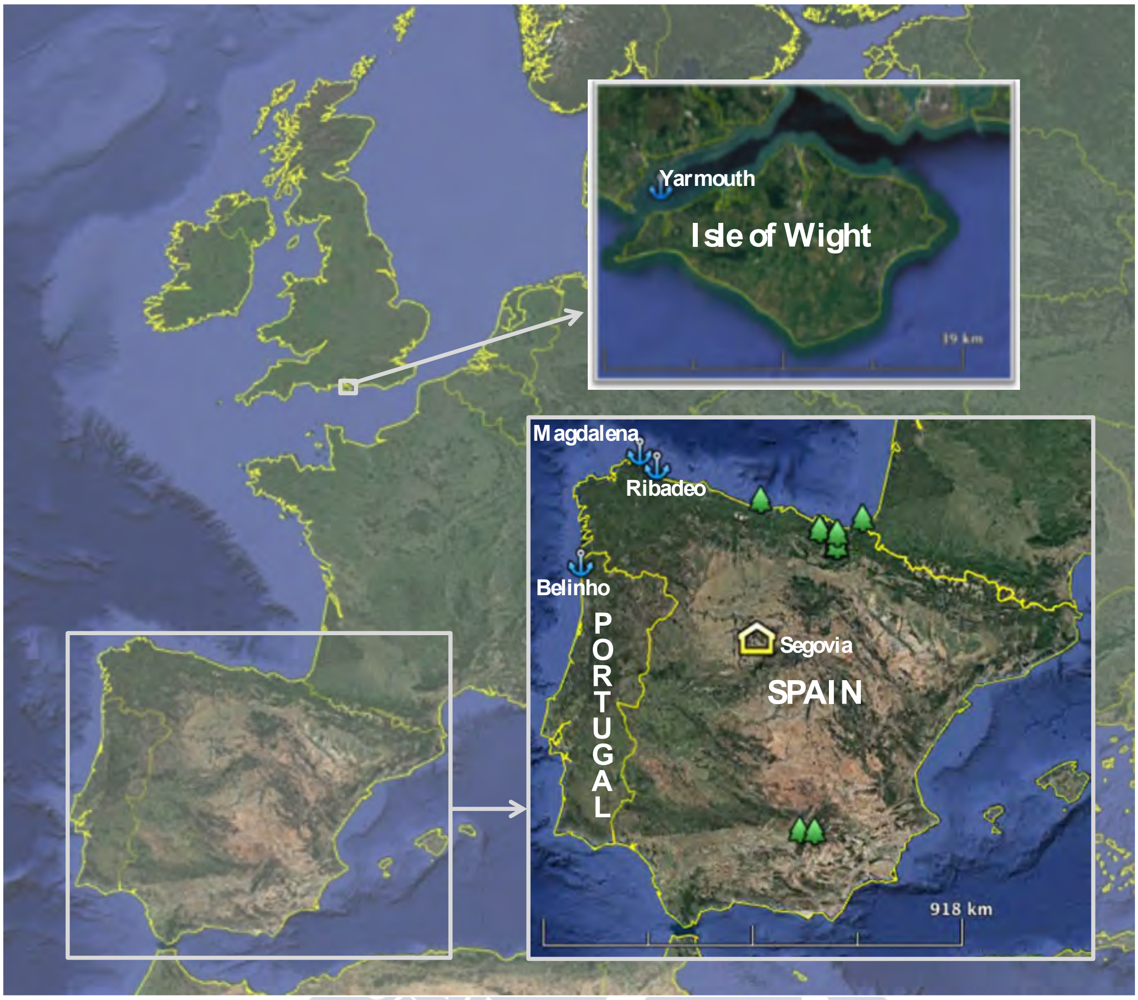

Figure.6. Map of the sampling locations of the living trees (tree symbols, green) and shipwrecks (anchor symbols, blue).

1.3.2. Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared (FTIR) spectroscopy is an easy to use and fast technique that can provide detailed information about the molecular structure of wood constituents (Moore and Owen 2001, Łucejko et al. 2015). It is the most commonly used infrared spectroscopy technique. It is based on vibrational excitations (stretching or bending) of bonds within a molecule, which are induced by radiation that is passed through an interferometer (Harwood and Claridge 1997, Stuart 2004). FTIR spectra consist of absorbance (or transmittance) in ordinates and wavenumbers in abscissae (Figure 7). Spectra were recorded over the mid-infrared region (4000-400 cm^{-1}). In general, there are much less bands in the range of 4000-1800 cm^{-1} than in the range of 1800-400 cm^{-1}; for this reason the latter is also referred to as the fingerprint region. The interpretation of FTIR spectra is facilitated by the assignment of the bands to vibrations of particular molecular structures (examples in Table 1). Typical FTIR spectra of woody samples show the absorbances of the main chemical compounds. High absorptions values in the region 1100-950 cm^{-1} due to C-O-C stretching; lower values at 1750-1650 cm^{-1} due to carbonyl bond vibrations, and a broad band at 3600-3200 cm^{-1} due to O-H stretching mainly from polysaccharide contents. Absorption peaks in the range of 1600-1400 cm^{-1}

are due to vibrations of aromatic structures, mostly lignin compounds, which also generate C-H out of plane vibrations in the range of 900-800 cm^{-1}.

FTIR spectroscopy has been widely used in wood chemistry studies, as it provides detailed information of functional groups abundances and other structural features (Bodirlau and Teaca 2009, Chen et al. 2010, Popescu et al. 2010, Allison 2011, Esteves et al. 2013). Molecular characteristic differences were found for softwoods and hardwoods related to guaiacyl and syringyl lignin content; also the relative proportions of polysaccharide and lignin showed differences between soft- and hardwood (Evans 1991, Barker and Owen 1999, Pandey 1999, Colom and Carrillo 2005). FTIR analysis also provided a suitable alternative for wood classification according to species and growing location (Rana et al. 2008, Carballo-Meilán et al. 2016, Traoré et al. 2018). Several studies assessed wood properties and quality according to changes due to treatment or degradation processes. FTIR spectra of treated wood permitted to elucidate the chemical changes due to the application of techniques to improve wood properties and qualities (Tjeerdsma and Militz 2005, Mohebby 2008, Esteves et al. 2013). Also, changes in the FTIR signal due to degradation of wood (by brown-rot and white-rot fungi) were associated to the structural alteration of carbohydrates and lignin on the molecular scale (Pandey and Pitman 2003, Mohebby 2005). Furthermore this technique has been shown to be useful for the studies of archaeological wooden artefacts. In archaeological contexts, the minimum sample manipulation (limited damage and indestructive) is highly appreciated (Łucejko et al. 2015, Traoré et al. 2016). The most common characteristic of shipwreck wood degradation shown by the FTIR technique is related to the decrease of the relative proportion of carbohydrate compounds (Borgin et al. 1975, Gelbrich et al. 2008, Pizzo et al. 2015).

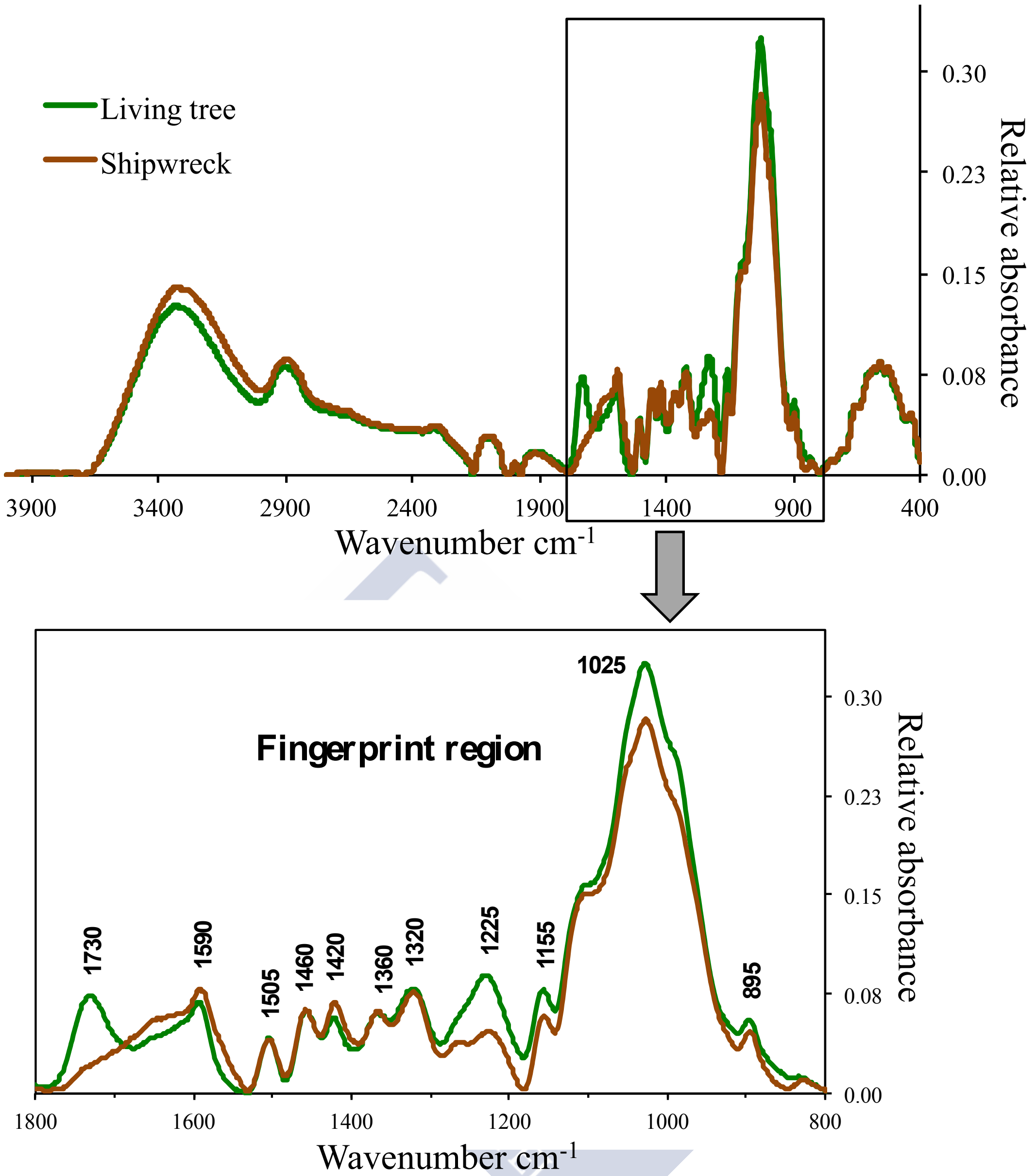

Figure 7. Examples of FTIR spectra of living tree wood (green lines) and shipwreck wood (brown lines)

Table 1. Examples of infrared bands (within the fingerprint region) and their related molecular bond assignments.

Bands (cm^{-1})	Band assignments	References
900	CH deformation of beta-glycosidic linkages in cellulose	Evans et al. 1992, Faix and Böttcher 1992
1025	C-O stretching in primary alcohols in cellulose	Popescu et al. 2007
1155	C-O-C asymmetric stretching in cellulose and hemicellulose	Faix and Böttcher 1992, Popescu et al. 2007
1225	OH vibration in guaiacyl ring, C-C, C-O, and C=O stretches in lignin	Chen et al. 2010, Zhou et al. 2015
1315	CH_2 wagging in crystalline cellulose	Colom and Carillo 2005, Popescu et al. 2007
1360	C-H deformation in cellulose and hemicelluloses	Evans et al. 1992, Mohebby 2008
1425	C-H asymmetric deformation in methoxyl, aromatic skeletal vibrations, lignin	Faix 1992, Popescu et al. 2007
1465	C-H asymmetric deformation in methoxyl for lignins, asymmetric in $-CH_3$ and CH_2 in pyran for hemicellulose	Popescu et al. 2007, Chen et al. 2010
1510	C=C stretching of the aromatic ring, C=O bond vibrations in extractive compounds	Popescu et al. 2007, Zhou et al. 2015
1590	Skeletal vibrations from the C-C	Kubo and Kadla 2005, Vahur et al. 2011
1730	C=O carbonyls in ester groups and acetyl group in xylan	Bodirlau and Teaca 2009, Zhou et al. 2015

1.3.3. Pyrolysis-Gas Chromatography-Mass Spectrometry (Py-GC-MS)

Pyrolysis-Gas Chromatography-Mass Spectrometry (Py-GC-MS) is a technique that combines three devices: a pyrolyser, a gas chromatographer and a mass spectrometer. Pyrolysis is defined as a chemical reaction caused by a short time heating in an inert atmosphere at high temperatures, in the range 400-900 ºC (Moldoveanu 1998, Łucejko et al. 2015). Temperatures below 400 ºC induce only a small thermal degradation that leaves most biopolymers largely intact, whereas temperatures above 900 ºC induce drastic changes in chemical composition that eliminates the majority of the targeted diagnostic molecular structures (Mohan et al. 2006, Nonier et al. 2006, Kaal et al. 2012). The thermal impact provokes the molecular fragmentation of the original compounds into pyrolysis products (pyrolysates). The interface between the pyrolyser and the GC inlet is kept at a constant temperature (e.g. 300 °C) to avoid the condensation of the pyrolysis products and to maintain them in gaseous state (Kaal 2011, Schellekens 2013). The pyrolysates are transported by the flow of an inert gas (usually helium) to the chromatographic column where compounds are separated depending on their boiling point and characteristics that define their interaction with the stationary phase (the "packing" of the capillary GC column). Meanwhile, an ionization process starts in the mass spectrometer, which is equipped with an ion trap detector where separation takes place based on the mass to charge ratio (*m*/*z*) of the ions. The signal from the detector is amplified and interpreted by an electronic data system, and generates a mass spectrum. Compound identification is facilitated by molecular characteristics of mass provided in databases (NIST mass spectra library) and literature (Boon 1989, Moldoveanu 1998, Nierop and Buurman 1998, González-Vila et al. 2001, Dignac et al. 2006, Buurman et al. 2009). Even if Py-GC-MS is a destructive technique, it presents the advantage of minimal preparation and use of only a very small amount (10 to 100 µg) of sample (Shedrinsky and Baer 2006).

The pyrolysis products of woody samples (Figure 8, Table 2) have a characteristic distribution of the major products derived from cellulose, hemicellulose, lignin and extractives (Evans and Milne 1987, Faix et al. 1987, van Bergen et al. 2000). Numerous studies have demonstrated that Py-GC-MS is a powerful technique to characterise lignocellulosic materials. It has also been successfully used to characterise the main chemical contents in woody stems of vascular plants (Demirbas and Arin 2002, Kaal et al. 2007, Łucejko et al. 2009). Other studies permitted to elucidate differences between softwood, hardwood and grass plants according to the structure of lignin (Sáiz-Jiménez and de Leeuw 1986, Faix et al. 1987, Pouwels et al. 1987). Variability in tree stem (bark, sapwood and heartwood) was indicated using this technique, showing high contents of extractives in heartwood and high polysaccharides content in sapwood (Campbell et al. 1990, Challinor 1995, Lourenço et al. 2015). Also, pyrolysis products of lignin, carbohydrates and extractive compounds enabled to discriminate between various oak species (Challinor 1995, Nonier et al. 2006). This technique presented a potential to evaluate the modification in wood properties due to treatment or degradation processes (Martínez et al. 2005, Grinins et al. 2013, Herrera et al. 2014). Py-GC-MS has also been widely used for archaeological wood studies, and specifically in the case of waterlogged (shipwreck) woods. It permitted to elucidate the importance of environmental conditions in the degradation process (Colombini et al. 2007, Traoré et al. 2016). Under waterlogged conditions carbohydrates are altered more intensely than lignin structures (Colombini et al. 2007, Tamburini et al. 2015). Interestingly and intuitively, some studies highlighted that degradation progresses from the outer surface (in contact with the degradation agent) to the inner part of the wood (Wilson et al. 1993, Tamburini et al. 2015).

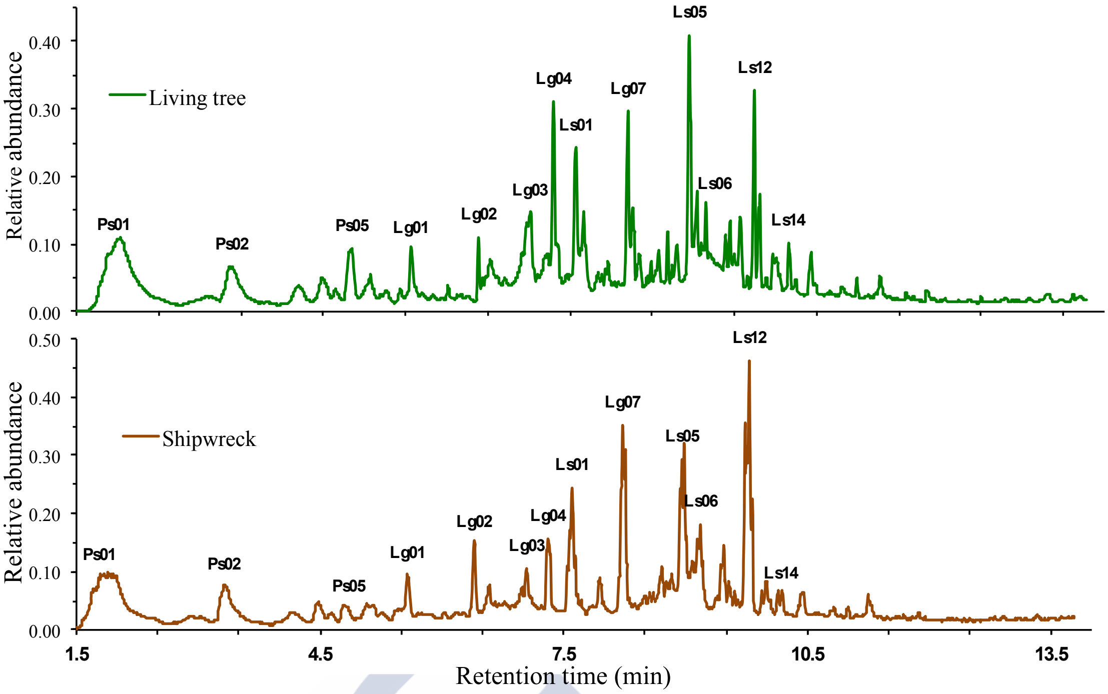

Figure 8. Examples of total ion chromatograms obtained from living tree wood (upper panel) and shipwreck wood (lower panel). Peak labels refers to products listed in Table 2.

Table 2. Example of list of pyrolysis products from wood samples

Code	Pyrolysis products	m/z	RT (min)
Ps01	acetic acid	60	1.655
Ps02	3/2-furaldehyde	95+96	3.325
Ps05	4-hydroxy-5,6-dihydro-(2*H*)-pyran-2-one	114+58	4.846
Lg01	guaiacol	109+124	5.572
Lg02	4-methylguaiacol	123+138	6.387
Lg03	4-ethylguaiacol	137+152	7.04
Lg04	4-vinylguaiacol	135+150	7.305
Lg07	$C_{3:1}$-guaiacol (*trans*)	164+149	8.223
Ls01	syringol	154+139	7.595
Ls05	4-vinylsyringol	180+165	8.945
Ls06	$C_{3:1}$-syringol	194+179	9.162
Ls12	$C_{3:1}$-syringol (*trans*)	194+179	9.754
Ls14	4-(propan-2-one)syringol	167+210	10.19

1.3.4. Multivariate data analysis

The application of multivariate data analysis (MVDA) to data obtained with FTIR and Py-GC-MS techniques revealed to be very important to obtain insights about the chemical structure and composition of wood materials (Faix and Böttcher 1993, Mohan et al. 2006, Łucejko et al. 2015). MVDA is a toolbox that potentially enables to extract useful information through statistical and mathematical calculations (Ferraz et al. 2000, Eriksson et al. 2013). It is mostly based on pattern recognition from the numerous variables provided by FTIR and Py-GC-MS data. Principal component analysis (PCA) and discriminant analysis (DA) are two useful and widely used methods of MVDA. PCA transforms a dataset by removing inter-correlation between variables and providing new variables, named components, that account for most of the variation existing in the original dataset (Dytham 2011, Eriksson et al. 2013). The application of PCA enables to obtain an overview of the patterns and major trends in the data matrix (observations in rows and variables in columns), highlighting relationships between variables and often grouping among observations (Labbé et al. 2006, Eriksson et al. 2013). DA extracts canonical functions, which are linear combinations of a given set of original variables, which support and maximise the separation of samples that were previously assigned to two or more groups. It permits to explain differences between groups of observations and also to determine the significance of variables for the existing group patterns (Carballo-Meilán et al. 2014, Acquah et al. 2016).

PCA and DA have been applied to wood FTIR and Py-GC-MS data, aiming to reduce dimension and provide patterns related to the main chemical characteristics (Evans and Milne 1987, Boon 1989, Carballo-Meilán et al. 2016, Traoré et al. 2016). The analysis of FTIR and Py-GC-MS data of various wood species with PCA permitted, for example, to characterise and separate softwoods and hardwoods according to their carbohydrate and lignin composition (Modugno et al. 2008, Chen et al. 2010). By using PCA on infrared data, Labbé et al. (2006) classified various wood samples according to species and determined the effect of high temperature (300-350 ºC) on wood polysaccharide contents; while Rana et al. (2008) used PCA on FTIR data to classify beech trees from different growth habitat. PCA based on pyrolysis products also helped to highlight degradation pathways in archaeological woods and determine the intensity of degradation (Tamburini et al. 2014, Traoré et al. 2017). Similarly, stepwise DA has shown to enable accurate separation of woods from different species and locations (Carballo-Meilán et al. 2014, Traoré et al. 2018). Among wood pyrolysis products, methoxyphenol patterns have been shown to be sufficient to differentiate between woods and geographical origin of various *Eucalyptus* species (Galetta et al. 2014).

1.4. Scope, objective and outline of this thesis

The research done in the framework of this thesis aimed to contribute to investigate shipbuilding and the commercial network of wood trade in the Iberian Peninsula between the 16th and the 18th century. The available historical and archaeological archives present limitations to elucidate the use of wood resources, but they provide relevant information about the potential forests that were exploited and specific techniques for shipbuilding of Iberian shipyards. In the ForSEAdiscovery project, the chemistry of wood was among the wood science disciplines that were considered potentially complementary to other disciplines (historical and archaeological) in order to answer the main questions related to the management of forest resources in the Iberian Peninsula. Several studies of wood chemistry characterisation with analytical techniques such as FTIR and Py-GC-MS highlight the relevance of the information they provide, although the complexity of wood chemical structure is associated to tree taxonomy and environmental factors (Fritts 1976, Creber and Chaloner 1984, Dobbertin 2005, Gratani 2014). In the particular case of archaeological woods, the storage condition (in seawater or marine sediment for shipwreck woods for example) is an additional factor to be taken into account (Eriksson et al. 1990, Blanchette 2000, Pandey and Pitman 2003). In fact, degradation processes make chemical studies of archaeological wood truly challenging due to the limitations associated with degradation factors. However, the use of MVDA offers an alternative to surpass these limitations (Faix and Böttcher 1993, Mohan et al. 2006, Łucejko et al. 2015).

The main objective of this thesis was to explore to which extent wood organic chemical composition can be of assistance for enhanced understanding of the characteristics and properties of wood, those related to species and environmental conditions of forests' growing location in particular. Within this framework, focus was placed to the potential of the application of FTIR and Py-GC-MS to generate new insights on living tree and archaeological wood chemical composition. In this perspective, we were mostly interested in the following specific aspects:

i) Initially, the analytical techniques (FTIR and Py-GC-MS) were combined with MVDA in order to develop specific methodologies/protocols for wood characterisation;

ii) Attention was paid to assess the variability within and between wood types according to species and growing locations;

iii) Identification of relevant chemical parameters (structures, molecules, indices, etc.), including those of diagenesis, for the appropriate evaluation of archaeological woods;

iv) Use of MVDA methods to develop models for identification of archaeological woods.

To report the achievement of the established objectives, the remaining sections of this thesis are subdivided. Firstly, we provide a General Discussion consisting on a synoptic view of the main subject and a General Conclusion with the main output of the research developed for this thesis. References are listed at the end of this part. The second section contains the scientific contributions (articles in peer-reviewed international journals).

2. GENERAL DISCUSSION

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2. GENERAL DISCUSSION

2.1. Chemical fingerprint of wood from living trees

2.1.1. FTIR spectroscopic characteristic of wood materials

Along this study, FTIR performed on thin wood fragments from individual ring showed that this technique provides high-resolution spectra. Thin fragments (< 1 mm thick) tend to give better resolutions. In general, signals related to polysaccharide and lignin compounds dominated the spectra, which is logical as wood is mainly composed of lignocellulose (Fengel and Wegener 1984, Sjöström 1993). However, there are also signals related to the organic "extractive" compounds. Diverse inter- and intra-molecular bonds between and within molecules and polymers are recognised in infrared spectra. As usual, few peaks can be identified in the FTIR range between 3500-2700 cm^{-1}, with the exception of the broad hydrogen bond vibrations for O-H (at 3300-3800 cm^{-1}) and the two narrow absorption peaks of the C-H (at 3000-2800 cm^{-1}) bonds. The mixture of inter- and intra-molecular hydrogen bonds induces the broadening of the O-H peaks at these wavenumbers, whereas the narrow C-H peaks are due to the occurrence of aliphatic fragments (Coates 2000, Kubo and Kadla 2005, Popescu et al. 2010). In the fingerprint region (1800-800 cm^{-1}), spectra appear very complex due to overlapping and/or combinations of various vibrations from the wood components. Since polysaccharides are the most abundant, high peaks appear for C-O bonds vibration near 1030-1020 cm^{-1}. The main signals for lignin compounds are related to vibrations in the aromatic structures, for example at bands near 1590 and 1425 cm^{-1} for C-C and C-H bond deformations, respectively. The extractive compounds also present signals from aromatic structures and other molecular structures such as carbonyl. For the fingerprint region, exploration of the second derivative spectra appeared paramount to identify the numerous underlying vibrations that cannot be witnessed from original spectra, as the second derivative spectra permit to enhance the apparent resolution and amplify small differences (Boeriu et al. 2004, Huang et al. 2008, Popescu et al. 2009, Zhang et al. 2016). The analysis of FTIR data of wood is carried out through the use of various infrared parameters. Among others, the relative intensities of peak absorption bands, which are calculated according to the whole spectrum or according to specific absorption bands. Numerous research results also highlighted the relevance of the use of relative intensities to characterise molecular composition of wood samples (Evans 1991, Pandey 1999, Colom and Carillo 2005, Popescu et al. 2010, Poletto et al. 2012). The complexity associated to wood composition does not permit to get most of the characteristics in depth by the common use of relative intensities. The convolution of infrared peaks raises the need for not only the second derivative, but also the application of MVDA methods that allow extracting meaningful information on wood chemistry (Ferraz et al. 2000, Chen et al. 2010, Acquah et al. 2016, Traoré et al. 2016, 2018).

2.1.2. Characteristic wood pyrolysis products

The application of Py-GC-MS to wood fragments indicated that the constant size of the samples is of importance for maintaining reproducibility and general performance. The proportion of extractive compounds showed to interfere with the coherence of the results. Depending on the type of samples (sound or archaeological), constant weight of wood chips was essential for comparing samples and obtaining consistent results. The pyrolysis data revealed compounds derived from polysaccharides (cellulose and hemicellulose) as the major pyrolysis products, followed by lignin-derived compounds (phenols, guaiacols and sometimes syringols). Smaller relative proportions are related to extractive compounds such as tannins or resinous substances. Indeed, the pyrolysis products recorded in the framework of this thesis represent typical products of wood samples (Faix et al. 1990 and 1991, Moldoveanu 1998). In general, the polysaccharide products, mainly acetic acid, furans and pyrans (Nonier et al. 2006, Łucejko et al. 2009) have shorter retention times due their low molecular weight in compounds with lignin products. The pyrolysis of wood also leads to the formation of anhydrohexose products, such as levoglucosan with relatively higher molecular weight and a longer retention time. Levoglucosan is a pyrolysis marker for cellulose and its predominance among polysaccharide-derived products may be due to the crystalline structure of cellulose (Shafizadeh 1982, Pouwels et al. 1987, Traoré et al. 2016, 2017). The absence of crystalline structure in hemicellulose makes it easier to degrade thermally (Faix et al. 1990, Mohan et al. 2006). Among the pyrolysis products of wood, there are large proportions of acetic acid that is generated by deacetylation of polysaccharide products, mainly hemicellulose compounds (Alén et al. 1996, Martínez et al. 2005, Traoré et al. 2017). It has been shown that pyrolysis contributes to deesterification of the acetylated hemicellulose (Pouwels et al. 1987, Mohan et al. 2006). Two main groups according to the structure of the phenylpropanoids characterise pyrolysis products derived from lignin, which appear less amenable to thermal degradation because of their heavier molecular weight and their aromatic structures (del Río et al. 2001, Zhao et al. 2014, Lourenço et al. 2015). Guaiacyl (methoxyphenol) products generally elute before the syringyl (dimethoxyphenol) products. Furthermore, other phenolic compounds are also largely lignin products generated via the cleavage of ether and carbon-carbon linkages (Faix et al. 1990, del Río et al. 2001, Mohan et al. 2006). Pyrolysates of extractive compounds comprise various groups of products that vary according to the origin, such as, tanins, terpenoids and fatty acids. There are many reaction pathways responsible for the formation of these products (Alén et al. 1996, Scalarone et al. 2003, Colombini et al. 2007).

2.1.3. Multivariate data analysis of wood chemical composition

For each factor (taxonomic and environmental) that influences wood forming processes, there are chemical parameters that can be potentially associated (proxies). Multivariate data analysis methods are well known to be relevant tools to handle data from complex systems like wood (Faix and Böttcher 1993, Allison 2011). FTIR and Py-GC-MS are techniques that provide a wealth of information on the chemical composition of wood materials, but the interpretation of FTIR and Py-GC-MS data is challenging due to the multitude of bands/peaks/compounds that can be studied (usually tens to hundreds). Additionally, the overlapping of lower intensity signals (peak absorption) by the higher ones also presents a possible meddling on spectral features. In wood FTIR spectra, signals related to a given structure are not only resulting from specific functional groups within the molecule, but also from vibrations from other nearby molecules (Allison 2011). As for Py-GC-MS, it appears difficult to determine the reaction pathways leading to the formation of pyrolysis products. These, among others, are reasons why the use of MVDA methods is necessary for wood studies on the complicated pyrolysis chemistry (Shin et al. 2001). Statistical methods allow to develop simplified ways through factors that gather specific patterns and trends into relevant parameters for enhanced characterisations. Multivariate data analysis methodology allows to focus on specific indicators and to improve the level of information obtained.

In this work, the application of principal components analysis (PCA) to FTIR and Py-GC-MS data shows the potential of the application of this chemometric approach. Indeed, PCA permits to agregrate complex signals into simplified indicators of the chemical composition of wood. In the particular case of FTIR data, PCA were applied to the transposed data matrix (samples as columns and spectral bands as rows), permitting to pay more attention to variations of the spectroscopic signal within wood samples. The transposed data matrix for PCA has been applied in other disciplines (e.g. López-Merino et al. 2012), but for wood spectroscopic data this approach appeared to be novel. In fact, the extracted PCA factors permitted to get better insight associating degradation process and relative storage environmental conditions between two types of archaeological wood (shipwreck wood and construction wood). Actually, PCA components can provide qualitative information about the wood contents. They can provide chronological information related to tree rings, as chemical contents vary between and within wood samples. Furthermore, the PCA factors may also reflect the type of wood (soft- and hardwood, early- and latewood, sound and archaeological wood). Already, several studies focussed on wood molecular structure and provided qualitative and quantitative information through the application of PCA to wood FTIR and/or Py-GC-MS data (Ferraz et al. 2000, del Río et al. 2001, Chen et al. 2010). Also, the use of PCA provides understanding about microscopic features of wood through molecular structure of its chemical contents (Hori and Sugiyama 2003, Labbé et al. 2005, Meier et al. 2005). The application of PCA allowed to shed light on chemical differences and similarities between samples by assessing the state of degradation of various archaeological woods (Łucejko et al. 2009, Tamburini et al. 2014, Traoré et al. 2016, 2017). Even though PCA has been used for wood species and provenance identification, discriminant analysis (DA) appears to be the most appropriate for such purposes (Rana et al. 2008 and 2010, Carballo-Meilán et al. 2014, Traoré et al. 2018). Discriminant functions are based on combinations of variables that maximise the correct classification of wood samples on a priori defined groups (as species or growing locations). Results in this thesis show that carbohydrate and lignin contents are relevant compounds for DA, albeit that wood carbohydrate contents appear to be particularly appropriate due to their molecular structure and relatively large proportion in wood samples. The use of DA is not as common as PCA but it has also been used in recent studies for wood identification. The application of DA to infrared spectral data permitted to classify samples according to plant parts (branch, stem, leaf) and also according to the quality of wood (Casale et al. 2010, Pastore et al. 2011, Acquah et al. 2016, Lang et al. 2017). In similar research studies, DA has been applied to wood FTIR spectra to successfully distinguish wood samples from various species and origins (Hobro et al 2010, Carballo-Meilán et al. 2014 and 2016, Traoré et al. 2018).

2.2. Sources of variation in chemical composition of living wood

2.2.1. Softwood and Hardwood contents

The outcomes from the application of FTIR and Py-GC-MS indicated the diversity of differences between softwood and hardwood samples. The most common dissimilarity between the two types of wood is related to the structure of lignin. Chemical data from the two analytical techniques showed similar results. Guaiacyl units constitute the main aromatic structure of lignin compounds in pine (softwood). Whereas in oak (hardwood), the aromatic structure of lignin is mainly constituted by both guaiacyl and syringyl units. Several investigations on wood showed this difference (between softwoods and hardwoods) related to the molecular structure of lignin compounds (Challinor 1996, Di Blasi et al. 2001, Asmadi et al. 2010, Duca et al. 2016). Regarding wood polysaccharide contents, variations in the molecular structure of hemicellulose compounds also indicate differences between softwoods and hardwoods. Pyrolysis studies found that 5-hydroxymethyl-2-dihydrofuraldehyde-3-one is present in pinewood, whereas it is absent in pyrolysates of oak wood. Also, the FTIR data showed higher absorptions related to vibrations in carbonyl bond in hardwood than softwood. Furthermore, numerous studies applying FTIR and/or Py-GC-MS found variations in the relative amount of hemicellulose content between the two types of wood (Pandey 1999, Unger et al. 2001, Chen 2014, Duca et al. 2016). Finally, the organic extractive compounds also suggest differences between softwoods and hardwoods. For instance, specific terpenoid compounds were detected in pine samples. Analyses on oak wood show an occurrence of phenolic compounds as extractive compounds. These results are in accordance with studies that stated resin compound as specific to pine species (Holmgren et al. 1999, Nielsen et al. 2009, Ribechini et al. 2009) and (ellagi)tannins as specific polyphenolic constituents of oak (Fernández de Simón et al. 1996, Cadahía et al. 2001, Pandey 2005, Humar et al. 2008).

2.2.2. Sapwood and Heartwood

Differences between sapwood and heartwood are also evident in chemical composition. Independent of wood species, the proportion of extractive compounds appears higher in heartwood than in sapwood. FTIR and Py-GC-MS data reveal that heartwood chemical composition is influenced by the prevalence of chemical signal related to extractive compounds, which contribute to the darker colour of this part of wood according to literature (Holmgren 1999, Pandey 2005). These variations are intimately associated to the structural characteristic of these wood constituents. Heartwood is the part of wood composed of dead cells and where maintenance substances (or heartwood substances) are stored, which contribute to wood's mechanical functions (Hillis 1987, Campbell et al 1990, Taylor et al. 2002). Definitely, extractives are the main chemical compounds that differentiate between sapwood and heartwood. Similar conclusions are stated in several previous studies (Holmgren et al. 1999, Sundqvist 2002, Nuopponen et al. 2003, Esteves et al 2005). Also, sapwood and heartwood show differences according to their polysaccharide content, with higher proportions in sapwood (Bertaud and Holmbom 2004, Metsä-Kortelainen and Viitanen 2010). Furthermore, heartwood appears to be chemically more stable than sapwood. Plus, heartwood is recognised as a conservative taxonomic tissue that involves detailed information (family, genus, and even the species) about the tree to which it belongs (Seikel et al. 1965, Hillis 1987, Mosedale and Savill 1996, Mosedale et al. 1998). These details strongly suggest heartwood samples as the most appropriate material for wood chemical studies aimed at species and provenance identification.

2.2.3. Wood species and growing locations

Wood species and growing location are two distinct but interdependent aspects that generate variability in specific chemical features of wood (Pettersen 1984, Poletto et al. 2012). The application of MVDA to FTIR and Py-GC-MS data proved to be relevant for studies involved with differentiation of various wood species of the same genus. Heartwood polysaccharide and lignin fingerprints contain sufficient information for species discrimination between *Pinus nigra* and *Pinus sylvestris*. Again, considering wood polysaccharide and lignin content, this approach enabled to separate the four oak species (*Quercus faginea*, *Quercus petraea*, *Quercus pyrenaica* and *Quercus robur*) studied in this thesis. The obtained results strongly support the usefulness of wood chemical composition for species discrimination. Similarly, the combination of MVDA with wood chemical data suggests the possibility to separate between wood samples from different growing locations. In fact, wood chemical fingerprint is defined by various factors related to trees' taxonomy and the growing location; for that reason the environmental conditions of the growing location need closer attention. The formation of xylem and phloem requires solar radiation that regulates changes in wood tissues (Barij et al. 2007, Zhang et al. 2016). Photosynthesis processes induce reactions that result in changes in chemical structure during lignin biosynthesis (González-Vila et al. 1999, McKendry 2002, Moura et al. 2010, Santos et al. 2012). Also, the strength of intra- and intermolecular hydrogen bonds controls the rigidity of polysaccharide chains and regulates differences between wood according to species and origin (Timell 1964, Sjöström 1993, Higuchi 1997, Klemm et al. 2004, Colom and Carrillo 2005, Rana et al. 2008, Poletto et al. 2014).

2.3. Shipwreck wood

2.3.1. General considerations on wood structure and properties

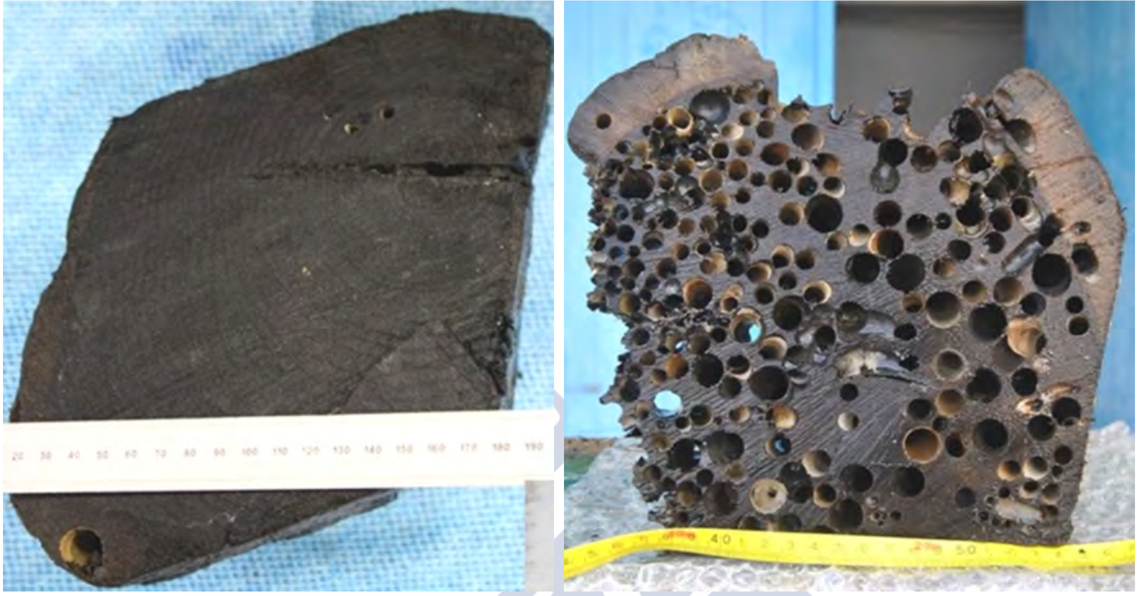

Figure 9. Oak wood samples of framing elements; Left: samples in good condition (from the Yarmouth shipwreck). Right: samples attacked by shipworm (from the Ribadeo shipwreck). Photographs by Sara Rich.

The shipwreck samples studied in this thesis exhibited different types of alterations, which are reflected, for instance, by changes in colour and appearance of wood. The archaeological wood samples are all characterised by darkening, the intensity of which varies according to the origin of the wooden artefacts. Alterations of the texture could also be witnessed, which are due to the influence of seawater inducing hygroscopic phenomenon on wood such as swelling (Fojutowski et al. 2011). More frequently, archaeological wood samples present physical damages that are reflected by multiple holes made by shipworms (Figure 9). All changes observed in these types of sample are attributed to numerous overlapping factors, related to seawater and micro- and macro-organisms (Jordan 2001, Williams 2005). The discoloration of the waterlogged wood samples is attributed to the loss of fluorescence and birefringence properties of cell walls (Hoffmann and Jones 1989). In fact, structural changes influence the appearance of wood, with the most noticeable parameter being colour. Under seawater condition, wood quickly changes colour because of the degradation of cell walls and pit membranes (Schweingruber 2007, Blanchette 2010, Oron et al. 2016). Many studies focussed on colour changes on woody material. However, only few studies focus on waterlogged wood, among which, many have been associated with the physical changes and the leaching of chemical constituents (Zborowska et al. 2007, Franceschi et al. 2008, Sandak et al. 2014, Eriksen et al. 2015, Liu et al. 2017). Actually, it has been shown that the activity of tunnelling shipworms depends on wood chemical contents

(Kim and Singh 2000, Jordan 2001, Pizzo et al. 2013, Eriksen et al. 2017). Wood polysaccharides are chemical compounds that are necessary as a source of energy to maintain numerous biological agents. Wood decay can be a visible phenomenon, nevertheless the pattern of degradation can also be recognised from chemical changes (Gelbrich et al. 2008, Kránitz et al. 2016).

2.3.2. Chemical composition of shipwreck woods

The degradation of the biomolecular constituents of the shipwreck woods studied in this thesis resulted in a decrease in the polysaccharide contents and a relative increase of lignin. This outcome is in agreement with what has been widely found in literature, about the relatively low abundance of polysaccharides in waterlogged archaeological woods (Wilson et al. 1993, van Bergen et al. 2000, del Río et al. 2002, Tamburini et al. 2014, Łucejko et al. 2015, Pizzo et al. 2015). In fact, polysaccharides appear to be the most vulnerable wood chemical compounds under seawater conditions. In such storage environment, there are two degradation processes recognised as the most influential. On one hand, the polysaccharides are subject of hydrolysis, enhanced by the swelling of the secondary cell walls after a prolonged stay of wood underwater conditions (Colombini et al. 2007, Kránitz et al. 2016). On the other hand, microbial agents preferentially attack polysaccharides over lignin (Hedges 1990, Jordan 2001). Furthermore, the research in this thesis permitted to point out the effect of the waterlogged conditions on chemical properties of specific molecules. Levoglucosan is commonly known as an important chemical marker of wood degradation, but our results indicated that it is not absolute evidence. Several authors stated that simultaneous occurrence of varied degradation patterns generally contribute to selective degradation of wood chemical compounds (van Bergen et al. 2000, Giachi et al. 2003, Tamburini et al. 2017, Zoia et al. 2017). In fact, this has been proved also by the chemical characterisation of the wood collected from an ancient building to which other type of degradation process was attributed (Traoré et al. 2016).

Under waterlogged conditions, certain carbohydrate structures resist the degradation processes, whereas others do not, and the same is observed for lignin. This has become clear both from FTIR absorption bands and specific pyrolysis products, showing that polysaccharides and lignin compositional changes (not only relative contents) can be studied by these techniques, if adequate MVDA methods are applied. This had hitherto not been investigated thoroughly. More specifically, high proportions of the pyrolysis product 3-hydroxy-2-methyl-2-cyclopenten-1-one (*m*/*z*: 55+112) were recorded for numerous shipwreck woods. Similar results have been found by other researchers, who suggested this compound as originating from a polysaccharide precursor that is relatively well-preserved during wood decay (Sáiz-Jiménez et al. 1987, Łucejko et al. 2009, Heigenmoser et al. 2013). This may refer to the crystalline structure associated with cellulose compounds, which plays an important role on the rate of wood decay (Sundqvist 2002, Lionetto et al. 2012). The results found in this thesis also indicated low abundances of numerous pyrolysis products, such as homovanillin and homosyringaldehyde (*m*/*z* 137+166 and *m*/*z* 167+196, respectively) due to lignin modification. Likewise, earlier studies showed very low content of some lignin derivative pyrolysis products (Colombini et al. 2009). It is likely that these products are from lignin precursors that are relatively sensitive to microbial degradation (Bugg et al. 2011, de Gonzalo et al. 2016). As a matter of fact, the molecules that display particular characters could be considered as specific chemical markers for shipwreck woods. Based on them, we proposed novel proxies for shipwreck wood preservation (SWPIs). The SWPIs consist of the most important molecules (in statistical terms) that are the least amenable to degradation processes. The results showed that they are adequate for studies about archaeological waterlogged woods. Therefore the SWPIs could be used as well as the most used parameters (ratios between specific pyrolysis products) to evaluate the chemical composition of decayed woods (van der Heijden and Boon 1994, Kaal et al. 2015, Schellekens et al. 2015).

In archaeological samples, the proportion of the organic extractive chemicals was relatively low. The decrease in the amount of extractive compounds in shipwreck woods is attributed to the very long time in contact with seawater. Wood extractives have been detected in relatively small amounts in numerous studies on waterlogged woods (Giachi et al. 2003, Gelbrich et al. 2008). Due to their chemical structure, many of them (such as flavonoids, condensed tannins and some terpenoids) are water-soluble (Nzokou and Kamdem 2004) and gradually leach from the wood. The removal of these compounds intensifies water penetration that enhances the swelling phenomenon and also the degradation of other wood components (Mantanis et al. 1995, Unger et al. 2001). Although the multiple degradation factors under seawater, it is important to remind that, there is still no evidence about the influence of micro-organisms on extractive contents of underwater archaeological woods. Furthermore, others research works demonstrated that the demethylation undergone by certain lignin compounds contributes to the formation of phenolic structures lacking methoxylic groups (van Bergen et al. 2000, Giachi et al. 2003).

2.3.3. Chemical fingerprinting of species and provenance for shipwreck woods

Identification of species and origin of wood represent important issues among the most frequent research questions associated to shipwreck woods studies (Bridge 2012, Rich et al. 2017). The attempt of species identification in this thesis indicated that most of the oak shipwreck woods belong to *Quercus robur* and/or *Quercus petraea*, species that are found in the Iberian Peninsula but also across Europe (Eaton et al. 2016). Furthermore, some of the shipwreck wood fragments were shown to belong to *Quercus pyrenaica* or *Quercus faginea*, which are oak species that are mostly found in the Iberian Peninsula (Olalde et al. 2002, Nieto Quintano et al. 2016). Notwithstanding the ecological distribution of wood species can be associated to this approach to designate likely source areas and support the statistical predictions. These results suggest Iberian forests as potential source for timber used to build the studied shipwrecks. A local source is likely according to the historical information, which locates the source of timbers to nearby the shipyard, in general terms (Albion et al. 1926, Allevato et al. 2009). Nevertheless, this outcome looks subjective due to many aspects. On one hand, the commercial network of timber during the early modern age and the scarcity of wood resources in the Peninsula are necessary to be taken into account (Crespo Solana 2015, Reichert 2016, Kumar 2018). And on the other hand, the state of preservations of these archaeological woods needs special consideration (Colombini et al. 2007, Traoré et al. 2017). The diversity of degradation factors appears as an additional factor that makes any attempt of chemical characterisation of shipwreck woods difficult (van Bergen et al. 2000, Kránitz et al. 2016). Nevertheless, the results in this thesis show the suitability of MVDA methods for this matter. It is evident that even after long periods of decay, specific chemical markers of tree taxonomy and growing location can still be recognised from chemical fingerprinting (Wilson et al. 1993, Giachi et al. 2003). Definitely, the molecular structure of lignin compounds in shipwreck pine (softwood) and oak (hardwood) wood samples remain different, even after centuries under seawater. This can be explained by the fact that lignin compounds are the least affected chemicals under waterlogged conditions (Robinson et al. 1987, Gelbrich et al. 2008). Certainly, further wood chemical contents remain unaffected and valid for identificationof archaeological wood. The use of chemical composition provides an additional way for obtaining information on species and provenance of archaeological woods, complementary to other tools such as wood anatomy analysis or dendrochronological assessment. Since this approach appears to be novel in the archaeological context, similar studies are lacking which compromises the level of discussion on this issue.


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3. CONCLUSIONS

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Wood consists of a complex mixture of chemical compounds with diverse molecular structure that varies at different scales, among others, between wood tissues, tree taxonomy and also environmental factors of the growing location. Besides the well-known dissimilarity between softwood and hardwood species, the differentiation between various wood species from the same genus is among the most relevant outcomes of this thesis. Furthermore, it has been shown that the chemical composition of wood varies between sapwood and heartwood according to the extractive compounds, as well as according to the two main wood constituents (carbohydrate and lignin compounds). Wood polysaccharide and lignin contents have been indicated as potential markers to discriminate between species and origin of the wood.

In the archaeological context, the storage conditions and related degradation factors also contribute to large changes in wood molecular composition. The extent of the changes depends on time and on various degradation pathways, which make most of the studies on archaeological wooden artefacts a real challenge. Under seawater conditions, after an extended period, the extractive compounds are the first to be affected. Polysaccharide compounds are also highly degraded due to the combination of long time exposures and the impact by microorganisms. However, the research developed in this thesis suggests the existence of well-preserved polysaccharide features that remain even after centuries under seawater. Lignin compounds also seem to be resistant to degradation processes, mostly due to their molecular structure, but there are particular lignin compounds that are subjected to drastic changes. These outcomes lead to infer that even after extended periods of degradation, part of the chemical signals could be exploited to identify wood species and provenance.

In the framework of this thesis, the use of FTIR and Py-GC-MS techniques was found to be noteworthy, as they provide detailed information about wood chemical composition. As a matter of fact, the two techniques produce complementary information. Furthermore, the combined application of MVDA methods to wood chemical data from these two techniques proved to allow getting better insight about wood chemical composition. Beside the extraction of signals from wood specific chemical contents, PCA components permitted to evaluate the rate of degradation between and within wood samples from living trees, as well as from archaeological shipwrecks. PCA also enabled to identify well-preserved compounds that were later used to establish new proxies for the characterisation of archaeological wood preservation state. Additionally, the application of discriminant analysis permitted to develop statistical models to classify heartwood samples according to species and growing location. In the same perspective, it was possible to assign the probable species to various shipwreck woods and then the possible provenance of the timber used for shipbuilding.

4. RECOMMENDATIONS


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4. RECOMMENDATIONS FOR FUTURE RESEARCH

- The size of the samples appeared to affect the quality of the obtained data. It has been remarked that thinner samples provide better quality of FTIR spectra. Also, when applying Py-GC-MS, constant weight is essential for comparing samples, especially if wood chips are considered. Attention should also be paid to the amount of samples by type of wood species; for instance, the quantity might be bigger for the archaeological wood than for living tree wood samples.
- FTIR and Py-GC-MS are powerful techniques to process samples but for the later in particular, the quantification of pyrolysis products appears as a bottleneck in the processing pipeline as manual quantification is time-consuming. Therefore, automatic quantification or generation of prediction models that can allow estimating pyrolysis data from infrared spectra would be of help. Moreover statistical evaluation of total ion current and/or individual partial ion currents, and auto-quantification procedures also would be of help to increase the analytical throughput of the Py-GC-MS technique, as employed by Tolu et al. (2015) and Gerber et al. (2016).
- To increase wood species and provenance identification level, molecular records from other sites would be interesting. For this purpose, wood samples could be from species and geographical scale that have not been considered in the ForSEAdiscovery project.
- In the particular case of shipwrecks, a representative number of samples is recommended, since it is known that hundreds of individual trees are used to build a single ship.
- Data obtained from FTIR and Py-GC-MS are known to comprise much details related to tree taxonomy and environmental factors of the growing location, then the application of additional MVDA methods (as those based on path analyisis or random forests) could provide novel understanding on wood chemical composition.
- Implementing working approaches that allow to compare wood chemistry with other characteristic parameters used in wood studies. For instance, the establishment of a chemical chronology could be an approach to integrate wood chemical composition with dendrochronology or dendroclimatology.


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5. REFERENCES


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6. ACKNOWLEDGMENTS


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6. ACKNOWLEDGMENTS

First and foremost I thank my supervisors for offering me this wonderful opportunity. Many thanks Antonio Martínez Cortizas and Joeri Kaal, from the whole of my heart, for making this dream come true. Thank youso much; I really appreciated your helps, the time dedicated and the good character and willingness to discuss, to comment and to solve doubts. You increased my passion for this working area. I will keep on hardworking.

I would also like to express my sincere thankfulness to my wonderful colleagues, Noemí Silva, Marta Pérez, Olalla López, Teresa Taboada, Cruz Ferro, Rebeca Tallón, Luis Rodríguez, Nerea Piñeiro and Marcela Rial; You offered me very enjoyable times and helpful supports, I have learned a lot from you. You are the best!

My gratitude to all the members of the ForSEAdiscovery project specially to my mates fellows (ESRs and ERs) of the project, it has been an amazing family. Many thanks to the troika Ignacio, Ana and Nigel for their good supervision and advises throughout the project execution. I would like to thank Tomas; I learned from you that trees have to be sampled with the feeling. Linar, Fadi (May your soul rest in peace), Marta and Peter we were not just multidisciplinary researchers but also multicultural thinkers, it was great to spend these wonderful moments together. I will not forget our first team-working outcome, TREES HAVE MANY SIDES.

I would like to express my sincere thanks to the members of the research groups (the Forest Ecology and Forest Management group in Wageningen UR, and Laboratoire Interdisciplinaire des Environnements Continentaux in Nancy) that facilitated my stays during my secondments. Many thanks to Ute Sass-Klaassen and Anne Poszwa for your suggestions and advises.

Finally, many thanks to my family, friends, and other loved-ones, your unconditional supports also contributed for making this dream come true.

7. MANUSCRIPTS


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MANUSCRIPT 1

Application of FTIR spectroscopy to the characterization of archeological wood

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 153, 63-70.
https://doi.org/10.1016/j.saa.2015.07.108

JCR IF (2016): 2.536, Q2 in Spectroscopy

MANUSCRIPT 2

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

Journal of Analytical and Applied Pyrolysis 126, 1-13.
https://doi.org/10.1016/j.jaap.2017.07.003

JCR IF (2016): 3.471, Q1 in Spectroscopy

MANUSCRIPT 3

Differentiation between pine woods according to species and growing location using FTIR-ATR

Wood science and technology 52, 487-504.

https://doi.org/10.1007/s00226-017-0967-9

JCR IF (2016): 1.509, Q1 in Materials Science, Paper & Wood

MANUSCRIPT 4

A promising approach for provenance studies of wood from Iberian shipwrecks: FTIR and Py-GC-MS in combination with multivariate statistical modelling

Under review at *Journal of Archaeological Science*


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A promising approach for provenance studies of wood from Iberian shipwrecks: FTIR and Py-GC-MS in combination with multivariate statistical modelling

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Abstract

Archaeological wood fragments from four Iberian shipwrecks (Belinho, Magdalena, Ribadeo and Yarmouth) and living tree cores of four oak species (*Quercus faginea*, *Quercus petraea*, *Quercus pyrenaica* and *Quercus robur*) were analysed by Fourier Transform Infrared spectroscopy (FTIR) and pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS). The use of multivariate statistical methods (principal components analysis and discriminant analysis) to the data obtained with both techniques enabled to differentiate between the oak species studied and to identify the species of the archaeological wood samples analysed. Most of the samples from the Magdalena shipwreck exhibited FTIR and Py-GC-MS signals that resembled those of both *Q. petraea* or *Q robur.* As the geographical distribution of these species is concentrated in Northwest Spain, where this frigate is known to have been built, use of local forest resources is suspected. The chemistry of the fragments from the Belinho, Ribadeo and Yarmouth shipwrecks were more similar to wood from *Q. faginea* and *Q. pyrenaica,* also pointing to forest from the Iberian Peninsula as most likely source. Our results show that FTIR and Py-GC-MS, in combination with multivariate statistical methods, facilitate a promising tool for provenance studies of archaeological woods.

Key words: Iberian shipwrecks; *Quercus* sp.; provenancing; wood chemistry; FTIR; Py-GC-MS; multivariate statistics.

1. Introduction

Forest resources were crucial for economic, political, and social development in Europe during the modern period (Chew 2001, Richards 2006, Creasman 2008, Crespo 2011). Various research approaches within the international ForSEAdiscovery project have shown the importance of interdisciplinary collaboration in studies about environmental history (Polónia et al. 2017). The three main disciplines (history, archaeology and wood sciences) involved in this project aim to provide new insights about the Iberian shipbuilding economy, which has been presented as the lifeblood of the European expansion overseas during the Age of Discovery (Creasman 2008, Castro 2008a). For wood provenance issues, history and nautical archaeology methodologies focussed on shipbuilding policies in the Iberian Empire, whereas the assignment to wood science was to extract novel information from wood structural (tree rings and vessels) and chemical compositions (organic and inorganic contents).

In archaeological investigations of shipwrecks, some of the most fundamental questions are related to the age and origin of the wooden artefacts. In this respect, dendroprovenancing has proven very successful in nautical archaeological contexts. It permitted to determine the age and origin of numerous shipwrecks (Nayling 2008, Domínguez-Delmás et al. 2013). Nevertheless, studies on tree ring series have limitations due to the lack of available regional chronologies, plus sometimes tree ring series have no connection with local environmental factors (Bridge 2012, Rich et al. 2016). Moreover, even though studies on strontium (Sr) isotopes showed to be invaluable to trace the provenance of ancient wooden artefacts (English et al. 2001, Rich et al 2015, 2016), this (expensive and time-consuming) approach has strong limitations for marine shipwreck woods due to the bias imposed by the isotopic Sr signature of seawater (Hajj et al. 2017). Alternatively, molecular and structural information obtained by e.g. Fourier-Transform Infrared Spectroscopy (FTIR) or pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS), in combination with multivariate statistic methods, have been used to distinguish wood samples from various geographic origins (Colom and Carillo 2005, Rana et al 2008, Xu et al. 2013, Santoni et al. 2015) and also provide other information on archaeological wood composition (Colombini et al. 2007, Pizzo et 2015, Łucejko et al. 2012). Furthermore, FTIR and Py-GC-MS are powerful, easy to use and cheap techniques that are therefore more attractive for provenance studies where large numbers of samples need to be analysed. However, the size of the datasets created so far is rather limited.

In previous studies of the ForSEAdiscovery project, multivariate statistical methods with FTIR and Py-GC-MS were combined to show the impact of storage conditions on the nature and intensity of wood degradation (diagenesis) (Traoré et al. 2016) and novel molecular proxies of wood preservation were proposed (Traoré et al. 2017a). Furthermore, we were able to differentiate between the living wood samples according to species and growing location (Traoré et al. 2017b).

Now that the proxies of degradation of shipwreck wood as obtained from FTIR and Py-GC-MS are known, and that there are strong indications of the usefulness of the approach for provenancing signals, in the present study a large number of extant and shipwreck wood samples are considered. Special attention is given to the geochemical signature of oak woods from four shipwrecks of Iberian typology (Castro 2008b), as determined using FTIR and Py-GC-MS. Living tree samples from four oak species (*Q. pyrenaiaca*. *Q. robur*, *Q. petraea* and *Q. faginea*) that have been collected in five different forests in Spain are also studied. Given the amount and complexity of the molecular data obtained, we applied Principal Component Analysis (PCA) and Discriminant Analysis (DA) to extract the main geochemical signals and determine whether it is possible to identify wood species and provenance of the shipwreck wood.

2. Material and methods

2.1. Sample descriptions

Sixteen living trees of four different oak species were sampled from five forests located between 600 and 950 m above sea level (m a.s.l.) in northern Spain: in Cantabria (Monte Aa: MAA) and in the Basque country (Gordoa: GOR, Jugatxi: JUG, Oteo: OTE and San Vicente de Arana: SVA). *Quercus pyrenaica* and *Q. petraea* samples were collected from GOR and JUG, *Q. robur* from JUG and MAA and *Q. faginea* from OTE and SVA. The wood cores were retrieved using an increment borer at breast high from old trees, most of them with ring number above 250. At all sites we collected two wood cores (from individual trees) for each species.

The shipwreck timbers used in this work were collected during sampling campaigns (from June 2015 to May 2016) carried out by the nautical archaeologists team of the ForSEAdiscovery project (http://forseadiscovery.eu/). The wood fragments from framing and planking structures were essentially identified (by the anatomical features) as deciduous oak (*Quercus* subg. *quercus*). Three of the shipwrecks were found in different locations of the coasts of the Iberian Peninsula (Belinho: BEL, Magdalena: MAG and Ribadeo: RIB) and one nearby the Isle of Wight (Yarmouth: YAR). Although three of the shipwrecks (BEL, RIB and YAR) exhibit characteristics that are typical of vessels from Iberian shipyards, the historian archives do not provide further information about their origin. The Magdalena frigate is the only one for which significant historical data is available; it was built and launched in July 1773 in the royal shipyard of Ferrol (northwestern Spain) and had sunk in the Viveiro estuary in 1810 (also in northwestern Spain) (Ministerio de Defensa 2011, Lema Mouzo 2014). The number of wood fragments studied was mainly based on their suitability to the analytical techniques to obtain a continuous record, as most shipwreck woods were severely damaged by shipworms. Six wood cores from BEL, two from MAG, four from RIB and five from YAR were considered suitable for assessment. These samples presented between 40 and 135 rings and none of them had signs of a bark edge.

Before chemical analysis, all the samples (living trees and shipwreck woods) were prepared as described previously (Traoré et al. 2017a).

2.2. Analytical techniques

The conditions of the FTIR analysis are described in Traoré et al. 2017a,b). Briefly, FTIR spectra were recorded on a Cary 630 (Agilent Technologies) scanning in the 4000-400 cm^{-1} wavenumber range. The FTIR spectra were recorded on individual rings throughout the cross sections (wood core for living tree and wood fragment for shipwreck samples) with a separation distance of 1 cm between measured rings. In order to increase the consistency of the discriminant models for species differentiation, we added supplementary spectra from individual rings belonging to the heartwood part for living trees wood cores. In total, more than a thousand FTIR spectra were recorded for this study. Since it is a more time- and effort-demanding technique, Py-GC-MS was performed on eight to ten individual rings throughout the cross sections, amounting to a total of 305 measurements. Pyrolysis-GC-MS of wood chips (< 1 mg) was performed using a CDS Pyroprobe 5000 (for pyrolysis at 650 ºC for 10 s) coupled to an Agilent 6890 gas chromatograph and 5975B mass selective detector (details in Traoré et al. 2017a,b).

2.3. Data analysis

For FTIR, we focused on the fingerprint region (between 1800 and 800 cm^{-1}), and also considered the second derivative spectra for band selection. Principal component analysis (PCA) was applied to the relative absorbance spectra of living trees using selected bands as variables, to synthesize the main spectral signals of the woods. Then, discriminant analysis (DA) was performed with the FTIR bands that showed the highest loadings for the extracted PCA factors in order to determine whether the shipwreck samples had the signature of sapwood or heartwood. In a similar way, a second PCA and DA were performed using all the identified pyrolysis products as variables. In a third DA we used FTIR and Py-GC-MS data together to check whether a combination of both techniques was more efficient in the identification of the wood type (sapwood/heartwood).

Furthermore, we applied a stepwise DA with FTIR data from heartwood samples only, in order to discriminate between the four oak species. To assess the accuracy of the DA model, we randomly split our data in training and validation sets, with a distribution of 80 % and 20% of the size of the original dataset, respectively. Although the size of the Py-GC-MS dataset of the heartwood samples of living trees was limited, we applied an exploratory DA to see if it was consistent in the identification of the four oak species we studied. Lastly, we used the DA model based on living tree samples to assign the shipwreck woods to the tree species considered. We excluded in this analysis those variables that showed to be controlled by degradation/preservation dynamics.

One-way ANOVA tests were applied to assess the significance of the observed differences. All statistical tests were done using SPSS 20.

3. Results and Discussion

3.1 General description of FTIR spectra and Py-GC-MS chromatograms and effects of diagenesis

3.1.1 FTIR

The average absorption spectra in the fingerprint region (1800-800 cm^{-1}) of the shipwreck and living trees samples are presented in **Fig. 1a**. The absorption bands are predominantly associated with lignocellulosic constituents. Variations in absorption intensity are easier to identify in the second derivative spectra (**Fig. 1b**), as noticed in previous investigations (Boeriu et al. 2004, Huang et al. 2008, Popescu et al. 2009, Zhang et al. 2016). The bands at 1740, 1370, 1320, 1155, 1025 and 895 cm^{-1} are specific to molecular bond vibrations in polysaccharide molecules (cellulose and hemicellulose), whereas bands at 1590, 1505, 1460, 1420, 1265 and 1230 cm^{-1} are related to bond vibration in lignin molecules and aromatic extractive compounds (Faix and Böttcher 1992, Schwanninger et al. 2004).

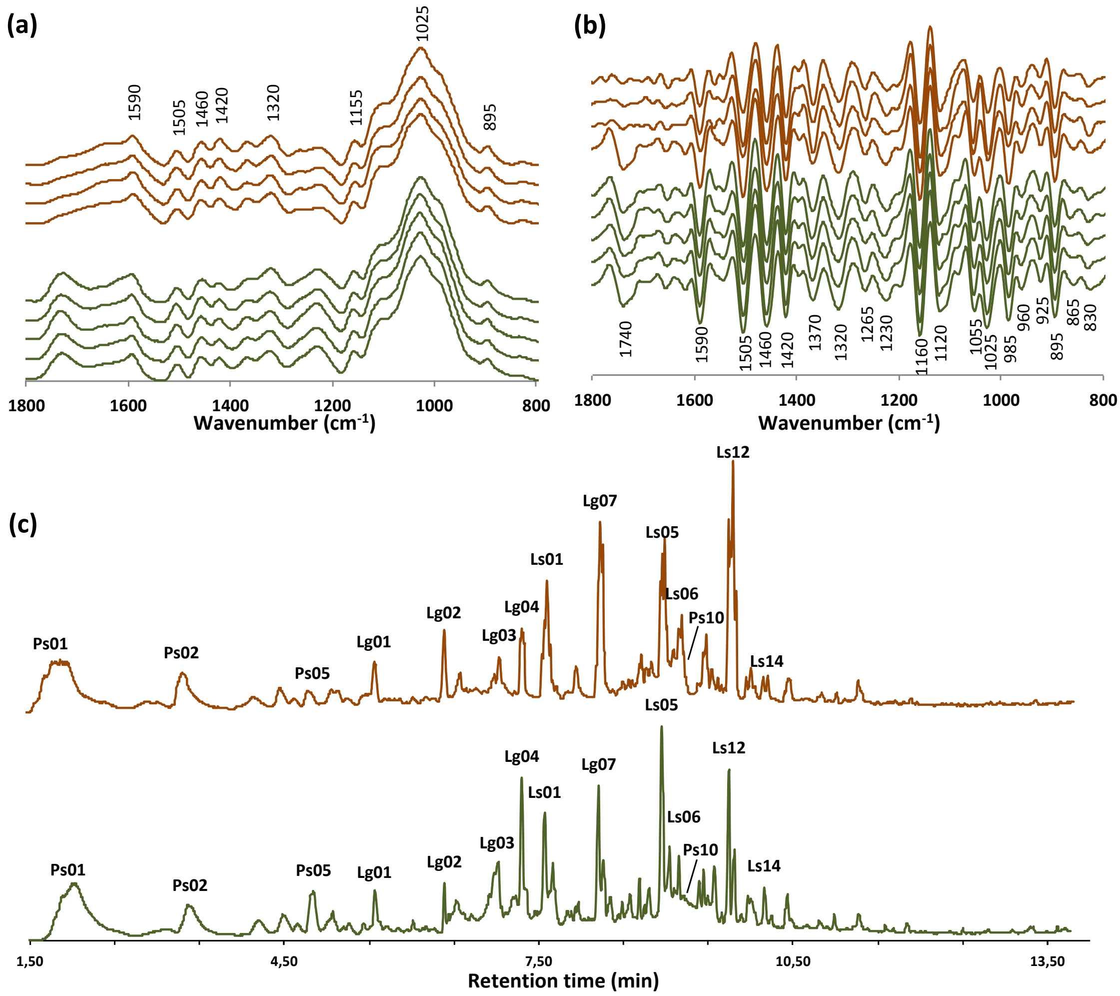

Fig. 1. Examples of FTIR and Py-GC-MS spectra of shipwreck wood (brown, upper lines) and extant wood (green, lower lines): (**a**) FTIR average absorption spectra, (**b**) FTIR second derivative spectra and (**c**) Py-GC-MS total ion chromatograms (peak labels are explained in Table 2; Ps = polysaccharide, Lg = guaiacyl lignin, Ls = syringyl lignin).

Table 1: Average and standard deviation of relative intensities of the main absorption bands of the fingerprint region for extant (*n*=134) and shipwreck woods (*n*=171). ([1] For the dominant molecular structure, see Traoré et al. 2017b; asterisks denote significant differences, * for *P*<0.05 and ** for *P*<0.01)

Bands (cm^{-1})	Assignment [1]	Extant		Shipwreck		Enrichment types	**Sig.**
		Ave	SD	Ave	SD		
830	Lignin	0.01	0.00	0.01	0.00	Extant	**
865	Lignin	0.02	0.01	0.01	0.01	Extant	**
895	Polysaccharide	0.06	0.01	0.05	0.01	Extant	**
925		0.06	0.01	0.05	0.01	Extant	**
960	Lignin	0.16	0.02	0.15	0.02	Extant	**
985	Polysaccharide	0.26	0.04	0.22	0.03	Extant	**
1025	Polysaccharide	0.33	0.04	0.28	0.03	Extant	**
1055	Polysaccharide	0.28	0.03	0.25	0.03	Extant	**
1080	Polysaccharide	0.18	0.02	0.17	0.02	Extant	**
1120		0.14	0.01	0.13	0.02	Extant	**
1160	Polysaccharide	0.08	0.01	0.06	0.01	Extant	**
1230	Lignin	0.09	0.02	0.05	0.01	Extant	**
1265	Lignin	0.06	0.01	0.04	0.01	Extant	**
1320	Polysaccharide	0.08	0.01	0.08	0.01		
1370	Polysaccharide	0.06	0.01	0.06	0.01	Shipwreck	**
1395	Polysaccharide	0.03	0.00	0.04	0.01	Shipwreck	**
1420	Lignin	0.06	0.01	0.07	0.01	Shipwreck	**
1460	Lignin	0.06	0.01	0.06	0.01		
1505	Lignin	0.04	0.01	0.04	0.01		
1540	Lignin	0.00	0.00	0.01	0.01	Shipwreck	**
1560	Lignin	0.01	0.00	0.04	0.01	Shipwreck	**
1590	Lignin	0.07	0.01	0.08	0.01	Shipwreck	**
1620		0.05	0.01	0.06	0.01	Shipwreck	**
1655	Polysaccharide	0.04	0.00	0.05	0.01	Shipwreck	**
1725	Polysaccharide	0.07	0.01	0.03	0.01	Extant	**
1740	Polysaccharide	0.07	0.02	0.02	0.01	Extant	**

The relative intensities for the main bands (in Table 1) correspond to those at 985, 1025 and 1055 cm^{-1}, and can be assigned to C-O bond stretching in polysaccharides. Their relative absorbance is significantly (*P*<0.01) higher in living trees than in shipwreck woods (Table 1). Bands at 1725 and 1740 cm^{-1}, from carbonyl bonds, that are mainly associated with hemicellulose (Colombini et al. 2007, Traoré et al. 2016), also showed significantly (*P*<0.01) higher intensities in living trees, pointing towards the higher polysaccharide content in living wood in comparison with shipwreck materials. Giachi and co-workers (2003) found that shipwreck wood degradation has a particularly strong effect on holocellulose content (cellulose and hemicellulose), due to biological attack. The storage environment of shipwrecks favours the water saturation of the spaces for polysaccharides in wood, although carbohydrates are known to be hydrophilic (Wilson et al. 1993). However, several other bands that correspond to signals from polysaccharides, such as the C-H vibration at 1370 cm^{-1} and the C-O stretching at 1395 cm^{-1}, were significantly ($P<0.01$) more intense in the shipwreck wood than in the living trees. These bands probably represent relative recalcitrant functional groups in polysaccharides or are formed during diagenesis (Saiz-Jimenez et al. 1987, Traoré et al. 2017a).

Regarding bands related to lignin (Table 1), the signals assigned to aromatic C-C skeletal vibrations, 1590 cm^{-1}, and C-H asymmetric deformation in methoxyl groups, 1420 cm^{-1}, were significantly ($P<0.01$) stronger in shipwreck samples, which may be explained by selective preservation of lignin under anoxic waterlogged conditions (Wilson et al. 1993, Colombini et al. 2009). Hence, even though the absorbance data show the overall effect of decay on the relative proportion of polysaccharides (decrease) and lignin (increase), some specific bands may show opposite trends due to changes in the chemistry of the different components.

3.1.2 Pyrolysis-GC-MS

Examples of pyrolysis chromatograms are given in **Fig. 1c** and the pyrolysis products used for the semi-quantification are listed in Table 2. These can be subdivided in several main groups, i.e. (a) carbohydrate products (acetic acid, furans/furaldehydes, pyrans and anhydrosugars) which originate predominantly from holocellulose; (b) 2-methoxyphenols (guaiacols, G) from guaiacyl lignin; (c) 2,6-dimethoxyphenols (syringols, S) from syringyl lignin; (d) phenols and catechols from lignin, degraded lignin and tannins; (e) C_{16}-C_{22} fatty acids and (f) two unidentified compounds.

The carbohydrate products are the most abundant group in most samples, i.e. 55.4% (average of $n=134$ samples) ± 8.6% (standard deviation) of TQPA in extant wood and 51.9 ± 12.3% ($n=171$) in shipwreck wood (Table 2). Even though this difference is significant ($P<0.01$), the effect of decay on the shipwreck woods' polysaccharides pyrolysis fingerprint is smaller than expected. Within this group (Table 2), acetic acid (Ps01), 2,3-dihydro-5-methylfuran-2-one (Ps03) and 4-hydroxy-5,6-dihydro-(*2H*)-pyran-2-one (Ps05) are enriched in extant wood, whereas 3/2-furaldehyde (Ps02), 5-methyl-2-furaldehyde (Ps04), 2-hydroxy-3-methyl-2-cyclopenten-1-one (Ps06), 3-hydroxy-2-methyl-*4H*-pyran-4-one (Ps07), 5-hydroxymethyl-2-tetrahydrofuraldehyde-3-one (Ps08) and 1,4:3,6-dianhydro-α-D-glucopyranose (Ps09) are enriched in shipwreck wood; indicative of the effects of decay on polysaccharide composition. However, one of the most frequently used proxies of intact cellulose, i.e. levoglucosan (Ps10), accounts for approximately 18 % in both types of wood, with no significant difference. In a recent study, Tamburini et al. (2017) found that a simultaneous occurrence of different degradation pathways could induce the failing of the commonly used parameters for archaeological wood studies. In a previous study (Traoré et al., 2017a), we showed that Ps10 and Ps05 were more abundant relative to other carbohydrate products (Ps03 and Ps06) when the intensity of decay was low, and used these compounds in a "shipwreck wood preservation index" for polysaccharides (SWPI-Ps). The SWPI-Ps is significantly higher ($P<0.05$) for extant wood (0.82 ± 0.12) than for shipwreck wood (0.79 ± 0.09) (Table 2), but the difference is considered too small (too much overlap in the ranges) for it to be a robust proxy of polysaccharide preservation.

Table 2: Average and standard deviation of the relative abundances of pyrolysis products in living trees and shipwreck samples (asterisks denote significant differences, * for $P<0.05$ and ** for $P<0.01$)

Code	Pyrolysis products	m/z	RT (min)	Extant		Shipwreck		Enrichment	Sig.
				Ave	SD	Ave	SD		
Ps01	acetic acid	60	1.655	13.67	8.82	8.30	2.70	extant	**
Ps02	3/2-furaldehyde	95+96	3.325	9.45	.22	11.99	7.85	ipwreck	**
Ps03	2,3-dihydro-5-methylfuran-2-one	55+98	4.145	3.64	.80	3.13	0.98	xtant	**
Ps04	5-methyl-2-furaldehyde	109+110	4.508	1.11	.55	1.47	0.91	ipwreck	**
Ps05	4-hydroxy-5,6-dihydro-(2*H*)-pyran-2-one	114+58	4.846	7.70	.85	4.15	2.57	xtant	**
Ps06	2-hydroxy-3-methyl-2-cyclopenten-1-one	112+55	5.11	1.08	.41	1.82	0.62	ipwreck	**
Ps07	3-hydroxy-2-methyl-4H-pyran-4-one	126	5.872	0.19	.06	0.42	0.12	ipwreck	**
Ps08	5-hydroxymethyl-2-tetrahydrofuraldehyde-3-one	57+70	6.399	0.57	.26	1.87	0.97	ipwreck	**
Ps09	1,4:3,6-dianhydro-α-D-glucopyranose	69	6.656	0.51	.16	0.85	0.40	ipwreck	**
Ps10	levoglucosan	60	9.344	17.52	7.41	17.93	9.22		
Lg01	guaiacol	109+124	5.572	1.98	.65	1.95	0.95		
Lg02	4-methylguaiacol	123+138	6.387	1.18	.61	1.32	0.63	ipwreck	*
Lg03	4-ethylguaiacol	137+152	7.04	0.70	.32	0.67	0.33		
Lg04	4-vinylguaiacol	135+150	7.305	3.55	.31	3.06	1.31	xtant	**
Lg05	$C_{3:1}$-guaiacol	164+149	7.59	0.27	.13	0.46	0.19	ipwreck	**
Lg06	4 propylguaiacol	137+166	7.658	0.13	.05	0.20	0.09	ipwreck	**
Lg07	$C_{3:1}$-guaiacol (*trans*)	164+149	8.223	1.27	.57	2.64	1.26	ipwreck	**
Lg08	homovanillin	137+166	8.317	1.10	.51	0.52	0.29	tant	**
Lg09	4-acetylguaiacol	151+166	8.503	0.45	.15	0.56	0.29	ipwreck	**
Lg10	Unknown Guaiacol	166+151	8.592	0.87	.48	0.46	0.28	xtant	**
Lg11	4-propan-2-one-guaiacol	137+180	8.815	0.95	.25	1.03	0.28	ipwreck	*
Ls01	syringol	154+139	7.595	3.80	.91	3.60	1.89		
Ls02	syringol isomer (?)	154+139	7.668	1.10	0.36	0.73	0.29	extant	**
Ls03	4-methylsyringol	168+153	8.192	2.67	.45	2.93	1.58		
Ls04	4-ethylsyringol	167+182	8.701	0.92	.36	1.08	0.54	ipwreck	**
Ls05	4-vinylsyringol	180+165	8.945	6.37	.07	5.71	2.19	xtant	**
Ls06	$C_{3:1}$-syringol	194+179	9.162	0.54	.26	1.10	0.39	ipwreck	**
Ls07	4-propylsyringol	167+196	9.194	0.21	.07	0.43	0.20	ipwreck	**
Ls08	$C_{3:1}$-syringol	194+179	9.453	0.56	.20	1.24	0.48	ipwreck	**
Ls09	syringaldehyde	182+181	9.583	1.71	.69	1.27	0.61	xtant	**
Ls10	C_3H_3-syringol	192+177	9.676	0.15	.10	0.19	0.09	ipwreck	**
Ls11	C_3H_3-syringol	192+177	9.713	0.09	.05	0.10	0.05	ipwreck	**
Ls12	$C_{3:1}$-syringol (*trans*)	194+179	9.754	3.59	.57	7.93	3.57	ipwreck	**
Ls13	homosyringaldehyde	167+196	9.816	1.87	.98	0.80	0.51	xtant	**
Ls14	4-(propan-2-one)syringol	167+210	10.19	1.99	.67	1.85	0.62		
Ls15	dihydrosinapyl alcohol	168+212	10.818	0.22	0.10	0.27	0.11	ipwreck	**
P01	phenol	94+66	4.648	0.71	.36	0.91	0.34	ipwreck	**

P02	2/3-methylphenol	107+108	5.281	0.30	.16	0.38	0.14	ipwreck	**
P03	4-methylphenol	107+108	5.447	0.52	.30	0.79	0.31	ipwreck	**
P04	C_2-phenol	107+122	6.039	0.30	.18	0.33	0.14		
P05	C_2-phenol	107+122	6.181	0.18	.11	0.24	0.11	ipwreck	**
P06	catechol	110	6.516	1.65	.77	1.28	0.58	xtant	**
P07	Methoxycatechol	125+140	7.035	2.35	.65	1.67	0.63	xtant	**
X01	unidentified compound	59	7.819	0.04	.03	0.23	0.14	ipwreck	**
X02	unidentified compound	132	8.483	0.09	.08	0.04	0.05	xtant	**
Fa01	fatty acid C_{16}	60	11.005	0.13	.07	0.09	0.08	xtant	**
Fa02	fatty acid C_{18}	60	11.944	0.02	.01	0.02	0.02		
Fa03	fatty acid C_{20}	60	12.794	0.01	.01	0.00	0.00	xtant	**
Fa04	fatty acid C_{22}	60	13.609	0.03	.02	0.01	0.02	xtant	**
	Σ polysaccharides			55.44	.60	51.93	12.35	tant	**
	SWPI-Ps [a]			0.82	0.12	0.79	0.09	extant	*
	Σ S lignin			25.78	7.47	29.21	8.80	shipwreck	**
	SWPI-Ls [b]			0.11	0.06	0.05	0.05	extant	**
	Σ G lignin			12.45	3.34	12.87	4.17		
	SWPI-Lg [c]			0.13	0.05	0.07	0.05	extant	**
	Σ phenols/catechols			6.01	.02	5.60	1.57	tant	*
	Σ fatty acids			0.19	0.08	0.13	0.10	extant	**

[a] $\text{SWPI-Ps} = \frac{\text{Ps05+Ps10}}{\text{Ps03+Ps05+Ps06+Ps10}}$

[b] $\text{SWPI-Ls} = \frac{\text{Ls13}}{\text{Ls01+Ls04+Ls05+Ls06+Ls08+Ls12+Ls13}}$

[c] $\text{SWPI-Lg} = \frac{\text{Lg08}}{\text{Lg01+Lg03+Lg04+Lg06+Lg08}}$

The sum of syringyl lignin products is slightly more abundant ($P<0.01$; Table 2) in the pyrolyzates of shipwreck (29.2 ± 8.8% of TQPA) than in those of extant woods (25.8 ± 7.5%). The major S products are (in decreasing order of average proportion) $C_{3:1}$-syringol (*trans*) (Ls12), 4-vinylsyringol (Ls05), syringol (Ls01) and 4-methylsyringol (Ls03). The extant wood samples' pyrolyzates have significantly higher ($P<0.01$) proportions of 4-vinylsyringol, syringaldehyde (Ls09) and homosyringaldehyde (Ls13), whereas the shipwreck wood samples are enriched ($P<0.01$) in 4-ethylsyringol (Ls04), the $C_{3:1}$-syringols (Ls06, Ls08, Ls12), the C_3H_3-syringols (Ls10, Ls11) and 4-propylsyringol (Ls07), and this combination of compounds is in agreement with the SWPI of syringyl lignin (SWPI-Ls; Traoré et al., 2017a). Indeed, SWPI-Ls shows a strong contrast between extant (0.112 ± 0.056) and shipwreck wood (0.045 ± 0.045), and is expected to be a useful proxy for identifying differences in diagenetic alteration between the different shipwrecks.

The guaiacyl lignin products account for 12.9 ± 4.2% and 12.5 ± 3.3% in shipwreck and extant wood pyrolyzates, respectively, which are not significantly different (Table

2). The series of compounds detected is similar to the syringyl equivalents, with 4-vinylguaiacol (Lg04), $C_{3:1}$-guaiacol (*trans*) (Lg07), guaiacol (Lg01) and 4-methylguaiacol (Lg02) being the most abundant. Extant wood is more prolific in Lg04, Lg08 and an unidentified guaiacol (Lg10), whereas shipwreck wood pyrolyzates have larger proportions of $C_{3:1}$-guaiacols (Lg05, Lg07), 4-propylguaiacol (Lg06) and 4-acetylguaiacol (Lg09). The large difference in SWPI of guaiacyl lignin (SWPI-Lg) between extant (0.127 ± 0.052) and shipwreck wood (0.068 ± 0.049) suggests that SWPI-Lg can be used to track decay intensity.

The sum of phenols (hydroxybenzenes) and catechols (dihydroxybenzenes) accounts for 5.6 ± 1.6% in the pyrolyzates of shipwreck wood, and 6.0 ± 2.0% in those of extant wood. Most of the phenols (P01, P02, P03 and P05) are more abundant ($P<0.01$) in shipwreck wood, probably indicative of an effect of lignin degradation. The catechols (P06 and P07) are more abundant ($P<0.01$) in the pyrolyzates of extant wood, which is probably indicative of a dominant tannin source and that this tannin becomes selectively depleted due to leaching of tannins after exposition to seawater (Fojutowski et al. 2011, Kaal et al. 2012).

The minor compounds that were quantified are fatty acids (shipwreck: 0.1 ± 0.1%; extant: 0.2 ± 0.1%) and unidentified products (shipwreck: 0.3 ± 0.1%; extant: 0.1 ± 0.1%).

3.2 Chemical differences between sapwood and heartwood

3.2.1 Evidence of sapwood and heartwood in living trees

The first principal component of the PCA applied to the FTIR data ($PC1_{FTIR}$, 39.3% of the total variance) of living trees was found to reflect differences between sapwood and heartwood (**Fig. 2a**). The bands with high loadings (absolute value higher than 0.5) for $PC1_{FTIR}$ are provided in Table 3. The highest positive loadings are observed for bands assigned to polysaccharides (985, 1025, 1080 cm^{-1} etc.; Table 3), suggesting higher polysaccharides content in the sapwood. The largest negative loadings are associated to bands assigned to lignin (1560, 1590, 1620 cm^{-1} etc., Table 3), suggesting a higher lignin content in heartwood.

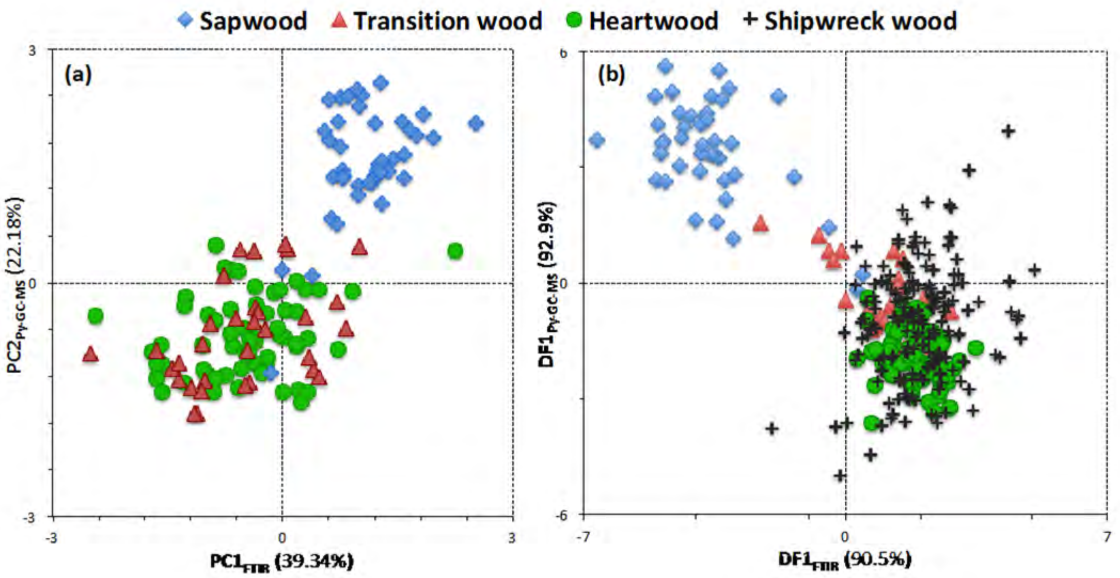

Fig. 2. Sapwood and heartwood differences: (**a**) factor scores of the first principal component of PCA for FTIR and Py-GC-MS data of living tree samples and (**b**) scores of the first function of the DA on FTIR and Py-GC-MS data.

Analogously, the second principal component ($PC2_{Py\text{-}GC\text{-}MS}$, 22.2% of the total variance) extracted with Py-GC-MS data is also related to differences in composition between sapwood and heartwood (Fig. 2a). Again, polysaccharide pyrolysis products (PS01, Ps03, Ps06, etc.) show large positive loadings (Table 3) and are enriched in sapwood, whereas lignin is significantly more abundant in heartwood as indicated by the negative loadings of many lignin compounds (Lg08, Ls13, Ls14, etc.; Table 3).

Fig. 2a shows that the PCA factors that account for the differences ($P<0.01$) between sapwood and heartwood ($PC1_{FTIR}$ and $PC2_{Py\text{-}GC\text{-}MS}$) are in general agreement. Most sapwood samples are associated with positive scores on $PC1_{FTIR}$ and $PC2_{Py\text{-}GC\text{-}MS}$ whereas samples from heartwood and transition zones have negative scores. This implies that most samples assigned to the transition zone are chemically more similar to heartwood. If sapwood and heartwood samples are considered separately, there is no correlation between the PCA scores of the two methods (FTIR and Py-GC-MS), and the transition zone samples do not plot between the sap- and heartwood samples, indicating that the contrast in chemistry between the two sections of the wood has a binary rather than a continuous character. Several studies suggest that differences between sapwood and heartwood are reflected by a progressive decrease of holocellulose with a corresponding increase in lignin from sapwood to heartwood (Bertaud and Holmbom 2004, Esteves et al. 2005, Lourenço et al. 2015). In fact, Ryan and co-workers (1995) stated the considerable consumption of maintenance energy by living cells in the sapwood explains its higher content of polysaccharide than heartwood. Also, application of PCA to wood polysaccharide and lignin contents permitted to differentiate between sapwood and heartwood in living trees (Metsä-Kortelainen and Viitanen 2010, Traoré et al. 2017a). Similarly, PCA enabled to separate between sapwood and heartwood using wood extractive compounds (Sundqvist 2002, Nuopponen et al 2003).

There are significant differences in the aforementioned preservation indices (SWPI) between sapwood (low SWPI) and heartwood (high SWPI) of the living plants. For the polysaccharide index (SWPI-Ps), Ps05 (in SWPI-Ps) is more abundant in heartwood than in sapwood. Analogously, homosyringaldehyde (Ls13), which is important in SWPI-Ls, is more abundant in heartwood than in sapwood (ANOVA P), whereas the analogous guaiacyl compound homovanillin (Lg08) for SWPI-Lg as well. The fact that the same compounds that are used as preservation index (due to rapid loss upon decay) are concentrated in the sapwood section of wood cores is in agreement with common knowledge that heartwood is more durable than sapwood (Taylor et al. 2002).

Finally, it is worth mentioning that large variations in polysaccharide abundances within living tree cores were observed, especially of acetic acid (Ps01). This variability is mainly associated with the remarkably large differences between sapwood and heartwood chemical composition. Sapwood is more prolific ($P<0.001$) in acetic acid ($n=41$, 24.1 ± 7.2%) than heartwood ($n=61$, 7.5 ± 3.2), notwithstanding the transition zone between sap- and heartwood with intermediate levels ($n=32$, 12.0 ± 5.1%). These results show that there are strong differences in carbohydrate and lignin pyrolysis fingerprints between sap- and heartwood within extant wood cores, which has to be taken into account.

3.2.2 Identification of sapwood and heartwood in shipwreck wood

Visual recognition of sap- and heartwood sections in shipwreck wood appeared difficult. Therefore, we applied a discriminant model that was generated from data of living trees to the shipwreck samples' data with the aim to differentiate between sapwood and heartwood.

The DA using FTIR bands that were associated with $PC1_{FTIR}$ (**Table 3**, **Table A.1**) correctly classified 92.9% of the living tree sapwood samples as sapwood ($n=41$), 77.4% of the samples in the transition zone as transition wood ($n=32$), and 90.2% of the heartwood samples as heartwood ($n=61$). This shows unambiguously that FTIR can be used to distinguish sap- and heartwood materials, and also transition but with a slightly weaker performance. The application of this model to shipwreck woods, of which the wood section is unknown, suggests that 91.2% of the shipwreck samples correspond to heartwood (as determined by the smaller Euclidean distance to the centroid of the heartwood samples), with a group membership probability of 0.98 ± 0.07 (average ± SD). The other samples were attributed to the transition zone and none were related to sapwood.

As for the DA using compounds with elevated loadings on $PC2_{Py\text{-}GC\text{-}MS}$ (**Table 3**, **Table A.1**), 90.5% of the sapwood samples ($n=41$) were correctly classified as sapwood, 80.6% of the transition zone wood ($n=32$) and 85.2% of the heartwood samples ($n=61$). Similar to FTIR, this DA model suggests that the vast majority of shipwreck samples (88.3%) are from the heartwood zone, with an average group membership probability of 0.98±0.07. Samples attributed to sapwood and transition zone were 10.5% and 1.2%, respectively.

Table 3: Loadings of the first principal component (PC1) obtained with FTIR and Py-GC-MS. For Py-GC-MS, compounds codes are explained in Table 2.

	$PC1_{FTIR}$		$PC2_{Py\text{-}GC\text{-}MS}$	
	Band (cm^{-1})	Loadings	Compound	Loadings
Positive loadings	830	0.80	Ps01	0.78
	865	0.71	Ps03	0.91
	895	0.79	Ps06	0.76
	925	0.85	Ps07	0.64
	960	0.86	Lg05	0.55
	985	0.94	Ls06	0.74
	1025	0.86	Ls08	0.55
	1055	0.84	Ls09	0.72
	1080	0.86	Fa01	0.51
	1120	0.63		
Negative loadings	1320	-0.81	Ps02	-0.65
	1395	-0.62	Ps05	-0.74
	1560	-0.67	Lg03	-0.58
	1590	-0.84	Lg08	-0.88
	1620	-0.82	Lg11	-0.50
			Ls13	-0.72
			Ls14	-0.63
			X02	-0.57

Figure 2b shows the scores plot of the first discriminant function of the DA on FTIR data plotted against the same function of the DA using Py-GC-MS data. Sapwood samples for living trees were associated to negative scores for FTIR and positive scores for Py-GC-MS function, and none of the shipwreck samples was clustered into this group. The heartwood and transition zone samples of living trees occupy the same area in this dimension with positives score for FTIR and negative scores for Py-GC-MS. All shipwreck samples were associated to this group. These results suggest that indeed virtually all timber corresponds to heartwood with some adjacent transition wood material.

Our results show that the combination of PCA and DA supports the interpretation that most, if not all, shipwreck wood materials correspond to heartwood and transition wood. This can be explained by either preferred use of heartwood as timber (Taylor et al. 2002), which is not surprising, as heartwood materials are known to be more resistant and durable than sapwood (Hillis 1987, Schultz et al 2007, Saito et al. 2008). Sapwood is enriched in specific compounds (polysaccharide for example) that are more susceptible to biological attack; which may explain why under erosion conditions timbers lose relatively quickly the sapwood part (Nayling 2008). These two reasons are not necessarily exclusive as the preferred use of heartwood and the preferential loss of relative labile constituents may operate simultaneously. Nevertheless, in following sections none of the shipwreck samples is considered as sapwood due to the fact that most of them have a heartwood fingerprint.

3.3. Compositional differences between oak species

3.3.1. Discriminant analysis applied to FTIR data of living trees heartwood

Stepwise DA applied to the extended heartwood spectral dataset (*n*=480, i.e. 30/tree core), divided into 80% training set and 20% validation set, provided three canonical discriminant functions (**Fig. 3, Table 4**). The first discriminant function ($F1_{FTIR}$), which explains 58.0% of the total variance, shows clear differences between *Q. petraea* and *Q. robur* with negative scores, and *Q. faginea* and *Q. pyrenaica* with positive scores (Fig. 3a). Furthermore, the discriminant scores of *Q. faginea* are significantly (*P*<0.01) higher than those of *Q. pyrenaica*. The second discriminant function ($F2_{FTIR}$; 24.1% of total variance) shows differences between *Q. pyrenaica* (high negative scores) and *Q. faginea* (lower negative scores) (*P*<0.01) and for *Q. pyrenaica* and *Q. robur*. The third function ($F3_{FTIR}$), which explains 17.9% of the total variance, shows significant differences (*P*<0.01) between all oak species except for the combination of *Q. petraea* and *Q. faginea* (**Fig. 3**).

Table 4: Post Hoc Tests to the scores functions from discriminant analysis of FTIR and Py-GC-MS from heartwood of living tree samples (Student-Newman-Keuls: the harmonic mean of the group sizes is used). Suffices t and v after botanical species indicate training and validation datasets for the discriminant analysis, respectively.

Datasets	Sample	N	Function 1	Function 2	Function 3
FTIR	*Q. pyrenaica*_t	96	0.61b	-1.54a	0.38c
	*Q. pyrenaica*_v	24	0.67b	-1.18a	0.45c
	*Q. robur*_v	24	-1.45a	0.71c	1.08d
	*Q. robur*_t	96	-1.2a	0.74c	1.2d
	*Q. petraea*_v	24	-1.66a	-0.15b	-1.13a
	*Q. petraea*_t	96	-1.54a	-0.04b	-1.02a
	*Q. faginea*_t	96	2.13c	0.83c	-0.59a,b
	*Q. faginea*_v	24	2.3c	0.97c	-0.32b
Py-GC-MS	*Q. pyrenaica*	13	3.33c	-2.73a	0.07b
	Q. robur	17	-2.08a	0.04b	1.60c
	Q. petraea	17	-2.44a	-0.43b	-1.47a
	Q. faginea	14	2.41b	3.01c	-0.22b

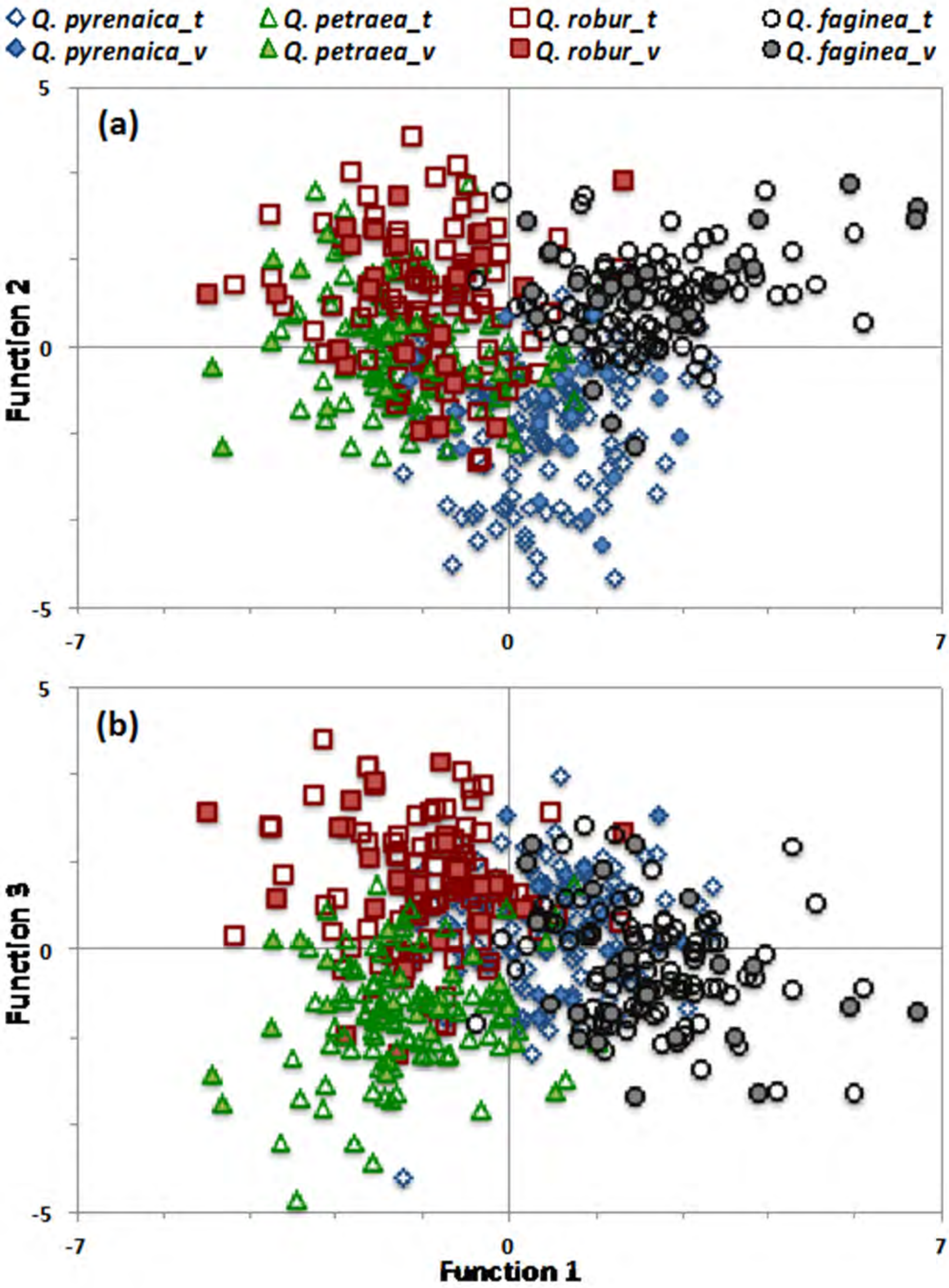

Fig. 3. Plot of the canonical functions obtained by the discriminant analysis using the FTIR absorption bands (t for training set and v for validation set): (**a**) for F1 and F2 and (**b**) for F1 and F3.

The probabilities of group membership in **Table 5** indicate the discriminant model from the FTIR data to differentiate between *Q. pyrenaica*, *Q. robur*, *Q. petraea*, and *Q. faginea* of the living wood is consistent. Very high probabilities (between 0.80 and 0.95) were found for all the species, both for the training and the validation sets. *Q. faginea* samples showed the highest probabilities of correct identification, with 91% for the training set and 75% for the validation set. *Q. robur* samples presented the lowest percentage of correct classification, with 75 and 71% for the training and validation sets, respectively. The lower values for *Q. robur* could be due to its similarity to *Q. petraea*. These two oak species are known to present similar morphological and physiological traits, distribution, and they can also naturally hybridise (Eaton et al. 2016); a reason why they are often treated as one taxon in dendrochronology (Haneca et al. 2009), even though some chemical and anatomical differences have been observed (Feuillat et al. 1997).

Table 5: Classification results of group prediction (probabilities of group memberships): * t for training set and v for validation set of the discriminant analysis input data.

Dataset	Samples*	*Q. pyrenaica*	*Q. robur*	*Q. petraea*	*Q. faginea*
FTIR	Extant_t	83.33 (0.86)	75.00 (0.89)	82.29 (0.86)	90.62 (0.92)
	Extant_v	79.16 (0.80)	70.83 (0.92)	75.00 (0.82)	75.00 (0.95)
	Shipwrecks	-	15.79 (0.57)	59.65 (0.71)	24.56 (0.75)
Py-GC-MS	Extant	92.31 (0.99)	82.35 (0.87)	76.47 (0.92)	92.86 (0.94)
	Shipwreck	24.56 (0.93)	44.44 (0.91)	30.99 (0.90)	-

The bands that allowed for the discrimination between the oak species are provided in **Table 6** with their standardized canonical coefficients for each function. Even though the discriminant model includes most of the selected bands, some appear to be particularly important for species separation and they could be considered as indicators. The bands at 985 and 1160 cm^{-1} (assigned to polysaccharides) and the bands at 1230, 1420 and 1460 cm^{-1} (assigned to lignin) were related to *Q. pyrenaica,* whereas the polysaccharide bands at 1080 and 1370 cm^{-1} were associated with *Q. petraea.* The wood samples from *Q. faginea* appear to be characterized by bands at 1320 and 1740 cm^{-1}, both due to molecular vibrations in polysaccharide compounds. It is interesting that differences between oak species can be associated to the main wood components (polysaccharides and lignins), although until now, extractive compounds were the most used chemicals as principal markers for various hardwood (oak) species (Fernández de Simón et al. 1996, Guchu et al. 2006). Few studies reported on differences between heartdwood of different species on the basis of polysaccharide and lignin chemistry (Hobro et al. 2010, Carballo-Meilán et al 2014, Carballo-Meilán et al 2016).

Table 6: Standardized Canonical Discriminant Function Coefficients (* 1, 2, 3 and 4 denote the association of FTIR bands and/or pyrolysis products to for *Q. pyrenaica*, *Q. robur*, *Q. petraea* and *Q. faginea* respectively). For Py-GC-MS, compound codes (polysaccharides and lignin only) are explained in Table 2.

FTIR discriminant functions				Py-GC-MS discriminant functions			
Bands* (cm^{-1})	F1	F2	F3	Compounds*	F1	F2	F3
830	-0.25	-0.02	-1.96	Ps01^{2}	-0.03	0.56	0.26
895	1.07	3.43	2.45	Ps02^{4}	1.14	2.29	0.27
925	-0.46	-3.07	0.65	Ps03^{4}	1.40	0.63	0.83
960^{1}	-2.89	2.70	-3.31	Ps04^{3}	-0.36	-0.57	-0.8
985	4.39	-3.45	2.83	Ps05^{2}	-0.39	0.34	0.18
1025	-3.87	1.50	-4.88	Ps06^{2}	-0.71	-0.54	0.36
1055	3.95	0.36	6.21	Ps07^{1}	1.90	-0.53	1.4
1080^{3}	-0.9	-1.21	-1.28	Ps08^{3}	-0.34	0.71	-0.54
1120	-2.59	0.68	-2.00	Ps09^{2}	-0.23	-0.36	0.21
1160^{1}	0.51	-0.25	1.37	Ps10^{4}	2.99	0.40	0.93
1230^{1}	1.34	-1.11	2.06	Lg01^{2}	-2.05	1.37	0.56
1265	-0.31	1.31	-1.7	Lg02^{1}	0.67	-0.99	1.66
1320^{4}	0.88	0.35	-1.07	Lg03^{3}	-0.56	2.98	-1.25
1370^{3}	-1.70	-0.90	-2.58	Lg04^{1}	1.91	-1.25	-1.15
1395	0.25	1.07	1.75	Lg05^{3}	-0.77	-2.92	-1.18
1420^{1}	0.32	-0.36	1.36	Lg06^{4}	0.39	0.56	1.88
1460^{1}	0.9	-0.79	0.62	Lg07^{4}	0.63	3.08	2.40
1560	0.88	0.39	0.46	Lg08^{2}	-0.38	-1.11	0.60
1620	1.20	-1.09	-0.37	Lg09^{3}	-0.73	0.25	-0.53
1655	-0.43	0.91	-0.78	Lg10^{3}	-0.57	-0.50	-1.83
1725	-4.53	-1.75	2.85	Lg11^{1}	1.24	-0.03	-0.36
1740^{4}	3.02	3.44	-1.02	Ls01^{3}	-0.10	0.79	-1.82
				Ls02^{4}	1.18	0.24	1.34
				Ls03^{3}	-2.14	-2.74	-0.74
				Ls04^{4}	3.18	3.81	-3.93
				Ls05^{1}	0.95	-1.79	2.68
				Ls06^{3}	-1.39	3.02	-3.03
				Ls07^{2}	-1.23	-2.29	1.76
				Ls08^{3}	-0.23	-2.17	-0.4
				Ls09^{3}	-1.20	0.05	-0.38
				Ls10^{1}	0.06	-0.47	1.81
				Ls11^{3}	-0.69	0.41	-0.63
				Ls12^{4}	2.52	1.45	0.65
				Ls13^{4}	0.39	0.88	0.87
				Ls14^{1}	1.01	-1.57	1.33
				Ls15^{3}	-0.62	0.06	-0.22

3.3.2. Discriminant analysis applied to Py-GC-MS data of living trees heartwood to differentiate between oak species

For the DA applied to the Py-GC-MS dataset, sapwood was omitted for the aforementioned big contrast in pyrolysis fingerprints and unlikely relevance for provenancing. The resultant heartwood dataset (*n*=61;) was too small to create training and validation subsets. The DA provided three discriminant functions explaining 56.5, 32.0 and 11.5% of the variance ($F1_{Py\text{-}GC\text{-}MS}$, $F2_{Py\text{-}GC\text{-}MS}$, $F3_{Py\text{-}GC\text{-}MS}$), respectively (**Fig. 4, Table 3**). Similar to $F1_{FTIR}$, $F1_{Py\text{-}GC\text{-}MS}$ shows a clear separation between *Q. petraea* and *Q. robur* with negative scores, and *Q. faginea* and *Q. pyrenaica* with positive scores (Fig. 4a). $F2_{Py\text{-}GC\text{-}MS}$ separates ($P<0.01$) between *Q. pyrenaica* (negative scores) and *Q. faginea* (positive scores) (Fig. 4a). Also similar to FTIR, no differences were shown for *Q. robur* and *Q. petraea* on $F1_{Py\text{-}GC\text{-}MS}$ or $F2_{Py\text{-}GC\text{-}MS}$, whereas $F3_{Py\text{-}GC\text{-}MS}$ does contrast ($P<0.01$) between these two species.

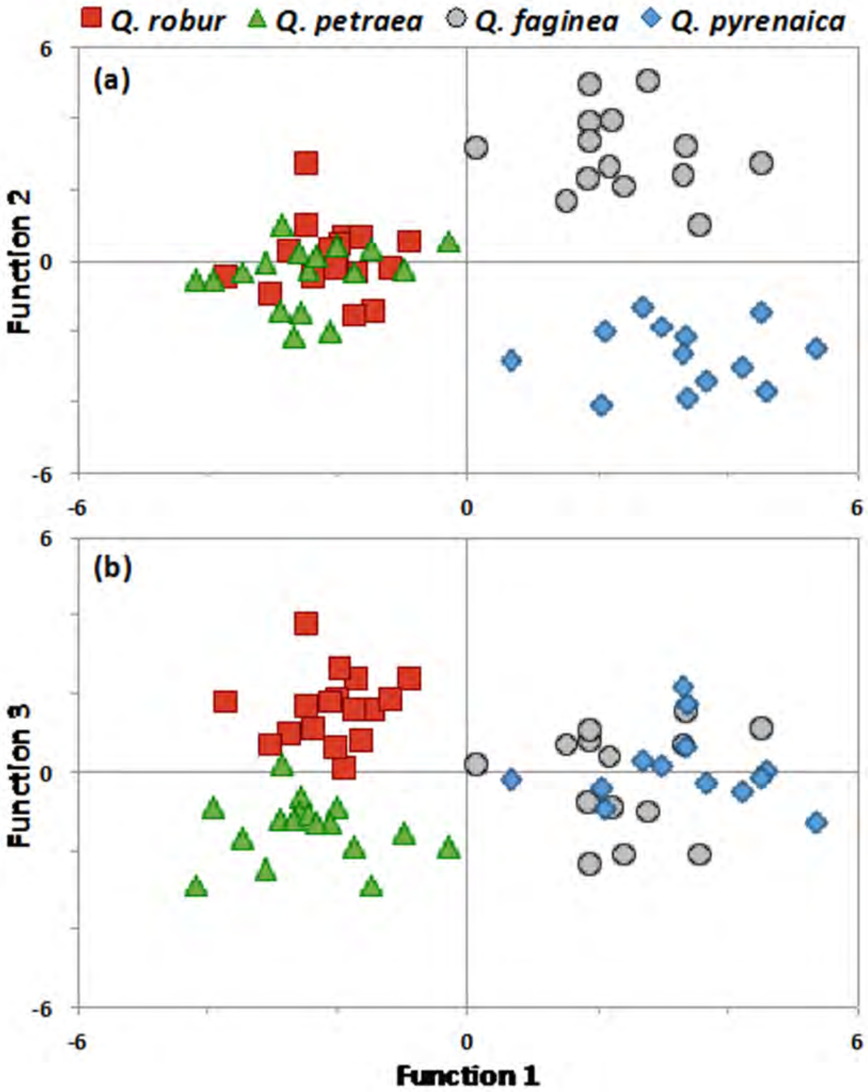

Fig. 4. Plot of the canonical functions obtained from the discriminant analysis for oak species, applied to the Py-GC-MS dataset: (**a**) for F1 and F2 and (**b**) for F1 and F3.

From the standardized canonical coefficients of the pyrolysis products (in **Table 6)**, several compounds can be considered as possible markers. More specifically, Lg04 and Ls14 were associated to *Q. pyrenaica*; Lg01 and Ls07 to *Q. robur*; Ls03 and Ls06 to *Q. petraea* and Ps02, Ls04 and Ls12 to *Q. faginea*. The discriminant models based on FTIR and Py-GC-MS data provide very similar F1-F2-F3 scores differentiation of the species, with a consistent pattern of separation between the analysed oak species (**Fig. 3** and **4**). This reveals the possibility to identify oak species using information only on the main wood chemical compounds (holocellulose and lignin). The good discrimination and the high similarity of the DA are not surprising. Several studies on wood chemistry reported that these two techniques are complementary (Wilson et al. 1993, Uçar et al. 2005, Kaal et al. 2012). Nevertheless, it is noted that the DA for Py-GC-MS could not be validated and group membership probabilities could not be determined, so that the value of this DA remains uncertain.

3.4. Species assignation to shipwreck wood samples

For shipwreck wood it is essential to consider possible transformations related to underwater degradation processes first. The PCAs (FTIR bands and pyrolysis products) applied to full datasets (living trees and shipwreck) permitted to identify variables that are proxies of the intensity of diagenesis (Supplementary data: **Fig. A.1, Table A.2**). Due to the major influence of diagenesis on wood chemistry, such effects may bias species allocation assignment if they would have effects on bands that are relevant for species identification of living wood. Hence, in order to assign an oak species to the shipwreck samples, we applied a new DA on living trees data but excluding the variables (FTIR bands and pyrolysis products) that are controlled by degradation.

The DA on FTIR data (extended dataset) resulted in the correct classification of 58.5 % of original grouped cases of the extant wood. On the basis of this DA, all the shipwreck samples were assigned to a *Quercus* species with probabilities of group membership between 0.53 and 0.71. A relatively large proportion of the samples were classified as *Q. petraea* (59.6%), followed by *Q. faginea* (24.6%) and *Q. robur* (15.8%). None of the samples was classified as *Q. pyrenaica*. Most of the shipwreck samples showed to highly correspond to *Q. petraea* wood (**Fig. 5a**). Nevertheless two wood fragments from Belinho trend towards *Q. faginea* and one from Yarmouth towards *Q. robur*.

The analogous DA model for Py-GC-MS, omitting compounds of which the variation is controlled by diagenesis, correctly classified 85.2% of the dataset, and the probabilities of group membership for the shipwreck samples were between 0.91 and 0.93. Samples were assigned to *Q. robur* (44.4%), *Q. petraea* (31.0%) and *Q. pyrenaica* (24.6%). None of the samples was assigned to *Q. faginea* (**Fig. 5b**). Woods from Belinho were classified either as *Q. robur* or *Q. petraea*. Timbers from Ribadeo were classified as *Q. robur,* except for one sample that showed a larger probability to belong to *Q. pyrenaica*. *Quercus robur* and *Q. petraea* dominate the two samples from

Magdalena. Yarmouth samples were either classified as *Q. robur* or *Q. pyrenaica*, but one of them showed a larger probability to belong to *Q. petraea* (**Fig. 5b**).

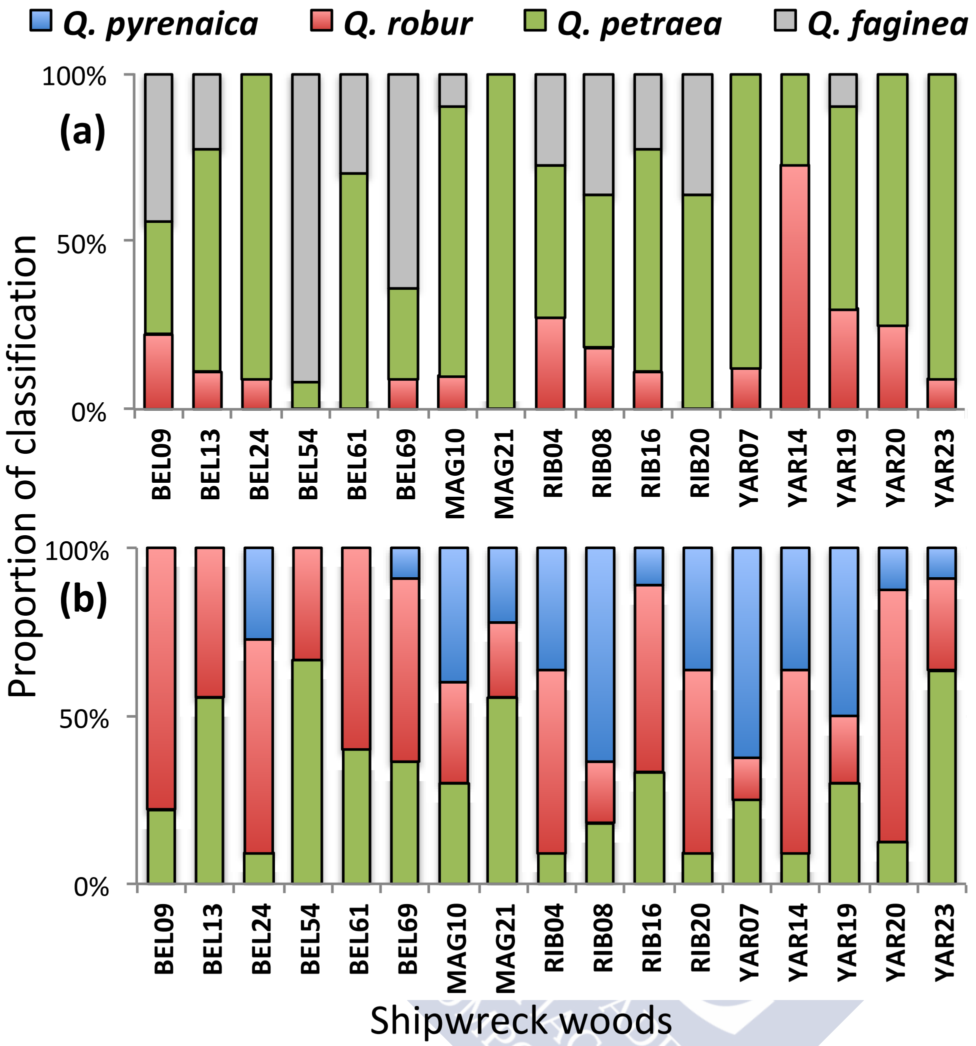

Fig. 5. Shipwreck wood classification results of the discriminant analysis on (**a**) FTIR absorption bands and (**b**) Py-GC-MS products.

The exclusion of FTIR bands associated with diagenesis considerably decreased the percentage of correct classification of extant wood, which was not the case for the pyrolysis products dataset. This can be explained by the fact that FTIR bands are usually associated to various wood components, they (excluded bands) also could contain signal related to the wood species. Whereas pyrolysis products are, in general, more directly related to a given wood component (Pandey 1999, Colom and Carillo 2005). However, there is agreement between the FTIR and Py-GC-MS models for shipwreck wood species identification. In both cases, about 75% of the shipwreck samples were classified as *Q. petraea* or *Q. robur*. This might be related to the similarity between the two species, already discussed in earlier sections, and hence that the relationship between *Q. petraea* and *Q. robur* is retained upon diagenesis. Nevertheless, there was no consistency between the methods regarding *Q. faginea* (assigned to many samples after FTIR) and *Q. pyrenaica* (assigned to many samples after Py-GC-MS). This suggests that the biomarkers for *Q. faginea* and *Q. pyrenaica* could be highly sensitive to the degradation processes, resulting to a lack of consistency between FTIR and Py-GC-MS results.

The DAs of both FTIR and Py-GC-MS strongly suggest that the two fragments of the Magdalena shipwreck are from *Q. petraea* and *Q. robur*. For Belinho, the Py-GC-MS DA model also likely suggests fragments of Belinho belonging to *Q. petraea* and *Q. robur* species, whereas the DA of FTIR suggested a considerable number samples from *Q. faginea* species (e.g. BEL54 and BEL69, Fig. 5). All the fragments from the Ribadeo and Yarmouth shipwrecks were assigned to *Q. petraea* and/or *Q. robur* according to the FTIR DA model, but a fragment from Ribadeo and another from Yarmouth were allocated to *Q. pyrenaica* according to the Py-GC-MS DA model. In spite of the discrepancies between the two techniques, it can be concluded that *Q. petraea* and *Q. robur* are the most probable sources of most of the analysed shipwreck wood fragments. The co-occurrence of *Q. petraea* and *Q. robur* in a given wood core –which is, obviously, impossible– shows that these species cannot be reliably distinguished due to the weakness of the third functions of the DA models considered ($F3_{FTIR}$ and $F3_{Py\text{-}GC\text{-}MS}$, which distinguish between *Q. petraea* and *Q. robur* in the extant wood), and therefore the taxon allocation could be better referred to as "*Q. petraea/Q. robur* type". The dominance of the *Q. petraea/Q. robur* type reveals a homogenous structure of the ships. We assume that the number of fragments studied by shipwreck is far from being representative regarding the quantity (about thousand of trees) of wood used to build a ship. However it is well known that homogeneity can be taken as an indicator of the original structure of the ship (Haneca et al. 2005, Allevato et al. 2009, Domínguez-Delmás et al. 2013).

3.5. Species and provenance of shipwreck wood samples

Our findings highly suggest that European forests were the sources for the analysed shipwreck woods. *Quercus petraea* and *Q. robur* are the most abundant and distributed species among the native *Quercus* species found in Europe (Ducousso and Bordacs, 2003). In this sense, the studied shipwrecks show the typical characteristics of the Iberian shipyards (Castro 2008a, 2008b). For shipbuilding, timber supplies used to be located not far from the shipyard (Albion 1926, Allevato et al. 2009); hence, Iberian forests are suggestive to be the potential source for our archaeological samples. However, there is no certainty about the exclusive origin of wood material from the Iberian Peninsula, because the scarcity of wood resources resulted in the establishing of timber trade networks during the age of discovery (16th to 18th centuries) (Creasman 2008, Gallagher 2016). The FTIR and Py-GC-MS DA models classified samples from Magdalena as *Q. petraea/Q. robur*. Thus, forests from the NW of the Iberian Peninsula are a likely source (both oak species being found there). The wood fragments identified as *Q. pyrenaica* or *Q. faginea* from Belinho, Ribadeo and Yarmouth shipwrecks, also suggest Iberian forests as the possible source of the timber. These two species are among the group of the so-called Iberian oaks, because they are highly abundant in the

Peninsula (Olalde et al. 2002, Petit et al. 2002, Hernández-Santana et al. 2008, Nieto Quintano et al. 2011). *Quercus pyrenaica* or *Q. faginea* woods could have been used for the construction of the ship, but also in later restoration carried out with wood supplied from Iberian forests.

4. Conclusions

The chemical composition of wood as determined by FTIR and Py-GC-MS clearly contains detailed information on the type of wood and, on the basis of known biogeographical distributions of oak species, the origin of shipbuilding timbers. Besides the ability of chemical characterization of the different types of wood (sapwood, heartwood and transition zone), PCA and DA performed on FTIR and Py-GC-MS data enabled to differentiate between the four oak species studied. The known resemblance between *Q. robur* and *Q. petraea* was highlighted by their chemical similarities. Additionally, our findings suggest that this similarity remains in archaeological woods. Diagenesis is an important factor that alters the chemical composition of wood, but its characterization permitted to avoid, to some extent, its influence on the identification of species in shipwreck woods. According to our results, most of the shipwreck samples belonged to European oak species *Q. robur* and/or *Q. petraea*. This is in agreement with the possible source of timber for the Magdalena frigate, indicated from Iberian forests. The identification of Iberian oak (*Q. pyrenaica* or *Q. faginea*) in wood fragments from Belinho, Ribadeo and Yarmouth shipwrecks, also highly suggests Iberian forests as probable timber source for these ships. Hence, wood chemical characterization appears to be paramount in archaeological studies due to the possibilities to provide additional information related to the state of conservation, the species and also the possible provenance of woods.

Acknowledgements

This study was developed within the framework of the ForSEAdiscovery project funded from the People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme (FP72007-2013) under REA grant agreement nº PITN-GA 2013-607545.

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Appendix A. Supplementary data

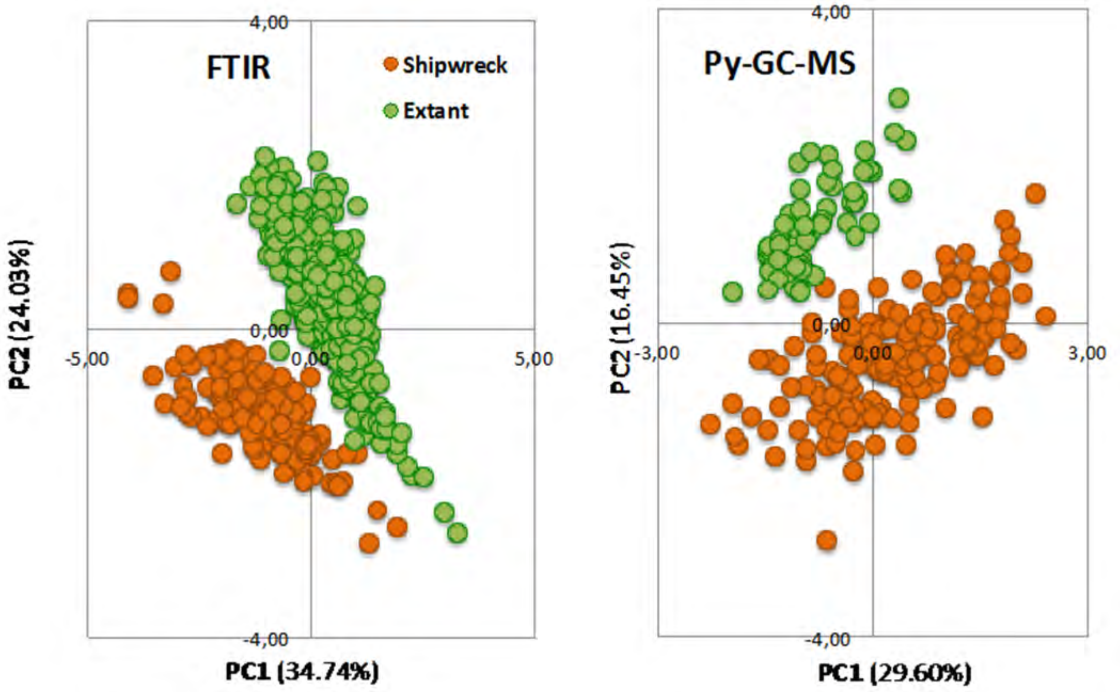

Fig. A.1. Component scores plot of the principal components (PC1 and PC2) from the PCA applied to FTIR and Py-GC-MS data, showing the differences between extant and shipwreck wood samples.

Table A.1: Classification results of group memberships of discriminant analysis for sapwood and heartwood identification for FTIR and Py-GC-MS.

		Predicted Group Membership (%)		
		Sapwood	Transition wood	Heartwood
FTIR	Sapwood	92.9	4.8	2.4
	Transition wood	0	77.4	22.6
	Heartwood	0	9.8	90.2
	Shipwreck wood	0	8.8	91.2
Py-GC-MS	Sapwood	90.5	9.5	0
	Transition wood	3.2	80.6	16.1
	Heartwood	0	14.8	85.2
	Shipwreck wood	10.5	1.2	88.3

Table A.2: Component loadings corresponding to the principal components (PC1 and PC2, from PCA) that are related to degradation/preservation dynamics: loadings of bands (FTIR) or compounds (Py-GC-MS) with high communalities values (>0.70) are given in bold. **For Py-GC-MS, compound codes are explained in Table 2 of the regular paper.**

Bands (cm^{-1})	Communality	PC1	PC2	Compounds	Communality	PC1	PC2
1740	**0.89**	0.43	0.84	**Lg06**	**0.77**	0.88	0.02
1725	**0.88**	0.36	0.87	**Ls06**	**0.77**	0.76	-0.44
1560	**0.87**	-0.83	-0.42	**Lg05**	**0.76**	0.85	-0.19
1265	**0.84**	0.26	0.88	**Ls05**	**0.75**	0.71	0.5
1230	**0.84**	0.39	0.83	**Lg04**	**0.74**	0.65	0.57
960	**0.83**	0.74	-0.54	**Ls04**	**0.74**	0.78	0.37
895	**0.80**	0.78	-0.44	**Lg03**	**0.74**	0.66	0.56
985	**0.79**	0.82	-0.34	**Ls07**	**0.71**	0.8	-0.27
1160	**0.75**	0.62	0.61	**Lg07**	**0.70**	0.81	-0.23
1540	**0.74**	-0.76	-0.41	**Ls08**	**0.70**	0.69	-0.46
1620	0.65	-0.66	0.47	Ls01	0.67	0.68	0.45
1655	0.63	-0.76	-0.24	Lg08	0.66	-0.49	0.65
1025	0.61	0.76	-0.2	Lg01	0.65	0.64	0.5
925	0.61	0.61	-0.49	Ls03	0.63	0.75	0.27
1590	0.60	-0.7	0.34	Ls12	0.60	0.69	-0.37
1055	0.57	0.72	-0.22	Lg02	0.58	0.7	0.32
1395	0.54	-0.74	-0.05	Ps07	0.58	0.17	-0.74
1320	0.54	-0.09	0.73	Ls13	0.58	-0.51	0.57
865	0.5	0.71	0.03	Ps08	0.54	-0.2	-0.71
1120	0.21	0.43	-0.14	Ps05	0.51	-0.58	0.42
14609	0.09	-0.13	0.27	Ps06	0.42	0.34	-0.55
1505	0.11	-0.11	0.32	Ps10	0.39	-0.57	-0.26
1420	0.43	-0.33	-0.57	Ps04	0.14	-0.28	-0.25
1080	0.46	0.58	-0.35	Ps09	0.26	-0.03	-0.51
830	0.19	0.27	-0.34	Ps02	0.23	-0.47	-0.04
1370	0.29	-0.54	0.06	Lg10	0.16	-0.31	0.26
				Ls11	0.19	0.43	-0.04
				Ls02	0.19	-0.16	0.4
				Ps01	0.02	0.06	-0.14
				Ls10	0.21	0.42	-0.18
				Ps03	0.16	0.09	-0.39
				Lg09	0.11	0.33	-0.02
				Ls09	0.03	-0.18	0.01
				Ls15	0.13	0.3	-0.19
				Lg11	0.26	0.32	0.39
				Ls14	0.28	0.01	0.53