



The scent of an archaic wreck: minimally invasive characterisation of archaeological finds

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Abstract

The chemical characterisation of archaeological finds necessitates a continuous focus on the development of nano/micro-invasive methodologies. These methodologies yield insights into the historical background, production processes, and conservation state of the items, often revealing astonishing properties of materials that have been unexpectedly preserved over the centuries. This work aims to characterise the molecular composition of a putative historical resin recovered during an archaeological excavation in Campese Bay off the island of Giglio in the 1980s. The resin was sampled from a trade amphora used for wine transport; it was likely employed as a coating to ensure better waterproofing of the containers. The characterisation was performed by detecting specific molecular markers using a GC–MS direct injection approach. In addition, we investigated the volatile fraction of the same sample through solid-phase microextraction (SPME) coupled with GC–MS to address the need for a fast, solvent-free, and field-compatible sample preparation method.

Rizzo Emanuela and Morelli Marco have contributed equally to this work.

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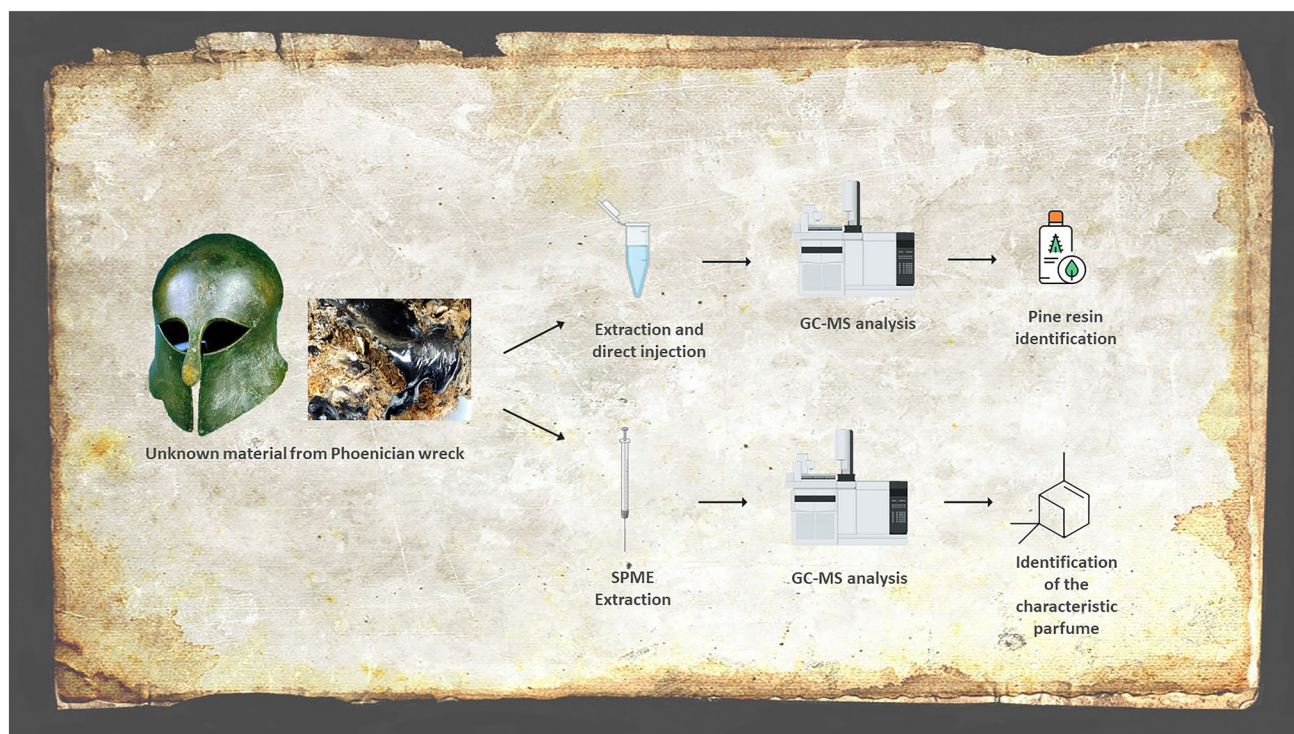
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Graphical abstract



Keywords SPME · GC–MS · Archaeological finds · Micro-invasive analyses

1 Introduction

1.1 State of the art

The need for minimally invasive, non-destructive analysis and the use of small amounts of sample is an increasingly important goal in the molecular characterisation of cultural heritage materials (Morelli et al. 2024). These analyses can provide information about manufacturing techniques, historical context, the conservation state of the artefacts, as well as any unexpectedly preserved properties of various materials over the centuries.

Despite the availability of a wide range of analytical techniques for the protection of cultural heritage artefacts, the challenges posed by the limited amount of samples available and the accessibility of monuments, hinder the wider application of these analytical tools (Moropoulou et al. 2013). The use of nano- and micro-invasive techniques that can be applied to small sample amounts is, therefore, necessary (Colantonio et al. 2022).

It has been shown that chromatographic techniques hyphenated with mass spectrometers (such as GC–MS, pyrolysis gas chromatography mass spectrometry [Py–GC/MS], and LC/MSMS) are the most efficient and accurate

analytical techniques for adequately characterising the chemical composition of both natural and synthetic polymers (binders) in microsamples (Brunetti et al. 2017). The ability to unambiguously identify different compounds by MS detection based on their mass spectra makes GC–MS particularly suitable for studying unknown matrices and/or tracking ageing and degradation pathways (Bonaduce et al. 2017).

A suitable nano-invasive technique, less commonly used in the field of cultural heritage, is solid-phase microextraction (SPME). This technique was developed to address the need for a fast, solvent-free, and field-compatible sample preparation method, allowing for the combination of sample extraction and pre-concentration in a single step using a very small amount of extraction solvent compared to the sample volume (Pawliszyn 2001).

In this work, we show two different approaches for the characterisation of the organic components of a putative resin discovered on a Phoenician shipwreck using micro-invasive analytical techniques. The first protocol involves an extraction method that requires the minimum amount of sample (about 500 µg), followed by GC–MS analysis. The second approach, aimed at characterising the volatile components, utilises a single-step sample extraction and

pre-concentration through the SPME technique, followed by a GC–MS analysis.

1.2 Archaeological context

The analysed putative resin comes from a collection of artefacts sampled during an archaeological excavation. In the 1980s, a significant shipwreck was identified and explored through an underwater excavation in Campese Bay off the island of Giglio, led by Mensun Bound of Oxford University. Some preliminary reports have been published (Bound 1991), but the wreck and the entirety of the finds are still to be published. The wreck contained a mixed cargo of East Greek, Greek, and Etruscan wares, suggesting a dating of the ship around 580–570 BCE. An international project led by the University of Naples Federico II with Alessandro Naso as the Principal Investigator, has been initiated to conduct a comprehensive study of the finds. This includes laboratory analysis of both organic and inorganic artefacts. The presence of a presumed vegetable resin on the wreck may be linked to trade amphorae discovered during excavations, which were reserved for wine transport and usually coated with pine resin to improve the waterproofing of the containers, as evidenced by several Corinthian trade amphorae from the seventh century BC (Davide Petriaggi 2023).

2 Materials and methods

2.1 Materials

The fibres used for volatile fractions (SPME) analysis were purchased from Supelco (Bellefonte Park, USA). Sulphuric acid, hexane, chloroform, and methanol were purchased from Sigma, and ammonium bicarbonate from Carlo Erba.

2.2 Samples

The historical sample consists of putative pine resin (Fig. 1) discovered in the wreck described in Sect. 1.2. The control samples for the SPME analysis were collected from a maritime pine tree, from which two fractions were analysed: the resin and the needles.

2.3 Material characterisation (extraction and derivatization)

An extraction protocol was performed to recover the nonpolar compounds (lipids and diterpenic acids). 1 mL of hexane (C_6H_{14}) was added to the historical sample. The extraction was conducted in an ultrasonic bath at room temperature for 5 min followed by agitation for 10 min. The sample was

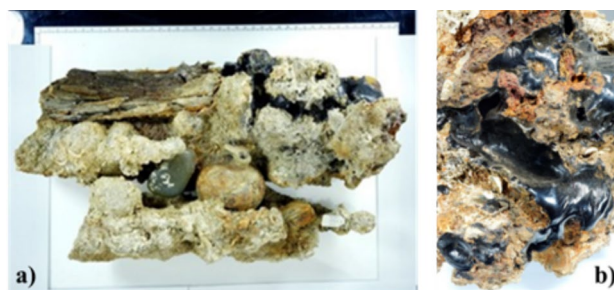


Fig. 1 Image of the material discovered in the shipwreck (a) and a detail of the resin (b)

then centrifuged at 10,000 RPM for 10 min to recover the supernatant.

To enhance analyte volatility for GC–MS analysis, transesterification was carried out by combining 150 μ L of sulfuric acid and 850 μ L of methanol with 1.0 mL of chloroform at 95 $^{\circ}$ C for 16 h. Subsequently, the pH was adjusted to neutrality by adding 2.0 mL of a 100 mg/mL ammonium bicarbonate solution (Melchiorre et al. 2020). The chloroform phase containing the reaction products was recovered, and any excess salts were removed with water. The resulting solution was dried under a nitrogen flow, then suspended in 1.0 mL of n-hexane and centrifuged to remove any residual solid reagents. A 1.0 μ L aliquot of the hexane supernatant was used for GC–MS analysis.

2.4 Analysis of volatile compounds (SPME)

Extraction and desorption of molecules were carried out by SPME using 2 cm 50/30 μ m divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre (Supelco). Adsorption occurred for 30 min at 60 $^{\circ}$ C, and the fibre was then exposed to a GC injector at a temperature of 230 $^{\circ}$ C for 3 min (desorption).

2.5 GC–MS

All gas chromatography analyses were performed using an Agilent GC 6890 coupled with a 5973 MS detector.

For the non-volatile fractions, the method employed involved an initial temperature of 70 $^{\circ}$ C for 2 min, then reaching 230 $^{\circ}$ C with an increase of 20 $^{\circ}$ C/min, 240 $^{\circ}$ C, and 270 $^{\circ}$ C. The maximum temperature reached by the instrument was 325 $^{\circ}$ C (Rizzo et al. 2024).

The mass spectra assignment was based on a direct match with the spectra of the NIST library. Identifications were considered reliable if the correlation match index was higher than 95%.

For the volatile fractions, the GC injector was maintained at 230 $^{\circ}$ C, while the analyser was kept at 250 $^{\circ}$ C. The collision energy was set to a value of 70 eV, and fragment ions

generated were analysed in the mass range of 20–450 m/z. The oven temperature was held at 60 °C for 3 min and then increased to 150 °C at 10 °C/min, increasing to 230 °C at 14 °C/min, and finally to 280 °C at 15 °C/min, held for 5 min for a total separation time of 23 min.

The identification of each compound was based on a combination of retention time and mass spectrum matching using Ms Search-Nist 05 library software.

3 Results

3.1 Historical sample characterisation

The molecular characterisation of the historical sample was performed through extraction and subsequent GC–MS analysis. The data obtained are summarised in Table 1.

According to literature studies, abietic acid is the major resin acid, followed by its hydrogenation products (dehydroabietic acids) (Zeiss 1948). In particular, among the diterpenic resins, dehydroabietic acid and 7-oxo-dehydroabietic acid are characteristic of ageing resins (Russo and Avino 2012). 10,18-Bisnorabieta-8,11,13-triene is another specific marker of conifer resin identified in the sample (Barberis et al. 2019); it is an isomer of norabietadiene and is interpreted as successively degraded abietane-type diterpenoids (Dimitrakoudi et al. 2011). Regarding pimarane-type resin acids, the most common one is pimaric acid (Alonso-Esteban et al. 2022). The identification of these chemical markers allows us to confirm the resinous nature of the unknown material. In particular, the identified molecules are compatible with conifer resin.

Moreover, one of the identified substances is androst-2,16-diene, whose androstenone-like structure is associated with the pine resin perfume (Schreiner et al. 2018). Nevertheless, sterols are known constituents in the extractable fraction of wood (Sjöström 2013). Lastly, another compound identified is retene, which is formed by the thermal

degradation of resin compounds in the wood (Ramdahl 1983).

3.2 Volatile fraction analysis

The pine resinous nature has been further validated through an SPME analysis using two control samples. This method was chosen because the sample still preserved the characteristic perfume of pine resins.

However, olfactory perception can vary due to individual factors, so it was necessary to identify scientific parameters to be able to confidently assert that these properties had been preserved for centuries in an extreme environment (such as the deep sea).

A sample of maritime pine resin and the needles from the same tree were used as control samples.

The analysis of the volatile fraction of the three samples was performed through extraction and characterisation by SPME coupled with GC–MS, as described in “Materials and methods”.

Prominent volatile compounds found in conifer resins and needles include α -pinene, camphene, sabinene, β -pinene, δ -3-carene, and limonene (Wilson et al. 2023). In addition, longifolene (Colombini and Modugno 2009) and myrcene (Zavarin et al. 1971) are recognised as volatile markers of pine trees.

As expected, many of these compounds were identified in the control samples (Table 2). Specifically, maritime pine resin comprises monoterpenes (α -pinene, β -pinene, α -terpineol, and limonene), as well as sesquiterpenes (β -caryophyllene, α -humulene, longifolene, α -cubebene, and germacrene D) (Alonso-Esteban et al. 2022). While in pine needles, it is also common to detect bornyl acetate, camphene, linalool, myrcene, and α -phellandrene (Sahin and Yalcin 2017). The molecular markers identified by SPME and GC–MS in the control samples correspond to those documented in the literature.

Several of the markers detected in the controls could not be identified in the historical sample due to its antiquity and state of degradation. However, the presence of α -Pinene, endo-borneol, and m-Cymen allows us to confirm the conifer resin origin of the historical sample (Budiman and Arifta 2015; Wilson et al. 2023).

In addition, most of the compounds listed in the table contribute to the scent, depending on the type of plant. For pine, the main components responsible for the smell are α -pinene, β -pinene, and longifolene. Borneol is often attributed to the smell of rosemary and chrysanthemums. Both α -pinene and borneol are also identified in historical resin, confirming its resinous nature and the preservation of its characteristic odour for centuries underwater (Upadhyay and Singh 2021).

Table 1 GC–MS profile of the historical sample. Each compound is provided with the retention time at which it was identified

RT (min)	Identified compound
18.92	Androst-2,16-diene
19.53	Methyl-dehydroabietate
19.93	10,18-Bisnorabieta-8,11,13-triene
20.98	Methyl-abietate
21.11	Retene
21.35	Pimaric acid
21.81	Methyl-dehydroabietate derivative
22.39	Dehydroabietic acid
23.29	7-Oxo-dehydroabietic acid

Table 2 GC–MS profiles of volatile fraction of the three analysed samples. Each compound is provided with the retention time at which it was identified

RT (min)	Identified compound	Historical resin	Maritime pine resin	Pine needle
6.83	3-Carene		x	
7.05	α -Pinene	x	x	x
7.33	Camphene		x	x
8.03	β -Pinene		x	x
8.18	β -Myrcene			x
8.42	α -Phellandrene		x	
8.92	D-Limonene		x	x
9.43	γ -Terpinene			x
10.09	Linalool		x	
10.50	α -Camphenol		x	
10.63	cis oxide limonene		x	
10.71	L-Pinocarveol		x	
10.79	Camphor			x
10.80	cis-Verbenol		x	x
11.12	Endo-Borneol	x		
11.61	Myrtenol			x
11.98	Verbenol		x	
12.41	m-Cymen, 5-tert-butyl	x		x
12.89	Bornyl acetate			x
13.83	α -Longipinene		x	x
14.16	Cyclosativene		x	
14.36	Sativene		x	
14.48	Cedrene		x	
14.49	Longifolene		x	x
14.73	Caryophyllene		x	
15.14	Humulene		x	

4 Conclusion

For the characterisation of cultural heritage materials, it is essential to utilise micro-invasive techniques that can be applied to minimal sample amounts and do not require invasive sampling. In this article, we propose two approaches for the molecular characterisation of an unknown historical material discovered during an archaeological excavation in Campese Bay off the island of Giglio in the 1980s. The data obtained from the GC–MS analysis of the historical material enabled the identification of conifer resin. In order to confirm the pine resinous nature of the historical material and verify the origin of its characteristic perfume, retained underwater for hundreds of years, an SPME analysis was conducted. SPME is a microextraction technique that simplifies the sample preparation step and offers several advantages over traditional methods, including sensitivity and a limit of determination comparable to techniques based on liquid extraction (Pawliszyn 2001). Due to its requirement for a minimal sample amount for analysis, SPME is particularly advantageous for identifying volatile compounds from cultural heritage samples.

In fact, the limited sample amount available for analysis presents a significant challenge in the characterisation and analysis of historical materials.

The results obtained through this analytical strategy have allowed for the identification of compounds that contribute to the scent of some plants. This not only validated the results obtained previously but also demonstrated the efficiency of the proposed micro-invasive protocol.

Author contributions A.C. conceived the project, reviewed, and edited the manuscript. A.C., C.M., A.A. and A.N. coordinated and supervised the activities. A.N. provided the samples and the historical background. E.R., M.M. and C.M. carried out the experimental activities. E.R. and M.M. analysed and interpreted experimental data and wrote and edited the manuscript. A.A. provided the MS setup. All the authors read and approved the final manuscript.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

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References

- Alonso-Esteban JJ, Carocho M, Barros D, Velho MV, Heleno S, Barros L (2022) Chemical composition and industrial applications of Maritime pine (*Pinus pinaster* Ait.) bark and other non-wood parts. *Rev Environ Sci Bio/technol* 21(3):583–633. <https://doi.org/10.1007/s11157-022-09624-1>
- Balasubramanian S, Panigrahi S (2011) Solid-phase microextraction (SPME) techniques for quality characterization of food products: a review. *Food Bioprocess Technol* 4:1–26. <https://doi.org/10.1007/s11947-009-0299-3>
- Barberis E, Manfredi M, Marengo E, Zilberstein G, Zilberstein S, Kosolapov A, Righetti PG (2019) Leonardo's donna nuda unveiled. *J Proteom* 207:103450. <https://doi.org/10.1016/j.jprot.2019.103450>
- Bonaduce I, Ribechini E, Modugno F, Colombini MP (2017) Analytical approaches based on gas chromatography mass spectrometry (GC/MS) to study organic materials in artworks and archaeological objects. *Analytical chemistry for cultural heritage*. Springer, Cham, pp 291–327. https://doi.org/10.1007/978-3-319-52804-5_9
- Bound M (1991) The Giglio Wreck: a wreck of the Archaic Period (C. 600 BC) off the Tuscan Island of Giglio: an account of its discovery and excavations: a review of the main finds. Hellenic Institute of Marine Archaeology
- Brunetti B, Miliani C, Rosi F, Doherty B, Monico L, Romani A, Sgamellotti A (2017) Non-invasive investigations of paintings by portable instrumentation: the MOLAB experience. *Analytical chemistry for cultural heritage*. Springer, Cham, pp 41–75. https://doi.org/10.1007/978-3-319-52804-5_2
- Budiman A, Arifita TI (2015) Continuous production of [alpha]-Terpineol from [alpha]-Pinene Isolated from Indonesian Crude Turpentine. *Mod Appl Sci* 9(4):225. <https://doi.org/10.5539/mas.v9n4p225>
- Colantonio C, Lanteri L, Ciccola A, Serafini I, Postorino P, Censorii E, Rotari D, Pelosi C (2022) Imaging diagnostics coupled with non-invasive and micro-invasive analyses for the restoration of ethnographic artifacts from French Polynesia. *Heritage* 5(1):215–232. <https://doi.org/10.3390/heritage5010012>
- Colombini MP, Modugno F (2009) Organic mass spectrometry in art and archaeology. Wiley
- Davidde Petriaggi B (2023) Recuperati dagli abissi: Il relitto alto-arcaico del Canale d'Otranto. Gangemi Editore, Rome
- Dimitrakoudi EA, Mitkidou SA, Urem-Kotsou D, Kotsakis K, Stephanidou-Stephanatou J, Stratis JA (2011) Characterization by gas chromatography—mass spectrometry of diterpenoid resinous materials in roman-age amphorae from northern Greece. *Eur J Mass Spectrom* 17(6):581–591. <https://doi.org/10.1255/ejms.1155>
- Melchiorre C, Ioio LD, Ntasi G, Birolo L, Trojsi G, Cennamo P, Barone Lumaga MR, Fatigati G, Carpentieri A (2020) A multidisciplinary assessment to investigate a XXII dynasty wooden coffin. *Int J Conserv Sci* 11(1):25–38
- Morelli M, Rizzo E, Perlingieri C, Moracci M, Somma PI, Carpentieri A, Marino G (2024) Molecular characterization of varnish organic components in historical paintings: a microinvasive GC-MS study. *Rend. Acc. Sc. Fis. Mat.* 91. Ahead of print
- Moropoulou A, Labropoulos KC, Delegou ET, Karoglou M, Bakolas A (2013) Non-destructive techniques as tool for the protection of built cultural heritage. *Constr Build Mater* 48:1222–1239. <https://doi.org/10.1016/j.conbuildmat.2013.03.044>
- Pawliszyn J (2001) Solid phase microextraction. Headspace analysis of foods and flavors: theory and practice. Springer, Boston, pp 73–87. https://doi.org/10.1007/978-1-4615-1247-9_6
- Ramdahl T (1983) Retene: a molecular marker of wood combustion in ambient air. *Nature* 306(5943):580–582
- Rizzo E, Morelli M, Carpentieri A, Marino G (2024) Molecular characterization of painting layer binders in a 13th-century wooden sculpture. *Rend. Acc. Sc. Fis. Mat.* 91. Ahead of print
- Russo MV, Avino P (2012) Characterization and identification of natural terpenic resins employed in “Madonna con Bambino e Angeli” by Antonello da Messina using gas chromatography–mass spectrometry. *Chem Cent J* 6:1–10. <https://doi.org/10.1186/1752-153X-6-59>
- Sahin HT, Yalcin OU (2017) Chemical composition and utilization of conifer needles: a review. *J Appl Life Sci Int* 14(3):1–11. <https://doi.org/10.9734/JALSI/2017/37076>
- Schreiner L, Bauer P, Buettner A (2018) Resolving the smell of wood-identification of odour-active compounds in Scots pine (*Pinus sylvestris* L.). *Sci Rep* 8(1):8294
- Sjöström E (2013) Wood chemistry: fundamentals and applications. Elsevier
- Upadhyay SK, Singh SP (eds) (2021) Bioprospecting of plant biodiversity for industrial molecules. Wiley, Hoboken, NJ
- Wilson TM, Ziebarth EA, Carlson RE (2023) Essential oil from naturally exuded *Pseudotsuga menziesii* var. *glauca* (Pinaceae) resin. *J Essent Oil Plant Compos* 1(3):213–219. <https://doi.org/10.58985/jeopc.2023.v01i03.27>
- Zavarin E, Snajberk K, Lee CJ, Henley M, Mirov NT (1971) The composition of terpenoid hydrocarbons from *Pinus monophylla* wood oleoresin. *Phytochemistry* 10(8):1857–1862. [https://doi.org/10.1016/S0031-9422\(00\)86450-X](https://doi.org/10.1016/S0031-9422(00)86450-X)
- Zeiss HH (1948) The chemistry of the resin acids. *Chem Rev* 42(1):163–187

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