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A multi-analytical approach using FTIR, GC/MS and Py-GC/MS revealed early evidence of embalming practices in Roman catacombs

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Abstract:

During the second-third century, cremation was progressively abandoned for inhumation in the Roman Empire and was accompanied by new funeral practices. Recent archaeological excavations in the catacombs of Saints Peter and Marcellinus in Rome revealed thousands of formerly undiscovered skeletons of individuals plastered and methodically stacked in previously unknown and inaccessible rooms. By setting up and applying a multi-analytical approach to characterize chemically all amorphous materials surrounding the skeletons, we investigated this important cultural change regarding the treatment of death.

Chemical characterization of the amorphous samples was achieved using FTIR spectroscopy and gas chromatography / mass spectrometry (Py-GC/MS and GC/MS) and by comparison with reference samples from known origin. This allowed for the unambiguous identification of precious and exotic resinous substances involved in the embalming process of the bodies. Amber, sandarac and frankincense, which were sourced from widespread locations, were used as part of the funerary treatment. This first evidence of such highly prized commodities in burial process provides us with new insight into funerary practices as well as commercial networks in the Roman Empire during the first centuries A.D..

Keywords:

Roman catacombs; embalming; natural resinous substances; plaster; FTIR spectroscopy; (Py)-GC/MS

1. Introduction

The Christian catacombs of Saint Peter and Saint Marcellinus were established along the ancient Roman road, *via Labicana,* located to the south-east of Rome. The majority of the galleries were gradually cleared from the end of the nineteenth century until the 1970s [1]. However, a small area of the first catacombs level, located centrally with respect to the network of underground galleries, remained inaccessible because of the substantial amounts of debris preventing entry. Investigations carried out in this area under the direction of the Vatican's *Pontificia Commissione di Archeologia Sacra* revealed a new funerary space markedly different from the traditional spatial organisation observed in the other sections of these catacombs, and indeed, in other Roman catacombs. By contrast with the normal structure of catacombs with straight galleries provided with *loculi* (simple individual burials), *arcosolida* (more elaborate burials) or *cubicula* (small chambers in which few individual burials are regrouped), the newly examined burial area was found to be divided into seven rooms each comprising hundreds of individuals methodically stacked (Fig. 1.). The radiocarbon dating of bones from these catacombs provided a calibrated age between 28 and 132 A.D., a date that is very early in the known history of the catacombs [2].

An archaeological investigation of the human remains in the catacombs of Saint Peter and Marcellinus revealed the preservation of anatomic connections of skeletons. This demonstrated that the 3000 bodies were buried in a very short timeframe following a mortality crisis event. The absence of injuries on the bones, as well as the sex and age ranges for this population, correlate with an epidemic, making these catacombs the largest archaeological evidence of such health crisis in Antiquity. The radiocarbon dates correspond to the well-known Antonine plague (165-180 A.D.) [3]. The field investigation also revealed that most of the buried bodies were individually wrapped in a cloth accompanied by amorphous materials (Fig. 1.).

Fig. 1. View of the mass grave inside the catacombs of Saints Peter and Marcellinus (Rome); skeletons of plastered individuals methodically stacked are shown (Photo by D. Castex – PACEA)- *Color should be used for this figure (online only).*

In order to understand the nature of the treatment applied to these individuals, a multi analytical investigation was performed on the amorphous materials surrounding all the bones and the main findings are presented in this paper.

2. Materials and Methods

2.1 Archaeological samples

A white substance was found in large quantities around the bones in the catacombs of Saint Peter and Marcellinus. A microscopic observation revealed that for some of the white samples, small shiny-reddish particles and a diffusely distributed yellow coloured material were embedded in the matrix (Fig. 2.). Black-brown amorphous materials were also present in significant amount. A total of 47 samples were taken for chemical investigation.

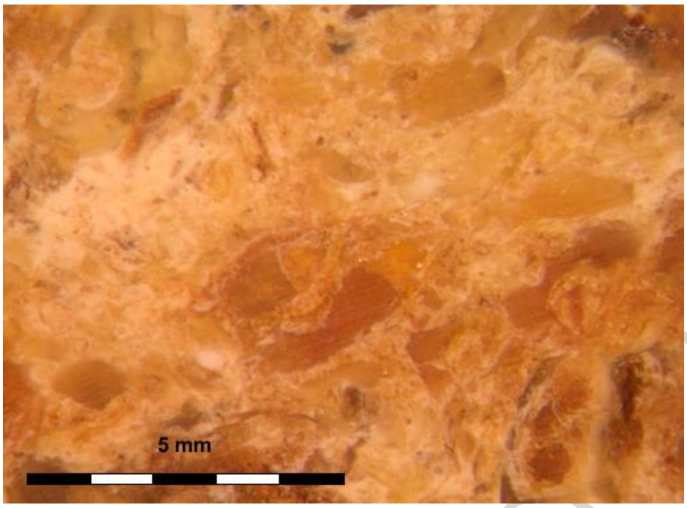

Fig. 2. Microscope photograph of the white substance with small shiny-reddish particle inclusions used to plaster individuals in the catacombs of Saint Peter and Marcellinus- *Color should be used for this figure (online only).*

2.2 Reference materials

Reference materials of gypsum, fossil resins, sandarac and frankincense were collected and where possible the provenance was recorded (details in Table A1).

2.3 Chemicals and standards

All solvents used were HPLC analytical grade. The derivative agents N,O-bis(trimethyl)silyltrifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane and hexamethyldisilazane (HMDS) were purchased from Sigma Aldrich (Milan, Italy).Tridecanoic acid and hexadecane, used as an internal standard, were purchased from Sigma Aldrich (Milan, Italy). Succinic acid (purity ≥ 99%) was purchased from Fluka (Switzerland).

2.4 Methods

2.4.1 Fourier Transform infrared (FTIR) spectroscopy

FTIR analyses were performed using a microspectrometer Spectrum 2000 (Perkin Elmer, Waltham, Massachusetts, USA). A few micrograms of sample were placed in a diamond cell and then placed into the instrument. All analyses were performed in transmission mode. Spectra were collected from 400 to 4000 cm^{-1} with 128 scans of acquisition with a resolution of 4 cm^{-1}.

2.4.2 Gas chromatography / mass spectrometry (GC/MS)

2.4.2.1 Sample preparation

Samples were saponified and then the neutral and acidic fractions were extracted as illustrated in Fig. 3. This protocol is based on a combined GC/MS analytical procedure developed for the characterization of glycerolipid, waxy, resinous and proteinaceous materials in a paint microsample [4].

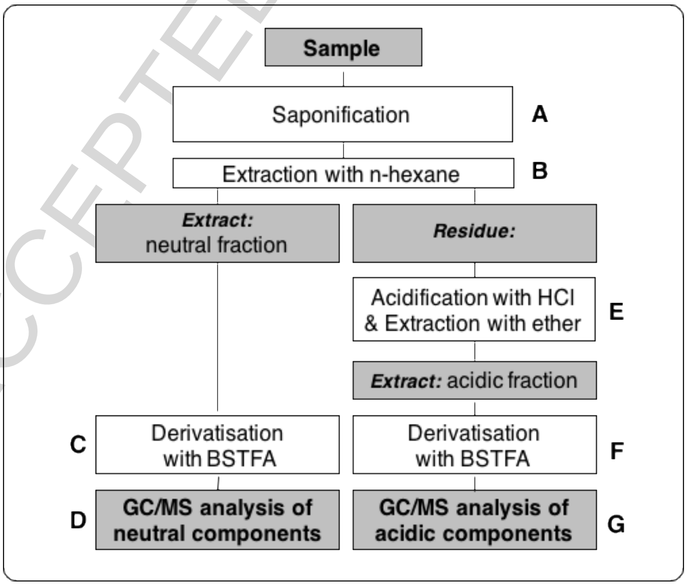

Fig. 3. Analytical procedure for GC/MS analyses

Step A: Saponification

The sample (2 mg) was subjected to saponification with 1 mL of 10 weight % potassium hydroxide in ethanol at 60°C for 120 min.

Step B: Extraction of the neutral fraction

After saponification, the hydroalcoholic solution was diluted in doubly distilled water. Neutral organic molecules (neutral fraction) were extracted three times by 200 μL of hexane. The hydroalcoholic solution was then kept for step E.

Step C: Derivatisation of the neutral fraction

An aliquot of the neutral fraction was evaporated to dryness under a nitrogen stream and subjected to derivatization with 20 μL of BSTFA at 60°C for 30 min in presence of 100 μL of isooctane (solvent). 5 μL of tridecanoic acid solution (135 µg/g of isooctane) was added as derivatization internal standard. Finally, 10 μL of hexadecane solution (80µg/g of isooctane) was added as injection internal standard.

Step D: GC/MS analysis of the neutral compounds

2 μL of the isooctane solution of neutral compounds was analysed by GC/MS. The analysis of the neutral fraction allows the neutral terpenoid compounds, sterols, alcohols and alkanes to be determined.

Step E: Extraction of the acidic fraction

The residue of the n-hexane extraction was acidified with hydrochloric acid (HCl, 6N) and then extracted three times with 200 μL of diethyl ether. The extract contained acidic organic molecules (acidic fraction).

Step F: Derivatisation of the acidic fraction

An aliquot of the acidic fraction was then subjected to the same procedure for derivatisation as described for the neutral fraction.

Step G: GC/MS analysis of the acidic compounds

2 μL of the isooctane solution of derivatised acidic compounds was analysed by GC/MS. The analysis of the acidic fraction allows for the determination of monocarboxylic acids, dicarboxylic acids, hydroxy acids and terpenoid acids.

2.4.2.2 Instrument and analytical conditions

The instrumentation consisted of a Trace GC gas chromatograph (ThermoFinnigan, USA) equipped with a programmed temperature vaporisation (PTV) injection port and a mass spectrometric detector with an ion trap analyser ThermoFinnigan Polaris Q. Chromatographic separation was performed using a chemically bonded fused silica capillary column HP-5MS (Hewlett Packard): stationary phase 5% phenyl – 95% methylpolysiloxane, 0.25 mm internal diameter, 0.25 μm film thickness, 30m length, connected to a 2m long deactivated fused silica capillary pre-column (internal diameter 0.32 mm).

The PTV injector was used in splitless mode at 300°C. The carrier gas was He (purity 99.9995%) at a constant flow of 1.2 mL/min. The chromatographic oven was programmed as follows: 80°C, isothermal for 2 min, 10°C/min up to 200°C, 200°C, isothermal for 4 min, 6°C/min up to 280°C, 280°C, isothermal for 40 min. The mass spectrometer was used in electro-ionisation mode at 70 eV. The temperatures of the ion source and the interface were fixed at 230°C and 280°C, respectively. The analysed mass interval selected was 50–650 a.m.u..

2.4.3 Pyrolysis–GC/MS

2.4.3.1 Sample preparation

A few micrograms of solid sample (identical to the sample used for FTIR and GC/MS analyses) were placed into a quartz tube, which was introduced into a spiral filament. 2 µL of HMDS was used as a derivative agent and was added before pyrolysis.

2.4.3.2 Instrument and analytical conditions

The Py-GC/MS analyses were carried out on a HP 6890N gas chromatograph (Agilent Technologies, USA) with a PTV injector and coupled to an HP 5973 quadrupole-mass spectrometer (Agilent Technologies, USA). On-line pyrolysis was performed with a CDS Pyroprobe 5000 Series (Analytical Inc., USA). The pyrolysis products were separated on a HP-5MS (Hewlett Packard) column with a stationary phase of dimethylpolysiloxane containing 5% phenyl, an internal diameter 0.25 mm, 0.25 µm film fused column and length 30 m. The column was preceded by a capillary pre-column in deactivated silica, internal diameter 0.32 mm and length 2 m.

The samples were pyrolysed with a temperature program from 50°C to 480°C at 20°C/ms. The temperature of the transfer line Py-GC and the PTV injector were 280°C and 300°C, respectively. The gas carrier was helium (He, 99,999%) with a constant flow of 1.3 mL/min. The chromatographic oven was programmed as follows: initial temperature 40°C, isothermal for 5 min, then 10°C/min up to 300°C followed by an isothermal stage at 300°C for 15 min. The mass spectrometer was used in an electro-ionisation mode at 70 eV. The temperatures of the ion source and the interface were fixed at 230°C and 280°C, respectively. The analysed mass interval selected was 50–600 a.m.u..

3. Results and discussions

FTIR analyses revealed that the dominant component of the white material used to plaster the bodies was gypsum. Figure 4 shows an example of FTIR spectra for the white archaeological materials (spectrum B) compared to a reference sample of gypsum (spectrum A). Both spectra are characterised by a broad band at 1140 cm^{-1} corresponding to v_3 S-O stretching and two narrow bands at 603 and 674 cm^{-1} corresponding to v_4 S-O bending. The broad band around 3400 cm^{-1} and the double band around 1650 cm^{-1} are attributable to O-H stretching and O-H bending of adsorbed water, respectively [5-7].

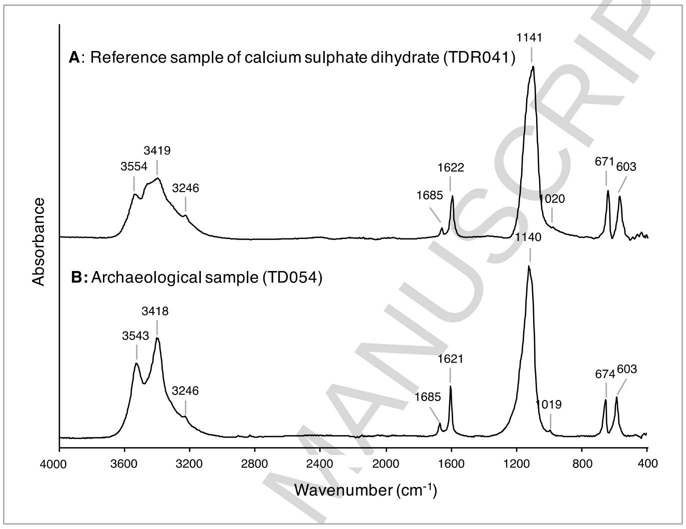

Fig. 4. Fourier transform infrared (FTIR) spectrum of the white material used to plaster the bodies compared to a reference of calcium sulphate dehydrate analysed in the same conditions

FTIR analyses also revealed that the inclusions in the plaster had a resinous nature (Fig. 5, Table 1.). More specifically, in the region between 1300 and 1100 cm^{-1}, bands caused by carbon-oxygen single bonds can be used as a fingerprint of a specific fossil resin [8-10]. The resin present as small inclusions in gypsum was identified as succinite, also known as Baltic amber, due to the presence of the characteristic ‘Baltic shoulder’, corresponding to the presence of a succinic acid ester, within the FTIR spectrum [11] (Fig. 6.). FTIR analyses also confirmed the presence of a second diterpenoid resin.

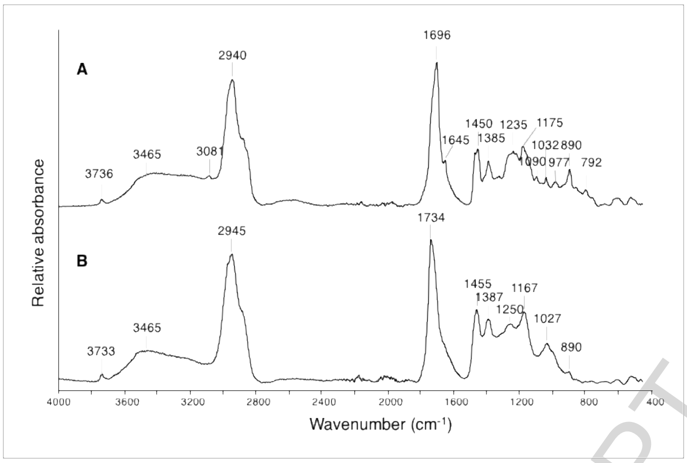

Fig. 5. Infrared spectra of the two types of resinous materials identified in the catacombs of Saints Perter and Marcellinus. Spectrum A: FTIR spectrum corresponding to the resin embedded in gypsum; Spectrum B: FTIR spectrum corresponding to the succinite resin present as small inclusions in gypsum. The assignment of bands in these spectra is reported in Table 1.

Table 1. Assignments of FTIR bands observed in spectra of resinous samples (*v*: stretching; δ: deformation; γ: out-of-plane bending)

Spectrum A	Spectrum B	Assignment of vibrational modes
3465	3465	*v*(O-H)
/	3081	*v*(=C-H)
2945	2940	*v*(C-H), CH_2, CH_3
1734	1696	*v*(C=O)
/	1645	*v*(C=C)
1455	1450	$\delta_{as}(CH_3)$, $\delta(CH_2)$
1387	1385	$\delta_s(CH_3)$
1250	1235	γ(OH) alcohol &*v*(C-O) ether

1167	1175	*v*(CO)
1027	1032	δ_s(C-H cyclohexane)
/	977	δ_s(C-H cyclohexane)
890	890	out of plane $\delta(CH_2)$ of the exomethylene functionality in C8-C20
/	792	$\delta(CH_2)$

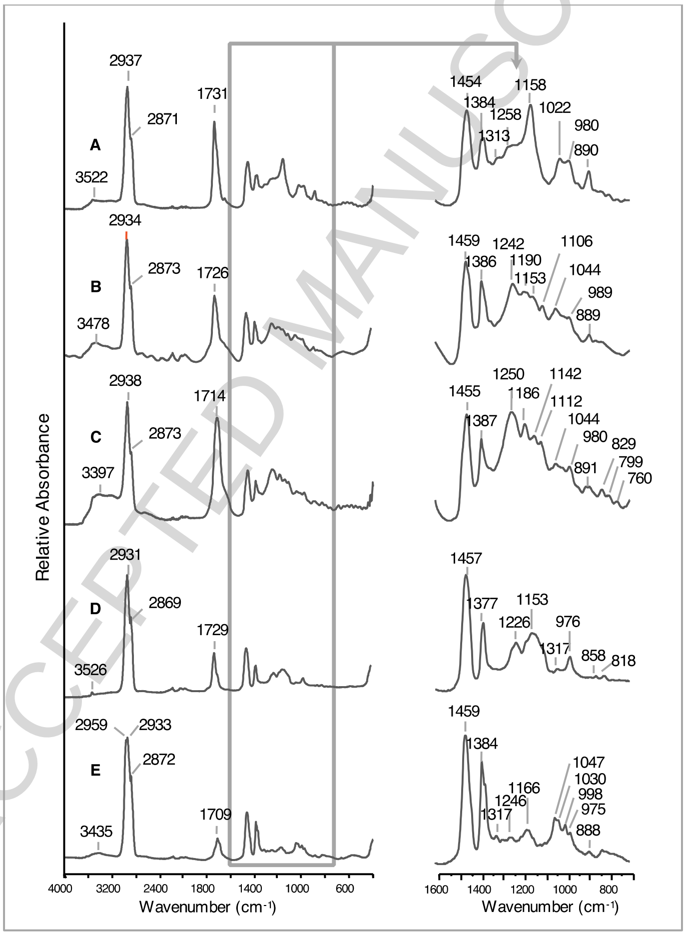

Fig. 6. FTIR spectra of reference fossil resins with certified geological origin including A: succinite, B: simetite, C: Apenine amber (Bologna), D: Apenine amber (Modena), E: Vicenza amber. The assignment of bands is reported in Table 1.

Subsequent investigations of the resins by Py-GC/MS (Fig. B1, Table B1) and GC/MS (Fig. 7, Table 2) were performed to deeply characterise each resin. The principal peak on the pyrograms at 21.5 min corresponds to the succinic acid, which is the main biomarker of succinite. Other small peaks correspond to pyrolytic markers of the polymeric fraction and to diterpenoid markers as identified in Table B1 [12, 13]. Pyrogram A obtained for the resin embedded in the gypsum is more complex and numerous diterpenoid compounds with pimarane skeleton such as sandaracopimaric and isopimaric acids were identified. They are characterised by a mass peak at *m/z* 374 and a base peak at *m/z* 121. These compounds can be considered as markers for the presence of sandarac and Manila copal [14-16]. Their presence is confirmed by the identification of other molecules such as communic, agathic and agatholic acids.

After solvent extraction, the acidic and neutral fractions were analysed by GC/MS (Figure 7, Table 2). Sugiol (g) in the acidic fraction and dehydroferruginol (j) and ferruginol (k) in the neutral fraction were identified. The fragmentation of these phenolic abietanes produces mass ions at *m/z* 247 and 261. These molecules are characteristic diterpenes of the *Cupressaceae* family [17-20]. Agatholic and agathic acid (h and i), already identified in Py-GC/MS analyses, were present in the acidic fraction. Sandaracopimaric acid (f), which is the most characteristic pimaradiene compound of sandarac resin, was also identified in the acidic fraction with a mass peak at *m/z* 374 [16, 19, 21, 22].

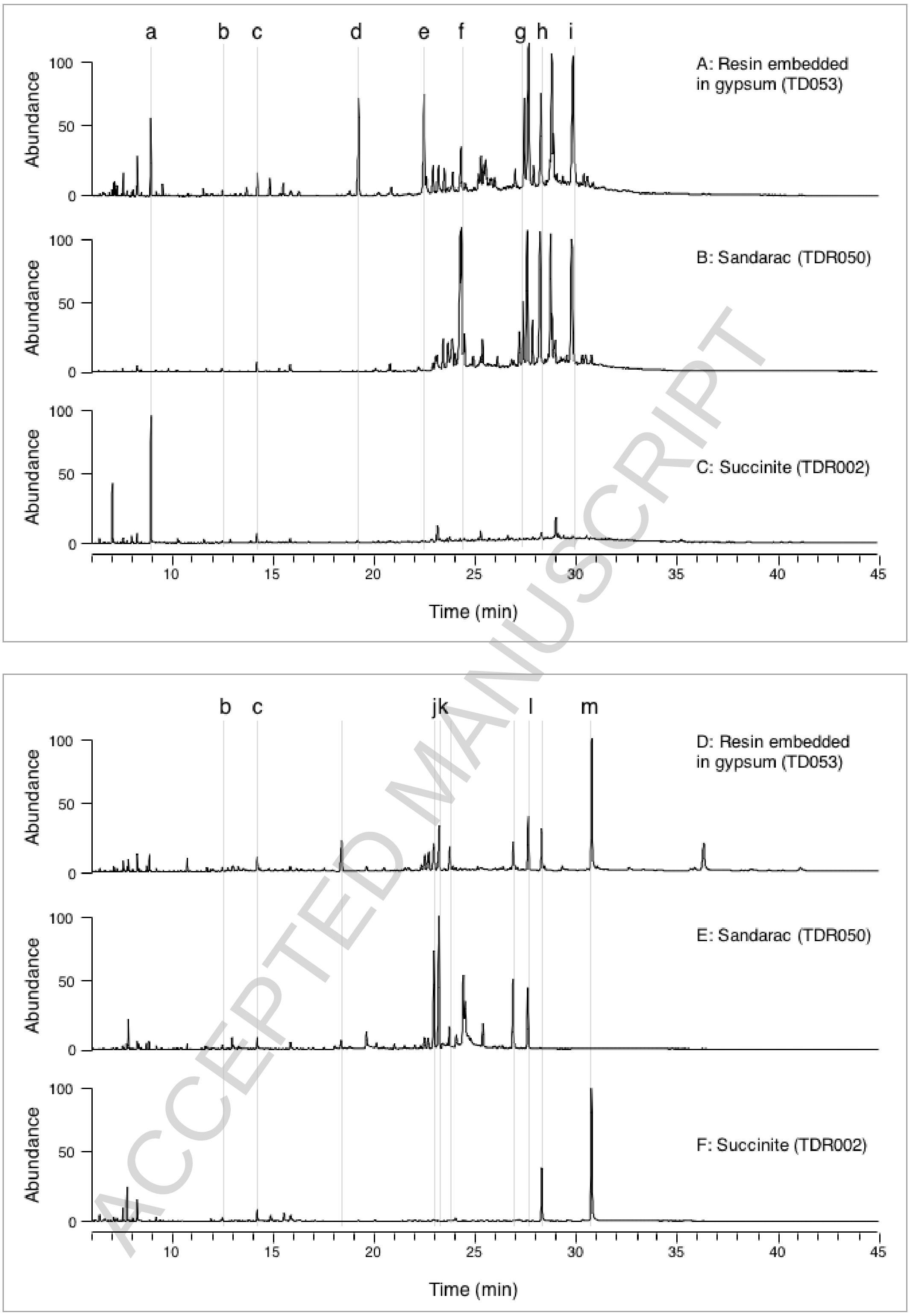

Fig. 7. Typical total ion current chromatograms of the acid (A-C) and neutral (D-F) fractions obtained by GC/MS for sample of the resin embedded in gypsum, a reference of sandarac and a reference of succinite. Identified diterpenes are listed in Table 2.

Table 2. Principal markers (TMS derivatives) identified by GC/MS in the resinous samples

Label	Compound	Class
a	succinic acid	Diacid
b	hexadecane [*Internal Standard*]	Alcane
c	tridecanoic acid [*Internal Standard*]	Fatty acid
d	hexadecanoic acid (*palmitic acid*)	Fatty acid
e	octadecenoic acid (*oleic acid*)	Fatty acid
f	isopimaric and sandaracopimaric acid	Diterpene
g	sugiol	Diterpene
h	agatholic acid	Diterpene
i	agathic acid	Diterpene
j	dehydroferruginol	Diterpene
k	ferruginol	Diterpene
l	monopalmitin	Monoglyceride
m	monostearin	Monoglyceride

These chromatographic analyses confirmed the presence of succinite (Baltic amber) thanks to the pyrolytic profile dominated by succinic acid [23]. In addition, both methods revealed the presence of sandarac, particularly with the confirmation of the presence of agathic and agatholic acids [16, 21].

FTIR analyses of the black substances produced profiles very similar to humic substances (Figure 8). The spectrum shows four major absorbance peaks around 3310, 1630, 1420 and 1070 cm^{-1}. According to the literature, the broad intense band at about 3300 cm^{-1} mainly represents the stretching vibrations of H-bonded hydroxyl (O-H) groups of phenols, water and amine (N–H) stretching. The shoulder at about 2960 cm^{-1} is attributed to aliphatic C-H stretching, indicating the presence of CH_3 and CH_2 groups. The broad band in the 1640-1600 cm^{-1} region was assigned primarily to the C=C vibration of alkenes and aromatic and to a smaller extent to C=O vibrations of amides and carboxylates. The band around 1419 cm^{-1} may have originated from aliphatic (C–H) deformation of CH_2 or CH_3 groups or to OH deformation and C-O stretching of phenolic OH groups. The strong band in the region 1080-1040 cm^{-1} could be attributed to C–O stretching vibrations of polysaccharides. All these vibration bands demonstrate the organic nature of the black material and the profile corresponds to humic substances [24-27].

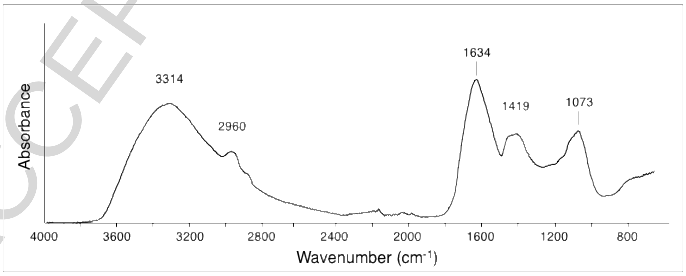

Fig. 8. FTIR spectrum obtained for the black samples recovered in the catacombs of Saints Peter and Marcellinus

Complementary analyses by Py-GC/MS (Fig. C1, Table C1) and GC/MS (Fig. 9, Table 3) were performed. The pyrogram is dominated by 5 classes of compounds common to all black samples analysed: sugars, aromatic and phenolic compounds, fatty acids and triterpenes. The polyphenols, sugars and aromatic acid can be correlated to the macromolecules present in humus. They are important precursors in the formation of humic substances. They follow an oxidative polymerisation catalysed both by enzymes and abiotic (inorganic) catalyst [28]. The fatty acids present in the samples are palmitic ($C_{16:0}$), stearic ($C_{18:0}$) and oleic ($C_{18:1}$) acids. These fatty acids are common to a large range of materials such as fats and plant materials and are not diagnostic of specific substances [29, 30]. The pyrogram also presents intense peaks attributable to 24-norleana-3,12-diene and 24-norursa-3,12-diene. These compounds can be correlated to the presence of frankincense. Indeed, the specific oleane and ursane skeletons of these pyrolysis products indicate a relationship with the characteristic markers of frankincense: α-boswellic acid, β-boswellic acid and their acetates [31]. GC/MS analyses confirmed the presence of frankincense resin with the 6 triterpenes identified (l, m, n, o, p and q) [31-37]. Four aromatic compounds were also identified (a, c, e, f). Three of these are cinnamic acid derivatives, which are important building blocks in lignins [38]. Analyses also revealed the presence of palmitic (g), oleic (h) and stearic (i) acids and two monoglycerides, namely monopalmitin (j) and monostearin (k). As already mentioned, these compounds are quite common in archaeological samples and consequently they are not diagnostic of a particular material.

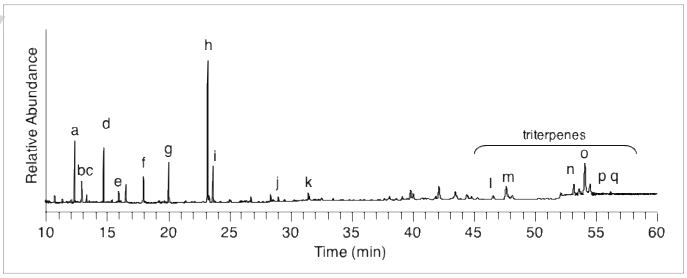

Fig. 9. Example of chromatographic profile for the acidic fraction of black samples.

Table 3. Principal biomarkers (TMS derivatives) identified by GC/MS in black samples

Label	**Compound**	**Class**
a	cinnamic acid	Aromatic
b	hexadecane [*Internal Standard*]	Alcane
c	hydroxybenzoic acid	Aromatic
d	tridecanoic acid [*Internal Standard*]	Fatty acid
e	p-methoxycinnamic acid	Aromatic
f	p-hydroxycinnamic acid	Aromatic
g	hexadecanoic acid (or palmitic acid)	Fatty acid
h	octadecenoic acid (or oleic acid)	Fatty acid
i	octadecanoic acid (or stearic acid)	Fatty acid
j	monopalmitin	Monoglyceride
k	monostearin	Monoglyceride
l	α-boswellic acid	Triterpene
m	β-boswellic acid	Triterpene
n	3-*O*-acetatyl-α-boswellic acid	Triterpene

o	3-*O*-acetatyl-β-boswellic acid	Triterpene
p	11-keto-α-boswellic acid	Triterpene
q	11-keto-β-boswellic acid	Triterpene

From these results, two hypotheses were investigated. First, these black substances could be a mixture of frankincense resin (used in the embalming treatment) and materials arising from the degradation of the bodies. The second hypothesis was that the bark from the *Boswellia* tree had been used and degraded producing black humic substances. These black materials were very homogeneous with the presence of the biomarkers of the frankincense resin consistently detected. For the degradation of a large number of bodies for which frankincense resin had been applied on the skin, some compositional variation, such as in the frankincense concentration and in the products of decomposition, would be expected. Moreover, the black material consistently contained coumaryl and furfural structures that could be linked to lignin and carbohydrates, respectively. These are not present in the frankincense resin but are characteristic of wood materials [39]. The combination of analytical results and archaeological observations thus confirms the use of the bark from *Boswellia* trees.

Conclusion

The chemical characterization of the amorphous materials from the catacombs of Saints Peter and Marcellinus provided unexpected and novel information about the funeral treatments used during this period. The bodies were packed with gypsum and in three of the rooms investigated, succinite and sandarac were mixed with the mineral. By comparison, in one of the rooms investigated, the succinite was not mixed with gypsum. This observation demonstrates different treatments for the preparation of the bodies, which may be related to different social levels or different phases in the epidemic crisis. At that time, amber was a very precious and expensive material. Pliny the Elder wrote in his Natural History that “the price of a figurine in amber, however small, exceeded that of a healthy slave” [40]. Generally, amber objects found by archaeologists come from the graves of Roman women. They consist in toilet articles, pendants, knife and mirror handles, figurines and small containers and vases [41-43]. Many beliefs were also associated with amber, such as the protective effect against evil, and may explain its use in the catacombs [44]. The identification of kilograms of succinite powder in the catacombs of Saints Peter and Marcellinus is an important discovery for the study of funeral practices. This is the first time that this fossil resin, from the Baltic region, has been identified as an ingredient for embalming treatment and its use has not been previously reported. Its presence demonstrates the exceptional character of the funeral treatment performed in these early dated catacombs. The discovery of sandarac mixed with amber and gypsum is another important result of this research. Sandarac is an imported resin obtained from conifers of the family *Cupressaceae*. African sandarac comes from *Tetraclinis articulate*, which is a genus occurring primarily in the mountains of Algeria and Morocco. African sandarac resin was used by Mediterranean peoples from ancient times until the Middle Ages for medicinal purposes [42]. Later, sandarac was used as a painting varnish as reported in painting treatises until the 19th century [16]. It was also employed as an additional ingredient for enhancing the hardness of coating film [16]. Sandarac was possibly added to gypsum for this property, while the function of the powder of amber was undoubtedly more symbolic.

The analyses carried out on the brown-black substances revealed the presence of another unexpected precious material: the bark from the *Boswellia* tree. In ancient times, frankincense was harvested by making incisions in the bark of the trunk or branches of the *Boswellia* tree. Generally, the resin exudes and is collected when it hardens sufficiently. The bark of the *Boswellia* tree is a product that was also traditionally harvested. Instead of making incisions, portions of the bark may simply be scraped away with a special tool [42]. As for the frankincense resin, the bark of *Boswellia* trees was undoubtedly imported and no information has been previously reported about the use of this material in a funeral context. All these organic materials identified in the catacombs were imported from different locations such as the Baltic region, Yemen and North Africa (Fig.10.). This emphasizes the exceptional nature of the funeral treatments employed in these catacombs. Not only have these materials not been previously observed in burials, their sources are diverse. This study also demonstrates scientifically, for the first time, the packing practices in Rome as described by authors in the classic literature. Green, in his article about Christian cemeteries in Rome mentions that in the catacombs "embalming was employed on occasions; more frequently the dead were swathed in a shroud surrounded by lime or plaster" [45]. On the contrary, nothing is written in the classical literature about the presence of precious resins such as sandarac or succinite mixed with the plaster and the use of bark of resinous trees has never been reported for funeral treatments.

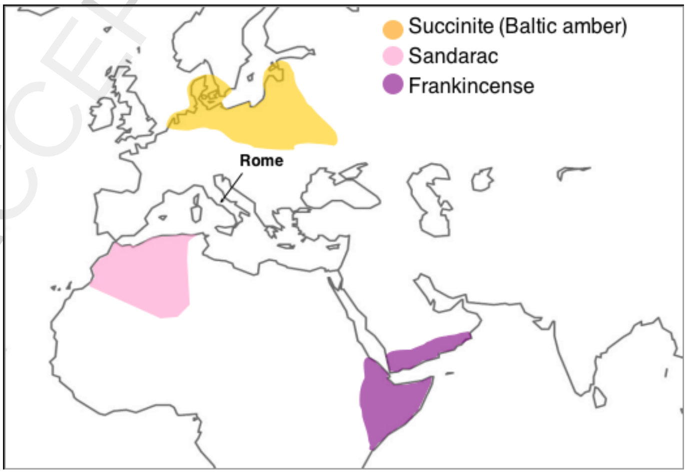

Fig. 10. Map showing the provenance of resinous substances recovered in the catacombs of Saint Peter and Marcellinus (succinite deposit around the Baltic region and the regions in

which resin yielding trees for sandarac and frankincense are grown). - *Color should be used for this figure (online only).*

This research demonstrates that important information is trapped in the amorphous materials found in the catacombs, and more generally in archaeological burials. Their nature can be revealed by chemical characterization. For the catacombs investigated for this research, these results revealed new and highly significant information about the rich embalming procedure used to prepare this population, who died as a result of one of the largest identified epidemic crisis thus far.

Other researchers started to apply the method developed by the author[46]on Roman burials from UK [47, 48]. It is expected that applying a similar approach to other archaeological sites will allow us to learn more about funeral treatments, particularly for this important period when cremation was abandoned and replaced by more elaborated practices and learn more about commercial exchanges for all these substances.

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Appendices

Appendix A

TableA1.

List of all reference materials used for comparison. Samples of gypsum, fossil resins, sandarac and frankincense were collected and where possible the provenance was recorded.

Material	Provenance	Supplier	Lab reference
Calcium sulphate dihydrate	/	Fisher (Rodano, Italy)	TDR041
FOSSIL RESINS			
Succinite	Baltic	Unipi (Pisa, Italy)	TDR001
Succinite	Baltic	C2RMF (Paris, France)	TDR002
Succinite	Baltic	C2RMF (Paris, France)	TDR003
Succinite	Baltic	Le pietre delle meraviglie (Milano, Italy)	TDR009
Succinite	Baltic	Unife (Ferrara, Italy)	TDR013

Succinite	Baltic	Unife (Ferrara, Italy)	TDR014
Succinite	Danzic, Poland	Museo Civico (Vicenza, Italy)	TDR032
Succinite	Kongsberg, Russia	Museo Civico (Vicenza, Italy)	TDR033
Succinite	Baltic	Getty (Los Angeles - USA)	TDR037
Succinite	Germany	Unipd (Padova, Italy)	TDR044
Succinite	Russia	Unipd (Padova, Italy)	TDR045
Succinite	Danzic, Poland	Unipd (Padova, Italy)	TDR046
Succinite	Bitterfeld, Germany	Unipd (Padova, Italy)	TDR047
Succinite	Bitterfeld, Germany	Polska Akademia Nauk Museum Zimi (Varsavia, Poland)	TDR051
Succinite	Bitterfeld, Germany	Polska Akademia Nauk Museum Zimi (Varsavia, Poland)	TDR052
Succinite	Klesow, Ukraine	Polska Akademia Nauk Museum Zimi (Varsavia, Poland)	TDR053

Succinite	Klesow, Ukraine	Polska Akademia Nauk Museum Zimi (Varsavia, Poland)	TDR054
Succinite	Jantarnyj, Russia	Polska Akademia Nauk Museum Zimi (Varsavia, Poland)	TDR055
Zigburgite	Bitterfeld, Germany	Polska Akademia Nauk Museum Zimi (Varsavia, Poland)	TDR056
Glessite	Hoyerwerda, Germany	Polska Akademia Nauk Museum Zimi (Varsavia, Poland)	TDR057
Gedanite	Bitterfeld, Germany	Polska Akademia Nauk Museum Zimi (Varsavia, Poland)	TDR058
Gedanite	Mikoszewo, Poland	Polska Akademia Nauk Museum Zimi (Varsavia, Poland)	TDR059
Goitschite	Bitterfeld, Germany	Polska Akademia Nauk Museum Zimi (Varsavia, Poland)	TDR060
Gedano-Succinite	Pomerania, Poland	Polska Akademia Nauk Museum Zimi (Varsavia, Poland)	TDR061
Apenine	Scanello (Bologna, Italy)	Museo di Storia Naturale e del	TDR00

Amber		Territorio di Calci (Pisa, Italy)	8
Apenine Amber	Monterenzio (Bologna, Italy)	Museo Civico (Vicenza, Italy)	TDR028
Apenine Amber	Monterenzio (Bologna, Italy)	Museo Civico (Vicenza, Italy)	TDR029
Apenine Amber	Monterenzio (Bologna, Italy)	Museo Civico (Vicenza, Italy)	TDR030
Apenine Amber	Loiano (Bologna, Italy)	Unimore (Modena, Italy)	TDR031
Apenine Amber	Castelvecchio Prignano (Modena, Italy)	Museo Civico (Vicenza, Italy)	TDR023
Apenine Amber	Castelvecchio Prignano (Modena, Italy)	Museo Civico (Vicenza, Italy)	TDR024
Apenine Amber	Castelvecchio Prignano (Modena, Italy)	Museo Civico (Vicenza, Italy)	TDR025
Apenine Amber	Castelvecchio Prignano (Modena, Italy)	Museo Civico (Vicenza, Italy)	TDR026
Vicenza Amber	Calcara (Vicenza, Italy)	Museo Civico (Vicenza, Italy)	TDR019
Vicenza Amber	Calcara (Vicenza, Italy)	Museo Civico (Vicenza, Italy)	TDR020
Vicenza Amber	Calcara (Vicenza, Italy)	Museo Civico (Vicenza, Italy)	TDR021
Vicenza Amber	Calcara (Vicenza, Italy)	Museo Civico (Vicenza, Italy)	TDR022

Simetite	Sicily, Italy	Museo Civico (Vicenza, Italy)	TDR016
Simetite	Sicily, Italy	Museo Civico (Vicenza, Italy)	TDR017
SANDARAC			
Sandarac	Callitris Quadrivalvis, Maroc	Orto Botanico (Palermo, Italy)	TDR049
Sandarac	Callitris Quadrivalvis, Maroc	Zecchi (Firenze, Italy)	TDR050
Sandarac	MCallitris Quadrivalvis, Maroc	eBay	TDR075
FRANKINCENSE			
Frankincense	unknown	C2RMF (Paris, France)	TDR069
Frankincense	unknown	C2RMF (Paris, France)	TDR070
Frankincense	unknown	C2RMF (Paris, France)	TDR071
Boswellia bark	Africa	eBay	TDR074
Boswellia bark	Oman, Yemen	Unipi (Pisa, Italy)	TDR076

Appendix B

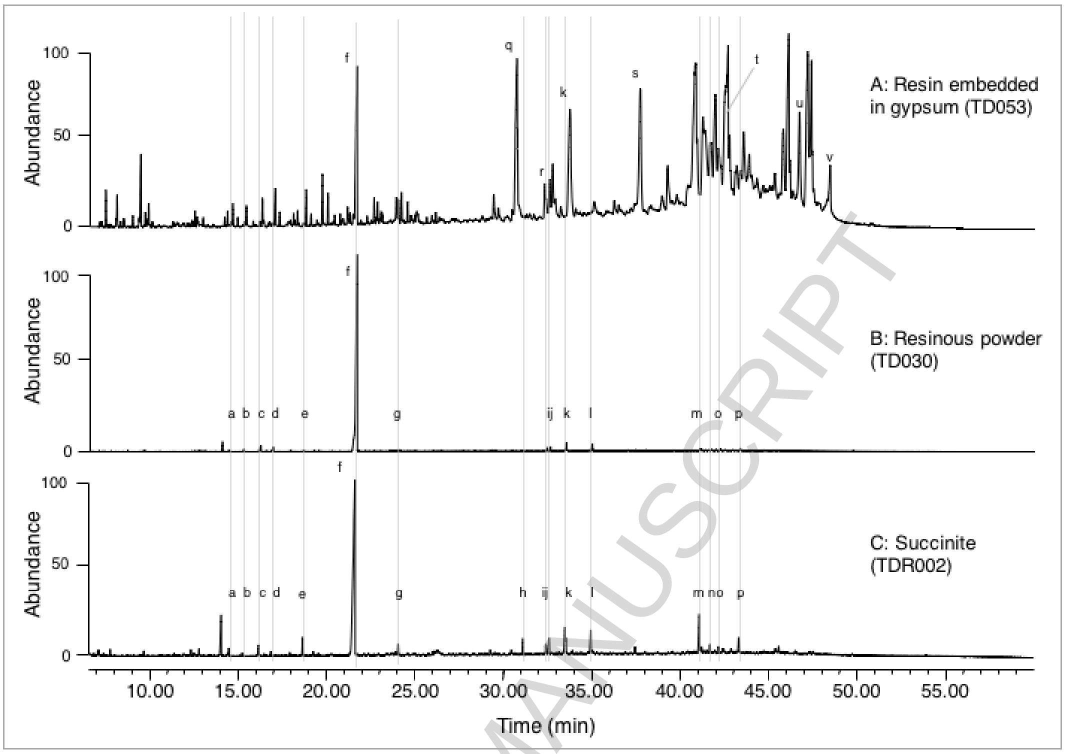

Fig. B1.

Examples of pyrograms for both resinous materials (Traces A and B) identified by FTIR and a reference sample of succinite (Trace C). The principal molecules identified are listed in Table B1.

TableB1.

Principal markers identified in the pyrograms of resinous materials

Label	Compound
a	acetic acid TMS derivative
b	fenchol
c	camphor
d	borneol
e	borneol TMS derivative
f	succinic acid TMS ester
g	(4a*S*)-1,4a,6-trimethyl-5-methylene-1,2,3,4,4a,5,8,8a octahydronaphthalene (1*R*,4a*S*)-1,4a,5,6-tetramethyl-1,2,3,4,4a,5,8,8a octahydronaphthalene
h	1,4a,6-trimethyl-1,2,3,4,4a,7,8,8aoctahydro naphthalene-1-carboxylic acid (TMS) 1,4a,6-trimethyl-1,2,3,4,4a,5,8,8aoctahydro naphthalene-1-carboxylic acid (TMS)
i	(1,4a,5,6-tetramethyl-1,2,3,4,4a,7,8,8a octahydronaphthalen-1-yl)methanol (TMS)
j	(1,4a,6-trimethyl-5-methylene-1,2,3,4,4a,5,8,8a octahydronaphthalen-1-

	yl)methanol (TMS)
k	1,4a,5,6-tetramethyl-1,2,3,4,4a,7,8,8a octahydronaphthalene-1-carboxylic acid (TMS) 1,4a,6-trimethyl-5-methylene-1,2,3,4,4a,5,8,8a octahydronaphthalene-1-carboxylic acid (TMS)
l	structure undefined
m	levopimaric acid TMS ester
n	18-levopimaric acid TMS ester
o	pimaric acid TMS ester
p	dehydroabietic acid TMS ester
q	naphtalene 1,2,3,4,4a,7,8,8a-octahydro-1(・),4a(β), 6 trimethyl,1(β)-carboxylic acid TMS derivative
r	naphtalene 1,2,3,4,4a,7,8,8a-octahydro-1(・),4a(β),5,6 tetramethyl,1(β)-carboxylic acid TMS derivative
s	hexadecanoic acid TMS derivative
t	communic acid TMS derivative
u	agatholic acid TMS derivative
v	agathic acid TMS derivative

Appendix C

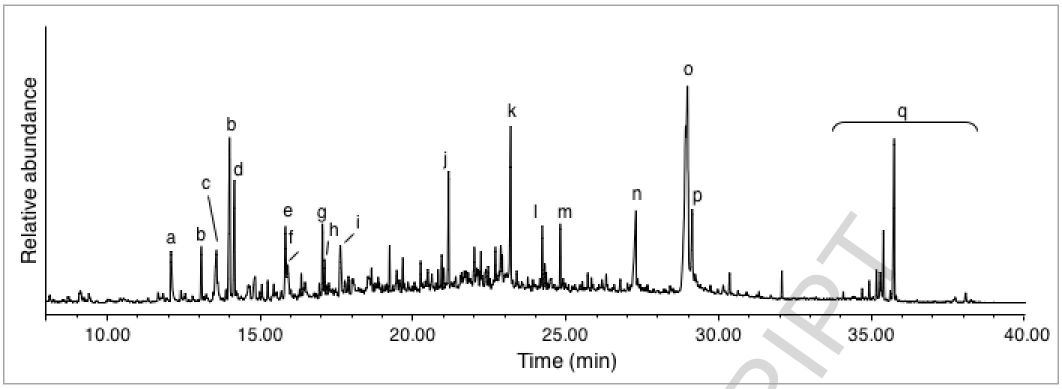

Fig. C1.

Typical pyrogram obtained for the black materials (Sample TD042). The principal molecules identified are listed in Table C1.

Table C1.

Principal biomarkers identified by Py-GC/MS in black samples

Label	Compound	Class
a	5 methyl furfural	Sugar
b	trimethylbenzene	Aromatic
c	phenol	Phenolic
d	1 methyl-4-(1-methyl ethyl) benzene (or Cymene)	Aromatic
e	1 methyl-4-(1-methyl ethenyl) benzene (or Cymenyl)	Aromatic
f	methyl phenol (or p-Cresol)	Phenolic
g	tetramethylbenzene	Aromatic
h	1-ethenyl-4-methoxy benzene	Aromatic
i	ethylphenol	Phenolic
j	dimethylnaphtalene	Aromatic
k	1,6,7-trimethylnaphtalene	Aromatic
l	1,6-dimethyl-4-(1 methylethyl)naphthalene	Aromatic
m	7 ethyl-1,4-dimethyl azulene	Aromatic
n	hexadecanoic acid (palmitic acid)	Fatty acid
o	octadecenoic acid (oleic acid)	Fatty acid
p	octadecanoic acid (stearic acid)	Fatty acid
q	triterpenes of frankincense	Triterpene

Highlights

- Amorphous materials used to embalm the bodies were characterized by a multi-analytical approach.
- Provenance of the amber was identified by both spectroscopy (FTIR) and mass spectrometry (Py-GC/MS and GC/MS).
- Diterpenoid compounds with pimarane skeleton such as sandaracopimaric and isopimaric acids confirmed the presence of sandarac.
- Frankincense bark was identified for the first time in archaeological context.