

# A natural resins reference collection to identify organic compounds in archaeological samples

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**Abstract** – Natural resins have been employed since the prehistory as an adhesive in the manufacturing processes and operative chains of several tool. For this study several type of natural resins in different preservation condition were collected or acquired to be analysed by GC-MS to create a specific reference data base to identify organic compounds in archaeological materials. The analytical approach employed to build the data base is defined and the quality assurance and quality control results are described. The results obtained show the reliability and strength of the developed method.

## I. INTRODUCTION

Due to their different properties, organic materials like resins have been employed since the prehistory, as antibacterial or to fix arrowheads made of rocks like chert. During the prehistory, it was easy to obtain resins in the environment, and these were often processed, heated or mixed, with other raw materials and used for example as adhesive or to create a small film to protect other artefacts[1]. Sometimes organic compounds incrustations are found in the chert objects surfaces, so ones extracted they can be identified by mass gas chromatography (GC-MS). This technique is commonly used for the analysis of volatile organic compounds like natural resins, as it allows the characterization of the different organic molecules with small amounts of sample. It is currently considered one of the most robust methods for the characterization of natural resins [2] being able to characterize several compounds [3]. One of the most important issues is that there are few data about the differences between natural fresh resins and degraded ones. The composition of natural resins is quite complex, and it is not entirely known yet due to the variety of the plant species; it may also depend on the climate and on the characteristics of the soil [4] and can change through time easily due to oxidation or polymerization processes.

Furthermore, there are many different resins as they can

vary a lot in the compounds found due to the type of tree, the environment and the age. Useful information about the resins employed in antiquity has been previously reported [5], reducing the number of natural resins to be identified. In this work, a set of natural resins and bee wax (often used in the past as adhesives or as elements of oil-resin media) were employed as reference materials to characterize unknown substance. Due to the complexity of the samples, they were treated with two different extractions to separate the neutral compounds from the acid fraction [2]. In the acidic fraction fatty acids and acidic terpenoids are recovered, thus achieving the identification of lipids and other particular resin components; in the neutral fraction hydrocarbons, sterols and neutral terpenoids are determined, finally the presence of both resins and waxes is evaluated [3].

## II. OBJECTIVES

The objectives of our paper are to present the results of the organic compounds found in a set of natural resins and create a data base to compare these with future archaeological samples that are made for example of adhesives or pitches.

## III. MATERIALS AND METHODS

### A. Reference samples

Some natural resins and waxes were purchased while others were obtained through natural extraction. Their provenance is summarized in Table 1.

### B. Gas chromatography-Mass Spectrometry (GC-MS)

Gas chromatography-mass spectrometry analyses were carried out by a gas chromatograph, model 7890 A (Agilent Technologies), incorporating a low polarity HP-5 (5%-phenyl)-methylpolysiloxane capillary column (30 m x 0.25 mm x 0.25  $\mu$ m) for the efficient separation of compounds. Detection was carried out using a simple quadrupole mass spectrometer (5975C inert XL EI/CI MSD, also from Agilent Technologies), using an electron impact ionization source at 70 eV. The autosampler cou-

Table 1. Description of the different samples with the brand and number

Sample	Description
RN1K/7	Amber Rosin. Brown and brittle resin.
RN1K/4	Sandaraca Juniper Resin. Fragments of a yellowish-white color.
RN1K/16	Burgundy resin. Large orange fragment.
RN1K/9	Benzoin gum. In pieces. Small light orange fragments with some small dark-colored pieces.
RN1K/1	Dammar resin. Fragments of a yellowish-white color.
RN1K/5	Copal-Manila resin. Mixture of yellowish fragments and dark pieces.
CR	Natural coniferous resin fresh
AR	Natural prunus persica resin.
WAX	Bee Wax

pled to the system was a 7683 model. The MSD Enhanced data analysis software was used for data acquisition.

The analyte extraction for chromatographic analysis was performed by weighing about 5 mg of powdered sample into a test tube, to which 1 mL of solvent (MeOH/H<sub>2</sub>O, 1:1) was added, followed by sonication for 10 min. The sample was heated at 70 °C for 2 hours. The first fraction to analyse (neutral fraction) was separated by liquid-liquid extraction with hexane, using 1 mL for each sample in triplicate. The hydroalcoholic solution was stored. The hexane solution was evaporated to 0.5 mL under N<sub>2</sub> flow. The basic hydroalcoholic solution was acidified and the acidic organic compounds, including terpenoid acids and fatty acids, were extracted three times with 1 mL of diethyl ether. After solvent evaporation, it was derivatized with BSTFA + TMS 2%. The sample was dissolved in 1 mL hexane. Hexadecane was employed as an internal standard for this method.

The chromatograph oven was programmed as follows: 50 °C maintained for 2 min, 50-220 °C at 8 °C/min, 220 - 260 °C at 2 °C/min, and 260-300 °C at 10 °C/min maintained for 5 min.

#### IV. RESULTS AND DISCUSSION

The results of some natural resins and their most important peaks are shown in Table 2. In Figure 1, to compare the extraction method, it can be seen the chromatograms of the neutral and acid fraction from the pine resins samples. The organic compounds found correspond to terpenes and some fatty acids transformed in methyl esters due the reaction of transesterification, but the signals obtained are lower than the fatty acids obtained derivatized with BSTFA-TMS in the acidic fraction. This behaviour can be mostly observed in the natural resins.

During the extraction of fatty acids of waxes and oils, most of their fatty acids were extracted in the neutral fraction. The conditions of the sample also affects their compounds, as the fresh sample (B2) had higher ratio of

fatty acids and it also had a higher ratio of abietic acid than other abietic derivatives compared to the other two samples which were aged or burnt. Abietic acid is the most important compound in pine resins. It is a cyclic diterpene produced especially in conifers. The treatment of these plants gives rise to a modification of the composition (i.e through combustion and environmental factors) and, therefore, the analysis of this gives us information about the treatment of their corresponding resins [6,7]. Some of the compounds identified, despite being neutral, or methylated derivatives, have been detected in the acid fraction (such as methyl dehydroabietate). On the other hand, fatty acids have also been identified. These last fatty are lipids present in oils, in this case belonging to coniferous species. These are molecules insoluble in water and very stable at high temperatures. The characterization of these fatty acids can be classified and form taxonomic groups [8]. Specifically, we have identified derivatives of palmitic acid (C16:0) and stearic acid (C18:0), which are quite common in plant and animal oils, and other acids that could be used as biomarkers like abietic acid for coniferous resins or sandaracopimaric acid for sandaraca resins. Some of the other analysed resins include the dammar resin from the dipterocarpaceae family. This resin is composed mainly of polysesquiterpenes whose molecules follow the cadinane skeleton. Some of these sesquiterpenes have been found in the acid part, but most of them are found in the neutral part. Generally, oils containing D-Germacrene are accompanied by sesquiterpenes such as cadinane and muurolane, so D-Germacrene can be the precursor of the other terpenes through an acid-catalyzed cyclization reaction [9].

As a resin that also comes from the coniferous family, like pine one, we have identified some of the compounds also in the dammar resins, so a material made of this material could be confused with a pine resins. For this reason, both the acid fraction and neutral fraction are extremely important. Acid fraction can be helpful to distinguish a sample from different kinds of tree families, but between resins of same tree species there could be similarities which could misclassify the sample. Different tree species

Table 2. List of compounds identified in the neutral and acid fraction of the analyzed samples. The compounds found in the samples are marked with an X.

Compound	CR	RN1K/5	RN1K/1	RN1K/9	RN1K/4	RN1K/16
Hexadecane (PR)	X	X	X	X	X	X
Palmitic Acid, TMS(1)	X	-	X	-	-	-
Stearic Acid, TMS(2)	X	-	X	-	-	-
Pimaric Acid, TMS (3)	X	X	X	-	-	X
Palustric Acid, TMS (4)	-	-	-	X	-	X
Methyl hydroabietate (5)	X	-	-	-	-	-
Dehydroabietic acid, TMS (6)	X	X	X	-	-	X
Abietic Acid, TMS (7)	X	-	X	-	-	X
Neoabietic Acid, TMS (8)	X	-	X	-	-	X
Isopimaric Acid, TMS (9)	X	-	X	-	X	X
Agathalic Acid, TMS (10)	-	X	-	-	-	-
Methyl agathic acid, TMS (11)	-	X	-	-	-	-
Acetoxy agathic acid, TMS (12)	-	X	-	-	-	-
Cinnamic Acid, Methyl ester (13)	-	-	-	X	-	-
Sandaracopimaric Acid, TMS (14)	-	-	-	-	X	-
Dehydroagathic Acid, TMS(15)	-	-	-	-	X	-
Dihydroagathic Acid, TMS (16)	-	-	-	-	X	-
Alpha-pinene (17)	X	X	-	-	X	-
Camphene (18)	X	X	-	-	-	-
Beta-pinene (19)	X	-	-	-	X	-
Ortho-cymene (20)	X	X	-	-	-	-
D-Limonene (21)	X	x	-	-	-	-
Camphor (22)	X	-	-	-	-	-
Borneol (23)	X	-	-	-	-	-
Alpha-terpineol (24)	X	-	-	-	-	-
Palmitic Acid, Methyl ester (25)	X	-	X	-	-	X
Stearic Acid, Methyl ester (26)	X	-	X	-	-	X
Methyl abietate (27)	X	-	X	-	-	-
Retene (28)	X	-	-	-	-	-
Copaene	-	X	-	-	-	-
Alpha-cubebene	-	-	X	-	-	-
Alpha-murolene	-	-	X	-	-	-
Alpha caryphyllene	-	-	X	-	-	-
D-germacrene	-	-	X	-	-	-
Alpha-selinene	-	-	X	-	-	-
Delta-cadinene	-	-	X	-	-	-

can be differentiated easier like the case of benzoim gum, a balsamic resin obtained mainly from Asian trees by making deep incisions in the bark. It stands out for having a characteristic smell of vanilla. Most studies characterize this resin with monolignol-type aromatic compounds (lignin monomers) from cinnamic acid, which in turn comes from a natural amino acid (phenylalanine) and with benzoate derivatives. The cinnamic acid peak shown in Figure 2 is the most important found peak, helping to differentiate a sample made of this material compared to other resins [10].

Finally, the analysis of the beeswax, both in the acid fraction and in the neutral fraction show the same peaks, this is why only the chromatogram of the acid fraction is of interest showing linear hydrocarbons and some relevant derivatized acid. Although not being a resin of vegetable origin, objects made by natural wax are of great interest in archaeology. Wax is a natural product whose composition is based on hydrocarbons, fatty acids, alcohols and esters. As is shown, most of the found peaks are linear hydrocarbons with a higher ratio of the compounds with a number of odd carbons [11].

## V. CONCLUSIONS

The use of the proposed procedure allows the characterization of terpenoid resins, oils, lipids and waxes present in incrustation especially in archaeological objects. The method provides satisfactory recoveries for the mentioned compounds with the identification of both neutral and acidic components of the natural materials studied. Dammar and mastic resins undergo significant oxidation and polymerization due to natural aging with the production of oxidized compounds containing tetrahydrofuranic and lactone groups. These transformations drastically decrease the amount of compounds available for gas chromatographic analysis and make the particular differences in composition existing between fresh dammar and mastic resins less evident. The characterization of the natural resins and waxes has been satisfactory in all the cases since the most intense peaks have been identified in the chromatograms, which provide a complete information about each of the resins. In pine resins, many compounds derived from abietic acid, and its oxidation-reduction cycle have been identified, as well as numerous terpenes. Dammar is principally composed of sesquiterpenes and triterpenes that have been identified in the two fractions analyzed, so the neutral fraction is necessary to differentiate from other coniferous tree resins, like pine resins or sandarac, which is composed mainly of diterpenes

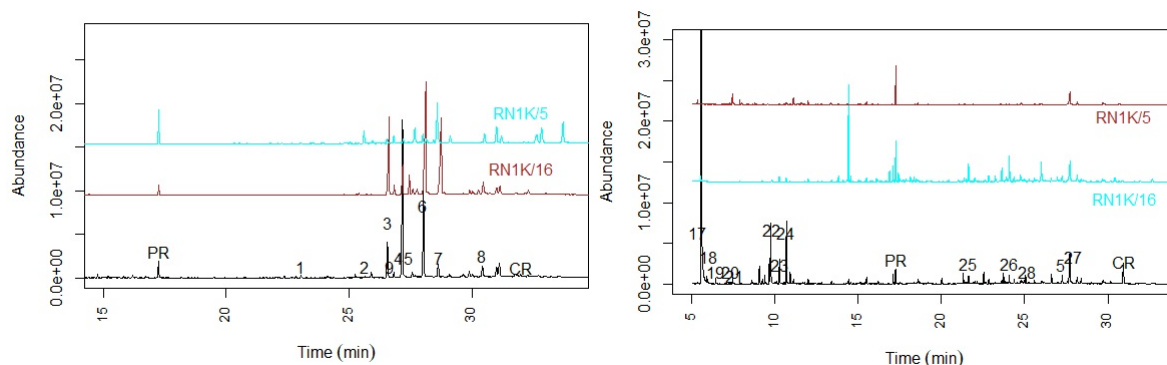


Fig. 1. Figure Chromatograms of the acidic fraction (A) (left) and the neutral fraction (N) of the samples (right) of the resin samples CR, RN1K/16, RN1K/5.

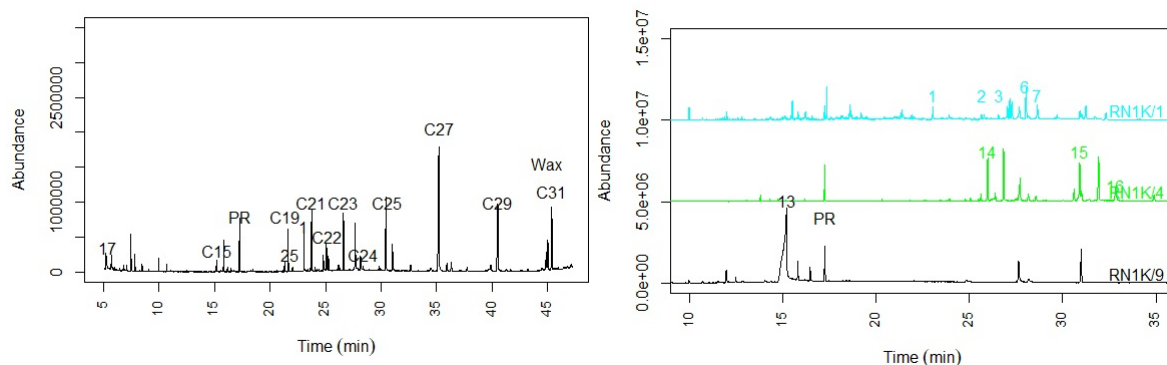


Fig. 2. Figure Chromatograms of some of the samples. Left: the acid fraction of the beewax sample Right: acid fraction of RN1K/1, RN1K/4 and RN1K/9

whose structures derives from labdane, in addition to some monoterpenes. In benzoin gum, aromatic compounds derived from cinnamic acid and benzoic acid which can only be identified in the acid fraction. Finally, beeswax is composed mainly of linear hydrocarbons with odd carbons and fatty acids.

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## VII. CITATIONS AND REFERENCES

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