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Chemical Characterization of Embalming Materials of Four Ibis Mummies from the Musée des Confluences, Lyon

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Abstract

The aim of this research work is to determine the chemical composition of the organic matter present in balms and impregnated textiles used for mummification of animals as well as to obtain information about the natural substances used and their alteration and/or degradation processes. Due to the sophisticated and heterogeneous nature of organic materials used in ancient Egyptian mummification a dual analytical approach, consisting of spectroscopic (FT-IR) and chromatographic (GC-MS) techniques, was applied. Four balms from votive ibis mummies belonging to the Egyptology collection of the Musée des Confluences in Lyon (France) were studied. Several substances have been characterized such as fatty matter like castor oil, beeswax and wood tar of a diterpenic resin belonging to the Pinaceae family. The obtained results were compared to those described in the literature.

Keywords: Alteration and ageing; Ancient Egypt; Animal Mummies; Infrared spectroscopy; Gas Chromatography coupled to Mass Spectrometry.

1. Introduction

While anthropogenic body preservation is a phenomenon that could be seen in past communities ranging from today's northern Chile to modern Japan (Arriaza et al., 1998; Sakurai et al., 1998), the deliberate mummification of animals appears to be a ritual practice exclusively limited to societies in Ancient Egypt. Thanks to the large amount of data gathered from numerous archaeological excavations it was discovered that Ancient Egyptians mummified millions of animals from the 16th Century BC (New Kingdom) until the 4th Century AD (Roman period) (Abdel-Maksoud and El-Amin, 2011; Bard, 2007; Porcier et al., 2019; Richardin et al., 2017), of which many of them have been classified as "votive mummies" offered to gods and goddesses (Ikram, 2017). However, ancient writers such as Herodotus, Strabo and Pliny the Elder, indeed mention that animals were subjected to this ritual practice, but in addition, provide scarce information about the organic commodities used during mummification processes (Abdel-Maksoud and El-Amin, 2011; Bard, 2007; Brettell et al., 2017; David, 2008; Ikram, 2017)

More importantly, up to this date, there are only two scientific articles with published data regarding information, obtained through chromatographic and spectroscopic techniques, about the organic substances used in animal mummification practices (Brettell et al., 2017; Buckley et al., 2004).

In this regard, taking into account the importance of the study of mummified animal remains in general, and their vital role in social, economic, religious and political context of Ancient Egyptian discourse, the relevance of this research paper lies in contribution of new comparable dataset to the already existing and narrow corpus of knowledge concerning organic substances used in mummification of animals in Ancient Egypt.

This study dealt with a dual analytical approach based on Fourier Transform Infrared Spectroscopy (FT-IR) and Gas Chromatography coupled to Mass Spectrometry (GC-MS) analysis of the embalming agents used in mummifying four votive ibis mummies from the Egyptology collection of Musée des Confluences in Lyon, France. These mummified remains were already studied in traditional archaeological manner and a calibrated C^{14} dating was realized (Porcier et al., 2019; Richardin et al., 2017). FT-IR allows to identify different materials (both organic and inorganic) on the basis of infrared absorption, so this analytical tool permits

initial screening of the samples (Font et al., 2007; Izzo et al., 2013; Łucejko et al., 2012; Ménager et al., 2014). However, FT-IR analysis is not sufficient for delicate molecular identification of precise ingredients, especially in complex and aged mixtures, present in embalming matter used for mummification of animal mummies. Therefore, GC-MS is often used as a powerful complementary analytical technique (Evershed et al., 2002; Jones et al., 2018), most notably for molecular analysis, thus demonstrating invaluable help in identifying specific compounds called molecular markers which are diagnostic in their nature and can be found in ancient balms or produced during ageing and/or other alteration processes (Łucejko et al., 2012).

Due to their amorphous and chemically complex nature, the primary aim of this paper was to ascertain the chemical composition of the embalming material applied on four votive ibis mummies using FT-IR and GC-MS analysis, as well as to compare the obtained results from these two complementary analytical techniques. Furthermore, more specific aims included comparing the results from this work with those from other published research papers which allowed assessment of the composition of the organic balms, as well as to ascertain possible similarities and/or differences in mummification of ibis mummies from different archaeological sites and time periods.

2. Materials and methods

2.1. Archaeological samples

Wrapping samples from four votive ibis mummies were selected from the collection of the Musée des Confluences in Lyon (France). The specimens (MHNL 90002472, 90002475, 90002486, 90002491) were acquired by the museum in the early 20th century. However, little is known about the origin of these mummified animals, with the exception of specimen 90002491 which comes from Roda (Upper Egypt). At that time, these mummified remains were studied by Dr. Louis Lortet, director of the Natural History Museum of Lyon, and the naturalist Claude Gaillard (Lortet and Gaillard, 1903).

Votive mummies under study are in a variable state of conservation. The mummy MHNL 90002472, partially unwrapped a century ago, consists solely of linen textiles in which it was initially wrapped, while the body of the animal itself is absent (Figure 1a). On the other hand, mummy MHNL 90002475 is intact and initial investigation established that the body of

the ibis is present (Figure 1b). Finally, two other mummies are in a poor state of conservation (MHNL 90002486, 90002491), partially damaged, some of the original linen textile is missing, the biological tissues are conserved partially, damaged by necrophagous insects, and most bones are visible (Figure 1c and 1d). Finally, these four votive mummies were chosen because of their state of preservation, which made it possible to carry out invasive analysis and to collect 50-200 mg of sample in order to characterize the organic matter present in balms. However, high historical value of these archaeological findings had necessitated certain levels of restraint during sampling. Worth mentioning is that one of the four mummies (MHNL 90002491) had been dated to the Greco-Roman period (92 cal BCE-65 cal CE) (Porcier et al., 2019; Richardin et al., 2017). Sampling of balms, corresponding to black amorphous substances, were realized on ibis referred MHNL 90002475 and MHNL 90002486 and sampling of impregnated textiles were done on mummies MHNL 90002472 and MHNL 90002491.

2.2. Solvents and reagents

All solvents and reagents were of analytical grade. Tetrahydrofurane (THF), hexane and N,*O*-Bis(trimethylsilyl)trifluoroacetamide/Trimethylchlorosilane (BSTFA/TMCS) were supplied by Sigma-Aldrich. Ethanol, dichloromethane (DCM) and diethyl ether were supplied by Merck.

2.2. Fourier Transform – Infrared Spectroscopy (FT-IR)

Archaeological samples were mixed and homogenized with 100 mg of KBr (VWR International, USA) and then pressed under 10 T/cm^{-2} to obtain a KBr pellet. Analyzes were performed with a Thermo-Nicolet iZ10 FT-IR spectrometer in transmission mode with OMNIC software. All FT-IR spectra were collected in the middle infrared (400 to 4000 cm^{-1}) recording 32 scans.

2.3. Gas Chromatography – Mass Spectrometry (GC-MS)

GC-MS analyses were performed with a Thermo Scientific Focus gas chromatographic system composed of a Thermo Scientific Al 3000 auto-sampler coupled with an ITQ 700 ion trap mass spectrometer (Thermo Fisher Scientific). A GC fused silica capillary column Thermo trace GOLD TG-5MS (5% diphenyl / 95% dimethylpolysiloxane, 30 m length × 0.25 mm i.d. × 0.25 µm film thickness) was used. The carrier gas was helium with a constant flow of 1 $mL.min^{-1}$. 1µL of each sample was injected in splitless mode. Mass spectra were recorded in electron impact mode with an electron ionization voltage of 70 eV, an ionization time of 25,000 µs and a mass range of 40–650 *m/z,* while the temperature of the injector was set at 250 °C. Transfer line, ion trap and manifold temperatures were respectively set at 300 °C, 200 °C and 50 °C The separation was achieved with the following temperature program: 50 °C with a 2 min hold, increased at 10°C.min−1 to 200 °C, then increased at 2.5 °C.min−1 to 310 °C, then increased at 8°C.min-1 to 330 °C and held isothermally for 3 min. All of the injections were realized in triplicate. Peak identification was implemented following established protocol in the form of comparison with mass spectra and retention times of pure standard compounds, as well as using NIST database (NIST MS Search 2.0)

2.4. Preparation of samples

10 mg of sample was extracted with 1 mL of dichloromethane and ultrasound was applied during time period of 10 min. Then the solution was centrifuged at 6000 rpm for 10 min. The supernatant was set aside to extract the solid pellet again. This step was repeated 2 times. The three fractions were collected, combined and evaporated to dryness under a stream of nitrogen. All of the extractives were trimethylsilyled with 200 µL of BSTFA/TMCS (99/1 v/v) and heated at 70°C for 30 min, the mixture was again evaporated to dryness and then dissolved in 0.2 to 1 mL of a hexane/DCM mixture (1/1, v/v) and filtered on a polytetrafluoroethylene cartridge (PTFE, 0.45 µm, VWR) (Mezzatesta et al., 2020a). 1 µL of the solution was injected in GC-MS apparatus.

2.5. Saponification

10 mg of sample was extracted with 3 x 1 mL of THF aided by sonification (5 min) and then centrifuged at 6000 rpm (5 min). The supernatant was set aside to extract the solid pellet again. The solvent extracts were combined and then 2 mL of a solution of potassium hydroxide KOH 10% in MeOH/H_2O (9/1, v/v) were added. The mixture was magnetically stirred and

heated at 65°C during 1 hour. After evaporation 3 mL of pure water was added with 1 mL of HCl 5 M. The aqueous phase was washed with 3 x 5 mL of diethyl ether. The organic phases were combined and dried with anhydrous sodium sulphate, then filtered on filter paper. Excess reagent was evaporated to dryness under a stream of nitrogen. Trimethylsilylation was applied. After evaporation, the derivatized sample was solubilized in hexane/DCM mixture (2/1, v/v) and filtered on PTFE cartridge before injection in GC-MS (Mezzatesta et al., 2020a).

2.6. Solid-Phase Extraction (SPE)

10 mg of sample was extracted with 1 mL of hexane/THF (1/1; v/v) aided by ultrasound for 5 min and then centrifuged at 6000 rpm for 5 min. The supernatant was set aside in order to extract the solid pellet again and these steps were repeated 3 times. The three fractions were combined and evaporated to dryness under a stream of nitrogen, then redissolved with 500 µL of hexane/THF (1/1; v/v). This solution is called charging. In parallel, a Strata® 200 mg / 3 mL SPE cartridge (Phenomenex) is calibrated with 4 mL of hexane. The initial 500 µL charge is deposited on the cartridge, then a first elution is carried out with 4 mL of hexane and collected in a hemolysis tube (fraction 1 for presence or absence of markers for bitumen, such as hopanes and steranes). A second elution is carried out with 4 mL of EtOH and then collected in another tube (fraction 2 for presence of resins). Finally, a final elution is carried out with 4 mL of DEE and 2% AcOH (fraction 3 for presence of fatty acids). Each fraction is evaporated to dryness under a stream of nitrogen. After trimethylsilylation with, fractions 1 and 2 were solubilized with 60 µL of a hexane/DCM mixture (2/1; v/v) while fraction 3 was solubilized with 1.5 mL of hexane/DCM (2/1; v/v). The three fractions were then filtered on a PTFE cartridge (0.45 µm) before respective injections in GC-MS apparatus (Mezzatesta et al., 2020b).

3. Results

3.1. FT-IR results

According to identified diagnostic and specific infrared bands (Table 1) such as hydroxyl (~3400 cm^{-1}), methylene (~2960-2850 cm^{-1}) and carbonyl (~1750-1650 cm^{-1}) functional groups (Bellamy, 1980), all analyzed spectra of samples 90002472, 90002475, 90002486 and 90002491, unambiguously indicated the presence of organic material corresponding to terpenic compounds and fatty matter (plant oil and/or animal fat). The resulting FT-IR spectra of the four analyzed samples have shown a similar standard

transmittance profile specific for diterpenic resins extracted from Pinaceae family (Scalarone et al., 2002). Presence of diterpenic resin has been confirmed according to the several infrared signals specific to these compounds (Figure 2). Namely, hydrocarbon skeleton structures, present in tricyclic structures in diterpenoids, emit strong stretching C-H vibrations modes of $=CH_2$ and CH_3 groups displayed at ~2957-2872 cm^{-1} and ~2920-2850 cm^{-1} (Font et al., 2007; Izzo et al., 2013). Regarding the specific carbonyl C=O stretching band occurring between ~1720-1710cm^{-1}, the absorption frequency also suggests the presence of a plant resin in all analyzed samples (Łucejko et al., 2012). In terms of further assessments (presence of either diterpenic and/or triterpenic compounds), several spectroscopic studies on fresh resins have shown that absorption band of C=O carbonyl group produces its maximum in range between 1705-1690 cm^{-1} depending on terpenic resin type (Ménager et al., 2014). Notably, as a diagnostic marker, location of the carbonyl band is used to distinguish between different types of terpenic compounds, in a way that C=O band for diterpenoids is always located below 1700cm^{-1}, and for triterpenoids it lies above this frequency (Bruni and Guglielmi, 2014). However, it is important to note that this "artificial border" can shift in degraded archaeological objects, especially in complex aged mixtures such as balms used for mummification (Ménager et al., 2014). Furthermore, in samples 90002486 and 90002491, shoulder band around ~1740-1735 cm^{-1} (serving as diagnostic frequency for presence of methyl esters) together with methylene ($=CH_2$) and methyl (CH_3) groups bending at respectively 1463-1454 cm^{-1} and 1384-1373cm^{-1} (diagnostic frequencies for diterpenic pine resins present in all four samples) as well as with bending vibrations from COOH at ~1173 cm^{-1}, suggest the presence of a mixture of exceedingly oxidized and dehydrogenated molecules (Font et al., 2007). These molecules are normally found in coniferous pine resins and they have been detected in the studied balms. On the other hand, the occurrence of fatty matter in the samples referred 9002475, 90002486 and 9002491 was confirmed through the presence of rocking C-H band at ~720 cm^{-1} and stretching C-O band between ~1170-1160 cm^{-1}. In all four samples these signals are valuable because they indicate the presence of the characteristic long linear molecular chains of fatty acids present in plant oils and animal fats (Font et al., 2007; Ménager et al., 2014).

Mixtures of oils, resins and beeswax are difficult to separate solely based on FT-IR analysis due to the overlapping of these signals. The characteristic bands of beeswax at 2920 cm^{-1}, 2850 cm^{-1}, 1740 cm^{-1} and 1173 cm^{-1} reflecting respectively the C-H bonds, as well as C=O and C-O bonds of the ester function were observed in samples 90002486 and 90002491 (Cuní et al., 2012; Svečnjak et al., 2015). As already discussed in previous lines, all of these bands could be also coming either from plant oils, or resinous substances.

The presence of proteins was possible in the samples 90002475 and 90002486 most notably due to FT-IR bands centered at ~1635 cm^{-1} (Amide I group) and 1540 cm^{-1} (Amide II group). Nevertheless, it is essential to underline here that GC-MS analysis was performed in order to target fatty matter and resinous compounds and therefore possible presence of proteins was not further assessed.

3.2. GC-MS results

The results of the gas chromatography-mass spectrometry analysis are summarized in Table 2. Analyzed data showed that primary commodities present in all four extracts from the embalming substance of ibis mummies are of organic nature originating most certainly from mixture of plant oils and/or animal fats, plant resins and beeswax.

3.2.1 Presence of beeswax

Fresh beeswax found in nature is defined as a complex mixture of different chemical compounds. This product made from the genus *Apis* is composed of long-chain fatty acids (C_{24}-C_{34}), *n*-alkanes (C_{23}-C_{33}) and mono-, di- and tri-esters, as well as some hydroxy-polyesters which are highly diagnostic of the presence of this substance (Clark, 2006; Ikram, 2017). However, when compared to fresh beeswax, in archaeological beeswax several differences are observed which can occur due to the human processing of this material in the past, microbial action or any other processes occurring over time which could alter the original chemical structure of this substance. It is important to mention that in archaeological samples beeswax can undergo oxidation thus yielding monocarboxylic acids and long chain alcohols. Furthermore, these long chain alcohols can undergo through process of oxidation, hence forming long chain fatty acids.

In this study presence of beeswax was observed in samples 90002486 and 90002491 without saponification preliminary step (Figure 3) through detection of several long-chain monocarboxylic fatty acids, while in samples 90002472 and 90002475, diagnostic markers essential for this substance were not identified in any of the analyzed chromatograms.

The characteristic fatty acid profile of beeswax (Buckley and Evershed, 2001; Heron et al., 1994), observed in samples 90002486 and 90002491, consisted of several fatty acids (FA) ranging from C_{22} to C_{32}, with lignoceric acid (C_{24}, tetracosanoic acid) as the most abundant

type, followed by cerotic (C_{26}, hexacosanoic acid), montanic (C_{28}, octacosanoic acid), melissic (C_{30}, triacontanoic acid) and lacceroic (C_{32}, dotriacontanoic acid) acids. *n*-Alkanes were not detected in chromatograms of samples 90002486 and 90002491 and a possible explanation for this particular phenomenon could be that the loss of alkanes happened due to extensive heating (Heron et al., 1994) of the embalming material (in support of this hypothesis the presence of retene have been also detected, see the below section). With the purpose of confirming either presence or absence of beeswax, saponification was performed as a preliminary step. Results obtained through saponification are in accordance with aforementioned data, that being the presence of beeswax was confirmed in samples 90002486 and 90002491, and absent in 90002472 and 90002475.

3.2.2 Identification of resins in balms

In this study, chemical markers of diterpenic carbon-structures diagnostic for resins coming from coniferous trees were identified in all analyzed ibis' mummies (Figures 3). Further chemical analysis focused on interpretation of these diterpenic markers revealed clearly the presence of dehydroabietic acid (molecular peak $M^{\bullet+}$ 372 and base peak fragment ion of *m/z* 239 B^+), 7-oxodehydroabietic acid ($M^{\bullet+}$ 386 and fragment ion of *m/z* 253 B^+), 15-hydroxy-7-oxo-dehydroabietic acid ($M^{\bullet+}$ 460 and fragment ion of *m/z* 445 B^+), 3-hydroxydehydroabietic acid and 7- hydroxydehydroabietic acid in the sample 90002491 (Figure 3). These oxidized and dehydrogenated abietane structures serve as markers for presence of coniferous diterpenic resins obtained from trees belonging to the Pinaceae family (Brettell et al., 2017; Buckley and Evershed, 2001; Colombini et al., 2000; Degano and Colombini, 2009). Normally, in fresh diterpenic resins of coniferous trees, dehydroabietic acid is present but as a minor constituent unlike abietic acid which acts as a primary and most abundant component. However, with natural alteration processes (ageing), abundance of dehydroabietic acid starts to decrease and, other compounds are starting to appear like already mentioned 7-oxo-dehydroabietic acid which is the major oxidized diterpenoid, after dehydroabietic acid, present in the analyzed ibis' samples. These highly oxidized compounds indicate an advanced state of oxidation of conifer resin.

In addition to these results it is important to note that in all samples methyl esters derivatives were detected such as 7-oxo-dehydroabietic methyl ester ($M^{\bullet+}$ 328 and fragment ion of *m/z* 253 B^+) and 15-hydroxy-7-oxodehydroabietic methyl ester. These methylated derivatives

occur during the preparation of wood tar with releasing of methanol during pyrolysis of the resinous wood (Colombini et al., 2005; Izzo et al., 2013). Retene ($M^{\bullet+}$ 234 and fragment ion of *m/z* 219 B^+) was also identified in samples 90002486 and 90002491. This last compound is considered as a marker for pine pitch because when the resin is heated to high temperatures or distilled in a low oxygen environment to obtain a pitch, aromatisation, demethylation, and defunctionalisation reactions occur in the abietadiene and pimaradiene acids (Colombini et al., 2005). These phenomena lead to the formation of a series of products as norabietatrienes and tetrahydroretene, with retene as the final stable product of all these transformations. Furthermore, we can argue that in samples 90002486 and 90002491 combined abundance of methyl esters with presence of retene indicated that a high-temperature pre-application processing of this resin was applied (Brettell et al., 2017; Jones et al., 2018). On the contrary, presence of retene was not confirmed in samples 90002472 and 90002475, meaning that resinous material employed for mummification was subjected to low-temperature pre-application heating. Additionally, we can argue that this particular ingredient used for mummification of these mummies, had to be imported from outside of the territory of Ancient Egypt and such claims are already reported in ancient texts from mid-first century AD (Chapman, 2016).

3.2.3 Presence of plant oil and/or animal fat

Thanks to the written historical sources (great number of ancient Egyptian texts identifying oils and fats as fundamental components of perfumes, cosmetics and medicines) (Clark, 2006; Ikram, 2017) associated with archaeological data (lipids preserved in canopic pottery jars used during mummification), researchers were able to recognize to importance of these substances in mummification process (Łucejko et al., 2017; Ménager et al., 2014). Based on these data, it was observed that certain plant oils and animal fats were convenient as a foundation for applying and/or mixing other ingredients, while others were most likely used due to their hydrophobic and drying properties (Clark, 2006).

With regard to the presence of markers for plant oils and/or animal fats in the ibis mummies, analyzed data revealed a great number of linear saturated monocarboxylic fatty acids in the C_6–C_{32} range, with myristic ($C_{14:0}$, $M^{\bullet+}$ 300 and diagnostic fragment ions of *m/z* 75, 117, 285), palmitic ($C_{16:0}$, $M^{\bullet+}$ 328 and diagnostic fragment ions of *m/z* 75, 117, 313) and stearic ($C_{18:0}$, $M^{\bullet+}$ 356 and diagnostic fragment ions of *m/z* 75, 117, 341) acids as the most abundant

acidic components in obtained chromatograms. A general approach in differencing between both fresh and degraded remains of oils and fats, in archaeological samples, is to calculate the ratio of $C_{16:0}$ and $C_{18:0}$ fatty acids (Eerkens, 2005; Evershed, 2008; Evershed et al., 2002). However, this common method is not exactly best suited when it comes to analysis of balms applied in Ancient Egypt mummification processes (Ménager et al., 2014). The ratio between these two fatty acids can be altered due to lipid thermal degradation and what is more likely, embalmers could have using a mixture of different oils and/or fats, thus "contaminating" proportion between these essential compounds. Moreover, $C_{16:0}/C_{18:0}$ ratio could be influenced by the presence of beeswax which contains high levels of palmitic acid.

Unsaturated fatty acid profile was observed only in sample 90002475 through the presence of $C_{18:1}$ (oleic acid). In the case of $C_{18:1}$, as it is most common in plant sources, specifically in large quantities in vegetable oils (e.g. olive oil), it can also be constitutive part of animal fats, hence this particular unsaturated fatty acid does not provide enough information for precise determination of the origin of this compound (Clark, 2006; Evershed, 2008; Evershed et al., 2002), as it is possible for instance with erucic and gondoic acids (biomarkers for radish oil) (Copley et al., 2005).

Furthermore, the presence of odd chain fatty acids in archaeological samples, should also be taken into particular consideration. Namely, non-negligible quantities of $C_{15:0}$ ($M^{\bullet+}$ 314 and diagnostic fragment ions of *m/z* 75, 117, 299) and $C_{17:0}$ ($M^{\bullet+}$ 342 and diagnostic fragment ions of *m/z* 75, 117, 327) could be indicative of presence of ruminant fat in the original embalming recipe (sheep, goat, cattle, etc.) applied in mummification of samples 90002472, 90002475, 90002486 and 90002491 (Donato et al., 2013; Eerkens, 2005; Lantos et al., 2018, 2017; Łucejko et al., 2017). In an extensive and extremely comprehensive study conducted by Eerkens (2005), it was strongly suggested that quantifying the ratios between odd ($C_{15:0} + C_{17:0}$) and even chain fatty acids ($C_{12:0} + C_{14:0} + C_{16:0} + C_{18:0}$) could provide insights whether we can denote or deny the presence of ruminant fat in studied samples. In the aforementioned research, it was pointed out that the calculated values that are higher than 0.04 are distinctive for ruminant animals, which was the case in all four analyzed samples of ibis mummies (~0.05). Nevertheless, we should be careful with this, given the fact that the presence of $C_{15:0}$ and $C_{17:0}$ could result from soil bacterial degradation of lipids within the body of the animals. Indeed, contamination from body fluids of animal mummies is often an issue in this type of analyses.

In the light of the foregoing discussion, it would seem difficult to achieve a more precise plant oil or animal fat origin assessment, present in studied balms, based exclusively on presence of the fatty acid profile in the analyzed samples. Embalmers could have been using a mixture of different plant oils and animal fats, and more importantly, due to degradation pathways of major diagnostic unsaturated components, which is confirmed by the presence of short chain dicarboxylic acids in the studied samples. Regarding the presence of the aforementioned dicarboxylic acids it was clearly observed in gas chromatograms of every sample that they represent the second most abundant components in the acidic profile. Further assessment of data identified presence of α, ω – dicarboxylic acids ("diacids") in the C_4 to C_{12} carbon chain length range, with azelaic (diC_9, nonanedioic acid) as the most abundant one together with suberic and sebacic diacids. Dicarboxylic acids are naturally not present in plant oils, resins, waxes and animal fat; they are formed as a result of several different oxidative degradation mechanisms of the double bond(s) in mono-, or poly-unsaturated fatty acids. Thus, the position of the double bond is directly related to the carbon chain length of the diacids formed. Therefore, the presence and the amount of diC_9 (azelaic acid, originating from C_{18} fatty acid with double bond between carbons 9 and 10 like linoleic and linolenic acids) combined with presence of diC_8 (suberic acid) and diC_7 (pimelic acid) acids are indicators of degraded vegetable oils, thus suggesting that a siccative or semi-siccative plant oil high in oleic acid was used during mummification of all four samples that are the subject of this study (Brettell et al., 2017; Colombini et al., 2005; Copley et al., 2005; Degano and Colombini, 2009; Jones et al., 2018; La Russa et al., 2014; Łucejko et al., 2017, 2012; Tchapla et al., 2004).

Additionally, the presence of ricinoleic acid (12-hydroxy-9-cis-octadecenoic acid) acting as a diagnostic compound of castor oil was identified only in sample 90002491 ($M^{\bullet+}$ 442 and fragment ion of *m/z* 187 B^+). However, the fatty acids distribution in this sample is not dominated by this chemical marker, which in this case acts as a minor constituent (Figure 4). The explanation for this phenomenon can be explained through interpretation of the resulting fatty acid profile which originates most probably from a mixture with other plant oils and/or animal fats that were included in the formulation of this specific balm applied in mummification of mummy 90002491 (Tchapla et al., 2004).

4. Discussion

Up to this date, only few scientific articles dealing with animal mummification are available to the scientific community (Brettell et al., 2017; Buckley et al., 2004) and total number of four balms from other votive ibis mummies were chemically characterized. Therefore, it is important to realize a comparison between the ibis balms of this study and those described in the specialized literature. Unlike the ibis mummies that are the subject of this study, in article published by Buckley *et al.* (2004) it was argued that embalming agents consisted, mainly, of hydrolyzed plant sugar gum used for securing the mummy wrappings. Additionally, only trace quantities of fatty acids were detected with high amounts of C_{16} to C_{18} ratios, which, according to authors of that paper, indicated oil of plant origin (Buckley et al., 2004; Buckley and Evershed, 2001). However, it is important to emphasize the fact that the mummy from Buckley *et al.* study originate from XXVI-XXX dynasty period (664-343 BC) as opposed to sample 90002491 (Roda) which was dated to the very end of the Ptolemaic period (92-65 cal BC) (Richardin et al., 2017). Given the fact that both of these samples originate from archaeological sites in Lower Egypt it is possible to consider the difference in their embalming strategy. According to numerous research papers, it is argued that the increase in use of conifer resin exudates in everyday and religious life of Egyptian societies started with Ptolemaic and Roman periods possibly due to relative abundance and ease of extraction (Colombini et al., 2000). Number of research works, dealing with human mummification practices, demonstrated that in majority of cases, presence of conifer resins acts in a certain way as indispensable part of embalming material that were applied during mummification due to their highly valued antimicrobial properties (Buckley and Evershed, 2001; Colombini et al., 2000; Degano and Colombini, 2009; Ikram, 2017).

On the other hand, results of three ibis mummies published by Brettel *et al.* (2017), are more similar with those obtained in this study. For two mummies it was determined that they belong to Ptolemaic (Inv. Num. 165, Sakkara site) and Roman period (Inv. Num.162). As in this study, paper from Brettel *et al.* showed that the main component of the analyzed balm contained either plant oil or animal fat, or combination of both. Also, the diterpenoids were confirmed through presence of Pinaceae resin in sample 471, while in sample 162 triterpenoids were observed through presence of *Pistacia spp.* resin which contrasts results obtained in this study with the absence of triterpenoids in all of the samples. In this study the presence of retene, observed in samples 90002486 and 90002491 which acts as a diagnostic marker for extensive heating, was not detected in any of the samples studied by Brettel *et al.* This difference begs the question of how they then mixed and process the various material needed for embalming the

413 bodies of the animal? Furthermore, through additional comparison of data between these two
414 research works, we can clearly notice another thing that implies the possibility of different
415 approach in embalming strategy through presence or absence of either bitumen or beeswax.
416 Namely, in this study no hydrocarbon diagnostic biomarkers characteristic for bitumen were
417 observed. The absence of diagnostic markers such as steranes and hopanes was confirmed in
418 all chromatograms obtained by complementary experiments using Solid-Phase Extraction
419 protocol (Mezzatesta et al, 2020b). This SPE protocol was performed on all of the samples and
420 it permitted to clearly confirm the occurrence or the absence of bitumen in the studied
421 archaeological samples.

422 Beeswax was detected through presence of several long chain monocarboxylic saturated
423 fatty acids (C_{26}, C_{28}, C_{30}, C_{32}). In contrast to this, in Brettel *et al.* paper, presence of bitumen
424 was observed in samples 165 and 471, while beeswax was absent in the analyzed
425 chromatograms.

426 **5. Conclusion**

427 The chemical analysis of organic embalming material employed in Late Ptolemaic/Early
428 Roman Egyptian mummification of ibis animals from the Collection of the Musée des
429 Confluences in Lyon (France) was performed. A dual analytical approach was employed in
430 order to characterize the organic matter present in balms and impregnated textile of four votive
431 ibis mummies.

432 In first step, the resulting FT-IR spectra have shown the presence of mixture of terpenic
433 compounds and fatty matter (plant oil and/or animal fat). A standard transmittance profile of
434 diterpenic resins extracted from Pinaceae family was more particularly observed.

435 In a second step, the GC-MS technique has confirmed and clarified certain results.
436 Beeswax was observed in samples 90002486 and 90002491. Concerning these two samples,
437 the combined abundance of methyl esters with presence of retene indicated that high-
438 temperature pre-application processing of the resinous matter was applied, while in samples
439 90002472 and 90002475 resin was subjected to lower temperature, which is in accordance with
440 the corresponding FT-IR results of these archaeological samples.

441 Moreover, some indicators of degraded plant oils suggested that a siccative or semi-
442 siccative plant oil was used in the preparation of these balms. At last, a diagnostic marker of
443 castor oil was identified only in sample 90002491. According to FT-IR results, proteins were
444 present in samples 90002475 and 90002486. GC-MS analysis was performed in order to target

lipid/resinous compounds and therefore the presence of proteins was not further assessed. Additionally, the origin of proteinous sources are complicated because they could be a contaminant originating from the body fluids.

Results of this study showed that applied balms were complex mixtures, consisting of plant oils and/or animal fats, conifer resins and beeswax in certain cases, which when compared to other animal and human mummies confirm that similar range of substances were used for mummification during Late Ptolemaic and early Roman period, but with possibility of different approach in methodology of embalming the body of the animal.

To conclude, this research study offers a new comparative dataset in a still evolving field, thus contributing to the development of scientific work of Egyptian votive animal mummies.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

This research was financially supported by Marie Skłodowska-Curie Innovative Training Network, The ED-ARCHMAT European Joint Doctorate, H2020-MSCA-ITN-EJD ED- ARCHMAT Joint Doctorate (Project ESR10, grant agreement no 766311) and it was carried out in connection with MAHES Program supported by the Agence Nationale de la Recherche from the « Investissement d'Avenir » program ANR-11-LABX-0032-01 LabEx ARCHIMEDE. The authors of this work are grateful to Dr. Didier Berthet, curator at the Musée des Confluences in Lyon (France) for giving us the opportunity to work on samples which are part of the collection of animal mummies. The authors acknowledge the anonymous reviewers for their careful reading of our manuscript and their many insightful comments and suggestions.

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598 **Table 1**

599 FT-IR data and interpretation of the obtained results

600

90002472	90002475	90002486	90002491	Corresponding bond	Source
3418	3408	3419	3420	OH stretching	Organic material
	3278				
	3067				
2960	2957	2958	2960	CH_3 stretching	Organic material
2920	2924	2920	2920	CH_2 stretching	Organic material
	2873			CH_3 stretching	Organic material
2850	2850	2850	2850	CH_2 stretching	Organic material
		1740	1740	(C=O) stretching	Organic material (ester bond)
1715	1713	1713	1716	(C=O) stretching	Organic material
1635	1635	1649		(OH) bending (C=C) stretching Amide I	Presence of proteins
	1540	1546		(CH_3) bending Amide II	Presence of proteins
1457	1455	1463	1457	(CH) bending	Organic material
		1413	1415	(CH) bending	Organic material
1374	1384	1383	1382	(CH) bending	Organic material
1315	1315				Calcium oxalate
1163	1171	1172	1173	(C-C) stretching	Organic material
1112		1119	1113		
1032	1038	1035	1032	Si-O stretching	Clay minerals
	720	724	720	C-H rocking	Organic material

601

602

603

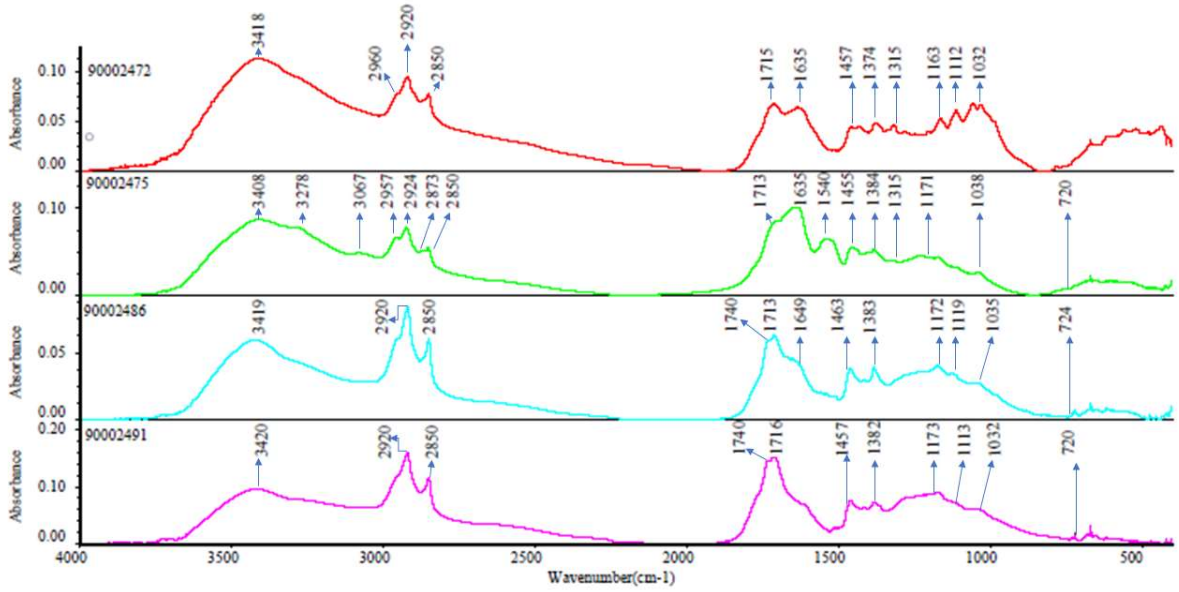
604 **Table 2.** List of identified compounds obtained from GC-MS analysis of embalming substances
605 from ibis' mummies (**D:** dicarboxylic acids; **S:** Saturated fatty acids; **U:** Unsaturated fatty acids;
606 **R:** Resinic acids; **H:** Hydroxycarboxylic acids)

No	Class	Identified compounds	Samples			
			90002472	90002475	90002486	90002491
1	S	Caproic acid (C6:0)				✓
2	S	Enanthic acid (C7:0)		✓	✓	✓
3	S	Caprylic acid (C8:0)			✓	✓
4	D	Succinic acid	✓	✓	✓	✓
5	S	Pelargonic acid (C9:0)	✓		✓	✓
6	D	Glutaric acid	✓	✓	✓	✓
7	S	Capric acid (C10:0)	✓	✓	✓	✓
8	D	Adipic acid	✓	✓	✓	✓
9	S	Undecylic acid (C11:0)		✓	✓	
10	D	Pimelic acid	✓	✓	✓	✓
11	S	Lauric acid (C12:0)	✓	✓	✓	✓
12	D	Suberic acid	✓	✓	✓	✓
13	S	Tridecylic acid (C13:0)	✓	✓	✓	✓
14	D	Azelaic acid	✓	✓	✓	✓
15	S	Myristic acid (C14:0)	✓	✓	✓	✓
16	D	Sebacic acid	✓	✓	✓	✓
17	S	Pentadecanoic acid (C15:0)	✓	✓	✓	✓
18	S	Palmitic acid (C16:0)	✓	✓	✓	✓
19	D	Dodecanedioic acid		✓	✓	
20	S	Margaric acid (C17:0)	✓	✓	✓	✓
21	U	Oleic acid (C18:1)		✓		
22	R	Retene			✓	✓
23	S	Stearic acid (C18:0)	✓	✓	✓	✓
24	S	Nonadecanoic acid (C19:0)	✓	✓	✓	✓
25	R	Dehydroabietic acid	✓	✓	✓	✓
26	H	Ricinoleic acid				✓
27	S	Arachidic acid (C20:0)	✓	✓	✓	✓
28	R	3-hydroxy-dehydroabietic acid				✓
29	R	7-hydroxy-dehydroabietic acid				✓
30	R	7-oxodehydroabietic methyl ester	✓	✓	✓	✓
31	R	7-oxodehydroabietic acid	✓		✓	✓
32	S	Behenic acid (C19:0)		✓	✓	✓
33	R	15-Hydroxy-7-oxodehydroabietic methyl ester	✓	✓	✓	
34	R	15-Hydroxy-7-oxodehydroabietic acid	✓			✓
35	S	Lignoceric acid (C24:0)			✓	✓
36	S	Cerotic acid (C26:0)			✓	✓
37	S	Montanic acid (C28:0)				✓
38	S	Melissic acid (C30:0)				✓
39	S	Lacceroic acid (C32:0)				✓

607

608 **Figure 1.** Photographs of the 4 ibis mummies of the Musée des Confluences: (a) MHNL
609 90002472; (b) MHNL 90002475; (c) MHNL 90002486; (d) MHNL 90002491 (© Program
610 MAHES).
611

612



613

614 **Figure 2.** FT-IR spectra of samples 90002472, 90002475, 90002486 and 90002491
615

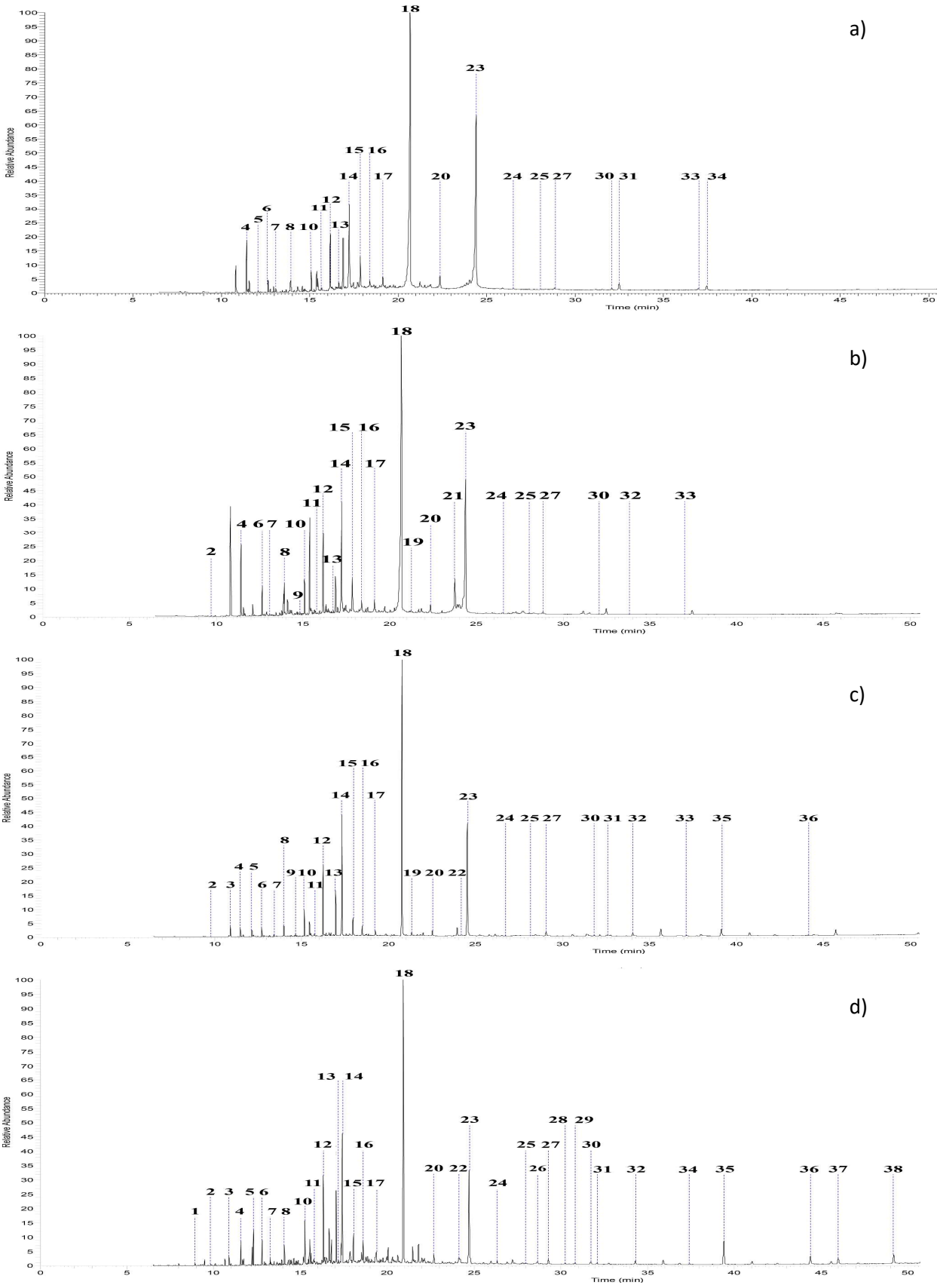


Figure 3. Total ion current chromatograms of samples 90002472(a), 90002475 (b), 90002486 (c) and 90002491 (d). All the compounds were identified as their TMS derivatives.