



HAL
open science

Chemical characterization of embalming materials of four ibis mummies from the Musée des Confluences, Lyon

Milan Marković, Elodie Mezzatesta, Stéphanie Porcier, Catherine Vieillescazes, Carole Mathe

► To cite this version:

Milan Marković, Elodie Mezzatesta, Stéphanie Porcier, Catherine Vieillescazes, Carole Mathe. Chemical characterization of embalming materials of four ibis mummies from the Musée des Confluences, Lyon. *Journal of Archaeological Science: Reports*, 2020, 34, pp.102624. 10.1016/j.jasrep.2020.102624 . hal-03173554

HAL Id: hal-03173554

<https://hal.science/hal-03173554v1>

Submitted on 19 Apr 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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

3 Milan Marković^{1*}, Elodie Mezzatesta¹, Stéphanie Porcier^{2,3}, Cathy Vieillescazes¹, Carole
4 Mathe^{1*}

5 ¹IMBE UMR 7263/ IRD237, Avignon University/ CNRS/ IRD/ Aix-Marseille university,
6 Restoration Engineering of Natural and Cultural Heritage, Faculty of Sciences, Campus Jean-
7 Henri Fabre, 301 rue Baruch de Spinoza BP 21239, 84916 Avignon Cedex 9, France.

8 ²ASM UMR 5140 “Archéologie des Sociétés Méditerranéennes”, CNRS/ UPV/ MCC/
9 INRAP, LabEx ARCHIMEDE, F-34090 Montpellier, France

10 ³HiSoMA UMR 5189 “Histoire et Sources des Mondes Antiques”, CNRS, F-69007 Lyon,
11 France

12 *Corresponding authors:

13 Milan Marković:

14 Phone: +33-490-142-247.

15 Email address: milan.markovic@univ-avignon.fr

17 Carole Mathe:

18 Phone: +33-490-144-454.

19 E-mail address: carole.mathe@univ-avignon.fr

21 **Abstract**

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

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

35

36 1. Introduction

37 While anthropogenic body preservation is a phenomenon that could be seen in past
38 communities ranging from today's northern Chile to modern Japan (Arriaza et al., 1998; Sakurai
39 et al., 1998), the deliberate mummification of animals appears to be a ritual practice exclusively
40 limited to societies in Ancient Egypt. Thanks to the large amount of data gathered from
41 numerous archaeological excavations it was discovered that Ancient Egyptians mummified
42 millions of animals from the 16th Century BC (New Kingdom) until the 4th Century AD
43 (Roman period) (Abdel-Maksoud and El-Amin, 2011; Bard, 2007; Porcier et al., 2019;
44 Richardin et al., 2017), of which many of them have been classified as "votive mummies"
45 offered to gods and goddesses (Ikram, 2017). However, ancient writers such as Herodotus,
46 Strabo and Pliny the Elder, indeed mention that animals were subjected to this ritual practice,
47 but in addition, provide scarce information about the organic commodities used during
48 mummification processes (Abdel-Maksoud and El-Amin, 2011; Bard, 2007; Brettell et al.,
49 2017; David, 2008; Ikram, 2017)

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

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

59 This study dealt with a dual analytical approach based on Fourier Transform Infrared
60 Spectroscopy (FT-IR) and Gas Chromatography coupled to Mass Spectrometry (GC-MS)
61 analysis of the embalming agents used in mummifying four votive ibis mummies from the
62 Egyptology collection of Musée des Confluences in Lyon, France. These mummified remains
63 were already studied in traditional archaeological manner and a calibrated C¹⁴ dating was
64 realized (Porcier et al., 2019; Richardin et al., 2017). FT-IR allows to identify different materials
65 (both organic and inorganic) on the basis of infrared absorption, so this analytical tool permits

66 initial screening of the samples (Font et al., 2007; Izzo et al., 2013; Łucejko et al., 2012;
67 Ménager et al., 2014). However, FT-IR analysis is not sufficient for delicate molecular
68 identification of precise ingredients, especially in complex and aged mixtures, present in
69 embalming matter used for mummification of animal mummies. Therefore, GC-MS is often
70 used as a powerful complementary analytical technique (Evershed et al., 2002; Jones et al.,
71 2018), most notably for molecular analysis, thus demonstrating invaluable help in identifying
72 specific compounds called molecular markers which are diagnostic in their nature and can be
73 found in ancient balms or produced during ageing and/or other alteration processes (Łucejko et
74 al., 2012).

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

83

84 **2. Materials and methods**

85 **2.1. Archaeological samples**

86 Wrapping samples from four votive ibis mummies were selected from the collection of
87 the Musée des Confluences in Lyon (France). The specimens (MHNL 90002472, 90002475,
88 90002486, 90002491) were acquired by the museum in the early 20th century. However, little
89 is known about the origin of these mummified animals, with the exception of specimen
90 90002491 which comes from Roda (Upper Egypt). At that time, these mummified remains were
91 studied by Dr. Louis Lortet, director of the Natural History Museum of Lyon, and the naturalist
92 Claude Gaillard (Lortet and Gaillard, 1903).

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

97 the ibis is present (Figure 1b). Finally, two other mummies are in a poor state of conservation
98 (MHNL 90002486, 90002491), partially damaged, some of the original linen textile is missing,
99 the biological tissues are conserved partially, damaged by necrophagous insects, and most
100 bones are visible (Figure 1c and 1d). Finally, these four votive mummies were chosen because
101 of their state of preservation, which made it possible to carry out invasive analysis and to collect
102 50-200 mg of sample in order to characterize the organic matter present in balms. However,
103 high historical value of these archaeological findings had necessitated certain levels of restraint
104 during sampling. Worth mentioning is that one of the four mummies (MHNL 90002491) had
105 been dated to the Greco-Roman period (92 cal BCE-65 cal CE) (Porcier et al., 2019; Richardin
106 et al., 2017). Sampling of balms, corresponding to black amorphous substances, were realized
107 on ibis referred MHNL 90002475 and MHNL 90002486 and sampling of impregnated textiles
108 were done on mummies MHNL 90002472 and MHNL 90002491.

109

110

111 **2.2. Solvents and reagents**

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

116

117 **2.2. Fourier Transform – Infrared Spectroscopy (FT-IR)**

118 Archaeological samples were mixed and homogenized with 100 mg of KBr (VWR
119 International, USA) and then pressed under 10 T/cm² to obtain a KBr pellet. Analyzes were
120 performed with a Thermo-Nicolet iZ10 FT-IR spectrometer in transmission mode with OMNIC
121 software. All FT-IR spectra were collected in the middle infrared (400 to 4000 cm⁻¹) recording
122 32 scans.

123

124

125

126 **2.3. Gas Chromatography – Mass Spectrometry (GC-MS)**

127 GC-MS analyses were performed with a Thermo Scientific Focus gas chromatographic
128 system composed of a Thermo Scientific AI 3000 auto-sampler coupled with an ITQ 700 ion
129 trap mass spectrometer (Thermo Fisher Scientific). A GC fused silica capillary column Thermo
130 trace GOLD TG-5MS (5% diphenyl / 95% dimethylpolysiloxane, 30 m length × 0.25 mm i.d.
131 × 0.25 μm film thickness) was used. The carrier gas was helium with a constant flow of 1
132 mL.min⁻¹. 1 μL of each sample was injected in splitless mode. Mass spectra were recorded in
133 electron impact mode with an electron ionization voltage of 70 eV, an ionization time of 25,000
134 μs and a mass range of 40–650 *m/z*, while the temperature of the injector was set at 250 °C.
135 Transfer line, ion trap and manifold temperatures were respectively set at 300 °C, 200 °C and
136 50 °C The separation was achieved with the following temperature program: 50 °C with a 2
137 min hold, increased at 10°C.min⁻¹ to 200 °C, then increased at 2.5 °C.min⁻¹ to 310 °C, then
138 increased at 8°C.min⁻¹ to 330 °C and held isothermally for 3 min. All of the injections were
139 realized in triplicate. Peak identification was implemented following established protocol in the
140 form of comparison with mass spectra and retention times of pure standard compounds, as well
141 as using NIST database (NIST MS Search 2.0)

142 **2.4. Preparation of samples**

143 10 mg of sample was extracted with 1 mL of dichloromethane and ultrasound was
144 applied during time period of 10 min. Then the solution was centrifuged at 6000 rpm for 10
145 min. The supernatant was set aside to extract the solid pellet again. This step was repeated 2
146 times. The three fractions were collected, combined and evaporated to dryness under a stream
147 of nitrogen. All of the extractives were trimethylsilyled with 200 μL of BSTFA/TMCS (99/1
148 v/v) and heated at 70°C for 30 min, the mixture was again evaporated to dryness and then
149 dissolved in 0.2 to 1 mL of a hexane/DCM mixture (1/1, v/v) and filtered on a
150 polytetrafluoroethylene cartridge (PTFE, 0.45 μm, VWR) (Mezzatesta et al., 2020a). 1 μL of
151 the solution was injected in GC-MS apparatus.

152 **2.5. Saponification**

153 10 mg of sample was extracted with 3 x 1 mL of THF aided by sonification (5 min) and
154 then centrifuged at 6000 rpm (5 min). The supernatant was set aside to extract the solid pellet
155 again. The solvent extracts were combined and then 2 mL of a solution of potassium hydroxide
156 KOH 10% in MeOH/H₂O (9/1, v/v) were added. The mixture was magnetically stirred and

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

163 **2.6. Solid-Phase Extraction (SPE)**

164 10 mg of sample was extracted with 1 mL of hexane/THF (1/1; v/v) aided by ultrasound
165 for 5 min and then centrifuged at 6000 rpm for 5 min. The supernatant was set aside in order to
166 extract the solid pellet again and these steps were repeated 3 times. The three fractions were
167 combined and evaporated to dryness under a stream of nitrogen, then redissolved with 500 µL
168 of hexane/THF (1/1; v/v). This solution is called charging. In parallel, a Strata® 200 mg / 3 mL
169 SPE cartridge (Phenomenex) is calibrated with 4 mL of hexane. The initial 500 µL charge is
170 deposited on the cartridge, then a first elution is carried out with 4 mL of hexane and collected
171 in a hemolysis tube (fraction 1 for presence or absence of markers for bitumen, such as hopanes
172 and steranes). A second elution is carried out with 4 mL of EtOH and then collected in another
173 tube (fraction 2 for presence of resins). Finally, a final elution is carried out with 4 mL of DEE
174 and 2% AcOH (fraction 3 for presence of fatty acids). Each fraction is evaporated to dryness
175 under a stream of nitrogen. After trimethylsilylation with, fractions 1 and 2 were solubilized
176 with 60 µL of a hexane/DCM mixture (2/1; v/v) while fraction 3 was solubilized with 1.5 mL
177 of hexane/DCM (2/1; v/v). The three fractions were then filtered on a PTFE cartridge (0.45 µm)
178 before respective injections in GC-MS apparatus (Mezzatesta et al., 2020b).

179

180 **3. Results**

181 **3.1. FT-IR results**

182 According to identified diagnostic and specific infrared bands (Table 1) such as
183 hydroxyl (~3400 cm⁻¹), methylene (~2960-2850 cm⁻¹) and carbonyl (~1750-1650 cm⁻¹)
184 functional groups (Bellamy, 1980), all analyzed spectra of samples 90002472, 90002475,
185 90002486 and 90002491, unambiguously indicated the presence of organic material
186 corresponding to terpenic compounds and fatty matter (plant oil and/or animal fat). The
187 resulting FT-IR spectra of the four analyzed samples have shown a similar standard

188 transmittance profile specific for diterpenic resins extracted from Pinaceae family (Scalarone et
189 al., 2002). Presence of diterpenic resin has been confirmed according to the several infrared
190 signals specific to these compounds (Figure 2). Namely, hydrocarbon skeleton structures,
191 present in tricyclic structures in diterpenoids, emit strong stretching C-H vibrations modes of
192 =CH₂ and CH₃ groups displayed at ~2957-2872 cm⁻¹ and ~2920-2850 cm⁻¹ (Font et al., 2007;
193 Izzo et al., 2013). Regarding the specific carbonyl C=O stretching band occurring between
194 ~1720-1710cm⁻¹, the absorption frequency also suggests the presence of a plant resin in all
195 analyzed samples (Łucejko et al., 2012). In terms of further assessments (presence of either
196 diterpenic and/or triterpenic compounds), several spectroscopic studies on fresh resins have
197 shown that absorption band of C=O carbonyl group produces its maximum in range between
198 1705-1690 cm⁻¹ depending on terpenic resin type (Ménager et al., 2014). Notably, as a
199 diagnostic marker, location of the carbonyl band is used to distinguish between different types
200 of terpenic compounds, in a way that C=O band for diterpenoids is always located below
201 1700cm⁻¹, and for triterpenoids it lies above this frequency (Bruni and Guglielmi, 2014).
202 However, it is important to note that this “artificial border” can shift in degraded archaeological
203 objects, especially in complex aged mixtures such as balms used for mummification (Ménager
204 et al., 2014). Furthermore, in samples 90002486 and 90002491, shoulder band around ~1740-
205 1735 cm⁻¹ (serving as diagnostic frequency for presence of methyl esters) together with
206 methylene (=CH₂) and methyl (CH₃) groups bending at respectively 1463-1454 cm⁻¹ and 1384-
207 1373cm⁻¹ (diagnostic frequencies for diterpenic pine resins present in all four samples) as well
208 as with bending vibrations from COOH at ~1173 cm⁻¹, suggest the presence of a mixture of
209 exceedingly oxidized and dehydrogenated molecules (Font et al., 2007). These molecules are
210 normally found in coniferous pine resins and they have been detected in the studied balms. On
211 the other hand, the occurrence of fatty matter in the samples referred 9002475, 90002486 and
212 9002491 was confirmed through the presence of rocking C-H band at ~720 cm⁻¹ and stretching
213 C-O band between ~1170-1160 cm⁻¹. In all four samples these signals are valuable because
214 they indicate the presence of the characteristic long linear molecular chains of fatty acids present
215 in plant oils and animal fats (Font et al., 2007; Ménager et al., 2014).

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

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

227

228 3.2. GC-MS results

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

233

234 3.2.1 Presence of beeswax

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

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

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

253 type, followed by cerotic (C₂₆, hexacosanoic acid), montanic (C₂₈, octacosanoic acid), melissic
254 (C₃₀, triacontanoic acid) and lacceroic (C₃₂, dotriacontanoic acid) acids. *n*-Alkanes were not
255 detected in chromatograms of samples 90002486 and 90002491 and a possible explanation for
256 this particular phenomenon could be that the loss of alkanes happened due to extensive heating
257 (Heron et al., 1994) of the embalming material (in support of this hypothesis the presence of
258 retene have been also detected, see the below section). With the purpose of confirming either
259 presence or absence of beeswax, saponification was performed as a preliminary step. Results
260 obtained through saponification are in accordance with aforementioned data, that being the
261 presence of beeswax was confirmed in samples 90002486 and 90002491, and absent in
262 90002472 and 90002475.

263

264 3.2.2 Identification of resins in balms

265 In this study, chemical markers of diterpenic carbon-structures diagnostic for resins
266 coming from coniferous trees were identified in all analyzed ibis' mummies (Figures 3). Further
267 chemical analysis focused on interpretation of these diterpenic markers revealed clearly the
268 presence of dehydroabietic acid (molecular peak M⁺ 372 and base peak fragment ion of *m/z*
269 239 B⁺), 7-oxodehydroabietic acid (M⁺ 386 and fragment ion of *m/z* 253 B⁺), 15-hydroxy-7-
270 oxo-dehydroabietic acid (M⁺ 460 and fragment ion of *m/z* 445 B⁺), 3-hydroxydehydroabietic
271 acid and 7- hydroxydehydroabietic acid in the sample 90002491 (Figure 3). These oxidized and
272 dehydrogenated abietane structures serve as markers for presence of coniferous diterpenic
273 resins obtained from trees belonging to the Pinaceae family (Brettell et al., 2017; Buckley and
274 Evershed, 2001; Colombini et al., 2000; Degano and Colombini, 2009). Normally, in fresh
275 diterpenic resins of coniferous trees, dehydroabietic acid is present but as a minor constituent
276 unlike abietic acid which acts as a primary and most abundant component. However, with
277 natural alteration processes (ageing), abundance of dehydroabietic acid starts to decrease and,
278 other compounds are starting to appear like already mentioned 7-oxo-dehydroabietic acid which
279 is the major oxidized diterpenoid, after dehydroabietic acid, present in the analyzed ibis'
280 samples. These highly oxidized compounds indicate an advanced state of oxidation of conifer
281 resin.

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

285 occur during the preparation of wood tar with releasing of methanol during pyrolysis of the
286 resinous wood (Colombini et al., 2005; Izzo et al., 2013). Retene (M^{+} 234 and fragment ion of
287 m/z 219 B^{+}) was also identified in samples 90002486 and 90002491. This last compound is
288 considered as a marker for pine pitch because when the resin is heated to high temperatures or
289 distilled in a low oxygen environment to obtain a pitch, aromatisation, demethylation, and
290 defunctionalisation reactions occur in the abietadiene and pimaradiene acids (Colombini et al.,
291 2005). These phenomena lead to the formation of a series of products as norabietatrienes and
292 tetrahydroretene, with retene as the final stable product of all these transformations.
293 Furthermore, we can argue that in samples 90002486 and 90002491 combined abundance of
294 methyl esters with presence of retene indicated that a high-temperature pre-application
295 processing of this resin was applied (Brettell et al., 2017; Jones et al., 2018). On the contrary,
296 presence of retene was not confirmed in samples 90002472 and 90002475, meaning that
297 resinous material employed for mummification was subjected to low-temperature pre-
298 application heating. Additionally, we can argue that this particular ingredient used for
299 mummification of these mummies, had to be imported from outside of the territory of Ancient
300 Egypt and such claims are already reported in ancient texts from mid-first century AD
301 (Chapman, 2016).

302

303 3.2.3 Presence of plant oil and/or animal fat

304

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

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

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

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

334 Furthermore, the presence of odd chain fatty acids in archaeological samples, should
335 also be taken into particular consideration. Namely, non-negligible quantities of C_{15:0} (M⁺ 314
336 and diagnostic fragment ions of *m/z* 75, 117, 299) and C_{17:0} (M⁺ 342 and diagnostic fragment
337 ions of *m/z* 75, 117, 327) could be indicative of presence of ruminant fat in the original
338 embalming recipe (sheep, goat, cattle, etc.) applied in mummification of samples 90002472,
339 90002475, 90002486 and 90002491 (Donato et al., 2013; Eerkens, 2005; Lantos et al., 2018,
340 2017; Łucejko et al., 2017). In an extensive and extremely comprehensive study conducted by
341 Eerkens (2005), it was strongly suggested that quantifying the ratios between odd (C_{15:0} + C_{17:0})
342 and even chain fatty acids (C_{12:0} + C_{14:0} + C_{16:0} + C_{18:0}) could provide insights whether we can
343 denote or deny the presence of ruminant fat in studied samples. In the aforementioned research,
344 it was pointed out that the calculated values that are higher than 0.04 are distinctive for ruminant
345 animals, which was the case in all four analyzed samples of ibis mummies (~0.05).
346 Nevertheless, we should be careful with this, given the fact that the presence of C_{15:0} and C_{17:0}
347 could result from soil bacterial degradation of lipids within the body of the animals. Indeed,
348 contamination from body fluids of animal mummies is often an issue in this type of analyses.

349 In the light of the foregoing discussion, it would seem difficult to achieve a more precise
350 plant oil or animal fat origin assessment, present in studied balms, based exclusively on
351 presence of the fatty acid profile in the analyzed samples. Embalmers could have been using a
352 mixture of different plant oils and animal fats, and more importantly, due to degradation
353 pathways of major diagnostic unsaturated components, which is confirmed by the presence of
354 short chain dicarboxylic acids in the studied samples. Regarding the presence of the
355 aforementioned dicarboxylic acids it was clearly observed in gas chromatograms of every
356 sample that they represent the second most abundant components in the acidic profile. Further
357 assessment of data identified presence of α, ω – dicarboxylic acids (“diacids”) in the C₄ to C₁₂
358 carbon chain length range, with azelaic (diC₉, nonanedioic acid) as the most abundant one
359 together with suberic and sebacic diacids. Dicarboxylic acids are naturally not present in plant
360 oils, resins, waxes and animal fat; they are formed as a result of several different oxidative
361 degradation mechanisms of the double bond(s) in mono-, or poly-unsaturated fatty acids. Thus,
362 the position of the double bond is directly related to the carbon chain length of the diacids
363 formed. Therefore, the presence and the amount of diC₉ (azelaic acid, originating from C₁₈ fatty
364 acid with double bond between carbons 9 and 10 like linoleic and linolenic acids) combined
365 with presence of diC₈ (suberic acid) and diC₇ (pimelic acid) acids are indicators of degraded
366 vegetable oils, thus suggesting that a siccativ e or semi-siccativ e plant oil high in oleic acid was
367 used during mummification of all four samples that are the subject of this study (Brettell et al.,
368 2017; Colombini et al., 2005; Copley et al., 2005; Degano and Colombini, 2009; Jones et al.,
369 2018; La Russa et al., 2014; Łucejko et al., 2017, 2012; Tchapla et al., 2004).

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

378

379 4. Discussion

380 Up to this date, only few scientific articles dealing with animal mummification are
381 available to the scientific community (Brettell et al., 2017; Buckley et al., 2004) and total
382 number of four balms from other votive ibis mummies were chemically characterized.
383 Therefore, it is important to realize a comparison between the ibis balms of this study and those
384 described in the specialized literature. Unlike the ibis mummies that are the subject of this study,
385 in article published by Buckley *et al.* (2004) it was argued that embalming agents consisted,
386 mainly, of hydrolyzed plant sugar gum used for securing the mummy wrappings. Additionally,
387 only trace quantities of fatty acids were detected with high amounts of C₁₆ to C₁₈ ratios, which,
388 according to authors of that paper, indicated oil of plant origin (Buckley et al., 2004; Buckley
389 and Evershed, 2001). However, it is important to emphasize the fact that the mummy from
390 Buckley *et al.* study originate from XXVI-XXX dynasty period (664-343 BC) as opposed to
391 sample 90002491 (Roda) which was dated to the very end of the Ptolemaic period (92-65 cal
392 BC) (Richardin et al., 2017). Given the fact that both of these samples originate from
393 archaeological sites in Lower Egypt it is possible to consider the difference in their embalming
394 strategy. According to numerous research papers, it is argued that the increase in use of conifer
395 resin exudates in everyday and religious life of Egyptian societies started with Ptolemaic and
396 Roman periods possibly due to relative abundance and ease of extraction (Colombini et al.,
397 2000). Number of research works, dealing with human mummification practices, demonstrated
398 that in majority of cases, presence of conifer resins acts in a certain way as indispensable part
399 of embalming material that were applied during mummification due to their highly valued
400 antimicrobial properties (Buckley and Evershed, 2001; Colombini et al., 2000; Degano and
401 Colombini, 2009; Ikram, 2017).

402 On the other hand, results of three ibis mummies published by Brettell *et al.* (2017), are
403 more similar with those obtained in this study. For two mummies it was determined that they
404 belong to Ptolemaic (Inv. Num. 165, Sakkara site) and Roman period (Inv. Num.162). As in
405 this study, paper from Brettell *et al.* showed that the main component of the analyzed balm
406 contained either plant oil or animal fat, or combination of both. Also, the diterpenoids were
407 confirmed through presence of Pinaceae resin in sample 471, while in sample 162 triterpenoids
408 were observed through presence of *Pistacia spp.* resin which contrasts results obtained in this
409 study with the absence of triterpenoids in all of the samples. In this study the presence of retene,
410 observed in samples 90002486 and 90002491 which acts as a diagnostic marker for extensive
411 heating, was not detected in any of the samples studied by Brettell *et al.* This difference begs the
412 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 (C₂₆, C₂₈, C₃₀, C₃₂). 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

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

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

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

456

457 **Conflict of interest**

458 The authors declare that they have no conflict of interest.

459

460 **Acknowledgements**

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

470

471

472 **References**

473

- 474 Abdel-Maksoud, G., El-Amin, A.R., 2011. A review on the materials used during the
475 mummification processes in ancient Egypt. *Mediterr. Archaeol. Archaeom.* 11, 129–150.
- 476 Arriaza, B.T., Cardenas-Arroyo, F., Kleiss, E., Verano, J.W., 1998. South American
477 mummies: culture and disease, in: *Mummies, Disease & Ancient Cultures*. Cambridge
478 University Press, Cambridge, pp. 190–237.
- 479 Bard, K., 2007. *An introduction to the archaeology of ancient Egypt*. Blackwell Publishing,
480 Oxford.
- 481 Bellamy, L., 1980. *The infrared spectra of complex molecules, vol. 2, advances in infrared*
482 *group frequencies*. Chapman and Hall Ltd., London and New York.
483 [https://doi.org/10.1016/s0022-328x\(00\)85832-5](https://doi.org/10.1016/s0022-328x(00)85832-5)
- 484 Brettell, R., Martin, W., Atherton-Woolham, S., Stern, B., McKnight, L., 2017. Organic
485 residue analysis of Egyptian votive mummies and their research potential. *Stud. Conserv.*
486 62, 68–82. <https://doi.org/10.1179/2047058415y.0000000027>
- 487 Bruni, S., Guglielmi, V., 2014. Identification of archaeological triterpenic resins by the non-
488 separative techniques FTIR and ¹³C NMR: The case of Pistacia resin (mastic) in
489 comparison with frankincense. *Spectrochim. Acta - Part A Mol. Biomol. Spectrosc.* 121,
490 613–622. <https://doi.org/10.1016/j.saa.2013.10.098>
- 491 Buckley, S.A., Clark, K.A., Evershed, R.P., 2004. Complex organic chemical balms of
492 Pharaonic animal mummies. *Nature* 431, 294–299. <https://doi.org/10.1038/nature02849>
- 493 Buckley, S.A., Evershed, R.P., 2001. Organic chemistry of embalming agents in Pharaonic
494 and Graeco-Roman mummies. *Nature* 413, 837–841. <https://doi.org/10.1038/35101588>
- 495 Chapman, S.L., 2016. *The embalming ritual of late period through Ptolemaic Egypt*.
496 University of Birmingham.
- 497 Clark, K.A., 2006. *Tracing the Evolution of Organic Balm use in Egyptian Mummification*
498 *via Molecular and Isotopic Signatures*. University of Bristol.
- 499 Colombini, M.P., Giachi, G., Modugno, F., Ribechini, E., 2005. Characterisation of organic
500 residues in pottery vessels of the Roman age from Antinoe (Egypt) 79, 83–90.
501 <https://doi.org/10.1016/j.microc.2004.05.004>
- 502 Colombini, M.P., Modugno, F., Silvano, F., Onor, M., 2000. Characterization of the Balm of
503 an Egyptian Mummy from the Seventh Century B.C. *Stud. Conserv.* 45, 19–29.
504 <https://doi.org/10.2307/1506680>
- 505 Copley, M.S., Bland, H.A., Rose, P., Horton, M., Evershed, R.P., 2005. Gas chromatographic,
506 mass spectrometric and stable carbon isotopic investigations of organic residues of plant
507 oils and animal fats employed as illuminants in archaeological lamps from Egypt.
508 *Analyst* 130, 860–871. <https://doi.org/10.1039/b500403a>
- 509 Cuní, J., Cuní, P., Eisen, B., Savizky, R., Bové, J., 2012. Characterization of the binding
510 medium used in Roman encaustic paintings on wall and wood. *Anal. Methods* 4, 659–
511 669. <https://doi.org/10.1039/c2ay05635f>
- 512 David, R., 2008. Egyptian mummies: an overview, in: David, R. (Ed.), *Egyptian Mummies*
513 *and Modern Science*. Cambridge University Press, Cambridge, pp. 10–21.

- 514 Degano, I., Colombini, M.P., 2009. Multi-analytical techniques for the study of pre-
515 Columbian mummies and related funerary materials. *J. Archaeol. Sci.* 36, 1783–1790.
516 <https://doi.org/10.1016/j.jas.2009.04.015>
- 517 Donato, P., Dugo, P., Mondello, L., 2013. Chapter 9 - Separation of Lipids, Liquid
518 Chromatography. Elsevier Inc. <https://doi.org/10.1016/B978-0-12-415806-1.00009-7>
- 519 Eerkens, J.W., 2005. GC-MS analysis and fatty acid ratios of archaeological potsherds from
520 the western Great Basin of North America. *Archaeometry* 47, 83–102.
521 <https://doi.org/10.1111/j.1475-4754.2005.00189.x>
- 522 Evershed, R.P., 2008. Organic residue analysis in archaeology: The archaeological biomarker
523 revolution. *Archaeometry* 50, 895–924. <https://doi.org/10.1111/j.1475-4754.2008.00446.x>
- 525 Evershed, R.P., Dudd, S.N., Copley, M.S., Berstan, R., Stott, A.W., Mottram, H., Buckley,
526 S.A., Crossman, Z., 2002. Chemistry of Archaeological Animal Fats. *Acc. Chem. Res.*
527 35, 660–668.
- 528 Font, J., Salvadó, N., Butí, S., Enrich, J., 2007. Fourier transform infrared spectroscopy as a
529 suitable technique in the study of the materials used in waterproofing of archaeological
530 amphorae. *Anal. Chim. Acta* 598, 119–127. <https://doi.org/10.1016/j.aca.2007.07.021>
- 531 Heron, C., Nemcek, N., Bonfield, K.M., Dixon, D., Ottaway, B.S., 1994. The Chemistry of
532 Neolithic Beeswax. *Naturwissenschaften* 81, 266–269.
533 <https://doi.org/10.1007/s001140050069>
- 534 Ikram, S., 2017. Animals in ancient Egyptian religion: belief, identity, power, and economy,
535 in: Abarella, U., Rizzetto, M., Russ, H., Vickers, K., Viner-Daniels, S. (Eds.), *The*
536 *Oxford Handbook of Zooarchaeology*. Oxford University Press, Oxford, pp. 1–15.
537 <https://doi.org/10.1093/oxfordhb/9780199686476.013.30>
- 538 Izzo, F.C., Zendri, E., Bernardi, A., Balliana, E., Sgobbi, M., 2013. The study of pitch via gas
539 chromatography-mass spectrometry and Fourier-transformed infrared spectroscopy: The
540 case of the Roman amphoras from Monte Poro, Calabria (Italy). *J. Archaeol. Sci.* 40,
541 595–600. <https://doi.org/10.1016/j.jas.2012.06.017>
- 542 Jones, J., Higham, T.F.G., Chivall, D., Bianucci, R., Kay, G.L., Pallen, M.J., Oldfield, R.,
543 Ugliano, F., Buckley, S.A., 2018. A prehistoric Egyptian mummy: Evidence for an
544 ‘embalming recipe’ and the evolution of early formative funerary treatments. *J.*
545 *Archaeol. Sci.* 100, 191–200. <https://doi.org/10.1016/j.jas.2018.07.011>
- 546 La Russa, M.F., Ruffolo, S.A., Belfiore, C.M., Comite, V., Casoli, A., Berzioli, M., Nava, G.,
547 2014. A scientific approach to the characterisation of the painting technique of an author:
548 The case of Raffaele Rinaldi. *Appl. Phys. A Mater. Sci. Process.* 114, 733–740.
549 <https://doi.org/10.1007/s00339-013-7866-1>
- 550 Lantos, I., Orgaz, M., Panarello, H.O., Maier, M.S., 2017. Preliminary molecular evidence of
551 feasting in the Inca site of Fuerte Quemado-Intihuatana, Catamarca, Argentina. *J.*
552 *Archaeol. Sci. Reports* 14, 580–590. <https://doi.org/10.1016/j.jasrep.2017.06.031>
- 553 Lantos, I., Palamarczuk, V., Orgaz, M., Ratto, N., Maier, M., 2018. Exploring the culinary
554 uses of Santa María and Belén painted vessels from the Late Intermediate Period in
555 Catamarca, Argentina. *J. Archaeol. Sci. Reports* 18, 660–667.

- 556 <https://doi.org/10.1016/j.jasrep.2017.03.019>
- 557 Lortet, L., Gaillard, C., 1903. La Faune Momifiée de l’Ancienne Égypte, Archives du
558 Museum d’Histoire naturelle de Lyon. Lyon.
- 559 Łucejko, J., Connan, J., Orsini, S., Ribechini, E., Modugno, F., 2017. Chemical analyses of
560 Egyptian mummification balms and organic residues from storage jars dated from the
561 Old Kingdom to the Copto-Byzantine period. *J. Archaeol. Sci.* 85, 1–12.
562 <https://doi.org/10.1016/j.jas.2017.06.015>
- 563 Łucejko, J.J., Lluveras-Tenorio, A., Modugno, F., Ribechini, E., Colombini, M.P., 2012. An
564 analytical approach based on X-ray diffraction, Fourier transform infrared spectroscopy
565 and gas chromatography/mass spectrometry to characterize Egyptian embalming
566 materials. *Microchem. J.* 103, 110–118. <https://doi.org/10.1016/j.microc.2012.01.014>
- 567 Ménager, M., Azémard, C., Vieillescazes, C., 2014. Study of Egyptian mummification balms
568 by FT-IR spectroscopy and GC-MS. *Microchem. J.* 114, 32–41.
569 <https://doi.org/10.1016/j.microc.2013.11.018>
- 570 Mezzatesta, E., Perraud, A., Vieillescazes, C., Mathe, C., 2020a. GC-MS and PCA analyses of
571 diterpenoids degradation state in 21 human mummies of Ancient Egypt dating from New
572 Kingdom to Graeco-Roman Period. *J. Cult. Herit.* - IN PRESS corrected proof.
573 <https://doi.org/10.1016/j.culher.2020.09.008>
- 574 Mezzatesta, E., Perraud, A., Vieillescazes, C., Mathe, C., 2020b. A new approach to analyze
575 balms from Egyptian human mummies by Solid Phase Extraction and Gas chromatography
576 coupled to mass spectrometry. *J. Sep. Sci.* - Revised manuscript submitted (Reference:
577 [jssc.202000746.R2](https://doi.org/10.1016/j.jssc.202000746.R2))
- 578 Porcier, S., Richardin, P., Louarn, G., Ikram, S., Berthet, D., 2019. Datations par le carbone
579 14 de 63 momies animales du musée des Confluences à Lyon (France), in: Porcier, S.,
580 Ikram, S., Stéphanie, P. (Eds.), *Creatures of Earth, Water, and Sky: Essays on Animals in*
581 *Ancient Egypt and Nubia*. Sidestone Press, Leiden, pp. 283–292.
- 582 Richardin, P., Porcier, S., Ikram, S., Louarn, G., Berthet, D., 2017. Cats, Crocodiles, Cattle,
583 and More: Initial Steps Toward Establishing a Chronology of Ancient Egyptian Animal
584 Mummies. *Radiocarbon* 59, 595–607. <https://doi.org/10.1017/rdc.2016.102>
- 585 Sakurai, K., Ogata, T., Morimoto, I., Long-Xiang, P., Zhong-Bi, W., 1998. Mummies from
586 Japan and China, in: Cockburn, A., Cockburn, E., Reyman, T.A. (Eds.), *Mummies,*
587 *Disease & Ancient Cultures*. Cambridge University Press, Cambridge, pp. 308–336.
- 588 Scalarone, D., Lazzari, M., Chiantore, O., 2002. Ageing behaviour and pyrolytic
589 characterisation of diterpenic resins used as art materials: Colophony and Venice
590 turpentine. *J. Anal. Appl. Pyrolysis* 64, 345–361. [https://doi.org/10.1016/S0165-2370\(02\)00046-3](https://doi.org/10.1016/S0165-2370(02)00046-3)
- 592 Svečnjak, L., Baranović, G., Vinceković, M., Prđun, S., Bubalo, D., Gajger, I.T., 2015. An
593 approach for routine analytical detection of beeswax adulteration using ftir-atr
594 spectroscopy. *J. Apic. Sci.* 59, 37–49. <https://doi.org/10.1515/JAS-2015-0018>
- 595 Tchaplá, A., Méjanelle, P., Bleton, J., Goursaud, S., 2004. Characterisation of embalming
596 materials of a mummy of the ptolemaic era. Comparison with balms from mummies of
597 different eras. *J. Sep. Sci.* 27, 217–234. <https://doi.org/10.1002/jssc.200301607>

598 **Table 1**
 599 FT-IR data and interpretation of the obtained results
 600

90002472	90002475	90002486	90002491	Corresponding bond	Source
3418	3408 3278 3067	3419	3420	OH stretching	Organic material
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 (C22:0)		✓	✓	✓
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)				✓

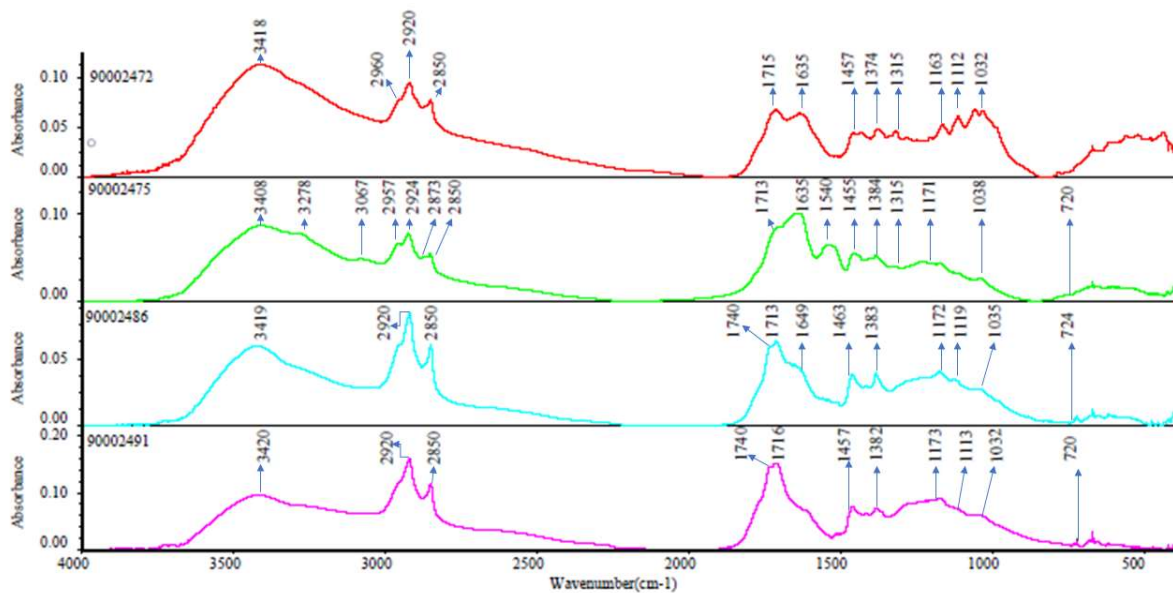


607

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

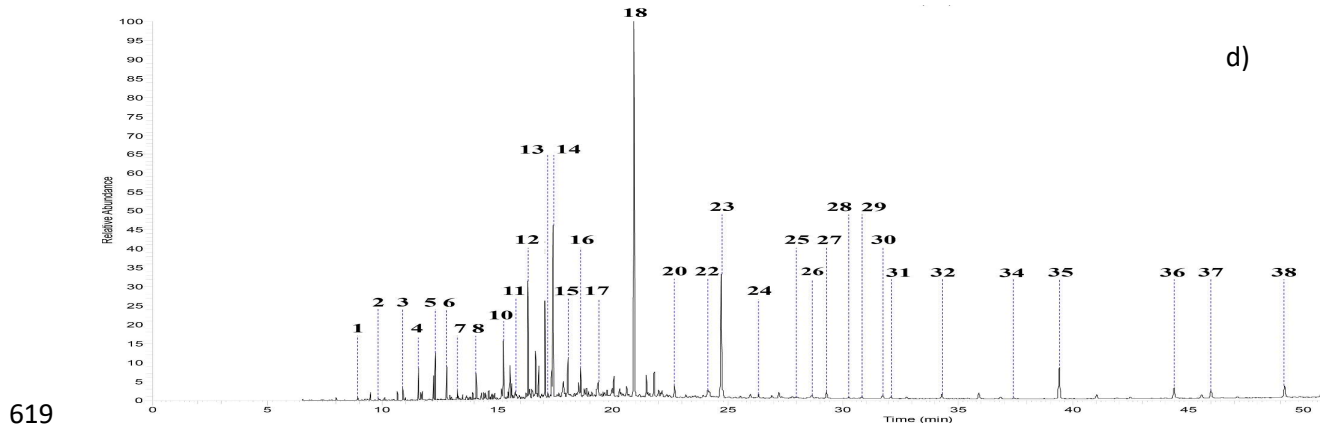
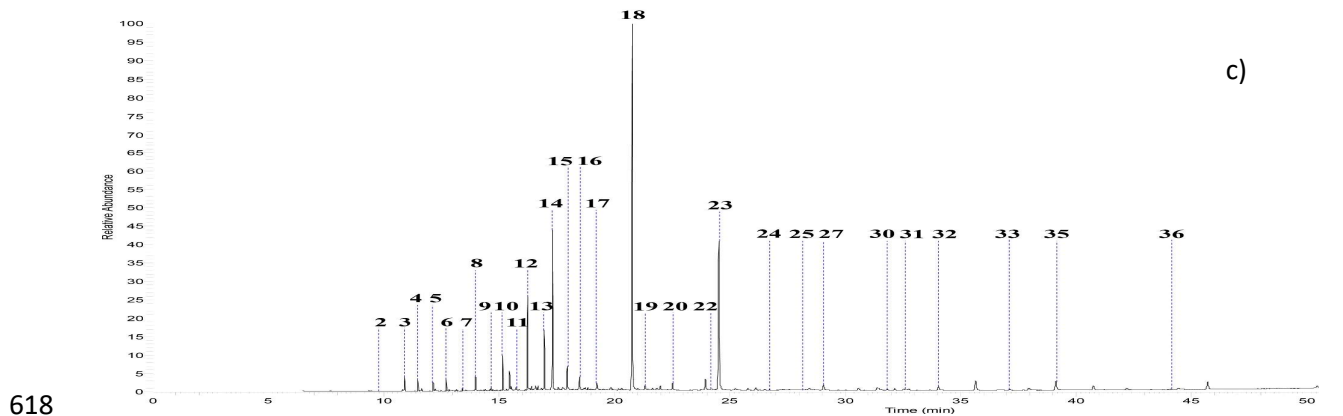
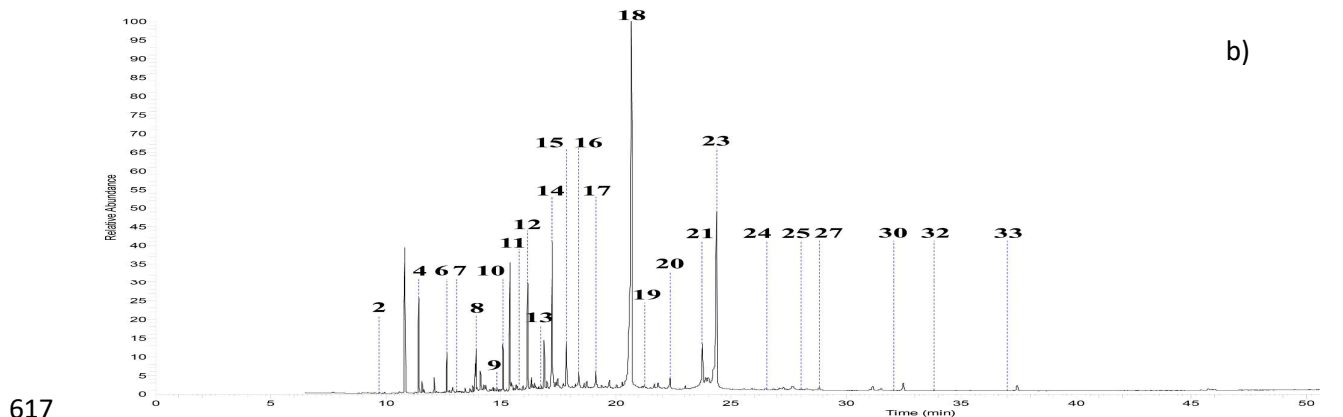
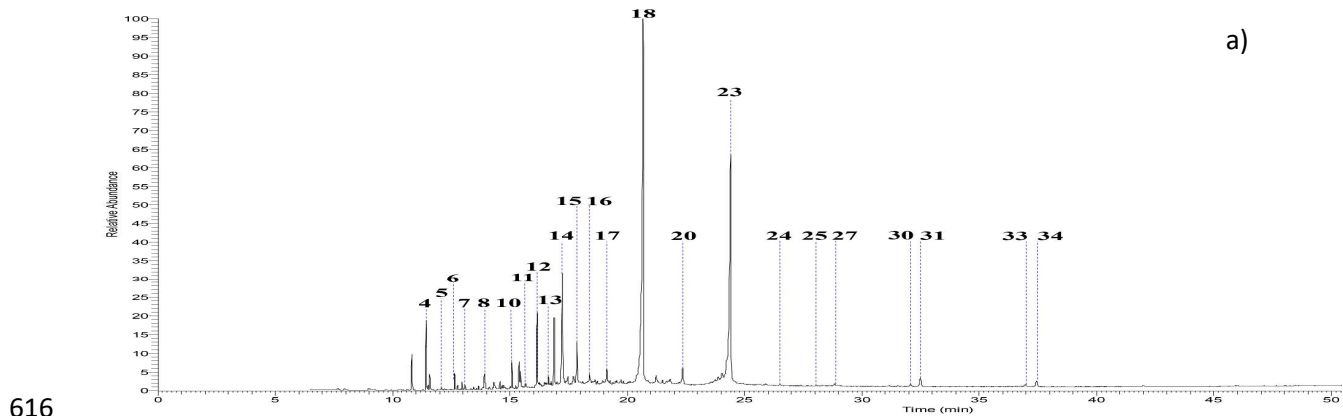
612



613

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

615



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