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1 **Anthropological and analytical studies of two Egyptian human mummies' heads from Deir-el-Medineh**

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11

12 **Abstract**

13 This paper focused on a multidisciplinary study of mummification balms' constituents from two Egyptian
14 mummies' heads belonging to the Osteology collection of the Confluences museum in Lyon (France). These
15 individuals come from the same necropolis, Deir el-Medineh in Upper Egypt. They were coated with particular
16 substances not often encountered in studies of mummification balms. The objective was to identify the
17 composition of the two types of samples found on each individual: an amorphous material resembling mummy
18 balm and a sandy substance. The knowledge of the anthropology and the mummification practices associated to
19 analytical chemistry study of the balms permitted to obtain a global characterization of these persons. The FTIR
20 and GC-MS results notably permitted to observe (i) the presence of fat and beeswax within the amorphous black
21 samples and (ii) the presence of natron, fatty matter and mastic resin within the sandy samples; this types of
22 samplings are uncommon in ancient Egypt.

23 **Keywords:** Ancient Egypt, mummification practices, GC-MS, FTIR.

24 **1. INTRODUCTION**

25 Mummification was a fundamental part of funerary practice in ancient Egypt. The Egyptians believed rebirth in
26 the afterlife and, in order to be able to access this afterlife, it was necessary to preserve the body [1]. The
27 application of balms was a key part of the mummification process in ensuring successful and lasting preservation
28 of the body. A wide variety of natural substances can be used in the formulation of balms, including plant oils or

29 animal fats, di- or triterpenic resins, bitumen or even beeswax [2,3]. Given the molecular richness of these
30 materials, their identification within an archaeological sample represents a real challenge. Indeed, these materials
31 can undergo oxidation, degradation and alteration processes, naturally with time (aging) or due to anthropic
32 factors, such as heating temperature. Degraded molecules can play an important role in species identification. To
33 carry out this work, it was necessary to use different analytical techniques, each providing complementary
34 information, such as Fourier transform infrared spectroscopy (FTIR) and gas chromatography coupled to a mass
35 spectrometer (GC-MS) [4]. The aim of this study was to provide a better knowledge of the mummification
36 process for these two individuals by analyzing the chemical composition of their mummification balms. Two
37 individuals were chosen because of the unusual but identical appearance of their balm.

38 **2. MATERIALS AND METHODS**

39 **2.1. Archaeological samples**

40 The Osteology collection of the Confluences museum in Lyon (France) contains about 250 Egyptian mummies'
41 heads and skulls, which were collected at the end of the 19th century. Louis Lortet and Ernest Chantre brought
42 back from their excavations in Egypt mummies' skulls and heads. All the mummified heads constitute a
43 important collection for the anthropologic study of ancient Egypt. The choice to take only the heads of the
44 mummies to come back in France corresponded to the anthropologists' practices at the end of the 19th century
45 and much knowledge about individuals has unfortunately been lost forever. Two individuals were selected for
46 this study: Nos. 30000150 and 30000306.

47 **2.2. Infrared spectroscopy**

48 A Thermo Scientific™ Nicolet™ iN10 and iZ10 Fourier transform infrared spectrometer in transmission mode
49 and a DTGS (Deuterated Triglycine Sulfate) detector were used. The main iN10 module is an integrated
50 microscope for imaging and the iZ10 add-on module that allows analysis of KBr pellets without imaging. For a
51 transmission analysis, the sample is placed in the infrared beam, the energy generated is measured to give a
52 spectrum. The DTGS detector is a pyroelectric thermodetector, which means that it reacts to a change in
53 temperature due to a variation in electrical polarization. It is sensitive from 4000 to 400 cm^{-1} and operates at
54 room temperature. All the resulting spectra were interpreted from the bands obtained and their respective wave
55 numbers, but also with the help of a database containing spectra of reference materials and according to
56 scientific literature.

57 2.3. Solvents and reagents

58 All solvents and reagents were of analytical grade. Hexane and *N,O*-
59 bis(trimethylsilyl)trifluoroacetamide/Trimethylchlorosilane (BSTFA/TMCS) were supplied by Sigma-Aldrich.
60 Dichloromethane was supplied by Merck.

61 2.4. Gas Chromatography – Mass Spectrometry (GC-MS)

62 2.4.1. Apparatus

63 GC-MS analyzes were performed with a Thermo Scientific Focus gas chromatographic system composed by a
64 Thermo Scientific AI 3000 auto-sampler coupled with an ITQ 700 ion trap mass spectrometer (Thermo Fisher
65 Scientific). A GC fused silica capillary column Thermo trace GOLD TG-5MS (5% diphenyl / 95%
66 dimethylpolysiloxane, 30 m length × 0.25 mm i.d. × 0.25 μm film thickness) was used. The carrier gas was
67 helium with a constant flow of 1 mL.min⁻¹. 1 μL of sample was injected in splitless mode. Mass spectra were
68 recorded in electron impact mode with an electron ionization voltage of 70 eV, an ionization time of 25,000 μs
69 and a mass range of 40–650 *m/z*. The injector temperature was set at 250 °C. Transfer line, ion trap and manifold
70 temperatures were respectively set at 300 °C, 200 °C and 50 °C The separation was achieved with the following
71 temperature programme: 50 °C with a 2 min hold, increased at 10°C.min⁻¹ to 200 °C, then at 2.5 °C.min⁻¹ to 310
72 °C, finally increased at 8°C.min⁻¹ to 330 °C and held isothermally for 3 min.

73 2.4.2. Preparation of samples

74 10 mg of material was extracted with 1 mL of dichloromethane (DCM) and ultrasonic was delivered for 10
75 minutes. The solution was then centrifuged for 10 minutes at 6000 rpm. The supernatant was left aside and the
76 solid pellet could be extracted once again. This phase was carried out twice more. Under a stream of nitrogen,
77 the three portions were collected, mixed, and evaporated to dryness. The extract was trimethylsilylated in 200 μL
78 of BSTFA/TMCS (99/1 v/v) and heated at 70°C for 30 minutes. The mixture was then evaporated to dryness and
79 dissolved in 0.2 to 1 mL of a hexane/DCM mixture (1/1, v/v). 1 μL of the solution was injected in the GC-MS
80 apparatus.

81 3. RESULTS AND DISCUSSION

82 The mummy's head No. 300000150 (Fig 1A) is a mature adult man from Deir-el-Medineh. Concerning the
83 mummification practices, there were a brain removal by trans-ethmoidal way with nasal and endocranial stuffing
84 by a heterogeneous mixture.



85

86 The mummy's head No 30000306 (Fig 2A) is a young adult woman from Deir-el-Medineh. The skull is
87 characterized by a *post-mortem* bilateral temporoparietal and left occipital fracture line. Concerning the
88 mummification practices, there were a brain removal by ethmoidal way.

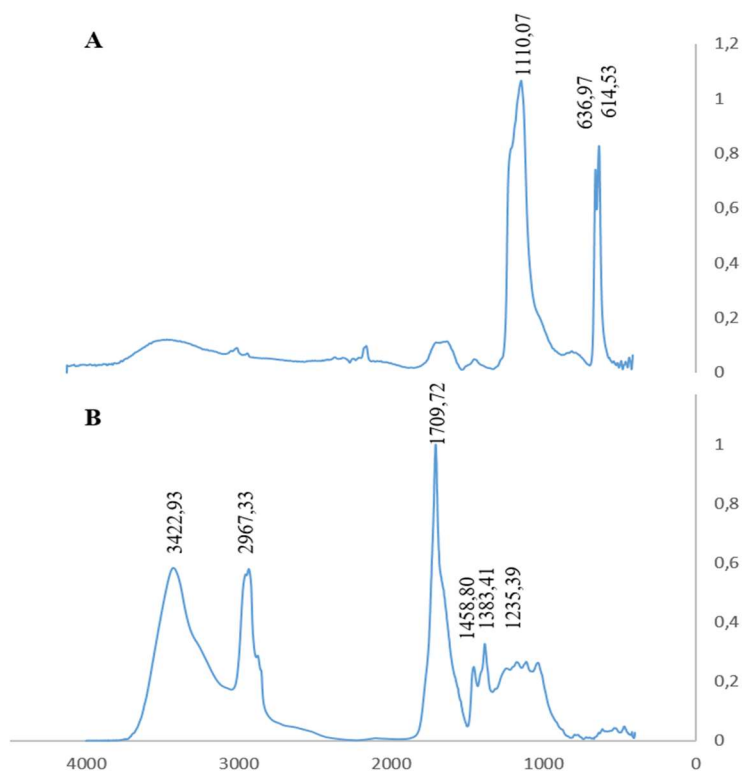


89

90 For both of the individuals, two samplings were realized; the first one were balm matter, a black, shiny
91 amorphous substance commonly observed on mummies and the second one were colored sand grains not often
92 encountered. For the balm matter, the sample Nos. 30000150a (Fig 1B) comes from left ear and the sample No.
93 30000306a (Fig 2B), right temple. For the sandy matter, the samples Nos. 30000150b (Fig 1C) and 30000306b
94 (Fig 2C) are respectively present inside the surface of the skulls. Radiocarbon dating was not made because of
95 the degradation state of the tissues.

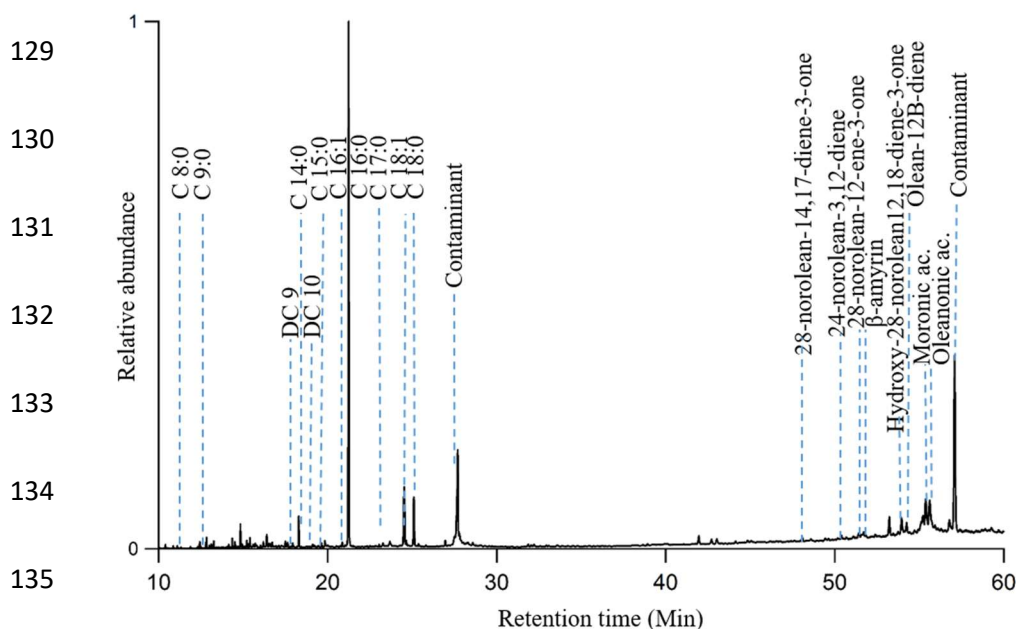
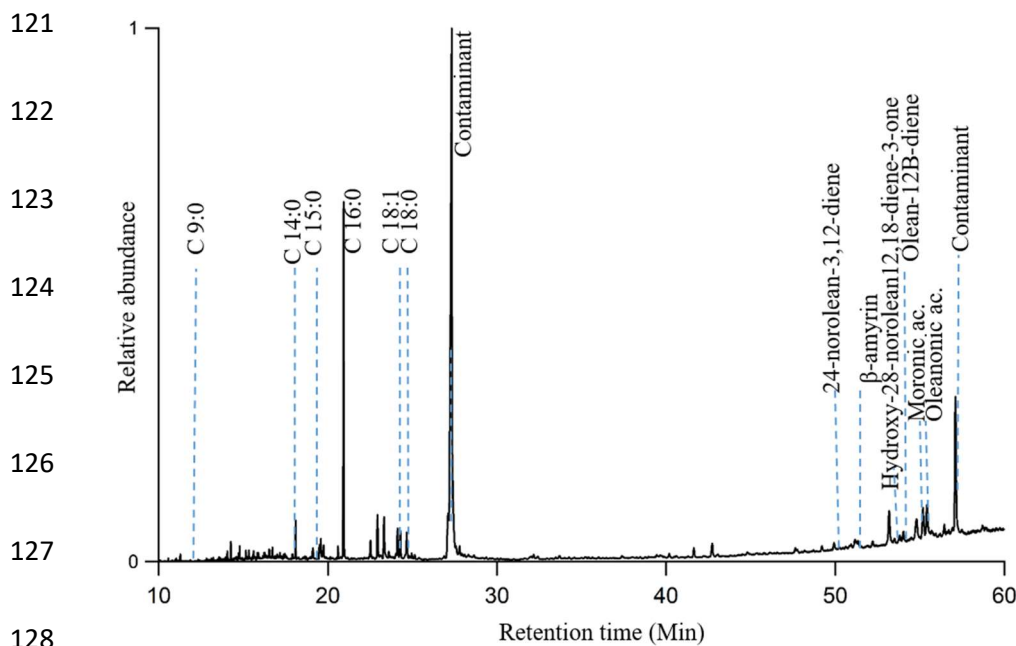
96 The analyzes by gas chromatography of balms' samples Nos. 3000150a and 30000306a provided a very high
97 proportion of fatty matter. It has been possible to identify lauric, myristic, palmitic, stearic, palmitoleic and oleic
98 acids, and also beeswax and traces of diterpenic resin. This mixture of substances is very common in the
99 formulation of balms [5,6]. It is however possible to note that the chromatograms of these two samples coming
100 from two different individuals are perfectly similar.

101 For the two other samples Nos. 30000150b and 30000306b, a first approach was realized by infrared
102 spectroscopy given the heterogeneity of the matters and their sandy aspect. The figure 3 presents the FT-IR
103 analyzes of the material grains of the sample 3000306b.



104

105 Two different spectra were obtained by analyzing the material grains of the archaeological sample. The infrared
 106 spectrum (Fig 3A) is characteristic of an inorganic compound because it has several bands between 1200 and
 107 600 cm^{-1} and does not absorb between 4000 and 1200 cm^{-1} . Two bands at 1150 and 1110 cm^{-1} and two others at
 108 640 and 614 cm^{-1} correspond to the elongation vibrations of the S-O bond of sodium sulfate. It is one of the main
 109 constituents of natron. Many bands are visible on the spectrum (Fig 3B), including bands characteristic of
 110 organic matter: the elongation bands at 3420 cm^{-1} , specific of O-H bonds, around 2960 cm^{-1} characteristic of C-
 111 H bonds, at 1705 cm^{-1} for the C=O bond, and the deformation bands at 1460, 1380 and 1235 cm^{-1} of these bonds
 112 [7]. The comparison of this spectrum with the data bank of the apparatus shows a correlation with more than
 113 80% with mastic resin. These results are identical for the sample 30000150b. Thus, it was possible to conclude
 114 that these sand grains were composed of two types of substances: an inorganic matter attributed to natron and an
 115 organic one. The organic part was analyzed by gas chromatography. In samples Nos. 30000150b and 30000306b
 116 (Fig 4), numerous fatty acids, monocarboxylic fatty acid (MC), were detected indicating the presence of fatty
 117 substances. This type of fatty acids indicates an unaltered fatty matter. In addition, triterpenic compounds, such
 118 as moronic and oleanonic acids were identified. All these molecules allow to define the formulation of these
 119 samples: fatty substance probably a plant oil or an animal fat in mixture with the resin of *Pistacia lentiscus* also
 120 called mastic resin.



4. Conclusion

The two individuals Nos. 30000150 and 30000306 have embalming substances with identical composition. This chemical balm composition was already described in the literature [8]. However, balm matter can be distinguished from sandy matter. The "balm" part is composed mainly of fat and beeswax, while the "sand" part contains natron, fatty substance and mastic resin. According to the authors' knowledge is the first time that balm matter associated to sandy matter were studied. It is possible to hypothesize that the embalming of these individuals was carried out in the same embalming atelier in Deir-el-Medineh with a particular know-how.

144

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173

Figure captions

Figure 1: Mummy No. 30000150 (A), balm sampling (B), sandy matter (C).

Figure 2: Mummy No. 30000306 (A), balm sampling (B), sandy matter (C).

Figure 3: Infrared spectrum from mummy No. 3000306a.

Figure 4: TIC chromatograms from mummy Nos. 30000150b and 30000306b.