# Chemical analyses of Egyptian mummification balms and organic residues from storage jars dated from the Old Kingdom to the Copto-Byzantine period

**Jeannette Łucejko[1,3], Jacques Connan[2], Erika Ribechini[3], Francesca Modugno[3], Sibilla Orsini[3]**

[1]Institute for the Conservation and Valorization of Cultural Heritage, National Research Council of Italy, Via Madonna del Piano, 10, I-50019 Sesto Fiorentino (Italy)

[2] 23 rue Saint-Exupéry, 64000- Pau, France

[3]University of Pisa, Department of Chemistry and Industrial Chemistry, Via Moruzzi 13, I-56124 Pisa (Italy)

## Abstract

Twenty three samples of Egyptian organic materials, spanning from the Old Kingdom to the Copto-Byzantine Period, were investigated by gas chromatography-mass spectrometry. The sample set was comprised of ten balm samples from human mummies, three balms from shrews, and ten samples of residues scraped from jars and amphora from storehouses.

This research program was undertaken with two main goals:

Firstly to provide complementary data on the mummification balms from both humans and animals with an emphasis on the occurrence of bitumen in mummification mixtures.

Secondly to explore whether the jar residues were mixtures that were used for mummification purposes or whether they were pure ingredients stored for various uses including ritual practices.

The analysis highlighted that the most abundant constituents of the mummification balms were: fats or oils, beeswax, conifer resin, pitch, mastic resin, castor oil, and bitumen. Balms from animal mummies were not found to be significantly different from the balms from human mummies. Residues from potsherds appeared to belong to two categories: pure products (fats and castor oil) and mixtures containing fats, Pinaceae resin and pitch, mastic resin, and castor oil, i.e. the constituents also identified in mummification balms. The mixtures were thus residues of preparations for ritual practices and embalming.

This study demonstrates that bitumen is underestimated by the chemical approach currently applied in most archaeometric studies of Egyptian organic residues, which are better suited for the identification of lipids and resinous materials. We thus applied a specific analytical design, targeted at bitumen. Bitumen from the Dead Sea was conclusively identified using the present day bitumen from the Dead Sea floating blocks, as well as several bitumens from mummification balms and bitumen lumps unearthed from the archaeological site of Tell Yarmouth near Jerusalem in Israel.

**Keywords:** Egypt, balms, mummies, Old Kingdom, Copto-Byzantine, organic residues, potsherds, storage jars, GC-MS, fatty acids, bitumen, conifer pitch, beeswax, conifer resin, castor oil, mastic resin.

## 1. Introduction

Recent advances in analytical techniques have enabled the chemical composition of various archaeological residues of organic materials from ancient Egypt to be investigated, in order to identify the ingredients used to prepare human and animal mummies (Buckley et al., 2004; Buckley and Evershed, 2001; Colombini et al., 2000; Ménager et al., 2014; Ménager et al., 2013; Perraud, 2012) and to investigate funerary artefacts such as boxes for canopic jars, ushabtis, Osiris statuettes, and coffins (Charrié-Duhaut, et al., 2007, Serpico, 2001). Even burial food, such as meat and poultry from Pharaonic tombs has been investigated (Clark, et al., 2013).

The various recipes used in mummification can be revealed by collecting data on the residues of organic materials stored in jars and amphoras. Such residues may be either ingredients stored for the subsequent preparation of mummification balms, or mixtures which were prepared and used for ritual or funerary purposes.

The aim of the study was to provide new data on the chemical composition of various mummification balms ranging from the New Kingdom to the 30$^{th}$ dynasty, i.e. between 1550 and 342 BC, and on several organic residues, scraped from the inside of a stone vase from the Old Kingdom, as well as from potsherds of jars and amphora covering the time range from the New Kingdom (1552-1069 BC) to the Copto-Byzantine period (395-645 AD).

The molecular profile of the balms was determined by gas chromatography-mass spectrometry (GC-MS). We adopted two different sample pre-treatments. The first is commonly used for characterising lipids and resinous materials in mummification balms, and is based on saponification, the extraction of neutral and acidic fractions, and derivatisation

before injection in the GC-MS (Lucejko, et al., 2012). For some samples, we also adopted a specific analytical procedure designed for the analysis of petroleum and rock extracts and for the identification of bitumen (Connan, 2012, Connan and Dessort, 1991).

The main question raised by the organic residues stuck on various potsherds from the various containers was: were they examples of the storage of pure organic materials (foodstuff? ingredients for special uses?) or mixtures of several natural products prepared for a specific purpose, for instance for embalming practices?

## 2. Experimental

### 2.1 Samples

The samples are described in Table 1 and are subdivided into three classes:

1- Samples from the embalming of human mummies. Ten samples were collected from various parts of the mummies (skull, chest, shroud) with an emphasis on samples inside the skull (Fig. 1). One sample came from a Duamoutef canopic jar and was associated with embalmed viscera. The dates of the mummies spanned from the New Kingdom (1550-1070 BC) to the Third Intermediate Period (22$^{nd}$ dynasty, 945-715 BC). The mummies all came from the Thebes area.

2- Samples from the embalming of animal mummies. Three samples were collected from the remains of shrews in the area of Guizeh, and dated from the 30$^{th}$ dynasty (380-342 BC),

3- Resin-like samples scraped from containers: one sample was extracted from a stone vase from the Old Kingdom, which was discovered in a pit near Saqqara. The other nine samples were scraped from potsherds of jars and amphoras dated between the New Kingdom and the Copto-Byzantine period.

As references for the Dead Sea bitumen (Table 2), present-day specimens of pure bitumen floating on the Dead Sea surface, and archaeological bitumen, unearthed from the Tel Yarmouth site in Israel were used. To complete the references for a comparison data were also added on bitumen identified as Dead Sea bitumen in balms of Egyptian mummies (Connan, 2005, Macke, et al., 2002 ).


Fig. 1. The location of sample # 2774

**Table 1** Information on the analysed samples

| Sample number | Sample | Dynasty | Date range | Area | Context |
|---|---|---|---|---|---|
| **1896** | Balm of human mummy collected along the thoracic vertebra, on the right side of a man wrapped in bandages | New Kingdom | 1550-1070 BC | Thebes | Burial vault |
| **2504** | Red balm at the level of the neck, probably originally in the mouth. It was also located on the palate | XXIe or XXIIe | 1069-945 or 945-715 BC | Thebes | Tomb excavation |
| **2759** | Balm from human mummy | XXI-XXIIe | 1069-715 BC | Thebes | Tomb excavation |
| **2678** | Balm from a canopic jar with a jackal head (Duamoutef) containing remains of viscera wrapped in linen soaked with "black resin" | XXIIe | 945-715 BC | Thebes | Burial vault |
| **2679** | Balm stuck to the linen of the shroud. Black balm smeared over the shroud to ensure adhesion | XXIIe | 945-715 BC | Thebes | Tomb excavation near surface |
| **2680** | Balm in the mouth. Red balm filling the mouth, pressed agianst the tongue, retracted into the back of the mouth | XXIIe | 945-715 BC | Thebes | Tomb excavation near surface |
| **2681** | Balm from the thoracic cavity. The bottom of the left chest cavity is lined with a black balm, poured hot when embalming and forming a horizontal surface | XXIIe | 945-715 BC | Thebes | tomb excavation near surface |
| **2770** | Balm from human mummy originating from the excerebration hole in the skull | XXIIe | 945-715 BC | Thebes | Tomb excavation |
| **2771** | Balm in the skull (sag in the skull that runs from front to back) | XXIIe | 945-715 BC | Thebes | Tomb excavation |
| **2774** | Balm collected deep inside the pharynx, which is completely obstructed by the black material | XXIIe | 945-715 BC | Thebes | Tomb excavation |

| | | | | | |
|---|---|---|---|---|---|
| **2702** | Balm from a mummified shrew | XXXe | 380-342 BC | Giza | Necropolis excavation |
| **2703** | Balm from a mummified shrew | XXXe | 380-342 BC | Giza | Necropolis excavation |
| **2704** | Balm on linens from a shrew | XXXe | 380-342 BC | Giza | Necropolis excavation |
| | | | | | |
| **1811** | Deposit in a stone vase discovered in a pit | Old Kingdom | 2686-2181 BC | Saqqarah | Pit excavation |
| **2785** | Yellow powder with mineral grains | New Kingdom | 1552-1069 BC | Thebes | Temple storehouse |
| **2795** | Deposit scraped from a potsherd | New Kingdom | 1552-1069 BC | Thebes | Temple storehouse |
| **2775** | Reddish amorphous material scraped from a potsherd | XIX-XXe (Rammesid period) | 1296-1069 BC | Thebes | Temple storehouse |
| **2776** | Amorphous material scraped from a potsherd | XIX-XXe (Rammesid period) | 1296-1069 BC | Thebes | Temple storehouse |
| **2779** | Amorphous material on a jar handle | XIX-XXe (Rammesid period) | 1296-1069 BC | Thebes | Temple storehouse |
| **2783** | Gray flakes scraped from a potsherd | XIX-XXe (Rammesid period) | 1296-1069 BC | Thebes | Temple storehouse |
| **2786** | Yellow/Orange amorphous material scraped from a potsherd | Copto-Byzantine period | 395-645 AD | Thebes | Temple storehouse |
| **2790** | Deposit scraped from the bottom of an amphora | Copto-Byzantine period | 395-645 AD | Thebes | Temple storehouse |
| **2794** | Deposit scraped from the bottom of an amphora | Copto-Byzantine period | 395-645 AD | Thebes | Temple storehouse |

**Table 2** Gross composition, isotope and molecular data of samples used as references for bitumen. Sat.:saturated hydrocarbons; Aro.: aromatic hydrocarbons.

| Sample number | Type of sample | Date | Extractable organic matter (% by w./ sample) | Sat. (%) | Aro (%) | Resins (%) | Asphaltenes (%) | $\delta^{13}C$ sat. | $\delta^{13}C$ aro. | $\delta^{13}C$ res. | $\delta^{13}C$ asp. | GA /C31R | GA /C30αβH | Ts/Tm | C29 20S/R | ppm C30 hopane | % C27 | % C28 | % C29 | Origin of bitumen (Macke, et al., 2002 ) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 69B-Dead Sea | Geological sample | | 98.7 | 1.8 | 10.2 | 21.9 | 66.1 | | | | -29.9 | | 0.44 | 0.04 | 0.71 | | 40 | 35 | 25 | |
| 68B | Geological sample | Dead Sea | | | | | | | | | -29.4 | | 0.44 | 0.06 | | | | | | |
| 69A | Geological sample | Dead Sea | | | | | | | | | -29.9 | | 0.44 | 0.05 | | | | | | |
| Yarmouth-2233 | Archaeological bitumen | 1100-1200 BC | | 0.1 | 1.2 | 7.6 | 91.2 | | -29.7 | | -29.2 | 1.56 | 0.46 | 0.07 | 0.64 | 1682 | 32.7 | 40.6 | 26.8 | |
| Yarmouth-2234 | Archaeological bitumen | 2650-2200 BC | | 0.4 | 0.5 | 4 | 95.2 | | -30.4 | | -29.3 | 1.32 | 0.47 | 0.08 | 0.7 | 387 | 33 | 41.1 | 26 | |
| Yarmouth-2235 | Archaeological bitumen | 2650-2200 BC | | 0.2 | 0.5 | 4.2 | 95.1 | | -30.1 | | -29.3 | 1.56 | 0.43 | 0.07 | 0.65 | 824 | 33.4 | 41.2 | 25.4 | |
| Yarmouth-2236 | Archaeological bitumen | 2650-2200 BC | | 0.1 | 0.6 | 4.9 | 94.4 | | -29.9 | | -29.3 | 1.63 | 0.46 | 0.07 | 0.66 | 1194 | 33.4 | 40.3 | 26.4 | |
| Yarmouth-2239 | Archaeological bitumen | 2650-2200 BC | | 0.3 | 0.5 | 4.1 | 95.2 | | -29.7 | | -29.4 | 1.63 | 0.44 | 0.07 | 0.67 | 568 | 34.2 | 39.7 | 26.1 | |
| Yarmouth-2249 | Archaeological bitumen | 2650-2200 BC | | 0.8 | 1.1 | 4.8 | 93.3 | | -29.7 | | -29.4 | 1.53 | 0.45 | 0.08 | 0.75 | 325 | 33.8 | 40.5 | 25.7 | |
| Yarmouth-2250 | Archaeological Bitumen | 2650-2200 BC | | 0.3 | 0.7 | 5.4 | 93.6 | | -29.6 | | -29.3 | 1.58 | 0.44 | 0.07 | 0.67 | 750 | 33.7 | 40.3 | 26 | |
| Yarmouth-2252 | archaeological bitumen | 1300-1400 BC | | 0.4 | 1.3 | 9.5 | 88.8 | | -29.7 | | -29.4 | 1.62 | 0.45 | 0.07 | 0.68 | 509 | 32.8 | 40.8 | 26.5 | |
| 237 | Mummy | 40-405 AD | | | | | | | | | -26.7 | | 0.49 | 0.04 | | | | | | Dead Sea |
| 255 | Mummy | 1105-800 BC | | | | | | | | | -24.5 | | 0.51 | 0.07 | | | | | | Dead Sea |
| 254 | Mummy | 50-405 AD | | | | | | | | | -25 | | 0.42 | 0.05 | | | | | | Dead Sea |
| 323 | Mummy | 753-404 BC | | | | | | | | | -25.2 | | 0.39 | 0.08 | | | | | | Dead Sea |
| 238 | Mummy | 50-405 AD | | | | | | | | | -25.8 | | 0.16 | 0.06 | | | | | | Dead Sea |
| 322 | Mummy | 753-404 BC | | | | | | | | | -23.3 | | 0.38 | 0.1 | | | | | | Dead Sea? |
| 252 | Mummy | 50-405 AD | | | | | | | | | -24 | | 0.29 | 0.11 | | | | | | Dead Sea? |

### 2.2 Sample treatment

All the archaeological samples were subjected to an analytical procedure based on gas chromatography-mass spectrometry (Colombini, et al., 2003) for the identification of acyl-lipids, waxes, resinous materials in the same micro-sample (procedure A). Samples (1-5 mg) were subjected to saponification with 10% hydroalcoholic KOH. Neutral organic components were extracted with n-hexane and, after acidification, the acidic organic components were then extracted from the residual solution with diethyl ether. Aliquots of both extracts were derivatised with N,O-Bis(trimethyl)-silyl-trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (Sigma) using isooctane as a solvent. 2µl were analyzed by GC-MS using hexadecane and tridecanoic acid as internal standards.

The samples were subjected to a second sample pre-treatment (procedure B) to quantify free alkanes, aromatic and polar compounds of a bituminous material in order to recognise the geographical origin. A brief description of Procedure B is reported in the literature (Connan, 1999, Connan, et al., 2013, Connan, et al., 2006b). In brief, the dichloromethane extract was deasphalted using hexane. The deasphalted fraction was separated into saturated hydrocarbons, aromatic hydrocarbons and resins by gravity flow column chromatography using a 100-200 mesh silica gel support activated at 400°C prior to use. Hexane, dichloromethane and dichloromethane /ethanol (50:50) were used to elute saturate, aromatic and NSO fractions, respectively. Following solvent evaporation, the recovered fractions were quantified gravimetrically. The $C_{15+}$saturated hydrocarbon fraction was subjected to molecular sieve filtration (Union Carbide S-115 powder) after the technique described by West et al. (1990). An aliquot of the total alkane fraction was kept to give access to the n-alkane distribution.

### 2.3 Apparatus

Procedure A: a Milestone ETHOS microwave was used. The program applied for the extraction consisted of a first step of two minutes during which the temperature was increased gradually to 60 °C with a power of 550W, followed by a second step of three minutes, which maintained a temperature of 60 °C with a power of 500W.

The GC-MS instrumentation consisted of a 6890N Network GC System (Agilent Technologies, Palo Alto, CA, USA) equipped with a PTV injector and coupled to a 5973 MS detector with a quadrupole analyzer.

MS parameters: electron impact ionisation (EI, 70 eV) in positive mode; ion source temperature 230 °C; scan range 50-700 *m/z*; interface temperature 280 °C.

GC separation was performed on an HP-5MS column (J&W Scientific, Agilent Technologies, stationary phase 5% phenyl–95% methylpolysiloxane 30m length, 0.25 mm i.d., 0.25 µm film thickness) connected to a deactivated fused silica precolumn (J&W Scientific, Agilent Technologies, 2 m length , 0.32 mm i.d.).

GC conditions: the PTV injector was used in splitless mode at 300 °C and the chromatographic oven was programmed as follows: 80 °C, for 2 min isothermal, 10 °C/min up to 200 °C, 4 min isothermal, 6 °C/min up to 280 °C, 40 min isothermal; constant He flow 1.2 ml/min, injector temperature 280 °C.

Peak assignation was based on the interpretation of mass spectra and a comparison with reference compounds and materials, with library spectra (NIST 1.7), and with spectra reported in the literature.

Procedure B: Gas chromatography (GC-MS) analysis of hydrocarbon fractions was performed using an HP5890 chromatograph (split injection) interfaced to an HP5971 mass spectrometer. An HP-2 column (50m length, 0.2 mm i.d., 0.11µm film thickness) was used for chromatographic separation. GC conditions: the chromatographic oven was programmed from 150°C to 325°C at 2°C/min, and then held for 10 min. The spectrometer was run in the selected ion mode (SIM), monitoring ions at *m/z* 177, 191, 205, 217, 218, 221, 231 and 253. In order to determine the concentrations of biomarkers, a deuterated internal standard (d4-C29ααα20R sterane, Chiron lab, Norway) was added to the $C_{15+}$branched/cyclic hydrocarbon fraction. Response factors (RFs) at *m/z* 221 for the deuterated standard to hopane (*m/z* 191) and sterane (*m/z* 217) authentic standards were found to be approximately 1.4 and 1.0, respectively. The concentration of individual biomarkers was determined using the following equation: Conc.(ppm) = (peak height biomarker) (ng standard)(RF)(mg B/C fraction). Stable carbon isotope ratios ($\delta^{13}$C in ‰ vs. VPDB) of the $C_{15+}$saturates, $C_{15+}$aromatics, NSO and asphaltenes were determined

using Sofer's combustion technique (Sofer, 1980) along with a Finnigan Delta E isotope mass spectrometer. Uncertainty is ± 0.05‰.

## 3. Results and discussion

### 3.1 Identification of resinous and lipid materials via procedure A

The samples submitted to saponification and extraction (procedure A) showed some differences in the molecular patterns of both the acidic and neutral fractions. For example, Figures 2 to 5 show the chromatograms of both the acidic and neutral fractions for balm samples #2678, #2679, #2680 and #2681, respectively. Table 3 lists the components identified in the various samples and their possible origins.

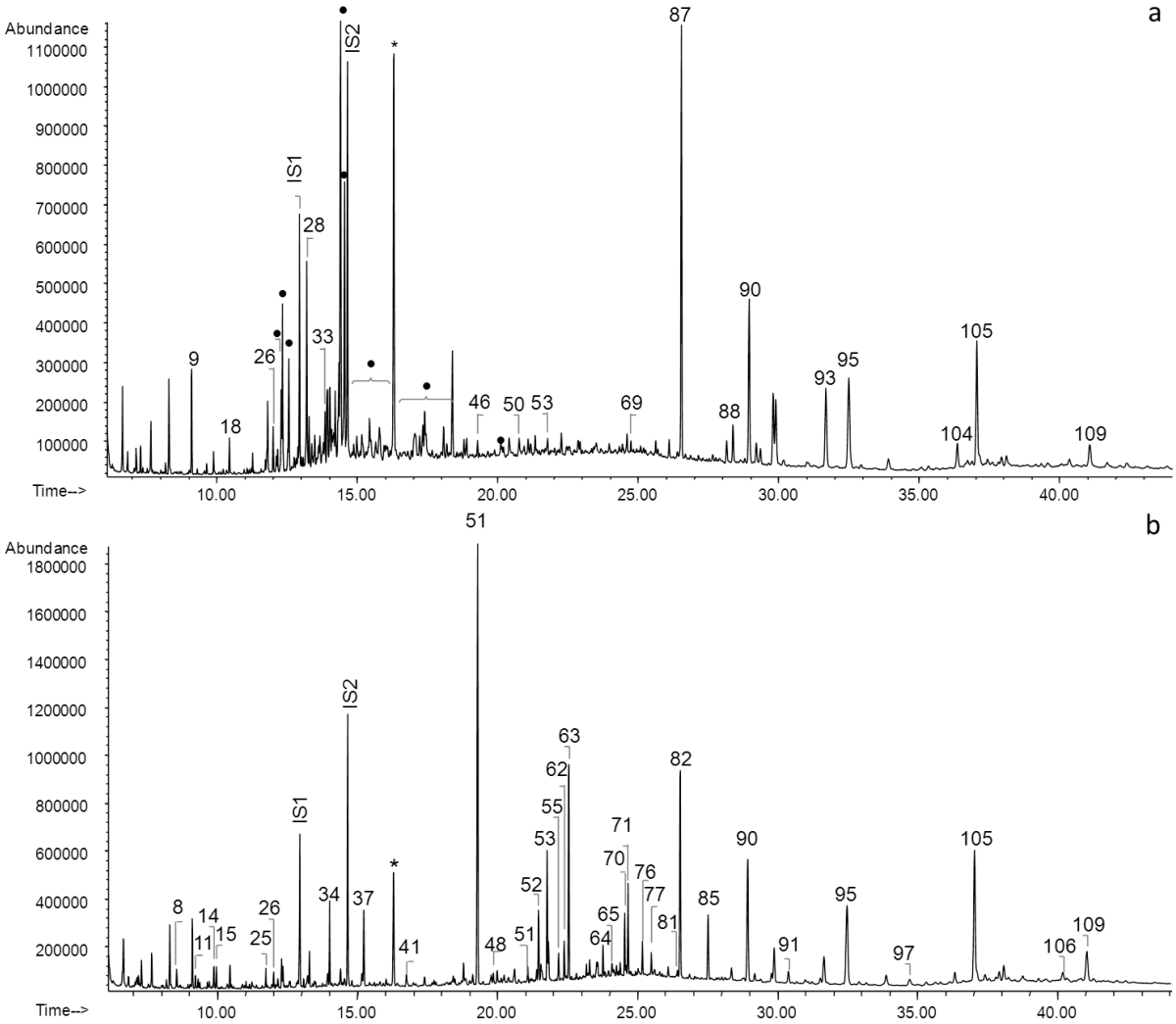



**Fig. 2.** Total ion current chromatogram of a) neutral and b) acidic fraction of sample #2678. (IS1 = hexadecane, IS2 = tridecanoic acid). The acidic and alcoholic species are present as TMS-derivatives, "•": sesquiterpenes, "*": phthalate contamination. The numbers refer to Table 3

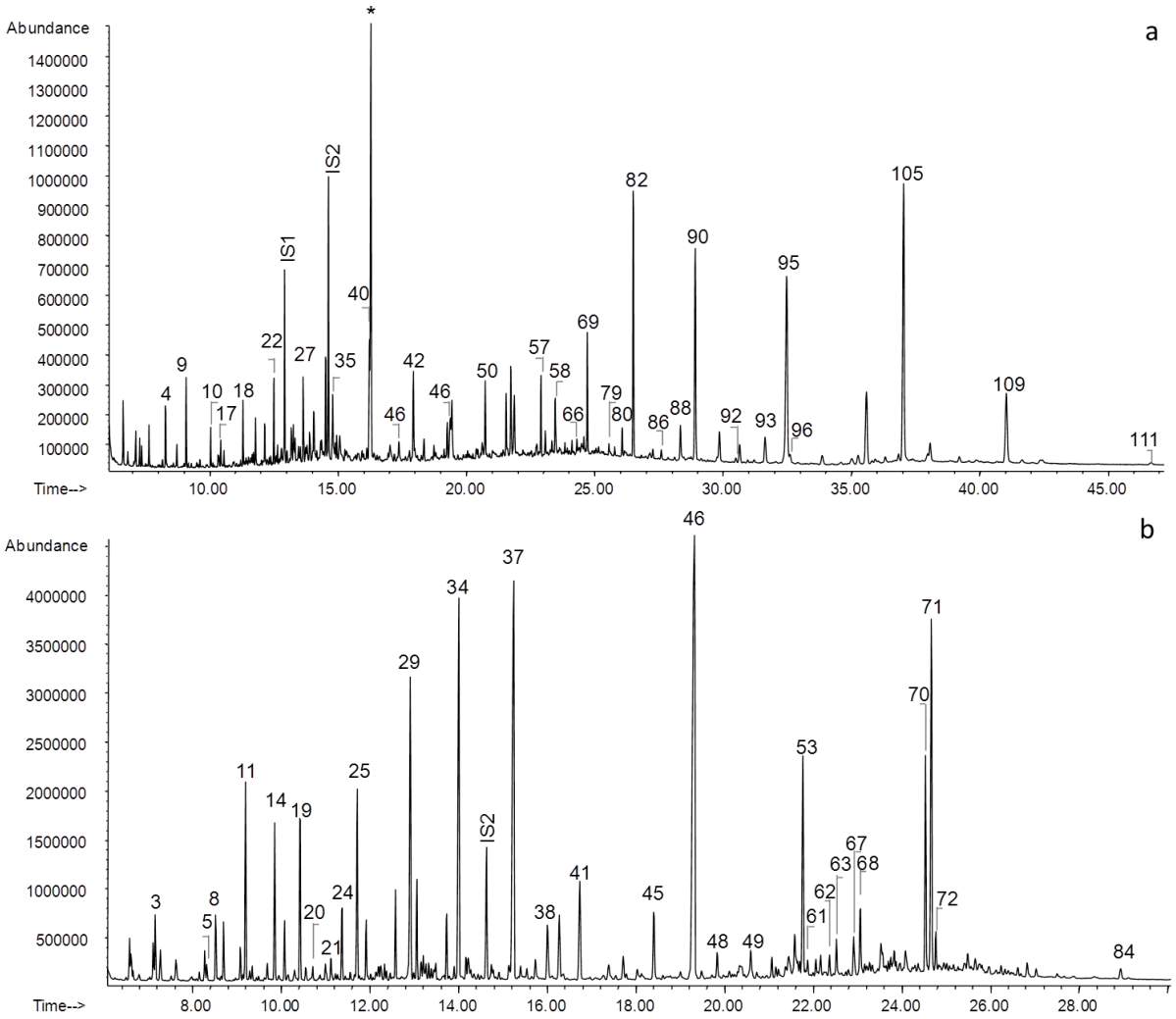



**Fig. 3** Total ion current chromatogram of a) neutral and b) acidic fraction of sample #2679. (IS1 = hexadecane, IS2 = tridecanoic acid). The acidic and alcoholic species are present as TMS-derivatives. "*": phthalate contamination. The numbers refer to Table 3

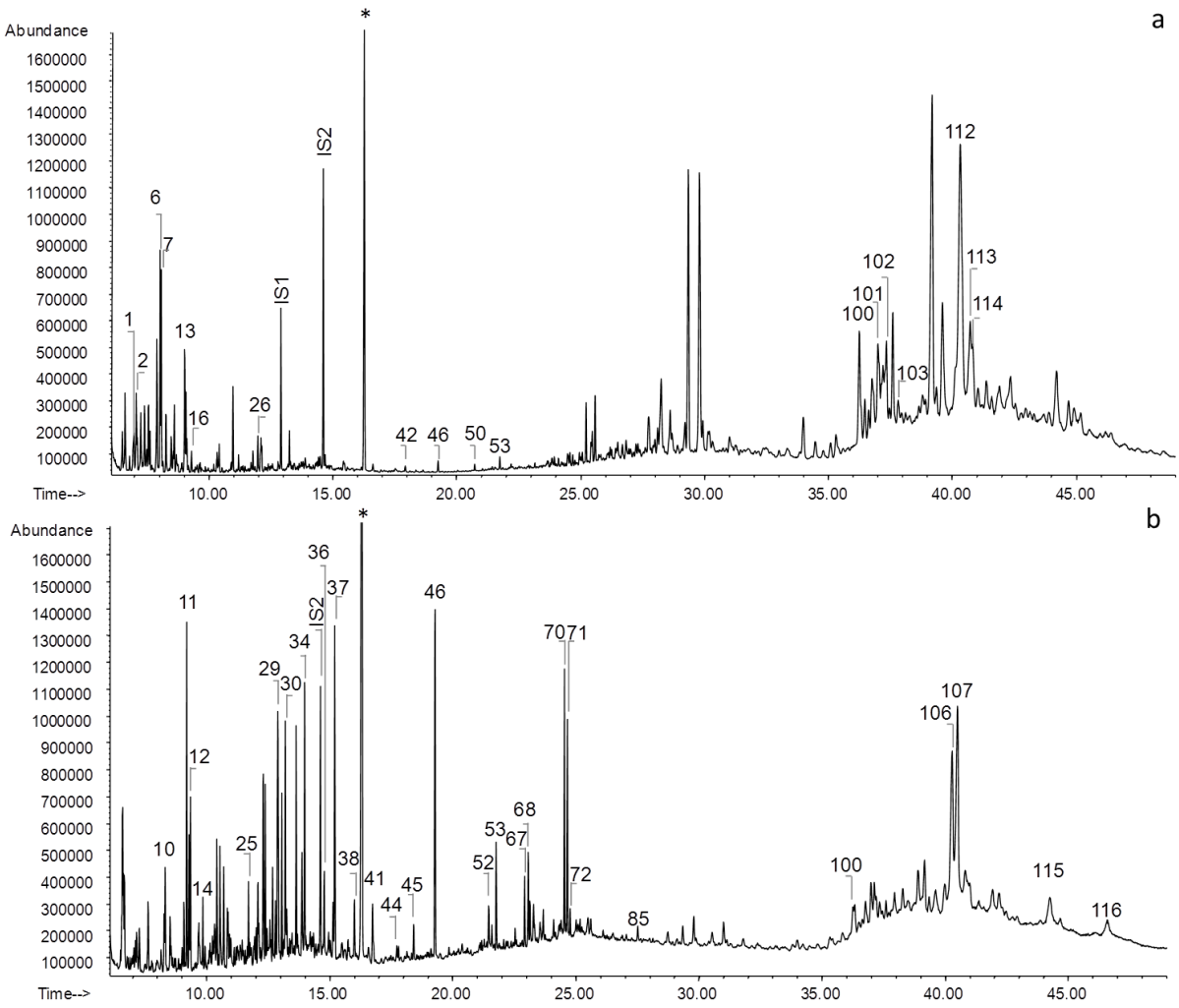



**Fig. 4** Total ion current chromatogram of a) neutral and b) acidic fraction of sample #2680. (IS1 = hexadecane, IS2 = tridecanoic acid). The acidic and alcoholic species are present as TMS-derivatives. "*": phthalate contamination. The numbers refer to Table 3

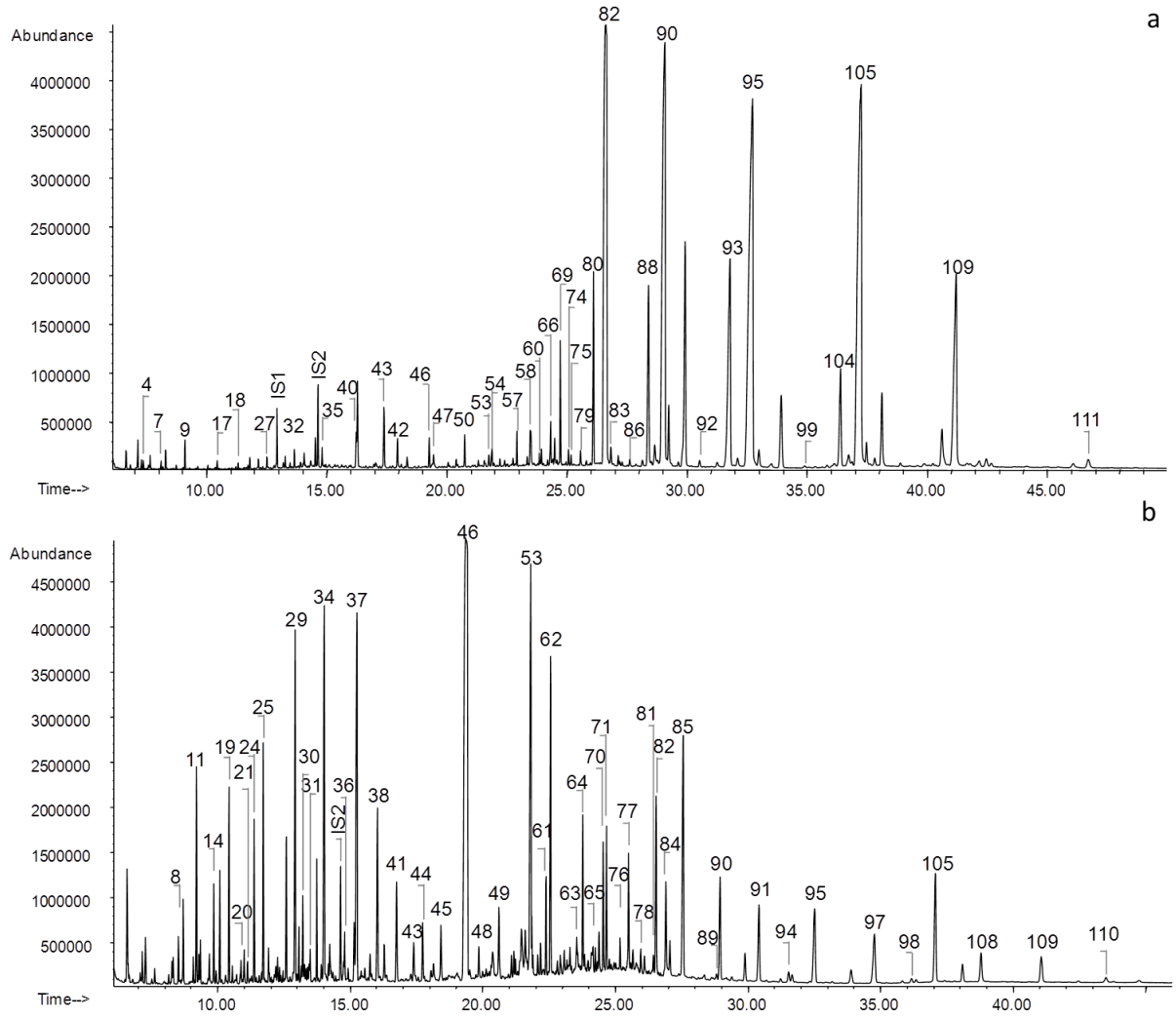



**Fig. 5** Total ion current chromatogram of a) neutral and b) acidic fraction of sample #2681. (IS1 = hexadecane, IS2 = tridecanoic acid). The acidic and alcoholic species are present as TMS-derivatives. "*": phthalate contamination. The numbers refer to Table 3

## Table 3. Peak identification for the chromatograms in Figures 2-5

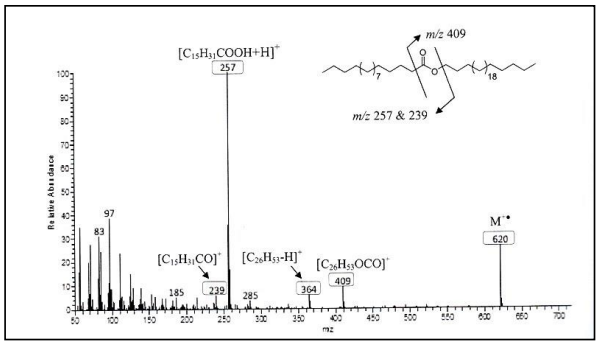
| N° | Identified compound | 2678 | 2679 | 2680 | 2681 | N° | Identified compound | 2678 | 2679 | 2680 | 2681 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | Pinocarveol | | | √ | | **59** | Pentadecanedioic Acid | | | | √ |
| **2** | Camphor | | | √ | | **60** | Eneicosanol | | | | √ |
| **3** | Heptanoic Acid | | √ | | | **61** | 14-Hydroxy Hexadecanoic Acid | √ | √ | | √ |
| **4** | Octanol | | √ | | √ | **62** | 15-Hydroxy Hexadecanoic Acid | | | | √ |
| **5** | Benzoic Acid | | √ | | | **63** | 16-Hydroxy Hexadecanoic Acid | √ | √ | | √ |
| **6** | Berbenone | | | √ | | **64** | Eicosanoic Acid | √ | | | √ |
| **7** | Borneol | | | √ | √ | **65** | Hexadecanedioic Acid | √ | | | |
| **8** | Octanoic Acid | √ | | √ | √ | **66** | Pentacosane | | √ | | √ |
| **9** | Tridecane | √ | √ | | √ | **67** | 11,12-Dihydroxy-Hexadecanoic Acid | | √ | √ | |
| **10** | Nonanol | | √ | | √ | **68** | 11,12-Dihydroxy-Hexadecanoic Acid | | √ | √ | |
| **11** | Butanedioic Acid | √ | √ | √ | √ | **69** | Docosanol C22OH | √ | √ | | √ |
| **12** | Dimethyl Butanedioic Acid | | | √ | | **70** | 9,10-Dihydroxy-Octadecanoic Acid | √ | √ | √ | √ |
| **13** | Myrtenol | | | √ | | **71** | 9,10-Dihydroxy-Octadecanoic Acid | √ | √ | √ | √ |
| **14** | Nonanoic Acid | √ | √ | | √ | **72** | 11,12-Dihydroxy-Octadecanoic Acid | | √ | √ | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **15** | 2-Methyl Benzoic Acid | √ | | | | **73** | 11,12-Dihydroxy-Octadecanoic Acid | | √ | √ | |
| **16** | Verbenone | | | √ | | **74** | 7-Oxo-Dehydroabietic Acid Methylester | | | | √ |
| **17** | Tetradecane | √ | | | √ | **75** | Hexacosane | | | | √ |
| **18** | Decanol | | √ | | √ | **76** | 7-Oxo-Dehydroabietic Acid | √ | | | √ |
| **19** | Pentanedioc Acid | | √ | | | **77** | Docosanoic Acid | √ | | | √ |
| **20** | 6-Hydroxyhexanoic Acid | | √ | | √ | **78** | 7-Oxo-Didehydroabietic Acid | | | | √ |
| **21** | Decanoic Acid | | √ | | √ | **79** | Tricosanol | | √ | | √ |
| **22** | Undecanol | | | | √ | **80** | Heptacosane | | √ | | |
| **23** | Pentadecane | | | | √ | **81** | Tricosanoic Acid | √ | | | |
| **24** | Heptanedioic Acid Methylester | | √ | | √ | **82** | Tetracosanol | √ | | | √ |
| **25** | Hexanedioic Acid | √ | √ | √ | √ | **83** | Dehydroabietic Acid Methyl Ester | | | | √ |
| **26** | Cuparene | √ | | √ | | **84** | 15-Hydroxy-Dehydroabietic Acid | | √ | | √ |
| **27** | Dodecanol | | √ | | √ | **85** | Tetracosanoic Acid | √ | | √ | √ |
| **28** | Longiborneol | √ | | | | **86** | Pentacosanol | | √ | | √ |
| **29** | Heptanedioic Acid | | √ | √ | √ | **87** | Eicosanedioic Acid | | | | √ |
| **30** | 4-Hydroxybenzoic Acid | | | √ | √ | **88** | Nonacosane | √ | √ | | √ |
| **31** | Dodecanoic Acid | | | | √ | **89** | Pentacosanoic Acid | | | | √ |
| **32** | Tridecanol | | | | √ | **90** | Hexacosanol | √ | √ | | √ |
| **33** | Calamenene | √ | | | | **91** | Hexacosanoic Acid | √ | | | √ |
| **34** | Octanedioic Acid | √ | √ | √ | √ | **92** | Heptacosanol | | √ | | √ |
| **35** | Tetradecanol | | √ | | √ | **93** | Hentriacontane | √ | √ | | √ |
| **36** | Vanillic Acid | | | √ | √ | **94** | 24-Hydroxytetracosanoic Acid | | | | √ |
| **37** | Nonanedioic Acid | √ | √ | √ | √ | **95** | Octacosanol | √ | √ | | √ |
| **38** | Tertadecanoic Acid | | √ | √ | | **96** | 3-Hydroxycholestestane | | √ | | |
| **39** | Hexadecenoic Acid | | √ | √ | √ | **97** | Octacosanoic Acid | √ | | | √ |
| **40** | Pentadecanol | | √ | | √ | **98** | 26-Hydroxyhexacosanoic Acid | | | | √ |
| **41** | Decanedioic Acid | √ | √ | √ | √ | **99** | Nonacosanol | | | | √ |
| **42** | Hexadecanoic Acid | | √ | √ | √ | **100** | Nor-B-Amyrone | | | √ | |
| **43** | Hexadecanoic Acid Methylester | | √ | | √ | **101** | A-Amyrine | | | √ | |
| **44** | Pentadecanoic Acid | | | √ | √ | **102** | Lupeon | | | √ | |
| **45** | Undecanedioic Acid | | √ | √ | √ | **103** | Lupeol | | | √ | |
| **46** | Hexadecanoic Acid | √ | √ | √ | √ | **104** | Tritriacontane | √ | | | √ |
| **47** | Heptadecanol | | | | √ | **105** | Triacontanol | √ | √ | | √ |
| **48** | Dodecanedioic Acid | √ | √ | | √ | **106** | Moronic Acid | √ | | √ | |
| **49** | Heptadecanoic Acid | | √ | | √ | **107** | Oleanonic Acid | | | √ | |
| **50** | Octadecanol | √ | √ | √ | √ | **108** | Triacontanoic Acid | | | | √ |
| **51** | Tridecanedioic Acid | √ | | | | **109** | Dotriacontanol | √ | √ | | √ |
| **52** | Octadecenoic Acid | √ | | √ | √ | **110** | Dotriacontanoic Acid | | | | √ |
| **53** | Octadecanoic Acid | √ | √ | √ | √ | **111** | Tetratriacontanol | | √ | | √ |
| **54** | Nonadecanol | | | | √ | **112** | 20,24-Epoxy-25-Hydroxydammaren-3-One | | | √ | |
| **55** | Tetradecanedioic Acid | √ | | | | **113** | Hydroxydammarenone | | | √ | |
| **56** | Nonadecanoic Acid | | | | √ | **114** | Oleanonic Aldehyde | | | √ | |
| **57** | Eicosanol | | √ | | √ | **115** | Masticadienoic Acid | | | √ | |
| **58** | Retene | | √ | | √ | **116** | Isomasticadienoic Acid | | | √ | |

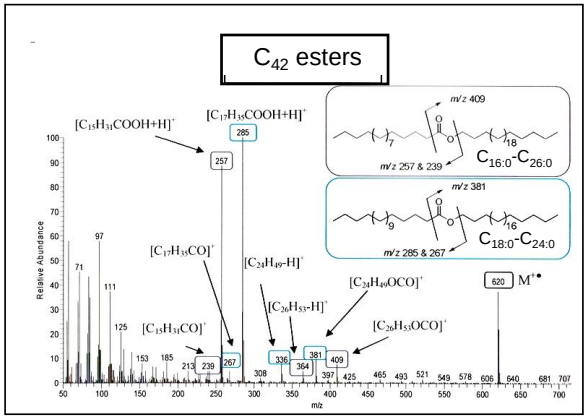
The main acidic components in all the samples were long chain (9-32 carbon atoms) linear monocarboxylic acids. The most abundant were palmitic acid (hexadecanoic acid, C16:0) and stearic acid (octadecanoic acid, C18:0). The length of the acylic chains suggested that they originated from the saponification of both glycerolipids (contained in drying oils) and cerides (long chain esters contained in natural waxes). In all the samples, except for #2785, #2786, #2790 and #2794, the presence of long-chain monocarboxylic fatty acids along with the co-occurrence in the neutral fraction of long chain alcohols and alkanes, indicated the presence of natural waxes (see Fig. 5). In fact, natural waxes are complex lipid mixtures

mainly consisting of long chain esters (cerides) of fatty acids with long chain alcohols, free fatty acids, hydroxy acids, alcohols, diols, and alkanes.

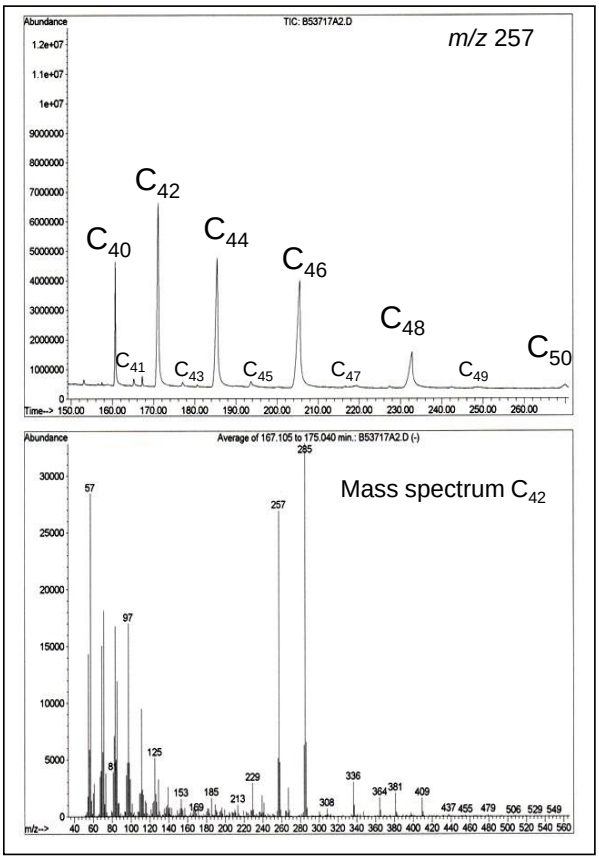
The molecular profile varies according to the type of wax and the degree of ageing (Evershed, et al., 1997, Heron, et al., 1994, Regert, et al., 2001). Here the hydroxy acids were mainly (ω-1)-OH-acids, with (ω-1)-OH-hexadecanoic acid as the most abundant. This is in line with the identification of the wax as beeswax together with the peaks corresponding to lignoceric acid (tetracosanoic acid, C24:0) in the fatty acid profile of almost all the samples. However although beeswax is likely to be the main constituent added, other wax esters originating from plant epicuticular waxes were also present, as shown in other archaeological cases. They were clearly detectable when comparing the mass spectra of long-chain esters with those published by Bastien et al. (2011) in her study on the organic residues from 30 vases excavated from tombs in the Deir el-Medineh necropolis, dated from the reign of Thoutmosis III (1500 BC) (Fig. 6). For instance a comparison of the mass spectrum of C42 esters in both pure beeswax (Fig. 6a) and a sample from Deir el-Medineh (Fig. 6b) shows that if the beeswax ester is the single hexacosanoyl palmitate, the esters in Deir el-Medineh sample are a mixture of hexacosanoyl palmitate and octacosanoyl stearate. Similar coelutions were observed in the balm scraped from the inside of the Amenhotep mummy, a royal scribe from the reign of Amenhophis III (Figs. 6c and d). This characteristic has also been reported by Ribechini, et al.(2008) in the chemical study of residues from seven unguentaria from the archaeological site of Oplontis (Naples, Italy). These glass ointment jars are dated between the 1$^{st}$ century BC and the 1$^{st}$ century AD.




Mass spectrum of the palmitate d'hexacosanoyle ($C_{42,}$ $C_{16:0}$-$C_{26:0}$)




Mass spectrum of the $C_{42}$ esters from the Deir el-Medineh archaeological samples characterized by a lipid basis




Mass fragmentogram *m/z* 257 and mass spectrum of the $C_{42}$ esters from the Amenhotep mummy (#413, 1450 BC)

Fig. 6. Mass spectrum of the $C_{42}$esters. a) beeswax. b) in a residue scraped from a vase of Deir-el-Medineh necropolis (1500 BC). d) in a balm from the Amenhotep mummy (1450 BC). Mass fragmentogram *m/z* 257 of the C40-C50 esters from the Amenhorep mummy (1450 BC).

The second most abundant compounds in the samples after monocarboxylic acids were α,ω-dicarboxylic acids ranging from 5 to 24 carbon atoms, with azelaic acid (nonandioic, diC9) as the most abundant, along with suberic (octandioic, diC8) and sebacic (decandioic, diC10) acids. Dicarboxylic acids are not by nature present in waxes, oils and fats, but can be formed during curing and ageing as a result of preferential oxidation and bond cleavage at the double bonds in the acylic chain. The prevalence of azelaic acid is thus an indication of the predominance of oleic acid (9-octadecenoic acid, C18:1) in the original material, which had undergone degradative oxidation, and is also highlighted by the presence of 9,10-hydroxy-octadecanoic acid.

The lipid profile thus seems to indicate that a plant oil or a mixture of plant oils was present in the mixtures (Copley, et al., 2005). We were unable to identify the type of plant oil due to the unspecificity of the fatty acids, except for samples #2504, #2759, #2771, #2775 and #2795

which showed a peak of ricinoleic acid (12-hydroxy-9-cis-octadecenoic), which is a marker of castor oil obtained from the seeds of *Ricinus communis* L. (Euphorbiaceae). This acid has been previously identified in Egyptian mummy samples (Tchapla, et al., 2004).

The presence of non-negligible amounts of odd chain length fatty acids and cholesterol indicates that animal fats, which could result from either lipid tissues from the body or from another source, were present in the samples. Odd-carbon-numbered straight-chain fatty acids (in particular pentadecanoic acid, C15:0 and heptadecanoic acid, C17:0) and the corresponding branched chain fatty acids suggest the presence of animal fats (in particular ruminant fats such as sheep, cattle, goats, etc.), or that the lipids had undergone bacterial degradation.

Another significant component of many of the analyzed samples (except for samples #2679, #2770, #2774, #2783 and #2795) was the terpenic fraction: sesquiterpenes, diterpenes and triterpenes. Sesquiterpenes, which can derive from resin, wood or wood extracts, were very evident in the chromatograms of the neutral fractions. Diterpenes, above all dehydroabietic and 7-oxo-dehydroabietic, highlight that an ingredient in the material used for embalming came from a resin exuded from plants of the *Pinaceae* family (Mills and White, 1994).

Large amounts of phenolic compounds (such as acetovanillone, hydroxybenzoic and vanillic acids) and of condensed aromatic hydrocarbons (PAHs), mainly phenanthrene, were revealed in many of the samples. The concomitance of these two classes of compounds suggests that one component of the mummification balm was obtained from combustion or partial dry-distillation of wood tar, tar, oil or wood. Phenanthrenes include retene (1-methyl-7-isopropyl phenanthrene) and abietatrienone, indicating that the pitch was obtained from Pinaceae wood. This evidence is also supported by the presence of methyl-dehydroabietate, which derives from the reaction of dehydroabietic acid with methanol produced during the dry distillation of resinous wood.

The presence of phenols in mummification balms has been tentatively attributed to the “cedar oil” mentioned by Pliny the Elder and Herodotus (Brai, et al., 2007, Koller, et al., 2005), in association with sesquiterpenes. Pliny the Elder describes indeed the process used in the production of liquid pitch from wood, called "cedrium" in Syria and for mummification in Egypt. The extract of Atlas cedar wood (Cedrus atlantica), a widespread conifer in the Atlas range in North Africa, contains sesquiterpenoids, such as junipene, cadalene, cadinatriene (calamene), cuparene and α-curcumene (Koller, et al., 2003), in addition to the characteristic compound hexahydro-benzotetramethyl-cycloheptadiene (Buckley, et al., 2004). Recent analyses of a residue from a Thinite jar (3100-2700 BC) have revised this diagnosis with the

conclusion that “cedrium” was not prepared from real cedar but from Juniper species (Sarret, et al., 2015).

In the samples herein discussed the occurrence of cuparene and related compounds compatible with a substance originating from the Cedrus species was not observed, and the overall molecular profiles points out the presence of a Pinaceae wood pitch, but it is not possible to hypothesize the specie of origin.

In six samples (#2504, #2678, #2680, #2770, #2775, #2704 and #2779), the high retention time region was characterized by the presence of triterpenoids in the neutral as well as in the acidic fractions (Fig. 4). Moronic, oleanonic, iso-masticadienonic and masticadienonic acids were observed in the acidic fraction, while nor-β-amyrone, hydroxydammarenone and 17-nor-oleanone, were found in the neutral fraction (Fig. 4a). All these compounds are characteristic biomarkers of mastic resin. Mastic resin, obtained from the *Pistacia genus*, was widespread in the Mediterranean in ancient times, and has been found to be an ingredient in chemical balms of mummification in ancient Egypt (Buckley, et al., 2004, Colombini, et al., 2000, Lucejko, et al., 2012).

### 3.2 Bitumen analysis *via* procedure B

Bitumen was detected directly in five samples by procedure A as listed in Table 5. However due to the method used to analyse the samples, it was not possible to quantify the bitumen biomarkers. Consequently the actual amount of bitumen in Egyptian artefacts (balms from human and animal mummies, coffins, canopic jars, etc.) is underestimated. To overcome this problem the dedicated protocol of procedure B (Connan, et al., 2006a) was used. The focus was thus on the isolation of saturated hydrocarbons. To investigate their profile the diagnostic mass fragmentograms corresponding to the *m/z* 217 and 218, and *m/z* 177 and 191 of steranes and terpanes were extracted and are shown in (Fig. 7). A comparison of the mass fragmentograms *m/z* 191 and *m/z* 217 or 217+218 obtained for sample #2680 with procedures A and B is presented in Fig. 7. The figure shows that although steranes and terpanes were not detected with procedure A, they were recorded with procedure B. In addition, the well-defined fingerprints enable the sterane and terpane ratios to be calculated and for the bitumen to be identified as coming from the Dead Sea.

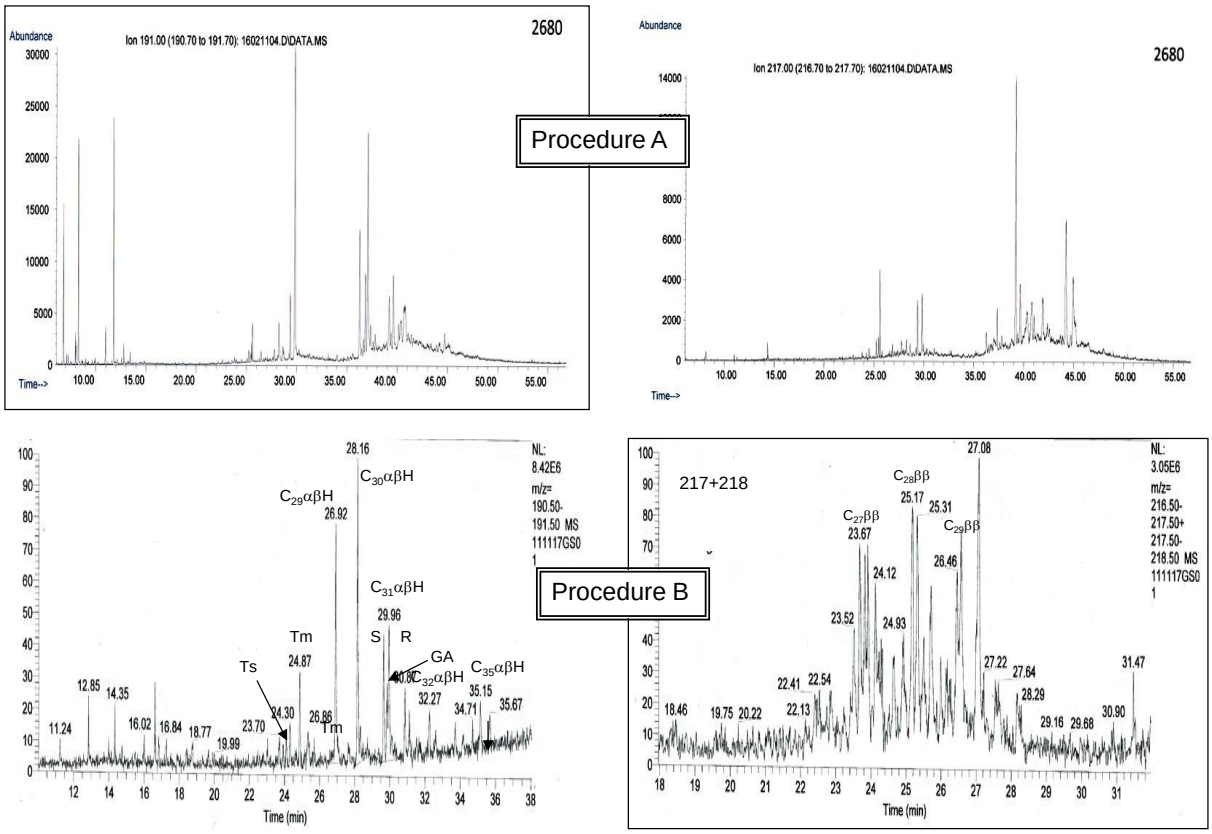



Fig. 7 Mass fragmentograms *m/z* 191 (terpanes) and *m/z* 217+218 (steranes) obtained for sample #2680 with procedures A and B.

In the four samples examined by this specialized protocol, namely #2680, #2681, #2771, #2774, the fossil biomarkers, steranes and terpanes were clearly identified. Their terpane distribution is characterized by the occurrence of the complete family of C19-C28 tricyclopolyprenanes, a low Ts/Tm ratio and a high amount of gammacerane (Fig. 8a). In addition the sterane distribution (Fig. 8a, Table 4) looks similar to that of Dead Sea bitumen exemplified by samples of the floating blocks from the Dead Sea and by lumps of pure bitumen excavated from Tell Yarmouth and covering different periods from 1100 BC to 2650 BC (Table 2). The composition of ββsteranes (Tables 2 and 4) reproduced in a ternary diagram (Fig. 8b) confirms that our four mummy samples are in good agreement with the references, both the present day bitumen from the Dead Sea and the archaeological bitumen from Tell Yarmouth. The plot of the diagnostic terpane ratios GA/C30αβH vs. Ts/Tm (Fig. 9) matches well with the reference Dead Sea bitumen. However the distribution of balms from mummies are slightly more scattered than the reference data. Balms

from mummies from this study showed higher values in Ts/Tm and GA/C30αβH, which suggests that the Dead Sea bitumen may have been affected by an incipient biodegradation. However the biodegradation effects here are limited and do not hamper the recognition of diagnostic Dead Sea molecular characteristics.

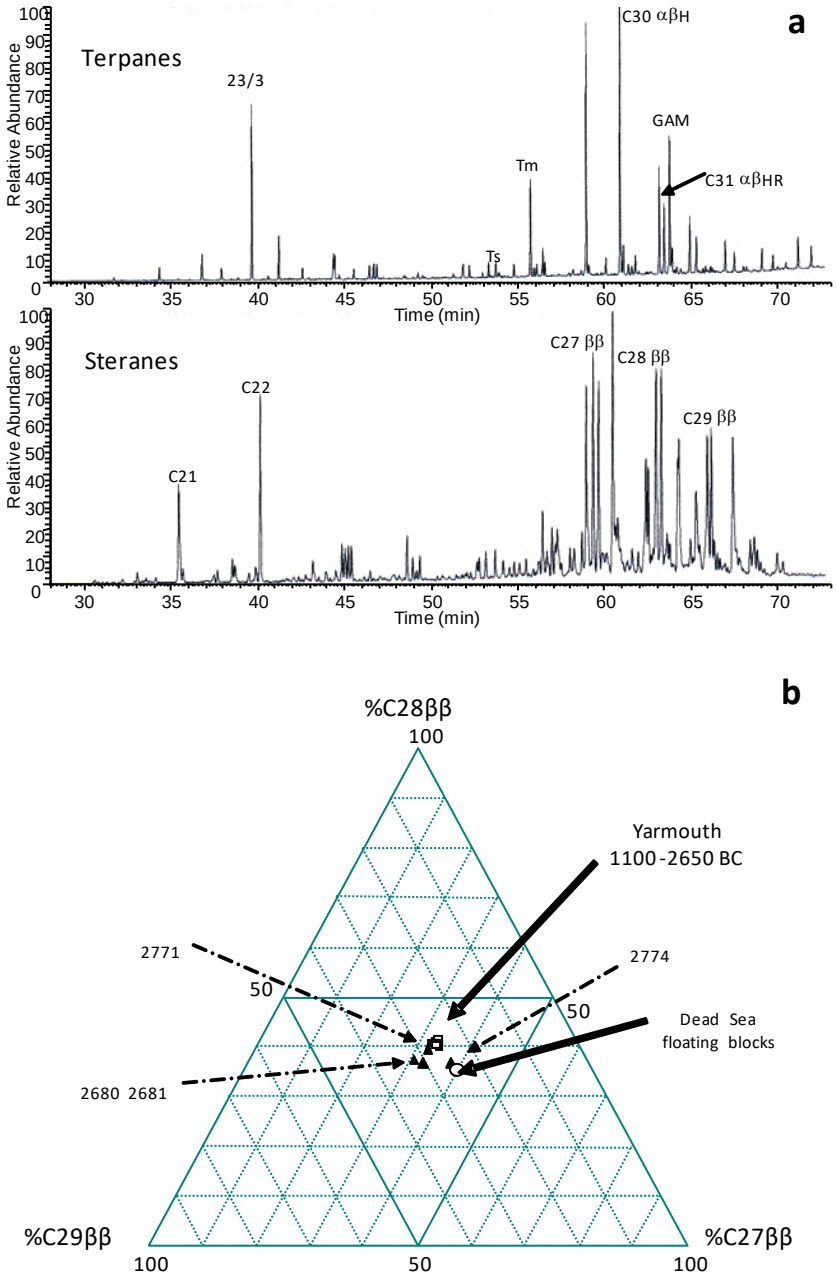



Fig. 8. a) Mass fragmentograms *m/z* 191 (terpanes) and *m/z* 217 (steranes) obtained for sample #2774, b) Composition of ββ steranes in a ternary diagram.

Table 4 Gross composition, isotope and molecular data of samples #2680, #2681, #2771, #2774. Sat.:saturated hydrocarbons; Aro.: aromatic hydrocarbons.

| Sample number | Type Of Sample | Date | Extractable organic matter (% by w./ sample) | Sat. (%) | Aro (%) | Resins (%) | Asphaltenes (%) | $\delta^{13}C$ sat. | $\delta^{13}C$ aro. | $\delta^{13}C$ res. | $\delta^{13}C$ asp. | GA /C31R | GA /C30αβH | Ts/Tm | C29 20S/R | ppm C30 hopane | % C27 | % C28 | % C29 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2771 | Mummy | | 2.2 | 0.4 | 1 | 11.7 | 86.9 | -28 | -25 | -24.6 | -25.5 | 1.59 | 0.44 | 0.09 | 0.26 | 481 | 32.4 | 39.6 | 28 |
| 2774 | Mummy | | 3 | 0.3 | 0.6 | 11.2 | 87.9 | -26 | -26 | -25.3 | -25.1 | 1.96 | 0.51 | 0.13 | 0.56 | 192 | 37.7 | 36.8 | 25.5 |
| 2680 | Mummy | | | | | | | | | | | 1.75 | 0.45 | 0.24 | 0.27 | | 32.7 | 36.8 | 30.5 |
| 2681 | Mummy | | | | | | | | | | | 2 | 0.61 | 0.09 | 0.35 | | 31 | 37.2 | 31.8 |

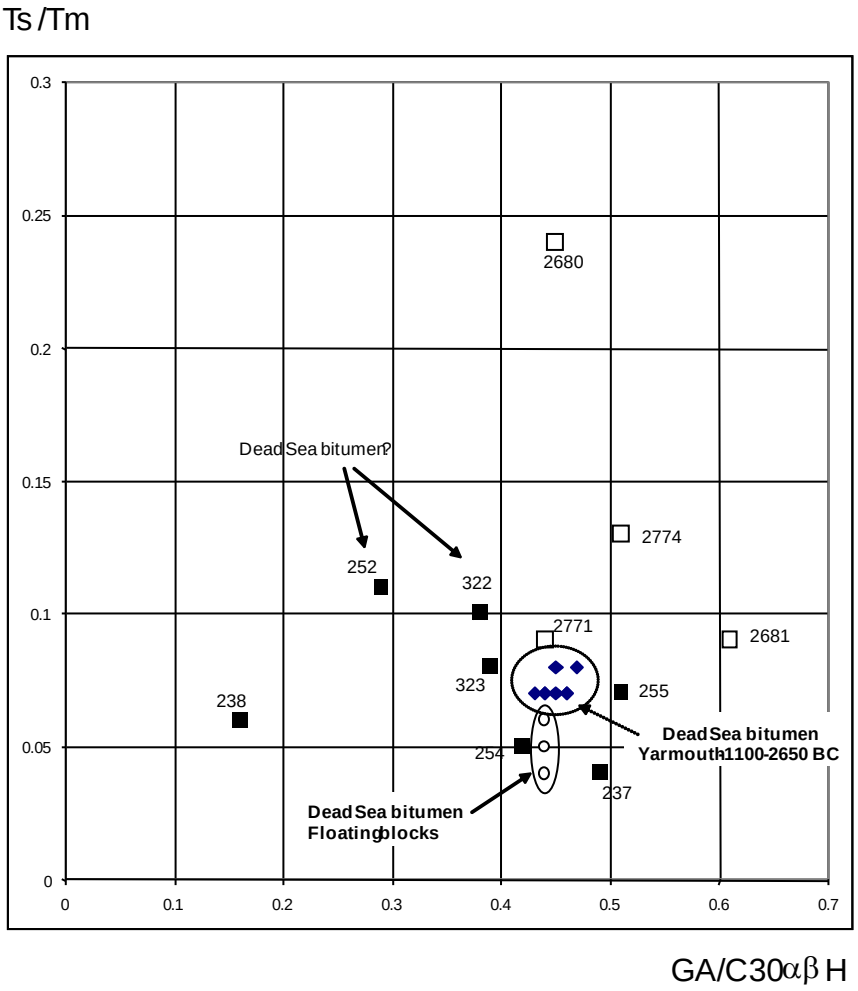



**Fig. 9** Plot of Ts/Tm vs. GA/C29αβH: comparison of the four selected samples (#2680, #2681, #2771, #2774) analyzed with references from Dead Sea floating blocks, mummies and bitumen from Tell Yarmouth (Table 2).

## Table 5- List of materials identified in analysed samples

| Samples | Glycerolipids | Beeswax | Pinaceae resin | Castor oil | Pinacea pitch | Triterpenoids | Bitumen | |
|---|---|---|---|---|---|---|---|---|
| | Fatty acids | Long chain alcohols; acids and hydroxyacids | Didehydroabietic dehydroabietic; 7-oxo-dehydroabietic; 15-hydroxy-7-oxo-dehydroabietic; 15-hydroxy-dehydroabietic acids | Ricinoleic acid | Retene ; PAH-polycyclic aromatic hydrocarbon; 18-norabietatriene; 19-norabietatriene; abietatrienone; methyl-dehydroabietate | Nor-β-amyrone; α-amyrine; lupeon, lupeol; 20,24-epoxy-25-hydroxydammarenone; hydroxydammarenone; oleanonic aldehyde; moronic, oleanonic, masticadienoic, isomasticadienoic acids | Hopanes ; steranes ; gammacerane | |
| **procedure** | **A** | | | | | | | **B** |
| 1896 | x | x | | | | | x | |
| 2504 | x | x | | x | | x | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 2759 | x | x | x | x | x | | x | |
| 2678 | x | x | x | | | x | x | |
| 2679 | x | x | | | x | | | |
| 2680 | x | x | x | | | x | | x |
| 2681 | x | x | x | | x | | | x |
| 2770 | x | x | | | | x | x | |
| 2771 | x | x | x | x | x | | | x |
| 2774 | x | x | | | | | | x |
| | | | | | | | | |
| 2702 | x | x | x | | x | | | |
| 2703 | x | x | x | | x | | x | |
| 2704 | x | x | x | | | x | | |
| | | | | | | | | |
| 1811 | x | x | | | x | | | |
| 2785 | x | | x | | x | | | |
| 2795 | x | x | | x | | | | |
| 2775 | x | x | | x | | x | | |
| 2776 | x | x | x | | x | | | |
| 2779 | x | x | | | | x | | |
| 2783 | x | x | | | | | | |
| 2786 | x | | x | | x | | | |
| 2790 | x | | x | | x | | | |
| 2794 | x | | x | | x | | | |

## 4. Conclusions

Our investigations show that the examined balms from animal mummies were not significantly different from the balms from human mummies. The constituents identified in both types of balms were: fats or oils of uncertain origin, beeswax, plant waxes, castor oil, conifer resins but also pitch derived from the pyrolysis of conifer wood, mastic resin and castor oil (Table 5). Our results are consistent with recently published findings by other authors.

The residues isolated from potsherds of jars and amphorae were found to be both pure products and mixtures. As pure natural products, the following were identified: fat in a stone vase from the Old Kingdom discovered in a pit from the Sakkara area, castor oil on a potsherd dated from the New Kingdom, Thebes area, and fat from the Ramesside period on a potsherd from the area of Thebes. In the seven remaining samples, the organic residue stuck on potsherds was composed of a mixture which was likely to have been prepared for ritual purposes, including mummification. These mixtures were very similar to those identified in balms from mummies and contained: fat, pinaceae resin, pinaceae pitch, mastic resin, castor oil. The mixtures dated from the Copto-Byzantine period were based on fat, Pinaceae resin and pitch: above all

conifer products mixed with oil and fat. Other samples, dated from the New Kingdom and Ramesside period were more diversified, including not only Pinaceae products but also Pistacia resin, castor oil, beeswax and fat.

This study suggests that the methods used by most researchers tend to underestimate the presence of bitumen in mummy balms. In fact procedure A, optimised to analyse the majority of organic constituents present in mummification balms, such as acyl-lipids, waxes and resinous materials, led to the qualitative detection of bitumen only in few cases. Therefore a specifically optimised procedure was necessary to quantitatively study the molecular pattern of bitumen constituents, in order to identify the geographical origin of the bituminous materials. This enabled us to identify bitumen in samples where it had not been identified by the saponification method. The bitumen in the balms was identified as Dead Sea bitumen (Connan, et al., 2006a, Macke, et al., 2002 ).

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We would also like to thank Philippe Schaeffer and Estelle Motsch who gave their support by analyzing two of the balms from mummies (#2680 and #2681) which enabled us to identify Dead Sea bitumen in these samples.

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