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## Characterization of Egyptian Human Mummy Balms by chemometric treatments of infrared data --Manuscript Draft--

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<b>Abstract:</b>	<p>This study presents the efficiency of an original method to characterize mummification balms. Currently, long protocols are used to extract each substance from mummification balms by using techniques such as gas chromatography and mass spectrometry. The SIMPLISMA approach using a simple algorithm combined with chemometric treatment, principal component analysis, was used to interpret the 47 Fourier Transform Infrared spectra. This treatment gave 6 pure variables and their corresponding extracted concentration profiles. The first pure spectrum corresponded to residual species, the second allowed the identification of polycyclic aromatic hydrocarbons, molecules which have a pyrolytic origin. Proteins were characterized, and they could be considered as markers of a good conservation of individuals. Finally, two pure spectra showed the same natural substance, fat, but on the one hand unaltered and on the other hand degraded fatty matter. This fast, simple and non-invasive approach allowed the composition of each balms studied as well as their state of conservation/degradation to be characterized.</p>
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To attention to Editors of Talanta

Avignon, August, 31<sup>st</sup> 2020

**Object / Cover letter**

Dear Editor,

Please find enclosed our manuscript entitled "Characterization of Egyptian Human Mummy Balms by chemometric treatments of infrared data" by Elodie Mezzatesta, Nathalie Dupuy, Carole Mathe for a submission in Talanta.

This paper deals with a new approach consisting to a fast, simple and non-invasive study allowed the composition of each balms studied as well as their state of conservation/degradation to be characterized, using Simplisma algorithm and Principal Component Analysis.

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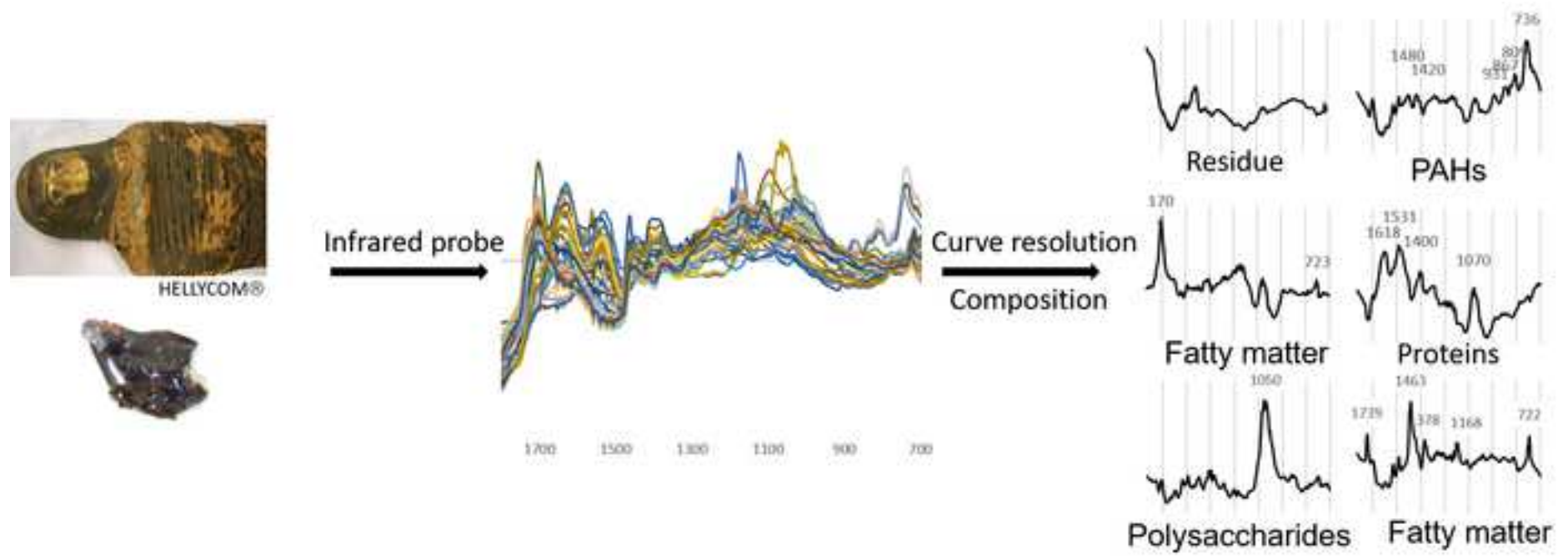
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Novelty statement

This paper deals with a new approach consisting to a fast, simple and non-invasive study allowed the composition of each balms studied as well as their state of conservation/degradation to be characterized, using Simplisma algorithm and Principal Component Analysis.

## Highlights

- Study of Egyptian embalming substances by MIR spectroscopy.
- Use of curve resolution method for the elucidation of balm composition Egyptian mummies
- Use of infrared spectroscopy as probe to estimate the conservation state of Egyptian mummies.



1 **Characterization of Egyptian Human Mummy Balms by chemometric treatments of**  
2 **infrared data**

3

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10

11 **Abstract**

12 This study presents the efficiency of an original method to characterize mummification balms.

13 Currently, long protocols are used to extract each substance from mummification balms by

14 using techniques such as gas chromatography and mass spectrometry. The SIMPLISMA

15 approach using a simple algorithm combined with chemometric treatment, principal component

16 analysis, was used to interpret the 47 Fourier Transform Infrared spectra. This treatment gave

17 6 pure variables and their corresponding extracted concentration profiles. The first pure

18 spectrum corresponded to residual species, the second allowed the identification of polycyclic

19 aromatic hydrocarbons, molecules which have a pyrolytic origin. Proteins were characterized,

20 and they could be considered as markers of a good conservation of individuals. Finally, two

21 pure spectra showed the same natural substance, fat, but on the one hand unaltered and on

22 the other hand degraded fatty matter. This fast, simple and non-invasive approach allowed the

23 composition of each balms studied as well as their state of conservation/degradation to be

24 characterized.

25

26 *Keywords: SIMPLISMA ; MIR ; PCA ; Ancient-Egypt ; Mummification ; Balms.*

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## 28 1. Introduction

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3 29 Balms in the mummification process are used for thousands of years in the history of ancient  
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5 30 Egypt. Given the importance of this funerary practice, the study and chemical characterization  
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7 31 of balms take on their full meaning, but also allow them to be placed in an Egyptian context. It  
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9 32 is therefore interesting to study each substance used in the formulation of the balm. A wide  
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11 33 variety of natural substances is used in the preparation of balms, such as plant oils, animal  
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13 34 fats, resins, beeswax and even other additives such as henna or spices [1–5]. These  
14  
15 35 substances have certain properties such as being hydrophobic, antibacterial or odoriferous.  
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17 36 Each material used in the formulation of a balm is subject to a succession of chemical reactions  
18  
19 37 linked to its evolution over time, from the moment it is obtained to the ageing of the balm, but  
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21 38 it can also undergo reactions linked to the preparation, such as heating. Compounds of  
22  
23 39 alteration can have an important part in the identification of plant species. The molecular  
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25 40 composition of samples can therefore provide information on the nature of the constituents,  
26  
27 41 the state of conservation of the sample and the treatment undergone by the material during  
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29 42 the preparation and/or the preparation of balms [6–8].  
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34 43 Different analytical techniques are used for the chemical characterization of balms depending  
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36 44 on the nature of the constituents. Inorganic materials are analyzed by using spectroscopic  
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38 45 tools such as Raman spectroscopy, energy dispersive X-ray spectroscopy and X-ray  
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40 46 diffraction. Gas chromatography coupled to mass spectrometry is mainly used for the analysis  
41  
42 47 of the organic part. The use of this separative technique is often preceded by a pre-treatment  
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44 48 of the samples, such as saponification reactions, extractions with hexane, dichloromethane or  
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46 49 diethyl ether and derivatization reactions (e.g. trimethylsilylation) [9–16]. Chromatographic  
47  
48 50 analyzes often provide a fine chemical characterization but it is also a laborious and  
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50 51 discriminating technique. Fourier transform infrared spectroscopy (FT-IR) is also used in this  
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52 52 archaeological context. It is a fast and relevant vibrational technique for the analysis of both  
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54 53 organic and inorganic matters, i.e. it allows the mineral part to be characterized and different  
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56 54 classes of organic materials to be highlighted within the same sample [10,17]. FT-IR requires  
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moreover a small amount of sample to obtain an imprint of the overall chemical composition of each individual balm and FT-IR can be non-destructive and non-invasive [18]. This spectroscopic tool is increasingly used for the study of cultural heritage artefacts because of all of these advantages and thus it is a technique of choice for analyzing mummy balms.

This global analytical technique is nevertheless restrictive due to the limited identification of the main components. The obtained spectra are the result of the additivity of all the spectral bands of all the molecules present. Owing to the complex chemical composition of mummification balms, in this study a curve resolution method for self-modeling analysis named SIMPLISMA is used. This mathematical method allows pure spectra and associated "contributions" of different substances present in balms without prior information of their existence to be obtained.

This study deals with the ATR-FTIR spectra obtained of 47 balms samples from 35 human Egyptian mummies belonging to the collection of the Confluence Museum (Lyon, France). The aim of this study was to determine a fine chemical composition of the balms in terms of fatty matter, beeswax, bitumen, polysaccharides, proteins, resinous material and their degradation products.

## 2. Materials and Methods

### 2.1. Archaeological samples

Within the framework of a multidisciplinary research project entitled *Human Egyptian Lyon Confluences Mummies (HELYCOM)-Mourir pour renaître*, mummification balms of human mummies were analyzed. A total of 47 samples belonging to the Egyptian collection of the Confluences Museum in Lyon (France) were considered (Table 1). The mummies studied were female and male dating from Predynastic period to the Roman Period and coming from different necropolises such as Thebes, Esna, Kom-Ombo and Deir-el-Medineh. The weight of the samples varied from 20 to 200 mg and they were collected at different location points on



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2 82 the mummies: on the mummified skin, on the textile covered by balms, or directly in the balms  
3 83 mass.

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## 5 6 7 85 2.2. Infrared spectroscopy

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10 86 FT-IR analyzes were performed on a Nicolet™ iS10 with a MCT detector cooled with liquid  
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12 87 nitrogen. Spectra were acquired in Attenuated Total Reflectance mode with a diamond crystal  
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14 88 and using Omnic Nicolet software. 100 scans of symmetrical interferograms were averaged  
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16 89 and the spectrum was calculated from 1800 to 700  $\text{cm}^{-1}$  at 4  $\text{cm}^{-1}$  spectral resolution. Each  
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18 90 analysis was repeated for take into account the heterogeneity of the samples.

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## 20 21 22 92 2.3. Chemometrics

### 23 24 25 93 2.3.1. SIMPLISMA

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27 94 The method used for self-modeling mixture analysis the SIMPLISMA method described in the  
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29 95 literature [19–24]. This interactive method is used for self-modeling mixture analysis by  
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31 96 resolving mixture data into pure component spectra and concentration profiles without the help  
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33 97 of prior information about the mixture. When overlapping spectral features are present in  
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35 98 spectroscopic data, this tool is unable to resolve broad spectral components and separate  
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37 99 spectral absorption bands characterizing one component. Its concept is based on the  
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39 100 determination of pure variables (e.g., a wavelength or a wavenumber in spectroscopic terms)  
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41 101 that have received the contributions from only one component and an optimization using least-  
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43 102 square method [25]. In order to obtain the proper resolution of the mixture data, user interaction  
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45 103 is required to deal properly with features such as noise, peak shift, and instrument drift [26].  
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47 104 Some applications of SIMPLISMA were already reported in the literature, e.g., on Fourier  
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49 105 transform Raman spectra for the time resolved activation of hydrogen peroxide by a nitrile [27],  
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51 106 gamma irradiated polymers [28], FT-IR microscopy spectra of a polymer laminate, and  
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59 107 pyrolysis mass spectra of biomaterials (feedstock) [19,20,22,25,23,24]. The fitting of the

108 SIMPLISMA results is calculated using the Relative Residual Sum of Squares (RRSSQ),  
109 square sum between the calculated and the original spectra. The SIMPLISMA data treatments  
110 were performed with Matlab 7.8 software. The up-ward shift of the baseline due to the  
111 fluorescence is corrected with Savitzky Golay derivative pre- processing.

### 112 2.3.2. PCA analysis

113 PCA is a descriptive statistical method whose purpose is to present, in graphical form, the  
114 maximum amount of information contained in a data table with the values of  $p$  quantitative  
115 variables for  $n$  individuals. In this study, the algorithm used to calculate eigenvalues is NIPALS  
116 (Non-linear Iterative Partial Least Squares) developed by Herman Wold in 1966 [29]. The  
117 infrared data were reprocessed by the Unscrambler software by applying certain corrections  
118 to eliminate instrumental variations. The first one is a correction of the baseline because the  
119 variations of the baseline participate in a variance and therefore, they can affect the different  
120 spectra. The second correction is normalization (Standard Normal Variate SNV) which allows  
121 the intensity of each spectra is at the maximum to be checked.

122 Kaiser-Meyer-Olkin test is used to validate sampling adequacy [30]. Kaiser, H.F, mentioned  
123 that "*the test measures sampling adequacy for each variable in the model and for the complete*  
124 *model. The statistic is a measure of the proportion of variance among variables that might be*  
125 *common variance*" [31]. The lower the proportion, the more suited the data is to Factor  
126 Analysis. KMO returns values between 0 and 1. A rule of thumb for interpreting the statistic  
127 KMO values less than 0.6 indicate the sampling is not adequate. In our PCA, the KMO equal  
128 0.773 [32].

129

## 130 2.4. Gas Chromatography coupled to Mass Spectrometry

### 131 2.4.1. Solvents and reagents

132 All solvents were of the highest purity grade. Hexane, tetrahydrofurane (THF) and N,O-Bis  
133 (trimethylsilyl)trifluoroacetamide/Trimethylchlorosilane (BSTFA/TMCS) were supplied by

134 Sigma-Aldrich. Ethanol, dichloromethane (DCM) and diethylether (DEE) were supplied by  
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2 135 Merck.  
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#### 6 137 2.4.2. Samples extraction

9 138 10 mg of the sample were extracted with 1 mL of dichloromethane (DCM) using ultrasounds  
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11 139 for ten minutes and then centrifuged at 6000 rpm for ten minutes. The supernatant was  
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13 140 removed, and this step was repeated twice. The three fractions were combined and evaporated  
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15 141 to dryness under a stream of nitrogen. Trimethylsilylation was realized with N,O-  
16 142 Bis(trimethylsilyl)trifluoroacetamide 99%/Trimethylchlorosilane 1% (BSTFA/TMCS) at 70°C for  
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18 143 thirty minutes. The mixture was evaporated to dryness under a stream of nitrogen, it was then  
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20 144 dissolved in 0.2 to 1 mL of hexane/dichloromethane (1/1, v/v) and finally it was filtered on a  
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22 145 0.45 µm PTFE cartridge filter (VWR). 1 µL of the solution was injected into GC-MS.  
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#### 29 147 2.4.3. GC-MS conditions

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32 148 A Thermo Scientific Focus gas chromatographic system composed by a Thermo Scientific AI  
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34 149 3000 auto-sampler coupled with an ITQ 700 ion trap mass spectrometer was used (Thermo  
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36 150 Fisher Scientific). The GC column was a fused silica capillary column Thermo trace GOLD TG-  
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38 151 5MS (5% diphenyl / 95% dimethylpolysiloxane, 30 m length × 0.25 mm i.d. × 0.25 µm film  
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40 152 thickness). Helium was the carrier gas with a constant flow of 1 mL.min<sup>-1</sup>. 1 µL of each sample  
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42 153 was injected in splitless mode. The injector temperature was set at 250 °C. Mass spectra were  
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44 154 recorded in electron impact mode with an electron ionization voltage of 70 eV, an ionization  
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46 155 time of 25,000 µs and a mass range of 40–650 *m/z*. The transfer line, ion trap and manifold  
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48 156 temperatures were respectively set at 300 °C, 200 °C and 50 °C. Detected compounds were  
49  
50 157 identified from interpretation of their mass spectra with reference to NIST database, retention  
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52 158 time and published literature. The oven temperature started at 50 °C with a 2 min hold, increase  
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54 159 of 10°C.min<sup>-1</sup> up to 200 °C, increase of 2.5 °C.min<sup>-1</sup> up to 310 °C, increase of 8°C.min<sup>-1</sup> up to  
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56 160 330 °C and held isothermally for 3 min. The total analysis time is 67 minutes.  
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2 162 3. Results and discussion  
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5 163 The first part of this work was to determine the characteristic bands of the main chemical  
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7 164 functions of the various natural substances which could be present in mummification balms  
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9 165 and that could be characterized in the mid-infrared. These substances were identified using  
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11 166 literature and fresh reference materials. As a reminder, the analyses were carried out between  
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13 167 1800 and 700  $\text{cm}^{-1}$ , this zone was chosen because from it is the fingerprint region. The IR  
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15 168 assignments of the bands are provided in supplementary material according to the literature  
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17 169 (Socrates, 2001). Despite their different locations on the human mummies and the number of  
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19 170 deceased individuals studied, balms samples have similar MIR spectra. A broad band (3700–  
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21 171 3000  $\text{cm}^{-1}$ ) is due to OH stretching vibrations ( $\nu\text{OH}$ ) (oleuropein, cellulose, organic acids, etc.)  
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23 172 and the two bands (3000–2800  $\text{cm}^{-1}$ ) from symmetric and asymmetric CH stretching vibrations  
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25 173 ( $\nu\text{CH}$ , alkyl). The 1800–1500  $\text{cm}^{-1}$  region corresponds to  $\text{C}=\text{O}$  and  $\text{C}=\text{C}$  stretching  
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27 174 vibrations (esters, acid, carboxylate, proteins, aromatic ring). The 1500–1200  $\text{cm}^{-1}$  range is  
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29 175 very complex with especially CH ( $\delta\text{CH}$ , alkyl), and OH deformation vibrations as CO stretching  
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31 176 vibrations (phenols). The intense bands between 1150 and 950  $\text{cm}^{-1}$  corresponds mainly to  
32  
33 177 the endocyclic and exocyclic C–O stretching vibrations of carbohydrates. Absorption bands in  
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35 178 the range 900–746  $\text{cm}^{-1}$ (C–H) are characteristic of the number of adjacent hydrogen atoms  
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37 179 on the aromatic ring.  
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46 181 Each FT-IR experiment was realized on 47 balms samples in duplicate so the SIMPLISMA  
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48 182 treatment was applied to the 94 spectra. This treatment gave 6 pure variables and their  
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50 183 corresponding extracted concentration profile. A pure variable is defined as a variable whose  
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52 184 intensity mainly results from one of the components in the mixture under consideration [21].  
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54 185 Extracted contribution profiles do not represent a real concentration but the contribution of  
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56 186 each pure component in each spectrum. Concentration profiles at zero do not indicate an  
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58 187 absence of this substance but they rather traduce that it is not the primary information within  
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188 the spectrum of this sample, meaning that this pure spectrum is surely characterized by  
189 another substance. This phenomenon can be explained by the fact that the infrared is an  
190 additive analysis of the data.

191 The first pure spectrum corresponded to instrumental contribution (baseline, water  
192 contribution) as well as residual species such as the mineral, so it is not more detailed in this  
193 study.

194 The second pure spectrum (Fig 1.A) was formed by taking the band at  $736\text{ cm}^{-1}$  as the main  
195 one. Other bands of lower intensity could also be observed at respectively 1480, 1420, 931,  
196 867 and  $809\text{ cm}^{-1}$ . All of these bands correspond to the vibrations of the C-H aromatic bonds  
197 [8]. These bonds were characterized by numerous bands depending on the nature of the C-H.

198 In view of the bands present which are characteristic of aromatic C-H bonds and the total  
199 absence of bands characteristic of the presence of oxygen atoms, this pure spectrum could be  
200 attributed to pure Polycyclic Aromatic Hydrocarbons (PAHs). These compounds can appear  
201 after a reaction of carbonization and their presence have been already described in balms [8].

202 The sample concentration profile corresponding to this pure PAHs' spectrum was in large  
203 proportion in the samples referred 44, 45, 46, 52 and 63 (Fig 1.B). These results were  
204 confirmed by chromatographic analyzes. PAHs molecules were detected in these samples by  
205 gas chromatography coupled to mass spectrometry. In order of elution, PAHs compounds  
206 were identified to naphthalene, acenaphthene, phenanthrene, anthracene, fluoranthene,  
207 pyrene, triphenylene, benzanthracene, perylene, benzopyrene, benzoperylene and  
208 dibenzopyrene, with a relative proportion of 95% in all of the corresponding chromatograms.

209 This relative percentage was calculated from the area of all the peaks detected. The origin of  
210 these PAHs can be determined by considering the peak area ratios of phenanthrene  
211 (Phe)/anthracene (Anth) and fluoranthene (Fluo)/pyrene (Pyr). These four molecules are stable  
212 over time and undergo, in an archaeological context, few variations in their concentrations.  
213 Thus, the ratios  $\text{Phe}/\text{Anth} > 30$  and  $\text{Fluo}/\text{Pyr} < 1$  indicate a petrogenic origin of PAHs and the  
214 ratios  $\text{Phe}/\text{Anth} < 10$  and  $\text{Fluo}/\text{Pyr} > 1$  indicate a pyrolytic origin of PAHs. These molecules are

215 notable markers of weathering processes [33], for example, under high temperature  
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2 216 phenanthrene will form anthracene and thus this phenomenon conduces to decrease the ratio  
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4 217 Phe/Anth [34]. Regarding all of the ratios calculated, PAHs come from a combustion of organic  
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6 218 matter, therefore a pyrolytic weathering process of anthropogenic origin. However, no visible  
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8 219 traces of combustion can be observed on the deceased individuals. Two hypothesis can be  
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11 220 formulated: either the deceased individuals were burned before wrapping, or they were already  
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13 221 wrapped before burning and wrapped again.

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16 222 The third pure spectrum extracted (Fig 2.A) showed several intense bands around 1650 and  
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18 223 1550  $\text{cm}^{-1}$  corresponding to the vibrations of the amide functions which may come from  
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21 224 proteins [35]. The concentration profile associated with this spectrum (Fig 2.B) showed that 12  
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23 225 samples (6, 8, 11, 13, 17, 20, 21, 34, 50, 61, 64 and 68) contained a significate proportion of  
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25 226 proteins. The presence of proteinous material was confirmed by specific Amido Black 10B tests  
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27 227 [36]. There are different possible sources of protein such as the human body of the deceased,  
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30 228 an additional ingredient added in the balm or an external contamination. However, this  
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32 229 substance was found in high proportion in the samples that had the least amount of alteration.  
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34 230 Proteins could therefore be markers for the well preservation of the mummy.

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37 231 The fourth pure extracted spectrum (Fig 3.A) showed an extremely intense band at 1050  $\text{cm}^{-1}$   
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39 232 which was characteristic of the presence of polysaccharides. Figure 3.B showed the  
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42 233 concentration profile of each sample for the pure polysaccharide spectrum. Polysaccharides  
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44 234 could be obtained from a variety of sources. They could come from the textile of the strips, the  
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46 235 gommo fraction of a resinous material or also from a gum like Arabic gum. The presence of  
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49 236 polysaccharides in all samples was therefore perfectly explainable.

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52 237 The two last pure extracted spectra and their two respective concentration profiles were  
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54 238 presented in Figure 4. Both of these pure extracted spectra (Fig.4A and 4C) characterized the  
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56 239 same natural substance (i.e. fatty matter or wax, such as beeswax) but in different state of  
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59 240 conservation. Figure 4A presented a high intensity band at 1704  $\text{cm}^{-1}$  (Fig 4.A) which was often

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241 observed in mummy balms corresponding to the C=O function of the carboxylic acids. This  
242 chemical group was generally found in the free fatty acids coming from altered and/or degraded  
243 fatty matter. In the figure 4C, the first observable band was at 1740 cm<sup>-1</sup>, characteristic of the  
244 vibrations of the C=O bond of the esters that could come from the triglycerides of plant oils,  
245 animal fats or waxes as well as beeswax [37]. A band was present at 1168 cm<sup>-1</sup>, interpreted to  
246 the C-O bond of the ester and carboxylic acid functions [38]. Moreover, other bands were  
247 observed at respectively 1463, 1378 and 722 cm<sup>-1</sup> corresponding to C-H bonds of aliphatic  
248 compounds. These bonds come from the long carbon chains of beeswax and plants oil or  
249 animal fats in the form of triglycerides [39]. Both concentration profiles presented  
250 heterogeneous positive and negative profiles with similar intensities (Fig. 4B and 4D). All  
251 samples contained fatty matter or beeswax well conserved or not, exception to those  
252 containing PAHs or another predominant substance. These results were illustrated by Principal  
253 Component Analysis (PCA) and these associated loadings in order to facilitate their readability.  
254 PCA indicated that 2 principal components (PCs) explained 79% of the variability with 55% for  
255 PC1 and 24% for PC2. The obtained results were illustrated in the figure 5 and figure 6. Three  
256 distinct groups have been formed representing samples with a significant proportion of  
257 altered/degraded (group No. 1) or well conserved (group No. 2) fatty matter or beeswax and  
258 the group no 3 contained samples representative by proteins, polysaccharides... However,  
259 exceptions were observed for the samples referred 6, 39, and 50.

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260 GC-MS analyses allowed these interpretations to be corroborated. The group No. 1 included  
261 samples with a high proportion of short carbon chain monocarboxylic free fatty acids and many  
262 dicarboxylic fatty acids (Table 2). The formation of short-chain fatty acids and dicarboxylic  
263 acids corresponds to a dehydration reaction of fatty matter. This reaction is natural and  
264 corresponds to rancidity [40]. It is an oxygenation of unsaturated fatty acids by oxygen from  
265 the air. Several radical chemical reactions form a hydroperoxide intermediate to ultimately  
266 generate short monocarboxylic acids (C) and dicarboxylic acids (DC). The dicarboxylic acids  
267 formed generally have between 4 and 13 carbons; azelaic acid DC 9 is the acid mostly formed.

268 The diacids formed depend on the position of the unsaturation of the fatty acids initially present  
1 [41]. The group No. 2 included samples with few degradation marker, i.e. dicarboxylic fatty  
2 269 acids (Table 3). These groups were therefore a good match for the respective concentration  
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4 270 profiles of the degraded (group No. 1) and the undegraded fatty matters (group No. 2).  
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#### 12 273 4. Conclusion

15 274 Owing to the various natural substances present in mummification balms associated to several  
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17 phenomena of alteration and/or degradation, the interpretation of FT-IR results needs great  
18 275 care. In addition, due to the chemical complexity of these archaeological samples, it can be  
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20 276 difficult to characterize all their materials. In this study, the SIMPLISMA algorithm was applied  
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22 277 with success to extract and to characterize several ingredients used in balms formulation. It  
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24 278 was possible to identify the presence of PAHs, proteins, polysaccharides and fatty matter or  
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26 279 beeswax well conserved or degraded/altered. Moreover, it was also possible to consider and  
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28 280 to compare a significant number of samples with a few experiments and to compare them. GC-  
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30 281 MS was performed to corroborate and to validate the previous interpretations. The obtained  
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32 282 extracted pure spectra associated to their respective concentration profiles provided precious  
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34 283 and fine results. Infrared analyses coupled with SIMPLISMA and PCA analysis also allowed  
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36 284 the presence a set of chemical families present in complex matrices such as balms to be  
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38 285 determined. This work allowed various substances present in mummies' balms to be  
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40 286 highlighted. PAHs, the only compounds that do not correspond to natural substance but they  
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42 287 have an anthropic origin. Mummy balms containing PAHs were apart and they were pointed  
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44 288 out by SIMPLISMA. However, some substances were not detected at all: mineral matter,  
45  
46 289 bitumen, and resins. Middle FT-IR is not the method of choice for mineral analysis. Bitumen is  
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48 290 a non-major constituent in these balms and is already difficult to detect without an extraction  
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50 291 protocol specific to this chemical family. Plant resins are however in great quantity in certain  
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52 292 balms but this product has no characteristic bands. Resinous materials have common bands  
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294 with the free fatty acids. For all of these reasons, it was not possible to discriminate them and  
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2 295 mathematically the algorithm did not manage to extract the resinous substances.  
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4 296 This study presented the efficiency of the method and it proposed an original approach to  
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6 297 characterize mummification balms. It could be interesting to use a portable infrared device,  
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8 298 which is non-invasive and requires no sampling, as a first approach with the parallel use of  
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10 299 SIMPLISMA. This approach would give a clear idea of the composition of the balms of very  
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12 300 different samples and with different state of conservation (deterioration/damage).  
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19  
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## 40 312

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430 Figure legends

431 Figure 1 : Second extracted spectrum (A) and second associated concentration profile (B)  
432 obtained by SIMPLISMA performed on all spectra of mummies samples.

433 Figure 2 : Fourth extracted spectrum (A) and fourth associated concentration profile (B)  
434 obtained by SIMPLISMA performed on all spectra of mummies samples.

435 Figure 3 : Fifth extracted spectrum (A) and fifth associated concentration profile (B) obtained  
436 by SIMPLISMA performed on all spectra of mummies samples.

437 Figure 4 : Third extracted spectrum (A) and third associated concentration profile (B) and sixth  
438 extracted spectrum (C) and sixth associated concentration profile (D) obtained by SIMPLISMA  
439 performed on all spectra of mummies samples.

440 Figure 5: Principal component analysis (PCA) performed on spectra of 47 mummies samples.

441 ● Group 1: samples with positive concentration profiles in figure 4B; ● Group 2: samples with  
442 positive concentration profiles in figure 4D; ● Group 3: samples with negative profiles in both  
443 of the figures 4B and D.

444 Figure 6: Associated loading of PC1 (A) and PC2 (B).

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447 Table legends

448 Table 1: Presentation of 47 samples of balms from 35 Human mummies.

449 Table 2: Fatty matter molecules detected, by gas chromatography and mass spectrometry, on  
450 all samples of cluster 1.

451 Table 3: Fatty matter molecules detected, by gas chromatography and mass spectrometry, on  
452 all samples of cluster 2.

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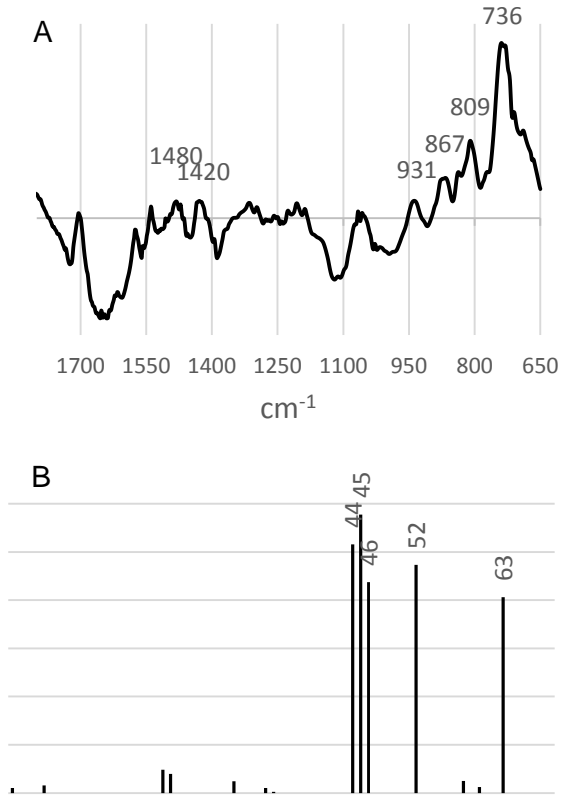
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Figure 1 : Second extracted spectrum (A) and second associated concentration profile (B) obtained by SIMPLISMA performed on all spectra of mummies samples.



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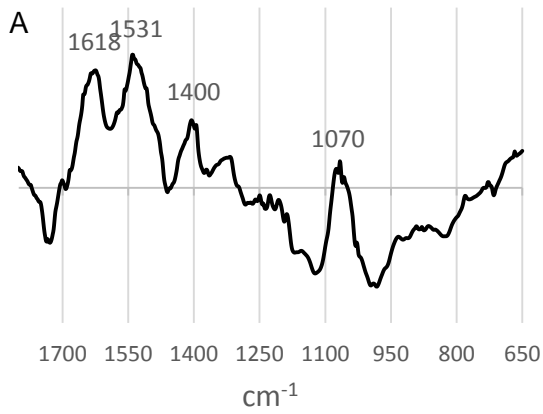
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497 Figure 2 : Fourth extracted spectrum (A) and fourth associated concentration profile (B)  
498 obtained by SIMPLISMA performed on all spectra of mummies samples.

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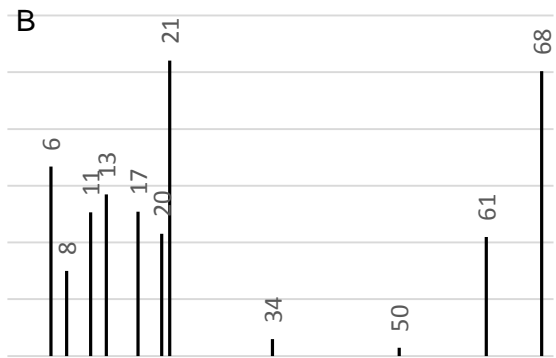
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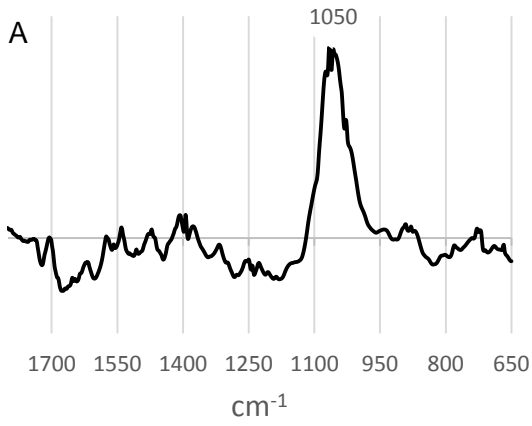
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Figure 3 : fifth extracted spectrum (A) and fifth associated concentration profile (B) obtained by SIMPLISMA performed on all spectra of mummies samples.

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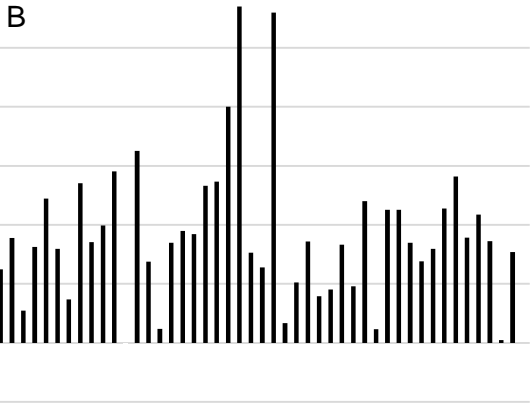
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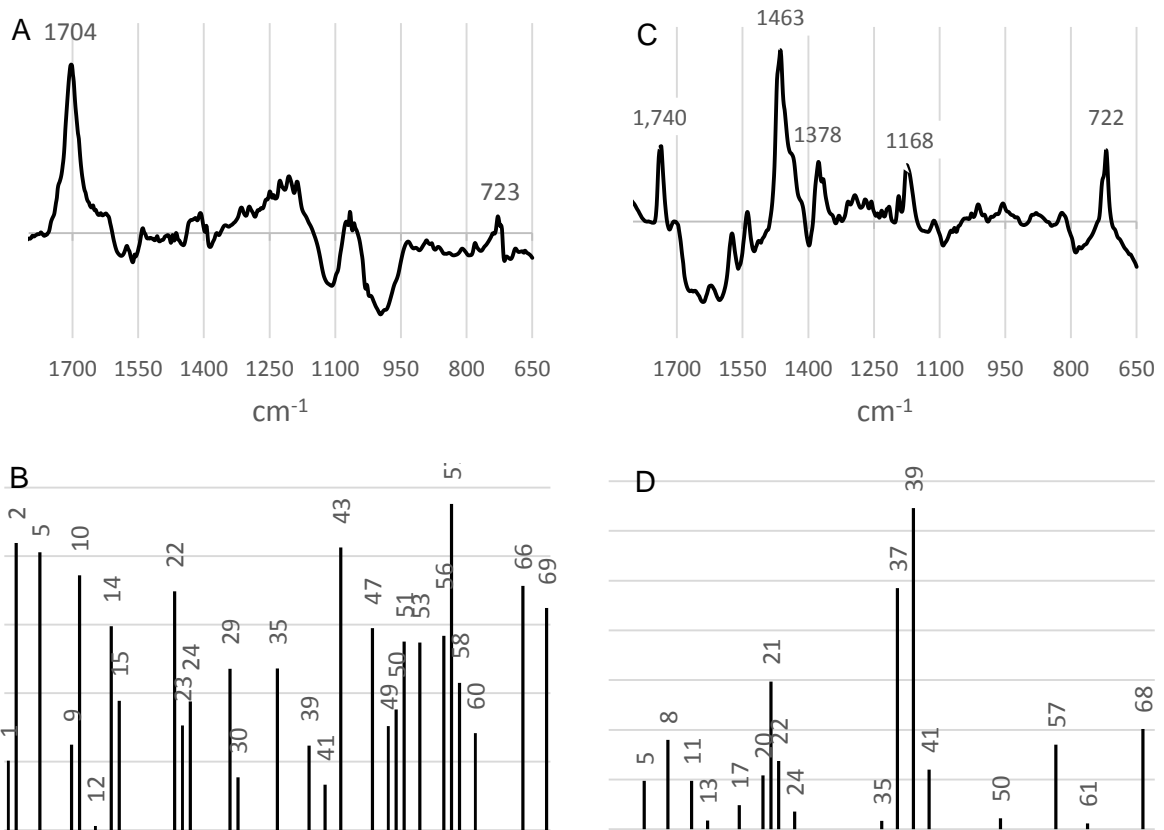
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Figure 4 : Third extracted spectrum (A) and third associated concentration profile (B) and sixth extracted spectrum (C) and sixth associated concentration profile (D) obtained by SIMPLISMA performed on all spectra of mummies samples.



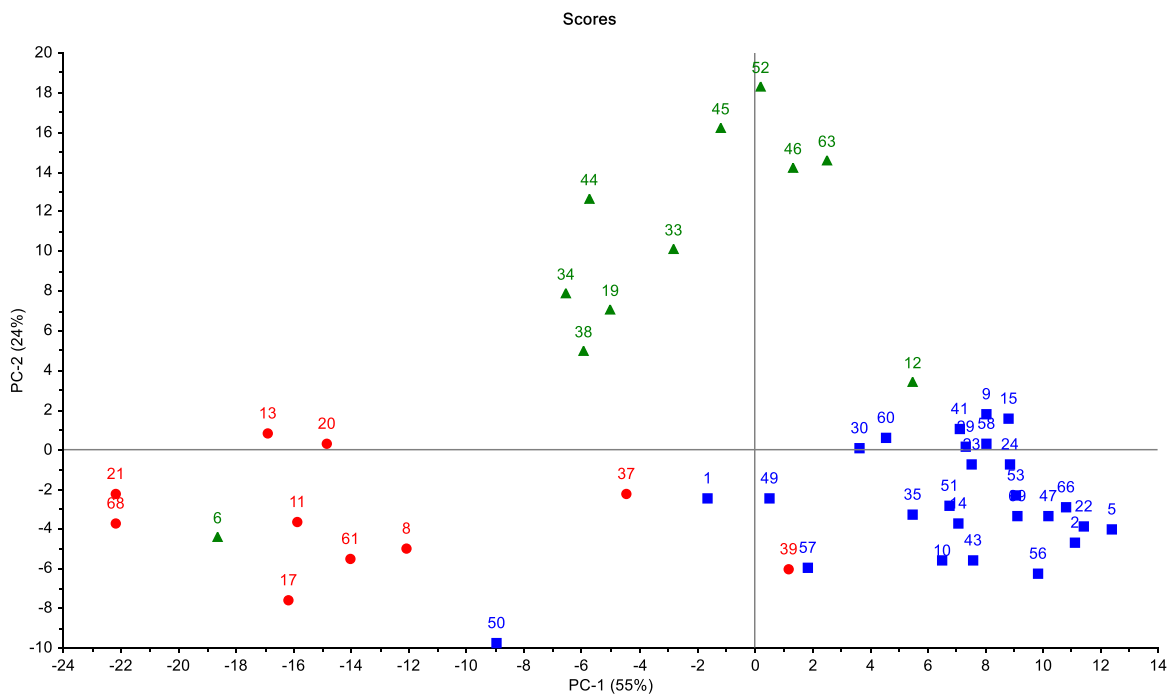


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4 594 Figure 5: Principal component analysis (PCA) performed on spectra of 47 mummies  
5 595 samples.

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9 597 positive concentration profiles in figure 4D; ● Group 3: samples with negative profiles in both  
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11 598 of the figures 4B and D.



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Figure 6: Associated loading of PC1 (A) and PC2 (B).

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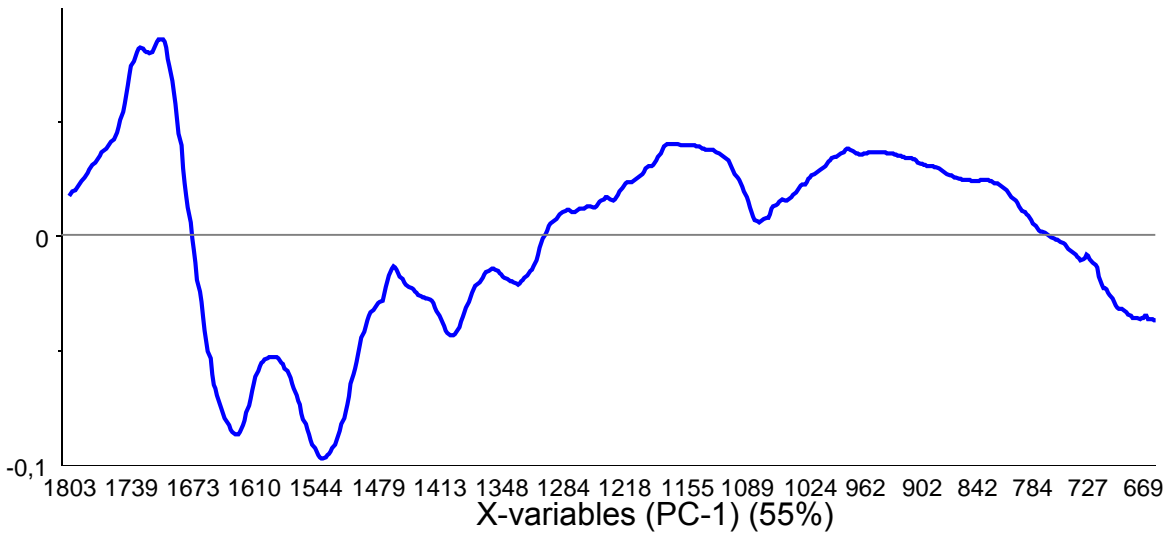
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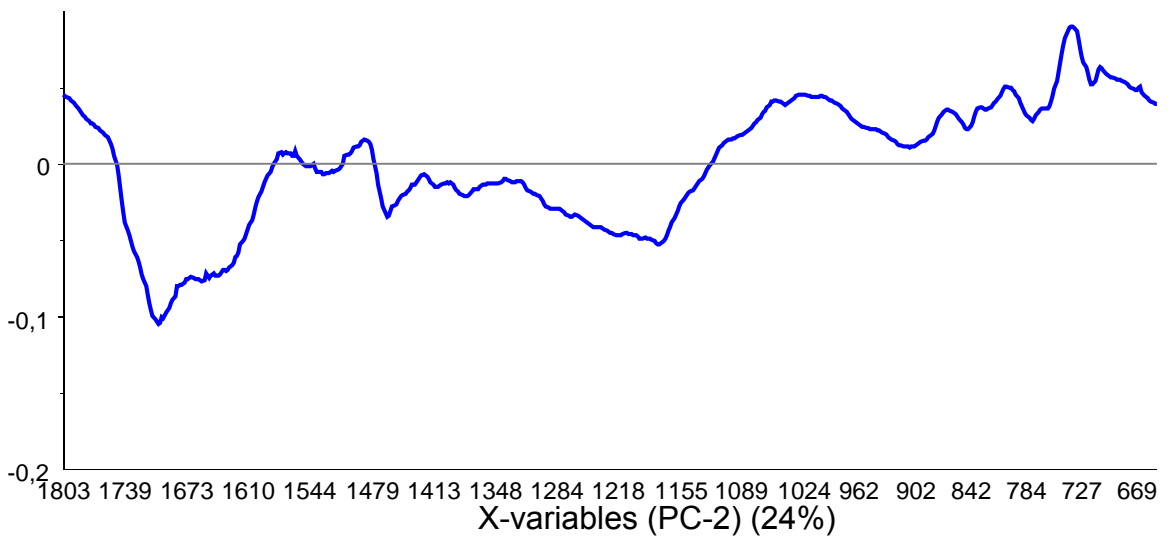
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646 Table 1: Presentation of 47 samples of balms from 35 Human mummies.

	Inventory	N°	Sex	Age	Localisation	Dating <sup>14</sup> C
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3	<b>30000104</b>	29	Female	Adult	Unknown	Late Period to Ptolemaic Period
4	<b>30000107</b>	68	Female	Elderly	Gournah	Unknown
5	<b>30000110</b>	14	Male	Adult	Thebes	Graeco-Roman Period
6	<b>30000111</b>	34	Male	Elderly	Gournah	Graeco-Roman Period
7	<b>30000114</b>	1	Female	Elderly	Thebes	Ptolemaic to Roman Period
8	<b>30000125</b>	5	Male	Adult	Gournah	Late Period to Ptolemaic Period
9	<b>30000144</b>	6	Male	Mature	Unknown	Ptolemaic to Roman Period
10	<b>30000146</b>	35	Female	Adult	Thebes	Unknown
11		22				
12	<b>30000133</b>	23	Male	Adult mature	Gournah	Late Period to Ptolemaic Period
13		24				
14	<b>30000139</b>	15	Female	Young adult	Gournah	Ptolemaic to Roman Period
15	<b>30000142</b>	63	Male	Adult	Gournah	Late Period to Ptolemaic Period
16	<b>30000143</b>	12	Male	Adult	Thebes	Third Intermediate Period to Late Period
17		37				
18	<b>30000148</b>	17	Male	Elderly	Thebes	Unknown
19		19				
20	<b>30000150</b>	20	Male	Adult	Deir el-Medineh	Unknown
21		21				
22		33				
23	<b>30000152</b>	2	Female	Teenager	Thebes	Graeco-Roman Period
24	<b>30000286</b>	10	Male	Mature	Esneh	Third Intermediate Period to Late Period
25		11				
26	<b>30000306</b>	30	Female	Young adult	Deir el-	Unknown
27	<b>30000364</b>	13	Female	Adult	Unknown	Unknown
28		8				
29	<b>90001402</b>	9	Male	Adult	Unknown	Third Intermediate Period to Late Period
30		38				
31	<b>90001635</b>	39	Female	Young adult	Unknown	Predynastic
32	<b>90001169</b>	41	Male	Young adult	Thebes	Late Period to Ptolemaic Period
33	<b>90001259</b>	47	Female	Adult	Kôm Ombo	Ptolemaic to Roman Period
34	<b>90001598</b>	53	Female	Adult	Unknown	Nouvel Empire
35	<b>90002978A</b>	60	Male	Adult mature	Gournah	Unknown
36	<b>90001627</b>	61	Unknown	Child	Rôda	Late Period to Ptolemaic Period
37	<b>90001948A</b>	44	Male	Adult	Gournah	New Kingdom to Third Intermediate Period
38		45				
39	<b>90001948A</b>	46	Male	Adult	Gournah	New Kingdom to Third Intermediate Period
40	<b>90001949A</b>	49	Female	Elderly	Gournah	Graeco-Roman Period
41	<b>90001950A</b>	52	Female	Adult	Gournah	Late Period to Ptolemaic Period
42	<b>90001951A</b>	50	Unknown	Adult	Gournah	Unknown
43		51				
44	<b>90001953A</b>	58	Male	Adult	Gournah	New Kingdom to Third Intermediate Period
45	<b>90001956A</b>	56	Female	Young	Gournah	Unknown
46	<b>90001957A</b>	57	Unknown	Adult	Gournah	Third Intermediate Period to Late Period
47		43				
48	<b>90001257</b>	69	Male	Adult	Gournah	Unknown
49	<b>90001597</b>	66	Female	Adult	Unknown	Late Period to Ptolemaic Period

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650 Table 2: Fatty matter molecules detected, by gas chromatography and mass spectrometry,  
 651 on 9 samples of cluster 1.

Ech	Monocarboxylic fatty acid	Dicarboxylic fatty acid	degraded beeswax
1	8:0, 9:0, 14:0, 15:0, 16:0, 18:1, 18:0	5, 7, 8, 9, 10	-
2	8:0, 9:0, 10:0, 14:0, 16:0, 18:1, 18:0	4, 5, 6, 7, 8, 9, 10, 11, 12	-
5	8;0 9:0, 10:0, 14:0, 16:0, 17:0, 18:1 (tr), 18:0, 20:0, 22:0, 24:0	5, 6, 7, 8, 9, 10	26:0, 28:0, 30:0
9	8;0 9:0, 10:0, 12:0, 14:0, 15:0, 16:0, 17:0, 18:0	7, 8, 9	24:0, 26:0, 28:0
10	8:0, 9:0, 10:0, 14:0, 15:0, 16:0, 17:0, 18:1, 18:0	5, 6, 7, 8, 9, 10	-
14	8:0, 9:0, 10:0, 14:0, 15:0, 16:0, 18:1, 18:0, ricinoléique	5, 6, 7, 8, 9, 10	-
15	8:0, 9:0, 10:0, 12:0, 14:0, 15:0, 16:0, 18:0	7, 8, 9, 10	-
22	8;0 9:0, 10:0, 14:0, 16:0, 17:0, 18:1, 18:0	9	-
23	8;0 9:0, 14:0, 16:0, 17:0, 18:0	9	-
24	8:0, 9:0, 10:0, 14:0, 16:0, 18:0	5, 6, 7, 8, 9, 10	-
29	8:0, 9:0, 14:0, 16:0, 18:1, 18:0	6, 7, 8, 9	-
30	6:0, 8;0 9:0, 14:0, 15:0, 16:1 16:0, 17:0, 18:1 (tr), 18:0	9, 10	-
35	9:0, 12:0, 14:0, 15:0, 16:0, 18:0	7, 9	-
41	8;0 9:0, 13:0, 14:0, 15:0, 16:0, 18:1, 18:0, 24:0	6, 7, 8, 9, 10	26:0 (tr), 28:0 (tr)
43	9:0, 10:0, 12:0, 14:0, 15:0, 16:0, 17:0, 18:1, 18:0, 20:0, 24:0	4, 5, 6, 7, 8, 9, 10	26:0, 28:0
47	8;0 9:0, 12:0, 13:0, 14:0, 16:0, 18:1, 18:0, 24:0	4, 5, 6, 7, 8, 9	26:0, 28:0, 30:0
49	9:0, 14:0, 15:0, 16:1, 16:0, 17:0, 18:1, 18:0, 20:1, 20:0, 22:1, 22:0	6, 7, 8, 9, 10, 11	-
50	9:0, 14:0, 15:0, 16:0, 17:0, 18:1 (tr), 18:0	7, 8, 9, 10	-
51	9:0, 12:0, 14:0, 15:0, 16:1, 16:0, 17:0, 18:0, 20:0, 22:0, 24:0	5, 6, 7, 8, 9, 10, 11	-
53	9:0, 14:0, 16:0, 18:1 (tr), 18:0	6, 7, 8, 9, 10	-
56	9:0, 10:0, 12:0, 14:0, 15:0, 16:0, 18:1, 18:0, ricinoléique, 20:0, 22:0, 24:0	5, 6, 7, 8, 9, 10, 11	-
57	9:0, 10:0, 14:0, 15:0, 16:1, 16:0, 17:0, 18:1, 18:0, ricinoléique, 20:0, 22:0, 24:0	5, 6, 7, 8, 9, 10	-
58	9:0, 10:0, 12:0, 14:0, 15:0, 16:0, 18:1 (tr), 18:0, 20:0, 22:0, 24:0	5, 6, 7, 8, 9, 10, 11	-
60	9:0, 12:0, 14:0, 15:0, 16:1, 16:0, 18:1, 18:0, 20:0, 22:0, 24:0	5, 6, 7, 8, 9, 10, 11	-
66	9:0, 14:0, 16:1 (tr), 16:0, 18:0	4, 5, 6, 7, 8, 9, 10, 11	-
69	9:0, 14:0, 15:0, 16:0, 17:0, 18:1, 18:0, 20:0, 22:0, 24:0	5, 6, 7, 8, 9, 10	-

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656 Table 3: Fatty matter molecules detected, by gas chromatography and mass spectrometry, on  
 1 657 9 samples of cluster 2.

Ech	Monocarboxylic fatty acid	Dicarboxylic fatty acid
<b>8</b>	9:0, 14:0, 15:0, 16:0, 17:0, 18:1, 18:0	9
<b>11</b>	14:0, 15:0, 16:0, 18:1, 18:0	-
<b>13</b>	9:0, 14:0, 16:0, 18:1 (tr), 18:0	-
<b>17</b>	14:0, 15:0, 16:0, 18:1, 18:0	-
<b>20</b>	9:0, 10:0, 12:0, 13:0, 14:2, 14:1, 14:0, 15:0, 16:1, 16:0, 18:1, 18:0, 17:1, 17:0, 18:1, 18:0, 20:1, 20:0, 22:0, 24:0	9
<b>21</b>	9:0, 12:0, 14:0, 15:0, 16:0, 18:1, 18:0	9 (tr)
<b>37</b>	14:0, 16:0, 18:0	-
<b>39</b>	9:0, 12:0, 13:0, 14:0, 15:0, 16:0, 18:1, 18:0, 24:0	-
<b>68</b>	9:0, 14:0, 16:0, 17:0, 18:1, 18:0	-

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**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Table 1: Presentation of 47 samples of balms from 35 Human mummies.

Inventory	N°	Sex	Age	Localisation	Dating <sup>14</sup> C
<b>30000104</b>	29	Female	Adult	Unknown	Late Period to Ptolemaic Period
<b>30000107</b>	68	Female	Elderly	Gournah	Unknown
<b>30000110</b>	14	Male	Adult	Thebes	Graeco-Roman Period
<b>30000111</b>	34	Male	Elderly	Gournah	Graeco-Roman Period
<b>30000114</b>	1	Female	Elderly	Thebes	Ptolemaic to Roman Period
<b>30000125</b>	5	Male	Adult	Gournah	Late Period to Ptolemaic Period
<b>30000144</b>	6	Male	Mature	Unknown	Ptolemaic to Roman Period
<b>30000146</b>	35	Female	Adult	Thebes	Unknown
	22				
<b>30000133</b>	23	Male	Adult mature	Gournah	Late Period to Ptolemaic Period
	24				
<b>30000139</b>	15	Female	Young adult	Gournah	Ptolemaic to Roman Period
<b>30000142</b>	63	Male	Adult	Gournah	Late Period to Ptolemaic Period
	12				
<b>30000143</b>	37	Male	Adult	Thebes	Third Intermediate Period to Late Period
<b>30000148</b>	17	Male	Elderly	Thebes	Unknown
	19				
<b>30000150</b>	20	Male	Adult	Deir el-Medineh	Unknown
	21				
	33				
<b>30000152</b>	2	Female	Teenager	Thebes	Graeco-Roman Period
	10				
<b>30000286</b>	11	Male	Mature	Esneh	Third Intermediate Period to Late Period
<b>30000306</b>	30	Female	Young adult	Deir el-	Unknown
<b>30000364</b>	13	Female	Adult	Unknown	Unknown
	8				
<b>90001402</b>	9	Male	Adult	Unknown	Third Intermediate Period to Late Period
	38				
<b>90001635</b>	39	Female	Young adult	Unknown	Predynastic
<b>90001169</b>	41	Male	Young adult	Thebes	Late Period to Ptolemaic Period
<b>90001259</b>	47	Female	Adult	Kôm Ombo	Ptolemaic to Roman Period
<b>90001598</b>	53	Female	Adult	Unknown	Nouvel Empire
<b>90002978A</b>	60	Male	Adult mature	Gournah	Unknown
<b>90001627</b>	61	Unknown	Child	Rôda	Late Period to Ptolemaic Period
	44				
<b>90001948A</b>	45	Male	Adult	Gournah	New Kingdom to Third Intermediate Period
<b>90001948A</b>	46	Male	Adult	Gournah	New Kingdom to Third Intermediate Period
<b>90001949A</b>	49	Female	Elderly	Gournah	Graeco-Roman Period
<b>90001950A</b>	52	Female	Adult	Gournah	Late Period to Ptolemaic Period
	50				
<b>90001951A</b>	51	Unknown	Adult	Gournah	Unknown
<b>90001953A</b>	58	Male	Adult	Gournah	New Kingdom to Third Intermediate Period
<b>90001956A</b>	56	Female	Young	Gournah	Unknown
<b>90001957A</b>	57	Unknown	Adult	Gournah	Third Intermediate Period to Late Period
	43				
<b>90001257</b>	69	Male	Adult	Gournah	Unknown
<b>90001597</b>	66	Female	Adult	Unknown	Late Period to Ptolemaic Period

Table 2: Fatty matter molecules detected, by gas chromatography and mass spectrometry, on 9 samples of cluster 1.

Ech	Monocarboxylic fatty acid	Dicarboxylic fatty acid	degraded beeswax
1	8:0, 9:0, 14:0, 15:0, 16:0, 18:1, 18:0	5, 7, 8, 9, 10	-
2	8:0, 9:0, 10:0, 14:0, 16:0, 18:1, 18:0	4, 5, 6, 7, 8, 9, 10, 11, 12	-
5	8;0 9:0, 10:0, 14:0, 16:0, 17:0, 18:1 (tr), 18:0, 20:0, 22:0, 24:0	5, 6, 7, 8, 9, 10	26:0, 28:0, 30:0
9	8;0 9:0, 10:0, 12:0, 14:0, 15:0, 16:0, 17:0, 18:0	7, 8, 9	24:0, 26:0, 28:0
10	8:0, 9:0, 10:0, 14:0, 15:0, 16:0, 17:0, 18:1, 18:0	5, 6, 7, 8, 9, 10	-
14	8:0, 9:0, 10:0, 14:0, 15:0, 16:0, 18:1, 18:0, ricinolérique	5, 6, 7, 8, 9, 10	-
15	8:0, 9:0, 10:0, 12:0, 14:0, 15:0, 16:0, 18:0	7, 8, 9, 10	-
22	8;0 9:0, 10:0, 14:0, 16:0, 17:0, 18:1, 18:0	9	-
23	8;0 9:0, 14:0, 16:0, 17:0, 18:0	9	-
24	8:0, 9:0, 10:0, 14:0, 16:0, 18:0	5, 6, 7, 8, 9, 10	-
29	8:0, 9:0, 14:0, 16:0, 18:1, 18:0	6, 7, 8, 9	-
30	6:0, 8;0 9:0, 14:0, 15:0, 16:1 16:0, 17:0, 18:1 (tr), 18:0	9, 10	-
35	9:0, 12:0, 14:0, 15:0, 16:0, 18:0	7, 9	-
41	8;0 9:0, 13:0, 14:0, 15:0, 16:0, 18:1, 18:0, 24:0	6, 7, 8, 9, 10	26:0 (tr), 28:0 (tr)
43	9:0, 10:0, 12:0, 14:0, 15:0, 16:0, 17:0, 18:1, 18:0, 20:0, 24:0	4, 5, 6, 7, 8, 9, 10	26:0, 28:0
47	8;0 9:0, 12:0, 13:0, 14:0, 16:0, 18:1, 18:0, 24:0	4, 5, 6, 7, 8, 9	26:0, 28:0, 30:0
49	9:0, 14:0, 15:0, 16:1, 16:0, 17:0, 18:1, 18:0, 20:1, 20:0, 22:1, 22:0	6, 7, 8, 9, 10, 11	-
50	9:0, 14:0, 15:0, 16:0, 17:0, 18:1 (tr), 18:0	7, 8, 9, 10	-
51	9:0, 12:0, 14:0, 15:0, 16:1, 16:0, 17:0, 18:0, 20:0, 22:0, 24:0	5, 6, 7, 8, 9, 10, 11	-
53	9:0, 14:0, 16:0, 18:1 (tr), 18:0	6, 7, 8, 9, 10	-
56	9:0, 10:0, 12:0, 14:0, 15:0, 16:0, 18:1, 18:0, ricinolérique, 20:0, 22:0, 24:0	5, 6, 7, 8, 9, 10, 11	-
57	9:0, 10:0, 14:0, 15:0, 16:1, 16:0, 17:0, 18:1, 18:0, ricinolérique, 20:0, 22:0, 24:0	5, 6, 7, 8, 9, 10	-
58	9:0, 10:0, 12:0, 14:0, 15:0, 16:0, 18:1 (tr), 18:0, 20:0, 22:0, 24:0	5, 6, 7, 8, 9, 10, 11	-
60	9:0, 12:0, 14:0, 15:0, 16:1, 16:0, 18:1, 18:0, 20:0, 22:0, 24:0	5, 6, 7, 8, 9, 10, 11	-
66	9:0, 14:0, 16:1 (tr), 16:0, 18:0	4, 5, 6, 7, 8, 9, 10, 11	-
69	9:0, 14:0, 15:0, 16:0, 17:0, 18:1, 18:0, 20:0, 22:0, 24:0	5, 6, 7, 8, 9, 10	-

Table 3: Fatty matter molecules detected, by gas chromatography and mass spectrometry, on 9 samples of cluster 2.

<b>Ech</b>	<b>Monocarboxylic fatty acid</b>	<b>Dicarboxylic fatty acid</b>
<b>8</b>	9:0, 14:0, 15:0, 16:0, 17:0, 18:1, 18:0	9
<b>11</b>	14:0, 15:0, 16:0, 18:1, 18:0	-
<b>13</b>	9:0, 14:0, 16:0, 18:1 (tr), 18:0	-
<b>17</b>	14:0, 15:0, 16:0, 18:1, 18:0	-
<b>20</b>	9:0, 10:0, 12:0, 13:0, 14:2, 14:1, 14:0, 15:0, 16:1, 16:0, 18:1, 18:0, 17:1, 17:0, 18:1, 18:0, 20:1, 20:0, 22:0, 24:0	9
<b>21</b>	9:0, 12:0, 14:0, 15:0, 16:0, 18:1, 18:0	9 (tr)
<b>37</b>	14:0, 16:0, 18:0	-
<b>39</b>	9:0, 12:0, 13:0, 14:0, 15:0, 16:0, 18:1, 18:0, 24:0	-
<b>68</b>	9:0, 14:0, 16:0, 17:0, 18:1, 18:0	-

Figure 1 : Second extracted spectrum (A) and second associated concentration profile (B) obtained by SIMPLISMA performed on all spectra of mummies samples.

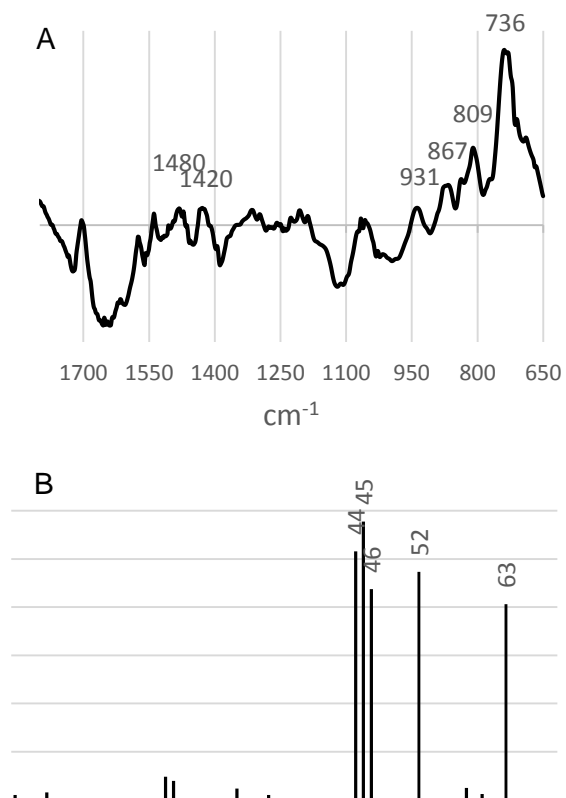


Figure 2 : Fourth extracted spectrum (A) and fourth associated concentration profile (B) obtained by SIMPLISMA performed on all spectra of mummies samples.

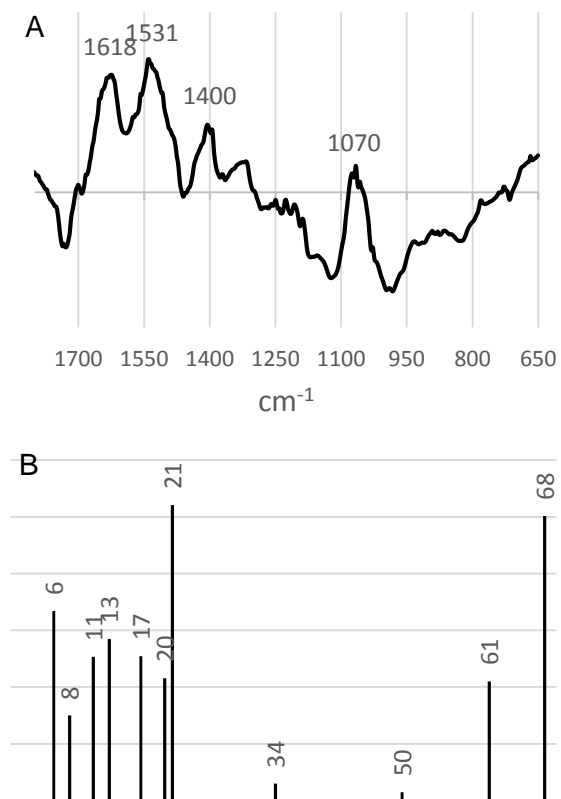


Figure 3 : fifth extracted spectrum (A) and fifth associated concentration profile (B) obtained by SIMPLISMA performed on all spectra of mummies samples.

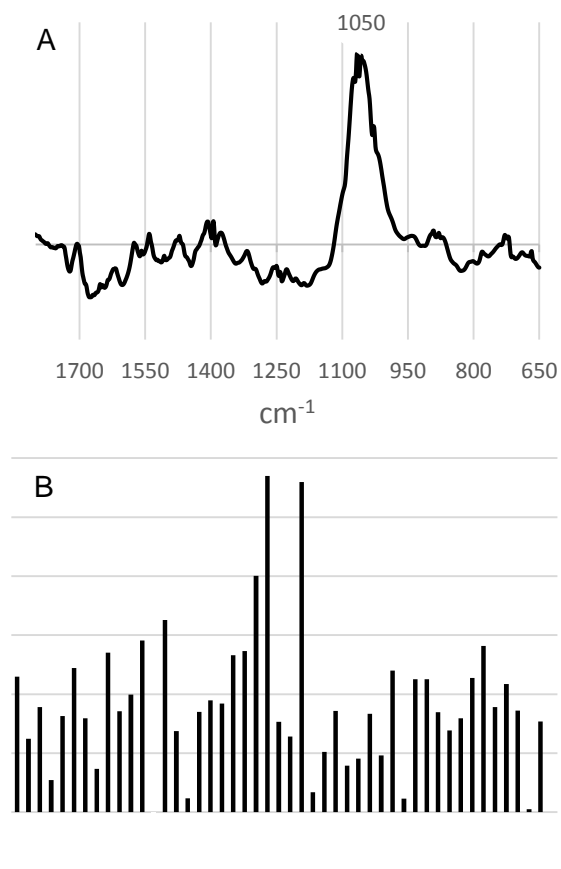


Figure 4 : Third extracted spectrum (A) and third associated concentration profile (B) and sixth extracted spectrum (C) and sixth associated concentration profile (D) obtained by SIMPLISMA performed on all spectra of mummies samples.

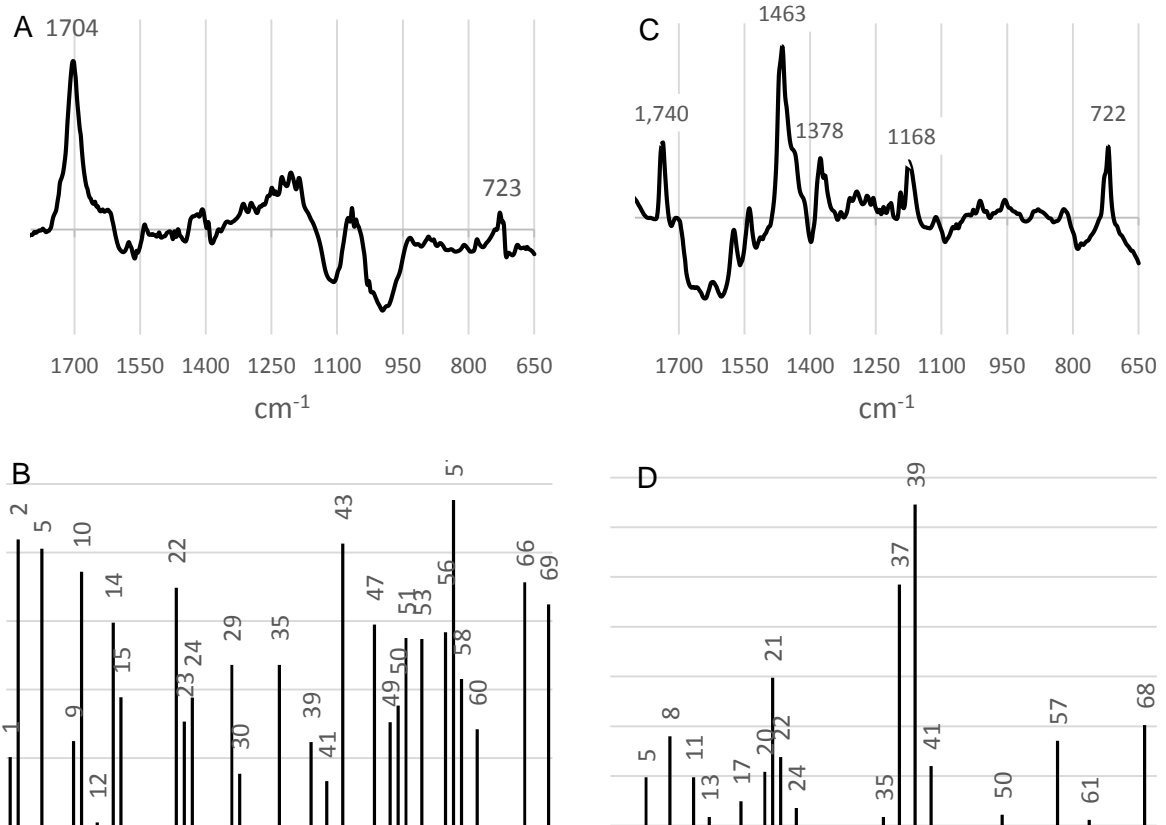




Figure 5

Figure 5: Principal component analysis (PCA) performed on spectra of 47 mummies samples.

- Group 1: samples with positive concentration profiles in figure 4B; ● Group 2: samples with positive concentration profiles in figure 4D; ● Group 3: samples with negative profiles in both of the figures 4B and D.

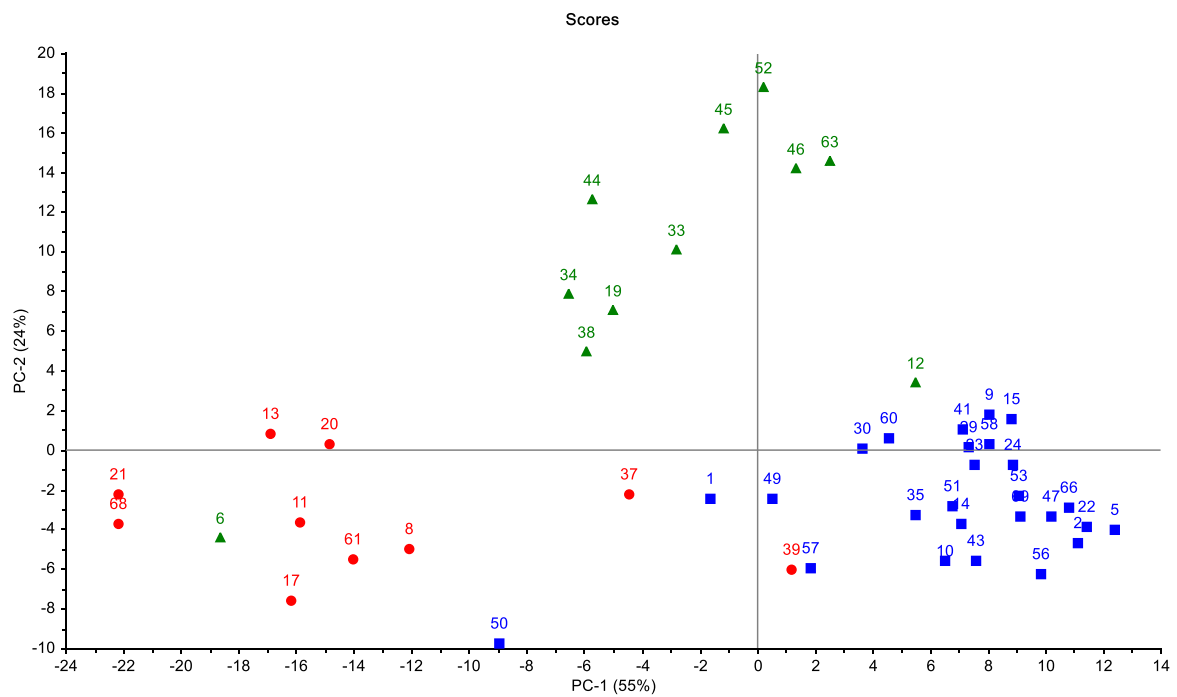
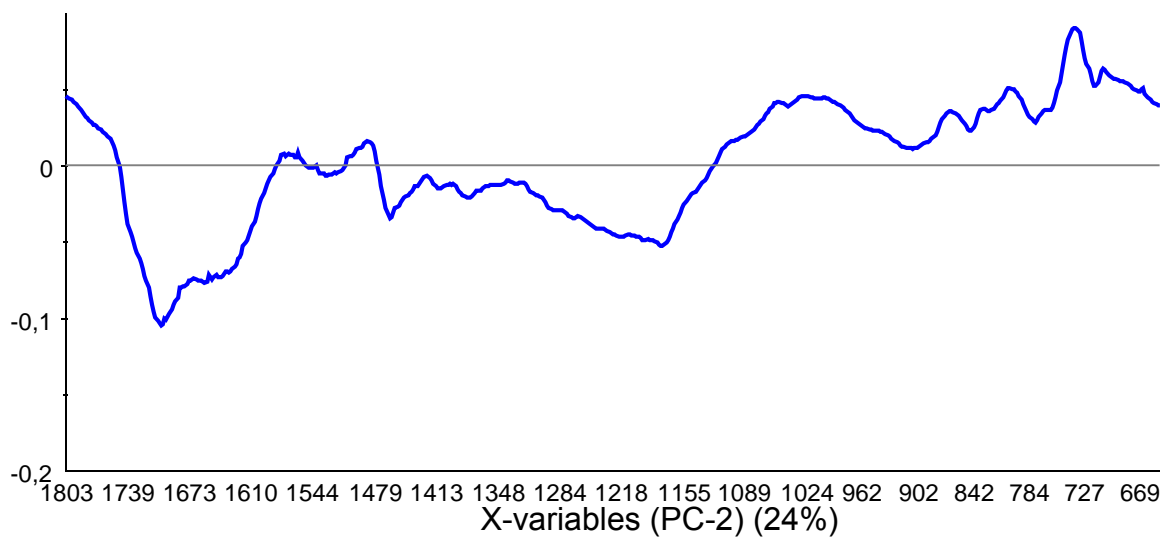
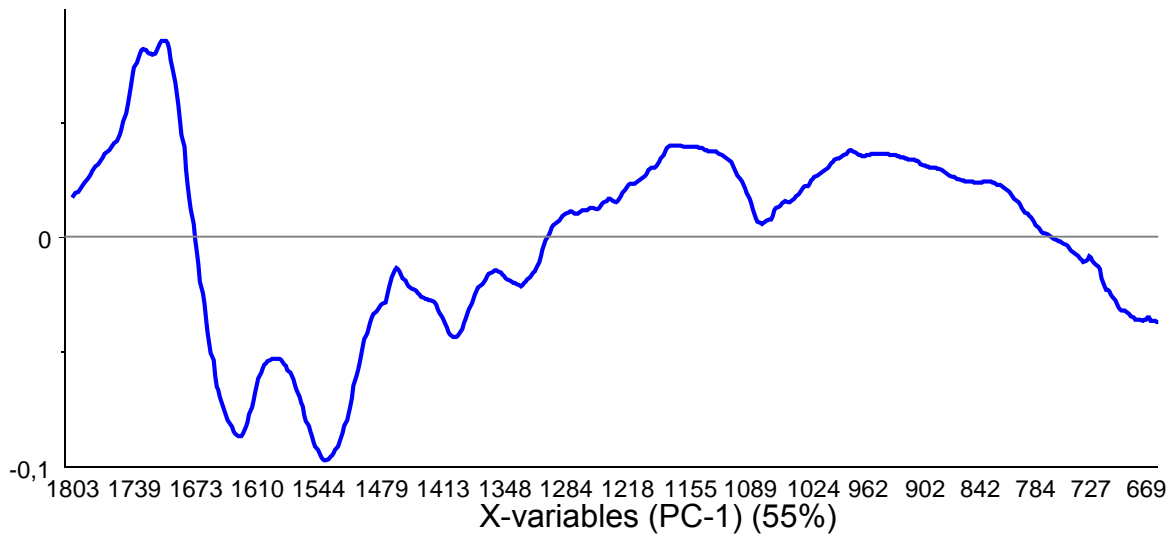


Figure 6: Associated loading of PC1 (A) and PC2 (B).





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