



Evaluation of a characterization method of Egyptian human mummy balms by chemometric treatments of infrared data

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

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Abstract:	<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, 31st 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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Sincerely yours,

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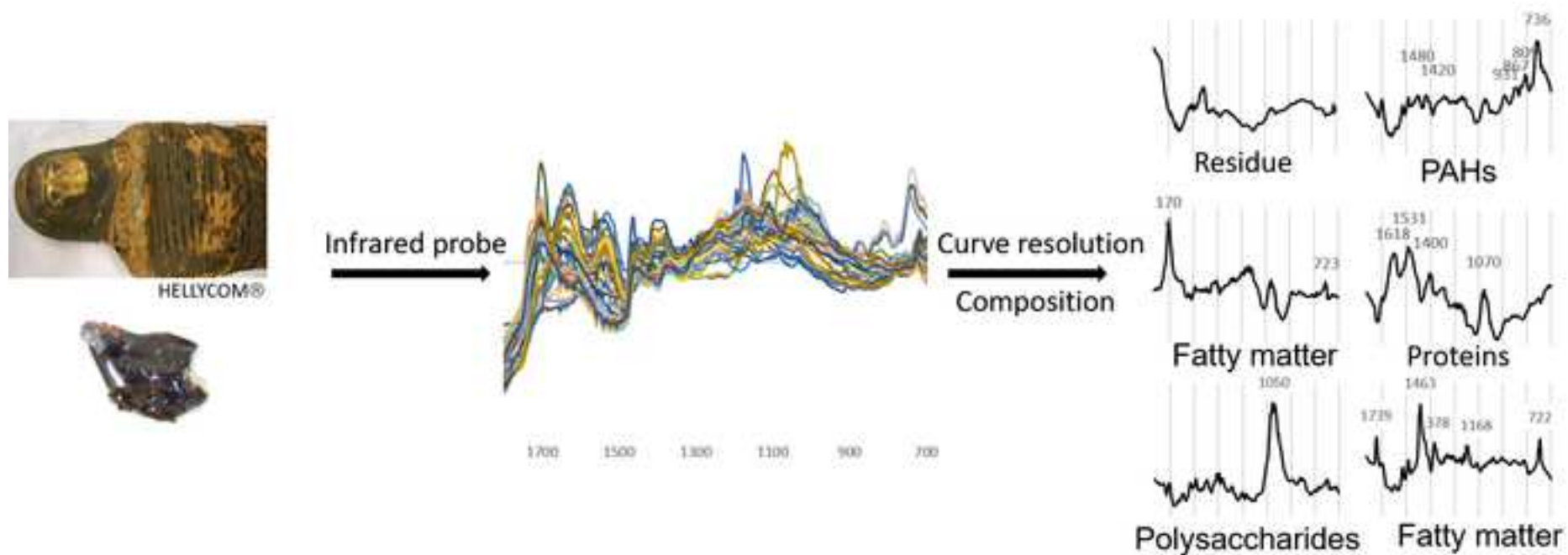
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Novelty statement

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

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22 23 Abstract

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1. Introduction

Balms in the mummification process are used for thousands of years in the history of ancient Egypt. Given the importance of this funerary practice, the study and chemical characterization of balms take on their full meaning, but also allow them to be placed in an Egyptian context. It is therefore interesting to study each substance used in the formulation of the balm. A wide variety of natural substances is used in the preparation of balms, such as plant oils, animal fats, resins, beeswax and even other additives such as henna or spices [1–5]. These substances have certain properties such as being hydrophobic, antibacterial or odoriferous. Each material used in the formulation of a balm is subject to a succession of chemical reactions linked to its evolution over time, from the moment it is obtained to the ageing of the balm, but it can also undergo reactions linked to the preparation, such as heating. Compounds of alteration can have an important part in the identification of plant species. The molecular composition of samples can therefore provide information on the nature of the constituents, the state of conservation of the sample and the treatment undergone by the material during the preparation and/or the preparation of balms [6–8].

Different analytical techniques are used for the chemical characterization of balms depending on the nature of the constituents. Inorganic materials are analyzed by using spectroscopic tools such as Raman spectroscopy, energy dispersive X-ray spectroscopy and X-ray diffraction. Gas chromatography coupled to mass spectrometry is mainly used for the analysis of the organic part. The use of this separative technique is often preceded by a pre-treatment of the samples, such as saponification reactions, extractions with hexane, dichloromethane or diethyl ether and derivatization reactions (e.g. trimethylsilylation) [9–16]. Chromatographic analyzes often provide a fine chemical characterization but it is also a laborious and discriminating technique. Fourier transform infrared spectroscopy (FT-IR) is also used in this archaeological context. It is a fast and relevant vibrational technique for the analysis of both organic and inorganic matters, i.e. it allows the mineral part to be characterized and different classes of organic materials to be highlighted within the same sample [10,17]. FT-IR requires

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

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

2.2. Infrared spectroscopy

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

2.3. Chemometrics

2.3.1. SIMPLISMA

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

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

2.3.2. PCA analysis

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

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

2.4. Gas Chromatography coupled to Mass Spectrometry

2.4.1. Solvents and reagents

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

Sigma-Aldrich. Ethanol, dichloromethane (DCM) and diethylether (DEE) were supplied by Merck.

2.4.2. Samples extraction

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

2.4.3. GC-MS conditions

A Thermo Scientific Focus gas chromatographic system composed by a Thermo Scientific AI 3000 auto-sampler coupled with an ITQ 700 ion trap mass spectrometer was used (Thermo Fisher Scientific). The GC column was a fused silica capillary column Thermo trace GOLD TG-5MS (5% diphenyl / 95% dimethylpolysiloxane, 30 m length × 0.25 mm i.d. × 0.25 µm film thickness). Helium was the carrier gas with a constant flow of 1 mL.min⁻¹. 1 µL of each sample was injected in splitless mode. The injector temperature was set at 250 °C. Mass spectra were recorded in electron impact mode with an electron ionization voltage of 70 eV, an ionization time of 25,000 µs and a mass range of 40–650 *m/z*. The transfer line, ion trap and manifold temperatures were respectively set at 300 °C, 200 °C and 50 °C. Detected compounds were identified from interpretation of their mass spectra with reference to NIST database, retention time and published literature. The oven temperature started at 50 °C with a 2 min hold, increase of 10°C.min⁻¹ up to 200 °C, increase of 2.5 °C.min⁻¹ up to 310 °C, increase of 8°C.min⁻¹ up to 330 °C and held isothermally for 3 min. The total analysis time is 67 minutes.

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3. Results and discussion

The first part of this work was to determine the characteristic bands of the main chemical functions of the various natural substances which could be present in mummification balms and that could be characterized in the mid-infrared. These substances were identified using literature and fresh reference materials. As a reminder, the analyses were carried out between 1800 and 700 cm^{-1} , this zone was chosen because from it is the fingerprint region. The IR assignments of the bands are provided in supplementary material according to the literature (Socrates, 2001). Despite their different locations on the human mummies and the number of deceased individuals studied, balms samples have similar MIR spectra. A broad band (3700–3000 cm^{-1}) is due to OH stretching vibrations (νOH) (oleuropein, cellulose, organic acids, etc.) and the two bands (3000–2800 cm^{-1}) from symmetric and asymmetric CH stretching vibrations (νCH , alkyl). The 1800–1500 cm^{-1} region corresponds to $\text{C}=\text{O}$ and $\text{C}=\text{C}$ stretching vibrations (esters, acid, carboxylate, proteins, aromatic ring). The 1500–1200 cm^{-1} range is very complex with especially CH (δCH , alkyl), and OH deformation vibrations as CO stretching vibrations (phenols). The intense bands between 1150 and 950 cm^{-1} corresponds mainly to the endocyclic and exocyclic C–O stretching vibrations of carbohydrates. Absorption bands in the range 900–746 cm^{-1} (C–H) are characteristic of the number of adjacent hydrogen atoms on the aromatic ring.

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Each FT-IR experiment was realized on 47 balms samples in duplicate so the SIMPLISMA treatment was applied to the 94 spectra. This treatment gave 6 pure variables and their corresponding extracted concentration profile. A pure variable is defined as a variable whose intensity mainly results from one of the components in the mixture under consideration [21]. Extracted contribution profiles do not represent a real concentration but the contribution of each pure component in each spectrum. Concentration profiles at zero do not indicate an absence of this substance but they rather traduce that it is not the primary information within

the spectrum of this sample, meaning that this pure spectrum is surely characterized by another substance. This phenomenon can be explained by the fact that the infrared is an additive analysis of the data.

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

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

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

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

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

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

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

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

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

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

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

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

4. Conclusion

Owing to the various natural substances present in mummification balms associated to several phenomena of alteration and/or degradation, the interpretation of FT-IR results needs great care. In addition, due to the chemical complexity of these archaeological samples, it can be difficult to characterize all their materials. In this study, the SIMPLISMA algorithm was applied with success to extract and to characterize several ingredients used in balms formulation. It was possible to identify the presence of PAHs, proteins, polysaccharides and fatty matter or beeswax well conserved or degraded/alterd. Moreover, it was also possible to consider and to compare a significant number of samples with a few experiments and to compare them. GC-MS was performed to corroborate and to validate the previous interpretations. The obtained extracted pure spectra associated to their respective concentration profiles provided precious and fine results. Infrared analyses coupled with SIMPLISMA and PCA analysis also allowed the presence a set of chemical families present in complex matrices such as balms to be determined. This work allowed various substances present in mummies' balms to be highlighted. PAHs, the only compounds that do not correspond to natural substance but they have an anthropic origin. Mummy balms containing PAHs were apart and they were pointed out by SIMPLISMA. However, some substances were not detected at all: mineral matter, bitumen, and resins. Middle FT-IR is not the method of choice for mineral analysis. Bitumen is a non-major constituent in these balms and is already difficult to detect without an extraction protocol specific to this chemical family. Plant resins are however in great quantity in certain balms but this product has no characteristic bands. Resinous materials have common bands

with the free fatty acids. For all of these reasons, it was not possible to discriminate them and mathematically the algorithm did not manage to extract the resinous substances.

This study presented the efficiency of the method and it proposed an original approach to characterize mummification balms. It could be interesting to use a portable infrared device, which is non-invasive and requires no sampling, as a first approach with the parallel use of SIMPLISMA. This approach would give a clear idea of the composition of the balms of very different samples and with different state of conservation (deterioration/damage).

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Figure legends

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: 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).

Table legends

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

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

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

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

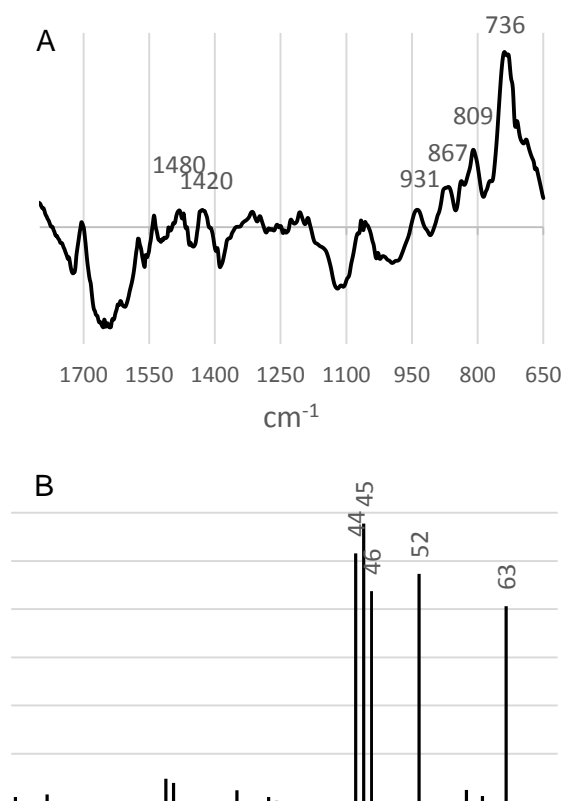


Figure 2 : Fourth extracted spectrum (A) and fourth associated concentration profile (B) obtained by SIMPLISMA performed on all spectra of mummies samples.

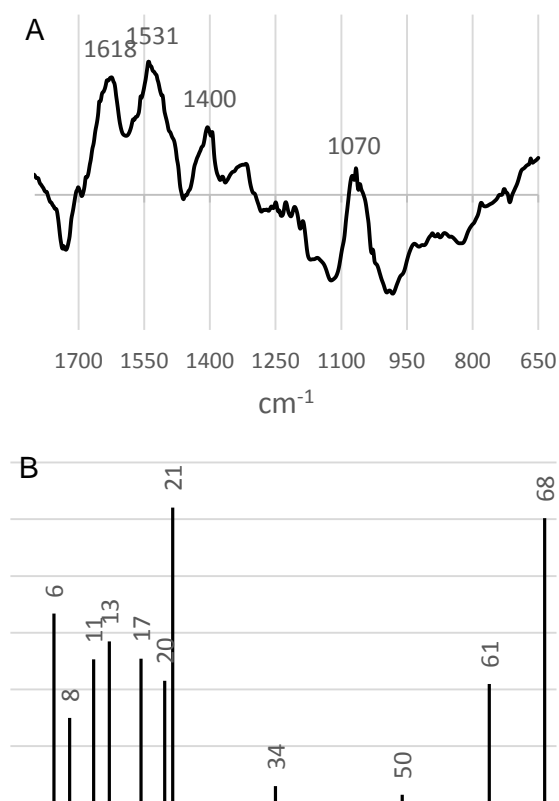


Figure 3 : fifth extracted spectrum (A) and fifth associated concentration profile (B) obtained by SIMPLISMA performed on all spectra of mummies samples.

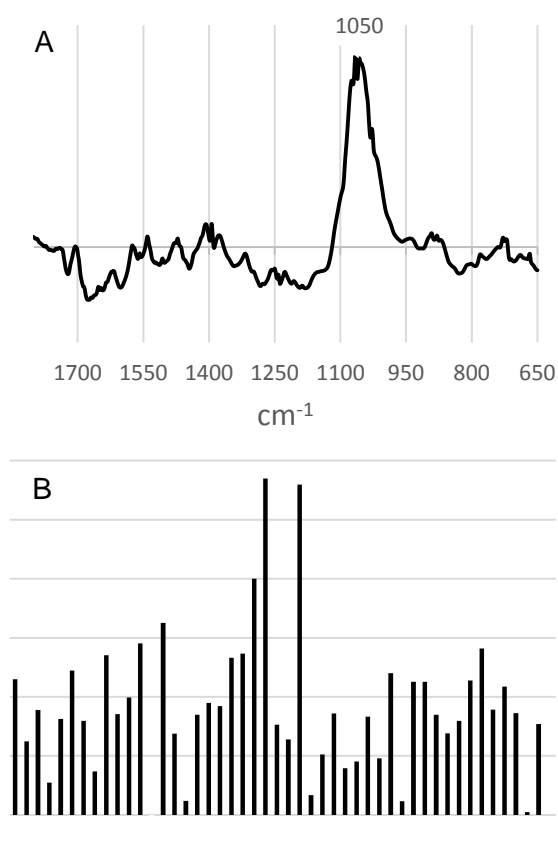


Figure 4 : Third extracted spectrum (A) and third associated concentration profile (B) and sixth extracted spectrum (C) and sixth associated concentration profile (D) obtained by SIMPLISMA performed on all spectra of mummies samples.

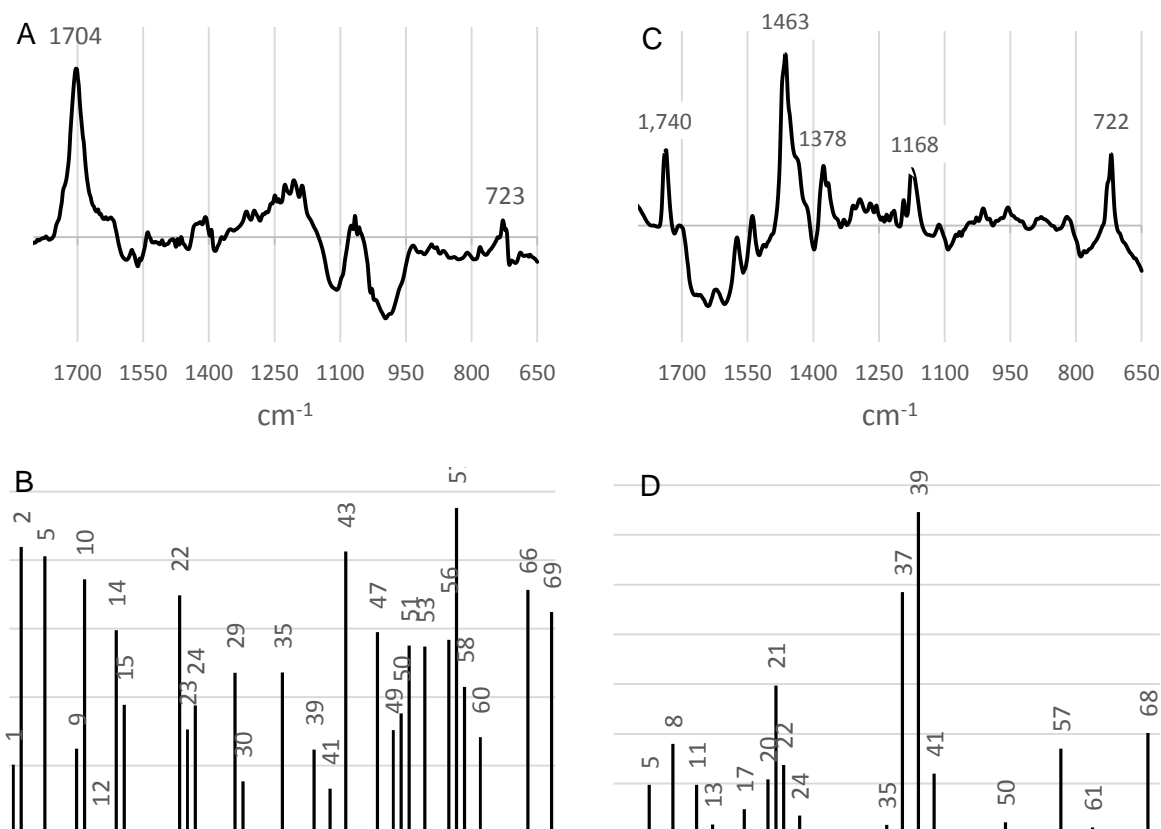
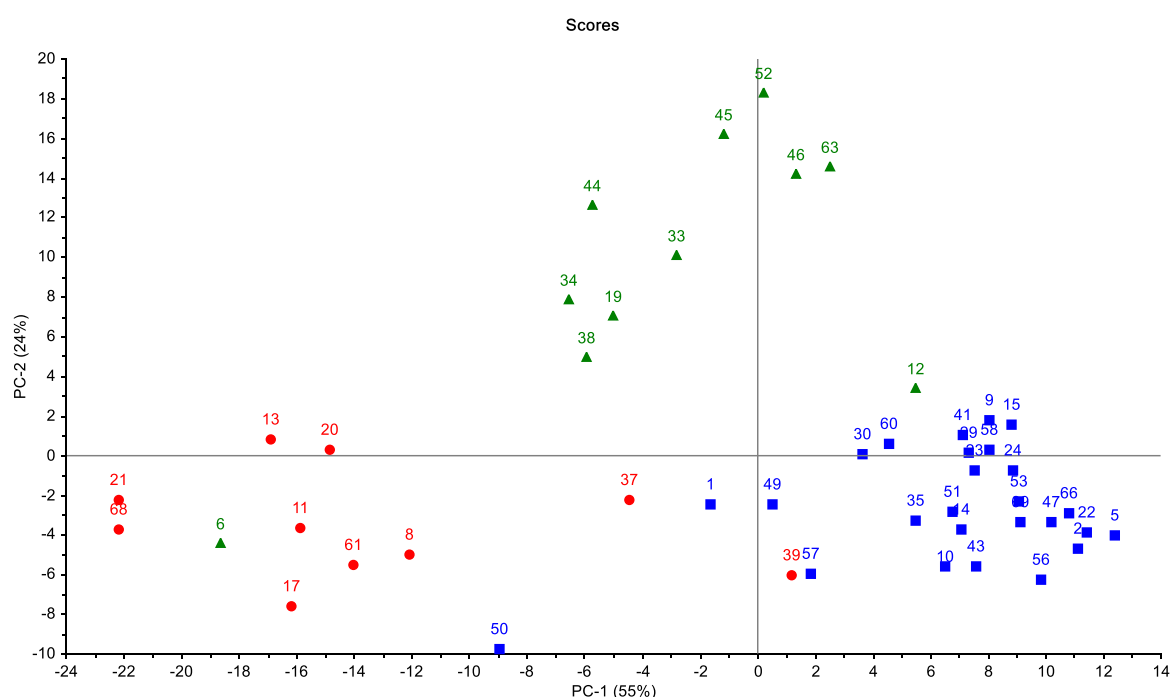


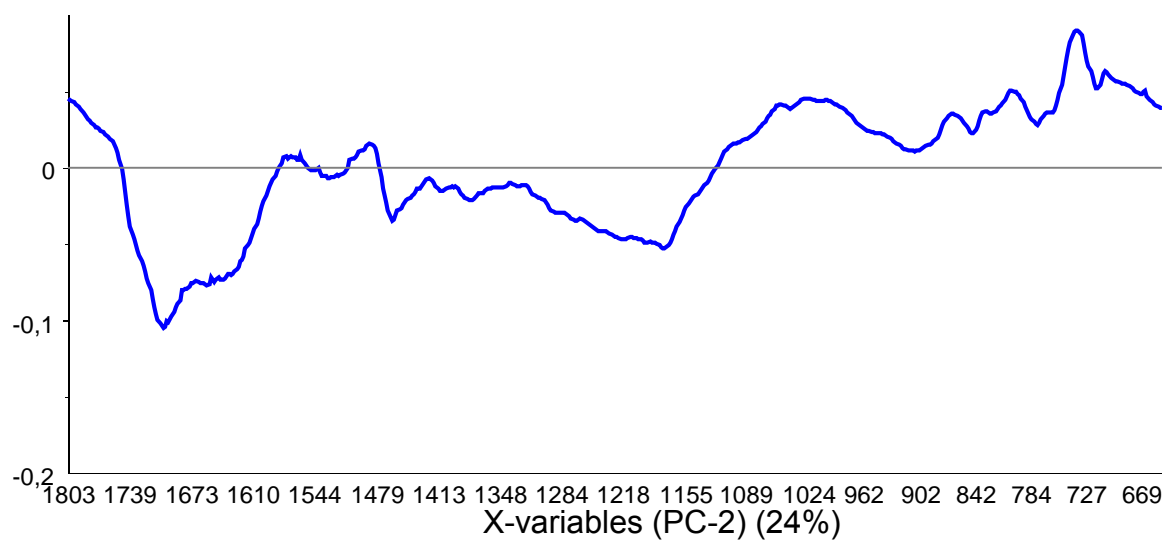
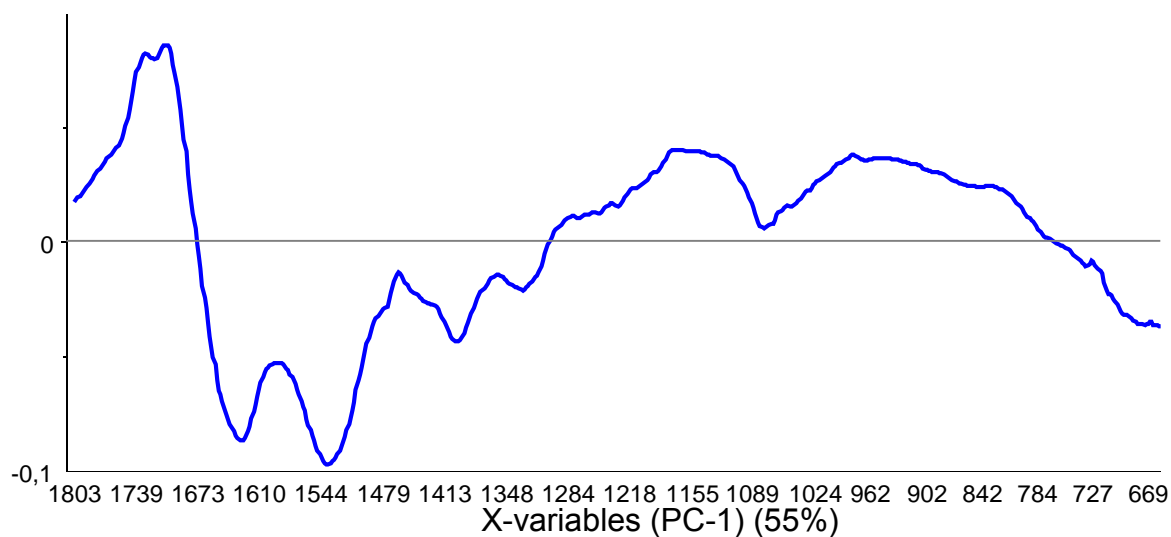
Figure 5: Principal component analysis (PCA) performed on spectra of 47 mummies samples.

● 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.



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



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

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

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

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

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30000146	35	Female	Adult	Thebes	Unknown
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30000148	17	Male	Elderly	Thebes	Unknown
	19				
30000150	20	Male	Adult	Deir el-Medineh	Unknown
	21				
	33				
30000152	2	Female	Teenager	Thebes	Graeco-Roman Period
	10				
30000286	11	Male	Mature	Esneh	Third Intermediate Period to Late Period
30000306	30	Female	Young adult	Deir el-	Unknown
30000364	13	Female	Adult	Unknown	Unknown
	8				
90001402	9	Male	Adult	Unknown	Third Intermediate Period to Late Period
90001635	38	Female	Young adult	Unknown	Predynastic
	39				
90001169	41	Male	Young adult	Thebes	Late Period to Ptolemaic Period
90001259	47	Female	Adult	Kôm Ombo	Ptolemaic to Roman Period
90001598	53	Female	Adult	Unknown	Nouvel Empire
90002978A	60	Male	Adult mature	Gournah	Unknown
90001627	61	Unknown	Child	Rôda	Late Period to Ptolemaic Period
	44				
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90001948A	46	Male	Adult	Gournah	New Kingdom to Third Intermediate Period
90001949A	49	Female	Elderly	Gournah	Graeco-Roman Period
90001950A	52	Female	Adult	Gournah	Late Period to Ptolemaic Period
	50				
90001951A	51	Unknown	Adult	Gournah	Unknown
90001953A	58	Male	Adult	Gournah	New Kingdom to Third Intermediate Period
90001956A	56	Female	Young	Gournah	Unknown
90001957A	57	Unknown	Adult	Gournah	Third Intermediate Period to Late Period
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90001597	66	Female	Adult	Unknown	Late Period to Ptolemaic Period

Table 2

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

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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	-

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

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

Figure 1

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

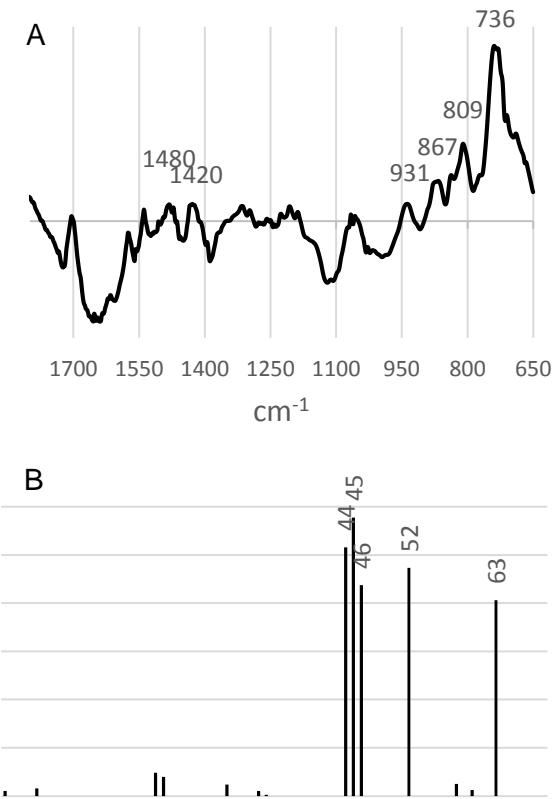


Figure 2

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

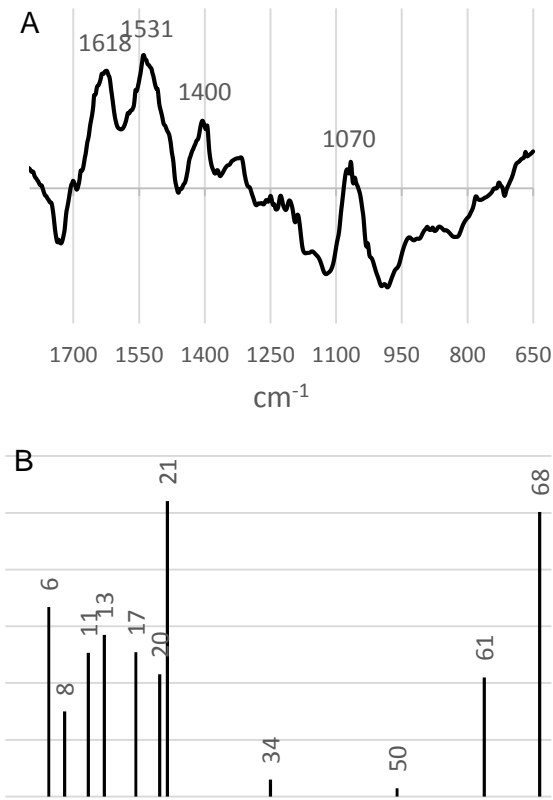


Figure 3

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

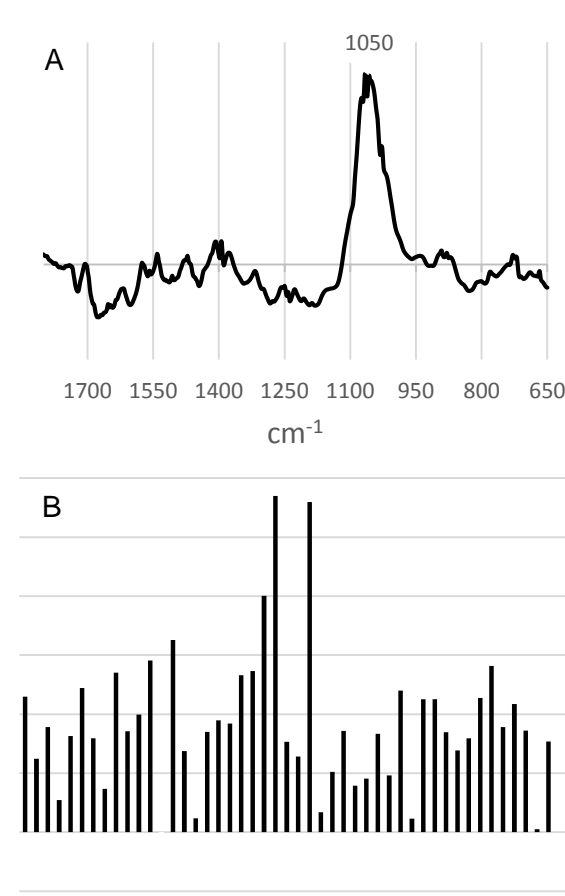


Figure 4

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.

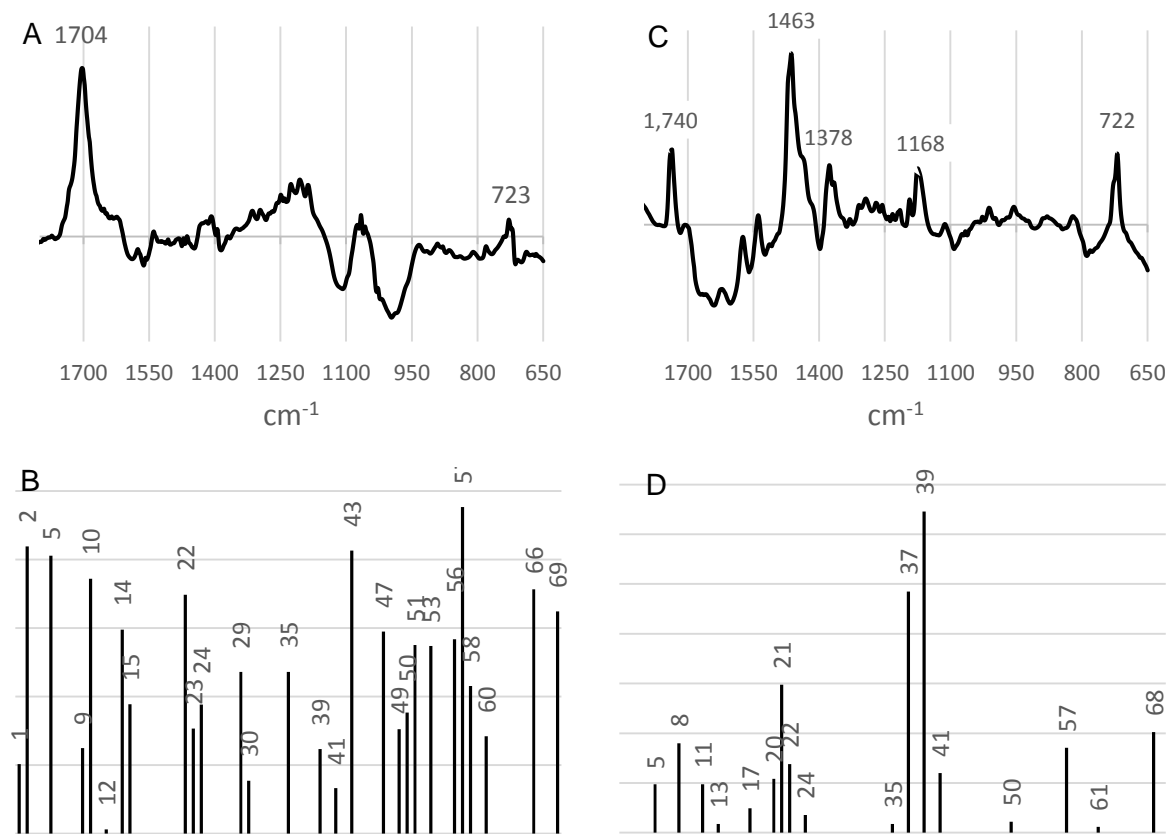


Figure 5

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.

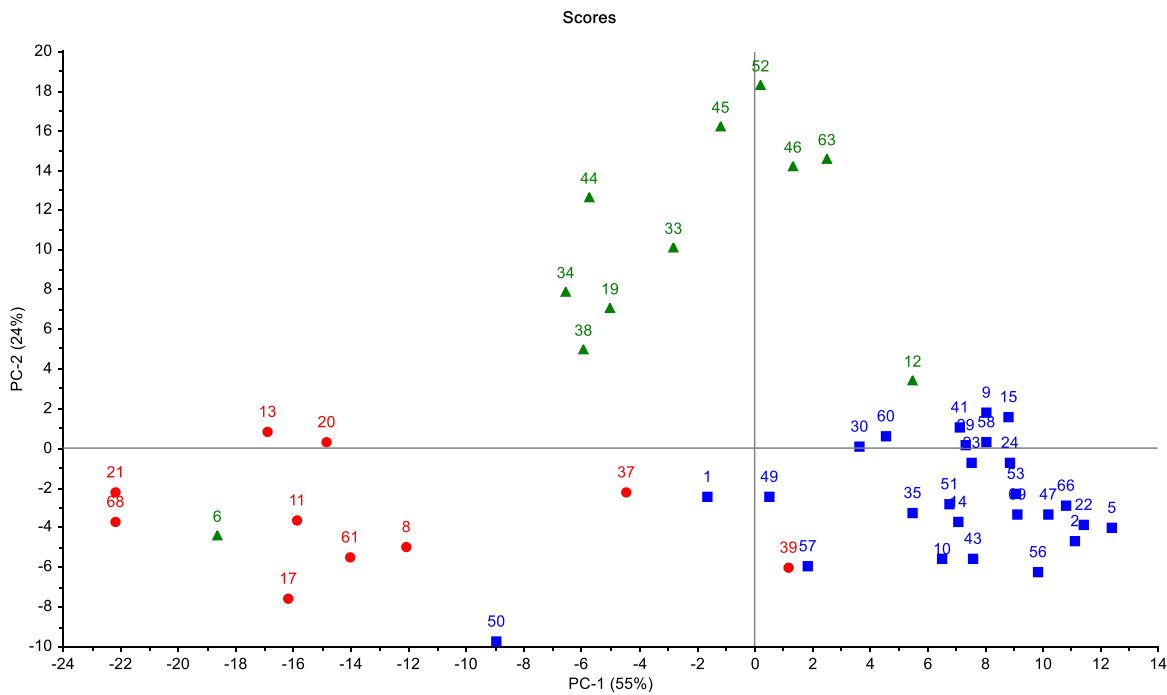
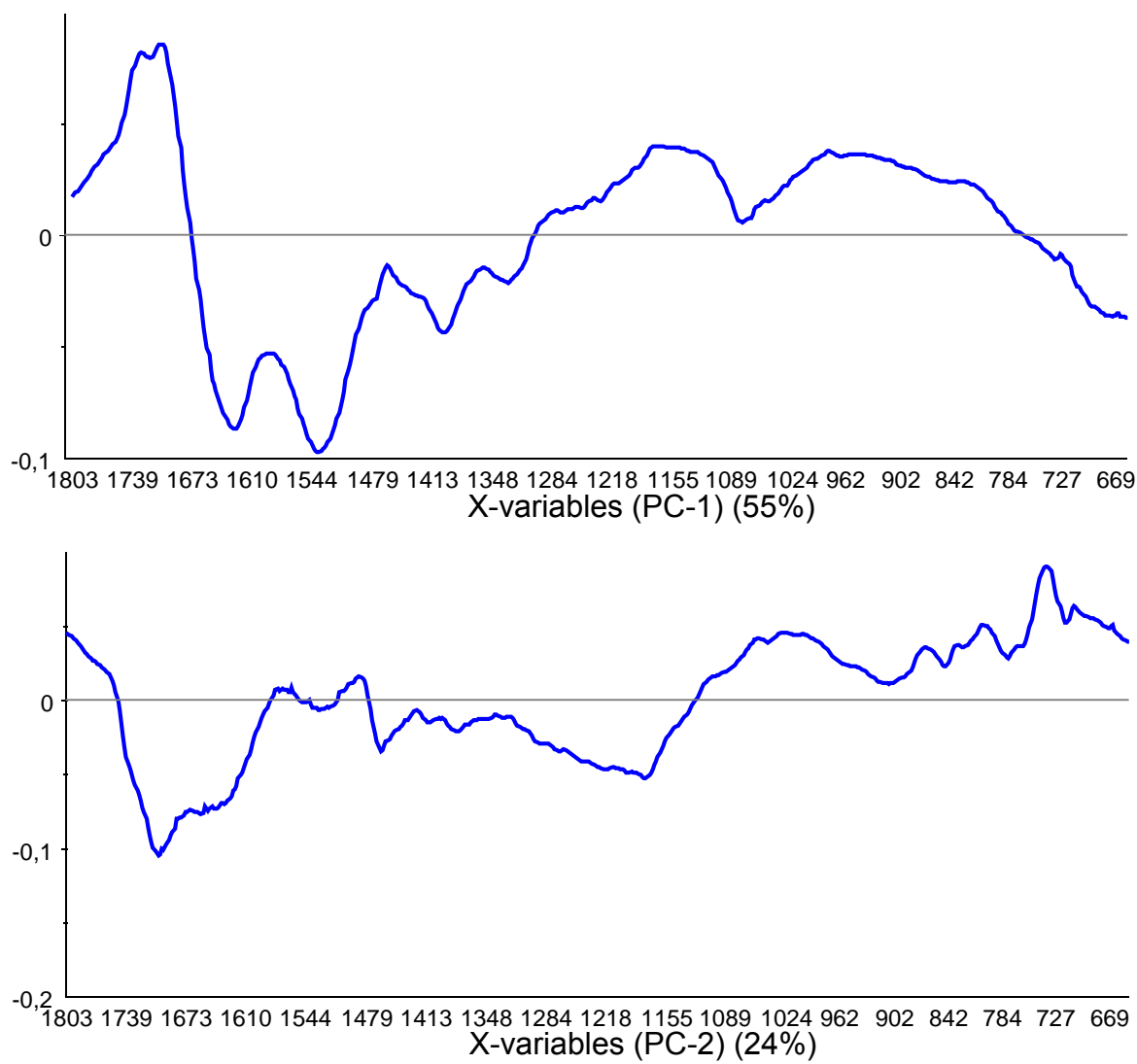
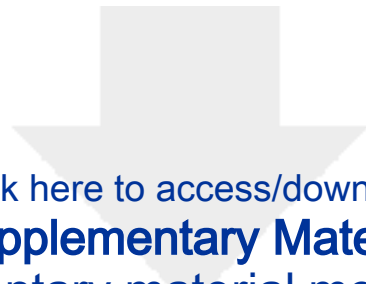


Figure 6

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





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Supplementary Material

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