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ORIGINAL PAPER

Detection of deterioration for biochemical substances used with Late Period mummy by GC-MS

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Abstract

Mummification was considered one of the most important processes used for the preservation of the body through ages in ancient Egypt for long times. Some natural materials were used to achieve the preservation goal. Some of these materials are natural resins which can be used alone as separate or as a mixture with others such as fat, wax, and oils. Some environmental factors (interior or exterior) were affecting these mixture components and lead to its degradation. This study aims to identify the chemical composition by gas chromatography mass spectrometry (GC/MS) and explain the deterioration mechanism of some mixture materials used in the preservation process of Late Period mummy. The results revealed that the preservative mixture samples contained resins pine pitch and mastic mixed with some biochemical substances such as fat and wax. The results also showed that the packing of the mummy's remains in plastic bags led to the presence of phthalate compounds as contaminants. Moreover, the degradation compounds of acetonitrile and 2-amino-n-isopropylbenzamide in the ancient tissue have been demonstrated with the sample taken from the cranial cavity. Additionally, phenanthrene compounds and its derivatives were identified, and this may be due to the use of pine pitch in the embalming mixture.

Keywords Mummification · Preservation · Resins · Biochemical substances · Gas chromatography mass spectrometry · Deterioration

Introduction

The ancient Egyptian mummification was a complex process, involving many different materials (Ben-Yehoshua and Ondřej Hanuš 2014). The resins, oils, fats, and beeswax were used in treating the bodies to achieve the preservation goal (Łucejko et al. 2010; Facchetti et al. 2012; Posh 2015). The advanced chemical analyses of different mummy samples proved the use of fats, oils, beeswax, sugar gum, petroleum bitumen, coniferous, *Pistacia*, and cedar resins in the mummification processes (Özen et al. 2016). Resinous materials were used in many purposes in mummification process such as filling the cranial cavity after extraction of the brain, smearing the internal and external parts of the body, for adhering of bandages and strips used for wrapping mummies, and using them as offerings between the layers of the wrappings (Abdel-Maksoud and El-Amin 2013; Jones et al. 2014; Brettell et al. 2015; Özen et al. 2016). Target for using resinous materials was to fill the porous of different parts of the body after the extraction of water by using natron salts. Additionally, the use of resins for the body preservation before the beginning of evisceration and desiccation processes proved that these materials were very important in mummification process (Jones et al. 2014). The application of natural resins played an important role in preservation of bodies, and without their application, the bodies would have deteriorated due to the absence of preservative material against some factors such as the humid environment of the tombs (Buckley and

Highlights
- GC/MS is an effective analysis technique for the identification of resins used in mummification processes in the Late Period in Egypt.
- Compounds of organic mixtures such as pine tar and mastic resins, as well as their essential oils, fat, and wax, were identified.
- Acetonitrile and 2-amino-n-isopropylbenzamide indicated the degradation of the ancient tissue taken from cranial cavity.
- The identification of phthalate compounds indicated contamination resulted from plastic bags used for the preservation of mummy's remains.

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Evershed 2001; Abd Elhay 2013; Khairat et al. 2013). Moreover, these resins had protected the soft tissues of the deceased body from biological attack. Also, these resins hide the decomposition smell by continuous release of their volatile aromatic components which are responsible for this preservation process (Brettell et al. 2015). Mastic and coniferous resins have anti-biological activities due to their triterpenes (Assimopoulou and Papageorgiou 2005; Tsokou et al. 2007; Jones et al. 2014). In the later periods of Egyptian history, used of resins was essential and common in the mummification process (Aufderheide et al. 2004). During these periods, mastic resin from the genus *P. lentiscus* and *P. terebinthus* (Colombini et al. 2000), beeswax, and coniferous resins were extensively used with corpses (Zesch et al. 2016). Mastic resin can be divided into an acidic fraction, a neutral fraction, and an unusual polymer constituent, 1,4-poly-β-myrcene (Sharifi and Hazell 2009; Kelegkouri 2014; Landau et al. 2014; Pulaj et al. 2016).

The presence of diterpene and diterpenoid compounds with abietane and pimarane skeletons in the archaeological sample refers to coniferous resins (Brettell et al. 2015), where dehydroabietic acid methyl ester and retene were identified by GC/MS in many archaeological organic samples (Evershed et al. 1997; Facchetti et al. 2012; Hanuš and Ben-Yehoshua 2013; Izzo et al. 2013; Jones et al. 2014). The physiochemical properties of the ancient organic residues and their changes from the preparation and degradation processes through time made their studying difficult and complex process (Izzo et al. 2013). The heating process of natural resins led to some changes in the chemical composition of the original materials (Colombini and Modugno 2009).

Moreover, in many cases, several types of compounds appeared which referred to many components in the preservative mixtures. Used of processed mixtures in the mummification process of dead bodies was confirmed by Jones et al. (2014) who identified some mixtures consisted of plant oil or animal fat as the main compound with far lesser amounts of a conifer resin and an aromatic plant extract/"balsam" as well as minor amounts of wax, plant gum/sugar (Jones et al. 2014). Facchetti et al. (2012) analyzed the balm material from the ceramic vessel dated back to Roman period and proved that the organic mixture consisted of *Pinaceae* resin, beeswax, and a fatty material. Resinous materials with other biochemical substances such as waxes, gums, oils, and bitumen were used for mummification processes in ancient Egypt (Colombini and Modugno 2009).

Analytical techniques played an important role for the identification and explanation of the deterioration mechanism of these mixture substances. Studying the chemical composition of the resins by GC/MS played a vital role in archaeology, and this may be due to the following reasons: it helps the specialists in archaeology and ethnography sciences to explain the deterioration process, and makes good plan for the preservation processes (Łucejko et al. 2010). This study aims to identify the chemical composition and explain the deterioration aspects and mechanisms of these samples taken from an Egyptian mummy dated back to Late Period.

Materials and methods

New resin samples

Myrrh, mastic, and frankincense resins were selected to be used as standards for the comparison with the archaeological samples. The resins selected were bought from the company Ragab El Attar, Cairo, Egypt. It should be noticed that the archaeological samples taken were similar to the standard of mastic resin, so the authors will show only the results of mastic resin, but results of myrrh and frankincense are excluded from the *Results and discussion* because they are not similar with the archaeological samples. It should be mentioned that the pine resin or tar identified is compared to the results obtained by international references in this field.

Archaeological samples

Two samples were taken from Sawa site (located just 15 km north-east of Zagazig city and about 1 km south of Saft el-Henna area, and degrees 31°–37° east longitude, and between degrees 30° and 33° north latitude). The mummy is unwrapped and belongs to a priest (Fig. 1) dated back to Late Period. It is preserved in the Anthropological laboratory, Research and Conservation of Antiquities Center, Egypt. One of these samples was taken from the cranial cavity (sample A)

Fig. 1 Remains of the priest mummy

and the other sample was taken from the external part of the body (sample B).

Gas chromatography mass spectrometry analysis

Agilent 6890 gas chromatograph equipped with an Agilent mass spectrometric detector, with a direct capillary interface and fused silica capillary column PAS-5 ms (30 m × 0.32 mm × 0.25-μm film thickness) at the Central Agriculture Pesticides Laboratory (CAPL), Ministry of Agriculture and Land Reclamation, Egypt, was used for analyzing the modern and archaeological samples. The analysis by GC/MS of the modern and archaeological samples was performed in accordance with Bruni and Guglielmi (2014) as in the following steps:

- A few milligrams of the samples were taken;
- The samples were ground to a fine powder;
- The samples were extracted twice, adding each time 5 ml of dichloromethane;
- After extraction by dichloromethane, the extracted sample was put in a vial and closed by Parafilm, then sonicated in ultrasonic machine for about 30 min;
- After sonicating, the combined extract was filtered by syringe microfilter;
- The solvent was removed under a gentle nitrogen stream;
- The sample was then subjected to saponification to separate and isolate the so-called acidic fraction, containing acid and other polar terpenic compounds. To this aim, the sample was treated with 1 ml of 10% KOH and kept stirred up for 4 h at 70 °C and for additional 16 h at room temperature;
- The hydrolysate was acidified with HCL to PH = 2, then the acidic fraction was recovered extracting three times with 1 ml diethyl ether;
- The solvent was evaporated and the residue retrieved again with 1ml chloroform.

The prepared modern and archaeological samples were injected under the following conditions: Helium was used as carrier gas at approximately 1.0 ml/min., pulsed splitless mode. The solvent delay was 3 min and the injection size was 1.0 μl. The mass spectrometric detector was operated in electron impact ionization mode with an ionizing energy of 70 e.v. scanning from *m/z* 50 to 500. The ion source temperature was 230 °C. The electron multiplier voltage (EM voltage) was maintained 1250 v above auto tune. The instrument was manually tuned using perfluorotributylamine (PFTBA). The initial temperature of the column was set to 60 °C and held for 1 min after the introduction of the sample, then increased to 70 °C at 20 °C/min, to 240 °C at 10 °C/min and up to 285 °C at 4 °C/min; the final temperature of 285 °C was held for 40 min. The injector temperature was set at 280 °C.

Results and discussion

The results of GC/MS analysis revealed that the identified compounds (Fig. 2; Table 1) in the archaeological samples were as follows:

Pine tar resin compounds

The GC/MS chromatogram (Fig. 2) of the archaeological samples showed the characteristic compounds of pine tar such as 7-isopropyl-1-methylphenanthrene (retene) and phenanthrene and 2-dodecyltetradecahydro (0.26, 1.68%) (RT 18.88, 27.69) in both A and B samples. Dehydroabietic acid methyl ester (0.24%) (RT 22.64) was detected in sample A. The myristic fatty acid (Fig. 2; Table 1) (1.27, 1.22%) (RT 14.406, 14.375) was identified in A and B samples respectively. Moreover, other fatty acid methyl ester was detected in sample A (Fig. 2a; Table 1) such as palmitic acid methyl ester, stearic acid methyl ester, and oleic acid methyl ester (0.70, 0.46, 0.52%, and RT 15.906, 17.807, 17.591, respectively). The presence of retene and dehydroabietic acid methyl ester refers to pine pitch since they appear when the resin is heated to high temperatures. The data revealed the presence of pine resin marker phenanthrene in sample B which is a derivative of retene. These compounds referring to pine pitch/tar were confirmed by Reunanen et al. (1989) and Egenberg et al. (2002). Polycyclic terpene hydrocarbons and acidic diterpenes were the characteristic compounds of pine pitch (Łucejko et al. 2010).

The characteristic components of pine tar, pine resin, and their deterioration products such as retene (RT 19.89), phenanthrene (RT 17.99), dehydroabietic acid methyl ester (RT 19.04), methyl dehydroabietate, methyl 7-oxodehydroabietate, and some methyl derivatives of fatty acids such as palmitic acid methyl ester (RT 16.62), stearic acid methyl ester (RT 18.53) with minor amounts of the 19- and 18-norabieta-8,11,13-trienes were identified by GC/MS in many archaeological organic samples (Evershed et al. 1997; Facchetti et al. 2012; Hanuš and Ben-Yehoshua 2013; Izzo et al. 2013; Jones et al. 2014).

It should be mentioned that the presence of methyl derivatives of fatty acids cannot be associated with the presence of methyl dehydroabietate, where the formation of methyl dehydroabietate (MDHA) is due to the pyrolysis of resinous *Pinaceae* woods (Brettell et al. 2017; Jones et al. 2018). In the other hand, thermal degradation for these kinds of woods leads to dehydrogenation, decarboxylation, and a higher degree of aromatization of the cyclic compounds and formation of DHA with retene (Reunanen et al. 1989; Egenberg et al. 2002; Brettell et al. 2017).

The identified compounds in the studied samples proved that pine tar/pitch was heated during preparation and application processes in addition to confirm that this pitch/tar which used in mixture was obtained from a destructive distillation of resinous wood, because during wood distillation, in fact,

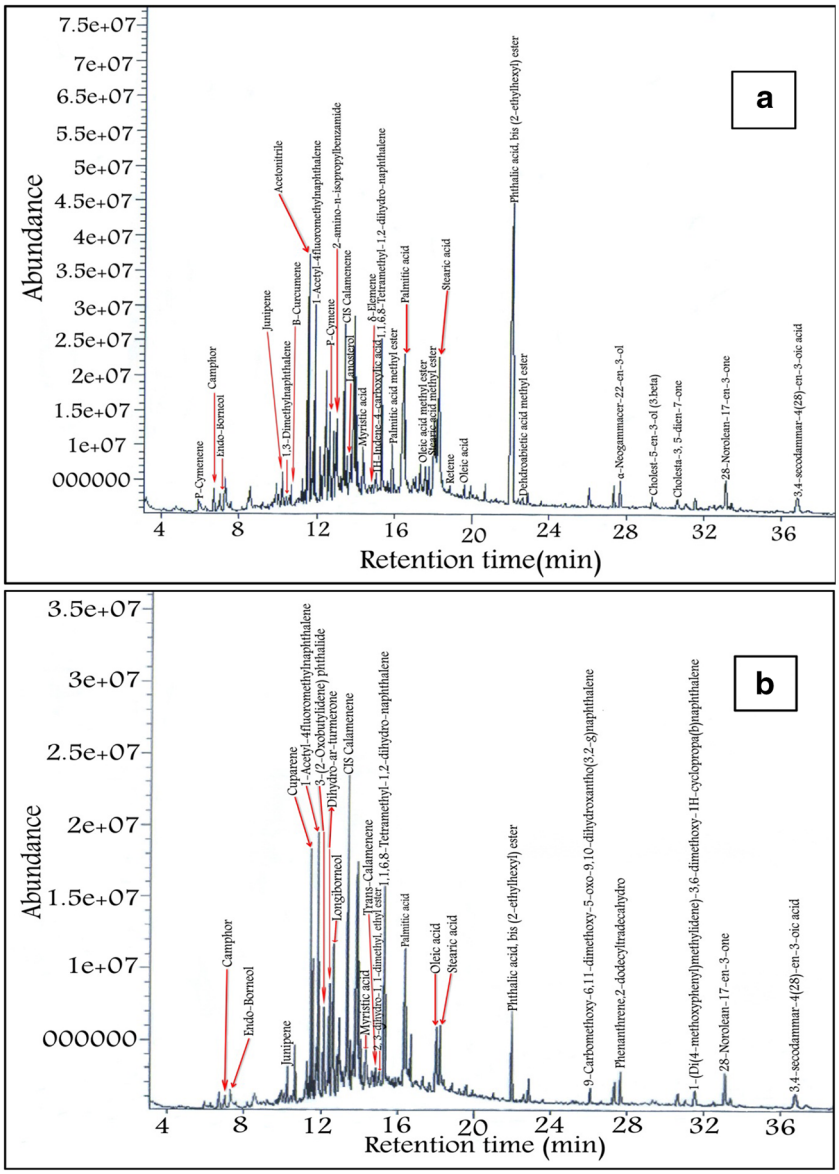

Fig. 2 The GC/MS chromatogram of the archaeological samples. **a** Sample from cranial cavity. **b** Sample from the external body

gaseous methanol was produced and reacted easily with the fatty acids to produce fatty acid methyl ester (Łucejko et al. 2010; Izzo et al. 2013). The presence of the dark shiny coating directly on the external body and the absence of tissue layer from the studied mummy (skeleton of the mummy) and low percentage of dehydroabietic acid confirmed that these mixtures were heated with high temperature during the preparation process. Jones et al. (2014) stated that the high abundance of retene (~ 10–30%), methyl dehydroabietate (~ 20–40%), and methyl 7-oxodehydroabietate with the norabietatrienes refers to the presence of high thermal-treated conifer tar. The absence or low abundance of these compounds (retene ~ 1%, methyl dehydroabietate ~ 2%, methyl 7-oxodehydroabietate trace, or absent) in fresh unheated conifer resin in addition to these compounds indicated the heating degree of pine resin in the preparation process before its application with linen wrappings and dead bodies. Also, the presence of oxidized diterpenoid resin acids and their methyl esters refers to the pitch resin obtained from the destructive distillation process of resinous wood, but the degraded abietane skeleton such as retene and methyl dehydroabietate compounds refers to the Pinaceae resins/pitch that have been strongly heated during its preparation (Koller et al. 2005; Izzo et al. 2013; Brettell et al. 2015). Myristic acid was naturally presented in tar samples (Egenberg et al. 2002).

The absence of abietic acid from the studied samples and presence of their degradation products were explained as follows:

The presence of the primary resin acids such as abietic acid in the archaeological residues proved the well-preserved state of the residues, but the absence of these acids may be due to the natural oxidation or thermal degradation during the

Table 1 The identified compounds in both archaeological samples

Terpene compounds in the archaeological samples					
RT	Matched compounds	Class of terpenes	Chemical formula	Composition by area (%)	
				Sample (A)	Sample (B)
5.922	P-Cymenene	Monoterpene	$C_{10}H_{12}$	0.57	---
6.704	Camphor	Monoterpene	$C_{10}H_{16}O$	0.55	0.54
7.002	Endo-borneol	Monoterpene	$C_{10}H_{18}O$	0.67	0.74
10.453	1,3-Dimethylnaphthalene	Monoterpene	$C_{12}H_{12}$	0.24	---
11.877	1-Acetyl-4-fluoromethylnaphthalene	Monoterpene	$C_{13}H_{12}O$	5.00	9.97
12.474	P-Cymene	Monoterpene	$C_{10}H_{14}$	3.86	---
12.646	Longiborneol	Monoterpene	$C_{15}H_{26}O$	---	6.26
15.360	1,1,6,8-Tetramethyl-1,2-dihydro-naphthalene	Monoterpene	$C_{14}H_{18}$	3.54	5.42
12.157	3-(2-Oxobutylidene)phthalide	Monoterpene	$C_{12}H_{12}O_2$	---	2.46
22.103	Phthalic acid bis(2-ethylhexyl) ester	Monoterpene	$C_{24}H_{38}O_4$	17.52	2.96
9.939	Neoisolongifolene	Sesquiterpene	$C_{15}H_{22}$	---	1.09
10.250	Junipene (Longifolene)	Sesquiterpene	$C_{15}H_{24}$	0.57	0.99
10.650	B-curcumene	Sesquiterpene	$C_{15}H_{24}$	0.95	---
11.489	Cuparene	Sesquiterpene	$C_{15}H_{22}$	---	9.94
12.462	Dihydro-ar-turmerone	Sesquiterpene	$C_{15}H_{22}O$	---	5.17
13.421	cis-Calamenene	Sesquiterpene	$C_{15}H_{22}$	3.53	10.17
14.699	δ-Elemene	Sesquiterpene	$C_{15}H_{24}$	0.17	---
14.864	trans-Calamenene	Sesquiterpene	$C_{15}H_{22}$	---	0.79
13.765	Lanosterol	Triterpene	$C_{30}H_{50}O$	0.51	---
18.881	Retene	Triterpene	$C_{18}H_{18}$	0.26	---
22.643	Dehydroabietic acid methyl ester	Triterpene	$C_{21}H_{30}O_2$	0.24	---
27.690	Phenanthrene, 2-dodecyltetradecahydro	Triterpene	$C_{26}H_{48}$	---	1.68
27.715	α-Neogammacer-22-en-3-ol	Triterpene	$C_{30}H_{50}O$	0.88	---
33.149	28-Norolean-17-en-3-one	Triterpene	$C_{29}H_{46}O$	1.28	2.28
36.842	3,4-Secodammar-4(28)-en-3-oic acid	Triterpene	$C_{30}H_{50}O_4$	1.35	1.95
Total percent (%)				41.7	62.41
Polymer compounds in the archaeological samples					
RT	Matched compounds		Chemical formula	Composition by area (%)	
				A sample	B sample
7.288	4′-Methylacetophenone		$C_9H_{10}O$	1.37	1.78
9.945	4,7-dimethyl-2,3-dihydro-1H-indene		$C_{11}H_{14}$	0.91	---
10.072	3,3-Dimethyl-1-indanone		$C_{11}H_{12}$	0.32	---
11.286	1-Methoxy-4-(phenylethynyl)benzene		$C_{15}H_{12}$	1.00	---
12.659	2,10,10-Trimethyltricyclo[7.1.1.0(2,7)]undec-7-en-6-one		$C_{14}H_{20}$	2.61	---
12.169	6-Methyl-4-indanol		$C_{10}H_{12}$	1.09	---
13.898	Styrene, 3,4-dimethyl		$C_{10}H_{12}$	6.71	---
15.080	1H-Indene-4-carboxylic acid,2,3-dihydro-1,1-dimethyl,ethyl ester		$C_{14}H_{18}O_2$	0.80	0.83
Total percent (%)				14.81	2.61
Proteinous compounds in the archaeological samples					
RT	Matched compounds		Chemical formula	Composition by area (%)	
				A sample	B sample
11.273	1-Aminoindan		$C_9H_{11}N$	---	1.01
11.585	Acetonitrile		$C_{11}H_{10}N_2O$	8.22	---
12.983	1H-Benzimidazol-2-amine		$C_7H_7N_3$	---	1.37
13.021	2-amino-n-isopropylbenzamide		$C_{10}H_{14}N_2O$	2.74	---
13.962	N,N-Dimethyl-2-(2-methylpyridin-4-yl) vinyl amine		$C_9H_{12}N_2$	---	15.27
22.879	4H-pyrane-3-carboxamide		$C_6H_5NO_3$	---	0.99

Table 1 (continued)

Terpene compounds in the archaeological samples				
26.126	Bis (4-amino-3-isobutyl-5-ethylphenyl)methane	$C_{15}H_{18}N_2$	0.49	---
Total percent (%)			11.45	18.64
Lipid compounds in the archaeological samples				
RT	Matched compounds	Chemical formula	Composition by area (%)	
			A sample	B sample
8.591	Nonanoic acid (pelargonic acid)	$C_9H_{18}O_2$	1.47	---
12.862	Hept-2-en-4-yn-oic acid methyl ester	$C_8H_{10}O_2$	---	0.97
14.406	Tetradecanoic acid (myristic acid)	$C_{14}H_{28}O_2$	1.27	1.22
15.906	Palmitic acid methyl ester	$C_{17}H_{34}O_2$	0.70	---
16.497	Hexadecanoic acid (palmitic acid)	$C_{16}H_{32}O_2$	8.89	6.84
17.74	Heptadecanoic acid	$C_{17}H_{34}O_2$	1.34	---
17.591	Oleic acid methyl ester	$C_{19}H_{36}O_2$	0.52	---
17.807	Stearic acid methyl ester	$C_{19}H_{38}O_2$	0.46	---
18.322	Stearic acid (octadecanoic acid)	$C_{18}H_{36}O_2$	12.36	2.47
19.90	Oleic acid	$C_{18}H_{34}O_2$	0.71	2.67
29.374	Cholest-5-en-3-ol(3.beta)	$C_{27}H_{46}O$	0.28	---
30.670	Cholesta-3,5-dien-7-one	$C_{27}H_{42}O$	0.57	---
Total percent (%)			28.57	14.17

--- Refers to that this compound is not found in this sample

preparation process of resins. The natural oxidation was characterized by increasing the level of the degradation products such as dehydroabietic and 7-oxo-dehydroabietic acids. Also, the appearance of oxidant diterpenoids with high degree in the archaeological organic residue may be due to the oxidant atmosphere which where the resinous materials were prepared and/or from the natural oxidation to these materials through time (Hanuš and Ben-Yehoshua 2013; Izzo et al. 2013; Jones et al. 2014; Brettell et al. 2015).

The presence of 7-oxo-dehydroabietic acid with high degree over dehydroabietic acid refers to the high oxidation process of the resin from *Pinaceae* family; in addition, time plays an important role in the appearance of some products which accompanied the main compounds of the mixture (Łucejko et al. 2010). Thermal degradation during the preparation process of resins leads to the reduction of abietic acid due to the dehydrogenation and decarboxylation processes which leads to the production of the mixture of diterpenoid hydrocarbons, methyl dehydroabietate, and dehydroabietic acid. Additionally, methyl dehydroabietate compound which was produced during the wood distillation especially when the methanol reacts with diterpenic acids and the absence of this compound refers to that pitch obtained by pyrolysis of resin alone (Izzo et al. 2013). The existence of such compounds, phenolic (such as acetovanillone, hydroxybenzoic, and vanillic acids), sesquiterpenes, and phenanthrene together in the sample, refers to the preparation process of the material which was used in the mummification process from combustion processes or partial dry distillation of wood tar, tar, oil, or wood (Łucejko et al. 2010). Finally, from this simple discussion, it was concluded that the absence of abietic acid from our result may be due to the thermal degradation with high temperature.

Also, naphthalene compounds (Fig. 2; Table 1) were identified in the studied samples: 1-Acetyl-4-fluoromethylnaphthalene (5.00, 9.97%); (1S,4S)-1,6-dimethyl-4-propan-2-yl-1,2,3,4-tetrahydronaphthalene (cis-Calamenene) (3.53, 10.17%); and 1,1,6,8-tetramethyl-1,2-dihydro-naphthalene (3.54, 5.42%) in both samples A and B. 1,3-Dimethylnaphthalene (0.24%) was found in sample A, and 9-carbomethoxy-6,11-dimethoxy-5-oxo-9,10-dihydroxantho(3,2-g) naphthalene and 1-(di(4-methoxyphenyl)methylidene)-3,6-dimethoxy-1H-cyclopropa (b) naphthalene (0.75,0.96%) were found in sample B. These compounds confirmed the presence of conifer in the mixtures (Koller et al. 2005). Additionally, the presence of polycyclic aromatic hydrocarbons like phenanthrene and methyl phenanthrene in our samples can be associated with the production of pine pitch in embalming mixture.

Essential oil of pine

The presence of many mono and sesquiterpene (Fig. 2; Table 1) specific for essential oil of pine resin in both two samples was recorded such as endo-borneol (0.67, 0.74%) (RT 7.002); 1, 1, 6, 8-tetramethyl-1, 2-dihydro-naphthalene (3.54, 5.42 %) (RT 15.360, 15.341); 1-acetyl-4-

fluoromethylnaphthalene (5.00, 9.97%) (RT 11.877, 11.864); and camphor (0.55, 0.54%) (RT 6.704) in both samples A and B; 1, 3-dimethylnaphthalene (0.24%) (RT 10.453) was found in sample A. Koller et al. (2005) and Tumen et al. (2010) proved that these compounds were from the essential oil of pine.

Mastic resin and its essential oil compounds

The identified data (Fig. 2; Table 1) represent that both samples contain mastic resin triterpene biomarkers such as 28-norolean-17-en-3-one (1.28, 2.28%) (RT 33.149/33.111) and 3,4-secodammar-4(28)-en-3-oic acid (1.35, 1.95 %) (RT 36.842/36.785) in samples A and B respectively. In archaeological residues by GC-MS in particular, acidic fraction of mastic contains a good percentage of oleanonic, moronic, isomasticadienonic, and masticadienonic acids, while the neutral fraction is composed of triterpenes of the oleanene, lupene, tirucallene, and dammarane series, in particular nor-β-amyrone, 28-norolean-17(18)-en-3-one, hydroxydammarenone, and oleanonic aldehyde (Łucejko et al. 2010; Brettell et al. 2015). Additionally, the presence of 28-norolean-17-en-3-one compound in the archaeological studied samples with the mentioned percentages refers to mastic resin having been burned during the preparation process (Colombini and Modugno 2009). 28-Norolean-17-en-3-one was from the characteristic compounds of mastic resin which was formed by the decarboxylation of oleanonic acid (Brettell et al. 2015).

Also, the presence of many mono and sesquiterpene (Fig. 2; Table 1) specific for essential oil of mastic resin has been recorded such as P-cymenene (0.57%) (RT 5.922), P-cymene (3.86%) (RT 12.474), δ-elemene (0.17%) (RT 14.699), B-curcumene (0.95%) (RT 10.650), lanosterol (0.51%) (RT 13.765), and α-neogammacer-22-en-3-ol (0.88%) (RT 27.715) were identified in sample A. trans-Calamenene (0.79%) (RT 14.864) was recorded in sample B. Camphor (0.55, 0.54%) (RT 6.704), cis-calamenene (3.53, 10.17%) (RT 13.421/13.409), and junipene (0.57, 0.99%) (RT 10.250/10.244) were recorded in samples A and B. P-Cymenene (0.59%) (Haloui et al. 2015), camphor (Tsokou et al. 2007; Backmann 2009), B-curcumene (Presti et al. 2008) were identified by GC/MS analysis from the main components of *Pistacia lentiscus* essential oil. Also, P-cymene was identified by this analysis from the composition of *Pistacia lentiscus* var. chia resin and its essential oil (Koutsoudaki et al. 2005). Lanosterol and dammaranes were identified from mastic resin compounds (Steigenberger 2013). cis-Calamenene (0.08%) (RT 12.125), trans-calamenene (1.48%) (RT 13.225), α-neogammacer-22-en-3-ol (1.16%) (RT 35.704), and 3, 4-secodammar-4(28)-en-3-oic acid (1.45%) (RT 36.708) were also identified from our reference mastic compounds. The high degradation especially oxidation process of *Pistacia* spp. resins from the archaeological sample was observed from ocotillones (oxidized dammaranes) which resulted from the oxidation process of dammaranes; on the other hand, the well-preserved state of these resins was noticed from the presence of resin acids (Brettell et al. 2015). The presence of oleanonic aldehyde in the archaeological sample refers to the degradation process as result from natural environmental interactions (Brettell et al. 2015).

Fat compounds

The absence of di- and monoacylglycerols in both archaeological samples indicated almost complete hydrolysis of the original acyl lipids that would have dominated the tissues at the time of death. However, in contrast to sample B, two steroidal compounds (Fig. 2; Table 1), cholest-5-en-3-ol (3.beta) and cholesta-3, 5-dien-7-one (0.28, 0.57%) (RT 29.374, 30.670), were observed as main components in the sample which were taken from cranial cavity.

The presence of lipid compounds from the archaeological sample compounds indicated the use of vegetable and animal fatty materials, where the animal fats were characterized from the odd chain length fatty acids and cholesterol (Łucejko et al. 2010). β-Sitosterol from plant sterols and cholesterol from the animal sterols were identified from archaeological samples (Jones et al. 2014). The degraded acyl lipid products can be derived from plant oils or animal fats or human body lipids, but these products which were identified in the archaeological samples were derived from the plant oils and animal fats (Jones et al. 2014). Abdel-Maksoud et al. (2019) found the same animal fat from the remains of the same studied mummy by studying the characteristic bands using FTIR analysis.

The presence of compounds 1H-indene-4-carboxylic acid and 2,3-dihydro-1,1-dimethyl ethyl ester (0.80,0.83%) (RT 15.080, 15.074) (Fig. 2; Table 1) in our archaeological A and B samples respectively refers to the autoxidation process to the major unsaturated components from the fatty acids. The lipids were characterized in the archaeological samples from the presence of mono- (C_{12} to C_{28}, with $C_{16:0}$ and $C_{18:0}$) and di-carboxylic fatty acids and the presence of linear and branched monocarboxylic fatty acids with 15 and 17 carbon atoms suggesting animal origin, especially animal fats (Facchetti et al. 2012). The characteristic saturated fatty acids (Fig. 2; Table 1) tetradecanoic (myristic acid), palmitic (hexadecanoic acid), stearic (octadecanoic acid) (1.27, 1.22; 8.89, 6.84; 12.36, 2.47%) (RT 14.406, 14.375; 16.497, 16.415; 18.322, 18.233), and characteristic unsaturated fatty acids Oleic (0.36, 2.67%) (RT 19.618, 18.036) were identified in both samples A and B respectively.

The identified compounds in the samples proved to contain lipids (from the plant or animal origin). These results were confirmed by Spangenberg et al. (2006) who divided the main fatty acids in plant and animal lipids into characteristic

saturated fatty acids: lauric, myristic, palmitic, and stearic acids; and the characteristic unsaturated fatty acids : palmitoleic, oleic, linoleic, and linolenic (Spangenberg et al. 2006). In addition, the presence of palmitic and stearic acids with high concentrations refers to the hydrolysis degradation process of lipids. The animal fats were identified with some human and animal remains at different archaeological sites (Evershed et al. 2002). Although phospholipids are susceptible to degradation and as such would not be expected to survive intact, the presence of the amides and proportion of the fatty acids seen in the aged tissues of both samples A and B may reflect the presence of phospholipids which is listed in Table 1. Since these amides were not seen in the total lipid extracts, they are presumed to derive thermolytically. In addition to lipids, some protein compounds were detected in sample A. Acetonitrile (8.22%) (RT 11.585) and 2-amino-n-isopropylbenzamide (2.74%) (RT 13.021) were detected in A sample. These proteinous origin compounds (which were found in A sample from the brain remains) are normally associated with the hydrolysis of proteins in the ancient tissues (Munson and Fetterolf 1987).

It should be mentioned that some wax compounds especially palmitic acid with percentage 8.89 and 6.84% and RT 16.497 and 16.415, and some long-chain n-alcohols as 3,4-secodammar-4(28)-en-3-oic acid ($C_{30}H_{50}O_4$) with percentage 1.35 and 1.95% and RT 36.842 (Fig. 2; Table 1) were identified in both samples A and B. This results were confirmed by Łucejko et al. (2010); Facchetti et al. (2012) and Brettell et al. (2017) who mentioned that high abundance of palmitic acid together with the presence of long-chain n-alcohols containing 24 to 32 carbon atoms referred to the presence of natural wax in the archaeological sample.

Additionally, the *n*-alkanols, long-chain fatty acids, and *n*-alkanes were from the major compounds of plant waxes (Jones et al. 2014). The saturated hydrocarbons, acids or hydroxy acids, and alcohols were from characteristic compounds of beeswax (Abdel-Maksoud and El-Amin 2011).

Contamination compounds from the plastic casings

Phthalic acid bis (2-ethylhexyl) ester with percentages 17.52 and 2.96% and RT 22.11 and 21.99 was identified in both samples A and B. Additionally, 3-(2-oxobutylidene) phthalide with 2.46% and RT 12.157 was detected in sample B. Phthalic acid esters (phthalates are the most commonly used plasticizers) are used as additives in plastics especially in PVC products to improve the polymer flexibility and to make these materials softer (Zou and Cai 2013; Gimeno et al. 2014; Xie et al. 2016). The presence of these phthalate compounds in the archaeological studied samples may act as contaminants from the plastic casings (Brettell et al. 2015). The phthalates can easily migrate from plastic which was used for packing of the remains of the priest mummy in the anthropological laboratory and transformed toward these archaeological remains (Jaworek and Czaplicka 2013; Xie et al. 2016).

Conclusion

This study supplied an interesting evidence of using the organic mixture in the preservation process of the external body and the cranial cavity of an ancient Egyptian mummy. The data indicated that oil and/or fat were used as base for mixing other materials. Additionally, most common resins employed were the conifer and mastic resins. The presence of pine/pitch and its essential oil in both samples can be confirmed by the presence of retene and dehydroabietic acid methyl ester which are products of chemical reaction between gaseous methanol and diterpenic acids during the distillation of wood. Mastic resin and its essential oils have been also found in both samples. The presence of mastic resin was confirmed by the presence of triterpene biomarkers: 28-norolean-17-en-3-one and 3, 4-secodammar-4(28)-en-3-oic acid. Some chemical biomarkers of bee wax were found in the sample from cranial cavity. Many identified compounds indicated degradation reactions taking place in such archaeological materials, for instance, the oxidation products of many unsaturated fatty acids and the degradation products of abietane (dehydroabietic acid, 15-dehydro-dehydroabietic acid, 15-hydroxy-7oxo-dehydroabietic acid, 7-oxo-dehydroabietic acid) and oleanene (28-nor-17(18) oleanen-3-one). Also, the presence of phthalate compounds may be considered an indication of contamination from plastic casing which was used for packing of the archaeological remains. Finally, the analysis of two archaeological samples from an ancient Egyptian mammy supplied an evidence of using a combination of mastic and pine resins and its essential oils in the mummification process. The data revealed many oxidation products of resin and its essential oils.

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