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MICROBIOLOGICAL, MORPHOLOGICAL AND SPECTROSCOPIC STUDY ON THE EFFECT OF RESINOUS MATERIALS IN THE PRESERVATION OF WRAPPING TEXTILES OF MUMMIES

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ABSTRACT

Resinous materials are considered the most important embalming agent, which help in the preservation of ancient Egyptians bodies over time. They differ in type and amount from one mummy to another based on the period, embalmer and the social position of the deceased. With the aim of evaluating the role of resinous materials in the preservation of the wrapping textiles of mummies in the present study, three wrapping textile fragments (Anc. 1, Anc. 2 and Anc. 3) were collected from three mummies dating back to the ancient Egyptian Late Period (525-343 BC). These fragments differ in their amounts of adherent resin. The three fragments were soaked in a mixture of dichloromethane and methanol (DCM: MeOH, 1:1 v/v) in order to extract resinous materials from the fibers of the linen textiles. Fourier Transform Infrared Spectroscopy (FTIR) analysis of the extracts proved that the Anc. 2 and Anc. 3 samples included resinous materials; while, the Anc. 1 sample did not contain any adherent resinous materials. Linen fibers from the three tested fragments were investigated by stereomicroscope, microbiological investigation, scanning electron microscope (SEM) and FTIR. The results showed that resinous materials have an important role in protecting the linen wrappings from microbial damage. They also preserved the fibers' morphological structure from deterioration caused by weathering. The FTIR results proved increasing oxidation of the Anc. 1 sample and the decreasing of its crystallinity index more than the other samples as a result of its direct contact with environmental deterioration factors.

KEYWORDS: Mummy wrappings, Resinous materials, Microorganisms, FTIR, Oxidation, Crystallinity, SEM

1. INTRODUCTION

Mummies are considered one of the hallmarks of the history of Egyptian civilization. They reach across time and space to tell us about their lives and cultures. Egyptian mummies consist of several organic materials that make it sensitive to deterioration. The deterioration of mummies is caused by several factors including environmental conditions in addition to physical and biological damage. Sometimes these factors occur in isolation, but they can also be present in combination (David, 2001). According to Cybulska et al. (2008), the chemical constitution, structure and other compounds of the object, such as embalming agents and finishes, can slow down or accelerate the deterioration processes.

Resinous materials are the most important embalming agent used in the many steps of the mummification process throughout different periods. The use of resinous materials in mummification was mentioned in several publications as follows: The inside and outside of the mummified body was prepared with all kinds of oils, aromatic resins, unguents and perfumes to prevent the reentry of moisture and to strengthen the skin (Taconis, 2005). The cranial cavity was filled with resin and/or linen immersed in resin (Salter-Pedersen, 2004). Linen immersed in melted resin was used for filling the cavities of ears and eyes. Hot liquid resin served to prevent the growth of bacteria and acted as a disinfectant and deodorant, such as sawdust mixed with resin (Iskander, 1980).

In ancient Egypt, mummification materials were expensive; and, some were imported from other countries, especially resinous materials. The bodies mummified by the most expensive materials were the most protected (Abdel-Maksoud and El-Amin, 2011). The type and amount of resinous materials differed according to the wealth and social position of the deceased. Therefore it is normal to find two mummies from the same period, same area and/or the same family with different mummification techniques in addition to different types and amounts of embalming agents. The prime examples are the mummies of Nes-Ptah, Barber of Amun, and his wife Ta-Bes, Songstress of Amun, who are in the collection of the Museum of Fine Arts, Boston (Marx and D'Auria, 1986).

From this viewpoint, it is worth mentioning that ancient Egyptian did not deal with mummification from just its material side. Tight connection between the objective/material and spiritual was clearly established in the ancient Egypt. Thus, this connection was reflected in their cultural products and their symbolic character (Ionesov and Kurulenko, 2015) as well as in the beauty retained in the mummification.

The beauty and attention to detail that so typified mummification received its full complement in the cases into which the bodies entered their final rest (Ionesov, 2015).

Actually, the mummification process was not only preserved the ancient Egyptians' bodies but also preserved their religious, rituals and way of thinking (e.g. Egyptian mummies were wrapped in linen textile because it was seen as a symbol of light and purity and as a demonstration of wealth (El-Gaoudy et al., 2011)).

Recently, several studies have dealt with resinous materials for the purpose of revealing their chemical components. The analyses were based on high performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), fast atom bombardment combined with mass spectrometry (FAB/MS), high resolution FAB/MS, FAB tandem mass spectrometry (FAB-MS/MS) and etc. These studies stated that resinous materials are complex molecular mixtures which contain products such as resin, wax, plant oil and animal fats (in some cases) used in the preparation of each balm. It was confirmed that coniferous resins/oil, mastic (*Pistacia* resin) in addition to beeswax and animal fats were used for embalming mummies (Connan et al., 1999; Klyis et al., 1999; Colombini et al., 2000; Buckley and Evershed, 2001; Koller et al., 2003; Stern et al., 2003).

The widespread use of plant oils indicates that the embalmers were aware of the special properties of unsaturated oils, which allow them to 'dry' or rather to polymerize spontaneously. This polymerization would have produced a highly cross-linked aliphatic network, which would have stabilized textile wrappings against degradation by producing a physico-chemical barrier that impedes the activities of microorganisms (Buckley and Evershed, 2001; Davies, 2011). Whereas the general purpose of resinous materials is for protection by isolating mummies from environmental conditions, the effect of these materials on the physical and chemical properties of mummy wrappings, especially after natural aging, is not completely clear. So, this study aims to evaluate the effect of resinous materials on the properties of the wrapping textiles of mummies, by comparing the analytical results of samples that are either covered or not covered with resinous materials.

2. MATERIALS AND METHODS

2.1. Fragments of mummy wrappings and modern linen sample for control

Fragments from the outer layer of the wrappings of three unknown Egyptian mummies were collected from an excavation area in El-Fayoum, Egypt (Fig. 1). The fragments date back to the Late Period (525-343

BC). The fragments were chosen based on the different amounts of attached resinous materials: each one contributing to the abovementioned aim of this study.

Modern un-bleached plain flax linen fabric was kindly supplied by (Linnet Co., Ltd.) to be used as control sample.

2.2. Visual assessment by Stereomicroscope

Stereomicroscope (SMZ800, Nikon Instruments Inc.) helped in the visual description of the condition of the collected samples.

2.3. Extraction of embalming agents

In order to (1) investigate and analyze the plain textile fiber by SEM and FTIR, avoiding any masking that might happen by the embalming materials; and (2) measure the embalming agents attached to fibers of tested fragments by FTIR, the extraction process was done for the three collected samples. 5mm² of each ancient sample was cut for use in this process. According to Charrié-Duhate et al. (2007) and Brettell et al. (2016), the cut samples were solvent and extracted in dichloromethane: methanol (DCM: MeOH, 1:1 v/v) three times aided by ultrasonication and centrifuged to facilitate separation of the soluble (embalming resin) and insoluble fractions. The solvent extracts were combined, and excess solvent evaporated under a steam of argon.

The extracted fibers were transferred in new tubes, and they were washed three additional times by the abovementioned process to be sure that all adherent materials were removed from the sample fibers.

2.4. Microbiological Investigation

In order to isolate microorganisms infecting our collected samples, small parts of the fibers from the collected fragments were inoculated upon 9 cm petri dishes containing different media: (1) Potato Dextrose Agar (PDA) with chloramphenicol (0.1%) for determining the fungal contamination (including xerophilic and hydrophilic); (2) Tryptic Soy Agar (TSA) for determining the bacterial contamination; and (3) Actinomycete Isolation Agar (AIA) for determining the actinomycetes. Inoculated dishes for bacterial and actinomycetes determination were incubated for five days at 35 °C. The inoculated dishes for determination of fungal contamination were incubated at 25 °C for ten days. The identification of isolated microorganisms was done at the Center of Fungal Consultation, Tokyo, Japan.

2.5. Investigation of the surface morphology by Scanning Electron Microscope (SEM)

The investigation of the surface morphology of the tested samples after resin extraction was performed on a Hitachi S-2460N SEM microscope using a high vacuum. The images were obtained in secondary electron image mode. The voltage was 25 kV, while the working distance was 23 mm. The surfaces of the samples were sputter coated with gold (SC-701AT; quick Auto Coater, Sanyu Electron Inc., Tokyo, Japan).

2.6. Determination of the cellulose oxidation and crystallinity index by Fourier Transform Infrared Spectroscopy (FTIR)

The spectral data of the three ancient samples (Anc. 1, Anc. 2 and Anc. 3) were collected by using the Fourier Transform Infrared (FTIR) spectrometer ((Nicolet iZ 10 module, Thermo Fisher Scientific Inc., United States) in order to determine alterations happened in fibers as compared with a modern sample. In this analysis, powdered fiber with potassium bromide (KBr) pellets was used for collecting spectra in transmission mode between 4000 and 400 cm⁻¹ at 4 cm⁻¹ resolution and 64 scans per spectrum. Three different spots of each sample were measured. The whole range of collected spectra was normalized to the maximum peak at 1050 cm⁻¹ by the Essential FTIR software.

The FTIR spectra, which were collected according to the abovementioned method, were used for determining the empirical crystallinity index (lateral order index (LOI)), by computing the ratio between the intensity of the bands at 898 cm⁻¹ (β -(1,4)- glycosidic bond in cellulose) and 1430 cm⁻¹ which is associated with the amount of the crystalline structure of cellulose.

3. RESULTS AND DISCUSSION

3.1. Visual assessment by Stereomicroscope

According to the visual assessment by stereomicroscope, the fragments were characterized depending on the resinous materials adherent with their fibers as: Anc.1 (no contamination by resin), Anc. 2 (contaminated by a thin layer of resinous material) and Anc. 3 (covered by a thick, black, resinous material). As shown in Fig. 2, the surface condition and woven structure of Anc. 1 appears to be irregular in contrast to Anc. 2 and Anc. 3. This may be a result of the resinous materials, which preserved the original woven structure of the textile.



Figure 1. Collected places for the investigated fragments from mummies' wrappings (Anc.1 and Anc. 2)

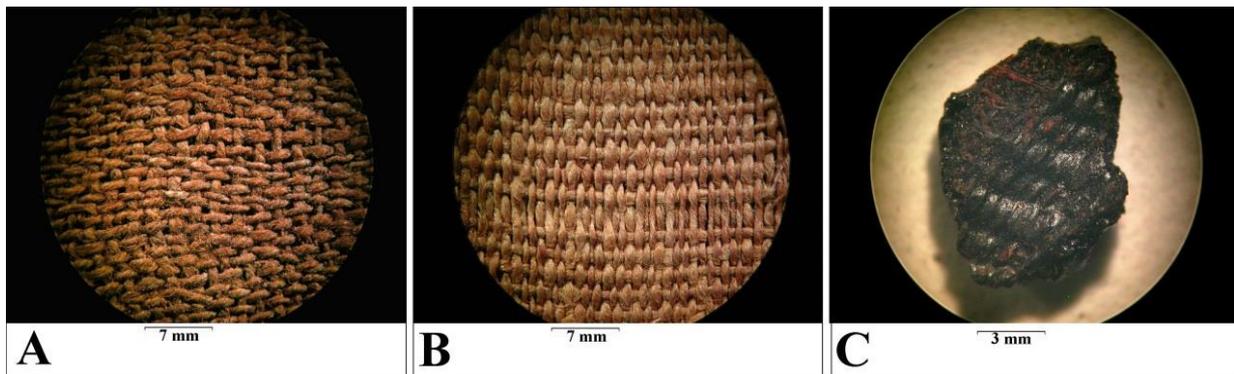


Figure 2. Stereo Photomicrographs of the ancient textile samples: (A) Anc. 1, (B) Anc. 2 and (C) Anc. 3

3.2. Microbiological Investigation

Table 1. Identified fungi isolated from the ancient textile samples

Sample	Isolated microorganisms	Colonies	Percentage (%)
Anc. 1	<i>Aspergillus ochraceus</i>	2	13.30
	<i>Aspergillus flavus</i>	2	13.30
	<i>Aspergillus sydowii</i>	1	6.70
	<i>Penicillium sp. 1</i>	5	33.30
	<i>Penicillium sp. 2</i>	4	26.70
	Actinomycete sp. 1	1	6.70
Anc. 2	<i>Aspergillus restrictus</i>	1	100%
Anc. 3	Actinomycete sp. 2	1	100%

The results revealed that microorganisms contaminated the three tested samples. Sample Anc.1 exhibited high contamination by fungi (*Penicillium* and

Aspergillus) and actinomycetes in comparison to samples Anc. 2 and Anc. 3 (Table 1). *Aspergillus* and *Penicillium* are known as the most common genera, which can grow on ancient and historical textiles (Abdel-kareem, 2010; Kavkler et al., 2015; Elserogy et al., 2016). *Aspergillus restrictus* is one of the xerophilic or xerotolerant species that grows at water activity $> a_w 0.6$. Water activity above $a_w 0.8$ allows growth of a wide variety of airborne fungi (Sterflinger and Pinzari, 2012). Depending on the isolated fungi from each sample, it can be stated that the water activity of Anc.1 is higher than the Anc. 2 and Anc. 3 samples. Grabić et al. (2013) mentioned that the increase in water activity made the materials' surface more suitable for fungal colonization. The difference in the water activity of the tested samples is based on the attached resinous materials. Actinomycetes were also isolated from Anc. 1 and Anc. 3 samples. They are classified as saprophytes that play a significant role in the breakdown of organic matter into more

readily assimilable nutrients (Abdul Hamid et al., 2015).

It is known that microbiological deterioration is one of the most important factors determining the durability of textile materials, which can be chemically assimilated (treated as a nutriment for microorganisms) or dissimilated (destroyed by metabolites produced by them). There are two main reasons for the susceptibility of ancient textile to biodeterioration; firstly, their constituent materials provide sources of many nutrients for microorganisms; secondly, many textile objects remain undisturbed for longtime, and therefore, infections could remain undetected for significant lengths of time. By this way, textiles could provide an undisturbed habitat and food source for microbes (Abdel-Kareem and Alfaisal, 2010). Most archaeological textiles stay in the soil where a variety of microorganisms can be found, such as bacteria, actinomycetes and fungi. Microbes emit acid radicals causing chemical processes typical of corrosion (Cybulska et al., 2008). According to Tomšič et al. (2007) and Kavkler et al. (2015), the decay happened due to natural aging factors (humidity, pH, light and temperature), which facilitate penetration of microbial enzymes.

As described above (Section 3.1.), samples Anc. 2 and Anc. 3 were contaminated and adherent by resinous materials. The Anc. 1 sample does not seem to contain visually any attached organic material. According to the results in Table 2, it was proven that resinous materials are likely to have played a role in the reduction of microbial contamination of the tested samples. The result of the present study agreed with several previous studies (e.g., Buckley and Evershed, 2001 and Davies, 2011), which discussed the resistance effect of resinous material. Significantly, chemical analysis of mummies shows how embalmers used a host of highly effective antimicrobial and antifungal materials to preserve the bodies and

provide safe transport to the afterlife. The components of the resinous materials, especially plant resins and oils, are not only hydrophobic but would have polymerised to produce a network capable of protecting fragile tissues and wrappings from microbial attack. Modern chemical analysis tells us that the protective wood pitches, resins and oils would have contained disinfectant phenolic compounds and molecules that inhibited microbial and fungal growth called terpenes (Davies, 2011).

3.3. Investigation of the surface morphology by Scanning Electron Microscope (SEM)

It was difficult to investigate the surface morphology of the tested sample fibers, which were covered with resinous materials (especially sample Anc. 3). The extraction process was done for the purpose of correctly determining the external condition of the fibers. SEM observation revealed a high degree of degradation of the Anc. 1 sample. The fibers are extremely damaged with transverse cracking in addition to dust interfering with the fiber as shown in Fig. 3A. The SEM image of Anc. 2 recorded the separation of the outer fibrils, which might have adhered with a thin layer of resinous material (before resin extraction) (Fig. 3B). This might have happened due to the difference between the coefficient of expansion and shrinkage of the textile fibers and resinous components causing the fibrils' separation. The investigation of Anc. 3 confirmed the role of resinous material in the preservation of the morphological structure and physical form of the textile fibers (Fig. 3C). The sample fibers look similar to the fiber of the modern sample; and, this is because of the thick layer of resinous material covering the fibers before extraction, which isolated them from the environmental factors, preserving the morphological form without any alteration.

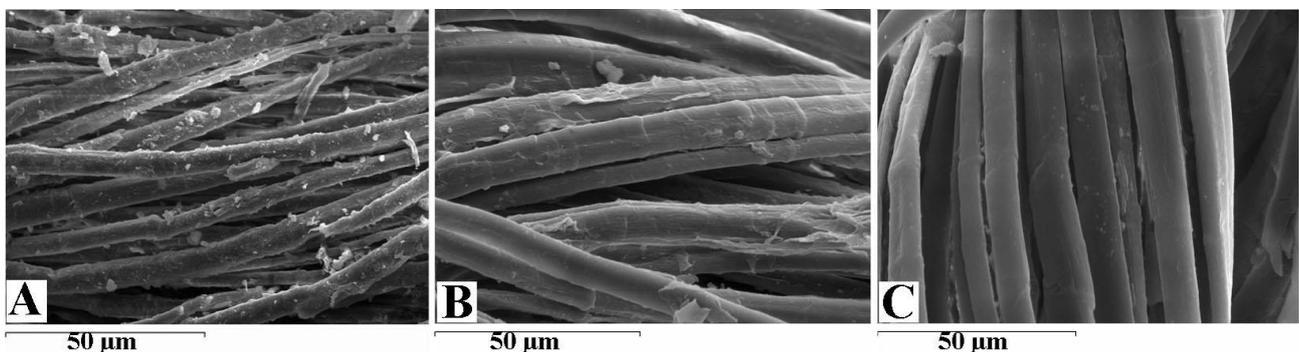


Figure 3. SEM images of the fibers after the extraction process: (A) Anc. 1, (B) Anc. 2 and (C) Anc. 3

3.4. Determination of the cellulose oxidation and crystallinity index by Fourier Transform Infrared Spectroscopy (FTIR)

3.4.1. Linen fibers after extraction

Since the FTIR is very important tool for detecting the functional groups, the ancient fibers were identified by the interpretation of its absorption spectra from FTIR spectroscopic analysis in comparison to modern (control) flax linen sample. The results confirmed that linen is the fiber that identified from the three ancient samples. The FTIR spectra of the modern (control) and three ancient textile samples after the extraction process are shown in Fig. 4. The FTIR data in Table 2 shows that there are significant changes between the modern and the ancient samples. The absorption bands at the region of 2900 cm^{-1} is assigned to (CH) bonds in aliphatic methylene

groups (Kavkler and Demšar, 2012). The ancient samples, Anc.1, Anc. 3 and Anc. 2 respectively, exhibited a decrease in the absorption band intensities in comparison to the control sample as an indication of oxidative and hydrolysis reaction (Tomšič *et al.*, 2007), which probably caused by the effect of burial and storage environment.

Table 2. FTIR data on control and ancient linen samples

Sample	Absorbency at			Absorbency Ratio (A2/A3)
	2900 cm^{-1} (A1)	1710 cm^{-1} (A2)	1639 cm^{-1} (A3)	
Cont.	0.560	0.108	0.276	0.391
Anc. 1	0.260	0.201	0.310	0.650
Anc. 2	0.390	0.159	0.340	0.470
Anc. 3	0.270	0.234	0.40	0.590

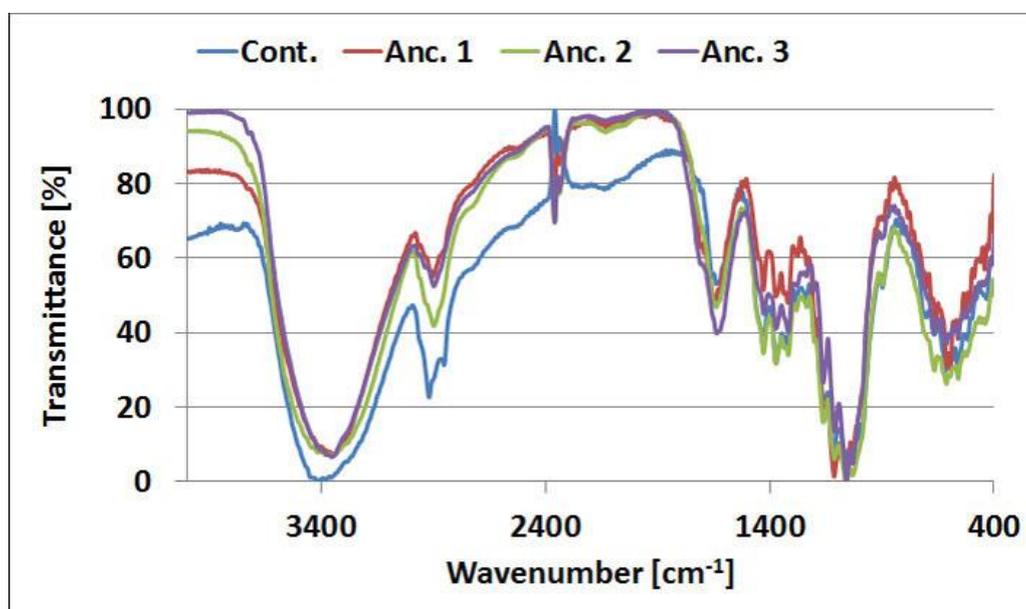


Figure 4. FTIR spectra of the three sample fibers after the extraction process

Since vibrational spectrum of cellulose is on the complex side, a good candidate to trace the changes in cellulosic materials caused by hydrolysis and oxidation are carbonyl groups ($-\text{COOH}$, $-\text{CHO}$, $-\text{CO}$) which occurrence on the broken ends of macromolecular chain (Łojewska *et al.*, 2006; Kavkler and Demšar, 2012; Kourkoumelis *et al.*, 2012). According to Abdel-Maksoud and El-Amin (2013) the region around $1750\text{--}1600\text{ cm}^{-1}$ was proved to be the most convenient for monitoring cellulose degradation. In the range of the absorption of carbonyl groups at the region around $1750\text{--}1600$, two remarkable absorption bands are found at 1710 cm^{-1} and 1639 cm^{-1} (Fig. 4). Ferrero *et al.* (1998) and Łojewska *et al.* (2005) reported that the absorbency ratio between the intensity of band at 1710 cm^{-1} and the intensity of band at 1639 cm^{-1} is a better index of chemical modification

induced by deterioration factors. As shown in Table 2, Anc. 1, Anc. 3 and Anc. 2 respectively exhibited a high absorbency ratio ($1710\text{ cm}^{-1}/1639\text{ cm}^{-1}$) in comparison to the control. The increase in the ratio of Anc. 1 more than the other ancient samples is related to its long-term direct contact with burial and environmental factors.

Using the FTIR data, several peak ratios were described earlier, highlighting the differences between the samples with regards to the relative amorphous and crystalline cellulose amounts. An empirical crystallinity index, also known as lateral order index (LOI), which is correlated with the overall degree of crystallinity in the cellulose, was defined as the ratio between the intensity of the absorption bands at 898 cm^{-1} (assigned to C–O–C stretching at β -1,4-glycosidic linkages in cellulose) and 1420--

1430 cm^{-1} which is associated with the amount of the crystalline structure of cellulose (Poletto et al.,

2014; Lourdin et al., 2016; Olaru et al., 2016; Auxenfans et al., 2017).

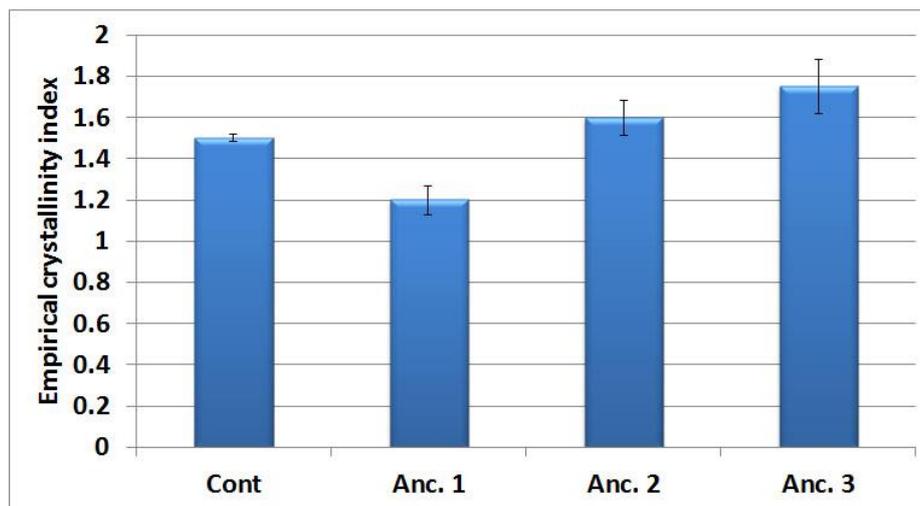


Figure 5. The empirical crystallinity index (lateral order index) of the control and the ancient linen samples

The increasing of the LOI of the ancient textile samples in comparison to the control sample was demonstrated (Fig. 5). It could be attributed to the fact that the environment conditions, especially the extremely high temperatures in the Egyptian desert, led to the degradation of amorphous regions and the reorganization of crystalline regions. Crystalline cellulose is the least susceptible to degrade (Gassan and Bledzki, 2001), and it is mainly affected by two factors: (1) UV irradiation (Bresee, 1986) and (2) hydrolysis by fungal enzyme in the long run (Kavkler and Demšar, 2012). These two factors appear to be found in the case of Anc. 1, which exhibited low crystallinity index in comparison to Anc. 2 and Anc. 3; because it is not covered with any materials, and several microorganisms are isolated from its fiber as shown in Section 3.2. Anc. 3 and Anc. 2 showed high crystallinity, which may be due to the resinous, protective layer covering its fibers.

3.4.2. Embalming Agents

According to Cartoni et al. (2003), Izzo et al. (2013), Sutherland et al. (2013) and Brettell et al. (2016), the FTIR spectra of extracted substances from Anc. 2 and Anc. 3 showed the characteristic absorption bands of natural resinous materials. A broad band in the 3500-3400 cm^{-1} region was recorded which is due to the stretching of OH groups. Methyl and methylene groups, referring to the hydrocarbon skeleton of the resin, give two absorptions in the ranges of 2960-2930 cm^{-1} and 2875-2850 cm^{-1} (C-H stretching), while 1467-1420 cm^{-1} and 1387-1360 cm^{-1} bands are due to the C-H bending (Fig. 6A and B). The main diagnos-

tic C=O stretching falls around 1700 cm^{-1} is demonstrating the presence of acid groups (Fig. 6A and B). The band at 1627 cm^{-1} were detected in Fig. 6A, which probably denotes the presence of calcium oxalates which likely derive from the gradual oxidative degradation of organic materials in the surface layers and their reaction with calcium-containing pigments and/or particulate dirt. Starching (C-O) weak band at 1238 cm^{-1} , which is attributed to terpenic components, was demonstrated in Fig. 6B. According to Ménager et al. (2014) C=O absorption band at fresh material analysis shows that terpenic resins present a maximum band at 1690 cm^{-1} . However, this band shifts during the degradation process.

Cartoni et al. (2003), who studied the characteristic of fresh and aged terpenic resins by micro-FTIR, stated that fresh mastic resin shows a broad band at 3430 cm^{-1} . In the C-H stretching region two bands, at 2948 cm^{-1} and at 2873 cm^{-1} , were observed. The main diagnostic C=O stretching falls at 1703 cm^{-1} . The finger-print region is characterised by the following bands: 1457 and 1381 cm^{-1} (medium), 1245 and 1164 cm^{-1} (weak). The ageing of mastic resin produces a broadening and a shifting of the diagnostic absorption bands. Significant changes occur in the range of 1700 cm^{-1} , where additional band fall at 1693 cm^{-1} .

By comparing the results of the current analysis with those from the previous studies, abovementioned, it could be stated that mastic (*Pistacia* resin) is the terpenic resin which was detected from the spectra of the extracts of Anc.2 and Anc. 3 samples.

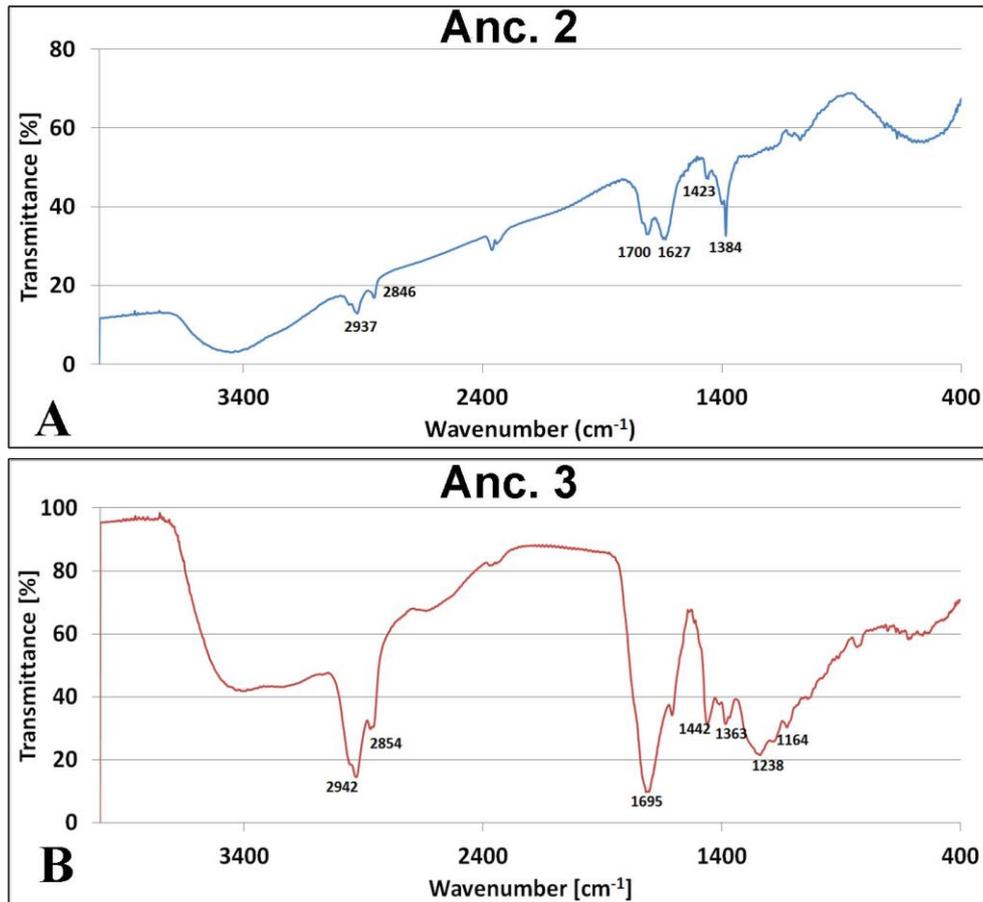


Figure 6. FTIR spectra of the extracts of samples: (A) Anc. 2 and (B) Anc. 3

4. CONCLUSIONS

Resinous materials are the most abundantly used in mummification. The type and amount depended on several factors, e.g., the social position of the mummified person and the embalmer. In some mummies, they cover the inside and outside of the body and wrapping textile; while, in others, they only cover the body. Microbiological investigation emphasized the role of resinous materials in protecting the linen fibers from microbial attack; while, SEM observation revealed the role of resinous materials in preserving the morphological structure and the outer shape of the linen fibers. FTIR results

demonstrated an increase of the absorbency ratio (1710 cm⁻¹/ 1639 cm⁻¹) of carbonyl bands intensities of Anc. 1 and the decrease of its crystallinity index more than Anc. 3 and Anc. 2 samples. This can be interpreted that the covered resinous materials protect the linen fibers (as shown with Anc. 2 and Anc. 3) from the mechanisms of deterioration by preventing the catalyzed factors of deterioration (oxygen and water) from penetration within the fibers. On the other hand, Anc. 1 did not contain resinous materials, and was affected by the oxidation and hydrolysis processes.

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