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Enzymatic Removal of the Oily Dirt from a Coptic Tunic using the Enzyme Lipase

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ABSTRACT

This article presents an extensive study on the use of the enzyme lipase to remove the oily dirt from a child's Tunic dated to the Coptic period. Furthermore, it presents interesting results about the effect of the enzymatic treatment on the mechanical and optical parameters of Linen using SEM, FTIR, XRD, CIE-Lab values and ASTM method D5035. To start the conservation procedure the restorers in the museum had previously used different solvent mixtures to remove the oily dirt from the Tunic, but the oily dirt showed high resistance, therefore a new suggestion of a different approach should come to the treatment plan. Lipase is considered nontoxic and is a less aggressive alternative compared to highly polar organic solvents and/or strong alkaline mixtures. The study was undertaken using Linen textiles in order to identify the optimum condition for the use of the enzyme, in relation to the time, the enzymes concentration and the temperature. The samples were immersed with Olive Oil. Then a process of artificial thermal ageing was applied to the samples for different periods of time. After that the enzyme was applied at different concentrations for different time periods. Finally, the removal of the enzyme residues from the textiles after the treatment was studied.

Keywords: Lipase, Dirt, Tunic, Textiles, removal, Linen, conservation

1. Introduction

Some archeological textile objects in museums contain fats, oils, and other greasy stains. Oily dirt containing unsaturated double bonds may be oxidized and form

hard stains. Oily dirt also causes other type of deterioration as weakness of the textiles at the edge of stains and acidity of textiles. In traditional method used to facilitate the dirt removal, a solvent mixture containing ammonia or another alkali to break down the oil network into smaller materials are often applied. This method is harmful and sometime not efficient. Several investigators have demonstrated the difficulty of the aged oily dirt removal from modern textiles used in daily life. (Agnes, 1998; Flury-Lemberg, 1988; Landi, 1992; Chi et al., 1998a)

Lipases are part of the family of hydrolyses that act on carboxylic ester bonds. The physiologic role of lipases is to hydrolyze triglycerides into diglycerides, mono glycerides, fatty acids, and glycerol. An enzyme's activity can be disrupted through any chemical, thermal or physical method that alters the tertiary configuration of the protein. (Wilson, 2000; Aymard and Belarbi., 2000; Owen, 1989; Blüher et al., 1997; Obendorf et al., 2001)

Lipases are often used in paper conservation for cleaning purposes, due to their ability to degrade aged oil films. It is safer than the conventional highly polar organic solvents or alkaline mixtures, and shows a more specific action, which poses less of a threat to the integrity of the work. The conditions required are mild and not harmful to the objective. On the other hand, for a number of years, conservators have used hydrolytic enzymes to clean polychrome surfaces. (Roberto et al., 1999; Blüher et al., 1997; Vokić, 2005)

Reverse micelles have been regarded as an excellent media for lipase enzymatic reactions containing polar compounds. Reverse micelles are consisted of a like organic phase and an querns phase dispersed in the organic solvent. This system became thermodynamically stable with the presence of an emulsifier such as the Sodium bis(2-ethylhexyl) sulfosuccinate (AOT) that has been widely used in reverse micellar enzymology. (Zhi et al., 2001; Carvalho et al., 1999)

This research presents an extensive and novel study of the use of lipase on the textile conservation to remove oily dirt from the Coptic tunic. We also studied the effect of the enzymatic treatment on the mechanical parameters (Tensile strength, Elongation and Crystallinity index) and color parameters such (ΔE), (ΔL*), (Δa*),(Δb*), (ΔC) and (ΔH) of linen fabric, because our object of research (the Coptic tunic) was made from linen fibers. Moreover, uncolored linen was the most commonly used fibers in the Coptic textiles (Ahmed, 2009). This work also focuses on the study of the removal of the enzyme residues from the textiles after residues from the textiles after the treatment.

2. Materials and Methods

2.1. Materials

- Lipase enzyme used was from *Candida Cylindracea* (62316 ,Fluka).
- Sodium bis(2-ethylhexyl) sulfosuccinate (AOT) (Sigma Co,323586).
- Olive Oil (Sigma Aldrich Chemical Co,47118).
- Egyptian linen fabrics supplied by Egylan Co., at the Second Industrial Zone, Alexandria, Egypt.
- The detergent Synperonic N (Lissapol N) 2-5%, non-ionic.

2.2. Samples preparation

The linen fabric was purified in the laboratory by scouring with a solution containing 2 g/L of detergent and sodium carbonate at a temperature of 60^0C for 15 min using a liquor ratio 1:50. The samples were thoroughly washed with tap water and dried at ambient conditions. (Micheal et al., 2005) In the present study the linen fabric samples were immersed in olive oil. The structures of the fabrics used for the experiments are shown in Table 1.

Table 1: Linen fabric Structure that were used in experiment part

	Structure	Color shade					Weight	Threads/cm		Elong-ation (mm)	Crystallinity Index
Linen	Plain Weave	L	a	b	C	H	g/m^2	Warp	Weft		
	1/1	64.03	1.90	9.47	9.66	78.68	105.7	11	16	7.1	85.78 %

2.3. Thermal ageing procedure

Ageing is the process of changing over time. Heating for 72 hours (3 days) at 120 ^{0}C is equivalent to about twenty- five years of ageing under normal conditions of ageing.(Feller, 1994) Textiles samples with oily dirt were hanged in a temperature controlled oven - a Fisher Isotemp oven (model 230F) - at 50 ± 1^{0}C for 1, 3, 6, 9, 12 and 15days. (Zhi et al., 2001)

2.4. Applying the enzyme

The modern textile samples were immersed with Olive oil, then cut to small pieces (2.5 × 2.5 cim), and placed in test tubes. In each tube 10 ml 50mM AOT/isooctane and 180 μL of lipase solution in Tris buffer (pH=7.5) were added. Then the tubes were incubated at different time intervals (0.5, 1.0, 1.5, and 2.0h) at room temperature (25 ^{0}C) and at a temperature of 37 ^{0}C. The enzyme concentration varied from (1, 10, 15, 20, 25, 30, 35, 40, 45, and 50U/ml) and initially the enzyme solution was added at each tube with stirring as well as without stirring. Each type of fabric sample had been previously aged for different periods (1, 3, 6, 9, 12, and 15 days) at 50 ^{0}C.

In order to monitor the hydrolysis of oily dirt, the fatty acid (Oleic acid) concentration that is liberated after the enzymatic treatment was measured Titrimetricly. 10 ml from every sample were placed into a 50 ml Erlenmeyer flask marked and then there were added: 3 ml of 95% Ethanol. Mix by swirling was followed and then 4 drops of 0.9% (W/V) Thymolphthalein Indicator Solution (TPH Indic) were added. Titration with 50 mM Sodium Hydroxide Solution (NaOH) took place until appearance of a light blue color.

2.5. Removal and deactivation of the enzyme.

The fabric samples were boiled in distilled water for 30 minutes to remove any finishing materials and after drying, the enzyme lipase was applied at a concentration of 60U/ml. The deactivation treatment was applied by using different methods at room temperature (25 ^{0}C).

1. Bathing the first sample in three bathes of clean distilled water for 10 minutes.
2. Bathing the second sample in three bathes of a mixture of Ethanol & distilled water (50: 50) for 10 minutes.

To determine the remaining activity of the enzyme after deactivation, each sample was transferred to a new test tube and supplemented with 0.65 ml of 0.05 M phosphate buffer (pH 7.2) and 0.1 ml 0.025 M para-nitrophenyllaurate (PNP-laurate) in absolute ethanol. The hydrolytic reaction was carried out at 65^{0}C for 30 minutes. After 30 min, 0.25 ml 0.1 M Na_2CO_3 was added. The mixture was centrifuged and the enzymatic activity was determined using a HITACHI. U-1100 – Spectrophotometer at 420 nm. This method was done according to Para-nitrophenyl-laurate (PNP-laurate) assay for lipases. (Sigurgisladottir et al., 1993)

2.6. Testing and Analysis

2.6.1. *Morphological study:*

The morphology of the surface of the untreated fabric in comparison to the enzymatically treated was investigated using Scanning Electron Microscope (SEM) - a Quanta 200 ESEM FEG from FEI.

2.6.2. ***Color Measurement***: The CIE-Lab values of the color changes were measured using double beam Optimatch spectrophotometer (Datacolor international Spectraflash SF450-UK). The colors are given in Commission Internationale de l'Eclairage (CIE) Lab coordinates, **L** corresponding to the brightness (100 = white, 0 = black), **a** to the red–green coordinate (positive sign = red, negative sign = green), and **b** to the yellow–blue coordinate (positive sign = yellow, negative sign = blue). The hue difference is given a positive sign when the hue angle **h** increases and a negative sign when **h** decreases.

$$L^*=116(Y/Yn)^{1/3}-16 \quad (1)$$

$$a^*=500[(X/Xn)^{1/3}-(Y/Yn)^{1/3}] \quad (2)$$

$$b^*= 200[(Y/Yn)^{1/3}-(Z/Zn)^{1/3}] \quad (3)$$

$$\Delta E^* = \{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2\}1/2 \quad (4)$$

$$\Delta H^* = \{(\Delta E^*)^2 - (\Delta L^*)^2 - (\Delta C^*)^2\}1/2 \quad (5)$$

(Wyszecki and Stiles., 2000; Booth., 1984)

2.6.3. ***Mechanical behavior:*** Mechanical parameters such as tensile strength and elongation were measured according to the ASTM method D5035 in the warp and weft directions. Linen, silk and cotton fabrics were cut into 30 cm strip length 5 cm width. Five samples per treatment set were tested and the breaking load averaged for each sample (Buschle-Diller et al., 1999; Tortora et al., 2007).

2.6.4. ***X-ray diffraction analysis***: X-ray diffraction measurements of enzymatically treated and untreated samples were carried out with a SIEMENS X-Ray Diffractometer – D 5000, given 40 Kv CU Ka, radiation of 30 mA. The diffractograms were recorded over $2\theta = 5^0$ to 30^0 continuously at a scan rate of 2^0/min. Crystallinity index (crystalline to amorphous ratio) can be calculated using the following equation: (Segal et al., 1959).

$$CrI = \frac{(I_{002} - I_{am})\ X\ 100}{I_{am}} \quad (6)$$

2.6.5. Fourier Transform Infrared Spectroscopy Analysis (FTIR):

The structural changes occurring in the fibers upon enzymatic treatment were monitored by FTIR. On the other hand, FTIR is a valuable method for the detection and the identification of organic components. (Mary, 1989) The FTIR analysis was carried out for untreated and treated fabric samples by using BRUKER – FTIR- TENSOR 27.

3. Results and Discussion

3.1. Effectiveness of lipase on oily dirt removal

The first step of the research was to choose the appropriate enzyme by identifying the substrate target. The second step was using traditional methods such as different types of solvents to remove the oily dirt from Tunic, but the oily dirt showed more resistance. The third step is to study the efficiency of the enzyme on removing the stains and to determine the best conditions for it regarding time, enzyme concentration, temperature and pH. We also studied the effect of enzyme on mechanical and optical parameters of linen. In this study, two different methodologies were applied to monitor the effectiveness of the lipase enzyme on oily dirt removal:

1) Investigation of the morphology of the surface of the fabric samples, using SEM before and after enzymatic treatment.
2) Calculation of the percentage of oily dirt hydrolyzed into fatty acid (Oleic acid) after lipase treatment.

Table 1 shows the linen fabric structure that was used in the experimental

part. The fabrics containing oily dirt turned to yellow when aged. We suggest that the presence of oil has greatly enhanced this yellowness. Nitrogenous impurities in the atmosphere contribute to the yellowing of the oils by forming hydro peroxide decomposition products of oil. These results are in agreement with results obtained by others studies. (O'Neill et al, 1962; Chi, Y., 1998a).

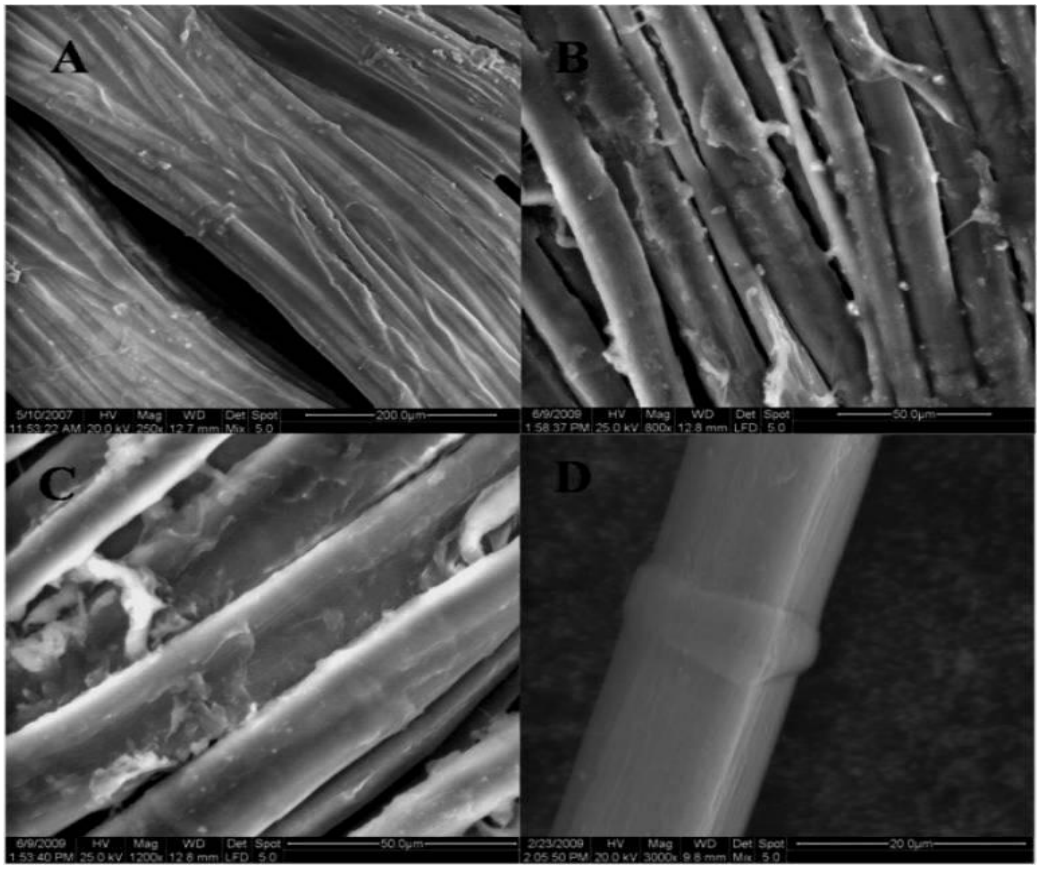

Fig.1. SEM photo of Linen coated with oily dirt after 12 days ageing before any enzymatic treatment (A) SEM photo of Linen coated with oily dirt after 12 days ageing after enzymatic treatment with concentration 5U/ml (B) SEM photo of Linen coated with oily dirt after 12 days ageing after enzymatic treatment with concentration 15U/ml (C) SEM photo of Linen coated with oily dirt after 12 days ageing after enzymatic treatment with concentration 40U/ml (D).

Fig.1A shows the linen samples that contain oily dirt after thermal ageing. It is clear that on the prolonged contact, viscous aged oils fill the smallest capillaries between fibers, and with oxidation continuously progressing, larger molecules that may chemically bond to the fabric are produced. In other words upon ageing, chemical changes take place in oily dirt, sometimes resulting in chemical bond formation between the fiber substrates and the oily dirt. Longer contact time between oils and fibers leads to a high degree of polymerization and covalent bonding of the oils with the fibers. These results are in agreement with results obtained by other studies. (Chi et al., 1998b)

Fig.1A-D show the difference between the samples before and after the enzymatic treatment. The effectiveness of the lipase seems to be influenced by differences in the physical structure of the fiber, meaning that lipase is very effective on oily dirt removal that is located on fiber surfaces and within the interfiber spaces of

the yarn structure. The use of lipase resulted in extensive cleaning of the fiber surfaces and interfiber capillaries, with high effectiveness for small capillaries and the center of the yarn bundle. These results are in agreement with results obtained by other similar studies. (Obendorf et al., 2001)

Studying the conditions affecting the performance of the enzyme in removing aged oil film, has allowed us to notice that the efficiency of the enzyme in the removal of aged oil film increases by increasing the enzyme concentration of the treatment solution. The increase on the concentration of the enzyme increases the number of free active sites that interact with more substrates molecules. On the other hand, increasing the concentration of enzymes increases the number of the successful collisions between the enzyme and the substrate. The rate of the reaction is directly proportional to the enzyme concentration and the higher the concentration of the enzyme the faster the reaction. Figs. 2 A- C

By studying the factor of time, it has been noticed that the longer the process of the treatment takes, the better the efficiency of the enzyme. Also, as for as temperature is concerned, the enzyme treatment at 37 ^{0}C results in better efficiency than applying it in room temperature (25^{0}C). as given in Fig. 2 D, an increase in temperature increases the enzymatic activity since the molecules now possess greater kinetic energy. These results are in agreement with results obtained by other studies who found that large lipase benefits were observed when the washing temperature was below or near the melting temperature of the oil, i.e., olive oil washed at 30^{0}C (Varanasi, A., 2001).

It has also been observed that the longer the time of the ageing for the samples immersed with oil dirt, the harder the process of removing the aged oil film. Results suggested that ageing caused chemical bond formation between the fiber substrates and the oily dirt. Longer contact time between oils and fibers allows a high degree of polymerization and covalent bonding of the oils with the fibers. See Fig. 2 E and F.

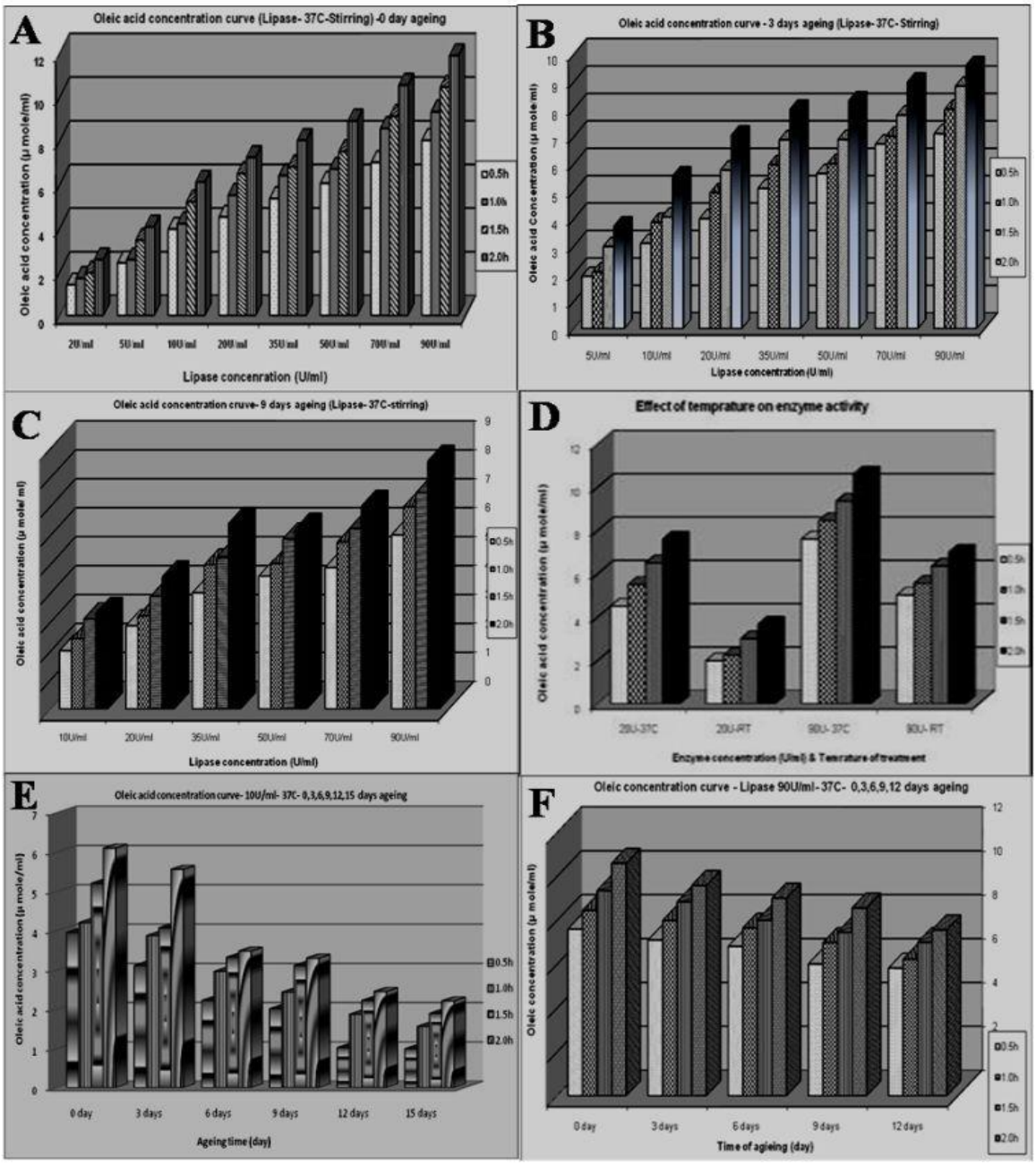

Fig.2. Oleic acid concentration curve after enzyme treatment with different enzyme concentration 2, 5, 10, 20, 35, 50, 70, and 90U/ml at 37 ^{0}C for different times 0.5, 1.0, 1.5 and 2.0h. Samples without ageing (A) Oleic acid concentration curve after enzyme treatment with different enzyme concentration 10 , 20, 35, 50, 70 and 90 U at 37 ^{0}C at different times 0.5, 1.0, 1.5 and 2.0h. Samples after 3 days ageing (B) Oleic acid concentration curve after enzyme treatment with different enzyme concentration 10 , 20, 35, 50, 70 and 90 U at 37 ^{0}C at different times 0.5, 1.0, 1.5 and 2.0h. Samples after 9 days ageing (C) Oleic acid concentration curve after enzyme treatment with enzyme concentration 20 and 80U/ml at 37 ^{0}C and Room temperature 25 ^{0}C for different times 0.5, 1.0, 1.5 and 2.0h (D) Oleic acid concentration curve after enzyme treatment with enzyme concentration 10U/ml at 37 ^{0}C for different times 0.5, 1.0, 1.5 and 2.0h. Samples after 0, 3, 6, 9,12 and 15 days ageing (E) Oleic acid concentration curve after enzyme treatment with enzyme concentration 90U/ml at 37 ^{0}C for different times 0.5, 1.0, 1.5 and 2.0h. Samples after 0, 3, 6, 9 and 12 days ageing (F)

3.2. Removing enzyme from textile after treatment

Shibayama et al., mentioned that literature search doesn't reveal any information about enzyme residues in textiles after rinsing. This study includes some interesting observations about the effectiveness of the rinses to remove enzyme residues from the textile. Table 2 presents Oleic acid concentration in the three rinsing solutions (distilled water bath or mixture of water and ethanol bath). Oleic acid concentration is considered an indicator of the amount of lipase in the existing solution. It was found that three rinses (either distilled water bath or a mixture of water and ethanol bath) are very effective to remove any enzyme residues from the fabric samples. These results are in agreement with Andrews et al., 1990 who found that two rinses are very effective to remove alpha amylase residues from paper. Deactivating the enzyme by heating is not approved because it may be harmful to the historical textiles. (Whaap , 2007)

Table 2: Oleic acid concentration observation

Parameter	Oleic acid concentration (baths in distilled water)		Oleic acid concentration (baths of ethanol & water (1:1)	
	absorbance at 420 nm	Oleic acid concentration (µ mole)	absorbance at 420 nm	Oleic acid concentration (µ mole)
First bath	0.463	19.37	0.403	16.86
Second bath	0.187	7.82	0.160	6.69
Third bath	0.036	1.50	0.034	1.42

3.3. Effect of Lipase treatment conditions on the crystallinity

XRD results of untreated and treated samples are presented in two ways.

The first way is to calculate the percentage of crystalline index of untreated linen sample and those treated by different enzyme concentration. One can see a slight decrease of crystallinity index, and results are presented in Table 3.

Table 3; Calculate the percentage of crystalline index of treated samples after enzyme treatment with different enzyme concentrations 20, 40, 60 U/ml at 37 ^{0}C for different times 1.0h, 3.0h.

Samples	Crystalline Area		Amorphous Area		Crystallinity index
	2 Θ	Counts	2 Θ	Counts	
Linen original	22.879^0	683	19.292^0	97.1	85.78 %
Linen- 20U- 1hr	22.959^0	419	19.106^0	74.2	82.29 %
Linen- 20U- 3hr	22.852^0	468	19.265^0	83.0	82.26 %
Linen- 40U- 1hr	22.879^0	514	19.504^0	93.6	81.78 %
Linen- 40U- 3hr	23.092^0	519	19.230^0	94.7	81.75 %
Linen- 60U- 1hr	22.852^0	526	19.425^0	93.2	82.30 %
Linen- 60U- 3hr	22.838^0	513	19.751^0	95.1	81.40 %

The Second way is the Wide Angle X-ray (WAXS) diffractograms of untreated and treated samples. This finding suggests that the treatment by using lipase enzyme does not particularly affect the size and shape of crystallites of the linen. Furthermore, the ratio of the crystalline and amorphous fractions barely changed, thus the enzymatic treatment did not result in considerable decrystallization in the linen cellulose. The

treatments slightly decrease the crystallite size of the longitudinal dimension, given in Fig. 3 A.

3.4. FTIR spectra of fabrics treated with lipase

The structural changes occurring in linen and silk fibers upon enzymatic treatment were monitored by Fourier Transform infrared spectral analysis (FTIR). Fig. 3B and Table 4 shows FTIR of raw linen samples after enzymatic treatment, one can observe the absorbance at bands intensity increases in the spectra of the sample treated in comparison with the spectra of the untreated sample, and this result reconfirms the partial dehydration hypothesis. The also study suggests that the treatment of the linen fiber with the lipase enzyme causes partial removal of concomitant substances (fats, pectins, and lignin) from cellulose. This finding is in agreement with (Shamolina et al., 2004) that found the treatment of linen after cellulase enzyme causes a partial removal of concomitant substances.

Table 4: FTIR of linen samples after enzymatic treatment

Band	Group	Band	Group
3336 cm^{-1}	peak due to OH groups	1053 cm^{-1}	peak due to -C-O-C groups
1642 cm^{-1}	peak due to-R-HC=O groups	1030 cm^{-1}	peak due to -C-O-C groups
1427 cm^{-1}	peak due to $-CH_2$ and $-CH_3$ groups	1002cm^{-1}	peak due to $–CH=CH_3$ groups
1204 cm^{-1}	peak due to -C-O-C groups	896 cm^{-1}	peak due to $–CH_2=$ C-R groups
1105 cm^{-1}	peak due to -C-OH groups	667 cm^{-1}	peak due to –C-OH groups

4.5. Effect of lipase on Linen color change

The results show that linen treated had a total color difference Δ E between 0.427 to 0.344. It is clear that there is a very slight increase in the brightness index ΔL. Samples also show very slight increase in the yellowness Δb* and redness Δa* with an increase in enzyme concentration. The angle (Δh) and color chromocity (ΔC) increased for treated linen. Table 5 All of these treated samples had color changes of ΔL, Δa, and Δb about 0.5 CIELab Unit, which cannot be detected by the human eye.

Table 5: Color change of samples after enzymatic treatment at different enzyme concentrations 20, 40, 60 U/ml at 37 ^{0}C for different times 1.0h, 3.0h

D65/10	Δ E	ΔL*	Δa*	Δb*	ΔC*	ΔH
Linen- Lipase- 20U- 1hr.	0.427	0.415	0.087	0.048	0.064	-0.076
Linen- Lipase- 20U- 3hr.	0.466	0.393	0.095	0.232	0.208	-0.140
Linen- Lipase- 40U- 1hr.	0.498	0.327	0.081	0.473	0.481	0.004
Linen- Lipase- 40U- 3hr.	0.418	0.280	0.103	0.292	0.307	-0.043
Linen- Lipase- 60U- 1hr.	0.456	0.367	0.155	0.369	0.393	-0.058
Linen- Lipase- 60U- 3hr.	0.344	0.313	0.118	0.382	0.204	-0.069

3.6. Effect of lipase on mechanical parameters of the samples

Tensile strength and elongation of untreated and treated linen samples by lipase enzyme are presented in Table 6. The linen samples treated showed significant improvement in elongation properties over untreated samples with an increase in enzyme concentration and treatment time. The results of the initial characterization (before enzymatic treatment) show that the treatment caused a slightly decreases in Tensile Strength.

Table 6: The mechanical parameters of treated samples after enzyme treatment with different enzyme concentrations 20, 40, 60 U/ml at 37 ^{0}C for different times 1.0h, 3.0h

	Samples	Tensile Strength (kg force)	E_b (mm)
Warp Direction	**Linen original**	56.780	7.112
	Linen –lipase- 20U- 1hr	51.964	9.123
	Linen –lipase- 20U- 3hr	50.320	10.313
	Linen –lipase- 40U- 1hr	49.097	11.741
	Linen –lipase- 40U- 3hr	48.821	13.535
	Linen –lipase- 60U- 1hr	47.139	17.239
	Linen –lipase- 60U- 3hr	46.742	19.289

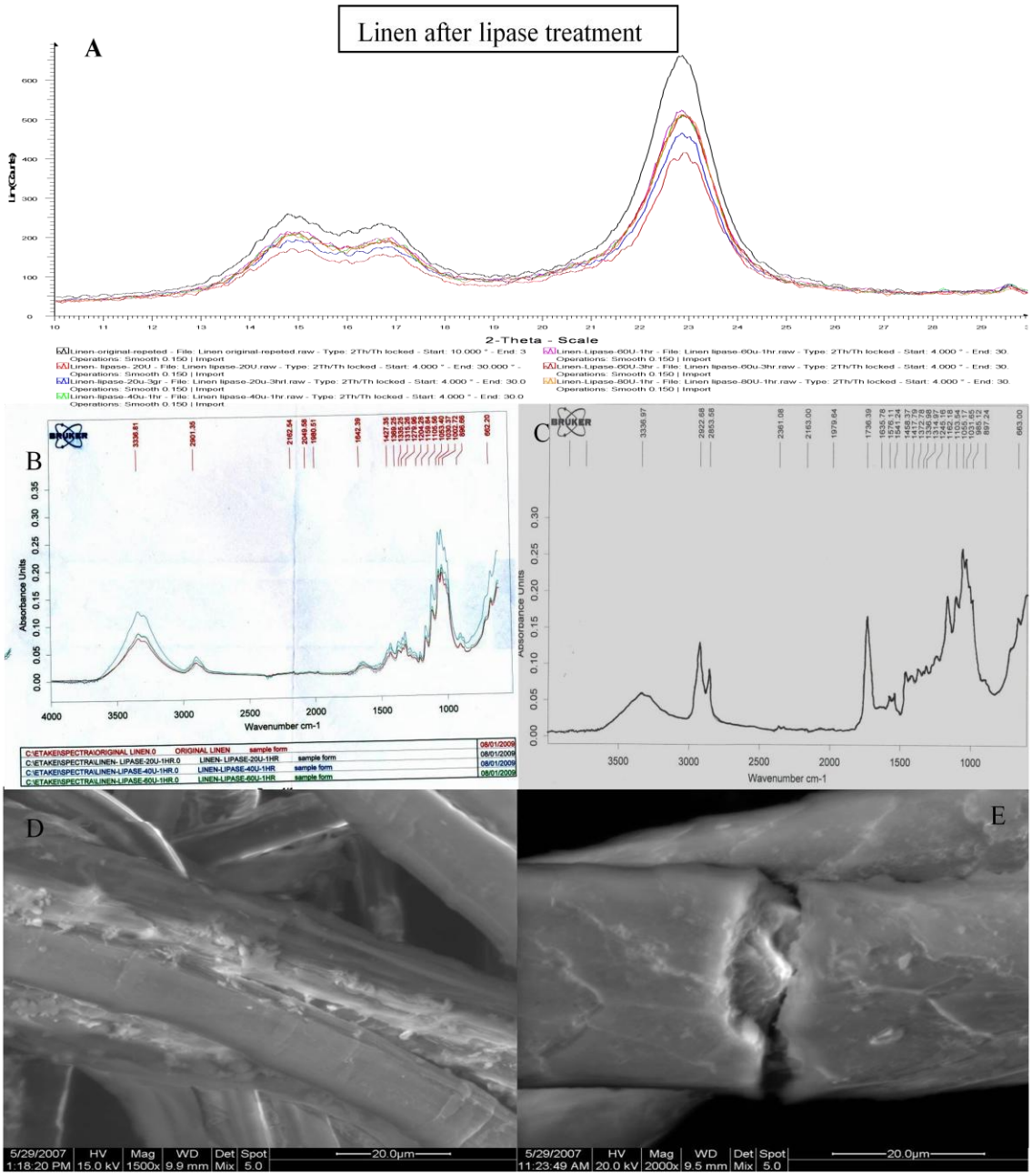

Fig.3. Wide Angle X-ray (WAXS) diffractograms of untreated linen samples and those treated with different enzyme concentration (A) FTIR spectra of treated linen samples before and the enzymatic treatment by using different enzyme concentrations 20, 40 and 60 U/ml at 37 ^{0}C for different time 1 and 3h (B) FTIR spectra for Coptic Tunic, that show the linen fibers contaminated with the oily dirt (C) wool fibers of ribbons decoration in Coptic tunic show some deterioration aspects (D) Linen fibers of Coptic tunic show some deterioration aspects in linen fibers (E)

4. Application part

4.1. Description of Coptic Tunic

A Tunic dated to the Coptic period, representing a child's Tunic with sleeves, decorated with ribbons was used in this study. This tunic carries registration number 1583 in Ismallia Museum and belonged to the Greek-Roman Museum collection in Alexandria. There is a big section of fabric missing on one side of the Tunic and in the hem. The Coptic tunic contained oily dirt and various types of deteriorations such as dust, grease, losses and weakened fibers. (Fig. 4)

Fig.4. Different details of Coptic tunic, we can see that the Coptic tunic contained oily dirt and various types of deteriorations such as dust, grease, losses and weakened fibers.

SEM Photos of examined Coptic Tunic are illustrated in Fig. 3D and E showing the linen and wool fibers that were identified from different parts of the Tunic. The fibers are extremely roughened, damaged, broken with transverse cracking and longitudinal splitting characterized by small scratches, small slits and holes. FTIR confirmed that the dirt on the different parts of tunic was of oily nature as shown in Fig.3C

4.2. Stability test of the colored ribbons

The dry fabric (the tunic) was softened by spraying distilled water, to counter its extremely dry condition. The next step was to test the stability of the colored ribbons to enzyme action and to wet cleaning by immersing a piece of cotton wrapped round a wooden stick into the cleaning solutions and placing it in contact with the colorful parts of the ribbons, each color individually tested. It was found that all the dyes were stable and did not bleed with either enzyme solution or wet cleaning solution. The final step was to apply a primary support to the Coptic Tunic by placing it between two webbed support fabrics, and stabilizing the fabric by fixing it to the support fabric, using appropriately thin needles and fine silk thread in order to protect the vulnerable part of the tunic from disintegrating during the different cleaning processes.

4.3. Enzyme application

According to our experimental part, the total volume of the enzyme solution (cm^3) was calculated according to the surface area of the object (cm^2). The enzyme solution with 50Mm Sodium bis(2-ethylhexyl) sulfosuccinate (AOT)/isooctane were placed in a wide glass dish and heated to 37^0C. Then the enzyme treatment was applied by dipping the affected areas of the Tunic into the enzyme solution for 25 minutes. The solution's temperature was monitored and maintained at 37 ^{0}C; enzyme concentration was 60U/ml. The fabric was gently agitated with a fine brush to ensure that the enzyme reached all areas for the effective removal of stains. Fig 5 A and B

Each oily stained section was treated in this way with fresh solution for 25 minutes. After the treatment, the enzyme excess had to be removed from the textiles. According to our experimental part and literature rinsing should be done with water of the same pH as the buffer used for the enzymatic treatment. This allows the enzymes to be rinsed out more effectively. A water bath was used in the next stage to remove dirt and dust as well as the enzyme residues. (Owen, 1984; Sandrine, 2002)

4.4. The wet cleaning procedure

This cleaning procedure used water with other detergent agents, to assist the cleaning process. The ratio was one part detergent Synperonic N to 100 parts of distilled water. The distilled water with soap was put into a large plastic basin, and the Coptic Tunic was placed in it. The water was agitated to allow it to penetrate between the fibers to release the dirt particles, for 15 minutes. The bath temperature was 30 ^{0}C. Then a second cleaning bath with distilled water only was applied for 10 minutes again with water agitation, and then a third bath with distilled water only, for 10 minutes. The wet-cleaning did succeed in extracting much of the remaining lipase. It also reduced the soiling, relaxed the fibers, removed the creasing and brightened the colors. Fig. 5C

4.5. The drying process

To dry the tunic without distortions we experimented with a Japanese tissue sandwich using different tissues (usugami or gampi) applied cross-grain or parallel-grain to the sample object with varying amounts of moisture and pressure. Consequently, the object was left uncovered to complete drying at ambient conditions. In air drying, the process can be shifted towards evaporation in several ways: by ensuring that the wet textile is setted in a place (i.e. workroom) that is sufficiently large in comparison to the size and surface area of the drying textile; and by ensuring that water vapor is removed as soon as it is formed e.g. with the help of dehumidifiers and /or effective ventilation. (Agnes, 1998; Shelley and Judith, 1979) (Fig. 5 D)

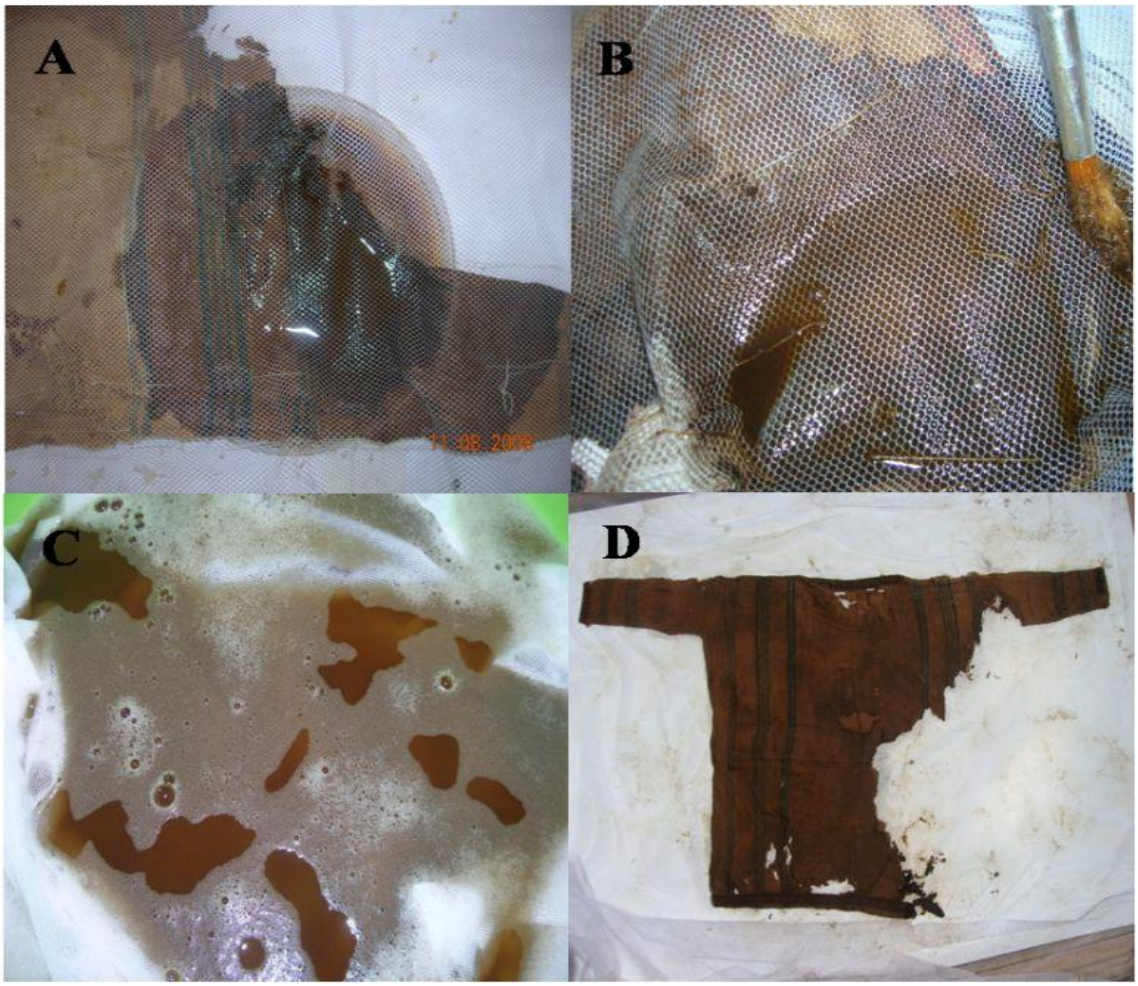

Fig.5. Local enzymatic treatment, a primary support to the Tunic by placing it between two webbed support fabrics (A and B) Wet cleaning procedure using water with detergent (C) Dry processes after wet cleaning (D)

4.6. The final support process

After completion of the tunic drying, the fabric webbing primary support was removed. A new linen support was prepared and washed well to remove chemical residues and prevent shrinkage at a later time due to humidity changes. Then the new linen support was ironed to remove creases and was placed inside the shirt between the outer and the inner layers. Tacking stitches were used with a very fine needle and fine silk thread to fix it into the tunic. In the beginning of the final stage, the edges of the tunic all around were attached by sewing with a small stitch technique (blanket stitch) and afterwards the edges of the missing and vulnerable parts were attached by small stitches. Similarly sized stitches were used to attach the body of the shirt. The sleeves were supported by attaching to new linen fabric (Fig.6). After completing the cleaning process and fixing the tunic, it could be displayed either on a mannequin made of inert material or on any other suitable three-dimensional viewing system. (Constance, 1970)

Fig.6. the Coptic tunic after conservation treatment: after removal of oily dirt, after wet cleaning and after support the tunic onto new linen fabric support

5. Conclusions

a) The enzyme lipase has been applied successfully and safely on the Archeological textiles to remove the oily dirt which is difficult to be removed by traditional methods.

b) For the removal of lipase residues from textiles after treatment, three water baths are required at room temperature (25 ^{0}C).

c) According to the results of the study there are no drastic changes on the mechanical and optical parameters of the textiles, except of the following:

   i. An improvement of the elongation, while there is a slight decrease on the tensile strength for Linen fabric.

   ii. A slight change in optical parameters such as (Δ E, Δa*, Δb*, ΔL*, ΔC, ΔH) for uncolored linen.

6. Recommendations

a) Further studies on the effect of lipase on mechanical and optical parameters of natural dyed fabrics are indicated.

b) Before any application by the lipase enzyme on historical textiles to remove oily dirt, an experimental part must be done to determine the optimal conditions (concentration, time and temperature) for the enzyme.

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