Journal of Cultural Heritage 12 (2011) 169–179

Available online at ScienceDirect www.sciencedirect.com

Elsevier Masson France EM|consulte www.em-consulte.com

Research paper

An investigation into the removal of starch paste adhesives from historical textiles by using the enzyme α-amylase

Harby E. Ahmed [a,*,b], Fragiskos N. Kolisis [a]

[a] Biotechnology Lab, Chemical Engineering School, National Technical University of Athens, 9 Iroon Polytechneiou, 15780 Zografou, Athens, Greece
[b] Conservation Department, Faculty of Archeology, Cairo University, El-Gamaa Street, 12613 Orman, Giza, Egypt

ARTICLE INFO

Article history:
Received 17 June 2009
Accepted 28 August 2010
Available online 3 February 2011

Keywords:
Enzyme
Starch
Textiles
Ageing
Treatment
Investigation
Activity
Layers
Conservation

ABSTRACT

The α-amylase enzyme has been reported during the last decade to be used for removal of the excess starch adhesive paste, which is usually used to fix textiles on paper, textiles, wood panels, or other rigid support materials. The final aim of this work was the application of α-amylase in order to remove the old starch from historical textiles in an attempt to conserve them under mild conditions. An extensive study was undertaken using various types of textiles in order to identify the optimum condition for the use of the enzyme, in relation to time, concentration, temperature and pH, before any other attempt. The first step was to simulate the textile ageing. The fabrics were coated with starch paste adhesive, and then a process of artificial thermal ageing was made on samples for different periods of time. After that the enzyme was applied to the samples, at different concentrations and at different intervals. This study also presents interesting results concerning the effect of the enzymatic treatment on the mechanical and optical parameters of linen, silk and cotton, dyed with madder or turmeric dye mordanted with $CuSO_4$ or ferric citrate. Finally, the removal of enzymatic residues from textiles after the treatment has been studied. The application section has been fulfilled by using the whole process in a piece of a historical carpet from fabric adhered with starch. This piece of carpet is in the museum of the Faculty of Applied Arts, Helwan University in Egypt.

1. Research aim

(1) The study of the use of α-amylase in the field of historical textiles conservation; removal of starch adhesive paste.
(2) The study of the removal of enzyme residues from textiles following the enzymatic treatment by simple and safe methods.
(3) The study of the effect of the enzymatic treatment on the mechanical parameters of fabrics such as tensile strength, elongation, and crystallinity index.
(4) The study of the effect of enzymatic treatment on the color changes of cotton fabric dyed with madder dye and turmeric dye (mordanted with $CuSO_4$ and ferric citrate).

2. Experimental

2.1. Introduction

The natural adhesive starch can often be found on historical or ethnographic textiles, e.g. when they have been adhered to a solid surface due to conservation procedure. Starch adhesive paste is often present in shrunk, cracked, rigid and brittle form due to the aged condition, and does not provide enough adhesion for effective support. It causes heavy damage to textile as a result of embrittlement, hardening, yellowness and acidity of historical textiles. Furthermore, starch is an attractive nutrient for a large number of fungi and bacteria that will decay textiles over time. It is often necessary to remove these adhesive materials from historical textiles because they may lead to deterioration of the textiles as they age [1–5].

Amylases, the enzymes specific for starch hydrolysis, have become widely accepted as some of the most useful reagents available to conservators. The application of enzymes on textiles is an efficient conservation method, and the least disruptive to the fibers. Extremely thick accretions, which traditionally would require inadvisably long time and repeated washes, are more efficiently removed by enzymes, because the last catalyze the degradation reaction of the components of the paste. Enzyme preparations can be dissolved in water and applied locally, as poultice or in overall treatments [6–17].

In 1979, Fletcher and Walsh used α-amylase to remove three prints by Whistler on exceptionally fine Japanese tissues from pulpboard mounts. Then in 1983, DeSantis presented a review of the conservation and enzymology literature in order to characterize

* Corresponding author.
E-mail addresses: harbyezzeldeen@yahoo.com (H.E. Ahmed), Kolisis@chemeng.ntua.gr (F.N. Kolisis).

1296-2074/$ – see front matter © 2011 Published by Elsevier Masson SAS.
doi:10.1016/j.culher.2010.08.001

the use of proteolytic and starch digesting enzyme in paper conservation. Furthermore, in 1984, Owen used α-amylase to facilitate a backing removal from a historical poster. In 1986, Chapman used α-amylase in a viscous medium (Laponte RD) to separate painted textiles that had been stuck to cardboard mounts with a starch paste. In 1990, Bott studied the removal of starch from a set of mid 17th century embroidered panels by using α-amylase. Also, Shibyama et al. used α-amylase to remove flour paste residues from a painted banner. In 2002, Sandrine presented a literature review of α-amylase used for adhesive removal in paper conservation. Also, in 2007, Whaap used α-amylase to remove starch residues from tow Coptic Tapestry. But none of these researchers had studied the effects of the enzymatic treatment on the fiber and dye characteristics (color changes, mechanical properties).

This research presents an extensive study of the use of α-amylase on textile conservation to remove starch adhesive paste. Moreover, we studied the effect of α-amylase on the mechanical and color parameters of linen, silk and cotton dyed fabric. This type of fibers were studied because they were the most commonly used fibers in the historical textile industries as well as the most of textile collections in the international museums made from linen, silk, wool and cotton fibers [18]. This paper focuses on studying the removal of enzyme residues from textiles following the treatment.

2.2. Materials and method

The materials used are:

- α-amylase enzyme from *Aspergillus oryzae*, type X-A. Code A6211 (Sigma). One unit of enzyme will liberate 1 mg of maltose from starch in 3 min-pH = 6.9 at 20 °C;
- starch from Riedel-De Haen AG. Seelze-Hannover, code 18727;
- Egyptian linen fabric supplied by Egylan Co., at the Second Industrial Zone, Alexandria, Egypt, Egyptian cotton;
- Greek silk fabrics supplied by Tsiakiris Co., Soufli, Greece, www.tsiakiris.gr;
- natural dyes such as madder dye and turmeric dye;
- mordents such as copper sulfate $CuSO_4 \cdot 5H_2O$ and ferric citrate (Fluka).

The colors are given in *Commission internationale de l'éclairage* (CIE L*a*b*) coordinates [19–21].

2.2.1. Fabric samples

The fabrics used in this work have the following identifications (Table 1).

2.2.1.1. Dyeing procedures. The dyeings were performed by the exhaustion method using a liquor ratio (LR) of 1:20 (1 g of fabric per 20 ml of bath). In the experiments, mordants ($CuSO_4 \cdot 5H_2O$ or ferric citrate) were added as concentrated solution (50 g/l) to give a final dye bath concentration of 2.5 g/l or 5 g/l. After dyeing, the unfixed dyestuff was removed by rinsing three times with cold water (5 min, room temperature [25 °C], LR 1:20) [22–25].

2.2.2. Application of adhesives

To apply the starch adhesive paste on the samples, the starch is applied as solution and it is introduced into the fibers (or to a yarn or textile) and then solidified [26].

2.2.3. Accelerated ageing

Prior to application of the enzymes on the fabric samples coated with starch, it is essential to be symmetrical to the historical textiles coated with starch as adhesive. The accelerated ageing was performed because we wanted to simulate the condition of a historical textile. Therefore, accelerated thermal ageing was used aiming to give the recent textile samples aging characters according to the following standard equations:

- heating paper or textiles for 72 h (three days) at 120 °C is equivalent to about 25 years of ageing under normal conditions;
- heating paper or textiles for six days at 120 °C is equivalent to about 50 years of ageing under normal conditions;
- heating paper or textiles for nine days at 120 °C is equivalent to about 55 years of ageing under normal conditions and so on [27].

Textile samples that had been applied with starch paste were hanged in a temperature-controlled oven. The samples were thermally aged at the temperature of 120 °C for different periods (one, three, six, nine and 12 days).

2.3. Enzymatic treatment

The samples that were coated with starch after ageing were cut up into small pieces (2 cm × 2 cm) and were put in test tubes. Then, were added to each tube 5 ml of enzyme solution (α-amylase in sodium phosphate buffer, pH = 6.9) and was incubated at different

Table 1
Fabrics stricture and color coordinates of linen, silk and cotton fabrics that used in experimental part.

Samples	Thread (cm)		Mechanical parameters		Weight (g/m²)	Crystallinity index (%)	Plain weave
	Warp	Weft	TST (kg)	Eb (mm)			
Uncolored linen	11	16	56.780	7.112	105.7	85.78	Plain 1/1
Uncolored cotton	25	29	61.920	26.448	66.5	88.87	
Uncolored silk	32	25	27.967	15.852	25.4	72.71	

Samples	L^*	a^*	b^*	C^*	h	X	Y	Z
Uncolored linen	64.03	1.90	9.47	9.66	78.68	31.64	32.83	28.47
Uncolored cotton	94.85	3.27	−9.9	10.52	288.11	84.41	87.23	109.0
Uncolored silk	89.87	0.574	6.211	6.214	84.8	72.34	76.02	73.53
Cotton-turmeric dye								
$CuSO_4$	74.30	5.26	45.07	45.38	83.34	46.57	47.18	18.16
Ferric citrate	72.54	4.80	35.97	36.29	82.40	43.77	44.47	21.31
no mordant	76.80	6.59	50.36	50.79	82.54	49.37	48.56	16.88
Cotton-madder								
$CuSO_4$	64.65	11.49	4.01	12.17	19.25	35.13	33.13	33.03
Ferric citrate	65.33	9.59	9.65	13.59	45.27	35.42	34.46	29.86
No mordant	60.38	14.10	4.60	14.83	18.05	30.70	28.55	27.54

TST: tensile strength; Eb: elongation.

time intervals (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 h) at room temperature (25 °C) and at a temperature of 40 °C. The enzyme concentration varied (1, 10, 15, 20, 25, 30, 35, 40, 45 and 50 U/ml) and was added at each fabric sample with stirring as well as without stirring.

In order to monitor the hydrolysis of the starch adhesive paste, the maltose concentration that is liberated after the enzymatic treatment was measured spectrophotometrically using the DNS method. Specifically, 0.5 ml from every sample were put in a new test tube and 0.5 ml of reagent DNS (3, 5-dinitrosalicylic acid) were added as well; the tubes were capped and placed in a boiling water bath for exactly 5 min; after that, 5 ml of deionized water were added and the mixture was recorded at the 540 nm wavelength against standard curve of maltose using a Hitachi U-1100-Spectrophotometer [28]. Maltose was measured from the equation determined from the standard curve Y = 9.63 X, (Y absorption, X concentration of maltose [mg/ml]). All samples were weighed before and after the treatment in order to calculate the percent of the weight decrease. The weight difference is due to the partial hydrolysis of the starch, caused by the amylase action.

2.4. Removal and deactivation of the enzyme

Enzyme deactivation after the treatment is considered as an important step in the conservation procedure; therefore a lot of investigators referred to this step [11,13–15,17,29]. The efficiency of rinsing α-amylases out of fabric samples after enzymatic treatment was measured according to an enzymatic assay [28]. We used an initial enzyme solution with concentration 60 U/ml and the total volume was 100 ml. There we added the fabric sample and let it for 10 min. Then the fabric samples were cut into small pieces (2 cm length and 2 cm width), divided into two groups and subjected to:

1. washing of the first group in three baths of distilled water for 10 min, for each bath;
2. washing of the second group in three bathes of a mixture of ethanol and distilled water 1:1 (V/V), for 10 min for each bath.

The determination of the amount of the enzyme residues removed from the samples was made as following, 1 ml from every bath was collected and put in new test tube; then1 ml of 1.0% (w/v) soluble starch solution was added and placed in water bath at 40 °C for 15 min. Amylase is expected to hydrolyze the starch to maltose. The amount of formed maltose is considered as an indicator of the amount of the enzyme in the solution. To determine the amount of liberated maltose, 0.5 ml from of the solution in every test tube were collected and put in new test tube, and then 0.5 ml of reagent DNS was added. The tubes were capped and placed in a boiling water bath for exactly 5 min. The samples were cooled on ice and 4 ml of deionized water were added, [28] then mixed by inversion and recorded at 540 nm using a Hitachi U-1100–spectrophotometer.

2.5. Testing and analysis

2.5.1. Morphological study

The morphology of the surface of the untreated in comparison to the enzymatically treated fabrics was investigated using scanning electron microscope (SEM) – a Quanta 200 ESEM FEG from FEI [30].

2.5.2. Color measurement

The CIE-Lab values of the color changes were measured using double beam Optimatch spectrophotometer (Datacolor international Spectraflash SF450-UK).

2.5.3. 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 widths. Five samples per treatment set were tested and the breaking load averaged for each sample [31].

2.5.4. 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 $2\theta = 5^\circ$ to 30° continuously at a scan rate of 2°/min. Crystalline index (crystalline to amorphous ratio) can be calculated using the following equation [32]:

$$\mathrm{CrI} = \frac{(\mathrm{I}_{002} - \mathrm{I}_{\mathrm{am}})}{\mathrm{I}_{\mathrm{am}}} \times 100$$

2.5.5. Fourier transform infrared spectral analysis (FTIR)

The structural changes occurring in the fibers upon enzymatic treatment were monitored by FTIR. The vibrational bands that appear in the infrared spectra provide information about the chemical functional groups of a sample, which leads to a general characterization of the material or even the identification of specific compounds. The infrared absorption spectra of the untreated and treated samples in the wavenumber range 500–4000 cm^{-1} with a resolution of 4 cm^{-1} were measured at room temperature with a Bruker–FTIR-Tensor 27 infrared spectrophotometer using the KBr pellet technique. The spectra were normalized by making the absorption of any spectrum vary from zero to one arbitrary unit. Such normalization is necessary to eliminate the concentration effect of the powder sample in the KBr disc [33,34].

3. Results and discussions

3.1. Effectiveness of amylase on starch adhesive paste removal

Before any application of enzymes on historical textiles in order to remove old adhesives that are hard to be removed by conventional ways, it is necessary to perform an integrated experiment on modern cloth samples. The objective will be to examine the effect of the enzymatic treatment on dyed fabrics, and to test how to remove the enzyme residues from textiles.

The fabrics structure (e.g. mechanical parameter–crystallinity index–weight of fabrics–thread numbers) and color parameters of linen, silk, and cotton dyed fabrics that were used in the experimental part are presented in Table 1.

Fig. 1A shows the digital photo of linen samples that covered with starch after thermal ageing of 120 °C for 12 days. While Fig. 1B shows the SEM photo for the same sample. We can notice that starch adhesive paste is often present in shrunk, cracked, rigid and brittle form due to their aged condition. In this study, three different methodologies were applied to monitor the effectiveness of α-amylase on starch adhesive paste removal:

1. Calculation of the percentage of starch hydrolyzed into Maltose after α-amylase treatment according to the enzymatic assay of α-amylase enzyme.
2. Investigation of the morphology of the surface of the fabric samples, using SEM before and after enzymatic treatment.
3. Comparing the weight of the fabrics coated with starch paste before and after enzymatic treatment. The amount of weight losses were calculated according to the following formula: $\% W_L = (W_1 - W_2)/W_2$ [35] (Fig. 2).

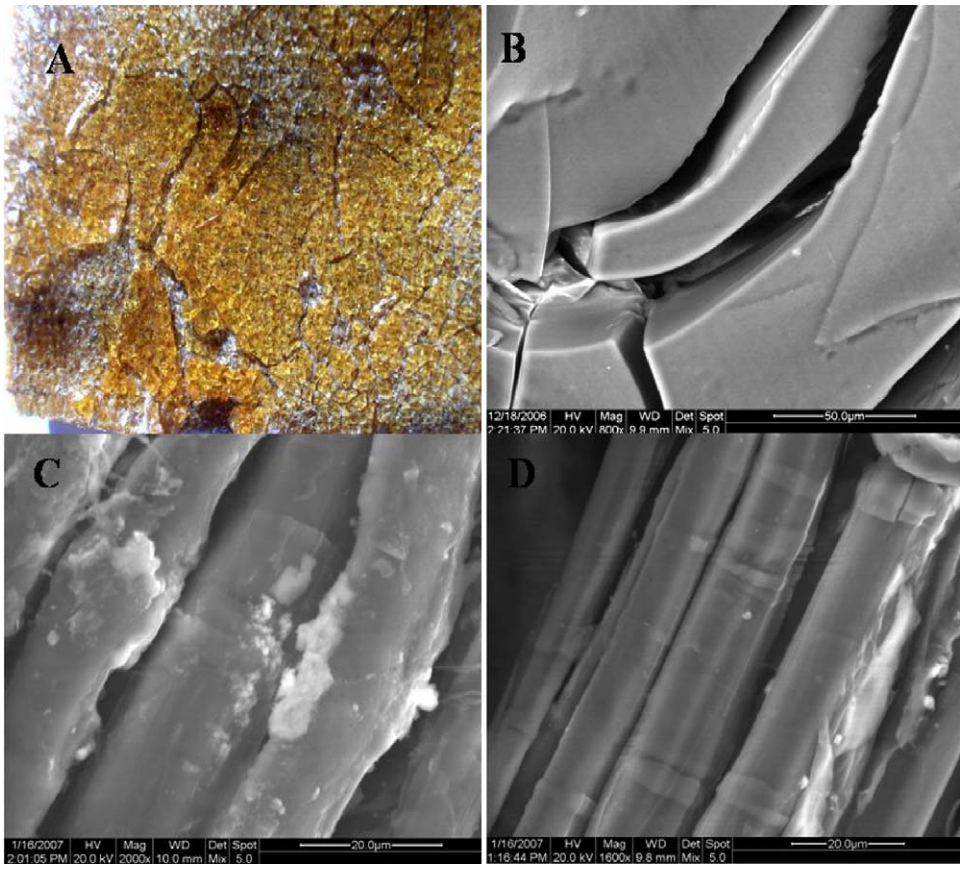

Fig. 1. A and B. Digital photo and SEM alternately for linen fabric that was coated by 40% starch concentration, after thermal ageing at 120 °C for 12 days. One can see that the starch is presents in shrunk, cracked, rigid and brittle from due to their aged condition. C and D. SEM of linen samples after enzyme application that was performed at concentrations 20 and 50 U/ml alternately for linen that coated by 40% starch, at 120 °C at 12 days. Treatment was done at 40 °C for 3.0 h with stirring.

3.1.1. Calculation of the percentage of hydrolyzed starch

Fig. 2A shows the maltose concentration curve after buffer or enzymatic application that was performed at the concentration of 5 U/ml. By comparing the samples treated with enzyme with that without any treatment, it is noticed that the latter is not effective in removing the starch. On the other hand, the enzyme has been very effective in removing the starch.

By studying the conditions affecting the performance of the enzyme in removing starch, it has been noticed that the efficiency of the enzyme in removing more amount of the hardened starch increases by increasing the enzyme concentration in the treatment solution. As you increase the concentration of the enzyme there is an increased in the number of the successful collisions, so the rate of reaction is directly proportional to enzyme concentration and the higher the concentration of the enzyme the faster the reaction. These results are presented in Fig. 2B and C.

Studying the effect of time also has been denoted; the longer the process of the treatment takes, the higher the efficiency of the enzyme to hydrolyze the hardened starch. It is also noticed that applying the enzyme in 40 °C resulted in better efficiency of the enzyme than applying it in 25 °C. Increasing the temperature can cause the enzymes to operate at a quicker pace and cause the enzymatic reaction to move along faster, while decrease in temperature can cause the enzyme to become sluggish and the reaction to slow down. On the other hand, if temperature is too high, the enzyme can fall apart, or denature due to the extreme heat. Most enzymes have an optimal temperature under which they can work efficiently and effectively. It has been also observed that the longer the time of ageing for the samples covered with starch, the harder the process of removing the starch becomes in the same conditions in which the enzyme works. Longer contact time between starch adhesive paste and fibers leads to a high degree of polymerization and covalent bonding of the starch with the fibers. In other words upon ageing, chemical changes take place in starch adhesive paste, sometimes resulting in chemical bond formation between the fiber substrates and the starch adhesive paste Fig. 2B and C.

3.1.2. The surface of fibers

The surface has been investigated using the SEM before and after applying the adhesive to the samples and after applying the enzyme. This is shown in Fig. 1A–D where the difference between the samples before and after the treatment can be noticed. On the other hand, we can see from SEM photos that the use of α-amylase resulted in extensive cleaning of the fiber surfaces, with high effectiveness for small capillaries and the center of the yarn bundle.

3.1.3. The samples weight

Weight has been compared before and after the treatment. Fig. 2 shows the decrease of starch weight (%) after enzymatic application. We can notice a direct correlation between the enzyme concentration and time of enzymatic treatment with the percentage of weight loss.

3.2. Removing enzyme from textile after treatment

Shibayama and Eastop mentioned that a literature search does not reveal any information about enzyme residues in textiles after rinsing [14]. This study includes some interesting observations

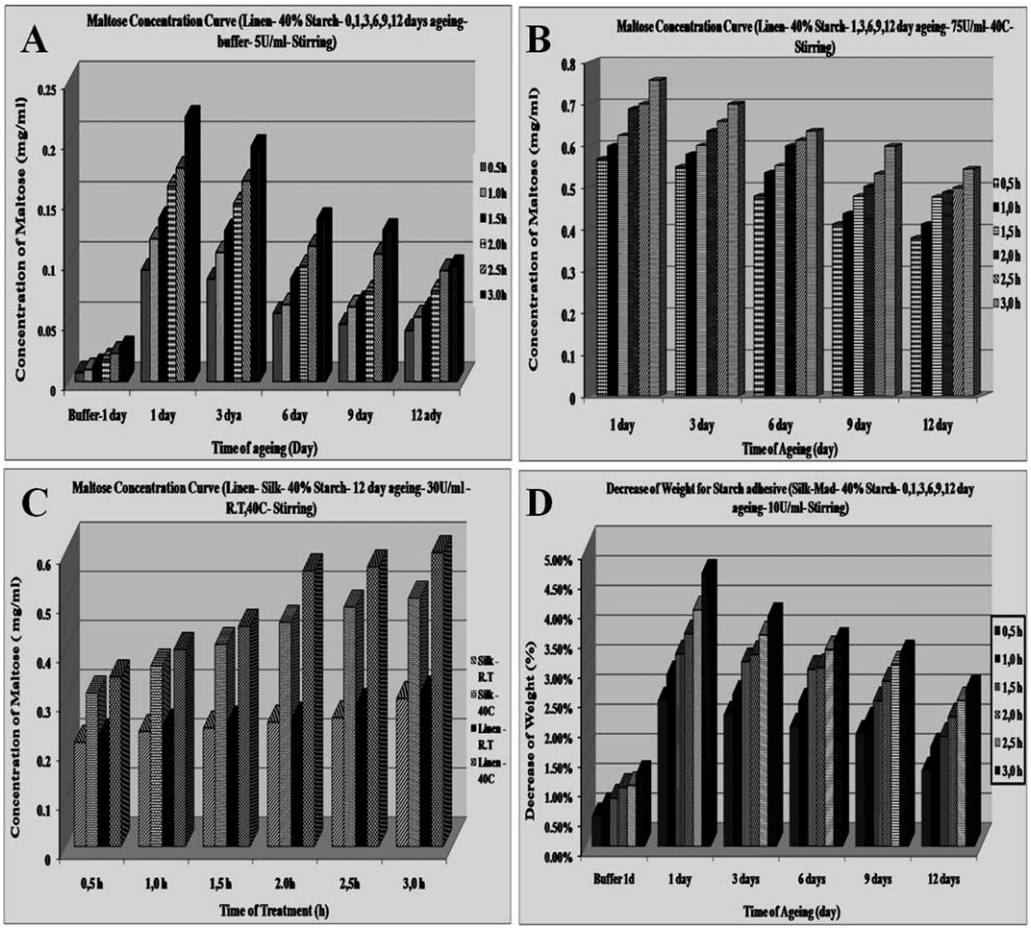

Fig. 2. A and B. Maltose concentration curve after enzyme application that was performed at concentration 5 and 75 U/ml alternately for linen fabric that coated by 40% starch, at 120 °C for one day to 12 days. Treatment was done at room temperature 25 °C and 40 °C for 0.5 h to 3.0 h with stirring. C. Maltose concentration curve after enzyme application that was performed at concentration 30 U/ml, for linen and silk fabric that coated by 40% starch, at 120 °C 12 days. Treatment was done at room temperature (25 °C) and (40 °C) for 0.5 h to 3.0 h with stirring. D. Decrease of starch weight (%) after buffer or enzyme application that was performed at concentration 10 U/ml, for silk fabric that coated by 40% starch, at 120 °C for one day to 12 days. Treatment was done at 40 °C for 0.5 h to 3.0 h with stirring.

about effectiveness of three rinses to remove enzyme residues from textile. Table 2 presents maltose concentration in the three rinsing solutions (distilled water bath or mixture of water and ethanol bath). Maltose concentration is considered as an indicator for the amount of α-amylase in rinsing 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., who found that two rinses are very effective to remove alpha amylase residues from paper [29].

3.3. *Effect of α-amylase treatment conditions on the crystallinity*

X-ray diffraction analysis (XRD) of untreated and treated samples is presented in two ways.

3.3.1. *The first way*

The first way presents the percentage of crystallinity index of untreated sample and those treated by different enzyme concentrations. There is a slight decrease of crystallinity index for linen. On the other hand, there is an increase of crystallinity index for cotton and silk after enzymatic treatment as presented in Table 3.

3.3.2. *The second way*

The wide angle X-ray (WAXS) diffractograms of untreated and treated linen, silk and cotton samples are presented in Fig. 3A–C that show a slight difference between the diffractograms of the treated and untreated fabrics due to the action of α-amylase. One can see that the treated linen showed a slight reduction in the peak intensity (counts) while the silk and cotton after enzyme treatment has a marked increase in the peak intensity (counts) in both the

Table 2
Maltose concentration observations.

Parameter	Maltose concentration (baths in distilled water)		Maltose concentration (baths of ethanol and distilled water (1:1))	
	Absorbance at 540 nm	Maltose concentration (mg/ml)	Absorbance at 540 nm	Maltose concentration (mg/ml)
Amylase 75 U	1.968	0.201		
First bath	0.926	0.096	0.774	0.080
Second bath	0.536	0.055	0.506	0.052
Third bath	0.184	0.019	0.147	0.015

Table 3
Crystallinity index of untreated and treated linen, cotton and silk after enzyme application with different concentrations 25, 50, 75 U/ml for different duration 1 and 3 h.

Samples	Crystalline area		Amorphous area		Crystallinity index (%)
	2θ (°)	Counts	2θ (°)	Counts	
Linen-control	22.879	683	19.292	97.1	85.78
Linen-amylase					
25 U–1 hr	22.998	454	19.671	87.9	80.63
25U–3 hr	22.874	451	19.272	80.7	82.10
50 U–1 hr	22.874	441	19.568	80.9	81.65
50 U–3 hr	22.936	538	19.382	92.9	82.73
75 U–1 hr	22.841	532	19.391	92.4	82.63
75 U–3 hr	22.998	449	19.692	81.4	81.87
Cotton-control	22.650	749	18.910	83.3	88.87
Cotton-amylase					
25 U–1 hr	22.801	760	18.667	86.6	88.60
25U–3 hr	22.782	752	18.077	82.4	89.04
50 U–1 hr	22.825	762	18.542	83.6	89.02
50 U–3 hr	22.744	754	18.504	84.2	88.84
75 U–1 hr	22.831	769	18.417	85.3	88.91
75 U–3 hr	22.812	778	18.605	86.4	88.89
Silk-control	20.440	192	12.960	52.4	72.70
Silk-amylase					
25 U–1 hr	20.58	201	12.491	56.2	72.03
25U–3 hr	20.713	216	13.056	61.0	71.48
50 U–1 hr	20.630	199	13.836	56,1	71.80
50 U–3 hr	20.481	203	12.890	56.6	72.36
75 U–1 hr	20.531	235	13.117	66.3	71.78
75 U–3 hr	20.605	233	13.365	63.3	72.83

U: enzyme concentration; hr: treatment time.

amorphous and crystalline regions. This finding suggests that the treatment by using amylase enzyme does not particularly affect the size and shape of crystallites of the linen and silk samples. Furthermore, the ratio of the crystalline and amorphous fractions barely changed, thus the enzymatic treatment did not result in considerable decrystallization in the linen, cotton and silk. The treatments slightly decrease the crystallite size of the longitudinal dimension for linen, while the treatments slightly increase the crystallite size of the longitudinal dimension for silk and cotton samples. Changes in crystallinity by amylase treatments may be indicated by the tensile properties of the samples. So there were no drastic changes on the size and shape of crystallites of samples (Table 3 and Fig. 3).

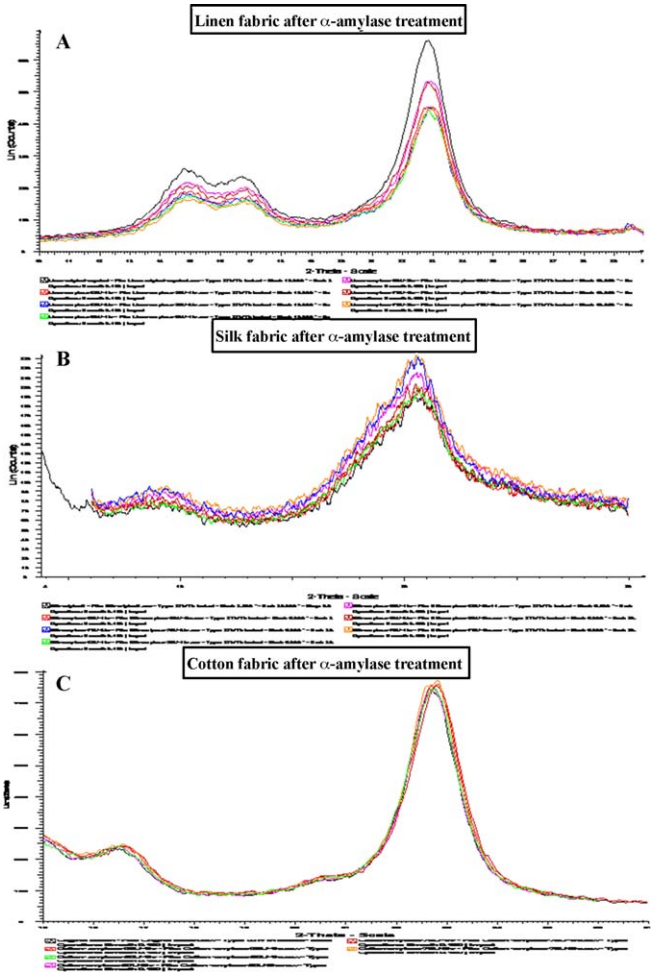

Fig. 3. A. Wide angle X-ray (WAXS) diffractograms alternately of linen after enzyme application that was performed at the concentration 25, 50 and 75 U/ml at 1 and 3 h. B. WAXS diffractograms alternately of linen after enzyme application that was performed at the concentration 25, 50 and 75 U/ml at 1 and 3 h. C. WAXS diffractograms alternately of linen after enzyme application that was performed at the concentration 25, 50 and 75 U/ml at 1 and 3 h.

3.4. FTIR spectra of fabrics treated with α-amylase

The spectra obtained for the control and the samples treated (uncolored linen, uncolored cotton, cotton dyed with madder and silk with madder) by α-amylase enzyme for different enzyme concentrations (10, 20, 30, 50 and 75 U/ml) at different duration (1 and 3 h) are reproduced in Table 4.

The related spectra showed that asymmetric and symmetric stretching modes of hydroxyl groups at 3336 cm^{-1} as well as C—O stretching bands at 1002, 1030, 1053, 1105, 1159 and 1205 cm^{-1} slightly increased as the concentration of the enzyme increased. These findings may result from opening some of the glucopyranose rings or/and splitting few of the hemiacetal bonds between the two glucopyranose rings C_1 and C_4 and formation of C_1—OH, C_4—OH and C_5—OH groups due to the swelling of cellulose as a result of its immersion in amylase solution. The effect of amylase enzyme solution sharply decreased in case of dyed cotton as the swelling effect decreased. Also, we suggest that the treatment of the cotton fiber and all the cellulosic fibers (cotton, linen) with α-amylase enzyme causes partial removal of concomitant substances (fats, pectins, and lignin) from cellulose. This finding is in agreement with Shamolina et al. who that found the treatment of linen and cotton after cellulase enzyme causes a partial removal of concomitant substances [37,38]. Furthermore, FTIR give no observed changes among the three tested samples indicating the good stability of silk to different concentration of amylase enzyme.

Generally, there are no drastic changes in the FTIR spectra among the treated and untreated samples. This is justified by the absence of new chemical groups and the fact that none of the existing groups was disappeared. Regarding to our aim, which is to conserve the fabrics, these results show that the enzymatic treatment caused no significant damage to the fibers (Fig. 4).

3.5. Effect of α-amylase on samples color

The results of the change in optical parameters of the uncolored cotton, uncolored linen and uncolored silk fabrics after α- amylase treatment with different concentrations for different times are demonstrated on Table 5. It is observed that there is a very slight increase in the brightness index ΔL^* of linen and cotton, while there is a slight decrease in the brightness of silk. Also there is a very slight increase in the redness of cotton and linen and a decrease in the redness of silk. The blueness very slightly increases after enzymatic treatment for both cotton and linen, while slightly decreases on silk. The angle (h) increases for treated cotton and silk, while decreases for treated linen. On the other hand color chromocity (ΔC) increases for linen, cotton and silk. All of these treated

Table 4
FTIR of linen and cotton dyed fabric after enzymatic treatment with different enzyme concentrations (25, 50, 75 U/ml) at different durations (1 and 3 h).

Wavenumber (cm^{-1})				Functional Groups
Linen raw	Cotton raw	Cotton dyed with madder dye	Silk dyed with madder dye	
3336	3334	3334	3279	Broad band due to bonded O—H stretching – in case of silk, this band overlapped N—H stretching
2901	2900	2900	2924.32	C—H stretching of saturated aliphatic groups
–	–	2162	2162	Overtones or combination bands
1642	1641	–	–	H_2O deformation band
–	–	1700	1705	C=O stretching of the dye
–	–	1643	1644	C=C stretching of the dye
–	–	–	1619	C=O stretching (amide I)
–	–	–	1514	C—N stretching + N—H bending (amide II)
–	–	–	1443	C—H bending (amide III) overlapped O—H bending
1427	1427	1427	–	O—H bending
1369	–	1360	–	C—H bending
1335	–	1335	–	
1315	1315	1315	–	
1279	–	1279	1230	C—O stretching of various hydroxyl groups
1204	1204	1204	1167	
1159	1160	1159	1068	
1105	1108	1108	–	
1053	1054	1053	–	
1030	1030	1029	–	
1002	1000	1000	998	
662	667	662	611	C—H out of plane

samples had color changes of ΔL, Δa, and Δb between 0.028 and 1.178 CIELab unit. So there were no drastic changes on the samples color.

Table 6 presents the color changes of colored cotton dyed with madder dye mordanted with $CuSO_4$ and ferric citrate, as well as without mordant after α- amylase treatment with different concentrations (25,50 and75 U/ml) for different times (1 and3 h). It is very important to investigate the changes of the colors because the historical textiles are of great importance and they need to keep their integrity after any treatment. We can notice that there is an increase in the brightness index ΔL^* for all the treated cotton dyed with madder dye. It is clear that there is a slight increase in the greenness with an increase in enzyme concentration for cotton dyed with madder dye mordanted with ferric citrate and without mordant, while there is an increase in redness for cotton dyed with madder mordanted with $CuSO_4$. Also one can see a slightly increase in blueness for all the cotton dyed with madder dye. The angle (h) is slightly decreased, as samples on the results of ΔH. All of these treated samples had color changes of ΔL, Δa, and Δb between 0.225 and 2.174 CIELab unit. So there were no drastic changes on the samples color.

Table 7 presents the color changes of colored cotton dyed with turmeric dye mordanted with $CuSO_4$, ferric citrate, as well as without mordant after amylase treatment with different enzyme concentrations for different times. There is an increase in the brightness index ΔL^* for all the treated cotton dyed with turmeric dye. On the other hand, there is a slight increase in the greenness for all the cotton dyed with turmeric dye. Also there is a slight increase in blueness for all the cotton dyed with turmeric dye. One can see a slight decrease in color

Table 5
Effect of α-amylase concentration on the brightness (L), red–green (a), yellow–blue (b) coordinates, the hue angle (h) and color chromocity (c) alternately of the uncolored cotton, uncolored linen and uncolored silk.

	ΔE	ΔL	Δa	Δb	ΔC	ΔH	Observation
Uncolored cotton							
25 U–1 hr	1.724	0.129	0.878	−1.178	1.683	0.348	Lighter redder bluer
25U–3 hr	0.610	0.028	0.347	−0.501	0.585	0.170	Redder bluer
50 U–1 hr	1.217	0.335	0.464	−1.074	1.165	0.102	Lighter redder bluer
50 U–3 hr	0.729	0.114	0.406	−0.595	0.695	0.194	Lighter redder bluer
75 U–1 hr	1.080	0.159	0.480	−0.954	1.057	0.153	Lighter redder bluer
75 U–3 hr	0.700	0.054	0.435	−0.545	0.656	0.137	Redder bluer
Uncolored linen							
25 U–1 hr	1.496	0.567	0.650	−0.936	1.073	−0.875	Lighter redder yellow
25U–3 hr	1.696	0.689	0.508	−0.840	0.583	−0.862	Lighter redder yellow
50 U–1 hr	1.878	0.488	0.712	−0.065	0.952	−0.793	Lighter redder yellow
50 U–3 hr	1.901	0.537	0.696	−0.753	1.211	−0.896	Lighter redder yellow
75 U–1 hr	1.789	0.544	0.740	−0.036	1.115	−0.899	Lighter redder yellow
75 U–3 hr	1.925	0.548	0.773	−0.571	1.408	−0.753	Lighter redder yellow
Uncolored silk							
25 U–1 hr	0.329	−0.119	−0.040	0.304	0.299	0.066	Darker yellow
25U–3 hr	0.596	−0.467	−0.001	0.371	0.370	0.032	Darker yellow
50 U–1 hr	1.513	−0.889	−0.005	1.224	1.219	0.106	Darker yellow
50 U–3 hr	1.305	−0.931	−0.116	0.906	0.913	0.031	Darker yellow
75 U–1 hr	1.226	−0.702	−0.048	1.006	1.007	0.041	Darker yellow
75 U–3 hr	1.474	−1.835	−0.410	1.965	1.998	0.200	Darker yellow

U: enzyme concentration; hr: treatment time.

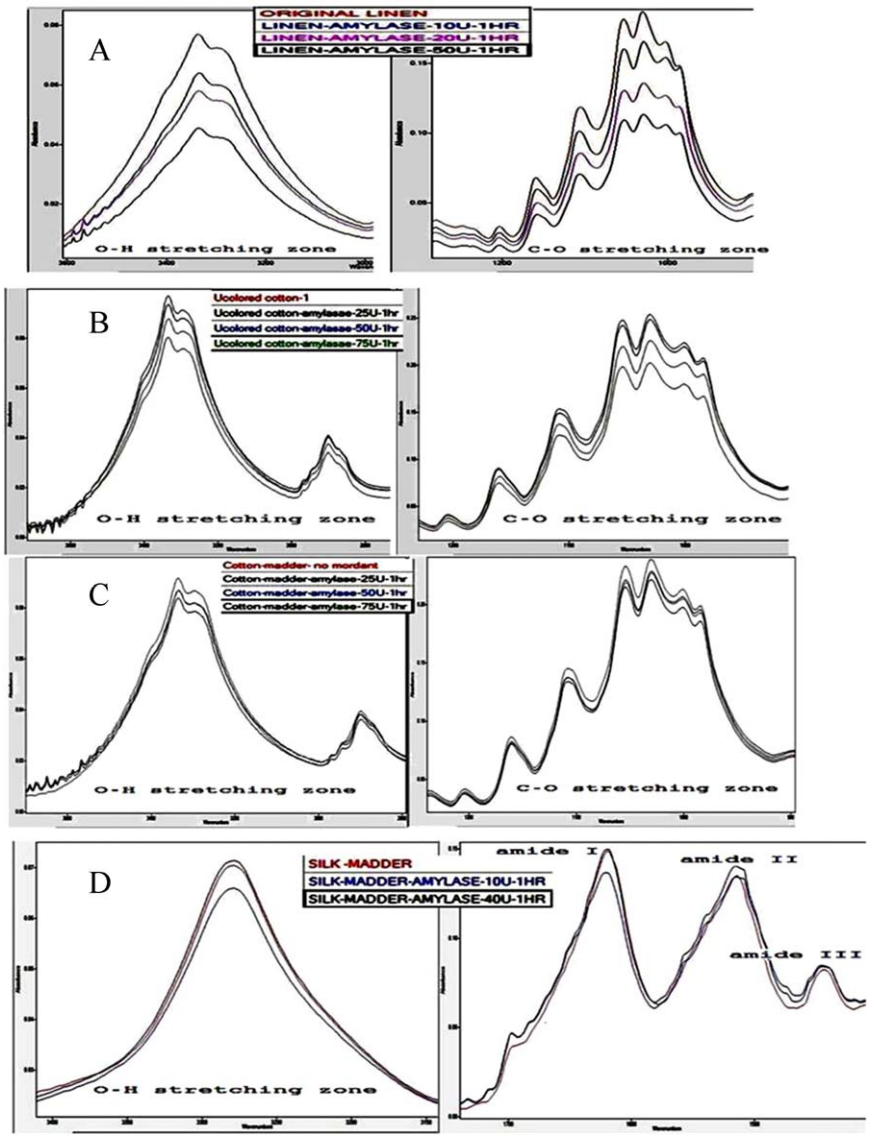

Fig. 4. A. FTIR of untreated and treated linen after α-amylase treatment with different concentrations at 1 h. B. FTIR of untreated and treated uncolored cotton after α-amylase treatment with different concentrations at 1 h. C. FTIR of untreated and treated cotton dyed with madder after α-amylase treatment with different concentrations at 1 h. D. FTIR of untreated and treated silk dyed with madder after α-amylase treatment with different concentrations at 1 h.

chormocity for all the cotton dyed with turmeric dye. All of these treated samples had color change less than of 2.5 CIELab unit.

3.6. Effect of α-amylase on mechanical parameters of the samples

Tensile strength and elongation of untreated and treated linen, cotton and silk fabric samples are presented in Table 8. The cotton, silk and linen samples that have been treated shows only slight improvement in elongation properties over untreated samples, with an increase in enzyme concentration and enzymatic treatment time. The results of initial characterization (before enzymatic treatment) show that the treatment caused an increase in tensile strength for cotton and silk samples, while it caused a slightly decrease in tensile strength of linen samples.

4. The application section

Following the interesting results obtained during the experimental part, the application part of the study has been undertaken by applying the enzyme on a sample of a historical carpet.

4.1. Object description

This sample is a part of a historical carpet that dates back to the Ottoman period, and was exhibited in the museum of the Faculty of Applied Arts, Helwan University, Egypt. The historical carpet is decorated by many geometric and plant decorations. We can notice from Fig. 5A that this carpet contains many colors and different decorations. It has much damage and most of the lost parts are in the center and near the edges. It can also be noticed that the piece of the carpet has some weaknesses in general. In addition, some of the parts of the carpet are not firmly connected, which may cause them to separate from the carpet easily.

4.2. Previous repairs

It is worth mentioning that the carpet is pasted and supported by backing cloth patches, which are adhered to it from behind

Table 6
Effect of α-amylase concentration on the brightness (L), red–green (a), yellow–blue (b) coordinates, the hue angle (h) and color chromacity (c) alternately of cotton dyed with madder without mordant and with ferric citrate, CuSO4.

	ΔE	ΔL	Δa	Δb	ΔC	ΔH	Observation
Cotton-madder – no mordant							
25 U–1 hr	3.012	2.575	−0.400	−1.510	−0.787	−1.350	Lighter less red less yellow
25U–3 hr	2.293	2.042	−0.835	−0.947	−1.072	−0.666	Lighter less red less yellow
50 U–1 hr	1.718	1.316	−0.821	−0.738	−1.002	−0.463	Lighter less red less yellow
50 U–3 hr	2.420	2.206	−0.580	−0.826	−0.794	−0.623	Lighter less red less yellow
75 U–1 hr	3.665	2.614	−1.772	−1.992	−2.230	−1.462	Lighter less red less yellow
75 U–3 hr	2.463	2.038	−1.332	−1.550	−2.174	−0.830	Lighter less red less yellow
Cotton-madder – $CuSO_4$							
25 U–1 hr	2.096	1.716	1.066	−0.493	1.169	−0.109	Lighter less red less yellow
25U–3 hr	1.348	1.244	0.225	−0.467	0.069	−0.513	Lighter less red less yellow
50 U–1 hr	1.498	1.439	0.229	−0.346	0.108	−0.401	Lighter less red less yellow
50 U–3 hr	1.862	1.776	0.440	−0.348	0.310	−0.468	Lighter less red less yellow
75 U–1 hr	1.370	1.169	0.301	−0.647	0.091	−0.708	Lighter less red less yellow
75 U–3 hr	2.530	2.513	0.252	−0.160	0.291	−0.069	Lighter less red less yellow
Cotton-madder – ferric citrate							
25 U–1 hr	2.845	2.386	−1.012	−1.012	−1.546	−0.114	Lighter less red less yellow
25U–3 hr	2.799	2.468	−1.108	−0.718	−1.287	0.296	Lighter less red less yellow
50 U–1 hr	3.240	2.744	−1.006	−1.358	−1.698	−0.288	Lighter less red less yellow
50 U–3 hr	2.586	1.879	−1.264	−1.248	−1.776	0.022	Lighter less red less yellow
75 U–1 hr	2.023	1.581	−0.918	−0.866	−1.261	0.045	Lighter less red less yellow
75 U–3 hr	2.737	2.057	−1.205	−1.345	−1.803	−0.097	Lighter less red less yellow

U: enzyme concentration; hr: treatment time.

by starch. The starch caused rigidity, cracking, brittleness, and shrinking to the carpet yarn and back due to their aged condition. Moreover, some of the carpet knots had been adhered together as one group by starch adhesive. On the other hand, there is yellowish discoloration of the carpet yarn (Fig. 5C). Spot tests were undertaken to identify this adhesive. Samples of the adhesive tested positive for starch in a starch indicator solution (0.1 g iodide and 1 g potassium iodine dissolved in 100 ml water) [13,14,17]. We confirmed this result by reinvestigating the adhesive by FTIR (Fig. 5B).

4.3. Total volume of the enzyme solution

According to our experimental procedure, the total volume of the enzyme solution (cm^3) was calculated according to the surface area of the object (cm^2). The total liquor was calculated as 150 ml of α-amylase solution with concentration of 25 U/ml.

4.4. Treatment of the piece of the historical carpet

The historical sample was subjected to many investigations using scanning electronic microscope, as well as the visual investigation. This is documented by photos before and after the treatment. A test was made on the piece of the carpet to see the dyes were fixed before applying the enzyme. This test was conducted by rubbing a piece of cotton wetted with the enzyme solution against every color in the historical piece of the carpet, and then observing the color of the piece of cotton after rubbing it. By doing this test, we proved that the dyes were fixed and ready to be treated by the enzyme.

Depending on the experimental section executed in the study, it had become clear to us that the optimum conditions in this case are enzymatic concentration at 25 U/ml at temperature $T = 40\,^{\circ}C$ and treatment time $t = 0.5$ h. The treatment of the historical piece has

Table 7
Effect of α-amylase concentration on the brightness (L), red–green (a), yellow–blue (b) coordinates, the hue angle (h) and color chromacity (c) of cotton dyed with turmeric without mordant, and with CuSO4 or ferric citrate.

	ΔE	ΔL	Δa	Δb	ΔC	ΔH	Observation
Cotton-safflower – no mordant							
25 U–1 hr	2.543	1.920	−1.180	−2.245	−2.060	0.686	Lighter less red less yellow
25U–3 hr	2.310	0.882	−1.132	−0.099	−0.133	2.130	Lighter less red less yellow
50 U–1 hr	2.691	2.913	−1.981	−1.152	−1.846	2.179	Lighter less red less yellow
50 U–3 hr	2.608	0.121	−1.145	−2.462	−1.569	0.583	Lighter less red less yellow
75 U–1 hr	2.615	2.050	−1.994	−2.831	−2.039	1.661	Lighter less red less yellow
75 U–3 hr	2.950	1.345	−1.497	−0.812	−1.072	2.397	Lighter less red less yellow
Cotton-safflower – $CuSO_4$							
25 U–1 hr	1.783	1.431	−0.282	1.025	−0.987	−0.395	Lighter less red less yellow
25U–3 hr	2.878	0.720	−0.846	−2.655	−2.523	−1.181	Lighter less red less yellow
50 U–1 hr	2.964	1.031	−0.327	2.759	−1.778	−0.005	Lighter redder yellow
50 U–3 hr	2.163	2.027	−0.078	−0.915	−0.899	−0.186	Lighter less yellow
75 U–1 hr	2.710	2.079	−0.016	−1.738	−1.736	−0.189	Lighter less yellow
75 U–3 hr	2.518	1.423	−0.279	−2.059	−2.077	−0.039	Lighter less red less yellow
Cotton-safflower – ferric citrate							
25 U–1 hr	2.752	2.654	−0.538	−0.487	−0.551	0.473	Lighter less red less yellow
25U–3 hr	1.850	1.798	−0.027	0.435	−0.428	0.089	Lighter yellow
50 U–1 hr	2.284	2.421	−0.387	−2.613	−2.420	0.892	Lighter redder less yellow
50 U–3 hr	2.505	2.413	−0.672	0.065	−0.019	0.675	Lighter less red
75 U–1 hr	2.394	2.362	−0.372	−0.099	−0.146	0.358	Lighter less red
75 U–3 hr	2.671	2.450	−0.521	−0.129	−0.188	0.713	Lighter less red less yellow

U: enzyme concentration; hr: treatment time.

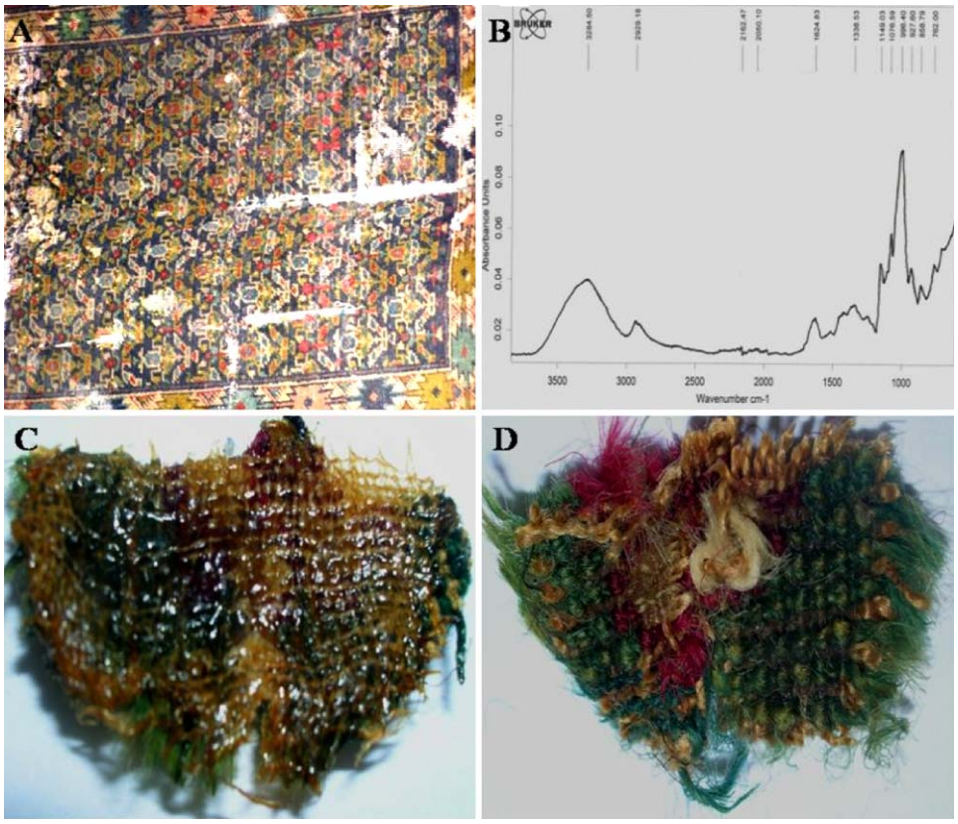

Fig. 5. A. The historical carpet, we can see different type of colors and decoration (geometric and plant decorations). It has much damage, lost parts and weaknesses in general. B. FTIR of starch that found in historical carpet. C. Carpet sample before enzyme treatment. D. Carpet sample after enzyme treatment.

Table 8
The mechanical parameters alternately of cotton, silk and linen samples after amylase treatment with different enzyme concentration 25, 50, 75 U/ml at 40 °C for different times 1.0 h, 3.0 h.

Warp direction		
Samples	TST (kg force)	Eb (mm)
Cotton-raw	61.920	26.448
Cotton-amylase		
25 U–1 hr	60.627	29.058
25U–3 hr	56.587	31.487
50 U–1 hr	63.010	29.802
50 U–3 hr	63.419	29.621
75 U–1 hr	64.727	29.232
75 U–3 hr	63.037	27.373
Silk-raw	27.967	15.852
Silk-amylase		
25 U–1 hr	27.881	15.901
25U–3 hr	28.005	16.301
50 U–1 hr	28.537	16.247
50 U–3 hr	28.423	16.769
75 U–1 hr	28.971	17.113
75 U–3 hr	29.318	17.023
Linen-raw	56.780	7.112
Linen-amylase		
25 U–1 hr	56.567	7.096
25U–3 hr	56.051	7.281
50 U–1 hr	56.234	7.392
50 U–3 hr	55.768	7.698
75 U–1 hr	56.113	7.440
75 U–3 hr	55.878	7.893

U: enzyme concentration; hr: treatment time; TST: tensile strength; Eb: elongation.

been done by fixing the temperature of a water bath at 40 °C, then the enzyme solution is put in a deep plate, and the plate is put in the water bath at 40 °C until the temperature of the enzyme solution reaches 40 °C. After that, the historical sample, which contains the adhesive starch, is put in the bath while making some stirring in the bath, to make sure that the enzyme solution has reached every part in the historical bath. While doing so, the temperature of the solution was observed and kept at the appropriate value. After half an hour, the piece is lifted out of the treatment solution. It can be noticed that the historical piece is separated from the backing cloth, and this indicates that the adhesive starch has loss.

It can be noticed that the piece after the treatment and in consequence after the removal of starch has regained some of its lost elasticity. In addition, it is seen that some of the threads on the edge of the piece, which was feebly attached to the historical piece, are separated from it after treatment. This means that weak sections should be fortified using the method of sewing to preserve all the sections of the piece (Fig. 5C and D).

4.5. Removal and deactivation of the enzyme

After treatment, the enzyme excess has to be removed from the textiles. According to the literature [39] and based on the previous results of the experimental part of this study, we proceeded to baths; rinsing was done with three water baths. The drying process was carried out by putting the object without cover to complete drying by air [40].

5. Conclusions

α-amylase is considered as an effective enzyme for the removal of starch adhesive paste even in relatively low temperatures (room

temperature). This is often very important because some historical textiles are very sensitive at temperatures higher than 25 °C.

For the removal of α-amylase residues from textiles after treatment, three water baths are required at room temperature (25 °C).

According to the results of this study, α-amylase causes:

a) an improvement of the mechanical parameters (tensile strength, elongation, and crystallinity index) for cotton and silk fabrics, on the other hand a slightly decrease in the same parameters for linen fabric;
b) a slight change in optical parameters such as (ΔE, Δa^*, Δb^*, ΔL^*, ΔC, ΔH) for cotton dyed with madder, or turmeric dye mordanted with CuSO4 and ferric citrate.

All these indications are significant because the properties of the historical textiles must not be altered. They need to keep their integrity after any treatment such as enzymatic treatment.

These results prove the ability of using α-amylase to remove the starch adhesive from archeological silk, linen and cotton fabrics having either madder or turmeric dye. This is because there are no drastic changes in the color shades or the hues as well as in the brightness values and the mechanical parameters.

Recommendations

There should be further studies on the effect of α-amylase on different dyes other than madder and turmeric dye mordanted with CuSO4 and ferric citrate.

This study was applied on a small scale (a piece of historical carpet). So a large-scale study is recommended (the whole historical carpet in faculty of art museum).

Acknowledgement

The authors would like to thank ETAKEI, GR for the use of their facilities in mechanical and optical parameters measurement. Harby E. Ahmed wishes to thank the State Scholarship's Foundation (Greece) for grant. Furthermore Prof Dr. Sawsan Darwish, Cairo University for her interpretation of FTIR.

References

[1] A. Balázsy, D. Eastop, Chemical Principles of Textile Conservation, UK, 1998.
[2] F. Lemberg, Textile Conservation and Research, Bern, 1988.
[3] S. Landi, The Textile Conservator's Manual, 2nd edition, London, 1992.
[4] I. Schwarz, A pre- package α-amylase poulticing system: Albertina–Kompresse, the book and paper group annual 9 (2000) http://aic.stanford.edu/sg/bpg/annual/v19/bp19-26.html.
[5] G. Banik, The Albertina compress. A ready-to-use enzyme compress for removing starch paste, Restauro 3 (1999).
[6] D. Sandrine, Enzyme used for adhesive removal in paper conservation: a literature review, J. Soc. Arch. 2 (2002) 187–195.
[7] D. Karlheinz, H. Waldman, Enzyme Catalysis in Organic Synthesis, A Comprehensive Handbook, Volume 1, WILEY-VCH, Germany, 2002.
[8] W. Christopher, On the use of enzymes for cleaning exterior stonework, Conservation news, United Kingdom Institute for Conservation of Historic and Artistic Works, 68 (1999).
[9] K. Wilson, Principles and Techniques of Practical Biochemistry, Fifth edition, Cambridge University press, UK, 2000.
[10] R. Gupta, K. Beg, P. Lorenz, Bacterial alkaline proteases: molecular approaches and industrial applications, Appl. Microbiol. Biotechnol. 59 (2002) 15–32.
[11] P. DeSantis, Some observation on the use of enzymes in paper conservation, J. Am. Inst. Conserv. 1 (1983) 7–27, http://aic.stanford.edu/jaic/articles/jaic23-01-002.html.
[12] J. Heuman, K. Garland, A poultice technique for the removal of cellulose nitrate adhesives from textiles, Conservator 11 (1987) 30–33.
[13] G. Bott, Amylase for starch removal from a set of 17th century embroidered panels, Conservator 14 (1990) 23–29.
[14] N. Shibayama, D. Eastop, Removal of flour paste residues from a painted banner with alpha-amylase, Conservator 20 (1996) 53–64.
[15] F. Whaap, The treatment of two coptic tapestry fragment, V&A Conservator J. 55 (2007) 11–13, www.vam.ac.uk/res_cons/conservation/journal/number_55/two_coptic/index.html.
[16] A. Lisa, A Fresh face for samuel gompers; methyl cellulose poultice cleaning, J. Am. Inst. Conserv. 28 (1989) 19–29.
[17] V. Chapman, Amylase in a viscous medium–textile applications, Conservator 10 (1986) 7–11.
[18] H. Ahmed, History of natural dyes in North Africa "Egypt", in: T. Bechtold, R. Mussakm (Eds.), Handbook of Natural Colorants, John Wiley & Sons Ltd, UK, 2009.
[19] F. Billmeyer, M. Saltzman, Principals of Color Technology, 3rd Ed., John Wiley & Sons, NY, 2000.
[20] R. Hunter, The Measurement of Appearance, John Wiley & Sons, NY, 1975.
[21] J. Rodgers, D. Thibobeaux, X. Cui, V. Martin, M. Watson, J. Knowlton, Instrumental and operational impacts on spectrophotometer color measurements, J. Cotton Sci. 12 (2008) 287–297, http://journal.cotton.org.
[22] H. Schweppe, Practical Hints on Dyeing with Natural Dyes, Washington DC USA, 1986.
[23] T. Bechtold, A. Turcanu, E. Ganglberger, S. Geissler, Natural dyes in modern textile dyehouses – how to combine experiences of two centuries to meet the demands of the future? J. Cleaner Production 11 (2003) 499–509.
[24] H. Ahmed, Y. Zidan, K. El-Nagar, Studies on dyeing with cochineal and ageing of silk dyed fabric, in: R. Janaway, P. Wyeth (Eds.), Scientific Analysis of Ancient and Historic Textiles: Informing Preservation, Display and Interpretation, AHRC Research Center for Textile Conservation and Textile Studies, First Annual Conference, UK, July 13–15, 2005, pp. 246–250.
[25] H. Schweppe, Practical Information for the Identification of Dyes on Historical Textile Materials, Washington DC USA, 1988.
[26] Z. Barampama, Effects of soaking, cooking and fermentation on composition, in-vitro starch digestibility and nutritive value of common beans, J. Plant Foods Hum. Nutr. 48 (1995) 349–365.
[27] F. Feller, Accelerated Aging Photochemical and Thermal Aspects, The Getty Conservation Institute, US, 1994.
[28] G. Miller, Use of dinitrosalisylic acid reagent for determination of reducing sugars, Analytical Chemistry 31 (1959) 426–428.
[29] T. Andrews, W. Andrews, C. Baker, An investigation into the removal of enzymes from paper following conservation treatment, The Book and Paper group annual 9 (1990), http://aic.stanford.edu/sg/bpg/annual/v09/bp09-01.html.
[30] J. Batcheller, Optical and scanning electron microscopy techniques for the identification of hair fibers from Romano- Egyptian textiles, in: R. Janaway, P. Wyeth (Eds.), Scientific Analysis of Ancient and Historic Textiles: Informing Preservation, Display and Interpretation, AHRC Research Center for Textile Conservation and Textile Studies, First Annual Conference, UK, July 13–15, 2005, pp. 51–57.
[31] P. Tortora, R. Merkel, Fairchild's Dictionary of textiles, 7 th Edition, Fairchild's Books & Visuals, 2007.
[32] L. Segal, J. Greely, A. Martin, M. Conrad, An Empirical method for estimating the degree of crystallinity of native cellulose using the X-ray diffractometer, Text. Res. J. 29 (1959) 786–794.
[33] M. Derrick, Fourier Transform infrared spectral analysis of natural resins used in furniture finishes, J. Am. Inst. Conserv. 28 (1989) 43–56.
[34] M. Baker, D. van der Reyden, N. Ravenel, FTIR Analysis of Coated Papers. The Books and paper Group annual 9 (1989), http://aic.stanford.edu/sg/bpg/annual/v08/bp08-01.html.
[35] N. Ozdil, E. Ozdogan, T. Oktem, Effect of enzymatic treatment on various spun yarn fabrics, FIBERS & TEXTILES in Eastern Europe 11 (2003) 57–62.
[37] J. Magoshi, M. Yoshiko, Physical properties and structure of silk. III. The glass transition and conformational changes of tussah silk fiberoin, J. Appl. Polym. Sci. 21 (1977) 2405–2407.
[38] I. Shamolina, A. Bochek, N. Zabivalona, E. Vlasova, B. Volchek, A. Sinitsin, Biochemical and physicochemical treatment of flax fibers, Russian J. Appl. Chem. 77 (2004) 1729–1732.
[39] A. Owen, Treatment and mounting of a Poster angleterre by A.M. Cassandre, J. Am. Inst. Conserv. 24 (1984) 23–32.
[40] S. Fletcher, J. Walsh, The treatment of three prints by whistler on fine Japanese, J. Am. Inst. Conserv. 18 (1979) 119–126, http://aic.stanford.edu/jaic/articles/jaic18-02-005.html.