# A Study on Using of Protease for Removal of Animal Glue Adhesive in Textile Conservation

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**ABSTRACT:** Animal glue has been used to fix historical textiles on paper, wood panels, or other rigid support materials. It is often present in shrunk, cracked, rigid, and brittle form because of the aged condition artifacts and may not provide enough adhesion for effective support causing damage to historical textiles. The biotechnological application of enzymes seems to be a very promising approach in the restoration of historical objects. In this experimental work, undertaken with modern linen and silk fabrics, interesting results have been obtained for the removal of animal glue by using the protease enzyme from *Aspergillus oryzae*. An extensive study was done in the enzymatic activity and efficiency for the removal of the animal glue from the textiles, as well as the effects of this treatment on mechanical and optical parameters of the textile fibers. The effect of protease on fibers is measured by Fourier transform infrared spectral analysis, scanning electron microscope, the CIE-Lab values, ASTM method D5035, and XRD. The results showed that using protease in adhesive removal presented good results with a safe and a short treatment time when compared with the conventional methods. No significant changes on the linen and silk fabrics are observed.
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**Key words:** conservation; animal glue; treatment; enzyme; hydrolyzes textiles; protease

## INTRODUCTION

Historically, animal glue has been widely applied as an adhesive for paper and textiles. It is often present in shrunk, cracked, rigid, and brittle form because of the aged condition and does not provide enough adhesion for effective support. The hard, solid animal glue may result in heavy damage to historic textiles as a result of embrittlement, hardening, yellowness, and acidity of historical textiles. Furthermore, animal glue is an attractive nutrient for a large number of fungi and bacteria that will decay textiles over time. Some natural adhesive can be removed by traditional methods as mechanical means. Sometime, water soluble adhesive can be removed by repeated washes. Generally, the reversal of some adhesive treatment can be achieved by swelling the adhesive, which can be achieved with solvents or by heat (warm air) to facilitate mechanical removal of the adhering support fabric. By these methods, the adhesive is not completely removed, with the result the textile becomes further impregnated with the polymers.[1–3]

The application of enzymes on textiles for removal of animal glue is an efficient conservation method and the least disruptive to the fibers. Extremely thick accretions, which traditionally would require inadvisably long and repeated washes for removal, are more efficiently removed by enzymes.[4–11]

Protease is a group of enzymes that catalyze the hydrolysis of peptide bonds in proteins into peptone, polypeptides, dipeptides, and finally amino acid. Protease is a nontoxic and environmental-friendly enzyme. Furthermore, protease is considered a commercial enzyme that is widely used in conservation.[7,12,13] After treatment, the excess enzyme has to be removed from the textile to avoid any further damage. The enzyme activity can be easily disrupted through any chemical, thermal, or physical methods that alter the tertiary configuration of the protein.[6,14,15]

The aims of this research were to study the use of protease in the field of historical textiles conservation to remove animal glue adhesive, to study the removal of enzyme residues from textiles following an enzymatic treatment by simple and safe methods, to study the effect of the enzymatic treatment on the chemical structure and the mechanical parameters of fabrics such as tensile strength, elongation, and

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crystallinity index, and finally to study the effect of the enzyme protease in color changes of linen and silk fabric.

## EXPERIMENTAL

### Materials

Protease from Aspergillus Oryzae P-4755 Type II (Sigma, Aldrich Munich, Germany). Egyptian linen fabrics supplied by Egylan Co. at the Second Industrial Zone (Alexandria, Egypt). Silk fabric supplied by TSIAKIRIS Co. (Soufli, Greece), www.tsiakris.gr. Animal glue supplied by S.P. Associates (Chennai, India), http://spassociates.tradeget. com.

### Application of adhesives

Preparing the glue adhesive: first, the animal glue and water are put together in a glass baker for 3 h. The concentration of glue must be 20%, and then we put the baker that contains the glue and water inside a water bath. After that, we raise the temperature of the water bath gradually to 50°C for 45 min only with continuing stirring till the solution becomes viscous. Then, we apply the glue as a liquid immediately after preparing it on the textiles by a suitable brush (twice). The glue starts to fill the gaps in between the yarns of textiles.[16,17]

### Thermal aging process

After applying the adhesive on the modern samples of textiles, we find that the glue solidifies. After that, industrial aging process is occurred for the samples of textiles that contain glue for different periods of time (1, 3, 6, 9, and 12 days) in modern controlled bakers. Some studies refer that the thermal aging process of 100°C for 3 days equals 25 years of aging in normal conditions. This is an experimental laboratory attempt for making the characteristics of modern samples like that of the old ones. The enzyme has been applied with various concentrations on the aging samples for studying the influence of aging and the age of samples on the efficiency of enzyme.[18] Textile samples that had animal glue adhesive applied were hanged in a temperature-controlled oven. The samples were thermally aged at a temperature of 100°C for different periods (1, 3, 6, 9, and 12 days).

### Enzymatic treatment

The fabric samples coated with animal glue adhesive were cut up into small pieces (2 cm × 2 cm) and put in test tubes. Then 5 mL of different concentration of protease in sodium acetate buffer, pH = 7.5, was added to each test tube. The samples were incubated at different 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 37°C. The enzymatic application was performed at different enzyme concentrations (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, and 35 U/mL) for each fabric sample with and without stirring.

To monitor the hydrolysis of animal glue adhesive paste, we measured spectrophotometrically the amino acid that is liberated after the enzymatic treatment. Specifically, 2 mL from every sample was withdrawn and put in a new test tube. Then 5 mL of 500-m*M* sodium carbonate solution ($Na_2CO_3$) was added. Finally, 1 mL of Folin and Ciocalteu's Phenol Reagent was added. The tubes were capped and mixed by swirling and then recorded at the 660-nm wavelength against standard curve of amino acid using a HITACHI U-1100-Spectrophotometer. Amino acid concentration was determined from the linear calibration curve $Y = 0.7004X - 0.0245$ (where $Y$ is the absorption, and $X$ is the concentration of amino acid). All samples were weighed before and after the treatment to calculate the percentage of the weight decrease. The weight difference is due to the partial hydrolysis of the animal glue caused by the protease action.

### Removal and deactivation of the enzyme

The efficiency of rinsing the enzyme protease out of fabric samples after the enzymatic treatment was measured according to an enzymatic assay.[19] We used an initial enzyme solution with concentration 20 U/mL, and the total volume was 100 mL. The fabric sample was added and left standing for 10 min. The fabric samples were then cut into small pieces (2 cm × 2 cm), divided into two groups, and subjected to: (1) washing the first group in four baths of distilled water (100 mL) for 10 min at room temperature (25°C) for each bath and (2) washing the second group in four bathes of a mixture of ethanol and distilled water 1 : 1 (V/V) (100 mL) for 10 min at room temperature (25°C) for each bath.

The amount of the enzyme residues removed from the samples was determined as follows: a sample of 50 μL from every bath was collected and put in new test tube; then 2 mL of 0.2% (w/v) Azo-Casein (as substrate to determine the enzyme activity) in 50 m*M* potassium phosphate buffer, pH = 7.5, was added. The assay was performed at 40°C for 10 min and terminated by addition of 2 mL of 0.1*M* trichloroacetic acid. After centrifugation at 13,000 rpm for 10 min, 2 mL of supernatant was added to 2 mL of 0.5*M* NaOH. The samples were left for 5 min at room temperature, and the absorbance was measured at 440 nm using a HITACHI U-1100 Spectrophotometer.

**TABLE I**
**Fabrics Structure, Crystallinity Index, and Color Coordinate of Linen and Silk Fabrics that Used in Experimental Part**

| Samples | Thread/cm | | Mechanical parameter | | Weight ($g/m^2$) | Plain weave |
|---|---|---|---|---|---|---|
| | Warp | Weft | T.St. | $E_b$ (mm) | | |
| Uncolored linen–control | 11 | 16 | 57.180 | 7.112 | 105.7 | Plain 1/1 |
| Uncolored silk–control | 32 | 25 | 27.967 | 15.852 | 25.4 | |

| | The CIE-Lab coordinators of the colors | | | |
|---|---|---|---|---|
| | $L^*$ | $a^*$ | $b^*$ | $C^*$ |
| Uncolored linen–control | 64.03 | 1.90 | 9.47 | 9.66 |
| Uncolored silk–control | 89.87 | 0.574 | 6.211 | 6.214 |

| | Crystalline area | | Amorphous area | | |
|---|---|---|---|---|---|
| | 2Θ | Counts | 2Θ | Counts | Crystallinity index (%) |
| Uncolored linen–control | 22.879° | 683 | 19.292° | 97.1 | 85.78 |
| Uncolored silk–control | 20.490° | 192 | 12.962° | 52.4 | 72.71 |

T.St. = Tensile strength; $E_b$ = Elongation.

### Testing and analysis

#### Morphological study

The surface morphology of the untreated samples was compared with the enzymatically treated fabrics using a scanning electron microscope (SEM)—Quanta 200 ESEM FEG from FEI.[20]

#### Color measurement

The CIE-Lab values of the color changes were measured using double beam Optimatch spectrophotometer (Datacolor international Spectraflash SF450-UK).[21,22]

#### Mechanical properties

Mechanical parameters, such as tensile strength and elongation, were measured according to the ASTM D5035 method in the warp and weft directions. Silk fabrics were cut into 30-cm strip length and 5-cm widths. Five samples per treatment set were tested, and the breaking load averaged for each sample.[23]

#### X-ray diffraction analysis

X-ray diffraction (XRD) 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 $2\theta = 5°$ to 30° continuously at a scan rate of 2°/min. Crystalline index (crystalline to amorphous ratio) can be calculated according to Segal et al.[24]

#### Fourier transform infrared spectral analysis

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 spectrometer using the KBr pellet technique.[25,26]

## RESULTS AND DISCUSSIONS

### Effectiveness of protease on the removal of animal glue adhesive paste

Table I shows the fabrics structure, crystallinity index, and color coordinate of linen and silk fabrics that were used in the study. Figures 1(A) and 3(A) show linen samples covered with animal glue adhesive paste after thermal aging of 100°C for 12 days. Animal glue adhesive fills the smallest capillaries between fibers. Three different methodologies were applied three ways to monitor the effectiveness of protease on animal glue adhesive paste removal:

1. calculation of the percentage of animal glue hydrolyzed into amino acid (tyrosine) after protease treatment according to the enzymatic assay of protease enzyme,
2. investigation of the morphology of the surface of the fabric samples using SEM before and after enzymatic treatment, and
3. comparing the weight of the fabrics coated with animal glue adhesive before and after enzymatic treatment. The weight losses were calculated according to the following formula: $\% W_L = (W_1 - W_2)/W_1$,

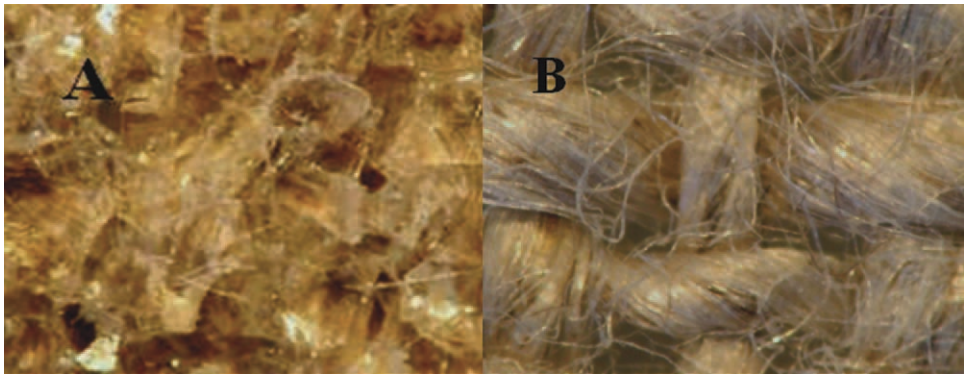


**Figure 1** A) Stereoscopy photo of linen fabric that coated with animal glue after 12 days aging before any enzymatic treatment. B) Stereoscopy photo of linen fabric that coated with animal glue after 12 days aging after enzymatic treatment with enzyme concentration 20 U/mL at 2 h. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

where $W_1$ is the weight of fabric that covered with animal glue adhesive before enzymatic treatment, and $W_2$ is the weight of fabric that covered with animal glue adhesive after enzymatic treatment.[27]

The percentage of hydrolyzed animal glue adhesive was calculated. By comparing the samples treated with enzyme with that treated only by buffer, it is apparent that the buffer is not effective in removing the animal glue adhesive. On the other hand, the enzyme has been very effective in removing the animal glue adhesive. Figure 2(A) shows the effect of varying the conditions on enzyme activity in removing animal glue adhesive. The efficiency of the enzyme, in removing greater amounts of the hardened animal glue adhesive, increases by increasing the enzyme concentration in the treatment solution. The rate of reaction is directly proportional to the enzyme concentration: the higher the concentration of the enzyme the faster the reaction. When the role of time was investigated, it was noticed that the longer the process of the treatment takes, the more efficiently the enzyme hydrolyzes the hardened animal glue adhesive.

The amino acid tyrosine must not exceed 2 μmole, because the device that reads the concentration is not equipped for reading values more than this rate. The amount of glue analyzed under the influence of the enzyme activity has been studied and through studying the surface of fibers by electronic microscope after every enzyme treatment, we find that the efficiency of enzyme in disposing of the adhesive becomes better when the concentration of enzyme is concentration of 20 units and in 37°C at least for linen and silk under the conditions provided in this study.

The ideal degree of temperature, which is recommended by enzymologist, is 37°C of the protease enzyme as each enzyme has an optimum temperature at which the enzyme works more efficiently. Thus, we have to use this degree of temperature to obtain this efficiency and ability to dispose of old adhesives, and at the same time, studying the efficiency and activeness of the enzyme in the

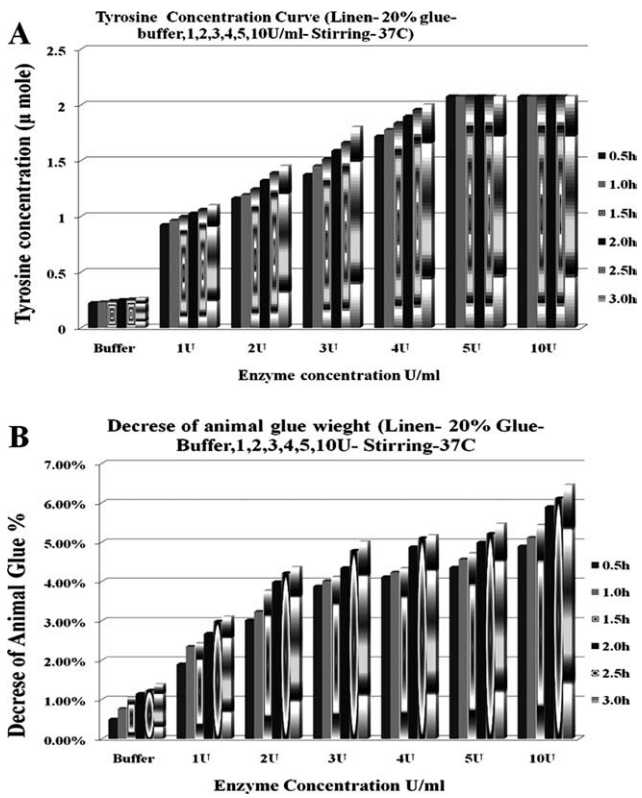


**Figure 2** A) Tyrosine concentration curve after buffer or enzymatic treatment that was performed at concentration 1, 2, 3, 4, 5, and 10 U/mL for linen fabric that coated by 20% animal glue adhesive. Treatment was done at 37°C from 0.5 to 3.0 h with stirring. B) Decrease of animal glue adhesive (%) after buffer or enzymatic treatment that was performed at concentration 1, 2, 3, 4, 5, and 10 U/mL for linen fabric that coated by 20% animal glue adhesive. Treatment was done at 37°C from 0.5 to 3.0 h with stirring.

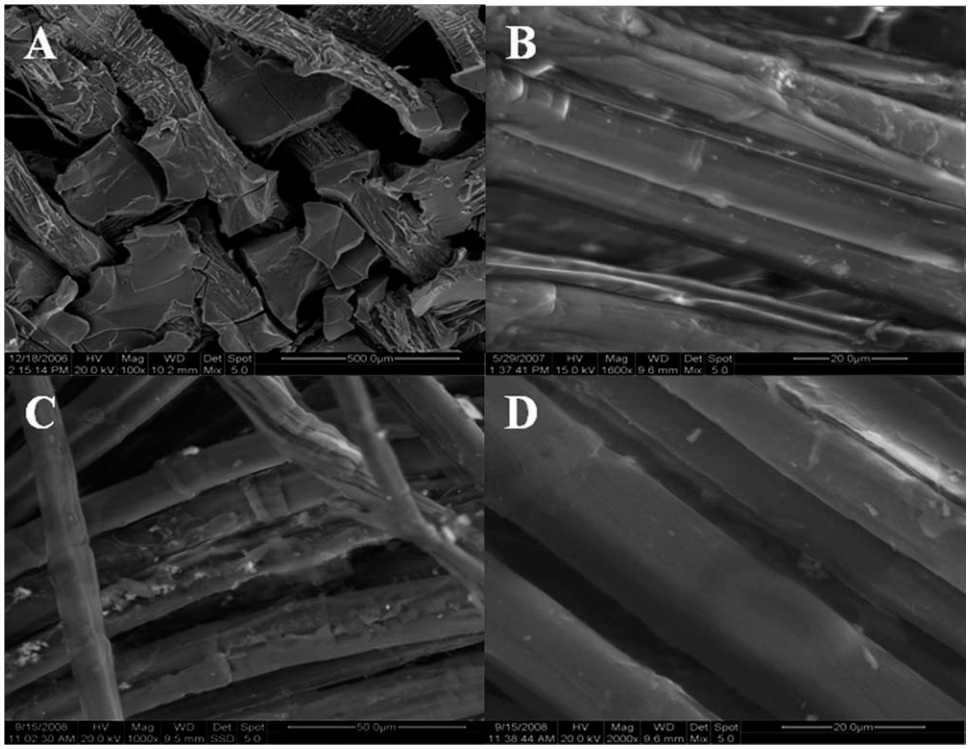


**Figure 3** A) SEM photo of linen that coated with animal glue after 12 days aging before any enzymatic treatment. B) SEM photo of linen that coated with animal glue after 12 days aging after enzymatic treatment 5 U/mL at 37°C for 1 h. C) SEM photo of linen that coated with animal glue after 12 days aging after enzymatic treatment 10 U/mL at 37°C for 1 h. D) SEM photos of linen that coated with animal glue after 12 days aging after enzymatic treatment 20 U/mL at 37°C for 1.5 h.

temperature of the room (25°C) and whether the application in this degree will affect on the efficiency of the enzyme or not. As in some cases, we cannot use high temperatures with the antiquities as it may affect passively on the safety of colors and fibers. So, we have to try to apply the enzyme in the optimum temperature recommended by enzymologists and study how to apply it in the room temperature if we need to do so. The increase in temperature helps the enzymatic reaction to proceed faster, while a decrease in temperature can cause the reaction to slow down.

The enzyme has been applied in different times such as 0.1, 0.1, 1.5, and 2 h to clarify the impact of the time of treatment on the efficiency of enzyme. From Figure 2, it becomes clear that the more the time of the application of the enzyme increases, the more the amount of decomposed adhesives increases under the impact of enzyme. Also, studying the influence of the time of treatment on fibers to give the researchers the chance to use the time of treatment that suits its condition and state. For example, some antiquities that contain weak layers of adhesives require short time for its treatment, and the amount of the required enzyme concentration is few. On the other hand, the calcified and thick layers require high concentration and more time for treatment. Through this study, it becomes clear that the application of the enzyme becomes effective when it is applied in concentration of 20 units and in 37°C, at least for linen and silk under the conditions provided in this study.

Examining the decrease in weight of the samples after applying an enzymatic treatment gives an indirect reference to the efficiency of the enzyme and its ability of removing animal glue adhesive as shown in Figure 2(B). The best conditions for this enzyme are at a concentration of 20 U/mL at 37°C for 1 h.

### Study of fiber morphology after enzymatic treatment

The surface of fibers was investigated using the scanning electronic microscope (SEM) and stereoscopy before and after applying the adhesive to the samples and after applying the enzyme. This is shown in Figures 1 and 3 where the differences between the samples before and after the treatment are seen. The SEM photos show that the use of the protease enzyme resulted in extensive removing of the animal glue paste from the fiber surfaces and interfiber capillaries, with high effectiveness observed for small capillaries and the center of the

**TABLE II**
**Tyrosine Concentration Observation**

| Parameter | Tyrosine concentration (baths in distilled water) for linen fabric | | Tyrosine concentration (baths of ethanol and distilled water (1 : 1) for linen fabric | |
|---|---|---|---|---|
| | Absorbance at 440 nm | Tyrosine concentration (μmole) | Absorbance at 440 nm | Tyrosine concentration (μmole) |
| Protease 20 U/mL | 0.711 | 0.472 | | |
| First bath | 0.317 | 0.196 | 0.243 | 0.144 |
| Second bath | 0.135 | 0.069 | 0.121 | 0.059 |
| Third bath | 0.067 | 0.021 | 0.041 | 0.003 |

yarn bundle. The direct relationship between the enzyme concentration and the degree of the removal of the animal glue adhesive from surface and inter-fiber capillaries is seen. The enzyme does not affect the morphological shape of the fibers. The enzyme does not cause any change in the form of the fiber, i.e., there is no swelling or inflation on any part of the fibers. This is evidence that the fiber is safe and in the protease enzyme solutions.

### Removing enzyme from the textile after treatment

This study includes some interesting observations about the effectiveness of three rinses to remove enzyme residues from the samples. Table II presents tyrosine concentration in the three rinsing solutions (distilled water bath or mixture of water and ethanol bath). Tyrosine concentration is considered as an indicator for the amount of the protease enzyme in the rinsing solution. It was found that only three rinses (either distilled water bath or a mixture of water and ethanol bath) are very effective to remove any enzyme residues from the linen or silk fabric samples.

### Effect of protease treatment conditions on the crystallinity

X-ray diffraction studies lead to understanding the crystalline structure and the degree of crystallinity. Any treatment with the potential to change fiber morphology may sometimes lead to crystallization or decrystallization. It was thought worthwhile to investigate changes because of the enzyme treatment on the different fibers.[28]

XRD results of untreated and treated samples are presented in two ways. The first way presents the percentage of the crystallinity index of untreated sample and those treated by different enzyme concentrations. As shown in Table III there is a slight decrease of crystallinity index for linen after enzymatic treatment.

**TABLE III**
**Crystalline Index of Enzymatically Treated Linen with Different Enzyme Concentrations (1, 5, 10, and 15 U/mL) at 1 and 3 h**

| Crystalline index of treated linen–enzyme concentrations (1, 5, 10, and 15 U/mL) at 1 h | | | | | |
|---|---|---|---|---|---|
| Samples | Crystalline area | | Amorphous area | | Crystallinity index (%) |
| | 2θ | Counts | 2θ | Counts | |
| Linen–original | 22.879° | 683 | 19.292° | 97.1 | 85.78 |
| Linen–protease: 1 U-1 h | 22.844° | 710 | 19.122° | 105 | 85.21 |
| Linen–protease: 5 U-1 h | 22.865° | 563 | 18.713° | 91.1 | 83.81 |
| Linen–protease: 10 U-1 h | 22.822° | 551 | 18.855° | 89.7 | 83.72 |
| Linen–protease: 15 U-1 h | 22.865° | 515 | 18.918° | 80.5 | 84.36 |
| Crystalline index of treated linen–enzyme concentrations (1, 5, 10, and 15 U/mL) at 3 h | | | | | |
| Samples | Crystalline area | | Amorphous area | | Crystallinity index (%) |
| | 2θ | Counts | 2θ | Counts | |
| Linen–original | 22.879° | 683 | 19.292° | 97.1 | 85.78 |
| Linen–protease: 1 U-3 h | 22.865° | 636 | 19.061° | 98.9 | 84.44 |
| Linen–protease: 5 U-3 h | 22.840° | 547 | 19.200° | 88.8 | 83.76 |
| Linen–protease: 10 U-3 h | 22.847° | 553 | 18.799° | 90.7 | 83.60 |
| Linen–protease: 15 U-3 h | 22.885° | 570 | 18.856° | 94.1 | 83.49 |

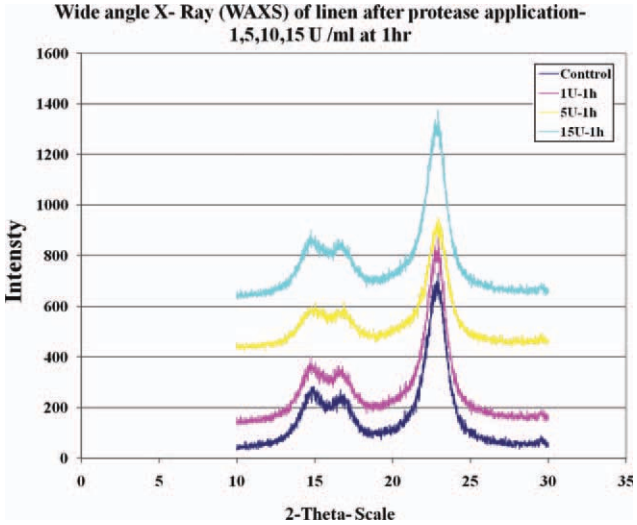


**Figure 4** Wide-angle X-ray (WAXS) diffractograms of linen after enzyme application that was performed at the concentration 1, 5, 10, and 15 U/mL at 1 h. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The second way is the wide-angle X-ray (WAXS) diffractograms of untreated and treated linen samples. Figures 4 and 5 illustrates WAXS diffractograms of linen after enzyme application at concentrations of 1, 5, 10, and 15 U/mL at 1 and 3 h showing the effect of the protease treatment conditions on the linen crystallinity. There is a slight difference between the diffractograms of the treated and untreated linen because of the action of protease enzyme. There were no drastic changes on the size and shape of the crystalline area of the samples.

### Effect of protease treatment on chemical structure

To confirm the results obtained by X-ray diffraction studies, we conducted Fourier transform infrared spectral analysis (FTIR) studies. The spectra obtained for the control and the samples treated by protease enzyme for different enzyme concentrations at different duration are reproduced in Figure 6. There is slight absorbance at 3336 $cm^{-1}$ (peak due to OH groups), 1642 $cm^{-1}$ (peak due to —R—HC=O groups), 1427 $cm^{-1}$ (peak due to —$CH_2$ and —$CH_3$ groups), 1204 $cm^{-1}$ (peak due to —C—O—C groups), 1105 $cm^{-1}$ (peak due to —C—OH groups), 1053 $cm^{-1}$ (peak due to —C—O—C groups), 1030 $cm^{-1}$, and 1002 $cm^{-1}$(peak due to —C—O—C groups). The 896 $cm^{-1}$ and 667 $cm^{-1}$ bands show that intensity increases in the spectra of the sample treated in comparison with the spectra of the untreated sample. 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 disappeared. Our aim, which is to conserve the fabrics, is met as these results show that the enzymatic treatment caused no significant damage to the fibers.

### Effect of protease on the color of linen samples

Colors differences of treated linen fabric after the enzyme protease treatment with different concentrations for different times are presented in Table IV. The linen samples had a total color change ($\Delta E$) about 1 CIE-Lab unit. The brightness value ($\Delta L$) increased about 1 CIE-Lab unit, showing the samples became slightly lighter. The redness value ($\Delta a$) had a color change between −0.010 and −0. 347, while the blueness ($\Delta b$) had color change between −0.064 and −0. 917. These changes cannot be detected by the human eye.

### Effect of the enzyme protease on mechanical parameters of the samples

Changes in the mechanical properties could be attributed to changes in crystalline orientation. The linen samples that have been treated with protease enzyme with different enzyme concentrations for different durations (1, 3, 5, 10, and 15 U/mL at 1, 3 h) show improvement in elongation properties over untreated samples, with an increase in enzyme concentration and enzymatic treatment time. The results of the initial characterization (before enzymatic treatment) show that the treatment caused a noticed increase in tensile strength of the linen samples. The increasing statistically is not massive, but the result is very positive as the improvement of the

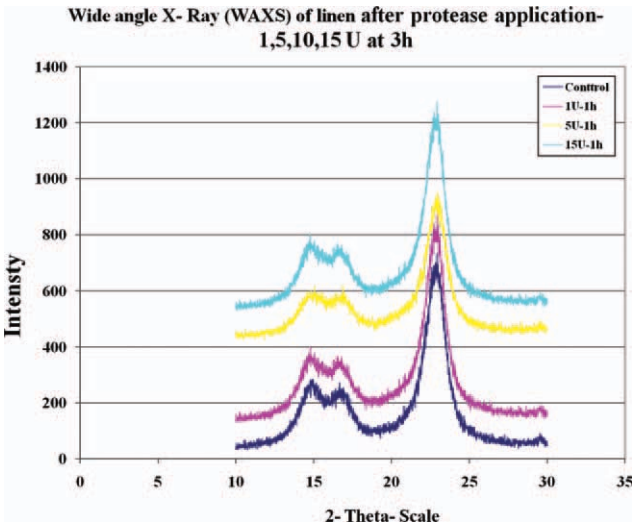


**Figure 5** Wide-angle X-ray (WAXS) diffractograms of linen after enzyme application that was performed at the concentration 1, 5, 10, and 15 U/mL at 3 h. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

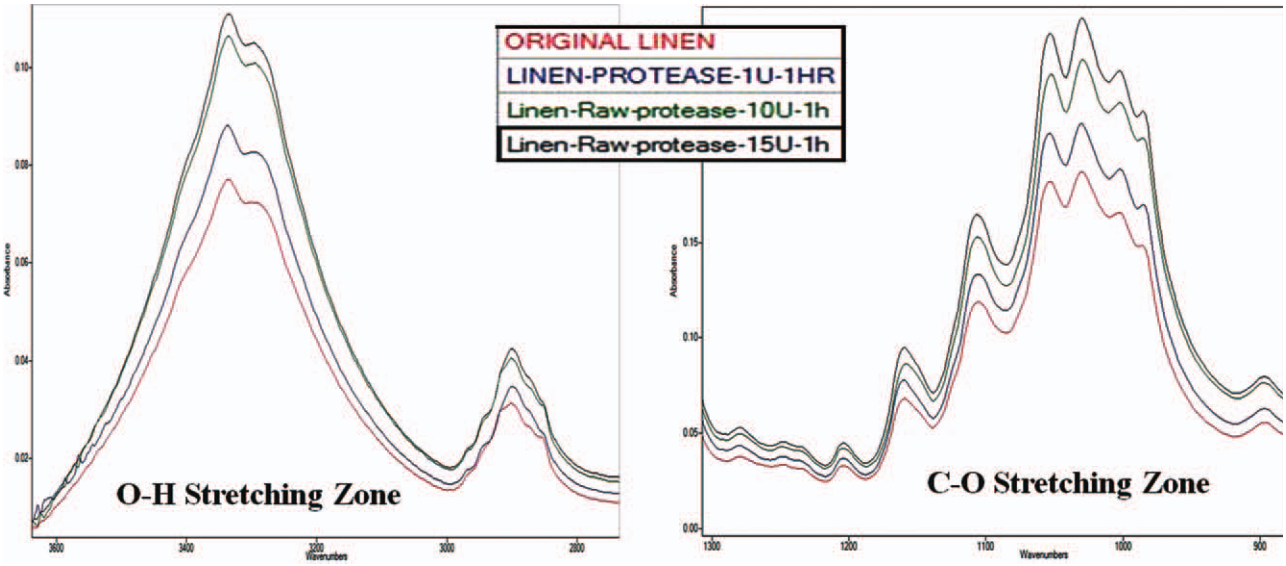


**Figure 6** FTIR of linen samples after protease treatment with different concentrations 1, 3, 10, and 15 U/mL at 1 h. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

**TABLE IV**
**Effect of Protease Concentrations on the Brightness (*L*), Red–Green (*a*), Yellow–Blue (*b*) Coordinates, the Hue Angle (*h*), and Color Chromaticity (*c*) of the Uncolored Linen**

| Uncolored Linen | $\Delta E$ | $\Delta L$ | $\Delta a$ | $\Delta b$ | $\Delta C$ | $\Delta H$ | Observation |
|---|---|---|---|---|---|---|---|
| Protease 1 U-1 h | 0.248 | 0.217 | −0.010 | −0.121 | −0.120 | 0.014 | Lighter less red less yellow |
| Protease 1 U-3 h | 0.588 | 0.445 | −0.071 | −0.378 | −0.355 | 0.146 | Lighter less red less yellow |
| Protease 3 U-1 h | 0.715 | 0.471 | −0.058 | −0.536 | −0.515 | 0.158 | Lighter less red less yellow |
| Protease 3 U-3 h | 1.093 | 1.029 | −0.347 | −0.126 | −0.196 | 0.312 | Lighter less red less yellow |
| Protease 5 U-1 h | 0.784 | 0.653 | −0.199 | −0.385 | −0.334 | 0.276 | Lighter redder less yellow |
| Protease 5 U-3 h | 1.317 | 0.915 | −0.233 | −0.917 | −0.945 | 0.046 | Lighter less red less yellow |
| Protease 10 U-1 h | 0.433 | 0.298 | −0.169 | −0.265 | −0.292 | 0.116 | Lighter less red less yellow |
| Protease 10 U-3 h | 1.183 | 1.079 | −0.215 | −0.433 | −0.465 | 0.130 | Lighter less red less yellow |
| Protease 15 U-1 h | 0.835 | 0.793 | −0.167 | −0.201 | −0.229 | 0.126 | Lighter less red less yellow |
| Protease 15 U-3 h | 0.208 | 0.067 | −0.188 | −0.064 | −0.098 | 0.171 | Lighter less red less yellow |

mechanical properties of fibers is better than the decrease of the mechanical properties (see Table V).

### Protease used to remove animal glue from silk fabrics

The previous methodology was followed to determine the extent of the efficiency to enzyme protease in removing the animal glue from the surface of silk textiles and also to study the effect of the enzyme on silk fibers. The examination was made by using the SEM. Figure 7(A) shows the surface of the silk textiles by the stereo microscope. By observing the samples before and after applying the enzyme, we note the extent of the efficiency of the enzyme in removing the animal glue. In Figure 7(B) the animal glue has been removed completely from the surface of the textile and from the spaces in between the wrap and weft yarn.

This result is also confirmed by use of the electron microscope survey for examining the surface of the silk textiles on which animal glue is applied before and after enzymatic treatment Figure 8(A–D). We can see the high efficiency of the enzyme in removing the animal glue from the surface of the textile

**TABLE V**
**Effect of Protease Enzyme Treatment on Mechanical Parameters Such as Tensile Strength and Elongation of Linen Samples after Different Concentration for Different Durations**

| Warp direction | | |
|---|---|---|
| Samples | T. St. (kg Force) | $E_b$ (mm) |
| Linen–control | 57.180 | 7.112 |
| Linen–protease: 1 U-1 h | 57.280 | 10.101 |
| Linen–protease: 1 U-3 h | 57.720 | 10.377 |
| Linen–protease: 3 U-1 h | 56.524 | 10.782 |
| Linen–protease: 3 U-3 h | 58.715 | 10.850 |
| Linen–protease: 5 U-1 h | 57.730 | 10.370 |
| Linen–protease: 5 U-3 h | 58.170 | 10.362 |
| Linen–protease: 10 U-1 h | 58.663 | 10.683 |
| Linen–protease: 10 U-3 h | 58.797 | 11.805 |
| Linen–protease: 15 U-1 h | 58.773 | 11.133 |
| Linen–protease: 15 U-3 h | 59.367 | 11.388 |

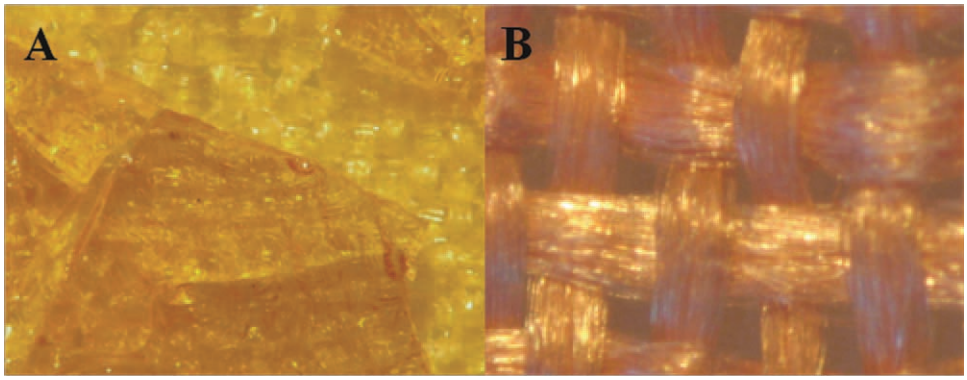


**Figure 7** A) Stereoscopy photo of silk fabric that coated with animal glue after 12 days aging before any enzymatic treatment. B) Stereoscopy photo of silk fabric that coated with animal glue after 12 days aging after enzymatic treatment with enzyme concentration 20 U/mL at 37°C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

and the spaces between the yarns as well. The best concentration for removing the animal glue from silk textiles is 20 U/mL for 1 h. The smoothness of the silk fibers helps in increasing the efficiency of removing animal glue and in removing the enzyme after the treatment. This method gives the same result as washing the fabrics three times, which was sufficient to remove the enzyme from the textiles. The animal glue has been removed completely from the surface of the yarn and from the spaces in between the warp and weft yarns as well.

### Effect of lipase treatment conditions on fiber crystallinity

XRD results of untreated and treated samples are presented in two ways. The first way is to calculate the crystalline index of untreated silk samples and those treated by different enzyme concentrations. There is a slight increase of the crystallinity index, and the results are presented in Table VI.

The second way is the WAXS of untreated and treated silk samples. The treated silk showed an

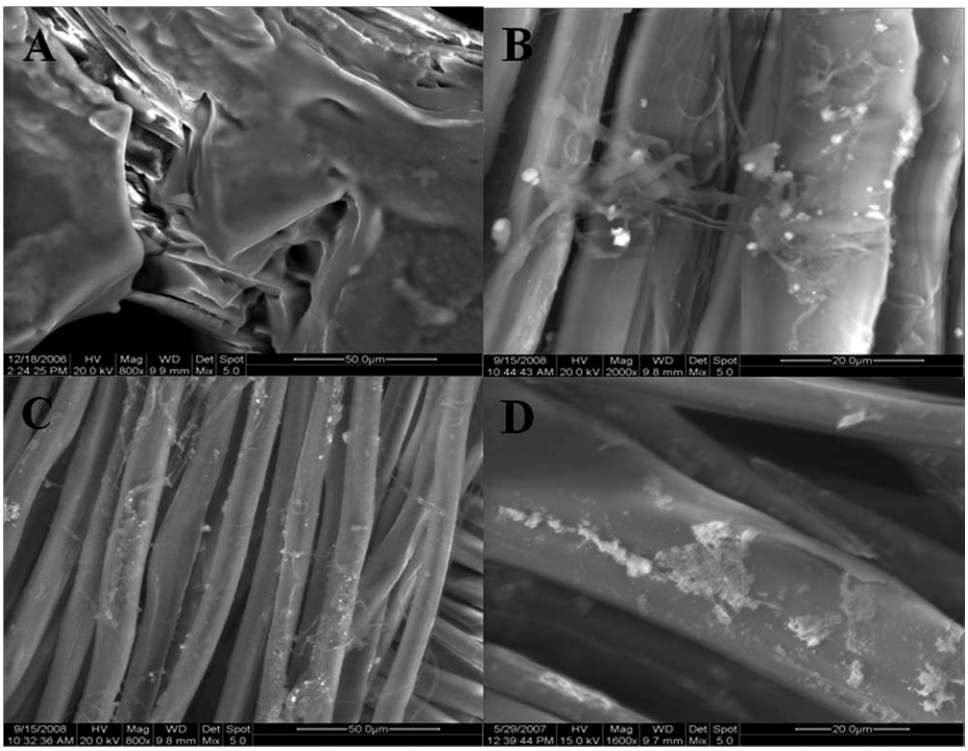


**Figure 8** A) SEM photo of silk that coated with animal glue after 12 days aging before any enzymatic treatment. B) SEM photo of silk that coated with animal glue after 12 days aging after enzymatic treatment 5 U/mL at 37°C. C) SEM photo of silk that coated with animal glue after 12 days aging after enzymatic treatment 10 U/mL at 37°C. D) SEM photos of silk that coated with animal glue after 12 days aging after enzymatic treatment 20 U/mL at 37°C.

**TABLE VI**
**Crystalline Index of Enzymatically Treated Silk with Different Enzyme Concentrations (5, 10, and 15 U/mL) at 1 and 3 h**

| Crystalline Index of treated silk with enzyme concentrations (5, 10, and 15 U/mL) at 1 h | | | | | |
|---|---|---|---|---|---|
| | Crystalline area | | Amorphous area | | |
| Samples | 2θ | Counts | 2θ | Counts | Crystallinity index (%) |
| Silk–original | 20.440° | 192 | 12.960° | 52.42 | 72.70 |
| Silk–protease: 5 U-1 h | 20.851° | 229 | 13.228° | 63.88 | 72.13 |
| Silk–protease: 10 U-1 h | 20.744° | 227 | 12.627° | 65.22 | 71.27 |
| Silk–protease: 15 U-1 h | 20.508° | 225 | 12.648° | 60.57 | 73.11 |
| Crystalline Index of treated silk with enzyme concentrations (5, 10, and 15 U/mL) at 3 h | | | | | |
| | Crystalline area | | Amorphous area | | |
| Samples | 2θ | Counts | 2θ | Counts | Crystallinity index (%) |
| Silk–Original | 20.440° | 192 | 12.960° | 52.42 | 72.70 |
| Silk–protease: 5 U-3 h | 20.529° | 243 | 13.808° | 66.53 | 72.63 |
| Silk–protease: 10 U-3 h | 20.357° | 249 | 13.550° | 65.23 | 73.81 |
| Silk–protease: 15 U-3 h | 20.423° | 231 | 12.518° | 62.17 | 73.09 |

increase in the peak intensity (counts). After the enzyme treatment, there is marked increase in the peak intensity (counts) in both the amorphous and crystalline regions. This finding suggests that the treatment using protease enzyme does not particularly affect the size and shape of crystallites of the silk fibers. Furthermore, the ratio of the crystalline and amorphous fractions barely changed, thus the enzymatic treatment did not result in decrystallization in the silk.

### Effect of protease treatment on chemical structure

Results in Figure 9 show the FTIR spectra of silk after protease treatment with different enzyme concentrations for different durations (5, 10, and 15 U/mL at 1, 3 h). We found that in the 3279 $cm^{-1}$(peak due to —OH groups), 1619 $cm^{-1}$ (peak due to —C=C— groups), 1514 $cm^{-1}$, 1443 $cm^{-1}$ (peak due to —$CH_2$ and —$CH_3$ groups), 1409 $cm^{-1}$(peak due to —OH groups), 1230 $cm^{-1}$(peak due to —C—O—C groups), 1168 $cm^{-1}$ (peak due to —C—O—C— groups), 1068 $cm^{-1}$(peak due to —C—O—C— groups), 997$cm^{-1}$(peak due to —CH=$CH_2$— groups), 974 $cm^{-1}$ (peak due to —CH=CH— groups), and 611 $cm^{-1}$(peak due to —C≡C—H— groups) band intensity increases slightly. 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

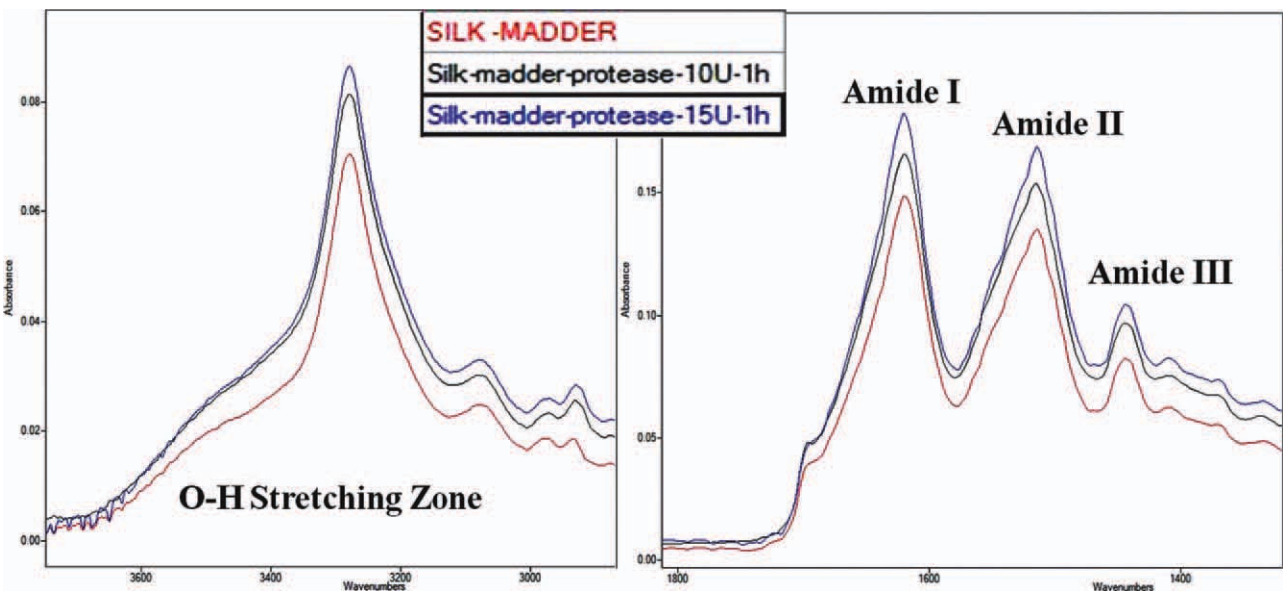


**Figure 9** FTIR of uncolored silk samples after protease treatment with different concentrations 10 and 15 U/mL at 1 h and 3 h. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

**TABLE VII**
**Effect of Protease Concentrations on the Brightness (*L*), Red–Green (*a*), Yellow–Blue (*b*) Coordinates, the Hue Angle (*h*), and Color Chromacity (*c*) of the Uncolored Linen**

| Uncolored Silk | $\Delta E$ | $\Delta L$ | $\Delta a$ | $\Delta b$ | $\Delta C$ | $\Delta H$ | Observation |
|---|---|---|---|---|---|---|---|
| Protease 3 U-1 h | 1.004 | 0.381 | −0.231 | −0.435 | 0.612 | 0.203 | Lighter less red less yellow |
| Protease 3 U-3 h | 1.131 | 0.681 | −0.451 | −0.371 | 0.236 | 0.487 | Lighter less red less yellow |
| Protease 5 U-1 h | 0.982 | 0.589 | −0.351 | −0.287 | 0.287 | 0.387 | Lighter redder less yellow |
| Protease 5 U-3 h | 1.023 | 0.785 | −0.447 | −0.784 | 0.867 | 0.239 | Lighter less red less yellow |
| Protease 10 U-1 h | 1.145 | 0.568 | −0.376 | −0.562 | 0.387 | 0.387 | Lighter less red less yellow |
| Protease 10 U-3 h | 1.088 | 0.987 | −0.465 | −0.690 | 0.461 | 0.296 | Lighter less red less yellow |
| Protease 15 U-1 h | 1.106 | 0.864 | −0.286 | −0.596 | 0.398 | 0.487 | Lighter less red less yellow |
| Protease 15 U-3 h | 1.271 | 0.874 | −0.346 | −0.629 | 0.476 | 0.208 | Lighter less red less yellow |

existing groups disappeared. These results show that the enzymatic treatment caused no significant damage to the fibers, which met our aim conserving the fabrics.

### Effect of protease on the color of silk samples

Color differences of treated silk fabrics after the enzyme protease treatment with different concentrations for different times are presented in Table VII. The results show these changes cannot be detected by the human eye; therefore, there were no drastic changes in the color.

### Effect of the enzyme protease on mechanical parameters of the samples

Changes in the mechanical properties could be attributed to changes in crystalline orientation. The silk samples that have been treated with protease enzyme, with different enzyme concentrations for different durations 1, 3, 5, 10, and 15 U/mL at 1, 3 h, show improvement in elongation properties over untreated samples, with an increase in enzyme concentration and enzymatic treatment time. The results of the initial characterization (before enzymatic treatment) show that the treatment has caused a very slight increase in tensile strength for silk samples (see Table VIII).

**TABLE VIII**
**Effect of Protease Enzyme Treatment on Mechanical Parameters such as Tensile Strength and Elongation of Silk Samples after Different Concentration for Different Durations**

| Warp direction | | |
|---|---|---|
| Samples | T. St. (kg Force) | $E_b$ (mm) |
| Silk–control | 27.967 | 15.852 |
| Silk–protease: 1 U-1 h | 27.872 | 20.109 |
| Silk–protease: 1 U-3 h | 27.861 | 20.724 |
| Silk–protease: 3 U-1 h | 27.990 | 21.260 |
| Silk–protease: 3 U-3 h | 27.105 | 22.689 |
| Silk–protease: 5 U-1 h | 27.895 | 21.936 |
| Silk–protease: 5 U-3 h | 28.119 | 22.238 |
| Silk–protease: 10 U-1 h | 28.309 | 22.818 |
| Silk–protease: 10 U-3 h | 27.402 | 22.911 |
| Silk–protease: 15 U-1 h | 28.330 | 22.150 |
| Silk–protease: 15 U-3 h | 28.258 | 23.538 |

## CONCLUSIONS

The use of protease enzyme resulted in extensive removal of animal glue paste from the fiber surfaces and interfiber capillaries, with high effectiveness for small capillaries at the center of the yarn bundle. 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. Furthermore, no significant change in the color and mechanical parameters of the samples was observed because of the protease enzyme treatment—at least under the conditions which were used in this study—indicating that the treatment did not markedly affect the silk and linen fabrics. The treated linen showed a slight reduction in the peak intensity (counts) in both the amorphous and crystalline regions, while the treated silk showed an increase in the peak intensity (counts) in both the amorphous and crystalline regions of the fibers. These results show the effectiveness of using protease enzymes to remove animal glue adhesive from archeological uncolored linen and silk fabrics having either madder, safflower, or mixture of madder and safflower dye. Further studies should be conducted on the effect of protease enzyme with different fibers and with other natural dyes.

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