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PROTEASE ENZYME USED FOR ARTIFICIAL AGEING ON MODERN COTTON FABRIC FOR HISTORIC TEXTILE PRESERVATION AND RESTORATION

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Abstract

Some of Historical textiles objects in Egyptian museums are containing different types of adhesives from previous restoration processes. Furthermore, they may contain some protein stains such as blood stains, which could involve more damage for the historical textiles. In the context of removing the adhesives by various methods, one may cause damage in the textiles, therefore the biotechnological application of enzymes seems to be a very promising approach in the restoration of historical objects. Our results show that enzyme removing is the most effective method, among all tested methods, in the removing of resistant old adhesives and stains. The tested enzymes for the removing technique solved the problems caused by other traditional removing techniques of resistant old adhesives from museum textiles. The main fibers of the tested objects were cotton fibers dyed with some natural dyes. Thus, the fibers that were used in this study were cotton, dyed with Turmeric dye, madder dye mordanted with alum, $CuSO_4$ or Ferric Citrate, as well as without mordant. Additionally,we studied the effect of the enzyme on the mechanical parameters of fibers (Tensile strength, Elongation, Crystallinity index), by FTIR, XRD and ASTM. Furthermore, the effect of enzymes on the morphology of the surface of the untreated and enzymatically treated dyed fabric was investigated by using SEM and Stereoscopy. The effect of enzymes as a function of enzyme concentration and time of treatment on the fabrics color parameters was extensively studied. There was no impact-destructive effect on cotton fibers after the enzyme treatment. Thus, we could conclude that the enzyme have a very slight effect on cotton fibers dyed with natural dyes.

Keywords: *Protease; removal; enzymes; cotton; natural dyes; SEM; FTIR*

Introduction

Many historical textiles were conserved by using adhesives, such as animal glue. Animal glue adhesive is often present in shrunk, cracked, rigid and brittle form due to the aged condition and does not provide enough adhesion for effective support. The hard, solid animal glue may cause damage to the fibers it had been applied to. Furthermore, there are many textile objects contaminated by different types of stains, such as blood. Some of the stains and adhesives are very resistant to removal by conventional methods. Thus, conservators search for new methods to remove resistant dirt and adhesives from heritage textiles [1-5].

Moreover, extremely thick accretions, which traditionally would require inadvisably long and repeated washes, are more efficiently removed by the enzymes, as they catalyze the

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degradation reaction of the constituent components of the paste. This efficiency leads to a safer treatment of the textiles due to the mild conditions that characterize an enzymatic reaction. The enzyme preparations can be dissolved in water and applied locally or in overall treatments. Adjustments of temperature and pH can also increase the enzyme efficiency [6-18].

This research presents an extensive study of the effect of protease on the mechanical parameters such as tensile strength, elongation and crystallinity index. Furthermore, the structural changes occurring in the fibers upon enzymatic treatment and the morphology of the surface of enzymatically treated fabrics of cotton fibers dyed with natural dyes, the most commonly used fibers in the contaminated historical textile objects were investigated before applying the enzyme to historical textile objects. Finally, the effect of protease on the color parameters of cotton dyed with madder, or turmeric dye was extensively studied. Between those parameters we included the total color change (ΔE), the change in color lightness (ΔL*), the change in the red-green coordinate (Δa*), the change in the yellow-blue coordinate (Δb*), the change in color chromocity (ΔC) and the change in color hue (ΔH) of Madder dye, Turmeric dye, safflower and mixture from madder with safflower mordanted with alum, $CuSO_4$ or Ferric Citrate, as well as without mordant.

Materials and method

Materials

The following materials were used for study:

- Protease from Aspergillus Oryzae P-4755 Type II (Sigma).
- Egyptian cotton fabrics supplied by El- Sharkia Spinning & Weaving Co. (SHARQATEX) - Zagazig, Egypt.
- Natural dyes such as Madder dye and Turmeric dye.
- Mordents such as $CuSO_4$ or Ferric Citrate (Fluka).

Methods

a. Dye and its extraction

The fibers are always entered in the wet state into a mordanting or dyeing bath to ensure that the liquor is taken up evenly. Cotton yarn should never be subjected to sudden changes of temperature. For this reason, the temperature of the mordanting or dyeing bath is warmed up slowly [19]. The dyeing with Madder dye or Turmeric dye was carried out according to the following steps:

- Prepare a 10% (w/v) dye in water solution
- Soaking the dyes in the distilled water for 24hr to extract the color from the powder.
- Heating the extract to boiling temperature for 2 hr with continuous stirring. It may require addition of water to compensate for the evaporated water during the heating process.
- Allow the extract to be cooled and then filtered many times to get a clear colored solution.

The dyeing was performed by the exhaustion method using a liquor ratio (LR) of 1:20 (1 g of fabric per 20mL of bath). The dyeing experiments were performed in beakers according to the temperature-dyeing diagram shown in figure 1. In the experiments mordants $CuSO_4$ or Ferric Citrate (Fluka), was added as concentrated solution (50 g/L). After dyeing, the unfixed dyestuff was removed by rinsing three times with cold water (5 min, room temperature, LR 1:20) [20- 22].

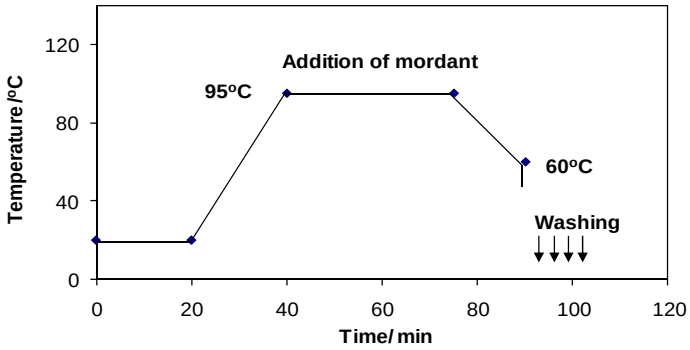

Fig.1. Temperature time diagram of the one-bath dyeing process

b. Enzymatic treatment

The samples were put into beakers then in each beaker we added 200mL of different solutions of Protease in a sodium acetate buffer, pH= 7.5. The samples were incubated for different time intervals (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0h) at a temperature of 37ºC. The enzymatic application was performed at different enzyme concentrations (1, 5, 10, and 15U/mL) for each fabric sample with stirring as well as without stirring.

Testing and analysis

a. Morphological study

The morphology of the surface of the untreated and enzymatically treated fabrics was investigated using tow microspore, as following:

- A Quanta 200 ESEM FEG from FEI Scanning Electron Microscope.
- The Stereomicroscope was a Zeiss Stemi DV4 (Germany) equipped with a digital camera. Small samples were taken from a fabric object from different parts and investigated under SEM and Steroscope, to show the kinds of fibers as well as the damage aspects on these fibers [23].

b. Color measurement

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

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

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

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

$$\Delta H^* = \{(\Delta E^*)^2 - (\Delta L^*)^2 - (\Delta C^*)^2\}^{1/2} \quad (4)$$

$$\Delta E^* = \{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2\}^{1/2} \quad (5)$$

Where Xn, Yn, and Zn are the values of X, Y, and Z for the illuminant that was used for the calculation of the X, Y, and Z of the sample [24-25].

c. Mechanical behavior

The mechanical parameters such as tensile strength and elongation of cotton were determined by using tensile testing machine according to the ASTM method D5035, in the warp and weft directions. Cotton fabrics were cut into 30/5 cm strips. Five samples per treatment set were tested and the breaking load averaged for each sample [26].

d. X-ray diffraction analysis

There were numerous attempts to examine and quantify the crystallinity of cellulose, such as infrared and x-ray. 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 2θ = 50 to 300 continuously at a scan rate of 20/min. The crystallinity index (crystalline to amorphous ratio) was calculated by using the following equation: [27].

$$CrI = \frac{(I_{002} - I_{am}) \cdot 100}{I_{am}} \qquad (6)$$

I_{002} is the maximum intensity (in arbitrary units) of lattice diffraction, while I_{am} is the intensity of the lattic diffraction in the same units at 2θ = 20, the angle that represents the amorphous scatter of fiber.

e. Fourier transform infrared spectral analysis (FTIR)

The structural changes occurring in the fibers upon enzymatic treatment were monitored by FTIR. The vibration patterns 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 to the identification of specific compounds [28-29]. FTIR analysis was carried out for untreated and treated fabric samples by using a BRUKER – FTIR- TENSOR 27. A small part of the samples were encased directly in sample holders and spectra were scanned from 4000 – 500 cm^{-1}

Results and discussions

From previous studies it is clear that the protease enzyme is very effective in removing this type of dirt and adhesive. It is very important to study the effect of the enzyme on cotton and silk fibers dyed with natural dyes. Table 1 presents the characterization of fibers dyed with natural dyes used in our experimental part.

Table 1. Fabric structure, color coordinates and crystallinity index of fabrics that were used.

Samples	Thread / cm		Mechanical parameters		Weight	Crystallinity Index	Plain weave
	Warp	Weft	T.ST (kg)	Eb (mm)	g/m2		
Uncolored Cotton	25	29	61.920	26.448	66.5	88.87 %	

Coordinate	L*	a*	b*	C*	H	X	Y	Z
Uncolored Cotton (Control)	94.85	3.27	-9.9	10.52	288.11	84.41	87.23	109.9
Cotton- Turmeric dye - CuSO4	74.30	5.26	45.07	45.38	83.34	46.57	47.18	18.16
Cotton- Turmeric- Ferric Citrate	72.54	4.80	35.97	36.29	82.40	43.77	44.47	21.31
Cotton- Turmeric –no mordant	76.80	6.59	50.36	50.79	82.54	49.37	48.56	16.88
Cotton-Madder- CuSO4	64.65	11.49	4.01	12.17	19.25	35.13	33.13	33.03
Cotton-Madder- Ferric Citrate	65.33	9.59	9.65	13.59	45.27	35.42	34.46	29.86
Cotton-Madder –no mordant	60.38	14.10	4.60	14.83	18.05	30.70	28.55	27.54

Parameters	Crystalline Area		Amorphous Area		Crystallinity index
	2 Θ	Counts	2 Θ	Counts	
Uncolored Cotton (Control)	20.490^0	192	12.962^0	52.4	72.71 %

Where T. ST = Tensile strength. Eb= Elongation

a. The effect of protease treatment conditions on crystallinity

X-ray diffraction studies lead us to understand the crystalline structure and the degree of crystalline portion. As any treatment that can change the morphology may sometimes lead to crystallization or decrystallization, it was thought worthwhile to investigate the changes caused by the enzyme treatment on the different fibers [30]. X-ray diffraction analysis (XRD) results of untreated and treated samples are presented in two ways.

The first way presents the percentage of the crystallinity index of an untreated sample and of samples treated with different enzyme concentrations. As shown in Table 2 there is a slight decrease of the crystallinity index for cotton.

Table 2. Crystallinity Index of treated Cotton

Samples	Crystalline area		Amorphous area		Crystallinity
	2θ	Counts	2θ	Counts	Index
Cotton – Original	22.650^0	820	18.910^0	98.1	88.05 %
Cotton - Protease -5U-1hr	22.650^o	789	19.046^o	92.7	88.25 %
Cotton - Protease- 5U-3hr	22.776^o	761	19.282^o	90.4	84.77 %
Cotton - Protease -10U-1hr	22.827^o	792	19.311^o	94.3	88.28 %
Cotton - Protease- 10U-1hr	22.790^o	790	19.074^o	88.3	88.18 %
Cotton - Protease- 15U-1hr	22.862^o	746	18.651^o	89.5	88.50 %
Cotton - Protease- 15U-3hr	22.825^o	770	19.021^o	93.2	87.90 %

The Second way is the Wide Angle X-ray (WAXS) diffractograms of untreated and treated Cotton samples. Figure 2 shows Wide Angle X-ray (WAXS) diffractograms of Cotton fibers after enzyme application at concentrations of 1, 5, 10, and 15 U/ml for 1 and 3 hr. It is clear that there is a slight difference between the diffractograms of the treated and untreated samples, due to the action of protease enzyme. One can see that the treated cotton fibers showed a slight reduction in the peak intensity in both the amorphous and crystalline regions. This crystallographic pattern behavior in cellulose was only observed due to a slight degradation in the crystallite structure and hence a decreased crystallinity. This finding is in agreement with previous studies [31]. No significant change was thus observed due to the protease enzyme treatment, indicating that the treatment does not have a bulk effect on cotton fibers.

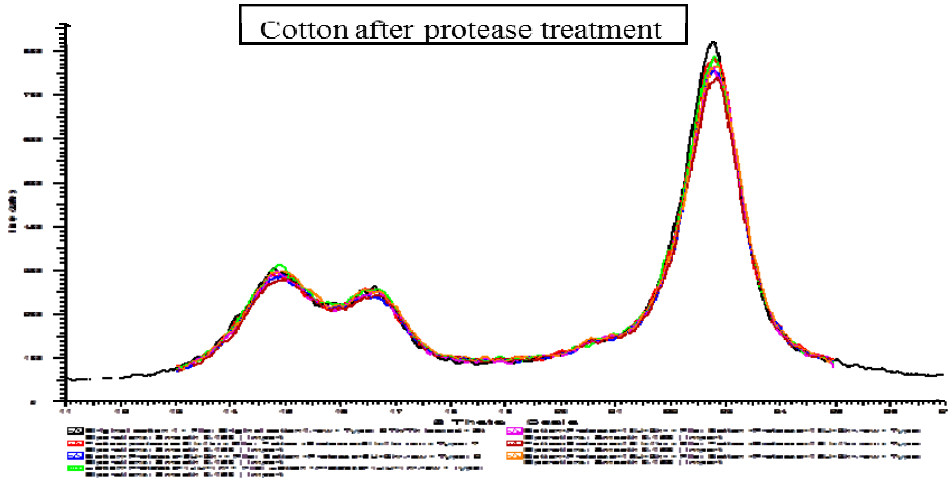

Fig. 2. Wide Angle X-ray (WAXS) diffractograms of cotton after enzyme application that was performed at the concentration 1, 5, 10, and 15 U/ml for 1 and 3 hr. One can see that the treated cotton showed a slight reduction in the peak intensity in both the amorphous and crystalline regions; for linen samples treated with different concentration (1, 5, 10, 15 U/ml) for different periods (1hr and 3 hr).

b. FTIR spectra of fabrics treated with protease

Figure 3 shows the FTIR spectra of cotton after protease treatment with different concentrations (1, 3, 10, 15U/ml at 1 and 3h). We noticed that there are some slight differences in the IR absorption characteristics in the 900 to 1100 cm^{-1} region and other regions. The IR absorption near the 900 to 1000 cm^{-1} region is found to be very sensitive to the amount of crystalline versus amorphous structure of cellulose; i.e. broadening of the band reflects a more disordered structure. Since the disorder of the cellulose structure is caused by the angle change around the β-glycosidic linkage and rearrangement of hydrogen bonds, the examination of the IR absorption charac-teristics of enzyme treated fibers in this region showed a slight broadening and a marked increase in intensity. This finding is in agreement with Janardhnan and Sain [31]. Also, we found peaks at 3334 cm^{-1} (due to -OH groups), 2900 cm^{-1} (peak due to –C-O-H groups), 1643 cm^{-1} (peak due to -R-HC=O groups), 1427 cm^{-1} (peak due to -CH2 and -CH3 groups), 1315 cm^{-1} (peak due to -OH groups), 1204 cm^{-1} (peak due to -C-O-C groups), 1159 cm^{-1} (peak due to -C-O-C groups), 1108 cm^{-1} (peak due to -C-OH groups),1053 cm^{-1} and 1029 cm^{-1} (peak due to -C-O-C groups) and 662 cm^{-1} slight increases in bands intensity.

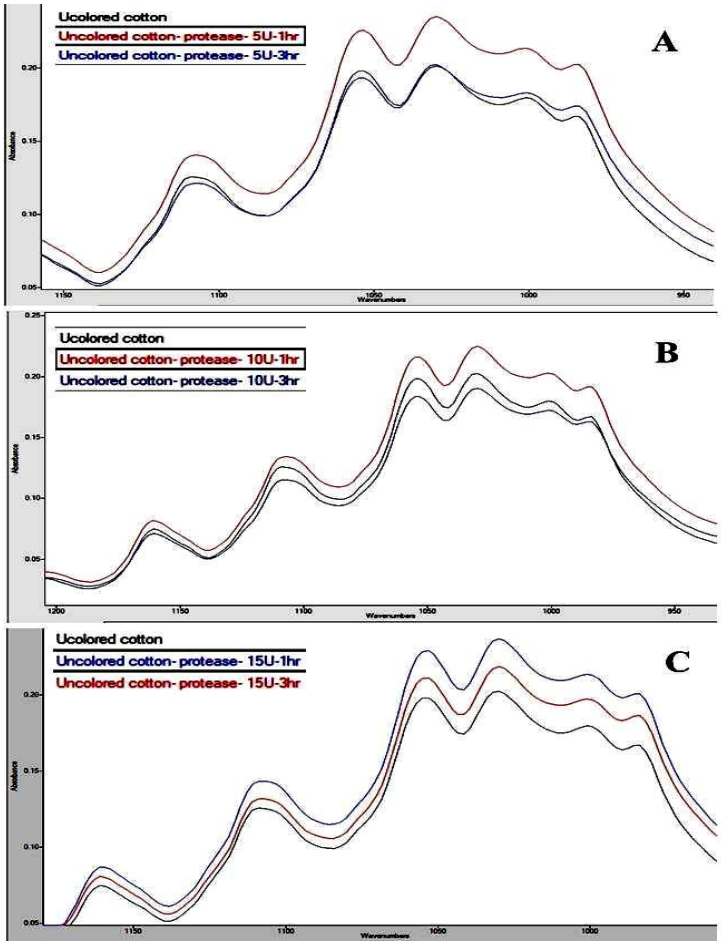

Fig.3. FTIR of Cotton samples after Protease treatment with different concentrations (5U/ml) at 1 and 3hr (A) FTIR of cotton after Protease treatment with different concentrations (10 U/ml) at 1 and 3hr (B) FTIR of cotton after Protease treatment with different concentrations (15U/ml) at 1 and 3hr (C).

This procedure can be used to reveal changes in the structural ordering of cellulose macromolecules after treatment of the fiber with enzymes. Also, we should mention that the treatment of the cotton fibers with protaese enzyme causes partial removal of concomitant substances (fats, pectins, and lignin) from cellulose. This finding is in agreement with Shamolina et al, (2004) who found that the treatment of linen and cotton after cellulase enzyme causes partial removing of concomitant substances [32-33].

c. The effect of protease on the sample color

Color differences of treated linen and cotton fabrics dyed with Madder dye or Turmeric dye mordanted with $CuSO_4$, Ferric citrate, as well as without mordant, after the enzyme protease treatment with different concentrations for different times are presented in Tables 3 - 5. The results in Table 3 show very little color change in uncolored cotton samples. We can see that the samples had a total color change (ΔE) of about 1 CIELab unit. The treated cotton became slightly darker, slightly redder. Also, it is clear that the treated cotton became slightly bluer. The hue is slightly increased in the treated cotton. All of the treated samples had color changes of about 1 CIELab Unit, which cannot be detected by the human eye.

Table 3. Effect of protease concentration on the brightness (L), red – green (a), yellow – blue (b) coordinates, the hue angle (h), and color chromacity (c) of the Cotton Raw

Uncolored Cotton	ΔE	ΔL	Δa	Δb	ΔC	ΔH	Observation
Protease 5U-1h	1.096	-0.565	0.279	-0.897	0.939	0.013	Darker redder bluer
Protease 5U-3h	0.957	-0.559	0.289	-0.721	0.775	0.049	Darker redder bluer
Protease 10U-1h	0.703	-0.035	0.252	-0.656	0.702	0.034	Redder bluer
Protease 10U-3h	1.117	-0.108	0.340	-1.058	1.112	0.005	Redder bluer
Protease 15U-1h	1.029	-0.205	0.318	-0.957	1.008	0.015	Darker redder bluer
Protease 15U-3h	0.943	-0.377	0.292	-0.813	0.864	0.024	Darker redder bluer

Table 4 presents the color changes of cotton dyed fabric with turmeric dye mordanted with CuSO4, after the enzyme protease treatment with different concentrations for different times. We can see that the samples show a slight whiteness, greenness, and yellowness. It is clear that both the color chromacity and the hue angle are increased. There are color changes colored cotton fabric dyed with turmeric dye, mordanted with ferric citrate, after the enzyme protease treatment with different concentrations for different times. One can see that the treated samples show a slight darkness, reddness and appear progressively blue. It is clear that both the color chromacity and the hue angle are decreased. The color changes for colored cotton fabric dyed with turmeric after the enzyme protease treatment with different concentrations for different times. One can see that the treated samples show slightly white, green and yellow tinting. It is clear that both the color chromacity and the hue angle are increased. All of the treated samples had color changes of about 3 CIELab Units, which cannot be detected by the human eye. Only the samples dyed with turmeric, mordented with Ferric citrate had color changes (Δ b and Δ C) of more than 3 CIELab units, which cannot be detected by the human eye.

Table 4. Effect of protease concentration on the brightness (L), red – green (a), yellow – blue (b) coordinates, the hue angle (h), and color chromacity (c) of the cotton dyed with turmeric dye without mordant.

Cotton-Turmeric – $CuSO_4$	ΔE	ΔL	Δa	Δb	ΔC	ΔH	Observation
Protease 5U-1h	1.302	0.701	-0.350	1.040	1.075	0.224	Lighter less red less yellow
Protease 5U-3h	0.909	0.534	-0.579	0.454	0.515	0.526	Lighter less red less yellow
Protease 10U-1h	2.073	1.456	-1.223	0.826	0.697	1.300	Lighter less red yellow
Protease 10U-3h	2.453	0.460	-1.518	2.087	2.106	1.807	Lighter less red yellow
Protease 15U-1h	2.218	1.667	-1.519	2.296	2.137	1.735	Lighter less red yellow
Protease 15U-3h	1.552	0.407	-1.390	0.557	0.414	1.439	Lighter less red yellow

Cotton-Turmeric – Ferric Citrate	ΔE	ΔL	Δa	Δb	ΔC	ΔH	Observation
Protease 5U-1h	6.240	-1.596	1.473	-5.850	-5.523	-2.428	Darker redder less yellow
Protease 5U-3h	7.870	-0.726	0.733	-7.802	-7.583	-1.970	Darker redder less yellow
Protease 10U-1h	6.164	-1.323	1.126	-5.914	-5.654	-2.067	Darker redder less yellow
Protease 10U-3h	5.834	-2.383	1.877	-4.984	-4.591	-2.698	Darker redder less yellow
Protease 15U-1h	5.462	-0.391	0.975	-5.361	-5.139	-1.308	Darker redder less yellow
Protease 15U-3h	4.344	-1.252	1.297	-4.496	-4.230	-2.001	Darker redder less yellow
Cotton-Turmeric – No mordant	**ΔE**	**ΔL**	**Δa**	**Δb**	**ΔC**	**ΔH**	**Observation**
Protease 5U-1h	3.758	3.326	-1.441	0.992	0.820	1.546	Lighter less red yellow
Protease 5U-3h	5.094	4.751	-1.831	0.169	0.037	1.839	Lighter less red yellow
Protease 10U-1h	4.670	3.475	-1.754	2.579	2.370	2.028	Lighter less red yellow
Protease 10U-3h	5.298	4.123	-1.802	2.796	2.582	2.097	Lighter less red yellow
Protease 15U-1h	5.719	3.675	-2.326	3.713	3.451	2.699	Lighter less red yellow
Protease 15U-3h	5.755	4.190	-2.422	3.114	2.847	2.731	Lighter less red yellow

Table 5 presents the color changes of cotton dyed fabric with madder dye, mordanted with $CuSO_4$, Ferric Citrate, as well as without mordant, after the enzyme protease treatment with different concentrations for different times. It is clear that the treated samples became slightly lighter, slightly greener, and slightly bluer. It is clear that both of the color chromacity and the hue angle are decreased. All of the treated samples had color changes of about 3 CIELab Units, which cannot be detected by the human eye.

Table 5. Effect of protease concentration on the brightness (L), red – green (a), yellow – blue (b) coordinates, the hue angle (h), and color chromacity (c) of the cotton dyed with madder dye without mordant.

Cotton-Madder – CuSO4	ΔE	ΔL	Δa	Δb	ΔC	ΔH	Observation
Protease 5U-1h	0.444	0.365	-0.058	-0.245	-0.023	-0.251	Lighter less red less yellow
Protease 5U-3h	0.587	0.293	-0.027	-0.508	-0.184	-0.475	Lighter less red less yellow
Protease 10U-1h	1.309	1.088	-0.568	-0.462	-0.685	-0.258	Lighter less red less yellow
Protease 10U-3h	0.984	0.937	-0.006	-0.301	-0.090	-0.287	Lighter less red less yellow
Protease 15U-1h	1.450	1.341	-0.104	-0.542	-0.267	-0.483	Lighter less red less yellow
Protease 15U-3h	0.442	0.102	-0.282	-0.324	0.168	-0.397	Lighter less red less yellow
Cotton-Madder– Ferric Citrate	**ΔE**	**ΔL**	**Δa**	**Δb**	**ΔC**	**ΔH**	**Observation**
Protease 5U-1h	1.877	1.746	-0.548	-0.419	-0.683	-0.097	Lighter less red yellow
Protease 5U-3h	1.950	1.453	-0.781	-1.040	-1.287	-0.186	Lighter less red yellow
Protease 10U-1h	2.925	2.598	-0.819	-1.066	-1.333	-0.177	Lighter less red yellow
Protease 10U-3h	2.605	1.651	-1.440	-1.410	-2.015	-0.034	Lighter less red yellow
Protease 15U-1h	1.688	0.927	-0.843	-1.131	-1.395	-0.208	Lighter less red yellow
Protease 15U-3h	1.965	1.457	-0.747	-1.085	-1.295	-0.245	Lighter less red yellow
Cotton-Madder– No mordant	**ΔE**	**ΔL**	**Δa**	**Δb**	**ΔC**	**ΔH**	**Observation**
Protease 5U-1h	1.170	0.638	-0.062	-0.979	-0.214	-0.957	Lighter less yellow
Protease 5U-3h	1.849	1.335	-0.475	-1.188	-0.785	-1.010	Lighter less red less yellow
Protease 10U-1h	2.686	2.265	-0.921	-1.111	-1.198	-0.805	Lighter less red less yellow
Protease 10U-3h	3.389	2.705	-1.199	-1.652	-1.598	-1.271	Lighter less red less yellow
Protease 15U-1h	2.698	2.300	-0.677	-1.238	-0.994	-1.002	Lighter less red less yellow
Protease 15U-3h	2.673	1.826	-1.260	-1.490	-1.620	-1.088	Lighter less red less yellow

d. The effect of the enzyme protease on the mechanical parameters of the samples

Changes in the mechanical properties could be attributed to changes in crystalline orientation. The cotton samples treated with protease enzyme of different concentrations and for different durations (5, 10, 15U/mL at 1, 3h) show a slight decrease in elongation and tensile strength, compared to untreated samples, as shown in table 6 and figure 4.

Table 6. Effect of Protease enzyme treatment on Mechanical parameters such as Tensile strength and Elongation of cotton samples after different concentration for different duration

	Samples	T. St. (kgf)	Eb (mm)
Warp Direction	**Cotton - Raw (Contorl)**	61.92	26.448
	Cotton - protease – 5U- 1h	61.613	28.045
	Cotton - protease – 5U- 3h	61.102	17.249
	Cotton - protease – 10U- 1h	59.872	17.941
	Cotton - protease – 10U- 3h	60.013	18.390
	Cotton - protease – 15U- 1h	59.11	17.491
	Cotton - protease – 15U- 3h	59.098	18.829

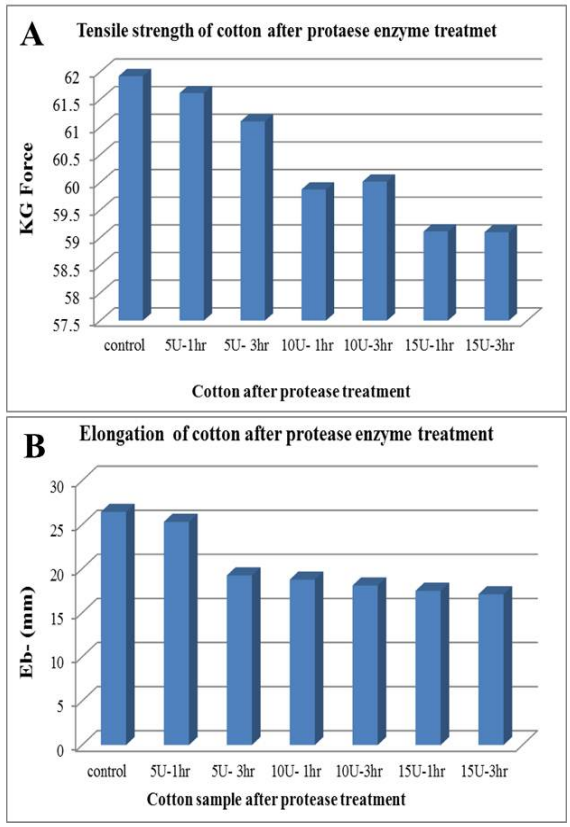

Fig. 4. Show the effect of Protease enzyme treatment on Mechanical parameters such as Tensile strength (A) and Elongation (B) of cotton samples after different concentration for different duration

e. Effect of enzyme on the fiber morphology

Scanning electron microscopy was done on the cellulose treated and untreated samples. The untreated fiber showed parallel ridges, characteristic to cotton fibers. However, a microphotography of the fiber after enzymatic treatment revealed smoothened surfaces. The enzyme peels off the cellulose, which results in the formation of protruding fibrils and the formation of more polished surfaces, as shown in figure 5.

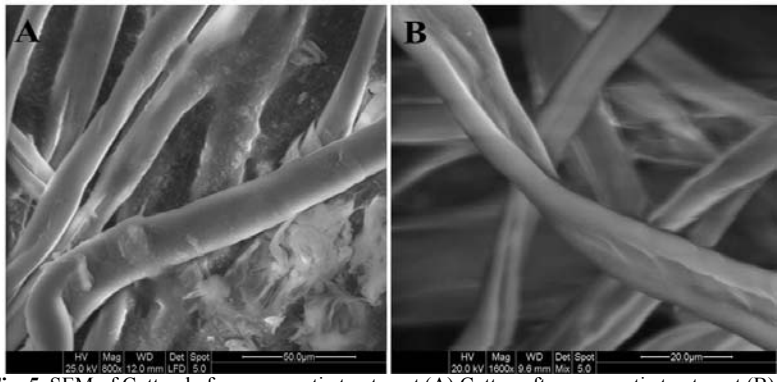

Fig. 5. SEM of Cotton before enzymatic treatment (A) Cotton after enzymatic treatment (B).

Conclusions

From our present study we concluded that:

- No significant change in the color and mechanical parameters of the samples was observed after the protease enzyme treatment, at least under the conditions which were used in this study, indicating that the treatment does not have a bulk effect on cotton fibers. A slight change was observed in optical parameters (such as ΔE, Δa*, Δb*, ΔL*, ΔC, ΔH) for uncolored cotton dyed with madder, turmeric dye, mordanted with $CuSO_4$ or ferric citrate.
- One can see that the treated cotton showed a slight reduction in the peak intensity (counts) in both the amorphous and the crystalline regions.
- These results prove the effectiveness of using protease to remove animal glue adhesive from colored cotton fabrics having either a madder or a turmeric dye.
- There should be further studies on the effect of protease on different dyes other than Madder and turmeric, mordanted with $CuSO_4$ or ferric citrate.

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