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RESEARCH ARTICLE

Effect of Extracted Egyptian Keratinase on the Properties of Native Coarse Wool

O. G. Allam^{1*}, S. Mowafi¹, A. Abou El -kheir¹, Azza M. Abdel-Fattah², A. Bendak¹

1. Textile Research Division

2. Chemistry Department

1, 2, National Research Centre, 33 Bohouth St. Dokki – Giza. Egypt. Affiliation ID: 60014618

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*Corresponding Author

O. G. Allam

Abstract

This work aims to study an economy approach to improve physical and chemical properties of native coarse wool fibre such as felt proofing, dyeability, and degree of whiteness & yellowness by using keratinase enzyme which is derived from extremophilic microorganism. It is isolated locally from leather wastes at Egyptian tanneries and identified phylogenetically as *Cyberlindnera fabianii* NRC3 aza. Pretreatment of wool fibre enhances the anti-felting properties as well as the dye exhaustion (%) as compared with the untreated one. These increases depend on many factors for enzyme such as: pH, concentrations, treatment time and temperature. Washing fastness of the pretreated dyed wool fibers with reactive dye was performed. The change of the chemical composition for the pretreatment wool fiber was investigated by using alkali solubility test and elemental analysis. Scanning electron microscopy was performed to investigate the morphological changes on the surface of the enzyme treated wool fibres.

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Introduction

Native coarse wool is mainly obtained by shearing fleece from the animals (sheep and goats and some alpaca). The type and content of vegetable impurities in wool are chiefly dependent on the country of origin and conditions of breeding. Vegetable impurities consist of dry vegetable matter, pods, and seeds (1). Approximately 8.5% of raw wool fibres contents are vegetable contamination. This implies that the contamination with vegetable material is too high for successful removal mechanically and that a more drastic chemical treatment is essential (2). Moreover, there are considerable amount of coarse wool deficiencies such as scouring, bleaching, felting. Chlorination and other oxidation methods without AOX (Absorbable organic halogen) are currently available to modify wool to improve its surface and whiteness. But a great disadvantage of these processes is their environmental pollution (3). Hydrogen peroxide (H₂O₂) is commonly used as bleaching agent. Hydrogen peroxide and proxy compounds damage wool fibres, due to progressive oxidation of disulphide bond ultimately forming cysteic acid. This chemical damage lead to adverse effects on the fibres mechanical properties (4-6). Felting of wool is an essential feature in industrial felt in the clothing fabric industry and undesirable in wool wet treatment, such as scouring and dyeing, and in home laundering, especially of knitted garments. Different methods have been developed for felt proofing of wool fibers; it can be classified into many methods by chemical means (7) or by biological means (8, 9) for example with synthetic resins such as Hercosett (10) or with a natural polymer such as chitosan (11)). Therefore, different methods of treatment are needed to improve these properties of wool in an environmentally friendly manner such as plasma, enzymes, etc...Enzymes, especially, are being researched and used extensively for the treatment of wool. The commonly used enzymes on wool are papain, pronase, trypsin, lipoprotein, lipase, and

keratinase (12). In the 1990s, attempts were made in Poland to lessen the undesirable effect of carbonization by using enzymes to remove vegetable impurities from wool (13 - 16). Over that enzymes can be used to reduce the concentration of chemicals, water and energy consumption. Scouring of wool fibres with an enzyme – based formulation provides efficient removal of natural impurities from wool without any fatal damage to the fibre (17). Also, it was possible to substitute conventional chlorine treatment by the enzymatic process, which enables to receive a fabric of the same level of anti-shrinking and anti-felting properties (18, 19). Knitted wool fabrics were treated with atmospheric argon plasma, enzyme (protease), chitosan and a combination of these processes produced high dyeing efficiency and shrink-resistance in wool fabric (20). Wool fibres have been modified with nordihydroguaiaretic using laccases in order to produce multifunctional wool textiles with improved wrinkle, shrink resistance and tensile strength in addition to antioxidant activity and UV stability and protection, (21). Recently Cardamone *et al* (22) studied the combined bleaching, shrinkage prevention and biopolishing of wool fabrics, a process that resulted in wool with excellent whiteness and a soft handle. Schumacher *et al* (23) found that protease treated wool had a higher degree of whiteness, lower felting tendency and improved dyeability. (24) Jovancic *et al* described a combined low-temperature plasma/enzyme wool shrink resist process resulting in a machine washable level of shrink resistance without excessive damage to the fibres. Microbial keratinase have become biotechnologically important enzyme since they target the hydrolysis of highly rigid, strongly cross-linked structural polypeptides “Keratin” recalcitrant. Keratins are insoluble fibrous proteins found in hair, wool, feather, nail, horns and other epithelial coursing contains beta helical coil which is linked through cysteins bridges. The bioconversion of insoluble feather keratin to soluble feather residue has high nutritional values and can be employed as a supplement for livestock feeds (25). It can use feather as a substrate to produce high amount of keratinase. Keratinase can degrade all the protein molecules, so it may also be used for detergent purpose. This enzyme can be an alternative to sodium sulfide, the major pollutant from tanneries, and may completely replace it. Its unique upon collagen enhances its industrial potential (26). It is therefore easy to convert the feather keratin into soluble crude protein through microbial fermentation technology by using pilot scale bioreactor (27) and soild state aerobic and anaerobic reactors to yeild byproducts (28). Keratinous wastes such as feathers, horns, nails and hair are generated in large quantities from various industries such as those that process leather and poultry (29). The feathers of poultry are recognized as an important bioresource with an annual production up to several million tons by the chicken industry in the world (30). Currently, only limited amount of feathers are utilized as feather meal in poultry feedstuffs .Therefore, degradation of feather keratin is a necessary step prior to application for this purpose. The purpose of the present study is to improve physical and chemical properties of native coarse wool fibers, after washing (with with tap water, well water and sea water to comparison in between them), such as felt proofing, dyeability, and degree of whiteness& yellowness. These is carried out by Egyptian keratinas enzymes which they isolated from leather wastes obtained from Egyptian tanneries that were identified phylogenetically as *Cyberlindnera fabianii* NRC3 aza .

2. Experimental

2.1. Materials

Raw Egyptian wool fleece fibres collected from south Sinai, average fibre diameter and length is 40.4µm and 70 mm were used.

2.2. Chemicals

Nonionic detergent (nonyl phenol ethoxylane, was supplied from Starch & Detergent Company, Alexandria, Egypt. All other chemicals and reagents used were laboratory grad.

2.3. Dyestuff

The used dye is a commercial sample; Lanazol Red 69 from Ciba (C.I. Reactive Red 84) based on α -bromo acrlamide. The structure is shown is figure 1.

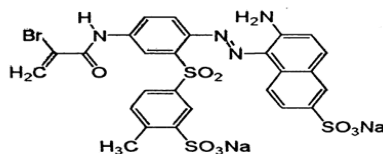


Figure 1: Molecular structure of C.I. Reactive Red 84

2.4. Preparation of Keratinases

2.4.1. Feather processing

Chicken feather were collected from poultry processing plant and soaked in a washing liquid with 1% detergent for degreasing for 12 h, they were washed carefully using distilled water 10 – 20 times until clean. Then, the cleaned feather were dried at 60 °C and crushed by grinding with a 1-mm pore sieve.

2.4.2. Fermentation of the selected strain for keratinase production (Inoculum preparation)

Cyberlindnera fabianii NRC3aza strain was grown on TSA slants at 37°C for 5 days. The spores were harvested with sterile distilled water containing 0.1% Tween 80 and transferred into the inoculum medium composed of (g/l) glucose, 10; peptone, 10; yeast extract, 3; CaCl₂.2H₂O₂ (31) . 2% of spore suspension containing 105 spores ml was used for SMF (submerged fermentation) inoculation and incubated at 37°C for 3 days on a shaker incubator (180 rpm).

2.4.3. Culture media

The basic liquid medium used for the enzyme production composed of (g/l) chicken feather, 20; NaCl, 0.5; KH₂ PO₄ , 0.7; K₂ HPO₄ , 1.4; MgSO₄ , 0.1, pH 7.2 (32). Fermentation was carried out by seeding 3-days inoculum of *Cyberlindnera fabianii* NRC3 aza (6 %, v/v) in a 250-ml Erlenmeyer flasks with 50 ml of the basic liquid medium containing chicken feather as the sole N and C source at 37°C and incubated on a shaker incubator at 180 rpm. Prior to assay the fermentation broth was centrifuged to separate the filtrate.

2.4.4. Preparation of Keratinases enzyme solution

Native chicken feathers (10 g) in 500 ml of dimethyl sulfoxide were heated in a reflux condenser at 100 °C for 2 h. Soluble keratin was then precipitated by addition of cold acetone (1 L) at –70 °C for 2 h, followed by centrifugation at 10 000 for 10 min. The resulting precipitate was washed twice with distilled water and dried at 40 °C in a vacuum dryer. One gram of quantified precipitate was dissolved in 20 ml of 0.05 mol/L NaOH. The pH was adjusted to 8.0 with 0.1 mol/L Tris and 0.1 mol/L HCl and the solution was diluted to 200 ml with 0.05 mol/L Tris-HCl buffer (pH 8.0) (33-36).

2.5. Method

2.5.1. Scouring

Native coarse wool (20g) was washed by using 1g/l nonionic detergent, 1g /l sodium carbonate at 50 °C for 30min., L.R 1:40 (o.w.f), with different types of water (tap water, well water or sea water).

2.5.2. Treatment of wool with prepared Keratinase (*Cyberlindnera fabianii* NRC3 aza)

Well water washed coarse wool fiber was treated with the specific concentration (0.25, 0.5, 0.75, 1% (o.w.f.) of phylogenetically as *Cyberlindnera fabianii* NRC3 aza aqueous solutions in stopper glass bottles using a liquor ratio of 1: 20 for (15, 30, 45, 60 min..) at (45,65, 85,95 °C) . The aqueous solutions bath was adjusted to pH 7, 9, 11, 13 using sodium carbonate solution to select the optimum degree. The fibres was then rinsed thoroughly with running water and air-dried.

2.5.3. Dyeing procedure

The untreated and treated native coarse wool fibers were dyed with C.I. Reactive Red 84. The dyebath was prepared by first pasting the needed amount of the dyestuff with small amount of water. This paste was then diluted by adding boiling water and was thoroughly stirred. The dyeing bath was adjusted to pH 4.5 using acetic acid solution, liquor ratio 1: 50. Dyeing was performed by exhaustion technique using a laboratory shaking apparatus (Julabo- Germany). The dyed samples were then withdrawn, thoroughly washed with hot and cold water, and air dried.

2.6. Measurements and analysis

2.6.1. Felt Test

The resistance of untreated as well as modified wool was assessed according to the IWTO 20–69 test method. The Aachener three-dimensional shaking machine was used to produce felt balls, the density of which was taken as a measure of the degree of felting.

2.6.2. Dye exhaustion (%)

Measurement of the amount of residual dye in the dyeing bath was determined spectrophotometrically using 6405 UV–visible Spectrophotometer (JENWAY, U.K.)

2.6.3. Washing fastness

The colorfastness of the dyed wool fiber was assessed according to the AATCC test 6–1975 using a launder meter (Technical Manual of AATCC 1972 & 1993).

2.6.4. Whiteness & yellowness Index

Measurement of whiteness & yellowness index was determined using Ultrascan Prospector photometer (Hunter Lab) Made in USA.

2.6.5. Mechanical Properties

The tensile testing (tenacity & elongation %) of the fibre before and after treatment was evaluated using an Instron Tensile Tester (USA) according to ASTM D 76 Standard Specification for Textile Testing Machines.

2.6.6. Alkali Solubility

The alkali solubility of the untreated as well as the modified wool fibers was evaluated using the standard method (ASTM D 1283-85). The alkali solubility of wool was determined according to the following relation:

$$\text{Alkali solubility\%} = \frac{\text{Weight of dry wool} - \text{Weight of dry residue}}{\text{Weight of dry wool}} \times 100$$

2.6.7. Elemental analysis

The amount of carbon, nitrogen, hydrogen, and sulphur, in the untreated and selected treated wool fibers were assessed using Elementary CHNS Analyser, Model Vario EL III, Germany.

2.6.8. Scanning electron microscopy

The surface morphology of the treated fibers was examined using scanning electron microscopy (SEM; Model JSM-5600LV, Jeol, Tokyo, Japan).

3. Results and discussion

3.1. Effect of washing using different types of water on impurities removal

Native coarse wool fibre (20g) was washed by using tap water, well water or seawater as a different types of water to comparison in between in presence of 1g/l nonionic detergent and 1g /l sodium carbonate and L.R 1:40 (owf). Washing step was carried out at 50 °C for 30min to remove impurities from wool. Lose in weight of wool after washing was calculated and data was tabulated in table 1.

Table1: Effect of washing of wool using of tap water, well water or seawater on removal of impurities

Types of Water	Wt _{before} (gm)	Wt _{after} (gm)
Tap water	20	12.2
Well water	20	12.0
Sea water	20	14.8

In table 1, it is clearly observed that, washing of wool by sea water resulting lose in weight by about 26% while by tap or well water cause lose in weight by about 39% . This result indicated that the removal of impurities from wool by well or tap water is better than that by sea water. So, it is economically to use well water in washing step to remove sufficient amount of impurities from wool surface.

Enzyme activity on native wool fibre is specific and mainly depends on the following factors:

3.2. Effect of wool treatment by enzyme at different pH values

Wool fibre was treated with 1 % keratinase at different pH values (7, 9, 11, and 13), 65°C for 1h., and liquor ratio 1: 20 then the samples undergo felting test as well as dyeing by reactive dye. Table 2 shows the effect of enzyme treatment at different pH values on the felt proofing and dye exhaustion of wool fibers.

Table 2: Effect of enzyme treatment at different pH values on the felt proofing and dye exhaustion of wool fibers

Sample	Felt ball diameter (cm)	Exhaustion %
Untreated	2.32	57.25
Washed	2.40	57.84
Washed and treated by enzyme at pH 7	2.68	63.533
Washed and treated by enzyme at pH 9	2.88	68.443
Washed and treated by enzyme at pH 11	3.02	77.889
Washed and treated by enzyme at pH 13	3.02	77.889

Dyeing condition: 1% C.I. Reactive Red 84 (o.w.f), at 85 °C, pH 4.5, for 1h, liquor ratio 1:50

Data of table 2 show that, treated wool fibers have anti-felting property as compared to the untreated ones. In addition, the dye exhaustion was enhanced. This is may be explained in term of the removal of wool scales under the effect of the enzyme treatment. The results clarify that, by increasing of the pH of the treatment enzyme bath, the antifelting and dye exhaustion (%) was improved and this may be owing to the activity of the keratinase increased by increasing ph.

3.3. Effect of treatment of wool by different concentration of enzyme

The effect of treatment of wool fiber by different concentrations of keratinase (0.25, 0.50, and 0.75, 1%) on the felting and dye exhaustion (%) was studied and the results are described in figures 1&2. In figure 1, it is observed an increase in felting ball diameter of the enzyme treated wool as compared to the untreated wool. Moreover, by increasing the concentration of the keratinase enzyme the diameter of the obtained felting ball increased till limited concentration (0.5%) and this is may be due to inactivation of enzyme by increasing its concentration (37) and also may be attributed to the relationship between rate of reaction and concentration of substrate which depends on the affinity of the enzyme for its substrate. The same results are shown in figure 2, where by increasing the treated enzyme concentration the dye exhaustion was increased in comparison with the untreated wool. Additionally, increasing the concentration of enzyme improved the dye exhaustion till limited concentration of treatment (0.5%)

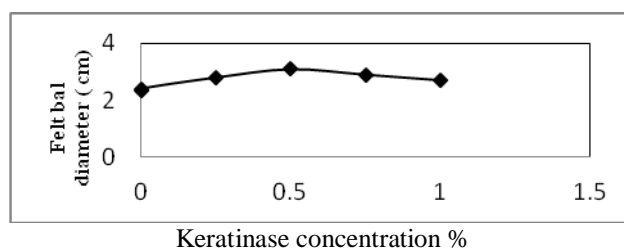


Figure 1: Aachen felt ball diameter of untreated and treated wool fibre at different concentration of enzyme (0.25, 0.50, 0.75, and 1%) (pH 11, at 65°C, for 1h) liquor ratio of 1: 20

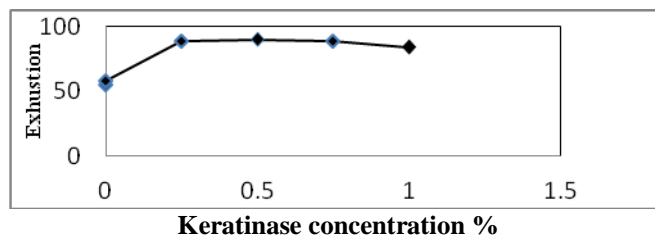


Figure 2: Exhaustion % of untreated and treated wool fibre at different concentration of enzyme (0.25, 0.50, 0.75, and 1%) (pH 11, at 65°C, for 1h) liquor ratio of 1: 20
Dyeing condition: 1% C.I. Reactive Red 84 (o.w.f), 85 °C, pH 4.5, 1h, liquor ratio 1:50.

3.4. Effect of treatment of wool by keratinase enzyme for different interval Treatment time

Figure 3&4 show the effect of treatment of wool fiber by 0.5% of keratinase, pH 11 at 65°C for different periods (15, 30, 45, 60and75min.), on the felt-proofing and dye exhaustion (%). There is increase in the diameter of the felting ball by increasing the time of the treatment by keratinase, see fig 3, but further increase in the treatment time (after 60min) has no effect. Furthermore, the dye exhaustion (%) of wool fiber increased by increasing the period of the treatment keratinase, see fig 4.

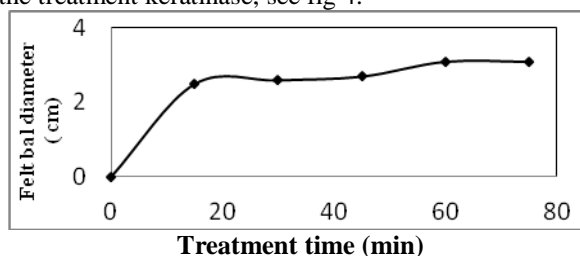


Figure 3: Aachen felt ball diameter of untreated and treated wool fibre by keratinase for different time (15, 30, 45, 60and75min.), pH 11, at 65°C liquor ratio of 1: 20

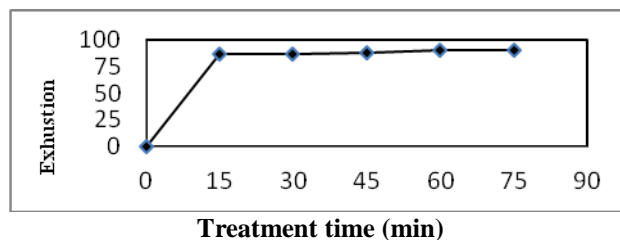


Figure 4: Dye Exhaustion % of untreated and treated wool fibre by keratinase for different treatment time (15, 30, 45, 60and75min.), pH 11, at 65°C liquor ratio of 1: 20
Dyeing condition: 1% C.I. Reactive Red 84 (o.w.f), 85 °C, pH 4.5, 1h, liquor ratio 1:50.

3.5. Effect of treatment of wool by keratinase enzyme at different temperature

Temperature plays an important role in biology as a way to regulate reactions. Enzyme activity increases as temperature increases, and in turn increases the rate of the reaction. This also means activity decreases at colder temperatures. All enzymes have a range of temperatures at which they are active, and there are certain temperatures where they work optimally. Figure 5&6 illustrate the effect of treatment of wool fiber by keratinase at different temperature (45, 65, and 85°C) on the anti-felting and dye exhaustion (%). Figure 5 shows that, by increasing the treatment temperature the anti-felting property of wool improved, but further increase in temperature resulting in decrease the diameter of felting ball and this is may be due to the deactivation of enzyme by increasing temperature above 65 °C .The same effect appears in figure 6, where the dye exhaustion (%) of the enzyme treated wool increased by increasing treatment temperature but after 65 °C the dye exhaustion reduce by increasing treatment temperature.

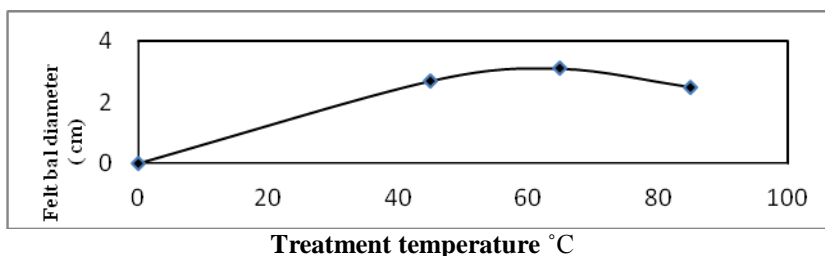


Figure 5: Aachen felt ball diameter of untreated and treated wool fibre by keratinase at different temperature (45, 65, 85°C.) PH 11, for 1h, liquor ratio of 1: 20

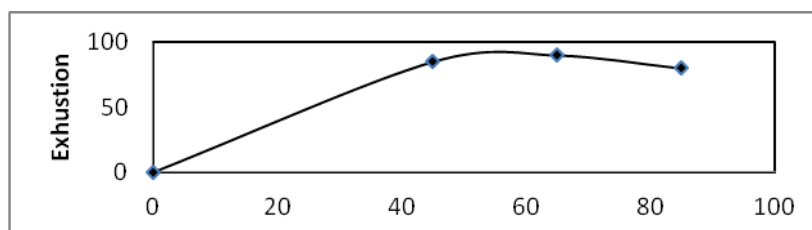


Figure 6: Exhaustion % of untreated and treated wool fibre by keratinase at different temperature (45,

65& 85 °C) pH 11, for 1h, liquor ratio of 1: 20

Dyeing condition: 1% C.I. Reactive Red 84 (o.w.f), 85 °C, pH 4.5, 1h, liquor ratio 1:50.

3. 6. Physical, Chemical, and mechanical analysis

The effect of the treatment by the said prepared keratinase enzyme on the physical, mechanical as well as chemical properties of wool fiber were studied and the results are concluded in table 3

Table 3: Whiteness, Yellowness, Tenacity and Alkali Solubility of untreated and treated wool fibres

Samples	Whiteness	Yellowness	Tenacity (g/den)	Elongation %	Alkali Solubility
-Untreated wool	-5.73	29.42	0.149	13.500	12.1
- well washed wool	9.46	19.98	0.149	13.500	11.9
- Treated wool with enzyme	32.67	15.76	0.207	6.916	10.3

Condition of enzyme treatment: Treated wool fibre for 60 min., pH 11, at 65°C and liquor ratio of 1: 20
 able 3 shows that, the whiteness of wool fiber increased after washing by about 40% and further increasing in whiteness was observed after treatment by keratinase. On the other hand, the yellowness of the enzyme treated wool was decreased as compared to the washed and untreated samples. Additionally, there is no significant change in the tenacity of the enzyme treated fiber and the untreated ones. Alkali solubility data show that, there are negligible changes after the enzyme treatment which indicated that, such treatment has no drastic effect on the fiber.

3.7. Elemental analysis

Carbon, hydrogen, nitrogen, and sulfur of the untreated as well as the enzyme treated wool fibers were measured and the data are summarized in table 4. It can be observed that, in table 4, there is no change in carbon, hydrogen, and nitrogen contents of the enzyme treated wool in comparison with the untreated fiber. Alternatively, sulfur content for the enzyme treated wool decreased by about 16% than the untreated sample and this is may be explained in the term of the enzyme effect on the descaling of wool surface that enriched by sulfur content. the high sulfur content is due to the high content of cysteine, a double amino acid containing two sulfur atoms in a disulfide bond : $\text{HOOCCH}(\text{NH}_2)\text{S CH}_2\text{CH}(\text{NH}_2)\text{COOH}$.

Table 4: Elemental analysis of untreated and keratinase treated wool fibres

Samples	N %	C %	S %	H %
Untreated wool fibre	14.38	44.22	2.444	5.18
Untreated & washing wool fibre	14.54	44.96	2.424	5.22
Treated wool fibre with enzyme	14.50	44.87	2.054	5.34

Condition of enzyme treatment: Treated wool fibre for 60 min., pH 11, at 65°C and liquor ratio of 1: 20

3.8. Scanning electron microscopy

The morphological structure of the washed untreated and enzyme treated wool was assessed (see fig 7&8) Figure 7 shows the normal scale surface of wool, while after enzyme treatment the wool scales were removed (see fig 8). The smooth surface of treated wool under the effect of enzyme, keratinase, resulting in an improvement of the anti-felting properties as well as the dyeability of the fiber.

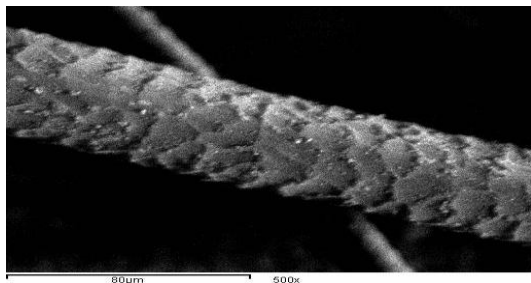


Fig 7: Scanning electron micrograph of washed untreated wool (x 500)

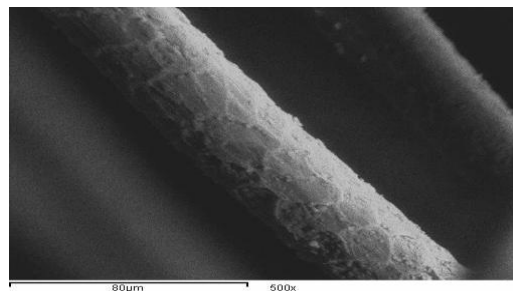


Fig 8: Scanning electron micrograph of wool fibre treated with enzyme (optimum conditions (x 500)

4. Conclusion

Coarse wool fiber was successfully cleaned by well water and undergoes treatment by keratinase enzyme. This enzyme was prepared from leather wastes at Egyptian tanneries and identified phylogenetically as *Cyberlindnera fabianii* NRC3 aza. Treatment of wool by the prepared keratinase enzyme under specific conditions improved its anti-felting property, dyeability due to the removal of wool scales under the effect of keratinase. The optimum conditions of wool treatment by keratinase is 0.5% concentration, pH 11, at 65°C for 1 h. Alkali solubility, whiteness and mechanical properties results show the insignificant deterioration effect of the applied enzyme on the treated wool. SEM shows the removal of wool scales besides the decrease in the sulfur content of the enzyme treated fiber comparing to the untreated ones that resulting in enhancing dyeability and felt-proofing of wool fiber.

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