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RESEARCH ARTICLE

ENZYMATIC STUDIES FOR IMPROVING WOOL QUALITY

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ABSTRACT

The Biotechnologists are interesting in the safe biotechnological products as an alternative to chemicals in many industrial applications. One of the interesting biotechnological products is the technical enzymes which can be used in textile industry. In this study, kertainolytic protease produced by *Bacillus amyloliquefaciens* MA20 is applied to improve wool fibers surface, tensile strength and water absorption. The wool straight off sheep was washing with triton X100 and hydrogen peroxide for scouring and bleaching respectively to facilitate the enzymes diffusion. Enzymatic treatment of wool fibers with keratinolytic protease was carried out at 50°C and 60°C which act as the maximum activity and stability of the enzyme. The various enzymatic treatments have been conducted with intervals time as follow: 1, 2, 3, 6, 9 and 12 hours. Scanning electron microscope (SEM) demonstrated smoother of wool surface in comparing with other untreated due to remove impurities. However, the tensile strength test proved increasing the tensile of wool. Water uptake techniques showed increasing in the wool water absorption and retaining after enzymatic treatment which have benefit for further application of dye on wool fibers. The best results of wool treated with keratinolytic protease produced by *B. amyloliquefaciens* MA20 were exhibited at 60°C and after incubation for 12 hrs. These results have important industrial applications of enzymatic wool finishing process which is environmental friendly in compared with chlorine treatment process.

INTRODUCTION

Wool is a natural protein fiber that has been widely used in different textile products such as carpets and blankets due to its high quality textile material. The typical commercial process for wool finishing is the chlorine-Hercosett method. This process leads to pollution of water with organic halogen (AOX) by-products (Silva *et al*., 2006). Hence, there is an urgent need using environmentally friendly, controllable and cheap processes for wool finishing. In recent years, the development of technology in the textile industry and increasing demand to wool made the researchers of worldwide have been investigated the eco-friendly ways to improve wool quality. Such efforts enable the substitute of wool chemical treatment by environmental friendly enzymatic process (Amara and Serour, 2008; Amara, 2013; Jus *et al*., 2007).

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Wool fiber consists of two major morphological parts: the cuticle and the cortex; wool cuticle cells are subdivided into two main layers, exocuticle and endocuticle (Plowman, 2003). The resistant of wool cuticle is owing to the covalent isopeptide crosslinks and covalently attached lipid (Heine and Hocker, 1995). The chlorination treatment alters the surface properties of the fiber by reducing its hydrophobic nature and enhances textile properties such as dye uptake, polymer adhesion in shrink resist treatments and electrical conductivity (Brack *et al*., 1999).

Due to the scale layers of wool, it tends to shrinkage and unsoftness. These scale layers affect the dying and water absorption of wool as well as, their disulfide bonds resists the wool degradation by protease enzymes. Therefore, the removing or decreasing these layers is main process of wool finishing (Cai *et al*., 2011). The wool never has been remained in the environment due its degradation by proteolytic enzymes which can be secreted by different microorganisms. Keratinolytic proteases were produced by different microorganisms using keratinous substrate such as hair, waste

wool, feather and whey milk- adairy byproduct (Alessandro *et al*., 2003; Roberta *et al*., 2006; Philippe *et al*., 1999; Prakasham *et al*., 2006; Gushterova *et al*., 2005). Enzyme process in wool finishing mainly depends on protease enzymes which are environmentally friendly and it has been utilized for the modification of wool fiber (Silva *et al*., 2005). So, many labs interested to use keratinolytic enzymes as promising tools to eliminate the outer surface of scales (Lenting *et al*., 2006).
In this study, the application of keratinolytic serine protease enzyme after hydrogen peroxide treatment to restrict the enzymatic activity on the outer surface of the scale layers for obtaining soft wool with high capability to retain water with limited impact on tensile strength was investigated. The impact of this method may be potentially applied in wool industry.

MATERIALS AND METHOD

Wool fiber

The wool fibers were collected from one of the Egyptian sheep (lamb sheep) living at New Borg El-Arab City in Alexandria governorate. The wool was straight off sheep before treating with chemical or biological materials.

Reagent and enzymes

The enzyme used in this study was the keratinolytic serine protease produced by *Bacillus amyloliquefaciens* MA20. The enzyme was produced and supernatant was obtained from the culture as described previously by (Hassan *et al*., 2013). The culture supernatant was used for further studies as crude enzyme source.

Measurement of keratinolytic protease activity

The protease activity was determined using casein Hammarstein as substrate at optimum conditions (pH 9 and 60°C for 15 min.) according to Hassan *et al*., (2013). The enzyme activity was calculated using tyrosine standard curve as described by Amara *et al*., (2013) where, one unit of alkaline protease was defined as one µmol of tyrosine liberated per min. All assays were carried out in triplicate and average values were reported.

Application of keratinolytic protease on wool fibers

Wool pre-treatments and enzymatic treatments

Wool fibers were subjected to a surfactant (scouring) or a surfactant and peroxide washing (bleaching) in order to enhance the proteolytic attack according to Silva *et al*., (2005) with some modification. Briefly, Wool was washed with 0.5% triton X100 as surfactant for 30 min at 40ºC and 100 rpm using shaking water bath. After washing process, the surfactant was removed from the fibers by washing with tap water, followed by distilled water. For the bleaching step, the wool fibers were immersed in 1% H_2O_2 for 1 hour at 55ºC and 100 rpm. Finally, the wool fibers were washed with distilled water about 3 times for removing any traces of hydrogen peroxide and allowed to air dry. The enzymatic treatment was conducted by incubating the wool fibers with crude enzyme diluted by glycine/ NaOH buffer pH 9. The final concentration of enzyme after dilution was defined as 25 U/ml. The treatment was allowed to proceed in water bath at the optimum temperature of enzyme (50, and 60ºC) for different intervals time (1, 2, 3, 6, 9 and 12 hrs). Finally, the fibers were washed many times with distilled water and exposed to 90ºC for 10 min in order to inactivate the proteolytic enzyme. The first control sample was the fibers which were washed with tap water; the second control was the fibers after washing and bleaching while the third control was the fibers treated with buffer pH 9 which haven't contained the enzymes. The fibers were balanced at 37ºC with a relative humidity of 60% for 48 hrs prior to further analysis according to Lenting *et al*., (2006).

Tensile strength resistance and dimensional stability

Tensile strength test was conducted using tensile tester (shimadzu, Japan) equipped with a load cell maximum capacity of 2 kgf as described before by Silva *et al*., (2005). Also, 100 mm/min of test speed, 10mm of gauge length and Tex of linear density was applied. The force of wool fibers samples was determined and the values of tensile test are given as the mean of triplicate experiment.

Morphological study using scanning electron microscope (SEM)

The morphology of the treated fibers with enzymes, washed fibers with tap water and fibers after washing and bleaching of wool fibers was investigated using a scanning electron microscope (Joel, Jsm-6360LA, Japan). The samples were fixed on glass slides and coated with gold using sputter coater. The golden-coated samples were scanned at 20 KV acceleration voltages and room temperature (Amara and Serour, 2008).

Water uptake

The ability of different treated wool to uptake water was examined. These represent treated samples at different temperatures with enzymes and samples before and after washing and bleaching were investigated. The water uptake was performed after embedding a known weight of wool fibers in water for 3 hrs (Lo Nostro *et al*., 2002). The water uptake of the used samples was calculated from the following formula:-

$$\text{Water uptake (\%)} = (W - W0 / W0) \times 100$$

Where W is the weight of the wool fibers after embedding in water at time t and W0 is the weight of the dry wool fibers.

RESULTS AND DISCUSSION

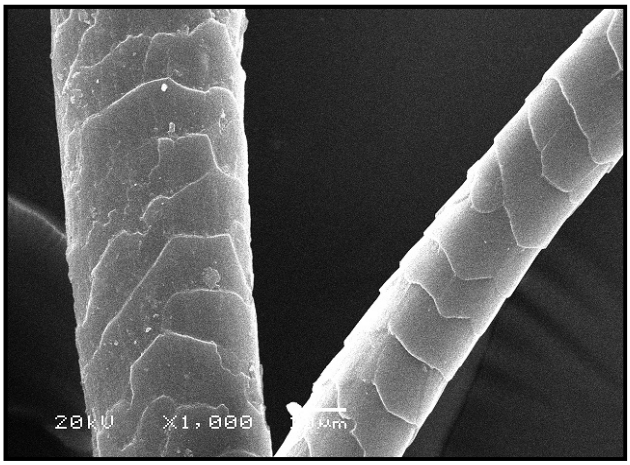
Keratinolytic protease enzymes have many medical and industrial applications such as Acne treatment, Removal of corns and calluses, feed additives, detergent, leathet dehairing and textiles industry **(**Gupta *et al*., 2013). One of the promising applications of keratinolytic proteases is finishing of wool process in industry. Brandelli, (2008) reported the ability of keratinases to degrade recalcitrant proteins. This exclusive feature to attack and degrade insoluble proteins paid attention of scientists to utilize them in dehairing of hides, textiles and keratin waste management (Gupta and Ramnani, 2006; Zambare *et al*., 2007; Shrinivas and Naik, 2011). Several produced keratinases by *Bacillus licheniformis*, *Bacillus thuringiensis* L11, *Chryseobacterium* L99, *Pseudomonas sp*., *Stenotrophomonas maltophilia* DHHJ, *Bacillus cereus* and

Fusarium sp. have been revealed to ameliorate wool fibers and their dyeing property (Liu *et al*., 2013; Infante *et al*., 2010; Lv *et al*., 2010; Cai *et al*., 2011; Cai *et al*., 2008; Sousa *et al*., 2007; Noriyuki *et al*., 2003). The main objective of this research was to investigate the influence of proteases produced by *Bacillus amyloliquefaciens* MA20 to change the morphology and physical properties of wool fibers in order to improve their properties.

Wool fibers straight off sheep were subjected to pre-treatment process as in textile industry using detergent and alkali solution because of the presence of fatty layer on the surface of wool fibers, being responsible for the strong hydrophobicity of wool (Silva *et al*., 2006; Hossain *et al*., 2008). .In this study, the crude wool from Egyptian sheep was washed and bleaching using triton X100 and H_2O_2 respectively to improve the contact of the keratinolytic protease with wool fibers. General protease enzymes can't degrade most keratinous substrate so, the used protease in wool treatment penetrate into the inner part of wool fibers and may cause damage to the fibers without degrading of cuticle layer. Consequently, the research in wool treatment was focused on keratinolytic proteases because it degrades wool fibers from outside part to inside as showed in this current research. Vasconcelos *et al*., (2006) demonstrated that the wool fibers were degraded after treatment with 100 u/ml of protease enzymes for 72 hrs. So; we used 25 U/ml if keratinolytic protease and incubated wool fibers with it for time intervals to avoid this problem and follow the effect of keratinolytic enzymes accurately. To optimize the incubation time of keratinolytic protease with wool fibers, the wool fibers were treated with the enzyme for intervals time (1, 2, 3, 6, 9 and 12 hrs) in optimum temperature and pH of the enzyme. The treatment was conducted at 50 and 60°C to utilize the high stability of the enzymes at 50°C (Amara, 2013). The morphology of wool fibers and their physical properties including tensile strength and percentage of water uptake were tested after crude keratinolytic proteases treatment in compare to the wild wool without treatment and to the wool which subjected to surfactant and bleaching processes.

Scanning electron microscope study

The wool samples were scanned using scanning electron microscope for studying the effects of each of the following treatments; washing and bleaching and the protease enzyme. The results have been evaluated using the Scanning Electron Microscope (SEM) to evaluate the effect of the proteases on the surface of the wool fibers. The results indicated that there are no changes on wool surface morphology after the pre-treatments (surfactant and bleaching processes) as in (Figure 1A and 1B) and these results are in agreement with Silva *et al*., (2005). There are many fragments on the surface of the crude wool. The fragments were removed by keratinolytic protease treatment for 3, 6, 9, and 12 hrs and increased with time hence, the surface of wool fibers were modified into smooth surface. Scanning electron microscope proved the effectiveness of the keratinolytic protease enzyme on the wool fibers compared with wild wool without treatment. The wool became smooth with increasing the time treatment and reach to maximum after 12 hrs. The enzymes showed high activity with stability at 60°C so; the potential results after treatment of wool fibers



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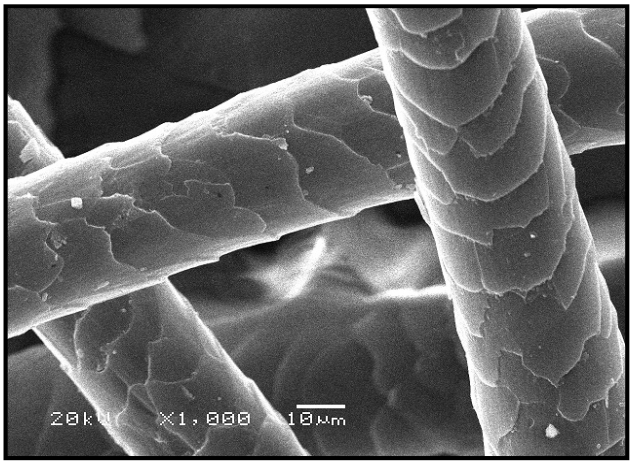
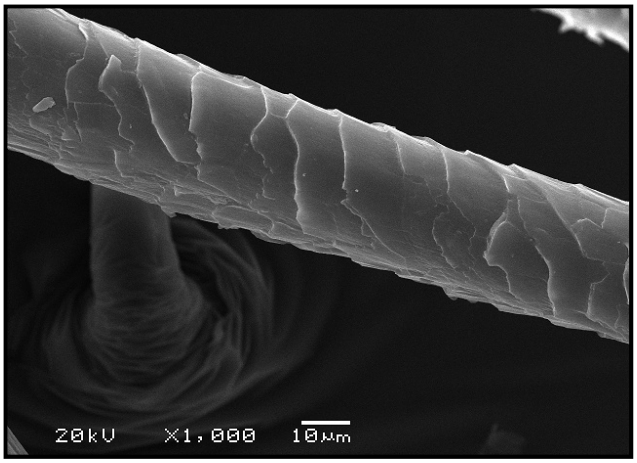


B

Figure 1. SEM of wool fibers at 1000X (A) indicates to the wild wool before processing while (B) indicates to the crude wool after washing and bleaching processes

with keratinolytic protease obtained from *B. amyloliquefaciens* MA20 were obtained after treatment at 60°C for 12 hrs and these results were better than treatment at 50°C (Figure 2, 3). Amara and serour described the effect of proteases enzymes which produced by *Bacillus licheniformis* and and *Geobacillus sp.* on wool fibers and showed that the enzymes improved the surface of wool by smoothing the wool fibers outer layer (Amara and Serour, 2008).



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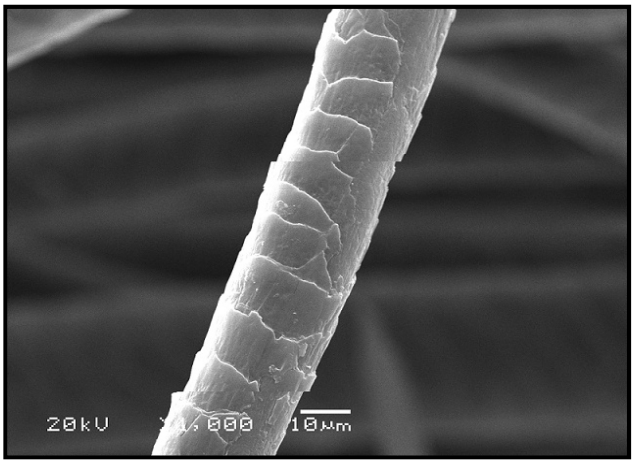

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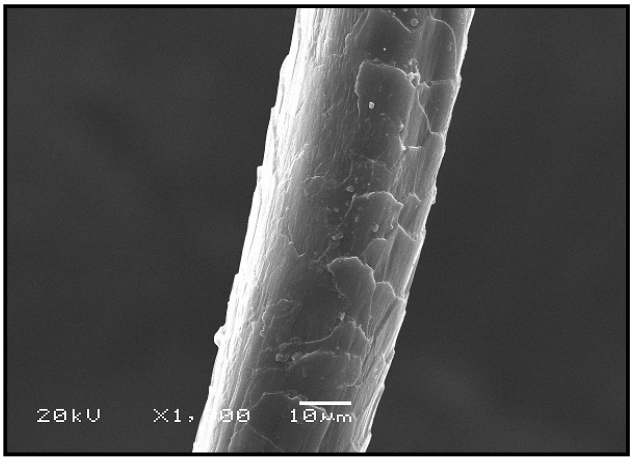

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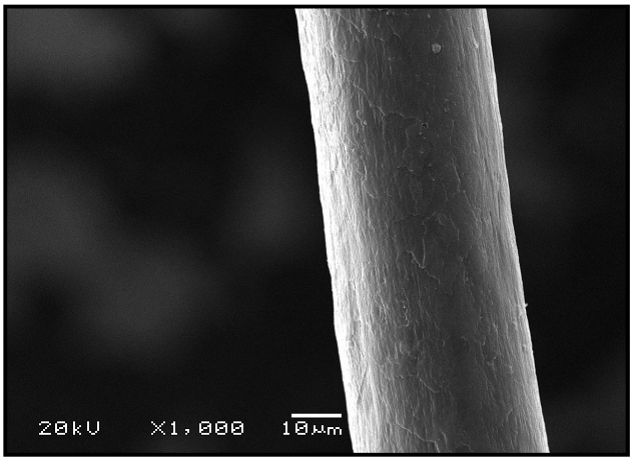
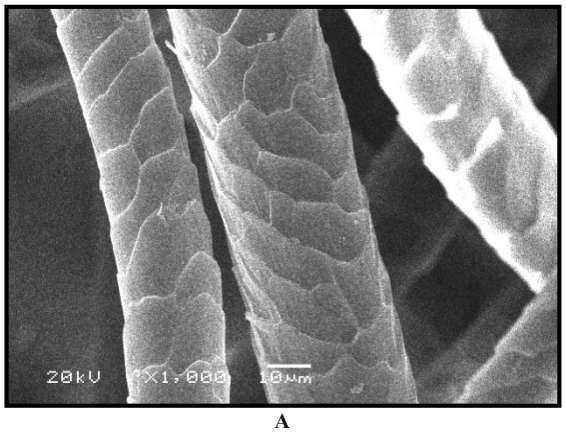


D

Figure 2. SEM of wool fibers after treatment with keratinolytic protease enzyme produced by *B. amyloliquefaciens* MA20 for intervals time at 50°C (A) wool fibers treated for 3 hrs, (B) wool fibers treated for 6 hrs, (C) wool fibers treated for 9 hrs, and (D) wool fibers treated for 12 hrs

In addition, the wool fibers which were treated with protease enzyme for 24 hrs were almost degraded because the enzyme was diffused into the inner part of fibers as reported before by Jus *et al*., (2007). Therefore, the time of the wool fiber exposure to the proteases is a critical step. These results suggested that the bio-finishing of wool fibers with different temperatures according to the industrial needed.



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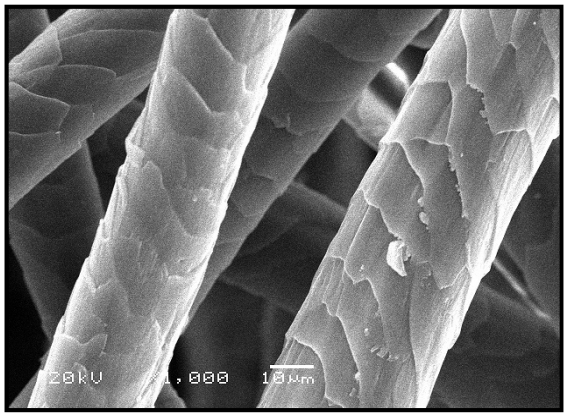

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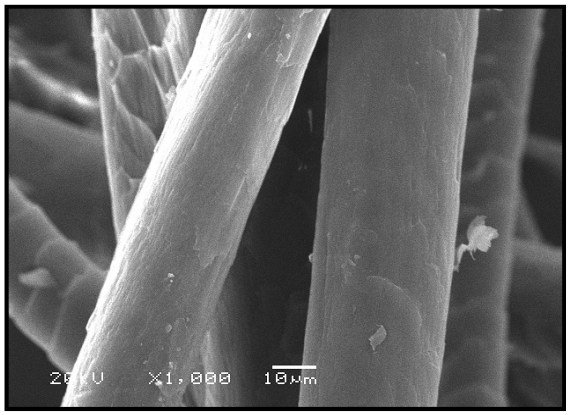

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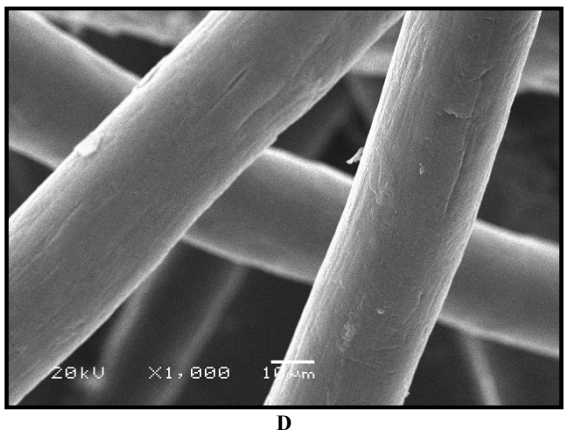

D

Figure 3. SEM of wool fibers after treatment with keratinolytic protease enzyme produced by *B. amyloliquefaciens* MA20 for intervals time at 60°C (A) wool fibers treated for 3 hrs, (B) wool fibers treated for 6 hrs, (C) wool fibers treated for 9 hrs, and (D) wool fibers treated for 12 hrs.

Tensile strength test of wool fibers

One of the important parts in manufacturing process involves making wool fibers is strong enough, particularly for the warp. Cai *et al.,* (2011) showed that the produced protease using keratinous wastes was capable of degrading the cuticle layers of wool fibers alone without any alteration in inner part of fibers. In addition, the results of some previous literatures showed that protease enzyme removed the fragment scales of wool fibers but there is no change in tensile characteristic of treated wool fibers by protease enzymes (Kim *et al*., 2005). On the other hand, the results of this study exhibit that the tensile strength of wool fibers was significantly increased compared to the wild wool. The tensile strength of untreated and treated wool with keratinolytic protease enzyme were measured using tensile machine. The tensile strength of wool fibers increased after treatment with the enzymes and indicated that it was increased against time revealed that keratinolytic proteases affected the surface wool fibers only and the impurities were removed and left pure wool fibers. The treated wool fibers were compared with crude wool fibers (reference sample 1) and wool after washing and bleaching (reference sample 2). Max force which is the expression of strength was obtained and it was 115.93 N (Newton) for wool treated with the enzymes from *B. amyloliquefaciens* MA20 after 12 hrs at 50°C while the max force was 125.78 N after 12 hrs at 60°C (Figure 4, 5). The results of tensile strength were approved with the results of SEM. The polypeptides and amino acids which degraded during treatment of wool fibers with keratinolytic protease diffused into wool fibers and attached to surface of fibers caused significantly increase in tensile strength.

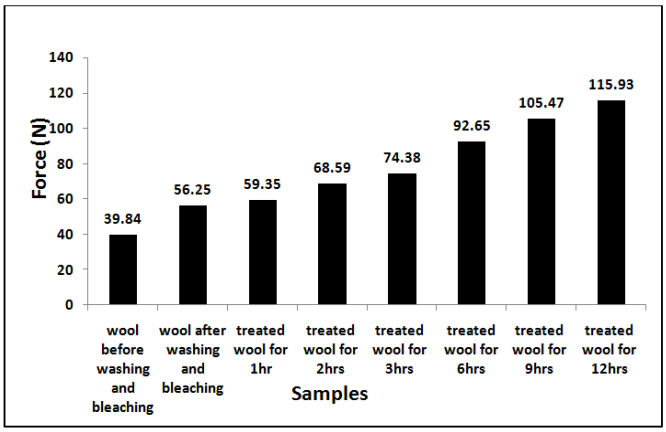

Figure 4. Tensile strength summary of wool after treatment with keratinolytic protease enzyme produced by *B. amyloliquefaciens* MA20 at 50°C for intervals time

Some researchers used mixture of polypeptides from keratin for utilizing their affinity to repair hair via forming film which made it very elastic consequently, the polypeptides can be used in improvement surface of fibers and their physical characteristic such as elasticity (Cai *et al*., 2007; Benson *et al*., 2009). In this study, the treatment of wool fibers was carried out using keratinolytic protease enzymes and the releasing polypeptides from hydrolyzed keratin in the same time. It is recommended to investigate the physical of wool fibers in all treatment steps for obtaining true results because the results which were depend on morphology of wool fibers only need to further examination.

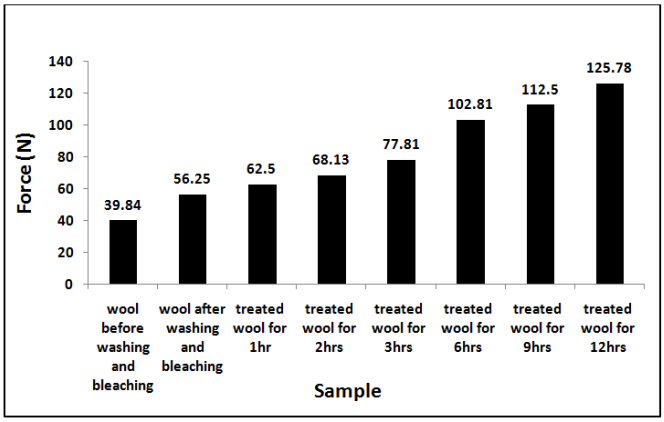

Figure 5. Tensile strength summary of wool after treatment with keratinolytic protease enzyme produced by *B. amyloliquefaciens* MA20 at 60°C for intervals time

Water uptake percentage of wool fibers

The protease enzymes from *Bacillus sp.* was applied to improve wool quality and their results indicated that increasing the time of wool treatment by protease led to enhancement the dye uptake of wool fibers as well as, the surface of wool became smoother due the removing scales of wool (Cai *et al*., 2007). In wool industry, the wool fibers stain with dyes for different clothes colour which acts as important step in industry. The treated wool with keratinolytic protease enzymes produced by *B. amyloliquefaciens* MA20 was tested for studying the ability of the dye to penetrate to the inner part of wool. This test was carried out by calculating the percentage of water uptake that wool can absorb and retain it. The water uptake (percentage) was 200.75% (w/w) for wool fibers treated with enzymes for 12 hrs at 50°C and was 204.23% (w/w) for wool fibers incubated with enzymes for 12 hrs at 60°C. The water uptake percentage indicated to increasing the water uptake of wool after treatment (figures 6, 7). In recent studies, extracting polypeptides from wool degraded by keratinolytic protease was conducted to modify the surface of wool fibers and the results showed improvement of dye uptake with a high level of shrink resistant after and before dying (Smith and Shen, 2011).

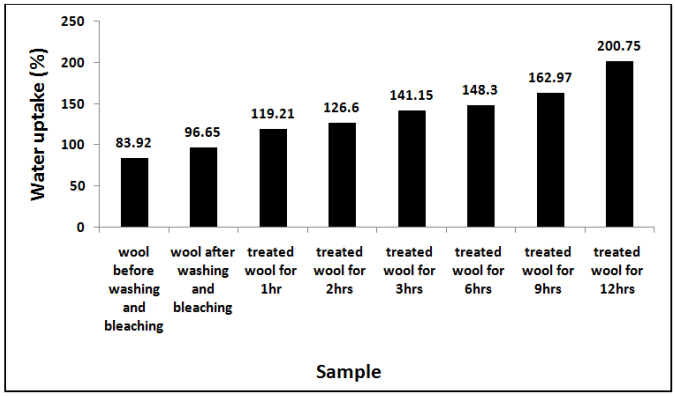

Figure 6. Water uptake percentage of wool after treatment with keratinolytic protease enzyme produced by *B. amyloliquefaciens* MA20 at 50°C for intervals time

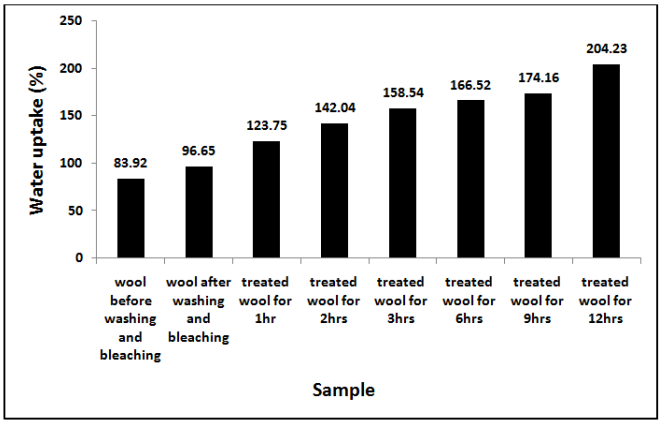

Figure 7. Water uptake percentage of wool after treatment with keratinolytic protease enzyme produced by *B. amyloliquefaciens* MA20 at 60°C for intervals time

Parvinzadeh, (2007) showed the ability of protease enzymes to make the wool fibers susceptible to dyes with high level compared to the untreated fibers. The water uptakes of wool fibers increased after keratinolytic protease treatment and these results were direct proportion with exposure time to enzyme. The ability of wool fibers treated with keratinolytic protease enzyme from *B. amyloliquefaciens* MA20 to reserve the absorbed water was increased significantly.

Conclusion

This study showed the important of protease enzyme in textiles industry. The presented results in this research demonstrated the pre-treatment process of wool fibers using triton X100 and hydrogen peroxide to promote the hydrolytic effect of keratinolytic protease enzymes. The keratinolytic protease which was investigated its effect on wool fibers was produced by *Bacillus amyliquifacins* in presence of wool due to enzyme specificity. The scales of wool fibers were removed and exhibited smooth after treatment with keratinolytic protease. The scan electron microscope proved the effect of enzymes without damage of the fibers and the tensile strength increased after incubation with enzymes for 12 hrs. In addition, the water uptake of fibers was increased significantly. These promising results caused by treatment with keratinolytic enzyme and polypeptides of wool hydrolysis in the same time. The traditional method for wool fibers treatment is Chlorine–Hercosett process which has severe effect in the environment through the effluent of hazard materials in water and they will need to forward the efforts to waste water treatment. The treatment process of wool fibers with keratinolytic protease and polypeptides could be conducted as a substitution to use Chlorine–Hercosett process. Eventually, it is recommended to use this cheap and potential process in wool industry.

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