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ARTICLE

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Investigation of effects of protease enzyme produced by *Bacillus subtilis* 168 E6-5 and commercial enzyme on physical properties of woolen fabric

Elif Demirkan[a], Dilek Kut[b], Tuba Sevgi[a], Meral Dogan[b] and Eren Baygin[a]

[a]Biology Department, Faculty of Arts and Sciences, Uludag University, Bursa, Turkey; [b]Department of Textile Engineering, Faculty of Engineering, Uludag University, Bursa, Turkey

ABSTRACT

Wool is one of the most important fibers in textile industry, and has been commonly used for producing value added products due to its properties of lightness, warmth, softness, and smoothness. However, the special scale structure in wool cuticle can cause felting shrinkage of wool fabrics. Proteases have been widely used to modify the surface of wool to prevent wool felting, due to their ability to catalyze the hydrolysis of peptide bonds in wool scales. Although the treatment of wool with proteases was considered as an environmentally friendly technique to provide wool fabrics with shrink resistance properties, proteases exhibited low efficacy in removing the cuticle scales because of the highly cross-linked barriers. In this study, wool fabric was treated with protease enzyme obtained from novel isolated bacteria and commercial protease enzyme, and the results were compared. The tear strength, pilling changes in ΔE values, whiteness and yellowness values of wool were controlled. Results showed that treatment with *Bacillus subtilis* 168 E6-5 protease enzyme yielded improvements in the physical properties of wool fabric compared with commercial enzyme.

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KEYWORDS
Bacillus; protease; wool; physical properties

1. Introduction

The use of enzymes in the textile industry is one of the most rapidly growing fields in industrial enzymology. In the textile industry, enzymes are increasingly being used to develop cleaner processes and reduce the use of raw materials and the production of waste. The enzymatic processes are performed at much lower temperatures and use less water compared with classical methods (Adrio & Demain, 2014). Various enzymes such as amylase, laccase, pectinase, lipase, protease, catalase, and xylanase are used in different textile processing steps. Proteases constitute one of the most important groups of industrial enzymes, accounting for approximately 60% of the total enzyme market (Sawant & Nagendran, 2014). Proteases (peptidases or proteolytic enzymes) are degradative enzymes that catalyze the cleavage of peptide bonds in other proteins and are classified according to their structure or the properties of their active site. There are several kinds of proteases, such as serine-, metallo-, carboxyl-, acidic-, neutral-, and alkaline proteases. Proteases are obtained from plants, animal organs, and microorganisms, with the majority obtained from bacteria and fungi. *Bacillus* species are the main producers of extracellular proteases, and industrial sectors frequently use *Bacillus subtilis* to produce various enzymes (Pant et al., 2015). Proteases are widely used in leather processing, the detergent industry, food industries, bioremediation processes, the pharmaceutical industry, the textile industry, waste processing companies, the film industry, and other sectors (Souza et al., 2015).

In textiles, natural wool clothing has the tendency to shrink, and felting is unwelcome by customers. Washable wool is today's requirement. Wool's felting properties are due to its scale-like surface structure (Doshi & Shelke, 2001). Wool fiber consists of three parts: the epidermis, cortex, and medulla layer. Wool fibers are highly resistant against enzymes compared with other protein fibers. The resistance comes from the disulfide (cysteine) bridges between the macromolecules. Disulfide bridges are reduced with reducing agents; wool keratin macromolecules are cleaved by trypsin and proteolytic enzymes such as papain as soon as they form polypeptides. The important point here is that enzymes work their way into wool fibers with large molecular structures and reveal the interior to the surface due to the shredding effects of the enzyme. The anti-felting effect of fibers can be good rather than harmful. Protease treatments can modify the surface of wool and silk fibers to provide new and unique finishes (Josephine et al., 2012). Protease enzymes can be used to produce an anti-felting effect, to remove the fiber ends protruding from the surfaces of woolen fabrics, to reduce pilling, and to increase brightness and smoothness. The combination of these effects in worsted wool causes weight loss to occur, and when using the selected pH, temperature, and process time, a reduction in enzyme concentration should be kept to

CONTACT Elif Demirkan edemirkan@uludag.edu.tr Biology Department, Uludag University, Faculty of Arts and Sciences, 16059 Gorukle, Nilufer-Bursa, Turkey.

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a minimum. The dyeability of wool fabric treated with the enzyme is increased. The enzymes were observed to work at lower temperatures. They could also be made bio-clean. Over the last several decades, the textile industry has greatly improved in environmental, economic and product quality aspects by using commercial enzyme preparations. Enzymes have become an indispensable part of textile processing and are used in a wide variety of applications in the modern textile industry (Shahid, Mohammad, Chen, Tang, & Xing, 2016).

Hartzell-Lawson and Dinger (2001) used protease enzymes to increase the end-use potential of coarse wool fibres. Schumacher, Heine, and Hocker (2001) found that protease-treated wool had a higher degree of whiteness, lower felting tendency, and improved dyebility. Mojsov (2017) treated the wool fabric with different concentrations of protease enzyme and observed the effects on wool fabric. Onar and Sarusik (2005) investigated environmentally friendly enzymatic treatment for wool fibre and fabric with different proteases and other enzymes.

Some researchers reported that enzyme pretreatment on dyed wool fabrics improved softness, handle, drape, pilling resistance, and change the colour (Riva, Algaba, & Prieto, 2002; Raja & Thilagavathi, 2011; Sharma, Sharma, & Shukla, 2011). Different proteolytic enzymes from *Bacillus lentus* and *Bacillus subtilis* in native and the soluble polymer polyethylene glycol (PEG)-modified forms were investigated and their influence on the modification of wool fibres were studied, as a result scanning electron microscope (SEM) images of wool fibres confirmed smoother and cleaner fibre surfaces without fibre damages using PEG-modified proteases (Jus, Schroeder, Guebitz, Heine, & Kokol, 2007). The fibre destruction is caused by the diffusion and the hydrolytic attack of the native proteases onto non-keratinous parts of the protein fibre (Heine et al., 1998; Shen, Bishop, Heine, & Hollfelder, 1999; Silva, Prabaharan, Gübitz, & Cavaco, 2005) showed that the native proteases diffuse deep into the wool fibre and causing no significant modifications on the outer fibre surface. The higher rate of native enzyme diffusion into the inner part of the fibre resulted in almost complete degradation of the fibre at longer incubation time (180 min) (Heine & Höcker, 2008). Infante et al. (2010) reported that the isolation of wool degrading *B. thuringiensis* L11, partial characterization of extracellular proteases, kinetics of production and potential application of bacteria for fabric treatment, and studies of physical properties on wool-fiber and -fabric have been showed promising results for the textile industry. Brier (2000) stated that the treatment of wool fabric with Perizym-AFW improved shrink resistance, whiteness, pilling behavior, dye ability, and wash ability. Schroeder et al. (2006) have demonstrated a moderate improvement in shrinkage behaviour of wool by enzymatic pretreatment, and have achieved successful results. The use of enzymes to improve white colour, shrinking behaviour, dyeing affinity (Cardamone & Damert, 2006), pilling behaviour (Mazzuchetti & Vineis, 2005) in the woollen sector is particularly interesting.

The aim of this work is to isolate *Bacillus* sp. with a high protease producing capacity from soils, to investigate the effects of different partially purified enzymes and commercial protease enzyme on the physical properties (weight loss, pilling, tear strength, and color) of woolen fabric, so that the effect of both enzymes will be compared.

2. Material and methods

2.1. Screening of potent protease-producing Bacillus sp

Bacillus sp. isolates from soil were screened for protease activity on the skim milk agar plates. Protease activity was observed from the zone of hydrolysis observed on agar surface. The diameter of clear halo zone around colonies was measured by ruler in mm. The best protease producing strain was selected for further experiments. Commercial enzyme was obtained from a biochemical plant (ORBA Biochemistry, Istanbul).

2.2. Taxonomic identification of Bacillus sp. strain

For the bacterial identification and phylogenetic analysis, genomic DNA was extracted from the chosen *Bacillus* sp. strain using the Qiagen Blood & Tissue kit (Qbiogene, Montreal, PQ, Canada) according to the manufacturer's protocol. The 16S rRNA gene amplification was performed using the universal primers 27 F:5′-AGAGTTTGATCCTGGCTCAG-3′and 1492 R:5′-ACGGCTACCTTGTTACGACTT-3′. The amplified sequences were determined by an automated ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). The resulting sequences were compared with the GenBank database (NCBI) using BLAST (Altschul, Gish, Miller, Myers & Lipman., 1990). The 16S rRNA gene sequence of the strain was aligned with those of *Bacillus* species by using the CLUSTAL W program (Thompson, Higgins, & Gibson, 1994). The phylogenetic analysis was done using the MEGA 6.0 software (Tamura, Dudley, Nei, & Kumar, 2007), and the tree obtained via the neighbor-joining method was analyzed for related organisms (Saitou & Nei, 1987).

2.3. Protease production

Protease production was carried out in a medium containing the following (% w/v): glucose-0.1, peptone-1, yeast extract-0.02, $MgSO_4$-0.01, $CaCl_2$-0.01, K_2HPO_4-0.05 (pH 7.0) (Quadar, Shireen, Iqbal, & Anwar, 2009). Glucose was sterilized separately and aseptically added to the flasks containing the liquid medium after cooling. The precultures were cultivated in Nutrient Broth medium (0.8% w/v) for 18 h. Then, overnight cultures with OD600 = 0.3 were inoculated at 1% in enzyme production media (150 mL in 500 mL Erlenmeyer flasks) and incubated at 37 °C for 16, 24, 40, 48, 64, and 72 h in a shaking incubator (150 rpm). At the end of each period, the cultures were centrifuged (6000 rpm, 10 min), and the supernatants were used to

Table 1. Fabric specifications.

Raw material	Areal density (g/m^2)	Ends/cm	Picks/cm	Count in Nm	
				Warp yarn	Weft yarn
100% Wool	150	37	31	44.5	44.8

determine the proteolytic activity. Bacterial biomass was determined by measuring the optical density at 600 nm.

2.4. Enzyme assay

The total protease activity was measured using a casein substrate by a modification of the Anson Method (Keay & Wildi, 1970). First, 1 mL of the culture supernatant was mixed with 1 mL of0.05 M phosphate buffer-0.1 M NaOH (pH 7.0 adjusted with phosphoric acid) containing 2% casein and incubated for 10 min at 37 °C. The reaction was stopped by adding 2 mL of 0.4 M trichloroacetic acid. After standing for 30 min at room temperature, the precipitate was removed by centrifugation, and the optical density of the assays was measured at 660 nm. A standard curve was generated using solutions of 0–60 µg/mL tyrosine. One unit of protease activity was defined as the amount of enzyme required to liberate 1 µg/mL tyrosine under the experimental conditions used.

2.5. Raw enzyme characterization

The effects of pH, temperature and the presence of various metal ions on the protease activity were determined. The enzyme samples were incubated for 30 min between 35 and 80 °C. The optimum pH for the enzyme was determined using the following buffers: 0.1 M glycine-HCl (pH 2.0 and 3.0), 0.1 M sodium acetate (pH 4.0–6.0), 0.1 M Tris-HCl (pH 7.0 and 8.0), and 0.1 M glycine-NaOH (pH 9.0 and 10.0).

The enzyme sample was incubated with some metal ions and potential inhibitors at the concentrations of 1 and 5 mM. The relative activities were expressed as percentages of the activity of the untreated control.

2.6. Partial purification of enzyme and its wool application

Three different partial purifications were done. In one of them, the raw enzyme was lyophilized for 48 h at −50 °C in a freeze-dryer (CHRIST Alpha 2-4 LD plus) to obtain a concentrated supernatant, and the lyophilized sample was then dissolved in distilled water. The sample was then dialyzed for 6 h with distilled water at 4 °C. Its protease activity was determined, and this sample was called as 'lyophilized enzyme (LE)'. In another partial purification, the raw enzyme was concentrated with ultrafiltration (Centriprep Centrifugal Filter Unit with Ultracel-10 membrane, MW cut-off 30.000 and 10.000). The concentrated solution was dialyzed against distilled water, and its protease activity was determined, and this sample is called as 'ultrafiltrated enzyme (UE)'. In the third purification, crude protease enzyme was precipitated with ammonium sulfate and dialyzed. The protease activity was determined and this sample is called as 'precipitated enzyme with ammonium sulphate (PEAS)'. Commercial enzyme (CE) was compared with these enzymes treatments.

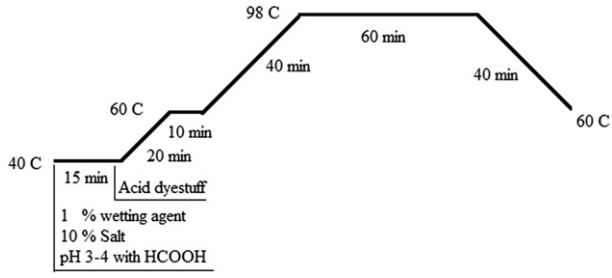


Figure 1. Dyeing diagram for wool fabric with acid dyestuff.

2.7. Wool material

The wool fabric used in all the trials was supplied by Yünsa fabric factory (Turkey). The specifications of the wool plain weave fabrics used in this investigation are given in Table 1. The raw wool fabric samples (50 cm × 50 cm) were used for the study.

2.8. Enzymatic treatments and wool pre-treatments

Protease treatments of wool fabric were carried out at 55 °C and pH 7, with 6 mL/L of protease at a liquor ratio of 25:1 for 1 h. The protease-treated wool fabrics were dipped in a rinsing bath at 90 °C for 5 min to inactivate the proteolytic enzyme and then dried at 40 °C.

2.8.1. Pre-scouring

The wool fabric was first scoured in 3 mL/L non-ionic detergent and 0.5 mL/L wetting agent at a liquor ratio of 25:1 for 20 min at 50 °C. A second scouring process was applied in 3 mL/L non-ionic detergent, 0.5 mL/L wetting agent and 3 mL/L bleaching agent in order to eliminate the impurities at a liquor ratio of 25:1 for 20 min at 50 °C.

2.8.2. Bleaching

Bleaching was performed for 1.5 h at 70 °C pH 7.5 with ammonia in a bath of H_2O_2 (50%) 20 ml/l, wetting agent 1 g/L, stabilizer 1 g/L, sodiumtripolyphosphate 1 g/L. The bleached fabric was washed four times (70 °C, 50 °C, cold water, and neutralization).

2.8.3. Dyeing

The wool fabrics were dyed with acid dyestuff. The dyeing diagram is shown in Figure 1. The treated wool samples were dyed in a blue shade with acid dye.

After these processes, some measurements (such as SEM and energy-dispersive X-ray spectroscopy (EDX) analyses,

weight loss, pilling test, tear strength test, color measurements) were applied to the wool samples.

2.9. Test methods

2.9.1. Scanning electron microscope and energy-dispersive X-ray spectroscopy analyses

To determine the extent of fibre damage and any changes to the external surface of the fibre, micrographs were taken using SEM of wool samples. The samples were analyzed by using SEM and energy-dispersive X-ray spectroscopy (EDX) by using ZEISS/EVO 40 electron microscope.

2.9.2. Weight loss analysis

The weight loss of the fabric after different treatments was determined. The fabrics were subjected to a process under the load of 9 kg in the Martindale abrasion tester. After 5000 and 10,000 cycles, the weight loss of the fabric was calculated as follows (Montazer & Ramin, 2010);

$$\text{Weight loss } \% = \frac{(\text{initial weight} - \text{secondary weight})}{\text{initial weight}} \times 100$$

2.9.3. Pilling analysis

The measurements were performed using the pilling box equipment and the standard of TS EN ISO 12945-1.

2.9.4. Tear strength analysis

The effect of the protease enzyme on the tearing strength of the wool fabric was measured by the tear strength test. Tear strength was determined according to the Elmendorf method. BS EN ISO 13937-1 standard is based on this test.

2.9.5. Color measurements analysis

The ΔE values, L, a, b values, whiteness and yellowness indexes of untreated and enzymatic treated wool fabrics were investigated by a spectrophotometer (Konica Minolta CM-3600 d, Japan) to determine the effects of different purification methods on the changes in ΔE, whiteness and yellowness values.

Results presented in this study are means of three independent determinations.

3. Results and discussion

3.1. Isolation and identification of protease-producing *Bacillus sp.* from soil

Overall, 343 *Bacillus* sp. strains isolated from soil samples were determined on the basis of morphological and biochemical characteristics (Table 2).

The proteolytic activities of all strains were assayed using skim milk agar and exhibited as the diameter of the clear zone in mm. In total, 71 *Bacillus* sp. strains were found to be extracellular protease producers. One isolate named *Bacillus* sp. E6-5, which displayed the largest clearing zone (10 mm), was selected (Figure 2). A phylogenetic tree based

Table 2. Morphological and biochemical tests for identification of *Bacillus* genus.

Test	Result
Shape	Rods
Gram staining	+
Spore formation	+
Motility	+
Starch hydrolysis	+
Catalase	+
Indol Production	−
Nitrate Reduction	+
Gas Production from Glucose	−
Acid Production from Glucose	+

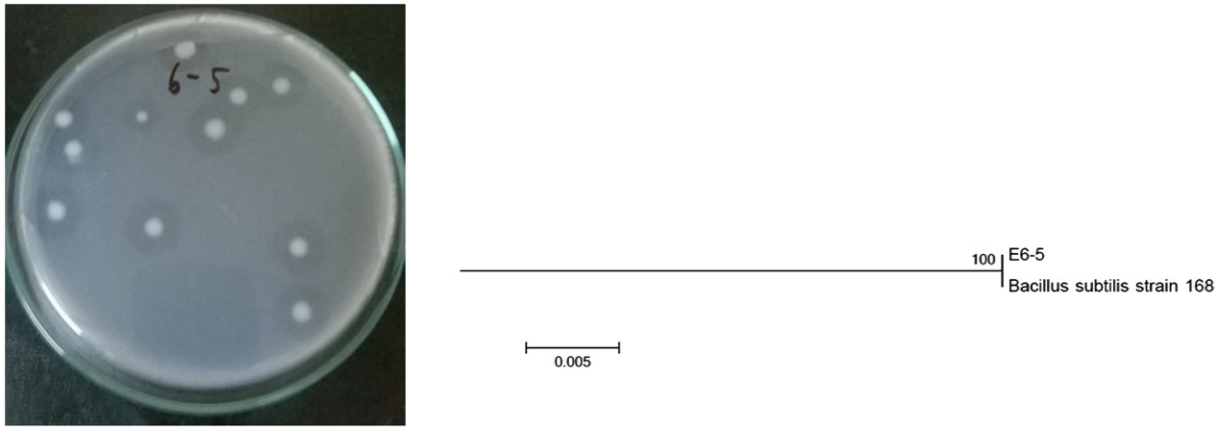


Figure 2. Clear zone by protease-producing *Bacillus* sp. E6-5. Phylogenic relationship of the 16S rRNA sequences of *Bacillus* sp. E6-5 with other type strains of *Bacillus*. Bar, 0.005 substitutions per nucleotide position.

on the 16S rRNA gene sequences from the *Bacillus* strains showed that the new isolate E6-5 shared 100% sequence identity with *Bacillus subtilis* strain 168 E6-5 (Figure 2). Thus, it was named *Bacillus subtilis* 168 E6-5 and checked by using a quantitative test of protease in liquid media. The enzyme activity was found to be 376 IU/mL at 48 h (Figure 3).

The raw protease enzyme was characterized. The activity of the enzyme was optimal at 55 °C and pH 8.0 (Figure 4). The protease activity was accelerated by inorganic compounds (Figure. 5). 5 mM concentration of inorganic compounds was more effective than 1 mM. Zn^{2+} stimulated protease activity of 14%. In most cases, the zinc ion is an essential cofactor for the observed biological function of metalloenzymes such as proteolysis (Butler, 1998). A catalytic zinc ion is located at the active site of an enzyme, where it participates directly in the catalytic mechanism, interacting with the substrate molecules undergoing reaction. This enzyme may be Zn-metalloprotease.

3.2. Enzyme activity measurements

The maximum enzyme activity value was obtained with these purification steps. The enzyme activity immediately after ultrafiltration was 1250 IU/mL; after ammonium sulphate precipitation, it was 523 IU/mL; and after the lyophylization and dialysis steps, it was 2000 IU/mL. The activity value of commercial protease enzyme was 405.200 IU/mL. To compare the commercial enzyme and the enzyme that was produced and purified using different methods, this value was diluted to an activity of 2000 IU/mL.

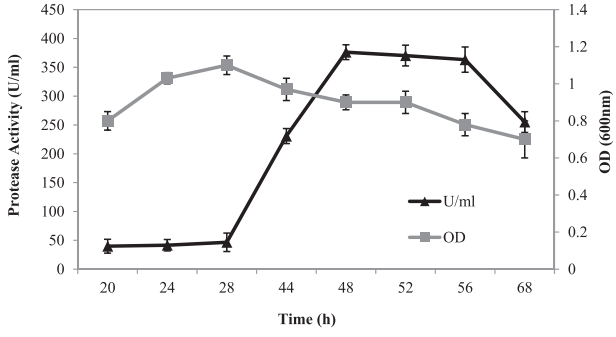


Figure 3. Production of protease by *Bacillus subtilis* 168 E6-5 with respect to time (h).

3.3. Scanning electron microscope and energy-dispersive X-ray spectroscopy analyses

Figure 6 shows SEM images of wool fabrics treated with protease enzymes produced by different purification methods and commercial protease enzyme, It did not showed any damage for all samples treatments, and no significant difference in the scale layer on surface of the wool fabric was observed. Furthermore, dissolution of the structure, such as fibrillary rupture, was not seen.

Smith and Shen (2011) reported the similar results with esperase, all SEM images showed that the wool fibres were still intact and were not severely damaged by the shrink-resist treatment in the presence of 3 μ/g native esperase. In another study with protease the SEM images of untreated and different treated wool fibres confirmed significant differences of effectiveness of enzymes and it is evident that noticeable damage and complete destruction of several fibres was caused after longer incubation time (180 min) (Jus et al., 2007). It was reported that the fibre destruction is caused by the diffusion and the hydrolytic attack of the native proteases onto non-keratinous parts of the protein fibre (Heine & Höcker, 2008; Rao, Tanksale, Ghatge, & Deshpande 1998; Shen et al., 1999; Heine et al., 1998).

Pooja and Fatima (2014) found that while the untreated wool fibre was rough and scales were sharp, the treated wool fibre surface scales got blunt and the smoothness of

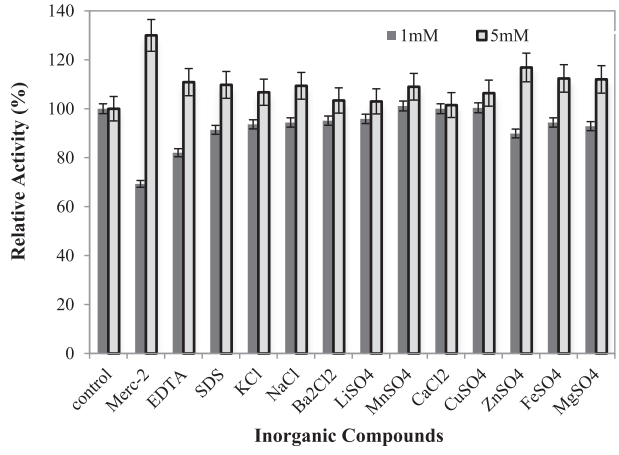


Figure 5. Effect of different metal ions and chemical reagents on relative activity (%) of the partial protease from *Bacillus subtilis* 168 E6-5.

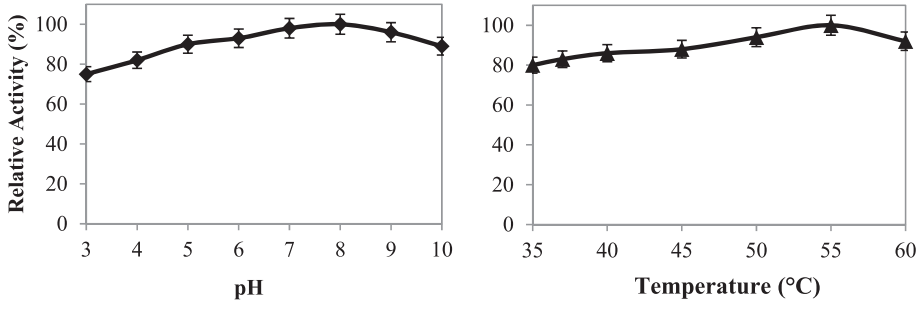


Figure 4. Effect of pH and temperature on protease production by *Bacillus subtilis* 168 E6-5.

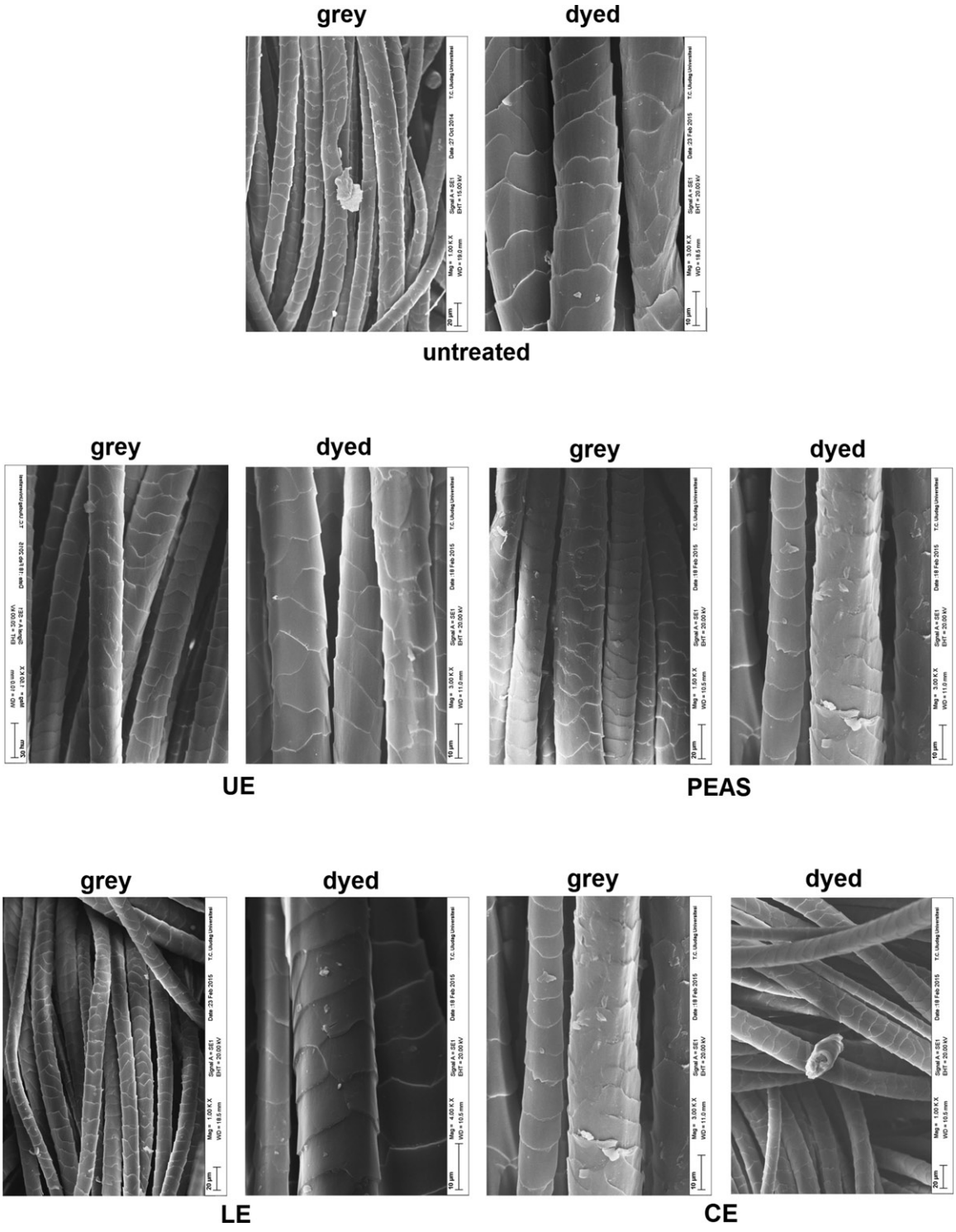


Figure 6. SEM micrograph for wool fabrics after enzymatic treatments and dyeing process.

Table 3. EDX results after the enzymatic treatments applied on wool fabric.

	EDX Analysis Atom.C (at. %)						
Treatments	EL AN	O 8	N 7	S 16	C 6	Aua 79	Pda 46	Total
Untreated (Control)		48.73	31.67	2.28	15.28	1.62	0.43	100.00
Treated with UE		47.70	33.55	2.33	13.70	2.06	0.66	100.00
Treated with PEAS		48.83	31.48	2.27	15.85	1.24	0.34	100.00
Treated with LE		39.05	37.58	3.24	12.94	5.10	2.09	100.00
Treated with CE		38.02	36.30	3.97	14.22	5.63	1.85	100.00

[a]Au and Pd element is used for coating the wool fabric.
CE: commercial enzyme; LE: lyophilized enzyme; PEAS: precipitated enzyme with ammonium sulphate; UE: ultrafiltrated enzyme.

Table 4. Results of weight loss measurements on wool fabrics treated with protease enzyme obtained by different purification methods.

	Undyed fabric weight loss (%)		Dyed fabric weight loss (%)	
Types of treatments	5000 cycles	10,000 cycles	5000 cycles	10,000 cycles
Untreated (Control)	3.13	3.58	3.14	4.33
Treated with UE	2.95	2.95	2.40	3.36
Treated with PEAS	3.01	4.02	2.90	3.73
Treated with LE	1.41	3.30	2.69	4.48
Treated with CE	1.44	2.4	2.84	3.79

CE: commercial enzyme; LE: lyophilized enzyme; PEAS: precipitated enzyme with ammonium sulphate; UE: ultrafiltrated enzyme.

surface was increased in high concentrations of enzyme and showed an improvement in the comfort factor of wool.

EDX was used to characterize the elemental composition of treated fabrics (Table 3). It is clear that, several elements which were built-in wool fabric were detected: Oxygen, Nitrogen, Sulphur, Carbon depicted in various ratios of different enzyme treated and untreated wool fabric. It was shown in EDX analysis values of LE and UE appear to be similar with commercial enzyme and confirm the effectiveness on wool fabric. Additionally the contrast between the values of raw wool fabric and enzyme treated support this effect.

Osman et al. (2014) found several elements (sulfur, silicon, calcium, iron, copper and zinc) depicted in different areas of the examined ancient fibers; sulfur, silicon and calcium were detected in large quantities. Nazari (2017) subjected the protease treated wool fabric to citric acid and Fe_3O_4 nanoparticles to enhance tensile strength, elongation, alkali resistance of the fabric and in order to characterize the elemental composition of treated fabrics EDX analysis was performed. EDX results were confirmed that the enzymatic wool fabrics could be modified by citric acid and Fe_3O_4 nanoparticles post-treatment.

3.4. Weight loss

In raw wool fabrics treated with protease enzymes obtained by different purification methods, weight loss was most observed in PEAS samples. Weight loss was also measured after dyeing with acid dyestuff. The most weight loss in wool fabric was found with the enzyme obtained from the end of LE. A protease enzyme under suitable conditions can make the hydrolysis of wool fabric. Due to the penetration of the enzyme into the internal layer of the wool may be losing weight after enzymatic hydrolysis. The activity value of the LE enzyme was higher than the other methods (Table 4).

Before enzyme treatment while 3.58% weight loss occurred in the raw fabric in 10,000 cycles, after treatment with ultrafiltration 2.95% weight loss, after ammonium sulphate precipitated enzyme treatment 4.02%, after lyophilized 3.30% weight loss and after commercial enzyme treatment 2,4% weight loss were obtained. One of the main purposes of enzymatic treatment is to remove short fibers on fabric structure and to remove the pilling problem that may occur during use. The weight loss of LE treated fabric was reduced comparable to the raw wool fabric. Since the enzyme had an effect on short fibers of fabric were removed, the after-friction the reduction of weight loss was in lesser rate. This result is parallel to the result obtained with commercial enzyme.

Mojsov (2017) showed that increasing the concentration of proteases and longer the time of proteolytic treatment, lead and increases loss of weight loss. As the weight loss increases, the tensile strength loss increases, which is due to the higher hydrolysis of protein chains occurring with higher concentrations of proteases.

In another study, the weight loss of wool treated with *Bacillus* sp. G51 proteases did not differ significantly from the control and this low weight loss of wool could be related with the moderate incubation conditions such as pH and temperature. (Iglesias, Sequeiros, Garcia, & Olivera, 2017) After wool treatment with native proteases from *Bacillus lentus* and *B. subtilis*, Jus et al. (2007) reported an increase in weight losses (4.79 and 14.67%, respectively, after 180 min incubation). High weight loss values have been associated with the diffusion of proteases inside the wool fiber causing undesirable hydrolysis and strength loss (Fu et al., 2015). It was reported that commercial processes aim for a 3–6% weight loss after enzymatic hydrolysis (Jovančić, Jocić, & Dumić, 1998).

In another study slight weight loss (6.31%) and smoother surface scales were obtained after enzymatic treatment (Pooja & Fatima, 2014). Montazer and Ramin (2010) found that the concentration of enzyme increases, the loss in weight also rises, the most changes in loss of weight occur for concentrations of 2–3% and an increased concentration of the enzyme has a considerable effect on the loss of weight. Partial hydrolysis of wool fibres by proteases can result in high strength and weight losses (Bishop, Shen, Heine & Hollfelder, 1998; Riva, Cegarra, & Prieto, 2008; Shen et al., 1999)

Montazer and Ramin (2010) found that increasing the concentration of proteases leads to a decrease in the tensile strength, which is in accord with the results of weight loss.

3.5. Pilling

The pilling results of wool fabric were investigated after applying the enzyme, and commercial enzyme. Figure 7 shows the results after a rotation of 30.000, as measured with the pilling box, and compares these results to the standard photos. Experimental fabrics were compared with standard photos at 5000 rotation intervals until reaching 30.000 rotations. In the fabric without any enzyme application, the pilling increased with the increasing rotational value. The pilling is mainly due to fibres with very high

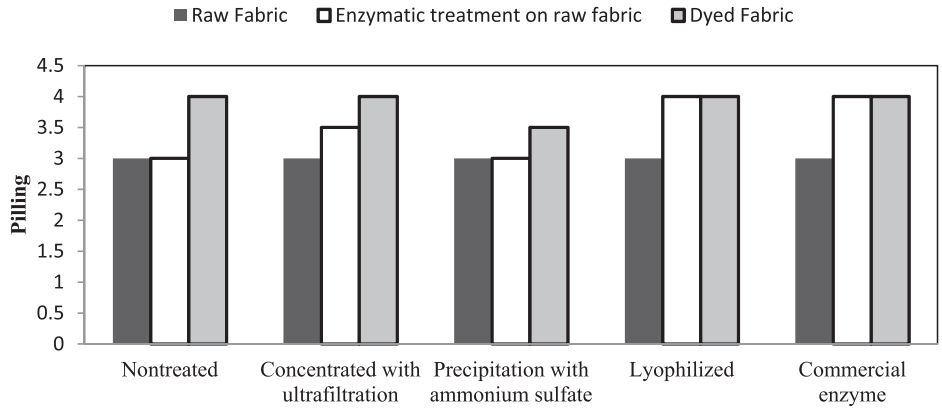


Figure 7. The pilling measurements of wool fabric after the rotation of 30.000.

lateral strength or bending strength or low brittleness. Pills don't form where fibers with low lateral strength such as wool, they can be easily scrubbed off.

As the cycle increased in the untreated woolen fabric, in the rate of pilling was increased. On the other hand, in the dyed fabric, there was no significant effect due to the rotational increase. This can be due to the short fibers that were not retained and shredded through the applied processes (washing, bleaching, and dying steps); therefore, pilling value might stay the same. The same healing effect was not seen in the pilling test results of the samples treated with PEAS. Both the enzymatic processes, the pretreatment and dyeing processes remove the short fibers on the surface, with mechanical effects applied to the fabric; therefore, no variance in the pilling values was observed. LE pretreatment on wool fabric improved pilling resistance. This is because of the higher activity value of LE than UE and PEAS samples that were used in treatments of wool fabric. This result shows that bacterial enzyme that was produced from *Bacillus subtilis 168 E6-5* and lyophilized shows remarkable effect on wool fabric similar to commercial protease enzyme and removes short fibers, significantly reducing the amount of fiber that can be transported to the surface (Figure 7).

Pooja and Fatima (2014) found the lowest concentration of enzyme (1 g/l) shows no pilling and the highest concentration (5 g/l) of enzyme showed moderate pilling. This suggests the idea of the pilling tendency depends on the increase in concentration of enzyme and the activity that was used in treatments of fabric, and may be due to weakening of fibres as proteolytic enzymes damage the surface cuticle and cortex region up to some extent. Enzyme can penetrate into the core of the fibres, and cause damage to the cortex region apart from damaging the surface cuticle (Dantyagi, 2006). Montazer and Ramin (2010) found that the pilling rate decreased with the enzymatic process and noted that the increased percent of enzyme concentration is inversely associated with the formation of pills, however, the higher the concentration of the enzyme, the more the weight and strength of the fabric decreases; therefore, they preferred 1% of the enzyme as the optimum concentration.

It was investigated the use of enzymes to reduce shrinkage (Cardamone & Damert, 2006), soften the product for handle improvement (Silva et al., 2005), increase pilling fastness (Silva et al, 2005; Cardamone & Damert, 2006), as well as improve the course and effectiveness of the dyeing and bleaching processes.

Table 5. Tear strength results on weft and warp direction for wool fabric.

	Warp direction(N)		Weft direction (N)	
Types of treatments	Grey fabric	Dyed fabric	Grey fabric	Dyed fabric
Untreated (Control)	44.14	43.05	50.92	47.80
Treated with UE	43.53	43.49	49.49	46.71
Treated with PEAS	43.91	43.12	49.51	44.07
Treated with LE	42.60	42.83	47.47	46.03
Treated with CE	43.47	42.74	47.81	46.63

CE: commercial enzyme; LE: lyophilized enzyme; PEAS: precipitated enzyme with ammonium sulphate; UE: ultrafiltrated enzyme.

3.6. Tear strength

Proteases can be used in environmentally friendly alternative processes for partial removal of cuticles (El-Sayed, Kantouch, Heine & Hocker., 2001; Heine & Höcker, 2008) or smoothing the edges, and in the last few years many studies on enzymatic improvement have been undertaken (Schroeder et al., 2006). However, partial hydrolysis of wool fibres by proteases can result in high strength and weight losses (Bishop et al., 1998; Riva et al., 2008; Shen et al., 1999).

In this study, after the purification processes of the protease enzyme, it was applied to the raw fabric, and a decrease was obtained in both the weft and warp directions. The decrease in the tear resistance of wool fabric after enzyme applications was compared with the purification methods applied, and the test results showed that the tear strength of all kind of enzyme treated wool fabric was reduced compared with untreated fabric (Table 5). However, it is obvious that the LE and commercial enzyme application has parallel effect. One of the most important reasons for preference for enzymes in textile applications is that it only affects the desired point and does not cause significant deformation or distortion in other parts (Mojsov, 2011). Our experimental study results also supported this approach.

Kotlińska and Lipp-Symonowicz (2011) suggests that enzymes are widely used in wool sector to improve the colour, shrinking behaviour, dyeing affinity, pilling behaviour,

Table 6. The whiteness and yellowness index values of the wool fabric with treated with different purified protease enzyme and nontreated.

Types of treatments	Stensby (Whiteness Index)	ASTM D1925 (Yellowness Index)	ΔE values
Untreated (Control)	49.360	24.158	–
Treated with UE	50.337	23.494	0.838
Treated with PEAS	49.689	23.960	1.317
Treated with LE	48.509	24.079	0.588
Treated with CE	48.664	24.099	0.402

CE: commercial enzyme; LE: lyophilized enzyme; PEAS: precipitated enzyme with ammonium sulphate; UE: ultrafiltrated enzyme.

and tensile strength. Since proteases are generally small enzymes, they are able to penetrate into the fibre easily and destroy the cortex, resulting in reduced tensile strength (Schroeder et al., 2006). It was found that the increase in the concentration of protease led to a decrease in the tensile strength in both warp and weft direction which was in accordance with the results of weight loss. As the weight loss increases, the strength decreases, due to the higher hydrolysis of protein chains occurring with higher concentrations of proteases (Pooja & Fatima, 2014). Montazer and Ramin (2010) showed that with an increasing enzyme concentration, the decreasing percent of the strength began to rise. The most changes in strength decline took place within a range of 2–3%. Increasing the concentration of proteases leaded to a decrease in the tensile strength, which is in accord with the results of weight loss.

3.7. Color measurements

The raw wool fabric was treated with enzymes that had been purified by various processes, and the whiteness and yellowness index values were measured. The whiteness value of the fabric treated with the UE was increased, whereas the whiteness value fabric treated with LE was decreased (Table 6). The EDX results indicate that both the decrease in the oxygen atom percentage and the increase in the sulphur and nitrogen level affect the degree of whiteness and yellowness (Table 3). Values of less than 1 are considered acceptable color difference values in color difference measurements after processing in commercial applications. Among the purification methods, in terms of color difference (ΔE), the best result was obtained with LE application. This value is very close to the value obtained with commercial enzyme.

Montazer, Pajootan, and Lessan (2012) found that samples treated with proteases were more whitened than raw fabric and the more a product is affected by the enzymatic concentration and period of time of the process, the more the fabric was whitened for the product treated was 2% for an operating period of 2 hours process.

4. Conclusion

Enzymes provide the desired effect on wool fabric without harming the material structure of the fabric. Hence, Proteinase-based treatments had a positive effect on fabrics and it is potential alternative to chemical treatments. The effects of protease from novel isolated *Bacillus subtilis* 168 E6-5 and commercial protease treatments of wool fabrics were compared and, *Bacillus* protease enzyme yielded improvements in the physical properties of wool fabric. It may be suitable for potential textile and other industrial applications.

Disclosure statement

No potential conflict of interest was reported by the authors.

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