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# Influences of Proteases and Trans-glutaminases on Wool

**Abstract**

*In this study wool fabrics were treated first with proteases (Savinase 16L) at different temperatures over various periods of time. The samples were then tested for some mechanical and physical properties. To overcome the strength loss of the fabric as a result of the protease treatment, the after-treatment with trans-glutaminases was examined. The results show that the felting behaviour of the proteases treated wool was improved and the degree of whiteness enhanced. Furthermore, the pilling performance of the samples was also improved; however, such treatment can cause irreversible and excessive damage to the fibre cuticle, with consequent high levels of weight and strength loss. The dyeing of untreated and enzyme treated wool with madder leads to an improved dyestuff uptake and a distinctive difference in the colour shade of the latter. In addition, treatment with T-Gases leads to a significant increase in tensile strength and may compensate for the damage caused by protease.*

**Key words:** *enzyme, mechanical and physical properties, dyeing, proteases, trans-glutaminases.*

## Introduction

Enzymes are biological compounds with protein structures. They add to the acceleration of chemical responses by reducing the activation energy to a minimum. The necessity of using more environmentally friendly processes has led to the replacement of conventional chemical treatments with enzymatic ones. In the case of wool fiber, there have been attempts to substitute conventional chlorine treatment with an enzymatic process capable of providing the fabric with the same characteristics (1, 2). In addition, this process can be carried out in mild conditions (T = 50 - 60 °C and pH = 5 - 8) (1).

The enzymes used in the textile industry are often with hydrolytic responses, creating properties such as anti-felting, an increase in absorption, a reduction in shrinkage, a decrease in dead fibers, an increase in whiteness properties and so on (3 - 7). The cleaning of protein stains is another property of the proteases enzyme.

The major characteristic of the enzymatic process with proteases is a hydrolytic attack that is not limited to the surface of fibers. The enzyme penetrates the fibers, causing the fabric strength to decrease undesirably. Thus, controlling hydrolysis with proteases plays a very important role in the enzymatic finishing of wool. However, the enzyme process with proteases bring about a decline in the strength of fibers.

The results of investigations show that Trans-glutaminase is a family of amino acid transferase which accelerates the acyl transfer between glutamine with peptides linkages and suitable primary amine. In most cases it brings about the formation of cross-link proteins. Therefore, if the Trans-glutaminase enzyme is used onwool under a temperature of 37 °C and pH = 8 alone or after proteases treatment, it will contribute to the rise in fabric and wool yarn strength (8, 9).

This study reports some changes brought about in wool fabrics due to the enzymatic process with proteases for different time periods and concentrations with respect to the strength, loss of weight, felting, solubility in alkaline, pilling, abrasion and absorbed drop. It also reports interesting results regarding, in particular, changes in abrasion with proteases concentration. Moreover, protease enzymes were studied in dyeing with madder with respect to how the absorption of dye increases.

## Materials

The wool yarns and fabric used, provided by the Iran Merinous Co., with their characteristics are indicated in ***Table 1***.

## Chemicals

This study used two types of enzymes: (EC 3.4.21.14) Savinase 16L of Novazyme product and Trans-glutaminase (EC 2.3.2.13), produced by Sigma Co. The first enzyme is of the proteases type and operates in an alkaline medium. It is obtained from the micro-organism "Basillus". The most useful pH for this enzyme is about 8 and the most useful temperature 50 °C. The Trans-glutaminase of a mammalian tissue (tTGase) needs $Ca^{2+}$ which has been isolated from the liver of a guinea pig (GPL). The best pH is about 8, and the best temperature 37 °C.

## Methods

### Enzymatic treatment with proteases

An 80 g sample, once it had been bleached and weighted, was placed in 50 °C temperature and pH = 8 using a 50 mM Tris-HCl buffer and L:G = 20:1 with proteases (***Table 2***).

### Enzymatic treatment with Trans-glutaminases

Woollen samples were treated with enzymes: 5 μg/ml TGase in 50 mM, Tris-HCl and 50 mM Dithiothreitol (DDT) as well as 50 mM $Ca^{2+}$ with pH = 8 at 37 °C for an hour and L:G = 20:1.

***Table 1.*** *Fabric characteristics.*

| Fabric | 100% wool |
|---|---|
| Weight, g/m² | 350 |
| Yarn count, Nm | 30/2 |
| Warp density, 1/cm | 16 |
| Weft density, 1/cm | 15 |
| Twist, t.p.m. | 550 |

***Table 2.*** *Different conditions of the enzymatic process.*

| Number | Enzyme conc., % | Time, min. |
|---|---|---|
| 1 | 0 | 60 |
| 2 | 0.5 | 60 |
| 3 | 1 | 30 |
| 4 | 1 | 60 |
| 5 | 1 | 120 |
| 6 | 1 | 180 |
| 7 | 2 | 60 |
| 8 | 2 | 120 |
| 9 | 3 | 60 |

### Dyeing

The untreated and treated samples with proteases were dyed with madder. Before dyeing, the samples were mordanted with Aluminum sulphate of 20% o.w.f and citric acid of pH = 4.5 and L : G = 40 : 1. The dyeing of mordanted fabric was carried out with madder of 50% o.w.f together with citric acid of pH = 4.5 and L : G = 40 : 1.

### Test methods

#### *Weight loss*

Samples with approximate dimensions of 10 × 10 $cm^2$ and weight of 3 g were left for 1 h at 150 °C and then put in a desiccator for balancing. These processes were done after the enzymatic process on the samples according to ASTM d 2720-94.

The loss of fabric weight can be calculated as follows:

$$\text{Weight loss} = (W_1 - W_2)/W_1 \times 100\%$$

where $W_1$ and $W_2$ are the weight of the fabric before and after the enzymatic process.

#### Alkaline solubility

1 g of the wool sample was put into a baker containing 1000 ml of sodium hydroxide of 0.1 N at 65 °C. After one hour of stirring, the contents were filtered. The filerated materials were dried at 110 °C after washing with distilled water several times. Once it had become cool in the desiccator, the weight reduction was calculated and the solubility in alkaline measured.

#### Water drop absorption

In order to measure the degree of drop absorption on the fabric before and after the enzymatic process using a dropper, the time for water absorption on the fabric was measured. To do this, we put the fabric wide open on the surface of a glass plate and dripped drops of water on it within a height of one cm. This was done 20 times, and the average time of drop absorption was reported.

#### Tensile strength

A Shirley Micro 250 was used to measure the tensile strength of the samples. Thus, samples of dimensions such as 20 × 5 cm were constructed. The speed was set at 1m/min and measurement was conducted under laboratory conditions in a warp direction. The method was CRE according to ISIRI 1147-1.

#### Felting

To do this, the samples were first marked 10 × 10 cm. Then they were subjected to milling under laboratory conditions in a soap bath of 1% with L : G = 40 : 1 for 45 minutes at 50 °C.

#### Fabric pilling

By using a Pilling Box, the samples' pills were investigated. This measurement was made at 20,000 r.p.m (ASTM D3514 – 09).

#### Fabric abrasion

In order to examine surface changes in the woollen fabrics and observe the amount of abraded fabric using a Martindale 2000 with a 12 kpa weight, the fabric surface was checked up to two warps or wefts to tear, and the number of abraded cycles were reported.

#### Dye absorption measurement

The level of dye absorption on the samples was obtained by measuring the level of reflectance and other functions. This was done using a spectrophotometer device (Data colour 98).

## Results

### Results of proteases enzyme concentration

***Table 3*** shows the effects of the proteases enzyme for 0.5, 1, 2 and 3% concentrations on the weight loss, strength loss, solubility in alkaline, felting, abrasion and drop absorption.

#### Weight loss

As ***Table 3*** shows, as the concentration of enzyme increases, the loss in weight also rises. The most changes in loss of weight occur for concentrations of 2 - 3%. Increasing the concentration of the enzyme has a considerable effect on the loss of weight. A protease enzyme can, under suitable conditions, make the hydrolysation of woollen fabric possible. Hence, an increasing rate of enzyme concentration causes an increase in the loss of weight of woollen fabric due to the penetration of the enzyme into the internal layer of the wool (Cortex).

#### Tensile strength

***Table 3*** shows that with an increasing enzyme concentration, the decreasing percent of the strength begins to rise. The most changes in strength decline take place within a range of 2 - 3%. Increasing the concentration of proteases leads to a decrease in the tensile strength, which is in accord with the results of weight loss. As the weight loss increases, the tensile strength decreases, which is due to the higher hydrolysis of protein chains occurring with higher concentrations of proteases. With a higher hydrolysis, the length of polypeptide chains in the proteins of wool decreases, and a lower energy is needed for the separation of chains and breakage of the fabric.

#### Felting

***Table 3*** highlights that samples have improved felting shrinkage with an increase in enzyme concentration. The least degree of changes in the following concentration was below 2%, and the more the concentration of enzyme increases, the more the felting percent decreases. Proteases can be used to reduce the felting shrinkage of woollen textiles through hydrolysing cuticle cells in wool, thereby reducing the coefficient friction between fibers. This makes fibers easy to move in relation to each other and decreases felting, which can be due to the removal of the edges of scales from wool fiber surfaces.

#### Alkaline solubility

As ***Table 3*** shows, the more concentrated the enzyme, the more the percent of weight loss rises in the dissolving of

***Table 3.*** *Effect of proteases concentrations on some fabric properties.*

| Samples | Weight loss, % | Tensile strength, N | Felting shrinkage, % | Abrasion, r.p.m. | Alkaline solubility, % | Water drop absorption, min |
|---|---|---|---|---|---|---|
| Untreated (Bleached) | 1.1 | 573.3 | 7.4 | 2065 | 5.6 | 66 |
| Treated without proteases (blank) | 1.4 | 566.0 | 7.3 | 2015 | 6.1 | 58 |
| Treated with 0.5% proteases | 2.0 | 551.4 | 5.9 | 2340 | 7.1 | 42 |
| Treated with 1% proteases | 3.1 | 532.7 | 4.4 | 2850 | 8.4 | 35 |
| Treated with 2% proteases | 4.3 | 511.5 | 3.9 | 1812 | 11.2 | 20 |
| Treated with 3% proteases | 5.5 | 468.9 | 3.6 | 1600 | 11.3 | 18 |

*Table 4. Effect of the treatment time of wool with 1% proteases on fabric properties*

| Time of enzymatic process, min | Weight loss, % | Tensile strength, N | Felting shrinkage, % | Abrasion, r.p.m. | Alkaline solubility, % | Water drop absorption, min |
|---|---|---|---|---|---|---|
| 0 | 1.0 | 573.8 | 7.3 | 2065 | 5.5 | 67 |
| 30 | 2.2 | 540.4 | 5.4 | 2410 | 7.2 | 40 |
| 60 | 3.1 | 532.7 | 4.4 | 2850 | 8.4 | 35 |
| 120 | 5.2 | 478.8 | 4.1 | 1925 | 10.5 | 29 |
| 180 | 7.0 | 452.5 | 3.1 | 1637 | 11.1 | 27 |

*Table 5. Colour coordinates of raw and 1% proteases pre-treated wool dyed with madder.*

| Samples | L* | a* | b* | C* | h* | DE | Batch is |
|---|---|---|---|---|---|---|---|
| Raw sample | 50.06 | 35.72 | 37.16 | 51.55 | 46.13 | - | - |
| Treated with 1% proteases | 45.69 | 37.56 | 34.52 | 51.01 | 42.59 | 5.43 | darker redder less yellow |

*Table 6. Color indices of different proteases treated wool fabrics.*

| Samples | L* | a* | b* | DE | Batch is |
|---|---|---|---|---|---|
| Raw | 85.08 | -0.07 | 16.66 | - | - |
| Blank | 84.48 | -0.03 | 16.21 | 0.76 | Darker/less yellow |
| 1%-1h | 85.35 | -0.25 | 15.16 | 1.54 | Lighter/greener/less yellow |
| 1%-2h | 85.32 | -0.18 | 15.02 | 1.88 | Lighter/greener/less yellow |
| 1%-3h | 85.31 | -0.23 | 14.80 | 1.67 | Lighter/greener/less yellow |
| 2%-1h | 85.05 | -0.27 | 14.91 | 1.77 | greener/less yellow |
| 2%-2h | 86.18 | -0.78 | 11.89 | 4.98 | Lighter/greener/less yellow |
| 3%-1h | 85.41 | -0.22 | 14.68 | 2.01 | Lighter/greener/less yellow |

woollen fabrics in alkaline, representing a higher solubility of wool in alkaline solution. This means that proteases hydrolyses the polypeptide chains of proteins in wool fibers and reduces the stability of wool against the alkali, which can be a disadvantage of protease treatment. The results are in accord with the those for the tensile strength. As both the tensile strength and alkali solubility are affected by increasing the proteases, this means that some of the linkages between chains of the polypeptide have been broken.

## Abrasion

***Table 3*** shows that the abrasion resistance of wool fabric with at 1% concentration has grown more than that of raw fabric and blank and has reached 2850 r.p.m, but at higher concentrations within a range of 2 - 3%, the percent of abrasion resistance has decreased such that the abrasion rotation reaches 1600 r.p.m at 3%. This can be caused by the further penetration of proteases into the internal layers of the wool (Cuticle) as well as by the hydrolysis of CMC as a result of damage to the fibers and spontaneous degradation of cells.

## Water drop absorption

The results in ***Table 3*** show that once the percent of concentration has increased, the amount of time for the water drop to be absorbed decreases, which means that proteases treatment helps to remove some of the hydrophobic compounds on wool surfaces. Overall, the proteases enzyme can change the surface properties of fabric, such as the moisture and dye absorption.

The results show that the optimum concentration of enzyme to achieve the final properties desired is 1%. Loss of weight within this range is 3%, the decline in strength 10% while the rotation of abrasion increases by about 38%. The shrinkage of the fabric declines by 3% more than that of raw fabric because of its felting property. To account for the changes given above, it can be proposed that the rate of enzymatic hydrolysis is dependant on the enzyme concentration i.e. if the enzymatic concentration increases, there is more enzyme penetration into the fiber, contributing to the irreversible decline in fabric strength. Hence, controlling the enzyme concentration plays a very important role in the finishing of wool.

## Time of the enzymatic process with proteases

***Table 4*** shows the effect of the enzymatic process with proteases for a time period of 30, 60, 120 and 180 minutes.

***Table 4*** shows that adding time to the process tends to increase the percentage of weight loss. It is observed that after 30 minutes, there is a slight slope in the weight loss diagram. Within this limit we face a weight loss of about 1.2%. Afterwards, the weight loss rises if the processing time rises to 60 or 180 minutes. It can also be seen that the longer the process, the more the percent of the strength drops, until it reaches 21% after 180 minutes. ***Table 4*** also shows that the more the time increases, the lower the felting shrinkage will be. The most changes can be observed after 1 to 3 hours, which can indicate that the longer the time, the more the solubility in alkaline increases, reaching its maximum at 180 minutes. The abrasion of woollen fabric increases for samples that are exposed to enzymatic hydrolysis for longer periods of time; tearing also occurs more quickly with additional processing time. Furthermore, an increase in the period of time of the enzymatic process leads to the fabric being able to absorb drops of water more quickly. The best time to achieve an ultimate property is 60 minutes. Time plays a very important role in fabric hydrolysis. The more enzyme there is, the more chance there is for protein destruction, causing loss of weight and the strength to decrease. In any case, with an increase in the time of the process, the strength of the fabric decreases remarkably, the weight of the fabric decreasing simultaneously.

In this section the effect of the enzymatic process on the creation of pills on woollen fabric was investigated. The results show that the pilling rate decreases with the enzymatic process. Of course, it should be 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, 1% of the enzyme is preferred as the optimum concentration.

## Dye absorption and whiteness

Images of untreated samples and those treated with the proteases enzyme, as mentioned in section 'Dyeing process', are shown in ***Figure 1***. As ***Figure 2*** shows, the longer the enzymatic process, the more the natural dye (madder) absorption level rises in the fabric. Likewise, samples treated with proteases are more whitened than raw fabric prior to dyeing. ***Table 6*** shows that the more a product is affected by the enzymatic concentration and period of time of the process, the more the fabric is whitened in such a way that the biggest difference for the product treated is 2% for an operating period of 2 hours process (***Figure 2***).

These results presented in ***Table 5*** entirely correspond to the visual observations, and it can be seen that a sample treated with an enzyme becomes darker than a raw sample after dyeing. Likewise, samples treated with an enzyme (before dyeing) become whiter and brighter than raw samples.

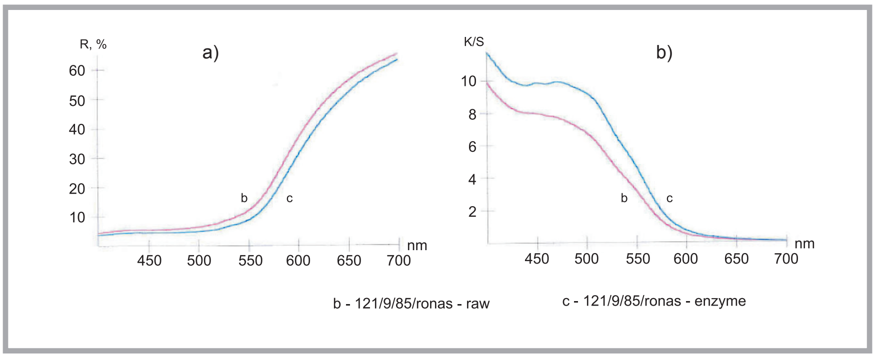


***Figure 1.*** *a) Reflectance of raw and 1% proteases treated wool fabric dyed with madder. b) K/S of raw and 1% proteases treated wool fabric dyed with madder.*

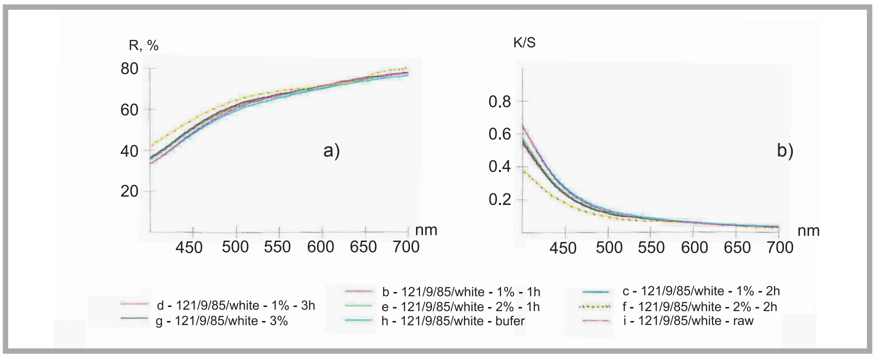


***Figure 2.*** *a) Reflectance of different proteases treated wool fabrics, b) K/S of different proteases treated wool fabrics.*

## Effect of Trans-glutaminases on wool yarns pre-treated with proteases

It is known that proteases can used to decrease the shrinkage and pilling of woollen fabrics through the hydrolysis of cuticle cells; however, these functions are accompanied by the reduced strength of fibers. Hence, the ability of the enzyme to recover the strength of wool with proteases or, in other words, reduce the negative effects on wool has been considered. A sample of yarn was treated with a 1% o.w.f enzyme of Savinase (proteases) processed in a Tris-HCl buffer with 5 μg/ml TGase and 5 mM $Ca^{2+}$ (added for activation of the TGases enzyme) and 5 mM DDT (dithiothreitol). The pH was determined as 8 and the temperature set at 37 °C. One blank sample was treated under the same conditions without TGase. These samples were compared with three other samples including raw wool, wool yarn treated with proteases alone and a blank sample without proteases with pH = 8 at a temperature of 50 °C with a Tris-HCl buffer. All samples were tested for their strength, the results of which are shown in ***Table 7***.

***Table 7.*** *Effect of trans-glutaminases on the mechanical properties and alkaline solubility of raw and different proteases pre-treated wool yarns.*

| Samples | Load at peak, N | Elongation at peak, mm | Strain at peak, % | Energy at peak, N/m | Alkaline solubility, % |
|---|---|---|---|---|---|
| Raw sample | 6.1 | 71.8 | 23.9 | 0.31 | 8.1 |
| Proteases blank | 5.7 | 66.7 | 22.1 | 0.26 | 8.6 |
| Treated with 1% proteases | 4.0 | 26.9 | 8.9 | 0.05 | 13.2 |
| T-Gases blank | 5.6 | 33.7 | 11.0 | 0.11 | 9.3 |
| Treated with T-Gases pre-treated with 1% protease | 5.3 | 58.4 | 19.4 | 0.20 | 10.3 |

As ***Table 7*** shows, wool yarns treated with T-Gases may decrease the disadvantage of the proteases process as the tensile strength property increases. The elongation and strength of samples treated with T-Gases are higher compared with their related control samples. The effect may be the opening up of the wool fiber structure by the proteases, which would facilitate T-Gases cross-linking via ε(γ-glutamine)lysine bridges.

The effect of Trans-glutaminases enzyme on wool yarn pre-treated with proteases was studied. The results showed that the alkaline solubility of treated yarns decreased after both proteases and trans-glutaminases treatment. However the rate of decrease is lower after trans-glutaminases treatment. This indicates an increase in the strength of yarn after the trans-glutaminases treatment. As a consequence, the increase in the lifecycle expected was accompanied by an increase in bonds derived from the function of Trans-glutaminases.

## Conclusion

An increased concentration of enzyme and time of the process have an effect on the level of wool proteolythic hydrolysis, causing the product to lose weight, strength, shrinkage, reduced pilling, increased whiteness and dye absorption. Therefore, in order to prevent wool from severe destruction and to achieve the properties desired, one must select a concentration of the enzyme that results in an acceptable loss of weight and strength. The temperature, pH, and L : G are other influential parameters that influence wool; however, the L : G effect is negligible and can be compensated with other parameter changes, such as the enzyme concentration, temperature and pH.

Removing the surface of scales in the enzymatic hydrolysis process causes pilling to decrease, bringing about an increase in the resistance against abrasion for 1% proteases; therefore, increasing the proteases concentration decreases the abrasion resistance.

The pretreatment of wool with the proteases enzyme results in higher dye absorption with madder. A complete evaluation of the results obtained from this research shows that the optimum condition for the fabric tested is 60 minutes for a 1% concentration of the proteases enzyme with L : G = 20 : 1 below a temperature of 50 °C at pH = 8. When Transglutaminase is applied to bleached wool or proteases pre-treated wool, this results in an increase in strength of the fabric and wool yarn. This result shows that T-Gases can alleviate the negative effects of proteolytic, which reduces the strength of fabric. By considering the solubility of wool yarns treated with T-Gases in alkaline, it is found that the tensile strength of samples increases considerably.

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