Application of enzymes in wool dyeing

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INTRODUCTION

The study on use of enzymes as auxiliary agents in wool dyeing was carried out in the field of a research on environmentally friendly dyeing processes.
In this work the possibility of reducing the temperature of conventional wool dyeing using an enzymatic treatment was studied, in order to reach exhaustion values comparable to those obtained with the standard procedure at 98°C. The aim was to develop a laboratory method that could be transferred at industrial level, reducing the energy consumption and fiber damage caused by the prolonged exposure to high temperature.
In recent years, some researchers focused their studies on enzymes for different reasons. First, the progress of biotechnology found new enzymes very specific for wool substrate and with well-defined activity profiles, which are available at low cost on a commercial scale. Moreover, the pressure from institutions of environmental control on industries to replace conventional processes with others with low environmental impact and low power consumption oriented textile research towards products and treatments based on enzymes.
It is particularly interesting for a wool dyeing process to consider proteolytic enzymes, belonging to the hydrolase class, protease or peptidase subclass, which are able to hydrolyse the peptide bond in proteins and polypeptides constituting the wool fiber.
Wool structure can be represented as a set of compact protein units, cuticolar and cortical cells, surrounded by a keratin cell membrane, all dipped in an intercellular cement and held together by it. The cell membrane consists of a chemically resistant protein layer and a lipidic layer. These two components, together with the intercellular cement, constitute a region known as the “cell membrane complex "(CMC). Then other histological intermediate structures are present between cuticle and cortical cells, very important for the diffusion of molecules between the outside and inside of fiber [1].
Cuticle constitutes a real barrier to the transfer of dye molecules from aqueous solution. Indeed, the wool fibers, for comparison with natural or synthetic fibers with homogeneous texture as cotton or polyamide, show a significant deviation from the theoretical model of dye diffusion in a process of dyeing (Fick’s law), due to that barrier effect.[2] A good wool dyeing process must provide a satisfactory exhaustion of dye bath and an adequate penetration of dye in the fiber, with the practical advantages of a good wet fastness and a uniform colouration.
The conventional methods for wool dyeing are based on long times with temperatures close to dye-water phase boiling point, in order to ensure good results of dye penetration and levelling. These methods can damage the fibers, with bad effects on the characteristics of the finished material.
The extent of the damage that can be present on dyed wool is a function of pH and time-temperature profile of dyeing cycle. When the wool is maintained at temperatures near 100°C in acid ambient for prolonged times, the structure of the fiber is gradually damaged by hydrolysis of peptide bonds. Such damage can be minimized by reducing the contact time or, better yet, reducing the dyeing temperature.

The positive effects of lower temperature on the quality of dyed wool are a lot and also involve economic considerations related to saving energy. This aim must be achieved maintaining the same levels of dye fastness and levelling. In this context, the enzymatic pre-treatment of wool is an important experimentation field.
All known enzymes are large molecules made of globular proteins whose properties are due to the tridimensional structure of their polypeptide chains.[3] They are present in all living cells, where have vital functions, as in the processes of catabolism, anabolism, cell growth and reproduction, energy production and conservation.
Enzymes act as catalysts, lowering the energy level required to ensure that a specific reaction takes place. Instead of inorganic catalysts, enzymes are very specific: in fact they catalyze the reaction involving a single type of chemical bond in a given compound.
Enzymes act in mild operating conditions, usually in a pH range between 5 and 8, at temperature around 30°C at atmospheric pressure. This minimizes secondary reactions, often involved with other types of catalysis, and the formation of secondary products or toxic residues. Furthermore, enzymes are very selective, in fact they play the catalytic role binding the substrate in a specific area of the protein, called catalytic active site, made up of several dozens of aminoacid side chains.
From an environmental point of view enzymes are interesting because they are already active in small doses and highly biodegradable. In the case of wool dyeing, the use of enzymes can reduce the amount of auxiliary agents, usually poorly biodegradable.
For this study, proteases are particularly interesting, because they are able to change the wool fiber structure acting mainly at the level of surface non-keratin proteins and modifying sites of access for dye at the interface between cuticolar cells and CMC, as well as lipases, which can improve the wetting of the fibres degrading part of the outer layer of the esocuticolar membrane.
Some authors have improved the dyeing affinity of wool fiber with a pre-treatment with a protease enzyme of bacterial or vegetal origin, and then with chitosane, or with an enzymatic pre-treatment in oxidizing ambient. It changes the wool fiber surface so as to give antifelting effect and tinctorial affinity even at low temperature.[4,5]
The problems of enzymatic activity conservation with time and temperature as well as of fiber damage due to proteolytic attack are still only partially resolved. The enzyme action must be gradually changed in concentration and possibly directed at the surface of the fibers, to avoid uncontrolled degradation of the CMC and irreversible damage to the fibers as disintegration and fibrillation.
Regarding it, Sawada et al suggested a dyeing process where in the same bath a protease, in a system of reverse micelles, coexisted with the dye, resulting in a limitation of wool fiber damage due to a reduced enzymatic activity. However, the enzyme structure, in the operating conditions adopted, adsorbed the dye molecules and consequently limited their migration on the fiber.[6]
In previous works [7,8] the effect on wool dyeing of several proteases, in commercial formulation, were tested and Multifect Neutral (Genencor) gave the better results in term of bath exhaustion and fiber properties so in this study it was chosen for a deeper investigation.
For each dyeing isotherm, apparent activation energy and adsorption rate constants were determined. The protection of wool fiber properties during the treatments were also investigated. In order to evaluate a possible fiber damage, the dyed samples were subjected to observation by scanning electron microscopy (SEM) and at the same time the loss of tensile strength and elongation were determined. The attack degree of the enzyme was controlled by SDS-Page analysis and even colour fastness tests were carried out.

EXPERIMENTAL

Materials

Dyed material was pure wool yarn 2/79 Nm, previously washed for 10 min in 1g/l solution of surfactant ECE and 10ml/l NH_3 (33%) with manual bath agitation, followed by rinsing first in lukewarm, then in cool water to completely eliminate foam which might affect the uniform migration of dye on the fabric.
The dye chosen was Turquoise Telon M5-G by Dystar. It has a low affinity for wool so it should highlight the enzyme effect on fiber. It is a disolfonate acid dye that presents a maximum absorbance peak at 617nm.
The enzyme used was Multifect Neutral, kindly supplied by Genencor inc, Palo Alto (CA) USA. It is a bacterial protease working at neutral pH, produced by the controlled fermentation of a genetically modified bacillus (Bacillus amyoliquefacens). Its activity is greatest at 40-60°C and its action is effective for pH values from 6 to 8, with a maximum corresponding to pH 7. The enzyme stability starts to fall at 70°C and become nothing at 80°C. The enzyme has an activity equal to 1600 AU/g. Activity in AU/g expresses the enzyme ability to hydrolyse azocasein substrate at pH 7.5 for 5 minutes at 30°C.
Influence of surfactants was tested with Avolan S, by Bayer, while for acidification acetic acid laboratory grade was used.

Dyeing process

The first aim of this study was the determination of exhaustion curves of the dye bath, with and without enzymatic pretreatment, at different dyeing temperatures: 98°C, 85°C, 75°C, 65°C. They were determined with Teintolab apparatus (Comeureg), a dyeing diagnostic equipment that allows the on-line control of the dyeing parameters such as pH, temperature, dye exhaustion.
An amount of wool of 26 g was treated with liquor ratio 1:50, in order to be close to industrial process.
For what concern tests with enzyme, wool yarn was put in 1g/l Multifect Neutral water solution, with a measured pH of 6.4-6.5, under agitation. Bath temperature was 50°C, maintained for 20 minutes, during which the enzyme acted. Then 1% dye o.w.f. was introduced and pH was adjusted to 4 adding the proper amount of acetic acid. In this way enzyme became denaturalised and ambient was favourable for dye exhaustion. Temperature was then raised at the final desired value in 20 minutes and maintained for 65 minutes, always under movement; finally the bath was cooled at 50°C.
With probes in dyeing bath, Teintolab can collect all the process parameter once a minute. In particular, bath exhaustion percentages are determined by spectrophotometric measurements, considering pure water and solution just after dye introduction as references for 100% and 0% exhaustion respectively.
Tests without enzyme were led in the same way, while for tests with surfactant agent in dyeing solution 1g/l Avolan was added.

Enzyme effect evaluation

To evaluate the effect on wool dyeing produced by the enzymatic treatment, adsorption rate constants and apparent activation energy were determined for every dyeing process.
Adsorption rate constants were determined from exhaustion curves, by the adjustment of experimental values to Cegarra-Puente modified empirical kinetic equation:

$$\ln\left[-\ln\left(1-\frac{E_t^2}{E_\infty^2}\right)\right] = a\ln t + a\ln K$$

where E_t is the dye concentration in the fiber at the time t, E_∞ the dye concentration at the equilibrium, K the adsorption rate constant, t the dyeing time.

Arrhenius equation was used for apparent activation energy calculation:

$$K_T = K_0 e^{-\frac{E}{RT}}$$

where K_T is the adsorption rate constant at the temperature T, E the apparent activation energy, K_0 the frequency factor, R the ideal gas constant (R = 1,9858 cal/mol°K), T the absolute temperature (°K).[9]
Moreover, electrophoresis SDS-PAGE analysis was carried out on samples subjected to dyeing with enzyme pretreatment and on a wool reference in order to obtain the molecular weight distribution and to highlight any degradation.
The SDS-PAGE was performed according to the Laemmli's method using XcellLock Mini-Cell (Invitrogen) that ensures a separation between 3 and 188KDa. Gel was fixed and coloured with a Comassie Blue R250 solution for 2 hours and decoloured with acetic acid and water.

SEM analysis

The surface morphology of the fabrics was examined by SEM with a Leica (Cambridge, UK) Electron Optics 435 VP scanning electron microscope with an acceleration voltage of 15 kV, a current probe of 400 pA, and a working distance of 20 mm. The samples were mounted on aluminum specimen stubs with double-sided adhesive tape and sputter-coated with gold in rarefied argon with an Emitech (Kent, UK) K550 sputter coater with a current of 20 mA for 180 s.

Physical tests

In order to evaluate the possible fiber damage, loss of tensile strength and elongation were determined by Uster Tensorapid Tester dynamometer according to UNI EN ISO 2062. For each sample, 50 measurements were led in order to calculate a significant average value.

Colour fastness determination

For each sample dyed with the different dyeing process, colour fastness to domestic washing (UNI-EN ISO 105-C01), artificial light (UNI-EN ISO 105-B02), rubbing (UNI-EN ISO 105-X12) and perspiration (UNI-EN ISO 105-E04) were carried out. These are very important parameters related to the quality of the material produced.

RESULTS AND DISCUSSION

Exhaustion curves

Exhaustion curves of dyeing with enzymatic pre-treatment at different temperatures are compared with the curve obtained with traditional process in Figure1.
It is evident that at 85°C with enzyme and at 98°C without enzyme final values of exhaustion very closed were reached, in fact at 85°C 93% bath exhaustion was reached, a good value even if at 98°C we had total bath exhaustion. The enzymatic effect is also evident on the kinetics. In fact, at all investigated temperatures, the initial slopes of the curves are higher than that of the curve obtained without enzyme. It means that Multifect significantly increases dyeing kinetics, fundamental aspect regarding a possible reduction of total dyeing times. Moreover in Figure 2 a comparison between exhaustion curves at 85°C with and without enzyme is reported, and the enzyme effect, that brought the final exhaustion percentage from 75% to 93%, is evident.
Tests with Avolan S were conduced in order to evaluate a possible interaction between surfactant and enzyme, reducing its effect. In Figure 3 curves obtained with 1g/l of Avolan in dyeing solution in presence and in absence of enzyme are compared. It is evident that the surfactant presence did not affect enzyme action that however enhanced the final exhaustion.

Enzyme effect evaluation

From exhaustion curves, adsorption rate constants (Table 1) and apparent activation energies (Table 2) were determined.
At each isotherm, dye without enzyme has lower adsorption rate constants values compared to the corresponding enzyme-treated. So it can be said that the action of the enzyme pretreatment helps dye adsorption on the fibers.
Apparent activation energy can be defined as the value of energy necessary to dye molecules to overcome the resistance of the fibers, coming into them. So a low E value means a lower barrier to the dye penetration into the fibers and less dependence on temperature.
In conclusion, we can say that enzymatic pretreatment produces a decrease in wool fiber resistance to dye diffusion.
Electrophoresis was carried out on samples subjected to enzymatic pretreatment and thermal dyeing and on a wool reference, in order to evaluate molecular weight distribution and to highlight any degradation.
Electrophoresis track (Fig. 4) of analyzed samples is quite comparable to that of reference wool: this means that there was no degradation on wool protein due to enzyme pretreatment and justifies the good mechanical performance found.

SEM analysis

Wool fibers treated at different temperatures, with and without enzyme, were submitted to SEM analysis. Results, reported in Figures 5-7, refer to process at 98°C and 85°C without enzyme, and 85°C with 1g/l Multifect Neutral. These samples were chosen in order to highlight possible damages caused by enzymatic action, regardless of the adopted temperature.
Enzyme acts firstly on CMC, that is non keratin wool component, where cysteine concentration and disulfide bonds are lower and the fiber structure is more vulnerable.

Looking the SEM results, we can notice that there are no damaged fibers in none of the considered samples, in fact a CMC disintegration causes epicuticle detachment making the fiber smooth, easily visible in the microscopic analysis.

Physical tests

Wool yarns dyed at different temperatures with and without enzyme pretreatment were tested.
Results are reported in Fig 8 and show that both tensile strength and elongation suffered a great loss after dyeing at 98°C, regardless of the enzyme presence, while at lower temperature with enzymatic pretreatment the degradation was reduced. It means that high temperature effect was more destructive than enzyme attack to wool structure, affecting with more importance the mechanical properties of wool yarns.

Colour fastness determination

In table 3-5 results of dyeing fastness are reported.
It has to be said that the used dye, Telon Turquoise M-5G, presents a difficult exhaustion with low fastness in wet ambient. Results obtained are so to be interpreted in order to highlight the positive effect of enzyme use, even if they could be considered not so good. Generally, washing fastness was better on high temperature dyed samples: dye degradation worsens, decreasing temperature, from 1 to 2 grades. Staining on wool did not change in the presence of enzyme and at different temperatures, while staining on cotton decreased from ½ to 1 grade decreasing temperature and after enzyme action. The value at 85°C is already stable. Perspiration fastness showed a similar behavior. The progressive worsening of these values could be due to enzyme effect which improves exhaustion, so the dye amount on the fiber is higher but not all of this is chemically bonded to the fiber, a fraction is just superficially adsorbed and not very resistant to wet action.
In order to improve wet fastness, all process parameters as dye type and concentration, liquor ratio, etc. have to be chosen in the right way, but it will be the aim of further studies.
In table 5 results of light fastness tests are reported and no effect of enzymatic treatment on light fastness was shown

CONCLUSIONS

The comparison of the results can indicate a dyeing process at 85°C with enzymatic pretreatment at 50°C as an alternative to traditional one. In this way, in fact, high values of bath exhaustion are coupled with low mechanical properties loss, while the dyed fabrics maintain satisfactory fastness values. Moreover it was proved that enzymatic action was unaffected by the presence of a surfactant agent in dyeing bath.
Good mechanical properties of dyed fabrics were confirmed by SEM analysis and SDS electrophoresis that did not evidence structural degradation of the wool, while dyeing kinetics improvement due to enzyme presence was confirmed by calculations of adsorption rate constants and apparent activation energies.

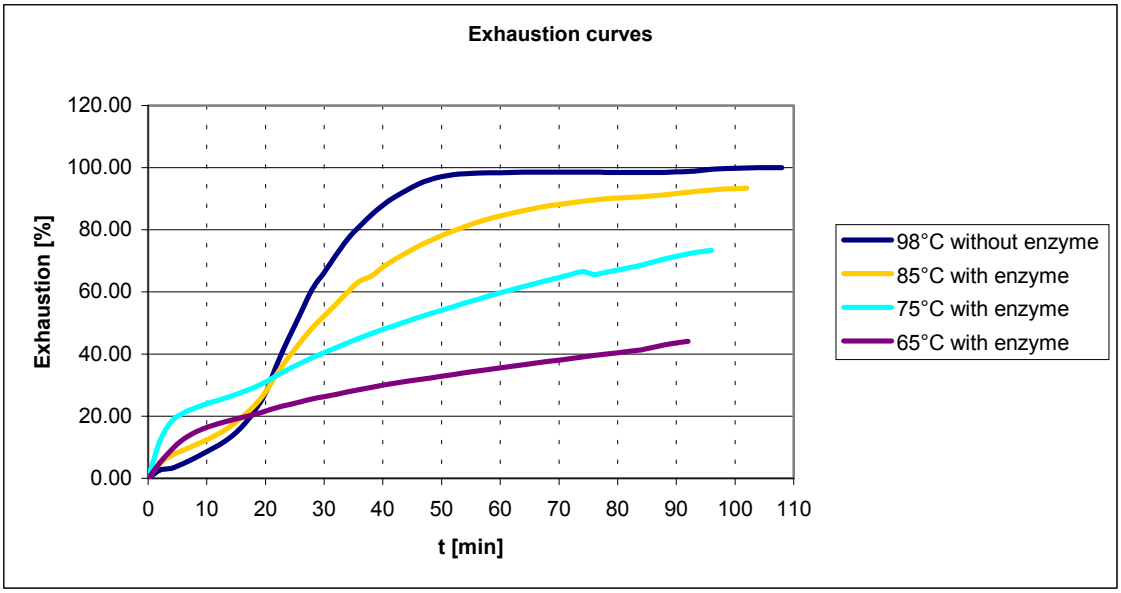

Figure 1: exhaustion curves at different temperatures, with and without enzymatic pretreatment

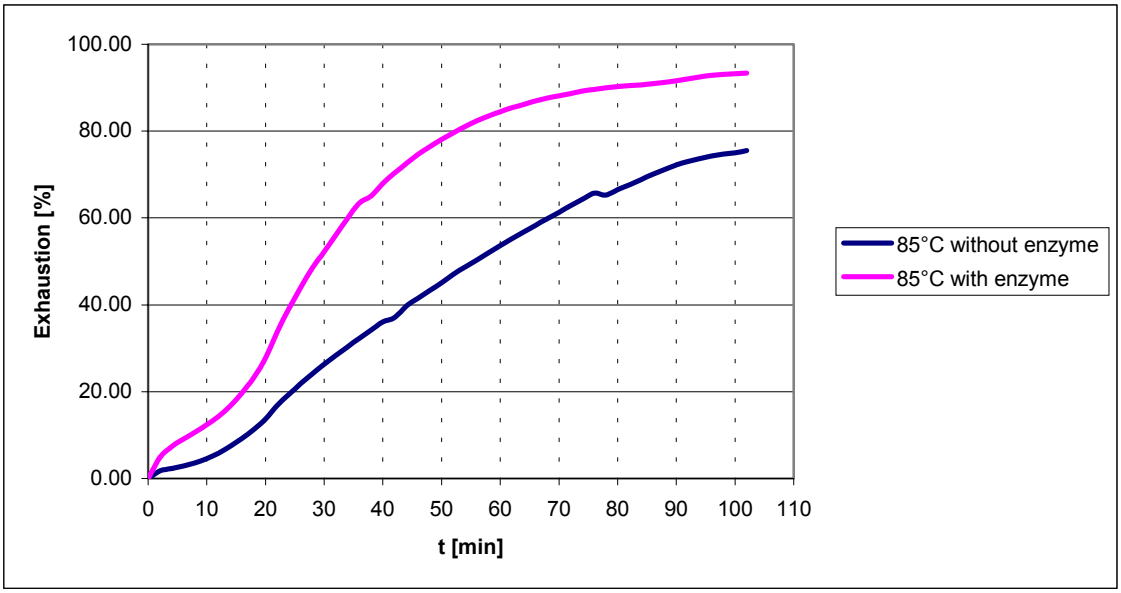

Figure 2: exhaustion curves at 85°C with and without enzymatic pretreatment

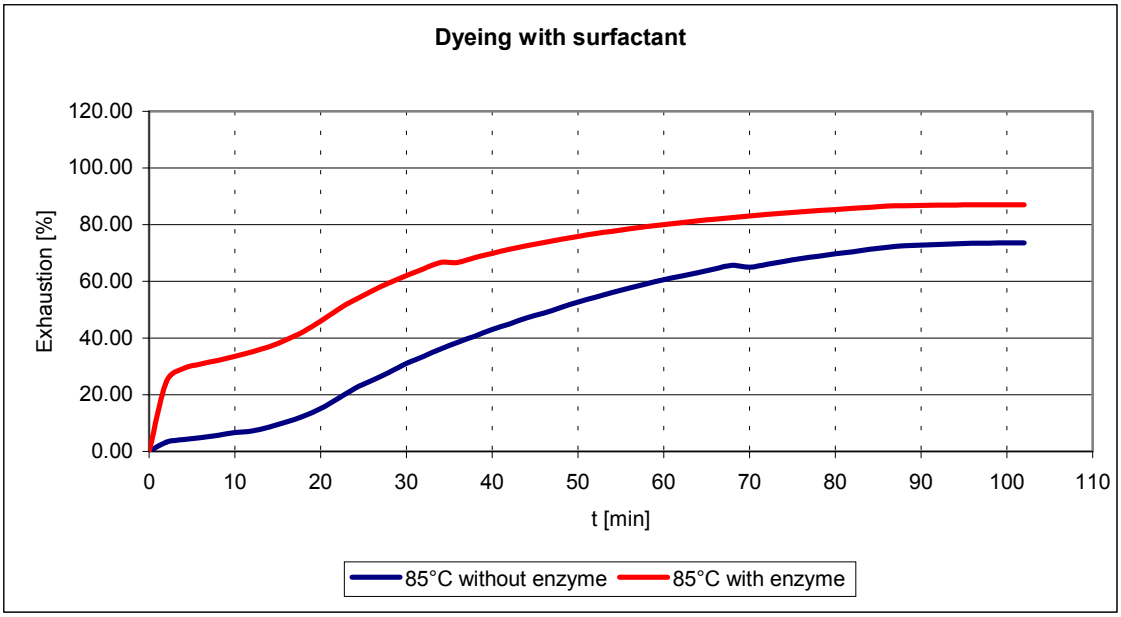

Figure 3: influence of surfactant presence

	Without enzyme	With enzyme
T(°C)	K (min^{-1})	K(min^{-1})
65	$1.8\ 10^{-3}$	$2.08\ 10^{-2}$
75	$1.44\ 10^{-2}$	$1.93\ 10^{-2}$
85	$1.52\ 10^{-2}$	$2.01\ 10^{-2}$
98	$2.24\ 10^{-2}$	$2.77\ 10^{-2}$

Table 1: adsorption rate constants

	E (kcal/mol)
Without enzyme	17.21
With enzyme	2.13

Table 2: apparent activation energies

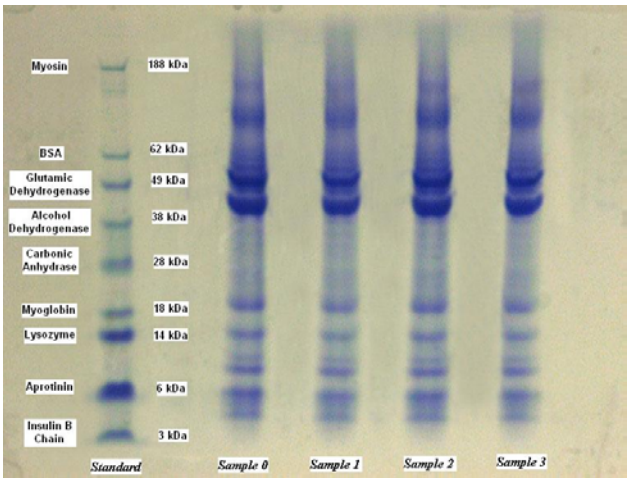

Figure 4: electrophoresis tracks. 0_untreated wool; 1_98°C without enzyme; 2_85°C without enzyme; 3_85°C with enzyme.

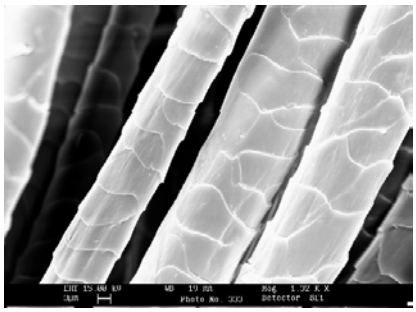

Figure 5: 98°C without enzyme

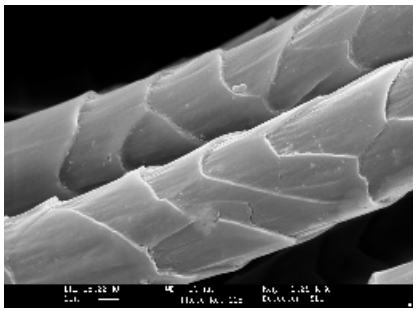

Figure 6: 85°C without enzyme

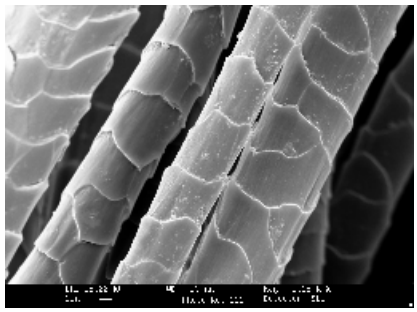

Figure 7: 85°C with enzyme

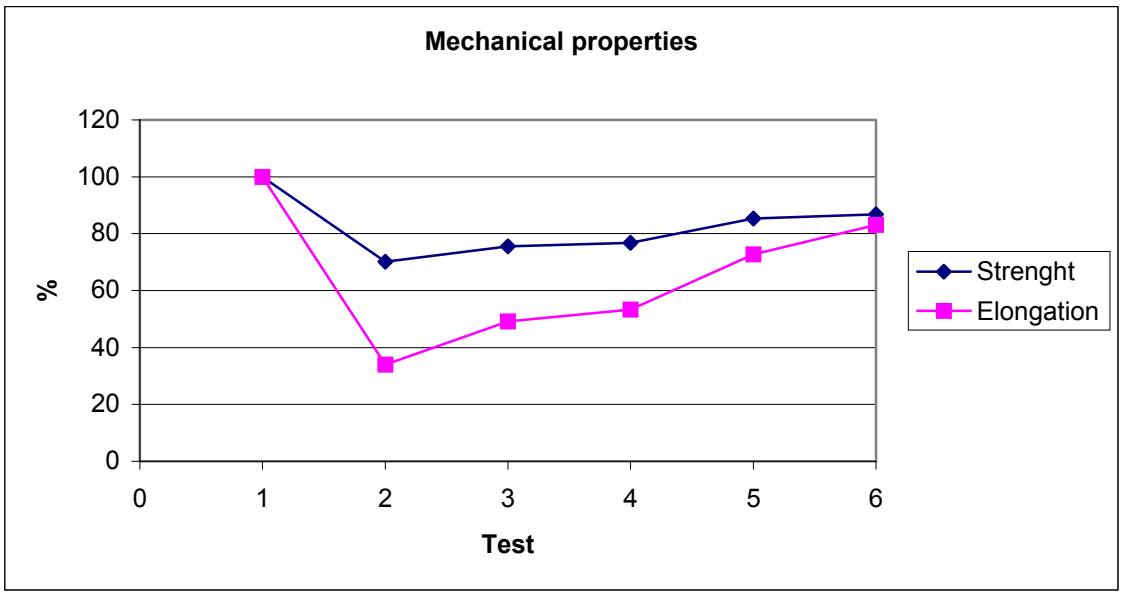

Figure 8: mechanical properties comparison. 1_untreated wool; 2_98°C without enzyme; 3_98°C with enzyme; 4_85°C with enzyme; 5_75°C with enzyme; 6_65°C with enzyme.

	Dye degradation		Staining on wool		Staining on cotton	
Temperature	No Enz.	Enz	No Enz.	Enz	No Enz.	Enz
98°C	4	3-4	4-5	4-5	4	3-4
85°C	4	4	4-5	4-5	2-3	3
75°C	2	1-2	4-5	4-5	3-4	2-3
65°C	1	1	4-5	4-5	3	3

Table 3: washing fastness

	Dye degradation		Staining on wool		Staining on cotton	
Temperature	No Enz.	Enz	No Enz.	Enz	No Enz.	Enz
98°C	4-5	4-5	4	4	4	3-4
85°C	4	4	3	3	2-3	2
75°C	3-4	3-4	2-3	2	2	1-2
65°C	3-4	4	2-3	2-3	2	1-2

Table 4: perspiration fastness

	Dye fastness	
Temperature	No Enz.	Enz
98°C	4-5	5
85°C	5	5
75°C	5	5
65°C	5	5-6

Table 5: light fastness

References

1) Gallico, L. 2000. *La lana*. Eventi e progetti Editore. IX, 225-232.
2) Bona, M., Isnardi., F.A., Straneo, S.L. 1981. *Manuale di tecnologia tessile* , Zanichelli Editore.
3) Neurath H. 1989. *Proteolitic Enzymes* (cap 1-4) , Beynon R.J., Bond J.S. ed ., IRL Press.
4) Cardamone, J.M., Damert, W.C. 2006. *Low-temperature Dyeing of Wool Processed for Shrinkage Control*. Textile Research Journal 76, 78-85.
5) Onar, N., Sarusik, M., 2005. *Use of enzymes and chitosan biopolymer in wool dyeing*. Fibres & Textiles in Eastern Europe.13, No.1 (49).
6) Sawada, K., Ueda, M. 2001. *Enzyme processing in reverse micellar solution*. J of Biotechnology 89, 263-269.
7) Riva, A., Alsina , J.M., Prieto, R. 1999. *Enzymes as auxiliary agents in wool dyeing*. JSDC 115, 125-129.
8) Ferrero F., Periolatto M., Mossotti R., Zerbola M., Innocenti R. *Applicazione degli enzimi nella tintura della lana*. Tinctoria 104 (5) 16-23 (2007).
9) A Riva, I Algaba and R Prieto, "*Dyeing kinetics of wool fabrics pretreated with a protease*", Coloration Technology, 118 (2002).

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