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Influence of protease on dyeing of wool with acid dyes

Research Article

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Abstract: The application of enzymes in the wool dyeing process is an important research goal in order to reduce the environmental impact and costs of this finishing process. The work has focused on the possibility of reducing the temperature of conventional dyeing, using an enzymatic pretreatment with a neutral protease to improve the diffusion of the dye into the fibers. After the identification of the optimal dyeing process, the study goes on transferring the method to an industrial application. For this reason the influence of a leveling agent added to the dyeing bath was considered and further tests with an industrial dyeing recipe were performed. In order to evaluate possible fiber damage, samples treated with enzyme were observed by scanning electron microscopy (SEM) and analyzed by gel electrophoresis (SDS-PAGE). Moreover, the variation of tensile strength and elongation of dyed samples were determined. In addition, color fastness to domestic laundering, perspiration and rubbing were carried out. Finally, color measurements and fiber section analysis were performed. The results show the possibility, thanks to the pretreatment with the investigated enzyme, to obtain a homogeneous and solid dyeing on fibers maintaining good mechanical properties, already working at 85°C instead of 98°C currently used in industry.

Keywords: Wool • Dyeing • Enzymes

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1. Introduction

Most textile finishing operations are carried out by wet processes. Among the textile treatments, the dyeing step generates waste water of great chemical complexity due to huge variety of fibers, dyes, process aids and finishing products [1]. Dyeing processes, in fact, involve the use of many chemical substances (dyestuffs, salts, acids, silicones and surfactants) that are typically difficult to purify biologically; these chemicals increase the environmental impact of textile industry waste water and may result in allergies for the end-users of the manufactured products [2]. Moreover, the relatively high temperature of these processes can weaken the fiber structure and consequently impart detrimental chemical and mechanical properties on the fabrics.

Research has been conducted with the aim to improve dyeing in terms of kinetics and final dye bath

exhaustion with good fastness of dyed materials at lower temperatures than those used in conventional processes [3]. Such methods should enable an easy technological transfer to the textile industry and should be suitable to work on actual industrial dyeing equipment with low environmental impact.

For wool textiles, the Sirolan LTD® (Low Temperature Dyeing) method enables to work at a lower temperature (85-90°C) than the conventional dyeing, or else at boiling but for a much shorter time. It was reported that this can cut electricity demand in the dyeing process by around 20% and greenhouse emissions by a similar proportion [4]. The above interesting results are achieved by an alkaline pretreatment of wool by an auxiliary called Valsol LTA-N, consisting mainly in synthetic alkoxylate derivatives of *N*-alkyl betaine or sulphobetaine [5], which are able to extract lipids from the cell membrane complex (CMC) of wool speeding up the intercellular diffusion of dyes [6]. Bayer introduced Baylan NT 02 as an auxiliary

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for dyeing wool at low temperature (85°C) or with a shorter dyeing time at 98°C. This product is a nonionic preparation of alkyl and aryl polyglycoethers. Finally Martí *et al.* proposed a new wool dyeing procedure at low temperature using liposomes and demonstrated that these auxiliaries yielded a lower contamination load of baths than Valsol LTA-N and Baylan NT 02 with the same good results of dye exhaustion at 80°C [7]. On the other hand, wool dyeing has been the subject of many investigations concerning the use of enzymes, since it was observed that they produce an improvement of dye absorption by wool fiber. Enzymes are already active in small doses and highly biodegradable [8]. They are very selective and act in mild operating conditions, usually in a pH range between 5 and 8, at 30-50°C, under atmospheric pressure. Moreover they are able to reduce or eliminate the amount of synthetic auxiliary agents, usually poorly biodegradable.

For wool dyeing, 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 dyes at the interface between cuticular cells and CMC. Hence considerable research aimed at improving dye uptake by using proteases in wool dyeing has been conducted [9-16]. However, the enzyme action should be carefully controlled to avoid uncontrolled degradation of the CMC and damage of the morphological and mechanical properties of the fibers [17-21].

In previous work [22] we tested the effect on wool dyeing of several proteases, in commercial formulation, and Multifect Neutral (Genencor) gave the best results in term of bath exhaustion and fiber properties. Indeed, the aim of the study was an investigation of enzyme-assisted wool dyeing in order to reduce dyeing temperature or dyeing time, minimizing the fibre damage and maintaining satisfactory dyeing fastness [23].

After the identification of the optimal conditions for the dyeing process, the present study goes on transferring the method to an industrial application. For this reason the influence of a leveling agent addition to the dyeing bath was considered because in industrial practice fastness values are often improved by such type of auxiliaries. Moreover further tests with an industrial dyeing recipe were performed.

2. Experimental Procedure

2.1. Materials

The textile material used was pure wool yarn 2/79 Nm, plied yarn S 825 turns per meter, single yarn Z 717 turns per meter, previously washed for 10 min in 1 g L⁻¹ solution of ECE surfactant and 10 mL L⁻¹ 33% ammonium

hydroxide under 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 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 amyloliquefacens*). The activity of protease was measured according to Silva *et al.* [24] using casein as substrate. One unit of activity (U) is defined as the amount of enzyme that hydrolyzes casein to produce the colour equivalent to 1 µmol of tyrosine per minute at pH 7.5 and 37°C. An initial enzyme activity of 80 U g⁻¹ was determined and tested before each fiber treatment. It was observed that the enzyme activity is greatest in the range 40-60°C and its action is effective for pH values from 6 to 8, with a maximum corresponding to pH 7. The enzyme loses 40% of its original activity at 70°C and inactivates completely at 80°C, as we confirmed by activity measurements.

At first the dye chosen was Telon Turquoise M5-G (C.I. Acid Blue 185) by Dystar, already used in the preliminary study. It is a copper phthalocyanine trisulfonate acid dye working at pH 4, that presents a maximum absorbance peak at 617 nm. It has a low migrability on wool so it highlights the enzyme effect on fiber. The influence of a leveling agent was tested by introducing Avolan S (manufactured by Bayer), that is a mixture of non-ionic and cationic surfactants. Acetic acid of laboratory grade was used for acidification.

Further tests were carried out using a dyeing recipe with a dye triad: Blue RAW (C.I. Acid Blue 80), Milling Red BB (C.I. Acid Red 114) and Yellow R (C.I. Acid Yellow 25) by Lamberti s.p.a., Albizzate, Italy. These own to acid milling dyes, which show great affinity for wool fibers and yield dyed products with good washing fastness. Moreover they work at pH 6, a value reached simply by adding the same dyes to the dyebath, so no acid addition was necessary in dyeing process.

2.2. Dyeing process

The first aim of this study was the determination of exhaustion curves of the dye bath in the temperature range between 98°C and 75°C with and without enzymatic pre-treatment. They were determined in a Teintolab apparatus (Comeureg, France), a dyeing diagnostic equipment that allows the on-line control of the dyeing parameters such as pH, temperature, dye exhaustion. With probes in the dyeing bath, Teintolab is able to collect all the process parameters once per 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.

An amount of wool of 26 g was treated with a material:liquor ratio of 1:50. In tests with enzyme pretreatment, wool yarn was put in a Multifect Neutral water solution with a concentration of 4 U g⁻¹ yarn, at pH 7, under agitation. Bath temperature was maintained at 50°C for 20 min, during which time the enzyme acted. Moreover, enzyme activity was investigated during whole pre-treatment. In fact, at the end an activity loss of approximately 4% was found.

For dyeing with Telon Turquoise, the pH was adjusted to 4 by adding acetic acid in the appropriate amount. Then 1% dye on weight of fiber (o.w.f.) was introduced. In this way the enzyme was inactivated and the conditions were favorable for dye exhaustion. Tests without enzyme pretreatment were carried out in the same way, while tests with leveling agent in dyeing solution were performed by adding 0.8% o.w.f. Avolan S.

For dyeing with the triad, the same procedure was followed unless pH adjustment was necessary, hence the enzyme denaturation was achieved only by temperature rising. In this case 1% o.w.f. of dye mixture was introduced, with a concentration of 0.067 g L⁻¹ of each dye.

In any case, after dye addition the temperature was raised to the final desired value in 20 min and maintained for 65 min, always under agitation; finally the bath was cooled at 50°C. This cycle was very close to that applied in industrial practice.

2.3. Fiber analysis

The surface morphology of the fibers 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.

In order to obtain the molecular weight distribution of the proteins and to highlight any degradation, SDS-PAGE was carried out on samples treated at 98°C and 85°C with or without enzyme pretreatment and on a wool reference. Samples were dissolved in a dithiothreitol/urea solution in pH 8.6 buffer, under a nitrogen atmosphere for 4 h. The protein concentration of the wool extracted was determined by Bradford protein assay method (BioRad), using bovine serum albumin as standard. The SDS-PAGE was performed according to the Laemmli's

method [25] using XcellLock Mini-Cell (Invitrogen), on 12% polyacrylamide gels.

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

For each dyed sample color fastness to domestic washing (UNI-EN ISO 105-C01), perspiration (UNI-EN ISO 105-E04) and wet rubbing, with a SDL crockmeter (UNI-EN ISO 105-X12), were evaluated.

Color measurements were performed according to ISO 7724-2:1984, measuring the spectral reflectances with a spectrophotometer (Spectraflash SF600XV) from Datacolor (Switzerland), under CIE standard illuminant D₆₅ and a 10° observer. In order to evidence the dye diffusion inside the fibers, dyed fibers cross sections were examined with an optical microscope (Axioscop Zeiss, magnification 400x).

3. Results and Discussion

3.1. Exhaustion curves

The influence of enzymatic pretreatment is evidenced by the exhaustion curves with Telon Turquoise plotted in Fig. 1 for each dyeing processes. In order to compare the different dyeing processes with and without enzyme pretreatment, final bath exhaustions reached were reported in Table 1. All enzyme pretreated yarns were characterized by values significantly higher than the ones dyed at the same temperature but without pretreatment. Moreover, the lower the temperature, the greater the difference between final bath exhaustion of pretreated and untreated samples at the same dyeing temperature. However, the dyeings at 85°C of the enzyme pretreated yarns reached final bath exhaustion very close to that of the reference (98°C).

From the exhaustion curves, times of half-dyeing were calculated and reported also in Table 1. The results show that the enzyme pretreatment produces a faster rate of dyeing with dye used. In fact, time of half-dyeing is the time required by a fiber to absorb half the amount of dye absorbed at equilibrium, so the smaller it is, the faster is the dyeing. Comparing the values found, it can be said that the enzymatic pretreatment greatly enhances dyeing kinetics at 85°C with a 37% reduction of half dyeing time.

In Fig. 2 the exhaustion curves with Telon Turquoise and addition of Avolan S are compared. At 98°C it can be seen that the final bath exhaustion reached with Avolan S is just 95% while 100% was reached with the

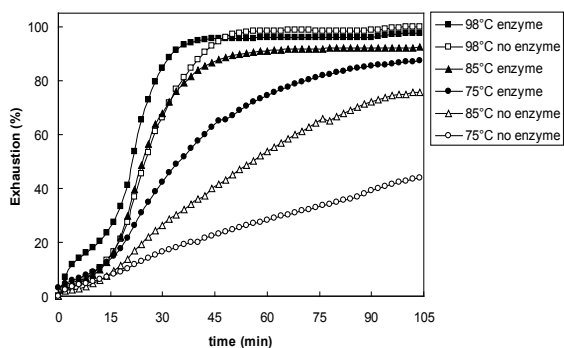


Figure 1. Exhaustion curves of Telon Turquoise on wool with or without enzyme pretreatment.

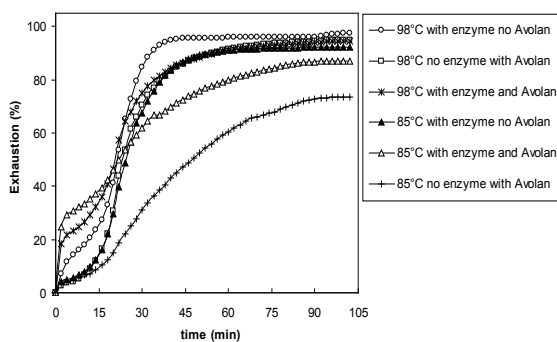


Figure 2. Exhaustion curves of Telon Turquoise on wool dyed with Avolan S, with or without enzyme pretreatment.

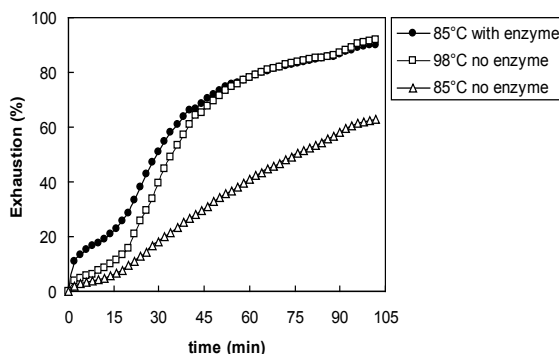


Figure 3. Exhaustion curves with dye triad on wool, with or without enzyme pretreatment.

conventional process without auxiliary. It could be due to the affinity of Avolan S for dye molecules: some of them in fact can remain bonded to the auxiliary in the bath even at the equilibrium, without reaching the fibers. However the leveling effect is due to this affinity, so the loss in final exhaustion reached is balanced by the enhanced dyeing fastness and leveling grade.

Tests with Avolan S were also carried out in order to evaluate a possible interaction between surfactant and enzyme, reducing its effect [26]. From the comparison of the curves obtained with 0.8% Avolan o.w.f. in dyeing solution with and without enzyme pretreatment at 85°C it is evident that the auxiliary presence did not influence the enzyme effect, however the final exhaustion

Table 1. Final bath exhaustion reached and times of half dyeing. A) with Telon Turquoise, B) with Telon Turquoise and Avolan S, C) with dye triad.

| Sample | T (°C) | Final exhaustion (%) | | Time of half dyeing (min) | |
|--------|--------|----------------------|----------------|---------------------------|----------------|
| | | with enzyme | without enzyme | with enzyme | without enzyme |
| A | 98 | 97.4 | 99.9 | 20.4 | 25.8 |
| | 85 | 93.2 | 76.8 | 24.0 | 44.3 |
| | 75 | 89.7 | 45.1 | 32.5 | 49.9 |
| B | 98 | 94.6 | 95.0 | 18.4 | 23.5 |
| | 85 | 87.0 | 73.6 | 17.7 | 36.1 |
| C | 98 | - | 92.3 | - | 33.0 |
| | 85 | 90.1 | 62.7 | 27.0 | 47.0 |

was enhanced from 73.6% to 87.0%, with a relative enhancement of 15% and half-dyeing time reduction of 51%. Even in this case the activity loss of enzyme was found practically complete (2% residual).

In Fig. 3 exhaustion curves with dye triad are compared and even in this case the final bath exhaustion improvement at 85°C, with enzymatic pretreatment (from 62.7% to 90.1%) and the kinetic effect due to the enzyme, are evident. Moreover it can be noticed that with enzymatic pretreatment the exhaustion values reached at 85°C are very close to that obtained at 98°C with the conventional process.

3.2. SEM analysis

Wool fibers treated with and without enzyme were submitted to SEM analysis. The images, reported in Fig. 4, refer to samples dyed both at 85°C, one without enzyme pretreatment and the other pretreated with 4 U g⁻¹ yarn Multifect Neutral. These samples were chosen in order to highlight possible damages caused by enzymatic action, regardless of the adopted temperature.

From the SEM results, it can be seen that there is no marked damage on the fibers in the enzyme treated sample, in fact a CMC disintegration causes epicuticle detachment, making the fiber smooth, easily visible in the microscopic analysis. However, the effect of the enzyme can be seen even if limited to the surface of the treated wool fibers. In particular, these fibers dyed at 85°C compared to untreated ones show a roughness modification due to lifting and smoothing of the scales.

3.3. Gel electrophoresis

Fig. 5 shows the electrophoresis gel of samples subjected to thermal process with or without enzymatic pretreatment together with a wool reference, in order to evaluate molecular weight distribution of proteins and to highlight a possible degradation of wool. In fact,

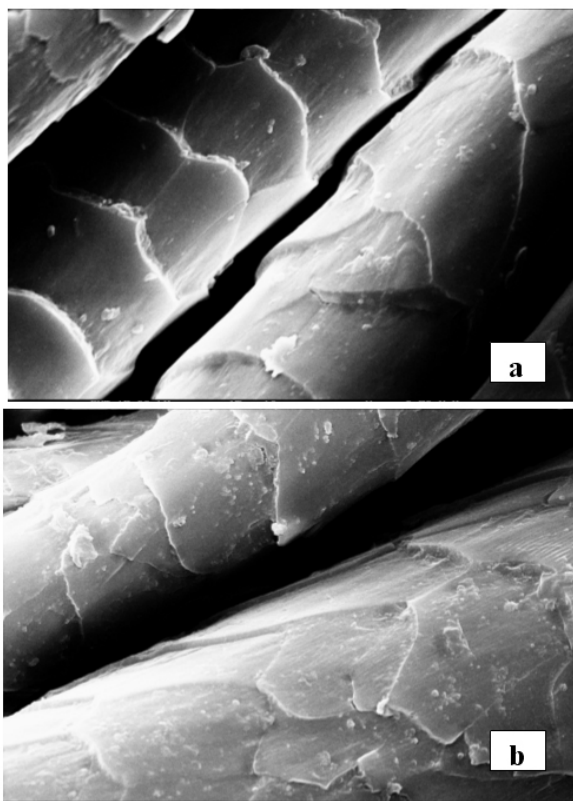


Figure 4. SEM images of wool fibers dyed at 85°C: a) without enzyme, b) with enzyme. Magnification ratio 2,500x.

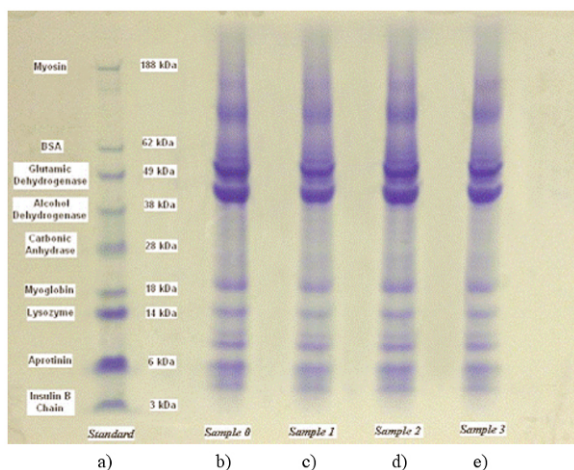


Figure 5. Electrophoretic separation patterns (SDS-PAGE) of wool samples: a) standard, b) untreated, c) dyed at 98°C without enzyme, d) dyed at 85°C without enzyme, e) dyed at 85°C with enzyme.

the enzyme acts firstly on CMC, that is a non keratin wool component, where cystine concentration and disulfide bonds are lower and the fiber structure is more vulnerable. Electrophoresis track of analyzed samples is quite comparable to that of reference wool. This means that there was no degradation of wool proteins due to enzyme pretreatment.

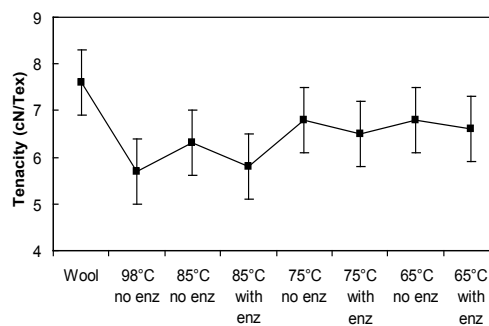


Figure 6. Tenacity measurements on wool yarn dyed in different conditions.

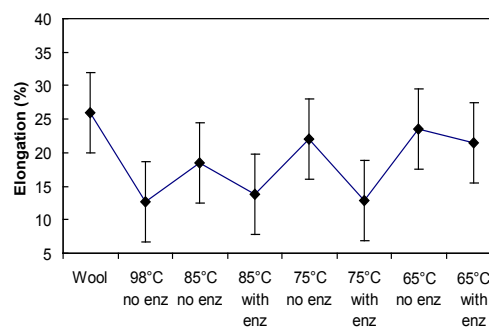


Figure 7. Elongation measurements on wool yarn dyed in different conditions.

3.4. Mechanical tests

Wool yarns dyed at the investigated temperatures with and without enzyme pretreatment were dynamometrically tested. The results, reported in Figs. 6 and 7, show that both tensile strength and elongation suffered a great loss after dyeing at 98°C, while at lower temperature the degradation was lower. It is noteworthy that at 85°C with enzyme pretreatment the tensile strength was about the same as at 98°C with conventional dyeing, while the elongation loss was even slightly lower. Moreover, from the results it is evident that the percentage loss of textile strength ascribable to the enzyme attack is lower than that due to temperature.

3.5. Color fastness determination

Washing and perspiration fastness tests on samples dyed with Telon Turquoise after enzyme pretreatment, as reported in Tables 2 and 3, show values identical or slightly lower than those obtained with the conventional dyeing procedure. However the wet rubbing fastness (Table 4) is much lower indicating an uneven distribution of dye across the fiber section.

Higher fastness values are shown in any case by the sample dyed in the presence of Avolan S. Even if Telon Turquoise has low wet fastness, dyeing with leveling agent improves this value because of a better dye penetration inside the fibers. Fastness to domestic

laundering increases 1 grade while fastness to acid perspiration increases more than 2 grades. Moreover, fastness to wet rubbing after enzyme pretreatment reaches the same values as the conventional process, showing the importance of the action of the leveling agent. Concerning dyeing with dye triad, fastness tests gave optimal results without any differences between the analyzed samples. Dyes used in this test have in fact a great affinity for wool fibers and the enzymatic pretreatment does not affect this characteristic.

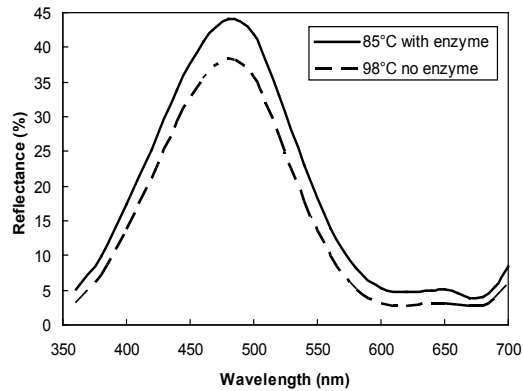


Figure 8. Datacolor spectra for wool fibers dyed with Telon Turquoise and Avolan S.

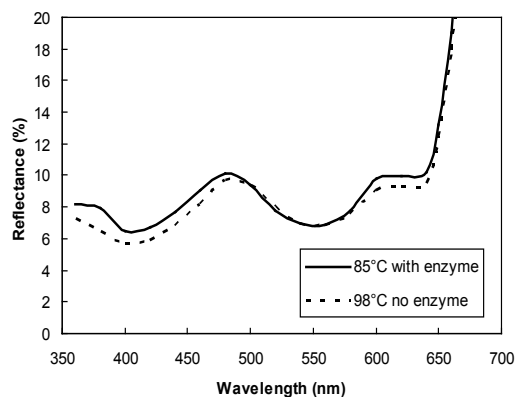


Figure 9. Datacolor spectra for wool fibers dyed with dye triad.

3.6. Color measurements

The color measurements were performed on samples dyed at 98°C without enzymatic pretreatment and at 85°C with pretreatment, both with leveling agent and with dye triad.

In Fig. 8 reflectance curves related to dyeing with Telon Turquoise and Avolan S are reported and they show the enzyme presence shifts the curve to higher values than the curve at higher temperature. This means that the sample dyed at 85°C is brighter than the reference at 98°C as confirmed by the final bath exhaustion reached which indicated a slightly lower value at 85°C. This difference was confirmed by color strength (K/S values), calculated using the Kubelka–Munk equation [27], of the reference sample (K/S = 18.26) and the sample dyed at 85°C (K/S = 21.01). Nevertheless the color difference can be compensated by adjusting the dye concentration. Moreover the curves related to the samples dyed with triad are almost overwritten, as shown in Fig. 9. In this case there is no brightness difference between the two analyzed samples, showing that the amount of the dyes on both the samples are similar, as confirmed by the close final bath exhaustion reached.

3.7. Fiber sections analysis

The fiber section analysis was performed on samples dyed with Telon Turquoise with and without enzymatic pretreatment and in presence of Avolan S at 85°C and 98°C. The images related are reported in Fig. 10.

Samples can be considered representative of the “population” of fibers. Analyzing the images it can be noticed that they are quite similar, in fact fibers with and without homogeneous dye distribution across the section as well as others with a more or less intense coloration are present in all samples in equal degree. It is also evident that the most of fibers show a homogeneous dyeing, indicating a good dye penetration inside the fibers. Moreover, the similarity of the images shows that the improvement of dyeing kinetics due to enzymatic pretreatment does not cause ring dyeing or other leveling problems.

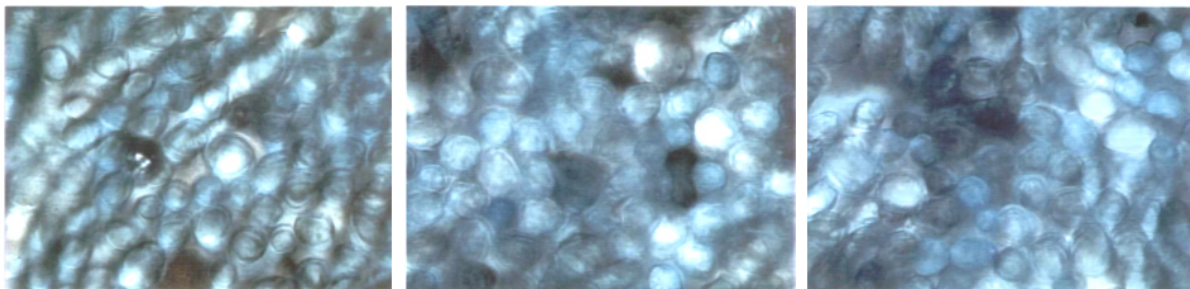


Figure 10. Cross section fibers analysis of wool fibers dyed with Telon Turquoise: a) 98°C without enzyme, b) 85°C without enzyme, c) 85°C with enzyme.

Table 2. Washing fastness. A) wool dyed with Telon Turquoise, B) wool dyed with Telon Turquoise and Avolan S, C) wool dyed with triad

| Sample | T (°C) | Dye degradation | | Staining on wool | | Staining on cotton | |
|--------|--------|-----------------|-------------|------------------|-------------|--------------------|-------------|
| | | without enzyme | with enzyme | without enzyme | with enzyme | without enzyme | with enzyme |
| A | 98 | 4 | 3-4 | 4-5 | 4-5 | 4 | 3-4 |
| | 85 | 4 | 4 | 4-5 | 4-5 | 2-3 | 2-3 |
| B | 98 | 4-5 | 4-5 | 4-5 | 4-5 | 4 | 3-4 |
| | 85 | 4 | 4 | 4-5 | 4-5 | 3-4 | 3-4 |
| C | 98 | 4-5 | - | 4-5 | - | 5 | - |
| | 85 | 4-5 | 4-5 | 4-5 | 4-5 | 5 | 5 |

Table 3. Acid perspiration fastness. A) wool dyed with Telon Turquoise, B) wool dyed with Telon Turquoise and Avolan S, C) wool dyed with triad

| Sample | T (°C) | Dye degradation | | Staining on wool | | Staining on cotton | |
|--------|--------|-----------------|-------------|------------------|-------------|--------------------|-------------|
| | | without enzyme | with enzyme | without enzyme | with enzyme | without enzyme | with enzyme |
| A | 98 | 4-5 | 4-5 | 4 | 4 | 4 | 3-4 |
| | 85 | 4 | 4 | 3 | 3 | 2-3 | 2 |
| B | 98 | 4-5 | 4-5 | 5 | 5 | 5 | 4-5 |
| | 85 | 4-5 | 4-5 | 5 | 4-5 | 4-5 | 4-5 |
| C | 98 | 4-5 | - | 4-5 | - | 5 | - |
| | 85 | 4-5 | 4-5 | 4-5 | 4-5 | 5 | 5 |

Table 4. Wet rubbing fastness. A) wool dyed with Telon Turquoise, B) wool dyed with Telon Turquoise and Avolan S, C) wool dyed with triad

| Sample | T (°C) | Dye degradation | |
|--------|--------|-----------------|-------------|
| | | without enzyme | with enzyme |
| A | 98 | 4-5 | 3 |
| | 85 | 4 | 1-2 |
| B | 98 | 4-5 | 4-5 |
| | 85 | 4-5 | 4-5 |
| C | 98 | 4-5 | - |
| | 85 | 4-5 | 4-5 |

4. Conclusions

The comparison of the results confirms that a dyeing process at 85°C after enzymatic pretreatment at 50°C is an alternative to conventional one at 98°C. In this way, high values of bath exhaustion and lower times of half dyeing are coupled with satisfactory mechanical properties. SEM observations showed only a small surface modification, while SDS-PAGE analysis did not evidence any degradation of wool proteins due to enzyme pretreatment.

The addition of a leveling agent such as Avolan S, in concentration 0.8% o.w.f., resulted in the final bath exhaustion being slightly lowered, but fastness values of dyed samples are significantly improved, without affecting the enzyme effect. Dye penetration inside fibers is good, as confirmed by a homogeneous distribution on the fibers cross section in all the analyzed samples.

Good results in terms of final bath exhaustion and kinetic enhancement due to the enzymatic pretreatment were found even in dyeing with an industrial recipe based on a dye triad. In this case fastness and leveling are good as a result of the high affinity of the dyes for wool, so the auxiliary addition is not necessary.

Color measurements carried out on samples dyed with Telon Turquoise showed some color differences ascribable to different dye amount fixed on fibers. Nevertheless these differences can be opportunely balanced adjusting the dyeing recipe.

The main advantage of this process may be due to energy saving (about 20%), with a consequent reduction of greenhouse emissions, achieved with addition only of a commercial biodegradable enzyme to the commonly used dyeing equipments and recipes. However, a correct economical balance should take into account the additional costs due to enzyme (about 0.45 € kg⁻¹) and increase of process time. On the other hand the enzymatic pretreatment may have other beneficial effects on wool properties such as hydrophilicity increase and antifelting improvement [28].

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