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INFLUENCE OF ENZYME PRE-TREATMENTS ON NATURAL DYEING OF PROTEINIC SUBSTRATES

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Abstract: *This study presents dyeing behavior aspects with Allium Cepa infusion of 100% wool yarns and 70% wool/30% Angora mohair blended yarns. Variants of yarns that were pre-treated according to the classical procedure of scouring were compared with variants of yarns that were subjected to proteolytic enzyme pre-treatment. The pre-mordanting technique with potassium alum was experimented. The influence of pre-treatments on natural dyeing was studied in terms of dyeing intensity, colour fastness to washing, artificial light, acid and alkaline perspiration, physical-mechanical and physical-chemical characteristics. Colour measurements have evidenced an increase of the tinctorial affinity to natural dye of wool fibre subjected to preliminary enzyme treatment followed by pre-mordanting. In the case of wool-Angora mohair blended yarns the tinctorial affinity to natural dye does not improve. Colour fastness to washing, acid and alkaline perspiration is good and very good for all the variants of treatment applied. Pre-mordanting has not positively influenced the colour fastness to light. Preliminary processes applied either in the classical or in the enzyme variant followed by pre-mordanting and dyeing induced a decrease of the breaking strength of 100% wool yarns by approx. 20% in the case of classical treatment and by 16% in the case of enzyme treatment while they do not negatively influence the breaking elongation values. For wool-Angora mohair blended yarns the enzyme treatment was much more aggressive compared to the classical one, leading to much lower values of the breaking strength, compared to the raw witness yarns, the decreases reaching up to 40%.*

Key words: *natural dyes, wool yarns, Angora mohair, Allium Cepa, mordanting*

1. INTRODUCTION

The current concerns for the environment as well as the norms and regulations in force have led to a growing demand for natural products in various industrial and industry-related branches. At present, natural dyes are reintroduced in the textile dyeing operations, because the synthetic dyes are associated with the emission in the environment of high amounts of chemical substances that are harmful for both the environment and human health. Numerous researches have evidenced the antifungal, antibacterial, antioxidative and UV protection properties of textile materials dyed with various natural dyes [1-3].

The development of efficient enzyme-assisted pre-treatments could improve the fibre sorption capacity for natural dyes and colour fastness. Enzymes were used for years in the textile industry because they activate under mild conditions and are biodegradable, being substrate-specific [4-5]. Both in natural dyeing and in textile chemical finishing, various methods to apply enzymes on different textile substrates such as cotton, wool, silk, synthetic fibres were approached [6-7]. Enzymes can facilitate diffusion and adsorption of natural dye at the surface and inside the fibre, acting especially at the surface level (cuticle layer) by modifying points of access to dyes. Another benefit of applying the enzyme treatments is the potential reduction of energy consumption of the dyeing process that could derive from the reduction of the bath temperature permitted by a higher enzyme induced affinity of the textile substrates. This study presents some aspects of the influence of enzyme pre-treatment, pre-mordanting and natural dyeing on the tinctorial capacity and on the characteristics of 100% wool yarns and 70% wool/30% Angora mohair blended yarns.

2. EXPERIMENTAL PART

2.1 Materials

100% wool yarns and 70% wool/30% Angora mohair blended yarns were developed in cooperation with SC STOFE Buhuşi SA, Romania. The natural dye was used as an extract in aqueous solution, obtained from dried scales on bulb of *Allium Cepa*. Potassium alum purchased from Sigma Aldrich was used for the mordanting process. Preliminary enzyme treatment was done with Perizym AFW proteolytic enzyme (Textilchemie & Dr. Perty), obtained from a combination of several types of proteases, with high specificity for proteinic fibers. Scouring were executed with a tensioactive product, a low foam non-ionic product based on polyethoxilated fatty alcohol Imerol JSF (Clariant) and Peristal WEB (Dr. Petry GmBH) was used as buffer solution.

2.2. Preliminary treatments

In order to determine the influence of the preliminary treatment and of the natural dyeing on the characteristics of 100% wool yarns and 70% wool/30% Angora mohair blended yarns, laboratory experiments were conducted using enzyme pre-treatment with proteolytic enzyme, in comparison with the classical scouring with non-ionic detergent. Treatments were conducted on Ugolini laboratory equipment (Hm 1:20), as follows:

***Classical preliminary treatment* (L_1)**

The yarns that are object of this study were scoured with a solution containing 2 g/L non-ionic scouring product (Imerol JSF), at a temperature of 40^{o}C, for 30 minutes. After this operation the yarns were subjected to repeated rinsing with warm water at 40^{o}C and with cold water and afterwards dried freely at room temperature.

***Enzyme preliminary treatment* (L_2)**

Prior to the natural dyeing, alternative treatment with Perizym AFW proteolytic enzyme product was carried on in order to improve wool tinctorial affinity and to reduce the felting capacity, effects that can be obtained by hydrolysis attack of protease on the scaled cuticle layer. 100% wool yarns and 70% wool/30% Angora mohair blended yarns were subjected to preliminary washing according to the classical scouring process, after having been immersed in a treatment bath at 30^{o}C, in which 3 g/L Peristal WEB (buffer solution) and 0.5 g/L Imerol JSF were added previously. After a checking of the bath pH (at a temperature of 70^{o}C) at 8.5 and its eventual correction with ammonia, Perizym AFW (2 g/L) enzyme was added. After the enzyme-catalysed reaction, with a duration of 30 minutes, the samples were squeezed, then in a first step rinsed at 40^{o}C in a bath acidulated with formic acid (pH=4) to deactivate the enzyme and subsequently rinsed with warm and cold water. The samples treated as such were freely dried at room temperature.

2.3. Preparation of the aqueous extract of *Allium Cepa*

To prepare the *Allium Cepa* extract, the mix resulted from adding 20 g of dried scales of the bulb to 1000 mL of purified water was boiled for 45 minutes. The resulted solution was left to cool for 24 h and afterwards filtered.

2.4. Mordanting

In this study the technique of pre-mordanting with metal salts was experimented. Mordanting was conducted on Ugolini laboratory equipment (Hm 1:30) at a temperature of 80°C for a duration of 45 minutes. Potassium alum - $AlK(SO_4)_2 \bullet 12H_2O$ was used as mordant with a concentration of 2% (owf). The yarns thus treated were successively rinsed in hot water at 60°C, 40°C and respectively in cold water, then were squeezed and freely dried at room temperature.

2.5. Dyeing with plant infusion

Dyeing of pre-mordanted yarn variants was done with the obtained infusion, without additional dilution. Yarns that were not mordanted were also dyed for comparison purposes. The dyeing operation was conducted on Ugolini laboratory equipment (Hm 1:40), at a temperature of 90°C (the temperature gradient up to the dyeing temperature was 2°C/min) for 75 minutes. The pH of the dyeing bath was adjusted by adding 1 mL/L acetic acid, until its value reached 5. The samples were subsequently rinsed with hot water at 60°C, soaped with a solution of 2 g/L Kemapol SR 40 detergent and then successively rinsed with hot water at a temperature of 60°C, 40°C and respectively cold water, squeezed and dried.

2.6. Colorimetry measurements

In order to establish the efficiency of preliminary treatment on the natural dyeing, colour measurements were conducted using Microflash spectrophotometer from Datacolor, according to standard ISO 105 J03:2001.

2.7. Determination of dyeing fastness

The influence of preliminary treatments on the natural dyeing was determined by evaluating colour fastness to washing, light, acid and alkaline perspiration. Standard SR EN ISO 105-C 10: 2010 was used to determine fastness to washing, in respect of colour change in the washing solution and colour staining on the multifibre standard, the assessment was made on the grey scale basis. Dyeing fastness to acid (pH=5.5) and alkaline (pH=8) perspiration was evaluated on the basis of standard SR EN ISO 105-E 04: 2013. Dyed samples were tested in respect of their fastness to artificial light, using Xenotest Apolo-James Heal device, according to standard SR EN ISO 105-B02: 03, and the results were assessed based on the blue scale.

2.8. Determination of physical-mechanical and physical-chemical characteristics

In order to evidence the influence of preliminary enzyme treatment and of natural dyeing on the integrity of the analysed yarns, the main physical-mechanical characteristics were determined in comparison with raw yarns (untreated), as well as with the yarns preliminary treated according to the classical method. For these determinations SR EN ISO 2060/97 (RT, 65% humidity) and respectively SR EN ISO 2062:2010 standards were used. The alkali solubility index of keratin fibres was determined by the solubilisation of yarns for 1h in a NaOH 0.1N solution, at a temperature of 65^{o}C, after having first been scoured of fats by extraction in Soxhlet with oil ether and ethylic alchool, in accordance with STAS 8398-69 standard.

2.9. Electron microscopy

The influence of enzyme preliminary treatment and of natural dyeing on the scaled cuticle layer of keratin fibres that compose a yarn was evaluated with electron microscopy, a Quanta SEM 200-FEI being used for the determination

Table 1 presents the codification of the yarn variants considered in the study.

Table 1: *Codification of the yarn variants considered in the study*

Code	Fibre composition of yarns/Applied treatment
L-1	Raw 100% wool yarns
L-7	Raw 70% wool-30% Angora yarns
L_1-1	100% wool yarns pre-treated according to L_1 variant and dyed (without mordanting)
L_2-1	100% wool yarns pre-treated according to L_2 variant and dyed (without mordanting)
L_1-7	70% wool-30% Angora yarns pre-treated according to L_1 variant (without mordanting)
L_2-7	70% wool-30% Angora yarns pre-treated according to L_2 variant (without mordanting)
L_1-1Al	100% wool yarns pre-treated according to L_1 variant, mordanted and dyed
L_2-1Al	100% wool yarns pre-treated according to L_2 variant, mordanted and dyed
L_1-7Al	70% wool-30% Angora yarns pre-treated according to L_1 variant, mordanted and dyed
L_2-7Al	70% wool-30% Angora pre-treated according to L_2 variant, mordanted and dyed

3. RESULTS AND DISCUSSIONS

3.1. Colour measurements

Objective colour assessment conducted by a trained observer under standard conditions (D65 average daylight) have evidenced the evenness of natural dyeing for the 100% wool yarns and for the wool-mohair blended yarns. Trichromatic values and colour differences obtained for the yarn variants considered in the study are presented in Table 2.

Table 2: *Colour measurements determined for 100% wool yarns and wool-Angora mohair blended yarns*

Variant code	X	Y	Z	Colour difference					
				DL*	DC*	DH*	DE*	Rating	Observations
L_1-1	19.63	16.86	7.33				REFERENCE		
L_2-1	23.08	20.01	8.80	3.76	1.23	0.57	3.99	3	light, saturated, yellow
L_1-1Al	21.03	18.57	4.23				REFERENCE		
L_2-1Al	19.96	17.53	4.70	-1.25	-4.21	-1.91	4.79	3	dark, unsaturated, red
L_1-7Al	18.19	16.35	4.33				REFERENCE		
L_2-7Al	18.72	16.61	4.21	0.34	1.59	-0.82	1.82	4-5	light, saturated, red

Obs: *Positive values of DL* indicate lighter samples compared to the reference; negative values of DL* indicate darker samples compared to the reference. Positive values of DC* indicate samples that are more saturated compared to the reference; negative values of DC* indicate samples that are more unsaturated compared to the reference. DE* represents the total colour difference between sample and reference.*

The data presented in Table 2 show a lightness difference between the witness sample scoured through the classical method followed by pre-mordanting (variant L_1-1Al) and the protease treated sample followed by pre-mordanting (variant L_2-1Al), with negative values for the latter. Negative values obtained for DL* reflect a darker colour for 100% wool yarns pre-treated with protease followed by pre-mordanting with potassium alum, thus evidencing an increase of wool fibre tinctorial affinity to *Allium Cepa* natural dye. In the case of wool yarns that were not pre-mordanted (variant L_1-1 compared to L_2-1), there was no evidence of an increase of tinctorial affinity in case of enzyme treatment applied prior to the natural dyeing with *Allium Cepa*. In the case of wool-Angora mohair blended yarns subjected to enzyme treatment and pre-mordanting (L_2-7Al variant) tinctorial affinity does not improve, the values obtained for DL* being sensitively equal with those obtained for the classical variant of pre-treatment (variant L_1-7Al), evidencing that Perizym AFW protease acts predominantly on the scaled cuticle layer of the wool fibre and does not show efficiency on the Angora mohair fibre.

3.2. Physical-mechanical and physical-chemical characteristics

The results for the physical-mechanical indices and the alkali solubility for 100% wool yarns that were subjected to classical or enzyme pre-treatment, pre-mordanted and dyed with *Allium Cepa* infusion, compared to those obtained for raw yarns are presented in Table 3.

Table 3: *Physical-mechanical and physical-chemical characteristics determined for 100% wool yarns and 70% wool-30% Angora mohair blended yarns variants*

Variant code	Physical-mechanical characteristics				Physical-chemical characteristics
	Linear density (Nm)	**Breaking strength** (N)	**Breaking elongation** (%)	**Breaking length** (km)	**Alkali solubility (%)**
L-1 (raw)	24.2/1	1.61	5.51	3.90	6.1
L_1-1Al	22.8/1	1.28	7.44	2.92	9.37
L_2-1Al	24.9/1	1.35	6.93	3.37	9.43
L-7 (raw)	5.8/1	6.58	10.23	3.80	10.67
L_1-7Al	5.8/1	5.36	20.58	3.10	10.83
L_2-7Al	5.7/1	3.97	12.11	2.29	16.61

An analysis of the data reveals that the preliminary processes, followed by pre-mordanting and dyeing, induce a decrease of the breaking strength of 100% wool yarns by approx. 20% in the case of classical treatment and by 16% in the case of enzyme treatment. Breaking elongation is not negatively influenced, and it has values that are higher for both variants of treatment, compared to the value obtained for raw yarns. For wool-Angora mohair blended yarns it is a fact that enzyme treatment is much more aggressive compared to the classical one, leading to much lower values of the breaking strength, compared to the raw witness yarns, the decreases reaching up to 40%.

The analysis of alkali solubility values shown in Table 3, for variants of 100% wool yarns subjected to classical pre-treatment (variant L_1-1Al) or enzyme pre-treatment (variant L_2-1Al), pre-mordanted and naturally dyed, reveals a slight increase of the value of this parameter, compared to the one obtained for raw yarns. The values obtained indicate the formation of new bonds, respectively the modification of wool fibre configuration, without partial solubilisation of the molecular chains. It is known that an index of alkali solubility above 20 % indicates a break of disulphide bonds.

For wool-Angora mohair blended yarns subjected to enzyme pre-treatment, pre-mordanted and naturally dyed (variant L_2-7Al) the values obtained for alkali solubility are higher compared to classical treatment (variant L_1-7Al). This behavior can be explained by the labile character and high reactivity of Angora mohair fibre components of the yarn to the chemical agents, compared to the wool fibre. The Angora mohair fibre has a less ordered cortical structure, which is made of *ortho* and heterotype para (*ortho→para* transition cells) randomly distributed, in contrast with wool, which has a highly orientated bilateral structure (*para* and *ortho*-cortical cells) [8-9]. This explains the higher values of alkali solubility and the lower values of breaking strength of wool-Angora mohair blended yarns, compared to the variants of 100% wool yarns similarly treated.

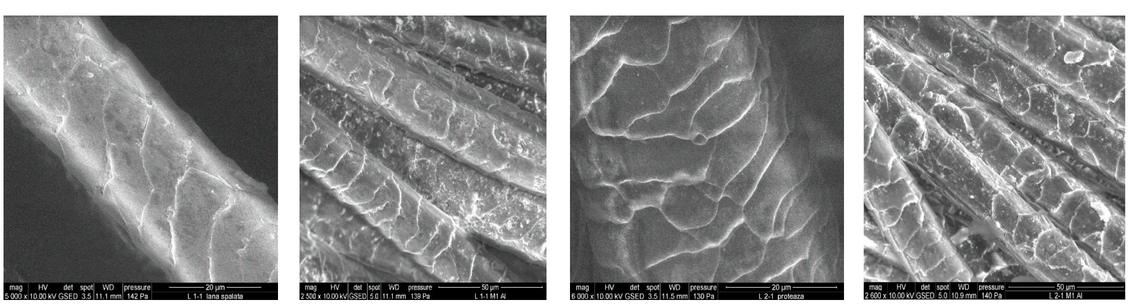
The values of colour fastness when 100% wool yarns and wool-Angora mohair blended yarns are dyed with *Allium Cepa* infusions, being pre-mordanted with potassium alum are shown in Table 4. From the point of view of colour change in the washing solution and colour staining on the multifibre standard, the colour fastness to washing is generally good, with 4/4-5 rating. A comparison of the dyed yarns from the standpoint of fibrous blend reveals a decrease of the fastness to washing of wool-Angora mohair blended yarns compared to the 100% wool yarns, irrespective of the preliminary treatment method applied, thus evidencing a lower fixation of the natural dye on the Angora mohair fibre. Dyeing fastness to acid or alkaline perspiration are good and very good for all the variants of treatment applied, the rating obtained for both colour change and colour staining on the multifibre standard are between 4-5/5. Colour fastness to light is poor, for all the variants experimented. The pre-mordanting treatment with potassium alum did not influence positively the colour fastness to light of *Allium Cepa* natural dye.

Table 4: *The colour fastness obtained for 100% wool yarns and for 70% wool-30% Angora mohair blended yarns dyed with Allium Cepa infusion*

Code	Colour fastness												
	Washing, 40ºC				Acid perspiration pH=5.5				Alkaline perspiration pH=8				Light 50ºC, RH 45% 50 h
	Change in colour	Staining			Change in colour	Staining			Change in colour	Staining			
		CO	PA	WO		CO	PA	WO		CO	PA	WO	
L_1-1	4-5	4-5	5	5	5	4-5	5	5	5	4-5	5	5	2-3
L_2-1	4-5	4-5	4-5	5	5	4-5	5	5	5	4-5	4-5	5	1-2
L_1-1Al	4-5	4	4	4-5	4	4-5	5	5	4-5	4-5	4-5	5	2-3
L_2-1Al	4-5	4	4-5	4-5	4	4-5	5	5	4-5	4-5	5	5	2-3
L_1-7Al	4	4	4	4	4-5	4-5	5	5	4-5	4-5	5	5	2
L_2-7Al	4-5	4	4	4	4-5	4-5	5	5	4-5	4-5	4-5	4-5	2

Observations: Evaluation on grey scale: *rating 5- very good fastness; rating 4/4-5-good fastness; rating 3/3-4-moderate fastness; rating 2/2-3-low fastness; rating 1/1-2-very low fastness;* ***Evaluation on a blue scale:*** *rating 8-exceptional fastness; rating 7-excellent fastness; rating 6-very good fastness; rating 5 good fastness; rating 4-acceptable fastness; rating 3- moderate fastness; rating 2- low fastness; rating 1-very low fastness*

The electron microscopy images obtained at various resolutions, for variants of 100 % wool yarns subjected to classical or enzyme preliminary treatment, followed by pre-mordanting with potassium alum and dyeing with *Allium Cepa* infusion are presented in Figure 1. The analysis of electronic images recorded on 100% wool yarns that were subjected to preliminary enzyme treatment reveals the presence of erosion area of wool fibre scales, differentiated from one part of the surface to the other, with rare spacings and detachments of the scales from the fibre body. Wool fibers that compose the yarn, having been pre-treated according to the classical variant have a smooth aspect, with intact scaled cuticle layer and do not present any degradation.



a. b. c. d.

Fig. 1: *Electronic images obtained for: a- L_1-1, b- L_1-1Al, c- L_2-1, d.- L_2-1Al*

4. CONCLUSIONS

This study presents some aspects of the influence of enzyme preliminary treatment with protease, pre-mordanting with potassium alum and natural dyeing with aqueous extract of *Allium Cepa* have on the tinctorial capacity and characteristics of 100% wool yarns and of 70% wool/30% Angora mohair blended yarns. Colour measurements have evidenced an increase of the tinctorial affinity to *Allium Cepa* natural dye of wool fibre subjected to preliminary enzyme treatment followed by pre-mordanting. In the case of wool-Angora mohair blended yarns that were subjected to enzyme treatment and pre-mordanting, the tinctorial affinity to natural dye does not improve, which is an evidence that protease acts predominantly on the scaled cuticle layer of the wool fibre and is not efficient on the Angora mohair component of the yarn. Preliminary processes applied either in the classical or in the enzyme variant and followed by pre-mordanting and dyeing induce a decrease by up to 20% of the breaking strength of 100% wool yarns, while they do not negatively influence the breaking elongation values. The values of alkali solubility indicate the formation of new bonds, respectively the modification of wool fibre configuration, without partial solubilisation of molecule chains. In the case of wool-Angora mohair blended yarns there is evidence that the enzyme treatment is much more aggressive in comparison with the classical one, leading to higher values of alkali solubility and to lower values of breaking strength (decrease by 40% compared to the raw witness yarns). This behaviour can be explained by the labile character and high reactivity specific to Angora mohair fibres that compose the yarn, due to a less ordered cortical structure, in contrast with the wool, which has a highly ordered bilateral structure. Colour fastnesses to washing, acid and alkaline perspiration are good and very good, for all the variants of treatment applied, the ratings obtained for both colour change and colour staining on the multifibre standard ranging between 4/4-5/5. Colour fastnesses to light are low for all the experimented variants. Pre-mordanting with potassium alum has not positively influenced the colour fastness to light in the case of *Allium Cepa* natural dye. Electron microscopy has allowed the evaluation of the effects of preliminary enzyme treatment and of natural dyeing on the state of the scaled cuticle layer of wool fibres, evidencing erosion areas of wool fibre scales, with rare spacings and detachments from the fibre body. Microscopic images have not evidenced areas of degradation of the cortical layer of wool fibres or of the fibrils exposure areas.

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