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Imparting anti-shrink functionality to wool by individual and simultaneous application of keratinase and papain

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In this study, a pure enzymatic process has been used to impart anti-shrink properties to wool using keratinase and papain applied individually and simultaneously. Both the enzymes have shown to reduce the shrinkage tendency when applied individually, but combined application results in minimum shrinkage. Along with the reduction in shrinkage tendency it is desired to keep the loss in tensile strength to a low level. It is found that the loss in tensile strength could be kept below 10%. The effect of enzyme treatment on other properties like dyeability, wash fastness, light fastness and moisture regain is also studied. SEM study shows that the maximum scale removal is obtained when both the enzymes are applied simultaneously. When the two enzymes are applied individually, papain shows higher efficacy in terms of scale removal than that with keratinase. Infrared spectrophotometric studies using FTIR show that there is no difference in the absorption bands observed in the IR spectra, thus indicating that the enzyme treated wool is not chemically altered, i.e. no new functional groups are introduced in the wool as a result of the enzyme treatment.

Keywords: Anti-shrink, Keratinase, Papain, Wool

1 Introduction

The main disadvantage of wool as a textile fibre is the tendency of woolen garments to change dimension during laundering. This change in dimensions usually results in shrinkage. There are two factors which cause shrinkage in woollen garment, namely relaxation shrinkage and felting shrinkage. The felting shrinkage is the main reason for the shrinkage of wool garments. It is caused by the scales on the surface of the wool fibres, as a result of directional frictional effect (DFE)[1]. This felting tendency has been overcome by the use of shrink resist treatments which reduce the frictional difference by either removing or modifying the surface geometry of the scales. The problem with these treatments is that the extent of severity required to achieve significant anti-shrink properties causes drastic loss in tensile strength and alteration of other fibre properties indicating extensive fibre damage. Conventional shrink-resist treatments utilize an oxidizing agent to modify the scales followed by a polymer coating to further reduce the frictional difference. The most commonly used efficient oxidizing agents are chlorine based e.g. nascent chlorine, dichloroisocyanuric acid (DCCA), etc. The chlorine-Hercosett is the most successful anti-shrink treatment available for woollen tops. Other oxidizing agents for imparting anti-shrink properties are oxygen-based, such as permonosulphuric acid (PMS). But the level of anti-shrink properties achieved by it is lower than that achieved by chlorine-Hercosett treatment, thus resulting in lower popularity and acceptability of such treatments. Novel treatments tried on wool include use of enzymes and electrical discharge treatments. The search for newer techniques has been driven by need for developing chlorine–free anti-shrink treatment, as the use of chlorine produces absorbable organic halides (AOX) in the effluents which are known to be toxic and non-biodegradable. The use of electrical discharge techniques like plasma and UV has been found successful to some extent but are not economically viable and thus are not commercially used. Enzymes provide the best alternative for imparting anti-shrink treatments. They are biodegradable, non-toxic and ecofriendly with no problem of AOX generation. The main advantages of enzyme treatment are that, it is carried out under milder treatment conditions causing less degradation to wool and their specificity enables the treatment to be concentrated more on the surface causing lower degradation of wool cortex with possible higher strength retention than conventional treatments[1-5].

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There are many enzymes capable of imparting anti-shrink treatment to wool. Most of the enzymes belong to proteases class and are capable of digesting the wool surface, thereby modifying the frictional behaviour of wool. The commonly used enzymes on wool include papain, pronase, trypsin, lipoprotein lipase, and keratinase. These enzymes are used in combination with other treatments like hydrogen peroxide bleach, pulse corona discharge, ozone and permonosulphuric acid[6–16].

The present study was carried out with the objective of developing an enzymatic process for imparting shrink-resist properties to wool. A pure enzymatic process using papain and keratinase, either individually or in combination was tried. These enzymes were selected so as to impart best possible effect on scales with minimum damage to wool. Papain has been the enzyme of numerous studies carried out for imparting shrink resist properties to wool and it attacks mainly at alanine, valine, leucine, isoleucine, phenylalanine, tryptophan and tyrosine; it has shown high activity on wool too. Wool cuticle possesses high sulphur content in the form of disulphide linkages; the latter resists the attack of chemicals and enzymes thus making it hydrophobic. Keratinase preferentially attacks these disulphide cross-linkages imparting shrink resist functionality to wool.

2 Materials and Methods

2.1 Preliminary Requirements

100% pure wool fabric made of 2/44 Nm warp and 1/44 Nm weft with GSM of 165 having 2/2 twill weave was used in this study. Two enzymes were used for the trial, viz. papain (Shree Ganesh Industrial Enzymes) and keratinase (Department of Biotechnology, Jaypee Institute of Information Technology). All other chemicals used were of laboratory grade.

2.2 Enzymatic Treatment of Wool

The study was divided into two phases. In phase 1, both the enzymes were applied separately onto wool to optimize the conditions of treatment and to ascertain if there is any possibility to apply both the enzymes under common application conditions in phase 2. In phase 2, the combined enzyme application was carried out and the treatment conditions were varied using the optimized conditions as achieved in phase 1 as the extremes. Box-Behnken design was used to obtain the experimental plan and statistical tools were used for the analysis of the data. The parameters used for experiment are shown in Table 1 for both keratinase and papain when applied individually and in combination.

On completion of the enzyme treatment, the samples were taken out, washed in running cold water, immersed in boiling water for 10 min to deactivate the enzyme and then again washed in cold water. Finally, the samples were squeezed, oven dried at 100°C for 30 min and were conditioned for 1 day before conducting any further testing.

2.3 Testing of Samples

The samples in phase 1 were evaluated primarily in terms of shrinkage potential and loss in tensile strength. To optimize the treatment conditions, the maximum permissible area shrinkage was fixed at 6%. This limit was derived from the Woolmark specification AW-1-'Flat woven, pile woven and pressed felt apparel products' which mentions maximum total shrinkage as 3% each in both warp and weft directions that comes out to be approximately 6% when calculated in terms of area shrinkage. Similarly, the maximum permissible strength loss due to enzymatic treatment was decided to be kept at 10%. Dimensional change and tensile strength were evaluated as per the AATCC test method 99-2004 and ASTM D5035-11 method respectively. The optimized samples from individual and combined enzyme application as well as untreated wool were dyed using five 1:2 metal complex dyes to assess changes in dyeability. The dyed samples were subsequently evaluated for wash fastness through AATCC test method 61-2006 and light fastness through exposure to xenon arc. Assessment of

Table 1—Levels of factors for treatment of wool with keratinase and papain individually and in combination

Parameter	Keratinase treatment	Papain treatment	Combined application
Keratinase conc. % (owf)	0.2, 0.3, 0.4	–	0.2, 0.3, 0.4
Papain conc. % (owf)	–	0.2, 0.3, 0.4	0.2, 0.3, 0.4
*p*H	5.0, 6.5, 8.0	5.0, 6.5, 8.0	As obtained from individual application
Temperature, °C	40, 55, 70	40, 55, 70	As obtained from individual application
Time, min	60, 90, 120	60, 90, 120	60, 90, 120
MLR	1:30	1:30	1:30

changes in moisture regain was also carried out on four samples, viz. untreated, optimized samples for both the enzymes applied individually and simultaneously. The SEM imaging was carried out to ascertain the changes on the surface of the wool as a result of the enzymatic treatment, and FTIR spectra of the four optimized samples were recorded to study the effect of enzymatic treatments on the chemical constitution of wool.

3 Results and Discussion

3.1 Enzymatic Treatment

The results of the experiments using Box-Behnken design for the keratinase and papain are initially evaluated using statistical tools. The response surface plots for keratinase are studied and it is observed that the area shrinkage decreases with the increase in concentration of the enzyme, *p*H and time. The area shrinkage decreases up to *p*H 8 and may continue the same trend beyond *p*H 8 ; hence the *p*H was fixed at 8 as there is a possibility of severe damage to wool at higher *p*H. In case of temperature, a peak is obtained in the range 50-65°C. Using regression model estimation it is possible to get the optimized treatment combination which causes minimum area shrinkage with loss in tensile strength of < 10%. This optimized treatment condition is shown in Table 2. The input parameters, i.e. enzyme concentration, *p*H, temperature and time (Table 2) are predicted as optimum by regression model while the output parameters, such as area shrinkage, loss in tensile strength in both warp and weft directions are the predictions from regression equations.

Table 2—Optimum conditions for the treatment of wool with keratinase and papain individually and in combination

Parameter	Optimized value		
	Keratinase	Papain	Keratinase + papain
Enzyme conc. % (owf)	0.3	0.4	0.399 ≈ 4 (keratinase) and 0.3 (papain)
*p*H	8.0	6.979≈7	7.8
Temperature, °C	50	70	70
Time, min	120	120	70
Area shrinkage, %	2.84	2.37	2.56
Loss in tensile strength (warp), %	9.20	7.79	9.39
Loss in tensile strength (weft), %	10.00	7.54	10.00

Similarly, the response surface plots of papain are studied and it is observed that the area shrinkage decreases with increase in enzyme concentration, temperature and time. The area shrinkage decreases with increase in temperature up to 70°C. Although it is possible that the area shrinkage may further decrease with increase in temperature beyond 70°C, further trials are not carried out at temperature beyond 70°C, as it is desirable to process wool under milder conditions of treatment. Here again the regression model estimation is used and the optimized conditions for papain are ascertained (Table 2). From the optimized conditions for the application of both the enzymes, it is observed that both the enzymes are most efficient under different conditions of temperature and *p*H. Keratinase and papain are most efficient at *p*H 8 & temperature 50°C and *p*H 7.0 & temperature 70°C respectively. Thus, a Box-Behnken experimental setup for 5 factors is designed so as to establish a common application condition for both the enzymes applied simultaneously. The levels of the each of the factors for the Box-Behnken setup are shown in Table 3.

Data obtained for combined application of enzymes are analyzed using regression model estimation technique and the optimized conditions along with their predicted responses are ascertained (Table 2).

3.2 Comparison in Performance of Enzymes

3.2.1 Changes in Area Shrinkage and Tensile Properties

The changes in area shrinkage and loss in tensile strength in both warp and weft directions are shown in Table 4. The input parameters are those, which the regression model has shown to be optimum. Hence, there is no difference in the input parameters shown in Tables 2 and 4. The output parameters are the actual values obtained when wool is treated with the enzymes as per the optimum conditions for both the enzymes applied individually and simultaneously. Hence, the data is somewhat different from Table 2. It is observed that papain is more active in reducing the shrinkage potential of wool. In combination, a

Table 3—Levels of factors for Box-Behnken experimental setup used for combined application of enzymes

Parameter	Level
Keratinase conc., % (owf)	0.2, 0.3, 0.4
Papain conc., % (owf)	0.2, 0.3, 0.4
*p*H	7.0, 7.5, 8.0
Temperature, °C	50, 60, 70
Time, min	60, 90, 120

synergism is observed in the action of the enzymes. The area shrinkage caused by combined treatment is less than that obtained by using kratinase and papain independently. When loss in tensile strength is compared, it is found that keratinase causes higher loss in strength than papain. This is attributed to the fact that keratinase ruptures the disulphide linkages in wool, thereby causing higher loss in tensile strength. The loss in tensile strength caused by combined application is higher than that caused by papain but lower than that caused by keratinase.

The optimized enzyme concentrations (0.4% keratinase and 0.3% of papain, owf) obtained in earlier experiments for individual enzyme applications are also applied in combined enzyme application to facilitate comparison of responses. The responses are predicted from the regression equations obtained for individual application of both keratinase and papain (Table 5). The objective of showing this data is to highlight the synergism in enzyme action when applied in combination rather than in individual application under identical treatment conditions except their concentrations. This indicates that although the treatment conditions are not optimum for both the enzymes (as shown by the predicted values for the enzymes applied individually), the enzymes when applied simultaneously under the identical conditions have shown improvement in the results.

It is observed that there is drastic reduction in area shrinkage when the enzymes are applied simultaneously as compared to those when the enzymes are applied individually. This shows that although both the enzymes are sensitive to changes in treatment conditions, the effect of change in temperature on keratinase is more than the effect of changes in *p*H on papain. The tensile strength loss observed for keratinase treated wool is higher than that for papain treated wool, which is again attributed to the attack of keratinase on the disulphide linkages despite the conditions of application is not maintained at optimum level. However, the loss in tensile strength observed in the case of combined enzyme application is lower as compared to those observed in cases of individual enzyme applications.

3.2.2 Changes in Dyeing and Fastness Properties

The spectrophotometric evaluation data for four woollen samples dyed with five 1:2 metal complex dyes are shown in Table 6.

In general, enzyme pre-treatment produces lighter shades upon dyeing than that on untreated samples, except for Cololan Olive Green BGL, and for keratinase treated sample dyed with Cololan Black RL, wherein darker shades are produced. There has been slight change in the tone of the dyeing also. Most of the dyeings have shifted towards greener side. The samples dyed with Cololan Black RL as well as the combined treated sample dyed with Cololan Red BRL have shifted towards the redder side. Similarly few samples have shown a shift towards the yellower side. All the samples dyed with Cololan Yellow AGLN and papain treated sample dyed with Cololan Red BRL have shown a shift towards the bluer side.

The wash and light fastness of the dyed wool (Table 7) substantiate enzyme inaction on these

Table 4—Changes in properties of wool treated under optimized conditions for both the enzymes applied individually and simultaneously

Parameter	Treatment conditions		
	Keratinase	Papain	Combined
Keratinase conc., % (owf)	0.32	-	0.4
Papain conc., % (owf)	-	0.4	0.3
*p*H	8.0	7.0	7.8
Temperature, ^{o}C	50	70	70
Time, min	120	120	70
Area shrinkage, %	2.91	2.68	2.08
Loss in tensile strength (warp), %	9.67	6.54	8.62
Loss in tensile strength (weft), %	9.34	7.12	9.04

Table 5—Changes in properties of wool treated with enzymes applied individually and simultaneously under optimized conditions for both the enzymes applied simultaneously

Parameter	Keratinase treatment	Papain treatment	Combined treatment
Keratinase conc. % (owf)	0.4	-	0.4
Papain conc., % (owf)	-	0.3	0.3
*p*H	7.8	7.8	7.8
Temperature, ^{o}C	70	70	70
Time, min	70	70	70
Area shrinkage, %	6.33*	5.28*	2.08$^{\#}$
Loss in tensile strength (warp), %	11.0*	9.76*	8.62$^{\#}$
Loss in tensile strength (weft), %	12.1*	9.5*	9.04$^{\#}$

*Predicted responses from regression equations.

$^{\#}$Responses obtained from actual sample.

Table 6—Spectrophotometric evaluation of untreated and enzyme treated wool dyed with five 1:2 metal complex dyes

Treatment	*K/S*	L	a	b	ΔL	Δa	Δb	ΔE	Colour status
Cololan Yellow AGLN (CI Yellow 241)									
Untreated	15.39	62.40	13.39	58.20	—	—	—	—	—
Keratinase treated	13.56	62.50	12.57	56.59	0.10	-0.82	-1.61	1.81	Lighter, Greener, Bluer
Papain treated	13.06	63.54	12.57	57.19	1.14	-0.82	-1.01	1.73	Lighter, Greener, Bluer
Combined treatment	13.54	63.38	12.59	57.69	0.98	-0.80	-0.51	1.37	Lighter, Greener, Bluer
Cololan Orange FBL (CI Orange 142)									
Untreated	13.04	46.65	41.70	39.21	—	—	—	—	—
Keratinase treated	13.29	46.90	41.63	40.12	0.25	-0.07	0.91	0.95	Lighter, Greener, Yellower
Papain treated	12.60	47.43	41.42	39.85	0.78	-0.28	0.64	1.05	Lighter, Greener, Yellower
Combined treatment	12.71	47.23	41.12	39.64	0.58	-0.58	0.43	0.93	Lighter, Greener, Yellower
Cololan Red BRL (CI Red 362)									
Untreated	13.80	38.66	40.94	20.36	—	—	—	—	—
Keratinase treated	13.78	38.72	40.89	20.67	0.06	-0.05	0.31	0.32	Lighter, Greener, Yellower
Papain treated	12.60	38.94	40.88	20.58	0.28	-0.06	-0.09	0.29	Lighter, Greener, Bluer
Combined treatment	13.61	38.82	40.97	20.56	0.16	0.03	0.20	0.27	Lighter, Redder, Yellower
Cololan Olive Green BGL (CI Green 104)									
Untreated	9.88	29.47	-7.03	4.49	—	—	—	—	—
Keratinase treated	10.30	28.63	-7.44	4.55	-0.84	-0.41	0.06	0.94	Darker, Greener, Yellower
Papain treated	10.11	29.01	-7.43	4.56	-0.46	-0.40	0.07	0.62	Darker, Greener, Yellower
Combined treatment	9.85	29.06	-7.38	4.56	-0.41	-0.35	0.07	0.54	Darker, Greener, Yellower
Cololan Black RL (CI Black 194)									
Untreated	15.19	22.75	-0.01	-2.81	—	—	—	—	—
Keratinase treated	16.29	21.93	0.08	-2.72	-0.82	0.09	0.09	0.83	Darker, Redder, Yellower
Papain treated	14.51	23.23	0.07	-2.72	0.48	0.08	0.09	0.49	Lighter, Redder, Yellower
Combined treatment	10.30	23.82	0.01	-2.78	1.07	0.02	0.03	1.07	Lighter, Redder, Yellower

properties as compared to those of untreated wool. While light fastness grades are excellent (7) for all four types of dyed wool, wash fastnes grades are found to be excellent (5), except showing a marginal fall to 4-5 for wool dyed with Cololan Olive Green BGL.

3.2.3 Changes in Moisture Regain

The effect on moisture regain of four samples, viz. untreated, keratinase, papain and combined enzyme treated woolfabrics are shown in Table 7. The moisture regain of all the samples increases upon treatment with enzyme; maximum increase being shown by papain treated fabric, followed by combined enzyme-treated ones, and the minimum increase by keratinase treated fabric. This is attributed to the attack of enzymes on the cuticle scales on wool surface which are hydrophobic in nature. The papain in addition to attacking the scales also attacks the interior of the fibre, thus facilitating penetration of the moisture into the interior of the fibre. The keratinase attacks the scale only and gives the lowest increase in the moisture absorption. The combined treatment obviously shows intermediate improvement in moisture absorption due to action of papain and keratinase in these two different mechanisms.

Table 7—Moisture regain properties of untreated and enzyme treated wool

Sample	Moisture regain, %
Untreated	11.3
Keratinase treated	12.2
Papain treated	13.5
Combined treated	12.6

3.2.4 SEM Study

SEM studies were carried out on four samples, viz. untreated, keratinase treated, papain treated and combined enzyme treated wool (Fig. 1). As anticipated, it is observed that the enzymes damage the cuticle scales on the surface of wool. Papain causes higher removal of scales than keratinase and

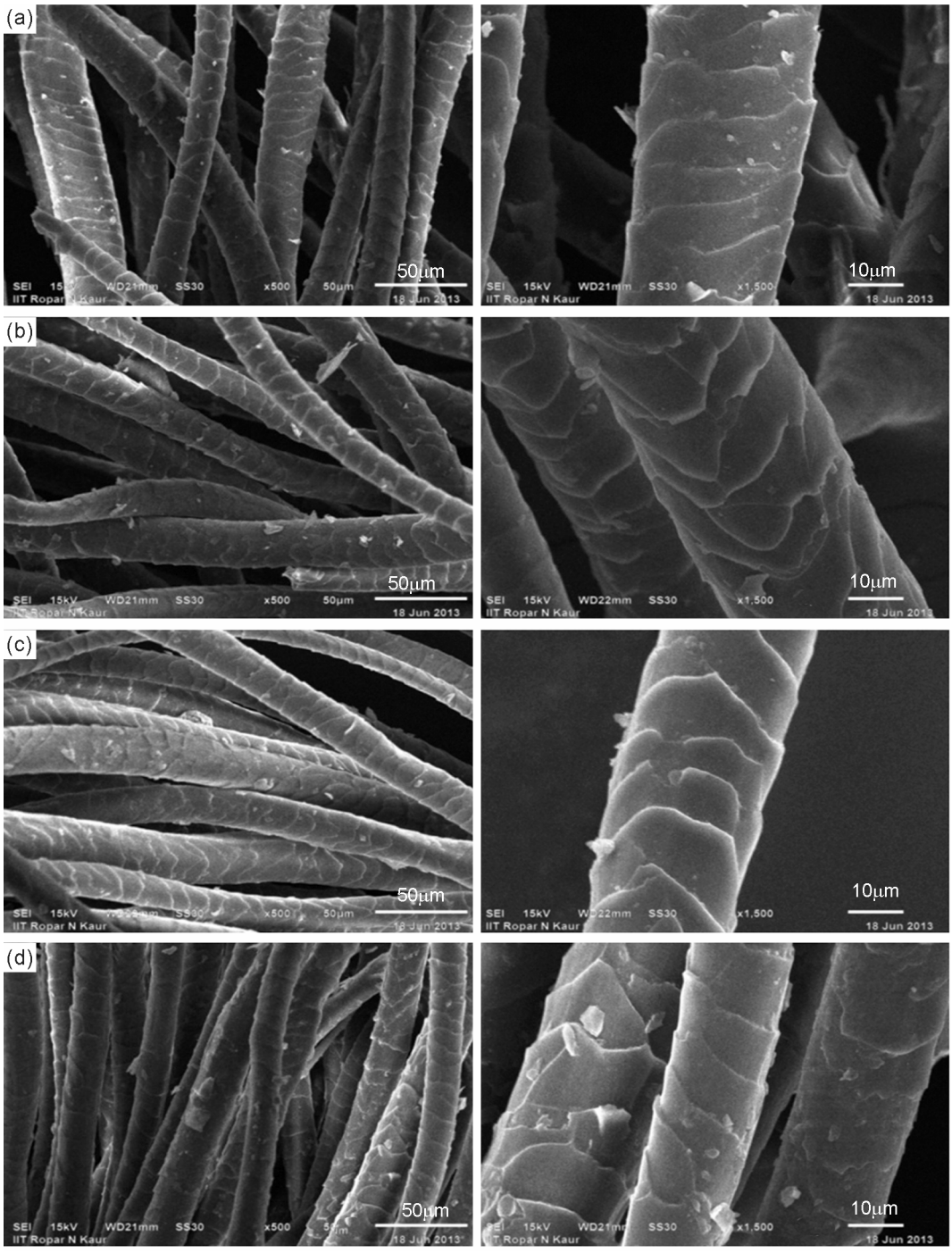

Fig. 1—SEM micrographs of wool (a) untreated, (b) keratinase treated, (c) papain treated, and (d) treated with combination of enzymes (Micrographs on LHS are magnified by ×500 while those on RHS are by ×1500)

that the combined application is most effective in removal of the surface scales yielding a smoother surface. As expected, the removal of the scales has direct impact on the felting shrinkage behavior of the wool, the combined treatment giving the least amount of felting shrinkage followed by papain-treated and then keratinase-treated sample. All the samples have shown drastic reduction in the felting shrinkage behavior as compared to the untreated samples in which the scales have not been removed.

3.2.5 FTIR Study

The infrared spectra of the untreated and enzyme treated wool show no such perceptible shift of absorption bands attributable to various functional groups. It is not possible to accurately predict the quantitative change in intensity of these functional groups which might have been caused by the enzymatic treatment but it is evident that no new functional groups are introduced by the enzymatic treatment.

4 Conclusion

Both keratinase and papain are able to reduce the felting shrinkage tendency of wool when applied individually or simultaneously. The best results are obtained in combined application when the area shrinkage is reduced to 2.08%, which is lower than those with either enzyme applied individually. The tensile strength loss is lower than 10% in all the cases. It is concluded that the keratinase causes higher strength loss than papain, because the former attacks the disulphide crosslinks. The dyeability study shows some effect of enzyme treatment on the K/S as well as lightness values but the trend is not uniform. The colour difference values (ΔE) of the enzyme treated wool are lower than 2 which is the industrial acceptable standard for color difference. The tonal differences are also observed as a result of enzyme treatment but no uniform trend is observed. The fastness tests show that there is no effect of enzyme treatment on the fastness properties. Papain causes the highest increase in the moisture regain, whereas keratinase causes the least increase in the regain value. This is attributed to papain causing higher damage to wool surface as well as the interior. From the SEM study it is concluded that maximum scale removal is obtained in the case of combined treatment. In terms of efficiency it is found that papain causes more scale removal than keratinase. The infrared spectra show no change as a result of the enzyme treatment, thus it is concluded that no new functional groups get introduced because of enzyme treatment.

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