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Use of Enzymes and Chitosan Biopolymer in Wool Dyeing

Abstract
The importance of bio-processes and mild chemical so-called 'soft chemistry' processes have been increasing of late. Especially for expensive and fashionable material such as wool and silk, the compatibility with the environment and protecting these fibres' natural properties during their treatments have been investigated. In this study, we used proteases of different origins to treat wool-woven fabric. Proteolytic treatments were carried out with various commercial protease preparations such as Perizym AFW, Alcalase 2.5L, Savinase 16L and Papain. After proteolytic treatments, the samples were evaluated with regard to the loss of tensile strength, weight loss, degree of whiteness and alkali solubility. The samples were then dyed with different classes of dyestuffs such as reactive dyes, levelling acid, milling acid and metal complex dyestuffs. The effects of these proteolytic treatments on colour strength and various fastness values were evaluated. Moreover, some samples treated with proteases were then treated with chitosan, a polysaccharide-based cationic biopolymer, and dyed. Next, the effects of the treatment with chitosan on colour strength were determined.

Key words: *wool, protease, chitosan, dyeing.*

Abbreviations

LanRed2G: Lanasol Red 2G
LanRed2B: Lanaset Red 2B
NeoRedAG: Neolan Red AG
NeoRedGRE: Neolan Red GRE 200%
ErioRedA3G: Erionyl Red A3G
LanYelCE: Lanasol Yellow CE
Cont: Control (treatment without protease before dyeing or chitosan treatment)
Per: Perizym AFW
Alc: Alcalase 2.5L
Sav: Savinase 16L
Pap: Papain
Chit: Chitosan
Cont+Chit: Control+Chitosan
Per+Chit: Perizym AFW+Chitosan
Alc+Chit: Alcalase 2.5L+Chitosan
Sav+Chit: Savinase 16L+Chitosan
Pap+Chit: Papain+Chitosan
WF_{Co}: Colour staining fastness to washing for cotton fabric
WF_{W}: Colour staining fastness to washing for wool fabric
RF_{D}: Dry rubbing fastness
RF_{W}: Wet rubbing fastness
LF: Light fastness.

Introduction

Wool fibre, based on a protein called keratin, consists of two major morphological parts: cortex cells (90% of the wool's weight) and the surrounding cuticle cells (10% of the wool's weight). The surface of the cuticle cells is highly hydrophobic due to covalently bound fatty acids [1,2].

The morphology of the wool fibre surface plays an important role in textile finishing processes. The covalently bound fatty acids and the high amount of disulphide bridges make the outer wool surface highly hydrophobic. Especially in the printing and dyeing of wool, the hydrophobic character of the wool surface is disturbing. Diffusion of the hydrophilic dyes at and into the fibres is hindered [3]. For this reason, the hydrophilicity and dyeability properties of the wool fibre should be developed. To this end, conventionally oxidative agents (such as hydrogen peroxide) and reductive agents (such as sodium hydrosulphite) have been used in wool treatment. In recent years, an environmentally friendly enzymatic treatment for wool fibre has been investigated.

With this aim in mind, proteases such as Papain, Pronase [3], Streptomyces Fradiae Protease (SFP), Bactosol WO liq., Alcalase 2.0T [4], Savinase, Alcalase, Neutrase, Lipolase [5], Perizym AFW [6] have been used in literature. Wool samples were treated with different proteolytic and lipolytic enzymes at varying values of pH, ionic buffer strength, treatment time, temperature [5] and enzyme concentration [6]. The enzymatic treatment gave wool more rapid dye adsorption kinetics and a higher total adsorption than untreated wool samples with reactive dyes [7,8], acid dyes [4], or 1:2 metal complex dyes [6]. The researchers also determined that proteolytic treatment improved the whiteness of wool samples [8].

On the other hand, the effect of chitosan, a polysaccharide-based cationic biopolymer, on the dyeability of wool fibres has also been investigated over the last few years. A method for improving the dyeability of wool fabric with reactive dyes, proposed by Julia [9], involves pre-treating the fabric with an oxidising agent, applying chitosan to the fabric. Other authors reported that the dyeability of wool fabrics pre-treated with a chitosan/nonionic surfactant mixture improved, and their colour strength with reactive dye increased [10].

Some of the studies mentioned above determined the effects of separately treated wool fabrics with protease enzymes and chitosan biopolymer, but a literature review reveals a gap in research into the combined effects of proteases and chitosan on wool fabric.

In this study, we intended to improve the dyeability of wool fabric with proteolytic treatment and/or treatment with chitosan without causing any significant change to the nature of the wool fibre. At the same time, our aim is to achieve environmentally friendly wool fabric processes, and thus to diminish the wastewater charge from a wool plant. We thus used proteases of different origins (such as microbial and plant) in the proteolytic treatment of wool woven fabric. Proteolytic treatments were carried out with various commercial protease preparations such as Perizym AFW (specific combination), Alcalase 2.5L (microbial-based), Savinase 16L (microbial-based) and Papain (plant-based). After proteolytic treatments, the samples were evaluated with regard to the loss of tensile strength, weight loss, degree of whiteness and alkali solubility. The samples were then dyed with different classes of dyestuffs, such as reactive dyes, levelling acid, milling acid and metal complex dyestuffs. The effects of these proteolytic treatments on colour strength and various fastness values were evaluated. Increases in colour strength would provide dyestuff and cost saving. In addition, some samples treated with proteases were then applied with chitosan and dyed. Next, the effects of the treatment with chitosan on colour strength were determined. Also, we tried to determine the optimum treatment condition with regard to proteolytic treatment time, protease type, dye class, and treatment with and without chitosan while improving colour strength.

Table 1. Proteases used for treating wool woven fabric.

Enzyme type	EC number	Source
Perizym AFW	-	Highly specific combination of proteases
Alcalase 2.5L, Type DX	EC 3.4.21.62	A serine-type protease, produced by submerged fermentation of a genetically modified bacillus micro-organism. Declared activity: 2.5 U/mg.
Savinase 16L, Type EX	EC 3.4.21.62	A serine-type protease, produced by submerged fermentation of a genetically modified bacillus micro-organism. Declared activity: 16 U/mg.
Papain	EC 3.4.22.2	A protease enzyme from Carica Papaya. 3.1 U/mg

Table 2. Dyestuffs used in dyeing and their properties.

Dyestuff	C.I. number	Dye class
Lanasol Red 2G	C.I. Reactive Red 116	Reactive dye containing sulpho-group and 1 or 2 bromo-acrylamide reactive groups. pH: 5
Lanaset Red 2B	C.I. Acid Red 252, C.I. Acid Red 407	Combination of modified, tictorially strong 1:2 metal complex, acid milling and reactive dyes. pH: 4.5
Neolan Red AG	C.I. Acid Red 447, C.I. Acid Orange 94, C.I. Acid Red 252	Milling acid dye, metal-free. pH: 5.5
Neolan Red GRE 200%	C.I. Acid Red 183	1:1 metal complex dye containing sulpho groups. pH: 2.5
Erionyl Red A3G	C.I. Acid Red 151	Levelling acid dye. pH: 2.5-3
Lanasol Yellow CE	C.I. Reactive Yellow 039, C.I. Reactive Red 136	Metal free reactive dye especially developed for wool dyeing. pH: 5

Table 3. Recipes for proteolytic treatments.

Concentration	Chemical agents	pH	Liquor ratio	Temperature, °C	Time, min
2 g/l	Perizym AFW	8	20:1	70	15-30-60
0.5 g/l	Perlavin NIC				
0.8 ml/l	Sodium hydroxide				
2.5 % o.w.f.	Alcalase 2.5 L	8.5	20:1	55-60	15-30-60
1 g/l	Perlavin NIC				
0.8 ml/l	Sodium hydroxide				
2.5 %o.w.f.	Savinase 16L	9.5	20:1	45-50	15-30-60
1 g/l	Perlavin NIC				
0.8 ml/l	Sodium hydroxide				
6.7 g/l	Papain	6-7	7.5:1	65	15-30-60
1 % o.w.f.	Sodium bisulphide				
For pH 6-7	Sodium bicarbonate				

Table 4. Recipes for dyeing; bath additions (1 and 2*) are marked in Figure 1.*

Bath additions	Chemical agents	Const.	pH	Liquor ratio
2*	Lanasol Red 2G	1% o.w.f.	5	20:1
1*	Albegal SET	1% o.w.f.		
	Glauber's salt	7.5% o.w.f.		
	Ruco-Acid ABS	2 g/l		
	Miralan Q	1 g/l		
2*	Lanaset Red 2B	1% o.w.f.	4.5	20:1
1*	Albegal SET	1% o.w.f.		
	Glauber's salt	7.5% o.w.f.		
	Ruco-Acid ABS	2 g/l		
	Miralan Q	1 g/l		
2*	Neolan Red AG	1% o.w.f.	5.5	20:1
1*	Albegal SET	2% o.w.f.		
	Glauber's salt	5% o.w.f.		
	Ruco-Acid ABS	2 g/l		
2*	Neolan Red GRE 200%	1% o.w.f.	2.5	20:1
1*	Albegal SET	2% o.w.f.		
	Glauber's salt	7.5% o.w.f.		
	Sulphuric acid (% 96)	4-5% o.w.f.		
2*	Erionyl Red A3G	1% o.w.f.	2.5-3	20:1
1*	Ruco-Acid EPV-1629	2 g/l		
	Albegal SET	2% o.w.f.		
	Glauber's salt	7.5% o.w.f.		
2*	Lanasol Yellow CE	1% o.w.f.	5	20:1
1*	Albegal SET	2% o.w.f.		
	Glauber's salt	5% o.w.f.		
	Ruco-Acid ABS	2 g/l		

Experimental

Materials

The textile materials were 100% wool fabric, plain weave, 173 g/m^2, 29 ends/cm, 27 picks/cm. The protease enzymes listed in Table 1 and the dyestuffs listed in Table 2 were used in this study. The chitosan used in our experiments was supplied by Seafresh Ltd.

Methods

Application methods

Treatments were carried out in different ways, as stated below:

- proteolytic treatment before dyeing, and
- proteolytic treatment before chitosan application, then dyeing.

All proteolytic treatments were carried out by the exhaustion method in a Land-ometer (Atlas) under the optimum application conditions showed in Table 3. After proteolytic treatment, the dyeing processes were carried out in IR Dyeing Apparatus (Labortex) under the recommended dyeing conditions, with the recipes given in Table 4 and Figure 1. After the proteolytic treatments on some samples, the treatment with chitosan was carried out according to the recipe shown in Table 5. All treatments were carried out twice.

Investigation methods

Weight loss

Weight loss (%) was calculated from

$$[(W_1-W_2) \times 100]/W_1$$

where:

W_1 - the weight of the sample before treatment,

W_2 - the weight of the sample after treatment.

Strength loss

Tensile strength (in warp direction) was evaluated according to ASTM D5035-90 (strip test) with an Instron 4411 tester.

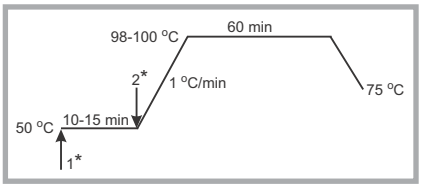

Figure 1. General dyeing graph (bath additions shown with 1 and 2* are given in Table 4).*

Table 5. Recipe for additive process with chitosan [9,16-18].

Conditions	Quantity
Chitosan	2.5% o.w.f.
Acetic acid	3 g/l
Liquor ratio	10:1
Temperature	30°C
Time	60 min

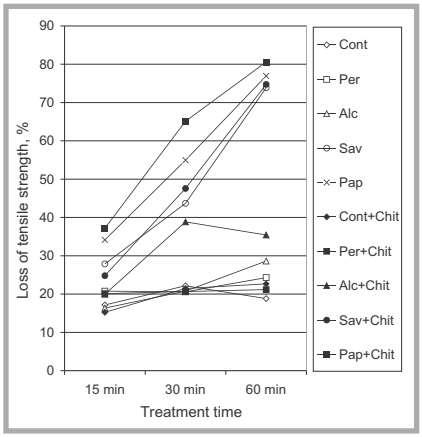

Figure 2. *Comparison of loss values of tensile strength of the samples treated with proteases and chitosan.*

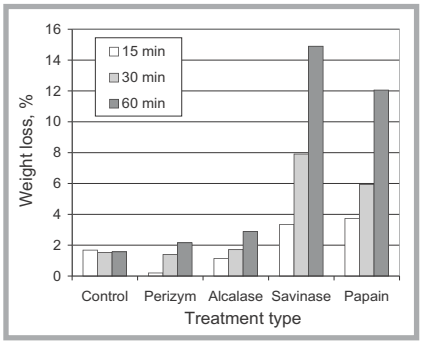

Figure 3. *Comparison of weight loss of samples treated with proteases.*

Table 6. *Whiteness degrees (Stensby formula) for the samples treated with proteases and chitosan.*

Treatment	15 min	30 min	60 min
Cont	47.87	47.93	48.00
Per	48.41	49.07	49.62
Alc	49.24	49.31	48.57
Sav	50.76	54.48	61.02
Pap	48.97	51.09	53.55
Cont+Chit	48.05	47.54	47.59
Per+Chit	48.85	49.51	50.16
Alc+Chit	49.06	49.57	49.02
Sav+Chit	51.65	55.38	61.20
Pap+Chit	49.40	51.12	54.84

Table 7. *The mean values and standard deviations of loss of tensile strength (%) of the samples treated with proteases and chitosan.*

Treatment	15 min		30 min		60 min	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
Cont	17.16	13.9	22.17	19.76	18.81	16.52
Per	20.76	7.52	20.58	14.83	24.31	11.96
Alc	16.35	20.15	20.76	14.91	28.63	9.97
Sav	27.88	13.79	43.70	16.56	73.88	10.61
Pap	34.16	11.74	54.95	16.57	76.96	11.26
Cont+Chit	15.24	9.6	21.44	14.88	22.66	12.05
Per+Chit	20.10	7.58	20.65	13.98	21.16	7.01
Alc+Chit	19.94	7.87	38.85	22.47	35.41	14.84
Sav+Chit	24.80	12.32	47.57	13.55	74.75	6.39
Pap+Chit	37.16	17.51	65.13	10.88	80.60	12.46

Table 8. *The mean values and standard deviation of weight loss (%) of samples treated with proteases.*

Treatment	15 min		30 min		60 min	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
Cont	1.682	1.02	1.525	0.87	1.571	1.15
Per	0.200	0.66	1.319	1.28	2.159	1.31
Alc	1.131	1.13	1.894	0.57	2.882	0.95
Sav	3.342	1.12	7.907	1.94	14.900	2.87
Pap	3.726	0.98	5.944	1.47	12.380	3.41

Table 9. *The K/S values of dyed wool samples after 15, 30, 60 min proteolytic treatment.*

Dye	Treatment	15 min	30 min	60 min	Dye	Treatment	15 min	30 min	60 min
Lanasol Red 2G	Cont	9.14	10.34	9.18	Lanaset Red 2B	Cont	10.73	10.58	11.34
	Per	15.65	17.7	17.70		Per	13.17	10.82	10.45
	Alc	13.59	16.36	15.64		Alc	9.10	9.71	10.98
	Sav	15.74	14.58	12.50		Sav	9.47	7.41	6.08
	Pap	17.37	17.94	16.98		Pap	12.14	10.11	8.97
Neolan Red AG	Cont	12.30	10.42	11.17	Neolan Red GRE 200%	Cont	12.62	14.68	15.85
	Per	12.53	12.37	11.98		Per	19.60	20.11	15.54
	Alc	11.51	11.17	10.02		Alc	17.14	16.95	17.97
	Sav	10.25	8.34	6.54		Sav	18.96	14.08	11.88
	Pap	11.61	11.08	8.82		Pap	20.26	18.28	15.25
Erionyl Red A3G	Cont	23.03	25.64	19.34	Lanasol Yellow CE	Cont	13.58	12.60	12.46
	Per	21.81	20.50	24.48		Per	11.55	14.59	16.12
	Alc	22.02	21.76	19.29		Alc	13.54	10.47	13.36
	Sav	19.02	15.55	14.15		Sav	11.41	10.39	9.44
	Pap	21.50	19.07	16.01		Pap	13.45	13.41	12.88

Alkali solubility

Alkali solubility values were determined by the IWTO-4-60 standard test method. The extraction in a soxhlet with dichloromethane for four hours at five cycles per hour was carried out in order to remove fatty matter from the samples. Then each sample was treated with 100 mL of 0.1 N NaOH solutions at 65°C for 60 minutes. The alkali solubility of the samples was calculated as a percentage of the original mass, according to the equation given below:

$$\text{Alkali solubility } \% = (M_1 - M_2)/M_1 \times 100$$

M_1 - mass of oven-dry samples before sodium hydroxide treatment, and

M_2 - mass of oven-dry samples after sodium hydroxide treatment.

Colour strength and whiteness degree

The degree of whiteness according to Stensby and the K/S values of dyed fabrics were measured by a Minolta (3200D) spectrophotometer.

Colour fastness

Colour fastness to washing was determined according to BS EN ISO C06 at 40°C, colour fastness to rubbing was determined according to BS EN ISO 105 X12, and colour fastness to light according to BS EN ISO B02.

Statistical analysis

A statistical analysis of the results was made with the SPSS statistics program with a 95% confidence interval. The ANOVA program was used for the analysis of variance (α=0.05).

Results

Effects of proteolytic processes on whiteness

The degrees of whiteness of fabrics according to Stensby are given in Table 6. As for the results, proteolytic treatment with Savinase 16L and Savinase+Chitosan

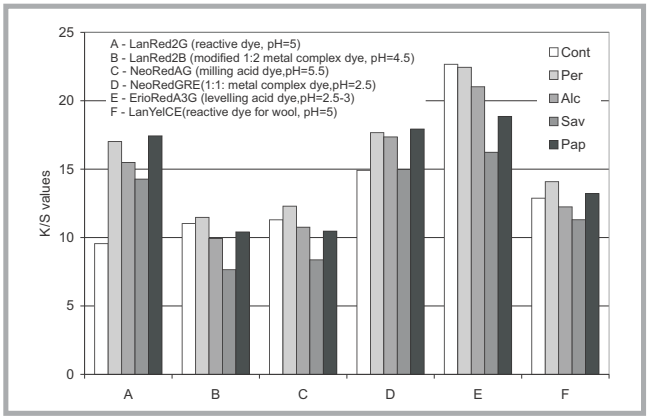

Figure 4. Comparison of K/S values of samples treated with proteases and then dyed.

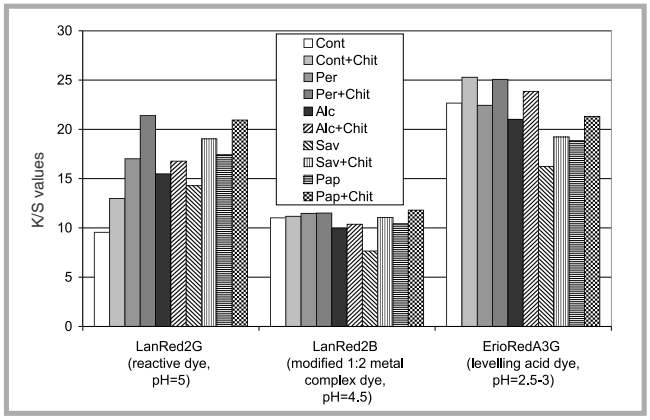

Figure 5. Comparison of K/S values of samples treated with proteases and chitosan.

combination caused the highest degree of whiteness when compared with the control fabric. The combined treatment with chitosan provided a slightly higher degree of whiteness when compared with the results of proteolytic treatment only.

Effects of proteolytic processes on tensile strength

Differences between the loss values of tensile strength corresponding to the protease types and the times of proteolytic treatment and their interactions were significant according to ANOVA (α=0.05). The longer the time of proteolytic treatment, the higher was the loss of tensile strength (Figure 2, Table 7). The loss values of tensile strength of samples treated with Alcalase 2.5L, Perizym AFW and control samples resemble each other; those of Savinase 16L and Papain after 60 minutes of proteolytic treatment time reached values of 70%. The highest loss of tensile strength was caused by treatment with Savinase+Chitosan and Papain+Chitosan. Chitosan treatment caused an increase in the loss of tensile strength.

Effects of proteolytic processes on fabric weight

The protease type, the time of proteolytic treatment and their interactions affected the values of weight loss, according to the result of analysis of variance (α=0.05). While the time of proteolytic treatment increased, the values of weight loss increased. Significant differences were obtained between weight loss values of the samples subjected to proteolytic treatment for 60 minutes (Figure 3 and Table 8).

Alkali solubility

The alkali solubility of wool fabrics indicates the extent of modification of the chemical properties. The higher the change in the values of alkali solubility, the greater was the modification of the fabrics [8]. The alkali solubility value of control samples was established as 8.5%. The alkali solubility values of the fabrics treated with Perizym AFW (7.5%) and Alcalase 2.5L (10%) resembled those of the control samples. For treatments with Savinase 16L and Papain, the higher values of approximately 13% were given. It was determined that the proteolytic treatments caused no great chemical damages for wool fabrics [11].

Effects of proteolytic processes on colour strength

There were significant differences between the K/S values (colour strength) corresponding to protease types, the time of proteolytic treatment and their interaction according to ANOVA (α=0.05). The means of K/S values are shown in Table 9, as well as a comparison of their K/S values in Figure 4.

K/S values for most of the dyestuff classes increased in the samples treated with proteases when compared with the control samples. For dyeing with Lanasol Red 2G (C.I. Reactive Red 116, pH: 5), there were no statistically significant differences in K/S values with regard to the times of proteolytic treatment. K/S values with regard to protease type increased in the following order: Savinase $\leq$ Alcalase $\leq$ Perizym $\leq$ Papain.

After 15 minutes of proteolytic treatment, Lanaset Red 2B obtained a higher colour strength, while no significant difference was noted after 30 and 60 minutes of proteolytic treatment. The K/S values increased with regard to proteolytic treatment in the following order: Savinase < Alcalase $\leq$ Papain < Perizym.

For Neolan Red, the highest K/S values were obtained on the samples pre-treated for 15 minutes. The longer the time of proteolytic treatment, the lower were the K/S values. The K/S values increased with regard to protease type pre-treated in the following order: Savinase < Papain = Alcalase < Perizym.

For Neolan Red GRE 200%, the highest colour strength was obtained after 15 minutes of proteolytic treatment. There was no significant difference between the K/S values corresponding to 30 minutes and 60 minutes of proteolytic treatment. There was no effect on the K/S values of protease type.

While dyeing with Erionyl Red A3G, when the time of proteolytic treatment was decreased from 60 minutes to 15 minutes, their K/S values increased. The colour strength increased in the following order: Savinase < Papain < Alcalase = Perizym. The K/S values of the control samples resembled those of the samples pre-treated with Perizym and Alcalase.

For Lanasol Yellow CE, the times of proteolytic treatment have no effect on colour strength. The K/S values with regard to protease type using the proteolytic treatment was affected in the following order: Savinase = Alcalase = Papain < Perizym.

Moreover, the K/S values of samples treated with chitosan and then dyed were evaluated. Dyestuff type, treatment type and their interactions have a significant effect on the K/S values of samples, which were treated with 4 different commercial proteases and chitosan, and then dyed with 3 different dyestuffs. Figure 5 shows a comparison of their means of K/S values. On the samples dyed with Lanasol Red 2G, significant differences were obtained between K/S values corresponding to 30 minutes and 60 minutes of proteolytic treatment. After 15 minutes of proteolytic treatment, lower K/S values were obtained. The K/S values increased with regard to the type of proteolytic treatment, and could be arranged in the following order: control < control + Chitosan < Savinase < Alcalase < Alcalase + Chitosan =Perizym

< Papain < Savinase + Chitosan < Papain + Chitosan = Perizym + Chitosan.

For Lanaset Red 2B dyestuff, the samples treated with proteases for 15 minutes and 30 minutes gave higher K/S values than the samples treated with proteases for 60 minutes. The K/S values increased in the following order: Savinase < Alcalase < Alcalase + Chitosan < Papain < Control < Savinase + Chitosan < = Control + Chitosan = Perizym = Perizym + Chitosan = Papain + Chitosan. The dyestuff did not provide any clearly increasing K/S values when compared with the corresponding values of other dyestuffs.

For Erionyl Red A3G dyestuff, 15 minutes of proteolytic treatment yielded higher K/S values. However, the K/S results for samples treated proteases were lower than those of the control samples. The K/S values increased with regard to the type of treatment in the following order: Savinase < Papain ≤ Savinase + Chitosan ≤ Alcalase ≤ Papain + Chitosan < Perizym ≤ Control ≤ Alcalase + Chitosan ≤ Perizym + Chitosan ≤ Control + Chitosan.

Colour fastness

The colour staining fastness to washing the cotton and wool, light and rubbing were determined. Tables 10 and 11 summarise the results of these tests. The values of fastness to washing, light and rubbing of the control samples and the samples treated with proteases did not differ from each other. The colour staining fastness values to washing for wool fabric were optimal (that is, 5). While the values of fastness to dry rubbing were well maintained, the values of fastness to wet rubbing decreased by 1-2 degrees of fastness with the increase in the time of proteolytic treatment. The samples treated with proteases and chitosan gave decreasing degrees of fastness to rubbing when compared with the fastness values for samples treated with proteases alone. The differences among washing fastness and light fastness values of the samples treated with proteases and chitosan were not significant.

Table 10. Values of colour fastness to washing, rubbing and light of dyed wool samples after 15, 30, 60 min of proteolytic treatment; ***for all three times, the value of WF_W equals 5.***

Dye	Treatment	15 min				30 min				60 min			
		WF_{Co}	RF_D	RF_W	LF	WF_{Co}	RF_D	RF_W	LF	WF_{Co}	RF_D	RF_W	LF
Lanasol Red 2G	Cont	4-5	4-5	3-4	6-7	4-5	4-5	4-5	6-7	4-5	4-5	4-5	6-7
	Per	4-5	4-5	4-5	6-7	4-5	4-5	4-5	6-7	4-5	4-5	4	6-7
	Alc	4-5	4-5	4-5	6-7	4-5	5	4-5	6-7	4-5	4	4	6-7
	Sav	4-5	4-5	4	6-7	4-5	4-5	4	6-7	4-5	3-4	2-3	6-7
	Pap	4-5	4-5	4	6-7	4-5	4	2-3	6-7	4-5	4-5	4-5	6-7
Lanaset Red 2B	Cont	4-5	4-5	4-5	6-7	4-5	4-5	4-5	6-7	4-5	4-5	4-5	6-7
	Per	4-5	4-5	4-5	6-7	4-5	4-5	4-5	6-7	4-5	4-5	4-5	6-7
	Alc	4-5	4-5	4-5	6-7	4-5	4-5	4-5	6-7	4-5	4-5	4-5	6-7
	Sav	4-5	4	3-4	6-7	4-5	4	3-4	6-7	4	4-5	4-5	6-7
	Pap	4-5	4-5	4	6-7	4-5	3-4	3	6-7	4-5	4	3	6-7
Neolan Red AG	Cont	4-5	4-5	4-5	3	4-5	4-5	4-5	3	4-5	4-5	4-5	3
	Per	4-5	4-5	4-5	3	4-5	4-5	4-5	3	4-5	4-5	4-5	3
	Alc	4-5	4-5	4-5	3	4-5	4-5	4-5	3	4-5	4	4	3
	Sav	4-5	4-5	4	3	4-5	3-4	3-4	3	4-5	3-4	2-3	3
	Pap	4-5	4	3-4	3	4-5	3-4	3	3	4-5	3-4	3	3
Erionyl Red A3G	Cont	4-5	4-5	4-5	3	4-5	4-5	4-5	3	4-5	4-5	4-5	3
	Per	4-5	4-5	4-5	3	4-5	4-5	4-5	3	4-5	4-5	4-5	3
	Alc	4-5	4-5	4-5	3	4-5	4-5	4-5	3	4-5	4-5	4	3
	Sav	4-5	4-5	4	3	4	3-4	3	3	4	3-4	2-3	3
	Pap	4-5	4	3	3	4-5	4	3	3	4	3-4	2-3	3
Neolan Red GRE 200%	Cont	4-5	5	4-5	6-7	4-5	4-5	4-5	6-7	4-5	4-5	4-5	6-7
	Per	4-5	4-5	4-5	6-7	4-5	4-5	4-5	6-7	4-5	4-5	4-5	6-7
	Alc	4-5	4-5	4-5	6-7	4-5	4-5	4	6-7	4-5	4-5	4	6-7
	Sav	4-5	4	4	6-7	4-5	3-4	3	6-7	4	4	2-3	6-7
	Pap	4-5	4-5	3-4	6-7	4	4	3-4	6-7	4-5	4	2-3	6-7
Lanasol Yellow CE	Cont	5	4-5	4-5	6-7	5	4-5	4-5	6-7	5	4-5	4-5	6-7
	Per	5	4-5	4-5	6-7	5	4-5	4-5	6-7	5	4-5	4	6-7
	Alc	5	4-5	4-5	6-7	5	4-5	4-5	6-7	5	4	4	6-7
	Sav	5	4	4	6-7	5	3-4	2-3	6-7	5	3	2-3	6-7
	Pap	5	4	3-4	6-7	5	3-4	3	6-7	5	3	2-3	6-7

Discussion

Protease enzymes specifically act on the peptide bonds of proteins and hydrolyse them. As a phenomenon, removal of the proteinaceous matter also causes covalently bound fatty acids to be removed, which consequently enhances the hydrophilicity of the wool surface [2,3,12-14].

The higher colour strength of the samples treated with proteases should be due to the higher hydrophilicity of the treated samples.

The higher whiteness index with the proteolytic treatment is caused by the decolourising action of the enzyme on the natural colorants present in the wool fibre [15].

As our results show, the longer the time of proteolytic treatment, the higher was the loss of tensile strength. After 15 minutes, the treatment with Perizym gave an acceptable loss of tensile strength values. By reducting the time of proteolytic treatment, the degradative effect of the treatment should be limited.

Additionally, the samples were treated with chitosan dissolved with acetic acid. The possibility of use residue acetic acid on the samples explained the additional reduction in tensile strength loss in the samples.

When the wool samples were treated with chitosan, the cationic groups of chitosan bonded with the anionic groups in wool. Moreover, in the wool dyeing process, bonds should be present between anionic dyestuffs in the dyeing bath and cationic chitosan absorbed in wool during the dyeing treatment. Consequently, the colour strength in the samples treated with chitosan increased.

In order to enhance chitosan sorption in wool fibres and increase the uniformity of its distribution, it is convenient to increase the wettability and anionic character of the wool surface [16]. By proteolytic pretreatment, the wettability of wool surface could be increased before chitosan treatment of wool samples. In this way, a combined proteolytic treatment and chitosan treatment should obtain the highest colour strength.

Conclusions

- Colour strength for most of the classes of dyestuff used in this study increased with proteolytic treatment independently of the protease type. Treatment with Perizym and Papain provided a greater increase in colour strength for most of the dye classes used.
- Generally, the optimum proteolytic treatment time was 15 minutes. Thus, a decrease in proteolytic treatment time saves time and increases the plant's productivity.
- For the optimum proteolytic treatment time (15 minutes), treatment with Perizym gave an acceptable loss of tensile strength values (20%, with the loss values of tensile strength of 17% for control samples) and weight loss (0.2%); the treatment with Papain gave unacceptable results, 34% and 3.7% respectively.
- The effect of proteolytic treatment on colour strength changed with the dyestuff class. For dyeing with Lanasol Red 2G, a reactive dyestuff, proteolytic treatment provided a greater increase

Table 11. *Values of colour fastness to washing, rubbing and light of dyed wool samples after 15, 30, 60 min of proteolytic treatment and then chitosan treatment.*

Dye	Treatment	15 min					30 min					60 min				
		WF_{Co}	WF_W	RF_D	RF_W	LF	WF_{Co}	WF_W	RF_D	RF_W	LF	WF_{Co}	WF_W	RF_D	RF_W	LF
Lanasol Red 2G	Cont	4-5	5	4-5	3-4	6-7	4-5	5	4-5	4-5	6-7	4-5	5	4-5	4-5	6-7
	Per	4-5	5	4-5	4-5	6-7	4-5	5	4-5	4-5	6-7	4-5	5	4-5	4	6-7
	Alc	4-5	5	4-5	4-5	6-7	4-5	5	5	4-5	6-7	4-5	5	4	4	6-7
	Sav	4-5	5	4-5	4	6-7	4-5	5	3-4	3	6-7	4-5	5	3-4	3	6-7
	Pap	4-5	5	4-5	4	6-7	4-5	5	4	2-3	6-7	4-5	5	4	2-3	6-7
	Cont+Chit	4-5	5	4-5	4	6-7	4-5	5	4-5	4	6-7	4-5	5	4-5	4-5	6-7
	Per+Chit	4-5	5	4-5	4-5	6-7	4-5	5	4-5	4	6-7	4-5	5	4-5	4	6-7
	Alc+Chit	4-5	5	5	4	6-7	4-5	5	4-5	4	6-7	4-5	5	4-5	4	6-7
	Sav+Chit	4-5	5	4-5	3	6-7	4-5	5	4-5	2-3	6-7	4-5	5	3-4	2-3	6-7
	Pap+Chit	4-5	5	4-5	3	6-7	4-5	5	4	3	6-7	4-5	5	4	2-3	6-7
Lanaset Red 2B	Cont	4-5	5	4-5	4-5	6-7	4-5	5	4-5	4-5	6-7	4-5	5	4-5	4-5	6-7
	Per	4-5	5	4-5	4-5	6-7	4-5	5	4-5	4-5	6-7	4-5	5	4-5	4-5	6-7
	Alc	4-5	5	4-5	5	6-7	4-5	5	4-5	4-5	6-7	4-5	5	4-5	4-5	6-7
	Sav	4-5	5	4	3-4	6-7	4-5	5	4	3-4	6-7	4	5	4	3-4	6-7
	Pap	4-5	5	4-5	4	6-7	4-5	5	3-4	3	6-7	4-5	5	4	3	6-7
	Cont+Chit	4-5	5	4	4-5	6-7	4-5	5	4-5	4-5	6-7	4-5	5	4-5	4-5	6-7
	Per+Chit	4-5	5	4-5	4-5	6-7	4-5	5	4-5	4	6-7	4	5	4-5	4-5	6-7
	Alc+Chit	4	4-5	4-5	4-5	6-7	4-5	5	4	4	6-7	4	5	4	4	6-7
	Sav+Chit	4-5	5	3-4	3-4	6-7	4-5	5	3-4	3-4	6-7	4	5	3-4	2-3	6-7
	Pap+Chit	4-5	4-5	4	4	6-7	4-5	5	3-4	2-3	6-7	4-5	5	3-4	2-3	6-7
Erionyl Red A3G	Cont	4-5	5	4-5	4-5	3	4-5	5	4-5	4-5	3	4-5	5	4-5	4-5	3
	Per	4-5	5	4-5	4-5	3	4-5	5	4-5	4-5	3	4-5	5	4-5	4-5	3
	Alc	4-5	5	4-5	4-5	3	4-5	5	4-5	4-5	3	4-5	5	4-5	4	3
	Sav	4-5	5	4-5	4	3	4	5	3-4	3	3	4	5	3-4	2-3	3
	Pap	4-5	5	4	3	3	4-5	5	4	3	3	4	5	3-4	2-3	3
	Cont+Chit	4-5	5	4-5	4	3	4	5	4-5	4	3	4-5	5	4-5	4-5	3
	Per+Chit	4-5	5	4-5	4	3	4-5	5	4	4	3	4-5	5	4	3-4	3
	Alc+Chit	4	5	4-5	4-5	3	4-5	5	4	4	3	4	5	4	3	3
	Sav+Chit	4	5	3-4	4-5	3	4-5	5	3-4	3	3	4	5	3	2-3	3
	Pap+Chit	4	5	4-5	3	3	4	5	3-4	3	3	4	5	3	2-3	3

in colour strength. Lanaset Red 2B, an acid dyestuff, did not provide any clear increases in K/S values when compared with the corresponding values of other dyestuffs.

- The values of fastness to washing, light and rubbing of control samples and samples treated with proteases did not differ from each other.
- Treatment with chitosan generally increased the colour strength when compared with wool samples pre-treated with proteases. The treatment with Perizym + Chitosan and Papain + Chitosan provided the highest colour strength values. For the optimum proteolytic treatment time (15 minutes), treatment with Perizym + Chitosan and Papain + Chitosan did not change the fastness values when compared with the fastness values of samples treated with Papain and Perizym alone. For the optimum proteolytic treatment time (15 minutes), treatment with Perizym + Chitosan (20%) did not result in any increase in the loss of tensile strength when compared with treatment with Perizym only (20%), while treatment with Papain + Chitosan (37%) increased the loss of tensile strength when compared with treatment with Papain only (34%).

In conclusion, the optimum treatment for improving the dyeability of wool in an environmentally friendly way is the treatment with Perizym + Chitosan for 15 minutes. In addition, proteolytic treatment provided a greater increase in colour strength for dyeing with a reactive dyestuff.

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