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A comparative study of two wool enzyme treatments

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The influence of enzyme concentration and treatment time on certain wool properties has been studied according to the Box-Hunter experimental design in order to better understand the wool modification caused by two different enzymatic multi-purpose formulations (enzyme A and enzyme B). It is observed that enzyme concentration and treatment time exert an influence on wool whiteness, shrink resistance, weight loss and urea bisulphite solubility. The enzyme concentration has a decisive influence on wool modification when enzyme A is applied, and on the treatment time when enzyme B is applied. Tensile strength properties and SEMs suggest that enzyme B can attack the non-keratinic parts of wool fibre structure. No significant degradation of cortical or even cuticular cells is observed after enzyme A treatment. Accordingly, enzyme A could be applied all over the experimental zone whereas in the case of enzyme B, the enzyme concentration over 5% and treatment time over 60 min should be avoided. FTIR/ATR analysis confirms that there is no significant change in the redox state of cystine disulphide bonds at the wool surface after the enzyme treatment.

Keywords: Enzymes, FTIR/ATR study, Physico-mechanical properties, Scanning electron microscopy, Wool

1 Introduction

There is currently an increased demand for environment-friendly wool treatments as an alternative to the conventional wool treatments that produce AOX byproducts. The use of enzymes to achieve wool shrink resistance, better whiteness and improved handle is of considerable interest[1-7]. Shrink-resistant wool is the major priority but if enzymes are applied at levels that provide the required shrink resistance, the wool fibres are often unacceptably damaged owing to irregular treatment[5, 8, 9]. Enzymes alone or in combination with hydrogen peroxide are also successfully employed in wool bleaching[10], as auxiliary agent in wool dyeing[11-15], for wool handle modification by reducing wool fibre stiffness and prickle[16, 17], and in wool carbonizing[18].

However, the knowledge of the specific action of enzymes in substrates with a heterogeneous morphological structure and a chemical composition, which are characteristics of wool, is still unsatisfactory. It is apparent that the results of enzymatic treatments, especially with proteases, can be unpredictable and may lead to unacceptable degradation of wool fibre. Consequently, it is essential to restrict the enzymatic action to the wool fibre surface or retard its action to avoid the enzyme diffusion into the wool. In other words, the enzymatic action on wool must be completely controlled[3]. The correct experimental design and statistical methods for analysis of the results can be very useful tool in investigating wool enzymatic treatment[8].

From both technical and practical points of view, it is often useful to optimize the enzymatic treatment conditions before its industrial use. The present work was, therefore, aimed at determining the influence of different commercially available enzyme multi-purpose formulations on selected wool properties. To this end, the effect of enzyme concentration and treatment time on the degree of whiteness, weight loss, urea bisulphite solubility and area shrinkage was investigated. The results were evaluated by a central rotatable design to follow the complex experimental conditions with more accuracy[19]. Moreover, it was

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expected that this approach should permit the objective evaluation of some similarity and differences between the enzymes used. Tensile strength properties of enzymatically-treated wool were also measured. Chemical and morphological changes in the fibre surface were investigated by FTIR/ATR spectroscopy and SEM respectively.

2 Materials and Methods

2.1 Materials

The knitted wool fabric (Pulligan International S.A., Spain) with a cover factor of 1.22 $tex^{1/2}$/mm was cleaned by Soxhlet extraction with dichloromethane, rinsed with ethanol and water and equilibrated in a conditioned room (20°C, 65%RH). Two specific enzyme multi-purpose formulations were employed in this investigation: (a) Bactosol WO (Clariant Iberica, Spain), a biocatalyst based on selected enzymes (new type hydrolase) which acts specifically on protein fibres, leading to proteolysis, esterolysis, lipolysis and keratinolysis (hereinafter called enzyme A); and (b) Biosoft PW (T. S. Chemicals, U K), a preparation of proteolytic enzymes proteases (hereinafter called enzyme B). All other chemicals and auxiliaries were of laboratory reagent grade.

2.2 Methods

2.2.1 Enzyme Treatment

Enzyme treatments were carried out by exhaustion method at a liquor-to-wool ratio of 15:1. The treatments were performed in round-bottomed flasks by thermostatically-controlled shaking bath OB 14 (Memmert, Germany) at 55°C and pH 9 using a Na_2CO_3/$NaHCO_3$ buffer. The level of agitation was low during the treatment. After the treatment, the wool samples were hand squeezed, rinsed in HCl at pH 4 for 5 min to stop the enzymatic action, then rinsed twice in distilled water and finally dried in air on a flat surface.

2.2.2 Experimental Design

The experimental levels of applied enzyme concentration (1-8 % o.w.f.) and treatment time (15-105 min) were calculated in accordance with the Box-Hunter central rotatable experimental design (Table 1). The complete central composite rotatable design for two variables at five experimental levels requires thirteen experiments and includes five repeated experiments carried out to obtain an estimate for the within-treatments variation[19, 20]. Further details about the central rotatable experimental design, analysis of the measured responses for each experimental condition, as well as the mode of obtaining the adjusted polynomial equations and so-called isoresponse (contour) diagrams can be found in the literature[8, 19-21].

Table 1—Experimental values of variables for different coded levels

Variable	Coded level				
	-1.41	-1	0	+1	+1.41
Enzyme concentration (x_1), %	1	2	4.5	7	8
Treatment time (x_2), min	15	30	60	90	105

2.2.3 Tests

Degree of whiteness (CIEGanz 82) was measured using a spectrophotometer Color-Eye 3000 (Macbeth, USA) with D65 illuminant and 10° observer. The higher the value of the degree of whiteness, the more white is the wool. Weight loss was determined on samples conditioned for at least 48 h at 20°C and 65% RH. The results are expressed as the percentage of the weight loss of the treated samples compared with an untreated sample. Urea bisulphite solubility (UB solubility) was determined in accordance with UNE 40.20572. It was calculated as the percentage of weight loss of the wool sample treated for 60 min with 50 ml of a standard urea bisulphite solution at 65°C. Area shrinkage was determined according to Woolmark TM 31 by the Wascator model FOM 71 washing machine using ISO 6330 5A wash cycle programme as a base to determine the total felting shrinkage of wool samples[22]. When the area shrinkage is lower than 8% after two 5A cycles, the wool can be considered as machine washable[23].

The chemical changes on the wool surface were determined using a FTIR Spectrophotometer 510 (Nicolet, Germany) in the ATR reflection mode with KRS-5 45° crystal. The spectra were normalized against the peak intensity of 1232 cm^{-1} (amide III). The peak intensity of each selected band frequency (1040, 1075 and 1124 cm^{-1} assigned to cysteic acid, cystine monoxide and cystine dioxide respectively) were compared with the corresponding peak intensity of untreated wool[24].

For SEM observations, untreated and enzymatically-treated wool samples were accordingly sputter-coated with a thin layer of gold and viewed at 15kV in the scanning electron microscope (Model 570, Hitachi, Japan).

Yarn tensile strength was determined using an Instron 5500R Tensile Tester in accordance with ASTM D 2256-80. Twenty repeated measurements per sample were carried out and the results were

expressed as tensile strength at maximum load rather than as load at break.

3 Results and Discussion

The complete experimental design and the results obtained for the degree of whiteness, urea bisulphite solubility, weight loss and area shrinkage for enzyme A and enzyme B as well as for the untreated sample are given in Table 2.

To obtain the regression coefficients and adjusted polynomial equations containing only the variables with a significance above 95%, the multiple regression analysis and analysis of variance (ANOVA) were employed with the aid of a computer program specially made for this purpose. Based on the data from the regression coefficients and adjusted polynomial equations, the isoresponse diagrams as projections of the response surfaces were drawn. The adjusted polynomial equations obtained for both enzymatic treatments are given in Table 3.

The results indicate that wool whiteness, when compared with that of untreated wool, is enhanced for all the combinations of enzyme concentration and treatment time (Table 2). As can be seen in Fig. 1, the whiteness is improved by increasing enzyme concentration and treatment time. The increase is

Table 2—Experimental conditions and results obtained for the parameters

Exp. no.	Level of variable				Response					
	Coded		Experimental		Whiteness CIE Ganz 82	UB Solubility, %	Weight loss[a], %	Area shrinkage, % 5A Wash cycles		
	x_1	x_2	x_1 %	x_2 min				1	2	3
					Enzyme A					
1	−1	−1	2	30	−4.8	51.5	0.40	27.9	44.2	55.0
2	+1	−1	7	30	−2.2	54.2	0.82	30.4	45.0	54.5
3	−1	+1	2	90	−3.7	50.9	1.04	24.7	44.2	52.4
4	+1	+1	7	90	2.2	55.9	1.65	26.0	42.8	53.5
5	−1.41	0	1	60	−1.4	49.2	0.58	27.4	45.7	54.5
6	+1.41	0	8	60	1.4	56.2	2.07	30.5	38.1	58.6
7	0	−1.41	4.5	15	−4.5	54.8	0.89	29.1	44.0	55.8
8	0	+1.41	4.5	105	−1.2	52.5	1.28	26.0	45.4	66.3
9	0	0	4.5	60	−1.7	54.0	1.25	29.7	48.8	58.9
10	0	0	4.5	60	−3.0	54.1	1.30	24.5	49.3	54.0
11	0	0	4.5	60	−3.4	53.0	1.26	28.9	48.9	58.7
12	0	0	4.5	60	−4.0	54.5	1.20	29.6	49.9	58.6
13	0	0	4.5	60	−3.2	52.7	1.10	32.4	49.5	59.6
					Enzyme B					
1	−1	−1	2	30	1.5	58.9	1.67	28.1	44.2	54.5
2	+1	−1	7	30	5.8	60.0	2.21	26.3	42.4	51.7
3	−1	+1	2	90	8.1	60.1	5.13	22.6	40.0	50.3
4	+1	+1	7	90	13.8	65.0	6.78	23.8	37.6	46.8
5	−1.41	0	1	60	4.4	57.3	2.42	26.7	43.7	52.6
6	+1.41	0	8	60	11.7	62.0	5.15	19.6	37.8	45.6
7	0	−1.41	4.5	15	4.1	57.4	1.39	29.4	42.6	54.5
8	0	+1.41	4.5	105	8.9	60.8	5.91	22.1	37.3	47.6
9	0	0	4.5	60	8.5	61.2	3.78	21.5	37.5	46.5
10	0	0	4.5	60	8.9	60.1	3.93	24.4	39.6	53.4
11	0	0	4.5	60	9.0	60.3	3.88	19.5	38.5	46.5
12	0	0	4.5	60	10.2	59.9	3.91	24.8	39.3	50.5
13	0	0	4.5	60	8.9	60.5	3.97	22.3	40.0	49.1
	Untreated sample				−5.3	49.5		33.8	57.0	63.9

[a]Compared to the untreated sample

Table 3—The adjusted polynomial equations for the parameters

Response	Enzyme A	Enzyme B
Whiteness	$-2.48+1.56x_1+1.29x_2+1.07x_1^2$ R=0.89 R^2=0.77 S.E.=1.38 F-ratio=4.70	$9.14+2.54x_1+2.68x_2-1.33x_1^2$ R=0.96 R^2=0.93 S.E.=1.10 F-ratio=24.91
UB solubility	$53.66+2.20x_1-0.48x_1^2+0.55x_1x_2$ R=0.94 R^2=0.88 S.E.=0.81 F-ratio=21.01	$60.40+1.59x_1+1.35x_2+0.96x_1x_2$ R=0.92 R^2=0.85 S.E.=0.98 F-ratio=7.98
Weight loss	$1.22+0.39x_1+0.25x_2$ R=0.91 R^2=0.83 S.E.=0.21 F-ratio=14.14	$3.89+0.76x_1+1.80x_2+0.27x_1x_2$ R=0.99 R^2=0.97 S.E.=0.30 F-ratio=110.93
Area shrinkage	$49.31-1.41x_1-3.53x_1^2-2.09x_2^2$ R=0.93 R^2=0.87 S.E.=1.44 F-ratio=19.34	$38.99-1.57x_1-2.06x_2^2+1.07x_1^3$ R=0.94 R^2=0.89 S.E.=0.97 F-ratio=16.93

R— Multiple correlation coefficient; R^2—Coefficient of determination; S.E.— Standard error of estimation, and F-ratio—Test variance/Error variance

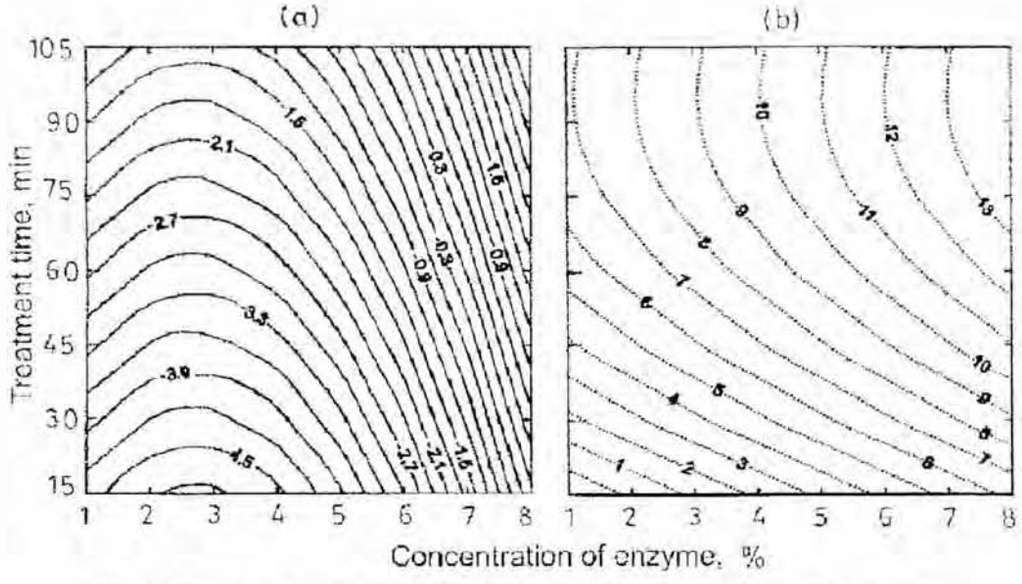

Fig.1—Whiteness degree obtained after treatment with (a) enzyme A; and (b) enzyme B

more pronounced with enzyme B, indicating that it is more effective than enzyme A (Fig. 1b). At the central point of experimental design (experiments from 9 to 13 in Table 2), the whiteness after treatment with enzyme B is considerably superior to that obtained after treatment with enzyme A, the difference being 11.62 CIE units (Table 3). This could be attributed to enzyme efficiency in eliminating the natural-coloured pigments of the wool surface which are bonded to the wool protein and lie mainly in the cuticle layer[2, 11].

The enzymatic treatments were only partially effective in relation to wool shrinkage reduction. The effect was slightly improved by increasing enzyme concentration and treatment time, as reported[5, 7, 25]. A comparative analysis of the shrink resistance obtained after treatment with enzymes A and B is shown in Figs 2a and 2b. It can be observed that wool shrink resistance after the enzyme treatments is fairly comparable. However, at a higher enzyme concentration and at a longer treatment time, the shrink-resist effect obtained by enzyme B is approximately 10% higher than the effect obtained by enzyme A (Tables 2 and 3).

It is clear that enzyme B is more effective than enzyme A not only in regard to whiteness improvement but also in regard to shrink resistance. However, this effect is associated with excessive fibre damage, expressed as weight loss (Figs 3a and 3b), and urea bisulphite solubility (Figs 4a and 4b). Both the parameters increase when the treatment time and enzyme concentration are raised. It is well known that urea bisulphite solubility of wool decreases as a result

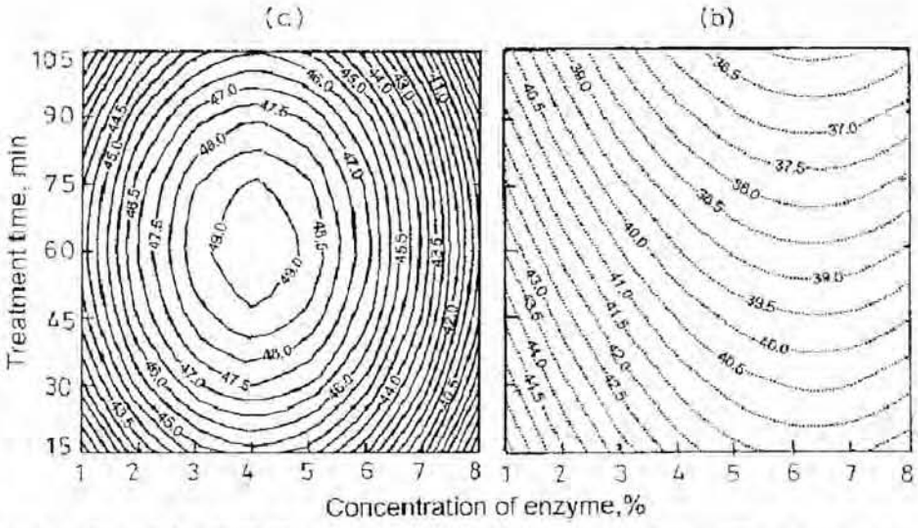

Fig.2—Area shrinkage obtained after 2×5A Wascator shrinkage test cycles after treatment with (a) enzyme A; and (b) enzyme B

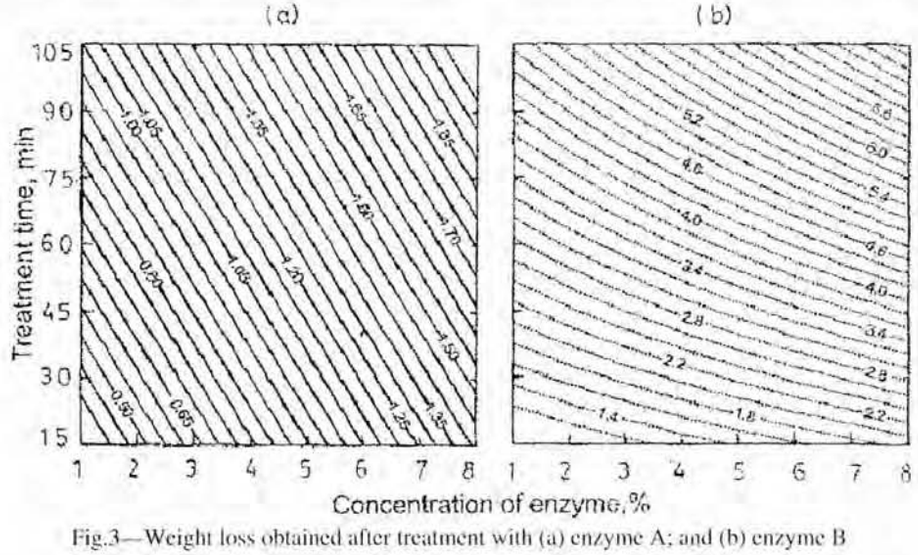

Fig.3—Weight loss obtained after treatment with (a) enzyme A; and (b) enzyme B

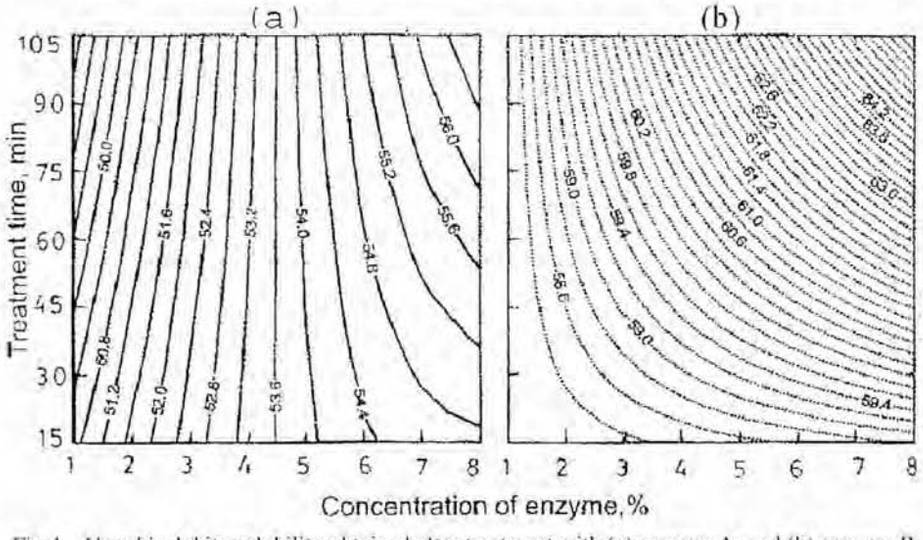

Fig.4—Urea bisulphite solubility obtained after treatment with (a) enzyme A; and (b) enzyme B

of alkaline treatments due to lanthionine and lysinoalanine formation[26] and increases because of the damage caused by acids or oxidising agents[27, 28]. In this case, increased urea bisulphite solubility after the enzyme treatment is probably due to hydrolysis of some of the wool peptide and isodipeptide bonds.

The results show that in the case of enzyme A, the concentration exerts a decisive influence on wool whiteness, urea bisulphite solubility, weight loss and shrinkage properties, whereas in the case of enzyme B, the treatment time plays an important role (Tables 2 and 3). Bearing in mind that a weight loss of 3-4.5% could be excessive for wool[5, 10], it is clear that enzyme A could be applied all over the experimental zone. By contrast, in the case of enzyme B, the concentration over 5% and treatment time over 60 min should be avoided.

The SEMs of wool treated under different conditions generally show that the enzymatic treatment is not uniform. Some fibres can be practically intact or slightly affected, whereas others are considerably damaged[5, 9]. To evaluate the effect of the different enzymatic treatments on the wool surface, SEMs were prepared (some of the most representatives ones are shown in Fig. 5). These SEMs also demonstrate that the enzymes produce different effects on the wool fibre surface.

Enzyme A significantly attacks the wool fibre only at higher concentrations. Some scale edges are raised at the enzyme concentration of 8% (Fig. 5d). The SEMs of wool treated with enzyme B show that even at the lowest enzyme concentration the wool fibres can be completely descaled (Fig. 5e). The biggest effect on the scale structure or on the fibre cortex was produced by a higher enzyme concentration (Figs 5f and 5g). However, the enzymatic treatment did not produce a uniform effect on wool (Fig. 5g).

As the proteases generally have a large molecule, they preferentially attack the highly swellable cell membrane complex (CMC), a non-keratinic part of wool fibre, by penetrating between cuticular scales, causing scale stripping and weakening of wool fibre[3,7]. Moreover, if the CMC is attacked by enzymes it will result in the liberation of individual cortical cells[29]. Since enzyme A leaves the wool scales intact and does not cause excessive damage to the wool (values of weight loss did not exceed 4.5%), it could be suggested that it produces superficial proteolysis or keratinolysis. By contrast, enzyme B removes the surface scales or even liberates individual cortical cells at higher enzyme concentrations. Therefore, it should be strongly controlled or used only at lower concentrations.

It is well known that the mechanical properties of wool fibre are closely related to the structure of the cell membrane complex[30, 31]. Hence, the mechanical properties such as tensile strength of wool remain unchanged after any treatment restricted to the wool surface[32]. The yarn tensile strength was determined (Table 4) to confirm this. At the central point of experimental design, the treatment using enzyme A does not cause the excessive tensile strength drop (2.83%) in contrast to the treatment with enzyme B (9.62%). Moreover, the tensile strength drop becomes progressive as the enzyme concentration is increased regardless of the enzyme used.

The results of FTIR/ATR measurements show that the enzymatically-treated wool exhibited poor changes in the redox state of -S-S- cystine bonds (Fig. 6). The peak intensity of cystine monoxide and cystine dioxide are slightly changed after enzyme treatments. Untreated wool has a certain amount of cysteic acid, probably due to weathering or photo-oxidation[33, 34]. The cysteic acid content slightly reduces on increasing the enzyme A concentration with respect to the untreated sample (Fig. 6a). The cysteic acid content of enzyme B treated wool also follows the same pattern (Fig. 6b). However, an increase in the enzyme B concentration does not give rise to significant differences in the cysteic acid

Table 4—Calculated yarn tensile strength properties after enzymatic treatments

Enzyme conc. %	Duration of treatment min	Enzyme A		Enzyme B	
		Tensile strength[a] cN/tex	Tensile strength drop, %	Tensile strength[a] cN/tex	Tensile strength drop, %
1	60	6.94±0.22	1.84	6.83±0.23	3.39
4.5	60	6.87±0.16	2.83	6.39±0.16	9.62
8	60	6.77±0.12	4.24	5.81±0.38	17.82
(Untreated)		7.07±0.20	—	7.07±0.20	—

[a] Errors are indicated at 95% confidence level.

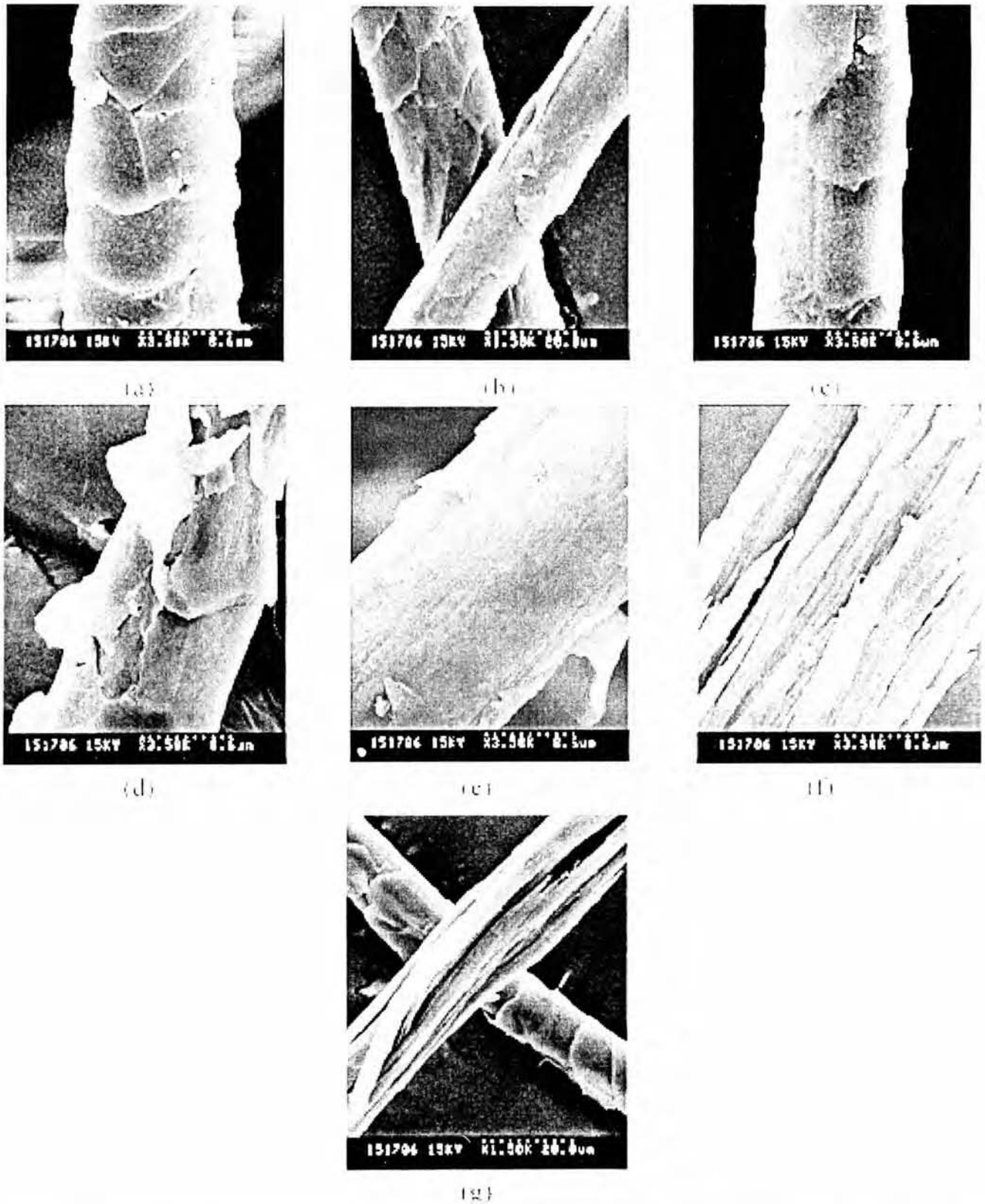

Fig.5—SEMs of wool: (a) untreated; (b-d) treated for 60 min with 1%, 4.5% and 8% respectively of enzyme A; and (e-g) treated for 60 min with 1%, 4.5% and 8% respectively of enzyme B

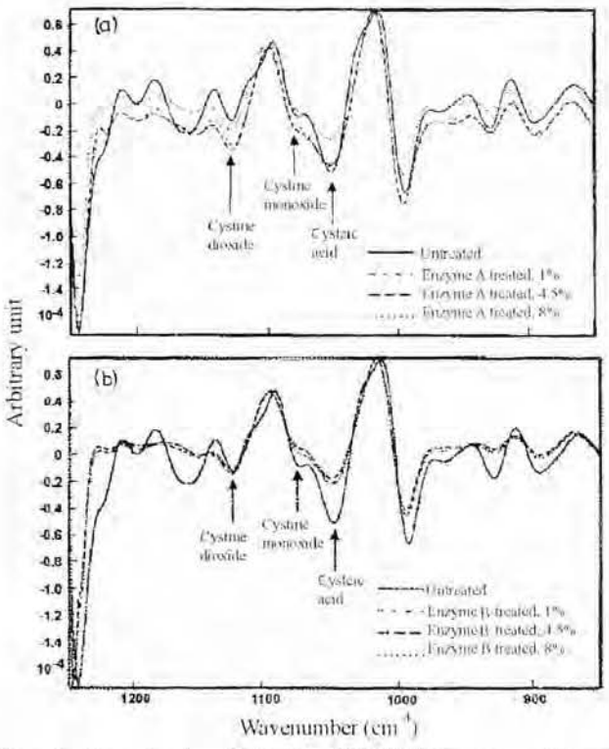

Fig. 6—Second-order derivative FTIR/ATR spectra of wool treated for 60 min with (a) enzyme A; and (b) enzyme B

content of wool. This is probably due to the loss of sulphur-rich material at the wool surface even at low enzyme concentrations (Fig. 5e).

Despite smoothening or descaling, only slight shrink resistance is obtained. It seems that to achieve a good level of shrink resistance, it is not only necessary to modify the cuticular scales but also to generate anionic groups, especially cysteic acid and/or cysteine-S- sulphonate groups, on the wool surface[7, 35].

To confer shrink resistance, an enzyme should be highly specific toward the outer sulphur-rich cuticular layer, preferably after the wool has been modified to enhance this specificy[6]. Disulphide bond splitting by an oxidative or sulphite pre-treatment of wool[27, 36-38] makes the wool fibre surface more accessible with the result that the consequent enzymatic attack on the cuticle is selectively activated. It has been found that as a consequence of a combined peroxide-enzyme treatment of wool, the enzyme action could be limited to the wool surface because of its ionic interaction with the new sulphonic groups formed on the wool surface[39].

4 Conclusions

Wool treatment with applied enzyme formulations exerts a positive influence on whiteness and shrink resistance, but has a detrimental effect on the physico-mechanical characteristics of the wool which are expressed as weight loss, tensile strength and urea bisulphite solubility. Wool suffers some damage owing to the type of enzyme, enzyme concentration and treatment time. Enzyme A could be applied all over the experimental zone (enzyme concentration 1-8% and treatment time 15-105 min), whereas in the case of enzyme B, the enzyme concentration over 5% and treatment time over 60 min should be avoided. FTIR/ATR analysis confirms the absence of significant changes in the redox state of cystine disulphide bonds after the enzyme treatments used in the study.

The SEMs suggest a clear difference between enzymes A and B with respect to the enzyme proteolytic activity on the wool surface as well as in the enzyme attack pathways. Enzyme A leaves the wool scales intact and does not cause excessive damage to wool. Thus, this enzyme preferentially produces a superficial proteolysis or keratinolysis. By contrast, enzyme B can attack the non-keratinic parts of wool fibre structure and should, therefore, be strongly controlled or used at lower concentrations.

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