Influence of enzimatic treatments on chitosan treated wool

Juliá, M.R.
CID CSIC, Barcelona Spain
Belobrajdic, L., Vázquez, A., Berrutti, A., Manancero, C., Varela, H.
Depto de Bioingeniería, UDELAR, Montevideo, Uruguay
De Giuda, M., Píppolo, D., Fernández, A., Martínez, A., Zinelli, T.
Sector Textiles, LATU, Montevideo, Uruguay

Introduction

Similar effects to those obtained on wool fibers subjected to an oxidative treatment (chlorination, hydrogen peroxide, etc.) are achieved when keratinase enzyme is applied on wool fabrics to obtain good handle properties and improve dyeing yield. This enzymatic treatment partially reduces the felting properties of wool during aqueous washing. The enzymatic extract is obtained using as culture media, agro-industrial by-products like whey and frigorifical wastes. With the application of this clean technology we substitute the use of contaminating products like chlorine and we are solving other important environmental problem: what to do with some agro - industrial waste products.

The present work develops the production of an enzymatic extract obtained using agro-industrial wastes that is appropriated to be used commercially. The treatment with this extract of wool fabrics be worsted or knitted substitutes contaminant processes as chlorination and potassium permanganate techniques. These processes are the cause of a more aggressive attack on the wool than the enzymatic one, being furthermore their use very dangerous for the health or the personnel that applies them. The high levels of contamination of the effluents when said processes are applied are also avoided

A new treatment which consists in chitosan application on oxidated wool was developed recently in order to reach the required limit values established by IWS Test method 31 (the shrinkage area after the 2nd wash cycle has to be less than 8%). However, due to the presence of the polymer on the fiber wool surface, the handle turns out to be not optimum.

2.0 Materials and methods

2.1 Microorganism and their maintenance

The trials were performed with an indigenous strain of *Bacillus* sp. selected in a screening program carried out by Bioengineering Department, Faculty of Engineering, Uruguay.

The strain was maintained on nutrient agar slant and subcultured every 3 months and stored at 4 °C.

2.2 Enzymatic extracts

The enzymatic extracts used were the ones identified as PK2 and PC. The first one is the extract obtained with the fermentation media detailed in 2.3. The second one is a commercial product of common use in Uruguay.

2.3 Growth medium and growth conditions

2.3.1 *Inoculation medium:* The following inoculation medium was used containing (gL^{-1}): glucose, 5; nutrient broth, 8; yeast extract, 3; K_2HPO_4, 0.7; KH_2PO_4, 0.3; $MnCl_2.4H_2O$, 0.1; $CaCl_2.2H_2O$, 0.3; $MgSO_4.7H_2O$, 0.01.

Fermentation medium: The composition of the culture medium was (gL^{-1}): glucose, 13; nutrient broth, 8; yeast extract, 3; K_2HPO_4, 0.7; KH_2PO_4, 0.3; $CaCl_2.2H_2O$, 0.3; $MgSO_4.7H_2O$, 0.01.

In all cases media were first adjusted to pH 7 by 0.1 N HCl or 0.1 N NaOH and then sterilized by autoclaving for 15 min at 121 °C.

2.3.2 *Shake flask experiments*: Fully slants of 12 h old *Bacillus* sp. incubated at 37 °C were scrapped off and suspended in 10 cm^3 of sterile saline solution. The cell suspension was shaken thoroughly to break up the aggregates and used for building up of inoculum in 1000 mL Erlenmeyer flasks containing 100 mL of inoculation medium. The inoculated flasks were incubated on an orbital shaker at 300 rpm and 37 °C for 12 hours. Inoculum was used to inoculate Erlenmeyers flasks of 2000 mL with 200 mL of fermentation medium. Culture temperature was 37 °C, shaking at 300 rpm. The concentration of cells at the beginning of fermentation was 0.2 gL^{-1}. Biomass was measured turbidimetrically at 650 nm.

2.4 Proteolytic activity measurement.

2.4.1 Coagulation Activity

The modified Arima technique was employed

The substrate used is a 10% (p/v) of skim powder milk in a 0.01M CaCl2 solution. This solution is shaked during 15 minutes in a magnetic shaker and then is filtrated through cotton fiber. To a 5.0 ml of this solution, previously incubated at 35°C during 30 minutes in a water bath, it is added 0.5 mL of an appropriate enzyme dilution. It is plugged with a rubber stopper and the essay tube is rotated manually in the water bath until is visible the appearance of little coagulums on the wall of the tube. Measure in seconds the time needed for coagulation. The quantity of enzyme that causes the coagulation of the substrate in 60 seconds is defined as 400 U. Just in case the times needed for coagulation are above 90 seconds the enzymatic extract whose activity is being evaluated must be diluted.

2.4.2 Proteolityc Activity on azocasein

Protease activity was assayed in triplicate by measuring spectrophotometrically the release of trichloroacetic acid-soluble peptides from 2% (w/v) azocasein in 50 nM Trizma buffer (pH8) at 35 °C for 30 min. One protease unit (U) is the amount of enzyme that hydrolyses 1μg azocasein per min under the assay conditions.

Se trabajó con una cepa autóctona de *Bacillus* sp. aislada por el Depto. de Bioingeniería, la que fue conservada a 4 °C en tubos inclinados de agar nutriente.

2.5 Wool fabric and top

Wool fabric with the following characteristics was used for the different tests:

warp yarns (ends) in fabric 3200; linear density 1/21; tpm 340 S; 100% wool; 23 μ
filling yarns (picks) in fabric 1700; linear density 1/21; tpm 340 S; 100% wool; 23 μ
mass per unit area: 300 g/m^2
width: 150 cm
Design: serge 2/1

2.6 Dyes

Drimalan F dyes:
Red Brillant Drimalan FB
Blue Brillant Drimalan FB

2.7 Dyeing of wool treated

Due to the modification of the fibre surface, shrink resistant and antifelt treated wool exhibits a change in the dyeing behaviuor with respect to the untreated wool. Since the dye begins in the lower temperature range and builds up more rapidly in the heating phase, the dyeing methods must be adapted to these conditions in order to avoid unlevelness.

Wool treated in this manner also exhibits much poorer wetfastness properties and particularly in medium to dark shades the requiered fastness properties can no longer be met with acid levelling dyes. For this reason, a careful selection of dyes is essential . The methods used was ***optilan:***

a)starting bath temperature 40°C
addition: 1 g/L sodium acetate
X% Drimalan F dyes
1% de Lyogeno FN liquid (amphoteric levellin agent, retards dyeing rate of Drimalan F dyes on wool treated)
pH = 5 acetic acid
b) increased temperature in 40 minutes to 85°C
c) maintain 30 - 60 minutes at 85 °C
RB = 1/100

2.8 Final exhaustation

The final bath exhaustation is determined at several wave lenghts, by using a spectrophotometer Unicam UV2.

2.9 Colour differences measurement

Test methods ITR TEX T023 Textiles Department (LATU)
Equipement : Elrepho 2000
Illuminat : day light D 65
Observer : 10 °
Spot : 12mm
White : Rx Ry Rz
82.22 81.65 77.38

The colour differences and tolerances between the references (fabric or top untreated) and the treated wool (fabric or top) was expressed as total difference (ΔE) using the CIE 1976 L*a*b* formula

2.10 Standard test methods for breaking load (raveled strip)

Test methods: Protocol TEX 004 Textiles Department (LATU) (based on ASTM D 5035-95)
Raveled strip
extension speed: 200 mm / min.
distance between jaws: 75 mm
jaws lenght: 75 mm
Equipment: JJ Lloyd M 5K (T43) CRE constant rate of specimen extension
specimens width: 25 mm
N° of specimens weftwise : 8
N° of specimens warpwise: 5
state of specimens: conditioned at 65 ±5 % HR and 20±2°C
The value of breaking strenght :
warpwise: kgf/25mm
weftwise: kgf/25mm

Specimens	*Direction*	*Expanded uncertanty*
Raveled strip	warpwise	± 2.82 kgf/25 mm
	weftwise	± 2.46 kgf/25 mm

2.11 Colourfastness to washing domestic and laundering

Test method: Protocol TEX 005 Textiles Department (LATU) (based on AATCC 61 1996)
Multifiber: N° 10 Testfabric
contents bands of:
wool, acrylic, polyester, nylon, cotton, acetate.
Evaluate the colour change of test specimens using the Gray Scale for Staining ISO R105/1 Part 3.
Uncertainty: one grade in the whole scale, uncertanty type B (interlaboratory)

Specimens are laid on a plataform with 45° angle for their evaluation
Grade 5: negligible or no color transfer
Grade 4 colour tranfer equivalent to Step 4 on the Gray Scale for Staining
Grade 3 colour tranfer equivalent to Step 3 on the Gray Scale for Staining
Grade 2 colour tranfer equivalent to Step 2 on the Gray Scale for Staining
Grade 1 colour tranfer equivalent to Step 1 on the Gray Scale for Staining

2.12 Dimensional changes in automatic home laundering of woven and knit fabrics

Test method: ITR TEX E029 Textiles Department (LATU)based on AATCC 135
Equipment : automatic home laundering Electrolux
Temperature : 40° C
laundering cicle : 3 (no wool cicle)
drying conditions: screen dry
Pressing: Singer steam press at 148°C

2.13 Fiber identification

Test method: Protocol Tex 406 a Textiles Department (LATU) (based on AATCC 20 A -1995)
Equipment: Microscope of polarized light Leitz Laborlux 12 Pol (T13) with photografic equipment.

2.14 Enzimatic treatment

The enzimatic treatment was done in a jigger (John Jeffreys), it was treated 8 m of fabric, with 1.0 L bath (RB = 1/3). The temperature was 60 °C, and the treatment was for 60 minutes. It was used the enzimatic complex PK2, with the following coagulant activities: 350, 436 and 1422 U/mL. The comercial complex PC, was used in 3%, (80 U/mL), this condition was recommended by the seller.
After the enzimatic treatment one rinse at 80°C for 10 minutes.
The residual coagulant activity in the dye bath was under the limit of detection.

2.15 Tratamiento con chitosan

2.16 Enzimatic - chitosan treatment

The treatment was done following 2.14, with the procedure 2.15.

3.0 Results and discussion

3.1 Fiber tenacity

The results obtained were:

Enzimatic Treatment	Coagulant activity (U/mL)	Fiber tenacity (cN/tex)

----	without treatment	13.6
PK2	2500	15.4
PC	80 (PC)	13.0

Table 1 Fiber tenacity with the enzimatic complex PK2 and PC

The tenacity was no modify by the enzimatic treatment.

3.2 Fabric breaking load (raveled strip)

The breaking load of the fabrics treated with the differents enzimatic procedures was determinated by the raveled strip method.

The results shows that the enzimatic treatment not modify the tensometric property. The lowering of the breaking force is of 4.0 kgf/25mm, but the combined and expanded uncertanty of the method is ± 2.82 kgf/25 mm. This lowering is more important in the commercial product (PC), figure 1.

Figure 1 Breaking force. Raveled strip method

3.3 Shrinkage in automatic laudering

The results are shown in figure 2.

The treatments are effective in reducing the shrinkage due to automatic home laundering (no wool cycle). This reduction is in the order of 65%. This improvement has no relation with the increase of the coagulant activity of the extract used.

Figure 2 Shrinkage of the fabric with the different enzimatic treatment

3.4 Colourfastness to washing domestic and laundering

The fabric dyed in blue and in red, had colorfastness to staining 5 or 4-5 in all the fibers of the Multifiber N° 10, and for all the treatments.

3.5 Cielab Colour Differences

The fabric was dyed in four different shades (2% y 0.2% Blue Brillant. Drimalan FB, and 2% y 0.2% Red Brillant Drimalan FB).

The results are shown in Table 2

Table 2

Colour	Enzimatic Extract	Coagulant Activity (U/mL)	DE
Blue 2%	PC	80	1.76
	PK2	350	0.88
	PK2	436	0.69
	PK2	1422	0.79
Pale Blue 0.2 %	PC	80	1.69
	PK2	350	1.88
	PK2	436	1.36
	PK2	1422	2.11
Red 2%	PC	80	0.57
	PK2	350	1.13
	PK2	436	1.24
	PK2	1422	1.21
Pale Red 0.2 %	PC	80	3.12
	PK2	350	2.39
	PK2	436	2.81
	PK2	1422	2.59

Table 2 Cielab differences

The difference is higher in the pale shades. In some cases the yield with the comercial product is higher. In other cases the yield improves when the coagulant activity increases (pale blue).

3.6 Dye bath exhaustation

The higher final exhaustation was obtained with the treated fabrics.

Result are detailed in Table 3

Table 3

Enzimatic extract	Coagulant Activity (U/mL)	Exhaustation (%) Red	Blue
----	s/t	75.2	93.2
PC	80	80.9	96.1
PK2	350	69.7	94.3
PK2	436	83.9	94.1
PK2	1422	85.0	95.1

Table 3 Final exhaustation of the dyeing bath

3.7 Shrinkage on the fabrics treated by the chitosan - enzimatic process

Enz.	C A (U/mL)	Shrinkage (%) Enzimatic Treatment			Enz. + Chitosan			Enz. + Oxid. + Chitosan		
		1c	2c	3c	1c	2c	3c	1c	2c	3c
----	s/t	17.1	32.8	45.9	9.2	21.1	37.7	3.3	9.6	20.0
PC	80	7.6	21.1	31.2	6.2	20.3	28.8	1.6	5.2	10.4
PK2	350	11.1	28.3	43.3	7.1	24.3	36.0	2.7	5.0	11.0
PK2	436	10.5	24.6	37.1	5.2	15.2	31.4	-1.2	2.3	3.9
PK2	1422	10.1	27.8	41.7	7.1	21.6	34.7	1.0	4.8	9.2

Table 4 Shrinkage in Wascator

When is applied the enzymatic treatment exclusively, in the four concentrations (80 to 1422 U/ml) it is apparent that the residual shrinkage diminishes with respect to the shrinkage of the non treated fabric. This reduction is superior to the one obtained when the alkaline-oxidative treatment is applied on non treated wool (for the second washing in this case the shrinkage reaches the value of 28.3 % and for the third washing goes up to 42,0 %)

When the enzymatic treatments (436 U/ml and 80 U/ml) or with chitosan alone are performed similar shrinkage results : 24.6% and 21.1 % respectively. are obtained.

When both treatments are applied sucesively machine washable products are obtained in all cases (shrinkage inferior to 8 % for the second wash).

It is important to remark that one of the treatments allows to obtain one product that even aster the third wash presents a residual shrinkage of only 3.9 %

3.8 K/S

4.0 Conclusions

- No one of the treatments has negative effects on the breaking load, as all the values obtained are comprised in the uncertainty of the method.

- The shrinkage obtained in home laundering machine (no wool cycle) with samples treated with enzymes was lowered up to 6.5 %.

- The visual observation of the dyed fabrics allows to establish that the dyeing yield is improved up to 10 %.

- The final exhaustion of the dyeing baths was up to 13 % superior with respect to the untreated sample, for the red dyed fabric. But the exhaustion for the blue dyed fabric reached only 2 % more.

- The washfastness obtained were as desired : grade 5 no staining.

- k/s

- The shrinkage results obtained when the enzymatic and the chitosan treatments are applied successively allows to affirm that this is a promissory procedure for obtaining articles that meet the IWTO requirements.