Properties of Cotton Fabrics Treated by Protease and Its Multienzyme Combinations

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ABSTRACT: A commercial serine-type protease preparation (Alcalase) was examined as a scouring agent for cotton fabrics. Application of the enzyme induced moderate changes in the composition of fibers that were mainly associated with the removal of protein and waxes. The relationship between the compositional modifications and structural transformations, which were reflected in the crystallinity index of the bioscoured cotton fibers, was demonstrated. The protease-treated textiles displayed superior whiteness and outstanding compressional resilience but exhibited a poor hydrophilicity and dyeing capacity. One-step scouring at neutral conditions, where proteolytic activity was supported by multienzyme combinations, could generate textiles with sufficient water absorbency and advanced performance. The implementation of the appropriate scouring conditions (concentration and combination of enzymes) could form fabrics with the desired physicochemical and micromechanical properties. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 114: 1567–1573, 2009

Key words: enzymes; fibers; mechanical properties

INTRODUCTION

The water absorbency and whiteness of raw cotton is limited because of noncellulosic components, such as fats, waxes, proteins, pectins, natural colorants, minerals, and water-soluble compounds, that are present in the primary wall and cuticle.[1,2] Removing these impurities from natural fibers in a scouring process is necessary for the subsequent dyeing and finishing steps.[3,4] Conventional scouring is accomplished with aqueous sodium hydroxide solutions, surfactants, and chelators at higher temperatures.[5] Efforts within the textile industry focus on replacing the traditional method with enzyme-based solutions, which provide for reduced water and wastewater costs, lower energy consumption, improved fabric quality, and compatibility with other processes, machinery, and materials.[6–8] Despite unquestionable advantages, enzymatic scouring has not yet been widely employed on a large scale because of unsettled issues, such as the higher cost of enzymes, incomplete removal of waxes, extended treatment times, and restricted applicability to dark-colored products.[9,10]

The implementation of proteases as scouring agents is well documented, but their efficiency is controversial.[11–13] The treatment of textiles with a wide range of proteases was not effective in improving their water absorbency.[9,14,15] However, a significant increase in the water retention capacity was reported for fabrics treated with a serine-type protease from *Bacillus subtilis,* which was suggestive of the effective removal of proteins from the lumen.[12] The superior wettability of textiles was also accomplished by scouring with other serine-type proteases, such as trypsin, chymotrypsin, and subtilisin.[16] Interestingly, cotton fabrics treated by this class of enzymes under lower protein concentrations and short treatment times were more resilient to compression and easier to shear but more rigid to bending than fabrics subjected to alkali treatment.

Several other enzymes, such as pectinases, lipases (L's), cutinases, cellulases, and xylanases (XLs) have been applied for the bioscouring of cotton textiles.[14,17–20] Alkaline pectinase is recognized as a key enzyme because it loosens the fiber structure by removing pectins between cellulose fibrils and facilitates the wash-off of waxy impurities.[6] Lipolytic and xylanolytic activities have been used as supportive agents in many bioscouring schemes.[13,18] The implementation of cellulases in bioscouring, although improving the softness of cotton fabrics, has been linked to typical fiber damage, which affects the strength and quality of the textiles.[21]

Improving the effectiveness of the scouring process has been attempted with the application of combinations of enzymes. Most of the research activities in this field has dealt with multistep processes,

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where, because of divergent optimal conditions, enzymes have been added successively.[13,19] The one-step scouring of cotton knitted fabrics with enzyme admixtures in a single bath is a promising alternative, which takes advantage of the synergistic effects between the selected biocatalysts. Reports on the implementation of one-step multienzyme scouring schemes for cotton fabrics is rather limited. Acidic conditions (pH 4.8) were maintained during treatment with a mixture of pectinase, L, XL, and cellulase,[21] whereas a combination of alkaline pectinase, cellulase, and XL or protease was applied at pH 9.0.[9]

The aim of this study was to assess the performance of a commercial serine-type protease from *Bacillus licheniformis* (Alcalase), as a scouring agent for cotton fabrics. Compositional analysis and an inclusive set of physicochemical and micromechanical properties formed the basis for characterization of treated fabrics. Improving the bioscouring process was attempted in one-step treatment by multienzyme combinations of Alcalase in a neutral environment, where enzymes retained most of their activity.

EXPERIMENTAL

Materials

Desized, plain woven (52 and 29 yarns/cm in the warp and weft directions, respectively) 100% cotton fabric (122 g/m^2), which was kindly provided by Thomoglou Textile Industry S. A. (Athens, Greece), was used throughout this study. The commercial enzyme preparations, which were kindly supplied by Novozymes A/S (Denmark), were Alcalase (*B. licheniformis* protease), Bioprep 3000L (endopectate lyase from genetically modified *Bacillus* sp.), Lipolase 100L (*Thermomyces lanuginosus* lipase), and Pentopan (β,1-4 xylanase from *T. lanuginosus* expressed in *Aspergillus oryzae* host cells). The enzyme activities detected in these preparations are given in Table I. Azocasein and other reagents were of the highest purity commercially available and were obtained from Sigma Chemical Co (St. Louis, MO).

Determination of the enzyme activities

The proteolytic activity was assayed spectrophotometrically with azocasein as a substrate.[22] One unit of activity was defined as the amount of enzyme required to produce a 0.1 increase in absorbance at 440 nm under the assay conditions (50°C and pH 8.0). L activity was determined against *p*-nitrophenyl propionate at pH 7.0 and 25°C with the aid of a microplate reader (Molecular Devices Corp., Sunnuvale, CA).[23] Pectate lyase (PL), polygalacturonase, and XL activities were determined as described.[24]

TABLE I
Main Specific Activities (U/mg of protein) Detected in the Commercial Enzyme Preparations

	Alcalase	Bioprep	Lipolase	Pentopan
Protease	3.78	0.14	–	
PL		11.93	–	
Polygalacturonase		2.19	–	
L		–	4.10	
XL		0.02	–	521.87

Scouring of the cotton fabrics

A fabric sample raveled to dimensions of 10 × 5 cm^2 (approximate weight = 3.0 g) was immersed in phosphate buffer (50 m*M*) at pH 7.0. A nonionic wetting agent (Sadopane SF 0.1% w/v) and the appropriate amount of enzyme(s) supplemented the solution. The liquor-to-fabric ratio was adjusted to 40 : 1, and the mixture was incubated at 50°C and 50 rpm for different treatment times. Inactivation of the enzyme(s) was carried out in hot distilled water; this was followed by extensive washing and air drying for at least 24 h before the fabric's properties were assessed.[21] The same procedure, but with the absence of enzyme, was applied for the reference fabrics, where conventional scouring was achieved by sodium hydroxide solutions (2.0%, w/v).[25] The experiments were carried out in duplicate.

Wettability drop test

Samples were tested at room temperature with AATCC test method 39-1980.[25] Ten readings were taken from different locations on the sample, and the average is reported.

Dyeing procedure

The dyeing of the fabrics was performed with a modified method of Canal et al.[26] with an Ahiba Polimat dyeing machine (Datacolor, Luzern, Switzerland).[25] The textiles were mixed at a 1 : 100 fabric-to-liquor ratio with a solution containing the reactive dye (Blue RD-HX, C. I. Blue 160, 10 g/L), electrolyte (Na_2SO_4, 70 g/L), and wetting agent (1 g/L). The temperature of the bath was increased slowly (0.5°C/min for approximately 30 min) and maintained at 90°C for 50 min. After the addition of Na_2CO_3 (20 g/L), the dyeing procedure was continued under alkaline conditions for 23 min at the same temperature. Finally, the dyed samples were washed with cold tap water until no residual color could be observed in the effluent.[27]

Whiteness and color strength

The whiteness index (WI) of the scoured samples and the color strength of the dyed fabrics were

determined with the aid of a Datacolor apparatus, according to methods described previously.[25]

Degree of polymerization (DP) and crystallinity index (CrI)

DP of the cellulosic component of textiles was estimated by viscosity measurements after dissolution in a copper ethylenediamine solution.[25]

The crystallinity of the cellulosic component of fabrics was determined by an X-ray diffractometer (Siemens D5000, Bruker-Axs, Karlsruke, Germany) with copper Cu Kα radiation. The generator intensity was 40 kV, and the generator current was 30 mA. Each sample was scanned from 10 to 30° in steps of 0.01°/s. The CrI was calculated according to the method proposed by Segal et al.[28] with the application of the following equation:

$$\mathrm{CrI} = \left(\frac{I_{002} - I_{\mathrm{am}}}{I_{002}}\right) \times 100 \quad (1)$$

where I_{002} is the peak intensity from the lattice plane and I_{am} is the peak intensity of amorphous phases.

Triplicate sets of data were used to establish the relative error associated with the X-ray diffraction method.

Chemical analysis of cotton fabrics

Compositional modifications of the scoured fabrics were identified with a multistep procedure, which included the drying of samples; extraction of oils, fats, and waxes; enzymatic proteolysis; and isolation of pectic polysaccharides by ammonium oxalate.[25] The chemical analysis was carried out in triplicate.

Kawabata evaluation system

The low-stress mechanical and surface properties of treated and untreated fabrics were assessed by a Kawabata Evaluation System (Kato Tech Co., Kyoto, Japan) under high-sensitivity conditions.[25] Three tests per direction were conducted to estimate the average values of extensibility (EMT) at 500 (gf/cm), shear stiffness (G; gf cm degree^{-1}), bending rigidity per unit length (B; gf cm^2 cm^{-1}), and compressional resilience (RC; %). The samples were conditioned at 20 ± 0.5°C and 65 ± 5% relative humidity before their properties were measured.

RESULTS AND DISCUSSION

Compositional changes

Partial removal of noncellulosic impurities from the cotton fabrics was achieved by a single Alcalase treatment. Increased enzyme concentrations detached higher amounts of the noncellulosic constituents, which were mainly proteins, waxes, and pectin (Fig. 1). Maximum total weight loss (0.9%) was observed for the highest protease concentration examined. This condition was associated with the dislodgement of 21.0 and 30.8% of the initial protein and wax contents, respectively. The removal of pectin was as high as 12.0% of the initial content, whereas the hemicellulosic content of the Alcalase-treated fabrics did not decrease. The results are in accordance with previous reports, where the implementation of high concentrations of proteases removed 17 and 50% of the protein content.[14] A major part of the proteins in cotton fibers is inaccessible to monoenzymatic proteolytic attack. As it has been reported, surface proteins cannot be extracted from the surface without destroying the structure of the cuticle.[12]

When the activity of protease was supported by other enzymes, increased amounts of noncellulosic components were removed (Fig. 2). Mixtures of Alcalase with pectinase or XL dislodged substantial amounts of protein (65.9 and 65.0%, respectively), which were much higher than the amounts removed by the individual enzyme preparations (Alcalase, 0.8%; Bioprep, 12.7%; Pentopan, 8.3%). The removal of pectin and hemicellulose, which are interconnecting materials between the cellulose microfibrils, would destabilize other constituents of the primary layer, such as proteinaceous molecules, and facilitate their removal.[29] Synergy between Alcalase and other enzymatic activities was also identified in the removal of other components. The highest degree of synergism was observed during the implementation of the protease and L combination for the removal of waxy components. The amounts of dissolved waxes with separate Alcalase and Lipolase preparations were 9.2 and 37.8% of the initial content,

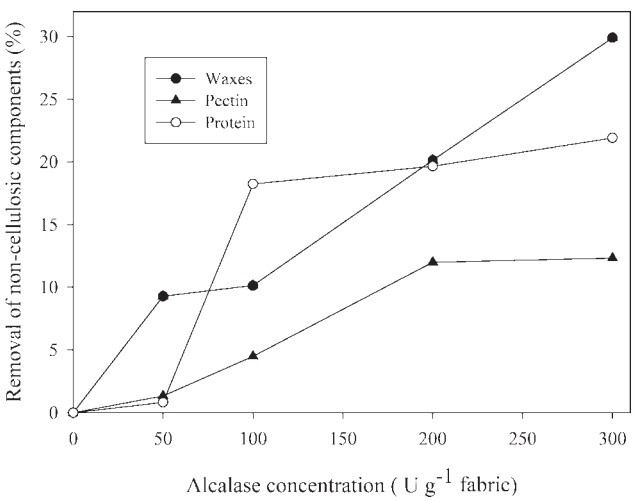

Figure 1 Effect of the protease concentration on the removal of (●) waxes, (○) pectin, and (▲) protein from cotton fabrics treated for 120 min.

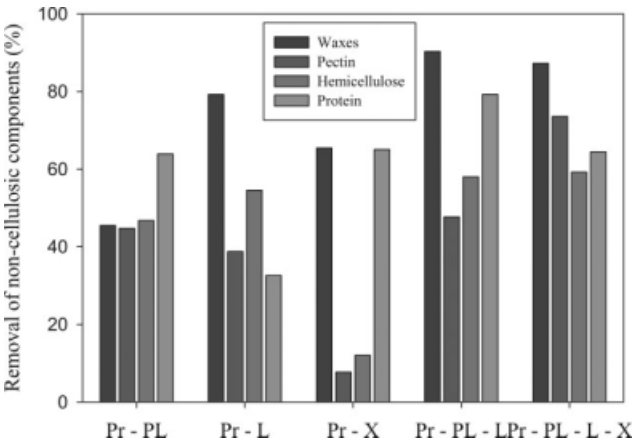

Figure 2 Effect of the one-step multienzyme scouring of cotton fabrics with protease combinations. The treatments were carried out with the addition of 50 U/g fabric from each of the preparations (Pr = protease).

respectively, which increased to 79.2%, when their combination was implemented. The results indicate that cleavage of peptidic bonds promoted the removal of waxy materials from cotton fibers, which was consistent with the potential involvement of lipoprotein molecules.

Physicochemical properties

The physicochemical properties of the cotton textiles treated by Alcalase and its combinations with commercial preparations of PL, L, and XL are presented in Table II. Under all of the conditions examined, the CrI of the bioscoured cotton fibers increased; higher values were observed for multienzyme combinations. Superior crystallinity after the scouring of natural fibers has been attributed to the removal of cementing noncellulosic materials, which probably leads to better packing and reorientation of cellulose chains.[30] An association between the compositional changes and structural modifications of the bioscoured fabrics was established. After estimation of the residual amounts of waxes (RW) and protein (RP), the prediction of CrI of the processed cotton fibers was feasible. Nonlinear regression analysis was used to derive the relevant equation, correlation coefficient, standard error of estimates, and p value, which were as follows:

$$\mathrm{CrI} = 89.6 - 3.3 \times \mathrm{RW} - 0.6 \times \mathrm{RW}^2 + 8.7 \times \mathrm{RP} - 9.6 \times \mathrm{RP}^2 + 4.4 \times \mathrm{RW} \times \mathrm{RP} \quad (2)$$

where R^2 is 0.99, the standard error of estimates is 0.15, and p is 0.003.

The DP of cellulose was marginally affected by the cellulase-free enzymatic treatments. The slightly decreased DP values, which were achieved after scouring with Alcalase and its combinations, were significantly higher than the corresponding values observed after conventional chemical treatment. The results verify that the bioscouring processes produced lower fiber damage because of their milder reaction conditions.[1]

The application of Alcalase as single scouring agent slightly improved the water absorbency of the cotton fabrics. Although the implementation of higher enzyme concentrations formed textiles with lower wetting times, these values were higher than 1 s, which is the maximum wetting time required for efficient dyeing and finishing.[5] As it has been reported, the hydrophilicity of the textiles was not improved by protease-mediated scouring and the removal of proteins.[9,14,15] Sufficiently low wetting times (<1 s) were achieved under bioscouring combinations of Alcalase with another supportive enzyme. Shorter times of multienzyme treatment (<30 min) were sufficient to form textiles with adequate hydrophilicity (data not shown).

TABLE II
Physicochemical Properties Observed for the Cotton Fabrics Treated for 2 h with Various Levels of Protease and Its Combinations

Type of treatment	CrI (%)	DP	Wetting time (s)	K/S	WI (%)
No enzyme	87.0 ± 0.5	2294 ± 13	12.5	2.70 ± 0.1	36.4 ± 0.7
50 U/g protease	88.2 ± 0.2	2182 ± 54	9.1	2.82 ± 0.2	43.5 ± 1.2
100 U/g protease	88.7 ± 0.7	2198 ± 34	7.8	2.81 ± 0.2	44.1 ± 2.7
200 U/g protease	89.2 ± 0.5	2163 ± 62	7.3	3.02 ± 0.3	44.9 ± 1.6
300 U/g protease	89.3 ± 0.3	2114 ± 53	6.4	3.07 ± 0.5	44.8 ± 1.1
Protease–PL	89.2 ± 0.2	2266 ± 68	<1	5.26 ± 0.3	40.2 ± 0.6
Protease–L	90.5 ± 0.2	2176 ± 33	<1	4.64 ± 0.3	42.6 ± 0.7
Protease–X	90.1 ± 0.3	2200 ± 53	<1	4.16 ± 0.3	40.1 ± 0.5
Protease–PL–L	90.6 ± 0.5	2243 ± 43	<1	4.96 ± 0.2	43.5 ± 0.6
Protease–PL–L–X	91.3 ± 0.4	2137 ± 21	<1	4.96 ± 0.5	45.1 ± 0.4
Alkali treatment	93.0 ± 0.1	1894 ± 18	<1	7.79 ± 0.1	54.9 ± 0.2

The treatments with enzyme combinations were carried out with the addition of 50 U/g of fabric from each of the preparations.

The dyeing performance of the cotton fabrics improved marginally after monoenzymatic treatment with Alcalase. Similar findings were reported for the interaction of protease-scoured cotton materials with reactive dyes.[15] The poor dyeing capacity of the textiles treated with various Alcalase concentrations was related to insufficient wetting times. The hydrophilicity of cotton is a key variable affecting the outcome of the dyeing process.[1] The higher levels of remaining pectic substances, which are present in more hydrophobic textiles, reduce the accessibility of dye molecules to the cellulose microfibrillar matrix.[26]

The implementation of multienzyme combinations created textiles with higher color depth (K/S) values. Optimal bioscouring conditions were achieved by protease and PL mixtures. The scouring of the cotton fabrics with a variety of noncellulolytic enzymes or their combinations improved their dyeing performance and resulted in higher values of CrI.[13,25] Despite a higher packing density of the amorphous regions due to the removal of cementing materials,[30] the widening of underlying cavities and increased absorbance capacity was reported for cellulosic textiles with higher CrI values.[31]

Whiteness was the property most affected by treatment of fabrics with Alcalase. Increased WI values were obtained after scouring with protease and its combinations. The decoloration of cotton by proteolytic attack has been attributed to the removal of pigments associated with proteinaceous molecules.[15] Interestingly, the whiteness of the textiles subjected to the single Alcalase treatment was equivalent or superior to that observed with multienzyme combinations. This was in accordance with previous results, which reported inferior whiteness after scouring with a protease–pectinase blend compared to that after a single protease treatment.[15] Similar findings for laccase-mediated scouring were related to the incomplete removal of natural pigments and lignin substances.[32]

Micromechanical properties

A comparison of the micromechanical properties displayed by the cotton fabrics after treatment with Alcalase and its combinations and those exhibited by the reference and alkali-treated fabrics is presented in Table III. The values of bending (B) and G that were observed for the Alcalase-treated fabrics compared favorably to those obtained after conventional alkali treatment. Bioscouring with proteases has been reported to increase the resistance of fabrics to bending deformation but reduce their resistance to shearing forces.[16] The ability of the fabrics to recover from compressional deformation was radically improved after Alcalase treatment. The highest

TABLE III
Micromechanical Properties of the Cotton Fabrics Treated for 2 h with Various Levels of Protease and Its Combinations

Type of treatment	B (gf cm^2 cm^{-1})			G (gf cm^{-1} degree^{-1})			EMT (%)			RC (%)
	Weft	Warp	Mean	Weft	Warp	Mean	Weft	Warp	Mean	
No enzyme	0.061 ± 0.002	0.083 ± 0.003	0.072	2.90 ± 0.10	3.20 ± 0.20	3.05	1.93 ± 0.09	3.32 ± 0.06	2.63	10.53 ± 1.33
50 U/g protease	0.109 ± 0.001	0.141 ± 0.001	0.125	3.30 ± 0.10	4.20 ± 0.10	3.75	2.85 ± 0.10	7.27 ± 0.18	5.06	27.61 ± 2.12
100 U/g protease	0.110 ± 0.001	0.141 ± 0.001	0.125	3.40 ± 0.05	4.30 ± 0.05	3.85	2.60 ± 0.10	5.73 ± 0.08	4.17	40.05 ± 2.53
200 U/g protease	0.109 ± 0.002	0.141 ± 0.001	0.125	3.75 ± 0.15	4.45 ± 0.05	4.10	2.15 ± 0.12	5.4 ± 70.10	3.81	50.22 ± 1.87
300 U/g protease	0.109 ± 0.001	0.133 ± 0.001	0.121	4.20 ± 0.10	4.60 ± 0.05	4.40	1.88 ± 0.10	5.49 ± 0.13	3.69	51.05 ± 0.93
Protease–PL	0.099 ± 0.002	0.100 ± 0.002	0.100	2.90 ± 0.30	3.85 ± 0.35	3.38	1.31 ± 0.15	2.79 ± 0.45	2.55	49.39 ± 1.04
Protease–L	0.099 ± 0.001	0.099 ± 0.002	0.099	2.90 ± 0.20	3.20 ± 0.10	3.05	0.63 ± 0.04	2.55 ± 0.10	1.59	40.25 ± 0.56
Protease–X	0.104 ± 0.003	0.137 ± 0.001	0.120	2.80 ± 0.10	3.20 ± 0.10	3.00	1.45 ± 0.04	5.48 ± 0.05	3.47	47.10 ± 0.77
Protease–PL–L	0.091 ± 0.002	0.110 ± 0.002	0.101	2.00 ± 0.10	5.10 ± 0.05	3.55	0.61 ± 0.10	2.05 ± 0.10	1.33	47.23 ± 0.84
Protease–PL–L–X	0.095 ± 0.002	0.108 ± 0.001	0.101	2.40 ± 0.10	5.55 ± 0.20	3.98	0.78 ± 0.07	2.20 ± 0.20	1.49	52.85 ± 1.54
Alkali treatment	0.104 ± 0.005	0.121 ± 0.001	0.113	3.60 ± 0.20	4.60 ± 0.10	4.10	2.10 ± 0.08	6.05 ± 0.25	4.08	54.30 ± 1.18

The treatments with enzyme combinations were carried out with the addition of 50 U/g of fabric from each of the preparations.

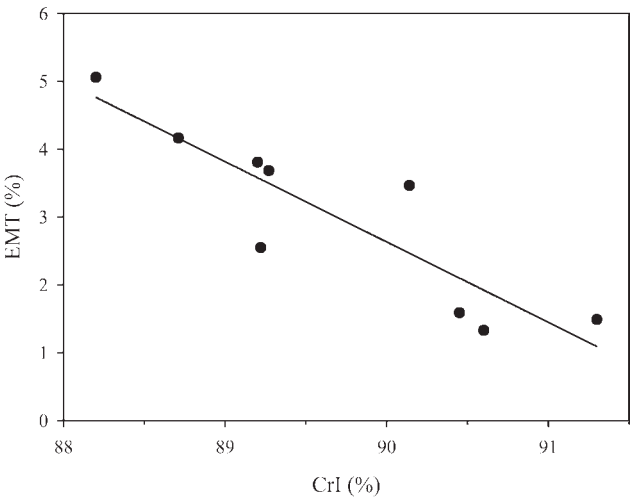

Figure 3 Relationship between the EMT of the cotton fabrics and the CrI of related fibers.

RC value, which was observed for the highest Alcalase concentration (300 U protease/g of fabric), was very close to the corresponding value obtained with alkali treatment and similar to the value reported when the cotton fabrics were treated with 1000 U/g of fabric of the commercial PL preparation (Bioprep).[25] The upgrading of the fabric's EMT was dependent on the amount of added protease; the highest EMT value, which considerably exceeded the value achieved with chemical scouring, was observed with the lowest protease concentration. An investigation of the relationship between the structural features and mechanical properties of the bioscoured textiles revealed that the CrI could be used as a single variable for the determination of their EMT (Fig. 3). The results obtained after linear regression analysis were in agreement with previous reports, where the micromechanical properties of cotton fabrics treated with PL could be predicted by the CrI of related fibers:[25]

$$\mathrm{EMT} = 109.2 - 1.2 \times \mathrm{CrI} \quad (3)$$

where R^2 is 0.79, the standard error of estimates is 0.6, and p is 0.001.

The mechanical properties of the multienzyme scoured fabrics diverged from those determined for the Alcalase-treated textiles. The values of B and G that were observed after scouring with combinations of enzymes were inferior to the corresponding values obtained with single-protease or alkaline treatments. Among the multicomponent conditions examined, the combination of protease with XL resulted in the highest B value; the same condition was associated with the highest EMT. Increased G values, which were comparable to those exhibited by the alkali-treated fabrics, were observed after scouring with multienzyme combinations of protease and PL. The RC of the bioscoured textiles improved regardless of the enzymatic combination implemented. An outstanding ability to be stretched under a tensile load was exhibited by the fabrics treated with the full mixture of enzymes.

The results indicate that the modification of the bioscouring conditions by the selection of the appropriate blend of enzymes could create fabrics with differentiated characteristics. Further investigation for optimized multienzyme combinations could assist in the development of low-cost and environmentally friendly bioscouring processes for high-quality textiles with desired physicochemical and micromechanical properties.

CONCLUSIONS

Noncellulosic impurities, mainly protein and waxes, were partially removed from cotton fabrics after scouring with commercial serine-type protease preparation. Treatment with Alcalase generated materials with significantly improved whiteness and outstanding micromechanical properties, which were superior to those obtained after conventional alkali treatment. The physicochemical properties of scoured textiles, such as hydrophilicity and dyeing performance, were advanced when protease was combined with other depolymerizing enzymes. The dislodgement of considerable amounts of protein (>60%) was achieved by means of cross synergism between protease and pectinase or XL, whereas the removal of waxes was facilitated by a protease and L combination. The assessment of the textiles' compositional or structural features could be used for the prediction of their micromechanical properties. One-step multienzyme scouring, where the appropriate blend of enzymes is selected, can form the basis for targeted compositional changes that produce cotton fabrics with desired physicochemical and mechanical properties.

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