Appl Biochem Biotechnol (2011) 165:748–759
DOI 10.1007/s12010-011-9293-0

Treatment of Bleached Wool with Trans-Glutaminases to Enhance Tensile Strength, Whiteness, and Alkali Resistance

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Received: 8 November 2010 / Accepted: 19 May 2011 /
Published online: 3 June 2011

Abstract Trans-glutaminases is known as a cross-linking enzyme for proteins. Wool is a proteinous fiber conventionally is treated through several processes to obtain the desirable characteristics. Bleaching is also one of the most important processes usually carried out by using an oxidizing agent in a conventional method. The tensile strength of wool yarns was reduced as a consequence of oxidative bleaching. Here, with the help of microbial trans-glutaminases (m-TGases), a novel bleaching process was disclosed in a way to obtain a bleached wool yarn with no significant reduction in the tensile strength. The results confirmed that the bleached wool yarns with H_2O_2 could be modified by m-TGases post-treatment. The m-TGases treatment on the bleached wool yarns improved the tensile strength and whiteness along with the higher alkali resistance.

Keywords Microbial trans-glutaminases · Bleaching · Wool · Tensile strength · Whiteness · Alkali solubility

Introduction

Wool is an important textile fiber due to their lightness, smooth handle, high moisture absorbance, and high fabric drape. Bleaching is a very common process that is usually done with reductive and oxidative agents. Reduction bleaches cause little damage to wool by comparison with oxidative methods. The preferred reductive bleaching treatment today utilizes stabilized sodium dithionite-based products or, alternatively, thiourea dioxide. Better whiteness can be achieved using formulations based on hydrogen peroxide at the expense of increased damage, particularly to cystine (CYS) [1].

Enzymes can be used for developing environmentally friendly alternative processes in the treatment of textile goods and they have gained widespread acceptance, and the applications of such technology are many and diverse, including industrial processing [2, 3]. There are many reports on application of enzymes on different textile goods [4–11].

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Trans-glutaminases (EC 2.3.2.13) are aminoacyltransferases that catalyze an acyl transfer between peptide-bound glutamine (acyl-donors) and a suitable primary amine (acyl acceptors). They are found widely in a host of different organisms including mammals, plants, fish, and microorganisms. In most instances, the result of their reaction is the crosslinking of proteins via intra or inters ε-glutamyl lysine isodipeptide bridges if the primary amine is the ε-amino group of the peptide-bound lysine. This leads to increased protein stability and increased resistance to chemical and proteolytic degradation. Trans-glutaminases can also modify proteins by the covalent incorporation of compounds containing primary amines (e.g., cadaverine), and if the polyamine is bi-functional, the crosslinking of proteins can also occur via *N*,*N* (glutamyl) polyamine bridges. If no primary amine is available, trans-glutaminases are also capable of deamidating peptide-bound glutamine to glutamic acid [2].

There have been several reports on application of trans-glutaminases on wool [2–5, 12]. However, there is no report on the application of trans-glutaminases on the bleached wool yarns. In this study, the wool yarns were bleached in conventional method by using hydrogen peroxide and then were treated by microbial trans-glutaminases (m-TGases) to revenge the negative influence of alkali bleaching process. Raw wool yarns were bleached with hydrogen peroxide (35%) by utilizing ammonia and caustic soda to adjust the pH of bath solution. Then those bleached wool yarns were treated with 20% *v*/*w* m-TGases for various lengths of times. The physical and mechanical properties of wool yarns such as tensile strength, elongation, whiteness, diameter, alkali solubility and weight reduction were measured before and after bleaching and enzymatic treatments. The results of scanning electron microscopy (SEM) and FTIR analysis were also reported.

Materials and Methods

Materials

The Iranian wool yarn with count of 2.04 Nm (two-ply) was selected as the substrate. Sodium carbonate, caustic soda, potassium persulphate, acetic acid, and ammonia were purchased from Merck Co. The m-TGases (EC 2.3.2.13) isolated from *Streptoverticillium mobaraense* was kindly supplied by Ajinomoto Co., Japan with 100-U/g activity, and industrial hydrogen peroxide (35%) from Merck Co. was also used in this study.

Scouring

The wool yarns were scoured using 1 g/L commercial anionic detergent and 0.5 g/L sodium carbonate at 60 °C for 30 min with L/G=40:1 (liquor to goods ratio), which was followed by rinsing with cold water.

Bleaching

The oxidative bleaching was carried out in alkali solution containing 8% (*v*/*v*) hydrogen peroxide and 16 g/L potassium persulphate as an activator, for 90 min at 60 °C. The bath was initially adjusted to pH 9.5 by using ammonia solution or caustic soda. The pH was kept constant throughout the bleaching process. The bleaching treatment was followed by rinsing with cold water.

M-TGases Treatments

It was shown that the optimum conditions for m-TGase treatment on wool is 20% m-TGase *w*/*w* (weight of m-TGase to weight of goods) at 37 °C in pH 9–10 with L/G (liquor to goods ratio)=40:1 [2, 7]. The same conditions were adopted in this research for wool treatment with m-TGase. The processing time, as an important factor which influences the mechanical properties of wool and economical point of view, was investigated between 45 and 60 min. Therefore, 5 g of wool was treated with 20% *w*/*w* at the mentioned conditions.

Inactivation of enzyme after enzymatic treatment was done in aqueous solution with pH 5 (adjusting with acetic acid) at 80 °C for 5 min to prevent hydrolyzing of wool and then the samples were rinsed with cold water.

Weight Loss

Five-gram samples of wool were first placed in an oven for 2 min at 100 °C and then left in the desiccators, and then their weight was measured. Weight loss (%) of samples after bleaching and enzymatic treatments was calculated by Eq. 1:

$$\text{Weight} \quad \text{loss}\% = \frac{(\textit{initial weight} - \sec \textit{ondary weight})}{\textit{initial weight}} \times 100 \tag{1}$$

Mechanical Properties

The intrinsic strength and elongation of the yarn samples were measured by Instron TM-SM at an elongation rate of 10 cm/min and gauge length of 10 cm. The tensile strength and elongation were calculated by Eqs. 2 and 3, respectively [13]:

$$\textit{Tensile} \quad \textit{strength} = \frac{\textit{Force}}{\textit{Linear density}} (g.f/tex) \tag{2}$$

$$\textit{Elongation}\% = \frac{(\textit{initial length} - \textit{secondary length})}{\textit{initial length}} \times 100 \tag{3}$$

Color

To evaluate the whiteness of the wool yarns after bleaching and enzymatic treatment, the CIE terms that include L* (lightness), a* (redness), b* (yellowness) were calculated on a color scale relative to CIE illuminant D_{65} for the 10° standard observer using spectrophotometer color eye XTH from GretagMacbeth. The specimens were packed at a sufficient thickness or in multilayer form to produce an opaque layer.

Alkali Solubility

The extent of fiber damage caused by bleaching was determined by the alkali solubility test. Alkali solubility is the weight loss of wool yarns after treatment with alkali solution and calculated by Eq. 4:

$$\textit{Alkalisolubility}\% = \frac{(\textit{initial weight} - \textit{secondary weight})}{\textit{initial weight}} \times 100 \tag{4}$$

One gram of wool yarn was weighed, and then dried by heating for 1 h at 110 °C. After it was cooled for 10 min, the sample was reweighed. The weighed wool was added to a 100-ml 0.1 N sodium hydroxide solution for 1 h at 65 °C. The wool yarn was filtered, rinsed six times with distilled water, and neutralized with acetic acid 1% and rinsed with distilled water for a further six times, then it was re-dried at 110 °C for 1 h, cooled, and reweighed [14].

Diameter

The wool fiber diameter may change during bleaching and enzymatic treatment; therefore, the changes were measured with Carl Zeiss microscope made in Germany. For each yarn sample, 200 fibers were taken, and the diameters were recorded [15].

Scanning Electron Microscopy (SEM)

The fibers' surfaces, before and after the treatments, were observed by SEM (Philips XL30) to investigate the fiber surface changes caused by bleaching and enzymatic treatment.

FTIR Analysis

A Nicolet FTIR instrument was employed to take the spectra between 400 and 4,000 cm^{-1} for analysis of wool fibers. The samples were mixed with an oven-dried KBr powder, pressed into pellets.

Results

The wool yarns have been coded to ease the identification. This was shown for the different wool processing in Table 1.

Table 1 Coding of different treated samples

Sample	Code
Raw scoured wool yarn	RW
Bleached wool yarn (pH adjustment with ammonia)	BWA
BWA treated with m-TGases for 45 min	BWA-TG45
BWA treated with m-TGases for 60 min	BWA-TG60
Bleached wool yarn (pH adjustment with caustic soda)	BWC
BWC treated with m-TGases for 45 min	BWC-TG45
BWC treated with m-TGases for 60 min	BWC-TG60
Sample	Code
Raw scoured wool yarn	RW
Bleached wool yarn (pH adjustment with ammonia)	BWA
BWA treated with m-TGases for 45 min	BWA-TG45
BWA treated with m-TGases for 60 min	BWA-TG60
Bleached wool yarn (pH adjustment with caustic soda)	BWC
BWC treated with m-TGases for 45 min	BWC-TG45
BWC treated with m-TGases for 60 min	BWC-TG60

Weight Loss

The loss of weight in wool processing is a usual phenomenon due to the loss of some tiny fibers or for removing of some fiber impurities (Table 2). The oxidative bleaching process of wool along with ammonia causes less damage in comparison with caustic soda. This leads to a lower weight loss on the wool yarns due to the more severe conditions of caustic soda. Here, the influence of enzymatic treatment on the bleached yarn has been investigated and reported. On this basis, the weight loss of the bleached yarn after m-TGases treatment in comparison with the bleached yarn was calculated and reported in Fig. 1. The bleached yarn lost about 6% of its weight after bleaching which can be due to the removal of the remaining impurities in the yarn. It should be mentioned that the coarse wool yarn has been used in this study that includes some impurities which may be due to the improper scouring treatment. The enzymatic treatment of wool also results in a weight loss of the wool yarns due to the alkali conditions; however, the dwell time of enzymatic treatment have a negligible effect on the weight loss of wool yarns (Fig. 1).

Tensile Strength and Elongation

The average values of tensile strength and elongation of different samples with their coefficient of variation (C.V. %) are presented in Table 3. The wool fiber can be chemically attacked by the oxidative bleaching agents (H_2O_2) that leads to decrease the tensile strength and elongation of yarns, but the enzymatic treatment by m-TGases remediated the wool yarns and improved the tensile strength and elongation of wool yarns (Figs. 2 and 3). This is due to the action of m-TGases which causes crosslinking of proteins via glutamine and lysine isodipeptide bridges and increases the tensile strength and elongation of wool yarns. The tensile strength and elongation of yarns increases more with 45 min enzymatic treatment in comparison with 60 min (Figs. 2 and 3). This can be related to the remaining of wool in alkali conditions for a longer time in 60 min leading to the breaking down of some disulfide bonds and reducing tensile strength and elongation of wool yarns.

Optical Properties

It was observed that the oxidative bleaching along with the caustic soda causes a higher lightness (L*) in the wool yarn in comparison with the ammonia. This can be related to the decomposition of hydrogen peroxide in that it is quicker in caustic soda solution than in ammonia solution. However, the enzymatic treatment on the both bleached wool yarns has

Table 2 Weight loss (%) of wool samples during different processes

Sample	Weight loss (%)	Initial weight (g)	Secondary weight (g)
RW	1.50	5.0618	4.9859
BWA	5.72	5.0729	4.7827
BWA-TG45	2.43	4.9609	4.8403
BWA-TG60	2.51	4.9374	4.8135
BWC	6.13	5.0212	4.7134
BWC-TG45	2.99	5.1193	4.9662
BWC-TG60	2.99	4.9813	4.8323

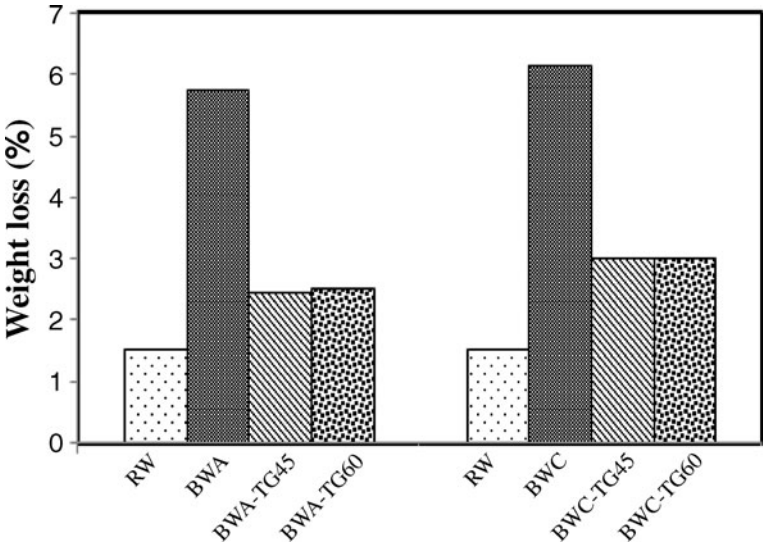

Fig. 1 Weight loss of wool yarn samples

led to the increasing of the yarn lightness. The dwell time of enzymatic treatment had a negligible effect on the wool lightness (Table 4 and Fig. 4).

Alkali Solubility

The alkali condition of the oxidative bleaching may break some peptide or disulfide bonds in the wool keratin that causes some damages to the wool and increases the alkali solubility.

Table 3 Average of tensile strength, elongation and diameter of different samples, and their coefficient of variation (C.V. %)

Sample	Average of tensile strength (g.f/tex)	Average of elongation (%)	Average of diameter (μ)
RW	6.28	21.60	30.30
C.V. %	12.60	11.04	35.53
BWA	4.89	14.40	27.00
C.V. %	8.97	10.62	37.27
BWA-TG45	6.91	17.00	22.40
C.V. %	11.53	9.54	33.45
BWA-TG60	6.44	16.50	22.10
C.V. %	11.56	11.48	30.61
BWC	4.61	15.50	26.70
C.V. %	9.80	9.35	40.12
BWC-TG45	5.52	16.30	22.10
C.V. %	6.29	8.59	37.56
BWC-TG60	5.19	14.80	21.90
C.V. %	5.32	11.00	34.19

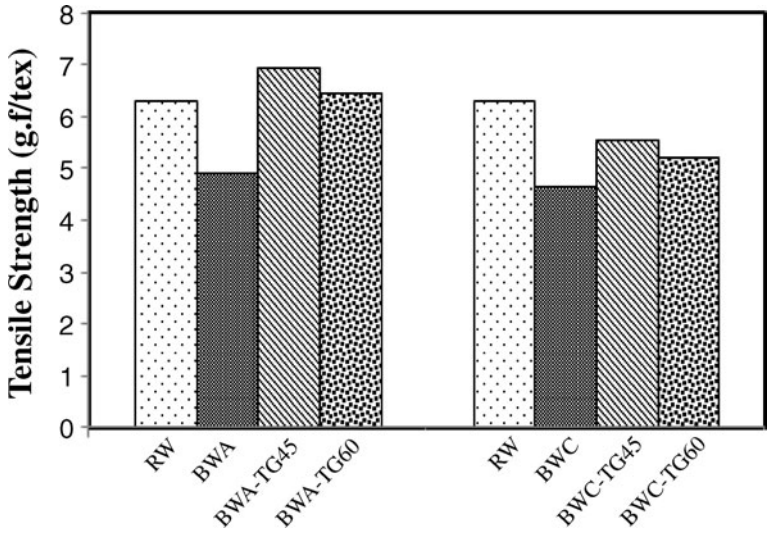

Fig. 2 Tensile strength of wool yarn samples

The results indicated that the m-TGases has crosslinked the wool proteins and remediated the damages caused by the oxidative bleaching and decreased the alkali solubility (Fig. 5).

Diameter

The average values of different sample diameters with their coefficient of variation (C.V. %) are presented in Table 3. It is observed that the treatment of wool yarns with different processing may decrease the medium wool fiber diameter due to the removing of some waxes and other impurities from each fiber and also because of destroying of some dead fibers. Enzymatic treatment decreased the wool diameter because of removing the dead

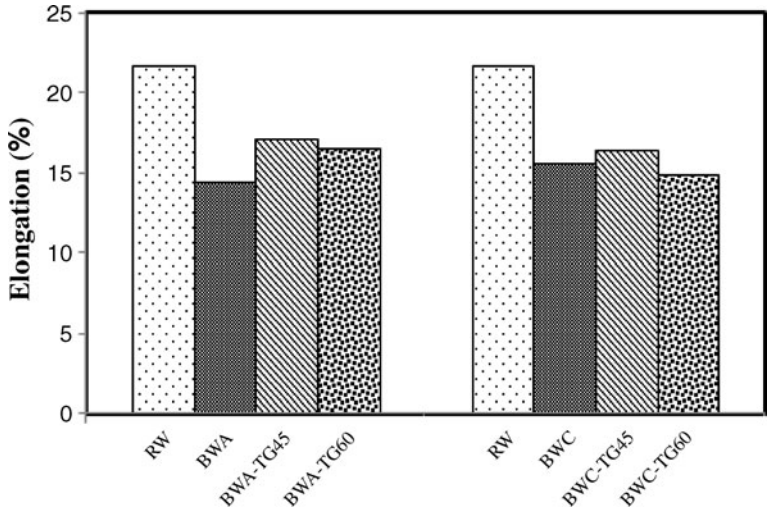

Fig. 3 Elongation of wool yarn samples

Table 4 Optical properties of different samples

Sample	L*	a*	b*
RW	70.09	2.20	12.2
BWA	76.31	0.93	14.12
BWA-TG45	77.16	0.06	14.27
BWA-TG60	80.11	−0.15	12.58
BWC	77.23	−0.32	10.51
BWC-TG45	77.59	−0.12	14.31
BWC-TG60	78.40	−0.53	14.18

fibers from wool yarns that are thicker than the other fibers. This means m-TGases has decreased the variation of wool fiber diameter (Fig. 6) that has led to the producing of a narrow distribution of wool diameter.

Scanning Electron Microscopy

The scanning electron microscopy (SEM) was used to investigate the surface changes of wool fibers during bleaching and enzymatic treatments. The raw wool after being washed only (Fig. 7a, a1) had no surface damages, as it could be expected. The oxidative bleached wool fibers (Fig. 7b, b1) appeared to have some surface damages that were indicated by damaged scales and the less even cuticle surface due to surface oxidation of wool and its alkali condition. Figure 7b, b1 also showed that surface impurities of scoured

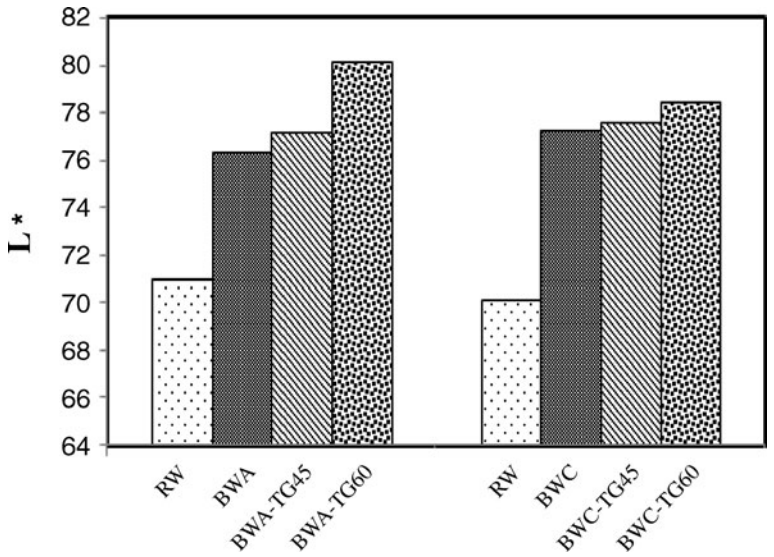

Fig. 4 The L* (lightness) of wool yarn samples

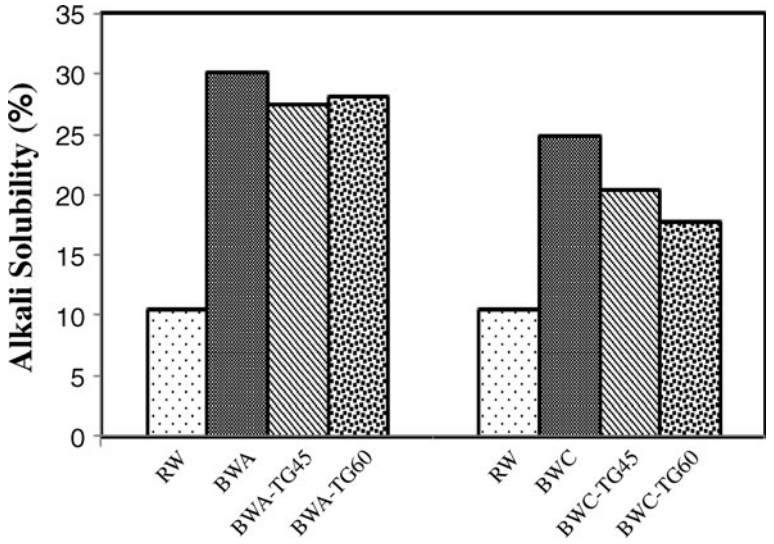

Fig. 5 Alkali solubility of wool yarn samples

wool had been removed by the bleaching process. The enzyme-treated wool (Fig. 7c, c1) also showed surface changes different from the bleached wool because of alkali condition of enzymatic treatment.

FTIR Analysis

Figure 8 illustrates the FTIR spectra of wool yarns. In the bleached samples compared with the raw sample, the intensity of the band at 1,035.6 cm^{-1} corresponding to S–O bonds,

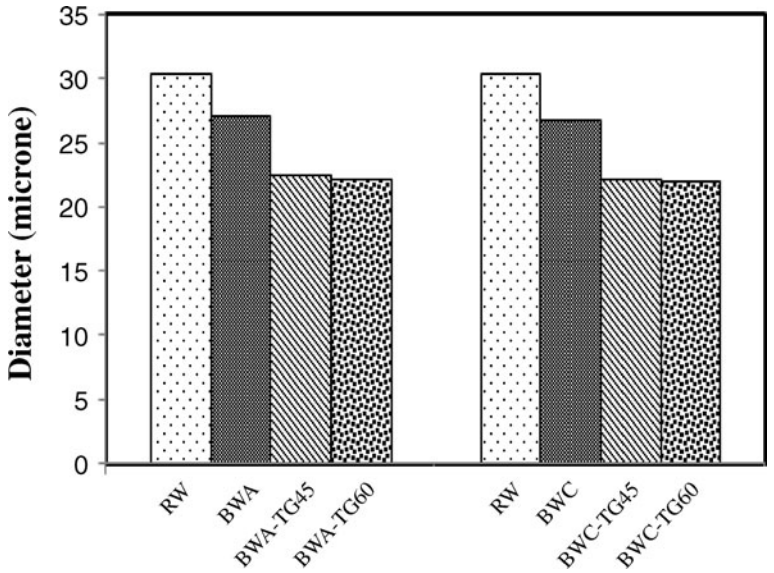

Fig. 6 Diameter of wool yarn samples

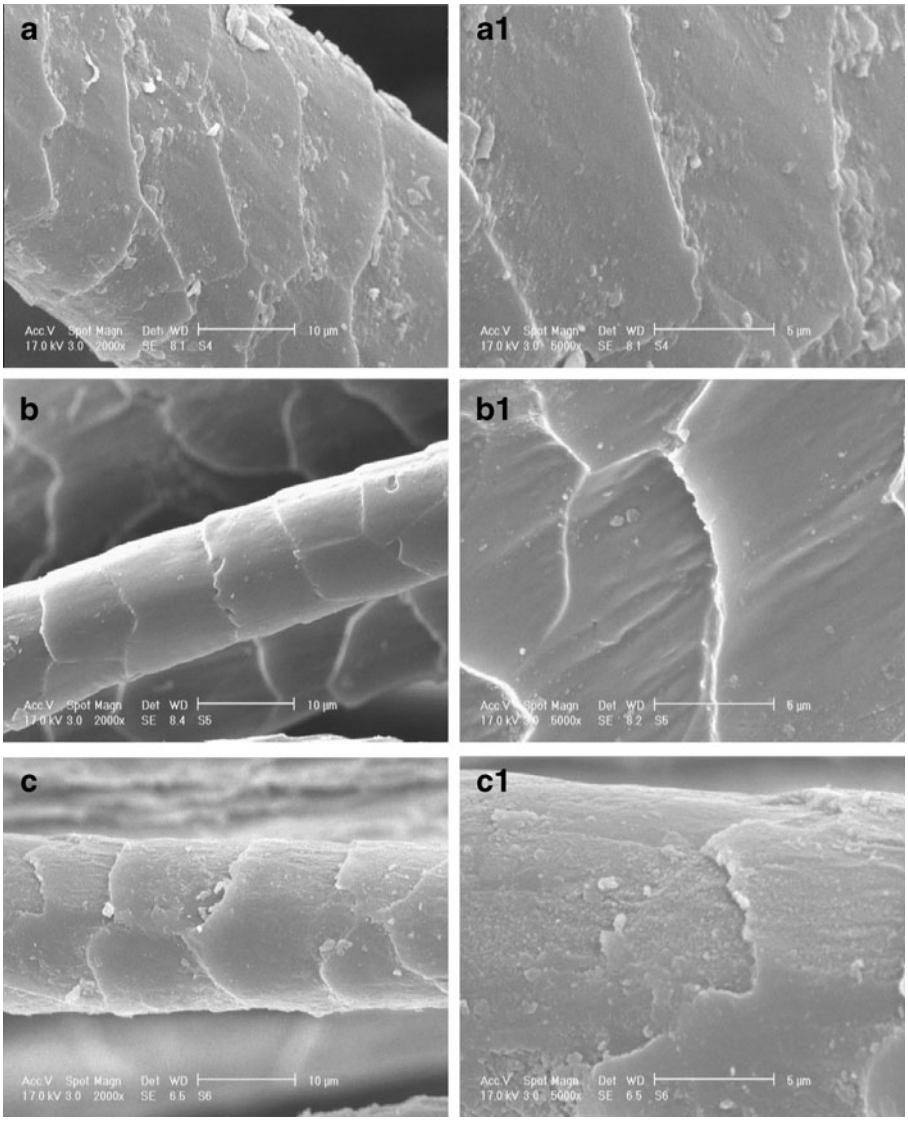

Fig. 7 Scanning electron microscopy (SEM) of different wool samples, (*a*, *a1*) scoured, (*b*, *b1*) bleached, (*c*, *c1*) enzyme-treated wool sample (×2,000, ×5,000)

increased due to the formation of cystic acid during the oxidation of wool under bleaching treatment conditions. The band intensity at 1,385.95 cm^{-1} relating to N–H bonds is higher for the enzyme-treated samples than bleached samples due to the crosslinking of wool by m-TGases. Cross-linking reaction of wool by m-TGases between two amino acids (glutamine and lysine) is shown in Fig. 9. The N–H bonds increases as a result of cross-linking wool.

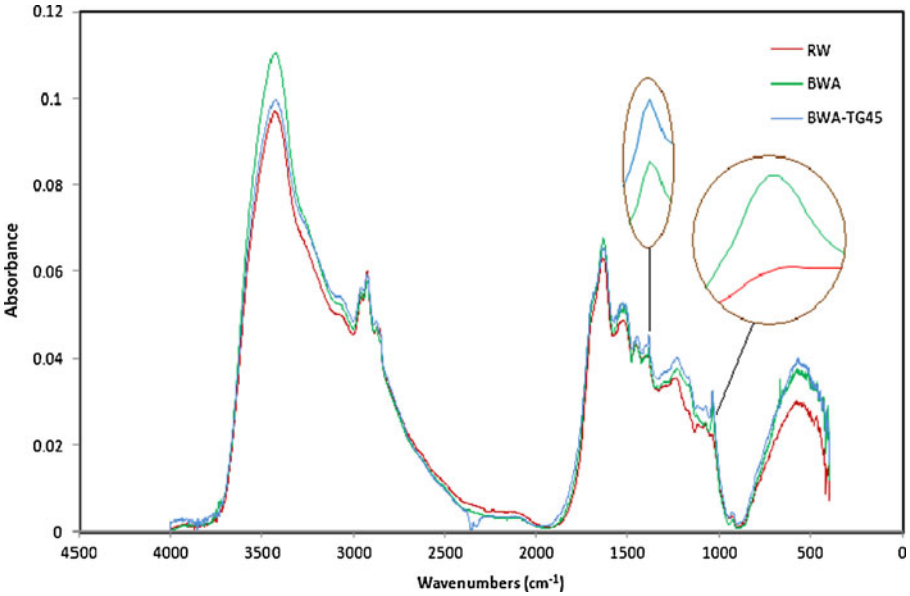

Fig. 8 FTIR spectrums of different wool yarn samples

Conclusion

In this study, the wool yarns were bleached by using hydrogen peroxide and then treated with m-TGases to improve its mechanical properties. The bleaching reduced the tensile strength and weight of the wool yarns due to oxidizing effects and caused surface damage confirmed by SEM images. The tensile reduction can be revenged by crosslinking of bleached wool through enzymatic treatment. Moreover, the SEM pictures show that enzymatic treatment also causes some surface changes to wool fibers due to the alkali

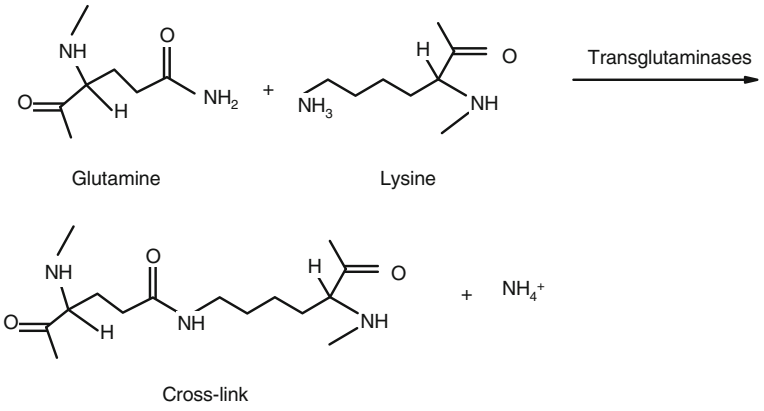

Fig. 9 Crosslinking of wool through covalent bond between different amino acids by m-TGases

conditions of the enzymatic treatment. The FTIR spectra confirmed the cross-linking effects of m-TGases on wool through the increasing of the band intensity at 1,385.95 cm^{-1} relating to N–H bonds. The results also indicated that 45-min m-TGase treatment on bleached wool is preferred because of higher tensile strength and lower diameter obtained on the treated yarns. These results encourage the post-treatment of bleached wool with m-TGases to produce a higher quality bleached wool.

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