

## Influences of Different Enzymatic Treatment on Denim Garment

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**Abstract** In current study, the effects of different enzymatic treatment including acid cellulases, neutral cellulases, and combination of laccases with cellulases on denim garment were investigated. The color changes of different samples were compared by colorimetric indices for the garment surface and back and also white pocket. Abrasion resistance, tensile strength, and crease recovery angle of the samples were measured within the standard methods. Also, surfaces of the treated samples were observed by SEM. The results showed that the combination of laccases with cellulases help to improve the lightness and decrease staining on both back of garment and on white pocket.

**Keywords** Cellulases · Laccases · Lightness · Washing · Denim

### Introduction

Denim fabric is usually produced with twill weave and indigo-dyed warp and white weft yarns [1]. Surfaces of the fabric play an important role in enzymatic decolorization of cellulosic fiber. This can have influences on the outset layers of cellulosic crystalline, and then the available part of cellulosic fiber increases and allows dye removal [2]. It is considerable that denim stonewashing process is treated on finished clothes; therefore, the staining on back of garment and white pocket is a basic problem. One of the major factors causes high indigo backstaining on denim fabrics (redeposition of dye on white yarns of denim) is the high ability of cellulases protein to bind cellulose. In this case, the basic mechanism of indigo redeposition should involve binding of dye to the enzyme molecules adsorbed on the surface of cellulose fibers. This mechanism implies that the enzyme molecules must have surface sites capable to bind indigo [3–5]. The structure of indigo molecule and its properties indicate that there may be two

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basic mechanisms of interaction between the dye and other molecules. First, the indigo molecule contains aromatic rings that can be involved in hydrophobic interactions. Second, the heterocyclic —NH— and =O groups may form hydrogen bonds with other molecules [6]. Ideal cellulases for application on denim (endoglucanase) are those with sites on the surface of protein globule capable of binding indigo with low adsorption ability on cellulose [7].

Backstaining is dependent on pH value and type of enzyme, according to recent researches; acid cellulases put stain on denim in acidic medium. The staining will be reduced with increasing a suitable auxiliary. Neutral cellulases put stain in pH=7 more than pH=5, and their effects are recognizable in pH=7. Overall, neutral cellulases are more important than acid cellulases due to decreasing of staining [8–11].

Laccases (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) have been reported as a bleach for indigo fabrics, and laccases-based systems are able to degrade indigo both in solution and on denim, leading to various bleaching effects on the fabric. The biodegradability of indigo and its industrial effluents by fungi has been studied. Tests of immediate biodegradability have proven that indigo can be classified as easily biodegradable by fungi, and toxicity is greatly reduced [5, 12, 13]. This enzyme finds commercial applications in oxidations of dyes, polymerization of lignin and lignosulfonates, preparation of musts and wastewater treatment to improve the whiteness in a conventional bleaching of cotton, and recently, bio-stoning [12, 14]. It is also used for modification of wool [15]. Laccases-based systems are able to degrade indigo both in solution and on denim, leading to various bleaching effects on the fabric [16].

We also reported the use of laccases with cellulases for repeatable bio-washing to save water, enzyme, and energy. By using this method, laccases help to discolor the effluent, and enzyme residuals of both cellulases and laccases are useful for repeated processing [16].

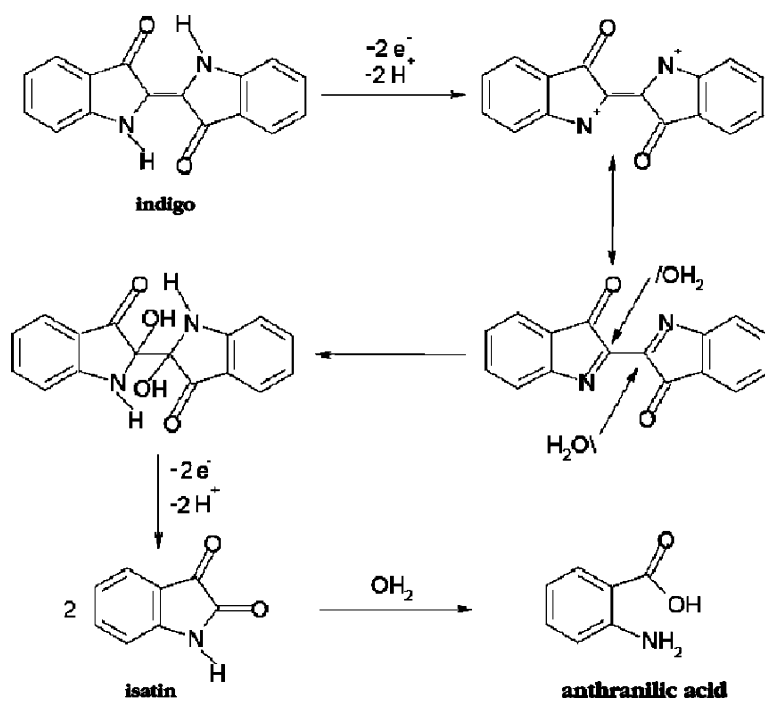
The indigo degradation product is isatin (indole-2, 3-dione) which further decomposes to anthranilic acid (2-aminobenzoic acid). Oxidation of substrates by laccases is believed to involve the reduction of molecular oxygen. The kinetics of indigo degradation with laccases shows that *Polyporus* sp. laccases are more effective due to the faster conversion of indigo into isatin, which further decomposes to anthranilic acid as a final reaction product [17, 18]. R. Campos and coworkers proposed the possible mechanism of indigo degradation with laccases [13]. The mechanism of indigo degradation by laccases was illustrated in Fig. 1.

Since the sewed clothes washing by stone and whiteness of white pocket and back garment are important, the staining should be prevented by indigo on white pocket and back garment during washing. In this study, we are trying to minimize the staining on white pocket and back of the denim by adding laccases in the washing bath. This may also partially increase the lightness of denim. Therefore, this research aims to apply different enzymatic conditions to reduce the backstaining and produce new effects.

## Experimental

### Materials

The denim fabric (Blue jean) used was 100% cotton with twill 2/1 weave construction, weft and warp count of 15 Nm with z twist, weft density of 20/cm, warp density of 26/cm, and fabric weight of 265 g/m<sup>2</sup>. The samples were selected into 30×50 cm<sup>2</sup> and sewed as leg form with two pockets, one on the face of fabric and the other on the back of the fabric that was white cotton fabric with specifications of plain weave and weft and warp count of 30 Nm open end yarn and weft density of 24/cm, warp density of 30/cm, and weight of 106 g/m<sup>2</sup> (Fig. 2).



**Fig. 1** Mechanism of indigo dye degradation by laccases [13]

**Fig. 2** Denim sample with one pocket on face and another (white pocket) on the back



Materials used were industrial acetic acid 70%, dispersing agent (Verlane N60) for preventing backstaining composed of polyacrylates and alkyl phosphonate with anionic structure from Rudolf Chemie Co.; anti-creasing (Rucolin JES) composed of polyacrylamide with nonionic structure from Rudolf Chemie Co., neutral cellulases (E.C. 3.2.1.4 with endocellulase unit 2450 ECU/g; Roglyr ultra 97655) from Rotta Co. which is soluble at 40–50 °C and has maximum activity at 55 °C and pH=7; acid cellulases (E.C. 3.2.1.4 with endocellulase unit 1500 ECU/g; Rucolase JEX) from Rudolf Chemie Co, soluble at 40–45 °C and has maximum activity at 55 °C and pH=5.5; laccases (E.C. 1.10.3.2 with laccase unit 120 LAMU/g; Denilite II) from Novo Chemie Co., soluble at 55–65 °C and has maximum activity at 60 °C and pH=4.5. Amylases (E.C. 3.2.1.1 with thermo-stable amylase unit 1200 NAU/g; Rotta-amylase 189-desizer) from Rotta Co. have a range of activity at 60–100 °C. A rotary drum washer with 5 kg capacity (steel, rpm=25) was used for desizing, stone washing, and washing.

### Desizing

The samples were desized for 15 min at 70 °C with pH=7 by 1 ml/l amylases. In all of the experiments, weight of samples was 450 g, LR=50:1; the amount of dispersing agent 2 g/l and anti-creasing 3 g/l were adjusted.

### Washing with Different Enzyme

The fabric samples treated with different concentrations of neutral cellulases at the temperature of 55 °C for 60 min and pH=7, acidic cellulase at the temperature of 55 °C for 60 min in pH=5.5, mixture of acidic cellulases and laccases at the temperature of 60 °C for 60 min in pH=5, and mixture of neutral cellulases and laccases at the temperature of 60 °C for 60 min in pH=7. The conditions of enzymatic treatments on each sample are reported in Table 1.

### Testing Methods

Each denim sample was cut to size 0.5×0.5 cm<sup>2</sup> and coated with gold by a Sputter Coater device from BAL-TEC Co., Switzerland. Microscopic pictures of the samples were produced with double zoom (25,500 μ) by an electron microscope device from Phillips Holland.

Colorimetric properties of samples were obtained by Datacolor with angle 10° and lamp D<sub>65</sub> (standard light). Each denim sample is composed of three parts (face, backs, and white pocket). The average values  $L^*$  (lightness),  $a^*$  (redness–greenness),  $b^*$  (yellowness–blueness), and  $\Delta E$  (color difference with desized sample) were reported. In addition, the whiteness index ( $W$ ) for white pocket was also reported.

The abrasion test was carried out by Martindale from Shirley with 10,000 rounds. The weight of samples was calculated before and after abrasion.

The weight of desized samples was measured before and after washing, and percentage of weight loss was calculated according to Eq. 1.

$$g = \frac{W_2 - W_1}{W_1} \times 100 \quad (1)$$

Where,  $g$ =percent of weight loss,  $W_1$ =fabric weight before treatment,  $W_2$ =fabric weight after treatment.

**Table 1** Different enzyme treatments on denim.

| Enzyme treatment                              | Enzyme concentration (%)<br>(O. W. G.) | Sample code | Temperature<br>(°C) | Time<br>(min) | pH  |
|---|--|-------------|---------------------|---------------|-----|
| Neutral cellulases                            | 3% weight of garment                   | 3NC         | 55 °C               | 60            | 7   |
|   | 6% weight of garment                   | 6NC         | 55 °C               | 60            | 7   |
|   | 9% weight of garment                   | 9NC         | 55 °C               | 60            | 7   |
|   | 12% weight of garment                  | 12NC        | 55 °C               | 60            | 7   |
| Acid cellulases                               | 1.5% weight of garment                 | 1.5 AC      | 55 °C               | 60            | 5.5 |
|   | 3% weight of garment                   | 3 AC        | 55 °C               | 60            | 5.5 |
|   | 6% weight of garment                   | 6 AC        | 55 °C               | 60            | 5.5 |
|   | 9% weight of garment                   | 9 AC        | 55 °C               | 60            | 5.5 |
|   | 12% weight of garment                  | 12 AC       | 55 °C               | 60            | 5.5 |
| Mixture of acid cellulases<br>and laccases    | 3%, 6% weight of garment               | 3Ac6L       | 60 °C               | 60            | 5   |
|   | 6%, 6% weight of garment               | 6Ac6L       | 60 °C               | 60            | 5   |
|   | 6%, 9%weight of garment                | 6Ac9L       | 60 °C               | 60            | 5   |
|   | 6%, 3% weight of garment               | 6Ac3L       | 60 °C               | 60            | 5   |
|   | 9%, 3% weight of garment               | 9Ac3L       | 60 °C               | 60            | 5   |
| Mixture of neutral cellulases<br>and laccases | 9%, 3% weight of garment               | 9Nc3L       | 60 °C               | 60            | 7   |
|   | 9%, 1% weight of garment               | 9Nc1L       | 60 °C               | 60            | 7   |
| Laccases                                      | 3% weight of garment                   | 3L          | 65 °C               | 60            | 4.5 |
|   | 6% weight of garment                   | 6L          | 65 °C               | 60            | 4.5 |
|   | 9% weight of garment                   | 9L          | 65 °C               | 60            | 4.5 |
|   | 12% weight of garment                  | 12L         | 65 °C               | 60            | 4.5 |

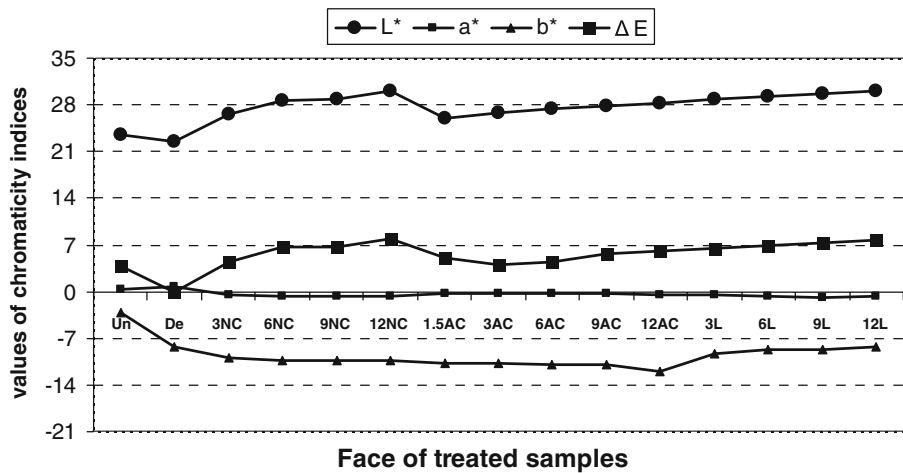
Each denim sample was cut to  $2.5 \times 5 \text{ cm}^2$ , and the crease recovery angle was measured according to ASTM standard. For each sample, these experiments were repeated three times, and the average was reported.

The strength of the samples was measured tensile tester (SDL ATLAS). These tests were repeated three times in warp direction, and the mean value was reported.

## Results and Discussion

### Effects of Cellulases and Laccases on Color Changes and Whiteness

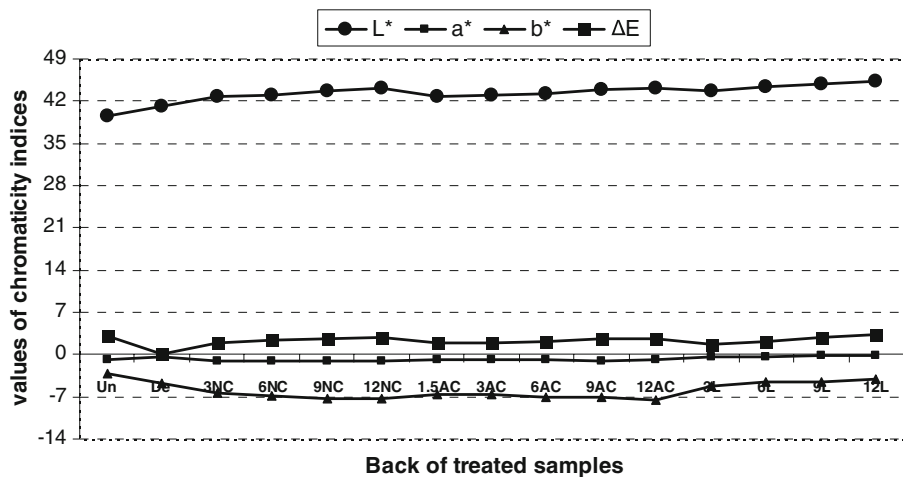
The results of chromaticity indices, color changes, and whiteness for treated samples with cellulases and laccases are illustrated in Figs. 3, 4, and 5. The results showed that  $L^*$  values of treated samples with laccases are higher than treated samples with cellulases. However, the  $L^*$  values of neutral cellulases are higher than acid cellulases.  $L^*$  values also showed that samples which were treated with laccases are lighter. The lightness will be increased by an increase of laccases concentration. The treated samples with laccases are greener than treated samples with cellulases. In other words, the treated samples with cellulases are redder than treated samples with laccases. Enzymatic treatment with laccases causes color changes from red to green as  $a^*$  decreases. The  $b^*$  values decrease according to laccases treatment of denim garment. This can be due to decrease of staining on denim garment. The blueness of treated samples decreases with the increase of laccases. Therefore, laccases



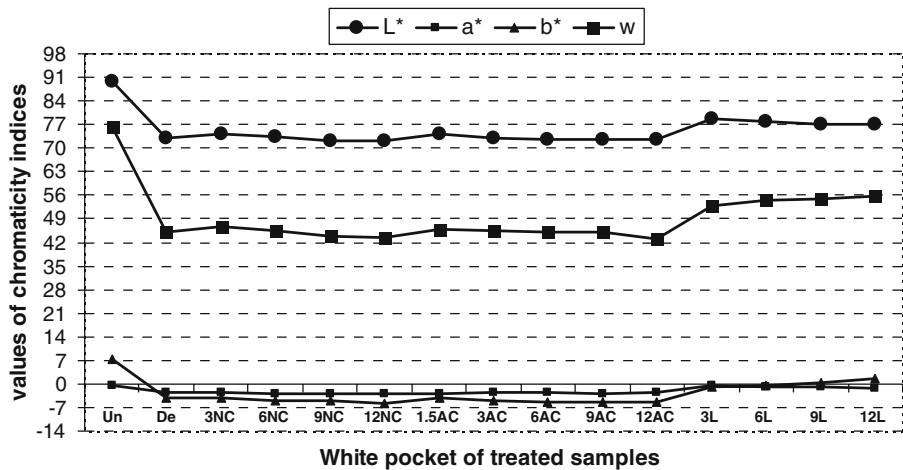
**Fig. 3** The colorimetric properties of cellulases and laccases treated samples face, *Un* untreated sample, *De* desized sample, *3NC* 3% neutral cellulases, *9NC* 9% neutral cellulases, *12NC* 12% neutral cellulases, *1.5AC* 1.5% acid cellulases, *3AC* 3% acid cellulases, *6AC* 6% acid cellulases, *12AC* 12% acid cellulases, *3L* 3% laccases, *6L* 6% laccases, *9L* 9% laccases, *12L* 12% laccases

causes color changes from blue to yellow and decreases staining on the garment. From the results of  $\Delta E$ , it can be considered that samples treated with laccases are recognized with more changes of color in comparing with the desized sample. Therefore, laccases can destroy the vat dye on the fabric surface but produce a yellowish color on the fabric surface.

As it can be seen in Fig. 4, the  $b^*$  values for back of samples show that increase of laccases can decrease backstaining. There is lower backstaining in laccases-treated samples than cellulases-treated samples. Treated samples with laccases have also a lower



**Fig. 4** The colorimetric properties of cellulases and laccases with treated samples back, *Un* untreated sample, *De* desized sample, *3NC* 3% neutral cellulases, *9NC* 9% neutral cellulases, *12NC* 12% neutral cellulases, *1.5AC* 1.5% acid cellulases, *3AC* 3% acid cellulases, *6AC* 6% acid cellulases, *12AC* 12% acid cellulases, *3L* 3% laccases, *6L* 6% laccases, *9L* 9% laccases, *12L* 12% laccases



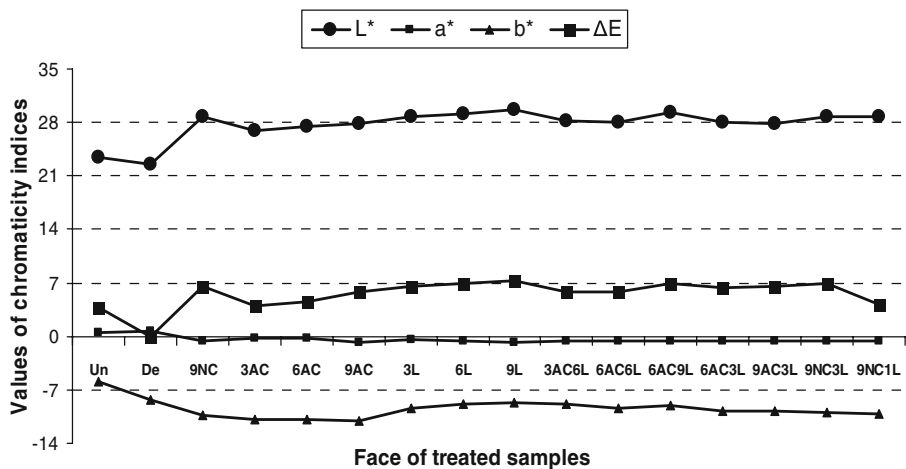
**Fig. 5** The colorimetric properties of cellulases and laccases treated with samples white pocket, *Un* untreated sample, *De* desized sample, *3NC* 3% neutral cellulases, *9NC* 9% neutral cellulases, *12NC* 12% neutral cellulases, *1.5AC* 1.5% acid cellulases, *3AC* 3% acid cellulases, *6AC* 6% acid cellulases, *12AC* 12% acid cellulases, *3L* 3% laccases, *6L* 6% laccases, *9L* 9% laccases, *12L* 12% laccases

backstaining than desized sample. The  $L^*$  values for back of samples will be increased with the increase of laccases and also are higher than cellulases-treated samples.

By increasing laccases in the washing process, the values of  $b^*$  become positive for the white pocket. This means that the garment color becomes yellow. Whereas, redeposited indigo on white pocket oxidized and the color changes toward yellow or white. Laccases is more effective than cellulases in reduction of staining on white pocket and even it can oxidize the indigo due to staining on white pocket completely. With high concentration of laccases, the changes of white pocket color happen from white to yellow. Whiteness factor shows that increase of laccases improves the whiteness of white pocket and also produces a whiter pocket than desized samples and treated samples with cellulases. The  $L^*$  values for pocket material proposes that the lightness of treated white pocket with laccases is more than cellulases (Fig. 5).

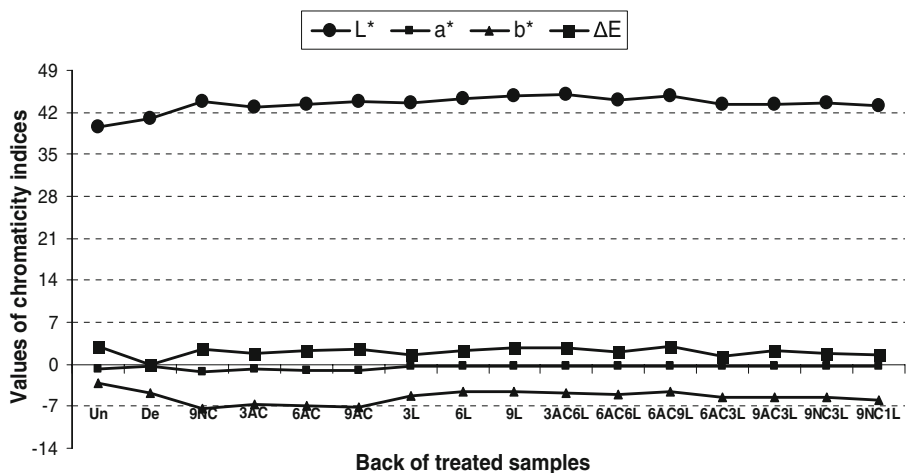
#### Effects of Co-application of Laccases with Cellulases on Color, Color Changes, and Whiteness

The results of chromaticity indices, color changes, and whiteness for treated samples with mixture of cellulases and laccases are illustrated in Figs. 6, 7, and 8. It is recognized that the treated samples with mixture of cellulases and laccases have the higher  $L^*$  than treated samples with only cellulases (Fig. 6). On the other hand, these samples have lower  $L^*$  than treated samples with only laccases. This illustrates that treated samples with laccases have the highest lightness, and the treated samples with a mixture of cellulases and laccases come afterwards in term of lightness. The lightness of samples increases by increasing laccases in mixture of enzymes. This can be known as a result of decreasing of staining on the samples, but increase of cellulases reduces the lightness. This is in accordance with the results of laccases alone as laccases destroy the blue vat dye.

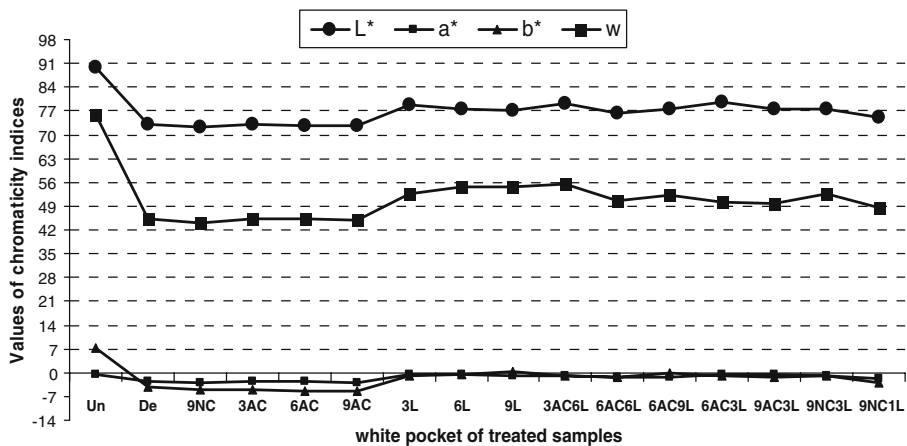


**Fig. 6** The colorimetric properties of mixture of cellulases and laccases treated with samples face, *Un* untreated sample, *De* desized sample, *3NC* 3% neutral cellulases, *9NC* 9% neutral cellulases, *12NC* 12% neutral cellulases, *1.5AC* 1.5% acid cellulases, *3AC* 3% acid cellulases, *6AC* 6% acid cellulases, *12AC* 12% acid cellulases, *3L* 3% laccases, *6L* 6% laccases, *9L* 9% laccases, *12L* 12% laccases

The treated samples with the mixture of cellulases and laccases are greener than treated samples with cellulases alone. With the increase of cellulases, the greenness decreases, but increase of laccases gains the greenness. This is due to the yellowish effect of laccases on the fabric. The value of  $b^*$  shows in Fig. 6 that treated samples with mixture of cellulases and laccases have a lower  $b^*$  than treated samples with cellulases alone and higher than treated samples with laccases alone. In other words, increase of enzymes reduces staining. However, this was opposite for the samples treated with higher amount of cellulases as the staining increases. The  $\Delta E$  value shows that any change in



**Fig. 7** The colorimetric properties of mixture of cellulases and laccases treated with samples back, *Un* untreated sample, *De* desized sample, *3NC* 3% neutral cellulases, *9NC* 9% neutral cellulases, *12NC* 12% neutral cellulases, *1.5AC* 1.5% acid cellulases, *3AC* 3% acid cellulases, *6AC* 6% acid cellulases, *12AC* 12% acid cellulases, *3L* 3% laccases, *6L* 6% laccases, *9L* 9% laccases, *12L* 12% laccases



**Fig. 8** The colorimetric properties of mixture of cellulases and laccases treated with samples white pocket, *Un* untreated sample, *De* desized sample, *3NC* 3% neutral cellulases, *9NC* 9% neutral cellulases, *12NC* 12% neutral cellulases, *1.5AC* 1.5% acid cellulases, *3AC* 3% acid cellulases, *6AC* 6% acid cellulases, *12AC* 12% acid cellulases, *3L* 3% laccases, *6L* 6% laccases, *9L* 9% laccases, *12L* 12% laccases

laccases and cellulases in mixture of enzyme have a remarkable change in sample color, and this color change has a potential fashion effect.

As Fig. 7 shows, the  $b^*$  values for samples back decreases with the increase of laccases in mixture of enzymes. This decreases the backstaining due to decomposition of blue vat dye by laccases. This also can decrease backstaining. In addition, increase of cellulases leads to increase of backstaining. Overall, treated samples with mixture of laccases and neutral cellulases have a lower backstaining in comparing with the treated samples with mixture of acid cellulases and laccases. Treated samples with mixture of laccases and cellulases also have a lower backstaining than treated samples with cellulases and higher than treated samples with laccases. The  $L^*$  values for back of samples increase with the increase of laccases in mixture of enzymes and also higher in comparison with cellulases-treated samples. This is because of the removal of deposited indigo on the back of the samples during treatment with laccases.

According to the results in Fig. 8, it can be considered that increase of laccases in the mixture of enzymes lead to increase of whiteness and lightness of the white pocket. This shows that staining decreases. This is due to the influences of laccases to decompose the blue vat dye. However, increase of cellulases leads to decrease of whiteness and lightness of white pocket. This comes out because of extraction of higher amount of blue vat dye from the fabric surface. The value of pocket whiteness for treated samples with a mixture of enzymes is higher than treated samples with cellulases and lower than treated samples with laccases. This is because of the different mechanism of these two enzymes. The laccases decompose the blue vat dye, but the cellulases degrade the cellulose that helps to releases the blue vat dye from the fabric to the washing bath and can deposited on the white pocket. For the white pockets, the values of  $b^*$  will be decreased with the increase of laccases in the mixture of enzymes. This means that lower staining has occurred. In contrast, with the increase of cellulases, the  $b^*$  value of samples of pockets become greater, and the pockets are more stained. Generally, the white pocket of the treated samples with the mixture of laccases and cellulases are greener than the laccases-treated samples and redder than the cellulases-treated samples.

**Table 2** Tensile strength and elongation of samples.

| Samples   | Elongation (mm) | Tensile strength (N) |
|---|-----------------|----------------------|
| Desized sample  | 59.98±2         | 1364±10              |
| Treated sample with neutral cellulase(6Nc)                  | 62.37±2         | 1046±9               |
| Treated sample with laccase(3L)                             | 71.84±2         | 1236±10              |
| Treated sample with mixture of laccase and cellulase(9Nc2L) | 70.517±2        | 1267±12              |

### Tensile Strength

The mean value of tensile strength and elongation for selected samples is illustrated in Table 2. The results revealed that a desized sample has a higher tensile strength with lower elongation. The lowest tensile strength is related to the treated sample with neutral cellulases. Laccases action was similar to cellulases and causes a decrease in tensile strength. However, the reduction of tensile strength is lower for laccases-treated samples. The tensile strength of treated samples with laccases noticeably decreases compared with desized samples. When samples are treated with a mixture of cellulases and laccases, the tensile strength will be reduced, but it is marginally higher than the sample treated with laccases alone. However, it is still much higher than the sample treated with neutral cellulases. This can be attributed to the lower effect of laccases when mixed with cellulases. The effectiveness of laccases on tensile strength reduction can be attributed to the acidic media (pH=4.5 at temperature 65 °C) and/or cellulose fiber oxidation.

### Crease Recovery Angle

The values of crease recovery angle of selected samples are illustrated in Table 3. The results characterized that the desized sample has the highest crease recovery angle. In other words, the desized sample is more wrinkle-resistant. Treatment of samples with cellulases or laccases reduces the fabric crease recovery angle. The differences between the value of crease recovery angle for treated samples with cellulases and mixture of laccases and cellulases are not significant. The crease recovery angle of treated sample with laccases alone is higher than other enzyme-treated samples but is lower than the desized sample. This is due to the action of laccases which decomposes the indigo dye of the fabric and has no influences on the cellulose. Finally, it can be also considered that enzymatic treatment reduces the wrinkle resistance as a result of acidic condition of laccases treatment and/or hydrolysis of cellulose by cellulases.

**Table 3** Crease recovery angle of different samples.

| Sample   | Crease recovery angle (°) |
|--|---------------------------|
| Desized sample   | 167±4                     |
| Treated sample with neutral cellulase (6Nc)                          | 148±3                     |
| Treated sample with acid cellulase (6Ac)                             | 151±4                     |
| Treated sample with laccase (3L)                                     | 160±5                     |
| Treated sample with mixture of laccase and acid cellulase (9Ac3L)    | 149±5                     |
| Treated sample with mixture of laccase and neutral cellulase (9Nc2L) | 151±4                     |

## Abrasion Resistance

The weights of selected samples are illustrated in Table 4 before and after abrasion test. The results revealed that the variation of weight for different samples is not significant. Therefore, abrasion of 10,000 cycles did not indicate a significant difference between samples. It can be considered that the abrasion resistance of samples is high. The highest weight loss is related to desized sample. It seems that the desized sample contains huge amounts of anchor fibers on surface that may subjected to abrasion and may be removed from the fabric surface. The lowest weight loss was related to the treated sample with neutral cellulases. This means that the neutral cellulases reduce the anchor fiber on the fabric surface, and consequently, the abrasion has a lower impact on the abrasion resistance of the fabric. For the treated sample with laccases, it can be observed that laccases treatment has a little impact on the abrasion resistance even at high concentration. This can be attributed to the mechanism action of laccases that has no action on the cellulose, and then the fabric surface remains unchanged.

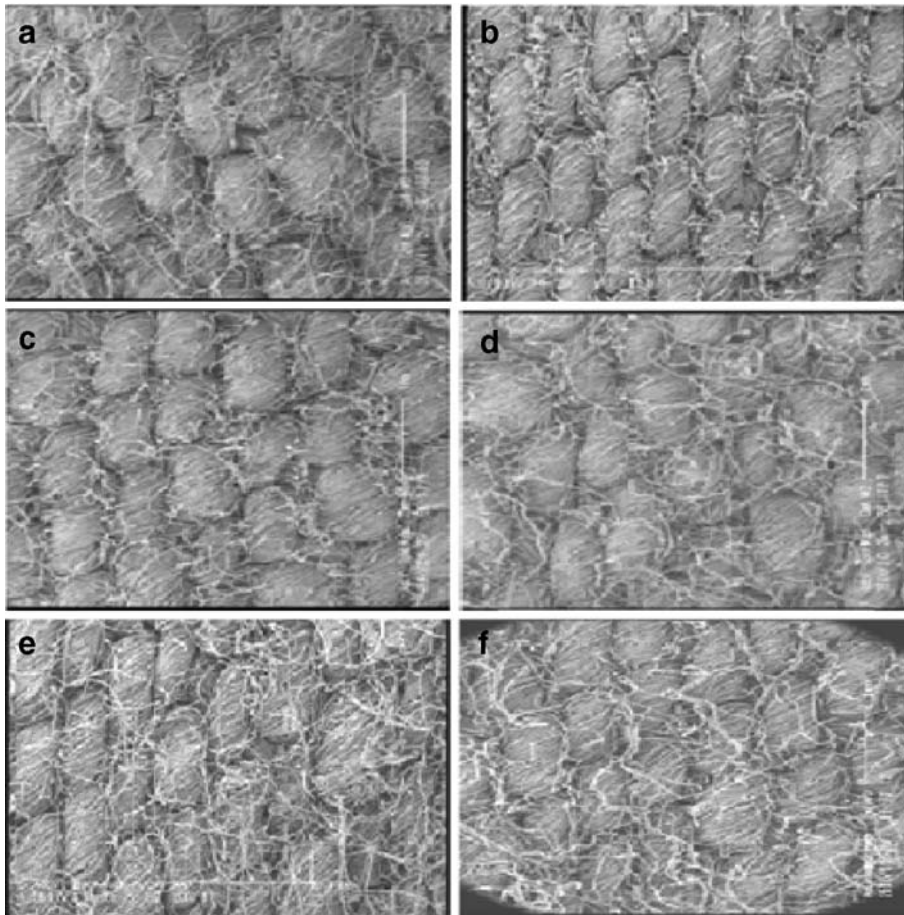
## SEM Observations

Microscopic pictures of sample surfaces are shown in Fig. 9. Figure 9a–c shows that the surfaces of desized sample are covered by the anchor fibers that are likely to be removed by the acid or neutral cellulases treatment. The picture of treated sample with laccases (Fig. 9d) shows that laccases is not able to remove the anchor fibers and only causes a change in color. Based on the fiber surfaces on the fabric samples, it can be suggested that the surfaces of the laccases-treated samples have similar surface as the desized samples, and the entanglement of fibers is more for those laccases-treated samples. It can be seen from Fig. 9e, f that between those treated samples with laccases and cellulases, the treated samples with lower concentration of cellulases have higher anchor fibers on the surface.

It can be observed from Fig. 10a that the fibers on the surface of desized sample have not been changed. This means that the fabric and also fibers of desized sample have not been altered as a result of desizing process. It can also be seen in Fig. 10b, c that the surfaces of outer fibers for the cellulases-treated samples have been damaged, and some fibers have been removed from the fabric surface. However, the inner fibers have been remained unchanged without any damage. In other words, treatment of the samples with cellulases can be only affected on the surface of outer fibers and has no influences on the

**Table 4** Percentage of weight loss for selected samples before and after abrasion.

| Samples  | Weight before abrasion (g) | Weight after abrasion (g) | Weight loss (%) |
|--|----------------------------|---------------------------|-----------------|
| Desized sample   | 0.4978                     | 0.4881                    | 1.94            |
| Treated sample with neutral cellulases (6Nc)                           | 0.4977                     | 0.4968                    | 0.18            |
| Treated sample with acid cellulases (6Ac)                              | 0.4951                     | 0.4923                    | 0.55            |
| Treated sample with laccases (12L)                                     | 0.5305                     | 0.5239                    | 1.24            |
| Treated sample with mixture of laccases and acid cellulases (6Ac9L)    | 0.5359                     | 0.5257                    | 1.82            |
| Treated sample with mixture of laccases and neutral cellulases (9Nc2L) | 0.4945                     | 0.4888                    | 1.15            |



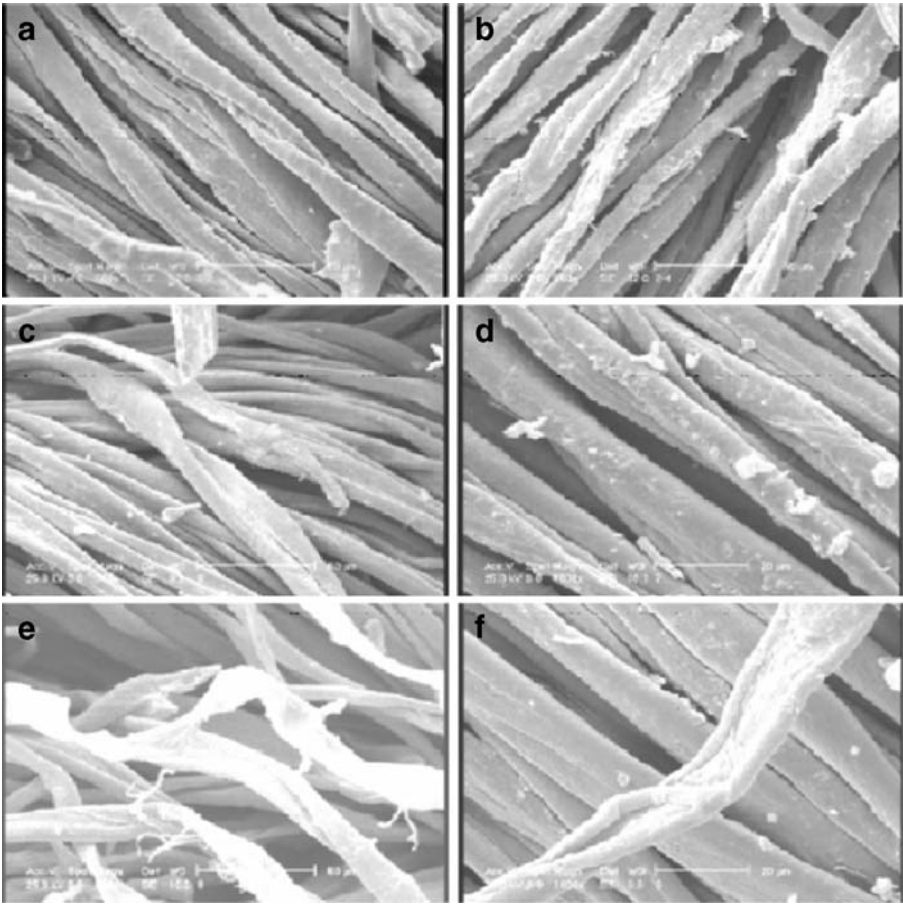
**Fig. 9** SEM pictures of different treated samples (25 $\times$ ): **a** desized, **b** neutral cellulases, **c** acid cellulases, **d** laccases, **e** mixture of laccases and neutral cellulases, **f** mixture of laccases and acid cellulases

inner fibers. This means that the cellulases have no chance to enter to the yarn construction during the enzymatic processing time to hydrolyze the inner fibers.

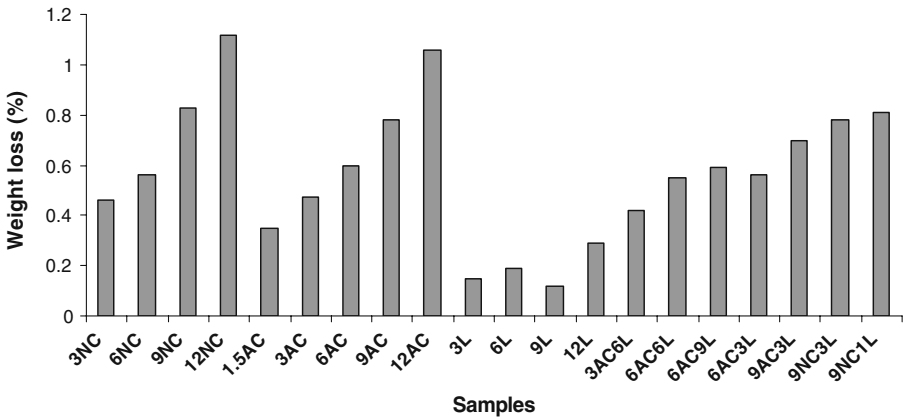
It can be interfered from Fig. 10d that the fiber surfaces of the treated sample with laccases has not been changed, and the fabric surface is similar to the fabric surfaces of the desized sample (Fig. 10a). The fiber surfaces of the treated sample with the mixture of laccases and cellulases (Fig. 10e, f) have less damage than those treated samples with the cellulases. This can be considered due to the reducing of cellulases activity when mixed with laccases. Overall, it can be considered that laccases with cellulases can reduce the fiber surface damage, and laccases alone has no significant impact on the cellulose which may leave the cellulose fiber surface unchanged.

#### Weight Loss Percentage during Washing

The percentage of weight loss for different samples is calculated according to formula 1 and reported in Fig. 11. The results showed that the weight loss of treated sample with laccases



**Fig. 10** SEM pictures of different treated sample (500×) **a** desized, **b** neutral cellulases, **c** acid cellulases, **d** laccases, **e** mixture of laccases and neutral cellulases, **f** mixture of laccases and acid cellulases



**Fig. 11** Weight-loss percentages for different treated samples

is less than the treated sample with cellulases. Therefore, cellulases can remove surface fiber and pills from the cellulosic fabric, in which may occur the highest weight loss among different samples. The samples treated with laccases indicated a little influence on surface of cellulosic fabric. For the treated samples with a mixture of cellulases and laccases, the weight loss increases with the increase of cellulases and also increases with the increase of laccases. However, the rate of weight-loss increase is low for laccases-treated samples. The values of weight loss for treated samples with the mixture of laccases and cellulases are less than treated sample with cellulases and more than those treated sample with laccases.

## Conclusion

Back of denim and white pocket staining are two important deficiencies which may occur during stonewashing and known as disadvantage for denim washing with cellulases. Discoloring of laccases was a remarkable concept in denim washing. Increasing neutral or acid cellulases concentration in the washing processes of denim garment makes a lighter denim color. However, this is related to the increase of staining on back and white pocket. In contrast, treated samples with laccases show lighter denim which becomes lighter by increasing laccases with lower backstaining. Increasing laccases in mixture with cellulases improves the lightness and decreases staining of both back and white pocket. In addition, by using over 9% of laccases, whiter pocket may be produced.

The lightness ( $L^*$ ) of treated samples has the following sequence:

Laccases > mixture of cellulases and laccases > cellulases

Staining reduction on back of denim and white pocket can be arranged as follows:

Laccases > mixture of cellulases and laccases > cellulases

The tensile strength of treated samples with neutral cellulases is less than desized sample and also treated sample with laccases. The tensile strength of treated sample with laccases is also less than desized sample, but the reduction in tensile strength is less than treated sample with cellulases.

The SEM pictures show that fibers of treated sample with cellulases have been damaged significantly, but fibers of treated sample with laccases were not affected. In other words, damages on the fibers are limited to the outer fibers, and inner fibers remained unaffected.

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