A comparative Study of Different Stone and Bio-stone Washing of Denim

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Abstract

Denim washing is known as one of the finishing treatment that has vast usage because of creating special appearance and updating clothes. Washing jean clothes are being developed and technology of denim washing is the main part of clothes Industry. In current study, comparison of different washing methods is considered. Different methods of denim washing including the use of pumice stone, neutral and acid cellulases, and also combination of pumice stone and cellulases. The change of color of resulting samples are compared by the reflecting colorimeter of inside garment, outside garment and pocket material after doing experiments. Tensile of samples is measured. XRD spectrum and crystalline degree also monitored. Furthermore, the surfaces of fibers in treated samples have been observed by SEM images. The result of experiments shows that neutral cellulases produced a fabric with higher lightness and increasing of enzyme adding to back staining. Denim treatment with 100% o.w.f pumice stone alone wasn't effective. However, combination of 100% pumice stone with cellulases showed a good washing effect.

Keywords: Denim, Stone washing, Back- staining, Lightness, Cellulases, Pumice stone

1. Introduction:

Denim produced from fabric with twill weave including the indigo dyed warp and white weft yarns [1]. Indigo dyed denim fabric usually stone washed for aged-look. The washing was done along with abrasion produced by pumice stones in the washing machine with or without oxidants (usually potassium permanganate). This operation will cause decolorization with a decrease in strength of cloth within allowed limitation. Stone washing effects can also be obtained by using cellulases and/or pumice stone. Surface of fabric play an important role in enzymatic decolorization of cellulosic fiber. This influences the outset layers of cellulosic crystalline and then the available part of cellulosic fiber increase and allows removal of dyes [2].

Traditionally, denim jeans manufacturers have washed their garments with pumice stones to achieve a soft handle as well as a desirable worn look, nowadays; the aged look is obtained by non-homogeneous removal of the indigo dye trapped inside the fibers by the cooperative action of the enzymes and mechanical factors such as beating and friction. Natural pumice stone is widely used in denim garment washing process has disadvantages, the difficulty of removing residual pumice from processed clothing items and the damage to the equipment by the overload of tumbling stones and the pumice stones and particulate material can also clog machine drainage passages and drains and sewer lines at the machine site. Denim washing with cellulases is thus a standard technique, providing an environmentally friendly process to achieve a desirable appearance and soft handle for fabrics. During the enzymatic treatment, the removed indigo dye can be redposited on the white yarn of denim fabric. This process is called back staining, and it can diminish the look of denim garment. An ideal bio-stoning enzyme would posse's high abrasive activity as well as low back staining. Since cellulases strongly adsorbed to their substrates, this phenomenon can be explained by dye binding to the bound enzyme protein [2, 3].

It is considering that in denim stonewashing sewed clothes are treated; therefore, staining on back of garment and white pocket is a basic problem. One of the major factors causing high indigo back staining on denim fabrics (re-deposition 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 re-deposition should involve binding of dye to the enzyme molecules adsorbed on the surface of cellulose fibers. This kind of mechanism implies that the enzyme molecules must have surface sites capable to bind indigo [4, 9].

The structure and properties of indigo molecule may cause the interaction mechanism also be existed with dyes and other molecules. The structure of indigo molecule and its properties indicate that there may be two basic mechanisms of interaction between the dye and other molecules. One, the indigo molecule contains aromatic rings that can be involved in hydrophobic interactions. Two, the heterocyclic –NH- and =O groups may form hydrogen bonds with other molecules [5].

Ideal cellulases for application on denim (endoglucanase) are the ones with sites on the surface of protein globule capable to bind indigo with low adsorption ability on cellulose [6].

Back staining dependant on pH value and type of enzyme, according to recent researches; acid cellulases stain denim in acidic medium. The staining will reduce with increasing suitable auxiliary. Neutral cellulases stain in pH=7 more than pH=5 and its effect is recognizable in pH=7. Overall, neutral cellulases are more important than acid cellulases due to lack of staining [6, 7, 15].

Cellulolytic enzymes are currently applied in textile processes where mechanical action is always present such as in jets or rotating drum washers. Cellulases offer an excellent alternative for stonewashing of blue denim garments and compare very well with commercial cellulases. The enzymes used to save time eliminate some disadvantages of stonewashing process such as damage to washers, and environment pollution. Furthermore, there is no handling of the bulky pumice when the washers are loaded and unloaded, and no removal of stones from the finished garments. Pumice stone and sand do not contaminate the floors and sewers [8].

Different articles about denim stone washing have been presented but a complete report about different amounts of pumice stone and cellulases and mixture of them has not been seen so far. Purpose of this research is to compare methods of stone washing in aqueous on denim garment and study the different characters of them. Thus, denim samples were treated with the different amounts pumice stone, neutral cellulases, acid cellulases and the mixture of them. In addition, the appearance and crystalline properties were studied.

2. Materials and Methods:

2.1 Materials:

Denim fabric (Blue jean) used, was 100% cotton with twill 2/1 weave construction and weft and warp no. of 15 Nm with z twist, weft density of 20/cm, warp density of 26/cm and fabric weight of 265 g/m^2. In order to preparing samples, the fabric with 30×50 cm^2 were selected and sewed as leg form with two pockets, one on 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 number of 30 Nm open-end spinning, and weft density of 24/cm, warp density of 30/cm and weight of 106 g/m^2 (Fig.1).

Fig.1. Picture of prepared sample showing two pocket one the face and other (white pocket) on the back.

Auxiliaries used, were: industrial acetic acid 70%, dispersing agent (Verlane N60) for preventing back-staining 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 (Roglyr ultra 97655) from Rotta. Co. which is soluble at 40-50°C and has maximum activity at 55°Cand pH=7, acid cellulases (Rucolase JEX) from Rudolf Chemie. Co, soluble at 40-45°C and has maximum activity at 55°C and pH=5.5. A rotary drum washer with 5 kg capacity (steel, r.p.m=25) was used for desizing, stone washing and washing.

2.2 Methods

2.2.1 Desizing:

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

2.2.2 Stone washing with neutral cellulases:

Four desized samples were treated for 60 min at 55ºC with pH=7 and different concentration of neutral cellulases including 3Nc (3% weight of garment), 6Nc (6% weight of garment), 9Nc (9% weight of garment) and 12Nc (12% weight of garment).

2.2.3 Stone washing with acid cellulases:

Five of desized samples were treated for 60 min at 55°C and pH=5.5 with different concentration of acid cellulases including 3Ac (3% weight of garment), 6Ac (6% weight of garment), 9Ac (9% weight of garment) and, 12Ac (12% weight of garment).

2.2.4 Stone washing with neutral cellulases along pumice stone:

Four desized samples were treated for 60 min at 55ºC with pH=7 and pumice stone (100% than weight of garment) along different concentration of neutral cellulases including 3NcPu (3% weight of garment), 6NcPu (6% weight of garment), 9NcPu (9% weight of garment) and 12NcPu (12% weight of garment).

2.2.5 Stone washing with acid cellulases along pumice stone:

Fore desized samples were treated for 60 min at 55ºC with pH=5.5 and pumice stone (100% than weight of garment) along different concentration of acid cellulases including 3AcPu (3% weight of garment), 6AcPu (6% weight of garment), 9AcPu (9% weight of garment) and 12AcPu (12% weight of garment).

2.3 Testing methods:

Each denim swatch was cut to size 0.5×0.5 cm^2 and coated with gold by Sputter Coater device from BAL-TEC.CO Switzerland. Microscopic pictures of samples were produced with double zoom (25,500µ) by electron microscope device from Phillips Holland.

Four selected samples were exposed to X-ray by XRD device from Siemens. Co with lamp Cu k_a. The degree of crystalline was calculated with use of equation1.

$$X = \frac{I_C}{I_C + I_A} \times 100 \qquad (1)$$

In this equation, X is degree of crystalline, I_A is area of amorphous and I_C is area of crystalline.

Colorimetric properties of samples were obtained by Datacolor with angle 10° and lamp D_{65} (standard light). Each denim swatch is composed from three parts (face, backs and white pocket). The average values L^*(lightness), a^*(redness-greenness), b^*(yellowness-blueness) and ΔE (color difference with desized sample) were reported. In addition, the whiteness index (W) for white pocket was also reported.

The strength of samples was measured with Instron from Shirley (Micro 250). These testes were repeated three times in warp direction and the average was reported.

3. Results and discussion

3.1 Color changes:

3.1.1 Study on effects of cellulases and pumice stone on index color and whiteness

The results of chromaticity indices color and whiteness illustrated for treated samples with neutral and acid cellulases and pumice stone in table1, 2. The L^* values of treated samples shown that with increasing of pumice stone, because of more abrasion increase the lightness. The results showed that L^* values of treated samples with cellulases are higher than treated samples with pumice stone, untreated sample and desized sample. This more lightness is because of effective hydrolyzing. Values of L^* of samples treated with cellulases showed that with increasing of cellulases concentration increase the lightness. Treated samples with cellulases along pumice stone are more lightness than treated samples with cellulases alone and treated sample with pumice stone alone. Thus, the lightness of sample increase with adds of enzyme concentration and pumice stone. Also with increasing of lightness, the redness of sample decrease. With increasing of abrasion, treated samples are bluer. Consequently, the effect of cellulases along pumice stone on denim sample is more than other treatment.

As from table1, 2 the b^* values of back samples are recognized treated samples back with pumice stone are more blue than untreated sample and desized sample. It is because of the removal of indigo dyes from garment surface and redposited it on sample back. With increasing of amount of pumice stone, back staining increases. Treated back samples with cellulases bluer than treated sample with pumice stone. Thus cellulases have high back staining on denim fabric due to high hydrolyze of enzyme and removal of indigo from denim surface. With add of cellulases concentration increase back staining and treated samples with cellulases along pumice stone have more back staining than treated sample with cellulases alone and treated sample with pumice stone alone. Thus pumice stone along cellulases remove indigo dyes effectively than other treatment.

The b^* values of samples pocket material are recognized that with increasing amount of pumice stone or cellulases concentration increase staining on white pocket and pumice stone along cellulases have more staining than other treated.

Tabale1. The colorimetric properties of neutral cellulases and pumice stone treated samples.

Samples	L^*	a^*	b^*	W
Untreated	**23.5**	**0.42**	**-4.93**	**-**
Desized	**22.51**	**0.77**	**-8.38**	**-**
1Pu	**24.74**	**0.04**	**-10.02**	**-**
2Pu	**25.71**	**-0.07**	**-10.39**	**-**
3Pu	**26.82**	**-0.34**	**-10.78**	**-**
4Pu	**26.83**	**-0.66**	**-11.41**	**-**
3NC	**26.63**	**-0.54**	**-9.97**	**-**
6NC	**28.74**	**-0.59**	**-10.24**	**-**
9NC	**28.77**	**-0.65**	**-10.27**	**-**
12NC	**30.10**	**-0.67**	**-10.31**	**-**
3NCPu	**29.50**	**-0.88**	**-9.99**	**-**
6NCPu	**30.64**	**-1.03**	**-10.33**	**-**
9NCPu	**30.70**	**-1.14**	**-10.34**	**-**
12NCPu	**31.93**	**-1.17**	**-10.50**	**-**
Untreated BS	**39.48**	**-0.81**	**-3.25**	**-**
Desized BS	**41.07**	**-0.38**	**-4.86**	**-**
1Pu BS	**41.35**	**-0.63**	**-5.75**	**-**
2Pu BS	**42.72**	**-0.73**	**-6.02**	**-**
3Pu BS	**43.96**	**-0.86**	**-6.24**	**-**
4Pu BS	**44.30**	**-1.25**	**-7.21**	**-**
3NC BS	**42.83**	**-0.98**	**-6.47**	**-**
6NC BS	**43.08**	**-1.06**	**-6.85**	**-**
9NC BS	**43.71**	**-1.18**	**-7.28**	**-**
12NC BS	**44.05**	**-1.19**	**-7.31**	**-**
3NCPu BS	**43.55**	**-1.25**	**-6.90**	**-**
6NCPu BS	**45.16**	**-1.32**	**-6.96**	**-**
9NCPu BS	**46.66**	**-1.34**	**-7.47**	**-**
12NCPu BS	**47.40**	**-1.27**	**-7.56**	**-**
Untreated PS	**89.88**	**-0.55**	**7.34**	**76.1**
Desized PS	**73.02**	**-2.50**	**-4.31**	**45.2**
1Pu PS	**75.41**	**-2.51**	**-3.05**	**48.9**
2Pu PS	**75.13**	**-2.58**	**-3.12**	**48.5**
3Pu PS	**74.55**	**-2.83**	**-3.91**	**47.6**
4Pu PS	**73.15**	**-3.58**	**-5.54**	**45.4**
3NC PS	**74.04**	**-2.63**	**-3.97**	**46.8**
6NC PS	**73.30**	**-2.90**	**-5.00**	**45.6**
9NC PS	**72.20**	**-2.92**	**-5.05**	**44.0**
12NC PS	**72.13**	**-2.99**	**-5.89**	**43.3**
3NCPu PS	**72.90**	**-2.21**	**-3.14**	**45.3**
6NCPu PS	**71.98**	**-2.96**	**-4.18**	**44.2**
9NCPu PS	**67.18**	**-3.03**	**-5.85**	**43.9**
12NCPu PS	**66.57**	**-2.87**	**-6.29**	**42.0**

The whiteness (w) of samples pocket material is shown that untreated sample pocket is whiter than desized sample pocket. Treated sample pocket with cellulases or pumice stone is less white than untreated sample pocket and whiter than desized sample due to removal of indigo (redposited during desizing) during stone washing treatment from pocket material. With add of pumice stone or cellulases concentration decrease whiteness. Treated samples pocket with pumice stone along cellulases are less whiteness than other treatment and treated samples pocket with cellulases are less whiteness than treated sample with pumice stone alone.

3.1.2 The comparison of acid cellulases with neutral cellulases on index color and whiteness

The results of chromaticity indices color and whiteness illustrated for treated samples with neutral cellulases in comparison with acid cellulases in table3.

The results showed that L^* values of treated samples with neutral cellulases are higher than treated samples with acid cellulases, thus treated samples with neutral cellulases are lightness than treated sample with acid cellulases that are due to high activity of this enzyme (current neutral cellulases).

The b^* values of back samples are recognized that back staining of treated samples with neutral cellulases and acid cellulases not differ but back staining of treated sample with acid cellulases slightly is higher.

The whiteness (w) of samples pocket material is shown that treated samples pocket with acid cellulases is bluer than treated samples pocket with neutral cellulases. Thus staining of current acid cellulases is higher than neutral cellulases.

Tabale2. The colorimetric properties of acid cellulases treated samples.

Samples	L^*	a^*	b^*	W
Untreated	**23.5**	**0.42**	**-4.93**	**-**
Desized	**22.51**	**0.77**	**-8.38**	**-**
3AC	**26.88**	**-0.27**	**-10.82**	**-**
6AC	**27.39**	**-0.28**	**-10.93**	**-**
9AC	**27.84**	**-0.31**	**-11.02**	**-**
12AC	**28.20**	**-0.43**	**-11.97**	**-**
3ACPu	**28.36**	**-0.58**	**-10.97**	**-**
6ACPu	**28.53**	**-0.59**	**-11.46**	**-**
9ACPu	**28.92**	**-0.62**	**-11.98**	**-**
12ACPu	**29.55**	**-0.42**	**-12.34**	**-**
Untreated BS	**39.48**	**-0.81**	**-3.25**	**-**
Desized BS	**41.07**	**-0.38**	**-4.86**	**-**
3AC BS	**42.97**	**-0.75**	**-6.60**	**-**
6AC BS	**43.33**	**-0.96**	**-6.97**	**-**
9AC BS	**43.92**	**-1.09**	**-7.19**	**-**
12AC BS	**44.11**	**-0.89**	**-7.61**	**-**
3ACPu BS	**44.46**	**-0.83**	**-6.83**	**-**
6ACPu BS	**44.57**	**-0.94**	**-7.05**	**-**
9ACPu BS	**44.84**	**-1.02**	**-7.15**	**-**
12ACPu BS	**45.23**	**-0.97**	**-7.67**	**-**
Untreated PS	**89.88**	**-0.55**	**7.34**	**76.1**
Desized PS	**73.02**	**-2.50**	**-4.31**	**45.2**
3AC PS	**73.03**	**-2.49**	**-4.98**	**45.4**
6AC PS	**72.47**	**-2.60**	**-5.27**	**45.1**
9AC PS	**72.61**	**-2.79**	**-5.33**	**44.9**
12AC PS	**72.60**	**-2.50**	**-5.53**	**43.2**
3ACPu PS	**74.24**	**-2.29**	**-4.87**	**44.3**
6ACPu PS	**73.63**	**-2.49**	**-4.92**	**43.4**
9ACPu PS	**73.43**	**-2.64**	**-5.11**	**42.8**
12ACPu PS	**74.45**	**-2.59**	**-5.55**	**41.0**

Tabale3. The comparison of neutral and acid cellulases on colorimetric properties and whiteness.

samples	L^*	a^*	b^*	W
3NC	**26.63**	**-0.54**	**-9.97**	**-**
6NC	**28.74**	**-0.59**	**-10.24**	**-**
9NC	**28.77**	**-0.65**	**-10.27**	**-**
12NC	**30.10**	**-0.67**	**-10.31**	**-**
3AC	**26.88**	**-0.27**	**-10.82**	**-**
6AC	**27.39**	**-0.28**	**-10.93**	**-**
9AC	**27.84**	**-0.31**	**-11.02**	**-**
12AC	**28.20**	**-0.43**	**-11.97**	**-**
3NC BS	**42.83**	**-0.98**	**-6.47**	**-**
6NC BS	**43.08**	**-1.06**	**-6.85**	**-**
9NC BS	**43.71**	**-1.18**	**-7.28**	**-**
12NC BS	**44.05**	**-1.19**	**-7.31**	**-**
3AC BS	**42.97**	**-0.75**	**-6.60**	**-**
6AC BS	**43.33**	**-0.96**	**-6.97**	**-**
9AC BS	**43.92**	**-1.09**	**-7.19**	**-**
12AC BS	**44.11**	**-0.89**	**-7.61**	**-**
3NC PS	**74.04**	**-2.63**	**-3.97**	**46.8**
6NC PS	**73.30**	**-2.90**	**-5.00**	**45.6**
9NC PS	**72.20**	**-2.92**	**-5.05**	**44.0**
12NC PS	**72.13**	**-2.99**	**-5.89**	**43.3**
3AC PS	**73.03**	**-2.49**	**-4.98**	**45.4**
6AC PS	**72.47**	**-2.60**	**-5.27**	**45.1**
9AC PS	**72.61**	**-2.79**	**-5.33**	**44.9**
12AC PS	**72.60**	**-2.50**	**-5.53**	**43.2**

3.2 Crystallinity:

Desized, neutral cellulases were selected to measure degree of crystallinity. The XRD spectrums of different samples are illustrated in Fig.2, 3. It is specified that desized sample (Fig.2) have a different diffraction pattern in comparison with the treated sample with cellulases (Fig.3).

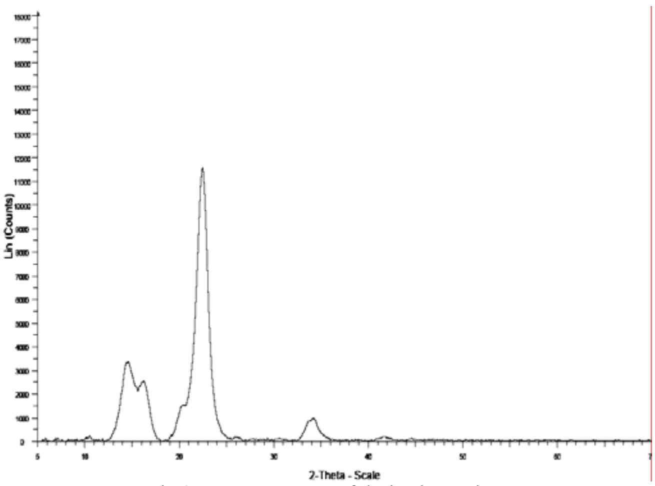


Fig.2. XRD spectrum of desized sample.

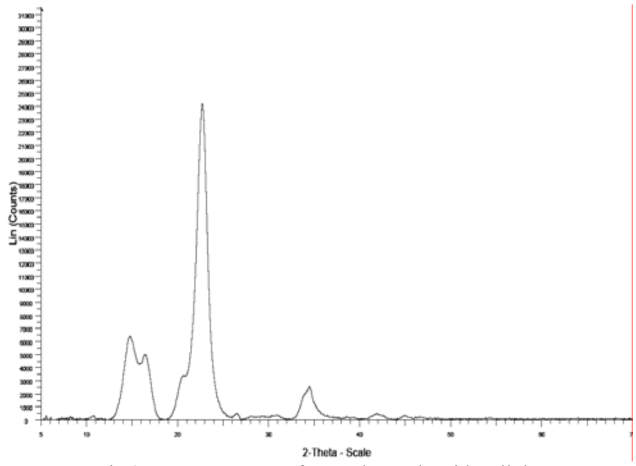


Fig.3. XRD spectrum of treated sample with cellulases

The percentage of crystallinity for different samples calculated and reported in Table 4.

Table 4. Crystallinity percentages of different samples

samples	Crystallinity (%)
Desized sample	**65**
Treated sample with neutral cellulases(6Nc)	**69**

As a result of cellulases treatment on the cellulose the percentage of crystallinity increases. This can be due to penetration of enzyme into amorphous region that may reduce the amorphous region.

3.3 Tensile:

The mean value of tensile and elongation for selected samples is illustrated in Table 5. The results revealed that desized sample has a higher tensile with lower elongation. The lowest tensile is related to the treated sample with neutral cellulases along pumice stone. The tensile of treated sample with pumice stone higher than treated sample with neutral cellulases and neutral cellulases along pumice stone thus pumice stone less damage to fabric in comparison with cellulases. Pumice stone along cellulases high damage to fabric. Treated sample with cellulases along pumice stone have a high elongation than other treatment.

Table 5. Tensile and elongation of samples

Samples	Elongation (mm)	Tensile (N)
Desized sample	59.98	1364
Treated sample with neutral cellulases (6Nc)	65.53	1046
Treated sample with pumice stone (3Pu)	62.37	1293
Treated sample with cellulases and pumice stone (6NcPu)	72.74	1015

3.4 SEM's pictures:

3.4.1 Study of fabric surface

Microscopic pictures of sample surfaces are shown in Fig. 4. Figures 4-a, 4-c and 4-d demonstrated that surfaces of desized sample covered by the anchor fiber which are likely removed by the acid or neutral cellulases treatment. The picture of treated sample with pumice stone (Fig4-c) showed that pumice stone is not able to remove the anchor fibers completely and just caused a change in color slightly. Therefore, from removal of surface fiber point of view, pumice stone treated samples are not many differences with desized sample and only more entanglement observed on the pumice stone treated samples. Pictures of treated sample with pumice stone along cellulases (Figures 4-e and 4-f) illustrated clearly that perfectly is able to remove the anchor fibers than cellulases treatment only. Neutral cellulases are able to remove the anchor fibers more effectively than acid cellulases.

3.4.2 Study of fiber surface

Microscopic pictures of surface samples are shown in Fig. 5. Surface of fibers can be are observed clearly in this figure. Picture of desized sample (Fig. 5-a) showed the fiber in the surface unchanged. This means that the fibers have not been damaged by desizing. It can be seen in pictures of treated samples with cellulases (Fig. 5-c and 5-d) that the surface of outer fibers was damaged and some fibers were broken. But inner fibers remain unchanged without damage. In other word, cellulases can only damage the surface of outer fibers and inner fibers has not been affected. This means that the inner fibers are not accessible for the cellulases.

It can be observed from Fig. 5-b that fiber surfaces for the treated sample with pumice stone was damaged but not like treated sample with cellulases. Fiber surfaces of treated sample with cellulases along pumice stone (Fig. 5-e and 5-f) are damaged higher than treated sample with cellulases alone. This is because of increasing of cellulases activity and pumice stone. Overall, it can be considered that neutral cellulases were damage higher than acid cellulases to surface of outer fibers.

4. Conclusion:

Considering to importance of stone washing finishing, it seems to have technical information in order to measuring of optimum conditions for stone washing process. Thus it is necessary to study of effect of different methods on physical and mechanical properties and appearance characterizes of denim garment. This current study, various methods of stone washing was applied and also the properties of treated denim garment was studied and reported.

The result of experimental are shown that with increasing of amount of pumice stone and cellulases concentration the lightness of denim garment increase. In comparison of applied different methods, increasing of lightness (L^*) of treated samples has the following sequence:

Neutral cellulases along pumice stone> acid cellulases along pumice stone > neutral cellulases> acid cellulases> pumice stone

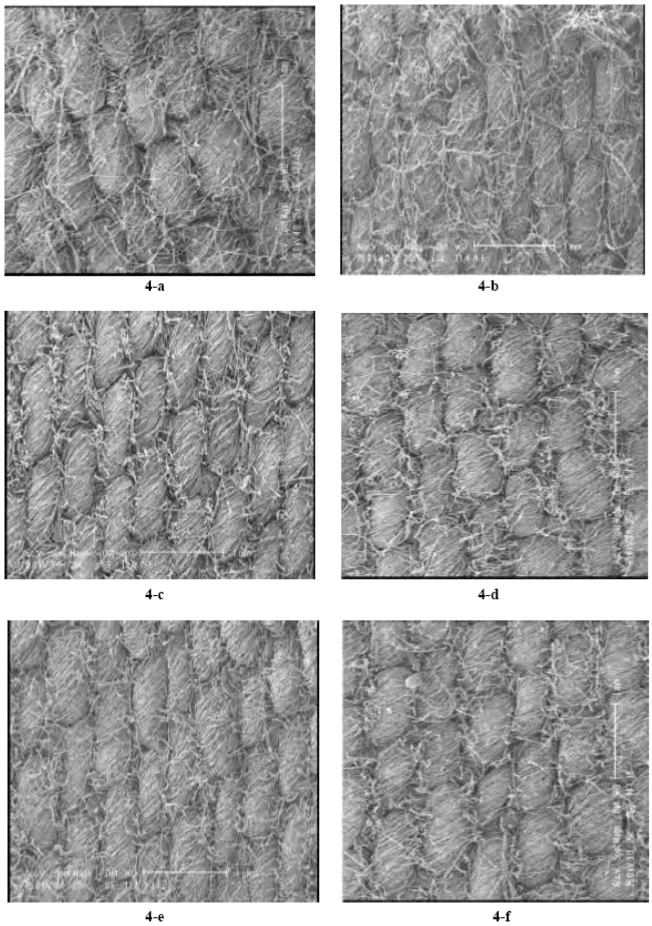
Alteration of b^* values of sample back or pocket material are shown that with increasing of amount of pumice stone and cellulases concentration the back staining of denim garment increase. Decreasing of staining on back of denim and white pocket can be arranged as following:

Pumice stone> acid cellulases> neutral cellulases> acid cellulases along pumice stone> neutral cellulases along pumice

The results of XRD patterns showed that treated sample with cellulases have a higher crystallinity percentage than untreated sample ore desized sample.

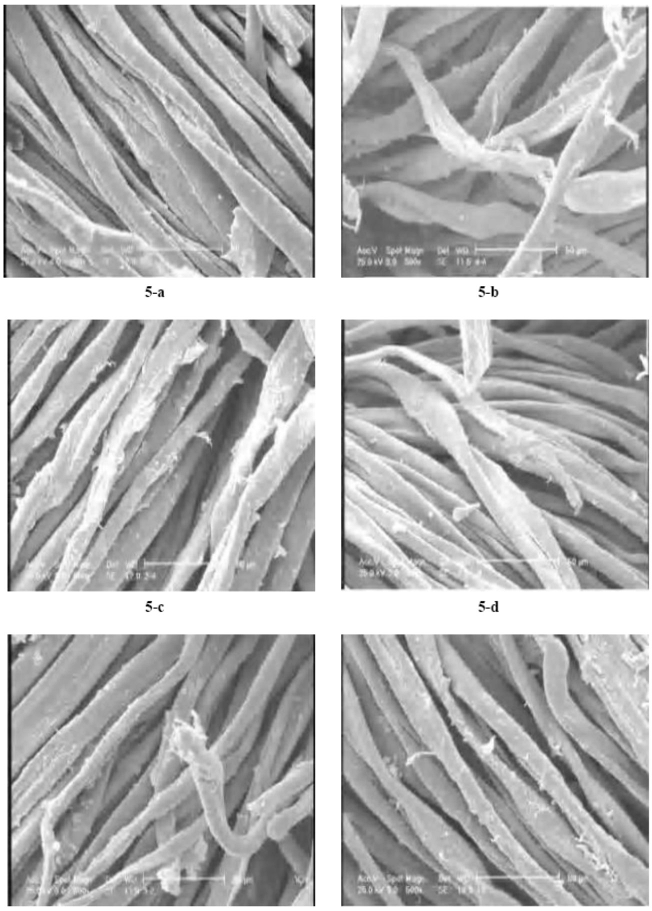
The tensile of treated sample with neutral cellulases along pumice stone is decreased than desized sample, treated sample with neutral cellulases alone and treated sample with pumice stone alone.

The SEM pictures showed that fibers of treated sample with cellulases along pumice stone damaged significantly than other treatment. Also damages on the fibers limited to the inner fibers and outer fibers remain unaffected. Pumice stone treated samples are not many differences with desized sample and only more entanglement observed on the pumice stone treated samples. Cellulases are able to remove the anchor fibers.



a- Desized sample, b- Treated sample with pumice stone, c- Treated sample with neutral cellulases, d- Treated sample with acid cellulases, e- Treated sample with neutral cellulases along pumice stone, f- Treated sample with acid cellulases along pumice stone

Fig.4. Microscopic pictures of different treated samples (25X)



a- Desized sample, b- Treated sample with pumice stone, c- Treated sample with neutral cellulases, d- Treated sample with acid cellulases, e- Treated sample with neutral cellulases along pumice stone, f- Treated sample with acid cellulases along pumice stone

Fig.5. Microscopic pictures of different treated samples (500X)

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