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DEGUMMING OF SILK USING PROTEASE ENZYME FROM *BACILLUS* SPECIES

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ABSTRACT
In this study, an attempt has been made to synthesize protease enzyme from bacillus species from the slaughter house waste and evaluate their suitability for degumming of silk yarn as against the commercial enzymes. The performance of enzyme treated yarn has also been compared with the yarns degummed by the conventional method ie with soap and soda. The degummed yarns were further dyed using natural dye turmeric in the presence and absence of mordants. The degummed samples were subjected to subjective evaluation like colour, luster and hand, dyed yarns for general appearance, depth of colour, evenness in dyeing and luster. Objective evaluation like Weight loss, Tensile strength, Elongation, Degumming efficiency, Colour fastness to washing, rubbing and to light were also carried out. The microscopic view of the degummed samples was obtained by SEM results.

KEY WORDS: degumming, dyeing, enzyme, protease, silk.

INTRODUCTION

Silk, the Queen of Textiles is a splendid gift of nature to the mankind known for its elegance, refinement, beauty and luxury. Silk has set the standard in luxury fabrics for several millennia. It is royal, splendid, exotic and sensuous in its radiance. Silk is a way of life in India. It offers periodical income and generates viable employment opportunities for marginal and small farm holdings round the year. Engaging 6.2 million people, sericulture plays a very important role as an important labour intensive and agro based cottage industry in India. It is the second largest producer of silk and contributes 18% of total raw silk production. India is making head way in becoming a supplier of silk garments and furnishing fabrics.

Silk is a continuous filament produced by the larva of certain insects, especially the silkworm, when constructing its cocoons. The silkworm secretes the silk as a viscous fluid from two large glands in the lateral part of the body. The fluid is extruded through a common spinneret to form a double filament cemented together. Degumming or Boiling-off is the process employed to remove the silk gum (sericin) enveloping the silk threads. Degumming of silk is traditionally carried out with soap or alkali. These methods have some major drawbacks, such as the degummed silk obtained is not uniform in quality, the strength loss is high and also the chemicals used cause environmental pollution. It is therefore, desirable to replace them by the eco-friendly alternatives and in this respect, the enzyme play a key role.

"Green is in" seems to be the buzzword in the textile industry after increased awareness and concern about the environment and pollution. Use of enzymatic methods is gaining momentum keeping in view the ill effects of the chemicals used for processing towards the global environment. It is only during the past few decades, the enzymatic degumming of silk has been developed along scientific lines. The action of enzyme can be controlled to avoid strength loss but at the same time obtain uniformly degummed silk.

Enzymatic degumming involves proteolytic degradation of sericin using specific protease which does not attack fibroin. Bacterial enzymes are being widely used because of its significant action on the fibroins. Protease is a class of enzymes which can catalyze hydrolysis of peptide bond. It can degrade protein into small molecules such as peptone, peptide and amino acid. The main sources of protease are bacterial, fungal, plant and animal origin. Among the various proteases, bacterial proteases are the most significant, compared with animal and fungal proteases. Protease treatments can modify the surface of wool and silk fibres to provide new and unique finishes.

Colour not only gives pleasant look to the clothing, but also expresses emotions and ideas. Being biodegradable and highly compatible with environment, the natural dyes are free from defects associated with synthetic dyes such as harmfulness of azo dyes to human body, pollution and waste water problem. It is well known that the rural folk dye the yarn by heating chopped leaves or flowers of the plant in water. A wide range of colours can be obtained from the use of these natural dyes. The silk yarns can be dyed using a wide range of natural dyes.

A study was made to isolate the *Bacillus* sp from the soil, screen the protease producing *bacillus* sp as well as substrate for the production of protease and study its application for silk degumming.

MATERIAL AND METHODS

Material

Mulberry silk yarn of denier 22 was used for the study.

METHODS

Enzyme production

Habitats that contain protein are the best sources to isolate the proteolytic microorganism. The organism was isolated from the slaughter house which contains a large amount of protein. They were collected in sterile polythene bags and

labeled after collection. They were stored at 4ºC until analyzed.

Media used for screening

The screening for protease producing bacteria was done using Casein agar medium which had the following composition.

Agar	- 2.0 g	Casein	- 1.0 g
NaCl	- 0.5g	Peptone	- 0.5g
Beef extract	- 0.3g	Distilled water	- 100 ml

Isolation of bacteria

Bacterial strains were isolated from the slaughter house waste. One gram soil was suspended in 10 ml of sterilized saline and subjected to serial dilution. 0.5 ml from an appropriate dilution was spread on a petriplate containing nutrient broth-casein-agar medium. The strain was selected on the basis of formation of a clear zone of casein hydrolysis on the petriplate and was transferred to nutrient agar slant for growth and maintenance.

Protease assay

Protease assay was determined by the method of Tsuchida *et al* **.**

Identification of bacteria

The bacterial colonies isolated from the slaughter house waste were identified and confirmed based on growth condition by various biochemical tests like gram staining, voges prokaurer test, citrate test, catalase test, nitrate test, starch hydrolysis and gelatin hydrolysis.

Optimization of cultural condition

Various parameters like pH, temperature, incubation time, nitrogen source, inoculum concentration was optimized

.**TABLE 1.** Optimization of cultural condition

Parameters	Variants	Optimum variant
pH	5-10	9
Temperature	17º C - 57º C at 10º C interval	37
Incubation time	24 – 120 hrs with 24 hrs interval	24
Nitrogen source	Beef extract, glycine,tryptone & peptone	Peptone
Inoculum concentration	1 – 5%	3%

Mass production of protease

The protease enzyme was produced in large scale by using fermentor, with a working volume of 2 liter capacity. 10 g/l of peptone; 5g/l of sodium chloride at pH 9 was optimized for protease production. The microbial source was inoculated aseptically into the production medium and incubated at 37ºC for 24 hours. After incubation, the fermented broth was centrifuged at 3000 rpm for 30 minutes. The supernatant was collected and preserved by using 3 to 4 drops of formaldehyde and was used as crude enzyme.

Optimization for degumming of silk yarn

Different parameters where optimized for finding the optimum degumming procedure

TABLE 2. Optimization for degumming of silk yarn

Parameters	Variants	Optimum variants
Enzyme concentration	4, 8, 12, 16 & 20 ml	20ml
Time interval	3, 6, 9, 12 & 24 hrs	6hrs
pH	5, 6, 7, 8 & 9	7
Temperature	25º C, 35º C, 45º C, 55º C & 65º C.	45º C

Degumming of silk using protease enzyme

Based on the procedure, 35.15g of silk yarn skein was degummed using 70 ml of the crude enzyme with the M: L ratio as 1:20. The enzyme was taken in a clean beaker and into it yarn washed with cold water was immersed. The beaker was placed in a hot air oven and the temperature was maintained at 45º C at pH 7 for duration of 6 hours. After the 6 hrs the degummed silk yarn was removed and washed thoroughly with hot and cold water, further dried and the weighted.

Conventional degumming of silk

The conventional degumming was carried out by taking 1000ml capacity stainless steel vessel and to it Marseille's soap 5gpl and soda 0.5gpl was added with M:L ratio as 1:20. 35.15g of yarn washed with soft water was immersed into the solution and was boiled for a period of 90 mins at 80 – 85°C in a water bath. After 90 mins the yarn was removed and washed with hot and cold water and then the yarn was dried and weighed.

Degumming using commercial enzyme

In the commercial enzyme degumming process, the enzyme bath was prepared by adding purified protease enzyme 50 ml, 0.5g sodium bicarbonate, 1g nonionic detergent with M :L ratio 1:20. 35.15g of silk yarn skein washed with soft water was immersed into the prepared enzyme bath. The temperature was maintained at 90°C for a period of 70 mins. The degummed silk yarn was removed and followed by washing, rinsing and drying.

Dyeing of yarn

Turmeric was used to obtain brilliant yellow shades and also it has an antimicrobial property, it was first dried for 8-10 hrs in an oven. The dried material was then crushed into small pieces and dried again. It was finally powdered and used for extraction. 100g of the extract was taken in a

beaker containing 2 litre of water and boiled for 1 hour. The solution was allowed to stand for sometimes until cooled and then filtered.

Optimization of dyeing variable

The dyeing M:L ratio was taken as 1:50 and the dyeing time 45 mins. Concentration of the mordant was fixed as 2%. To optimize the dye concentration the silk samples were dyed in 2% and 5%.

TABLE 3. Optimization of dyeing variable

S.No	Sample	Wt of the sample (gm)	Conc. Of dye (%)	Conc. of mordant (%)	M:L ratio	Time (min)	Temp (°C)
1.	CSM1 CSM2	1	2% 5%	2%	1:50	45	100
2.	OSM1 OSM2	1	2% 5%	2%	1:50	45	100
3.	ESM1 ESM2	1	2% 5%	2%	1:50	45	100
4.	CSC1 CSC2	1	2% 5%	-	1:50	45	100
5.	OSC1 OSC2	1	2% 5%	-	1:50	45	100
6.	ESC1 ESC2	1	2% 5%	-	1:50	45	100

CSM- Conventional Sample with mordant, OSM- Commercial Sample with mordant, ESM- Enzymatic treated Sample with mordant, CSC- Conventional Sample without mordant, OSC- Commercial Sample without mordant, ESC- Enzymatic treated Sample without mordant.

Dyeing in the absence of mordant's

The degummed silk yarn was dyed with extracted dye solutions M:L ratio of 1:50 in absence of mordants. The dyeing was carried out at 100°C for 45 mins in an open dye bath. After dyeing the dyed material was washed and finally dried.

Dyeing in the presence of mordant's

The degummed silk yarn was simultaneously mordanted with 2% alum and dyed keeping the material to liquor ratio of 1:50. The dyeing was carried out at 100°C for 45 mins in an open dye bath. After dyeing the dyed material was washed and dried.

Simultaneous mordanting

In this method the mordant and the dye were applied simultaneously in the same bath. The silk yarn was placed in the extracted dye bath and dyeing was carried out for 15 minutes. The required amount of mordant was added to the dye bath by lifting silk yarn and stirring. The yarn was then boiled in the solution for 30 minutes. The dyed silk yarn was removed from the dye bath, washed, rinsed and dried in shade.

EVALUATION

Subjective evaluation

All the degummed samples of equal size were mounted on black sheet and evaluated visually for its colour; luster and hand by a panel of judges consisting of 25 graduate students doing costume design and fashion, using a proforma. The dyed samples analyzed visually for general appearance, depth of colour, evenness in dyeing and luster by the same 25 under graduate students.

Objective evaluation

Standard yarn tests such as Weight loss, Tensile strength, Elongation, Degumming efficiency, Colour fastness to washing, rubbing and to light, were carried out in the original sample, conventionally treated, enzyme treated and commercial enzyme treated samples.

RESULTS AND DISCUSSION

Isolation and screening of bacteria

Bacterial colonies were isolated and a total of 24 isolates were screened for protease production. These bacterial isolates were plated over nutrient agar medium containing 0.4 % gelatin. Out of 24 strains, 11 strains showed good zone of clearance.

TABLE 4. Screening of bacteria

S.No	Bacterial colonies	Protease activity (U/ml/min)
1	BC1	0.1356
2	BC2	0.1539
3	BC3	0.1875
4	BC4	0.4213
5	BC5	0.6607
6	BC6	0.1580
7	BC7	0.1456
8	**BC8**	**0.9908**
9	BC9	0.4654
10	BC10	0.2446
11	BC11	0.2734

BC- Bacterial Colony

Identification of selected bacteria

The results of gram staining, motility and biochemical tests of selected bacterium BC8 are given in Table.

TABLE 5. Identification of bacteria

Characteristics	Isolated bacterial colony (BC8)
Gram staining	Positive
Motility test	Motile
Indole production test	Negative
Methyl red test	Negative
Voges Prokaurer test	Positive
Citrate utilization test	Positive
Catalase test	Positive
Oxidase test	Negative
Urea hydrolysis	Negative
Nitrate reduction	Positive
Starch hydrolysis	Positive
Gelatin hydrolysis	Positive
Fluorescence on King's B medium	Negative.
Growth at 18º C and 45º C	Good

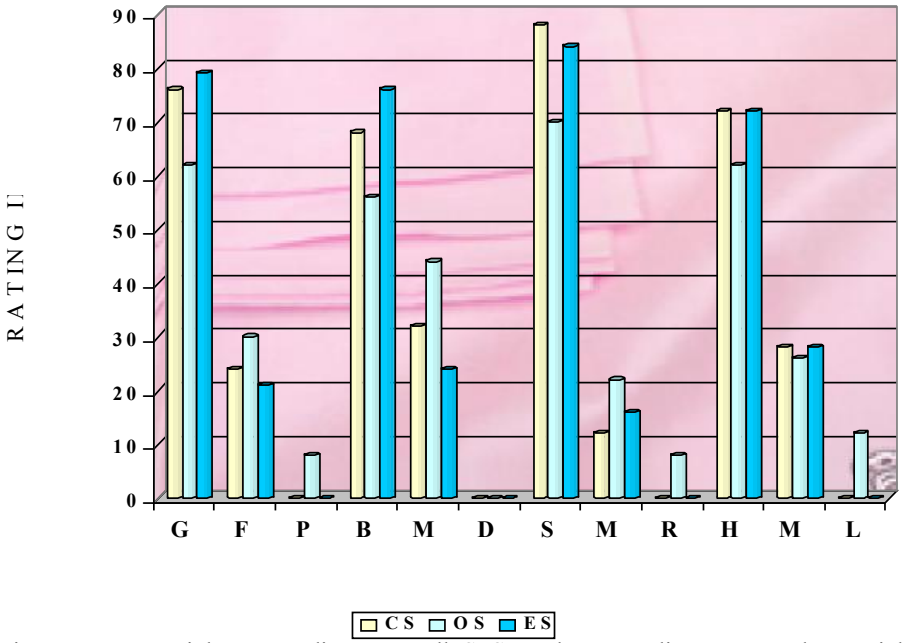
Based on the results the isolate bacterium BC8 was identified as ***Bacillus.***

Subjective evaluation

Visual evaluation of degummed silk yarn

The samples were evaluated visually for appearance, colour, luster and hand. While looking at the overall rating of the judges ES (Enzyme sample) were given the maximum rating followed by CS (Conventional sample) and finally OS (Commercial sample) which lacked in all the four aspects of visual evaluation. The silk yarns degummed using extracted protease enzyme produced by *bacillus* Sp was good in general appearance, whiteness, texture and luster.

FIGURE – I Visual evaluation

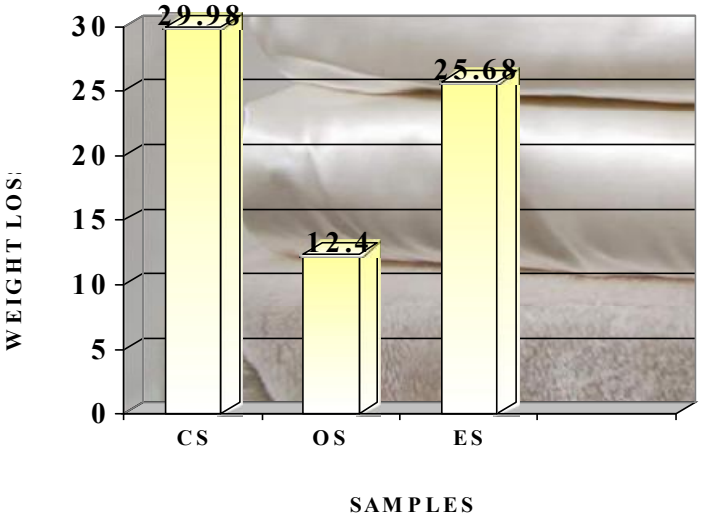


G- Good, F- Fair, P- Poor, B- Bright, M- Medium, D- Dull, S- Smooth, M- Medium, R- Rough, H- High, M- Medium, L- light.

Objective evaluation – weight loss

When comparing the weight of the yarns before and after treatment the weight loss% is more in samples CS and ES as 30 and 26 percent. While the weight loss in OS is relatively low, indicating inefficient degumming process.

FIGURE 2. Weight loss

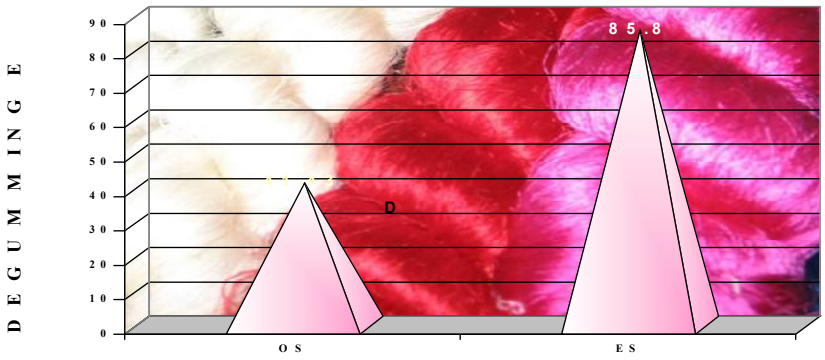


CS-Conventional Sample, OS- commercial, ES-Enzyme treated sample

DEGUMMING EFFICIENCY

The degumming efficiency of OS with CS and ES with CS, ES yielded the maximum result of 85.8% indicating efficient degumming. When comparing to commercial enzyme treated sample, protease enzyme treated gave the best result.

FIGURE 3. Degumming efficiency



OS- commercial, ES-Enzyme treated sample

Strength of degummed silk yarn

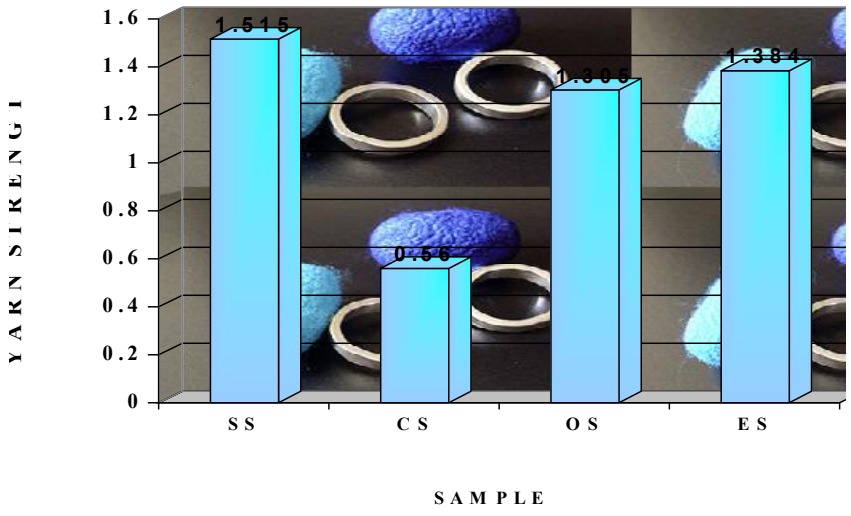
Table 6. features a minimum difference in strength of 9 percent between the samples ES and SS where as a maximum difference of 63 percent is seen between samples SS (Original sample) and CS respectively. From the results it could be said that the degumming has reduced the strength. The strength loss was minimum in enzymatic method and maximum in conventional method. Hence it could be concluded that the enzyme treatment reduces loss of tensile strength unlike the chemical treated one.

TABLE 6- Strength of degummed silk yarn

S.No	Sample Name	Yarn strength In (kgs)	Loss or gain over original		SD
		Mean	Actual	%	
1.	SS	1.515	-	-	0.113
2.	CS	0.560	-0.955	63%	0.0658
3.	OS	1.305	-0.21	13.8%	0.0864
4.	ES	1.384	-0.131	8.6%	0.0698

SS- Original sample, CS-Conventional Sample, OS- commercial, ES-Enzyme treated sample

FIGURE 4. Strength of degummed silk yarn



SS- Original Sample, CS-Conventional Sample, OS- commercial, ES-Enzyme treated sample

Elongation of degummed silk yarn

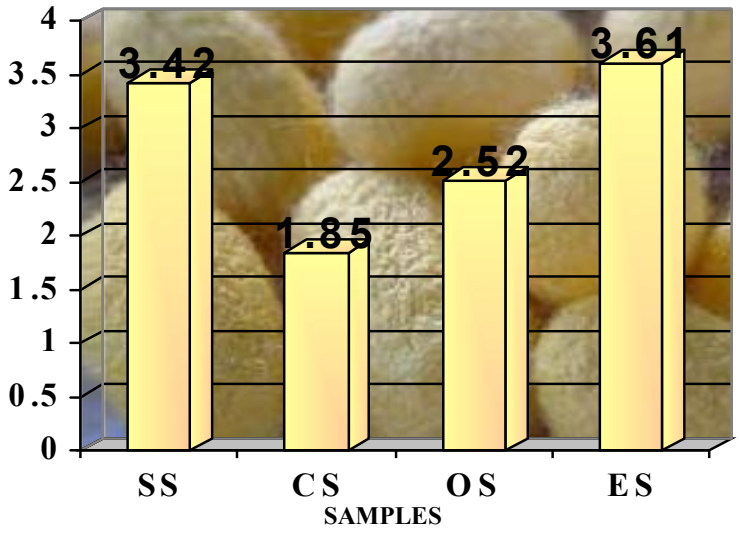
The yarn elongation had increased in protease enzyme treated sample and reduced in the other two methods when compared to the original.

TABLE 7. Elongation of degummed silk yarn

S.No	Sample Name	Yarn Elongation In (cms)	Loss or gain over original		SD
		Mean	Actual	%	
1.	SS	3.420	-	-	0.239
2.	CS	1.510	-1.91	55.8	0.113
3.	OS	2.520	-0.9	26.3	0.0698
4.	ES	3.610	0.19	5.5	0.0864

SS- Original sample, CS-Conventional Sample, OS- commercial, ES-Enzyme treated sample

FIGURE 5. Elongation of degummed silk yarn



Strength of dyed silk yarn

The yarn strength has increased by 47.2 and 48.5 percent in OS and ES samples respectively than the original sample. Among the degummed samples the sample CS has the minimum strength and ES has the maximum strength. From the results it could be concluded that the yarn degummed using protease from *bacillus* sp had better strength even after dyeing process.

TABLE 8. Strength of dyed silk yarn

S.no	Sample name	Yarn strength In (kgs)	Loss or gain over original		SD
		Mean	Actual	%	
1.	SS	1.1515	-	-	0.113
2.	CS	0.448	-0.7035	61.6	0.034
3.	OS	1.695	0.544	47.2	0.059
4.	ES	1.710	0.558	48.5	0.073

SS- Original sample, CS-Conventional Sample, OS- commercial, ES-Enzyme treated sample

Elongation of dyed silk yarn

The elongation is found to be maximum and minimum in samples ES and CS at 25 and 0.8% respectively. When comparing SS with CS, there is a minimum increase of 0.8% where as samples OS and ES show an increase of 24 and 25% respectively.

.**TABLE 9.** Elongation of dyed silk yarn

S.no	Sample name	Yarn elongation In (cms)	Loss or gain over original		SD
		Mean	Actual	%	
1.	SS	3.420	-	-	0.239
2.	CS	3.450	0.03	0.87%	0.273
3.	OS	4.250	0.83	24.2%	0.217
4.	ES	4.280	0.86	25.1%	0.210

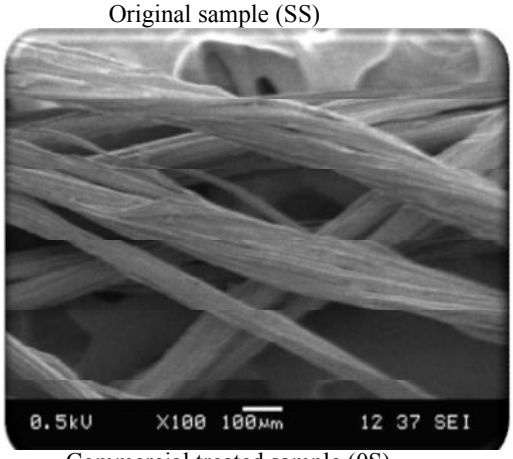
SS- Original sample, CS-Conventional Sample, OS- commercial, ES-Enzyme treated sample

Microscopic view

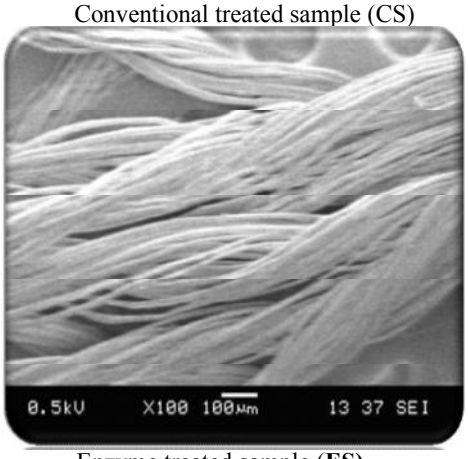
The scanning electron micrographs of the original and the treated samples are shown below. The samples CS and ES showed complete and uniform removal of sericin. The cleaning of the surface by the enzyme is responsible for the improvement in the luster of the treated samples. The sample OS gave fairly good results although the removal of the sericin was not very uniform. Compared to all the treated samples, protease enzyme treated samples showed a better removal of sericin.

FIGURE 6. Scanning electron microscope

Original sample (SS)

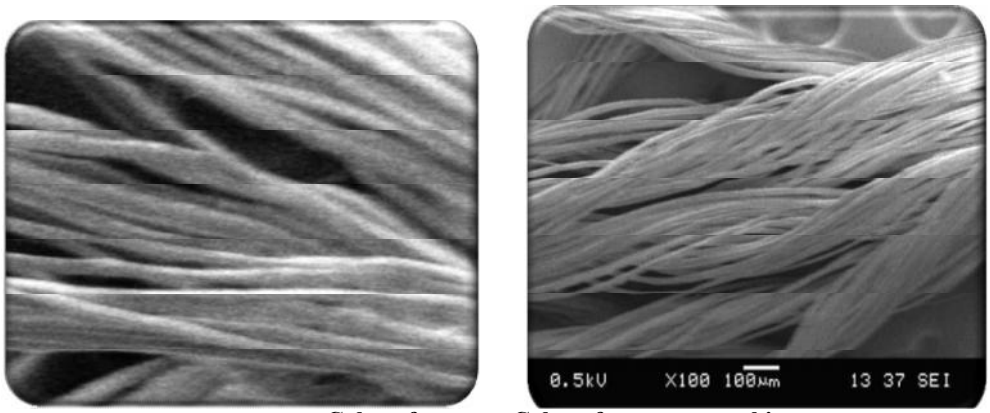


Conventional treated sample (CS)



Commercial treated sample (0S)

Enzyme treated sample (**ES)**



Colour fastness - Colour fastness to washing

TABLE 10. Colour fastness to washing

S.No	Samples	Washing	
		Colour	Staining
1.	CSM1	5	4/5
2.	OSM1	4/5	4
3.	ESM1	4/5	4/5
4.	**CSM2**	**5**	**5**
5.	OSM2	5	4/5
6.	ESM2	5	4/5
7.	CSC1	4/5	4/5
8.	OSC1	4	4
9.	ESC1	4/5	4/5
10.	**CSC2**	**5**	**5**
11.	OSC2	4/5	4/5
12.	**ESC2**	**5**	**5**

1-Very Poor, 2-Poor, 3-Moderate, 3/4-Fair, 4-Good, 4/5-Very Good, 5-Excellent

The above table illustrates that samples CSM1, CSM2, OSM2, ESM2, CSC2, and ESC2 are rated excellent and OSM1, ESM1, CSC1, ESC1 and OSC2 are rated very good for colour fastness to washing. Hence it could be concluded that all the samples were rated good to excellent in fastness to washing.

Colour fastness to rubbing

TABLE 11. Colour fastness to rubbing

S.No	Samples	Crocking			
		Wet	Staining	Dry	Staining
1.	CSM1	5	4/5	5	5
2.	OSM1	4/5	4	4/5	5
3.	ESM1	5	4/5	4/5	5
4.	**CSM2**	**5**	**5**	**5**	**5**
5.	OSM2	4	4/5	5	4/5
6.	ESM2	4/5	4/5	5	4/5
7.	CSC1	4/5	4/5	4/5	4/5
8.	OSC1	4/5	4	4	4/5
9.	ESC1	4/5	4/5	4/5	5
10.	**CSC2**	**5**	**5**	**5**	**5**
11.	OSC2	5	4/5	4/5	5
12.	**ESC2**	**5**	**5**	**5**	**5**

1-Very Poor, 2-Poor, 3-Moderate, 3/4-Fair, 4-Good, 4/5-Very Good, 5-Excellent

From the table it is evident that samples CSM1, ESM1, CSM2, CSC2, OSC2 and ESC2 are rated excellent and samples OSM1, ESM2, CSC1, ESC1 and OSC1 were rated good for wet crocking. CSM1, CSM2, OSM2, ESM2, CSC2 and ESC2 were rated excellent for dry crocking, OSC1 was rated 4 and others were rated 4/5 for dry crocking. From this it can be drawn that all the samples were rated good to excellent for fastness to crocking.

COLOUR FASTNESS TO LIGHT

Below Table 12 shows that generally all the samples are rated from good to very good except samples CSC2 and ESC2 which are rated excellent. Hence it could be concluded that the majority of the samples were rated good to very good for colour fastness to sunlight.

TABLE 12. Colour fastness to light

S.No	Samples	Light
1.	CSM1	4/5
2.	OSM1	4
3.	ESM1	4/5
4.	CSM2	5
5.	OSM2	4/5
6.	ESM2	4/5
7.	CSC1	4/5
8.	OSC1	4
9.	ESC1	4/5
10.	CSC2	5
11.	OSC2	4/5
12.	ESC2	5

1-Very Poor, 2-Poor, 3-Moderate, 3/4-Fair, 4-Good, 4/5-Very Good, 5-Excellent

CONCLUSION

The textile industry is the largest industry in terms of value, production and also in effluent generation. With the increasingly important requirement for textile manufacturers to reduce pollution in textile production, the use of enzymes in the chemical processing of fibres and textiles is rapidly gaining wider recognition because of their non-toxic and eco-friendly characteristics. As far as textiles are concerned, researchers emphasis on reduction of the use of harsh chemicals and reuse of effluent waste water. Hence this study, which was based upon the use of enzymes for degumming has thrown light on reduction of cost and energy. The use of proteolytic enzymes is a

better method because they remove the sericin without attacking the fibroin. Tests with high concentrations of enzymes show that there is no fibre damage and the silk threads are stronger than with traditional treatment. The results of the study prove the potentiality of enzymes in producing green label products, which in turn would be the right solution to solve environmental issues.

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