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# Optimization of Conditions for Enzymatic Pre-Treatment of Silk with Protease Enzyme

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**Abstract**

Hand woven khadi silk fabric was pretreated with proteolytic enzyme (Silkenz DGM Liquid). These enzymatic conditions were optimized without significantly affecting the physical properties. Optimized enzymatic conditions included pH media 8, enzyme concentration 10% (owf) and treatment time of 60 minutes.
**Key words : Enzyme, Optimization, Pre-treatment, Proteolytic, Silk.**

Due to global awareness for eco-friendly products the natural fibers are assuming importance. Silk is the most beautiful of all the natural filaments, acclaimed as queen of textiles. It neither possesses lusture nor softness due to the presence of gum or serecin. Hence silk is generally degummed either at yarn or fabric stage before it is converted into a final product. The degumming process may be considered primarily as a process of cleavage of peptide bonds of serecin by the hydrolytic or enzymatic method and its subsequent removal from fibroin by solubilization or dispersion in water (1). It is believed that a fraction of these waxes is soaponifiable and therefore can be removed with alkali or soap treatment, but these methods are believed to be harmful to fibroin. These also give rise to pollution problem. Enzymes are now being used as alternative degumming agents for the processing of silk. Being bio-degradable, enzymes are eco-friendly and results in uniform degumming which improves the wettability and whiteness of silk with minimum strength loss (2). With the increased awareness and regulation about environment concerns, many industrial enzymes for textiles have been developed. The enzymes are emerging as the obvious choice for wet processing as these are bio-degradable, work under mild conditions and save precious energy (3). These do not produce toxic effluents as these can be easily deactivated and disposed off. The use of enzymes is found to be safer, more efficient and specific in nature. The investigation was undertaken to optimize the catalytic action of proteolytic enzyme by optimizing the variables viz. pH of enzyme solution, concentration of enzyme and reaction time after analyzing their respective effect on the physical properties i.e. fabric thickness loss, GSM, weight loss, strength loss, flexural rigidity and moisture absorption.

## Methods

Hand woven, pure khadi silk fabric with the following specifications was used : Weave; plain, warp : 30 ends/cm, weft : 29 pick/cm, fabric mass: 105 g/m$^2$. A non-ionic surfactant, proteolytic enzyme procured from Rossari Biotech India Private Ltd., Mumbai and tris (hydroxymethyle) amino methane (tris HCI), a buffer solution was used.

A silk fabric was scoured to remove impurities. The samples were soaked in warm water using 0.5% of a non-ionic surfactant solution for 30 minutes, keeping MLR 1 : 20.

### *Optimization of Variables for Enzyme Treatment*

Trials were conducted to determine the optimum values of three variables—pH of the enzyme solu-

**Table 1.** Optimization of pH media for enzymatic pre-treatment on the basis various physical properties. CD* = Critical difference at 5% level of significance.

| pH | Per centage fabric thickness loss | GSM | Per centage weight loss | Per centage strength loss | | Overall flexural rigidity | Per centage moisture absorption |
|---|---|---|---|---|---|---|---|
| | | | | Warp | Weft | | |
| 8 | 1.670* | 99.850* | 4.554* | 5.242* | 5.364* | 68.972* | 71.150* |
| 8.5 | 2.532* | 98.120* | 5.410* | 5.829* | 5.977* | 64.762* | 73.532* |
| 9 | 3.554* | 95.320* | 6.166* | 6.279* | 6.427* | 61.490* | 76.634* |
| CD | 0.519 | 1.312 | 0.605 | 0.231 | 0.461 | 1.203 | 1.118 |

tion, enzyme concentration and treatment time. Deionized water was used throughout the experiment. The procedure followed for optimization is discussed below.

*Optimization of pH Media*

For optimization of pH the scoured samples were treated with 5% enzyme concentration (owf) at varied pH values of 8, 8.5 and 9. During the treatment a nonionic surfactant (1g/liter ) was added as a wetting agent. The treatment was carried out in launderometer at 50 ± 2C for 45 minutes. The fabric was taken out immediately, put in a hot water bath (80 C), stirred for 15 minutes to denature the enzyme and dried. The samples were then evaluated for percentage fabric thickness loss, GSM, weight loss, strength loss, flexural rigidity and moisture absorption. The pH at which the samples gaves the best result without significantly affecting the physical properties was considered the optimum.

*Optimization of Enzyme Concentration*

For optimization of enzyme concentration, 5, 10, 15, 20 and 25% of enzyme dosages (owf) were taken separately in five beakers. Buffer solution having optimum pH was added to each beaker. The enzyme concentration at which the sample gave the best results with regard to physical properties was selected for the final treatment.

*Optimization of Enzyme Treatment Time*

The buffer solution having optimum pH was poured into three beakers containing enzyme solution of optimum concentration. The treatment was carried out at 45, 60, 75 minutes in the lauderometer. The optimum treatment time was determined with regard to best results related to physical properties of fabric, namely percentage fabric thickness loss, GSM, weight loss, strength loss, flexural rigidity and moisture absorption, were tested using standard test methods (4). The change in physical properties of silk was statistically analyzed and the variables for the enzymatic pre-treatment were optimized for final treatment.

## Results and Discussion

*Optimization of Conditions for Enzymatic Pre-Treatment*

Optimum conditions for enzymatic pre-treatment were assessed by measuring percentage fabric thickness loss, GSM, weight loss, strength loss, flexural rigidity and moisture absorption.

*Optimization of pH media*

The results pertaining to the effect of pH media on various properties of the test specimens are given in Table 1. All the values of percentage fabric thickness loss, GSM, weight loss, strength loss, flexural rigidity and moisture absorption were significantly different from each other ($P \leq 0.05$), when treated with pH 8.0, 8.5 and 9.0. Thus pH 8.0 was taken as optimum pH for further study.

*Optimization of Concentration*

Table 2 shows that the values of treatment means at 10% enzyme concentration was not significantly different from 5% concentration but beyond 10% en-

**Table 2.** Optimization of concentrattion for enzymatic pre-treatment on the basis of various physical properties. CD = Critical difference, * = Significant difference at 5% level.

| Concen-tration | Percentage fabric thickness loss | GSM | Percentage weight loss | Percentage strength loss Warp | Weft | Overall flexural rigidity | Percentage moisture absorption |
|---|---|---|---|---|---|---|---|
| 5 | 2.028 | 99.573 | 4.583 | 5.423 | 5.673 | 69.260* | 71.606 |
| 10 | 2.101 | 99.040 | 4.685 | 5.506 | 5.683 | 67.306* | 72.053 |
| 15 | 2.825* | 97.82* | 5.005* | 5.840* | 6.040* | 62.913* | 73.056* |
| 20 | 3.115* | 96.09* | 5.540* | 6.006* | 6.083 | 58.756* | 74.136* |
| 25 | 3.405* | 94.93* | 6.020* | 6.086 | 6.276* | 56.786* | 75.920* |
| CD | 0.408 | 1.07 | 0.146 | 0.255 | 0.275 | 0.953 | 0.687 |

zyme concentration the difference was significant ($P \leq 0.05$) for all the values in terms of percentage fabric thickness loss, GSM, percentage weight loss and moisture absorption.

Percentage strength loss increased in both the warp and weft direction of the fabric. In warp direction, the values treated at 5 and 10% enzyme concentration were not significantly different from each other. However, the value at 10% concentration was at par with the value at 5% concentration. Percent strength loss was significant ($P \leq 0.05$ ) beyond 10% concentration upto 20%while a non-significant difference was observed between 20 and 25% enzyme concentration. Similarly, in weft direction, the values analyzed for percentage strength loss was observed at 15% enzyme concentration and showed a significant difference ($P \leq 0.05$) as compared to 10% enzyme concentration. No significant difference between 15 and 20% enzyme concentration was observed. Again there was a marked increase in percent strength loss, as revealed by a significant difference ($P \leq 0.05$) between 20 and 25% enzyme concentration. The value at 10% enzyme concentration was the optimum value in terms of percentage strength loss for both the warp and weft direction as the percentage strength loss increased rapidly after 10% enzyme concentration.

The flexural rigidity of the fabric decreased with the increase in enzyme concentration. Fabric appeared to be limp at higher enzyme concentrations. All the values were significantly different from each other ($P \leq 0.05$).

All the physical properties except flexural rigidity depicted 10% enzyme concentration as optimum enzyme concentration.

### *Optimization of Time*

A gradual increase in percentage of fabric thickness loss and moisture absorption was envisaged due to enzyme pre-treatment time increased from 45 to 60 upto 75 minutes. All the values differed significantly ($P \leq 0.05$) when the pre-treatment time was increased from 45, 60 and 75 minutes.

GSM of fabric decreased with an increase in treatment time. The difference of treatment means between 45 and 60 minutes was insignificant. Further, when the time was increased to 75 minutes a significant increase ($P \leq 0.05$) was found. Also, in percentage of

**Table 3.** Optimization of the time for enzymatic pre-treatment on the basis of various physical properties. CD= Critical difference, * = Significant difference at 5% level.

| Time | Percentage fabric thickness loss | GSM | Percentage weight loss | Percentage strength loss Warp | Weft | Overall flexural rigidity | Percentage moisture absorption |
|---|---|---|---|---|---|---|---|
| 45 | 1.910* | 98.220 | 4.665 | 3.427 | 3.717 | 68.445* | 72.640* |
| 60 | 2.805* | 98.181 | 4.788 | 3.898 | 4.145 | 64.535* | 74.247* |
| 75 | 3.555* | 95.870* | 5.228* | 4.823* | 4.916* | 61.630* | 75.292* |
| CD | 0.552 | 0.533 | 0.150 | 0.787 | 0.494 | 1.183 | 0.778 |

weight loss and strength loss both in warp and weft direction a slight increase was observed when pretreatment time increased from 45 to 60 minutes but further treatment upto 75 minutes led to a significant increase ($P \leq 0.05$) in the given physical properties.

A marked decline was observed among all the values of flexural rigidity when the treatment time was increased. All the values were significantly different from each other ($P \leq 0.05$). Considering weight loss and strength loss as an important physical properties, 60 minutes was the optimum time.

### *Conclusion*

All the parameters of enzyme treatment have considerable influence on various physical properties of the fabric. The results showed that the enzyme treatment reduced the fabric thickness, weight and strength of the fabric substantially. It improved the absorbency and made the fabric better for dye uptake. The treatment at pH 8, enzyme concentration 10% (owf) and treatment time 60 minutes was statistically found to be best.

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