Chemometric approach to optimize cellulase treatment of cotton knits

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Enzymatic treatment of the cotton knitted fabrics with cellulase enzyme Denifade has been carried out according to Box and Behnken response surface design with three independent variables, viz. enzyme concentration, duration of treatment and treatment temperature, at three levels of each variable. Enzymatic treatment results in weight and strength loss of the fabrics. However, the treated fabrics show less stiffness and improved wettability as well as higher dye uptake. The scanning electron micrographs show that the surface fibres are removed on enzyme treatment. The treatment conditions have been optimized with respect to enzyme concentration, duration of treatment and temperature of treatment. A comparative study with Cellosol cellulase enzyme has also been carried out.

Keywords: Enzyme, Denifade, Cellosol, Cotton knit

1 Introduction
The presence of protruding fibres on the surface of knitted cotton fabrics gives the fabric fuzzy appearance as well as reduces its lustre. These protruding fibres can be easily removed by treatment with cellulase enzyme which can degrade cellulose by catalyzing the hydrolytic cleavage of 1-4-β-glucosidic linkages of cellulose molecule. The process can be carried out under controlled conditions so as to minimize the degradation of the fabric. This process of eliminating the surface fibres is also known as 'biopolishing'. The removal of the surface fibres results in weight loss as well as strength loss. It is, therefore, necessary to maintain a compromise between the weight loss and the strength loss while removing the surface fibres.

In the present study, an attempt has been made to find the optimum conditions for the use of cellulase enzyme on cotton knitted fabrics by carrying out the experiments as per statistically designed experimental plan using indigenous commercial enzymes.

2 Materials and Methods
2.1 Fabric
For the optimization of treatment conditions, ready-to-dye 34s single knitted rib fabric was used. The optimized conditions were further investigated on 20s combed sinker knitted fabric.

2.2 Enzyme Treatment
The enzyme treatment was carried out in Atlas Launderometer and consisted of:

- Enzyme (Denifade of Advanced Biochemicals Ltd, Bombay). The enzyme activity was 407.4 FPU/ml. Concentrations of enzyme used are given in Table 1.
- Wetting agent—Lissapol N (10% owf)
- Citrate buffer—The stock solution was prepared by mixing 210 g citric acid monohydrate in 750 ml distilled water. Sodium hydroxide was added till 4.3 pH and then the stock solution was diluted to 1000 ml to get 4.5 pH. This is 1M citrate buffer of pH 4.5; when diluted to 0.05 M, pH should be 4.8.

| Table 1—Variables and their levels used in the experimental plan for enzyme treatment |
|---------------------------------|------------------|
| Variable                        | Level            |
|                                 |    +1  | 0  | -1    |
| Enzyme conc., % owf            |  18   | 10  | 2     |
| Time, min                      |  75   | 45  | 15    |
| Temperature, °C                |  60   | 50  | 40    |
2.3 Preparation of Samples

Fabric samples of 30 cm \( \times \) 30 cm (approx. 20g) were cut and conditioned at 30% RH for 18 h. The treatment was carried out maintaining the M:L ratio at 1:30. The enzyme-treated samples were washed thoroughly, dried and conditioned again before testing.

2.4 Design of Experiments

The experiments were carried out according to the Box and Behnken response surface design with three independent variables and three levels of each variable. The total number of test runs required is 15 instead of 27. Apart from the fewer test runs, it also offers the advantage of being rotatable. A design is rotatable if fitted models estimate the response with equal precision at all points in the factor space that are equidistant from the centre. Moreover, in this design, three experiments are carried out under identical conditions to find out the experimental error due to unaccounted factors.

The parameters selected as independent variable were:

- \( X_1 \) — Enzyme concentration,
- \( X_2 \) — Duration of treatment
- \( X_3 \) — Temperature of treatment

The levels of variables and the treatment conditions are given in Tables 1 and 2 respectively.

2.5 Tests

Weight loss was determined by measuring the difference in the weight of the conditioned untreated sample and the enzyme-treated sample. The Instron Universal Testing System No. 4202 was used for the assessment of strength of the samples. Bending length of the samples was determined on Shirley fabric stiffness tester.

Air permeability test FRX03300 was used for measuring air permeability. Wicking time was measured in terms of time taken for water to rise to 2.5 cm length on the strip of 10 cm \( \times \) 2.5 cm. Cambridge Steroscan was used to obtain the scanning electron micrographs.

Relaxation of cotton knits was done by Starfish project given by Heap Alba.°

2.6 Statistical Analysis

A quadratic polynomial was used to analyze the relationship of each response with the three independent variables.

\[
y = b_0 + \sum_{i=1}^{3} b_i X_i + \sum_{i=1}^{3} b_{ij} X_i X_j
\]

where \( b_0, b_i, b_{ij} \) and \( b_{ij} \) are the coefficients of the regression equation; \( i, j \) are integers with \( i < j \); and \( Y \) the response or dependent variable.

The best fitted equation was obtained by using the student t-test for significance of the estimated coefficients.

Three-dimensional surface plots were prepared to study the effect of variables on the responses. The optimum enzymatic conditions were selected from the predicted combinations and the experiments were repeated under these conditions to assess reproducibility.

The effect of Cellosoft enzyme (Novo Nordisk, activity 520 FPU/ml) on the properties of the two knitted fabrics was assessed and compared with the Denifade enzyme.

### Table 2—Treatment conditions and observed responses

<table>
<thead>
<tr>
<th>Expt No.</th>
<th>Enzyme conc. % owf ((X_1))</th>
<th>Time, min ((X_2))</th>
<th>Temp, °C ((X_3))</th>
<th>Wt. loss, % ((Y_1))</th>
<th>Strength loss, % ((Y_2))</th>
<th>Bending length, cm ((Y_3))</th>
<th>Gain in AP, % ((Y_4))</th>
<th>Wicking time, s ((Y_5))</th>
<th>Abrasion cycles ((Y_6))</th>
<th>Whiteness index ((Y_7))</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>15</td>
<td>50</td>
<td>2.4</td>
<td>4.2</td>
<td>1.4</td>
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<td>15.5</td>
<td>8.1</td>
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<td>231.0</td>
</tr>
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<td>227.0</td>
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<tr>
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<td>9.9</td>
<td>4.7</td>
<td>238.0</td>
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<td>13.0</td>
<td>8.2</td>
<td>203.0</td>
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<td>75</td>
<td>40</td>
<td>4.2</td>
<td>14.2</td>
<td>1.3</td>
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<td>14.9</td>
<td>7.0</td>
<td>207.0</td>
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<td>60</td>
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<td>8.1</td>
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<td>10</td>
<td>45</td>
<td>50</td>
<td>4.9</td>
<td>7.6</td>
<td>1.2</td>
<td>20.2</td>
<td>15.2</td>
<td>8.0</td>
<td>239.0</td>
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<tr>
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<td>10</td>
<td>45</td>
<td>50</td>
<td>5.5</td>
<td>6.3</td>
<td>1.2</td>
<td>20.5</td>
<td>14.9</td>
<td>8.2</td>
<td>238.0</td>
</tr>
</tbody>
</table>

AP—Air permeability; CW—Course-wise; and WW—Wale-wise
3 Results and Discussion

The results of all the experiments are given in Table 2 and the values for the control samples are given in Table 3.

3.1 Effect of Independent Variables on Different Responses

3.1.1 Weight Loss

The regression equation obtained for weight loss is,

\[ Y_i = 5.007 + 0.754 X_1 + 1.0272 X_2 + 0.193 X_3 \\
- 0.872 X_1^2 - 0.815 X_2^2 - 0.626 X_3^2 \\
+ 1.07 X_1 X_2 + 0.33 X_2 X_3 \quad \ldots (1) \]

\[ R^2 = 0.936; \text{ Standard error of estimate } = 1.337; \]

and F-ratio = 26.529

Spatial diagrams of the response surface are given in Figs 1 and 2. It is apparent that treatment time has the maximum effect on weight loss, followed by enzyme concentration. The treatment temperature within the range of 40-60°C does not result in any significant weight loss. All the three independent variables have a positive effect on the response, i.e. weight loss.

The interaction of enzyme concentration and treatment time is more pronounced at their higher values. A maximum weight loss of about 6% may occur at the highest level of enzyme concentration (18% owf) and at the highest level of treatment time (75 min).

Figs 1 and 2 show that the weight loss increases rapidly with the increase in enzyme concentration up to 10% owf. Subsequently, it levels off at higher concentration. Similarly, weight loss increases rapidly on increasing the treatment time up to 45 min and then levels off.

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Table 3—Effect of different cellulase enzymes on various properties of 34s single knitted rib and 20s combed sinker knitted fabric

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weight loss %</th>
<th>Strength loss %</th>
<th>Bending length cm</th>
<th>Air permeability at 400 Pa</th>
<th>Whitening index</th>
<th>Abrasion resistance cycle</th>
<th>Wicking time, s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34s Single Rib</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>—</td>
<td>—</td>
<td>1.7</td>
<td>1871.2</td>
<td>65.5</td>
<td>251.0</td>
<td>39.6</td>
</tr>
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<td>Denifade</td>
<td>4.6</td>
<td>7.5</td>
<td>1.2</td>
<td>2386.7</td>
<td>66.9</td>
<td>238.0</td>
<td>15.2</td>
</tr>
<tr>
<td>Cellosot</td>
<td>4.2</td>
<td>10.1</td>
<td>1.3</td>
<td>2519.8</td>
<td>65.9</td>
<td>232.0</td>
<td>14.3</td>
</tr>
<tr>
<td></td>
<td>20s Combed Sinker</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>—</td>
<td>—</td>
<td>1.7</td>
<td>854.3</td>
<td>58.2</td>
<td>99.5</td>
<td>38.8</td>
</tr>
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<td>Denifade</td>
<td>5.7</td>
<td>8.7</td>
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<td>966.1</td>
<td>60.8</td>
<td>96.0</td>
<td>30.5</td>
</tr>
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<td>Cellosot</td>
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<td>9.0</td>
<td>1.5</td>
<td>1020.8</td>
<td>59.1</td>
<td>96.0</td>
<td>27.9</td>
</tr>
</tbody>
</table>

CW—Course-wise; and WW—Wale-wise
3.1.2 Strength Loss

The regression equation obtained for strength loss is,

\[ Y_2 = 7.451 + 1.769 X_1 + 4.245 X_2 + 1.507 X_1^2 
   - 1.45 X_2^2 + 3.184 X_3 + 2.662 X_1 X_2 \]  

\[ R^2 = 0.900; \text{ Standard error of estimate } = 1.387; \]
and \( F \)-ratio = 21.890

This indicates that strength loss (\( Y_2 \)) is significantly dependent on all the three factors. Treatment time has the maximum effect followed by enzyme concentration.

Spatial diagrams of the response surface are given in Figs 3 and 4. The interaction of treatment time and enzyme concentration is more pronounced at their higher values. A maximum strength loss of 16% may occur at the highest level of enzyme concentration (18% owf) and at the highest level of treatment time (75 min).

Figs 3 and 4 show that the strength loss increases rapidly with increase in concentration beyond 10% owf at longer time periods of treatment.

3.1.3 Bending Length

The regression equation obtained for percentage decrease in bending length is,

\[ Y_3 = 30.326 + 3.394 X_1 + 4.112 X_2 + 1.544 X_3 
   - 3.088 X_1^2 - 5.332 X_2^2 + 3.890 X_1 X_2 
   + 1.996 X_2 X_3 \]  

\[ R^2 = 0.799; \text{ Standard error of estimate } = 2.696, \]
and \( F \)-ratio = 8.952

Spatial diagrams of the response surface are given in Figs 5 and 6. It is apparent that the treatment time has the maximum effect on decrease in bending length followed by enzyme concentration. The treatment temperature within the range of 40-60°C does not result in significant decrease in bending length. All the three independent variables have a positive effect on the response.

The interaction of enzyme concentration and treatment time is more pronounced at their higher values. A maximum decrease in bending length of about 36% may occur at the highest level of enzyme concentration (18% owf) and at the highest level of treatment time (75 min).

Figs 5 and 6 show that the decrease in bending length is more pronounced at enzyme concentration up to 10% owf. Subsequently, it levels off at higher concentration. Similarly, decrease in bending length is substantially more for the treatment time up to 45 min and then levels off.

3.1.4 Air Permeability

The regression equation obtained for percentage gain in air permeability is,

\[ Y_4 = 19.277 + 6.714 X_1 + 7.077 X_2 
   + 2.379 X_1^2 + 2.315 X_1 X_2 
   + 2.103 X_2 X_3 - 1.903 X_3 X_1 \]  

\[ R^2 = 0.827; \text{ Standard error of estimate } = 3.382, \]
and \( F \)-ratio = 12.180

Spatial diagrams of percentage gain in air permeability are given in Figs 7 and 8. It is evident
from the figures that the effect of enzyme concentration and treatment is more pronounced than that of treatment temperature and that air permeability increases linearly with increase in enzyme concentration or treatment time.

3.1.5 Wicking Time

The regression equation obtained for course-wise wicking time is,

\[ Y_5 = 14.833 - 0.657 X_1 + 0.844 X_2 + 0.347 X_3 - 1.207 X_3^2 + 1.240 X_3^3 - 1.845 X_3^4 + 0.517 X_1 X_2 + 0.719 X_3 X_1 \ldots (5) \]

\[ R^2 = 0.844; \text{ Standard error of estimate} = 0.674; \]

and F-ratio = 10.441

The regression equation obtained for wale-wise wicking time is,
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\[ Y_6 = 8.100 - 0.844 X_1 - 0.582 X_2 \\
- 0.503 X_1^2 + 0.326 X_1 X_3 - 1.034 X_1 X_3 \\
- 0.710 X_1 X_2 - 0.542 X_2 X_3 \\
+ 0.621 X_3 X_1 \]  
\[ R^2 = 0.843; \text{ Standard error of estimate} = 0.479; \]
\[ \text{and F-ratio} = 10.370 \]

It is apparent from Table 2 that enzyme treatment improves the wicking time but low coefficients of independent variables show that the process variables do not have much effect on the wicking time. However, the wicking time of untreated sample is substantially higher than that of the treated samples (Tables 2 and 3).

3.1.6 Abrasion Resistance

The regression equation obtained for percentage loss in abrasion resistance is,

\[ Y_7 = 5.976 + 3.436 X_1 + 3.088 X_2^2 + 4.88 X_2 \\
+ 2.192 X_3^2 + 3.785 X_1 X_2 + 3.088 X_3 X_1 \]  
\[ R^2 = 0.560; \text{ Standard error of estimate} = 3.638; \]
\[ \text{and F-ratio} = 3.966. \]

It is apparent that there is a poor correlation between percentage loss in abrasion resistance and process parameters.

3.1.7 Whiteness

The regression equation obtained for percentage increase in whiteness is,

\[ Y_8 = 2.932 - 0.657 X_3 - 2.487 X_1 \\
- 1.655 X_3^2 + 1.079 X_2 X_3 \\
- 3.238 X_1 X_3 \]  
\[ R^2 = 0.712; \text{ Standard error of estimate} = 1.432; \]
\[ \text{and F-ratio} = 7.914. \]

It is apparent from the equation that whiteness is not dependent on linear terms of independent variables. So, it is not possible to predict about the trend of whiteness but Table 2 indicates that generally there is an improvement in whiteness with increase in enzyme concentration.

3.2 Interaction between Responses

The change in various properties of enzyme-treated knitted fabric can be studied as dependent on the weight loss obtained. Hence, the regression analysis was carried out with weight loss as the independent variable and the various properties studied as dependent variables. A good correlation was observed only in the case of % decrease in wale-wise bending length and weight loss. The regression equation is as follows:

\[ Y_1 = 10.034 + 4.189 Y_1 \]  
\[ R^2 = 0.849; \text{ Standard error of estimate} = 2.339; \]
\[ \text{and F-ratio} = 79.576 \]

Fig. 9 shows that there is a linear correlation between per cent decrease in bending length and weight loss.

3.3 Optimization of Treatment Conditions

To find out the optimum conditions for the cellulase enzyme treatment on 34s single rib fabric, the estimated values of all 27 cases of those properties which showed excellent correlation with the process parameters \( R^2 \geq 0.800 \), namely weight loss, decrease in bending length (wale-wise), strength loss (yarn) and air permeability, were ranked. Even though the wicking time showed an improvement after the treatment, this property was not considered because the differences between the 27 cases were not significant. In case of strength loss, lower values were given higher ranks. These ranks were then added up for each case. Theoretically, the case having the maximum sum represents the optimum conditions having a weight loss more than 4.5%. There were five such cases. The cases in the order of decreasing sum of ranks are given in Table 4.

Table 4—Cases in the order of decreasing sum of ranks

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Enzyme conc.</th>
<th>Time (min)</th>
<th>Temperature (°C)</th>
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<tbody>
<tr>
<td>1</td>
<td>18</td>
<td>75</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>18</td>
<td>75</td>
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</tr>
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<td>3</td>
<td>10</td>
<td>75</td>
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</tr>
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<td>4</td>
<td>10</td>
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<td>50</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>45</td>
<td>60</td>
</tr>
</tbody>
</table>

Fig. 9—Correlation between weight loss and decrease in bending length
Fig. 10—Scanning-electron micrographs of untreated, Denifade-treated and Cellossoft-treated cotton knitted samples: (a) untreated yarn, (b) untreated fabric, (c) Denifade-treated yarn, (d) Denifade-treated fabric, (e) Cellossoft-treated yarn, and (f) Cellossoft-treated fabric.
Table 5—Results of dye uptake measurements

<table>
<thead>
<tr>
<th>Dye (Shade)</th>
<th>Fabric</th>
<th>Treatment</th>
<th>( \lambda_{\text{max}} ) nm</th>
<th>( % \text{R} )</th>
<th>( K/S )</th>
<th>Comparative colour strength %</th>
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<td>Remazol</td>
<td>34s</td>
<td>Untreated</td>
<td>580</td>
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<td>100.00</td>
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<td>Blue R</td>
<td>Single rib</td>
<td>Buffer</td>
<td>580</td>
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<td>Buffer</td>
<td>510</td>
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<td>510</td>
<td>14.61</td>
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<td>106.29</td>
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</table>

Of the five best treatment conditions, the treatment no. 4, i.e. cellulase enzyme at a concentration of 10% owf at 50°C for 45 min was chosen for further study since in this case the minimum amount of enzyme, time and temperature were required. Three repeats were performed at this condition and the average results are presented in Table 3.

Samples were also prepared with Cellosolve enzyme using the same treatment conditions as used for Denifade.

The effect of the two cellulase enzymes on various properties of 34s single knitted rib fabric and 20s combed sinker fabric are also given in Table 3. It is apparent from this table that different cellulase enzymes result in different properties, probably depending upon their chemical composition. However, Denifade enzyme shows better results as compared to Cellosolve enzyme. It is also apparent from this table that it is not possible to compare the properties of the two fabrics because of their different structures. In this case also, Denifade enzyme shows better results as compared to Cellosolve enzyme for 34s single knitted rib fabric and 20s combed sinker fabric.

3.4 Dye Uptake

The preliminary studies of dye uptake was carried out on 34s single rib fabric and 20s combed sinker knitted fabric. The results of the K/S measurements are given in Table 5. The results indicate that enzyme treatment results in higher K/S value. This shows that the enzyme-treated samples appear darker. In the case of Denifade-treated sample, there was an increase of approximately 13-17% in K/S value, while in the case of Cellosolve, the increase was 6-10%. Further studies are required to confirm this aspect.

3.5 SEM Studies

Scanning electron micrographs of the untreated and enzyme-treated (Denifade- and Cellosolve-treated under optimized conditions) samples are shown in Fig. 10. It is apparent from these micrographs that the untreated sample has more protruding microfibrils and fuzzy appearance than the Denifade- and Cellosolve-treated samples. The Denifade enzyme treated samples have more clear surface than Cellosolve enzyme treated samples.

4 Conclusions

4.1 Cellulase treatment of cotton knits reduces the surface hairiness of the fabric such that a carded fabric may appear like a combed one.

4.2 The cellulase treatment results in the decrease in the stiffness of the fabric which gets reflected in the decrease in the bending length of the fabric.

4.3 The wetting and air permeability properties also improve after cellulase treatment.

4.4 Dyeing with reactive dyes after enzyme treatment results in a deeper shade. It may be due to surface modification of knitted fabric. More studies are required to confirm this result.

4.5 For 34s single knitted rib fabric, treatment with 10% owf Denifade enzyme at 50°C for 45 min is recommended. About 4-6% weight loss accompanied with 6-9% strength loss results in improved surface and fabric properties by using the recommended conditions.

4.6 The Cellosolve enzyme was also used on 34s single knitted rib fabric using the same treatment conditions. Better results were obtained with Denifade enzyme as compared to Cellosolve enzyme.

4.7 Better results were obtained with Denifade enzyme as against Cellosolve enzyme when used on 20s combed sinker knitted fabric.

References