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〈論　　文〉

DEGUMMING OF SILK FABRIC WITH AN ENZYME DEGUMMASE

SHEETAL CHOPRA, SAMIR GARG and M. L. GULRAJANI
IIT, Delhi, India.

Abstract

Degumming with enzyme Degummase can be a viable alternative to the existing methods of degumming. Degumming, carried out with the conventional method of Marseille's soap 25% (owf) for 1.5h at boil gives a weight loss of about 24%. On comparison, degumming with 10% enzyme at 50℃ for 60 min gives a weight loss of about 22%. Treatment with enzyme reguires a much lower temperature and also the treatment time is shorter than the conventional process. Processing of silk at these conditions is likely to retain the lustre and softness of silk. To economise on the quantity of enzyme, conditions were optimised for a pad-store process. An enzyme concentration of 0.5% at 50℃ for a time period of 5~6h resulted in a weight loss of 22%. Though the concentration of the enzyme used was 20 times less than that used for long liquor method there was some strength loss. At 50℃ treatments carried out for 7~8 h resulted in some degradation. Strength loss occurs because enzyme in the absence of any stirring keeps acting at the place where it is deposited. Effect of temperature was more pronounced than time in degrading silk.

Key words : Silk degumming, enzyme.

INTRODUCTION

The specific action of the proteolytic enzymes for degradation of proteins and polypeptides forms the basis of their use as degumming agents on silk.

Enzymes are high molecular weight bioorganic catalysts. Unlike other chemical entities enzymes are characterised primarily on a functional basis. An enzyme is identified by what it does rather than its chemical composition since all enzymes are basically proteins. They are highly specific both in the reaction catalysed and in their choice of reactants which are called substrates. The enzyme molecule consists of a known chain of amino acids with a particular geometric conformation. Some amino acids in the protein structure of the enzyme form the active sites and this is where the substrate attaches itself to the enzyme molecules. Catalytic ability and specificity are thus the hallmarks of enzymes.

The protein nature of the enzymes explains many of its problems and properties. The protein nature and hence the catalytic properties are affected by temperature and pH changes, water activity, ionic strength and similar variables. The same factors which affect the ability of a protein to function as an enzyme also affect the manner in which it acts as an enzyme.

For proteins a special class of enzymes are used termed as proteases. These enzymes can hydrolyse the peptide bond (-CO-NH-) of proteins or protein fibres. On the basis of the conditions in which they are most active, protease are classified into three types; alkaline, acidic and neutral proteins. Some enzymes are specific in their action in the sense that they recognise and attack the amide linkages attached to only certain amino acids. The enzyme selected for the specific cleavage of a large polypeptide in general produces small peptides.

For degumming of silk three main types of enzymes have been used viz. trypsin, papain and bacterial enzymes. Processes of degumming silk with these have been recently reviewed (Gulrajani, 1992).

Degummase is a bacterial protease produced by a strain of *Bacillus subtilis*. It is a serine endopeptidase which acts on peptide bonds in the interior of the chain. Activity of an enzyme determines the concentration at which the enzyme is to be used. The use of Degummase isolated from *B. Cereus* has been investigated (Katagiri, 1941) for its hydrolytic action on sericin and other proteins.

In the present study an attempt has been made to evaluate enzyme Degummase as an alternative degumming agent and conditions have been optimised for its use using a long liquor method. A pad-store method has also been investigated and the results obtained by the two methods have been compared.

MATERIALS AND METHODS

Raw mulberry silk used for the study had the following specifications:

Ends/cm	–	49
Picks/cm	–	44
Area density	–	0.0045 g/cm^2
Warp denier	–	49.09
Weft denier	–	40.91

The degumming formulation consisted of:

(a) Enzyme (Degummase 1000 L manufactured by Advanced Biochemicals Ltd., Bombay). Activity of the enzyme was 800 Au/mg. Concentrations of the enzyme used are given in Table 1.

(b) Surfactant (Sandopan DTC) – 1 gpl

(c) Alkaline buffer – The stock solution was prepared by mixing 25 ml of 0.1 M sodium carbonate and 475 ml of 0.1 M sodium bicarbonate. pH obtained was around 8.75.

The degumming by the long liquor method was carried out in Atlas Launderometer and for the pad-store method, padding was carried out on padding mangle. After padding samples were wrapped in a polythene and stored at a constant temperature in the Launderometer.

1. Long Liquor Method

Fabric samples of 30 cm × 100 cm were cut and conditioned at 30% RH for 48 h. The conditioned samples were weighed and the treatment was carried out maintaining a M : L ratio of 1 : 30. The degummed samples were washed thoroughly, dried and conditioned.

The experiments were carried out according to the Box and Behnken Response Surface Design. The Box and Behnken Response Surface Design is used for experimenters with three independent variables, with three levels of each factor. The total number of test runs required are 15, instead of 27 (3^3). 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 of the design.

The parameters selected as independent variables were :

χ_1 = Concentration
χ_2 = Time
χ_3 = Temperature

The levels of the variables and treatment conditions are given in Tables 1 and 2, respectively. The dependent variables studied (i.e. responses) were,

y_1 = weight loss%
y_2 = crease recovery angle
y_3 = bending length (warp) cm
y_4 = bending length (weft) cm
y_5 = strength loss% (warp)
y_6 = strength loss% (weft)
y_7 = elongation-at-break (warp) cm
y_8 = elongation-at-break (weft) cm

Table 1. *Variables and their levels used in the experimental plan for degumming with enzyme degummase*

Variable	Level		
	+1	0	−1
X_1 (% owm)	18	10	2
X_2 (min)	90	60	30
X_3 (°C)	70	60	50

Table 2. *Conditions of 15 experiments according to box and Behnken Response Surface Design*

Case	Conc. (%)	Time (min)	Temp. (℃)
1	2	30	60
2	18	30	60
3	10	30	70
4	10	30	50
5	18	60	70
6	2	60	70
7	18	60	50
8	2	60	50
9	10	90	50
10	10	90	70
11	18	90	60
12	2	90	60
13	10	60	60
14	10	60	60
15	10	60	60

A quadratic polynomial was used to analyse the relation of each response with the three independent variables.

$$y = b_0 + \sum b_i\chi_i + \sum b_{ii}\chi_i + \sum b_{ij}\chi_i\chi_j$$

where b_0, b_i, b_{ii} and b_{ij} are the coefficients of the regression equation and i, j are integers. y is the response or dependent variable

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

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

2. Pad-store Method

For the pad-store method preparation of samples and their conditioning was done as for the long liquor and the treatment was carried out using the following conditions.

Degummase – 0.5%
$NaHCO_3$ – 5 gpl
Nonionic – 1 gpl
Surfactant

The samples were padded with this solution keeping 100% expression and stored for required time period. Subsequently they were washed thoroughly, dried and conditioned.

Initially treatments were carried out by varying temperatures (50, 55, 60℃) and time periods (60, 90, 120 min). Later more experiments were carried out for even longer time periods upto 8 h at a constant temperature of 50℃ and upto 6 h keeping the temperature constant at 60℃.

The treated samples were tested for change in mechanical properties. SEM studies were carried out to assess the damage caused during the treatment.

3. Test Methods

Weight loss (an indirect indication of degumming) was determined by measuring the difference in weight between the raw and the degummed samples.

The Instron Universal Testing System No. 4202 was used for assessment of tenacity and elongation of the samples.

Bending length of the degummed samples was determined using Shirley Fabric Stiffness Tester.

Shirley Crease Recovery Tester was used for measuring crease recovery angle.

Cambridge Stereoscan was used to obtain the scanning electron micrographs.

RESULTS AND DISCUSSIONS

1. Long Liquor Method

The best fitted regression equations for the various properties studied, with the adjusted multiple regression coefficient (R^2), standard error of estimate (S.E.) and F-ratio are given in Table 3. The observed and calculated values of all the 15 cases of Box and Behnken Response Surface Design are given in Table 4. The calculated values from the equations for all the possible 27 cases are given in Table 5.

It is apparent from Table 3 that weight loss ($\hat{Y}_1$) is affected by all the three independent variables investigated. A good correlation was also observed between weight loss and the process parameters. Spatial diagrams of the response surface were

Table 3. *Equations for different responses*

Regression equations	$\bar{R}^2$	S.E.	F-ratio
$\hat{y}_1 = 209.189 + 2.998\chi_1 + .351\chi_2 + 7.472\chi_3 - .034\chi_1^2$ $.002\chi_2^2 - .067\chi_3^2 - .007\chi_1\chi_2 - .022\chi_1\chi_3$	0.888	2.832	14.861
$\hat{y}_2 = 586.788 + 2.569\chi_1 + .272\chi_2 + 30.964\chi_3 - .290\chi_3^2$	0.811	17.075	16.061
$\hat{y}_3 = 14.1 - .275\chi_1 - .43\chi_3 + .004\chi_1^2 + .004\chi_3^2 + .002\chi_1\chi_3$	0.778	0.372	10.790
$\hat{y}_4 = 18.972 - .216\chi_1 - .044\chi - .546\chi_3 + .005\chi_3^2 + .003\chi_1\chi_3$	0.818	0.387	11.504

Table 4. *Observed and calculated responses*

Case	Conc (%)	Time (min)	Temp. (C)	Weight loss %		CRA		Bending length (warp) (cm)		Bending length (Weft) (cm)	
				Obs.	Calc.	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.
1	2	30	60	5.68	9.461	213.2	240.350	3.38	2.41	3.47	4.620
2	18	30	60	22.71	22.069	290.8	281.454	1.66	1.21	1.96	4.044
3	10	30	70	1.34	3.361	197.2	193.452	3.15	2.65	3.86	5.972
4	10	30	50	18.72	19.121	274.3	270.262	1.70	1.25	2.29	3.692
5	18	60	70	4.05	7.079	208.6	222.254	3.43	2.47	3.85	6.704
6	2	60	70	2.26	1.351	204.7	181.150	3.31	3.35	4.02	6.800
7	18	60	50	22.48	26.359	292.4	298.974	1.49	3.75	1.73	3.344
8	2	60	50	13.81	13.591	254.4	257.870	2.12	2.27	2.70	4.400
9	10	90	50	21.83	21.581	293.8	286.582	1.52	1.25	1.70	4.052
10	10	90	70	2.33	5.821	198.7	209.862	3.37	2.65	4.08	7.532
11	18	90	60	22.03	21.169	296.6	297.774	1.54	1.21	1.73	5.044
12	2	90	60	12.06	15.281	251.7	256.670	2.60	2.41	3.33	5.580
13	10	60	60	18.85	20.971	261.3	269.062	2.15	1.55	2.97	4.812
14	10	60	60	18.24	20.971	272.3	269.062	1.99	1.55	2.53	4.812
15	10	60	60	21.63	20.971	300.6	269.062	1.55	1.55	1.87	4.812

drawn based on the data from Table 3 and are reproduced in Fig. 1. It is evident from the figure that the effect of concentration and temperature are very pronounced, while time of treatment seems to have little effect on weight loss. Weight loss increases with increase in concentration. The increase is much more at lower temperature (50°C) than at 70°C. On the other hand with increase in temperature, weight loss decreases. At a lower concentration of the enzyme, as the temperature is increased upto 60°C, there is increase in weight loss but increasing the temperature further to 70°C results in considerable reduction in weight loss. The same trend was seen for treatments carried out for different time periods.

The extent of degumming rapidly falls between 60°C and 70°C. This may be due to accelerated denaturation of enzyme proteins at high temperatures.

The crease recovery angle ($\hat{y}_2$) is also affected by the three independent variables (Table 3). Effect of temperature is most pronounced while CRA is affected least by variations in time. Spatial diagrams of the response surface generated from the data obtained from the equation (Table 3) are given as Fig. 2.

CRA improves initially with temperature but reduces at higher temperature. With increase in concentration though, CRA increases but the increase is not much. With temperature, CRA increases upto 60°C but as temperature is increased further a decrease in CRA is seen. The effect of

Table 5. *Calculated values for various responses for all 27 cases*

Case	χ_1	χ_2	χ_3	y_1	y_2	y_3	y_4
1	2	60	60	14.171	248.510	2.406	5.100
2	2	60	50	13.591	257.870	2.266	4.400
3	2	60	70	1.351	181.150	3.346	6.800
4	2	30	60	9.461	240.350	2.406	4.620
5	2	30	50	8.881	249.710	2.266	4.220
6	2	30	70	3.359	172.990	3.346	6.020
7	2	90	60	15.281	256.670	2.406	5.580
8	2	90	50	14.701	266.030	2.266	4.580
9	2	90	70	2.461	189.310	3.346	7.580
10	10	60	60	20.971	269.062	1.550	4.812
11	10	60	50	22.151	278.422	1.250	3.872
12	10	60	70	6.391	201.702	2.650	6.752
13	10	30	60	17.941	260.902	1.550	4.332
14	10	30	50	19.121	270.262	1.250	3.692
15	10	30	70	3.361	193.542	2.650	5.972
16	10	90	60	20.401	277.222	1.550	5.292
17	10	90	50	21.581	286.582	1.250	4.052
18	10	90	70	5.821	209.862	2.650	7.532
19	18	60	60	23.419	289.614	1.206	4.524
20	18	60	50	26.359	298.974	0.746	3.344
21	18	60	70	7.079	222.254	2.466	6.704
22	18	30	60	22.069	281.454	1.206	4.044
23	18	30	50	25.009	290.814	0.746	3.164
24	18	30	70	5.729	214.094	2.466	5.924
25	18	90	60	21.169	297.774	1.206	5.004
26	18	90	50	24.109	307.134	0.746	3.524
27	18	90	70	4.829	230.414	2.466	7.484

time of treatment is not appreciable.

The relation between the treatment parameters and bending length in Table 3 shows that while the bending length in warp direction ($\hat{y}_3$) is independent of the time of treatment within the time periods investigated, the bending length in the weft direction ($\hat{y}_4$) is affected by all the three parameters investigated. However, the contribution of time of treatment is marginal even in the later case.

The spatial diagrams of the response surfaces in Figs. 3 and 4 are almost similar and indicate that as the concentration of enzyme increases the bending length decreases, having lowest value at 50°C and 18% enzyme conc. On the other hand as the temperature of treatment goes up from 50°C to 70°C the bending length goes on increasing irrespective of the enzyme conc. as stated above, at higher temperatures the weight loss is low hence the fabric

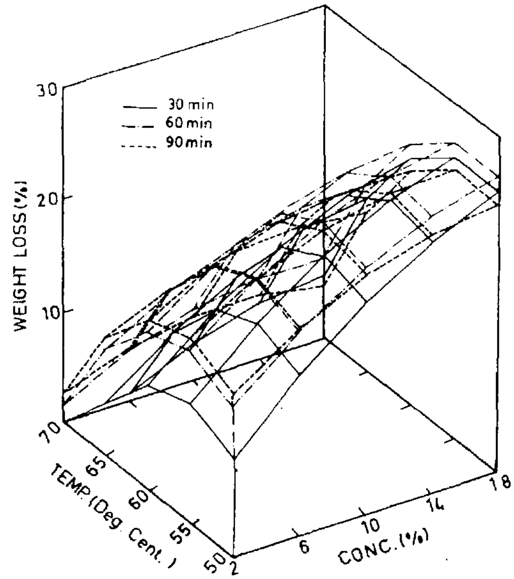

Fig. 1. *Spatial diagrams of weight loss against temperature and concentration for 30, 60 and 90 min treatment times.*

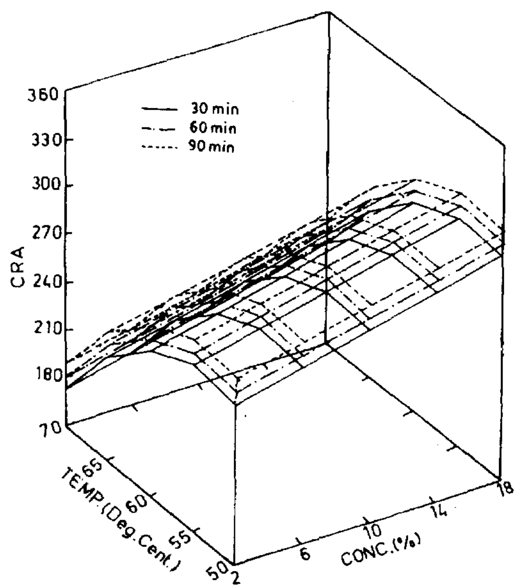

Fig. 2. *Spatial diagrams of Crease Recovery Angle (CRA) against temperature and concentration for 30, 60 and 90 min treatment times.*

may be stiffer. This observation is further substantiated by the fact that there is a linear relationship between weight loss and bending length as indi-

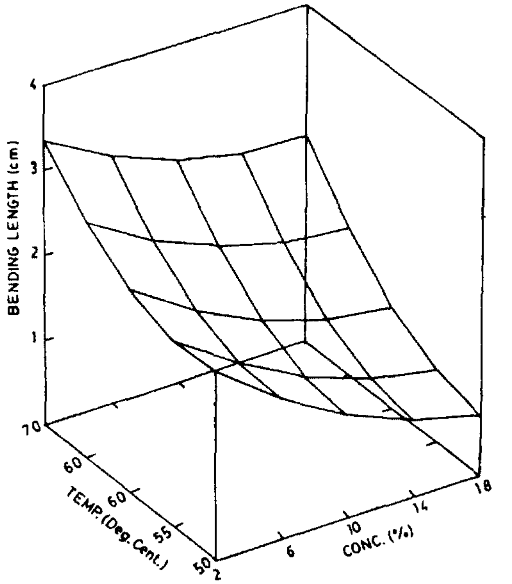

Fig. 3. *Spatial diagram of bending length (warp) against temperature and concentration.*

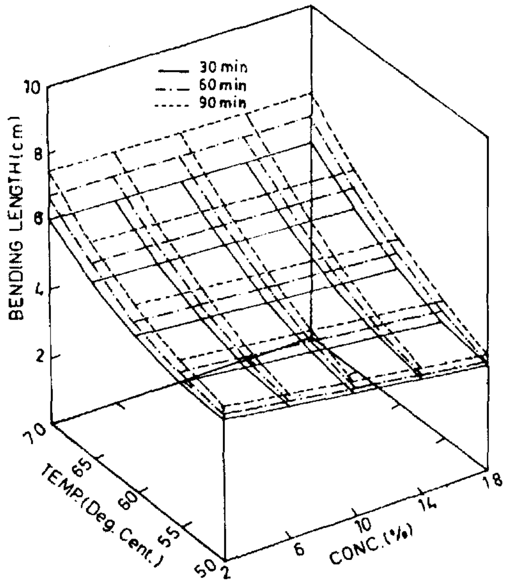

Fig. 4. *Spatial diagrams of bending length (weft) against temperature and concentration for 30, 60 and 90 min treatment times.*

cated later in this paper.

Poor correlation was observed between the influencing factors and the tenacity as well as elongation-at-break in both the warp and weft directions. Hence no best fit models were obtained.

The poor correlation shows that these responses also depend on factors other than the parameters studied. These can be the weave of the fabric, the type of fibre or the crimp in the yarn.

2. Interaction Between Responses

The change in various properties of degummed silk can be studied as dependent on the weight loss obtained on degumming. Hence regression analysis was carried out with weight loss as the independent variable and the various properties studied as dependent variables.

1) Bending Length vs. Weight Loss

Bending length in warp and weft direction showed excellent correlation with weight loss. This is logically consistent with the fact that with the increasing weight loss fabric becomes softer due to removal of outer sericin layer. The regression equations are as follows :

$$y_3 = 3.592 - 0.091y_1 \text{ (warp)}$$

$$\bar{R}^2 = 0.944 \text{ ; S.E.} = 0.186 \text{ ; F-ratio} = 238.534$$

$$y_4 = 4.234 - 0.1034y_1 \text{ (weft)}$$

$$\bar{R}^2 = 0.915 \text{ ; S.E. } 0.264 \text{ ; F-ratio} = 151.953$$

Fig. 5 shows how the bending length (warp and weft) varied with change in weight loss.

2) CRA vs. Weight Loss

Crease recovery angle shows excellent correlation with weight loss. The regression equation obtained is

$$y_2 = 190.307 + 4.596y_1$$

$\bar{R}^2$ of 0.976, S.E. of 6.145 and F-ratio of 560.131 is obtained. Fig. 6 shows the relation of CRA with weight loss.

Strength loss and elongation-at-break showed poor correlation with weight loss.

3. Optimization

In order to find out the optimum conditions of degumming of silk with Degummase, the estimated values of all 27 cases of these properties which showed excellent correlation with the process pa-

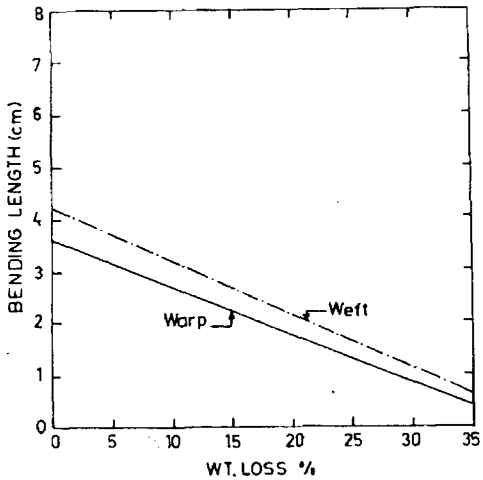

Fig. 5. *Plot of bending length (warp and weft) against weight loss*

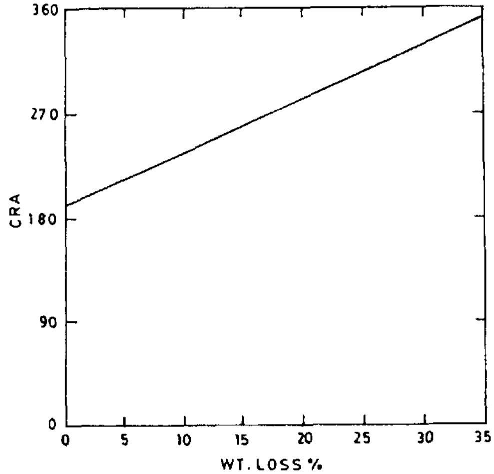

Fig. 6. *Plot of Crease Recovery Angle (CRA) against weight loss.*

rameters ($R^2 \geq 0.800$) were ranked. These were weight loss, bending length (weft) and crease recovery angle. In case of bneging length, 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 condition. The cases with expected weight loss greater than 22% in order of decreasing sum of ranks are :

1. 18% 60 min 50℃
2. 18% 30 min 50℃
3. 10% 60 min 50℃
4. 18% 30 min 60℃
5. 18% 60 min 60℃

Since the concentration of the enzyme is very high in the first, second, fourth and fifth cases, only the third case presents a viable alternative. It is also economical in terms of lower enzyme concentration comparatively and also process time.

Three repeats were performed at this condition and the average results obtained are presented in Table 6.

Table 6. *Results of the experiments performed under optimum conditions compared with control sample*

Property	Avg. value	Control sample
1. Weight loss (%)	21.8	-
2. Bending length (cm)		
Warp	1.665	3.20
Weft	2.13	3.28
3. Stress at break (kgf/g)		
Warp	70.45	64
Weft	79.41	76.12
4. Elongation at break (cm)		
Warp	1.120	1.322
Weft	0.9386	0.972
5. CRA	245.5	192.5

4. Pad-store Method

The above-mentioned studies show that a minimum concentration of 10% (owf) enzyme is required to degum this variety of silk. In order to work out the possibility of degumming with lower concentrations of enzyme experiments were performed by padding the fabric with enzyme solution and storing it at different temperatures for varying duration of time. The preliminary studies indicated that an enzyme concentration of 0.5% (owf) was sufficient to achieve reasonable amount of gum removal.

The results of this study carried out at three different temperatures for time periods varying from 30 to 120 min, in Table, show that a maximum of 19.95% weight loss is achieved by degumming for 120 min at 60℃. Moreover, this treatment results in about 38% strength loss.

It seems that during storage localized action of enzyme takes place resulting in the damage to the fibroin. In order to substantiate this observation further experiments were carried out by storing the enzyme padded fabrics at 50 and 60℃ for longer duration. The results of the experiments, in Table 8, confirm the earlier findings.

Pad-store process involves close contact of the enzyme with the fabric without any agitation, thus the enzyme keeps acting at the same place for the time the sample is stored. Thus an accompanied strength loss was observed even for treatments carried out for 60 min at 50℃. Degradation of the samples increased by increasing the temperature of treatment to 60℃. This was observed in the form of fibrillation of the silk filaments at certain localised areas.

The results of this study show that by padding silk with 0.5% enzyme solution and storing it for 120 min at 50℃ a weight loss of about 18.64% can be achieved without loss in strength.

5. SEM Studies

Scanning electron micrographs of the original and degummed samples (long liquor as well as pad-store) are reproduced in Fig. 7. The raw silk contains sericin which is seen as deposits on the surface of the filaments (Fig. 7a). Degumming by the long liquor method at the optimum conditions results in removal of most of the deposits and the filaments as is apparent from the electron micrograph in Fig. 7b. Pad-store treatment of the fabric at 50℃ for 2 h also resulted in a considerable removal of gum (Fig. 7c). However, treatment at 60℃ affects the silk also. Samples degummed for 2 h at this temperature showed signs of fibrillation at certain points where individual fibrils could be detected but were not separated (Fig. 7d). Treatment at 60℃ for even longer time period 3 h resulted in more fibrillation (Fig. 7e). This was also confirmed by observing accompanied strength loss. Treatment for still higher time of 5 h at this temperature resulted in more degradation (Fig. 7f).

Table 7. *Results of experiments of pad-store method*

S.No.	Time (min)	Temp. (℃)	Wt. loss(%)	Bending L (Weft)(cm)	Bending L (Weft)(cm)	CRA (o)	Br. elong. Warp(cm)	Br. elong. Weft(cm)	Br. stress Warp(kgf/g)	Br. Stress Weft(kgf/g)
1	30	50	12.54	2.012	2.882	225.9	1.450	1.077	73.45	83.69
2	30	55	14.83	1.8954	3.052	222.7	1.229	0.758	52.29	71.78
3	30	60	16.06	1.655	2.600	247.9	0.9687	0.761	63.03	78.79
4	60	50	15.81	1.8675	2.705	247	1.137	0.871	65.35	82.89
5	60	55	15.35	1.7325	2.715	263.1	1.113	0.745	53.5	70.47
6	60	60	18.65	1.5725	2.115	288.2	0.7727	0.428	49.68	58.56
7	120	50	18.64	1.705	2.492	261.5	1.023	0.783	66.05	80.74
8	120	55	18.84	1.5975	2.160	277.7	0.824	0.582	49.31	69.24
9	120	60	19.95	1.4575	1.742	294.5	0.607	0.332	39.78	46.52
	Control			3.20	3.88	192.5	1.322	0.972	64.00	76.12

Table 8. *Results of experiments performed for longer time periods*

S.No.	Time (min)	Wt. loss (%)	Stress Warp (kgf/g)	Stress Weft (kgf/g)	Br. elong. Warp (cm)	Br. elong. Weft (cm)	Str. loss Warp (%)	Str. loss Weft (%)
50℃								
1	420	21.58	50.63	64.81	0.767	0.760	20.89	14.85
2	480	20.72	51.16	54.65	0.787	0.772	20.06	21.63
60℃								
1	240	22.29	33.73	33.52	0.485	0.270	47.29	55.16
2	300	22.17	37.27	40.86	0.606	0.304	41.76	46.32

Figure 7. *Scanning electron micrographs of undegummed and degummed silk sample*
(a) X1500 Raw silk, (b) X1500 Long liquor optimum, (c) X1500 Pad-store method, 50℃ for 2 h, (d) X1500 Pad-store method, 60℃ for 2 h, fibrillation observed, (e) X1500 Pad-store method, 60℃ for 3 h, fibrils start coming out, (g) X1500 Pad-store method, 50℃ for 8 h, fibrils coming out.

Samples degummed at 50℃ for 8 h show only slight degradation as can be seen from Fig 7g.

摘 要

酸素精練으로 기준 정련방법이 대체될 수 있다. 마르세일비누(25% o.w.f)로 1.5시간 동안 煮沸하는 기존 방법으로 정련하는 경우 練減率이 24% 내외인데 비해 효소(10% o.w.f)로 50℃에서 1시간 精練하면 22%의 練減率을 얻었다. 酸素精練은 기존 방법에 비해 처리온도를 현저히 낮출 수 있고 처리시간을 단축할 수 있으며 이러한 방법으로 실크의 광택과 부드러운 촉감을 얻을 수 있었다.

酸素의 처리량을 절감하기 위한 Pad-store 방법이 구명되었는데 이 방법에서 효소처리 농도를 0.5%로 낮추고 50℃에 5~6시간 精練하면 練減率이 22%로 되었다. Pad-store 방법은 위의 용액처리 방법에 비하여 효소처리량을 20배 절감할 수 있었지만 처리絹의 强力이 감소되므로서 50℃에서 7~8시간 처리로 精練絹의 脆化가 일어난것으로 보인다. Pad-store 방법에서 絹의 强力이 감소된것은 精鍊과정중 효소가 작용하는 부위에서 교반작용이 없기 때문이며 실크의 脆化는 시간보다 온도의 영향이 큰 것으로 생각된다.

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