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Enzymatic treatment on cooking and reeling of muga silk (*Antheraea assama*) cocoon

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ABSTRACT

Cocoon is a protective shell made up of a continuous protenious filament spun by the mature silk worm prior to pupation. Cooking of cocoon is essential to secure adequate quantity of reeling ends to reach out a composite thread of high cohesion and size of the yarn. Cooking of muga cocoon with pure papain of concentration 0.05 % and Na_2CO_3, 0.20 % showed highest breaking load while cocoon treated with latex of concentration 0.05 % extracted from fresh green papaya and Na_2CO_3 0.15 % produced yarn of highest breaking load.

Key words : Enzyme, papain, Muga cocoon, Cooking and Reeling, *Antheraea assama*

The history of textile is an integral part of the history of civilization. There are many fibrous structures in nature but only few which have been classified as textile fibres. The chief natural fibres now in use are cotton, linen, wool and silk. Silk is produced by cultivated silkworm and was at one time the most priced of all the textile fibres. Traditionally, it originated in China about 2500 BC. According to Chinese legend, *si-ling-chi*, a Chinese emperor was the first to rear silkworm and spun silk thread, she made a rope of silk for her husband using the thread.

The north eastern region of India occupies an important position on account of its unique faunal and floral wealth. The climate is subtropical. The congenial atmosphere has made the region the natural home for many varieties of insects, moths and butterflies, particularly certain serigenous insects as well as there corresponding host plants. Therefore, the region can be called naturalistic paradise. The congenital atmosphere helps the healthy growth and development of the sericulture industry which covers mulberry, oak, tassar, eri and muga culture. Muga culture is predominant in this region and it is unique and confined particularly to the Brahmaputra valley. Muga silk is golden yellow in colour which makes it very attractive. No other silk has such unique colour in the natural state.

The scientific name of muga silk worm is *Antheraea assama* Westwood, belongs to Lepidoptera, family-Saturniidae. The muga is assiduously practised in the district of upper Assam and also in certain parts of lower Assam in a smaller measure. The important commercial muga growing areas are North Lakhimpur, Dhemaji, Dibrugarh, southeast of Sibsagar and south of Jorhat and chiefly reared by the Ahom community.

The muga silkworm is polyphagous and thrives on various endemic plants mostly belonging to the family Lauraceae. The commonest laurel is "som"(*Machilus bombycina or odoratissma*) in upper Assam and "sualu" (*litsaea polyantha*) used in lower Assam, are primary host plants. The secondary host plants for muga silkworm are mejankari (*Litseae citrata*), chapa (*Magnolia sphenocarpa*) etc. Sericulture has been practices for a long time in India but only during last decade the country has earned a good reputation in silk production. Indian enjoys the world monopoly for the fabulously famed golden yellow coloured muga silk which is multivoltine in nature.

Cocoon is a protective shell made up of a continuous portentous filament spun by the mature silk worm prior to pupation. Due to environmental changes in different seasons and also due to changes in location, the life cycle of muga silk worm such as egg, larva, pupa, cocoon and adult moth may vary from brood to brood and race to race, thus affecting the quality and quantity of silk. Reeling cocoon has to be subjected to process of shifting with the object of killing pupa inside the cocoon so as to obtain the continuous filament after cooking of cocoon. Cooking of cocoon is essential to secure adequate quantity of reeling ends to reach out a composite thread of high cohesion and size of the yarn to produce high quality fabrics. The swelling of fibres helps to release the filament from the crossing and facilitates the smooth unwinding nature of filament. The extent of solubility of sericin, optimum condition of boiling at different stages are necessary prerequisites for securing high percentage of recovery of silk from cocoon shell.

Cocoon cooking is most difficult and complicated process as it affects the quality of silk. Cocoon cooking is the process of softening and swelling of cocoon to obtain silk filament and thereby reeled out a composite thread of high cohesion (Sundermurthy and Narasingh, 1991). The muga cocoon cooking is the most complicated process as it affected not only by machineries but also other conditions such as chemicals, temperature, pressure and qualities of water used for cooking. The sericin present in muga cocoon is very ferocious, thus it needs proper cooking to remove it from the fibroin, *i.e.*, the actual fibre part to produce the quality silk. During cooking the sericin gets hydrolyzed in different solvents making the reeling process easier (Kim, 1988). Hydrolysis of protein can be carried out by treatment with acids, alkali and enzymes. Acids are non specific, trend to attack very vigorously and the reaction is not easily controllable, hence, it is not used for degumming of silk. Alkali attacks both sericin and fibroin. However, the difference of rate of hydrolysis is large enough to permit the control of the reaction. Hydrolysis of sericin is based on certain amino acids to different extent (Gulrajoni, 1989). Therefore, the investigation was carriedout on cooking and reeling of muga silkworm cocoon (*Antheracea assama*) because a very few studies have been done on this aspect.

METHODOLOGY

Selection of crop:

The two commercial crops of muga cocoon *viz.* (kotia and jethua) breed were selected for study.

Grading of cocoon:

Cocoon purchased in lots were tested for its quality.

Sorting of cocoon:

Sorting of cocoon was done by following the method suggested by CSTRI (1983). The good and defective cocoons were sorted out from the lot purchased. This was done on a table with good illumination. The defective cocoon percentages were calculated following formula:

$$\text{Defective cocoon percentage} = \frac{\text{Nos. of the defective cocoon}}{\text{Nos. of the whole cocoon}} \times 100$$

Conditioning and preparation of sample:

Prior to testing, the cocoon shell and the silk skins collected from cocoon treated under the different cooking conditions were conditioned moisture equilibrium at 65$\pm$ 2% relative humidity (R.H) and 25$\pm$2^{0}C temperature as per standard ASTM (1987) and IS method (1972).

Individual cocoon testing:

The weight of the cocoon, length and breadth of cocoon, shell ratio percentage, were determined accurately as suggested by CSTRI (2003) for testing individual cocoon. The weight of single cocoon and single shell weight were found out by using electronic balance for getting accurate result. The shell ratio percentages were calculated as:

$$\text{Shell ratio percentage} = \frac{\text{Weight of single shell}}{\text{Weight of whole cocoon}} \times 100$$

Cooking and reeling of muga cocoon:

Cooking of muga cocoon is done. papain, a proteolytic enzyme. is used for cooking of cocoon (Gulrajoni and Gupta, 1989). An experiment was designed for cooking of muga cocoon which has not been carried out by any researcher. This has been performed with the cocoon (jethua brood) of Jorhat district only to find out the efficiency of papain to cook the cocoon. The methods are as follows-

Pure papain:

The purified enzyme"papain", of High media, India of different concentrations (0.05%, 0.10 % and 0.15 %) was used to cook the cocoon along with low concentration of mild alkali (Na_2CO_3). Papain has been widely recommended for sericin removal process but its action was specific as it attacked on certain amino acids. Therefore sericin removal of silk with papain required a pretreatment to swell the sericin, which gave a satisfactory removal of sericin. Mild alkali in low concentration was used along with the papain (Gulrajoni and Gupta, 1989).

One hundred muga cocoons were boiled in an alkaline solution prepared from sodium carbonate and enzyme by keeping the material to liquor ratio 1:20. Cooking gas LPG was used for cooking or boiling of muga cocoon. When the solution reached the boiling point, the flame was lowered and kept at simmer till the fibre was easily traced out from the cocoon and the temperature at this stage was around 95^{0}C. The cocoons were stirred at 5 minutes of interval with the help of wooden spoon which helped in even cooking. The lid of the saucepan was used to cover the container during cooking to prevent excessive evaporation of water.

The general recipe followed for cooking is as below:

– Concentration of sodium carbonate solution- 0.10 %, 0.15 %, 0.30 % and 0.35% (on the basis of average wt. of whole cocoon).

– Water for cooking –pond water.

– Container for cooking – stainless steel of 5 litre capacity and of sandwich bottom was used.

– M:L - 1:20.

– Temperature of the cooking bath - 95^{o}C to boil.

– Enzyme -0.05 %, 0.10 % and 0.15 %.

Papain extracted from the latex of fresh papaya:

The latex of fresh papaya contains considerable amount of papain. Fresh and matured fruits of papaya were slitted diagonally several times and the fresh latex secreted from the slitted area was collected in an aluminum foil. The fresh latex containing papain was weighed and used in different concentrations to cook the cocoon along with lower concentration (Ramón, 1970). The remaining procedure of cooking was similar to above method.

Reeling of cocoon:

The cooked cocoons were reeled by the expert reelers in CSTRI power and pedal operated cum twisting machine in warm water (40^{0}C – 45^{0}C) was used during reeling material to liquor ratio 1:20.

Raw silk was reeled by collecting six ends of cocoon to make a filament yarn (FAO, 1972). The reeled yarn was again reeled in Approurvette to prevent from entangle of yarn and to get accurate length.

Data recorded during cooking and reeling of cocoon:

Cooking time:

Easy trace of fibre from the cocoon was possible when it cooked properly. Cooking time was recorded in minutes with the help of watch for each experiment.

pH of cooking media:

The pH of cooking media before and after cooking was recorded by pH meter (Perkin-Elmer) for each cooking experiment.

Dropping percentage:

The dropping percentage was calculated from the following relationship:

$$\text{Dropping percentage} = \frac{\text{No. of drops}}{\text{Total no. of cocoons}} \times 100$$

Coking efficiency:

The cooking efficiency was calculated from the following formula :

$$\text{Cooking efficiency (\%)} = \frac{\text{No. of cocoons from which filaments were traced out}}{\text{Total no. of cocoon cooked}} \times 100$$

Percentage ratio of raw silk to cocoon weight:

The percentage ratio of raw silk to cocoon was found out each set of cooking experiment (TBTS, 1975).

Observations during reeling:

Yarn breaking – Number of breaks of yarn during reeling were recorded.

Assessment of physical properties of fibre and yarn:

The muga silk fibre and yarn reeled from different cooking conditions were assessed for their physical properties.

Denier (size) of muga yarn :

The denier of the raw silk yarn was calculated as per IS method (1942— 1972) by using the formula :

$$\text{Denier} = \frac{\text{Total weight of reeled yarn (g)}}{\text{Total length of reeled yarn}} \times 100$$

Breaking load and per cent elongation:

It was performed according to ASTM procedure (D 2256 - 1987) using computer aided textile strength testing machine instron 1122. The gauge length used was 254 mm, cross head speed (100mm/min). The breaking load, the per cent elongation at the time of rupture, load elongation curve for different tests of each sample were found out from the computer attachment with the instrument.

Analysis of load vs elongation:

This was done by calculating load at varying elongations. The graphs were plotted and analyzed.

Tenacity of year:

Tenacity of year was calculated from the breaking load and denier of yarn (ASTM D 2256 -1987).

$$\text{Tenacity g/d} = \frac{\text{Breaking load (g)}}{\text{Denier (d)}} \times 100$$

Evenness of yarn :

The evenness (U %) was calculated as per ASTM procedure (D – 1425 1987) using Uster evenness tester model B-type. The test was performed at a speed of 25 meter /min and evaluation time was 150 seconds.

Colour of muga silk yarn :

The colour and luster of muga silk yarn treated under different cooking conditions were evaluated for its golden yellow colour using colour graph. The wave length used for the purpose was 610 nm (t max). Colour coordination of different yarn samples were found out and the result was recorded as follows:

L= Total Reflectance

B=Degree of Yellowness

W=White Index / Brightness

Cs= Colour Strength

Cost and return from production of yarn /kg :

The cost of per kg yarn was estimated by considering the expenditure in different heads which was done in consultation with economist and then the return from production of yarn per kg was estimated.

RESULTS AND DISCUSSION

Cooking reeling of muga cocoon was done with mild alkali (Na_2Co_3) and enzyme (pure papain and latex from papaya). Muga cocoon of jethua brood from Jorhat was cooked separately in optimum concentration of Na_2Co_3 (0.15 % and 0.20 %) along with pure papain and latex from fresh papaya and reeled in CSTRI muga reeling cum twisting machine. The yarns from different conditions were tested for physical properties (Tables 1, 2 and 3).

Cooking and reeling of muga cocoon:

From the study conducted on cooking and reeling performances cocoon (jethua) of Jorhat district cooked under optimum concentration of Na_2Co_3 with pure papain/latex (different concentration) in pond water at boiling point 95°C. Following results were observed in Table 1 and presented below:

– Cooking time decreased with the increased concentration of both pure papain and latex. The cooking time required in latex was comparatively more than pure papain. The cause might be the deposition of mineral content of latex on the surface of the fibre.

– Dropping percentage of cocoon was negligible in lower concentration of papain. However, the increased concentration of alkali along with higher concentration of papain increased the dropping percentage. Cooking efficiency percentage also decreased with the increased concentration of papain as well as latex.

– The percentage of ratio of raw silk to cocoon weight was found to be very good. Percentage ratio of raw silk to cocoon weight was found more in pure papain than with latex. However, the increased concentration of either papain or latex decreased the percentage ratio of raw silk to cocoon weight. The reason might be due to frequent yarn breaking in higher concentration of papain or latex which attacked some specific amino acids vigorously and thereby more dissolution of sericin from the actual fibre part *i.e.* fibroin.

Physical properties of muga silk yarn:

The following results have been observed from Table 2.

– The denier of yarn varied between 24.43 to 30.77.The higher concentration of papain resulted in high denier. The reason might be the frequent breaking of yarn during reeling.

Table 1 : Comparative cooking and reeling performance of cocoon (jethua) of Jorhat district cooked under optimum concentration of Na_2Co_3 with pure papain/latex (different concentration) in pond water at boiling point 95°C

Name of enzyme	Conc. of Na_2Co_3 (%)	Conc. of papain (%)	Cooking time (min)	pH of both before	pH of both after	Dropping (%)	Cooking efficiency (%)	% ratio of raw silk to cocoon wt.	Yarn breaking
Pure papain	0.15	0.05	20	10.3	7.0	1	99	19.79	-
		0.10	15	10.3	7.2	2	98	17.89	+
		0.15	15	10.4	7.4	5	95	16.31	+
	o.20	0.05	15	10.2	7.5	1	99	19.09	-
		0.10	15	10.3	7.6	2	98	16.72	+
		0.15	10	10.6	7.8	1	99	18.83	+
Latex	0.15	0.05	22	10.2	7.2	-	100	16.12	-
		0.10	20	10.9	7.5	2	98	15.13	+
		0.15	20	10.9	7.6	3	97	15.18	+
	0.20	0.05	20	10.5	7.4	1	99	15.79	-
		0.10	18	10.5	7.6	4	96	12.51	+
		0.15	16	10.6	7.8	10	90	9.71	+

(+) = Breaking; (-) = No breaking

* Crude papain content in fresh latex of optimum condition (0.05g) was found to be 0.0024 ec mole (enzyme activity)

Table 2 : Effect of pure papain and latex on physical properties of muga yarn reeled from cocoon (Jethua cocoon) of Jorhat district

Sr. No.	Conc. of Na_2Co_3 (%)	Conc. of papain (%) latex	Denier	Breaking load (g)	Tenacity (g/d)	Elongation (%)	Unevenness U (%)
1.	Pure papain (0.15)	0.05	27.95	121.40	4.34	20.08	11.25
2.		0.10	29.33	117.83	4.02	18.18	12.18
3.		0.15	30.65	106.70	3.48	27.26	12.95
4.	Pure papain (0.20)	0.05	26.43	140.10	5.38	26.88	10.98
5.		0.10	28.79	119.10	4.14	29.49	11.28
6.		0.15	30.04	105.20	3.50	26.46	11.70
1.	Latex of papaya	0.05*	26.60	148.50	5.58	19.28	10.20
2.	(0.15)	0.10	28.26	102.44	3.62	29.86	12.48
3.		0.15	27.14	100.20	3.69	29.15	12.80
4.	Latex of papaya	0.05*	28.26	142.00	4.93	21.26	10.29
5.	(0.20)	0.10	30.77	110.15	3.58	27.20	12.84
6.		0.15	29.12	120.20	4.13	31.38	12.21

*Crude papain content in fresh latex of optimum condition (0.05 g) was found to be 0.0024 μ mole (enzyme activity).

Table 3 : Effect of pure papain and latex on colour coordinates of muga yarn reeled from cocoon (Jethua cocoon) of Jorhat district

Sr. No.	Conc. of Na_2Co_3 (%)	Conc. of papain (%) latex	Total reflectance	Degree of yellowness (b)	Degree of brightness (W)	Colour strength (CS) %
1.	Pure papain (0.15)	0.05	66.58	17.92	10.12	101.67
2.		0.10	70.36	17.01	5.01	100.71
3.		0.15	67.90	17.05	9.38	66.18
4.	Pure papain (0.20)	0.05	66.18	18.33	9.04	102.65
5.		0.10	63.73	17.53	8.69	73.25
6.		0.15	65.29	18.04	8.89	69.82
1.	Latex of papaya	0.05*	68.38	17.01	11.39	93.34
2.	(0.15)	0.10	67.47	17.92	10.84	87.26
3.		0.15	67.89	17.71	9.57	85.21
4.	Latex of papaya	0.05*	70.28	17.56	13.84	95.77
5.	(0.20)	0.10	67.21	17.81	5.84	94.65
6.		0.15	67.20	17.78	6.80	73.46

Crude papain content in fresh latex of optimum condition (0.05 g) was found to be 0.0024 μ mole (enzyme activity).

– The results of colour coordinates were not very satisfactory in respect of brightness (w) and colour strength (CS %). The colour strength decreased more with the increased concentration of papain or latex. However, effect of the pure papain was better than latex. Latex contains minerals other than papain. These mineral had a direct bearing on the luster and finish of silk (Sane *et al.,* 1994). The presence of minerals during cooking also caused frequent breakages of silk yarn during reeling, too much of dropping of cocoon and many other troubles (Shamachery, 1998).

Conclusion:

Cooking of muga cocoon with pure papain of concentration 0.05 % and Na_2CO_3, 0.20 % showed highest breaking load while cocoon treated with latex of concentration 0.05 % extracted from fresh green papaya and Na_2CO_3 0.15 % produced yarn of highest breaking load. The colour strength (CS %) and degree of brightness concentration of papain was better than latex. The mineral present in latex had a direct bearing in colour, luster and finish of silk.

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