International Journal of
Biological Chemistry

Possible-efficacy of 26 kDa *Antheraea mylitta* Cocoonase in Cocoon-cooking

¹J.P. Pandey, ¹P.K. Mishra, ¹Dinesh Kumar, ¹A.K. Sinha, ¹B.C. Prasad, ¹B.M.K. Singh and ²T.K. Paul

¹Silkworm Physiology Laboratory, Central Tasar Research and Training Institute (Central Silk Board), P.O. Piska-Nagri, Ranchi-835303, India
²Post Cocoon Technology Laboratory, Central Tasar Research and Training Institute (Central Silk Board), P.O. Piska-Nagri, Ranchi-835303, India

Corresponding Author: Dr. J.P. Pandey, Central Tasar Research and Training Institute (Central Silk Board), P.O. Piska-Nagri, Ranchi-835003, India Tel: 91-09431168530

ABSTRACT

Generally, *Antheraea mylitta* cocoons cooking is carried out in alkaline condition by using soap, soda, H_2O_2, etc., which adversely affects the natural beautiful colour and softness of tasar silk. At eclosion stage, the emerging adults of tasar silkworm, *A. mylitta* exude a proteolytic enzyme 'cocoonase' which helps in softening anterior portion of cocoon shell and facilitates emergence of moths. Interestingly, cocoonase directly acts on the sericin protein without affecting the fibroin protein. It evidently indicates that, sericin is excellent natural substrate of cocoonase. This natural phenomenon engenders an idea to collect the cocoonase of *A. mylitta* and investigate its possible-efficacy in cocoon cooking. The SDS-PAGE analysis of freshly collected cocoonase (from emerging moths) showed molecular weight around 26 kDa. A simple technique for cocoonase collection from freshly pierced cocoons has been developed. Cooking of cocoon in cocoonase is concentration, pH, temperature and time dependent. Low concentration (1:15, 1:20, 1:25, 1:30 and 1:35) increases the cooking time and decreases the cooking efficiency. Higher concentration (1:5 dilutions) minimises the cooking time and increases the cooking efficiency. But cocoons were not fully reeled due to hardness in inner portion of the cocoons. Initial boiling of cocoon in water for 30 min followed by cooking in cocoonase (1:5) at 35-40°C temperature and 8.5 to 9.0 pH yielded comparatively better cooking efficiency with 50-55% silk recovery. Yarn obtained from the cocoons cooked in cocoonase preserve natural beautiful unique tasar silk colour, softness and lustre.

Key words: *Antheraea mylitta*, cocoonase, tasar silk, sericin, fibroin, proteolytic enzyme

INTRODUCTION

The silk fibre produced by silkworm is a composite material formed by fibroin protein surrounded by sericin protein which accounts around 67-75 and 22-25%, respectively (Prasong *et al.*, 2009; Srihanam *et al.*, 2009; Mahmoodi *et al.*, 2010; Prasong, 2011). The *Antheraea mylitta* cocoons have the utmost capacity of silk production being the largest among all the other known non-mulberry silk producing insects (Akai, 2000). Tasar cocoons are comparatively harder than cocoons of other sericigenous insects' and it's cooking and reeling is relatively complicated. Several methodologies have been used by investigators (Singh *et al.*, 2003; Mondal *et al.*, 2007;

Nakpathom *et al.*, 2009; Mahmoodi *et al.*, 2010; Teshome *et al.*, 2011) for cooking of cocoons and silk degumming. Insect proteases are bioactive enzymes and they perform many functions during post-embryonic development of insects (Kanost and Clem, 2012). Several enzymes i.e., lipase, protease etc., has been tried for cocoon cooking and degumming of silk (Gulrajani *et al.*, 2000; Singh *et al.*, 2003; Nakpathom *et al.*, 2009). But as a ruling practice, cooking of tasar cocoon is generally used to perform in alkaline solution by using soap, soda, H_2O_2, alkali etc., which adversely affects the natural colour and softness of tasar silk. Hence, we need to explore a new technique for tasar cocoon cooking and enzyme based cocoon cooking technique might be probable solution. As enzymes are known for specific and mild action hence it is expected that enzyme based cocoon cooking will be useful in preserving natural colour and softness of tasar silk in comparatively eco-friendly manner. It is reported that, several sericigenous insects including *A. mylitta* exude a proteolytic enzyme cocoonase as they near the final stages of their metamorphosis (Felsted *et al.*, 1973a, b). This proteolytic enzyme makes anterior portion of cocoon soft which facilitates the moth exit from cocoon (Liu and Li, 2002; Wang *et al.*, 2005a, b; Yu-Dan, 2008; Wu *et al.*, 2008; Wang, 2008; Yang *et al.*, 2009). This natural phenomenon generates an idea if cocoonase is having capacity to soft anterior portion of cocoon during adult emergence, then it can be utilize for cooking of tasar cocoons. Most of the basic studies on cocoonase have been made on various aspects by number of researchers (Felsted *et al.*, 1973a; Liu and Li, 2002; Wang *et al.*, 2005a, b; Yu-Dan, 2008; Wu *et al.*, 2008; Wang, 2008; Yang *et al.*, 2009) without much correlation with its possible-efficacy in cocoon cooking. Hence, in the present study efforts has been made to develop a method for *A. mylitta* cocoonase collection to investigate possible-efficacy of cocoonase in tasar silk cocoon cooking.

MATERIALS AND METHODS

Rearing of *Antheraea mylitta* (Lepidoptera: Saturniidae): The larvae of tropical tasar silkworm, *A. mylitta* Drury (Bivoltine Daba ecorace) were reared in outdoor conditions in rearing farm and fed on fresh leaves of Asan, *Terminalia tomentosa* W and A. After spinning stage, cocoons were collected from farm tree and stored at room temperature in grainage. These cocoons were allowed to get ready for adult emergence and utilised for collection of cocoonase. For cocoon cooking and reeling, *A. mylitta* DABA bi-voltine cocoons were used. Our rearing farm and laboratory are located at Latitude-23°.21'N, Longitude- 85°.20'E and an altitude of 652 m MSL, in the state of Jharkhand, India (Pandey *et al.*, 2010; Kumar *et al.*, 2011). This research work was conducted from 15th June 2010 to 15th July 2011.

Recognition of specific stage pupae: Specific stage of pupae was identified on the basis of change in pupae integument colour and softness. Pupae ready for adult eclosion were separated by change in integument colour from natural dark violet to black with its loosening. These pupae were allowed to emerge in natural condition and adult makes outlet in anterior portion (peduncle region) of cocoons.

Extraction and collection of proteolytic enzyme cocoonase: Freshly pierced cocoons were collected from the laboratory grainage. To collect the cocoonase, the anterior wet portion of the pierced cocoon (peduncle region) was washed and squeezed in pre-chilled 500 μL Tris buffer (pH = 8.5). It was further centrifuged at 12000 rpm at 4°C to minimise the impurity. Collected cocoonase in this manner was considered as stock solution. As per experimental need, cocoonase was diluted in different concentrations in Tris buffer (1:5, 1:10, 1:15, 1:20, 1:25, 1:30 and 1:35) and

utilised for cocoon cooking. Alternatively, cocoonase was also collected from eclosion stage (emerging moth) adults directly in 1.5 mL sterilised eppendorf tube and utilised for protein estimation, enzyme activity and SDS-PAGE analysis.

Silk cocoon: The cocoons produced by the tropical tasar silkworm *A. mylitta* Drury (Daba bi-voltine ecorace) were collected, hot air stifled for 2-3 h at 70°C and used in the cocoon cooking investigation.

Basic principal of the work and cocoonase activity assessment: In natural condition, to facilitate emergence, emerging adults secretes cocoonase which helps in softening anterior portion of cocoon shell by directly targeting sericin. It indicates that sericin is natural substrate of cocoonase. This natural phenomenon provokes us to use cocoon shell sericin as a substrate to investigate its possible-efficacy in cocoon cooking. Hence in the present study, to assess the enzyme activity, small pieces of cocoon shell/cocoons were kept in cocoonase which was collected from freshly pierced cocoons. A series of temperature (25, 30, 35, 40, 45 and 50°C), pH (6.0, 6.5, 7.0, 7.5, 8, 8.5, 9.0 and 9.5) and concentration (1:5, 1:10, 1:15, 1:20, 1:25, 1:30 and 1:35) dependent standardisation experiments were conducted to study the possible-efficacy of cocoonase in cocoon cooking. Cocoons were dipped in various concentrations of Cocoonase: Tris buffer (1:5, 1:10, 1:15, 1:20, 1:25, 1:30 and 1:35) to see the impact of enzyme on cocoons cooking efficiency and quality of yarn obtained from the cooked cocoons. Alternatively, cocoons were subjected to initial water boiling for 30 min followed by cooking in cocoonase to minimise the use of cocoonase with better cooking efficiency at different temperature regimes (25, 30, 35, 40, 45 and 50°C). After completion of cocoon cooking in cocoonase, the left over cocoonase solution was centrifuged at 12000 rpm at 4°C and reused again once by adding 10% of additional volume of cocoonase. Different pH (6.0, 6.5, 7.0, 7.5, 8, 8.5, 9.0 and 9.5) range of cocoonase: Tris solutions were made and cocoon cooking efficiency of enzyme was monitored at various temperature (25, 30, 35, 40, 45 and 50°C) and concentration (1:5, 1:10, 1:15, 1:20, 1:25, 1:30 and 1:35). In respective control experiments cocoonase was not added in cocoon cooking solutions.

Cocoon cooking and reeling: After standardisation of temperature (35-40°C), pH (8.5-9.0) and enzyme concentration (1:5), cocoons were subjected to water boiling for 30 min followed by cooking in cocoonase. In respective control experiments cocoonase was not added. As per ruling practice standard protocol cocoon cooking was conducted by boiling and steaming of cocoons (about 60 min) by adding soap (5-10 g L^{-1}) and H$_2$O$_2$ (10 mL L^{-1}) in water, this was considered as positive control for comparisons. Subsequent to cocoon cooking, the cocoon samples were taken out, semi-dried, deflossed and then single silk filament reeling was performed on an approved machine in the Post cocoon Technology Section of our Institute. The silk recovery percentage was calculated by using standard formula (Ramesha *et al.*, 2009):

$$\text{Recovery}(\%) = \frac{\text{Weight of reeled silk}}{\text{Weight of reeled silk+weight of waste silk}} \times 100$$

Microscopic observation of silk filaments: The Silk filaments obtained after cocoonase cocoon cooking as well as cocoon cooking in H$_2$O$_2$ and soap were subjected to microscopic morphological observation by using Steriozoom microscope. Comparative morphological evaluation of silk filament was done to see sericin content uniformity.

Estimation of protein: The protein estimation was carried according to Bradford's micro protein assay (Bradford, 1976) with slight modifications. Concentration of the protein sample was determined from a standard curve drawn using bovine serum albumin.

SDS-PAGE analysis and coomassie blue staining: The SDS-PAGE analysis was carried out according to procedure of Laemmli (1970). Its main components constitute of a 2.5% stacking gel (pH 6.8) and a 12.5% resolving gel (pH 8.8). The electrode buffer used for running the SDS-PAGE was prepared from Tris-glycine with 0.1% SDS (pH 8.5). The 20 µL of cocoonase was boiled for 5 min with equal volume 1X protein loading buffer. After boiling protein samples were loaded in gel and the resolved proteins were visualized by Coomassie blue staining as per standardized protocol with slight modifications.

Data analysis: The data was subjected to the statistical analysis by using Student's t-test. Microsoft Excel 2007 software was used to analyze the data.

RESULTS

It is found that, at eclosion stage, the emerging adults of tasar silkworm *A. mylitta* exude a bioactive enzyme cocoonase. This enzyme helps in softening of anterior portion of cocoon (peduncle region) shell and facilitate emergence of moth (Fig. 1a-c).

Interestingly, cocoonase directly acts on the sericin protein without affecting the fibroin protein in natural condition (Fig. 2). It evidently indicates that, sericin protein is excellent natural substrate of cocoonase. Technique for cocoonase collection from freshly pierced cocoons has been developed. By using this method, cocoonase was extracted by washing/squeezing anterior portion of freshly pierced cocoons (peduncle region) in Tris buffer (Fig. 3a-d). Initial result indicates that cocoonase collected from freshly pierced cocoons was active and it has softened the cocoon shell pieces within 24-36 h (Fig. 3e-f). Alternatively, cocoonase was also collected from eclosion stage (emerging moth) adults directly in 1.5 mL sterilised eppendrof tube and subjected to SDS-PAGE. The SDS-PAGE analysis of cocoonase showed molecular weight around 26 kDa (Fig. 4). By using this method almost pure form cocoonase was collected in active form. A series of standardisation experiments was conducted to study the potential-efficacy of cocoonase in cooking tasar cocoons.

Fig. 1(a-c): Secretion of proteolytic enzyme cocoonase by emerging *A. mylitta* moth. (a). Arrow showing initiation of cocoonase secretion and their action in making outlet in cocoon, (b). Arrow indicating point of cocoonase secretion and above portion indicates softening of anterior portion of cocoon shell after cocoonase secretion, (c) Arrow indicating exact point of cocoonase secretion in magnified view

Fig. 2: Pierced cocoons of *A. mylitta*, showing moth exit point (arrows) and intact fibroin/silk filaments
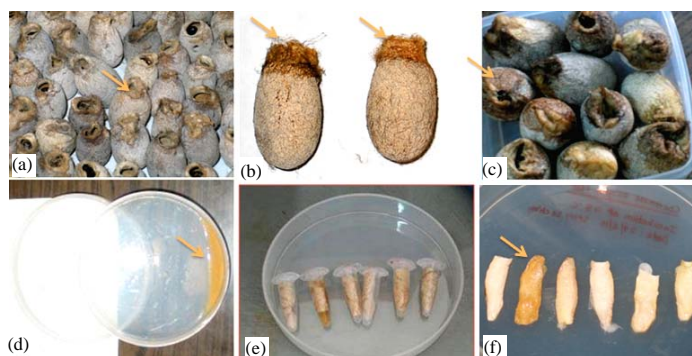

Fig. 3(a-f): Method for collection of *A. mylitta* cocoonase from freshly pierced cocoons and their activity in softening of tasar cocoon shell pieces. (a) Freshly pierced cocoon collected for cocoonase collection, (b) Arrows indicating anterior wet portion of freshly pierced cocoons due to cocoonase secretion, (c) Cocoons washing and extraction in Tris Buffer, (d) Extracted cocoonase in crude form after squeezing anterior portion of cocoons. (e) Impact of collected cocoonase in softening cocoon shell pieces in eppendrof tube, (f) Arrow showing softens cocoon shell pieces (natural substrate) due to activity of cocoonase

Collected cocoonase showed efficacy in softening cocoon. Loosening/softening of cocoon was found in cocoonase added cooking solution (Fig. 5a, d) but cocoon was intact in control where only buffer was added (Fig. 5b, c).

Effect of temperature on cocoon cooking activity of cocoonase: Various regimes of temperatures (25, 30, 35, 40, 45 and 50°C) were tested to see the impact of temperature on cooking of cocoon in cocoonase. Elevated temperature (45 and 50°C) adversely affected the cocoonase cocoon cooking efficiency but low temperature (25 and 30°C) not much affected its cooking efficiency. The
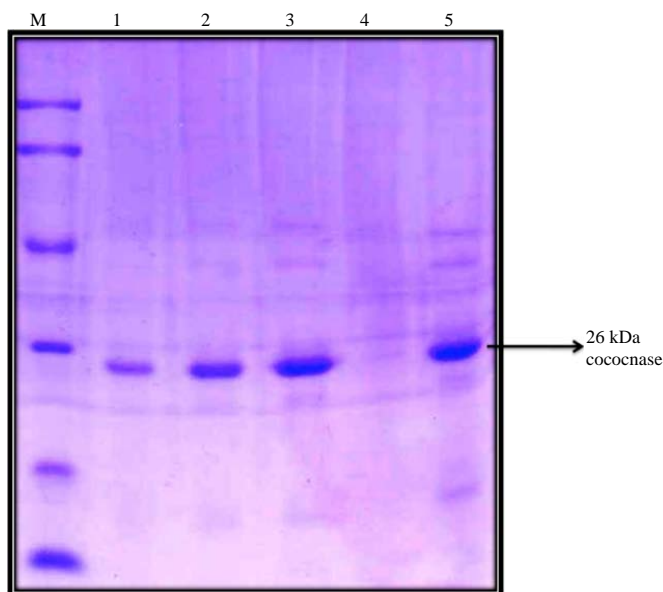
Fig. 4: 12.5% SDS-PAGE analysis showing 26 kDa protein band of cocoonase. Lane M: Protein molecular weight marker. Lane 1-5 showing 26 kDa band of cocoonase
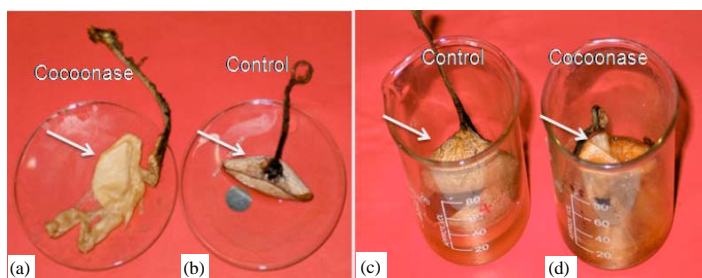

Fig. 5(a-d): Activity of cocoonase in softening of cocoon shell of *A. mylitta.* (a) Loosening of anterior portion of cocoon after cocoonase application. This enzyme showed activity in softening of tasar cocoons by targeting cocoons sericin. (b) Intact anterior portion of cocoon in control where cocoonase was not added, (c) Arrow showing complete intact cocoon in control where cocoonase was not added, (d) Possible-efficacy of cocoonase in cocoon cooking. Arrow showing Complete loosening/softening of cocoon was found after cocoonase application

35 to 40°C temperature range was found better for DABA bi-voltine cocoon cooking in cocoonase at 1:5 dilutions in Tris buffer at pH 8.5-9.0 and incubation time 24-36 h.

Effect of pH on cocoon cooking activity of cocoonase: Likewise, the different pH ranges (6, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0 and 9.5) were screened to find appropriate pH for cooking of cocoon in cocoonase. The pH 6, 6.5, 7.0, 7.5 and 8.0 were not found appropriate for cocoon cooking. The pH 9.5 and above, although showed comparatively better cooking efficiency but reeled yarn was

not able to retain natural beautiful colour of tasar silk. The pH range 8.5 to 9.0 was found comparatively appropriate for cocoon cooking in cocoonase.

Effect of cocoonase concentration on cocoon cooking activity: Various concentrations of Cocoonase: Tris buffer (1:5, 1:10, 1:15, 1:20, 1:25, 1:30 and 1:35) were used to see the impact of enzyme on cooking efficiency of tasar cocoons and quality of yarn obtained from the cooked cocoons. Cooking of tasar cocoons with cocoonase enzyme is concentration and time dependent. Low concentration (1:15, 1:20, 1:25, 1:30 and 1:35) increases the cocking time and decreases the cooking efficiency. Higher concentration (1:5 and 1:10) minimises the cooking time and increases the cooking efficiency. But after cocoon cooking, due to hardness in inner portion of the cocoons, they could not fully reeled leading to decrease in silk recovery percentage. When the cocoons were initially subjected to water boiling for 30 min, followed by cooking in cocoonase (1:5) at 35-40°C, comparatively better cooking efficiency with 50-55% silk recovery was found. The left over cocoonase solution can be reused once by adding remaining 10% volume of fresh cocoonase.

Effect of initial cocoon boiling in water on cooking activity of cocoonase: To minimise the hardness in cocoons inner portion, cocoons were subjected to initial water boiling for 10, 20 30 and 40 min followed by cooking in cocoonase (1:5) at 35-40°C. Initial boiling of cocoons for 20-30 min in water gives comparatively better cooking efficiency with 50-55% silk recovery.

Effect of cocoon cooking in cocoonase on silk recovery percentage and morphological features of silk filaments: Although, the silk recovery percentage in cocoonase cooking was less (50-55%) in comparison existing chemical based (H_2O_2 and soap etc.) cocoon cooking technology (60-65%) but yarn obtained from the cocoonase cooking maintain natural beautiful unique tasar silk colour, softness and lustre (Fig. 6b, d) in comparison to cocoon cooking in H_2O_2 and Soap where loss of natural colour and softening were observed (Fig. 6a, c). Microscopic observation of silk
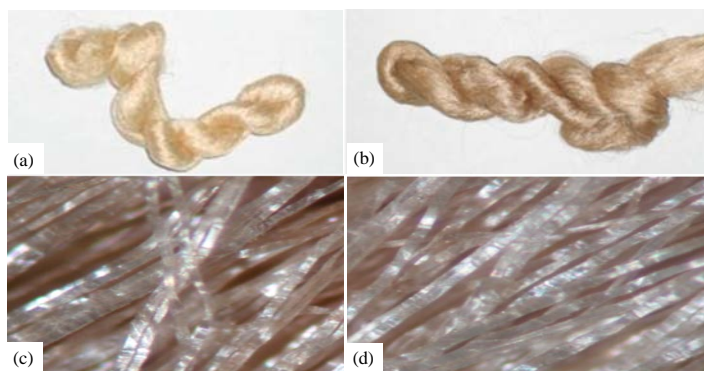

Fig. 6(a-d): Comparative morphological features of tasar silk reeled after cocoon cooking. (a) Loss of natural colour of tasar silk when cocoons were cooked in H_2O_2 and soap, (b). Retention of natural colour of tasar silk when cocoons were cooked in cocoonase, (c) Microscopic observation of silk filament reeled after cocoon cooking in H_2O_2 and soap, (d) Microscopic observation of silk filament reeled after cocoon cooking in cocoonase
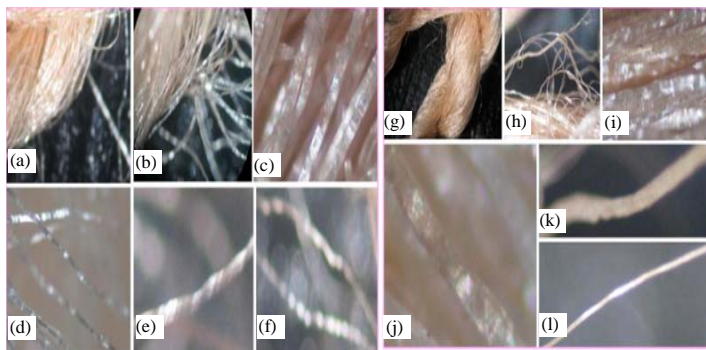
Fig. 7(a-l): Comparative morphological evaluation of silk filament to see sericin content uniformity. (a-f) Microscopic observation of silk filaments reeled after cocoon cooking in H_2O_2 and soap, (g-l) Microscopic observation of silk filaments reeled after cocoon cooking in cocoonase

filaments was conducted to see the uniformity of sericin in silk filaments (Fig. 7a, l) There is almost comparable sericin uniformity in silk filament was observed in cocoonase cooked (Fig. 7g-l) and H_2O_2 and soap cooked cocoons (Fig. 7a-f).

DISCUSSION

Insect proteases are bioactive enzymes and they perform many functions during post-embryonic development of insects (Kanost and Clem, 2012). Likewise, cocoonase secreted by *A. mylitta* is also a proteolytic enzyme (protease). At the end of larval stage silkworm starts silk spinning to form cocoons which is a composite material formed by fibroin proteins surrounded by a sericin protein. Although this tough and beautiful silk cocoons gives protection to pupae from adversity but at the end of pupal period it creates setback to eclosion stage moth to come out from cocoon. Therefore, nature has evolved an enzyme based well-organized mechanism for the escape of moth from their cocoon (Felsted *et al.*, 1973a, b).

In many sericigenous insects including *A. mylitta*, at eclosion stage, the emerging adults exude a proteolytic enzyme 'cocoonase' which helps in softening of cocoon shell and facilitates easy emergence of moths. Secretion of cocoonase during adult emergence is reported in several sericigenous insects by number of researchers (Felsted *et al.*, 1973a; Liu and Li, 2002; Wang *et al.*, 2005a; Yu-Dan, 2008; Wu *et al.*, 2008; Wang, 2008; Yang *et al.*, 2009). The catalytic properties of the cocoonase and kinetics of cocoonase zymogen have been also studied by researchers (Kafatos and Kiortsis, 1971; Kafatos, 1972; Hruska *et al.*, 1973). Likewise in *A. mylitta*, emerging moth gradually secretes the cocoonase which makes anterior portion of cocoon (peduncle region) soaked (Felsted *et al.*, 1973a). Since, cocoonase targets sericin protein of cocoon shell without affecting the fibroin protein hence it can be known as sericinase also. It is evidently clear that; sericin is excellent natural substrate of cocoonase. This natural happening engenders a hypothesis to collect *A. mylitta* cocoonase to see its possible-efficacy in cocoon cooking. But collecting bioactive enzyme from biological system was difficult task. The molecular study of cocoonase was also conducted by number of researchers (Wang *et al.*, 2005a; Yu-Dan, 2008; Wu *et al.*, 2008; Yang *et al.*, 2009), but they could not get cocoonase in large scale. Since, enzymes are known for

their repetitive reaction hence a simple method was developed to collect the cocoonase from freshly pierced cocoons by washing and squeezing the anterior portion of cocoon in Tris buffer (pH 8.5-9.0). Collected cocoonase showed possible-efficacy in softening cocoon shell pieces and cocoon in 24-36 h. Loosening of cocoon was found in cocoonase added cooking solution and intact cocoon was found in control where only buffer was added. These results indicate that, collected cocoonase from freshly pierced cocoons is in active condition and it is having capacity for cocoon cooking.

Alternatively, cocoonase collected from eclosion stage (emerging moth) adults in 1.5 mL eppendorf tube showed its purity in SDS-PAGE. Molecular weight of *A. mylitta* cocoonase (26 kDa) is more or less similar than other sericigenous insect's cocoonase (Kafatos and Kiortsis, 1971; Kafatos, 1972; Hruska *et al.*, 1973; Wu *et al.*, 2008; Yang *et al.*, 2009). Insect proteases are bioactive enzymes and they perform many functions during post-embryonic development of insects (Kanost and Clem, 2012). Since enzymes are known for mild action (Ajlia *et al.*, 2010) hence several enzymes i.e., lipase, protease etc. has been tried for cocoon cooking and degumming of silk (Gulrajani, *et al.*, 2000; Singh *et al.*, 2003; Nakpathom *et al.*, 2009). It is reported that temperature and pH shows crucial role in enzyme activity. In the present study different regimes of temperature and pH range were tried and it is found that cooking of cocoon in cocoonase is concentration, pH, temperature and time dependent. The 35 to 40°C temperature and 8.5 to 9.0 pH range was found comparatively suitable for cooking of cocoon in cocoonase. Likewise several proteolytic enzymes show good activity at this pH range. Since nature has evolved cocoonase to soften the cocoon shell which is tough materials hence it is assumed that higher pH is require for this enzyme to soften the anterior portion of cocoons. Various concentrations of cocoonase: Tris buffer (1:5, 1:10, 1:15, 1:20, 1:25, 1:30 and 1:35) was used to see the impact of enzyme on cocoon cooking efficiency and quality of yarn obtained from the cooked cocoons. Even after using higher concentration (1:5) of cocoonase, hardness in inner portion of the cocoons found which leads to affect the cooking efficiency. Hence it is assumed that due uneven cocoonase penetration and tasar cocoons hardness inner layer of cocoon could not soften. When cocoons were initially subjected to water boiling for 30 min, followed by cooking in cocoonase (1:5) comparatively better cooking efficiency and silk recovery was found. It is assumed that initial cocoon boiling affects the sericin and it happens due to high temperature and water soluble nature of sericin. After cocoon boiling, when cooking of cocoon was conducted in cocoonase it properly reaches to inner layer of cocoons which leads to improve cooking efficiency with 50-55% silk recovery. Due to cyclic nature and better thermo-stability of cocoonase the left over cocoon cooking solution was reused once by adding remaining 10% volume of fresh cocoonase. Several methodologies have been used by investigators (Singh *et al.*, 2003; Mondal *et al.*, 2007; Nakpathom *et al.*, 2009; Mahmoodi *et al.*, 2010; Teshome *et al.*, 2011) for cooking of cocoons and silk degumming. Present study is a different kind of method where cocoonase was used for cocoon cooking.

Microscopic observation of silk filaments was conducted to see the uniformity of sericin in silk filaments which is very important tools for silk strength indicator. Similarly based on morphology, structure and thermal properties characterization *Philosamia ricini* fibroin film was also studied (Srisuwan *et al.*, 2009). Almost analogous sericin uniformity in silk filament of cocoonase cooked and H_2O_2 and soap cooked cocoons, hence it is expected that silk filaments strength is also comparable. Tasar silk is having unique colour, inimitable quality which leads to immense demands at global level. But when cooking is carried out in alkaline condition by using soap, soda, H_2O_2, alkali materials etc., it adversely affects the natural beautiful colour and softness of tasar silk. Generally the cocoons are cooked in presence of strong alkali agent or other harsh chemicals

(Tikoo and Goel, 1987; Moon *et al.*, 1996). Since, the chemical methods reduce the quality of the tasar silk thread in many ways (Tikoo and Goel, 1987), an alternative method for the tropical tasar cocoon cooking based on the cocoonase may be developed for better results. The cocoonase may be used in cooking of the silk cocoon to soften it by decomposing or partially solubilising the silk gum sericin which is concerned in binding the fibroin silk strands together in the cocoon shell (Singh *et al.*, 2003). Present study substantiates the finding of other investigators (Prasong *et al.*, 2009; Srihanam *et al.*, 2009; Mahmoodi *et al.*, 2010; Prasong, 2011).

Although, the silk recovery percentage in cocoonase cocoon cooking is found less (50-55%) in cocoonase to existing chemical based (soap, soda, H_2O_2, etc.) cocoon cooking technology (60-65%), but yarn obtained from the cocoons cooked in cocoonase preserve natural beautiful unique tasar silk colour, softness and lustre. Out initial result indicates that tasar silk natural beautiful colour can be preserved by using cocoonase for cocoon cooking without any adverse impact on silk filaments due to mild and specific action of enzyme.

CONCLUSIONS

At eclosion stage, the emerging adults of tasar silkworm exude a proteolytic enzyme 'cocoonase' which helps in softening of cocoon shell and facilitates easy emergence of moths. Method for collection cocoonase form freshly pierced cocoons has been developed. The SDS-PAGE analysis showed its molecular weight around 26 kDa. Cocoonase showed efficacy in cocoon cooking by directly targeting sericin as a natural substrate without affecting the fibroin protein. The 35-40°C temperature and 8.5-9.0 pH range found better for cooking of cocoon in cocoonase. Initial boiling of cocoon in plain water for 30 min followed by cooking in cocoonase (1:5) at 35-40°C has enhanced cooking efficiency and resulted 50-55% silk recovery. Yarns obtained from the cocoonase cooking, maintain natural pretty unique tasar silk colour, softness and lustre. Further study is needed to get better cocoonase cooking efficiency and uniform sericin removal with higher or analogous silk recovery than the ruling practices.

ACKNOWLEDGMENTS

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