Silk fibroin extraction and quantification of silk powder from cocoons of Philosamia ricini (Eri) and Antheraea assamensis (muga)

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Abstract: ***Silk is a natural protein fibre and its utilisation is ubiquitous in the society as one of the most ancient and useful natural fibers in the world. The fibroin protein of silk may be resolubilized into an aqueous solution which can be regenerated into a desirable form. This regenerated silk fibroin solution is of great potential for use in biomedical applications in relation to tissue engineering, regenerative medicine and drug delivery applications. The present study was conducted for solubilization of fibroin extracted from Antheraea assamensis (muga) and Philosamia ricini (eri) using non conventional methods to obtain silk fibroin powder which could be utilised as biomedical resources or in cosmetics and food industry. Pure silk fibroin protein was extracted from P.ricini and A.assamensis silk cocoon by degumming method using aqueous Na_2CO_3 solution. This was followed by hydrolysis of silk fibroin in NaOH to obtain silk powder.***

Key words: Silk, fibroin, Antheraea assamensis, Philosamia ricini, degumming

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1. INTRODUCTION:

Silk is a proteinaceous secretion from a number of arthropods and is used for a variety of purposes such as for anchorage, to entangle prey or form protective nests and cocoons. Among the arthropods, nearly 400-500 species of insects are known to produce silk, but only a few are being commercially exploited. The silkworms are the commonest source of commercial silks.

The silk obtained from silkworms consists of 2 main kinds of proteins – the core protein fibroin, which is hydrophobic and fibrous in nature; and sericin, which is a hot water soluble glycoprotein that envelope the inner fibroin, imparting toughness and strength to the fibre [1]. Fibroin is the protein of interest for many workers in various fields. For biomedical use, fibroin has to be purified/extracted from the silkworm cocoon by removal of the sericin. Recent researches indicated that similar to the collagen, silk fibroin (SF) was found ideal for attaching animal cells cultured *in vitro*, and was also important for maintaining cell function [2, 3]. In fact, the silk fibroin fibres have been used as medical sutures over the past millennia[4]. Moreover, the finest silk powder, silk amino acids and hydrolyzed silk proteins (fibroin and sericin) are used in candy food products. The silk fibroin peptides, in the form of powder or gels, are also used as cosmetics due to their glossy, flexible, elastic coating power, easy spreading and adhesion characters[5,6]. Characteristics of the silk including biodegradability, biocompatibility, controllable degradation rates and versatility to generate different material formats from gels to fibres and sponges, have attracted interests in the field of biomaterials [7]. The non-inflammatory and non-immunogenic properties of fibroin too contribute to its wide applications in medically related applications.

Among silk producing insects, the silkworms have received special attention because of the tough mechanical properties of their products. These worms have been broadly classified into the mulberry (*Bombyx mori*) and the non-mulberry (*Philosamia ricini*, *Antheraea assamensis*, etc) silkworms. The mulberry silkworm, *B.mori* is the most common model insect for the study and production of silk and has been extensively exploited due to its easy domestication and availability, but very less work has been carried out on the non-mulberry silkworms because of their confinement to only certain parts of India, the North Eastern states being a major hotspot.

However, though a lot of literature is available for the solubilization of silk fibroin from *B.mori* silks, the methodologies for dissolution of silks from non-mulberry silkworm cocoons to obtain higher yields of fibroin remain unsolved till today [1]. Also, it has been seen that among the limited works, the conventional methods used for mulberry silks to obtain fibroin result in lower yields when used in case of non-mulberry silk fibroins [8]. In the present work, silk fibroin extraction methods have been applied on the cocoons of non-mulberry silkworms *P.ricini* (Eri) and *A.assamensis* (Muga) to standardize a protocol since such works on these varieties of silkworms are very scanty.

2. MATERIALS AND METHODS:

2.1. Collection of sample – Silk cocoons of *P ricini* and *A. assamensis* were collected from a local farm in the area of Ganeshnagar, near Bamunimaidan, Assam and State Muga Farm, Govt. of Assam, Khanapara respectively.

2.2. Degumming of silk cocoons and fibroin extraction – To obtain pure silk fibroin, the degumming process is very necessary so as to remove the sericin completely from the silk, leaving behind only the fibroin. The degumming and fibroin extraction protocols were carried out independently and separately for *P.ricini* and *A.assamensis*, following the generalized degumming methodology using Na_2CO_3.

Cocoons were cut into small pieces after disposing off the pupae. 2L of double distilled water was boiled into which 4.24g of Na_2CO_3 was added (0.02M solution of Na_2CO_3). After complete dissolution of Na_2CO_3, 5g of cocoon pieces were added to the boiling solution. Boiling was continued for 30 minutes with occasional stirring for complete removal of sericin. After 30 minutes, the fibroin was removed from the solution and placed in 1L of double distilled water and rinsed properly for 20 minutes. The water was then discarded, and the rinsing step was repeated for another three times. After the last wash, the silk fibroin was removed, squeezed well and spread out on a clean piece of aluminium foil to dry well.

The degumming process can be repeated for second time if the sericin does not get removed completely from the fibres.

The dry degummed silk fibroin was kept safely and can be stored indefinitely at room temperature. For long-term storage, the fibroin can be kept in a plastic bag or wrapped in aluminium foil.

2.3. Hydrolysis of silk fibroin in NaOH to obtain silk powder: The silk fibroin hydrolysis methodology by Mandal *et al.* [9] was followed, but with slight modifications.

For hydrolysis of silk fibres, 15g NaOH pellets were added to 25ml of distilled water. When approximately 70% of the NaOH pellets were dissolved, 1g of the dried degummed silk fibres was added and stirred with a glass rod. After dissolution of the fibres, 45ml water was added to the reaction mixture to stop further hydrolysis. The solution was then centrifuged at 3000 RPM for 5 minutes. The supernatant was discarded and another 40ml water was added to the fibroin and centrifuged again at 3000 RPM for another 5 minutes. This step was repeated 5-6 times to remove the excess remaining alkali. The pH of the solution was measured and adjusted to 7.0 using HCl. The neutralized fibroin solution was again centrifuged at 3,500 rpm for 5 minutes. This step was repeated 2-3 times. The fibres were then resuspended in PBS. Finally, the residual matter was transferred to a cavity block and allowed to air-dry for 3-4 days, keeping safe from dust etc. Silk fibroin powder was generated after complete drying.

The percentage of dissolution of silk fibroin from *P.ricini* and *A.assamensis* cocoons during the process of hydrolysis was obtained by using the following formula :-

% dissolution of silk fibroin = [1 – weight of remnants after dissolution ÷ initial weight of degummed fibre × 100]

3: RESULT

3.1. Silk fibroin powder: The following table represents the amounts of raw materials taken during the process of hydrolysis of silk fibroin and the resultant amounts of eri and muga silk fibroin powder obtained : -

Table 1. Quantities of raw materials taken and resultant amount of silk fibroin powder obtained from *P.ricini* (Eri) and *A.assamensis* (Muga) cocoons after the process of hydrolysis.

Name	Amount of fibre taken (gram)	Amount of NaOH taken (gram)	Amount of water taken (ml)	Net amount of silk fibroin powder obtained (gram)
P.ricini (Eri)	1	15	25	0.44
A.assamensis (Muga)	1	15	25	0.54

3.2. Percentage of dissolution of silk fibroin: Table 2 depicts the percentage dissolution of eri and muga silk fibroin. From the data, it has been evident that the percentage dissolution of eri silk fibroin (56%) was comparatively higher than that of muga silk fibroin (46%).

Table 2. Showing the % dissolutions of Eri and Muga silk fibroins.

Name	Net amount of silk fibroin powder obtained (gram)	% of dissolution of silk fibroin
P.ricini (Eri)	0.44	56
A.assamensis (Muga)	0.54	46

(a)

(b)

Fig. 3 Photographs showing silk fibroin powder obtained from silk fibroin solutions of (a) Eri and (b) Muga

4: DISCUSSION AND CONCLUSION:

Results of the present work show that the percentage (%) dissolution of silk fibroin (46%) from *A.assamensis* (Muga) is lower than the % dissolution of silk fibroin (56%) from *P.ricini* (Eri) silkworm. The differences in the dissolution percentages (%) indicate the greater tensile strength of muga silk than any other silk, as has been reported earlier by different authors [1, 8]. The results conform to reports on other silkworms such as *B.mori* by Ajisawa *et al.* [10] and many other workers. This suggests our non-conventional solubilization methods to be at par with the conventional methods so far followed. Our method holds significance that unlike the solubilization agents used in normal protocols, our agents are safer and cheaper.

Agents like LiBr, used conventionally, even though lead to complete dissolution of silk fibroin [11] and are used in producing silk hydrogels etc, is not fully acceptable as it is toxic and also known to cause air pollution due to its intense exothermic reaction. Some other agents like $CaCl_2$, $MgCl_2$, etc consume a lot of time to dissolve silks and may also need to be combined with other substances to obtain proper results.

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5. REFERENCES

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