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A COMPARATIVE STUDY ON EXTRACTION AND QUANTIFICATION OF SILK POWDER FROM DABA (TV), DABA (BV), AND RAILY COCOONS

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Abstract

Silk, a natural protein fiber, holds immense significance in society as one of the oldest and most versatile natural fibers worldwide. The fibroin protein present in silk can be dissolved in water, forming an aqueous solution that can be transformed into a desired form. This regenerated silk fibroin solution shows tremendous potential for various biomedical applications, including tissue engineering, regenerative medicine, and drug delivery. In this particular study, the objective was to solubilize fibroin extracted from three different sources, namely Daba(TV), Daba(BV), and Raily silk, using unconventional methods. The aim was to obtain silk fibroin powder that could be utilized as a valuable resource in the biomedical field, as well as in the cosmetics and food industries. To achieve this, pure silk fibroin protein was extracted from Daba(TV), Daba(BV), and Raily silk cocoons through a process called degumming. Aqueous Na2CO3 solution was used in this step. Subsequently, the silk fibroin was hydrolyzed in NaOH to obtain the desired silk powder.

Key words: Silk, fibroin, Daba(TV), Daba(BV) Raily , Degumming.

I**NTRODUCTION**:

Silk, a proteinaceous secretion from a number of arthropods, has a range of functions, including anchoring, entangling prey, and forming nests and cocoons for protection. Only a few of the 400–500 bug species known to generate silk among arthropods are used for commercial purposes. The most prevalent source of commercial silk is the silkworm.

The two primary types of proteins found in silk produced by silkworms are sericin, a hot water soluble glycoprotein that surrounds the inner fibroin and gives the fiber toughness and strength, and fibroin, which is the fiber's core protein and is hydrophobic and fibrous in nature [1]. For many professionals in numerous fields, fibroin is the protein of interest. Fibroin needs to be isolated from the silkworm cocoon and refined by removing the sericin before it can be used in biomedicine. According to recent studies, silk fibroin (SF), like collagen, was discovered to be perfect for connecting animal cells cultivated in vitro and was also crucial for maintaining cell function [2, 3].

Additionally, the best silk powder, silk amino acids, and silk proteins (fibroin and sericin) hydrolyzed are employed in confectionery food goods. Due to their glossy, flexible, elastic coating power, simple spreading and adherence characteristics, and availability in powder or gel form, silk fibroin peptides are also employed in cosmetics [5,6]. The biomaterials industry has shown interest in the properties of silk, such as its biodegradability, biocompatibility, predictable degradation rates, and adaptability in producing a variety of material formats, such as gels, fibers, and sponges [7]. The widespread use of fibroin in healthcare-related applications is further facilitated by its anti-inflammatory and anti-immunogenic capabilities.

Due to the strong mechanical qualities of their products, silkworms have drawn particular attention among insects that produce silk. These worms have been roughly divided into mulberry (Bombyx mori) and non-mulberry Daba(TV), Daba(BV), and Raily etc. silkworms. The mulberry silkworm, B. mori, is the most popular model insect for the study and production of silk and has been extensively exploited due to its easy domestication and availability, but very little work has been carried out on the Daba (TV), Daba(BV), and Raily silkworms due to their confinement to only certain parts of India, with Chhattisgarh being a major hotspot.

However, despite the abundance of research on the solubilization of silk fibroin from B. mori silks, methods for dissolving silks from non-mulberry silkworm cocoons to produce larger yields of fibroin are still a mystery [1]. Furthermore, it has been observed in the few studies that when non-mulberry silk fibroins are produced using the traditional procedures used to produce fibroin from mulberry silk, the yields are lower [8]. Since research on these tasar silkworm variations is fairly limited, methods for extracting silk fibroin from their cocoons have been used in the current effort to standardize a protocol.

MATERIALS AND METHODS:

Collection of sample – Silk cocoons of Daba(TV),Daba(BV) and Raily were collected from different places of Chhattisgarh state through the units of Central Silk Board,Govt of C.G

Degumming of silk cocoons and fibroin extraction - The degumming procedure is highly important to acquire pure silk fibroin since it fully removes the sericin from the silk, leaving just the fibroin. Following the generalized degumming methodology with Na2CO3, the fibroin extraction and degumming protocols were carried out independently and separately by Daba(TV), Daba(BV), and Raily.

Following the disposal of the pupae, cocoons were divided into minute pieces. 4.24g of Na2CO3 (0.02M solution of Na2CO3) was heated into 2L of double-distilled water. Five grams of cocoon pieces were added to the boiling solution after the Na2CO3 had completely dissolved. Sericin was completely removed by continuing to boil for an additional 30 minutes while stirring occasionally. The fibroin was taken out of the solution after 30 minutes and submerged in 1L of double-distilled water for 20 minutes of thorough rinsing. After throwing away the water, the rinsing procedure was performed four more times. After the last wash, the silk fibroin was taken out, thoroughly squeezed, and spread out on a fresh piece of aluminum foil to dry thoroughly.

The dry, degummed silk fibroin was kept secure and can be kept at room temperature indefinitely. The fibroin can be preserved in a plastic bag or wrapped in aluminum foil for long-term storage.

Silk fibroin hydrolysis in NaOH to produce silk powder:

The silk fibroin hydrolysis procedure described by Mandal et al. [9] was

followed, albeit with a few minor adjustments.Silk fibers were hydrolyzed using 15g of NaOH pellets and 25ml of distilled water. A glass rod was used to stir in 1g of the dry, degummed silk fibers once roughly 70% of the NaOH pellets had dissolved. In order to stop additional hydrolysis after the fibers had been dissolved, 45 ml of water was added to the reaction mixture. After that, the solution was centrifuged for five minutes at 3000 RPM. After discarding the supernatant, 40 ml of water was added to the fibroin, and it was centrifuged once more at 3000 RPM for an additional five minutes. . To eliminate the amount of residual alkali, this step was repeated 5–6 times. The pH of the mixture was determined and set at 7.0 using HCl. Once more centrifuged for five minutes at 3,500 rpm, the neutralized fibroin solution. Repeating this action two to three times. The fibers were afterwards re-dissolved in PBS. Last but not least, the leftover material was moved to a cavity block and allowed to air dry for 3–4 days while being protected from dust and other contaminants. After thorough drying, silk fibroin powder was produced.

Dissolution of silk fibroin

The following formula was used to calculate the proportion of silk fibroin that was dissolved during the hydrolysis process from Daba (TV), Daba (BV), and Raily cocoons:

Dissolution of silk fibroin

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

RESULT

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 Daba(TV),Daba(BV) and Raily silk fibroin powder obtained : -

Table 1. Quantities of raw materials taken and resultant amount of silk fibroin powder obtained from Daba(TV),Daba(BV) and Raily cocoons after the process of hydrolysis.

Name	**Amount of fibre taken (gram)**	**Amount of NaOH taken (gram)**	**Amount of watertaken (ml)**	**Net amount of silk fibroin powderobtained (gram)**
Daba TV	1	15	25	0.54
Daba BV	1	15	25	0.56
Raily	1	15	25	0.44

Percentage of dissolution of silk fibroin: Table 2 depicts the percentage dissolution of silk fibroin. From the data, it has beenevident that the percentage dissolution of Raily silk fibroin (56%) was comparatively higher than that of Daba (TV) and Daba (BV) silk fibroin (46%) and (44%).

Table 2. Showing the % dissolutions of Daba TV, Daba BV and Raily silk fibroins.

Name	**Net amount of silk fibroin powder obtained (gram)**	**% of dissolution of silk fibroin**
Daba TV	0.54	46
Daba BV	0.56	44
Raily	0.44	56

CONCLUSION AND DISCUSSION:

The current study's findings demonstrate that the percentages of silk fibroin that dissolve (46% and 44%) from Daba TV and Daba BV are lower than the percentages (56%) that dissolve silk fibroin from Raily silkworm. This implies that our unconventional solubilization techniques are comparable to the standard techniques that have been used up to this point. Our technology is significant because, in contrast to the solubilization agents employed in conventional procedures, our agents are more affordable and safe.

Conventionally used agents like LiBr, although though they completely dissolve silk fibroin [11] and are used to create silk hydrogels and other products, are not entirely acceptable because they are poisonous and have a history of polluting the air due to their strong exothermic reactions. To get the best results, some additional agents, such as CaCl2, MgCl2, etc., may need to be combined with other substances and take a long time to dissolve silks.

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