**An in-depth analysis of the production of silk sericin and its biological applications**

**Abstract**

Silk fibroin and sericin are the two types of proteins that make up silk. Twenty to thirty percent of the entire cocoon weight is made up of sericin. Sericin is a by-product of the silk industry that is organic, environmentally benign, proteinous, and biodegradable. To give raw silk luster, a degumming process must be used to remove the gooey sericin substance. Its solubility and molecular weight depend on how silk sericin was extracted. It has numerous uses in pharmacy, medicine, and cosmetics, including anti-hypertensive, wound-healing, antioxidant, and anti-aging effects.

**Keywords**- Sericin, Extraction, biological Application

**Introduction**

Drug and biomolecule delivery systems frequently utilize the natural biomaterial silk. Bombyx mori (silkworms) produce the protein sericin, which is used in the manufacture of silk. Most of this natural protein was taken out of the cocoon silk during the degumming process. Sericin and fibroin, two proteins that make up the majority of the two proteins that make up silk produced by silkworms, and the sericin protein serve as the glue that coats the silk strands and helps them adhere to one another. Another form of silk protein called sericin has 18 amino acids total, including all nine necessary amino acids, and is distinguished by having 32% serine.

Sericin contains 45.8% of the total recommended daily intake of hydroxy amino acids. Polar amino acid residues make up 42.3% of the total, whereas nonpolar amino acid residues make up 12.2%. Sericin makes up between 20–30% of the overall cocoon weight (1,2).

To generate high-quality cocoons, silkworms require a particular diet, and white mulberry leaves (Morus alba) have the nutritional value needed for this. Water makes up the majority of these leaves (81.72%), followed by fat (0.57%), protein (1.55%), fiber (1.47%), carbs (14.21%), and other insignificant substances (0.48%), like minerals (3,4). The silk glands in the cocoon produce the leaf proteins, which are then stored in the lumen and converted into silk fiber. This silk is discharged via the die hole after passing through the anterior gland during the pinning process. As a result, a delicate double fibroin filament with a sericin-coated surface is produced. Because it serves as a binder and preserves the structural integrity of this one, the final one aids in the formation of the silk cocoon. The generated oval-shaped structure serves as a sanctuary for the larva during its transformation into a pupa (5).

**life cycle – (Bombyx mori)**

The lepidopteran insect Bombyx mori has been tamed for more than 7,000 years. Due to the historical importance of silk production for the economy, this species' physiology has been extensively studied (6). The four phases of transformation in the silkworm life cycle are egg, larva, pupa or chrysalis, and adult (moths). Five stages, or ages, make up the larval stage. The initial phase of transformation is egg production. The male silkworm survives a little while longer after this event, while the female silkworm produces 300 to 400 eggs simultaneously and shortly after passes away (7). Eggs are incubated for around ten days during the second stage before hatching into larvae (caterpillars). Mulberry leaves are fed to larvae so they can store adequate nutrients and be able to shed their skin five times. It takes between four and six weeks for a caterpillar to form during this time. Following the end of the feeding period, the third stage, often known as the pupa or chrysalis, starts with the construction of the silkworm cocoons (8). This cocoon's purpose is to shield the pupa from predators, natural drying during metamorphosis, and microbial deterioration (9). Silk caterpillar continuously moves its head as an 8 or a S to create a cocoon around itself. To do this, they repeatedly bend and stretch their bodies. Several grams light and compact, cocoons are constructed of a single continuous filament of silk that ranges in length from 700 to 1500 m. These are built in around three days, at which point the silkworm sheds its skin for the last time and changes into a pupa. The two extremities of the cocoon, which have the least thickness overall, are ellipsoidal in shape. The silkworm secretes an alkaline substance that punctures these sites, allowing the invertebrate to emerge as a moth and complete the transformation.

 

Image 1(10) Image 2 (11)

 Image 3 (12)

**Properties of sericin**

The random coil and -sheet structures are present in sericin. In hot water, the random coil structure dissolves, and when the temperature drops, the random coil structure changes to a -sheet structure, which causes the gel to form (13).

Sericin has the ability to easily dissolve into water at 50 to 60o C and then transform back into a gel upon cooling. The isoelectric point of sericin is around 4.0 because there are more acidic than basic amino acid residues. When the sericin molecules change from a random coil to a sheet configuration, their solubility in water drops (14,15)

**Molecular structure**(16)



**Solubility of Sericin**(17) **(Table 1)**

|  |  |  |
| --- | --- | --- |
| Sr. No | Solvent  | Solubility (mg/ml) |
| 1 | Water  | 112 |
| 2 | Phosphate Buffer (pH 9-10) | 85 |
| 3 | 0.1 N HCl  | 78 |
| 4 | Ethanol | 35 |
| 5 | Methanol  | 25 |
| 6 | Acetone  | 28 |

**Component of Sericin**(18) **(Table 2)**

|  |  |  |
| --- | --- | --- |
| Sr No | Amino Acid  | Sericin  |
| 1 | Glycine (G) | 14 |
| 2 | Alanine (A) | 5 |
| 3 | Serine (S) | 33 |
| 4 | Tyrosine (Y) | 3 |
| 5 | Valine (V) | 3 |
| 6 | Aspartic acid (D) | 15 |
| 7 | Arginine (R) | 3 |
| 8 | Glutamic (D) | 8 |
| 9 | Isoleucine (I) | 1 |
| 10 | Leucine (L) | 1 |
| 11 | Phenylalanine (F)  | 1 |
| 12 | Threonine (T) | 8 |
| 13 | Cysteine (C) | 0 |
| 14 | Histidine (H) | 1 |
| 15 | Lysine (k) | 4 |
| 16 | Methionine (M)  | 0 |
| 17 | Proline (P) | 1 |
| 18 | Tryptophane (W)  | 0 |

**Isolation and Extraction of sericin**

By separating sericin from the fibroin component, sericin can be removed from silk. Since the silk business only uses the fibroin portion of silk, sericin must be removed, which is done by a degumming process, and then dumped into the effluent. Bombyx mori silkworm cocoons were degummed in order to release the sericin. The deflossing, cleaning, cutting into smaller pieces, and rinsing with distilled water of the cocoons. An appropriate amount of distilled water was added to the cocoons in a reagent bottle at a 1:20 ratio. It was then filtered, lyophilized (Christ Alpha 1-4 lyophilization, Osterodo, Germany), and autoclaved for 30 minutes at 121 0 C to produce dry powder (19).

## **Production volume of raw silk in India in the financial year 2022, by state (in metric tons)**

India has the distinction of being the only country in the world producing all the four commercially known silks, namely, Mulberry, Tasar (includes oak tasar), Eri and Muga.

 **Image 4**

**Biological Application of Sericin**

**Wound Healing-** Sericin promotes wound healing by activating collagen formation in wounds and inducing epithelialization. It also has strong hydrophilic qualities, is biocompatible, and is biodegradable. Sericin is also said to encourage fibroblast and keratinocyte adhesion and proliferation in human skin (20).

**Anti-Inflammatory Activity-** One of the stages of the healing process is inflammation, during which time necrotic tissues and potential pollutants present at the wound site are phagocytosed. Additionally, at this stage, inflammatory cells release growth factors and cytokines that attract the cells needed to build new tissue. The expression of metalloproteinases, which are in charge of degrading the extracellular matrix, is promoted by the unchecked, exuberant expression of inflammatory cytokines, therefore this phase must be managed. In this regard, biomaterials created for treating wounds must be able to manage the inflammatory response (21,32).

**Hypertension** -In isolated rat thoracic aorta, sericin-derived oligopeptides from silk cocoons were examined for the in vivo hypotensive effect and the underlying mechanism involved in vasodilation. The quick and temporary hypotensive activity caused by oligopeptides. Recent studies have shown that peptides generated from dietary proteins, such as egg white, soy protein, and -casein, can modify vasodilation and reduce blood pressure. Furthermore, fibroin hydrolysate has demonstrated that silk fibroin, the main protein in silk fiber, may have potential hypotensive and antioxidant effects. Dipeptides produced from silk fibroin, such as glycine-tyrosine, can have antihypertensive effects by inhibiting the angiotensin-converting enzyme (ACE)25.

**Cancer-** Studies have demonstrated that the 1,2-dimethylhydrazine agent is successfully suppressed when sericin is administered orally (by mouth) by rats and mice. The incidence of colorectal cancer decreased when this drug was eliminated because it promotes the growth of cancer26. Additionally, it has been noted that sericin stimulates the apoptosis factor, which causes cancer cells in rats to undergo apoptosis, and limits the proliferation of cloned tumor cells. Additionally, sericin, which the colon does not digest, has a potent antioxidant action that lowers oxidative stress and the development of colon tumors27.

**Antioxidant-** Sericin's antioxidant action has the potential to be very beneficial for your health. According to studies, this protein allows for a decrease in the amount of cancer cells as well as oxidative stress in human organs including the colon26,27.

**Cosmetics-** Sericin has become a useful economic resource for the manufacture of cosmetics because it has a great affinity for keratin. Skin moisturizers have been used to treat excessive trans-epidermal water loss, one of the reasons for dry skin. Sericin's primary ingredient, serine, is similar to the natural moisturizing factor (NMR) in human skin, which makes it a potent moisturizer (29). To stop water loss from the top layer of the skin, sericin gel is created. It creates a hydrating, shielding, anti-wrinkle film on the skin's surface, giving the skin an instantaneous, enduring, smooth, and silky feeling30.

**Anti-frosting agent-** Because of its anti-frosting action, sericin's anti-frosting characteristic can be used to coat a coating on the surface of refrigeration equipment31. A common anti-frosting technique that works well for deep freezers, refrigerators, refrigerated trucks, and ships is the use of sericin film. Furthermore, frost damage can be avoided by using coated film on roofs and roadways. It has also been claimed that sericin coating on surfaces made of different durable materials improves functionality32.

#### Drug Delivery-For local and systemic drug delivery, a variety of materials including scaffolds, films, hydrogels, fibers, foams, spheres, capsules, and microneedles can be employed. Sericin can be utilized as a carrier because it readily binds charged medicinal molecules or hydrophobic and hydrophilic medicines due to its amphiphilic property (polar side chains and hydrophobic domains). Sericin also has a long half-life in vivo and strong moisture absorption and desorption capacities, both of which are advantageous for use in drug delivery (22,23).

**Food additives and food packaging-** Silk Sericin is a prospective food additive due to its outstanding safety, biocompatibility, oxidation resistance, and simplicity of breakdown. Notably, the U.S. Food and Drug Administration (FDA) has included Silk Sericin and its derivatives to the list of substances that are generally recognized as safe (GRAS). Consuming Silk Sericin in vivo has been shown to increase the bioavailability of various elements, including Zn, Fe, Mg, and Ca, without changing the serum levels of these substances. Additionally, it has been hypothesized that dietary Silk Sericin prevents atherosclerosis, which is linked to high triglyceride levels and significantly low-density lipoprotein (VLDL) levels (24-26).

#### Cell Culture - The scale of cell culture is expanding along with the market need for cell culture medium as a result of the biomedicine sector's quick development. One of the primary ingredients in culture medium, foetal bovine serum (FBS), has taken up tens of billions of dollars on the market. However, there are many concerns with the development and use of FBS, including expensive costs, the possibility of viral infection, problems with animal ethics, and a lack of resources. Different types of cocoons' sericin and its hydrolysate may significantly boost cell division. Additionally, after contrasting how sericin and FBS affect cell culture. When it came to cell viability, the sericin protein and its hydrolysate groups compared favourably to the control (10% FBS), and there was no discernible change in the shape of the cells between the various groups. Additionally, cells cultured in sericin medium displayed comparable cell shape, comparable or higher cell survival, and a quicker population doubling time than cells cultured in FBS medium (27-29).

**Tissue engineering-** One of the main objectives of biomedical research is to create materials that can be employed as grafts, immobilizing matrices, and drug delivery systems (33). Gelatine and sericin from the silkworm Antheraea mylitta have successfully created films and scaffolds. Fabricated supports have uniformly spaced pores, strong compressive properties, and strong swelling properties. They also exhibit excellent porosity, minimal immunogenicity, and improved viability and attachment of cells. These characteristics make sericin potentially useful for the production of bio-polymeric grafts in the future because they are essential for tissue engineering and biomedical applications (34-36).

**Antimicrobial-** Antimicrobial activity of the serums was assessed against Gram-negative E. coli and Gram-positive S. aureus in bacterial growth inhibition experiments. Experimental research demonstrates the antibacterial efficacy of silk sericin, demonstrating a zone of inhibition at 370°C for bacteria and 300°C for fungi (30,31).

**Conclusion**

Sericin has become a widely used commercial resource in a variety of businesses, including those producing cosmetics, medications, and food as well as numerous functional biomaterials. The creation of biomedical, pharmacological, and food products could benefit from the use of sericin. Sericin has become a valuable biopolymer due to its many useful applications and environmentally favorable qualities, as can be seen. Many silk producers and processors still throw away this important sericin that is present in the effluent because they are unaware of its existence. Many scientists are researching this area to investigate the viability of sericin extraction and its potential uses. Sericin has a bright future, and research in this area will undoubtedly support silk and its related sectors.

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