THE SCIENCE WORLD
A Monthly e Magazine
ISSN:2583-2212

Review Article

March 2024 Vol.4(3),1144-1161

An Overview Of Silk Sericin A Versatile Biopolymer From *Bombyx Mori* Silk Cocoon

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https://doi.org/10.5281/zenodo.10889458

Abstract

Bombyx mori, the domesticated mulberry silkworm, lepidopteran molecular model and the most significant economic insect. It is emerging as a great molecular genetic resource for tackling biological challenges. During the final stage of larval development, the silkworm *B. mori* produces massive quantities of silk proteins. These proteins are stored in the middle silk gland and expelled through the spinneret at the end of the fifth instar. Silk fibroin and sericin are the two prime silk proteins in the silk cocoons. Silk fibroin is a fibrous protein composed up of heavy chain (H), light chain (L), and glycoprotein linked by disulfide bonds, as well as sericin, a macromolecular protein that acts as an adhesive substance to combine fibroin for the production of cocoons of silkworm *B. mori*. A brief synopsis of sericin is given in this review. The structure, content, solubility, genetics, and characteristics of silk sericin are the main topics of this review. Large amounts of sericin, a silk protein, are often discarded by the textile and sericulture industries during the degumming process of production silk from silk cocoons. Because sericin is highly hydrophilic and confers useful biological and biocompatible properties like antibacterial, antioxidant, anticancer, and anti-tyrosinase properties, it can be investigated for its application. Aspartic acid, glycine, and serine are among the beneficial amino acids found in sericin, which is derived from silk cocoons. The creation of films, coatings, and packaging materials has shown the effectiveness of using sericin in combination with other biomaterials.

Key words: Bombyx mori, Silkworm cocoons, Biopolymer and Sericin

1. Introduction

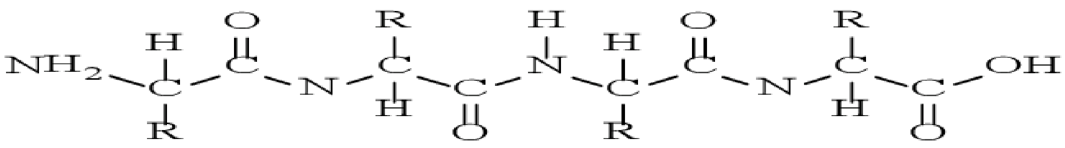
Sericulture is the practice of raising silkworms to produce silk, a valuable biomaterial. Silk is the elegant and most beautiful of all fibres, known as the queen of textiles. Commercial silks are produced by silkworm belongs to the families, *viz.*, Saturnidae (*Samia ricini*, *Antheraea assamensis*, *Antheraea mylitta* and *Antheraea proylei*) and Bombycidae (*Bombyx mori*). *Bombyx mori* also known as mulberry silkworm, produce a delicate creamise white silk fibre which is the primary commercial silk in the world. New developments in the sericulture derived products attracted many countries to this field. Silk is one of the most abundant naturally derived polymers, along with cellulose, chitosan, and collagen. It has also been recognized as a luxury raw material in the textile industry for thousands of years since the first discovery in Chinese and Indus civilizations nearly 2500 BC (Komatsu, 1980). Silk protein is synthesised by silk gland cells in silkworm larvae and stored in the lumen of silk gland before being transformed into fibre. The fiber is thin, long, light, and soft. It is well recognized for its water absorbency, dyeing affinity, thermotolerances, insulating characteristics, and luster (Mondal *et al.*, 2007). It is utilised as a raw material for producing extensive fabrics, drug delivery, artificial blood vessels and surgical sutures. To achieve the full potential of silks for such advanced applications, fundamental material knowledge is extremely important. Silkworms secrete silk as liquid during the process of spinning, it passes through the anterior gland and discharged out through the spinneret aperture (Shimizu, 2000) when it comes in contact with air, it turns into fibre. Recent studies have demonstrated the potential use of the *B. mori* cocoon and its two main proteins, fibroin and sericin, in the fields of polymers, biomaterials, cosmetics, and the food industry (Padol *et al*., 2012; Joseph and Raj, 2012). In the subject of sericulture, sericin has been ignored for a very long period. According to estimates, 400,000 tons of dried cocoons are produced worldwide, yielding 50,000 tons of sericin (Gulrajani, 2005). The majority of sericin is disposed of in wastewater (Fabiani, *et al.*, 1996). Enzyme, alkali, and acid are typically used to achieve the degumming process. The discharge of industrial effluent into the wastewater stream results in elevated levels of both chemical and biological oxygen demand (COD and BOD). As a result, the wastewater that the silk industry releases contaminate the environment and water. (Fabiani *et al.*, 1996). The economy and the environment would both benefit if sericin could be collected and utilized to create value-added products (Vaithanomsat and Kitpreechavanich, 2008). Removal and usage of sericin may have significant effects on the economy, society, and ecology; particularly in nations where sericulture is prevalent, such Brazil, India, and China. Sericin is quickly hydrolyzed, breaking down into smaller protein molecules that are easily distributed or dissolved in hot water, but it cannot dissolve in cold water (Gulrajani, 1988). Seri-waste and seri-by-products are now utilized to create value-added products. The silk fibers have excellent natural properties, which are comparable to the most advanced synthetic polymers, yet the manufacture of silk does not require laborious processing conditions. Consequently, the need for biocompatible and biodegradable materials demonstrates the expanding interest in using silk protein for non-textile applications across a wide range of scientific and medical fields, which supports the current review, which outlines the characteristics and biological uses of silk sericin derived from *B. mori* cocoons. Silk sericin is a natural *Bombyx mori* macromolecular protein. Sericin may be recovered for other applications throughout several phases of the production of raw silk and textiles. The recovery of sericin also decreases the environmental effect of silk production. Because of its characteristics, sericin protein is helpful. The protein resists oxidation, is UV-resistant, and readily absorbs and releases humidity.

2. Sericin Sources

Most common source of sericin is silkworm (Silva *et al*., 2022). Sericin has been extracted from wild non-mulberry silkworm cocoons such as *Philosamia ricini, A. mylitta* and *Cricula trifenestrata.* Sericin obtained from cocoons of *A. mylitta* has molecular weight 70, <200 and >200kDa. It shows similar secondary structure like sericin from *B.mori* but differ in biochemical and immunological properties (Kundu *et al.*, 2008).

3. Structure and composition of sericin

During cocooning, the two filaments, each originating from a silk gland, are wrapped by sericin layers to produce the silk thread (Padamwar and Pawar, 2004). Silk sericin is a natural glow like globular protein is made up of randomly coiled and β-sheet structures. Molecular motion, humidity, and temperature influence the random coil shape of β-sheet, causing the sol-gel transition to take place. If the water is hotter than 50–60°C, the protein becomes soluble. Solubility is decreased at lower temperatures, leading to random coil formation, and then into β-sheets, which leads to the development of a gel. Das *et al.*, 2022. More often than in an organized β-sheet structure, sericin is found as amorphous random coils. Random coil configurations can easily be transformed into a β-sheet structure due to repetitive mechanical stretching and absorption of moisture (Kludkiewicz *et al.*, 2009). Normally, sericin is partially unfolded, with 63 percent random coil and 35% β-sheet, without α-helix content (Kataoka *et al.*, 1977).



Where R = $^-CH_3$ or $^-CH_2C_6H_4OH$

Figure 1: Chemical structure of silk sericin.

Complex hydrophilic macromolecule is formed of the eighteen amino acids, polar groups like hydroxyl, carboxyl, and amino, and also has a mine and other types of amide functional groups that are able to crosslinking, polymerizing, and joining with other polymers. The carbon to oxygen ratio is 46.5 percent while the ratio of nitrogen to hydrogen is 16.5 percent. Das *et al.*, 2022. These 18 amino acids consist of all the necessary amino acids and contain 32 percent serine. In sericin, hydroxy amino acids make up 45.8% of the total. 12.2 percent of the residues are nonpolar amino acids and 42.3 percent are polar amino acids. About 20-30 per cent of the weight of the cocoon is contributed by sericin. Wrapping the fibroin is their primary function. When sericin is present, the fibers are rough and rigid; when it is removed, the fibers become soft and lustrous (Voegeli *et al.*, 1993, Shaw and smith, 1951).

Table 1. Amino acid composition of sericin.

Sl. No	**Amino acid**	**Sericin**
1	Glycine	14.7
2	Alanine	4.3
3	Serine	37.3
4	Tyrosine	2.5
5	Valine	3.5
6	Aspartic acid	14.8

7	Glutamic acid	3.4
8	Threonine	8.6
9	Leucine	1.4
10	Phenyl alanine	0.38
11	Proline	0.36
12	Methionine	0.76
13	Cystine	0.51
14	Lysine	2.4
15	Histidine	1.1
16	Arginine	3.5
17	Isoleucine	0.70
18	Tryptophan	0.20

Information derived from Kamili and Masoodi (2000); Shimura (1978; 1988);

4. Forms of sericin

4.1 Based on their degree of solubility, sericin can be classified into three fractions:

Sericin A- The outermost layer, sericin A, is insoluble in hot water. It has a nitrogen content of 17.2% as well as amino acids such as aspartic acid, glycine, serine, and threonine.

Sericin B- The middle layer, sericin B, hydrolyses in an acidic solution to produce tryptophan and the amino acid of sericin A. 16.8% of its content is nitrogen.

Sericin C- Sericin C, the innermost layer, is insoluble in hot water and can be treated with heated, diluted acid or alkali to separate it from fibroin. Along with the amino acids of sericin B, proline is also produced during acid hydrolysis. It also has 16.6% nitrogen and sulfur in it. (Shaw and smith *et al.*, 1951; Sprange, 1975).

4.2 Based on relative solubilities, sericin has been classified into several species. Other researchers have also named different fractions of sericin as sericin A and B, or sericin I, II, III, and IV, or S1, S2, S3, S4, and S5, and as α, β, and γ modification based on their dissolving behaviour (Komatsu *et al.*, 1980; Voegeli *et al*., 1993). The primary molecular shape of sericin that dissolves readily is a random coil, while the β-sheet structure is more challenging to dissolve. The molecular aggregation structure becomes denser and more crystalline due to recurrent moisture absorption, which results in decreased solubility. The three layers of the sericin structure are seen in the γ -ray investigation. There were some fiber direction filaments in the outer layer, cross-fibre direction filaments in the middle layer, and longitudinal filaments in the inner layer Wang *et al.,* 1985. The temperature at which sericin is cast also affects its structure. As the casting temperature is lowered, the sericin molecules adopt a β-sheet shape instead of a random coil configuration Zhu *et al.*, 1998.

4.3 Sericin is categorized according to its molecular weight, with the middle region of the silk gland serving as the site of synthesis by *et al*. (2002). The protein is composed of three big polypeptides called sericins, A, M, and P. The Ser1 gene encodes sericins P and M, which together make up the first and second sericin layers that round the fibroin (Takasu *et al.*, 2005). Prior to day 6 from the fifth instar, your transcripts are expressed in the middle and posterior regions of MSG but not in the anterior portion. The anterior region exhibits a high level of Ser2 gene expression, whereas the middle region shows little to no expression of the gene. From day 4- 6 of the fifth instar, expression is prominent (Takasu *et al*., 2010). The sericin A gene, encoded by the Ser3 gene, is primarily found in the inner and outer layer of the cocoon (Takasu *et al.,* 2007). It is scarce in the middle portion and generally found in the anterior section. The 5th instar marks the beginning of the Ser3 transcript signal, which intensifies until day 7. In *B. mori* cocoons, the sericin layers are made up of products from the Ser1 and Ser3 genes, but the proteins expressed by the Ser2 gene are not considered cocoon-related and are linked to larval silk (Takasu *et al.,* 2010). Before each instar transformation and before the cocoon is produced, silkworms spin a tiny bit of silk to secure the cocoon to an appropriate substrate (Kludkiewicz *et al.,* 2009).

5. Sericin genes in *Bombyx mori*: Sericin belongs to a glycoproteins family produced through alternate splicing of sericin genes (Michaille *et al.,* 1989), contributes to 25 to 30 percent of cocoon weight. The genes' expressions are temporally regulated according to larval development, which adds a little homogeneity between the exons and accounts for the substantial protein diversity (Kundu *et al.,* 2008 and Sehnal, 2008). The synthesis of sericin is regulated by at least three genes: Ser1, Ser2, and Ser3. The first gene to be found was Ser1, which is found in the Src locus of chromosome 11. It is a single copy that is approximately 23 kb long, has 9 exons and 8 introns and encodes four major mRNAs (10.5, 9.0, 4.0, and 2.8 kb) by alternative splicing (Okamoto *et al*., 1982 and Takasu *et al.,* 2007). Michaille *et al*., 1989 found the Ser 2 gene, a highly polymorphic gene composed of 13 segments ranging in size from 28 to 2574 bp and encoded two mRNAs (3.1 and 5.0-6.4 kb) by alternative splicing. Ser2 has been discovered to be the most complex and versatile gene known to encode silk proteins. Its gene organization resembled that of the Ser1 gene, particularly the first two exons encoding signal peptides are the same size (Kludkiewicz *et al*., 2009). Takasu *et al.* 2007 found the last gene associated in sericin synthesis, Ser3, which is also situated on chromosome 11, locus Src-2. This gene, which is around 3.5bp in size, comprises three exons and encodes a simple transcript of 4.5 kb (Gamo, 1983).

Alternative splicing contribute diversity to sericin proteins (Kunz *et al*., 2016). Northern blotting revealed Ser 1 gene is expressed by cells in the middle and posterior subpart and not by anterior subpart. Ser 2 and Ser 3 genes are expressed only in the anterior subpart of MSG (Takasu *et al*., 2010). Sericin genes are transcribed during intermolts and maximum levels of sericin transcripts are observed several days before the larva spins cocoon. At fifth instar stage 150 posterior cells of MSG transcribe Ser 1 gene while posterior most 42 cells transcribe 2.8 kb mRNA. Other 108 cells in MSG produce 4.0, 10.5 and 9.0 kb mRNA (Garel *et al.,* 1997). Michaille *et al* (1989) have reported three new genes strictly expressed in MSG named as MSGS-3, MSGS-4 and MSGS-5 but their structures are unknown. These genes encode 3500, 2950 and 450 nucleotide long m RNA.

6. General properties of Silk Sericin

6.1 Gelling Property

The solubility of the sericin random coil in hot water facilitates its conversion into a β -structure, which in turn enables the gelation process. However, this structure transforms into a β-sheet structure at low temperatures (10ºC, pH around 6-7), which aids in the creation of a three-dimensional network and encourages the construction of the sericin gel (Zhu *et al*., 1998; Huddar *et al*., 1985**)**. This behaviour is reversible when the sample is heated at 50–60ºC. On the contrary, the ability of sericin to gel can also be obtained through chemical crosslinking, such as using glutaraldehyde, which results in the creation of a stable β-sheet structure (Zhu *et al*., 1995; Hirabayashi *et al*., 1989; Padamwar *et al*., 2004).

6.2 Isoelectric pH

The isoelectric point of sericin is around 4.0 due to the presence of more acidic than basic amino acid residues (Voegeli *et al*., 1985).

6.3 Sol-Gel Transition

Sericin has the sol-gel property, readily dissolving in water at 50–60°C and gelling again upon cooling (Zhu *et al*., 1995)

6.4 Solubility of sericin: Specifically, sericin in the outer layer of the fiber is most soluble in heated water (α-sericin), whereas sericin in the inner layer of the fiber (next to fibroin) is insoluble in hot water (β-sericin). This indicates that the position of sericin within the silk fiber influences its water solubility (Mondal *et al* 2007).

The poly (Na acrylate) addition enhances the solubility of sericin, but the addition of formaldehyde, polyacrylamide, or resin finishing agents diminishes it (Kataoka, 1977; Ishizaka and kakinoki, 1980; Zhu *et al*., 1995).

6.5 Molecular Weight

The molecular weight of sericin extracted using 1% sodium deoxycholate solution and precipitated with an equal volume of 10% trichloroacetic acid ranges from 17100 to 18460 (Rassent, 1967). The results of the gel electrophoresis analysis of sericin extracted with hot water demonstrate a molecular weight of 24000. In contrast, the spray-drying approach yielded sericin with a molecular weight of 5000-50,000 and an enzyme action of 300-10,000 and 50,000 when extracted with aqueous urea at 100ºC (Tsubouchi *et al*., 1999).

7. Sericin Properties Favourable for Biomedical and Pharmaceutical Applications

Sericin dissolved in degumming bath has been discarded in the process of raw silk production. Recovery and using the sericin from the degumming bath have potential economic and environmental benefits (Takasu *et al*., 2002). Easy degradation of silk sericin in hot water and alkali during processing imparts difficulties in studying physicochemical properties of silk sericin. A fibroin deficient mutant strain of *B.mori* named 'Sericin hope' secretes only sericin. Intact sericin derived from 'Sericin hope' is useful as a biomaterial and for the determination of physicochemical properties (Teramoto and Miyazawa, 2005).

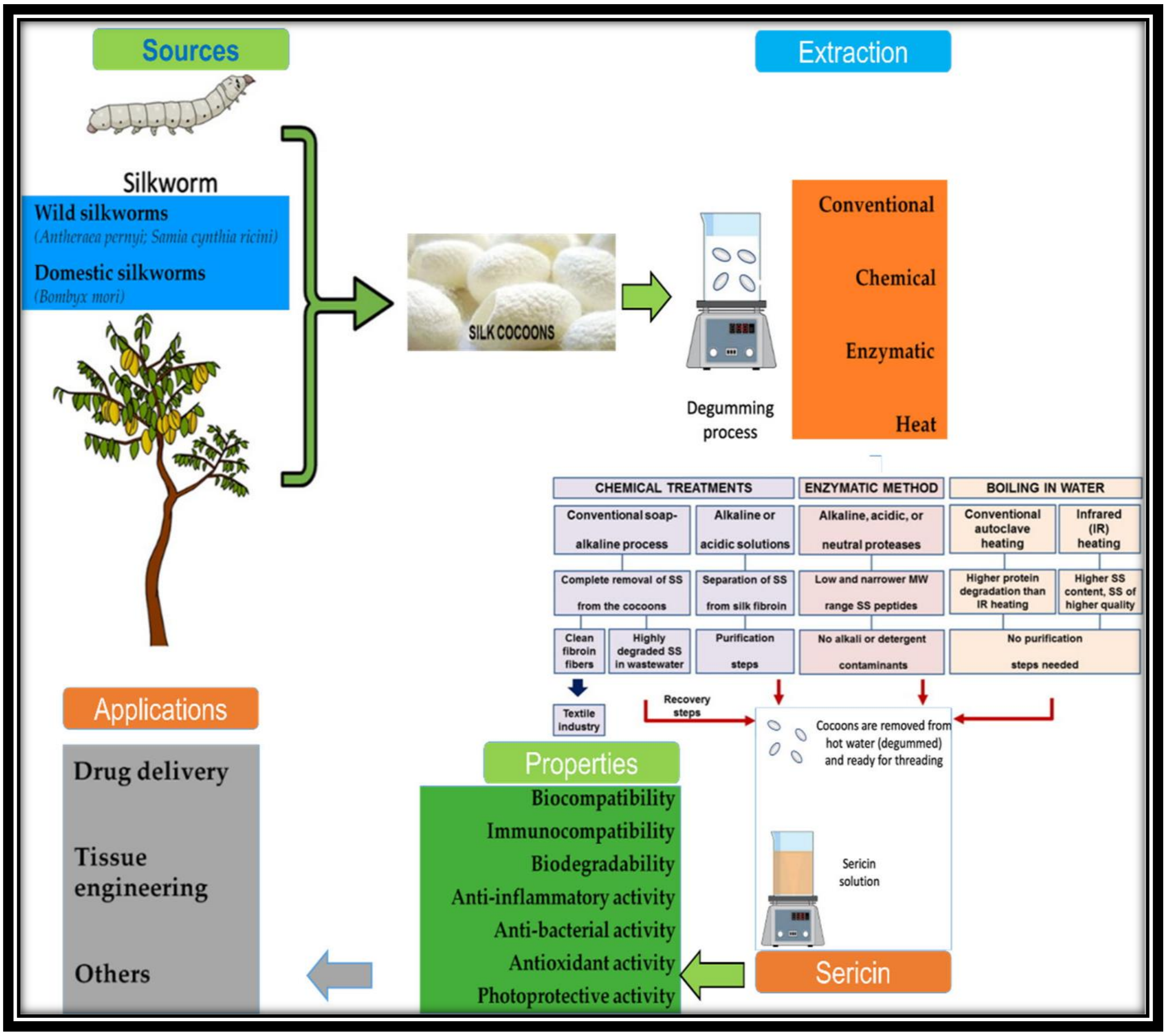


Figure 2. Graphical representation of the source, extraction process, attributes and application of sericin. Adopted from Silva *et al*. (2022); from Das *et al*. (2021) and from Lamboni *et al*. (2015),

Biodegradability, biocompatibility, anti-inflammatory, photoprotective activity, antioxidant, antibacterial, and immunocompatibility are among the most important and promising biological features of silk sericin (Figure 2).

7.1 Biocompatibility and Immunological Response: Biocompatibility is the primary prerequisite for any biomedical material. The moment a biomaterial interacts with the human body, it should not result in any negative outcomes. (Such as an immunological reaction) (Cacopardo, 2022). Sericin's biocompatibility has been proved in a number of investigations, as this specific protein lacks immunological activity. Numerous cell lines' culture medium containing sericin have demonstrated that this does not cause cytotoxicity, showing sericin is harmless for cells (Terada, *et al*., 2005). Furthermore, sericin doesn't cause immune reactions. Chlapanidas *et al*. (2013) demonstrated the activity of sericin derived from various silkworm strains in peripheral blood mononuclear cells. The findings showed sericin (in certain strains) encouraged a reduction in interferon-gamma production in vitro (IFN-γ). Similarly, there was no obvious effect on the release of interleukin 10 (IL-10) and tumour necrosis factor-alpha (TNF-α).

7.2. Biodegradability: A biodegradable biomaterial is capable of disintegrating into other substances by an organism via various biological processes. In most circumstances, biodegradable biomaterials are selected for biomedical product production since they are only retained in the human body until they perform their purpose, after which they are gradually and naturally eliminated. When biodegradable biomaterials are utilized to treat wounds, the benefits are obvious. By using a biodegradable wound dressing, the patient experiences less pain and discomfort and the newly formed tissue is not harmed. It also eliminates the need to replace or remove the dressing from the wound site (Holland *et al.*, 2019). Proteolytic enzymes that operate on the amorphous hydrophilic portions of the heavy and light chains of silk tissue break down the biodegradable polymer sericin both in vitro and in vivo. Examples of these enzymes include protease XIV, α-chymotrypsin, proteinase K, papain, matrix metalloproteinases, and collagenase (Holland *et al.*, 2019 and Cao and Wang, 2009). Amino acids are the by-products of sericin's breakdown, which the body absorbs without triggering an immunological reaction.

7.3. Anti-Inflammatory Activity: Inflammatory phase of the healing process involves phagocytosis of necrotic tissues as well as potential contaminants at the wound site (Koh *et al*., 2011). At this point, growth factors and cytokines secreted by inflammatory cells assemble the cells needed for the development of new tissue. However, since the uncontrolled, excessive synthesis of inflammatory cytokines stimulates the expression of metalloproteinases, which are accountable for the degradation of extracellular matrix, this phase needs to be regulated. That's why biomaterials meant for healing wounds need to be able to regulate inflammation (Anderson, *et al.*, 2008). In general, tests used to assess anti-inflammatory action frequently rely on measuring the expression or release of inflammatory cytokines, such as antitumor necrosis factor-alpha (TNF-α) and interleukin 1 (IL-1). The two most significant inflammatory mediators are TNF-α and IL-1β, as reported in the literature. Anderson *et al.* (2008) reported that Adhesion molecules, which are required for the proliferative phase, are triggered to express. Consequently, experiments conducted both in vitro and in vivo have shown that sericin regulates the release of TNF-α and IL-1β, two inflammatory cytokines (Kunz *et al.,* 2016).

7.4. Antibacterial Activity: If a biomaterial eliminates bacteria or inhibits their growth or capacity to multiply, it possesses antibacterial qualities (Zhao, 2011). Certain studies suggest that sericin's antibacterial properties could be attributed to the uncharged polar amino acid cysteine, which has sulfhydryl groups in its composition. These sulfhydryl groups may subsequently interact with oxygen or nitrogen to produce weak hydrogen bonds, which creates an extremely reactive chemical that influences a variety of enzyme reactions and metabolic functions of microorganisms. Empirical evidence indicates that sericin has antibacterial activity against both Gram-positive and Gram-negative bacteria. Research conducted by Ahamad and Kumar Vootla (2018) demonstrated that sericin possesses antibacterial properties against *Staphylococcus aureus*, *Escherichia coli*, and fungus such as *Aspergillus flavus* and *Candida albicans*. Similarly, Jassim and Al-Saree (2010) discovered that increasing the concentration of sericin (10-20 mg/mL) improved its inhibitory effect on the development of

Streptococcus pneumoniae, Pseudomonas aeruginosa, and *E. coli*. There have been reports of silk sericin's antibacterial and anti-biofilm properties. Recently, Aramwit (2020) and other authors conducted an in vitro study to evaluate sericin's ability to prevent biofilm formation and disrupt previously established biofilm (treatment). They discovered that sericin extracted with heat, urea, or acid degradation might prevent and/or lessen the formation of biofilms; the most potent antibiofilm action was exhibited by urea-extracted sericin (against *Streptococcus mutans*). This demonstrates the potential use of sericin as a biofilm inhibitor.

7.5. Antioxidant and Photoprotective Activity: Normal cellular metabolism produces reactive oxygen species (ROS), which can be dangerous in high doses. Since they are unstable, free radicals and ROS readily react with other molecules or groups within the body, resulting in cell or tissue destruction and causing numerous diseases (cancer, cirrhosis, ischemia reperfusion, *etc*.) (Takechi, 2014). Kato *et al.* (1998) found that sericin's antioxidant attribute derived from its capacity to scavenge ROS, reduce lipid peroxidation, and inhibit tyrosinase and elastase. Li *et al.* (2008) showed that Sericin may increase the activity of antioxidant enzymes such superoxide dismutase, catalase, and glutathione peroxidase. Sericin's antioxidant actions are linked to its high serine and threonine content, whose hydroxyl groups function as chelators for trace metals like copper and iron (Fatahian *et al.*,2021, Lamboni *et al*., 2015). Pigment molecules (e.g., flavonoids and carotenoids) that concentrate in sericin layers may be one source of sericin's antioxidant and anti-tyrosinase activity. Sericin has also been shown to have photoprotective properties because it can effectively absorb ultraviolet (UV) light and prevent oxidative damage by preserving redox equilibrium. It has previously been observed that topical application of *B. mori* sericin protects female hairless mice from UVB radiation-induced sunburn and tumor initiation (Li *et al*., 2008). Sericin has several amino-based groups rich in hydrogen, oxygen, and nitrogen, which allow for high absorption of UV radiation wavelengths under 200 nm (Zhaorigetu, *et al*., 2003).

7**.6 Anti-aging activity:** Sericin can boost moisturizing qualities and replenish natural moisturizing elements (Kundu *et al*., 2008). Sericin promotes the synthesis of collagen type I while suppressing apoptosis; - Nitrite, which results in oxidative stress and promotes the growth of b-cell lymphoma 2 (bcl-2), is regulated by sericin (Kitisin *et al*., 2013). Sericin inhibits apoptosis in UVB (30 mJ/cm2) irradiated human epidermal keratinocyte cells to inhibit the activation of caspase-3 (Gillis *et al*., 2009). At 12 weeks of age, mature male Wistar albino rats weighing between 257 and 395 g were given 30 mg of sericin powder through their thoraxes. Five animals (83.33%) in the sericin group had substantial fibrosis, fibroblastic activity, and collagen deposition (Yazicioglu *et al*., 2017).

8. Extraction Methods of Sericin from Silk Cocoons:

The process of degumming silk results in the breaking of peptide bonds caused by sericin hydrolysis and subsequent separation from fibroin. Since sericin is soluble in water and hydrophilic while fibroin is hydrophobic, it is possible to extract sericin from silk.

Table 3. Overview of the primary methods for extracting sericin from *B. mori* cocoons.

Extraction methods	**Approach**	**Advantages**	**Limitations**	**References**
Conventional	Detergents/soaps (e.g., Sodium bicarbonate and Marseille)	Effective	The degradation of sericin is high. Recovery from sericin is challenging. It is not environment friendly/effluent problems	Wang *et al*., 2019
Chemical	Alkaline solutions (e.g., sodium phosphate, sodium carbonate, sodium hydrosulfite and sodium silicate)	Rapid Low-cost Effective	Degradation of sericin occurs Recovery from sericin is challenging. Steps for purification are required. It is not conducive to the environment/effluent problems	Lamboni *et al.*, 2015 and Wang *et al*., 2019
	Acidic solutions (e.g., citric, tartaric, succinic acid).	Sericin is less degraded than When using alkaline solutions	Sericin is degraded Ineffective Purification steps are needed It is not environment friendly/effluent problems	Lamboni *et al.*, 2015 and wang *et al*., 2019
	Urea (with or without Mercapto ethanol)	Effective Time-consuming Sericin is poorly degraded	Steps for purification are required to remove the chemical impurities Toxic to cells	Lamboni *et al.*, 2015 and wang *et al*., 2019

Enzymatic	Proteolytic enzymes (e.g., bromelain, pancreatin, alkalase, savinase, degummase, papain, trypsin, etc.)	Effective Environment-friendly/no effluent problems	Expensive Sericin is degraded Time-consuming	Lamboni *et al.*, 2015, Wang *et al.*, 2019 and More *et al.*, 2018
Heat	Boiled in water (associated or not with high pressure by autoclaving)	Simple Low-cost Time-consuming No purification steps needed Environment-friendly/no effluent problems	Sericin is degraded (when Used at high temperatures) Fibroin is damaged Removes only the outer layer of the sericin.	Lamboni *et al.*, 2015 and Wang *et al.*, 2019

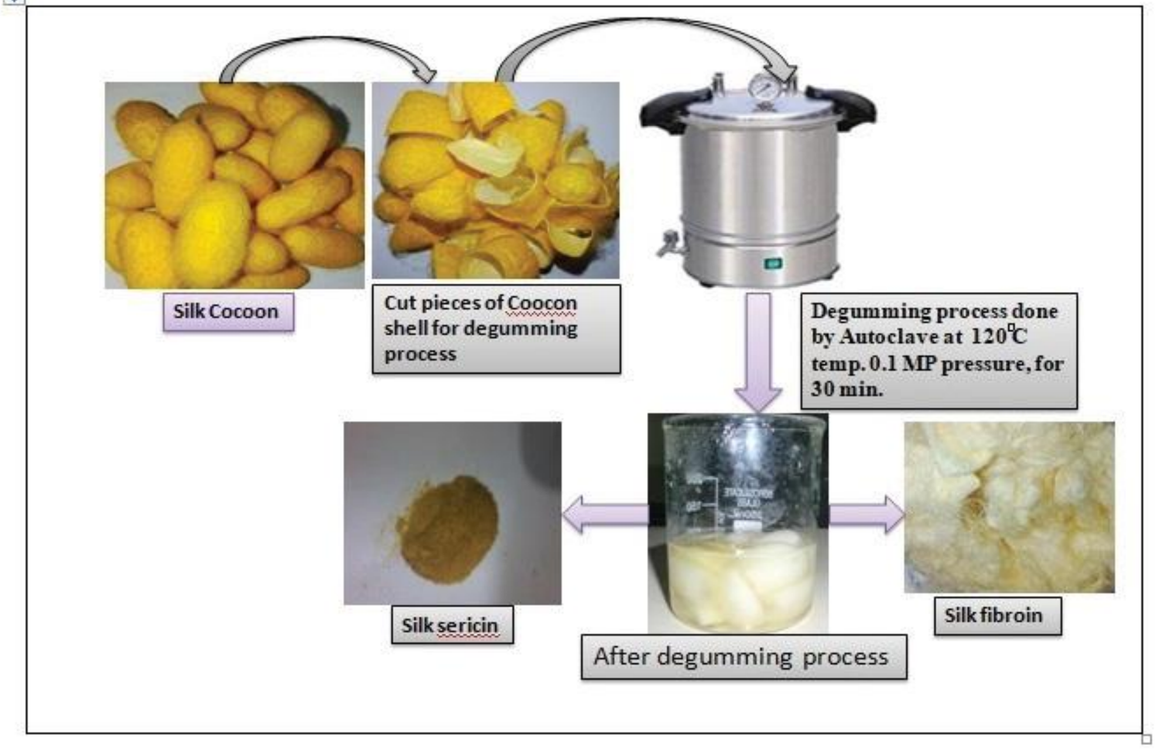


Figure 3: Schematic diagram of extraction process of sericin from silk cocoons (Adopted from Saha *et al.*, 2019).

9. Potential Applications of Sericin

Silkworm silk attracted attention for its biomedical application due to its biocompatibility, ease of chemical modification and slow in vivo degradation (Rockwood, *et al.*, 2011).

9.1 Biodegradable materials: Blending sericin with other resins can yield environmentally friendly biodegradable polymers (Annamaria *et al*., 1998). Polyurethane foams comprising sericin are stated to have superior moisture absorption and desorption characteristics (Aiba *et al.*, 1995). Polymer films, foams, molding resins, and fibers containing sericin (0.01-50% w/w) can be produced by reacting a blend including a polyol, tolylene, di-isocyanet, di-butyltin di-laurate (catalyst), and trichloromonofluoromethane (a blowing agent) in the presence of sericin.

9.2 Membrane materials: Membrane-based separations (such as reverse osmosis, dialysis, ultrafiltration, and microfiltration) are utilized in a variety of processes, including water desalination, ultrapure water manufacturing, the bioprocessing sector, and several chemical processes. Because sericin contains a significant quantity of amino acids with neutral polar functional groups, it is not easy to make membranes from pure sericin. However, membranes created from sericin that has been cross-linked, mixed, or copolymerized with other substances are easily made. Membranes that contain sericin are highly hydrophilic. A protein-containing synthetic polymer film for separating water from organics can be created by copolymerizing acrylonitrile, which is used to make some synthetic polymers, with sericin (Zhou *et al*., 2008).

9.3 Functional biomaterials According to research by Nakajima (1994), a sericin layer placed on top of liquid crystal can uniformly orient the molecules of the liquid crystal to produce high-quality, distortion-free displays. Due to its anti-frosting properties, sericin-coated film is applied to the exterior of refrigeration equipment (Tanaka and Mizuno, 2001). A common and efficient way to prevent frost in refrigerators, deep freezers, refrigerated trucks, and ships is to use coated sericin film. Frost damage can be avoided by using the coated film on roofs and roadways more frequently. To improve functioning, sericin protein can be applied to the surfaces of a variety of sturdy materials. Sericin can be used to protect an object's surface or to prepare pigments for art. Excellent weatherability, good permeability, and resistance to warping during drying characterize the substance that covered the sericin. Water-soluble polymers, particularly polyvinyl alcohol (PVA), combine well with sericin. It is claimed that a blended hydrogel consisting of sericin, fibroin, and PVA has extraordinary elasticity and moisture-absorbing and desorbing capabilities (Yoshii *et al*., 2000). In addition to being utilized in medical supplies and wound dressings, hydrogel can be used as a soil conditioner. Using glutaraldehyde as the cross-linking agent, Miyairi and Sugiura (1978) described a cross-linked sericin film for enzyme immobilization. The immobilized J-glucosidase on the cross-linked sericin film exhibits greater heat stability, electro-osmosis resistance, and stability than the free enzyme.

9.4 Medical biomaterials: The most common type of silk-like material utilized in biomedical applications, especially for sutures, is silkworm silk fibers. Silk fibers have been used for decades and have shown to be useful in a variety of therapeutic applications. Biocompatibility has also come under scrutiny due to certain biological reactions to the protein. created a wound dressing based on silk fibroin that could be peeled off without harming the newly produced skin and could accelerate healing. The non-crystalline fibroin film used as a wound dressing had a thickness of 10–100 μm and a water content of 3–16%. The wound dressing was subsequently produced using a combination of sericin and fibroin (Mondal *et al*., 2007). An excellent substrate for the proliferation of adherent animal cells is a membrane made of sericin and fibroin, which can be utilized as substitute of collagen. The adherence as well as the growth of animal cells on films composed of sericin and fibroin were studied by Minoura *et al*. (1995). Ensuring the composite membrane maintained a minimum of around 90% sericin was necessary for cell adhesion and proliferation The functional features of a film composed of sericin and fibroin are similar to those of the human cornea and it possesses outstanding oxygen permeability. The idea was to create article corneas using a film containing a combination of sericin and fibroin. Through the template polymerization of acrylic acid with silk sericin, a new mucoadhesive polymer has been created. Sericin and fibroin can be sulfonated to transform silk protein into a biomaterial having anticoagulant qualities. The silk protein's antioxidant activity was demonstrated by the suppression of in vitro lipid peroxidation by sericin. Moreover, it was discovered that sericin inhibits tyrosinase activity. These findings suggested that sericin is a valuable natural food and cosmetic component.

Keratin and the biopolymer sericin are very affinistic. One factor contributing to dry skin is excessive trans epidermal water loss (TEWL), which can be mitigated with the use of skin moisturizers. The natural moisturizing factor and silk sericin resemble one another (NMR). To make sericin gel, sericin solution is combined with carbopol and pluronic as a stabilizer to stop water loss from the skin's outer layer. It creates a protective, semi-occlusive, anti-wrinkle, moisturizing layer on the skin's surface that leaves the skin feeling instantly smooth and silky for a long time (Padamvar *et al*., 2005). Skin may absorb sericin naturally, which rejuvenates cells. It has been found that sericin can inhibit the processes of active oxygen, a major aging agent that causes wrinkles and dark spots. the application of oxygen-permeable membranes for contact lenses, prosthetic skin, etc. made from silk fibroin and silk sericin, which contain roughly 60% water.

Other applications for sericin include soil conditioner, coagulant for waste water purification, hygroscopic moisture-releasing polyurethane foams and their production for furniture and interior materials, medical composites of sericin, rice cooker additives to prevent colon cancer, fabric care compositions, makeup powders coated in sericin, dermatitis inhibitor, and wound protection. chewing gum, movies, and nail polish. In rats, sericin improves intestinal absorption of iron, zinc, calcium, and magnesium through dietary intake, indicating the potential use of this natural component in food manufacturing.

10. Conclusion

The recovery of sericin from wastewaters used in the silk industry is very important for the environment, society, and economy. As this review has indicated, sericin is a substance that can be used in a variety of biomedical and pharmacological applications because of its special qualities. silk sericin makes it possible to create commercially feasible, biodegradable and biocompatible products. Sericin has several advantageous properties and potential uses, but there are certain drawbacks that prevent it from being used in biomedicine. Firstly, the selection of an extraction process ensures a consistent physicochemical profile and enhanced biological performance. This method should also be scalable and sustainable enough to be used at the industrial level, and it may even be able to maintain the silk industry's economic competitiveness.

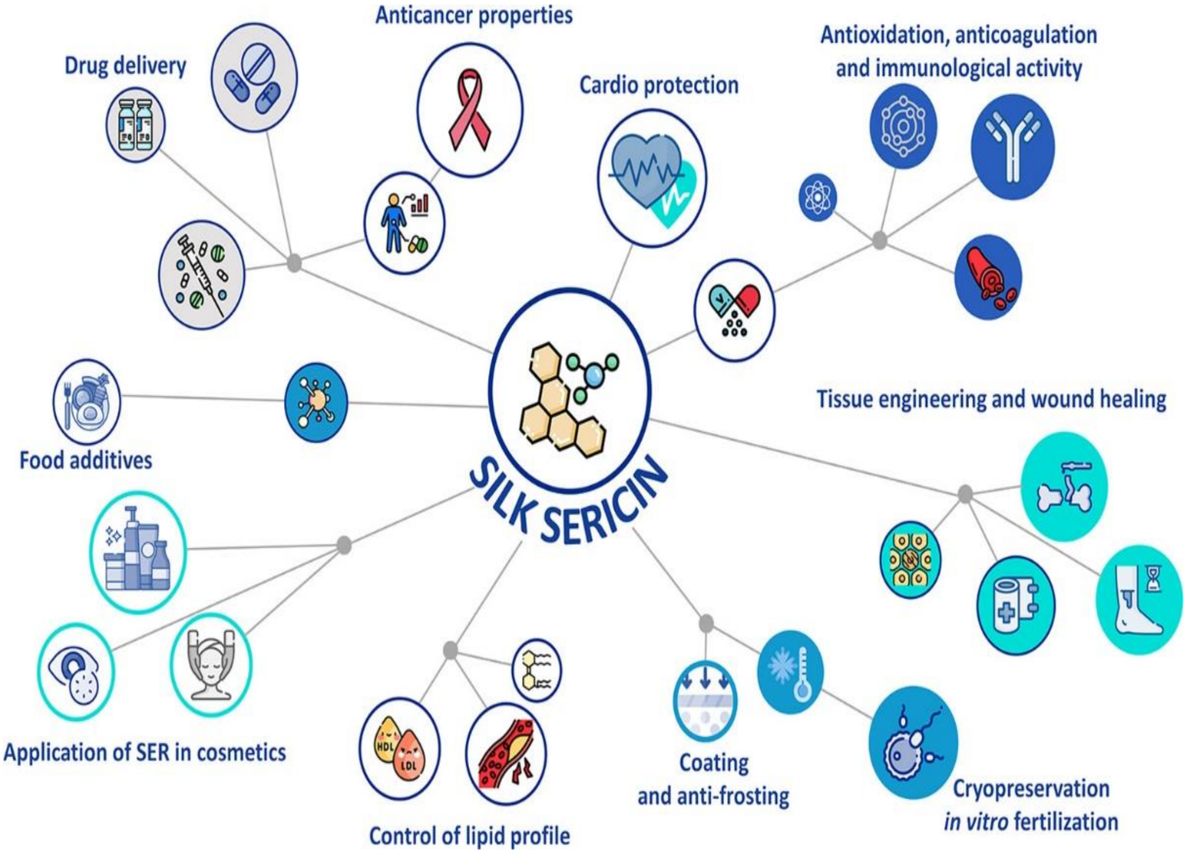


Figure 4 Major applications of sericin (Adopted from Marwa *et al.*, 2023)

Furthermore, because of its low mechanical properties and rheological characteristics, silk sericin cannot be used as a drug delivery system or as scaffolds for tissue engineering. As a result, the production of sericin-based scaffolds with desirable properties, often prepared in combination with other compounds. The sericin is obtained from silk cocoon waste through autoclave and centrifuge machine without using any chemicals. This technique could be helpful for the economy and environment.

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