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Advances in the Recovery of Silk Sericin from *Bombyx Mori* Cocoon Degumming Wastewater: Extraction Methods, Characterisation, and Emerging Applications: A Review

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***Abstract*—Silk sericin, a water-soluble globular protein secreted by the silkworm *Bombyx mori*, constitutes approximately 20–30% of the cocoon weight and functions as a natural adhesive that binds fibroin filaments during cocoon formation. Traditionally, sericin is discarded during the silk degumming process, generating protein-rich wastewater that contributes to environmental pollution. However, increasing scientific evidence has highlighted sericin as a valuable biomolecule owing to its excellent biocompatibility, biodegradability, antioxidant, antimicrobial, anti-inflammatory, and wound-healing properties, making it a promising material for biomedical, pharmaceutical, cosmetic, food, and tissue engineering applications. This review comprehensively summarises the current methods for sericin extraction, including physical, chemical, enzymatic, and wastewater recovery approaches. Physical techniques such as autoclave, boiling, high-temperature high-pressure, infrared heating, and microwave-assisted extraction are discussed with respect to extraction efficiency, protein integrity, environmental sustainability, and scalability. Chemical extraction methods employing urea, alkaline agents, acids, and detergents are critically evaluated for their high extraction yields alongside concerns regarding protein degradation, purification requirements, and environmental impact. Enzymatic degumming using proteolytic enzymes is highlighted as an eco-friendly alternative capable of preserving sericin bioactivity, although its industrial application remains limited by higher costs and longer processing times. The review further compares the advantages and limitations of each extraction strategy and discusses the influence of extraction conditions on sericin quality and functionality. Efficient recovery and utilisation of silk sericin not only minimises environmental pollution associated with silk processing but also transforms an industrial by-product into a high-value biomaterial. Future research should focus on developing sustainable, cost-effective, and scalable extraction technologies that preserve sericin's biological properties while supporting its broader industrial and biomedical applications.**

***Index Terms*—Silk sericin; Sericin extraction; Degumming; Sustainable extraction; Biomaterials; Wastewater recovery.**

I. INTRODUCTION

Sericulture is an agro-based industry, and its main objective is silk production. Including silk, sericulture by-products also have economic value and remarkable applications (Sharma *et al.,*2022). The *Bombyx mori* silkworm is mainly composed of two proteins, fibroin and sericin. Silk sericin has three layers: the outer layer, middle layer and inner layer (Cao T.T. and Zehang YQ 2016). Sericin is a glue-like protein that is water-soluble. Sericin is secreted by the silkworm *Bombyx mori* to cover the main silk fibre, i.e. fibroin. Sericin helps the thin fibroin fibres hold together to form a cocoon. The total weight of a cocoon contains about 20-30% of sericin (Masahiro *et al.,*2000). Sericin is removed from the silk fibres during the degumming process and discharged with silk processing wastewater. This can cause severe environmental pollution problems (Capar *et al.,* 2008). The silk textile industry is an industry where the use of water cannot be avoided, and this increases the amount of wastewater. This leads to the contamination of water due to the released wastewater in the environment (Fabiani *et al.,* 1996). The recovery of sericin could be used to develop value-added products, and this can be beneficial in the economic sector and to reduce environmental pollution (Vaithanomsat P and Kitpreechavanich V 2008). Silk sericin was previously considered a waste by-product in silk extraction. In recent years, sericin has been studied due to scientific interest as it has biological and pharmacological properties. Sericin-based materials have multifunctional applications in the upcoming decades, uses in various industries and in technological advancement as reported by (Yunong *et al.,* 2024).

Therefore, the present review aims to provide a comprehensive and critical evaluation of silk sericin, encompassing its biological origin, molecular composition, physicochemical properties, and functional characteristics. Particular emphasis is placed on the comparative assessment of conventional and emerging green extraction technologies, factors influencing extraction efficiency and protein quality, structure–property relationships, and recent advances in biomedical, pharmaceutical, cosmetic, agricultural, food, and environmental applications. In addition, the review highlights the role of sericin in supporting circular bioeconomy, identifies current research gaps, and proposes future directions for sustainable recovery and industrial commercialisation. By integrating recent scientific advances with critical analysis, this review seeks to provide researchers, industry stakeholders, and policymakers with a comprehensive framework for the sustainable utilisation of silk sericin as a high-value biomaterial.

BIOLOGY AND CHEMISTRY OF SILK SERICIN

Sericin is a natural protein produced by the silkworm *Bombyx mori.* Sericin is usually discarded in wastewater in the silk industry. But recent studies on sericin reveal its potential in different applications. Sericin has various applications due to its potential; sericin is used as a commercial protein in medical, cosmetics and pharmacology. Its structural composition and its solubility help to study the use of sericin. It has different properties such as biodegradability, biocompatibility, anti-inflammatory, etc. (Vidyashree S *et al*., 2024).

Sericin is a highly hydrophilic protein. It is a natural polymer with adhesive properties. Sericin helps to hold silk filaments together to maintain their structural integrity during cocoon formation. Sericin is a globular protein containing a random coil and β-sheets structure (Sharma *et al.,* 2022). The presence of polar amino acids along with hydroxyl groups leads to the hydrophilic nature of silk sericin. These groups play an important role in the structure of sericin and have importance in different biological activities (Gulrajani *et al*., 2005). It is a globular protein secreted in the middle region of the silk gland. The nature of silk sericin is dependent on the presence of 18 amino acids with polar groups like hydroxyl, carboxyl, and amino groups and high content of serine, aspartic acid and glycine (Kunz *et al.,* 2016). Silk sericin is a natural glue-like protein made up of random coiled and β-sheet structures. Sericin is a soluble protein; its solubility decreases at lower temperatures and leads to the formation of random coils and β-sheets; this helps to form a gel (Das *et al.,*2022). Understanding these biological processes is essential for optimising sericin recovery while preserving its native structure and bioactivity.

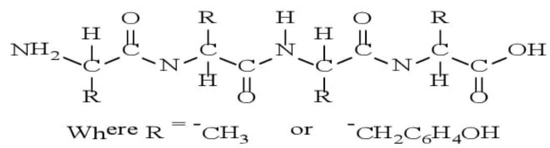

Figure 1: Chemical structure of silk sericin.

SILK SERICIN EXTRACTION

From previous research, we found that there are different methods of silk sericin extraction. Sericin can be obtained by separating it from fibroin fibres. The degumming process is done by several methods, such as by using the boiling method, by using urea with 2-mercaptoethanol, soap, alkali, etc.

II. METHODS OF EXTRACTION

Physical methods

Extraction of silk sericin by Hot Water

Another method of extraction of silk sericin was reported by Wu *et. al*. (2007) and Boraiah J. and Shivashankar M. 2024). Silkworm cocoons are boiled in distilled water for 2-3 hrs with constant stirring to separate sericin from fibroin in water. After this, the sericin was obtained, the condensation process was done, and water was evaporated at 50 °C. This can remove the excess water left in the solution, and the concentration of sericin increases. After this, the sericin extract condensed, 75% ethanol was added to the solution in the ratio of 1:10. Solution left for one night in the refrigerator at 4°C. Then the solution was sprayed on a glass plate and left at room temperature; there should be no moisture in the dried sericin on the plate. Then, dried sericin was scraped, and sericin was obtained in powdered form.

(Remi *et al*., 2022) used this boiling method and, with some modifications (Bhatt *et al.,* 2024), also used the same method. Cocoons were cut and boiled at 100°C for different time periods, 30, 60 and 90 minutes, and to obtain the sericin powder, the protocol of hot air-drying and freeze-drying was used.

As described by Capar (2012), the nanofiltration method was used to weigh and boil for 90 min. Then, a water solution was obtained and kept for cooling. After that, the microfiltration was done using glass fibre media with a pore size of 1µm. To remove the unwanted particles, a vacuum filtration apparatus was used.

Extraction of silk sericin using an autoclave

Silk sericin extraction by using an autoclave, 50 gm silk cocoons were cut into small pieces, then deionised water was added. And then autoclaved at 120°C for 30 min. Then, the fibroin mixed solution was filtered using Whatman filter paper. This helps to separate fibroin from silk sericin. After this, centrifugation was done to separate silk sericin from the sericin solution. Then, the collected sericin was dried at 100°C in an oven (Khalifaa *et al*., 2012; Saha J. *et al*., 2019). Again, this method is used, i.e., heating using an autoclave, as it is easy and doesn't harm the environment. But temperature should be proper as high temperature can cause damage to the sericin (Lamboni *et al*., 2015 and Wang *et al.*, 2019).

Cocoons were cleaned and distilled water was taken in a ratio of 1:30 (w/v). Autoclaved at 121° C for 60 min, then the sericin solution was centrifuged at 8000 g. After this, it was passed through a 0.4 µm filter, and it was kept in an oven to dry it, as it helps to evaporate the moisture and water in the solution. In the last series, sericin powder was collected (Battampara *et al*., 2025). Autoclave method (Rocha *et al*., 2017). Cocoons were cut into very small pieces and weighed; 15 g were taken, 1000 mL of ultra-pure water was added, and autoclaved at 120 °C for 60 min. To recover the sericin powder, the Hot air-drying method (Bharathi *et al*., 2020) sericin solution was spread on petri plates and kept in the hot air dryer at 70 °C to get the solution. Once the solution was dried to get sericin powder, the petri plates were scraped. These methods are also followed by (Bhat *et al*., 2024).

(Capar *et al*., 2008) used membrane filtration, cocoons were cut and autoclaved at 120°C for 1 hour. Then, filtration was done using glass fibre media 1.6µm. After this, to get the sericin precipitate, cold ethanol was used and kept for 24 h at 4°C. Precipitate was frozen at -80°C, then powder was obtained by lyophilising it.

Extraction of silk sericin by High Temperature and High Pressure (HTHP)

Extraction of sericin using the High Temperature and High Pressure (HTHP) method. As reported by Hossain *et al*. (2023), dry cocoons were cut into small pieces, 4g were taken, and 100 mL of deionised water in 500 mL steel autoclave. Autoclaved at two different temperatures, 90° and 110° C, for 15 and 30 min. After this mixture was filtered through filter paper, the filtrate was collected. The last freeze-drying method was used to obtain sericin powder.

(Gulrajani *et al*., 2008) prepared HTHP degumming liquor by boiling cocoons using a ratio (M: L) 1:40 at 115°C for 30 min. After this, the filtration was done using Whatman filter paper grade 1, and the ultrafiltration was done using a membrane system (Amicon 8000, Millipore). The spray drier model LU-227 India, by keeping its temperature at 180°C and atomization pressure 3.00 $kg/cm^{2,}$ used this method for the preparation of sericin powder.

Extraction of silk sericin by using Infrared (IR) Heating

Extraction of silk sericin by using Infrared (IR) Heating, reported by (Gupta *et al*., 2013) using an IR dying machine DLS 7000 (Daelim Scarlet, Korea) has six medium wave IR heating tubes. Once the aqueous liquor was obtained, it was dried by two methods, i.e. tray drying and spray drying. In tray drying, she kept the liquor in a glass tray and used a hot air oven (MMM Group, Canada) at 40 °C, and it was kept until the dry powder was obtained. In spray drying, she used a spray dryer LU-227 Advanced (Labultima, India) at a temperature of 110 °C and an atomization pressure of 3-4 kg/cm^2. This study was done at temperatures of 100, 110, 120 °C for 60, 90, 120 min. This process reported that sericin extraction can be obtained at high quality, and can reduce protein degradation.

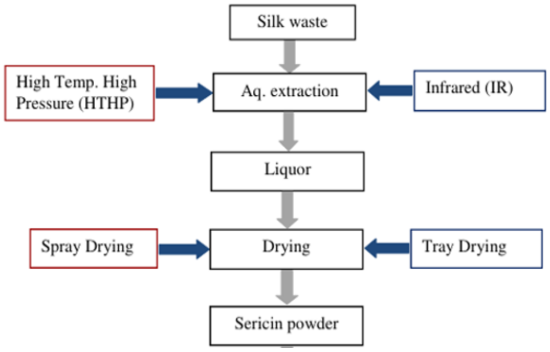

Fig 2: Systematic representation of the process of extraction of silk sericin given by Gupta *et al*. (2013).

Extraction of Silk Sericin Using Microwave Radiation
Silk Sericin Extraction using Microwave Radiation Method described by (Mohammad *et al*., 2010; Jenny Angel Stanislaus, 2020). Used the Microwave Model CE305CF (SUMSUNG) with the setup of power consumption 1400 W and operating frequency 2450 MHz. Cocoons were cut into small pieces and immersed in distilled water using a ratio of 1:50. After this, it was exposed to microwave radiation for different time periods 5,10,15 and 20 minutes, then it was filtered with the help of filter paper.

Extraction of silk sericin from wastewater through the Membrane Filters
Degumming wastewater was collected and directly dried using freeze-drying, and tray drying instruments were used for freeze-drying (Labconco Corporation: 77535-01, USA) and for tray-drying (Memmert: UM 500, USA). Ultrafiltration with the membrane (molecular weight 20,000-80,000 Da range, Millipore Co., USA) is used to get the concentration of sericin. The obtained sericin was freeze-dried. reported by Pilanee Vaithanomsat and Vichien Kitpreechavanich (2008).
According to Zahra M and Babak N (2017). The collected degumming wastewater was first filtered through filter paper to remove coarse impurities. The filtrate was then centrifuged at 3,000 rpm for 15 minutes to eliminate suspended particles. The clarified supernatant was concentrated using a rotary evaporator, followed by lyophilisation in a freeze dryer at −45 °C under a pressure of 20 mbar for 8 hours. Subsequently, ethanol was added to obtain a final concentration of 75% (v/v), and the mixture was incubated at room temperature to precipitate sericin. After precipitation, the ethanol was removed, and the resulting precipitate was freeze-dried to obtain purified sericin powder. Discharged water from silk reeling containing sericin was collected and kept at room temperature for 4 hours. After this, it was kept at 4°C overnight to allow sedimentation and obtain the precipitate of the glue-like protein sericin. Decantation of supernatant was done without disturbing the precipitate. Precipitate containing sericin was recovered and pre-cooled in ethanol at < 10 °C at a 1:4 ratio. The precipitate was treated with this and left to stand for 4 hours. Again, the supernatant was discarded, and the precipitate was centrifuged at 4000 rpm for 5 min. After this, the suspension of precipitate was done in the 1M Tris buffer at pH 9.0, and it was dialysed against the same buffer at 20°C for 48 hrs. By spinning the dialysed sericin solution at 10,000 rpm for 5 min to recover the suspension. It was preserved for the study described by Moorthy S. (2020).

III. CHEMICAL METHODS

Extraction of silk sericin by using Urea (with or without Mercapto ethanol)
As Takasu *et al*. (2002) reported, sericin extraction by aqueous urea. Cocoons were cut into pieces and weighed accurately, about 100mg, then placed in 8 M aqueous urea solution with or without 5% 2-mercaptoethanol that would be preheated at 40 to 80°C ratio of solvent to the cocoon shell of 30 (ml/g). Then, incubation was done with stirring and residual cocoons were separated, and the extract was centrifuged at 8,000 rpm (6869) for 10 min. After this, a clear sericin solution will be obtained. This method was also studied by Lamboni *et al.* (2015) and Wang *et al.* (2019), and the use of urea can increase the steps of purification because it contains chemical impurities.

Extraction of silk sericin by using Acid
According to Lamboni *et al*. (2015) and Wang *et al.* (2019), extraction can be done with the help of different acids, such as citric, tartaric, succinic acid, etc. Hydrolysis using such acids helps to hydrolyse the glue-like protein sericin by breaking the peptide bonds of amino acids and helps to degum the sericin in such acids (Silva *et al*, 2022 and Chirila *et al*., 2016). It is cost-effective and has low purity, as well as a high impact on the environment. (Kurioka *et al*., 2004 and Shiozaki *et al*., 2001) Also reported the use of citric

acid for the degumming process, chopped cocoons were boiled in 160 ml of 1.25% citric solution for 30 min, and tartaric acid and succinic acid were also used with the same conditions. Chopra and Gulrajani (1994) also described different methods of degumming for acid; they used tartaric acid, and in their conclusion, they reported that acidic degumming was not a satisfactory method.

Extraction of silk sericin by Alkaline solutions

Extraction of silk sericin is also done by using different alkaline solutions such as sodium carbonate, sodium phosphate, sodium silicate, sodium hydrosulfite, etc. These types of solutions also help to dissolve sericin or to separate the sericin from fibres (Silva *et al*., 2022 and Chirila *et al*., 2016). 0.1 to 0.5 % Na_2CO_3 is used in laboratory degumming, which contains sodium salts and separates salt from protein; it is difficult, as well as causes sericin degradation. As sericin is used in biomaterial applications due to such recovery of sericin, it was not suitable for some biomaterial applications (Cao *et al*., 2013 and Lamboni *et al*., 2015). Use of Na_2CO_3 is also studied by Wang and Zhang (2011) with the degumming condition pH 11.0, 100°C, 20 min. Fatahian *et al*. (2017) reported that cocoons were cut into small pieces and placed for boiling with the ratio of 1:30 L: R for different time intervals, 30, 60, 90 and 120 min at temperatures of 80°C and 60°C in the presence of sodium carbonate.

Extraction of silk sericin by Soap/ Detergents

Use of detergents and soap, e.g., sodium bicarbonate and Marseille soap, for the extraction of silk sericin. Chopra and Gulrajani (1994) used Marseille soap and reported that the use of soap can remove the sericin, but it is not cost-effective as more quantity of soap is required. Use of soap can decrease the purity, as it is hard to separate the sericin and soap and has more impact on the environment as it releases the soap in wastewater (Lamboni *et al*., 2015, Aad *et al*., 2024, and Gulrajani *et al*., 2008). The silk sericin can extracted using Marseille soap obtained from olive oil, degumming time 90-120 min and boiling temperature proper as hydrolysis with this soap is more (Silva *et al*., 2022 and Chirila *et al*., 2016) traces of soap remains in protein and sericin is also degraded.

Sedimentation method: Using ammonium sulfate in the degumming solution, sedimentation can be achieved (Fatahian *et al*., 2017) by adding 40g of ammonium sulfate per 100 ml of solution. Once sedimentation was done, it was followed by filtration, and to obtain powder sericin, it was dried at ambient temperature.

Enzymatic Extraction Methods:

There are several methods of silk sericin extraction. Use of enzymes for the degumming of sericin is also emerging, and it is discussed in various research papers. Enzymes, proteolytic enzymes - bromelain, pancreatin, alkaylase, degumming enzyme, papain, trypsin, savinase, etc. (Lamboni *et al*., 2015, Wang *et al*., 2019, More *et al*., 2018) are described, it's helpful for the environment and also has some limitations, such as being time-consuming and costly.

Enzymatic hydrolysis, described by (Padmawar *et al*., 2004 and Migule *et al*., 2020), involves extraction using enzyme alkylase or using 2-2.5g/L alkaline protease for 90 min at a temperature of 60°C with a pH of 10. Hydrolysis of trypsin at different temperatures, concentrations, and times. 1% trypsin hydrolysis is mostly complete in 10h and 32h at 37 and 20°C temperatures. By 4h treatment, with 1% and 8% trypsin solution, the obtained sericin were 26.4% and 28.7%, respectively (Padmawar *et al*., 2004)

Table 1: Advantages and limitations of silk sericin extraction technologies:

Extraction methods	By Using	Advantages	Limitations	Suitable Applications	References
Physical methods of extraction	Hot Water	Sustainable method Presence of bioactive properties.	A difference in quantity can be seen.	Laboratory extraction	(Boraiah *et al*., 2024 and Bhat *et al*.,2024)

	Autoclave	Maximum quantity and shows a high protein purity ratio.	High temperature Decreases bonding stability.	Biomedical materials	(Bhat *et al*., 2024)
	HTHP	Shows high thermal stability at low temperature and time. Environment-friendly.	Quantity varies with temperature and time.	Industrial wastewater recovery	(Hossain et al., 2023)
	Infrared (IR)	Chemical-free method and green process.	Large scale extraction has commercial and economic limitations.	Phapharmaceutical applications	Gupta *et al.,* (2013)
	Microwave	Environment friendly	Low efficiency	Cosmetic-grade sericin	(Mohammad *et al*.,2010)
Chemical methods of extraction	Urea (with or Without Mercapto ethanol)	Gives high yield effective	Requires time, needs purification and contains chemical impurities.	Textile processing Medical-grade sericin Textile processing	(Lamboni *et al*., 2015, Wang *et al*., 2019, and Silva *et al*., 2022)
	Acids	Good quantity	Low purity and causes pollution		(Silva *et al*., 2022 and Chirila *et al*., 2016)
	Alkaline	A high amount was obtained	Difficult to separate		
	Soap/Detergents	Remove sericin	Degrade sericin more with a larger amount of soap and cause pollution.		(Lamboni *et al.,* 2015, Aad *et al*., 2024, Gulrajani *et al.,* 2008)
Enzymatic methods of extraction	Proteolytic enzymes,papain, degumming enzyme, trypsin, pancreatin, savinase, alkaylase,	Environment friendly	Time consuming and costly	Medical-grade sericin	Lamboni *et al*., (2015)

	etc.				

Critical Comparative Analysis

Most published studies compare extraction methods primarily based on extraction yield. However, this criterion alone is inadequate because the biological functionality of sericin depends strongly on molecular weight, structural integrity, amino acid preservation, and absence of chemical contaminants. Emerging green technologies—including microwave-assisted extraction, HTHP processing, enzymatic extraction, membrane separation, and electrochemical recovery—offer a more balanced combination of environmental sustainability, protein quality, and industrial feasibility than conventional alkaline degumming. Future research should therefore emphasise integrated process optimisation using life-cycle assessment, techno-economic analysis, and standardised quality metrics rather than yield alone.

Table 3. Comparative evaluation of major silk sericin extraction technologies

Extraction method	Yield	Protein integrity	Environmental impact	Industrial scalability	Overall assessment
Hot-water	Moderate	Moderate	Low	Moderate	Good
Autoclave	High	High	Low	High	Excellent
HTHP	High	High	Low	High	Excellent
Microwave	Very high	Excellent	Very low	High	Excellent
Infrared	High	High	Low	Moderate	Very good
Chemical	Very high	Moderate to low	High	Excellent	Moderate
Enzymatic	High	Excellent	Very low	Moderate	Excellent
Membrane filtration	High (recovery)	Excellent	Very low	High	Excellent
Electrochemical recovery	Promising	High	Very low	Emerging	High potential

Seo *et al.* (2023) in their review reported the application of sericin in the food sector industries, and different applications of sericin when it combines with other biomaterials. And it has hydrophobic, hydrophilic amino acids, which help to explain its different properties, such as antibacterial, antioxidant, anti-inflammatory, anti-cancer, etc. Mallikarjuna *et al.* (2024) studied the bivoltine cocoons as their layers contain protein, and it has antimicrobial properties. Meerasri *et al*. (2023) in their study, they studied the sericin. They described that sericin protein can form sericin films and can be used in food and biomedical applications. Reis *et al.* (2025) findings suggest sericin's usefulness in wound healing and anticoagulation, although its low antioxidant and anti-inflammatory effects may limit broader biomedical applications.

Mandal *et al*. (2007) reported in a review about value-added biomaterial for medical applications, application of silk protein fibroin and sericin as a biomaterial, and other Seri byproducts. Silk sericin is a protein and has various properties that make it a biocompatible material. This is useful in medical, pharmaceutical and cosmetic applications (Padamwar and Pawar, 2004).

There are several methods of extraction of silk sericin using different chemicals, such as Alkaline degumming using sodium carbonate (Na_2CO_3), NaCl, Urea, Soap-Alkaline, Ammonium Sulfate and Acid Degumming, acids citric, tartaric, succinic, etc. According to Kabir *et al*. (2024), the use of chemicals can lead to environmental pollution, as well as being hard to separate sericin from alkali, soap, etc., and also has some limitations (Arango *et al*., 2020). Paladini *et al*. (2025) have discussed different methods of degumming in their review, including Boiling/Autoclave, soap, Alkaline, Acidic, urea, Enzymatic and also described the effects of different degumming methods on sericin.

IV. CONCLUSION

Silk is one of the industries that is helpful in the economic sector. Silkworm Bombyx mori is a mulberry silkworm that produces silk, and it consists of two proteins, i.e. fibroin and sericin. Fibroin is the main fibre used in the silk industry, while silk sericin is discarded during the degumming process. Silk sericin is a glue-like protein that helps to bind the silk fibres at the time of cocoon formation. Sericin is discarded in wastewater, but different studies indicate that sericin has some properties that are helpful in different fields. Extraction of sericin also helps to reduce the environmental effects, as it is released in wastewater. Sericin is a protein in nature and is used in the pharmaceutical industry, cosmetics, etc. In this review, the different methods of extraction of silk sericin were studied. There are several methods of extraction of sericin, such as physical, chemical, and enzymatic, etc. In this review, most of the methods are sustainable to reduce the use of chemicals, and in some methods, we studied that chemicals are also used to extract the silk sericin. The process of extraction can also affect the sericin properties and the amount of sericin. Sericin has several applications, and due to this, it is important to study the methods of sericin extraction to extract the proper sericin content.

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