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METHODS FOR SEPARATING SERICIN FROM COCOON PROCESSING FACTORIES WASTEWATER

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Abstract. This article discusses the issues of extracting sericin protein produced in the cocoon processing industry. The physicochemical properties of sericin and the functions of its constituent amino acids have been studied. Various technologies used in extracting sericin, their advantages and disadvantages, their ecological impact on the environment, and the significance of the extracted product in biomedical applications are reviewed.

Keywords: sericin, fibroin, cocoon industry, wastewater, protein, ecology, extraction.

Introduction. Today, efficient use of resources and ensuring ecological safety in the light industry, particularly in the sericulture sector, is one of the most pressing issues [1]. Silkworms have played an important role in the textile industry for centuries, producing silk - a natural protein product with diverse applications [2]. Silk consists of two main components: sericin and fibroin (Table 1).

- Fibroin is the main skeletal protein that gives the fiber strength and elasticity.

- Sericin is a natural protein located on the outer part of the silkworm cocoon that glues the fibroin fibers together.

Table 1

Molecular composition and structure of silk fiber

FIBROIN composition	**SERICIN composition**
1. 65–75% of cocoon	1. 25–35% of cocoon
2. Composed of glycine, alanine, serine amino acids	2. Rich in serine, aspartate, threonine hydrophilic amino acids
3. Heavy chain 350–390 kDa	3. Extracted from cocoon by degumming method
4. Light chain 25–30 kDa	

Sericin constitutes approximately 25–35% of silk, while the remaining 65–75% is fibroin. In the silk industry, producers boil cocoons to remove the sericin coating the outer layer of silk, obtaining fibroin fibers suitable for textiles, and the removed sericin is discarded into wastewater. The global volume of silk production exceeds 100,000 tons per year, meaning more than 30,000 tons of sericin would be wasted if not properly utilized. This waste represents an opportunity to optimize resources and achieve sustainable development in the silk industry [3].

Sericin is a silk protein adhesive that attracts attention due to the possibility of extracting it from textile waste, as it possesses antibacterial, anticancer, UV-resistant, and moisture-absorbing properties [4]. Sericin is a globular rubbery silk protein secreted by the Bombyx mori silkworm, binding fine fibroin fibers together to form a durable cocoon. Since silk is a valuable raw material for the textile industry, silk thread has long been produced through a series of procedures, starting from boiling cocoons in hot water, followed by traditional degumming using chemical agents such as Marseille soap. Sericin is soluble in water and is therefore discarded together with silk waste, leaving soft and lustrous fibroin fibers for subsequent processes such as dyeing [5]. Sericin has some unique properties including antibacterial, anticancer, and anti-inflammatory activity, as well as water purification capability; therefore, it is considered a potential raw material for several industries including biomedicine, cosmetics, food, and textiles [6]. Silk is produced in large quantities in Asian countries, where China is the world's leading producer and supplier, followed by India. In 2019, a total of 109,111 tons of silk were produced worldwide. This corresponds to 20,000–30,000 tons of sericin per year, as sericin constitutes 25–35% of cocoon weight, but it is currently lost into receiving water bodies. The price of sericin varies depending on its physical properties and purity; the average price of food/cosmetic ISO9001-certified sericin is 60 EUR/kg, while cell culture-grade sericin reaches up to 199 EUR/g [4–5]. Therefore, significant economic value can be achieved by recovering this protein.

Chemical composition and properties of sericin: Sericin is a water-soluble globular protein containing 18–20 types of amino acids. Its chemical composition and functional groups (carboxyl, hydroxyl, and amino groups) allow it to interact with other polymers, which enhances its antioxidant, moisturizing, healing, antibacterial, antimicrobial, UV-protective, and anticancer properties (Table 2). Physicochemically, sericin dissolves well in water, is sensitive to pH environment, and denatures at high temperatures [6–7].

Table 2.

Structure and function of the main amino acids in sericin composition

Amino Acid Name	Function
Serine	Provides hydrophilicity, supports water retention and moisturizing properties.
Glycine	Contributes flexibility and compactness to the sericin structure.
Aspartic acid	Adds negative charge, increases sericin's solubility in water and binding with metal ions.
Threonine	Supports hydrophilicity and protein stability, contributes to sericin's bioactivity.
Glutamic acid	Provides negative charge and increases water solubility; plays a

	role in chelation and bioactivity.
Tyrosine	Enhances aromaticity; contributes to antioxidant properties through free radical scavenging.
Alanine	Increases structural stability and compactness of sericin.
Proline	Provides rigidity to the protein structure, affecting mechanical properties.
Arginine	Adds positive charge; supports ionic interactions and enhances bioactivity (e.g., wound healing).
Lysine	Provides positive charge; plays a role in binding negatively charged molecules and surfaces.
Histidine	Enhances buffering capacity and contributes to antioxidant properties.
Cysteine	Forms disulfide bonds, stabilizes sericin structure; contributes to bioactive and antioxidant roles.
Phenylalanine	Increases hydrophobicity and aromaticity; contributes to structural stability.
Valine	Contributes to hydrophobic interactions and enhances protein stability.
Leucine	Plays a role in maintaining protein stability and hydrophobic core interactions.
Isoleucine	Increases structural stability through hydrophobic interactions.
Asparagine	Contributes to hydrophilicity and protein stability through hydrogen bonding.
Glutamine	Enhances hydration properties and supports protein stability.
Methionine	Plays a role in structural stability and serves as a precursor for bioactive molecules.
Tryptophan	Provides aromaticity and contributes to antioxidant properties

	through radical scavenging.

The main stages of cocoon processing are as follows (Figure 1):

Figure 1. Main stages of cocoon processing.

The silk sericin layer is carefully separated from the silk fibroin fibers during the degumming process. This complex process hydrolyzes or enzymatically breaks the peptide bonds of sericin, separating it from the silk fibroin matrix. Chemical, thermal, and biological methods have been used for sericin extraction (Figure 2).

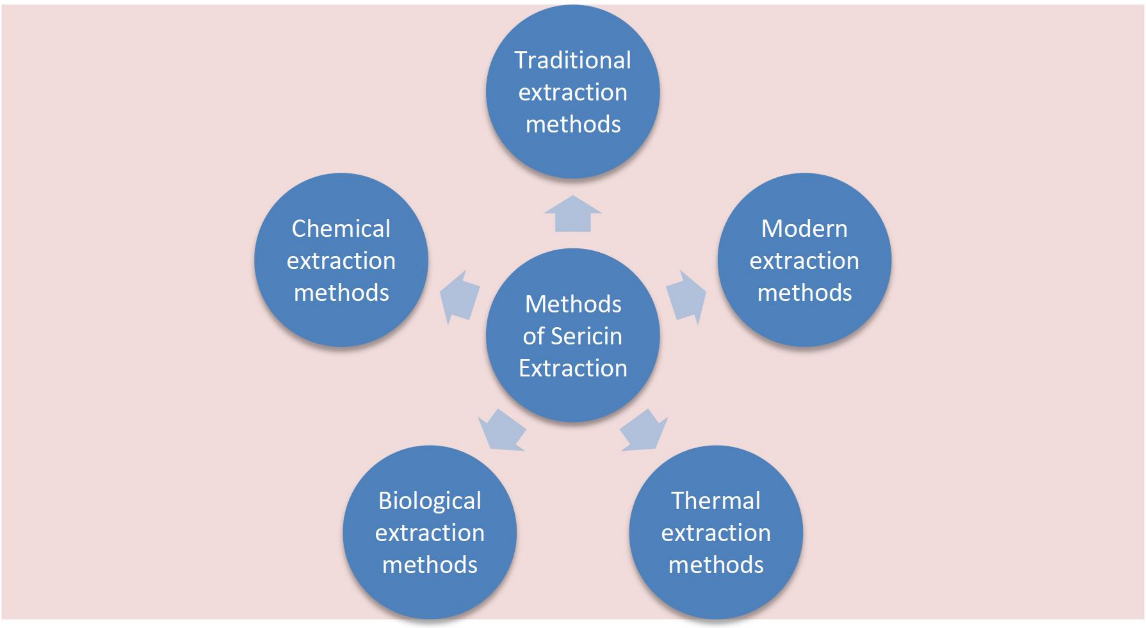

Figure 2. Sericin extraction methods.

Traditional extraction methods. For more than two centuries, silk has been degummed by boiling in soap solutions, particularly Marseille soap made from olive oil. The alkali from soap hydrolysis breaks down sericin from silk threads, and soap emulsifies it into water. Marseille soap is preferred for degumming at boiling temperature for 90–120 minutes for maximum hydrolysis. Marseille soap is effective but has economic disadvantages, such as problems related to water quality that may damage silk quality and the need for large amounts of soap that cause environmental concerns. Combinations of soap and alkali have been tested to accelerate degumming and reduce contamination; however, they are prone to hard water. Alkalis such as sodium silicate and sodium carbonate have been studied for their ability to improve degumming and maintain pH levels. However, recovering sericin from soap solution is difficult, which complicates the recovery process. Sericin, salts, and soap residues are present in degumming wastewaters, requiring thorough filtration and encouraging the exploration of alternative purification methods.

Chemical extraction methods. Chemical procedures that remove sericin without Marseille soap are widespread in industrial degumming methods. Citric, tartaric, succinic, and sodium carbonate, phosphate, silicate and hydrosulfite acids or bases are used for extraction. These compounds break peptide bonds between amino acids to hydrolyze sericin and dissolve it in acidic or alkaline solutions. Acid- and base-based extractions significantly degrade proteins. Urea-based extraction, typically with 2-mercaptoethanol, appears less harmful to sericin. This technology extracts up to 95% of silk fiber sericin without structural damage. Despite being effective, this method is expensive and time-consuming. Sericin extracted with urea is highly cytotoxic, making it difficult to use in biological applications. Despite advances, chemical residues after purification may reduce sericin purity. These extraction processes also harm the environment, as chemical pollutants in wastewater threaten ecological systems.

Biological extraction methods. This approach breaks sericin bonds using proteinases such as cocoonase. The method typically uses trypsin, papain, and bacterial proteases. Due to sericin's high lysine and arginine content, trypsin - a proteolytic enzyme - breaks the peptide bonds between these amino acids. Papain is effective for degumming cocoons due to its broad polypeptide specificity. Standardized bacterial enzyme alcalase and fungal proteases are economical, environmentally safe, and chemical-free. Enzyme concentration, treatment duration, and enzyme type affect the chemical properties of soluble sericin peptides, making enzymatic degumming successful. Enzymatic procedures are more expensive than traditional procedures but consume less energy, making them more sustainable.

Thermal extraction methods. Thermal extraction, carried out by boiling silk cocoons in hot water, is popular due to its simplicity and low chemical content. This process is carried out at 80°C–100°C under atmospheric pressure. High-pressure extraction in laboratory-scale autoclaves has extended capabilities for silk sterilization. Boiling sericin in water at ambient or high pressure guarantees extraction without mixtures, allowing the obtained sericin to be used directly without dialysis. Thermal extraction is the most popular method as it preserves many of sericin's characteristic properties, although it may degrade some at high temperatures or with prolonged exposure. The molecular weight of extracted sericin depends on temperature, pressure, and heating duration. Careful adjustment of these parameters controls sericin's molecular weight, enabling flexible extraction and use.

Modern extraction methods. Sericin can be separated from fibroin more efficiently and sustainably using new technologies. These new methods include infrared heating, microwave treatment, steam treatment, supercritical CO_2 liquid extraction, and ultrasonic treatment. These approaches showed promise in reducing extraction water use and supporting sustainability goals. However, temperature and processing time may affect the molecular weight of extracted sericin. Infrared heating was found to completely remove sericin from raw silk and deliver higher quality protein compared to standard procedures. Electromagnetic waves directly heat the material, extracting sericin and increasing its water solubility. Water molecules additionally remove sericin during energy transfer. Studies show that microwave degumming reduces extraction time, energy consumption, and chemical contamination. Microwave and infrared heating are more efficient and sustainable than acid, alkali, boiling, high-temperature, and high-pressure degumming. Due to their chemical-free nature, infrared and microwave heating can extract sericin with high purity and efficiency. Table 3 provides a comparison of sericin extraction methods in terms of solvents, environmental impact, and suitability for biomedical use.

Table 3

Comparison of sericin extraction methods

Extraction Method	Solvent / Separating Agent	Environmental Impact	Suitability for Biomedical Use
Traditional (soap-based)	Marseille soap + boiling water (90–120 min)	High (soap waste, water consumption)	Limited (residual impurities, poor consistency)
Chemical	Citric/tartaric/succinic acid; sodium carbonate/phosphate/silicate	High (chemical contaminants)	Limited (cytotoxicity, low biocompatibility)
Biological	Proteases (trypsin, papain, alcalase); enzymes from Bacillus under mild alkaline pH	Low (eco-friendly)	High (bioactive, minimal degradation)
Thermal	Boiling water (80–100°C), pressurized steam	Moderate	Moderate to high (minimal chemical use)
Modern	Infrared heating, microwave treatment, supercritical CO_2, ultrasonic treatment	Very low (green methods)	High (pure, bioactive, scalable)

Conclusion. Extracting sericin from the wastewater of cocoon processing plants is an important issue both ecologically and economically. Sericin is a valuable protein with high biological activity, and reprocessing it as secondary raw material further increases industrial efficiency. By introducing modern extraction technologies, wastewater treatment and environmental protection can be achieved. The purer and more natural sericin is preserved, the higher its value. For this reason, extracting sericin using biological and modern extraction methods - which have a lower environmental impact - yields very good results. Furthermore, sericin extracted using biological and modern extraction methods has broad potential for use in biomedicine.

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