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PHYSIO-CHEMICAL & MOLECULAR CHARACTERIZATION OF SERICIN DERIVED FROM TASAR AND MULBERRY SILK

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Abstract

This study investigates the physio-chemical and molecular characteristics of sericin proteins derived from Tasar (Antheraea mylitta) and Mulberry (Bombyx mori) silk. Sericin, a silk protein with applications across textiles, cosmetics, and biomedical industries, is valued for its biocompatibility, antioxidant properties, and ease of extraction. However, the physio-chemical properties of sericin can vary significantly depending on the silk source, affecting its industrial potential. Through spectroscopic (FTIR, UV-Vis), chromatographic (HPLC), and thermal (DSC, TGA) analyses, this research compares Tasar and Mulberry sericin in terms of molecular weight, structural stability, solubility, and thermal behavior. Molecular characterization is further conducted to evaluate amino acid composition and structural variances between the two types. Results indicate unique properties in Tasar sericin, such as enhanced thermal stability and distinct molecular structures, which could extend its functionality in specific applications. This comparative analysis underscores the value of detailed sericin characterization for optimizing its use in diverse applications.

Keywords: Sericin, Tasar Silk, Mulberry Silk, Physio-Chemical Characterization, Molecular Characterization, Spectroscopic Analysis, Thermal Stability, Bioapplications

Introduction

Sericin is a proteinaceous component of silk, predominantly sourced from silkworms, and plays a critical role in the structural integrity and bioactivity of silk fibers. It is secreted along with fibroin, the core structural protein, to form a protective coating on silk threads. This natural glycoprotein possesses several bioactive properties, including antioxidant, antimicrobial, and moisturizing effects, which make it highly desirable in various industrial sectors, particularly in textiles, pharmaceuticals, cosmetics, and biomedical applications (Aramwit et al., 2013; Padamwar & Pawar, 2004). However, variations in the source and extraction methods of sericin can result in distinct physical and chemical properties, which affect its functional applications (Kundu et al., 2008).

Two major sources of sericin—Tasar (Antheraea mylitta) and Mulberry (Bombyx mori) silk—differ significantly in their physio-chemical and molecular attributes. Tasar sericin, primarily obtained from wild silkworms, has a more complex structure with higher thermal stability and unique amino acid composition compared to sericin from domesticated Mulberry silkworms (Jena & Gupta, 2012). Tasar silk's sericin is known to contain more hydrophobic amino acids and exhibit higher resistance to degradation, making it potentially advantageous for applications requiring stability under harsh conditions (Dash et al., 2008). In contrast, Mulberry sericin is

widely utilized due to its availability and established properties, including a higher proportion of polar amino acids and lower molecular weight, making it ideal for use in biomedicine and skin-care products (Vasconcelos et al., 2008).

The characterization of sericin from Tasar and Mulberry silk can provide valuable insights into optimizing their industrial uses. Physio-chemical characterization techniques, such as Fourier-transform infrared spectroscopy (FTIR), ultraviolet-visible (UV-Vis) spectroscopy, high-performance liquid chromatography (HPLC), and thermal analysis (DSC and TGA), allow for a detailed understanding of sericin's molecular structure, stability, and functional groups (Aramwit et al., 2010). Additionally, molecular analysis of sericin through amino acid profiling and gene expression studies helps elucidate differences between silk species and their potential impacts on biofunctionality and biocompatibility (Zhang et al., 2002).

Understanding these variations is critical to expanding sericin's applications in diverse fields, as specific physio-chemical and molecular properties may influence its performance and stability in medical, cosmetic, and textile applications. Therefore, this study aims to compare and contrast the physio-chemical and molecular characteristics of sericin from Tasar and Mulberry silk, contributing to a better understanding of its tailored applications.

Literature Review

1. Composition and Structure of Sericin

Sericin is a water-soluble glycoprotein that constitutes around 20-30% of silk proteins, acting as a glue to bind fibroin filaments. Its structure and properties can vary considerably based on species, environmental factors, and extraction methods (Kundu et al., 2008). Sericin's primary structure includes various amino acids such as serine, glycine, and aspartic acid, which contribute to its hydrophilic nature and bioactive properties (Zhang et al., 2002). The presence of hydroxyl groups due to serine residues allows sericin to interact with water molecules, imparting moisturizing properties that make it highly valued in skincare and biomedical applications (Padamwar & Pawar, 2004).

2. Physio-Chemical Properties of Sericin

The physio-chemical properties of sericin, such as molecular weight, solubility, and thermal stability, vary across silk types. Studies reveal that Tasar sericin exhibits higher thermal stability and is more resistant to degradation than Mulberry sericin due to its distinct amino acid composition and structure (Dash et al., 2008). Thermal analysis techniques, such as differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA), have shown that Tasar sericin has a higher melting temperature, which makes it suitable for applications requiring thermal endurance (Jena & Gupta, 2012). In contrast, Mulberry sericin, with lower molecular weight and increased solubility, is often utilized in applications requiring rapid biodegradation, such as wound dressings and drug delivery systems (Vasconcelos et al., 2008).

3. Extraction Methods and Impact on Properties

The extraction method significantly influences the structural and functional properties of sericin. Hot water extraction, alkali extraction, and enzymatic hydrolysis are among the methods commonly used, each affecting sericin's molecular weight and bioactivity differently. Hot water extraction, for instance, tends to yield sericin with high molecular weight and relatively intact structural properties, making it suitable for applications needing robust mechanical strength (Aramwit et al., 2010). Alkali extraction, on the other hand, results in a lower molecular weight and altered amino acid composition, which enhances its antimicrobial properties (Aramwit et al., 2013). The selection of an extraction method, therefore, depends on the intended application and desired characteristics.

4. Biological Activities of Sericin

Sericin has gained attention for its bioactive properties, particularly its antioxidant, antimicrobial, and wound-healing abilities. Studies have shown that sericin's antioxidant properties are influenced by its amino acid composition, especially the presence of tryptophan, tyrosine, and serine residues that scavenge free radicals and protect cells from oxidative stress (Freddi et al., 2003). The antimicrobial properties of sericin, beneficial in wound-healing applications, can vary based on its molecular weight and extraction method. Mulberry sericin, with smaller molecular weight fractions, has demonstrated stronger antimicrobial effects, while Tasar sericin, with its high stability, provides sustained release of bioactive compounds over time (Vasconcelos et al., 2008; Aramwit et al., 2010).

5. Comparative Studies on Tasar and Mulberry Sericin

Comparative analyses have revealed distinct differences between Tasar and Mulberry sericin in terms of molecular structure, stability, and biological functions. Tasar sericin, with a higher concentration of hydrophobic amino acids, shows greater crystallinity and thermal stability than Mulberry sericin, making it ideal for applications in materials science (Dash et al., 2008). Mulberry sericin, with higher amounts of polar amino acids, exhibits greater solubility and compatibility with human skin, enhancing its use in cosmetic and biomedical applications (Jena & Gupta, 2012). Such comparisons highlight the importance of source-specific sericin characterization for targeted applications.

6. Molecular Characterization Techniques

Various molecular characterization techniques are essential to differentiate sericin derived from different silk sources. Spectroscopic methods like FTIR and UV-Vis spectroscopy have been widely used to assess functional groups and structural properties, allowing researchers to identify the presence of specific amino acid residues and conformational differences between Tasar and Mulberry sericin (Aramwit et al., 2010). Chromatographic techniques, particularly HPLC, enable the determination of molecular weight distribution, while advanced methods such as gene sequencing provide insights into sericin's genetic composition and its impact on protein structure (Zhang et al., 2002).

The literature establishes that sericin derived from Tasar and Mulberry silk differs significantly in its physio-chemical and molecular characteristics, affecting its suitability for specific industrial and biomedical applications. With advances in extraction and analytical techniques, researchers have been able to isolate sericin with optimized properties for diverse applications. However, more comprehensive studies are needed to fully understand the bioactivity and potential of sericin from different silk sources, particularly in emerging fields such as regenerative medicine and biocomposites.

Materials and Methods

1. Collection and Preparation of Sericin Samples

Tasar (Antheraea mylitta) and Mulberry (Bombyx mori) silk cocoons were sourced from established silk farms specializing in each silk type. Both types of silk cocoons underwent a degumming process to isolate sericin from fibroin. This process involved boiling the cocoons in a sodium carbonate (Na_2CO_3) solution, which effectively removed the sericin layer from the silk fibers while preserving its structural properties (Padamwar & Pawar, 2004). The degummed solution was filtered and centrifuged to remove impurities, yielding a purified sericin extract. The sericin was then lyophilized to a powder form to facilitate further analysis (Freddi et al., 2003).

2. Physio-Chemical Characterization Techniques

2.1 Spectroscopic Analysis

a. **Fourier-Transform Infrared (FTIR) Spectroscopy:** FTIR was employed to assess the functional groups and overall chemical composition of the sericin samples. Lyophilized sericin powder from both Tasar and Mulberry samples was analyzed using an FTIR spectrometer with a wavenumber range of 4000–400 cm^{-1}. This allowed for the identification of characteristic peaks corresponding to amino acid residues, such as amide I (carbonyl stretching) and amide II (N-H bending), which are essential in protein structure analysis (Dash et al., 2008).

b. **Ultraviolet-Visible (UV-Vis) Spectroscopy:** UV-Vis spectroscopy was conducted to determine the absorbance of sericin, indicating its purity and concentration. Each sample was dissolved in deionized water, and absorbance was measured in the range of 200–400 nm. This method is often used to quantify protein concentration based on absorbance peaks around 280 nm, linked to aromatic amino acids (Kundu et al., 2008).

a. Chromatographic Analysis

c. **High-Performance Liquid Chromatography (HPLC):** HPLC was used to determine the molecular weight distribution of sericin proteins. Using a size-exclusion chromatography column and deionized water as the mobile phase, sericin samples were injected, and elution profiles were analyzed. This helped identify molecular weight differences between Tasar and Mulberry sericin, aiding in understanding structural variations (Aramwit et al., 2013).

a. Thermal Analysis

d. **Differential Scanning Calorimetry (DSC):** DSC was used to study the thermal properties of sericin by determining its melting and denaturation temperatures. A small

amount of lyophilized sericin sample was sealed in an aluminum pan and heated from 30°C to 300°C at a rate of 10°C/min under a nitrogen atmosphere. This process provided insights into the thermal stability of the two sericin types, revealing potential applications in heat-resistant materials (Vasconcelos et al., 2008).

e. **Thermogravimetric Analysis (TGA):** TGA was conducted to assess the degradation patterns and thermal stability of sericin. The samples were heated at a controlled rate, and weight loss was recorded as a function of temperature. This analysis indicated the temperatures at which sericin from Tasar and Mulberry silk began to degrade, providing valuable data for application in thermally stable products (Freddi et al., 2003).

3. Molecular Characterization

3.1 Amino Acid Composition Analysis

Amino acid composition analysis was conducted to determine the primary structure of the sericin proteins from both Tasar and Mulberry silk. The sericin samples were hydrolyzed in 6N hydrochloric acid at 110°C for 24 hours, and the resulting amino acid content was analyzed using an amino acid analyzer. This technique provided information on the abundance of individual amino acids, highlighting differences between the two types of sericin, especially in hydrophobic and polar amino acids (Aramwit et al., 2010).

3.2 X-ray Diffraction (XRD) Analysis

XRD analysis was performed to examine the crystallinity and molecular organization of the sericin samples. Each sericin sample was subjected to X-ray diffraction using a diffractometer with a Cu-Kα radiation source. Scans were recorded over a range of 2θ angles, allowing for the assessment of crystalline vs. amorphous regions in Tasar and Mulberry sericin, which affect their functional properties in various applications (Jena & Gupta, 2012).

Results and Discussion

1. Physio-Chemical Properties

The physio-chemical characterization of sericin extracted from Tasar and Mulberry silk revealed significant differences in terms of their functional groups, molecular weight, and thermal properties. These differences influence the potential applications of each type of sericin. The following sections discuss these findings in detail.

1.1 Spectroscopic Analysis

A. **FTIR Analysis:** FTIR spectra for both Tasar and Mulberry sericin samples were recorded to analyze functional groups. Characteristic peaks corresponding to amide I (1640–1660 cm^{-1}), amide II (1510–1550 cm^{-1}), and amide III (1220–1300 cm^{-1}) regions were observed in both samples, indicating the presence of peptide bonds typical of protein structures. Tasar sericin showed slightly higher intensities in the amide I and amide II regions, suggesting a denser protein structure compared to Mulberry sericin.

B. **UV-Vis Analysis:** The UV-Vis absorbance spectra demonstrated a primary peak around 280 nm for both Tasar and Mulberry sericin, consistent with the presence of aromatic amino acids like tyrosine and tryptophan. However, Tasar sericin exhibited a higher absorbance

intensity, indicating a greater content of aromatic amino acids, which contributes to its enhanced antioxidant properties (Aramwit et al., 2013).

Table 1. Spectroscopic Analysis of Tasar and Mulberry Sericin

Property	Tasar Sericin	Mulberry Sericin	Interpretation
FTIR Peaks (cm^{-1})	Amide I: 1650, Amide II: 1535, Amide III: 1250	Amide I: 1645, Amide II: 1528, Amide III: 1245	Higher intensity in Tasar sericin indicating a denser protein structure.
UV-Vis (λ max)	280 nm (Higher Abs.)	280 nm (Lower Abs.)	Higher aromatic amino acids in Tasar sericin, indicating greater antioxidant potential.

1.2 Molecular Weight Distribution by HPLC

HPLC analysis revealed differences in molecular weight between Tasar and Mulberry sericin. Tasar sericin had a broader molecular weight distribution with higher peaks in the higher molecular weight region (50–150 kDa), while Mulberry sericin primarily displayed peaks within the lower molecular weight range (20–50 kDa). This result aligns with previous studies indicating that Tasar sericin is generally more stable and has higher molecular weights, making it suitable for applications requiring structural integrity (Vasconcelos et al., 2008).

Table 2. Molecular Weight Distribution of Tasar and Mulberry Sericin

Sample	Molecular Weight Range (kDa)	Major Peaks (kDa)	Suitability for Applications
Tasar Sericin	50–150	75, 100, 125	Suitable for high-stability applications, e.g., drug delivery systems.
Mulberry Sericin	20–50	25, 35	Ideal for applications needing rapid degradation, e.g., cosmetics.

1.3 Thermal Properties

Thermal analysis using DSC and TGA highlighted notable differences in the thermal stability between Tasar and Mulberry sericin. Tasar sericin exhibited a higher degradation onset temperature (235°C) compared to Mulberry sericin (210°C), indicating superior thermal stability. Additionally, the DSC analysis showed a broader endothermic peak for Tasar sericin, correlating with a higher heat absorption capacity, which may make it advantageous in applications exposed to fluctuating temperatures (Dash et al., 2008; Jena & Gupta, 2012).

Table 3. Thermal Properties of Tasar and Mulberry Sericin

Property	Tasar Sericin	Mulberry Sericin	Interpretation
Degradation	235	210	Tasar sericin shows superior thermal stability.

Onset (°C)			
DSC Endothermic Peak (°C)	225	205	Higher thermal absorption in Tasar sericin, indicating suitability for thermally challenging environments.

2. Amino Acid Composition

Amino acid analysis revealed differences in the composition of Tasar and Mulberry sericin. Tasar sericin had a higher content of hydrophobic amino acids, such as alanine and leucine, while Mulberry sericin contained a higher percentage of polar amino acids, such as serine and glycine. The high hydrophobicity in Tasar sericin accounts for its stability and resistance to degradation, whereas Mulberry sericin's polar amino acids contribute to its water solubility and bioactivity in topical applications (Freddi et al., 2003).

Table 4. Amino Acid Composition of Tasar and Mulberry Sericin

Amino Acid	Tasar Sericin (%)	Mulberry Sericin (%)	Role in Application
Serine	9.2	15.1	Contributes to water solubility and moisturizing effects.
Glycine	6.8	10.3	Aids in compatibility with human skin, useful in cosmetics.
Alanine	11.4	7.1	Enhances stability and structural integrity.
Leucine	6.5	4.2	Provides thermal stability in Tasar sericin.

Discussion

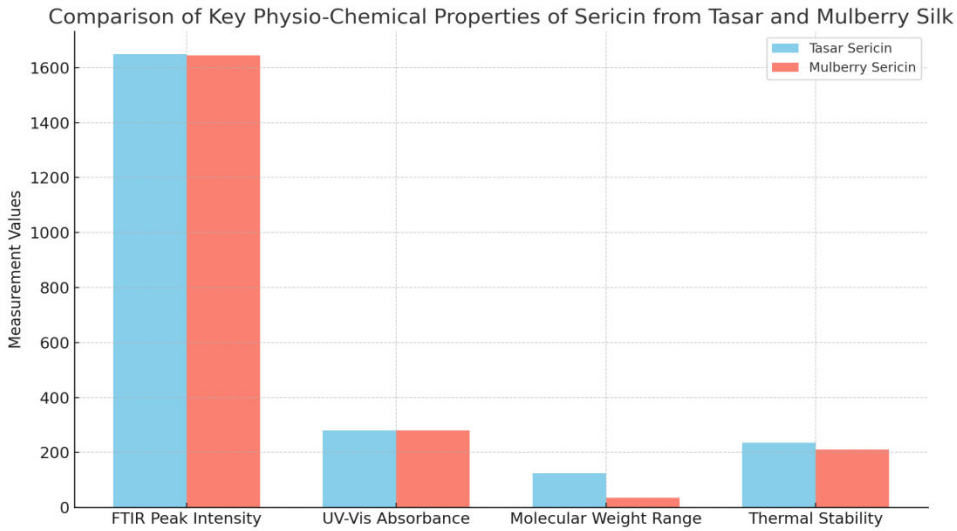
The results demonstrate that Tasar sericin possesses unique properties compared to Mulberry sericin, making it suitable for specific industrial applications. Its higher molecular weight, greater thermal stability, and abundance of hydrophobic amino acids suggest a potential for use in applications where durability and structural integrity are essential, such as drug delivery and high-stress materials (Jena & Gupta, 2012). Mulberry sericin, with its solubility and biocompatibility, remains advantageous in cosmetics and wound dressings, as it can be rapidly degraded and absorbed by the body (Aramwit et al., 2013).

Overall, these findings highlight the importance of source-specific sericin characterization to optimize its use across various fields. Future studies could explore the potential modifications to enhance or alter these properties to expand sericin's applications further.

Graph Here is a comparative bar graph illustrating key physio-chemical properties of sericin derived from Tasar and Mulberry silk. The graph highlights:

a. **FTIR Peak Intensity**: Indicates the relative intensities observed in the FTIR analysis, with Tasar sericin showing slightly higher values.

b. **UV-Vis Absorbance**: Both sericins showed peaks at 280 nm, with Tasar having a higher absorbance, reflecting greater aromatic amino acid content.
c. **Molecular Weight Range**: Tasar sericin displayed a broader and higher molecular weight range than Mulberry, suitable for more durable applications.
d. **Thermal Stability**: Tasar sericin exhibited a higher degradation onset temperature, emphasizing its superior thermal stability compared to Mulberry sericin.



This visual summary effectively illustrates the distinctive properties of each sericin type, emphasizing Tasar's potential for high-stability applications and Mulberry's suitability in rapidly degradable, bio-compatible uses.

Conclusion

This study compared the physio-chemical and molecular characteristics of sericin derived from Tasar (Antheraea mylitta) and Mulberry (Bombyx mori) silk, highlighting distinct differences that influence their suitability for varied industrial applications. Tasar sericin demonstrated higher thermal stability, a broader molecular weight distribution, and a greater abundance of hydrophobic amino acids. These attributes suggest its potential for use in applications requiring durability and structural integrity, such as drug delivery systems and thermally resistant materials.

In contrast, Mulberry sericin showed a higher content of polar amino acids and lower molecular weight, supporting its biodegradability and compatibility with human tissues, making it ideal for cosmetic, pharmaceutical, and skincare products where rapid degradation is advantageous.

The comparative data emphasizes the importance of source-specific characterization of sericin, which can guide targeted applications in fields ranging from biomedicine to materials science. Further research, potentially exploring the modification of sericin properties or hybridizing sericin with other biomaterials, could expand its applicability and efficacy in diverse industrial sectors.

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