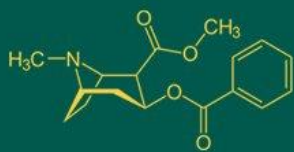


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Comparative Evaluation of Functional and *In vitro* Biological Properties of Silk Sericin from Mulberry, Eri and Tasar Cocoons

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Abstract

Silk sericin is a hydrophilic protein fraction of silk that is removed during conventional degumming and can therefore represent an underutilized resource. This study comparatively evaluated sericin recovered from mulberry (*Bombyx mori*), Eri (*Samia ricini*) and Tasar (*Antheraea mylitta*) cocoons using an identical autoclave-based extraction procedure. Cocoon samples were treated at 120 °C for 30 min in distilled water, followed by filtration and freeze-drying. Recovery yield, oil absorption capacity, water absorption capacity, foaming capacity, foam stability, emulsifying capacity and emulsion stability were assessed. *In vitro* DPPH radical-scavenging, α -amylase inhibitory and pancreatic lipase inhibitory activities were also compared. The obtained dataset showed the highest recovery for Mulberry sericin ($10.25 \pm 0.12\%$), followed by Eri ($9.00 \pm 0.10\%$) and Tasar ($6.60 \pm 0.08\%$). Mulberry sericin also recorded the highest oil absorption (0.89 ± 0.01 g/g), water absorption (0.95 ± 0.01 g/g), foaming capacity ($68.49 \pm 0.85\%$), foam stability ($64.10 \pm 0.72\%$), emulsifying capacity ($69.27 \pm 0.90\%$) and emulsion stability ($56.53 \pm 0.80\%$). Mulberry sericin further showed the highest DPPH radical-scavenging activity ($46.55 \pm 0.60\%$), α -amylase inhibition ($33.19 \pm 0.42\%$) and pancreatic lipase inhibition ($28.88 \pm 0.40\%$). The results indicate clear species-associated differences in sericin recovery and functional performance under the conditions tested. Because the biological assays were conducted *in vitro*, the observed antioxidant and enzyme-inhibition responses should be interpreted as preliminary functional evidence rather than therapeutic effects.

Keywords: Silk sericin, *Bombyx mori*, *Samia ricini*, *Antheraea mylitta*, functional properties, DPPH, α -amylase inhibition, pancreatic lipase inhibition

1. Introduction

Sericulture generates silk fibre as well as protein-rich fractions that are separated during silk processing. Silk consists principally of fibroin and sericin, with sericin forming a substantial fraction of the silk protein matrix. During degumming, sericin is solubilized and is commonly treated as a process by-product. Recovery and utilization of this fraction are increasingly relevant to resource efficiency and circular approaches in sericulture^[1-4].

Sericin is hydrophilic and contains a range of polar and non-polar amino-acid residues. These characteristics contribute to its ability to interact with water and lipid phases and have stimulated interest in functional formulations, films, coatings, cosmetics and biomaterial systems. Previous investigations have reported antioxidant and other biological activities for silk-derived proteins^[1-4].

Most investigations have focused on mulberry silk, whereas sericin from non-mulberry silks, including Eri and Tasar, has received comparatively less attention in direct comparative studies. Because silkworm species and extraction processes can influence sericin molecular characteristics and functional behaviour, comparative evaluation of different silk sources is warranted^[2, 4, 5].

The present study therefore compared sericin recovered from Mulberry, Eri and Tasar cocoons using the same extraction approach. The study focused on recovery yield, selected functional properties and three *in vitro* bioactivity indicators: DPPH radical-scavenging activity, α -amylase inhibition and pancreatic lipase inhibition. The objective was to establish a comparative baseline for the three sericin sources and identify the source showing the most favourable performance under the experimental conditions used.

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2. Materials and Methods

2.1 Sources of Sericin

Sericin was recovered from cocoons of three silkworm species: Mulberry (*Bombyx mori*), Eri (*Samia ricini*) and Tasar (*Antheraea mylitta*). The three sources were selected to permit direct comparison of species-associated differences in sericin recovery and functional characteristics.

2.2 Extraction of Sericin

Sericin was extracted using an autoclave-based aqueous method. 100 g cocoon samples were treated with distilled water (M/L ratio = 1:30) at 120 °C for 30 min in an autoclave. Following treatment, the extract was filtered to separate insoluble cocoon residues. The filtered aqueous extract was retained for preparation of sericin powder [2, 4].

2.3 Recovery of Sericin Powder

The filtered extract was freeze-dried (lyophilized) to obtain a dry sericin preparation. Recovery was expressed as the percentage of dry crude sericin powder obtained relative to the initial sample weight. The experiment was conducted in a completely randomized design with six replications.

Recovery (%) = (weight of crude dry sericin powder / weight of initial sample) × 100.

2.4 Functional Properties of Sericin

Oil absorption capacity (OAC) was assessed using a protein-functional-property approach adapted from Deng *et al.* [6]. Briefly, 0.2 g of protein sample was mixed with 10 g soybean oil and stirred for 30 min at ambient temperature. The mixture was centrifuged at 6000 rpm for 10 min, after which unabsorbed oil was removed. Water absorption capacity (WAC) was determined by mixing 0.2 g sample with 10 mL deionized water, followed by centrifugation at 6000 rpm for 10 min and removal of the supernatant [6].

Foaming capacity and foam stability were assessed using the reported homogenization procedure. Sericin (1 g) was dispersed in 25 mL deionized water and homogenized at 10,000 rpm for 5 min. Foaming behaviour is affected by protein solubility, molecular flexibility, adsorption at the air-water interface and interfacial film formation [7].

Emulsifying capacity and emulsion stability were determined by homogenizing sericin solution with soybean oil at a 1:1 (v/v) ratio. Emulsion stability was assessed after heating at 80 °C for 30 min. Sericin has been demonstrated to possess emulsifying activity, with interfacial behaviour influenced by amino-acid composition and molecular characteristics [8].

2.5 In vitro Biological Activities

2.5.1 DPPH Radical-Scavenging Activity

DPPH radical-scavenging activity was determined spectrophotometrically at 517 nm and expressed as percentage inhibition relative to the control. Silk sericin has previously been shown to scavenge DPPH and other radicals [9, 10].

2.5.2 α -Amylase Inhibitory Activity

α -Amylase inhibitory activity was determined using a starch-iodine colorimetric procedure. The reaction mixture contained sericin sample, sodium phosphate buffer and α -amylase, followed by starch as substrate. The reaction was terminated with HCl and absorbance was measured at 620

nm. The starch-iodine approach provides a quantitative basis for monitoring α -amylase activity [11].

2.5.3 Pancreatic Lipase Inhibitory Activity

Pancreatic lipase inhibitory activity was determined using p-nitrophenyl butyrate (pNPB) as substrate. The reaction was monitored at 405 nm by measuring p-nitrophenol formation. This spectrophotometric approach is widely used for screening pancreatic lipase inhibition [12].

2.6 Statistical Analysis

Data were analysed using analysis of variance (ANOVA) appropriate to the completely randomized design. Statistical significance was assessed at $p \leq 0.05$. Values are presented as mean $\pm$ the dispersion measure reported in the experimental dataset. Critical difference (CD) values at the 5% probability level are presented in the tables where available.

3. Results and Discussion

3.1 Sericin Recovery

Sericin recovery differed significantly among the three cocoon sources. Mulberry sericin exhibited the highest recovery ($10.25 \pm 0.12\%$), followed by Eri ($9.00 \pm 0.10\%$) and Tasar ($6.60 \pm 0.08\%$). The differences were statistically significant ($CD = 0.90$; $p \leq 0.05$), indicating that cocoon source had a significant influence on sericin recovery under the extraction conditions employed.

The observed variation in recovery may be associated with differences in cocoon composition, sericin-fibroin interactions, accessibility of the sericin fraction and the physicochemical characteristics of silk proteins. Previous studies have demonstrated that sericin recovery is strongly influenced by extraction temperature, extraction duration, solvent-to-material ratio and extraction methodology [2, 4]. Autoclave-assisted extraction can alter sericin molecular characteristics and recovery, emphasizing the importance of using identical conditions for comparative studies [4, 5].

The recovery values obtained in the present investigation should be considered crude sericin recovery yields rather than absolute sericin contents, because purity and total sericin content were not independently determined. Consequently, direct comparison with literature values obtained using different extraction protocols should be made cautiously.

Table 1: Recovery of sericin from Mulberry, Eri and Tasar cocoons

Species	Recovery (%)
Mulberry (<i>Bombyx mori</i>)	10.25 ± 0.12
Eri (<i>Samia ricini</i>)	9.00 ± 0.10
Tasar (<i>Antheraea mylitta</i>)	6.60 ± 0.08
CD ($p \leq 0.05$)	0.90

3.2 Oil and Water Absorption

Significant differences were observed in the oil absorption capacity (OAC) and water absorption capacity (WAC) of sericin obtained from the three cocoon sources. Mulberry sericin exhibited the highest OAC (0.89 ± 0.01 g/g), followed by Eri (0.84 ± 0.01 g/g) and Tasar (0.81 ± 0.01 g/g). WAC showed a similar trend, with values of 0.95 ± 0.01 , 0.90 ± 0.01 and 0.86 ± 0.01 g/g for Mulberry, Eri and Tasar sericin, respectively.

The observed OAC values indicate appreciable interaction of the sericin preparations with lipid molecules. Protein oil-binding capacity is influenced by hydrophobic groups, surface characteristics and protein conformation, whereas water absorption is associated with the availability and accessibility of hydrophilic groups [6, 7]. Sericin contains a high proportion of polar amino acids and therefore exhibits substantial interaction with water [2, 4].

Previous studies have reported measurable water- and oil-holding properties for sericin and demonstrated that these characteristics can change following enzymatic hydrolysis [7]. The higher OAC and WAC observed for Mulberry sericin may therefore indicate differences in the molecular characteristics of the preparations. However, because amino-acid composition, molecular-weight distribution and surface hydrophobicity were not determined, the precise molecular basis cannot be established.

Table 2: Oil and water absorption capacities of sericin

Species	Oil absorption (g/g)	Water absorption (g/g)
Mulberry	0.89 ± 0.01	0.95 ± 0.01
Eri	0.84 ± 0.01	0.90 ± 0.01
Tasar	0.81 ± 0.01	0.86 ± 0.01
CD (p ≤ 0.05)	0.0060	0.0058

3.3 Foaming Properties

Mulberry sericin exhibited the highest foaming capacity (68.49 ± 0.85%), followed by Eri (63.81 ± 0.68%) and Tasar (48.99 ± 0.55%). Foam stability showed a similar trend, with values of 64.10 ± 0.72%, 56.04 ± 0.65% and 35.88 ± 0.49%, respectively. The observed differences were significant at p ≤ 0.05.

Protein foaming properties are governed by solubility, molecular flexibility, adsorption at the air-water interface and the ability to form a cohesive interfacial film [7]. Sericin and its hydrolysates have been shown to possess foaming activity, while changes in molecular structure can modify foam formation and stability [7]. The relatively high foaming performance of Mulberry sericin may therefore indicate favourable interfacial behaviour of the extracted preparation. The substantially lower foaming capacity and stability of Tasar sericin suggest source-associated differences in its ability to form and maintain air-water interfaces.

Table 3: Foaming capacity and foam stability of sericin

Species	Foaming capacity (%)	Foam stability (%)
Mulberry	68.49 ± 0.85	64.10 ± 0.72
Eri	63.81 ± 0.68	56.04 ± 0.65
Tasar	48.99 ± 0.55	35.88 ± 0.49
CD (p ≤ 0.05)	1.05	1.27

3.4 Emulsifying Properties

Mulberry sericin showed the highest emulsifying capacity (69.27 ± 0.90%) and emulsion stability (56.53 ± 0.80%), followed by Eri sericin (58.52 ± 0.75% and 44.60 ± 0.70%) and Tasar sericin (45.41 ± 0.62% and 33.71 ± 0.58%), respectively. The differences were statistically significant at p ≤ 0.05.

The emulsifying behaviour of proteins is primarily associated with their ability to adsorb at the oil-water interface and form a protective interfacial layer. Protein solubility, flexibility, amphiphilicity and surface characteristics are important determinants of emulsification [7, 8]. Sericin has been directly demonstrated to function as an

emulsifier, and water-extracted sericin can form stable emulsions [8]. The higher emulsifying capacity and stability observed for Mulberry sericin may therefore reflect differences in the interfacial properties of sericin derived from the three cocoon sources.

Table 4: Emulsifying capacity and emulsion stability of sericin

Species	Emulsifying capacity (%)	Emulsion stability (%)
Mulberry	69.27 ± 0.90	56.53 ± 0.80
Eri	58.52 ± 0.75	44.60 ± 0.70
Tasar	45.41 ± 0.62	33.71 ± 0.58
CD (p ≤ 0.05)	1.37	1.55

3.5 DPPH Radical-Scavenging Activity

All three sericin preparations exhibited measurable DPPH radical-scavenging activity. Mulberry sericin showed the highest activity (46.55 ± 0.60%), followed by Eri (38.30 ± 0.52%) and Tasar (26.89 ± 0.41%). The differences were statistically significant (CD = 0.82; p ≤ 0.05).

The antioxidant activity observed in the present study is consistent with previous reports demonstrating radical-scavenging activity of silk sericin. Fan *et al.* demonstrated DPPH radical-scavenging activity of *Bombyx mori* sericin, while Kumar and Mandal showed that antioxidant performance differs among mulberry and non-mulberry sericins and is strongly influenced by extraction procedure and molecular characteristics [9, 10]. The comparatively higher DPPH response of Mulberry sericin suggests stronger radical-quenching behaviour under the present assay conditions. However, DPPH is a chemical *in vitro* assay and cannot by itself establish physiological antioxidant efficacy.

Table 5: DPPH radical-scavenging activity of sericin

Species	DPPH radical-scavenging activity (%)
Mulberry	46.55 ± 0.60
Eri	38.30 ± 0.52
Tasar	26.89 ± 0.41
CD (p ≤ 0.05)	0.82

3.6 α-Amylase Inhibitory Activity

The sericin preparations exhibited significant α-amylase inhibitory activity, with Mulberry sericin showing the highest inhibition (33.19 ± 0.42%), followed by Eri (28.12 ± 0.38%) and Tasar (22.05 ± 0.31%). The critical difference was 0.63 at p ≤ 0.05, confirming significant variation among the three cocoon sources.

The starch-iodine approach provides a useful *in vitro* method for assessing α-amylase activity [11]. The greater inhibition exhibited by Mulberry sericin indicates a comparatively stronger interaction with α-amylase under the conditions of the present assay. Differences may reflect variation in protein or peptide composition and associated extractable constituents. Nevertheless, the observed inhibition should be interpreted as *in vitro* enzyme-inhibitory activity rather than antidiabetic efficacy. Further studies involving enzyme kinetics, concentration-response relationships, peptide characterization and appropriate biological models are required.

Table 6: *In vitro* α-amylase inhibitory activity of sericin

Species	α-Amylase inhibition (%)
Mulberry	33.19 ± 0.42
Eri	28.12 ± 0.38
Tasar	22.05 ± 0.31
CD (p ≤ 0.05)	0.63

3.7 Pancreatic Lipase Inhibitory Activity

Pancreatic lipase inhibition was highest for Mulberry sericin ($28.88 \pm 0.40\%$), followed by Eri ($25.68 \pm 0.36\%$) and Tasar ($18.19 \pm 0.29\%$). The differences were statistically significant ($CD = 0.94$; $p \leq 0.05$).

The p-nitrophenyl butyrate assay provides a spectrophotometric means of measuring pancreatic lipase activity through formation of p-nitrophenol [12]. The stronger inhibitory response of Mulberry sericin may reflect differences in protein composition, peptide characteristics or other extractable constituents among the three cocoon sources. Previous experimental studies have also shown that sericin-containing silk proteins can influence lipid-related parameters in biological systems [13-15]. However, pancreatic lipase inhibition *in vitro* should not be interpreted as direct evidence of hypolipidemic or anti-obesity efficacy.

Table 7: *In vitro* pancreatic lipase inhibitory activity of sericin

Species	Pancreatic lipase inhibition (%)
Mulberry	28.88 ± 0.40
Eri	25.68 ± 0.36
Tasar	18.19 ± 0.29
CD ($p \leq 0.05$)	0.94

3.8 Overall Comparative Performance

Across the measured parameters, Mulberry sericin consistently recorded the highest values, whereas Tasar sericin generally showed the lowest values and Eri occupied an intermediate position. This consistent ranking suggests that the source of silk is an important factor influencing the functional behaviour of the recovered sericin preparation. At the same time, the present study does not establish whether the differences arise from intrinsic protein composition, molecular size, residual associated compounds or differences in extraction efficiency.

Table 8: Overall comparative performance of sericin from Mulberry, Eri and Tasar cocoons

Property	Mulberry	Eri	Tasar
Recovery (%)	10.25	9.00	6.60
Oil absorption (g/g)	0.89	0.84	0.81
Water absorption (g/g)	0.95	0.90	0.86
Foaming capacity (%)	68.49	63.81	48.99
Foam stability (%)	64.10	56.04	35.88
Emulsifying capacity (%)	69.27	58.52	45.41
Emulsion stability (%)	56.53	44.60	33.71
DPPH (%)	46.55	38.30	26.89
α -Amylase inhibition (%)	33.19	28.12	22.05
Pancreatic lipase inhibition (%)	28.88	25.68	18.19

4. Conclusion

The present comparative study demonstrates clear differences among Mulberry, Eri and Tasar sericin preparations obtained using the same autoclave-based extraction approach. Mulberry sericin showed the highest recovery and the most favourable performance across the selected absorption, foaming and emulsifying properties. It also produced the highest DPPH radical-scavenging, α -amylase inhibitory and pancreatic lipase inhibitory responses in the *in vitro* assays.

These findings identify Mulberry sericin as the strongest overall performer among the three preparations under the conditions evaluated and provide a useful comparative basis for future product-oriented studies. However, the biological

assays are screening-level *in vitro* tests and should not be interpreted as evidence of therapeutic efficacy. Future studies should characterize sericin purity, molecular-weight distribution, amino-acid/peptide composition and structural properties; establish concentration-response relationships and IC50 values; and evaluate safety and application-specific performance before making claims for nutraceutical, cosmetic or biomedical use.

5. Limitations and future research

Crude sericin recovery rather than purified sericin was evaluated; protein purity and residual non-protein constituents were not independently quantified.

The exact assay equations and calculation bases should be checked against the original laboratory worksheets before journal submission, particularly for OAC, WAC and foaming capacity.

Molecular weight, amino-acid composition, peptide profile and secondary structure were not characterized, limiting mechanistic interpretation.

The biological assays were *in vitro* screening assays and do not establish *in vivo* efficacy or therapeutic benefit.

Future work should include dose-response studies, IC50 values, structural characterization, purity assessment, safety testing and application-specific formulation studies.

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