Separation and physico-chemical analysis of sericin protein from silk

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Abstract. Sericin protein was extracted from silk using a water-based method. The impact of sericin on the aquatic environment and its behavior under various temperature conditions were thoroughly investigated. To assess the total protein content in the solutions derived from silk at different temperatures, the Lowry method was employed. Subsequently, sericin protein was isolated in its pure form. In order to gain deeper insights into the characteristics of sericin, both IR-spectrometric and UV-spectrometric analyses were conducted. These analyses provided valuable information about the molecular structure and properties of the sericin protein. Additionally, the study focused on determining the composition of amino acids present in the sericin protein. For this purpose, high-performance liquid chromatography (HPLC) was employed, allowing for precise quantification and identification of individual amino acids within the protein. Through these comprehensive analytical techniques and experiments, researchers aimed to unravel the properties, behavior, and potential applications of sericin protein, contributing to a better understanding of its role and impact in various environmental and temperature conditions.

1. Introduction

Silk cocoon fiber is a thin, strong, shiny, fiber, which is a natural textile raw material formed from the liquid that comes out of the two silk glands of the silkworm. Silk consists of two non-sticky, sericin-coated and glued fibers that surround the cocoon of the silkworm. Silk contains two types of proteins, fibroin and sericin [1, 2].

The content of sericin in raw silk fiber is 20-30%. It consists of 18 amino acids. There are different ways to separate sericin from silk. The solubility, molecular weight, and recovery of the gel-like properties of sericin depend on processing methods. Sericin is used as a high-potential biomaterial widely used in medicine, pharmaceuticals, cosmetics, biosorbents and other fields. Sericin is very suitable for biomedical and pharmaceutical applications [3-7]. In the pharmaceutical industry, the use of sericin protein is effective instead of ε-aminoenanoic acid, which plays an important role in the production of anticoagulants [8-11].

Sericin protein is also widely used in the production of cosmetics, creams, shampoos and face masks [12]. Sericin has a similar structure to fibroin, except that it is more soluble in hot water than fibroin. Due to the multipolar groups in the molecule, good hydration weakens intermolecular bonds, causing swelling and dissolution. The dissolved sericin molecule passes from the structure to the globular state [13, 14].

Sericin consists of a polar side chain consisting of hydroxyl, carboxyl and amino groups, which allow the formation of improved biodegradable materials through crosslinking, copolymerization and mixing with other polymers [8]. Some scientists have divided the cocoon capsule into two classes: α-sericin and β-sericin. The outer shell of the capsule consists of α-sericin and the inner layer of β-sericin. β-sericin contains less C and H and more H and O than β-sericin. The solubility of 9α-sericin is higher than the solubility of β-sericin in hot water [15-18].

The aim of this study was to select the most suitable method for separating the sericin protein from silk, conduct an IR spectrum analysis of the sericin, and study the sericin protein by UV spectrophotometry.

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2. Materials and methods

Preparation of sericin protein from silk by water extraction. Sericin is insoluble in alcohol, ether, acetone, benzene and similar solvents, but soluble in water, aqueous solutions of alkalis and acids. (less than pH=4. [12]. We chose the aqueous extraction method as the simplest and most efficient method for isolating sericin, and the essence of the method is explained as follows. Water enters the sericin, causing it to swell, separate, and partially melt. The melting of sericin is explained by the large number of polar groups in its chain.

(-NH, -NH_2, -OH, -COOH)

Sericin does not have a critical melting point; it consists of polydisperse molecules [13]. The upper layers of the cocoon contain shorter and longer internal sericin molecules, so the sericin in the upper layers of the cocoon starts to melt at 70°C. The inner layers melt at 80°C and above. The uniform distribution of polar groups in the elemental chain of sericin greatly facilitates the hydration and breaking of individual parts of the chains [14].

The light solubility of sericin is determined by the amount of substances released when the shell is boiled in water for 7.5 minutes.

Determining the amount of total proteins. The Lowry method was used to determine the total protein content in solutions obtained from silk under the influence of four different temperatures [17]. Samples with a color reaction are detected on a modern spectrophotometer at a wavelength of 670 nm. The results are presented in tabular form below:

IR spectrophotometry. The IR spectra of sericins were recorded on an IR Fourier spectrometer IRTracer-100 (SHIMADZU CORP., Japan, 2017) complete with an attenuated total internal reflection (ATR) MIRacle-10 attachment with a diamond/ZnSe prism (spectral range in wavenumber scale - 4000÷400 cm-1; resolution - 4 cm-1; sensitivity signal-to-noise ratio - 60,000:1; scanning speed - 20 spectra per second).

UV - spectrophotometry. 10 mg of sericin protein was measured, 10 mg of water was added, dissolved in an ultrasonic water bath for 5 minutes, and the composition was examined on a UV-1280 UV-VIS spectrophotometer at a wavelength of 400 nm.

HPLC analysis of PTK derivatives of amino acids. Synthesis of PTC (phenylthiocarbomayl) derivatives of amino acids was carried out according to the method of Steven A., Cohen Daviel

Identification of FTC-amino acids is carried out on an Agilent Technologies 1200 chromatograph on a 75x4.6 mm Discovery HS C18 column. Solution A: 0.14 M CH_3COONa + 0.05% TEA pH 6.4, B: CH3CN. Flow rate 1.2 ml/min, absorbance 269nm. Gradient %B/min: 1-6%/0-2.5min; 6-30%/2.51-40min; 30-60%/40.1-45min; 60-60%/45.1-50min; 60-0%/50.1-55min.

3. Results and discussion

The melting point of sericin is 12-15%, depending on the breed of silkworm and the original method of processing cocoons. It varies even within the same batch of cocoons. The average solubility of a series of 50 cocoon shells is 4.39%. The melting temperature of individual series of cocoon shells ranges from 2.51 to 6.29% [15]. Silk is completely devoid of sericin when boiled in water at 100 ^{0}C for one hour. With a decrease in temperature and an increase in the concentration of an aqueous solution, sericin turns from a sol (colloidal solution) into a gel, that is, it gelatinizes (Table 1).

Table 1. Dependence of temperature and duration of soaking in water on melting and swelling of the crust relative to the initial mass, %.

Continuous moistening, min	Water temperature, ^{o}C							
	45-50		65-70		90-95		Boiling	
	swell	melting	swell	melting	swell	melting	swell	melting
1	-	-	-	-	86.1	2.29	123.7	4.77
3	43,4	0.79	46.4	2.06	86.9	3.15	106.2	5.46
5	42,4	0.97	49.6	2.52	96.6	4.23	101.8	5.23
10	49,3	1.21	58.4	2.12	107.7	4.18	105.6	7.21
30	53,5	1.28	62.3	2.87	-	-	93.1	8.51

The results of electrophotometric studies and a study of the concentration of solutions and the intensity of light scattering showed that sericin in the outer layer of the shell is better soluble than in the inner layer. Sericin in the outer layer consists of a portion of large molecules, while the inner layer consists of a portion of smaller molecules (Figure 1).

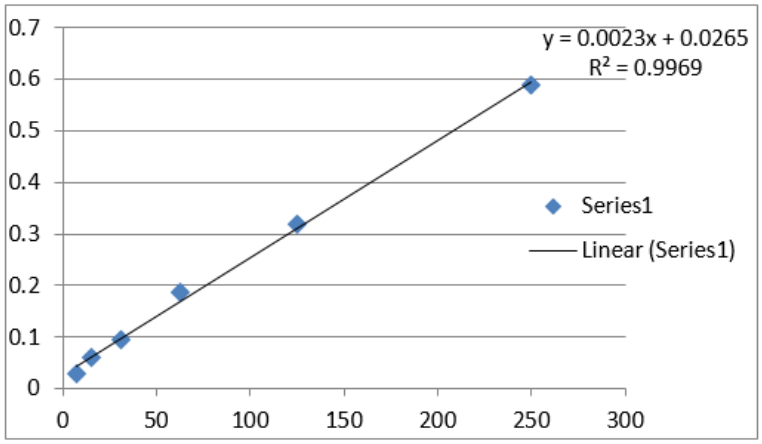

Fig. 1. Calibration curve for determination of the amount of protein by the Lowry method.

The total amount of proteins. Silk composition solutions exposed to four different temperatures were stained using the Lowry method for protein quantification, and the total protein content was measured on a UV-VIS spectrophotometer (Table 2 and Figure 2).

Table 2. The total amount of protein in the obtained samples.

Samples	Amount mkg/ml
sample control	0,00
sample 1 (45-50 °C)	214,9
sample 2 (65-70 °C)	363,6
sample 3 (90-95 °C)	680,4
Boiling point	793,9

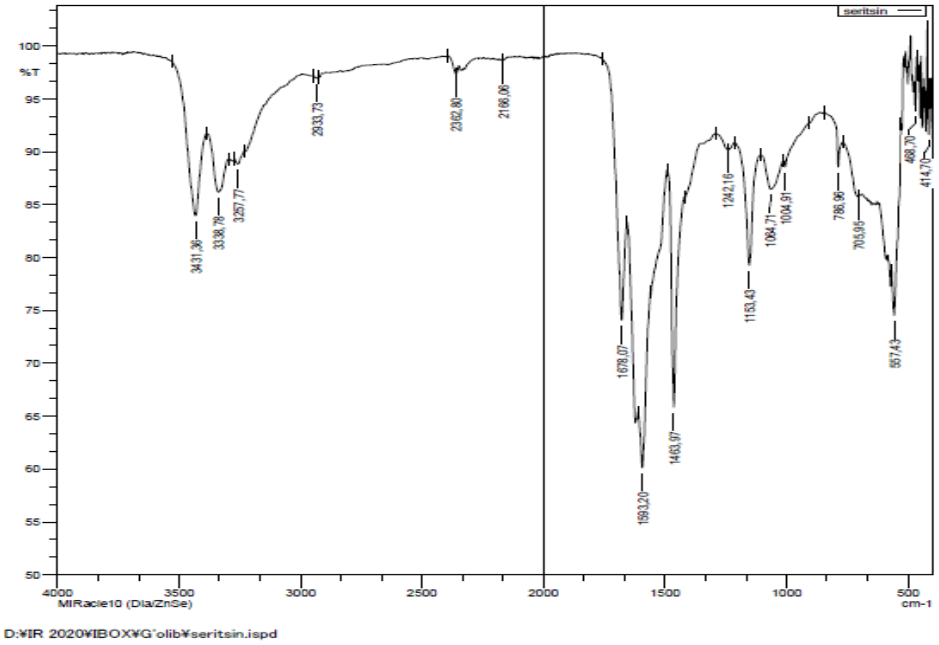

Fig. 2. IR spectrum of sericin protein.

Results IR spectrum of sericin protein. Valence vibration frequencies belonging to NH groups were observed in sericin at 3431-3339 cm^{-1}. The stretching vibration frequencies belonging to the C=O groups in the molecule were observed at 1678 cm^{-1}. At 1593 cm^{-1}, a vibration was observed corresponding to the frequency of bending vibrations of NH_2 groups. At 1464 cm^{-1}, the deformation frequencies belonging to the methylene groups ($-CH_2-$) appeared in the intense state; the vibration frequencies corresponding to the N-C bonds in the sericin molecule were observed with moderate intensity at 1153-1064 cm^{-1}.
In addition, vibrational frequencies corresponding to four alternating benzene derivatives were observed in the regions 787–706 cm^{-1} in Figure 3.

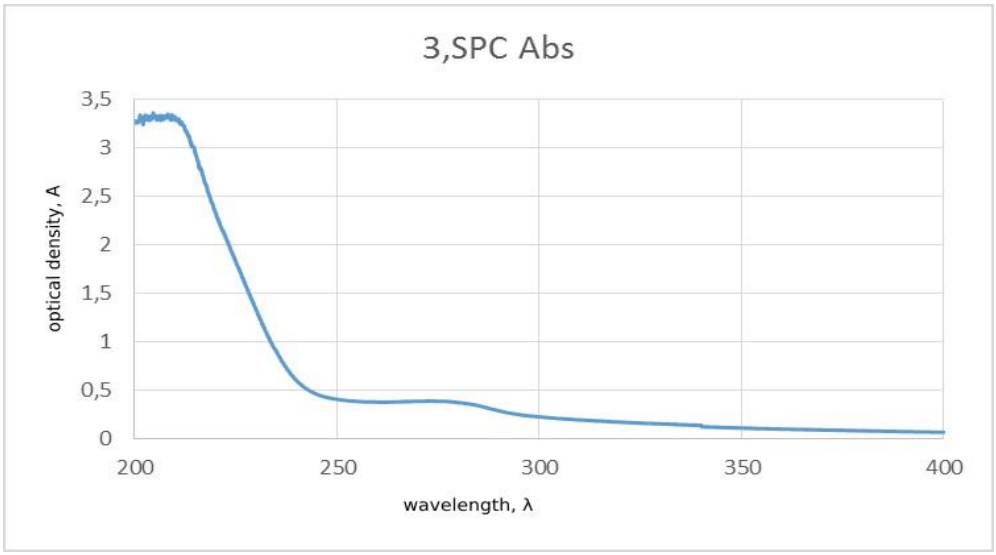

Fig. 3. UV spectral analysis of the sericin protein.

The results of the UV spectrum of the sericin protein. It is known from the literature that bands belonging to chromophore groups in the sericin protein show a very low absorption line. π→π* and n→π* - bands resulting from electronic transitions Figure 4 and Table 3).

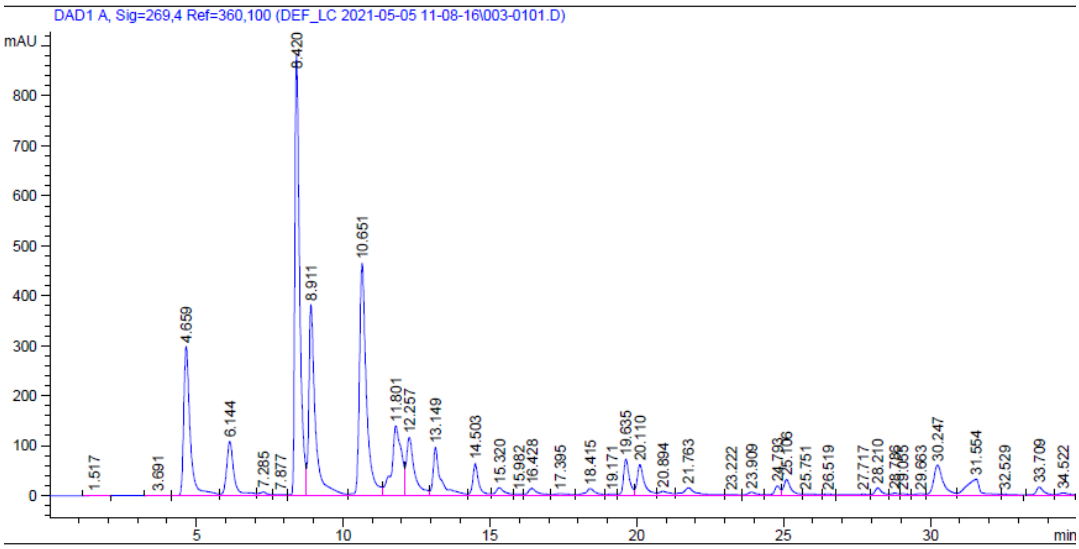

Fig. 4. HPLC analysis of PTC derivatives of amino acids.

The sericin protein has been found to contain mainly high amounts of the amino acid serine. In addition, glycine and threonine have been found to be significantly higher than other amino acids.

Table 3. The results of the study of the number of amino acids in the sericin protein.

Amino acids	Amino acid concentrations (mg/g)
Aspartic acid	74.4733
Glutamine acid	38.02548
Serene	124.8733
Glycine	67.66889
Asparagine	0
Glutamine	0
Cysteine	22.57732
Threonine	66.94187
Argenin	30.23438
Alanine	15.51818
Proline	7.235063
Tyrosine	23.56349
Valine	13.56771
Methionine	10.85398
Isoleucine	7.181743
Leucine	8.274313
Histidine	2.539063
Tryptophan	0
Phenylalanine	39.9443
Lysine HCl	12.01273
Total	**565.4851**

4. Conclusions

In this study, we isolated the protein sericin from silk by aqueous extraction. We found that water temperature has a direct effect on the release of sericin and other water-soluble biochemicals in silk. We have carried out various spectral analyzes of the pure sericin protein. In conclusion, the following can be said:

1. Sericin protein is a mixture of at least two modifications in an aqueous solution. They are divided into fractionated deposits.
2. Sericin is an amphoteric substance from a chemical point of view. The isoelectric point of sericin is 4.3 at pH=3.9, which indicates the high acidity of sericin.
3. Sulfuric acid, salts (copper sulfate, iron chloride), tungsten phosphorus, molybdic phosphoric acid are coagulated from aqueous solutions under the action of alcohol, acetone, etc.
4. Protein sericin is economical, given that the raw materials for isolating sericin are expensive and in short supply.
5. Sericin is widely used in the pharmaceutical industry as well as cosmetics.

References

1. S. K. Rajput and Mukesh Kumar. Singh Sericin – A Unique Biomaterial. IOSR Journal of Polymer and Textile Engineering (IOSR-JPTE) e-ISSN: 2348-019X, p-ISSN: 2348-0181, Volume 2, Issue 3, PP 29-35 (2015)
2. Padamwar M N and Pawar A P, Silk Sericin and its application, Journal of Scientific & Industrial Research, vol. 63, pp 323-329. (2004)
3. Komatsu K, silk (its formation, structure, character and utilization), the polymeric materials encyclopedia (CRC Press) (1996)
4. Wu, J,-H, Wang, Z, & Xu, S,-Y. Preparation and characterization of sericin powder extracted from silk industry wastewater. Food chemistry, 103, 1255-1262. (2007)
5. Gulrajani M.L. Sericin: A bio-molecule of value. Souvenir 20th congress of the international sericulture commission, Banglore, India 15-18th December 2005. Pp. 21-29. (2005)
6. Takasu, Y, Yamada, H., Tsubouchi, K. Isolation of three main sericin components from the cocoon of the silkworm, bombyxmori. Bombyx mori, bioscience, biotech, biochem., 66, 2715-2718. (2002)
7. Park, C.J.; Ryoo, J.; Ki, C.S.; Kim, J.W.; Kim, I.S.; Bae, D.G.; Um, I.C. Effect of molecular weight on the structure and mechanical properties of silk sericin gel, film, and sponge. Int. J. Biol. Macromol, 119, 821–832. [CrossRef] [PubMed] (2018)
8. Wang, R.; Zhu, Y.; Shi, Z.; Jiang, W.; Liu, X.; Ni, Q.-Q. Degumming of raw silk via steam treatment. J. Clean. Prod, 203, 492–497. [CrossRef] (2018)
9. Craig, C.L. Evolution of arthropod silks. Annu. Rev. Entomol, 42, 231–267. [CrossRef] (1997)

10. Ude, A.; Eshkoor, R.; Zulkifili, R.; Ari_n, A.; Dzuraidah, A.; Azhari, C. Bombyx mori silk fibre and its composite: A review of contemporary developments. Mater. Des, 57, 298–305. [CrossRef] (2014)
11. Kundu, S.C.; Dash, B.C.; Dash, R.; Kaplan, D.L. Natural protective glue protein, sericin bioengineered by silkworms: Potential for biomedical and biotechnological applications. Prog. Polym. Sci. 33, 998–1012. [CrossRef] (2008)
12. Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J. Protein measurement with Folin phenol reagent // *J. Biol. Chem.* 224p. (2011)
13. E.Egamberdiev, S. Turabdjanov, D. Mirzaeva, Kh. Khaydullaev, U. Sharipova, A. Shokhakimova, and O. Bakhtiyorov.: Effect of chitosan substance on the mechanical properties of paper obtained on the basis of flax cellulose. *E3S Web of Conferences* 371, 01045 (2023)
14. Ergashev Y.; Egamberdiev E.; Mirkhodjaeva D.; Akmalova G.; Umarova M.; Kholdarov R.: Obtaining a filter material used in gas and air purification. *E3S Web of Conferences* 371, 01012 (2023)
15. Arslanov, Sh.; Turabdjanov S.; Azimova, Sh.; Azimov D.; Sultankhojaeva N.; Egamberdiev E.: Physico-chemical properties and research of acids contained in oils of Uzbekistan. *E3S Web of Conferences*, 371, 01021 (2023)
16. Egamberdiev E.; Turabdjanov S.; Akmalova G.; Mukhtarova N.; Ayubova I.; Mirzakhmedova M.; Rakhmonberdiev G.: Obtaining paper from composition of different fibers and its analysis. *E3S Web of Conferences*, 371, 01004 (2023)
17. Ergashev Y.; Egamberdiev E.; Turabdzhanov S.; Akmalova G.; Isanova R.; Rashidov R.; Sobitov O.: Obtaining filter material from natural fiber composition and areas of its use. E3S Web of Conferences, 371, 01047 (2023)
18. Egamberdiev E.; Makhkamov A.; Rakhimjonov B.; Khusanov D.; Akmalova G.; Mirzakhmedova M.; Rahmonberdiev G. Effectiveness of cleaning of sunflower oil with filter material made from composition of organic and inorganic fibers. 3rd International Conference on Energetics, Civil and Agricultural Engineering, ICECAE 2022 Virtual, 187394 (2022)