INVESTIGATE THE CHARACTERISTICS AND ABILITY TO EXTRACT SERICIN FROM SILKWORM COCOONS USING HIGH TEMPERATURE

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SUMMARY

Sericin is a natural polymer found in the cocoon of the *Bombyx mori* silkworm, connecting with fibroin molecules through hydro bonds, constituting 25-30% of the total cocoon weight. Sericin is a hydrophilic protein with the ability to dissolve in hot water. To separate sericin from fibroin or the silkworm cocoon, degumming techniques can be employed and implemented through various methods such as heat, urea, acid, alkaline. The hot water extraction method is the most common due to the mentioned constraints for sericin extraction. Both time and temperature play important roles in the amount of extracted sericin. Therefore, the effects of high-temperature methods on sericin extraction were investigated in this study. Temperature ranged from 80 to 130°C and time extraction changed from 15-30-45-60 minutes was investigated. It was established that 120°C in 30 minutes extraction leads to the highest sericin content. Then, sericin from this extraction method was studied the characterization of secondary structure using Fourier transform infrared spectroscopy (FTIR), antibacterial and antioxidant properties. The presence of water and a high concentration of hydroxyl groups contributes to a broad absorption peak around 3273.57 cm^{-1}. Antibacterial properties against *Staphylococcus aureus* and *Escherichia coli* depend on sericin concentration, which affects the ability to prevent microorganisms from growing and reduce bacterial density. Antioxidant properties were determined by 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic) (ABTS) radical scavenging showed the strong antioxidant activity of sericin. It was suggested that sericin from silkworm cocoon has high potential source to apply in the food preservers and other industries.

Keywords: Antibacteria, *Bombyx mori*, extraction, high temperature, sericin.

INTRODUCTION

Silk is a natural fiber produced by silkworms, including those belonging to the *Bombycidae*, *Saturnidae*, and *Lasiocampidae* families, as well as spiders. *Bombyx mori*, a member of the *Bombycidae* family, is the source of mulberry silk, which is extracted from worms that have been fed mulberry leaves. Sericulture is the term used to describe the historical domestication that resulted in the need for human care when growing these silkworms. The two primary proteins involved in the structural makeup of the silk cocoon layer are sericin and fibroin. The primary component, fibrin, is a fibrous protein; the adhesive component, sericin, is a globular protein that encircles the fibers and bonds them together. The silk cocoon's ability to repel water is also enhanced by some impurities known as "non-sericin" ingredients, which include salts, sugars, and waxes. In terms of different kinds of silkworms and how the components are extracted according to the sources of nourishment, the main components of the cocoon are fibroin and sericin, along with other contaminants such colors, waxes, sugars, and phytochemicals. These constituents comprise roughly 75-83%, 17-25%, and 1-4 percent of the cocoon, in that order (Biganeh *et al*., 2022). Sericin is a hydrophilic amino acid macromolecule that consists of eighteen hydrophilic amino acids. Strong polar groups like amino, carboxyl, and hydroxyl groups are present in it, and these groups have the ability to copolymerize, create crosslinks, and combine with other polymers (Lamboni *et al*., 2015). Sericin's elemental composition analysis shows that it is composed of 6.9% sulfur, 0.9% hydrogen, 46.5% carbon, 16.5% nitrogen, and 31% oxygen. There is a relationship between the C/N ratio and solubility in hot water; a lower ratio corresponds to greater solubility in hot water (Jena *et al*., 2018; Kunz *et al*., 2016).

Heat, urea, acid, and alkaline represent the amino acid composition of sericin that was extracted using different techniques. The primary amino acid components of sericin are constant, despite minor differences in the percentage of amino acids recovered by various techniques. About 30% of sericin is made up of the amino acid serine, with the remaining 10-20% being aspartic acid and glycine. Methionine content in sericin drops with temperature, with considerably larger levels than in sericin extracted using other techniques. Furthermore, urea-extracted sericin has much less tyrosine than sericin extracted using other techniques. Moreover, the largest concentrations of sulfur-containing amino acids that can form double helix structures methionine and cysteine are found in sericin that has been heatedly removed (Aramwit *et al*., 2012).

Currently, research on sericin in the field of food technology is attracting attention from the scientific community and the food industry worldwide. Sericin, a protein derived from silk, is being explored and applied in various applications within the food industry.

MATERIALS AND METHODS

Materials

The *B. mori* silkworm cocoons used in the study are from silkworm farms in the Nam Dinh provice, Vietnam in the fall from September to ends in November.

Methods

Extraction sericin

A purified cocoon that is a cocoon that has been cleaned of silk and pupa inside the cocoon were chopped into strands with diameters 1-2 mm to increase extraction efficiency. 0.5 g of chopped silkworm cocoons was soaked into 30 mL of distilled water, high temperature degumming method is used to extract sericin from silkworm cocoons at various temperatures and time. After gumming, the sericin and fibroin-mixed solution was filtrated through Qualitative Filter Paper. The sericin is separated from the sericin solution using a centrifuge machine following filtration to obtain pure sericin in the final.

Determination sericin content

Estimation of sericin quality and quantity the protein content by Lowry's method. Folin-Ciocalteu reagent was diluted 5 times with distilled water before use. Based on the standard protein chart (BSA), the sericin content to be analyzed can be determined at 660 nm wavelength of a spectrophotometer.

FTIR analysis

To identify the functional groups found in sericin, the FTIR spectra of samples of the compound were recorded using an FTIR spectrometer (Jasco, Japan). Spectra in the range of 4000-400 cm^{-1} were acquired using the KBr pelleting process.

Evaluation of the antioxidant activity of sericin

$ABTS^{+}$ is a stable free radical cation. ABTS is soluble in water at a concentration of 7 mM. Prepare a potassium persulfate solution with a concentration of 2.45 mM. Prepare a cationic solution ABTS+, including 250 µL of ABTS (7 mM) and 250 µL of $K_2S_2O_8$ (2.45 mM). Allow the mixture to stand in the dark at room temperature for 12-16 hours before use. Stoichiometrically, ABTS and potassium on sulfate react in a 1:0.5 ratio, resulting in incomplete oxidation of ABTS, with absorbance peaking over 6 hours. The radical was stable in this form for more than two days when stored in the dark at room temperature. Dilute the ABTS solution with distilled water until the absorbance is 0.7±0.02 at 734 nm.

Evaluation of antibacterial activity of sericin

Escherichia coli (VTCC-B-482) and *Staphylococcus aureus* (VTCC-B-480) bacterial strains used in this study were purchased from the Institute of Microbiology and Biotechnology (Vietnam National University, Hanoi). *S. aureus* and *E. Coli* were cultivated in Peptone solution at 37°C for 24 hours. Sericin agar dilution was prepared by adding 0.56, 0.66, 0.75, 0.84, 0.94, and 1.03 mg sericin/mL. 100 µL of bacteria solution (OD660 nm = 0.01) was applied into petri plate. Then, 20 mL sericin agar dilution with various sericin concentration was applied. Mix the medium with the bacteria thoroughly by rotating the petri dish clockwise and counterclockwise. Let it stand for 15 minutes. Finally, the sample was incubated in an incubator at 37°C for 24 hours and read the results.

Statistical analysis

All the analysis are repeat at least 3 times to take the mean data and standard deviation is analyzed following ANOVA and SPSS with Ducan's Multiple Range test ($p < 0.05$) to distinguish the difference.

RESULTS AND DISCUSSION

Determination of the optimal temperature during the sericin extraction process

To investigate the effect of temperature on the extraction process of sericin with distilled water as solvent under temperature conditions of 80, 90, 100, 110, and 120, 130°C for a fixed time of 30 minutes. Based on the results in Figure 1.A, it showed that sericin concentration progressively was raised from low to high at under 100°C temperatures. At temperatures of 80°C, 90°C, and 100°C, there is a slight increase in sericin concentration. The sericin concentration obtained at the above temperatures is relatively low, and the sericin yield is correspondingly lower (Wang *et al*., 2021). This also indicated that molecular decomposition of sericin hardly occurred until treatment at temperatures (80, 90, and 100°C) for 30 minutes.

The concentration of sericin increased dramatically at extraction temperatures above 100°C. For instance, three times higher than below 100°C are 30.7±0.5, 37.17±0.6, and 30.5±0.4 mg/mL at 110°C, 120°C, and 130°C, respectively. The temperature increased from 110°C to 120°C, which increased the sericin content. But the

greater sericin content did not occur beyond 120°C. In comparison to the sericin concentration at 120°C, the concentration of sericin dropped to 130°C. It revealed that, in comparison to the temperatures examined, the sericin concentration is maximum at 120°C. The conclusion is consistent with Wang's earlier research in that processing temperature affects how sericin breaks down molecularly. Temperature may have an impact on how well sericin concentration is absorbed (Wang *et al*., 2021). Since the molecules' thermodynamics improve with temperature, the extraction procedure proceeded smoothly, and the amount of sericin that was extracted progressively rose as well. Since sericin is a protein, temperature and extraction time may cause it to become denatured. As a result, throughout the filtration and centrifugation stages, sericin would start to denature and be eliminated once the ideal sericin content extraction conditions were reached, resulting in a drop in sericin content.

Additionally, the results demonstrated that the recovered sericin concentration at 130°C was 30.25±0.4 mg/mL, which was much lower than the extraction at 120°C. Protein molecules underwent structural changes and lost activity as the temperature rose. The molecule dilated as a result of the temperature's influence because some functional groups (SH, NH_2, and COOH) were released when the secondary bonds were broken. Subsequently, the protein molecules create an unruly connection and precipitate.

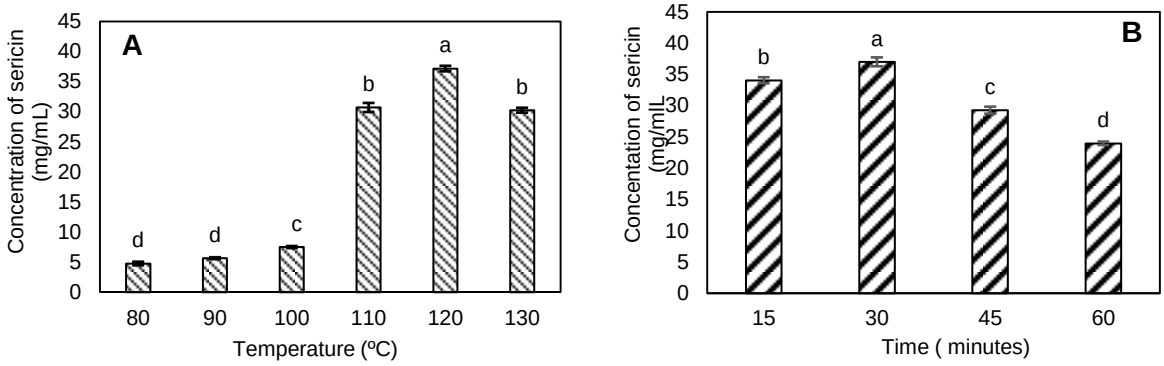


Figure 1. Sericin concentration from various temperature (A) and time (B) extraction

Error bars show SD derived from triplicate biological experiments. The letters show a significant ($p < 0.05$) difference between the mean.

Determine the optimal time during the sericin extraction process

According to Figure 1B's data, the sericin concentration increased from 15 to 30 minutes but subsequently reduced after 30 minutes of extraction at the times of 15, 30, 45, and 60 minutes. The concentration of sericin was only 34.05±0.51 mg/mL at 15 minutes, whereas at 30 minutes, it was measured to be 37.02±0.72 mg/mL. Nevertheless, the concentration of sericin dropped to 29.28±0.55 mg/mL and 23.96±0.28 mg/mL, respectively, after a longer extraction period of 45 and 60 minutes. The findings demonstrated that the sericin concentration started to gradually drop after 45 minutes and started to considerably drop after 60 minutes. It was proposed that the 30-minute interval in the survey at the time intervals yields the highest concentration of sericin. Similar to previous studies (Biswal *et al*., 2022; Saha *et al.*, 2019), 30 minutes was found to be the ideal duration for sericin extraction in this investigation. The autoclave's high pressure and temperature environment may have affected the sericin protein, causing protein chain separation and a decrease in molecular weight, which could account for the decreased sericin concentration after a longer extraction period. As the autoclaving extraction time increases, the molecular weight of protein will decrease. It is evident that meticulous management is necessary during the autoclave decalcification time, and that preserving the sericin structure is the preferred outcome. This demonstrated that protein denaturation and sericin molecular weight significantly decrease with extended duration (Biswal *et al*., 2022). As a result, it was demonstrated that the amount of sericin extracted is significantly influenced by the temperature and time of the extraction process.

FTIR Analysis

The C–O vibrations are linked to absorption maxima about 1238.075 cm^{-1} in the high temperature research of sericin. Water and a high hydroxyl group concentration cause sericin to absorb light, with a broad peak around 3273.57 cm^{-1}. Concurrently, observation bands at around 2363.8 cm^{-1} showed how the amino groups in the sericin structure's N–H vibrations were absorbed (Figure 2). FTIR spectral analysis is essential because it sheds light on how sericin solutions' gelation process is affected, which in turn affects the mechanical characteristics of sericin films. This knowledge is crucial for a variety of uses in food products containing sericin as well as for varying heat treatment techniques used in the creation of sericin materials including gels, coatings, films, fibers, etc.

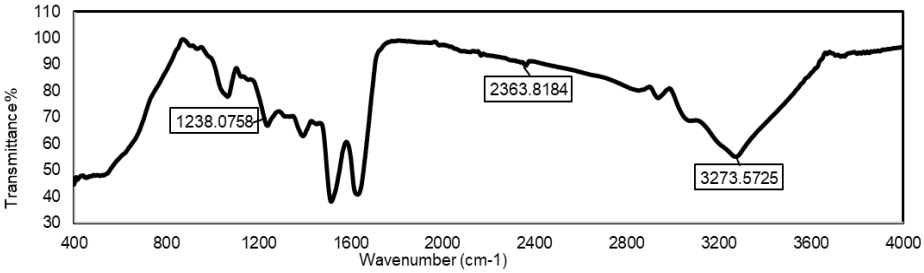


Figure 2. FTIR spectrum of sericin from high temperature extraction method

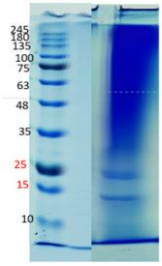


Figure 3. SDS-PAGE analysis of sericin samples

SDS – Page Analysis

The molecular weight distribution of the sericin sample was determined by the SDS-PAGE method, and the results are shown in Figure 3. Sericin extract of our group's sample showed diffuse bands in the molecular weight range of 13 kDa and 24 kDa. According to a number of studies, the extraction technique and source of sericin affect its molecular weight. Additionally, thermal extraction produces a faint band due to the decomposition of the polypeptide protein into smaller molecular-weight fractions (Sahu *et al*., 2016). According to research by Sahu *et al*, the molecular weight of the sericin was consistent with previous research using the boiling method. The evidence reported by Sprague *et al*, indicated that sercin is a mixture of at least 15 different polypeptide chains. However, because variable extraction and processing circumstances impact the complex components of sericin, the precise molecular weight of sericin has not been determined (Noosak et al., 2022). In addition, sericin also contains low-molecular-weight amino acids (< 20 kDa) that can be used in biological materials, health, cosmetics, and medicine production, while substances with high molecular weight are usually used as biomaterials, membranes, hydrogels, and complex polymers (Cao & Zhang, 2016).

Evaluation of the antioxidant activity of sericin

Figure 4's results demonstrate how sericin extract's capacity to scavenge radicals progressively increases with concentration. The capacity of sericin ABTS to scavenge free radicals is 30.22±0.72; 40.57±0.82; 49.91±0.51; 65.52±0.63; 77.63±0.74; and 88.22±0.38% at doses of 1, 2, 3, 4, 5, and 6 mg/100 mL. As a result, experimental findings demonstrated that the concentration at which sericin is extracted affects its capacity to scavenge free radicals (ABTS). The more sericin present, the more efficient it is at snaring free radicals. When proton radicals are scavenged, the typical absorption maximum of ABTS, a proton radical, lowers to 734 nm.

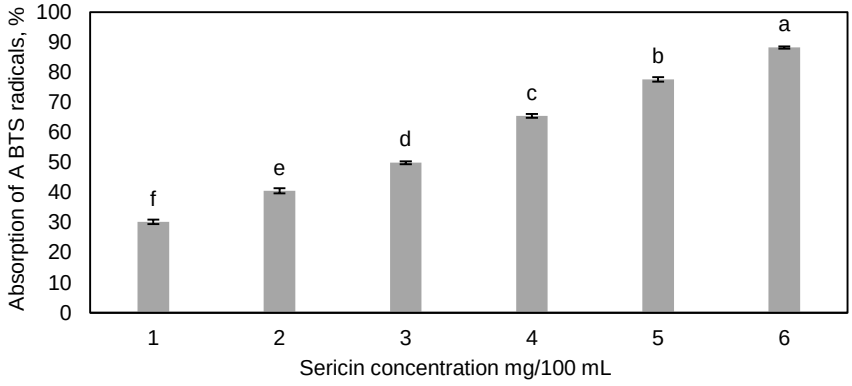


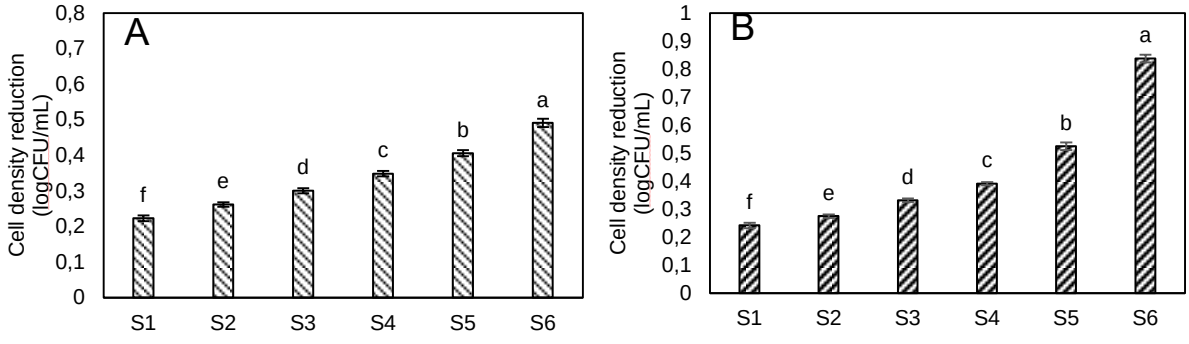
Figure 4. Antioxidant activity of sericin from high temperature extraction method

Error bars show SD derived from triplicate biological experiments. The letters show a significant ($p < 0.05$) difference between the mean

Both the radical scavenging capacity and the sericin extraction technique vary depending on the strain of silkworm cocoons. Since the structure and content of amino acids in proteins mostly determine their antioxidant capacity (Sangwong *et al*., 2016). The presence of sericin extract has been linked by some writers to anti-tyrosinase action, lipid peroxidation inhibition, and free radical neutralization (Miguel, Álvarez-López, 2020). The technique of extraction utilized affects the antioxidant ability of sericin protein.

Evaluation of antibacterial activity of sericin

The capacity to lower the *E. coli* bacteria's cell density from a low dilution of 0.56 mg sericin/mL medium to 1.03 mg sericin/mL medium was demonstrated in Figure 5A. This ability to lower the cell density also ranged from low to high, from 0.22 to 0.5 (log CFU/mL). This demonstrated that the ability to suppress *E. coli* cell density was positively correlated with sericin concentration. Stated differently, the capacity of E. coli to decrease cell density rose substantially with the amount of sericin present in the sample. This might be stated as follows: larger dilutions of sericin lead to higher concentrations of sericin, which in turn enhances the compound's ability to suppress *E. coli* growth.



Sample	S1	S2	S3	S4	S5	S6
mg sericin/mL	0.56	0.66	0.75	0.84	0.94	1.03

Figure 5. Cell density reduction in the various sericin concentrations in the A. *E. coli* and B. *S. aureus*

Error bars show SD derived from triplicate biological experiments. The letters show a significant ($p < 0.05$) difference between the mean

The capacity to decrease S*. aureus* bacterial cell density grew gradually when the concentration of sericin extract was 0.56, 0.66, 0.75, 0.84, 0.94, and 1.03 mg/mL medium, respectively, at 0.21, 0.24, 0.29, and 0.36 (log CFU/mL) (Figure 5B). Furthermore, there is a high ability to diminish the microbial density at 0.84 and 1.03 mg sericin/mL medium. In comparison to 0.56, 0.66, 0.75, and 0.84 mg sericin/mL medium, the findings of these sericin content ranged from 0.51 (log CFU/mL) to 0.79 (log CFU/mL) and demonstrated a two-fold reduction in microbial density. The results, as illustrated in Figure 5B, demonstrated that a higher dosage of sericin had a greater antibacterial impact on the tested gram-positive bacteria (*S. aureus*). According to some research, sericin's potent antibacterial qualities rely on its concentration, which influences its capacity to inhibit the growth of germs and lower the density of bacteria. The experiment described above is comparable to earlier research (Hong *et al*., 2019). Certain investigations also indicate that the degumming procedure had an impact on sericin's antibacterial qualities. Sericin from the water degumming technique has the capacity to suppress *S. aureus* bacteria that are found in food (Seo *et al*., 2023).

CONCLUSION

The results showed that the optimal sericin content of the distilled water solvent was 37.1 ± 0.72 mg/mL at the optimal temperature and time conditions of 120 °C for 30 minutes. Furthermore, the degumming method affects the sericin extraction process. To maximize sericin recovery, minimize protein damage, and protect the environment, extraction using the high temperature method is an effective choice

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KHẢO SÁT ĐẶC TÍNH VÀ KHẢ NĂNG TRÍCH LY SERICIN TỪ KÉN TẰM BẰNG NHIỆT ĐỘ CAO

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TÓM TẮT

Sericin là một loại polymer tự nhiên có trong kén của tằm *Bombyx mori*, liên kết với các phân tử fibroin thông qua liên kết hydro, chiếm 25-30% tổng trọng lượng kén tằm. Sericin là một protein ưa nước có khả năng hòa tan trong nước nóng. Để tách sericin khỏi fibroin hoặc kén tằm, kỹ thuật khử keo có thể được sử dụng và thực hiện bằng nhiều phương pháp khác nhau như sử dụng nhiệt, urê, axit hoặc kiềm. Trong đó, phương pháp chiết xuất bằng nước nóng là phổ biến nhất do những hạn chế đã đề cập đối với việc chiết xuất sericin bằng phương pháp khác. Cả thời gian và nhiệt độ đều đóng vai trò quan trọng trong lượng sericin được chiết xuất. Vì vậy, ảnh hưởng của phương pháp nhiệt độ cao đến việc chiết xuất sericin đã được tìm hiểu trong nghiên cứu này. Nhiệt độ dao động từ 80 đến 130°C và thời gian chiết thay đổi từ 15-30-45-60 phút đã được thí nghiệm. Người ta xác định rằng chiết xuất ở nhiệt độ 120°C trong 30 phút sẽ dẫn đến hàm lượng sericin cao nhất. Sau đó, sericin thu được từ phương pháp chiết này được phân tích đặc tính cấu trúc bậc hai bằng phương pháp quang phổ hồng ngoại biến đổi Fourier (FTIR), đặc tính kháng khuẩn và chống oxy hóa. Sự có mặt của nước và nồng độ cao của các nhóm hydroxyl góp phần tạo ra đỉnh hấp thụ rộng khoảng 3273.57 cm^{-1}. Đặc tính kháng khuẩn chống lại *Staphylococcus aureus* và *Escherichia coli* phụ thuộc vào nồng độ sericin, ảnh hưởng đến khả năng ngăn chặn vi sinh vật phát triển và làm giảm mật độ vi khuẩn. Đặc tính chống oxy hóa được xác định bằng phương pháp quét gốc tự do 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic) (ABTS) cho thấy hoạt tính chống oxy hóa mạnh của mẫu sericin thu được. Vì vậy, sericin từ kén tằm có tiềm năng ứng dụng cao trong bảo quản thực phẩm và một số lĩnh vực khác.

Từ khóa: Kháng khuẩn, *Bombyx mori*, chiết xuất, nhiệt độ cao, sericin.

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